Aalto-yliopisto
Insinööritieteiden
korkeakoulu

Iiro Lehtinen

Phosphorus Analysis from Drinking Water

Diplomityö, joka on jätetty opinnäytteenä tarkastettavaksi diplomi-insinöörin tutkintoa varten.

Espoossa 8.3.2017
Valvoja: Professori Riku Vahala
Ohjaaja: TkL Pirjo Rantanen

Aalto-yliopisto
Insinööritieteiden korkeakoulu

Aalto-yliopisto, PL 11000, 00076 AALTO
www.aalto.fi

Diplomityön tiivistelmä

Tekijä Iiro Lehtinen

Työn nimi Talousveden Fosforianalyysi

Koulutusohjelma Yhdyskunta- ja ympäristötekniikka

Pää-/sivuaine Vesi- ja ympäristötekniikka **Koodi** R3005

Työn valvoja Professori Riku Vahala

Työn ohjaaja TkL Pirjo Rantanen

Päivämäärä 8.3.2017 **Sivumäärä** 66 **Kieli** Englanti

Diplomityössä kehitettiin uusi menetelmä alhaisten kokonaisfosforipitoisuuksien analysoimiseksi talousvedestä. Uutta menetelmää hyödynnettiin analysoimalla pääkaupunkiseudun talousveden kokonaisfosforipitoisuus, jota verrattiin saman veden nitriitti- ja kokonaistyppipitoisuuksiin sekä orgaanisen hiilen pitoisuuksiin. Lisäksi diplomityössä arvioitiin fosforin vaikutusta nitrifikaatioon talousvesiverkostossa analysoimalla laboratoriomittakaavaisesta talousvesiverkostosta kerättyjä näytteitä.

Kokonaisfosforin analysoimiseen käytettävien yleisimpien standardimenetelmien määritysraja on 2 µg/l. Tehokkaat talousveden käsittelymenetelmät voivat kuitenkin tuottaa vettä, jonka kokonaisfosforipitoisuus on alle kyseisen määritysrajan. Työssä kehitetyllä menetelmällä talousvesinäytteen kokonaisfosforipitoisuutta väkevöitiin haihduttamalla suuremmaksi kuin standardimenetelmän (SFS EN ISO 6878) vaatima määritysraja. Lisäksi määritysrajaa laskettiin käyttämällä 10 cm kyvettiä standandardissa ohjeistetun 4 cm kyvetin sijasta. Menetelmästä kehitettiin mahdollisimman yksinkertainen työskentelyn helpottamiseksi sekä analyysin luotettavuuden varmistamiseksi. Talousveden kokonaisfosforipitoisuus kyettiin määrittämään luotettavasti suuremmista kuin 0.34 µg/l pitoisuuksista. Menetelmän luotettavuus tarkistettiin 2,5 µg/l kokonaisfosforia sisältävillä tunnetuilla näytteillä, joiden pitoisuuden keskiarvo oli 2.52 ± 0.122.

Pääkaupunkiseudun talousveden kokonaisfosforipitoisuus vaihteli välillä 0.6-2.3 µg/l ajanjaksolla 9.9.2014–2.6.2015. Vuodenajalla ei havaittu olevan vaikutusta kokonaisfosforipitoisuuteen. Kokonaisuudessaan kokonaisfosforipitoisuus verkostossa pysyi suhteellisen tasaisena etäisyyden ja vuodenajan suhteen, mikä viittasi verkoston vakiintuneeseen biologiseen tilaan. Kun verrattiin fosforipitoisuuksia verkostossa havaittuun nitriittiin, selkeää riippuvuutta ei kyetty todentamaan. Kokonaisfosforipitoisuuksien todettiin olevan niin matalia, että mikrobien käytettävissä oli fosforia noin 50-kertaisesti vähemmän kuin orgaanista ainetta tai kokonaistyppeä.

Laboratoriomittakaavan verkostosimulaattorista ajanjaksolla 23.4.2015–26.6.2016 kerätyistä vesinäytteistä analysoitiin kokonaisfosforipitoisuus käyttämällä standardimenetelmää (SFS EN ISO 6878). Menetelmän määritysrajaa laskettiin pitoisuuteen 0.68 µg/l käyttämällä 10 cm kyvettiä. Analyysitulokset osoittivat, että uusissa talousvesiverkostoissa fosforin puute voi estää tai hidastaa haitallisen nitrifikaation esiintymistä. Nitrifikaation ei kuitenkaan nähty lisääntyvän merkittävästi sen jälkeen, kun vedessä esiintyi fosforia yli 5 µg/l.

Avainsanat Fosfori, Talousvesiverkosto, Kokonaisfosfori, Menetelmän Kehitys

Aalto University
School of Engineering

Aalto University, P.O. BOX 11000, 00076 AALTO
www.aalto.fi

Abstract of master's thesis

Author Iiro Lehtinen

Title of thesis Phosphorus Analysis from Drinking Water

Degree programme Yhdyskunta- ja ympäristötekniikka

Major/minor Vesi- ja ympäristötekniikka **Code** R3005

Thesis supervisor Professor Riku Vahala

Thesis advisor Lic.Tech. Pirjo Rantanen

Date 8.3.2017 **Number of pages** 66 **Language** English

A novel method for analysing low total phosphorus concentrations from drinking water samples was developed. The new method was utilized in analysing the total phosphorus concentration of water samples collected from the Helsinki metropolitan regions drinking water distribution system (DWDS). The total phosphorus concentration was compared to corresponding samples nitrite- and total nitrogen concentrations as well as total organic carbon concentrations. Furthermore, the effect of phosphorus on nitrification occurrence in DWDSs was studied from water samples collected from a laboratory scale DWDS.

Current standard methods are able to determine total phosphorus concentrations which are above 2 µg/l. However, efficient drinking water treatment processes are able to reduce phosphorus concentrations below the 2 µg/l limit. The novel method was used to preconcentrate total phosphorus through evaporation. This raised the total phosphorus concentration above the limit required by the standard method SFS EN ISO 6878. Furthermore, the quantification limit was lowered by utilizing a 10 optical cell instead of a 4 cm. The method was developed to be as simple as possible, in order to ensure easy methodology and good reliability. The novel method was deemed to reliably determine total phosphorus concentrations above 0.34 µg/l from drinking water samples. The reliability was ensured by 2.5 µg/l known concentration total phosphorus analysis, that showed an average of 2.52 ± 0.122.

The total phosphorus concentration in the Helsinki metropolitan region ranged between 0.6-2.3 µg/l during 9.9.2014-2.6.2015. The total phosphorus concentration in the bulk water did not show any relationship to seasonality. The total phosphorus concentration remained fairly even in the DWDS, which indicated that the microbial growth had reached a mature state. When comparing the total phosphorus to the observed nitrite in the DWDS, no clear connection was observed. The total phosphorus concentration was determined to be roughly 50 times less than total organic carbon or total nitrogen in the DWDS.

Water samples collected from the laboratory scale DWDS during a period of 23.4.2015–26.6.2016 were analysed with the standard method SFS EN ISO 6878. The quantification limit was lowered to 0.68 by using a 10 cm optical cell. The analysis suggested that in young DWDSs, the lack of sufficient phosphorus could inhibit or delay unwanted nitrification occurrence. However, once sufficient phosphorus (5 µg /l) was available, nitrification was not seen to increase with increasing phosphorus concentration.

Keywords Phosphorus, DWDS, Total Phosphorus, Method Development

Foreword

What brought me to approach this thesis topic was my interest towards laboratory work, which has always fascinated me with its capability to bridge theory to practice. Furthermore, providing information that contributes to the safe keeping of the most precious natural resource humanity will ever possess, gave me motivation in no short supply.

I am grateful for my supervisor Professor Riku Vahala and tutor Pirjo Rantanen for providing me with this opportunity to take part in the ongoing drinking water distribution system research project. On top of that, the extraordinary support I received from my tutor truly was key in completing this thesis.

Although occasionally very long days had to be worked in the water laboratory, they were made ever so enjoyable by the support and guidance provided by Aino Peltola, Ari Järvinen, and Marina Sushko. With a big smile I also thank my friends and colleagues who have kept the work atmosphere light and forward going.

This thesis would have not been possible without the funding provided by the Maa- ja Vesitekniikan Tukisäätiö, that works to uphold the basic human right that should be available to all.

Lastly I would like to impart my deep gratitude towards my parents and brothers, who have provided me great comfort and stability.

Espoo 28.2.2017

Iiro Lehtinen

Table of Contents

Abbreviations

RO	Reverse Osmosis
TOC	Total Organic Carbon
AOC	Assimilable Organic Carbon
PE	Polyethene
PVC	Polyvinyl Chloride
MAP	Microbially Available Phosphorus
TP	Total Phosphorus
WTP	Water Treatment Plant
Calcareous fur	Precipitated calcium carbonate, often found on boiler surfaces
DWDS	Drinking Water Distribution System
Anthropogenic	Human Origin or Influence
Iron Tubercles	Crystallized form of Iron Rust $Fe(OH)_3$

1 Introduction

Centralized water production and its delivery trough a distribution network have been instrumental in allowing for healthy population growth of urban areas (Lee et al. 2005). Currently, using efficient water treatment methods, production of high quality drinking water is possible from almost any source of water. The delivery of water through centralized distribution systems has emerged as one of the most significant task of the water supply management (Katko 2013).

In addition to having problems related to their physical integrity, drinking water distribution systems (DWDSs) have often been seen to attract water quality degradation due to microbial growth. As of late, Miettinen et al. (1997) noticed that in certain types of water, including Finnish, the microbial growth in DWDS was controlled by the amount of phosphorus available for microbial growth.

This remark gives emphasis to the need for understanding the complex interactions of biological, chemical and physical events taking place beneath our feet. Quite interestingly, in terms of the total phosphorus contained in drinking water, the most utilized methods are often not able to report the actual concentration due to their methodological limitations. Thus the development of an easy to use and accurate analysis tool for total phosphorus, would allow water engineers to make decisions based on information that is more relevant.

The aim of this master's thesis was to research whether through the development of a previously conceived laboratory method of total phosphorus determination, it could be used to produce accurate and precise data on water quality changes in DWDSs. Testing focused on establishing the lower boundary total phosphorus concentration at which the analysis method could be used reliably. Along the testing of the analysis method, the results were used to study and discuss possible interactions of phosphorus in DWDS´s.

Additionally, the development process included the objective of analyzing water samples acquired from a pilot DWDS operated at the water engineering laboratory of Aalto University, and from the DWDS of the local municipal water utility. These analyses served as both testing of the novel methods under research, and as a source to provide novel information on DWDS system dynamics in terms of total phosphorus.

The fundamental aspect of the method under study focuced on preconcentrating the phosphorus contained in drinking water samples for total phosphorus analysis. The thinking behind the development was that by evaporating samples, the majority of the phosphorus contained in the water could be reduced into solid precipitate form. The solid precipitate, which in majority consist of calcium carbonate ($CaCO_3$), would contain the bound phosphorus, which could then be dissolved by utilizing a strong acid. The evaporation would bring the concentration per sample volume high enough for determination. Then by utilizing the molybdate colorimetric method SFS EN ISO 6878, total phosphorus could be determined. Due to the preconcentration and the possible undetected fraction of phosphorus, the actual sample concentration would be an unknown fraction of the real concentration. In order to reliably determine the actual concentration of total phosphorus found in water samples, the resulting yield concentration should consistent and significantly be close to the theoretical 100 %.

Furthermore, after establishing the range at which total phosphorus concentrations could exist in the studied drinking water, amendments to the original novel method plan were made. If the estimation of the amount of total phosphorus in the drinking water showed it to be sufficiently high, it could be enough to increases its concentration above the reliable quantification limit of the standard method. The key aspect would be that of that the phosphorus would still be contained in the liquid phase, thus avoiding the need for strong solvent utilization.

The literature review of Chapter 2 aims to establish the current knowledge on phosphorus interactions with the water cycle of earth. Furthermore, the chapter discusses the various aspects and components involved in the functioning of DWDS. Along the review, the significance of phosphorus to each component is presented. In addition, the relatively few different methods for analyzing phosphorus from raw or drinking water samples are presented. At the end, the fundamentals of the novel method development of this thesis are presented.

The materials and methods Chapter 3 provides information on the multiple sources of results that were a part of this thesis. This includes descriptions of a pilot DWDSs operated at the water laboratory and the Helsinki metropolitan regions DWDS, that was used to collect water samples. Additionally, the methods for testing and evaluating the functionality of the novel methods are presented.

Chapter 4 presents the results of this thesis. The results aim to present two main objectives. First, the results of the total phosphorus analyses from several water sources are presented. The results are then used to produce a range of total phosphorus concentration that could exist in the Helsinki regions drinking water. In addition, some results were obtained for discussion on the dynamics of phosphorus in DWDSs. Furthermore, the reliability and the degree of success on the usability of the novel method for total phosphorus determination is presented. Additionally, unpublished data from DWDS nitrification is used to minor extent to look at any possible relationship between the two.

The final Chapter 5 concludes the thesis` findings, and suggests possible research steps to further validate the novel method. The thesis overall served as major learning agenda in terms of both data analysis and laboratory methodology. The results presented in this thesis deepen the current information on drinking water phosphorus, as well as add information to previously suspected mechanics of DWDSs. Hopefully it will also work as a platform for further studies to benefit the field of water engineering.

2 Literature review

2.1 Phosphorus

In nature, phosphorus can be found as inorganic phosphates or bound to organic material. Organic phosphorus is found in organic structures like phospholipids, phosphoamides and sugar phosphates (Turner et al. 2005). The abundance of inorganic phosphates and organic phosphorus varies in different aquatic ecosystems, but it has been suggested that they could exist in equal proportions (Turner et al. 2005) Some the most common forms of inorganic phosphates are orthophophates such as HPO_4^{2-} and H_2PO4^- (Lehtola 2002).

All phosphorus on earth is either in particulate or soluble form (Lehtola 2002). Soluble phosphorus consists of inorganic polyphosphates, orthophosphates and dissolved organic phosphates (Lehtola 2002). However, the solubility of phosphorus in water is very low generally, depending on their molecular compound form. Solubility coefficients in water (20 °C) for $Ca_3(PO_4)_2$ and $Mg_3(PO_4)_2$ are 0.002 and 0.00026 (g/100g) respectively, whereas for example, the solubility of $Ca(NO_3)_2$ and $Mg(NO_3)_2$ in the same conditions are 121.2 and 69.5 (g/100g) respectively. Particulate phosphorus can be encountered as precipitates of phosphorus bound to organic material, crystalline minerals and reaction products with cations such as Fe^{2+}, Al^{3+} or Ca^{2+} (Lehtola 2002). Reactions with such cations may occur easily as orthophosphate carries normally a negative charge of either -1,-2 or -3 depending on environmental conditions. The non-ionic form of phosphate is phosphoric acid (H_3PO_4).

Phosphorus does not exist in its elemental form or in a gaseous phase in nature (UMN 2009). The form and distribution of phosphates is closely related to the pH of the surrounding environment (Werner 1996, Lehtola 2002). As can be seen from figure 1, in neutral conditions HPO^{2-}_4 and $H_2PO^-_4$ exist roughly in equal proportions, whereas when moving towards more acidic conditions the $H_2PO^-_4$ form becomes more prevalent (Werner 1996).

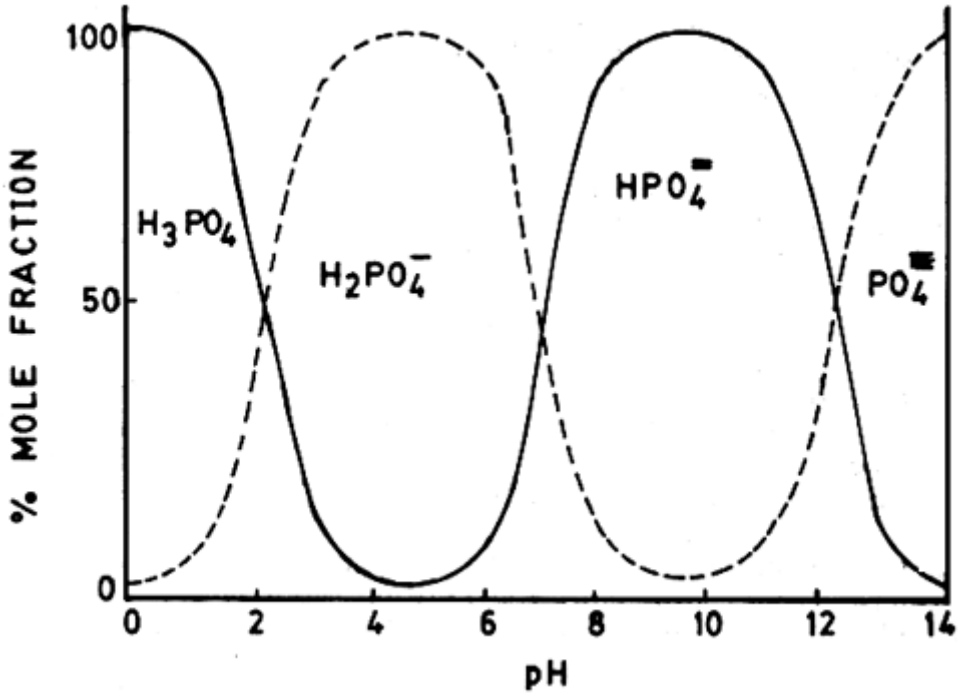


Figure 1. Relationship of pH and phosphate speciation (FAO 1987).

2.2 Phosphorus cycle in nature

The majority of phosphorus distributed in terrestrial ecosystems currently has and is being produced by the weathering of minerals, of which the most common is the apatite group $Ca_5(PO_4)_3(OH,F,Cl)$ (Schlesinger 2013). In terrestrial ecosystems, inorganic phosphorus is incorporated into organic material by plant uptake (figure 2). Roughly 500 x 10^{12} g of phosphorus is contained within the current land vegetation and 66000 x 10^{12} g is estimated to be bound in the soil (Schlesinger 2013). For this, Schlensinger 2013 has suggested a yearly 0.5 % turnover of phosphorus between soil and land vegetation. Turnover signifies the rate at which phosphorus is turned from particulate or organic form to inorganic phosphate (Withers 2008). Weathering causes the phosphorus bound to soil particles to eventually be carried towards larger waterbodies through waterways. As such pathways, rivers are currently moving the largest share of the phosphorus flux (21 x 10^{12} g/yr), whereas soil dust and seaspray contribute only 1 x 10^{12} g/yr (Smil 2002). Furthermore, phosphorus flows out to the seas, where it will support marine ecosystems with a roughly 90 % turnover per year in the surface layer of the sea (Schlensinger 2013). Eventually the phosphorus will be deposited into the ocean floor sediments, where approximately 7400 x 10^{15} g is currently stored. Per year, roughly the same amount of phosphorus that is deposited to the sediments is brought in by the river flux. The phosphorus cycle is completed as the ocean floors move according to the tectonic plate movements, thus raising new mineral deposits above sea level (Schlensinger 2013).

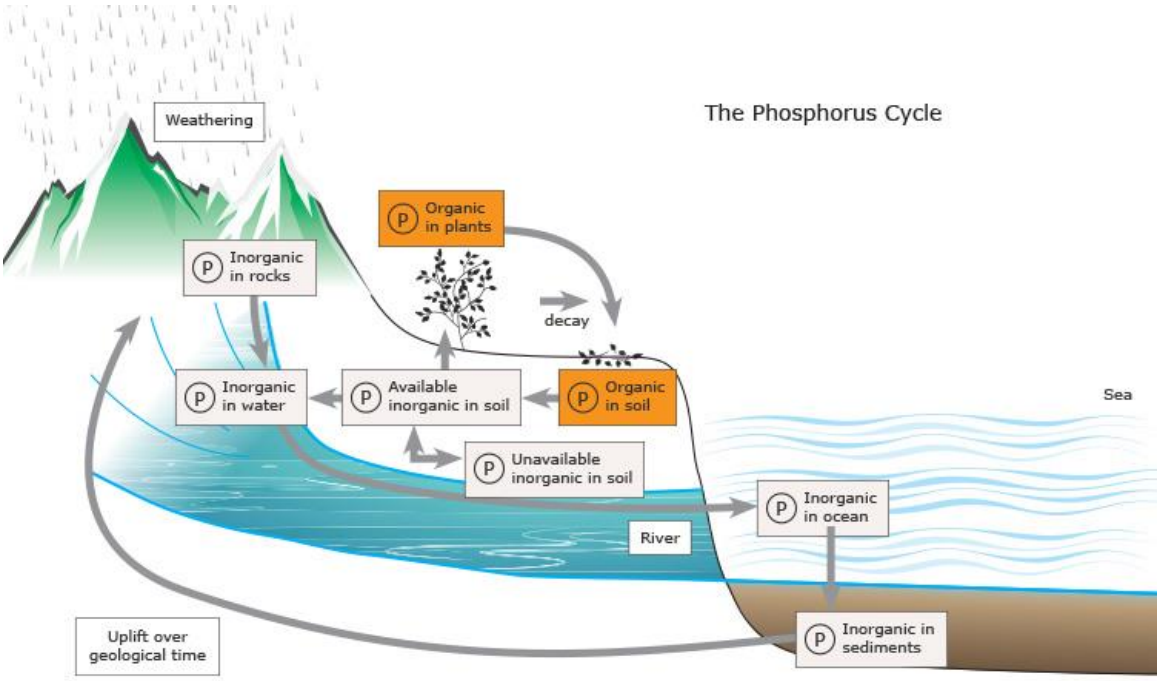


Figure 2. Phosphorus cycle (University of Waikato 2013).

In addition to the natural cycle of phosphorus, the input of anthropogenically derived phosphorus from fertilizer use and wastewater has caused significant changes to the natural phosphorus cycle. Bennett et al. 2001 estimates that the increase of phosphorus storage in terrestrial and freshwater ecosystems is more than 75 % greater compared to the preindustrial amounts. However, the latest trends have shown that in developed countries the amount of anthropogenically caused phosphorus storage to sediments has been decreasing. Coincidently reductions in the eutrophication of freshwater bodies due to improved wastewater treatment and fertilizer management have been seen (Bennett et al. 2001, EEA 2003).

