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Heavy metal removal by floating treatment wetlands: Plant selection

Licentiate thesis in Plant Physiology, by Maria Schück

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Front page: Floating treatment installation in Edsviken, Stockholm, placed next to a stormwater outlet.

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Abstract

Elevated levels of heavy metals and chloride are commonly found in stormwater, as a consequence of pollution from traffic, building material and industries, and the use of salt for deicing in wintertime. Floating treatment wetlands (FTWs), consisting of vegetated rafts that can be placed in stormwater ponds, may be able to reduce heavy metal and chloride concentrations, but until this date have mainly been used for nutrient removal in warm climates. Plants are essential in FTWs as pollutants are taken up into plant tissues, adsorbed to exposed plant surfaces, precipitated due to chemical interactions with root exudates or bound to plant litter.

The aim of the study was to examine: A) which plant species that should be used on FTWs in a cool climate for efficient heavy metal and chloride removal, and B) to identify plant traits that are connected to high pollutant removal capacity as a help for identification of additional suitable species.

Thirty-four wetland plant species, all growing in wild in Sweden, were used in the study. These were all grown hydroponically for 5 days in a solution containing 1.2 µg Cd L^{-1}, 68.5 µg Cu L^{-1} ¸ 78.4 µg Pb L^{-1}, 559 µg Zn L^{-1} and 55.4 mg Cl L^{-1}. *Carex pseudocyperus* and *Carex riparia* were found to quickly reduce the concentration of all added heavy metals, and keep the concentration low for the remainder of the exposure period. In addition, nine species were able to remove all metals except cadmium quickly. High removal capacity of metals was found to be connected to biomass traits, mainly large fine root and leaf biomass, and to transpiration, which is correlated with to leaf biomass. Twenty-three of the tested species have also been evaluated for their chloride uptake, and *Phalaris arundinacea* and *Glyceria maxima* were identified as the species with highest chloride removal capacity. Preliminary analysis show that the correlation between biomass and chloride removal capacity is weaker than for heavy metals.

In conclusion, the removal capacity of heavy metals and chloride differs between plant species, which can be explained by differences in the traits of the plants. The findings indicate that removal of both heavy metals and chloride can be achieved by FTWs in cold climates using a combination of native plants.

Sammanfattning

Förhöjda halter av tungmetaller och klorid är vanligt förekommande i dagvatten. Detta är orsakat av föroreningar från trafik, byggnadsmaterial och industriell verksamhet samt från användandet av vägsalt under vintrar. Flytande våtmarker som består av bevuxna flottar som kan placeras i dagvattendammar skulle potentiellt kunna minska koncentrationen av tungmetaller och klorid. Hittills har dock denna typ av våtmarker mestadels använts för att minska halterna av kväve och fosfor i vatten i länder med varmt klimat.

Växterna i den flytande våtmarken är nödvändiga för att reningen ska bli effektiv. De tar upp en del av föroreningarna genom rötterna och ackumulerar i växten. Föroreningarna fäster också på ytan på rötter och döda växtdelar samt fäller ut och sedimenterar genom kemiska förändringar i vattnet runt rötterna. Växternas rötter som hänger ned i vattnet minskar vattenhastigheten, vilket ger ökad sedimentation av föroreningar som är bundna till partiklar. Därefter kan växtdelarna och sedimentet transporteras från platsen och tas om hand på ett säkert sätt.

Syftet med denna studie var att: A) Undersöka vilka växtarter som kan användas i flytande våtmarker i kallt klimat för effektiv rening av tungmetaller och vatten, samt B) identifiera vilka egenskaper hos växterna som är kopplade till hög reningsförmåga hos växterna, för att på så sätt lättare kunna identifiera fler lämpliga arter.

Studien baseras på 34 arter av svenska våtmarksväxter. De odlades hydroponiskt, och exponerades i fem dagar för en lösning innehållande 1,2 µg Cd L^{-1}, 68,5 µg Cu L^{-1}, 78,4 µg Pb L^{-1}, 559 µg Zn L^{-1} och 55,4 mg Cl L^{-1}, en föroreningsmängd baserat på halterna i dagvatten från högtrafikerad väg.

Slokstarr (*Carex pseudocyperus*) och jättestarr (*Carex riparia*) visade sig snabbast minska koncentrationen av samtliga tungmetaller i lösningen. Ytterligare nio arter minskade snabbt koncentrationen av koppar, bly och zink, men inte kadmium. Växternas reningsförmåga visade sig korrelerat med mängden biomassa, framförallt mängden blad och tunna rötter, samt med växtens transpiration som i sin tur beror på mängden blad. Förmågan att rena klorid har analyserats hos 23 av arterna och rörflen (*Phalaris arundinacea*) och jättegröe (*Glyceria maxima*) visade sig vara de mest effektiva arterna. Preliminära analyser visar att korrelationen mellan saltupptag och biomassa är svagare än för tungmetaller.

Slutsatsen av denna studie är att växters förmåga att rena vatten från tungmetaller och klorid uppvisar stora skillnader, som kan förklaras genom skillnader i växternas egenskaper. Detta indikerar att rening av dagvatten från både tungmetaller och klorid i kallt klimat kan uppnås med flytande vårmarker med inhemska växter.

List of Papers

This thesis is based on the following papers, referred to in the text by their roman numerals:

I **Schück,** M. and Greger, M. 2019. Capacity of 34 wetland plant species to remove heavy metals from water. *Submitted to Environmental Science and Pollution Research*

II **Schück,** M. and Greger, M. 2019. Plant traits related to the heavy metal removal capacities of wetland plants. *Submitted to International Journal of Phytoremediation*

My contribution to both manuscripts was as follows: Planning and analysis of experiments and writing of the manuscripts were made by me with advice from my supervisor. I made all laboratory work.

Content

Introduction

Polluted stormwater

Stormwater, also known as urban runoff, is formed when rain and snowmelt water flows over surfaces that prevents infiltration, such as roads, cars, rooftops and industrial areas. The water may already have absorbed pollutants in the atmosphere, and is further polluted on the ground by dissolving or re-suspending the numerous substances found in urban environments. Many of these contaminants are threats to human health and environment, particularly aquatic biota (Makepeace et al. 1995).

The increasing urbanization of the world increases the volumes of stormwater (WWAP 2015). Precipitation that would been seen as a resource in an agricultural context becomes waste product that needs to be disposed of (Echols and Pennypacker 2015). Moreover, climate change is expected to increase both volumes and contaminant concentrations (Sharma et al. 2016). For a long time, the focus of stormwater management was to effectively manage the volumes to prevent flooding. However, increasing focus is put on improving the quality of stormwater to prevent downstream pollution (Liu et al. 2015). In addition, increasing interest for utilization of stormwater as irrigation also requires control and reduction of contaminant levels (Feng et al. 2012; Nnadi et al. 2015).

Heavy metals in stormwater

Heavy metals are ubiquitous contaminants in stormwater. The concentration of metals in stormwater shows wide variation and depends on the land use in the drainage basin, weather conditions and size of the metal particles (Borne et al. 2013; Liu et al. 2015). A large share of the metals originates from traffic where they are released by wear on cars, especially tires and brake linings, and combustion (Makepeace et al. 1995). The most frequently studied metals in traffic-related urban runoff are cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn), followed by chromium (Cr), cobalt (Co) and nickel (Ni). Roofing materials, industrial factories, fertilizers and natural sources also contribute to the pollution of metals in stormwater.

