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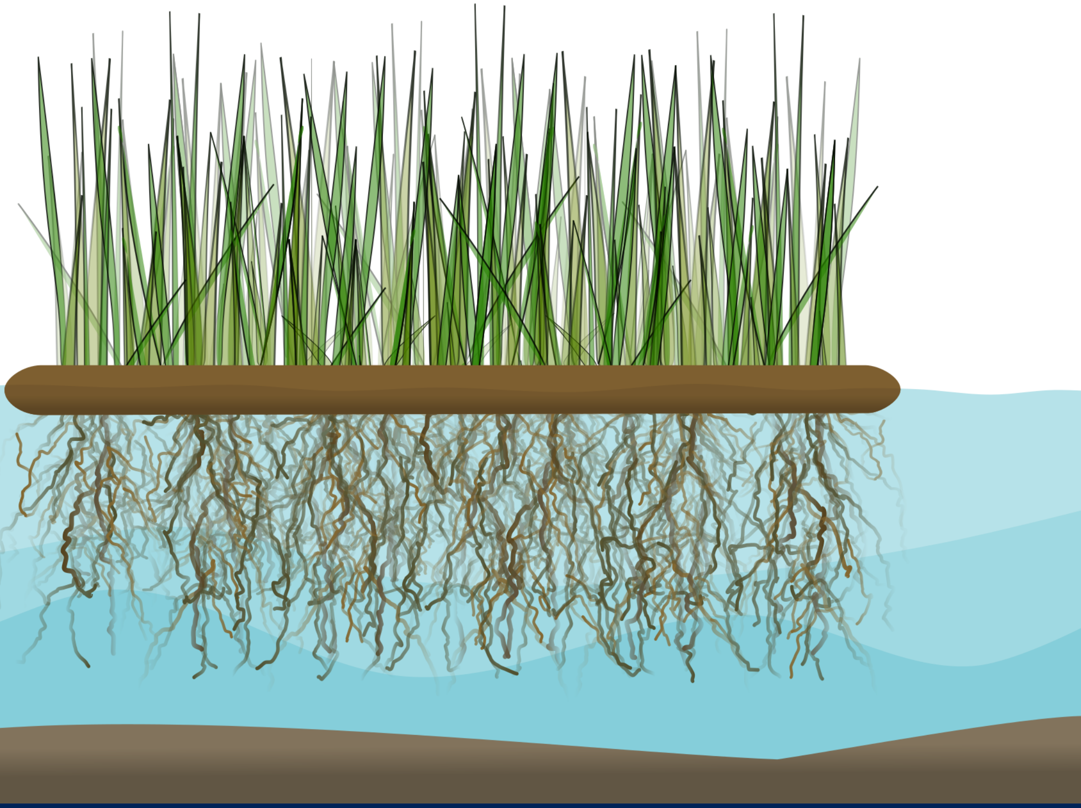
Floating treatment wetlands for stormwater management

Plant species selection and influence of external factors for heavy metal and chloride removal in a cold climate

Maria Schück



Doctoral Thesis in Plant Physiology at Stockholm University, Sweden 2022

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Maria Schück

Academic dissertation for the Degree of Doctor of Philosophy in Plant Physiology at Stockholm University to be publicly defended on Friday 7 October 2022 at 13.30 in Vivi Täckholmsalen (Q-salen), NPQ-huset, Svante Arrhenius väg 20.

Abstract

Stormwater, which consists of rainwater and snowmelt, often contains pollutants from vehicle traffic, building materials, and industries. These pollutants include chloride and heavy metals, which can cause several environmental issues, such as being toxic to biota at elevated concentrations. A relatively new water treatment method is floating treatment wetlands. These vegetated rafts have given promising results, mainly for nutrient removal in eutrophic watercourses in warmer climates. However, knowledge is lacking about their ability to remove chloride and heavy metals and their performance in a cold climate.

The aim was to identify plant species, intended for floating treatment wetlands, which efficiently can remove chloride and the heavy metals Cd, Cu, Pb, and Zn from water in a cold climate such as Sweden and to understand how changes in the environment affect the removal capacity of the plants. This was studied in various conditions by placing plants in water that contained chloride and heavy metals and measuring the concentration of chloride and heavy metals that remained in the water (plant removal capacity; **I, III, IV**) and the accumulation of removed chloride and heavy metals in the different plant parts (plant accumulation capacity; **III, V, VI**). In addition, traits of plants capable of high removal and accumulation were identified by correlating their capacity with their morphological characteristics (**II, III, VI**).

The results show that there are Swedish wetland plant species with a high ability to treat water containing chloride and heavy metals, even under varying conditions. Many species effectively reduced the levels of heavy metals in water, and the graminoid species *Carex pseudocyperus* and *Carex riparia* distinguished themselves by quickly and significantly decreasing the concentrations of heavy metals in the water (**I**). Hardly any species were effective chloride removers, but a few, including *Phalaris arundinacea*, removed large amounts of chloride (**III**). Species with a high removal and accumulation capacity of chloride and heavy metals generally had high total biomass, a large amount of leaf and thin root biomass, and high transpiration (**II, III, VI**). The absorbed heavy metals mainly accumulated in the roots, while chloride accumulated in the shoot tissue (**III, V, VI**). External factors affected the removal and accumulation capacities of the plants to varying degrees. Increased salinity in the water led to lower removal of Cd and Pb, and low temperature decreased the removal of all investigated heavy metals, but some species' removal capacities were less affected by the salt and the cold (**IV**). The plant's content of the heavy metals usually equilibrated with the surrounding water. This effect led to increases in the plant's uptake of heavy metals when their concentration in the water increased, but a release of some accumulated heavy metals if the concentration in the water sank (**V**). Under field conditions, uptake patterns differed (**VI**). The plants on floating treatment wetlands accumulated the most Cu followed by Zn, Pb, and Cd, and *P. arundinacea* distinguished itself through high growth and high uptake. The plants accumulated more in one of the stormwater ponds with no clear explanation.

This thesis shows that there is potential in a Swedish climate for floating treatment wetlands for the removal of chloride and heavy metals from polluted water. It will be essential to select species expected to achieve high removal capacity in the intended environment, such as *P. arundinacea*.

Keywords: *Floating treatment wetlands, Phytodesalination, Rhizofiltration, Phytoremediation, Stormwater, Heavy metals, Chloride, Plant traits, Carex riparia, Carex pseudocyperus, Phalaris arundinacea.*

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Till Tomas och Klara

Floating Island

Harmonious Powers with Nature work
On sky, earth, river, lake, and sea:
Sunshine and storm, whirlwind and breeze
All in one duteous task agree.

Once did I see a slip of earth,
By throbbing waves long undermined,
Loosed from its hold; — how no one knew
But all might see it float, obedient to the wind.

Might see it, from the mossy shore
Dissevered float upon the Lake,
Float, with its crest of trees adorned
On which the warbling birds their pastime take.

Food, shelter, safety there they find
There berries ripen, flowerets bloom;
There insects live their lives — and die:
A peopled world it is; in size a tiny room.

And thus through many seasons' space
This little Island may survive
But Nature, though we mark her not,
Will take away — may cease to give.

Perchance when you are wandering forth
Upon some vacant sunny day
Without an object, hope, or fear,
Thither your eyes may turn — the Isle is passed away.

Buried beneath the glittering Lake!
Its place no longer to be found,
Yet the lost fragments shall remain,
To fertilize some other ground.

Dorothy Wordsworth (1771-1855)

Abstract

Stormwater, which consists of rainwater and snowmelt, often contains pollutants from vehicle traffic, building materials, and industries. These pollutants include chloride and heavy metals, which can cause several environmental issues, such as being toxic to biota at elevated concentrations. A relatively new water treatment method is floating treatment wetlands. These vegetated rafts have given promising results, mainly for nutrient removal in eutrophic watercourses in warmer climates. However, knowledge is lacking about their ability to remove chloride and heavy metals and their performance in a cold climate.

The aim was to identify plant species, intended for floating treatment wetlands, which efficiently can remove chloride and the heavy metals Cd, Cu, Pb, and Zn from water in a cold climate such as Sweden and to understand how changes in the environment affect the removal capacity of the plants. This was studied in various conditions by placing plants in water that contained chloride and heavy metals and measuring the concentration of chloride and heavy metals that remained in the water (plant removal capacity; **I, III, IV**) and the accumulation of removed chloride and heavy metals in the different plant parts (plant accumulation capacity; **III, V, VI**). In addition, traits of plants capable of high removal and accumulation were identified by correlating their capacity with their morphological characteristics (**II, III, VI**).