2.3 Phosphorus as a nutrient for microbial growth

Microbes, as all other forms of life on earth, require phosphorus, nitrogen and organic carbon as macronutrients for their survival. Phosphorus is required for a wide range of biological functions, and can be found as a part of a variety of molecular structures such as DNA, RNA, cell structures and ATP. (Lehtola 2002, Rubulis et al. 2007). For example, exactly one P atom is attached to each DNA and RNA nucleotide (Stal et al. 2016).

The ratio between carbon, nitrogen and phosphorus needed for optimal growth in oceanic water for phytoplankton was suggested to be approximately 100:15:1 by oceanographer A.C Redfield in 1934. Loladze and Elser 2011, who suggested that the ratio for the need of P and N was caused by proteins to rRNA ratio of microbes, and supported the established Redfield ratio, furthered this theory. More recently, a publication by EPA (1994) suggested the ratio

to be 100:10:1. Environmental conditions, such as the availability of the nutrients, temperature and microbial specie have been seen to affect the actual ratio of carbon and phosphorus found in microbes (Martiny et al. 2013).

All organisms are able to convert organically bound phosphorus to inorganic phosphate by using alkaline phosphatase enzymes, although only some microbes and fungi can perform this conversion as an extracellular process (Lehtola 2002). Alkaline phosphatase is secreted when phosphorus deficiency occurs within the cell, and a high affinity transport system is used to transport phosphates against the surrounding concentration through the cell membrane (Lehtola 2002). Furthermore, the fraction of phosphorus that is in a soluble inorganic phosphate form can be utilized in its entirety by microbes (Lehtola 2002). During times of low phosphorus availability, some heterotrophic bacteria can store excess polyphosphates in granules within the cell for later utilization (Vadstein 2000, Seviour et al. 2010). When phosphorus is available in sufficient amounts in the surrounding environment, a low affinity transport system is used to transport phosphates through the cell membrane of bacteria (Lehtola 2002).

2.4 Phosphorus in freshwater

2.4.1 Phosphorus in raw water

In majority of the world countries drinking water is produced from either groundwater, fresh surface water or desalinated seawater. In terms of drinking water quality, the used raw water source can have a major impact on the required treatment methods. Groundwater has been noted to generally contain less of both dissolved and particulate matter as well as a lower initial bacterial count compared to surface water (Grey 2005). Phosphorus is introduced into these waterbodies by natural processes or by anthropogenic causes such as fertilization, treated wastewater effluent and runoff from impervious surfaces. The majority of the inorganic phosphates entering lakes, precipitate with metallic cations to insoluble forms and get stored in the lake sediments (Schlensinger 2013). However, under completely natural conditions, phosphorus inputs to freshwater waterbodies are small (Withers et al. 2008, Schlensinger 2013).

The amount of phosphorus found in raw water can be highly affected by the surrounding land use type, flow conditions and seasonality (Withers et al. 2008). Wastewater effluents, urban surface runoff and agricultural runoff can contain up to 20 mg/l, 0.4 mg/l and 10 mg/l respectively as total phosphorus concentration (Withers et al. 2008). Finnish lake waters have been shown to contain phosphorus in the range of 5-120 µg P/l with the average roughly at 10-15 µg P/l as total phosphorus (VHVSY 2004, Ympäristö.fi 2013). If phosphorus limited waterbodies contain more than 30 µg P/l of total phosphorus, they are considered to be eutrophicated according to the OECD trophic levels (Anderson et al. 2014).

2.4.2 Phosphorus removal from drinking water

Drinking water treatment methods aim to produce safe and good quality water from raw water (RIL 124-1 2003). Any unwanted substances in the raw water need to be removed due to their direct and indirect adverse effects for human health. In addition to many toxic or pathogenic substances that might exist in raw water, reductions in macronutrients such as carbon, nitrogen and phosphorus are often required in order to reduce the proliferation of microbes after treatment. On the other hand, phosphorus and its common phosphate forms have not been found to be harmful to humans in the concentrations encountered in normal raw waters (RIL 124-1 2003). As such, no regulations are generally placed on phosphorus concentration in the effluent drinking water.

However, phosphorus is often removed efficiently from raw water during normal water treatment plant (WTP) processes aiming for reductions in organic carbon (Lehtola 2002, Polanska et al. 2005). Both of the more readily available fractions of carbon and phosphorus for microbes, meaning the assimilable organic carbon (AOC) and microbially available phosphorus (MAP), have been shown to get effectively removed during drinking water purification.

Lehtola (2002) reported effective reductions of AOC and MAP using traditional methods of coagulation using iron- and alumn sulphates (equations 1 and 2) and soil filtration. Furthermore, Lehtola reported that activated carbon filtration reduces both AOC and MAP effectively. However, the same study noted also that ozonation might break down larger molecules of organic carbon and phosphate and result in increased AOC and MAP concentrations. This could possibly lead to increased microbial growth. Polanska et al. (2005) on the contrary saw a reduction in MAP concentration when using ozonation as a part of a water treatment processes. Okabe et al. (2002) noted that more advanced water purification methods, such as biological activated carbon filtration and biofilm membrane filtration also resulted in efficient reductions of both AOC and phosphate (PO_4^{3-}).

$$Al^{3+} + H_nPO_4^{3-n} \leftrightarrow AlPO_4 + nH^+ \quad (1)$$

$$Fe^{3+} + H_nPO_4^{3-n} \leftrightarrow FePO_4 + nH^+ \quad (2)$$

Reduction percentages are rarely monitored in terms of phosphorus concentration in WTPs, whereas the concentration in the effluent water is commonly better known. Juhna et al. (2007) and Lehtola et al. (2004) reported total phosphorus concentrations of tap water < 10 µg P/l and < 2 µg P/l in Riga, Latvia and Kuopio, Finland respectively. Helsinki Region Environmental Service Authority (HSY) reported total phosphorus concentrations ranging between <10 ug/l and 16 ug/l (unpublished HSY data 2010-2016). Thus, even though the concentration of phosphorus and other essential nutrient can be significantly reduced during water treatment, some amounts of them will remain in drinking water to support possible microbial growth (LeChevallier 1990).

2.4.3 Disinfection of drinking water

Traditionally the unwanted microbial growth in drinking water systems has been controlled with the addition of chlorine based disinfectants, such as chlorine dioxide, sodium hypochlorite or chloramines, to the effluent drinking water (Fang et al. 2010). The effectiveness of chlorination depends on the concentration and type of the used chlorine solution, contact time, water temperature and the targeted microbial species (Lehtola 2002). After addition, chlorine based disinfectants remain in the water as residual chlorine which will continually react with organic material and effectively inhibit the regrowth of microbes (van der Kooij et al. 2014). Typical chlorine concentrations for example in the Helsinki metropolitan region drinking waters vary between 0.03 and 0.3 mg/l (HSY 2016). However, there are negative aspects related to the use of chlorination as the disinfection and growth control method of drinking water. For example, the development of microbial resistance to chlorine based disinfectants has been observed to develop in biofilms over time (LeChevallier et al. 1990), and the possibility of forming harmful concentrations of disinfection by products (DBPs) (Wahman et al. 2014). DBPs can be formed in harmful concentrations when chlorine reacts with organic material and inorganic material (Richardson et al. 2014).

In order to reduce the formation of these carcinogenic DBPs, the use of chloramines over other free chlorine compounds has become more popular in recent years (Wahman et al. 2014). Chloramine reduces the DBP problem by being more stable and by having a longer dissipation time in the drinking water distribution system (DWDS) (Vikesland et al. 2001). Additionally, chloramines have been suggested to better protrude into biofilms, resulting in better disinfection efficiency (LeChevallier et al. 1988). Fang et al (2010) confirmed this suggestion when comparing the disinfection efficacy of monochloramine and free chlorine. They noticed that while the addition of 300 µg/l of phosphate phosphorus to a bioreactor increased the biofilm cell growth, it also resulted in a higher reduction of the biofilm cell counts when using monochloramine (0.5 and 2.0 mg/l) as the disinfectant. Furthermore, the same study found that the relative efficiency of both disinfectants was increased with the addition of phosphorus, possibly due to phosphorus induced structural changes to the biofilm matrix.

Furthermore, as the DWDS ages, the dissipation rate off chlorine based disinfectants might increase due to pipe wall biofilm dissipation (Ekeng et al. 2011). LeChevallier et al. (1988) reports that free residual chlorine concentrations below 1-5 mg/l might not be adequate to prevent biofilm growth in DWDSs. For this reason, the investigation of microbial growth dynamics and other possible control methods against harmful microbial growth are of great interest.

2.5 Drinking water distribution systems

2.5.1 Structure and relation to phosphorus

DWDSs vary tremendously according to population densities and environmental conditions. Structurally DWDSs are considered to consist of all the components that are involved in the

delivery of potable and non-potable water to consumers, industry and fire utilities (Committee on Public Water Supply Distribution Systems: Assessing and Reducing Risks 2006). This includes infrastructure such as pipes, storage tanks, valves and pumps (Committee on Public Water Supply Distribution Systems: Assessing and Reducing Risks 2006). Large water mains are commonly used to transport large quantities of water from the water treatment plant to a water storage tank (Gray 2005). From there on, distribution pipelines are used to deliver the water to the consumer, commonly forming a looping network to improve reliability and flow (Gray 2005). Commonly used pipe materials include polyvinyl chloride (PVC), polyethylene (PEH), ductile iron and concrete, steel and copper (Katko 2013). Most pipe types do have unique problems, for example, ductile iron pipes tend to form iron tubercles which have been shown to provide growth platforms for microbes in DWDSs (Katko 2013, Chen et al. 2013). In part, this might have induced a change in municipal design so that the majority of new water pipes installed today in Finland are made of durable and flexible plastics (Katko 2013). Furthermore, DWDSs are considered to be one of the largest fractions of the water utilities` capital, and as such require significant financial investments to establish and upkeep. It is estimated that roughly 80 % of the cost of operation in Finnish water utilities, is related to DWDSs (Katko 2013). Thus the upkeep and maintenance of DWDSs should be considered from the aspect of investment value protection, as well as the public health aspect. This poses a multitude of challenges, to which the ultimate goal is the continuous delivery of safe and good quality drinking water to the consumer (RIL 124-1 2003).

In terms of structure, DWDS can influence nutrient concentrations in water through various ways. Figure 3 shows a corroded drinking water pipe, which has accumulated significant amounts of inorganic deposits. Chen et al. (2013) noticed that these deposits can leach increased amounts of nutrients to the water, most likely resulting in higher bacterial growth rates. Studies have noted that the addition of phosphate, naturally or by human addition, can reduce and inhibit the corrosion and scaling of cast iron and copper pipes (Comber et al. 2010). Additionally, Chen et al. (2013) noticed that the stagnation of drinking water during low flow conditions in the distribution system triggered a release of nutrients including phosphorus from iron tubercles and deposits. Furthermore, new plastic pipes have been shown to leach phosphorus into drinking water until the pipes have been properly flushed (Lehtola et al. 2004).

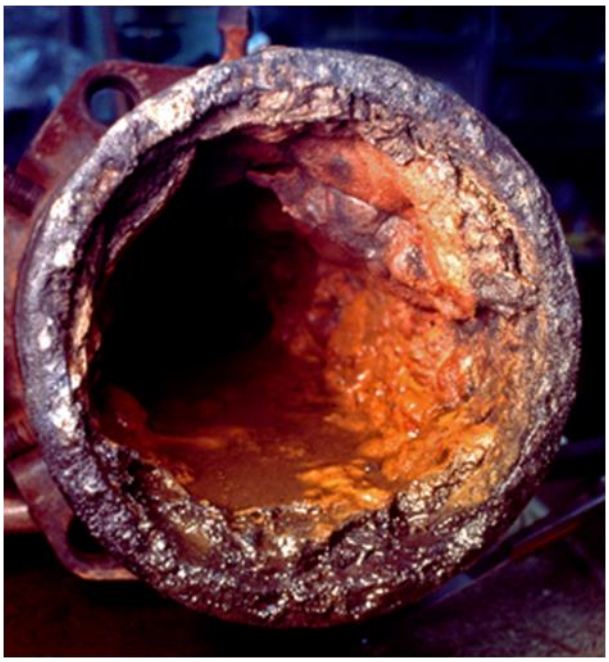


Figure 3. Biofilm growth in a drinking water supply pipe (ProEconomy 2015)

In general, due to biological and physiochemical processes occurring during water distribution, the water quality at the consumers tap is lower than it was at the water treatment plant (Liu et al. 2013). The constantly changing conditions of DWDS in terms of pH, hydraulic conditions and stagnation periods, pipe material, natural ageing and temperature all create unique physical and biological environments, where microbiological regrowth can occur (Rozej et al. 2015, Lee et al. 2005).

2.5.2 Microbial growth and species

The growth of microbes can induce bio-corrosion of pipe materials, nitrification and even lead to the proliferation of pathogenic bacteria (Fang et al. 2010, Okabe et al. 2002, and Liu et al. 2013). Additionally, the regrowth of both autotrophic and heterotrophic bacteria can significantly reduce the amount of dissolved oxygen in the drinking water, which for example makes it possible for microbes to reduce sulfides into hydrogen sulfide gas (Grey 2005). In terms of microbial growth, DWDS can be separated into four phases which are bulk water, biofilm, suspended solids and loose deposits (figure 4) (Liu et al. 2013). Biological stability i.e. the level of regrowth in water is dependent largely on the available nutrient levels such as AOC, phosphorus, nitrogen, magnesium, potassium as well as the physical properties of the water and residual chlorination level (Liu et al. 2013, van der Kooij et al.2014, Seviour et al. 2010). In terms of microbial growth, the different phases can function in widely different ways depending on the conditions, and thus it is important to investigate microbial growth in all phases to build a complete picture of the DWDS ecology and species.

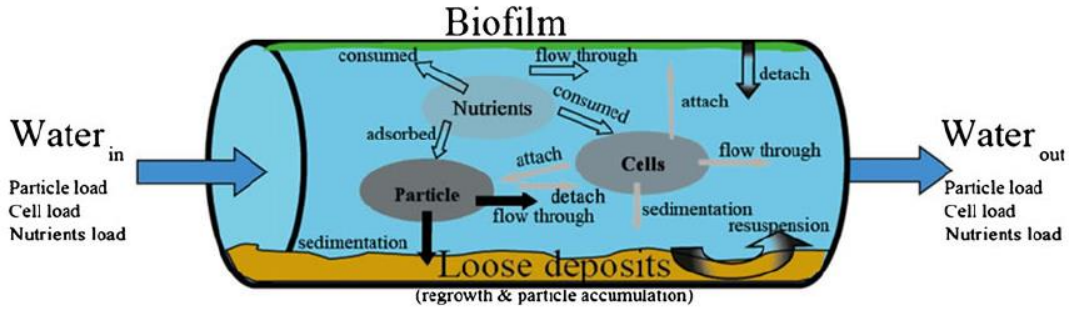


Figure 4. The four phases of DWDSs and their interaction pathways (Liu et al. 2013)

Several different types of microbes, including bacteria, protozoa, algae and fungi can inhabit the four phases of DWDSs. Furthermore, both heterotrophic and autotrophic bacteria do profilitate in the niches created within the DWDSs (Berry et al. 2006). For example, Martiny et al. (2003) studied the process of biological growth development in DWDSs, and found that in a three-year-old pilot DWDS species such as *Planctomyces*, *Acidobacterium* and *Pseudomonas* were abundant. Naturally, a part of the microbial biota in the DWDSs is pathogenic towards humans. These include species such as *Legionella* spp, *Helicobacter* spp. and *Pseudomonas aeruginosa*. (Fang et al. 2010, Berry et al. 2006). Studies have shown that pathogenic bacteria can proliferate well in most of the four phases found in DWDSs, and furthermore be protected against disinfection by other non-harmful microbial growth (Liu et al. 2013). However, it should be noted that the majority of waterborne outbreaks occur due to water contamination through flooding or surface runoff in disinfected water supply systems (Miettinen et al. 2001).

Nitrification and ammonia oxidizing bacteria (AOB) have received more attention in regards to their activity in DWDSs due to the adverse effects of nitrites and nitrates. The occurrence of nitrification in the DWDSs has been well established at least in the US, Canada, Europe and Australia (Wahman et al. 2014). Previous studies have identified species such as *Nitrosomonas* spp. and *Nitrospira* spp. to be thriving in the biofilm and bulk water phases of several DWDSs (Wahman et al. 2014). In fact, Martiny et al. (2003) noted that after some 250 days of pilot DWDS biofilm growth, roughly 78 % of the microbial community was of a specie related to *Nitrospira*. However, the population started to diminish with the aging biofilm until it was no longer detected after 571 days of growth. This describes well the dynamic community structure of DWDSs.

Interestingly the mechanisms of phosphorus uptake vary between different microbes (Jansson 1988). Bacteria for example use two different transport systems to aquire phosphorus from the surrounding environment (Lehtola 2002). The Pi system is used when phosphorus is sufficiently present, but in situations of low phosphorus availability a more effective system called the Pst system is turned on (Lehtola 2002). This induces the production of alkaline phosphatase as described in chapter 2.3 (Lehtola 2002). On the otherhand the phosphorus uptake process of algae and fungi are not still not known in such detail as with bacteria (Beever and Burns 1981). However, some studies have discovered that bacteria tend to be more efficient in phosphorus uptake in comparison to algal species (Jansson 1988). Furthermore, some studies have speculated that there could exists a symbiotic relationship between algae and bacteria in terms of phosphate uptake rates (Tittel 2012)

In addition to the biological and physiochemical characteristics of DWDSs, the disinfection methods used can have a significant impact on the microbiological community diversity (Berry et al. 2006). For example, the addition of chloramine to drinking water as a disinfectant has been shown to result in higher growth rates of ammonia oxidizing bacteria (AOB) (Wahman et al. 2014).

2.5.3 Biofilms

It has been suggested that the majority of microbial growth in DWDSs occurs primarily in the biofilm phase (Servais et al. 2004). This biomass is commonly found growing on the inner surfaces of the pipes (van der Kooij 2014). Essentially biofilms are a collection of bacteria, which are surrounded by an exopolysaccharide layer (EPS). This layer is secreted by the bacteria for the purpose of attachment, nutrient storage and protection (van der Kooij et al. 2014). For example, the protecting layer of biofilms has been shown to better resist the bactericidal effects of chlorine in comparison to free-floating growth (Butterfield et al. 2002). Generally biofilm formation occurs in several stages, where first a micro-organism adheres to a surface material, then starts secreting the EPS layer and finally multiplies continuously leading to the release of new bacteria (figure 5) (van der Kooij et al. 2014). Berry et al. 2006 suggests that it may take several years of biofilm development until a steady state in terms of microbial species and growth rate is achieved. During this time the structural development of the biofilm phase can vary greatly. Biofilms have been noted to initially form as a thin layer just a few micrometers thick, and from there to start, forming thicker clusters colonies (Martiny et al. 2003). Additionally, speciation can vary during the development of the biofilm, as new niches for growth are formed and changed (Martiny et al. 2003) For example as the thickness of the biofilm increases, anaerobic zones can be created within the biofilm and speciation in the different layers may occur.

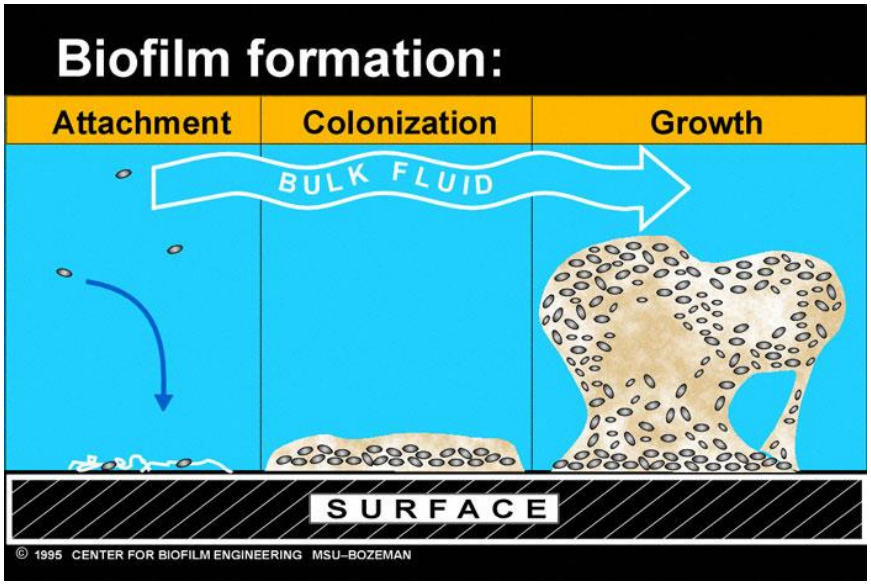


Figure 5. The growth phases of a microbial biofilm (Onisei et al. 2008).

Biofilm development in DWDSs is dependent on several factors such as temperature, pipe material roughness, flow conditions, chlorination, nutrient availability and corrosion products (Okabe et al 2002, Lehtola et al. 2004, van der Kooij et al. 2014). Traditionally it has been thought that carbon and especially AOC is the main limiting nutrient in drinking water systems. However, if the natural waters contain large amounts of AOC, other nutrients such as phosphorus have been proposed to be controlling the microbial growth in DWDSs biofilms (Lehtola et al. 2002). As such, phosphorus has been shown to be a limiting nutrient in drinking water at least in countries such as Finland, Japan and South-Korea (Miettinen et al. 1997, Kasahara et al. 2004 and Jang et al. 2012). Furthermore, phosphorus addition has been shown to result in the changing of the biofilm composition, as Fang et al. (2010) reported increasing cell count but decreasing EPS formation in the biofilm phase. Similarly, Kasahara et al. (2004) and Jang et al. (2012) reported one order of magnitude higher cell counts in biofilm densities, when adding phosphorus into drinking water.