The metals are present in particulate and dissolved forms, where the latter is more toxic due to its bioavailability (Morrison 1989). The distribution between dissolved and particulate fraction varies with metal, season and road management practices. Cadmium, Cu and Zn have larger dissolved fraction than Pb (Revitt and Morrison 1987; Lara-Cazenave et al. 1994). The dissolved fraction is larger in winter in cold climates and correlates with the volume of suspended solids (Hallberg et al. 2007; Westerlund 2007; Westerlund and Viklander 2008). Using salt for deicing further increase the dissolved fraction compared to using sand or gravel (Westerlund 2007). The standard separation method for particulate and dissolved fraction is filtering through a 0.45 um filter, but the dissolved fraction can be divided even further to better describe its toxicity (Revitt and Morrison 1987).

Heavy metal removal techniques in stormwater

Heavy metals cannot be degraded to less toxic compounds, in contrast to many organic pollutants. Thus, remediation methods have to focus on removing them from environments or chemical forms where they pose a threat to biota, and instead concentrate the metals where they are less bioavailable. This could include immobilizing by binding them to soil or organic particles, or accumulating them in something that could safely be removed and disposed of, as sediments or plants.

Common treatment practices for stormwater include infiltration in swales, constructed wetlands, stormwater ponds, or treatment in wastewater treatment plants (Liu et al. 2015). However, direct discharge to the recipient is most common. Swales and other infiltration practices efficiently bind both particulate and dissolved metals to the filter material, but might be limited in capacity. Stormwater ponds can on the other hand handle large volumes and efficiently remove particulate pollutants, but have only little impact on dissolved metals and nutrients. It is important to properly design and manage the treatment systems to prevent clogging or resuspension of settled metals (Vymazal 2008; Kadlec and Wallace 2010).

Stormwater in Sweden

The main sources for metals in stormwater in Sweden are traffic and industrial activities, and highest contaminant levels are expected in urban areas with high traffic intensity (Lindgren 2001). The pollution load varies with season. The water volumes and contaminant levels are higher during winter, caused by low evaporation and increased wear on roads due to studded tires and deicing with sand. The sub-zero temperatures during winters often causes delays of transport of contaminants as they are retained in ice and snow (Westerlund and Viklander 2008).

The responsibility to collect, delay and treat stormwater is divided between land owners and municipalities in urban areas (Billberger 2011). The Swedish Transport Administration is responsible for stormwater from roads and railways owned by the state. However, the planning, design and construction of stormwater treatment systems are mainly carried out by subcontractors such as landscape architect firms, environmental and technical consult companies and construction companies. All these stakeholders need access to state of the art knowledge and further development of stormwater treatment methods to be able to plan and construct sustainable stormwater treatment system that meets the demands of the society (Blecken 2016). Sustainable stormwater management should consider both quantitative, qualitative and amenity aspects, which often is achieved by open or semi-open systems (Stahre 2008).

There are no national requirements for stormwater quality, but the Water Framework Directive (2000/60/EC) commits all members of EU to achieve good ecological and chemical status for all water bodies. As stormwater has been identified as a major impact on water quality, the Water Framework Directive indirectly affects the stormwater management (Viklander et al. 2019).

Stormwater ponds, grass swales and constructed wetlands are common treatment practices in Sweden (Blecken 2016). Traffic-related runoff is mainly treated by grass swales that surround roads in rural environments, whereas highway runoff is mainly treated in ponds or reservoirs that have low capacity to remove dissolved metals. Combined systems for stormwater and wastewater are common in urban environments, which puts a strain on the wastewater treatment facility during large precipitation events. In the capital, Stockholm, 50% of the stormwater is discharged directly to a water body, and the remaining 50% is treated together with wastewater in wastewater treatment facilities (Vall 2015).

Phytoremediation and rhizofiltration

Phytoremediation is a collective term for technologies that use plants to remove or stabilize pollutants in the environment (Raskin et al. 1994). The most well know of these technologies are phytoextraction, phytodegradation, phytostabilization and rhizofiltration (Arthur et al. 2005). In phytoextraction, plants accumulate the pollutants from soil, followed by plant harvest that removes the pollutant from the site. Phytodegradation consists of degradation of organic compounds to less toxic products by root-mediated processes. Phytostabilization focuses on immobilizing the pollutants in the soil to prevent leakage to ground water, a suitable technique in sites where removal is not possible.

Rhizofiltration, the technique used in this study, describes the remediation in water by plant roots. These processes include the absorption of pollutants by plant roots and following concentration of pollutants in the plant, as well as precipitation and adsorption of pollutants to the roots of the plant (Raskin et al. 1994). The plants used for rhizofiltration are either free-floating plants like duckweed (*Lemna* spp.) or water hyacinth (*Eichhornia crassipes*), or hydroponically cultivated plants.

Metal uptake and accumulation in plants

Plants accumulate heavy metals by absorption of ions from the surrounding medium with their roots. Further transportation inside the roots occurs either through the apoplast or symplast, and long distance transport to other parts of the plants uses xylem translocation. Most heavy metals remain in the roots of the plants, although allocation to the shoot occurs to a varying degree.

The fact that plants accumulates heavy metals might seem strange at first glance. Why would plants take up these substances known for their toxicity to the environment, including both plants and humans? There are two reasons. Firstly, some of the heavy metals are actually essential nutrients for plants, which means they are necessary for the plants to complete their life cycle. Copper and zinc both belongs to this category, meaning that plants need to accumulate these and therefore have developed high affinity uptake mechanisms. Copper is a component in many enzymes involved in redox reactions, including key functions such as photosynthesis, respiration and N and C metabolism (Marschner 2012). Zinc in plants is found in a wide range of enzymes, often in a catalytic or co-catalytic role, as well as free ions. Secondly, the plants cannot prevent uptake of heavy metals. The absorption of metal by the roots is a passive process in which the plants are not able to discriminate between essential and non-essential metals as cadmium and lead. Some plants have developed exclusion mechanisms as physical barriers that reduce the uptake, but all plants absorb some non-essential heavy metals. Much of the absorbed heavy metal remains in the apoplast, whereas some passes the cell membrane through transport proteins, which transports both essential and non-essential metals due to their similarity in size and charge. The transport of non-essential metals allows the plant to decrease the concentration in the roots, where it might reach toxic levels, by transporting the metal to other parts where it causes less damage. Some of the transporters have been identified as P1B-ATPases, COPT-transporters and the ZIP protein family (Guerinot 2000; Williams and Mills 2005; Pilon 2011).