The results show that there are Swedish wetland plant species with a high ability to treat water containing chloride and heavy metals, even under varying conditions. Many species effectively reduced the levels of heavy metals in water, and the graminoid species *Carex pseudocyperus* and *Carex riparia* distinguished themselves by quickly and significantly decreasing the concentrations of heavy metals in the water (**I**). Hardly any species were effective chloride removers, but a few, including *Phalaris arundinacea*, removed large amounts of chloride (**III**). Species with a high removal and accumulation capacity of chloride and heavy metals generally had high total biomass, a large amount of leaf and thin root biomass, and high transpiration (**II, III, VI**). The absorbed heavy metals mainly accumulated in the roots, while chloride accumulated in the shoot tissue (**III, V, VI**). External factors affected the removal and accumulation capacities of the plants to varying degrees. Increased salinity in the water led to lower removal of Cd and Pb, and low temperature decreased the removal of all investigated heavy metals, but some species' removal capacities were less affected by the salt and the cold (**IV**). The plant's content of the heavy metals usually equilibrated with the surrounding water. This effect led to increases in the plant's uptake of heavy metals when their concentration in the water increased, but a release of some accumulated heavy metals if the concentration in the water sank (**V**). Under field conditions, uptake patterns differed (**VI**). The plants on floating treatment wetlands accumulated the most Cu followed by Zn, Pb, and Cd, and *P. arundinacea* distinguished itself through high growth and high uptake. The plants accumulated more in one of the stormwater ponds with no clear explanation.

This thesis shows that there is potential in a Swedish climate for floating treatment wetlands for the removal of chloride and heavy metals from polluted water. It will be essential to select species expected to achieve high removal capacity in the intended environment, such as *P. arundinacea*.

Svensk sammanfattning

Dagvatten som består av regnvatten och smält snö, innehåller ofta föroreningar från fordonstrafik, byggnadsmaterial och industrier. Dessa föroreningar inkluderar tungmetaller och klorid, som kan orsaka flera problem i miljön om halterna är förhöjda, däribland att vara giftiga för levande varelser. En relativt ny metod för vattenrening är flytande våtmarker som har givit lovande resultat framförallt för rening av övergödda vattendrag i varmare klimat. Kunskap saknas dock gällande deras förmåga att rena tungmetaller och klorid, samt hur väl de fungerar i kallt klimat.

Syftet med avhandlingen var att identifiera växtarter som effektivt kan rena vatten från klorid (Cl) och tungmetallerna kadmium (Cd), koppar (Cu), bly (Pb) och zink (Zn) i ett kallt klimat som Sveriges. Växter av dessa arter är tänkta att placeras i flytande våtmarker i dagvattendammar för att förbättra dammarnas rening av dagvatten. Dessutom syftade avhandlingen till att studera hur reningsförmågan hos växter påverkas av olika miljöfaktorer. Detta undersöktes genom att placera växter i vatten som innehöll tungmetaller och klorid i olika miljöförhållanden, och därefter mäta den kvarvarande koncentrationen av tungmetaller och klorid i vattnet (växternas reningsförmåga, **I, III, IV**) och koncentrationen av tungmetaller och klorid i olika delar av växten (växternas ackumuleringsförmåga, **III, V, VI**). Dessutom identifierades karaktärsdrag hos de växter som hade hög reningsförmåga genom att korrelera växters reningsförmåga med deras morfologiska egenskaper (**II, III, VI**).

Resultaten visar att det finns svenska våtmarksväxter med hög förmåga att rena vatten från tungmetaller och klorid även under varierande förhållanden. Många arter minskade effektivt halten av tungmetaller i vatten, och halvgräsarterna slokstarr och jättestarr utmärkte sig genom att snabbt och tydligt minska halten tungmetaller i vattnet. Några få arter gav effektiv saltrening, men ett par, däribland rörflen, tog upp stora mängder klorid. Arter med hög reningsförmåga av både tungmetaller och klorid hade generellt sett hög totalbiomassa, mycket blad och tunna rötter, samt hög transpiration. De upptagna metallerna lagras framförallt i rötterna, medan klorid mestadels lagras i skottdelarna av växten. Växternas reningsförmåga påverkades i olika grad av olika externa faktorer. Ökad salthalt i vattnet ledde till lägre rening av Cd och Pb, och låg temperatur gav lägre rening av samtliga undersökta tungmetaller, men att vissa arters reningsförmåga blev mindre påverkade av saltet och kylan. Växtens innehåll av tungmetaller balanserade sig oftast med det omgivande vattnet. Detta ledde till att växternas upptag av tungmetaller ökade om föroreningshalten ökade, medan delar av det som tagits upp släpptes ut om tungmetallshalten i det omgivande vattnet sjönk. Under fältförhållanden fanns det tydliga skillnader i upptagsmönster.Växterna tog upp mest Cu följt av Zn, Pb och Cd. Mer metaller togs upp i ena dammen som försöket genomfördes i, utan tydlig orsak. Rörflen utmärkte sig genom hög tillväxt och högt upptag jämfört med de andra arterna.

Avhandlingen visar att det i ett svenskt klimat finns potential för att rena förorenat vatten från tungmetaller och klorid med hjälp av flytande våtmarker. Det kommer att vara viktigt att välja de arter som har hög reningsförmåga i den tilltänkta miljön. Eftersom växterna har olika styrkor rekommenderar vi att en blandning av arter används i flytande våtmarker för att ge en stabil rening under varierande förhållanden. Om bara en art kan väljas, renar rörflen (*Phalaris arundinacea*) bra under de flesta förhållanden och har hög tillväxt, men den renar relativt långsamt enligt studie I, har något sämre Cu- och Pb-rening enligt studie IV samt låg har Cl-tolerans under näringsfattiga förhållanden enligt studie III. Även om denna avhandling har haft målet att finna växter till flytande våtmarker för dagvattenrening i Sverige så täcker den in många aspekter som är relevanta när växter används för rening i andra system. Sammantaget ger avhandlingen en god bild av växternas reningsförmåga ur många olika aspekter, vilket är nödvändigt för att kunna använda växtbaserad vattenrening på ett optimalt sätt. Fortsatta studier bör titta på skördemetodik, långtidseffektiviteten i fält hos flytande våtmarker, samt dessa arters förmåga att rena andra typer av föroreningar.

List of papers

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

I **Maria Schück**, Maria Greger. 2020. Screening the Capacity of 34 Wetland Plant Species to Remove Heavy Metals from Water. *International Journal of Environmental Research and Public Health* 17 (13) DOI: 10.3390/ijerph17134623

II **Maria Schück**, Maria Greger. 2020. Plant traits related to the heavy metal removal capacities of wetland plants. *International Journal of Phytoremediation* 22 (4), 427-435 DOI: 10.1080/15226514.2019.1669529

III **Maria Schück**, Maria Greger. 2022. Chloride removal capacity and salinity tolerance in wetland plants. *Journal of Environmental Management* 308, 1–10. DOI: 10.1016/j.jenvman.2022.114553

IV **Maria Schück**, Maria Greger. Influence of salinity and temperature on removal of heavy metals and chloride from water by wetland plants. *Manuscript*

V **Maria Schück**, Maria Greger. Effect of tissue concentration on accumulation and distribution of Cd, Cu, Pb, and Zn in *Carex pseudocyperus* L. *Manuscript*

VI Emre Boynukisa, **Maria Schück**, Maria Greger. Differences in metal accumulation from stormwater by three plant species growing in floating treatment wetlands in a cold climate. *Manuscript*

My contribution to the studies were as follow:

I was the main responsible for experimental design of all studies, with feedback from my main supervisor Maria Greger (MG). I conducted all experimental work, and analyzed all samples with a few exceptions: MG analyzed half of the Cl^- samples of study III, and all Cd, Cu, and Pb samples of study V. Emre Boynukisa (EB) analyzed the metal content of all plants and water samples of study VI. I wrote the first draft of the manuscripts of all studies, except for the first draft of study VI that EB wrote. I participated in finalizing the manuscripts of all studies with feedback from MG.