However, microbial growth in DWDSs is a result of complex interactions. Vahala (2002) discussed the possibility that a low phosphorus concentration in the bulk water phase might not limit growth in the biofilm due to the recycling of nutrients from dead biofilm material. Furthermore, by reducing the corrosion of pipe materials with the addition of phosphate into DWDSs, reductions in the growth of heterotrophic bacteria have been observed, indicating that the physical and chemical properties of the DWDS might play an even more important role in controlling the microbial growth (Appenzeller et al. 2001). In addition, Batte et al. (2003) did not observe any increase or decrease in the biofilm cell counts, when testing the effect of phosphate addition on biofilms grown in Canadian drinking water. However, large scale experiments on the effects of phosphorus do sufficiently indicate that phosphorus in drinking water does play a major role in the growth dynamics of biofilms in DWDS in certain regions of the world (Lehtola et al. 2004)

2.5.4 Effects of nitrogen

The global nitrogen cycle is a fundamental nutrient cycle that supports all life on earth. Nitrogen is ubiquitous in all biospheres, and it has multiple pathways through which it can move from one phase to another. Nitrogen fixing bacteria in the soil are able to transform gaseous nitrogen into ammonia. Furthermore, commonly used nitrogen fertilizers introduce large amounts of nitrates into agricultural lands, from where due to high degree of leachability nitrogen is washed rapidly to larger waterbodies that can act as a drinking water supply. (Schlesinger 2013)

Because high concentrations of nitrate and nitrite can pose severe health hazards to human beings, their levels in drinking water are usually regulated by law (Keeney et al. 2001). If ammonium (NH_4) is found in drinking water in any form, a risk of nitrification occurring in the DWDS does exist (Wahman et al. 2014). Nitrification in drinking water is started first by AOB, which are autotrophic bacteria that obtain their energy for growth from nitrification and cell carbon from carbon dioxide (Seviour et al. 2010). AOB convert ammonia into nitrite (equation 3) which subsequently can be oxidized into nitrate (equation 4) by a few nitrite oxidizing bacteria (NOB) species (Wahman et al. 2014). Both steps release energy for microbial growth. Although nitrifying bacteria themselves are not known to be pathogenic, nitrification has been shown to effect drinking water quality by consuming available oxygen,

reducing pH and causing disinfectant depletion (Wahman et al. 2014). In order for AOB growth to be likely in DWDSs, conditions such as the availability of nitrogen (natural ammonium or chloramine), slow water velocities and biofilm formation are needed (Wahman et al. 2014). AOBs are commonly slower to grow than heterotrophic bacteria, due to the relatively small energy gains from the oxidation of ammonia (Wahman et al. 2014).

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad (3)$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \quad (4)$$

2.6 Drinking water analysis

2.6.1 Water quality

Drinking water quality is often regulated by national laws, which set maximum allowed limits to various substances commonly found in drinking water to ensure the safety of drinking water. For example, the World Health Organization (WHO) *provides Guidelines for Drinking Water Quality*, which can be used as a framework to establish case specific water safety plans (LeChevallier and Au 2004). Furthermore, the European Union requires through the Drinking Water Directive 98/83/EC, that its member countries provide clean and wholesome water for consumption (Drinking Water Directive 98/83/EC 1998). This directive mandates that the member countries must monitor and test 48 microbiological, chemical and indicator parameters. The directive uses own scientific committee and the the above mentioned WHO guidelines in general to establish the required quality standards (Drinking Water Directive 98/83/EC 1998). In order to monitor the fulfilling of the safe conditions, appropriate analytical methods have been developed for each parameter. Reliable and good methods of water analytics are efficient, accurate and reproducible. Common water quality analyses required in many countries include measurements of: TOC, nitrate, color, turbidity, odor, pH and a wide range of elemental substances.

2.6.2 Microbial water quality

In addition to the analysis of inorganic substances, the qualitative and quantitative determination of microbiological species is of paramount interest for maintaining high quality drinking water. Currently used methods for estimating the biological stability in any of the four phases in DWDS include heterotrophic plate counting (HPC), adenosine triphosphate (ATP) assay, flow cytometry method (FCM) and total cell counting (TCC) for example (Liu et al. 2013). Each method has their advantages and disadvantages, but as noted by Liu et al. (2013), it is strongly recommended that a combination of multiple appropriate methods should be used to increase reliability. Furthermore, the most reliable microbiological analyses determine both cell count and cell activity (Liu et al. 2013).

2.6.3 Phosphorus

When looking at phosphorus contained in drinking water, a commonly used parameter is the total phosphorus concentration. However, phosphorus, as mentioned before, does exists in multiple forms of particulate, dissolved, organically bound and inorganic. Each fraction behaves in their own way in terms of chemistry, which makes the accurate determination of phosphorus more challenging.

A standardized method of molybdate colorimetry, SFS EN ISO 6878, is commonly used in European water analyses when wanting to estimate the total phosphorus concentration in water. Peroxodisulphate is used as a reagent coupled with high temperature boiling to oxidize all possible organic phosphorus, regardless of its form in a sample, into soluble orthophosphate form. Following the oxidation, the orthophosphate reacts with acid molybdate and antimonyl tartrate to form an antimony phosphomolybdate complex (Lehtola 2002). Addition of ascorbic acid reduces the complex, resulting in the coloration of the orthophosphate. Measuring the absorbance of light passing through this sample with spectrophotometry will allow for the calculation of the concentration of orthophosphate present. The standard method can be used to analyze total phosphorus concentrations as low as 5 µg P/l, when using a spectrophotometric cuvette length of 1-5 cm. However, the accuracy of this method does suffer when samples contain high amounts of organic material for example. Furthermore Turner et al. (2005) noted that some organic phosphorus fractions might not react with the acid molybdate, thus remaining undetected.

The standard method (SFS EN ISO 6878) does include a method where with a solvent extraction in combination with the addition of ascorbic acid and acid molybdate the orthophosphate in a sample can be extracted into the solvent phase. The solvents used are hexan-1-ol and ethanol. The orthophosphate is then measured with spectrophotometry. The detection limit is about 0.5 ug/l for the method. However, without digestion, any organophosphorus compounds might not be extracted and thus remain undetected by the method.

Furthermore, when determining the portion of phosphorus that is readily available for utilization by micro-organisms, the phosphorus content can be analyzed as MAP (Lehtola 1999).This analysis is based on a microbial bioassay, which uses the correlation between microbial growth and phosphorus concentration to estimate the fraction of phosphorus readily available for microbes (Lehtola et al. 1999). The method uses the microbial specie of P. Fluorescens, which is allowed to grow in a nutrient solution, and further incubated and enumerated on R2A-agar spread plates (Lehtola 2002). The growth rate is estimated by colony counting. Under normal conditions, the share of MAP of total phosphorus can vary between 20 and 100 % (Lehtola et al. 1999). For example, Lehtola et al. (2004) reported MAP levels of 0.62-3.39 µg MAP/l in a Finnish drinking water system, while the total phosphorus concentration was reported to be < 2 µg P/l. The reported determination limit of the method is 0.08 µg P/l (Lehtola 2002). However, this method requires several days to complete the analysis, and it is often available in specialized laboratories only.

Currently used drinking water treatment methods are able to reduce the phosphorus content efficiently to minute concentrations. However, as previous research has shown, even additions as low as of 1-5 µg P/l of phosphorus have resulted in the increased growth of heterotrophic bacteria in DWDSs (Miettinen et al. 1997). Thus, there exists a need for the development of a more accurate phosphorus analysis method for below 5 µg P/l concentrations.

2.7 Novel total phosphorus determination methods

There does not exists much literature on any attempts on developing a new total phosphorus determination method. Mostly, literature shows that some novel methods have been tested for the determination of various fractions of phosphorus in water. The majority of the literature on drinking water analyses, report total phosphorus concentrations that are lower than the used method detection limit, which can limit the reliability of the studies that focus on phosphorus specifically.

Preconcentration of phosphorus fractions in water samples has been suggested as method of overcoming the low detection limit problems of the currently used techniques (Turner et al. 2005). Turner et al. has suggested that in order to concentrate the organic fraction of phosphorus, methods such as reverse osmosis, ultrafiltration, lyophilization, precipitation with lanthanum and ultraviolet photo-oxidation could be used. However, the majority of the methods described involve the utilization of expensive filter materials, complex chemistry, large sample quantities or the use of high power UV-lamps.

Even though the molybdate colorimetric method for total phosphorus has been noted to underestimate the organic phosphorus fraction and overestimate the inorganic faction, it is still used widely due to its relative simplicity, low cost and general accuracy (Turner et al. 2005). As such, developing new methods for phosphorus analyses with the molybdate colorimetry as the core could come with many benefits of the already established method. A novel method should aim to be simple in terms of practical execution, have low cost, take as little time as possible to complete and produce reliable results.

3 Materials and methods

3.1 Pilot drinking water distribution system

A pilot DWDS was run for 18 months (521 days) during the time period of 8.1.2015-28.6.2016, in order to study the effects of microbial growth on water quality. The pilot consisted of two identical parallel systems P1 and P2, which were run consistently throughout the study period (figures 6 and 7). The pilot system was located at Aalto University water laboratory facilities (Espoo, Finland). A 25 mm diameter polyethene tubing (Pipelife, Finland) was used as the piping with a total length of 22 m. The wall thickness of the pipe was 2.3 mm. Constant water circulation was upheld by 45 Watt Iwaki displacement pumps (Fukushima, Japan). The flow of the system was initially adjusted to 900 l/h until 26.2.2015, when the flow of both systems was adjusted to roughly 100 l/h. The flow was between laminar and turbulent in both systems, with Reynolds number roughly at 1700. Water consumption from a DWDS was simulated by emptying the system in one week intervals, and subsequently restarting with new inlet water. The pilot system space was kept at an even average temperature of 18 ±1 ° C. The bulk water (30 l) was contained in two tanks of 50 liters each. The system included online measuring of pressure to determine flow with Semiconductor MXP5050DP (Freescale, Japan) and temperature with pt-100/620S (Nokeval, Finland), located at the inlet of the DWDS. During the weekly inlet water change, the bulk water tanks and all equipment involved in the system were cleaned thoroughly and rinsed with reverse osmosis (RO) water so that only microbial growth from the pilot system itself would be able to proliferate. Prior to starting the pilot system, shock chlorination to all surfaces of the water tanks was performed. Additionally, the system was flushed for 24 hours with tap water and then with RO water for 22 days.

Sampling was performed weekly on both the pilot inlet water and outlet water. Outlet water samples were taken first, after it had circulated in the DWDS for the duration of one week. After changing the inlet water, it was allowed to circulate for 10 minutes in the DWDS before sampling. Samples were taken directly from the 30 liter water containers after thorough mixing. Duplicate samples were taken into 500 ml plastic bottles, and frozen for later analysis.

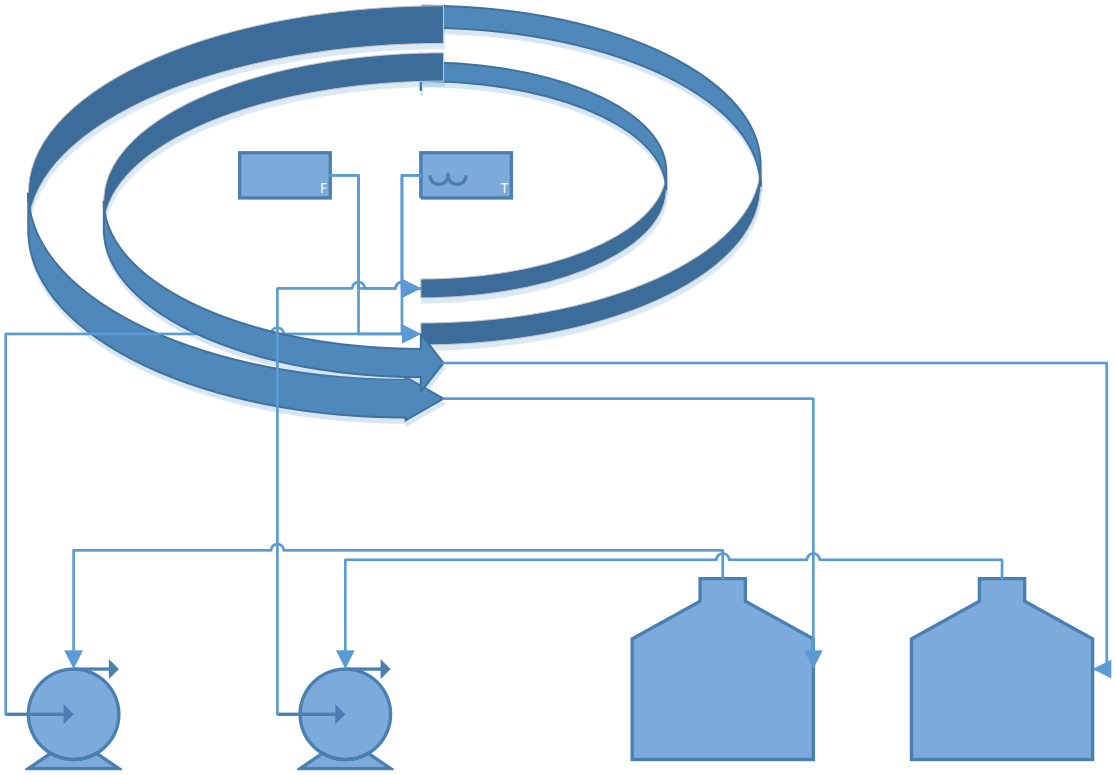


Figure 6. A schematic of the pilot DWDS with F = flow meter and T = temperature meter.

Figure 7. The used pilot DWDS setup.

3.2 Pilot inlet water

The inlet water (S1 and S2) used in the pilot was made from tap water that was produced by the local municipal water treatment utility (HSY). HSY services drinking water to roughly one million inhabitants of the capital region of Finland. The utility uses surface water from Lake Päijänne as its raw water supply, which is transported through a 120 km long tunnel to the treatment facility. The raw water is abstracted from the depth of 25 meters to maintain an even temperature throughout the year. The raw water is treated either at one of two drinking water treatment facilities located in Helsinki. Both WTPs use identical treatment methods. Raw water is first treated with chemical coagulant (iron III sulfate) to agitate the precipitation of organic material, which is then removed by gravity and sand filtration. Secondary treatment includes ozonation and addition of carbon dioxide for alkalinity control. Following this, the water is filtered through an active carbon filtration systems and disinfected with UV-light. Finally, chloramine is added to water and pH is corrected if necessary. The drinking water produced is distributed to the user via the DWDS in the metropolitan area. The DWDS is roughly 3000 km in total length, with an average age of 40 years (HSY 2015). The tap water used as the base for the pilot inlet water was taken from an outlet located in the water laboratory facility. The water quality parameters of the tap water before any spiking are presented in table 1.

Table 1. Physico-chemical and microbial characteristics of the water laboratory tap water (n = 14-44).

Parameter, unit	Mean ± SD	Max	Min
HPC, cfu/ml	273±608	2400	0
pH	7.85±0.10	8.02	7.60
TOC, mg/l	1.80±0.14	2.08	1.47
Turbidity, FNU	0.17±0.04	0.22	0.11
Hardness, °dH	2.85±0.26	3.08	2.17
Alkalinity, mmol/l	0.65±0.10	0.72	0.37
N-tot, mg/l	0.57±0.03	0.64	0.49
NH_4-N, mg/l	0.06±0.04	0.14	0.00
NO_2-N, mg/l	0.05±0.02	0.09	0.02
NO_3-N, mg/l	0.33±0.05	0.42	0.25
Total Cl_2, mg/l	0.19±0.09	0.33	0.06

Before entering the pilot, the tap water was dechlorinated and tempered by a stagnation period of 1 week. Additionally, during the operation of the pilot, a 75 day test was run, where the inlet water was diluted with RO water in 50:50 ratio. The test run simulated a lower concentration of organic matter of drinking water. Due to this dilution, the inlet water was amended to maintain correct hardness and alkalinity. Hardness was adjusted by addition of 35.9 g/l Ca^{2+} as $CaCl_2x2H_2O$ and 7.60 g/l Mg^{2+} $x7H_2O$ as $MgSO_4$. Alkalinity was adjusted by addition of 3.17 g/l HCO_3^- as $NaHCO_3$.

The inlet water was amended once a week to facilitate controlled experimental changes. Ammonium was spiked starting from 8.1.2015 to have a final concentration of 0.12 mg/l NH_4-N. Between 4.8.2015 and 27.1.2016, the NH_4 concentration was adjusted to 0.2 mg/l NH_4-N. From 2.2.2016, the ammonium concentration was amended to be 0.8 mg/l NH_4-N until 26.6.2016. After the initial spiking, NH_4-N was added every second week.

Between 4.8.2015 and 27.1.2016, the pilot system was used to perform nitrification rate tests with varying nitrite and ammonium concentrations, with both tap water matrix and diluted tap water matrix. Ammonium tests were run with the addition of 5 ml of 4.56 mg/l ammonium chloride (NH_4Cl) in both inlet water situations. Nitrite tests were done with addition of 5 ml of 10.93 mg/l potassium nitrite (KNO_2) in both inlet water situations. Between days 3.11.2015 and 15.12.2015 nitrate was added as 5 ml of 5.59 mg/l sodium nitrate ($NaNO_3$), in order to simulate nitrate levels commonly found in drinking water.

Phosphate in final concentration of 5 µg P/l PO_4-P was added from 23.4.2015 as 1.5 ml of 100 mg/l of monopotassium phosphate (KH_2PO_4). Furthermore, the phosphate concentration was raised to 20 µg P/l PO_4-P as 6 ml of 100 mg/l KH_2PO_4 from day 2.2.2016 until 26.6.2016. During 4.8.2015-27.10.2015, phosphorus was added to the pilot tanks as 0.15 ml of 4.39 g/l KH_2PO_4. In between 3.11.2015 and 27.1.2015, phosphorus was added as 0.5 ml of 1.34 g/l NaH_2PO_4 x H_2O.

3.3 Water analyses

Majority of the performed water analyses were done according to standard methods (table 2). The pH measurement was performed with an electronic continuously measuring glass electrode (Metrohm, Switzerland). TOC was analysed with a TOC-VCPH total organic carbon analyzer (Shimadzu, Japan). Nitrite, nitrate and total nitrogen were determined with an FIA automatic sampler Fiastar 5000 (Ordior, Finland) and spectrophotometric analyzer UV-1201 (Shimadzu, Japan). Turbidity was determined with a Hach 2100AN analyzer (Hach, USA). Hardness was determined from the sum of calcium and magnesium concentrations.

Table 2. Basic analysis methods of the performed water analyzes. Modifications explained in text.

Parameter, unit	Method	Year
HPC	9215-C	2000
pH	SFS 3021	1979
TOC	SFS-EN 1484	1997
Turbidity	SFS EN ISO 7027	2000
Hardness	SF 2340-B	1997
Alkalinity	SFS EN ISO 9963-1	1996
N-tot	SFS EN ISO 13395	1996
NH4-N	SFS 3032	1976
NO2-N	SFS EN ISO 13395	1996
NO3-N	SFS EN ISO 13395	1996
Total Cl2	4500-Cl	2000
P-tot	SFS EN ISO 6878	2004

Total phosphorus was determined by a modified ammonium molybdate spectrophotometric method (SFS EN ISO 6878). The measurements were done with the modification of utilizing 880 nm wavelength and 10 cm cuvette instead of 5 cm cuvette. These modifications were used in all calibration curves and sample analyzes, where the standard method was used. The used spectrophotometer was UV-1201 (Shimadzu, Japan). Total phosphorus concentrations in samples were calculated using the linear regression curves shown in figure 8. The equations of the curves were used to solve for the concentration at x, and sample dilutions were accounted with a multiplying factor. The regression curves were redone at appropriate intervals. Along with all samples analyzed using only the SFS EN ISO 6878 standard method, a 5 µg P/l was carried to monitor the accuracy and precision of the method. The sample with a known concentration was done by addition of KH_2PO_4 into RO water.

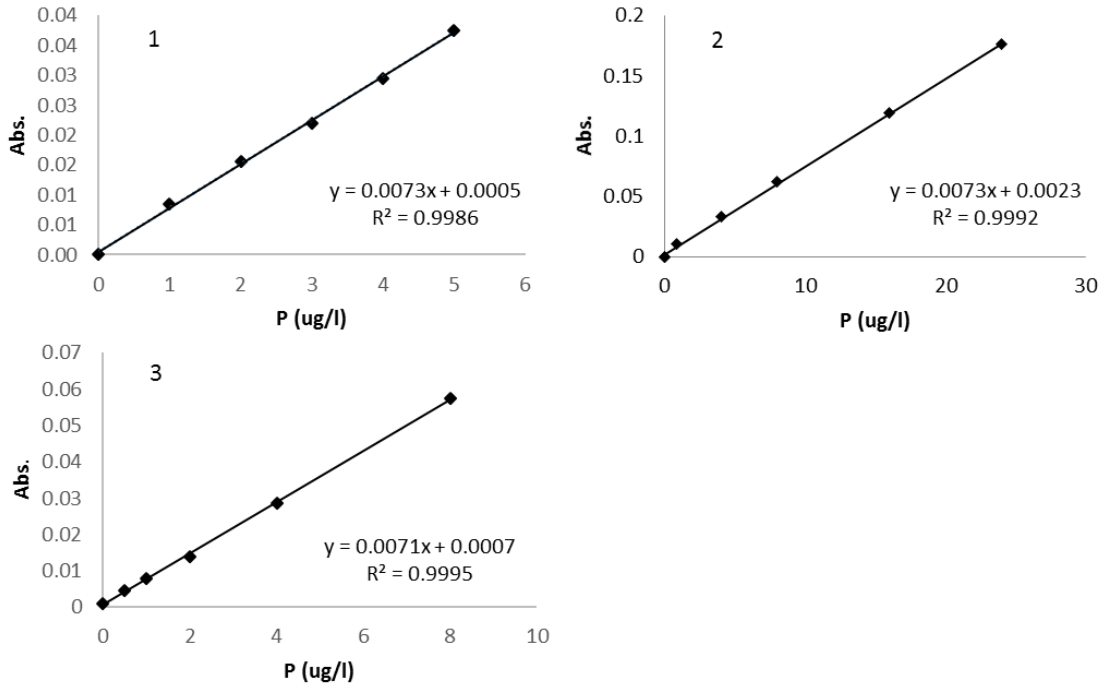


Figure 8. Linear regression curves 1, 2 and 3 with SFS EN ISO 6878 on 12.5.2016, 28.6.2016 and 30.9.2016 respectively.

3.4 Tap water analyses

3.4.1 Water laboratory outlet drinking water

During the pilot operation, water samples were collected from a tap at the water laboratory outlet for total phosphorus analysis. The results of these total phosphorus analyses are indicated in text with the abbreviation "WLO", to distinguish them from other samples originating from tap water outlets. These samples were collected on the same days, as were samples of the pilot water. Water was run for 15 minutes before samples were collected to 500 ml plastic bottles. The samples were frozen for later analysis. Total phosphorus analysis was performed for the samples according to SFS EN ISO 6878 method.