Heavy metal toxicity and tolerance

Both essential and non-essential metals become toxic above a certain level, varying both between metal and plant species. Plants have several detoxification mechanisms to mitigate the effects. Some plants, known as hyperaccumulators, are able to accumulate very high levels of metals without damage. These species are found in metal-rich soils, and as a trade-off for metal tolerance they are often small plants (Arthur et al. 2005). For phytoremediation purposes they are often not interesting due to their small size and specific growing requirements. Another type of coping strategy is demonstrated by excluders (Raskin et al. 1994), which are able to prevent most of the uptake and translocation to aboveground tissue. As many phytoremediation practices builds upon harvest of shoots, excluders are not useful for removing metal from the ground. Instead, they can be used to stabilize the soil and prevent leakage to groundwater (phytostabilization).

Regular plants accumulate metals to varying extent, but lack the extreme characteristics of hyperaccumulator and excluder plants. Even though their concentration in the tissue is lower than in hyperaccumulators, they can be interesting for phytoremediation purposes if they combine metal uptake with large growth, and preferably also translocation to the shoot, leading to a high removal per plant (Ladislas et al. 2015; Vymazal 2016). It is also easier to find plants of this type that has less specific growing requirements, making phytoremediation practices possible in a wide range of conditions. The metal concentration of stormwater is expected to have low toxicity to plants, making regular plants useful in rhizofiltration of stormwater.

Chloride uptake and accumulation in plants

Chloride is an essential mineral nutrient element to plants, necessary for generation of O_2 in photosynthesis and osmoregulation of stomata, among other processes. Chloride ions are absorbed from water by plant roots, and thought to be transported mainly through the symplast and xylem. Chloride channels mediate transport in and out of cells. About 0.2-0.4 mg Cl g^{-1} dry weight is needed for optimal growth, however plants generally contain 10-100 times more Cl than this, a sign of “luxury consumption” (Marschner 2012). Toxic concentrations of chloride differs between plant species, with chloride sensitive species developing toxicity reactions to concentrations above 4-7 mg Cl g^{-1} dry weight (White 2001).

Halophytes are groups of plants that are able to withstand high Cl concentrations as they have developed tolerance mechanisms as excretion, avoidance and accumulation (Sruthi et al. 2017). They have also commonly developed biochemical protection mechanisms as osmoprotectants and antioxidants, which also can protect plants from heavy metal stress. For this reason, halophytes has been suggested for phytoremediation of heavy metals (Manousaki and Kalogerakis 2011).

Floating treatment wetlands

The method of floating treatment wetlands (FTWs) has been proposed for increasing the removal efficacy of stormwater ponds (Kerr- Upal et al. 2000; Borne et al. 2013). FTWs consist of rafts with emergent plants grown hydroponically, thereby providing direct contact between plant roots and the polluted water (fig 1) (Headley and Tanner 2012). The rafts commonly consist of plastic materials to provide stability and buoyancy, and an organic planting medium. Compared to conventional constructed wetlands, the FTWs require no additional land use as they can be placed in existing ponds. Moreover, the FTWs tolerate large variation in water level, a common problem for plants in conventional wetlands. The FTWs remove pollutants by increased sedimentation, direct uptake into plants, sorption and plant-mediated microbial processes (Headley and Tanner 2012; Pavlineri et al. 2017). The lack of soil or sediment surrounding plant roots forces the plants to absorb nutrients and pollutants directly from the water, creating a system which quickly can react to high pollution loads caused by rain or snowmelt events. Most FTW studies so far have focused on removal of nitrogen (N) and phosphorous (P), and meta-analyses show mainly efficient removal throughout varying nutrient concentrations, climate conditions and experimental designs (Wang and Sample 2013; Pavlineri et al. 2017). Other pollutants removed by FTWs include metals, organic pollutants, solids, pathogens and algae (Chang et al. 2012; Winston et al. 2013; Keizer-Vlek et al. 2014; Walker et al. 2017; Olguín et al. 2017; Rehman et al. 2018; Tara et al. 2019).

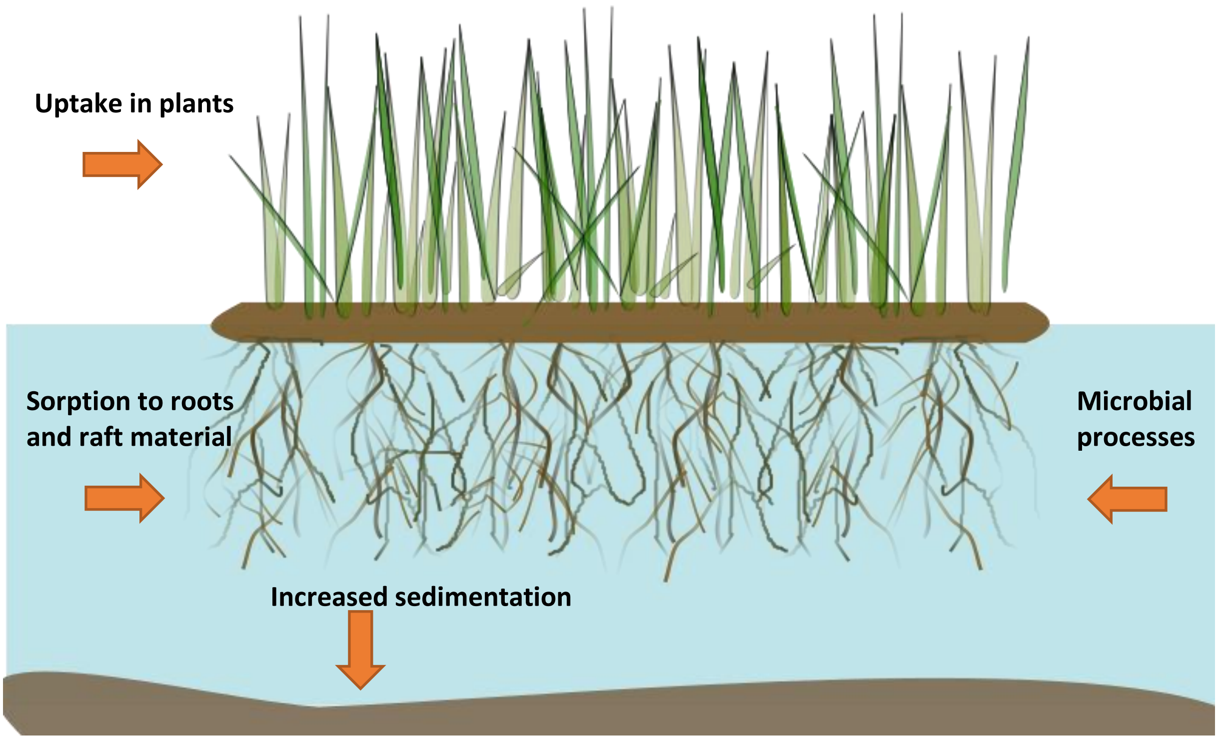


Fig 1. Removal pathways for pollutants of floating treatment wetlands.

Plants in FTWs

Plants are a key component in FTW systems (Tanner and Headley 2011). The plants used on FTWs in studies display a wide range of species, of various sizes, geographic origin and genetic background. Mainly wetland plants have been used, as they are able to tolerate oxygen deficiency that commonly is found below the FTW (Borne et al. 2014). Even so, plant survival can be challenging, as showed in the early study by (Smith and Kalin 2000) that found all of their 2000 *Typha angustifolia* seedlings dead due to the oxygen consumption caused by the decay of the peat they had used as planting medium. Coconut coir, living sphagnum moss and mineral material as volcanic gravel have been used with more success for establishment of plants in the floating wetlands.