Contents

Abbreviations

AAS	atomic absorption spectrometry
AMD	acid mine drainage
BOD	biological oxygen demand
COD	chemical oxygen demand
CP	*Carex pseudocyperus*, cyperus sedge
CR	*Carex riparia*, greater pond sedge
DO	dissolved oxygen
DW	dry weight
EC	electric conductivity
Eh	redox potential
FAAS	flame AAS
FTWs	floating treatment wetlands
FW	fresh weight
GFAAS	graphite tube AAS
IC	ion chromatography
PA	*Phalaris arundinacea*, reed canary grass
PAH	polycyclic aromatic hydrocarbons
PET	polyethylene terephthalate
PSII	photosystem II
ROL	radial oxygen loss
ROS	reactive oxygen species

Introduction

Human life, and the civilizations we have created, cause an increased redistribution of many natural resources that otherwise would have a slower turnover. The speed and magnitude of these redistributions may cause environmental problems, as we see with climate change, deforestation, overfishing, and landscape degradation. Among these problems are salinization and the pollution caused by the redistribution of heavy metals from bedrock to air, soil, and water, where they can be toxic to almost all lifeforms. However, during the last decades we humans have had an increasing interest to use tools from nature to fight these problems, showing that we are not only their cause but also could be their solution. One such example is phytoremediation, where the natural capacity of plants is used to remove, degrade or stabilize pollutants from water, soil or air (Arthur et al., 2005; Raskin et al., 1994). This study concerns which plants are best suited to remove chloride and heavy metals from polluted water in a cold climate, to determine if plants on floating treatment wetlands could function for water treatment of road runoff in Swedish conditions.

Polluted road runoff and wastewater

Humans affect the natural water cycle in many ways. Besides a large consumption of mainly freshwater, we affect the paths of water through the landscape by creating hard surfaces, such as rooftops, industrial areas, and roads, which prevent rain and snowmelt from infiltrating directly into the ground. Instead of infiltrating, the water flows on the surface. In cities and on roads, railways, and airport runways, this can result in large water volumes, which must be managed to prevent flooding and accidents. This water is commonly referred to as stormwater, or in case of road-related stormwater, as road runoff.

Common practice to handle stormwater is to discharge it directly into the recipient (lake/stream/sea) via water inlets and plumbing. Ponds are sometimes built to collect larger volumes to delay the water flows and thus to prevent flooding. Since the water commonly contains pollutants from the air and the surfaces it has flown over, increasing demands for treatment to reduce the concentration of these pollutants prior to discharge have led to a need for new management practices. Common pollutants in stormwater include nutrients, organic matter, litter, organic pollutants such as oils and polycyclic aromatic hydrocarbons (PAH), and inorganic pollutants such as heavy metals (Nnadi et al., 2015; Schmitt et al., 2015; Sharma et al., 2016; Vincent and Kirkwood, 2014). Currently, common stormwater management methods include treatment in wastewater plants, treatment in constructed wetlands, and infiltration methods such as biofilters or grass swales.

However, the current methods for stormwater treatment all have several drawbacks. Wastewater plants often lack the capacity to handle the large volumes of rain or snowfall. Additionally, the lower ionic strength, higher P concentration, and higher COD of stormwater compared to domestic wastewater negatively affect the treatment efficacy of the wastewater plants by decreasing the flocculation stability, which results in reduced ability to remove particles, and in some cases also in reduced removal of N (Wilén et al., 2006). Constructed wetlands require large areas, are sensitive to changes in water level as it may either drown or dry out the plants, and some studies indicate that the long-term removal of nutrients and heavy metals Cd and Pb is low (Blecken, 2016; Gill et al., 2017; Headley and Tanner, 2012). Infiltration beds require large areas, suitable geo-hydrological conditions, and may clog (Blecken, 2016; Feng et al., 2012). Stormwater ponds only remove dissolved substances to a low extent (Alm et al., 2010; Barbier et al., 2018). In many of these systems, removal of the pollutants is not possible or requires intrusive methods such as dredging which risks remobilizing the pollutants.

Human activities such as industries, agriculture, mining, and households also create polluted water, commonly referred to as wastewater. The pollutant types and concentrations directly relate to the origin of the wastewater. The treatment methods vary, but wastewater plant treatment is typical for highly concentrated effluents.

Heavy metal pollution

Heavy metals are metal elements, often characterized by high density (Pourret et al., 2021). The group includes a wide range of substances. Some of these are toxic to almost all life forms even in low concentrations, such as mercury (Hg), lead (Pb), and cadmium (Cd). Others are essential in low concentrations for biota such as iron (Fe), copper (Cu), and zinc (Zn) but are toxic at high concentrations. Nonetheless, many toxic heavy metals are widely used in the world today.

Being chemical elements, heavy metals cannot degrade into harmless substances, compared to organic pollutants that ideally degrade into water and carbon dioxide. Heavy metals are thus persistent in nature and tend to bioaccumulate. The main management strategy is to collect and store them where they cause least harm. Although much effort is invested in reducing the use of toxic heavy metals and careful handling of heavy metal waste, much eventually ends up in the soil, water, and atmosphere. Approximately 40% of lakes and rivers globally are estimated to be polluted with heavy metals (Zhou et al., 2020).

Heavy metals in stormwater

The composition and concentration of heavy metals in stormwater depend on land use and precipitation patterns in the drainage basin. Concentrations detected in a number of studies in stormwater in Sweden are summarized in Table 1 (for a comprehensive review of global concentrations and trends, see Huber et al. 2016). A large share of the heavy metals in stormwater originates from vehicle traffic, including wear on brakes, tire linings, road markings, galvanized metal structures as guardrails, and fuel combustion in engines (Makepeace et al., 1995; Moghadas et al., 2015). Other sources include industrial pollution, roofing materials, fertilizers, and natural sources. The metals have several chemical forms, commonly divided into particulate and dissolved forms. Dissolved forms, less than 0.45 µm in size, are more bioavailable and thus more toxic (Revitt and Morrison, 1987).

Table 1. Metal and chloride concentrations detected in stormwater ponds and stormwater runoff in two Swedish studies. All metal concentrations are given in µg L^{-1} and chloride concentrations in mg L^{-1}. Data references: a - Alm et al. 2010, b - Bäckström et al. 2003.

Metal	**Type**	**Larbo stormwater pond, yearly average** [a]	**Tibble stormwater pond, yearly average** [a]	**Svaneberg road runoff, summer** [b]	**Svaneberg road runoff, winter** [b]	**Norsholm road runoff, summer** [b]	**Norsholm road runoff, winter** [b]
Cd	Total	0.16	0.16	0.05	0.11	0.06	0.53
	Dissolved	0.08	< 0.05	0.02	0.04	0.04	0.26
	% dissolved	48	-	40	39	64	49
Cu	Total	30	20	13	39	18	126
	Dissolved	10	6	6	16	9	64
	% dissolved	34	30	43	41	48	51
Pb	Total	7	9	7	9	6	21
	Dissolved	0.1	0.2	0.5	0.4	0.5	0.6
	% dissolved	1	2	7	5	8	3
Zn	Total	175	130	89	124	130	287
	Dissolved	58	17	50	53	92	112
	% dissolved	33	14	56	43	71	39
Cl	Total	260	51	12	490	16	3490

The distribution between dissolved and particulate fractions varies between metals, where Pb has the smallest available fraction and Zn generally the largest (Table 1). Moreover, the share of the dissolved fraction is increased by rain rather than by snowmelt events, low pH, high salinity and high concentration of dissolved organic material in the water (Mueller et al., 2012; Viklander et al., 2019; Westerlund, 2007). Furthermore, the dissolved fraction can be divided into several subfractions, as by size (e.g. bound to colloids (3 kDa-0.45 µm) and the truly dissolved fraction (< 3kDa) (Lindfors et al., 2021)), or by speciation (e.g. bound to organic materials, in aqueous complexes, or as free ions (Behbahani et al., 2021)). For Cd, Cu and Zn, the smaller and less bound fractions generally dominate over the larger fractions bound to colloids, while the opposite is found for Pb (Bäckström et al., 2003; Behbahani et al., 2021; Lindfors et al., 2021; Mueller et al., 2012). Cadmium and Zn are mainly present as free metal ions, whereas Cu forms small complexes with dissolved organic matter, and is to a very extent present as free ions in water and instead binds to colloids, forming large but still dissolved complexes.