3.4.2 Addition method on tap water

Furthermore, total phosphorus analysis by addition method was performed on tap water collected from the water laboratory outlet on 27.6.2016. The analysis was performed to evaluate the accuracy and precision of the SFS EN ISO 6878 with 10 cm cuvette on low phosphorus

concentrations, and furthermore to overall estimate the tap water total phosphorus concentration. The analysis was performed by creating a sample series of increasing phosphorus concentration. The calculated concentrations were between 5 and 30 (µgP/l) + tap water concentration, done with the addition of KH_2PO_4. The analysis was performed on tap water collected on the day, from the water laboratory tap outlet after letting the water run for 15 minutes.

3.4.3 HSY drinking water distribution system

The modified novel method (see Chapter 3.6) was used to analyze total phosphorus from samples obtained from various tap water outlet locations in the HSY DWDS between 9.9.2014 and 2.6.2016. Four sampling days were analyzed. The abbreviations of the sampling locations, and the distance that the drinking water has travelled along the network from the water treatment facility are shown in table 3. The distances were not measured physically, but estimated from maps of the distribution network. The samples were collected from tap water outlets after running water for roughly 15 minutes. Samples were collected into 500 ml plastic bottles, and frozen for later analyses. Sampling point PI 2 was added to the sampling plan during the sampling period. It was located at the outlet of the Pitkäkoski WTPs water treatment process line 1. The sampling point PI is located at Pitkäkoski WTPs´ water analysis laboratory, which is connected to the outgoing drinking water line after an estimated 500-600 meters of piping. Pipe diameters were not known.

Table 3. Location, abbreviation and distance of the sampling points in HSY DWDS.

Location	Abbr.	Distance (km)
Pitkäkoski WTP, laboratory Kuninkaantammentie 17	PI	0
Pitkäkoski WTP, Kuninkaantammentie 17	PI 2	0
Suomen ympäristökeskus, Hakuninmaantie 6	SY	0.68
Edita Oyj, Hakuninmaantie 2	ED	0.77
Shell Kaivoksela, Kaivokselantie 1	SH	2.6
Myyrmäen urheilutalo, Myyrmäenraitti 4	MY	4.2
Neste Oil Rajatorppa, Vapaalantie 1	NR	5.9
Neste Oil Keimola Itä, Hämeenlinnanväylä 601	NK	11.5
Katriinan sairaala, Katriinankuja 4	KA	17

3.5 Novel analysis method for total phosphorus

The predevelopment for this method was done in an earlier unpublished work by Sophie Aubry (2015), which tested and optimized parameters such as oven temperature, acid concentration, pH, technique of evaporation and dissolution of the precipitate (Appendix 1). The novel method can be separated into three distinct phases. First the sample concentration was increased through evaporation. One liter samples were evaporated in beakers (Duran, Germany) with an oven SL-180 (Pol-Eko Aparatura, Poland) or Binder (Binder, Germany), at

the temperature of 80 °C and fan speed of 90 %. The sample volume was completely evaporated. The beakers were allowed to cool to room temperature in desiccators. All glass instruments were washed with acid solution Deconex, 25 Organacid and alkaline detergent Deconex 22 LIQ-X (Borer, Switzerland) and rinsed with RO water.

The second phase aimed at dissolving the calcareous fur precipitated at the bottom and sides of the beakers. 10 ml of 2 M hydrochloric acid (HCl) was used to dissolve the fur using a glass pipette. A technique of even spread and coverage was emphasized. The contact time between the acid and the fur was 10 minutes. After transferring the used acid precipitate solution to a 50 ml borosilicate autoclave bottle (VWR, USA), the remaining dissolved fur was rinsed from the beaker with 30 ml of RO water, aiming for maximum coverage of the container. A glass funnel was used to guide the liquids into the containers.

The final phase of the method was the analysis of the total phosphorus concentration in the samples using SFS EN ISO 6878 standard method with 10 cm cuvette and 880 nm wavelength. Before the samples were autoclaved, the pH was corrected to approximately seven. This was done with the addition of 8 M NaOH or 2 M HCl, and tested by using a plastic pipette with pH paper (Merck, Germany). After measuring the absorbance, the total phosphorus concentration was calculated using calibration graphs done according to SFS EN ISO 6878.

This method was tested on three occasions. First the method was tested on RO water based phosphorus additions with concentrations ranging between 0.2 and 10 (µg P/l). The second test was a tap water based test at very low phosphorus concentrations of 0.02-1.0 (µg P/l). The final test was also done as tap water based additions of phosphorus with concentration of 0.2-0.8 (µg P/l). Phosphorus was added as KH_2PO_4 on all occasions. The results were calculated by subtracting the absorbance of the zero sample, which was the unknown total phosphorus concentration in tap water.

3.6 Modified novel analysis method for total phosphorus

The previously described method was amended so as to increase the concentration of total phosphorus in drinking water samples with only a factor of two. This could bring the total phosphorus concentration above the quantification limit of the standard SFS EN ISO 6878 method utilizing the 10 cm cuvette, but still possibly minimize the calcareous fur build up.

First, water samples of 300 ml were measured and evaporated, in similar oven conditions mentioned in Chapter 3.5, to approximately 150 ml volume or less. 400 ml beakers (Duran, Germany) were used as vessels in the oven. To measure the 300 ml and 150 ml volumes, 500 ± 3.75 and 250 ± 1.0 (ml) measuring cylinders (Duran, Germany) were used. The beakers were allowed to cool down in desiccators, and while cooling down the beakers were covered with watch glasses to stop contamination. If the samples evaporated under 150 ml, RO water was used to fill the samples to the 150 ml mark. Furthermore, the samples were analyzed using SFS EN ISO 6878 and the total phosphorus concentration was calculated using the standard calibration graph established in Chapter 3.3. Any dilutions of the samples were taken in to account in calculations. Along with all samples analyzed using the modified novel analysis method, a water sample of 2.5 µg P/l was carried to monitor the accuracy of

the method. The known concentration sample was done by addition of KH_2PO_4 into RO water.

3.7 Pilot drinking water distribution system biofilm

As the final step of the analysis on the pilot DWDS, biofilm samples were collected. The biofilm collector consisted of 5 individual pipes, each 60 cm in length, in both pilot lines P1 and P2. The collectors were made of the same pipe material as the rest of the pilot DWDS. All collectors had shutoff valves at both ends. The collectors were harvested on two different occasions. The first harvest was sent for processing on 16.2.2016 and the second 28.6.2016. Thus, the first harvest accumulated biofilm growth for roughly 14 months and the second collector for roughly 4 months. The biofilm collectors from both pilot systems were sent to a third party analysis laboratory for biofilm separation (THL, Kuopio, Finland). The separation method was done by scraping the inner surface of the collectors, using glass beads and shaking. The collected biofilm solid material was stored in 250 ml plastic bottles with bulk water liquid from the pilot and frozen until analysis. During analysis care was taken to properly mix the suspended solids in the liquid, and to pipette the samples homogenously into further analysis. The samples were used to determine the total phosphorus concentration in the biofilm. The sample analyzes were done according to SFS EN ISO 6878.

In order to estimate the phosphorus concentration per the organic material of the biofilm, suspended solids (SS) and volatile suspended solids (VSS) were analyzed according to SFS-EN 872 (2005) and SFS 3008 (1990). The used filter paper was Whatman GF/C. The VSS was determined solely from the 28.6.2016 collector. Additionally, the phosphorus concentration was also estimated per pipe surface area.

3.8 Quantification limits and statistical analyses

A quantification limit was determined for the SFS EN ISO 6878 with 10 cm cuvette, the novel analysis method for total P and the modified novel analysis method for total P. The calculations were done according to Shrivastava and Gupta (2011) (equation 5). Quantification limit determined through this equation sets the limit to be ten times the background noise level. For each analysis set, a quantification limit was determined from the blanks and the calibration curve. Average limits of respective analysis methods were reported.

$$QL = \frac{10 * Std\ 0}{b} \quad (5)$$

Where,

QL = Quantification limit

Std 0 = Standard deviation of the blank sample

b = slope of the regression line of the calibration curve

Additionally, comparisons in the differences of total phosphorus levels in the pilot system were calculated with excel software, using the statistical tool pack. Student´s two-way paired t-tests were used to test for significant differences.

4 Results and discussion

4.1 Pilot drinking water distribution system

The total phosphorus concentration for each pilot inlet line (S1 and S2) and outlet waters (P1 and P2) are shown below (figure 9). The average total phosphorus concentration for the pilot systems at Period 1 (23.4.2015-19.1.2016) was P1 = 4.7 ± 1.2, S1 = 6.5 ± 1.1, P2 = 5.3 ± 2.0 and S2 = 6.7 ± 0.7 µg P/l. Similarly, for the Period 2 (2.2016-26.6.2016) the averages were P1 = 26.9 ± 1.5, S1 = 29.0 ± 1.1, P2 = 24.2 ± 1.7 and S2 = 29.2 ± 1.0 µg P/l. The inlet total phosphorus concentrations were higher than the calculated 5 and 20 µg P/l in both periods, which might be explained by the input of phosphorus already contained in tap water (see chapter 5.3). In addition, phosphorus might have been recirculated from the biofilm to the outlet water, thus increasing the concentration.

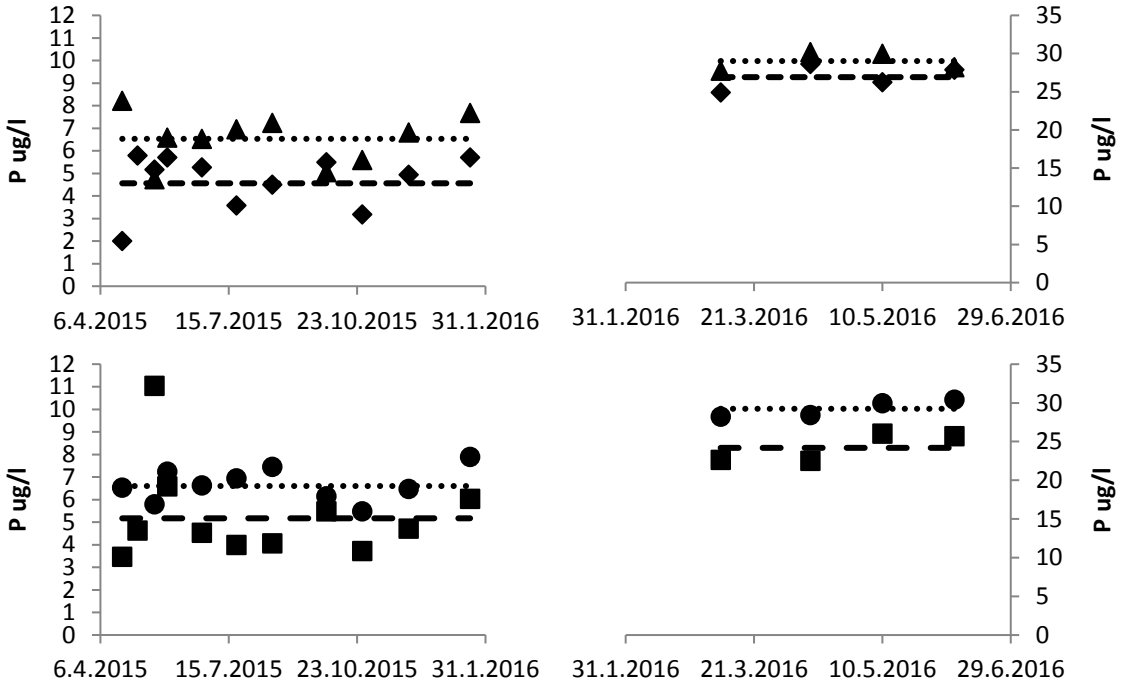


Figure 9. Total phosphorus concentration in period 1 and 2, with horizontal averages in the pilot 1: P1 (♦) and S1 (▲) and pilot 2 P2 (■) and S2 (●) (SFS EN ISO 6878 with 10 cm cuvette).

The uptake of phosphorus in the DWDS was calculated from the difference between the inlet and outlet water samples. For the period 1 pilot 1 had an average weekly phosphorus reduction of 1.88 µg P/l and pilot 2 1.36 µg P/l. In both systems, the difference was statistically significant (Two-way Student´s T-test $p<0.005$). Thus, microbial organisms were likely utilizing phosphorus. As the biofilm was still probably developing and the microbial growth in any of the four phases had not most likely stabilized, it is possible that phosphorus was being

used for either cell growth or cellular storage. However, it should be noted, that without more specific studies such as ATP or enzyme activity analyzes the specific utilization of the phosphorus cannot be stated.

For the pilot run period of 2 the phosphorus addition was increased. This resulted in that pilot 1 had an average weekly phosphorus reduction of 2.10 µg P/l and pilot 2 5.00 µg P/l (Figure 9). Both were statistically significant (Two-way Student´s t-test $p<0.005$). When comparing the reduction rate of the pilot 2 between the two additions phases, the higher reduction rate at period 2 suggests the possibility of mass transfer limitation of phosphorus during period 1. Lehtola et al. (2006) suggested that the biofilm growth of a pilot DWDS was being mass transfer limited by phosphorus, when testing the effect of varying flow speeds in DWDSs. In the pilot 1 similar change in the reduction rate was not seen.

In advance to the addition of any phosphate to the pilot inlet water, the main objective of nitrification in the pilot DWDS had not occurred. After the addition of 5 µg P/l of phosphate, both pilot systems started nitrifying ammonium into most likely and in majority to nitrite within 3-8 weeks. Figures 10 and 11 show the uptake of ammonium nitrogen in both pilot systems. This supports Lehtolas et al. (2002) findings that even 5 µg P/l or lower phosphorus concentrations can cause increases in biological growth and activity. Lehtola et al. (2002) found as well that phosphorus poor drinking waters cannot support maximal biofilm growth with just the recycling of existing low concentration (<2 µg P/l) of phosphorus. Any additional changes from the increased (20 µg P/l) total phosphorus concentration were not seen in ammonium uptake.

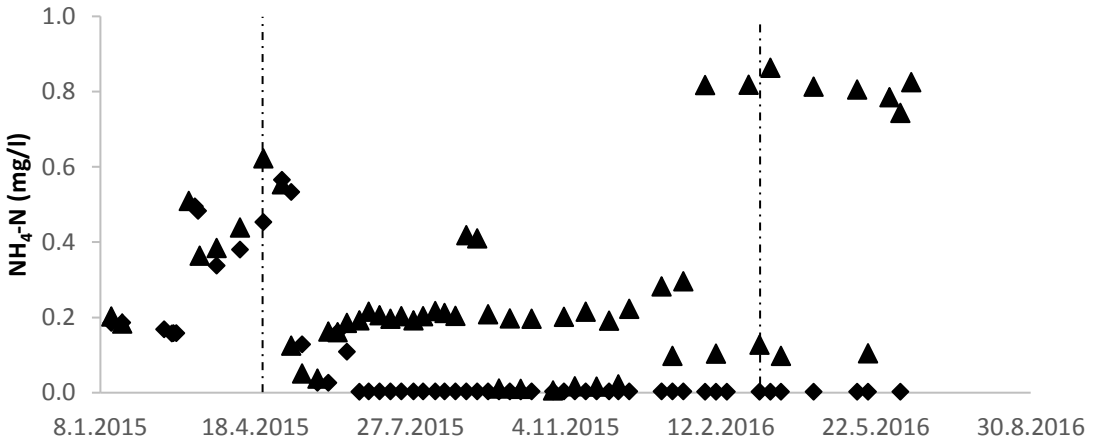


Figure 10. Ammonium nitrogen concentration in the pilot P1 (♦) and S1 (▲). Dashed vertical line indicates beginning of phosphate addition.

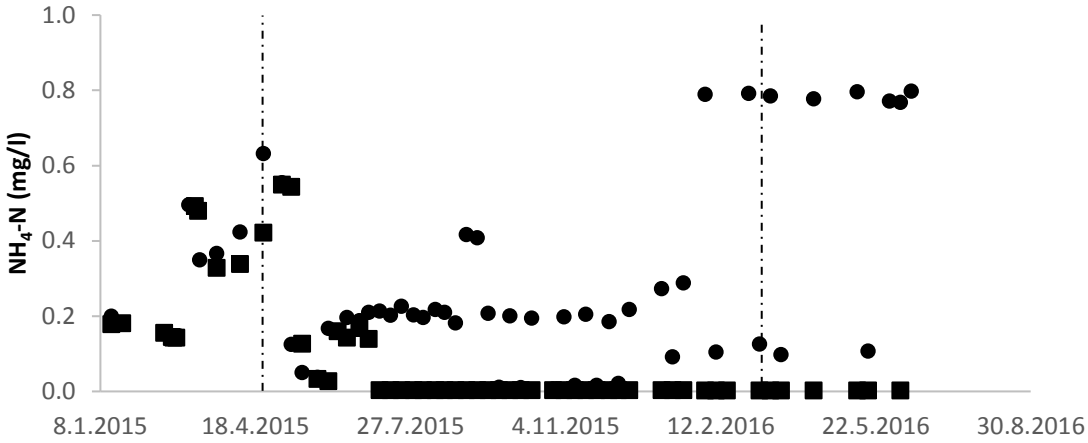


Figure 11. Ammonium nitrogen concentration in the pilot P2 (■) and S2 (●). Dashed vertical line indicates beginning of phosphate addition.

The clear change in ammonium concentration after phosphate addition indicated the possibility of phosphorus limitation in the inlet water, especially for the nitrifiers. In this sense, the inhibited or slow initialization of nitrification could be due to heterotrophic bacteria competing for the phosphorus. It has been noted that some Heterotrophic bacteria are able to reproduce 2-3 generations in one hour, whereas species like *Nitrosomonas* and *Nitrospira* have been noted to be able to only produce a generation in 5-10 hours (Salkinoja-Salonen et al. 2002). This can create situations where the heterotrophic biomass grows over the nitrifiers, possibly inhibiting their ability to obtain essential nutrients. Before any additions of phosphorus, it is highly likely that the growth of all bacteria may have been limited by the lack of available phosphorus.

The quantification limit for the used analysis method (SFS EN ISO 6878 with 10 cm cuvette) was calculated to be 0.68 µg P/l when using calibration curve 1 (figure 8) and 0.66 µg P/l when using calibration curve 2 (figure 8) (equation 5). All of the samples analyzed from the pilot water were above this limit. The sample of pilot P2 on day 18.5.2015 was a possible outlier, with roughly 11 µg P/l total phosphorus concentration when tested with Grubbs test (Z-score 2.66 > critical value 2.29). However, there is a possibility that the high value seen on 18.5.2015 was due to a detached biofilm material in the water sample. This could have occurred possibly due to changing flow conditions or just as a natural detachment. It is important to note that the SFS EN ISO 6878 method does not distinguish between the soluble and bound fractions of phosphorus. If a biofilm particle detaches from the pipe surface, its nutrient uptake does not stop but the spatial change can affect the phosphorus uptake.

4.2 Pilot drinking water distribution system biofilm

The determination of total phosphorus from the biofilm harvester samples showed higher concentrations of phosphorus than in any of the bulk water analysis performed. The concentration was determined from the combination of the bulk liquid and the biofilm particles that were contained in it. The biofilm samples showed roughly 1 log higher total phosphorus concentrations than were detected with any unspiked tap water samples. From the harvests of 16.2.2016, the average total phosphorus concentration was 73 ± 1.8 µg P/l, and the harvests taken on 28.6.2016 it was 52 ± 0.3 µg P/l. The maximum and minimum values ranged between 42 and 82 (µg P/l). The biofilm harvester taken on 16.2.2016 showed a higher total phosphorus concentrations compared to the second harvester most likely due to the longer period available for biofilm growth before the first harvest. Before the measurements, a visual examination of the sample liquids showed that the sample of pilot 2 16.2.2016 had the highest amount of particulate matter and the darkest color, which correlated with the highest measured total phosphorus concentration.

Furthermore, the average concentration of phosphorus calculated per pipe surface area was 0.12 µg/cm^2 and 0.09 µg/cm^2 for the 16.2.2016 and 28.6.2016 samples respectively. Figure 12 shows the total phosphorus concentration per pipe surface area for each pilot line on both sampling days. The phosphorus amount in the pipe walls of the longer growth period (16.2.2016) is 0.03 ug higher than in the 28.6.2016 harvest. Again, the longer growth period most likely allowed the biofilm to grow in thickness, resulting in higher phosphorus within the cell structures. When considering biofilm samples, the phosphorus contained in the organic material is of great interest. Previous literature has noticed that the microbial growth in DWDSs mainly occurs in the biofilm phase, and furthermore that micro-organisms have a tendency to store nutrients such as phosphorus in the biofilm matrix (Servais et al. 2004). This nutrient storing can keep microbial growth up during times of starvation, rendering improvements in water treatment technologies less effective against microbial proliferation. The 28.6.1016 harvesters contained roughly 0.009 µg P/µg VSS, or 0.85 % w/w. This is slightly less than the typically reported 1.5-2.5 % (w/w) phosphorus content of biomass (Saravanamuthu 2009). However, due to the low concentration of organic material in the biofilm samples, the P/VSS concentration should be only considered as an indicative value.

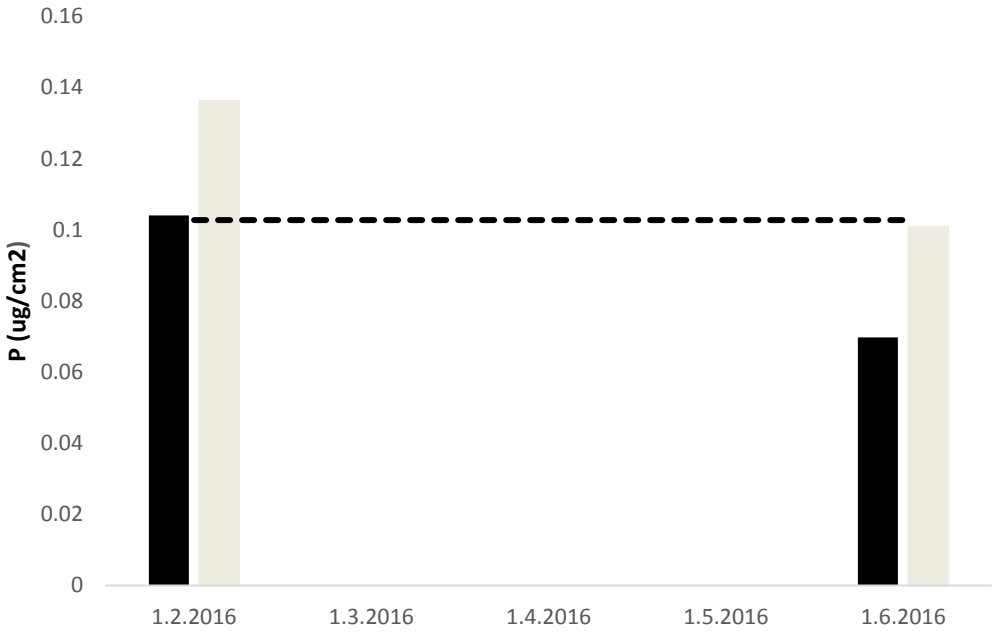


Figure 12. Biofilm harvester total phosphorus per pipe surface area analysis with pilot 1 (■) and pilot 2 ( )Dashed line represents average value (SFS EN ISO 6878 with 10 cm cuvette).