The plants chosen for FTWs should be native to avoid invasive species, and able to survive in the climate conditions at the site (Wang and Sample 2014). Tolerance of elevated pollutant levels in their tissues is also necessary (Bonanno and Cirelli 2017). The water quality itself can also be a challenge for plant survival, as it might contain low nutrient levels, low pH or high concentration of pollutants dependent on the source. Chloride from deicing salt, commonly present in stormwater in cold climates, is toxic for plants at elevated levels but can be tolerated to various extents. Chloride also affects the availability of pollutants, such as most heavy metals, in water. The availability can either be increased or decreased depending on metal, temperature and presence of sediment (Greger 1995; Fritioff et al. 2005; Du Laing et al. 2008).

Metal and chloride removal by FTWs

Heavy metal removal by FTWs has only been investigated in a few field and mesocosm studies. These studies have shown reduced concentration of heavy metals in the outlet in most cases (Revitt et al. 1997; Tanner and Headley 2011; Borne et al. 2013), but sometimes negligible reduction for one or several investigated metals (Van de Moortel et al. 2010; Borne et al. 2013;

Ladislas et al. 2015) or even higher concentration of zinc compared to inlet concentrations have been measured in an early study (Revitt et al. 1997). The removal could be further improved by the use of growth promoting and dye degrading bacteria (Tara et al. 2019).

Increased metal concentrations in the sediment was found in all studies where it was investigated (Borne et al. 2013; Ning et al. 2014). This effect is due to changes in the chemical conditions of the water caused by root and microbial processes, and by reduction of water velocity by the root mass, which makes smaller particles sediment and prevents resuspension (Smith and Kalin 2000; Tanner and Headley 2011). During the FTW treatment the metal concentrations in the plants increased, but according to Borne et al. (2014) and Ladislas et al. (2015), the contribution of plant uptake to the total metal removal was small. However, only a small number of species have been tested so far in FTWs, and none of these studies have considered plant selection as a tool for increasing efficacy of metal removal.

Chloride removal by FTWs has to my knowledge never been studied. Some FTW trials have been conducted in saline water, and salinity negatively affected the accumulation of Cu and Zn in aboveground parts of *Phragmites australis* on a FTW (Huang et al. 2017).

Fig 2. Floating treatment wetland installation inside floating baffles in Rönningesjön, Täby outside Stockholm. Stormwater with increased levels of phosphorous passes the FTWs before it is released into the lake.

FTWs in cold climate

Low temperatures and short vegetation periods are expected to reduce the removal capacity of FTWs. When temperature falls, the biological activity of plants and biofilm formed on plant roots ultimately ceases. The removal mechanisms, still active during winter, will be reduction of water velocity by plant roots, adsorption to roots and binding to organic matter provided by the plants (Van de Moortel et al. 2010; Headley and Tanner 2012). However, spring and autumn temperatures might be beneficial for FTW performance. van de Moortel et al. (2010) found that the FTW removal effect for N and P was optimal at temperatures between 5 °C and 15 °C, compared with lower and higher temperatures. No clear trends were seen for metal removal and temperature in the same study. The plant uptake of N and P in low can temperatures can be increased using cold tolerant species (Zou et al. 2016), which likely also applies for other pollutants.

Winter temperature also affects plant survival, limiting the number of plant species that can tolerate the weather. The exposed conditions on a FTW might further decrease survival, however, Wang et al. (2015) studied the survival of plants on FTW that were encased in snow and ice during winter, and did not see any negative impact on plant survival.

The first Swedish FTWs were installed 2013 in Rönningesjön, Täby, outside Stockholm (fig 2). The 150 m^2 system, surrounded by floating baffle curtains, treats stormwater with the aim to reduce the P load to the eutrophic lake. This system was studied in a bachelor thesis the year after installation, the only scientific study of a FTW made in Sweden (Dunér and Myhrberg 2014). Only 9.4% P removal was found, which was explained by low P load, the newly installed FTW that had not reached its full potential, and a sampling technique (spot sampling) that gives a less reliable result than continuous sampling. Regardless of the low removal, this FTW system has been followed with a handful of other installments, for stormwater remediation of N, P and particles in ponds, lakes and the sea.

Aim

This licentiate thesis is part of the PhD project ”Sustainable stormwater treatment with floating treatment wetlands”. The combination of increasing amounts of legislation and other directives regarding responsibly for stormwater management, and increasing volumes of polluted stormwater due to heavy rains and urbanization, have created a need for new management practices (Vall 2015; Blecken 2016; Swedish Environmental Protection Agency 2019). By utilizing the ability of plants to clean the environment, known as phytoremediation, the project aims to provide the scientific basis for sustainable metal removal from stormwater by FTWs in cold climate.

The full project includes identification of suitable plant species, quantification of the species removal capacity under varying conditions, development of FTWs based on sustainable materials, and tests of the FTW system to evaluate long-term removal efficacy. The outcome of the project is expected to be utilized within a near future by stormwater treatment stakeholders, as building and water management companies, municipalities etc.

The aim of this licentiate thesis was to identify which plants that could be useful for metal removal from stormwater by FTWs in a cold climate. The specific aims of the study were to answer the following questions:

- What is the removal capacity of heavy metals and chloride for plants grown in hydroponic conditions?
- Which plant traits are connected to high removal capacity of heavy metals and chloride?
- Which plant species could be useful for metal removal from stormwater by FTWs in a cold climate?

Comments on Materials and Methods

Choice of plant species

Plants from 34 perennial herbaceous species, all growing in or near water, were used in the studies (see **Paper I** or **II** for a list of species). The majority of the species had large biomass, but some smaller species were also included. Only species naturally growing in Sweden were used since the project this thesis is part of aims to develop FTWs for metal removal suitable for Swedish climate, which has a short and intense vegetation season and cold winters. However, none of the plants are endemic to Sweden and many have a wide distribution, which means that the results from these studies will be useful for other conditions as well. Free-floating or submerged plant species were not included in the study since they are not able to grow on FTWs, although the latter has been shown to accumulate high amounts of metals (Fritioff and Greger 2003).

Metal and chloride concentrations used in the experiments

The hydroponic solutions used in the experiments contained 1.2 µg Cd L^{-1}, 68.5 µg Cu L^{-1}, 78.4 µg Pb L^{-1}, 559 µg Zn L^{-1} and 55.4 mg Cl L^{-1} added as $CdCl_2$, $CuCl_2$, $PbCl_2$, $ZnCl_2$ and NaCl. As chloride-based metal salts were used for preparation of the solution, no other anions were present at start that could interfere with the plants or metals. The metal concentrations were based on measurements on stormwater in Sweden (table 1) (Hallberg et al. 2007; Alm et al. 2010). The concentrations were similar to what have been used in other studies (Weiss et al. 2006; Ladislas et al. 2013). In contrast to natural stormwater, this solution did not contain other pollutants as particles, organic material or nutrients. Acid washed (5% HNO_3) plastic equipment was used to minimize binding of metals to surfaces.