Table 2. Estimated total heavy metal load, and stormwater share, on water recipients in Sweden. Data collected from Ejhed et al. (2010) and does not include the load from the approx. 400 stormwater ponds managed by the Swedish Transport Administration.

Gross load Sweden	**Cd**	**Cu**	**Pb**	**Zn**
All sources (ton/year)	5	190	92	949
Stormwater (ton/year)	0.7	38	20	112
Stormwater, %	15	20	22	12

In Sweden, most heavy metal pollution originates from non-point sources including stormwater (Ejhed et al., 2010). Stormwater contains heavy metals in low concentrations, but given the large volumes of stormwater generated, the total amount of pollutants is high and it is estimated to contribute 12-20 % of the yearly total load of Cd, Cu, Pb, and Zn in Swedish waterbodies (Table 2). Although there are no national guidelines for heavy metals concentration in stormwater, there are requirements for the recipients of stormwater. This creates a need for sufficient treatment of the stormwater not to decrease the recipient's water quality. The annual average value of bioavailable Cu and Zn cannot exceed 0.5 µg Cu L^{-1} and 5.5 µg Zn L^{-1} for the recipient to be considered to have good surface water status, which is the goal for all Swedish lakes and watercourses (HVMFS 2019:25, n.d.). Additionally, several municipalities in Sweden have adopted locally based requirements for heavy metal concentration in stormwater (Table 3). Notably, the drinking water can contain much higher concentrations of Cu than surface waters. The reason for this is likely that compared to Cd and Pb where the intake should be as low as possible, the recommended Cu dose for adults is 0.9-1.3 mg Cu day^{-1} (Livsmedelsverket, 2017).

*Table 3. Guidelines for heavy metal concentrations in surface water in Sweden, drinking water in the EU including Sweden, and examples of local guidelines for stormwater quality (Directive (EU) 2020/2184; HVMFS 2019:25; SLVFS 2001:30; Norrköping 2019; Miljöförvaltningen Göteborgs stad 2020). *Concentrations in drinking water within a parenthesis are considered "acceptable with remark" according to additional Swedish legislation.*

Maximum concentration	**Cd (µg L^{-1})**	**Cu (µg L^{-1})**	**Pb (µg L^{-1})**	**Zn (µg L^{-1})**	**Cl (mg L^{-1})**
Good surface water status of a recipient	-	0.5	-	5.5	-
Stormwater, Gothenburg municipality	0.9	10	28	30	-
Stormwater, Norrköping municipality	0.5	30	10	90	-
Drinking water	5	2000 (200)*	5	-	250 (100)*

Chloride pollution

Another type of anthropogenic pollution is increased salinity (salinization) of soil and water, caused by changed land use, irrigation, industrial waste, or deicing salt (Liang et al., 2017). The increased concentration of chloride (Cl), sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) ions negatively affects plants, animals, and farming (Sánchez and Matos, 2018).

Chloride in stormwater

Deicing salt, commonly consisting of NaCl (although also $MgCl_2$ and $CaCl_2$ are used to some extent), is often used on highly travelled roads in winter conditions to improve road safety. Most deicing salt is used in regions with cold climate as North America, Northern Europe and northern China, but local use in mountain regions with snowy conditions also occurs (Barbier et al., 2018; Billberger, 2018; Denich et al., 2013). When the snow and ice melt, the road salt follows the road runoff. Sodium and Cl^- cause different environmental problems. Sodium readily binds to soil mineral particles, replacing and thus remobilizing other cations, including the heavy metals Cd and Cu, by ion exchange (Bäckström et al., 2004). Sodium also weakens the soil structure, causing decreased aeration, increased leakage, and erosion (Edelstein et al., 2010). Chloride negatively impacts roadside vegetation, drinking water quality, and aquatic organisms (Blomqvist, 1998; Findlay and Kelly, 2011; Willmert et al., 2018). Chloride also damages infrastructure such as concrete and metal constructions (Denich et al., 2013; Hääl et al., 2006; Luping and Utgenannt, 2007).

Similar to heavy metals, the concentration of chloride in stormwater depends on land use and precipitation patterns in the drainage basin. In areas where deicing salt is used, concentrations are high in winter and early spring.

Levels of 11 000 mg Cl^{-} L^{-1} have been detected in road runoff in Sweden, and close to 700 mg Cl^{-} L^{-1} in stormwater retention ponds with a yearly average of 50 mg Cl^{-} L^{-1} (Alm et al., 2010; Lundmark et al., 2007; Semadeni-Davies, 2006). During summer, concentrations of 15 mg Cl^{-} L^{-1} and below are expected. Saline stormwater can also be caused by industrial release of chloride or by the inclusion of seawater (Sanicola et al., 2019; Szota et al., 2015). No guidelines specify the allowed concentration of Cl^{-} in surface waters or stormwater, but drinking water cannot contain more than 250 mg Cl^{-} L^{-1} and Swedish guidelines consider drinking water with Cl^{-} levels above 100 mg Cl^{-} L^{-1} "acceptable with remark" (Directive (EU) 2020/2184; SLVFS 2001:30).

Phytoremediation

Humans are not only polluters; we can also come up with new ways to clean polluted environments. Phytoremediation, developed since the 1980s, uses plants and plant-associated microorganisms to accumulate, stabilize or render toxic compounds harmless (Arthur et al., 2005; Raskin et al., 1994; Tonelli et al., 2022). The method can be efficient, flexible, cheap, environmentally friendly, and easy to manage. Its limitations include slow treatment speed compared to industrial methods such as "dig and dump" and "pump and treat", inability to handle extremely high concentrations as they poison the plants, and seasonal growth patterns which result in varying efficacy during the year (Rai, 2009). These drawbacks, together with the unfamiliarity of the methods to practitioners and legislation that favors industrial methods, might explain the low level of commercial implementations of soil phytoremediation (Ågren et al., 2021; Beans, 2017). Plant-based treatment of water, such as vegetated roofs, constructed wetlands, and raingardens, is much more common (Blecken, 2016; Gavrić et al., 2019; Stahre, 2008).

Several types of phytoremediation techniques exist (Fig. 1), and sometimes the terms are overlapping or have multiple definitions. Perhaps the most well-known technique is phytoextraction, where plants accumulate the target substance from the soil with the roots and transport the substance to the shoot, which is harvested to remove the substance from the site. Phytodesalination is the extraction of salt (sodium and/or chloride) from saline soils or water and is considered a subcategory of phytoextraction (Manousaki and Kalogerakis, 2009; Suaire et al., 2016). With phytodegradation, the plants decompose organic pollutants to ideally less toxic compounds within the plants (Arthur et al., 2005). Similarly, in phytostimulation, plant-associated microorganisms in the soil degrade organic pollutants (Souto et al., 2020). In cases where extraction or degradation is not possible (due to concentration, substance, or practical reasons), phytostabilization reduces the mobility of the substances in the environment by adsorption to root tissue, or transforming them into less bioavailable forms by precipitation, reduction in metal valence and sequestration within root tissues (Yan et al., 2020). Rhizofiltration is a term for all remediation activity performed by plant roots in water. It includes removal of toxic metals from polluted water by adsorption on plant root surfaces, absorption in plants including tissue accumulation (net effect of uptake (influx) and release (efflux)), and precipitation in water promoted by plants (Rai, 2009). This is similar to the soil phytoremediation techniques phytoextraction and phytostabilization but takes place in water.