As noticed by Inkinen et al. (2014), biofilm development can be practically impossible to prevent due to the small amounts of microbial nutrients and bacteria existing in drinking water. For this, the removal of the biofilm material and other soft deposits from the DWDSs has been suggested as a viable option for biofilm control. Lehtola et al. (2003) saw significant decreases in the growth rate of microbes in a DWDS, after utilizing pigging as a cleaning method in a municipal DWDS. Furthermore, the importance of time scale on any biolocial measurements is essential. It s highly expected that biofilm growth in a new pilot DWDs is rapid and prone to environmental changes. However, when looking at large and old DWDSs where biofilms are diverse and well established, any changes in the growth conditions might take effect in such a slow pace that measurements might not detect them.

4.3 Tap water analyses

4.3.1 Water laboratory outlet drinking water

Drinking water samples taken from the water laboratory tap during the pilot DWDS sampling days were analyzed to set up an initial frame of total phosphorus concentration context (figure 13). This frame describes the background level of total phosphorus concentration in the inlet waters S1 and S2. The average concentration was 0.85 ± 0.26 µg P/l. The background concentration could partially explain the higher than calculated total phosphorus concentrations in the inlet water samples.

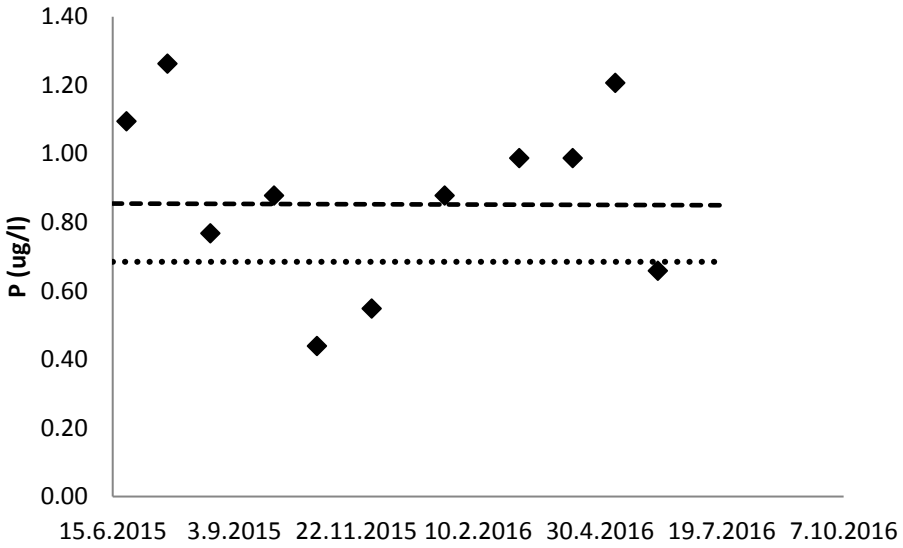


Figure 13. Total phosphorus of tap water from water laboratory outlet (♦). Dashed line represents average value and dotted line the quantification limit (SFS EN ISO 6878 with 10 cm cuvette).

The quantification limit for the tap water samples was the same as for the pilot water samples, as they were analyzed and calculated identically. However, some of the tap water samples presented were below the quantification limit. Based on these indications, in order to reliably analyze any other tap water samples, further development of the analysis method was deemed necessary.

4.3.2 Addition method on tap water

The analysis performed on the tap water further indicated the existence of a low, but significant amounts of phosphorus in tap water (figure 14). When the results were extrapolated to the x-intercept the zero response tap water phosphorus concentration was shown to be 0.58 µg P/l. This can be compared to the measured value of 0.61 µg P/l in a tap water only sample, indicating good accuracy.

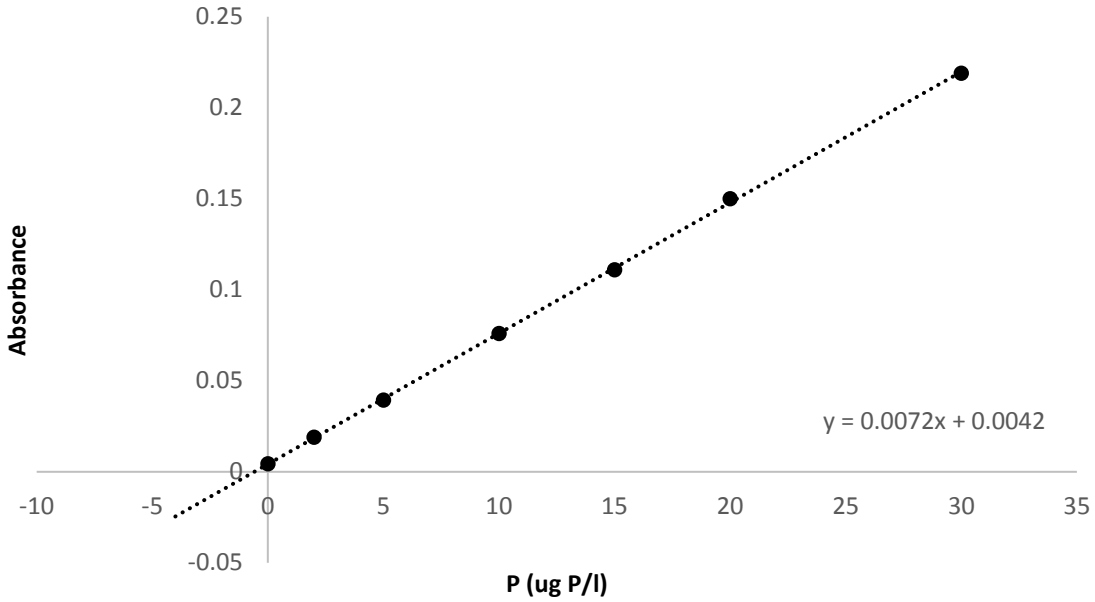


Figure 14. Addition method of total phosphorus in water laboratory tap water 27.7.2016 (SFS EN ISO 6878 with 10 cm cuvette).

The reliability of the results was also examined by comparison of the measured concentrations to the calculated concentrations (figure 15). Only slight deviation was seen at 20 µg P/l concentration, where the measured concentration was 20.46 µg P/l. Over all the standard method with the longer 10 cm cuvette and 880 nm wavelength was seen to give reliable results, even at low phosphorus concentrations. This supports the notion that the utilization of a longer cuvette could bring the range of reliable detection to >0.7 µg P/l as total phosphorus.

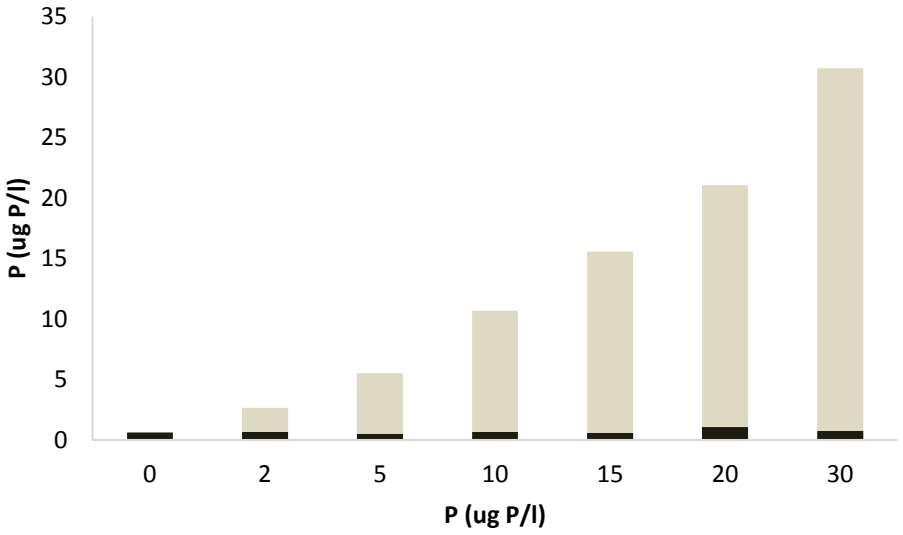


Figure 15. Comparison of yield ( ) and calculated share tap water ( ) total phosphorus, with increasing additions.

However, as the concentration was determined with the calibration curve 2, the quantification limit at 0.68 µg P/l was higher than the estimated values for tap water total phosphorus. This indicated that even though the standard method was seen to have an increased detection capability, it would still not be sufficient alone for drinking water analyses. For this reason, and especially because phosphorus concentrations have been noted to fluctuate in drinking water, it was seen as a necessary step to further develop the novel method. Furthermore, with low concentrations the capability of the spectrophotometer in use to differentiate the significant decimals, on the absorbance of tap water from the blank RO sample, was seen as a possible source of unreliability.

By using the reliability established by the standard method of SFS EN ISO 6878 with a longer 10 cm cuvette, it was hoped that any total phosphorus concentration in drinking water could be measured accurately and precisely if its concentration could be brought above 0.7 µg P/l for measurement (see Chapter 4.7).

4.3.3 HSY drinking water distribution system

The average total phosphorus concentration in the HSY DWDS was 1.1 ± 0.17 µg P/l during the sampling period of 9.9.2014-2.6.2015 (figure 16). The maximum and minimum measured concentrations were 2.3 and 0.60 µg P/l respectively. The results did not clearly indicate any seasonal trends in phosphorus concentrations, although more frequent sampling would be required to thoroughly investigate possible patterns. Similar average concentrations of <2 µg/l and <10 µg/l by Lehtola et al. (2004) and Juhna et al. (2007), have been reported as total phosphorus in drinking waters found in Finland and Latvia respectively.

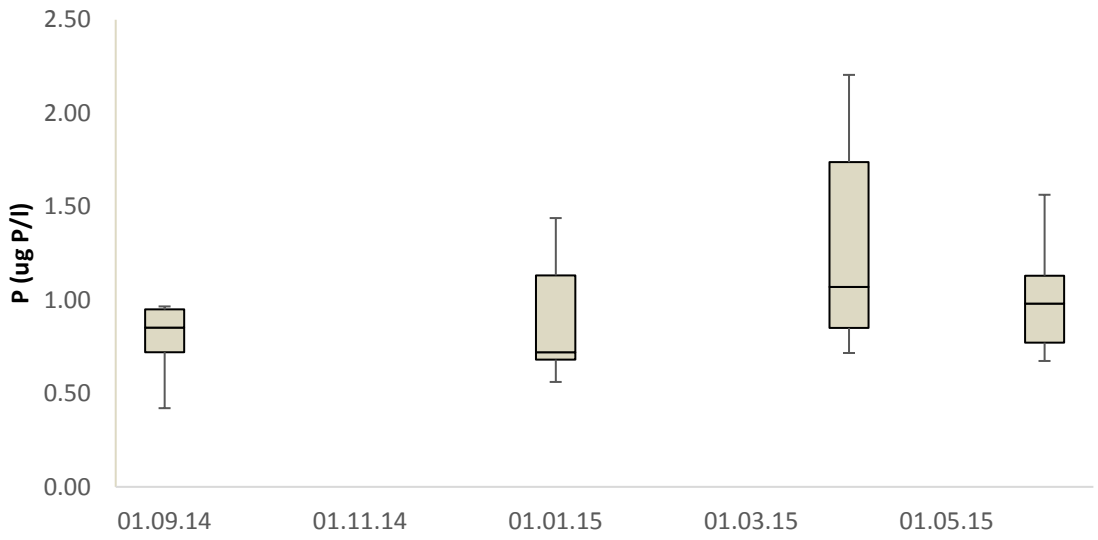


Figure 16. Total phosphorus concentration variation in HSY DWDS with time (Q2, median and Q1) with max and min whiskers (Modified Novel Analysis Method).

Interestingly, the total phosphorus analyses performed by MetropoliLab between 10.6.2014 and 21.4.2015 showed that the total phosphorus concentration in the drinking water of the two WTPs of HSY was higher than >10 µg/l in 55 % of the analyzed samples (table 4). In comparison to the data obtained in our research, this suggests a possibility that there is a rapid uptake in the DWDS after the WTPs. However mostly likely the increased accuracy of the used modified novel method is revealing more information on the total phosphorus concentrations of the same period.

Table 4. Drinking water total phosphorus at HSY WTPs. Unpublished data. Analyses performed by Metropolilab.

Sampling	Pitkäkoski WTP	Vanhakaupunki WTP
Date	Tot-P (mg/l)	Tot-P (mg/l)
2.2.2016	<0.010	<0.010
8.12.2015	<0.010	<0.010
4.8.2015	<0.010	<0.010
21.4.2015	0.015	0.016
20.1.2015	0.015	0.015
18.11.2014	0.014	0.013
30.9.2014	0.011	N.A
10.6.2014	0.011	0.011
18.2.2014	<0.010	<0.010

Additionally, the total phosphorus concentration has been noted to vary greatly according to the water source. Rubulis et al. 2007, found total phosphorus concentrations ranging between 7±5 and 32±34 µg P/l in a DWDS located in Latvia, with both surface and ground water used as the raw water source respectively.

The changes in total phosphorus concentration in the DWDS, in comparison to the distance from the treatment facility, showed no clear trend (figure 17). A minor trend of the bulk water phosphorus concentration of stabilizing towards 1 µg P/l was seen when the distance increased beyond 5 km from the WTP. Furthermore, it was noted that during the one-year sampling period, the pipeline between the WTP and the WTP water analysis laboratory was microbiologically active, and that ammonium was nitrified into nitrite (figure 18).

On the samples from 9.9.2014 and 2.6.2015, the concentration showed a slight trend of increase with distance, whereas samples taken on 20.1.2015 and 14.4.2015 showed a slightly decreasing trend, which can be seen from figure 20. There is a possibility that the differences in the trends were caused by changes in environmental conditions such as temperature, which has been noted to affect the microbial activity in the DWDS (LeChavallier 1990).

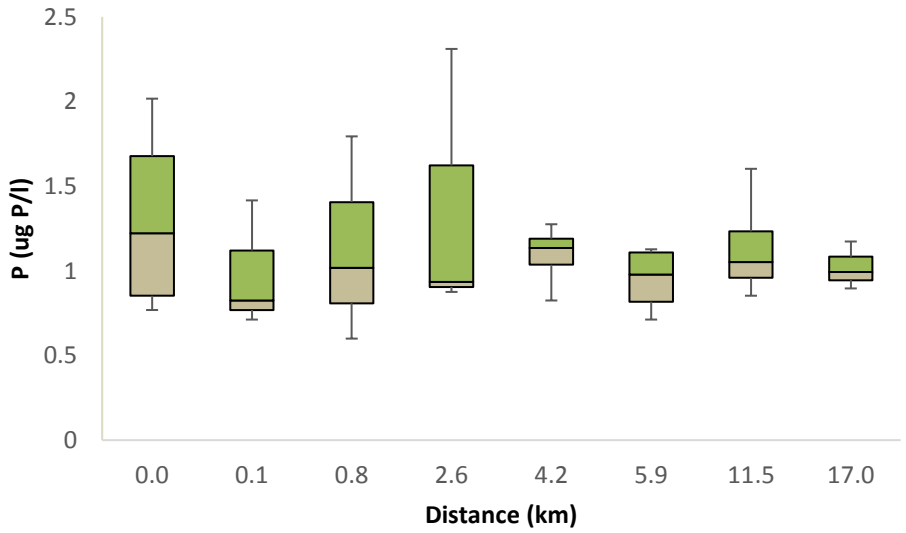


Figure 17. Total phosphorus concentration variation in HSY DWDS with distance (Q2, median and Q1) with max and min whiskers. Distance 0 = WTP production line and 0.1 = WTP water analysis laboratory. (Modified Novel Analysis Method).

Overall in terms of both spatial and temporal variation of phosphorus, the results indicate that the effect of microbial consumption of phosphorus cannot be seen. This is to say that phosphorus does not seem to be accumulating within the DWDS. This indicates that whatever the amount that the biofilm and loose deposits uptake from the water, roughly the same amount is extruded back into the water flow as loose deposits or particles.

Total phosphorus and nitrite nitrogen concentrations in the HSY DWDS were compared in figure 18. Nitrification can be seen to be actively occurring throughout the sampling period. Furthermore, as the distance in the DWDS increased, the nitrification of ammonium caused the nitrite concentration to increase until 6 km distance from the WTP. Further than 6 km, the nitrite concentration is decreasing on all sampling days, most likely due to conversion to nitrate. At 17 km distance, nitrite concentration is reduced to nearly zero on all sampling days. As nitrifying microbes have been shown to require more time and energy to reach a steady state, it can be deduced that heterotrophic biological activity is most likely occurring in the network as well.

The results did not indicate any clear connection between nitrification and phosphorus concentration. Phosphorus concentration can be seen to be decreasing at distances <0.77 km except on 14.4.2015 and 2.6.2015, whereas nitrite concentrations are increasing rapidly at the same distance except on 9.9.2014. However, as nitrifiers can possibly constitute only a fraction of the microbiota existing in the DWDS, it is very likely that the changes in phosphorus concentration cannot be seen through nitrifier growth. A more important aspect to consider is the average level of phosphorus present in the network that can support nitrification. Zhang et al. (2010) noted that at bulk water under 5 µg P/l, nitrification was restricted to below 40-60 % ammonium loss in premise plumbing systems. In this sense the measured concentrations indicate, that nitrification was being restricted by the low phosphorus amount available. However, nutrient storing into the biofilm matrix and other indirect conditions, such as release of phosphate from iron tubercles, can support higher microbial activity in low phosphorus conditions (Chen et al. 2013). Furthermore, the complex pathways of phosphorus utilization by microbes might mask the effect of phosphorus concentration. During high availability of phosphorus, microbes tend to store polyphosphates into the granules for utilization during low phosphorus availability (Lehtola et al. 2004). These mechanisms of phosphorus uptake during long periods of time can even out changes in the phosphorus concentration of the drinking water. It is possible that a matured biofilm that has filled all possible growth niches would not show any significant phosphorus uptake at any distance.

The phenomena of nitrification in the HSY DWDS has been previously studied by Vahala et al. 1999, where it was found that a granular activated carbon (GAC) filtration system (currently used at both HSY WTPs) caused an increase in nitrification. As mentioned before activated carbon filtration can effectively remove phosphorus from raw waters, which in turn could result in phosphorus limitation. As there is no clear correlation with phosphorus and nitrite, it is possible that nitrification is less dependent on nutrient limitations than other heterophic microbes.

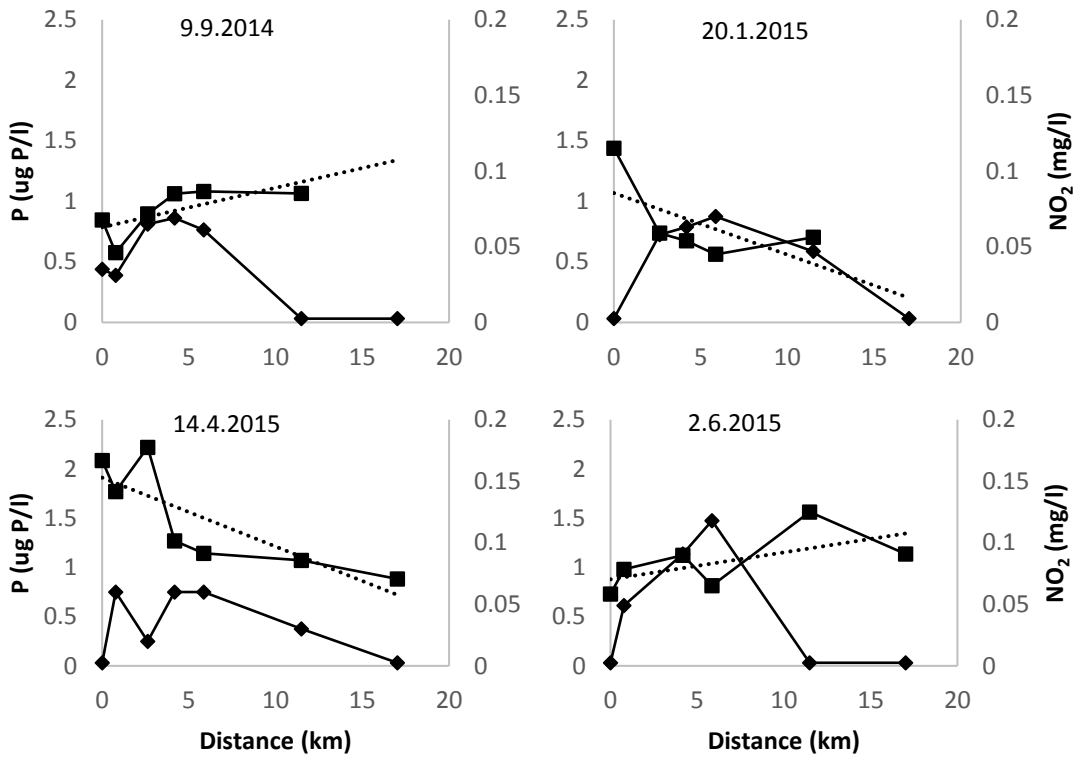


Figure 18. DWDS total phosphorus concentration (■) and nitrite nitrogen (♦) against sampling point distance from treatment facility. Dotted line indicates total phosphorus trend line.