Table 1. Detected levels of heavy metals and chloride in stormwater, and levels of stormwater used in studies.

Source	**Cd** (µg L^{-1})	**Cu** (µg L^{-1})	**Pb** (µg L^{-1})	**Zn** (µg L^{-1})	**Cl** (mg L^{-1})	**Reference**
Stormwater in stormwater ponds	<0.05-0.16	8.4-66	2.0-28	29-500	10-554	(Alm et al. 2010)
Stormwater from highly travelled road	1.05-1.18	251-611	62-118	98-102	4.16-885	(Hallberg et al. 2007)
Artificial stormwater	10	100	300	250	63	(Weiss et al. 2006)
Artificial stormwater	-	16	-	485	-	(Tanner and Headley 2011)
Artificial stormwater	10	-	-	500	-	(Ladislas et al. 2013)
Artificial stormwater in present study	1.2	68.5	78.4	559	55.4	**Paper I and II**

Use of hydroponic setup

Hydroponic experiments in a greenhouse was used as experimental setup. Compared with field conditions in a stormwater pond or similar environment, the pollution concentration, light, temperature and relative humidity can be controlled. The consistency of conditions allows an easy comparison between a multitude of species (Sricoth et al. 2018), and studies have shown correspondence in plant performance between hydroponic experiment and later field studies (Watson et al. 2003).

Analysis of metals

The metal concentrations in the samples were analyzed with atomic absorption spectrometry (AAS). The technique is based on the absorption patterns of light of particulate wavelengths by atomic metals in gaseous state. Furnace atomization was used for Cd, Cu and Pb due to their low concentration (detection limits 0.006, 0.04, and 0.06 µg L^{-1}, respectively), whereas flame spectrometry could be used for Zn analysis (detection limit 1 µg L^{-1}). A number of measures were made to avoid interference from chloride, other metals and plant exudates. Standard additions were used consistently with 3 additions for Zn, and 4 additions for Cd, Cu and Pb. Additions of 5 µl mL^{-1} sample of 65% HNO_3 was found to increase and stabilize the signal of especially Cu and Pb. As chloride affects speciation of metals in water, 2.2% NaCl solution was used as a modifier in the AAS furnace analysis to ensure a high chloride concentration in all samples even if the chloride present from start had been removed by the plants. Furthermore, to avoid contamination, only deionized water that had been further purified with an Elga Purelab Classic (Veolia Water Solutions & Technologies, Lane End, U.K.) was used for mixing of standards, modifiers and dilution of samples.

Analysis of chloride

Chloride analysis was performed on samples from 23 species and no-plant control using liquid chromatography of ions according to ISO 10304-1:2007, which has a detection limit of 0.1 mg Cl L^{-1}. Column PRP-X100 (Hamilton Company, Reno, U.S.) with pre-column IonPac AG9-HC (Dionex, Sunnyvale, U.S.) was used.

Metal and chloride content of the plant samples were not analyzed, since the studies aimed to quantify the removal from water and not the subsequent distribution of the pollutants in and on the plant.

Results and Discussion

Removal capacity of heavy metals and chloride for plants grown under hydroponic conditions

Heavy metal removal

Plants are able to remove heavy metals from water and decrease remaining metal concentration in the water to a minimum (**Paper I**; Rai et al. 1995; Rezania et al. 2016). The metals are taken up into plant tissues, adsorbed to exposed plant surfaces, precipitated due to chemical interactions with root exudates or bound to plant litter (Van de Moortel et al. 2010, 2012; Headley and Tanner 2012; Pavlineri et al. 2017). By utilizing these plant effects in polluted waters, as stormwater or wastewater, heavy metals can be removed from the water column to prevent downstream pollution. The retained metals can thereafter be removed by harvest of plant material and dredging of sediment.

The removal capacity of plants depends on a number of factors, both biotic factors related to the plants (as plant species, size and health), and abiotic as the concentration and mass of the pollutant, water velocity, temperature, pH and presence of other pollutants (**Paper II;** Juang et al. 2011; Weiss et al. 2014; Zou et al. 2016; Rezania et al. 2016). The planting medium also affects plant uptake (Weiss et al. 2006), which is avoided in hydroponic settings. Compared with plants grown in soil or other substrate, the roots are directly exposed to the pollutants, resulting in a higher uptake (Headley and Tanner 2012). Floating treatment wetlands (FTWs) is in fact a hydroponic growing system that utilize these effects in stormwater and other polluted waters. Stormwater has low concentration of pollutants, which results in lower metal uptake compared with experiments performed in higher metal concentration. On the other hand, the low concentration does not impair the plant health due to its low toxicity, making the system long-lasting (Walker et al. 2017; Olguín et al. 2017).

Removal of Cd, Cu, Pb and Zn

In **Paper I**, we studied the metal removal capacity of 34 wetland plant species, all growing in wild in Sweden. The plants were grown hydroponically and exposed during 5 days to a solution containing low concentrations of Cd, Cu, Pb, Zn and Cl. A large variation in removal capacity was found, with the best species able to remove up to 61, 86, 94 and 52% of Cd, Cu, Pb and Zn, respectively after 0.5 h exposure. Close to 100 % of the metals had been removed after 5 days of exposure, corresponding to 0.70, 14.4, 36.6 and 275 mg kg^{-1} biomass for Cd, Cu, Pb and Zn, respectively, for the best species. The removal likely consisted of both adsorption to root surfaces and accumulation in plant tissue due to adsorption through the roots.

The long-term removal capacity of the species in **Paper I** was not evaluated due to the relatively short exposure time and low volume of contaminated solution. If the contaminant concentration or volume of solution had been larger or the duration of the test longer, the plants had likely been able to remove more metal. Christofilopoulos et al. (2016) exposed plants to mixtures containing 15 mg Cd L^{-1} and 1250 mg Zn L^{-1}, corresponding to 1500 and 2500 times higher concentrations than in our study, and achieved accumulation of 38 g Cd kg^{-1} biomass and 6773 g Zn kg^{-1} biomass, but the plants suffered from severe physiological damage due to the toxicity of the mixture. Weiss et al. (2014) found continuous uptake in *Schoenoplectus tabernaemontani* for 3 weeks of exposure, after which growth of the plants was required to increase uptake. On the other hand, Ladislas et al. (2013) studied accumulation properties of *Carex riparia* and *Juncus effusus*, which were also included in our study, for 16 weeks of exposure. The accumulations found for Cd and Zn were similar to our findings for the two species (*Carex riparia*: 0.10 vs. 0.14 mg Cd kg^{-1} biomass and 37 vs. 34 mg Zn kg^{-1} biomass, *J. effusus*: 0.19 vs. 0.23 mg Cd kg^{-1} biomass and 42 vs. 62 mg Zn kg^{-1} biomass for their and our study respectively), indicating that short tests as ours are sufficient for determining removal capacity.