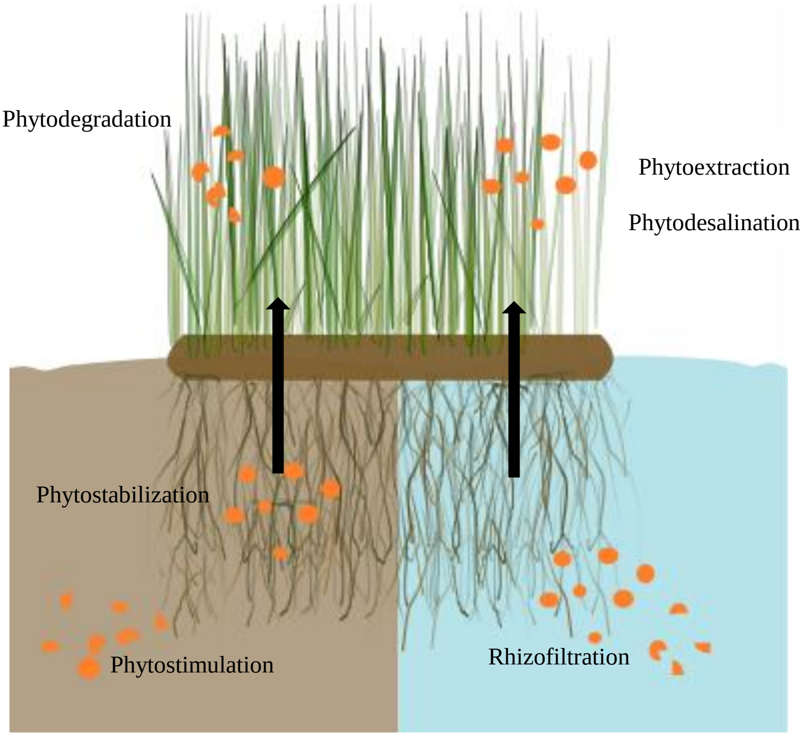

Figure 1. Phytoremediation techniques.

Plant properties enabling phytoremediation

Plants are the drivers of all phytoremediation techniques, using the plants' natural processes for nutrient accumulation, metabolism, and storage to treat pollutants.

Plants that accumulate and tolerate high concentrations of the target pollutant are suitable for phytoextraction (Arthur et al., 2005). If a plant species has strong protection against accumulation (i.e. exclusion) of the pollutant, it may be suitable for phytostabilization (Sricoth et al., 2018). The tolerance mechanisms including exclusion may have developed as an adaptation to life in an environment where the pollutant occurs naturally. Suitable plants for phytodegradation should produce high amounts of enzymes that degrade the target pollutant (Arthur et al., 2005). The same plant species can of course be used for several different techniques but is likely most suitable for one.

Because plant species differ in phytoremediation capacity, it is important to identify high-capacity plants to obtain effective phytoremediation. Besides species differences discovered when comparing different plants in an identical environment, they respond differently to environmental variations. Temperature, nutrient supply, insect infestation, water supply, light, salinity, and more are all factors that can all affect plant performance and thus their phytoremediation capacity. High levels of pollutants in the soil, sediment, or water may be toxic to the plant. This can be seen on a biochemical and cellular level, and is manifested by reduction of growth, seedling survival, leaf number, leaf area, root growth, and other morphological signs (Weis and Weis, 2004). Differences in phytoremediation capacity have also been found between ecotypes (Manousaki et al., 2014; Marchand et al., 2010; Redjala et al., 2009), clones (Fernández et al., 2014; Landberg et al., 2011; Maeda et al., 2006), plants of different age (Touchette et al., 2012), and between plants that previous were exposed to different levels of pollutants (Greco et al., 2012; Landberg et al., 2011). Explaining factors for these differences include genetic and epigenetic factors and developmental stages that result in differences expression levels of transporters or detoxifiers, or in morphology (Balafrej et al., 2020; Greco et al., 2012; Lange et al., 2017; Tian et al., 2017; Touchette et al., 2012).

Heavy metals in plants

The heavy metals Cu, Fe, Mn, Ni, and Zn are essential nutrients, which means that they are necessary for completing the lifecycle of all plants. Each essential metal has an optimal concentration range in plants. Plant growth is reduced below and above this range, and the plants show deficiency or toxicity symptoms (Fig. 2). During nutrient deficiency, plant growth will be restricted by the most deficient element, as essential elements cannot be replaced with another substance. Other heavy metals, like Pb, Cd, Cr, and Hg, are not essential for plants and are toxic if they disrupt metabolic processes. Below is a more detailed presentation of the specific metals that are relevant to this thesis.

Cadmium is a toxic element for almost all plants (Küpper and Andresen, 2016). However, due to its chemical similarity to other heavy metals (especially to Zn that has the same electron configuration), Cd^{2+} ions and Cd chelates are transported into plant root cells by non-specific heavy metal transporters, e.g., by the ZIP family of transport proteins or by Ca^{2+} channels (Jamla et al., 2021; Rizwan et al., 2019). The toxic effects are caused by the ability of Cd to bind to the amino acids cysteine and histidine, thereby replacing Zn^{2+} in several enzymes or by replacing Ca^{2+} in PSII and Mg^{2+} in rubisco and chlorophyll (Jamla et al., 2021; Küpper and Andresen, 2016). This results in non-functional molecules, leading to reduced photosynthesis and electron transport, and thereby reduced growth (Song et al., 2019). Toxic effects in the form of mutations have been detected in *Arabidopsis* at concentrations as low as 1 µg Cd L^{-1} (Kovalchuk et al., 2001). Plants that tolerate high Cd concentrations increase the barrier towards the polluted surroundings by increased lignification or formation of suberin lamellae in the root cell walls (Küpper and Andresen, 2016). Binding of Cd, and other metals, to the cell wall or sequestration in vacuoles after uptake, are protective measures as they thereby limit the ability of the metals to interfere with cellular processes (Fernández et al., 2014). Hyperaccumulation of Cd in plants is very rare but has been identified in a few species (Tian et al., 2017).

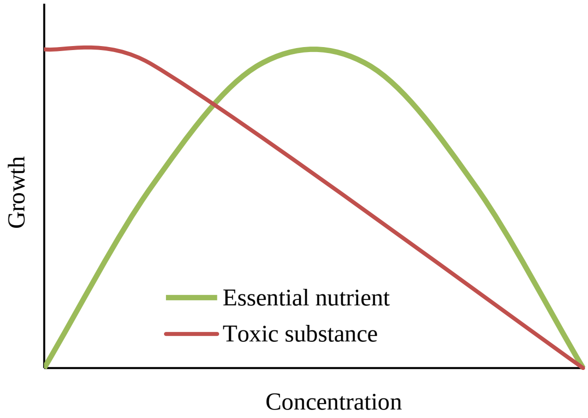

Figure 2. Effect on plant growth of essential heavy metal nutrients as Cu and Zn and toxic heavy metals as Pb and Cd.

<u>Copper</u> is an essential element involved in several redox reactions, especially those related to photosynthesis. It is also an activator of enzymes and part of the antioxidant CuZn superoxide dismutase that transforms the free radical superoxide $O2^{\bullet-}$ generated by abiotic stress, including heavy metal stress, to the less reactive H_2O_2.The optimal concentration range in plant tissue is approx. 4-20 µg Cu g DW^{-1}, and toxicity commonly occurs above 20-30 µg Cu g DW^{-1} leaf for many crop species (Broadley et al., 2011). When the plant is exposed to toxic concentrations, most Cu remains in the roots which results in stunted roots, whereas translocation to aboveground tissue is restricted. Copper transporter protein family (COPT) and heavy metal ATPases (HMA) among other regulate Cu transport between the apoplast and cytoplast and between cellular compartments (Wairich et al., 2022). The main cause of Cu toxicity is oxidative stress at high Cu concentrations, while lower concentrations damage photosystem II, causing reduced photosynthesis and increasing ROS production by Fenton-like and Haber–Weiss reactions (Küpper and Andresen, 2016; Wairich et al., 2022). Plants that tolerate high Cu concentrations commonly exclude Cu from passive influx by increased lignification of

roots or bind excessive Cu in cell walls (Broadley et al., 2011; Küpper and Andresen, 2016). Tolerance of Cu by hyperaccumulation of Cu has been found in a number of plants, of which 95% originate from copper-rich areas in central Africa (Lange et al., 2017).

Lead is toxic for all plants. However, Pb has low mobility in the plant as it efficiently binds to organic material. Most of the absorbed Pb is therefore bound in the roots to cell walls, to cell membranes, and to phytochelatines in vacuoles, and only minor amounts are transported to aboveground tissue (Fischer et al., 2014; Gupta et al., 2013; Jamla et al., 2021). Toxicity symptoms have been recorded in solutions with as little as 21 µg L^{-1} available Pb (Fischer et al., 2014). Uptake into the symplast, or release into the apoplast, of Pb is mediated by several transporters, including ABC transporters (Jamla et al., 2021). The toxic effect of Pb is mainly membrane disruption, which inhibits photosynthesis and growth and increases ROS levels. Tolerance mechanisms include high activity of enzymatic antioxidants, such as superoxide dismutase and catalase, to decrease the damage done by excessive ROS, and detoxification of Pb by binding to phytochelatines and further transport from the cytosol into the vacuole (Fischer et al., 2014; Gupta et al., 2013). Thickening of cell walls at apical tips where most Pb is taken up has been recorded, suggesting a barrier role of cell walls towards Pb uptake (Krzesłowska, 2011). A number of Pb hyperaccumulator species has been identified but these are generally small and grow slowly, making them unsuitable for phytoremediation (Gupta et al., 2013).