4.4 Drinking water total phosphorus concentration

The total phosphorus concentrations from all analyzed drinking water samples are summarized in table 5. The results suggest that an average concentration of total phosphorus in drinking water in the Finnish metropolitan area DWDS was 0.89 ± 0.17 µg/l. The available literature does mostly report total phosphorus concentrations below detection limits (<2 µg/l or <10 µg/l), which indicates that the results of this work estimate the concentration to be significantly lower. The results are also in line with Rubulis et al. (2007), who detected MAP concentrations in Latvian drinking waters ranging between 0.23 and 10.2 µg MAP/l, which can represent a 20-80 % fraction of the total phosphorus concentration. The obtained low concentration is supported by the notion that traditional water treatment techniques, especially aluminum/iron -precipitation, are able to efficiently reduce the phosphorus concentration during water treatment.

Table 5. Total phosphorus concentrations of all analyses from tap water samples.

Analysis	Sampling Date	No. of Samples	Tot-P (µg/l ± std.)
Drinking Water, Addition meth. (SFS EN ISO 6878 with 10 cm cuvette)	27.6.2016	7	0.70 ± 0.16
Drinking Water (WLO) (SFS EN ISO 6878 with 10 cm cuvette)	24.6.2015-7.6.2016	12	0.85 ± 0.26
HSY DWDS (Modified Novel Analysis Method)	9.9.2014-2.6.2015	27	1.11 ± 0.17
Average			0.89 ± 0.17

4.5 Drinking water distribution system nutrient ratio

The results of total nitrogen, TOC and total phosphorus analyses performed on samples collected from HSY DWDS and Aalto University´s water laboratory (WLO) are presented in table 6, figure 19 and figure 20. From these, the nutrient ratio of HSY DWDS can be calculated to be 2000:610:1 as C:N:P. This ratio clearly demonstrates the overwhelming amount of organic material when compared to phosphorus. In comparison, Lehtola et al. (2002) reported an AOC:MAP ratio of 252:1, in drinking water samples taken from a DWDS located in Eastern Finland. It has been established in literature, that Finnish natural waters do contain exceptionally large amounts of humic material, that needs to be removed during water treatment (RIL 124-1 2003). Based on the calculation, the growth limiting nutrient of drinking water in the metropolitan area of Helsinki could be phosphorus if the value is compared to the ratio of optimal growth (100:15:1) for aquatic micro-organisms. Furthermore, this result is in line with previous studies of Miettinen et al. (1997), who saw a strong microbial growth regulation due to phosphorus limitation of Finnish drinking waters. However, it should be noted that microbial growth is a complex process, where not only one nutrient for example can dictate growth. Heterotrophic bacteria are very fast growing, meaning that their carbon consumption rate is relatively high. It is possible that in local communities within the different biological phases of DWDSs, carbon could be the limiting nutrient even though the concentration of phosphorus in the water phase would suggest otherwise.

Table 6. Total nitrogen, TOC and total phosphorus concentration averages of all sample dates.

Average	TOC (µg /l)	Tot-N (µg /l)	Tot-P (µg/l)
HSY DWDS	1798 ± 124	532 ± 32	1.11± 0.17
Drinking Water (WLO)	1797 ± 175	566 ± 0.03	0.85 ± 0.26
Average	1798	549	0.91
Nurient ratio	2000	610	1

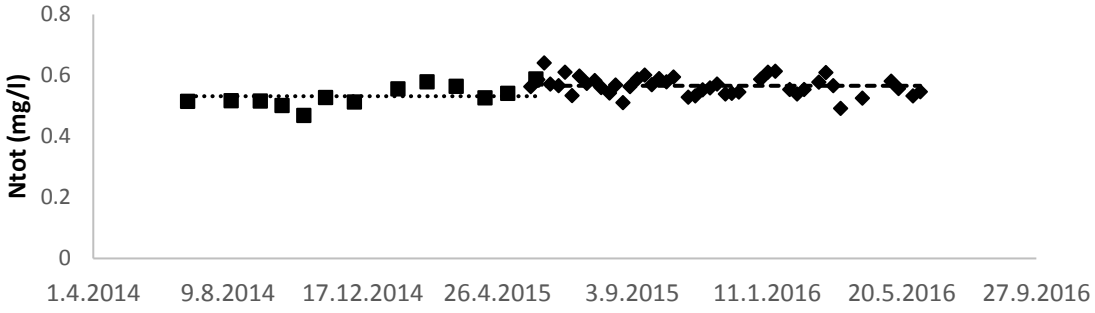


Figure 19. Total nitrogen concentration of WLO (♦) and HSY DWDS (■). Dashed lines indicate averages.

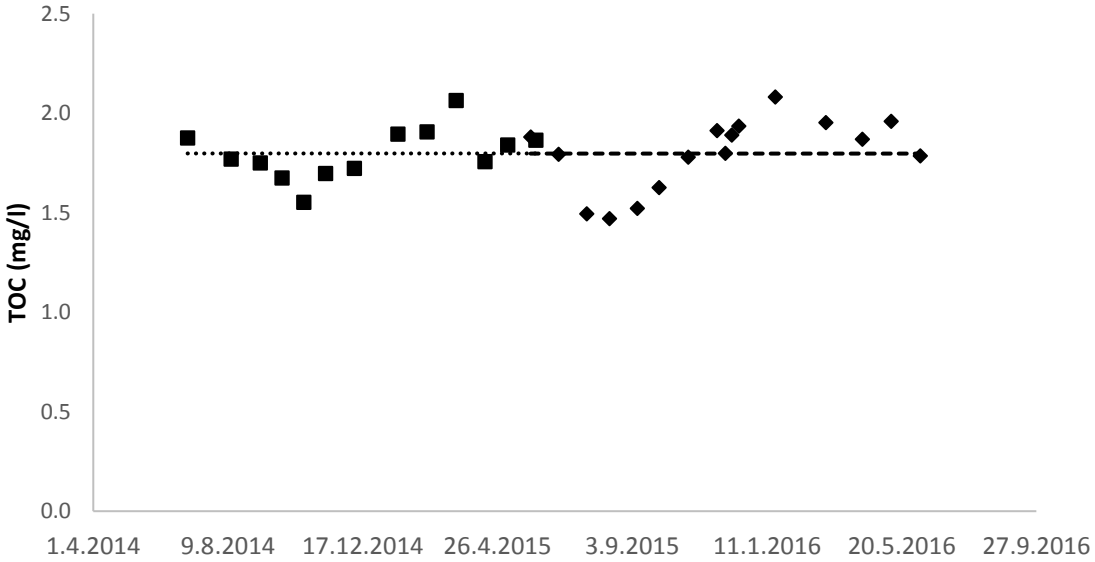


Figure 20. TOC concentration of WLO (♦) and HSY DWDS (■). Dashed lines indicate averages.

The Redfield ratio for optimal microbial growth was suggested to be 100:15:1 as C:N:P, which indicates that also in terms of nitrogen, the phosphorus concentration is very low in the DWDS. Furthermore, when comparing the nitrogen to organic material ratio, the abundance of organic material can be seen.

4.6 Novel analysis method for total phosphorus

4.6.1 Reverse osmosis water

The initial results of the novel method development showed promise as to the reliability of results when using RO water as the base. Figure 21 shows a linear relationship between the tested phosphate concentration and absorbance ($R^2 > 0.99$). However, it should be noted that the same run indicated that the method would result in increasingly higher yields when higher concentrations of phosphate were tested (figure 22). The yields varied between 105 and 123 % from smallest to highest concentration respectively. The yield overestimated the total phosphorus concentration consistently, with the accuracy increasing towards higher concentrations. The reason for the increasing yield remained unknown. Furthermore, the increasing yield made it impossible to establish a coefficient for any equation that could describe the actual total phosphorus concentration. The quantification limit calculated for the series was determined to be 1.1 µg P/l, which indicated a higher standard deviation of the blank samples.

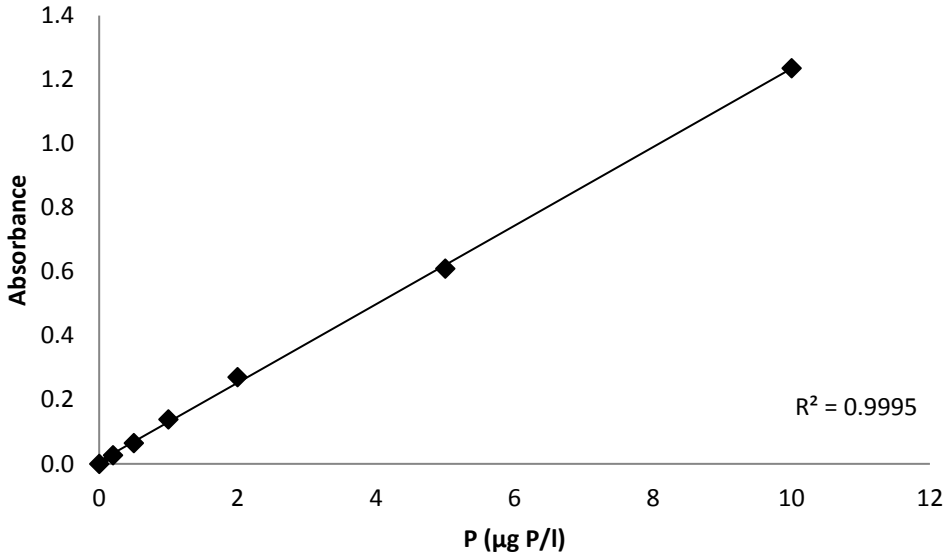


Figure 21. RO water absorbance (♦) against theoretical phosphate concentration (Novel Analysis Method).

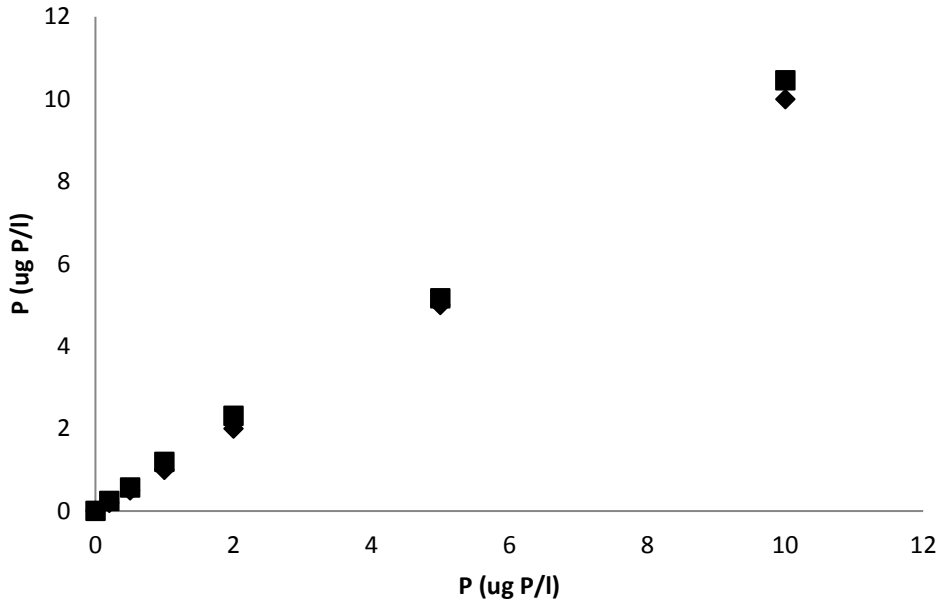


Figure 22. RO water total phosphorus yield (■) against calculated phosphate concentration (♦) (Novel Analysis Method).

4.6.2 Tap water 1

When moving to test the novel method with tap water as the base, there was a significant loss of precision and accuracy (Figure 23). The measured absorbance values had high variance and showed only weak linearity (R^2 = 0.71). Aubry (2015) saw far more consistent yield values (98-108 %) for the same test conditions except with a shorter wavelength of 700 nm and higher total phosphorus concentrations of 4-20 µg P/l. The yield values obtained here were significantly higher than the calculated input of total phosphorus concentrations, ranging between 78 and 821 %, without consistency (figure 24).

In addition, the quantification limit of tap 1 series was 2.0 µg P/l which was higher than the tested total phosphorus concentrations, indicating unreliability of the method. The resolution of the absorbance showed that there was no significant difference above 0.1 and 0.2 (µg P/l) concentrations. It is possible that the poor resolution of the absorbance was due to the very low additions of phosphate, which is why a further tap water based test with higher concentrations was proposed. Overall, the first tap water analysis could not be termed to be reliable enough for total phosphorus determination at very low total phosphorus concentrations.

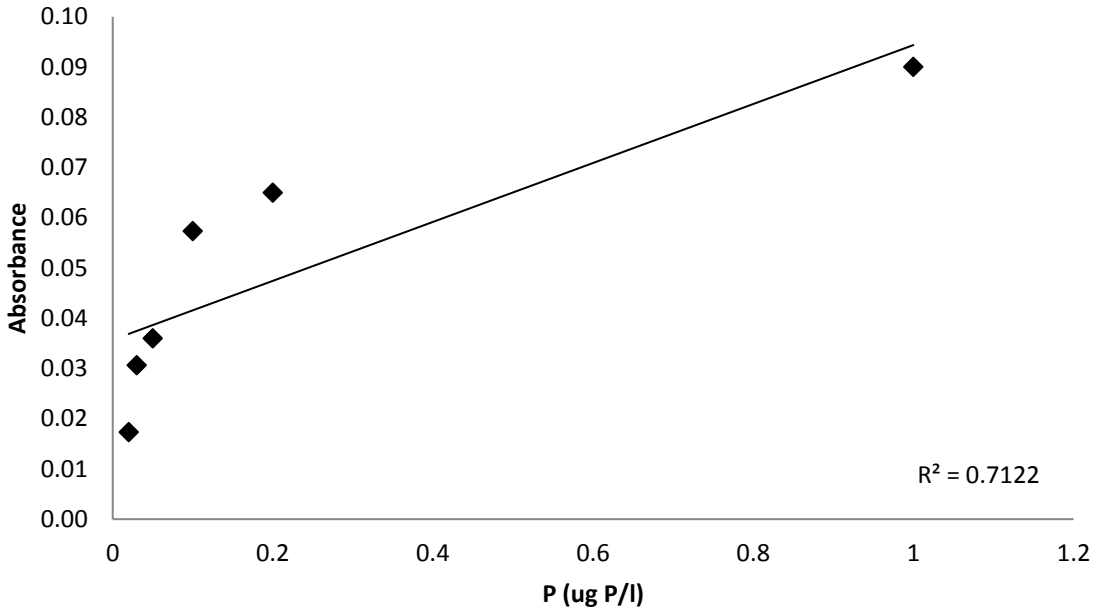


Figure 23. Tap water 1 absorbance (♦) against phosphate concentration (Novel Analysis Method).

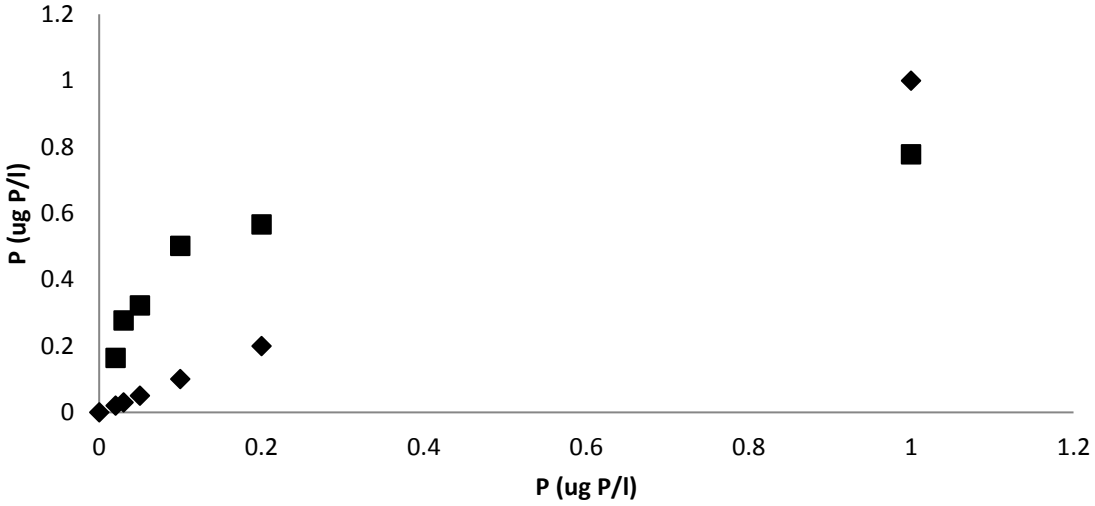


Figure 24. Tap water 1 phosphate yield (■) against calculated phosphate concentration (♦) (Novel Analysis Method).

4.6.3 Tap water 2

A second test, run with similar conditions to the tap water 1 analysis, indicated similar linearity (R^2 = 0.82), but resulted in lower yields of 61-106 %, in comparison to the respective phosphate addition (figures 25 and 26). The quantification limit for the series was calculated to be 22.9 µg P/l, which was higher than any of the analyzed total phosphorus concentrations. Furthermore, when looking at the resolution of the absorbance, there is no significant difference between 0.2 and 0.4 (µg P/l) or 0.6 and 0.8 (µg P/l) concentrations. Due to the insensitivity indicated by the tap water tests 1 and 2, the novel method was considered unreliable at the phosphorus concentrations of interest.

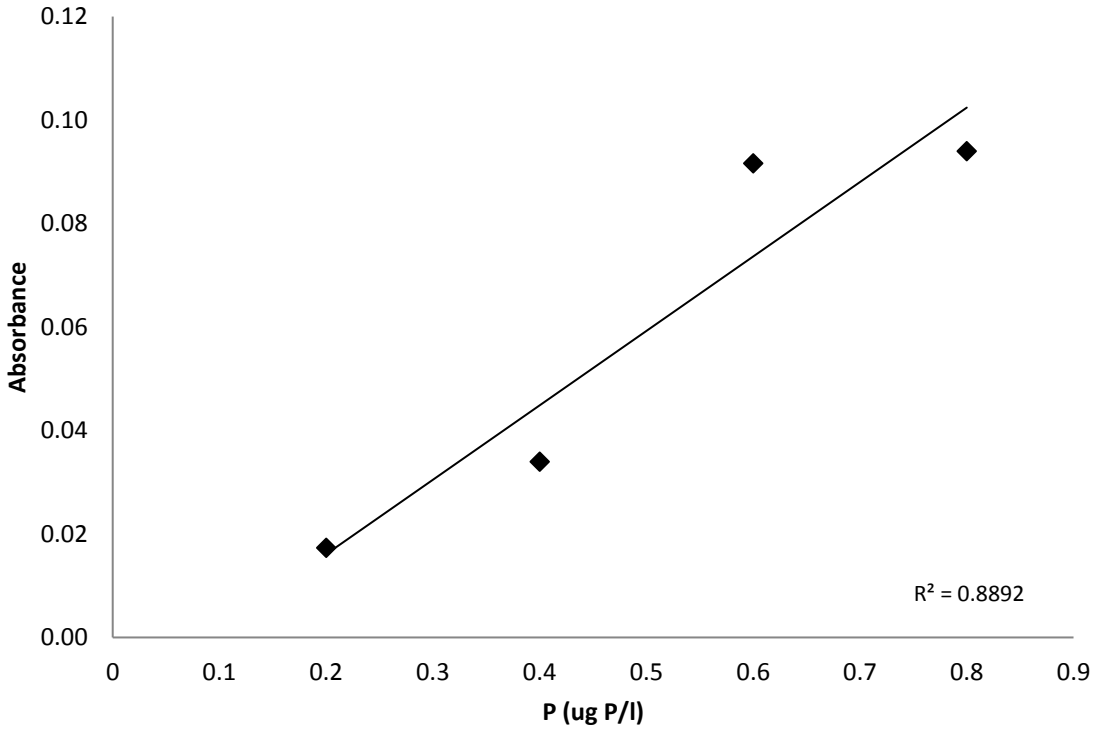


Figure 25. Tap water 2 absorbance (♦) against phosphate concentration (Novel Analysis Method).

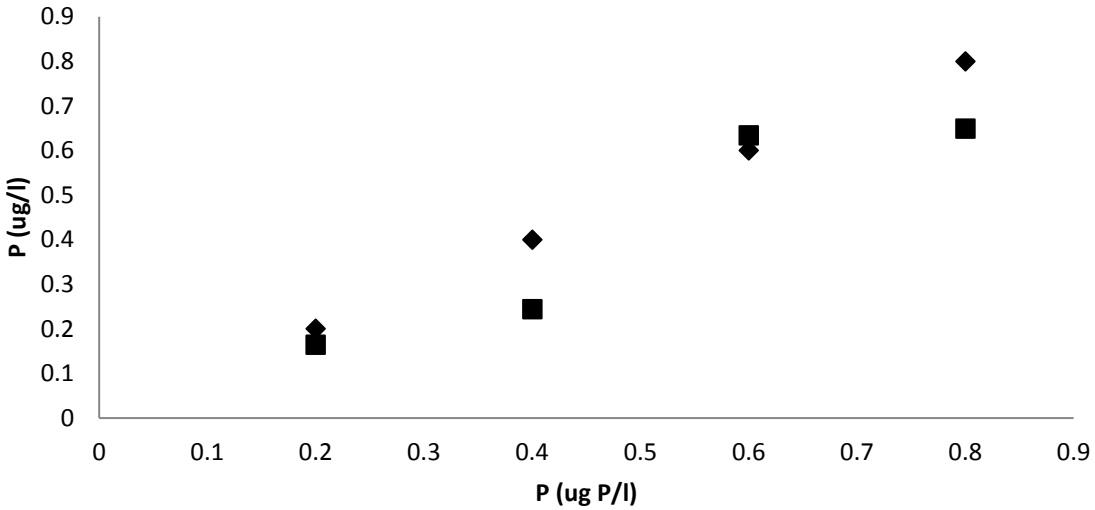


Figure 26. Tap water 2 actual total phosphorus yield (■) against calculated phosphate concentration (♦) (Novel Analysis Method).

Regardless of well performed optimization of the individual steps of the method by Aubry (2015) (Appendix 1), the measured absorbance did not overall show consistent linearity in terms of yield. Especially the unreliability of the method was outstanding when using tap water as the base. Even though the phosphorus concentration can vary in the tap water, it was considered that such variance should be insignificant, after running the tap for 15 minutes.

Possible errors, which could be causing the unreliability with the tap water, might be caused due to the difficulty of dissolving the calcareous fur (figure 26). Calcareous fur is virtually impossible to dissolve completely from any container. This was supported by the notion that the cleaning process of the fur from the beakers through physical effort required above normal strain.