The variation in species capacity in **Paper I** seemed to be connected to their morphology, which was explored in P**aper II**. Species with large removal capacity were found to generally have large biomass, mainly consisting of leaves and fine roots. Coarse roots and stem did not have any impact on removal capacity, whereas rhizome mass had a positive influence but only after 119h of exposure. Fine roots provide large surface area for uptake and adsorption of metals (Marschner 2012). The fine roots also affect the surrounding water by releasing organic matter and altering pH and oxygen levels, which can increase the precipitation and binding of metals to organic matter (Van de Moortel et al. 2010; Chen et al. 2016). Large leaves and transpiration are connected to large root biomass and also results in high water uptake, where metals follow the water stream into the roots (Marschner 2012).

Fate of heavy metals in plants

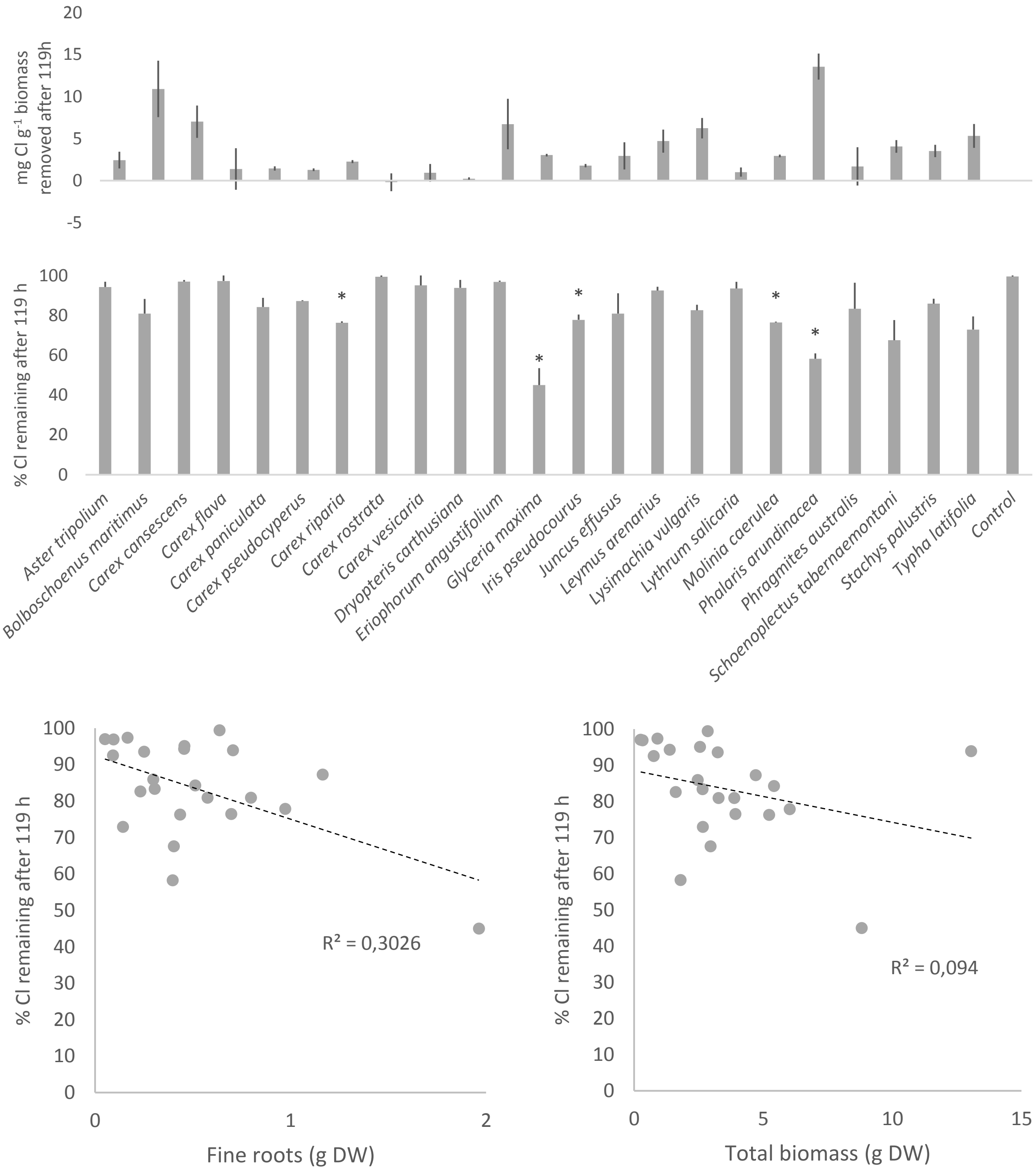
Although tissue concentrations of plants after metal exposure were not tested in our studies, it is extensively researched by others (as Fritioff and Greger 2003; Weiss et al. 2006; Ladislas et al. 2013; Huang et al. 2017) and can provide insights to the removal dynamics in our work. The removal processes in **Paper I** were fast, with a majority of the metals removed after 30 minutes of exposure by some species. At this point, the metals are mainly removed by adsorption to root surfaces and by absorption into the apoplast of roots (Marschner 2012). Further transport into cellular tissue and translocation requires more time, and likely had occurred to some extent after 119 h of exposure, where up to 100 % had been removed from the water. The translocation, i.e. transport and accumulation of metal from roots to shoots, will affect the harvest strategy of these plants in phytoremediation applications. For plant species with high translocation, shoot harvest will be sufficient, whereas root harvest or full-plant harvest will be required for other plant species to maximize metal removal from the site. Most common is that the majority of metals remain in the roots (Deng et al. 2004; LIU et al. 2010; Li et al. 2015).

Removal of other heavy metals

Besides Cd, Cu, Pb and Zn, considered the most important heavy metals in road runoff, Co, Cr and Ni have also received attention. The particulate fraction in stormwater of these metals are retained to a high degree in stormwater ponds (Schmitt et al. 2015), and plants have been found to accumulate dissolved fractions (Rezania et al. 2016). The dynamics are hence the same as for the more common heavy metals, and plants that have been able to remove Co, Cr or Ni have also accumulated these metals. Hence, it can be assumed that the species in **Paper I** would be able to remove also Co, Cr or Ni to a similar degree. Titanium, used in thermoplastic road markings as colorant (Lassen et al. 2015), might be a metal of interest for future phytoremediation studies as the microplastic pollution from traffic gains increasing attention (Magnusson et al. 2016).

Chloride removal

The contaminant mixture in **Paper I** contained 55 mg Cl L^{-1} in addition to heavy metals. Samples from 23 of 34 species in total have been analyzed (figs 3-6), and a complete analysis will be the base of a future paper. Although the Cl concentration was 1000 times higher than heavy metal concentration, a significant decrease of chloride concentration in water was seen. The average decrease was 8.2±1.0 mg Cl, corresponding to a removal of 16.5±2.0% (N=66±SE).



Figs 3-6. Chloride removal by plants. 3: Removed Cl per unit biomass, n=3±SE. 4: Remaining Cl in solution after 119 h exposure, %, n=3±SE. 5: Correlation remaining Cl after 119h and fine root biomass (DW). 6: Correlation remaining Cl after 119h and total biomass (DW). Each data point in Fig 5 and 6 represent average value for a species, n=3. 23 of the 34 species in the study have been analyzed so far.