Zinc is an essential element involved in membrane stability, detoxification of free radicals, and synthesis of plant hormones, among other functions (Broadley et al., 2011). Zinc forms strong tetrahedral complexes, and as such, it can be a part of enzymes and an enzyme activator. Uptake into plant tissue is mediated by ZIP (zinc-regulated transporter and iron-regulated transporter protein) transporter family and other heavy metal transporters (Eide, 2006; Guerinot, 2000; Küpper and Andresen, 2016). At least 15-20 µg Zn g DW^{-1} is required in the leaves and 100-150 µg Zn g DW^{-1} in meristematic tissue. Toxicity is common for leaf tissue concentrations above 300 µg Zn g DW^{-1}, but tolerant plants can withstand higher concentrations by transporting excessive Zn into the vacuole where it is sequestered (Balafrej et al., 2020). Toxicity is mainly caused by excessive Zn replacing other divalent cations, such as Mg^{2+} in chlorophyll, which inhibits photosynthesis and results in leaf chlorosis and reduced root growth (Küpper and Andresen, 2016). However, Zn toxicity is uncommon as its accumulation is tightly regulated. By the year 2020, 28 Zn hyperaccumulating plant species had been identified, none native to Sweden (Balafrej et al., 2020).

Chloride in plants

Chloride is an essential element for plants, required for osmotic adjustments and enzyme activity among other functions (White and Broadley, 2001). Chloride accumulation is generally much higher than the amount of Cl^- needed (Broadley et al., 2011), a feature which is utilized in phytodesalination. In low-saline conditions, active uptake of Cl^- into cells is facilitated by $Cl^-/2H^+$ symports, whereas in saline conditions, uptake is mediated by anion channels (Geilfus, 2018). Once accumulated, Cl^- is relatively mobile and is distributed between roots and shoots by both xylem and phloem (White and Broadley, 2001). While Cl^- is an essential nutrient in low concentrations, high salinity of the growth medium causes salt stress which manifests itself as water deficit in the plant due to osmotic stress. Moreover, high Cl^- accumulation reduces metabolic functions of the cells due to cytotoxic effects. The toxicity threshold for Cl^- varies between species and is approximately 4-7 and 15-50 mg Cl^- g DW^{-1} for Cl^--sensitive and Cl^--tolerant plant species, respectively. Chloride deficiency in plants is rare but can occur at tissue concentrations below 0.1-5.7 mg Cl^- g DW^{-1}.

Multiple Cl^- tolerance mechanisms exist in tolerant plants, including restricting accumulation by efflux, tolerating high tissue concentrations by compartmentalization in the vacuoles, and exudation through salt glands (Flowers and Colmer, 2008; Teakle and Tyerman, 2010; Wu and Li, 2019). Plants that tolerate Cl^- concentrations of 80 mM NaCl or above (i.e. 4657 mg NaCl L^{-1}, corresponding to 2836 mg Cl^- L^{-1}) are called halophytes (Flowers et al., 2021). They are of extra interest for phytoremediation research as some of their salt detoxification mechanisms can also lead to high accumulation and tolerance of heavy metals (Manousaki and Kalogerakis, 2011; Sruthi et al., 2017).

Box 1: A method with many names

Floating treatment wetlands (FTWs) is the most widespread term and the term I use in this thesis. However, multiple other names are used in the scientific literature. During my search for literature I came across the following 23 terms: Artificial floating bed, Artificial floating island, Artificial floating meadow, Artificial floating system, Artificial floating wetland, Constructed floating island, Constructed floating wetland, Ecological floating bed, Floating bed system, Floating bioplato, Floating constructed wetland, Floating hydroponic system, Floating island, Floating island system, Floating mat economic plant-based treatment system, Floating phytobed, Floating plant island, Floating treatment wetlands, Floating vegetated island, Floating vegetation mat, Floating treatment island, Hydroponic root mat, and Planted floating bed.

Floating treatment wetlands

A relatively recent application of phytoremediation is floating treatment wetlands (FTWs) (see page 11, *Box 1: A method with many names*), which have shown promising results for the remediation of polluted waters. They consist of rafts planted with emergent species (Fig. 3).

In the 1980s and 1990s, many constructed wetlands were created for the detention and treatment of water (Vymazal, 2022). Floating wetlands are a further development of these, which combine the wetland with hydroponic cultivation to provide a wetland that is more efficient for purification, that can withstand varying water levels, and that can be placed in existing water bodies such as ponds, lakes, and reservoirs (Headley and Tanner, 2012; Sharma et al., 2021; White and Cousins, 2013). Compared with the plants in a constructed wetland which are rooted in the sediment, the plant roots of an FTW are suspended in the water, providing direct contact between the substances in the water and the plant. This is suggested to increase treatment efficacy (Headley and Tanner, 2012).

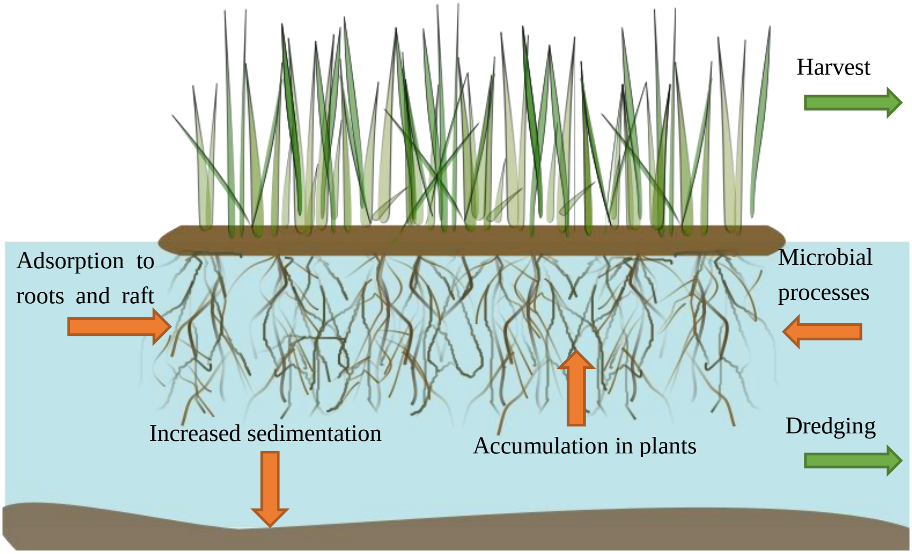

Figure 3. Principle and removal pathways for pollutants of floating treatment wetlands (FTWs).

The raft can be constructed in several ways, as long as they provide buoyancy, support to the plants, and anchoring possibility. Typical materials are various plastics, as in commercially available Beemats (Beemats LLC, New Smyrna Beach, FL) and Bio Haven floating islands (Floating Island International, Inc. Shepard, MT). Other types of constructions, predominately used in research projects, are buoyant frames consisting of drainage pipes (e.g., Wang and

Sample, 2014), bamboo (e.g., Zhao et al., 2012), glass foam (Fang et al., 2017) or stainless steel pipes (Aqua green Schwimmkampen, BGS Ingenieurbiologie und -ökologie GmbH, Germany), surrounding a plastic or metal net which supports the substrate. The substrate supports and stabilizes plant roots, provides surface area for biofilms together with the plant roots, and can, dependent on the material, also absorb pollutants. Organic substrates, such as soil, coconut coir, or peat, also provide nutrients and water-retaining capacity for newly established plants.