Controlling the pH of the samples before and after autoclave oxidation proved to be extremely laborious and time consuming. Each sample had to be individually tested and adjusted, with even the smallest acid or base addition causing a major change in pH. This was most likely due to the utilization of a strong acid in the dissolving phase. Additionally, the dissolving phase required a very consistent technique, which could be prone to human error. Furthermore, the task of evaporating the samples took anywhere between 24 and 48 hours with 15 samples. The duration depended heavily on the positioning and number of the beakers inside the oven, and uneven evaporation was occurring. This might pose problems if samples require quick analysis. After the evaporation was complete, the time needed to complete the analysis of total phosphorus took roughly four hours, when running series of 16-20 samples.

Figure 26. Calcareous fur residue after evaporating water samples completely.

4.7 Modified novel analysis method

The modifications of the sample evaporation amounts did reduce the accumulation of calcareous fur build up. Although some small specks of residue were visible on the bottoms of the beakers, their number was negligible in comparison to the completely evaporated samples (figure 27). Without the need for the utilization of a strong acid, the modified novel method was far more fluent in comparison to the novel method. Biggest sources for possible loss of accuracy came from the need of measuring the final evaporated sample sizes, requiring the transfer of samples between containers. Uniform methodology and routine should minimize this to a minute error. The evaporation time required for halving the sample size from 300 ml to roughly 150 ml was between 20 and 24 hours with 15 samples. Due to uneven evaporation of the samples, changing of the beaker positions in the oven might produce more even sample amounts at the end of the evaporation process. Even though the evaporation still required a long period, it was far more consistent and thus favorable.

Figure 27. Calcareous fur residue after evaporating 50 % of the sample amount.

4.8 Achieved improvements in the novel method

Table 3 shows a summary of the development of the novel analysis method, in terms of the parameters that had a major influence on the reliability of the method. Development phase 1 (Aubry 2015) showed good promise when analyzing high total phosphorus concentrations with a well-established technique. However, it lacked the capability to perform at the required low range of phosphorus concentrations.

The development phase 2, describes the achievements that have been discovered for the novel method during this thesis. Significant improvements in accuracy of the method were achieved through testing and changing many of the individual parameters. The most important improvement in comparison to currently used methods was achieved by the doubling of the concentration. By using the quantification limit of 0.68 µg P/l calculated for the SFS EN ISO 6878 with 10 cm cuvette, the lowest phosphorus concentration in a sample before concentration could be 0.34 µg P/l. In comparison to the total phosphorus concentrations seen in the previous results of HSY DWDS, this level of accuracy could be sufficient to cover the majority of phosphorus variation.

Table 7. The development stages summary for the novel method for total phosphorus determination. Dev. 1 refers to the previous work performed by Aubry (2015).

Parameter	Dev. Phase 1	Dev. Phase 2
Wavelength	700 nm	880 nm
Cuvette	1-4 cm	10 cm
Concentration Ratio	20:1	2:1
Quantification Limit	>20 µg P/l	>0.34 µg P/l
Evaporation	100 %	50 %
Dissolving	Strong Acid	Not Used

4.9 Reliability by known concentration

4.9.1 SFS EN ISO 6878 with 10 cm cuvette

The reliability of the standard method SFS EN ISO 6878 with 10 cm cuvette was monitored by carrying a 5 µg P/l a known concentration in each analysis series. From figure 28, it can be seen that the method produced an average of 4.95 µg P/l with an average deviation of 0.11 µg P/l from the calculated 5 µg P/l concentration. The maximum deviation was 0.37 µg P/l. Two-way Student´s T-test showed no statistical difference between the theoretical and the measured values (p=0.4). The improvements brought by the utilization of a longer cuvette and wavelength became apparent through increased resolution of the spectrophotometer, when looking at the various tests run by Aubry (2015), who tested 1 and 4 (cm) cuvettes (Appendix 1).

Figure 28. 5 µg P/l follow up of known phosphorus concentration (SFS EN ISO 6878 with 10 cm cuvette).

4.9.2 Modified novel analysis method

The accuracy and reliability of the modified novel analysis method was tested by carrying a total phosphorus concentration of 2.5 µg P/l (figure 29). The average concentration was 2.52 µg P/l and on average, the results deviated by 0.122 µg P/l from the calculated 2.5 µg P/l concentration. The observed low deviation indicates good reliability and accuracy of the method at low total phosphorus concentrations. Two-way Student´s T-test showed that there was no significant difference between the theoretical and the measured phosphorus concentrations (p=0.8). However, the low sample size should be pointed out, which might inhibit the confidence in the used Student´s T-test.

Figure 29. 2.5 µg P/l phosphorus concentration carried along HSY DWDS analyses (modified novel method)

5 Conclusions and future development

5.1 Phosphorus in drinking water distribution systems

The results from the pilot DWDS demonstrate that an addition of 5 µg P/l of phosphorus is sufficient to support microbial growth and furthermore establish unwanted nitrification. This suggests that nitrification in DWDSs could possibly be reduced by controlling the level of phosphorus leaving the WTPs. However, the results also suggested that in order for nitrification to occur in the DWDS, the concentration of phosphorus (0.85 µg P/l) found in the drinking water of the Aalto water analysis laboratory is not sufficient to initialize nitrification in newly developed microbial growth. It could be of interesting to see how varying phosphorus concentrations in drinking water would affect the time required for initialization of nitrification in DWDSs. Due to the slower growth rate of nitrifiers; it remained unclear whether nitrification could have been initialized after a longer period of time, when phosphorus could have accumulated into the biofilm matrix.

The analyses from the full size HSY DWDS indicate that the total phosphorus concentration varied between 0.60-2.6 µg P/l in the Helsinki metropolitan area. Even though both WTPs in the metropolitan area do reduce the phosphorus concentration of drinking water to very minute concentrations, the analyzed levels of nitrite suggested that unwanted microbial growth exists regardless. Interestingly there was no clear relationship seen between the amount of nitrite and phosphorus in the HSY DWDS regardless of the limiting nutrient status of phosphorus. This suggested that the amount of nitrification occurring could be controlled more strongly by other parameters than the amount of total phosphorus. Due to the complex effect of phosphorus on both microbial regrowth and physical properties in the DWDSs, additional improvements brought by reducing the phosphorus concentration in drinking water production should be considered critically. The difference between the seen phosphorus uptake in the pilot DWDS and the non-existent uptake of phosphorus in the HSY DWDS suggest that the biofilm in the HSY has reached a mature state, whereas the pilot DWDS showed clear signs of growth.

As this study has been able to quantify a general level of total phosphorus concentration in a full scale DWDS, it would be interesting to study whether and how individual water treatment processes affect the phosphorus concentration. Furthermore, studying the effects and changes in the microbiology of a mature biofilm growing within the DWDSs could help provide information on to what extent could reductions in phosphorus levels help reduce microbiological drinking water degredation. For example, an experimental set up of varying water treatment methods in combination with a new DWDS would allow to study the rate of biofilm proliferation. Furthermore, it would be highly interesting to know whether by controlling the total phosphorus concentration at the WTP, it would be possible to achieve a difference in DWDS biofilm structure and dynamics. As this thesis has pointed out that even at very low phosphorus concentrations microbial activity, including nitrification, does occur in DWDSs. This points to the notion that even if phosphorus is efficiently removed from drinking water, it will most likely support growth in the long term. Thus, future studies should also aim to study the long term impacts of changing water quality conditions.

The total phosphorus concentration in the HSY DWDS varied inconsistently, when looked at from both traveled distance and seasonality perspectives. However further studies with more frequent and widespread sampling could help to establish more reliably whether there exist spatial or temporal patterns in the phosphorus concentrations of DWDSs. This could be used to establish linkages between water quality changes or even estimating hot spots for DWDSs maintenance. Concentrations of below 1 µg P/l are already very minute in comparison to natural water or wastewater levels, and yet phosphorus is still performing as a key element in microbial water quality degradation.

Carbon, nitrogen and phosphorus are the essential macronutrients for microbial growth. This thesis concluded that with a C:N:P ratio of 2000:610:1 µg/l the drinking water flowing in the Helsinki Metropolitan area DWDS could be heavily phosphorus limited. However, phosphorus accumulation and recycling from the biofilm phase can possibly circumvent this inhibition for growth. The rate of regrowth of microbes could possibly be reduced by further minimizing the availability of the limiting nutrient at least in certain types of waters. This could bring about safer transportation of water to the consumers as well as slower physical biodegradation of the network.

5.2 Phosphorus analysis methods

A new method to analyze drinking water total phosphorus was developed, based on the currently used standard method SFS EN ISO 6878. The development of the novel method for total phosphorus analysis, showed great promise when analyzing the aimed low concentrations. Even though phosphorus as such does not pose a severe threat to human health, the indirect effects warrant the information to be as accurate and precise as possible. Precise information gives power to develop further tests and estimations on microbial growth, perhaps at some point resulting in accurate simulations of microbial growth in DWDSs.

The currently widely used MAP bioassay for evaluating phosphorus concentrations in DWDSs has great value when the interest lies on whether and how well a water sample can support microbial growth. However, it comes with the common problems of conventional microbiological assays, as it requires long periods of growh time and can only describe a certain fraction of total phosphorus. This is where the newly developed novel analysis method can provide more information. For this it would be of interest, how well could the novel method be implemented into monitoring schemes of drinking water analyses across various WTPs.

The difficulty involved in developing a new method came to light, when the highly varying and undesirable results started to occur. It is possible that a part of the inconsistency in the results could be explained by the varying laboratory methodology of different persons. However, this also prompted the need to discard such volatile procedure steps. As mentioned before, a good laboratory method is as simple as possible, requiring only the minimum of steps. At this stage, the novel method is working as well as initially aimed for, with the lowest total phosphorus concentrations analyzable below the current limit of 2 µg/l.

The novel method should be developed further by first establishing calibration graphs utilizing the novel method. After this, quantification limits should be re-evaluated in order to take into account the effect that the evaporation process might have on the accuracy and precision

of the end result. Further, as the development looked at and established several improvements in both the novel method and the standard method SFS EN ISO 6878. Especially the improvements of a 10 cm cuvette in combination with 880 nm wavelength should be considered to be used in future total phosphorus analyses for improved accuracy and precision.

Even though the results obtained in this thesis indicate that stopping microbial regrowth through nutrient limitation can be next to impossible, the future might bring with it changing water treatment strategies, where it is imperative to know the level of phosphorus at the microgram level. The drinking water that flows beneath our feet should always be regarded with high importance. Regardless of the ever-changing world around us, the need for clean water will always remain. Only through developing our understanding and gathering more information, can we safely move forward in the field of water resource management.

References

Anderson N., Bennion H., Lotter A. 2014. Lake eutrophication and its implications for organic carbon sequestration in Europe. Global Change Biology vol. 20:2741-2751.

Appenzeller B., Batte M., Mathieu L., Block J., Lahoussine V., Cavard J., Gatel D. 2001. Effect of adding phosphate to drinking water on bacterial growth in slightly and highly corroded pipes. Water Research. vol. 35:1100-5.

Aubry S. 2015. La nitrification dans les réseaux d'eau potable. Unpublished documents. Aalto University. Department of civil and environmental engineering. Water and Environmental Engineering.

Batte M., Mathieu L., Laurent P., Prevost M. 2003. Influence of phosphate and disinfection on the composition of biofilms produced from drinking water, as measured by fluorescence in situ hybridization. Candadian Journal of Microbiology. vol. 49:741.

Beever R., Burns D. 1981. Phosphorus Uptake, Storage and Utilization by Fungi. Advances in Botanical Research. vol. 8:127-219.

Bennett E., Carpenter S., Caraco N. 2001. Human Impact on Erodable Phosphorus and Eutrophication: A Global Perspective. Bioscience vol. 51:227-234.

Berry D., Xi C., Raskin L. 2006. Microbial ecology of drinking water distribution systems. Current Opinion in Biotechnology. vol. 17:297-302.

Butterfield P., Camper A., Ellis B., Jones W. 2002. Chlorination of model drinking water biofilm: implications for growth and organic carbon removal. Water Research. vol. 36:4391-4405.

Chen L., Jia R-B., Li L. 2013. Bacterial community of iron tubercles from a drinking water distribution system and its occurrence in stagnant tap water. Environmental Science Processes and Impacts. vol. 15:1332-1340.

Comber S., Casse F., Brown B., Gardner M. 2010. Phosphate treatment to reduce plumbosolvency of drinking water also reduces discharges of copper into environmental surface waters. Water and Environment Journal. vol. 25(2):266-270.

Committee on Public Water Supply Distribution Systems: Assessing and Reducing Risks. 2006. Drinking Water Distribution Systems – Assessing and Reducing Risks. The National Academies Press. ISBN: 10 0-309-66432-2.

Counsil Directive 98/83/EC. 1998. European Commission. [Online] Accessed on 19.2.2017. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31998L0083

EEA 2003. European Environment Agency. Phosphorus in Lakes. [Online] Accessed on 24.7.2016. Available at: http://www.eea.europa.eu/data-and-maps/indicators/phosphorus-in-lakes

Ekeng E., Agunwamba J. 2011. The Effect of Pipe Ageing of Different Diameter and

Pressure on Residual Chlorine. Journal of International Academic Research. vol. 11

EPA. 1994. Environmental Protection Agency. DIANE Publishing Company. Control of Biofilm Growth in Drinking Water Distribution Systems. Factors That Favor Biofilm Growh. Seminar Publication. DIANE Publishing. Online] Accessed on 11.8.2016. ISBN: 0788106449, 9780788106446.

Fang W., Hu J., Ong S. 2009. Influence of phosphorus on biofilm formation in model drinking water distribution systems. Journal of Applied Microbiology. vol. 106:1328-1335.

FAO. 1987. Food and Agriculture Organization of the United Nations. Fisheries and Agricultural Department. Kutty.M. Site Selection for Aquaculture. Chemical Features of Water. Phosphorus. [Online] Accessed on 20.8.2016. Available at: http://www.fao.org/docrep/field/003/ac183e/AC183E00.htm#TOC.

Gray N. 2005. Water Technology. An Introduction for Environmental Scientists and Engineers. 2nd Edition. Elsevier Butterford-Heinemann. ISBN: 0750666331. p.255-308.

HSY. 2016. Veden Laatu. Juomavesi ja Kloori. [Online] Accessed on 12.9.2016. Available at: https://www.hsy.fi/fi/asukkaalle/kodinvesiasiat/juomavesi/Sivut/Veden-laatu.aspx.

HSY. 2015. Toimintakertomus 2014. HSY:n tarkastuslautakunta 14.4.2015. Vesihuoltoverkosto HSY:n toiminta-alueella. [Online] Accessed on: 10.10.2016. Available at: https://www.hsy.fi/fi/tietoa-hsy/paatoksenteko/Documents/arviointikertomus2014.pdf.

Jang H-J., Choi Y-J., Ro H-M., Ka J-O. 2012. Effects of Phosphate Addition on Biofilm Bacterial Communities and Water Quality in Annular Reactors Equipped with Stainless Steel and Ductile Cast Iron Pipes. The Journal of Microbiology vol. 50:17-28.

Jansson M. 1988. Phosphate uptake and utilization by bacteria and algae. Hydrobiologia. vol. 170:177-189.

Juhna T., Birzniece D., Rubulis J. 2007. Effect of Phosphorus on Survival of *Escherichia coli* in Drinking Water Biofilms. Applied and Environmental Microbiology. vol.73:3755-3758.

Kasahara S., Maeda K., Ishikawa M. 2004. Influence of phosphorus on biofilm accumulation in drinking water distribution systems. Water Science and Technology: Water Supply vol. 4:389-398.

Katko S. 2013. HANAA! Suomen vesihuolto – kehitys ja yhteiskunnallinen merkitys. Suomen Vesilaitosyhdistys ry. ISBN: 978-952-5000-97-9. p.10-173.

Keeney D., Hatfield J. 2001. The Nitrogen Cycle, Historical Perspective, and Current and Potential Future Concerns. Nitrogen in the Environment: Sources, Problems and Potential Future Concerns. ISBN: 978-0-444-50486-9.

LeChevallier M., Au K.-K. 2004. Water Treatment and Pathogen Control. World Health Organization (WHO). IWAPublishing. ISBN: 92-4-156255-2.

LeChevallier M., Olson B., McFeters A. 1990. Assessing and Controlling Bacterial Regrowth in Distribution Systems. American Water Works Association. [Online] Accessed on 18.7.2016. ISBN: 0898674905, 9780898674903.

LeChevallier M. 1990. Coliform Regrowth in Drinking Water: A Review. Journal of the American Water Resources Association.74-86.

LeChevallier M., Cawthon C., Lee R. 1988. Inactivation of Biofilm Bacteria. Applied and Environmental Microbiology. vol. 54:2492-2499.

Lee E. Schwab K. 2005. Deficiencies in drinking water distribution systems in developing countries. Journal of Water and Health. vol. 3:109-127.

Lehtola M., Laxander M., Miettinen I., Hirvonen A., Vartiainen T,m Martikainen P. 2006. The effects of changing water flow velocity on the formation of biofilms and water quality in pilot distribution system consisting of copper or polyethylene pipes. Water Research vol. 40:2151-2160.

Lehtola M., Juhna T., Miettinen I., Vartiainen T., Martikainen P. 2004. Formation of biofilms in drinking water distribution networks, a case study in two cities in Finland and Latvia. Journal of Industrial Microbiology & Biotechnology. vol. 31:489-494.

Lehtola M., Nissinen T., Miettinen. I., Martikainen P., Vartiainen T. 2003. Removal of soft deposits from the distribution system improves the drinking water quality. Water Research vol. 38:601-610.

Lehtola M. 2002. Microbially available phosphorus in drinking water. National Public Health Institute. ISBN: 951-740-307-0.

Lehtola M., Miettinen I., Vartiainen T., Martikainen P. 1999 Mikrobeille käyttökelpoisen fosforin analysointi vedestä. Vesitalous 4/1999.

Liu G., Verberk Q., Dirk J. 2013. Bacteriology of drinking water distribution systems: an intergral and multidimensional review. Applied Microbiological Biotechnology. vol. 97:9265-9276.

Loladze I., Elser J. 2011. The origins of the Redfield nitrogen-to-phosphorus ration are in a homoeostatic protein-to-rRNA ratio. Ecology Letters. vol. 3:244-250.

Martiny A., Pham C., Primeau F., Vrugt J., Moore K., Levin S., Lomas M. 2013. Strong latitudinal patterns in the elemental ratios of marine plankton. [Online] Nature geoscience. vol. 6.

Martiny A., Jorgensen T., Albrechtsen H., Arvin E., Molin S. 2003. Long-Term Succession of Structure and Diversity of a Biofilm Formed in a Model Drinking Water Distribution System. Applied and Environmental Microbiology. vol. 69(11):6899-6907.

Miettinen I., Zacheus O., von Bonsdorff CH., Vartiainen T. 2001. Waterborne epidemics in Finland in 1998-1999. Water Science and Technology. vol. 43:67-71.

Miettinen I., Vartiainen T., Martikainen P. 1997. Phosphorus and bacterial growth in drinking water. Applied and Environmental Microbiology vol. 63:3242-3245.

Okabe S., Kokazi. T., Watanabe Y. 2002. Biofilm formation potentials in drinking waters treated by different advanced treatment processes. Water Science and Technolgy: Water Supply 2:4:97-104

Onisei D., Onisei D., Feier I., Rusu D., Stratul S-I. 2008. The Biofilm: Formation and Removal. Timisoara Medical Journal. vol. 54:1-2.

Polanska M., Huysman K,m Keer C.V. 2005. Investigation of microbially available phosphorus (MAP) in Flemish drinking water. Water Research. vol. 39:2267-2272.

ProEconomy 2015. Controllin Biofilms and Pathogens in Water Systems. [Online] Accessed on 17.10.2016. Available at: https://proeconomy.wordpress.com/2015/10/08/controlling-biofilms-and-pathogens-in-water-systems/.

Richardson S., Plewa M., Wagner E., Schoeny R., DeMarini D. 2014. Occurrence, genotoxicity, and carcinogenicity of related and emerging disinfection by-products in drinking water: A review and roadmap for research. Mutation Research. vol. 636:178-242.

RIL 124-1. 2003. Vesihuolto I. Karttunen E. Suomen Rakennusinsinöörien Liitto RIL r.y. ISBN: 951-758-431-8. p.215-260.

Rozej A. Cydzik-Kwiatkowska A. 2015. Structure and microbial diversity of biofilms on different pipe materials of a model drinking water distribution systems. World Journal Microbiological Biotechnology. vol. 31:37-47.

Rubulis J., Juhna. T. 2007. Evaluating the potential of biofilm control in water supply systems by removal of phophorus from drinking water. Water Science and Technology. vol 55:211-217.

Salkinoja-Salonen M., Aalto J-M. 2002. Mikrobiologian Perusteita. Helsingin Yliopisto. ISBN: 951-45-9502-5. p.185-255.

Saravanamuthu V. 2009. Water and Wastewater Treatment Technologies. The removal of phosphorus during wastewater treatment: A review. Eolss Publisher Co. Ltd. ISBN: 978-1-84826-194-5.

Servais P., Anzil A., Gatel D., Cavard J. 2004. Biofilm in the Parisian suburbs drinking water distribution system. Journal of Water Supply: Research and Technology – Aqua. vol. 53:313-324.

Seviour R., Nielsen P. 2010. Microbial Ecology of Activated Sludge. IWA Publishing. eISBN: 9781780401645.

Shrivastava A., Gupta V. 2011. Methods for the determination of limit of detection and limit of quantification of the analytical methods. Chronicles of Young Scientists. vol. 2:21-25.

Smil V. 2002. Phosphorus: Global Transfers. Encyclopedia of Global Environmental Change. John Wiley and Sons, Ltd. ISBN: 0-471-97796-9.

Stal L., Cretoiu M. 2016. The Marine Microbiome: An Untapped Source of Biodiversity and Biotechnological Potential. Springer International Publishing Switzerland 2016. ISBN: 978-3-319-32998-7.

Tittel J., Buttner O., Kamjunke N. 2012. Non-cooperative behaviour of bacteria prevents efficient phosphorus utilization of planktonic communities. Journal of Planktonic Research. vol. 34:102-112.