Plants were necessary for any chloride reduction to occur, as no-plant control had a negligible removal (0.3±1.3 mg Cl, corresponding to a removal of 0.4±2.4% (N=6±SE)). The average uptake per biomass was 3.7+±0.5 mg Cl, higher than the negligible accumulation found by Weiss et al. (2006) for *Glyceria grandis*, *Schoenoplectus tabernaemontani*, and *Spartina pectinate* after exposure to 63 mg Cl L^{-1}. No obvious reason to the better accumulation of *S. tabernaemontani* in our experiments could be found. Our chloride removal results were lower than for halophytes *Atriplex halimus* and *A. hortensis* exposed to 500 mg Cl L^{-1} solution that accumulated 13 and 9 mg Cl g^{-1} respectively in their shoots alone (Suaire et al. 2016), probably due to the higher Cl concentration in the solution. Even though the chloride concentration was almost 50 times lower than brackish waster, potentially toxic chloride levels, 4-7 mg Cl g^{-1} biomass (White 2001), were reached by glycophytes *Carex canescens* and *Eriophorum angustifolium*. Analysis of Cl concentration in water after 0.5h of exposure reveal a slower removal process than for heavy metals (not shown).

Eight of the analyzed species are halophytes (salt tolerant) and the remaining fifteen were glycophytes (salt sensitive), but no difference in Cl removal was found between the groups. Many of the species that removed a significant amount of chloride also efficiently remove Cu, Pb and Zn, but not Cd (fig 4, **Paper I**). The correlation between chloride uptake and biomass was also weaker than for metals (figs 5 and 6, **Paper II**). These findings indicate that other uptake mechanisms and underlying traits than for heavy metals control the removal of chloride.

Useful plant species for metal removal from stormwater by FTWs in cold climates

The importance of finding and choosing species

More than 374 000 plant species have been identified so far, and about 2000 new species are identified each year (Christenhusz and Byng 2016). A minimal share of these have been studied for phytoremediation purposes, and large differences in metal removal capacity have been found (Rai 2008; Rezania et al. 2016). These performance differences demonstrate the potential of studying new plant species to find better plants for water treatment systems as FTWs, biofilters and constructed wetlands where plants are a crucial component (Read et al. 2009; Kadlec and Wallace 2010; Headley and Tanner 2012).

Nevertheless, large scale screenings of species for phytoremediation are rare in literature. Often, one or a few species is studied, providing in-depth knowledge about metal uptake for these particular species. However, direct comparison of removal capacity of species between studies is notoriously hard due to differences in experiment design, making it difficult to identify the best species. Even figures as bioconcentration factors and effective uptake, that aims to provide a summarizing figure of a species' capacity, is impacted by the exposure time, concentration and mass of the metals, velocity of water etc. (Nyquist and Greger 2007, 2009; Juang et al. 2011; Weiss et al. 2014; Li et al. 2015). Comparisons between species in the same study is most reliable, whereas relative comparisons between studies is possible if at least one species is found in both studies.

Paper I overcame the comparison difficulties by screening the metal removal capacity of many species in identical settings. This set-up allows direct comparison between these species and bridges to other studies by including some species also studied by others, as the well-studied species Phragmites australis, Typha latifolia and Juncus effusus.

Another reason to study metal uptake of new species is the advantages of using local flora in phytoremediation, as these are non-invasive and tolerate the local climate and have thereby been recommended for FTW use (Wang and Sample 2014). Moreover, it is recommended to use perennial plants for multi-year phytoremediation installation to reduce workload and promote an early start in spring. Jabeen et al. (2009) claims that terrestrial plants are best suited for FTWs since they commonly produce large root systems. However, since low dissolved oxygen levels are commonly found beneath FTWs (Pavlineri et al. 2017), wetland plants, which are adapted to waterlogged soils or sediments, have been chosen for most FTW installations.

Most FTW studies and adjoining preparatory lab studies have been made in countries with a warm climate with high summer temperatures and mild winters. This is expected to increase the efficacy of FTWs as they remain biologically active through longer periods of the year, although temperatures above 15 °C was found to decrease the removal efficacy (Van de Moortel et al. 2010). The warm climate also influence the choice of plant species. The species used in FTW applications for metal removal until this date are *Acorus calamus*, *Arundo donax*, *Canna flaccida*, *Canna indica*, several *Carex* species, *Cyperus ustulatus*, *Iris pseudacorus*, *Juncus edgariae*, *Juncus effusus*, *Phragmites australis*, *Pontederia cordata*, *Schoenoplectus tabernaemontani* and *Typha orientalis* (Revitt et al. 1997; Van de Moortel et al. 2010; Tanner and Headley 2011; Beery 2013; Borne et al. 2013; Ning et al. 2014; Ladislas et al. 2015; Huang et al. 2017; Tara et al. 2019). The majority of the species used in previous studies cannot survive in cold conditions. For applications in a Nordic environment, other species must be considered.

Fig 8. *Carex riparia.*

Photo by Bertrant Bui /CC-BY-SA 2.0. https://commons.wikimedia.org/wiki/File:Carex_riparia_plant_(01).jpg

Species for a cold climate

Paper I was made using only plants that grow in the wild in Sweden, hence tolerating a cool climate with sub-zero degrees during winter. All species were perennial, and only a few of the species had previously been evaluated for metal or chloride phytoremediation of water. This study compared these species in regard to metal removal, and found a number of promising candidates for FTW applications.

Carex riparia and *C. pseudocyperus* (figs 7-8) stood out from the other species as they were able to reduce the concentration of all four metals already after 0.5 exposure, and kept the concentrations low throughout the test (**Paper I**). Both *C. riparia* and *C. pseudocyperus* are both tall sedge species that grows in nutrient rich lakes, ponds and ditches. Both species are found over the northern hemisphere, and while they are relatively uncommon species in Swedish nature, they can locally be highly abundant. Specimens from both species had large biomass, mainly consisting of fine root and leaves. None of the specimens had stems, and only *C. riparia* had a small amount of rhizomes. *Carex pseudocyperus* had never been studied for any phytoremediation purposes before, whereas *C. riparia* had been evaluated for Cd, Ni and Zn removal both in lab and in FTWs in stormwater by Ladislas et al. (2013, 2015). We can now, based on **Paper I**, confirm its high performance and also add high Cu and Pb removal capacity. *Carex riparia* was also able to reduce chloride concentrations.

Fig 7. *Carex pseudocyperus.*

In addition, a number of other species were able to achieve high and quick removal of Cu, Pb and Zn, but were slower in removing Cd. Many of these species in addition have a high Cl removal. These species represent a wide variety of clades, families and growing environments, which opens up for species choice adapted for local conditions. In saline environments the sea-living species *Bolboschoenus maritimus* and *Schoenoplectus tabernaemontani* could be suitable, whereas flowering species *Stachys palustris* and *Lythrum salicaria* could be useful in urban environments with aesthetic requirements. *Glyceria maxima* and *Carex paniculata* mainly grows in nutrient rich environments, and *Juncus effusus*, commonly used in FTWs, thrives in many conditions. For placement of FTWs in ponds with high hydraulic retention, where water turnover is slow, species that perform well after 119h of exposure in this test could be useful even though their uptake ability after 0.5 h of exposure is limited.