The use of FTWs for water treatment began already in the 1980s in Germany (Hoeger, 1988). Similar rafts were previously used to provide wildlife habitats (Kerr-Upal et al., 2000). The first scientific studies of FTWs include Dobberteen et al. (1991) who in 1988 began experimenting with rafts with *Typha angustifolia* and *T. latifolia* to investigate if they had any potential for water purification. Almost simultaneously, in 1989, Smith and Kalin (2000) investigated whether rafts with *T. latifolia* could purify acid mine drainage (AMD) from suspended solids. During the 1990s, some further studies followed, including Revitt et al. (1997a) who tested FTWs with *Phragmites australis* for heavy metal purification in airport runoff, and Nakamura (1999) who used FTWs as breakwater structures to protect the shoreline from erosion. Many studies followed during the early 2000s, but the interest and the number of studies increased sharply from 2006 and still continues to rise (Colares et al., 2020). By 2019, authors based in the United States and China had written 52 % of the FTWs studies. Floating wetlands can also form naturally, but it is a slow process unsuitable for phytoremediation (Headley and Tanner, 2012).

Treatment methods for heavy metals and chloride in FTWs

FTWs combine several treatment methods, and the plants are involved directly or indirectly in most of them. The phytoremediation technique of FTWs is rhizofiltration since all plant involvement depends on the roots, which interact with the water in several ways. Roots decrease water velocity, increasing the contact time between root and pollutant, and increasing sedimentation of heavy particles (Vymazal, 2011). The plants release oxygen through the roots, called ROL (radial oxygen loss), and consume oxygen during respiration, creating oxic (oxygen-rich) and anoxic (oxygen-free) pockets below the surface of the FTW (Chang et al., 2017; Urakawa et al., 2017). Root exudates favor the development of microbial communities (biofilms) on the plant roots, which can trap and sequester pollutants (Gupta et al., 2020; Ma et al., 2016). Additionally, substances adsorb directly to the plant root surface and can be taken up and accumulate within plant roots (Borne et al., 2014). The extent and relative importance of these processes, further described below, differs between metals.

Adsorption

Metals adsorb to the surface of plant roots but also to raft material, litter and biofilms (Fig 4). The process is passive, and the extent depends on surface area and chemical properties such as charge, pH, and salinity. Plants with large root systems promote adsorption since the larger area contains more adsorption sites (Rezania et al., 2016). Adsorbed metals can be removed from the treatment system by plant harvest or by removal of litter or raft material. Adsorption to surfaces, rather than cellular accumulation or sedimentation, has been reported as the main Pb removal mechanism in hydroponic systems (Sánchez-Galván et al., 2008). However, under field conditions, Pb plant absorption will likely be lower than this as Pb will also bind to the sediment and other substances in the water.

Figure 4. Biofilm containing pollutants formed on Carex riparia roots in ***study VI****.*

Accumulation

Plants on FTWs can remove dissolved heavy metals and chloride by plant uptake and subsequent accumulation in plant tissue. The accumulation is the net effect of uptake (influx) and release (efflux), two processes that occur simultaneously.

The uptake of small water-soluble substances, such as metal and chloride ions, into root cells from the surroundings is a multi-step process. The first step, movement between the surrounding water and the apoplast of roots, is a passive process driven by diffusion or mass flow. The plants can modify the conditions, and thereby uptake, by releasing plants exudates affecting the chemical conditions (e.g., pH, redox conditions) of the nearby environment (Ma et al., 2016) or restricting cell access by lignification and suberization of cell walls (e.g., Casparian bands and suberin lamellae) (Küpper and Andresen, 2016).

The next step, transport between the apoplast and the cell cytoplasm, is a selective process controlled by cell membrane proteins. Transport can take place against a concentration gradient if it is powered by energy or by simultaneous transport of another substance along its concentration gradient. Transmembrane transporters from several families mediate ion transport, including transport of the heavy metal ions Zn and Cu. As described in section *Accumulation*, due to the similarity of the ions, toxic heavy metals such as Cd and Pb can also be transported by these transport proteins.

Heavy metals and chloride, together with other substances, are further transported in the symplast between cells through plasmodesmata. The transport is driven by diffusion based on concentration gradients. The metals may remain in the cytoplasm, be transported into the vacuole for storage, or transported out of the cell and into the xylem for long-distance transport. This transport over membranes against a concentration gradient into either the vacuole, apoplast or xylem requires energy, whereas remaining in the cytosol does not cost any energy for the plant but may result in cytotoxic effects.

The heavy metals show different distribution patterns within the plant dependent on their chemical properties and functional role in the plant. In the roots, Cd, Cu, Pb, and Zn are mainly stored within the inner parts of the root (stele) (Vesk et al., 1999) and within cell walls (Loix et al., 2017; Vesk et al., 1999). In shoot tissue, these metals mainly accumulate in the xylem, mesophyll, and hypodermal tissue, and generally rather accumulate in cell walls than within the cells (Loix et al., 2017; Vesk et al., 1999). As plant parts senesce, metals may be released due to leakage, or remain bound in plant litter (Weis and Weis, 2004). Excessive chloride can be stored in roots and leaf sheaths to protect mesophyll cells, and within the cells, vacuolar compartmentalization may protect the cell from cytotoxic effects (Geilfus, 2018; Wu and Li, 2019; Zhang et al., 2020).

The extent and importance of plant accumulation of pollutants compared to other removal pathways (Fig. 3) in FTWs are debated, as reviewed by Bi et al. (2019) and Pavlineri et al. (2017). Studies on N and P removal display large variations, likely caused by differences in study layout. Factors that promoted high nutrient accumulation were the selection of plant species with high growth and tissue accumulation under experimental conditions, high pollution load, and a short duration of the studies. For heavy metals, far fewer data exist. An FTW mesocosm study estimated that the plant accumulation was 5 % for Cu and 13 % for Zn of the total removal by FTWs (Tanner and Headley, 2011). The study compared four species, finding that the total Cu removal and the share of accumulated Cu increased as plant biomass increased. This demonstrates the potential of plant selection for increased metal removal by FTWs. For field conditions, only Borne et al. (2014) have attempted to quantify plant accumulation compared to the total removal capacity of FTWs. They concluded that the accumulation was not dominant compared with other pathways, but refrained from further calculations as it was difficult to determine the timing of accumulation in relation to the changes in metal concentrations of the stormwater in the pond. Their low accumulation might have resulted from low metal loads, known to decrease plant accumulation (Bi et al., 2019). The metals and chloride that is not accumulated by plants could either be removed from the water by other pathways, or remain in the water and thus risk to pollute the recipient.

Microbial processes

The FTW plants promote the formation of biofilms on plant roots by providing root surfaces to attach to, by releasing root exudates, which provide energy and nutrients, and by creating a variety of oxygen levels favoring different types of microorganisms. The bacteria, fungi, and algae in the biofilms may be naturally present in the water (e.g., Gupta et al., 2020; Zhang et al., 2014) or added to the system by inoculation (e.g., Tara et al. 2019; Gao et al. 2020; Nawaz et al. 2020; Shahid et al. 2020). In a study performed on an FTW in Florida, USA, the microbial community in the biofilms differed in composition between roots and on artificial substrates of the raft (approx. 7-20 % overlap) and between species (approx. 70-80 % overlap between two compared species) (Urakawa et al., 2017).

The biofilm affects heavy metal removal in several ways. The microbes may accumulate metals in living cells (bioaccumulation) or dead cells (biosorption) (Ma et al., 2016). The accumulation of heavy metals in biofilms has a linear relationship with the concentration of free metals ions in the water as long as the pH is approx. pH 6-8, regardless of climate, sampling period, ecosystem, temperature, site, and biofilm composition (Laderriere et al., 2021). Microbes may affect the speciation of metals as microbe-mediated metal sulfide formation (described below). Additionally, microbes in the biofilms can indirectly promote plant removal processes by alleviating metal toxicity, promoting plant growth, and inducing defense mechanisms against pathogens (Ma et al., 2016). Inoculation of bacterial strains *Acinetobacter junii* strain NT-15, *Rhodococcus* sp. strain NT-39, and *Pseudomonas indoloxydans* strain NT-38 to *P. australis* plants in an FTW mesocosm system showed increased accumulation of several heavy metals, including Cd, Cu, Pb, and Zn, compared to plants without bacterial inoculation (Nawaz et al., 2020).