Turner B., Frossard E., Baldwin D. 2005. Organic phosphorus in the environment. CABI Pub 2005. E-Book. p.1-16.

UMN 2009. Busman L., Lamb J., Randall G., Rehm G., Schmitt M. The nature of phosphorus in soils. University of Minnesota. [Online] Accessed on 13.7.2016. Available at: http://www.extension.umn.edu/agriculture/nutrient-management/phosphorus/the-nature-of-phosphorus/.

University of Waikato. 2013. The Phosphorus Cycle. [Online] Accessed on 3.11.20166. Available at: http://sciencelearn.org.nz/Contexts/Soil-Farming-and-Science/Science-Ideas-and-Concepts/The-phosphorus-cycle

Vadstein O. 2000. Heterotrophic, planktonic bacteria and cycling of phosphorus: Phosphorus requirements, competitive ability and food web interactions. Advances in microbial ecology. vol. 16:115-167.

Vahala R. 2002. Two-Step Granular Activated Carbon Filtration in Drinking Water Treatment. HUT Laboratory of Water and Wastewater Engineering. ISBN: 951-22-6163-4.

Vahala R. Niemi R.M., Kiuru H., Laukkanen R. 1999. The effect of GAC filtration on bacterial regrowth and nitrification in a simulated water main. Journal of Applied Microbiology Symposium Supplement. vol. 85:178S-185S.

Van der Kooij, Dirk van der Wielen, Paul W. J. J. 2014. Microbial Growth in Drinking-Water Supplies - Problems, Causes, Control and Research Needs. IWA Publishing. [Online] Accessed on 13.7.2016. Available at: http://app.knovel.com/hotlink/toc/id:kpMGDWSPC1/microbial-growth-in-drinking/microbial-growth-in-drinking

VHVSY. 2004. Elämän vesi - veden kiertokulku -ympäristökasvatushanke. [Online] PDF. Accessed on 14.8.2016. Available at: http://www.vhvsy.fi/files/upload_pdf/2113/Veden_laatu.pdf

Vikesland P., Ozekin K., Valentine R. 2001. Monochloramine Decay in Model and Distribution System Waters. Water Research vol. 35:1766-1776.

Wahman D., Pressman J. 2014. Nitrification in Chloraminated Drinking Water Distribution Systems: Factors Affecting Occurrence. Comprehensive Water Quality and Purification. vol.2:283-294.

Werner S., Morgan J. 1996. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters. 3rd Edition. ISBN: 978-0-471-51185-4.

Withers P., Jarvie H. 2008. Delivery and cycling of phosphorus in rivers: A review. Science of the Total Environment. vol. 400:379-395.

Ympäristö.fi. 2013. Vesien tila. [Online] Accessed on 3.8.2016. Available at: http://www.ymparisto.fi/fi-FI/Kartat_ja_tilastot/Vesien_tila.

Appendices

Appendix 1. Search for a New Phosphorus Measuring Method by Evaporation. 10 pages
Appendix 2. Instructions on Novel Analysis Method for Total Phosphorus Determination. 2 pages.
Appendix 3. Verkostonäytteiden Analysointi. 2 pages.

Appendix 1. Search for a New Phosphorus Measuring Method by Evaporation

C° µg/l	Absorbance x 1000 (700 nm, cuvette 1cm)																					
	0	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
0	x																						
5		x	x																				
9		x	x																				
10		x	x	x																			
12			x	x																			
15				x	x	x																	
16					x	x																	
20								x	x														
22									x	x													
24							x			x													
26											x	x											
30												x	x	x									
35															x								
38																x	x						
40																	x						
42																		x	x				
45																				x	x		
50																						x	x

Chart 1

CAN WE OBTAIN ACCURATE PHOSPHORUS LOW CONCENTRATION MEASURES WITH 1 CM CUVETTE ?

- No : same absorbance for ≠ C° until 40 µg/L (high C°) (chart 1)

CAN WE OBTAIN ACCURATE PHOSPHORUS LOW CONCENTRATION MEASURES WITH 4 OR 10 CM CUVETTE ?

- No : same absorbance for ≠ C° (charts 2 and 3)

→ need to find a way to increase this concentration

C° µg/l	Absorbance x 1000 (700 nm, cuvette 4cm)											
	0	3	4	5	6	7	8	9	11	12	13	14
0	x											
2	x		x									
3	x											
4		x	x									
5							x					
6					x	x						
7		x	x									
8			x	x								
9			x	x								
10				x	x							
12							x	x				
14										x	x	
16										x		
18									x			x
20											x	

Chart 2

C° µg/l	Absorbance x 1000 (700 nm, cuvette 10cm)																			
	1	2	5	7	8	9	10	11	12	14	15	16	17	18	23	24	25	27	29	31	33	40
0	x	x																				
2			x		x																	
3				x		x																
4					x																	
5					x			x														
6							x															
7						x		x														
8									x	x												
9											x			x								
10												x	x									
12															x	x						
14																		x			x	
16																x	x					
18																			x			
20																				x		x

Chart 3

EVAPORATION 80°C, 90% AERATION [AIR CIRCULATION]

80°C => don't destroy OM and Phosphorus

- 39 hours to evaporate 1L (≈ 1,5 day)
- 65 hours to evaporate 2L (≈ 2,5 days)

A bit longer if the oven is full: 18 x 1L in oven => 2,5 days

Change places of beaker in the oven => reduce evaporation time

HOW TO RINSE BEAKERS ?

Which acid to choose?

- Rinsing beakers with H_2SO_4 => white precipitate ($CaSO_4$) => solubility in H_2SO_4 ↘ for pH = 2 or 3
- Test acid oxalic => precipitate does not dissolve
- Using HCl is ok to dissolve that !
  - Which concentration ?
    - 3M et 5M HCL => need to much NaOH to adjust pH (>3 ml)
    - 0,1 M HCL no enough strong
    - 1 & 2 M HCL => ok => test each => 2M : better yield
- Think to use ultrasound bath
- In fact it's impossible to dissolve it

=> same protocole for each sample => same amount dissolve

Rinse with acid

- **Material:** Glass Pasteur pipette, measuring cylinder (25mL), little beaker, 2M HCl, 3 timers

- Use 10 mL 2M HCl (put at least 15 mL in the measuring cylinder)
- Scrub in little back and forth in the side of the beakers => well spread of the acid
- Do entire turns like this going down, then shake the beaker a little => ↗ contact time
- Wait exactly 10 min before rinse with ROW

- Rinse Glass Pasteur pipet and measuring cylinder with acid contain by the little beaker between each sample
(I think it's not necessary to rinse if it's the same sample or standards)

- Fill the measuring cylinder to 15 ml

Rinse with ROW

- **Material:** Plastic Pasteur pipette, funnel with short tube, little beaker 30ml, measuring cylinder (MC) 50ml , blue autoclave bottle

- Used 30 mL to rinse (measure with MC and pipet in the beaker)
- First: pour acid in the blue bottle with the funnel, put ≈1,5 ml to the top with inclined beaker
- Rinse 3 time with 3 x ≈ 2,5ml (quasi full Pasteur pipet) shake well and pour. Put ≈1 ml to the top with inclined beaker
- Wait a little with little inclined beaker to have liquid going down, during this time, fill to 30 mL the MC to next rinse.
- Pour, put ≈1,5 ml to the top with inclined beaker
- Rinse the funnel with the rest of the ROW (≈1,5 ml)

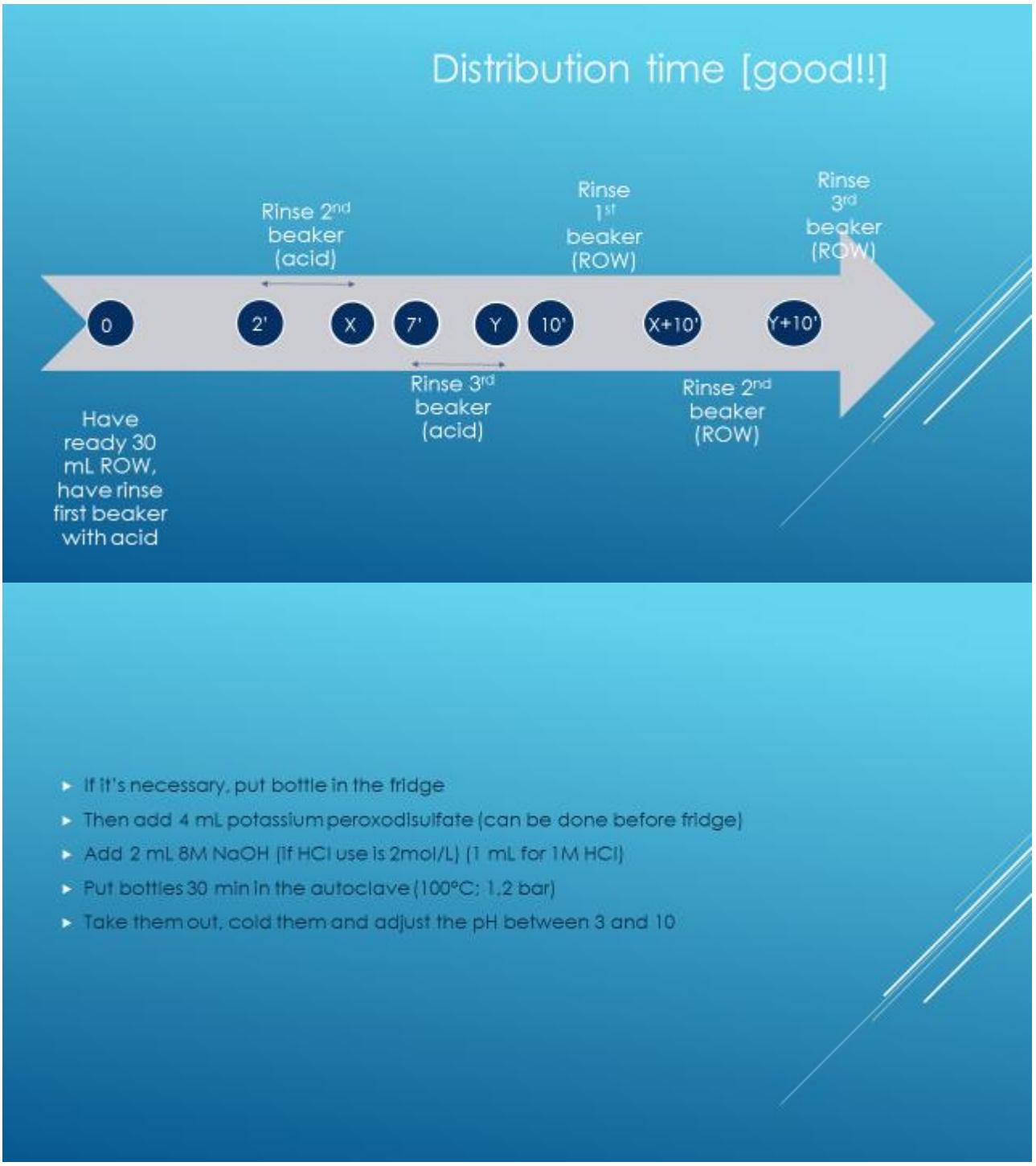
- Rinse pipet, little beaker and funnel with ROW between each beaker

Distribution time

- Fill with 30 ml ROW the measuring cylinder

- Take your time to rinse the first beaker with acid, start the 10' timer when it's done
- Clean glass pipet and MC with acid
- Fill MG to 15 or 20 mL of acid
- Start to rinse your 2nd beaker 2' after having start the 1st timer
- You must rinse your beaker, pipet and MC and fill it again in less than 5'
- Don't forget to start an other 10' timer after having rinsing this beaker
- Start to rinse your 3rd beaker about 7' after having start the 1nd timer, (max 3 min)
- Start a 3rd 10' timer

Rinse beaker in order with ROW and rinse pipet, beaker, funnel (about 5' each)


Distribution time [good!!]
Rinse 2nd beaker (acid)
Rinse 1st beaker (ROW)
Rinse 3rd beaker (ROW)
0
2'
X
7'
Y
10'
X+10'
Y+10'
Rinse 3rd beaker (acid)
Rinse 2nd beaker (ROW)
Have ready 30 mL ROW, have rinse first beaker with acid
If it's necessary, put bottle in the fridge
Then add 4 mL potassium peroxodisulfate (can be done before fridge)
Add 2 mL 8M NaOH (if HCl use is 2mol/L) (1 mL for 1M HCl)
Put bottles 30 min in the autoclave (100°C; 1,2 bar)
Take them out, cold them and adjust the pH between 3 and 10

HOW TO ADJUST PH ?

- **Material:** pH paper, Pasteur pipette, ≠ Volume pipette : (1 ml; 50µL ; 20µL etc) ; 8M NaOH, HCl and glass pipet
- In some bottle (≈1/6) (+ 1 by extra sample if there is):
  - Measure pH (usually pH is really low (<1))
  - Add 0,5 mL 8M NaOH (if 2M HCL) (0,4 mL 8M NaOH if 1M HCl)
  - Mix and measure pH again
  - Adjust it by adding 50µL by 50µL , it changes really fast !!
- In other bottles:
  - Add the firsts beakers necessary amount to adjust pH
  - Check pH in each
  - If necessary, add a little more of NaOH (20µL by 20µL)

If pH surpasses 10, correct it with drops of HCL

HOW TO MEASURE ABSORBANCE

- **Material:** ascorbic acid, ammonium molybdate, 1 & 2 mL pipet, 800mL ROW beaker, 800mL empty beaker, Pasteur pipet, 4 & 10 cm cuvette, 50 mL volumetric flask
- Pour blue bottles into 50 mL volumetric flasks
- Complete volumetric flasks 6 by 6 with 1 ml ascorbic acid, 2 ml ammonium molybdate and ROW to the mark (with Pasteur pipet)
- Mix very well and wait 1 to 2 minutes (especially if C° is high (if a dark blue coloring appears))
- During this waiting time, do the blank with 4 cm cuvette (V ≈ 13 mL) and ROW, and if necessary: pour the waste beaker and fill the 800 mL beaker with ROW

HOW TO MEASURE ABSORBANCE

- Measure the absorbance of 6 volumetric flasks with 4 cm cuvette, rinse well cuvette with ROW between each
- Do the blank with 10 cm cuvette (V ≈ 23 mL) and ROW
- Measure the absorbance of the 6 volumetric flasks with 10 cm cuvette, rinse cuvette well with ROW between each
- Don't waste solution, there is not enough to fill again one cuvette
- Rinse very well all cuvettes with ROW
- Continue with 5 (or 6) other volumetric flasks

SUMMARY

Evaporate beakers

Rinse beakers with 1M HCl then with ROW and pour all in blue bottles

Add 4mL potassium peroxodisulfate and a part of 8M NaOH

Put bottles 30 min in the autoclave

Adjust pH with 8M NaOH

Pour bottle into 50 mL volumetric flasks

Add 1 mL ascorbic acid and 2 mL ammonium molybdate

Measure absorbance at 700 nm with 4 and 10 cm cuvette

NOTE

- Don't shake beakers in the oven to avoid adding deposit on the sides
- Short tube funnel permit to prevent the tube to dip in the liquid
- A 10 ml beaker for cleaning stuff with acid permit to limit contamination at 1 beaker (in case of contamination in this little beaker)
- Take care not to fill the funnel and spill liquid in case of no air can go out of the blue bottle
- Change the funnel if drops remain adhered to it (it's certainly due to a phosphorus deposit)
- Try to make acid cover the bottom of the beaker after having add all the acid by:
  - Shaking it a little
  - Tilting it a little
- Add a part of 8M NaOH permit to prevent dirty the autoclave and injure plugs of the blue bottles

Appendix 2. Instructions on Novel Analysis Method for Total Phosphorus Determination

1. Evaporation of samples
   Transfer 1 L of sample into a beaker, with a 1 l measuring cylinder
   Oven 80 °C for X hours
   90 % ventilation
   Maximum of 18 beakers in the oven
   - > Only remaining white precipitate
2. Dissolving precipitate
   10 ml of 2 M HCl acid washing using a glass pipette (Rinse beaker sides also).
   Transfer acid/phosphorus liquid into a 50 ml autoclave bottle through a funnel.
3. Rinsing with reverse osmosis water (ROW)
   Utilize 30 ml of ROW for the following rinsing.
   Use a Pasteur pipette to rinse the empty acid/phosphorus beaker.
   Rinse the funnel used for transfer
4. Addition of oxidant
   Add 4 ml of potassium peroxodisulfate to the autoclave bottle
5. Adjust pH (to prevent corrosion of autoclave)
   Test initial pH of the autoclave bottle on a pH paper strip (usually very acidic).
   Insert 2 ml of NaOH base with a micropipette.
   Test pH (if 3-10 move to section 6)
   Insert 0,3 ml of NaOH base with a micropipette
   Test pH (if 3-10 move to section 6)
   Insert 0,05 ml of NaOH base with a micropipette
   Test pH (if 3-10 move to section 6)
   Repeat until pH is 3-10
   If pH goes/ is or moves over 10, use 0,05 ml of 2 M HCl acid to lower pH
6. Autoclave samples
   Make sure the autoclave bottles with the sample in them are correctly labeled.
   Autoclave the bottles for 30 min at 120 °C
   Cool down samples and prepare volumetric bottles for section 7
7. Follow SFS EN ISO 6878
   Cool to room temperature
   Test pH and adjust to 3-10 with NaOH/HCl (≈700 ug/l NaOH)
   Empty the autoclave bottles into 50 ml volumetric flasks
   Add 1 ml of ascorbic acid into each
   Within 30 sec, add 2 ml of acid molybdate solution
   Input glass stoppers to all and shake samples well
   Wait 10 minutes and start to measure sample absorbances (section 8)
8. Absorbance
   Have ROW water for rinsing
   Set up spectrophotometer to 880 nm and use 10 cm cuvette

Auto-zero the spectrophotometer by using ROW water in the cuvette
Move onto measuring the absorbance, starting from the 0 sample
Use maximum of 30 min to complete measurements

Appendix 3. Verkostonäytteiden Analysointi

Verkostonäytteiden Analysointi

Tarkoituksena on analysoida HSY:n verkostoalueelta kerättyjen talousvesinäytteiden kokonaisfosforipitoisuus. Verkostonäytteitä on kerätty aikavälillä 2014–2015, kahdeksasta eri kohteesta (taulukko 1). Analyyseistä saatujen tulosten perusteella voidaan tutkia onko fosforipitoisuudella ja vedenpuhdistamon etäisyyden välillä yhteyttä. Lisäksi kokonaisfosforin pitoisuuden vuodenaikaan perustuvaa vaihtelua pyritään arvioimaan. Näytteitä analysoidaan aluksi kolmelta näytepäivämäärältä, jotka ovat 9.9.2014, 20.1.2015 ja 2.6.2015. Menetelmän toimiessa, analysoidaan lisäksi näytteet päivämääriltä 9.12.2014 ja 14.4.2015.

Verkostonäytteiden kokonaisfosfori pitoisuuden oletetaan olevan alle luotettavan määritysrajan, kun käytetään SFS EN ISO 6878 mukaista molybdaatti menetelmään. Tästä syystä näytteet analysoidaan käyttämällä kehitteillä olevaa menetelmää, jossa haihduttamalla näytteitä voidaan nostaa kokonaisfosfori pitoisuus määritysalueelle. Tällä oletuksella, näytteet voidaan analysoida standardimenetelmällä käyttämällä näytteiden spektrofotometristä absorbanssia pitoisuuden määrittämiseksi. Tämän jälkeen voidaan laskennallisesti saada selville näytteiden todellinen kokonaisfosforipitoisuus.

Taulukko 1 (Pirjo Rantanen, Phase Two Research Plan)

	Kohde	Näytteen nimi	PKOO	IKOO	Etäisyys verkostoa pitkin km
		Pulloihin			
1	Pitkäkosken vesilaitos, Kuninkaantammentie 17	PI	6683507	25494268	0
2	Edita Oyj, Hakuninmaantie 2	ED	6683228	25493617	0,77
3	Shell Kaivoksela, Kaivoksentie 1	SH	6683487	25493028	2,6
4	Myyrmäen urheilutalo, Myyrmäenraitti 4	MY	6683664	25491773	4,2
5	Neste Oil Rajatorppa, Vapaalantie 1	NR	6682610	25490389	5,9
6	Neste Oil Keimola Itä, Hämeenlinnanväylä 601	NK	6689072	25491284	11,5
7	Katriinan sairaala, Katriinankuja 4	KA	6692696	25493039	17
8	Suomen ympäristökeskus, Hakuninmaantie 6	SY	6683018	25493917	0,68

Analyysi

1. Tarkoituksena on kaksinkertaistaa kokonaisfosforipitoisuus näytteissä. Lisäksi jokainen näyte analysoidaan kolmena kappaleena luotettavuuden parantamiseksi.
2. Valitaan näytepäivämäärä, josta analysoidaan näyte jokaisesta kohteesta.

3. Näytteitä haihdutetaan 50 % alkuperäisestä tilavuudesta
4. Näytteet analysoidaan menetelmällä SFS EN ISO 6878

Ohjeet

1. Mittaa 300 ml jokaista kohdenäytettä volumetrisellä sylinterillä dekantterilasiin (500ml).
2. Mittaa 300 ml käänteisosmoosivettä (nollat) volumetrisellä sylinterillä dekantterilasiin (500ml).
3. Mittaa 300 ml 5 ug/l PO4-P liuosta volumetrisellä sylinterillä dekantterilasiin (500 ml).
4. Aseta dekantterilasit uuniin tasaisesti. Aseta uunin lämpötila 80 °C ja tuuletus 90 %.
5. Tarkista näytteet maksimissaan 24 h kuluttua. Näytemäärät on oltava alle 150 ml, mutta ei missään nimessä täysin kuivuneita (Ei kattilasakkaa).
6. Ota näytteet pois uunista, kaada näytteet vuorollaan volumetriseen sylinteriin, jossa täydennät näytettä RO vedellä 300 ml:n asti.
7. Ota näytettä 40 ml autoklaavipulloon, pese mittasylinteri huolellisesti, kuivaa ja toista.
8. Analysoi näytteet SFS EN ISO 6878 kappale seitsemän mukaisesti.
9. Laske näytteille pitoisuudet menetelmän aikaisemman ROW standardisuoran mukaan, haihtuminen huomioon ottaen.
10. Taputa itseäsi selkään, kuivaa silmäsi, kyllä se siitä vielä :)