In contrast to metal removal capacity, where large species generally had similar removal capacity for all included metals, the chloride removal did not follow the same species pattern. *Carex pseudocyperus*, one of the two best species for heavy metal removal, had a low chloride removal. Instead, the best species for Cl removal were *Glyceria maxima* and *Phalaris arundinacea* that removed 55 and 42 % Cl from the solution after 119h of exposure, respectively (fig 4). *Phalaris arundinacea* also had the highest uptake per biomass (fig 3).

Fig 9. *Phalaris arundinacea*

The low mass of metals in the solution and the choice to not include any tissue concentration analysis meant that the test did not identify hyperaccumulators, as this requires high exposure and information about root-shoot distribution of the accumulated metal. However, no hyperaccumulators are expected to be found within the studied species in **Paper I**, as these commonly are small, slow-growing species mainly growing in specific environments (Baker et al. 2000; Jabeen et al. 2009).

Iris pseudacorus and *Phragmites australis*, commonly used species in FTW studies (Revitt et al. 1997; Keizer-Vlek et al. 2014; Wang et al. 2015; Tara et al. 2019), had low metal and chloride removal capacity, possibly explained by their low amounts of fine roots (**Papers I, II**).

Expected field performance

The species found to have high removal capacity in lab will likely also have high removal in field (Watson et al. 2003; Ladislas et al. 2013, 2015). After the plants have reached their maximal removal capacity, growth is necessary to increase the available storage capacity. Harvest can stimulate growth and at the same time remove metal-containing biomass from the system. Since much of the removal of metals by plants on FTWs takes place through sedimentation caused by retardation of water velocity and precipitation, the root mass is probably even more important that what has been found in lab-studies focusing on plant uptake, including our studies. The field conditions itself can also alter the morphology of the plant, as low nutrient concentrations generally promote root growth at the expense of shoot growth (Marschner 2012).

Fig 10. *Glyceria maxima.*

Identification of new plant species for rhizofiltration

A simple identification tool for promising plant species candidates for rhizofiltration, built on traits that easily can be measured in field, could streamline the search for efficient species. The close relationship between the morphology of the plant and its metal uptake ability, demonstrated in **Paper II**, could be used for this purpose. The identified morphology traits, as leaf, root and total biomass could easily be measured in field, whereas transpiration, also found to correlate with metal uptake, requires testing in a controlled environment. However, the morphology identification must be followed by verification of the metal removal capacity, as the underlying traits only explained about 70% of the variation (**Paper II**).

From a Nordic perspective, *Calamagrostis purpurea*, *Carex acutiformis* and *Poa palustris*, which were not included in the studies but have large root systems and large leaf mass (Mossberg and Stenberg 2018), could be of potential interest for use on FTWs and for evaluation of the model.

The traits found in **Paper II** could probably explain the performance of species used on in previous studies of FTWs. *Carex riparia* and *Juncus effusus* (Ladislas et al. 2015), *Carex virgata* (Borne et al. 2013) and *Cyperus ustilatus* (Tanner and Headley 2011) were all found to be efficient for metal removal. Based on photos and measurements from their studies, all of their specimens of these species had large biomass mainly consisting of leaves and fine roots.

Furthermore, the traits identified in **Paper II** as connected to metal removal have also been found to promote performance in studies of vegetation for biofilters (Read et al. 2009; Payne et al. 2018). Plants in biofilters accumulates nutrients and pollutants in their tissue, degrade organic pollutants by root processes and by promoting formation of microbial communities, and reduce clogging of filters, which secures infiltration capacity and long-time function (Read et al. 2008; Valtanen et al. 2017). These studies of Read et al. (2009) and Payne et al. (2018) focused on removal of N, P and other pollutants rather than heavy metals and chloride, had longer exposure time, used other plant species and different climate conditions compared with **Paper I**. Yet the same traits have been identified as critical as in **Paper II**, and these also seems to promote Cl removal but to a lower degree than metals. These similarities suggest that maximum exposure of roots to the surrounding medium and large leaf mass and transpiration, promotes vegetation-based water treatment systems, regardless of their target pollutants, design and climate. These similarities between studies performed in different systems also means that findings from studies made in non-hydroponic settings can be used for identification of species for FTWs.

Concluding remarks

It is apparent from this work that removal capacity of heavy metals and chloride differs between wetland plant species. These differences were shown to be explained to a large extent by differences in the morphology of the plants, where large plants with large root systems and leaves promotes heavy metal removal. These traits have also been found to promote removal of N and P in other plant-based water treatment system, suggesting that the same plant species can be used for remediation of many pollutants, regardless of treatment system. However, this work showed that chloride removal had a weaker correlation with biomass than the other pollutants had, and the underlying traits affecting chloride removal are yet to be found.

A number of species with high removal capacity of heavy metals and chloride were identified in this work, including *Carex pseudocyperus*, *C. riparia* and Phalaris *arundinacea*, all thriving in cold climates. Furthermore, the plant traits identified in this study as related to high removal capacity can be used to discover additional suitable species. The efficient removal of pollutants found in these studies indicate that removal of both heavy metals and chloride can be achieved by FTWs in cold climates using a combination of heavy metal and chloride removing native plants. Hence, the findings of this study contributes to development of sustainable stormwater systems which is requested by the society. However, further research of impact by species mixtures, previous pollutant exposure, salt, and low temperature on metal and chloride removal capacity are necessary before the findings of this study can be implemented by planners and constructors of FTWs.

Future perspectives

FTWs present an opportunity to reduce the pollution load from stormwater in an efficient, cost-effective and environmentally friendly way. The plants, which play a crucial role in the FTWs, have been shown to differ in removal capacity, and hence the pollutant removal by FTWs can be expected to increase if the most efficient plants are used. However, a number of questions remains regarding the performance of plants for metal and chloride removal, and these should be resolved before large-scale implementations in field are carried out. If these tests are successful, FTWs with plants selected for optimal metal and chloride removal should be a natural choice for stormwater remediation.

Salinity and temperature impact

The effect on removal capacity by higher salinity and lower temperature, alone and in combination, should be evaluated as they are expected to decrease the removal of metals. The extent of the decrease for the various pollutants is necessary to investigate in order to predict field removal over the seasons.

Pollutant distribution and harvest of plant tissue

The distribution of metals and chloride in and on the plant should be investigated, as this will impact the harvest strategy, another topic to be investigated. Theoretic predictions on parts to be harvested and harvest time have been made (Olguín and Sánchez-Galván 2012; Wang et al. 2014, 2015; Ladislas et al. 2015), but the practical experiments on how harvests affects metal uptake and plant survival are yet to be conducted.

Long-term removal capacity

The long-term performance of these plant species should be investigated to ensure that they are able to continuously remove metal during a whole season with repeated flushes of polluted stormwater. This can be tested in a controlled laboratory environment by either measuring the removal capacity after previous exposure to different levels of the metal, called ‘loading’ (Nyquist and Greger 2007), or as multiple additions of the metal during a longer time period (Sricoth et al. 2018). Ultimately, performance in field conditions for several seasons, either in mesocosms with natural stormwater or *in situ* in a stormwater pond should be evaluated.

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