Precipitation

Another removal pathway for heavy metals is the precipitation of dissolved metals as less soluble compounds, which later sediment. The FTW vegetation creates reducing and anaerobic conditions below the raft and provides organic matter, which supports the bacterial formation of metal sulfides (Gupta et al., 2020; Van de Moortel et al., 2011). Metal sulfides have low solubility; thus, sulfide formation effectively immobilizes the heavy metals as long as reducing conditions are maintained (Berggren Kleja et al., 2006). This occurs both in AMD (Gupta et al., 2020) and in natural waters (Van de Moortel et al., 2011). Other forms of precipitates such as metal hydroxides and carbonates may form in high-pH environments (Berggren Kleja et al., 2006).

Radial oxygen loss by wetland plant roots promotes the formation of metal plaques, consisting of precipitated iron hydroxides, on the root surface (Mei et al., 2014). Conflicting findings have been reported regarding whether the plaque promotes or decreases metal uptake of the plants (Tripathi et al., 2014).

Sedimentation

Plant roots suspended in the water column reduce water velocity and turbidity, allowing smaller particles, which may contain heavy metals, to settle in the sediment. Moreover, settling of metal plaques, other precipitates, plant litter, and biofilm with absorbed or adsorbed heavy metals will occur.

The speciation of heavy metals in the sediment, i.e. the change of the chemical forms of the metals, depends on metal and water characteristics including redox conditions and presence of other substances as iron (Fe) and manganese (Mn). In reducing conditions, common in the sediment beneath FTWs, metal-sulphide complexes form, which have low solubility and thus low bioavailability (Du Laing et al., 2008; Poot et al., 2007; Van de Moortel et al., 2011; Vymazal et al., 2010). Oxidation, for example caused by ROL from plant roots, or increased salinity, may promote release of heavy metals from the sediment (Du Laing et al., 2009). The redistribution of metals from the sediment below the FTW caused by turbation, including bioturbation, will be low (Van de Moortel et al., 2011).

Applications for FTWs

As described in section *Floating treatment wetlands*, the first experimental applications for FTWs were for coastline protection, removal of excess nutrients, suspended solids, and heavy metals from lakes, AMD, industrial wastewater, and airport runoff. Since then, FTWs use has been investigated for a wide range of pollutants and water sources. The strongest focus has been put on nutrient removal, especially of N and P (Bi et al., 2019). Other uses have been the treatment of excessive water levels by plant transpiration (Liberati et al., 2018), fecal coliforms (Olguín et al., 2017), sulphate (Gupta et al., 2020; Kiiskila et al., 2019), textile dyes (Nawaz et al., 2020; Tara et al., 2019), crude oil products (Rehman et al., 2019), and microplastic (Ziajahromi et al., 2020). Positive effects on water health parameters such as redox potential (Eh), electric conductivity (EC), dissolved oxygen (DO), chemical oxygen demand (COD), and biological oxygen demand (BOD) are common (Bi et al., 2019). The polluted waters have included wastewater from industries, mining, aquaculture, agriculture, polluted rivers, eutrophic lakes, and runoff from roads, airports, and buildings.

Heavy metal removal applications

A number of studies have evaluated heavy metal removal by FTWs on the mesocosm or field scale (Table 4). These were performed in several types of polluted waters in at least 13 countries on five continents, using over 50 different plant species, to evaluate the removal of 11 different heavy metals (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn). The outcomes of the studies varied, but plants accumulated metals unless the metal concentrations in the water were too low, and positive effects on the overall water quality were often observed. The variation in performance can likely be attributed to differences in metal concentrations, plant selection, climate conditions, measurement methods, duration, and the relationship between water volume and FTW size.

Notably, studies performed on road runoff are scarce, and no studies on metal removal have been performed north of 51°N (Sweden is located at 55-69 °N). Thus, to evaluate if FTWs could function in the treatment of Swedish road runoff, further studies are needed.

Table 4. Studies of heavy metal removal by FTWs in mesocosm or field scale. These studies were identified in Web of Science database by combining "metal" and any of the FTW synonyms in Box 1 (e.g. "floating treatment wetland" or "artificial floating bed"). Reviews, microcosm studies, and studies performed with free-floating plants were excluded.

Water type	**Species**	**Metals**	**Study system**	**Location**	**Reference**
Acid mine drainage	*Carex lacustris, Typha latifolia, Juncus canadensis*	Fe	Field trial	Ontario, Canada	Gupta et al. 2020
Acid mine drainage	*Chrysopogon zizanioides*	Al, Cr, Cu, Fe, Mn, Ni, Pb, Zn	Mesocosm	Illinois, USA	Kiiskila et al. 2017, 2019
Acid mine drainage	*Chrysopogon zizanioides, Phragmites australis*	Cu, Fe, Mn, Zn	Mesocosm	Alentejo, Portugal	Borralho et al. 2020
Domestic and industrial wastewater	*Typha domingensis*	Cd, Cr, Cu, Pb, Zn	Mesocosm	Rio Grande do Sul, Brazil	Bauer et al. 2021
Domestic and industrial wastewater	*Brachiaria mutica*	Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb	Mesocosm	Faisalabad, Pakistan	Ijaz et al. 2015

Water type	**Species**	**Metals**	**Study system**	**Location**	**Reference**
Domestic and industrial wastewater	*Phragmites australis, Typha latifolia*	Cd, Cr, Cu, Fe, Ni, Pb, Zn	Mesocosm	Varanasi, India	Kumari and Tripathi 2015
Domestic wastewater	*Carex spp, Lythrum salicaria, Phragmites australis, Juncus effusus*	Cu, Fe, Mn, Ni, Pb, Zn	Mesocosm	Gent, Belgium	Van de Moortel et al. 2010
Domestic wastewater	*Lycopersicum esculentum*	Cd, Cr, Ni, Pb	Mesocosm	West Bengal, India	Rana et al. 2011
Drinking water reservoir	*Phragmites australis*	Cr, Cu, Pb, Zn	Field trial	Shanghai, China	Huang et al. 2017
Drinking water	*Marsilea quadrifolia*	Zn	Mesocosm	Puducherry , India	Abbasi et al. 2018
Industrial wastewater	*Cynodon dactylon, Cynodon* sp., *Stenotaphrum secundatum, Arundo donax, Panicum dichotomiflorum*	Al, Cu, Fe, Mn, Zn	Field trial	Georgia, USA	Hubbard et al. 2011
Industrial wastewater	*Phragmites australis*	Cd, Cr, Fe, Ni	Mesocosm	Faisalabad, Pakistan	Tara et al. 2019
Industrial wastewater	*Phragmites australis, Typha domingensis, Leptochloa fusca, Brachiaria mutica*	Cd, Cr, Cu, Fe, Ni, Pb	Field trial	Chakwal, Pakistan	Afzal et al. 2019
Industrial wastewater	*Chrysopogon zizanioides*	As, Cd, Cr, Pb	Mesocosm	Maharashtra, India	Chandanshive et al. 2020
Industrial wastewater	*Chrysopogon zizanioides*	Cd, Pb	Mesocosm	Tamil Nadu, India	Davamani et al. 2021
River water	*Brachia mutica, Typha domingensis, Phragmites australis, Leptochloa fusca*	Cr, Fe, Mn, Ni, Pb	Mesocosm	Lahore, India	Shahid et al. 2019, 2020
River water	Mixture of 8 species	Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn	Field trial	Jiangsu, China	Ning et al. 2014
River water	Mixture of 15 species	Al, As, Cd, Cr, Cu, Pb, Mn, Zn	Field trial	Illinois, USA	Peterson et al. 2021

Water type	Species	Metals	Study system	Location	Reference
River water	*Pontederia cordata, Canna indica, Calla palustris*	As, Cd, Cr, Cu, Hg, Pb	Field trial	Zhejiang, China	Zhao et al. 2012
River water	*Iris pseudacorus, Iris sibirica, Canna sp.*	Cr, Cu, Pb, Zn	Field trial	Jiangsu, China	Liu et al. 2014
Stock solution	*Acorus calamus*	Cd, Cr, V	Mesocosm	Beijing, China	Lin et al. 2019
Stock solution (artificial industrial wastewater)	*Phragmites australis*	Cu, Fe, Mn, Ni, Pb, Zn	Mesocosm	Faisalabad, Pakistan	Nawaz et al. 2020
Stock solution (artificial stormwater)	*Carex dipsacia, Carex virgata, Cyperus ustulatus, Eleocharis acuta, Juncus edgariae, Schoenoplectus tabernaemontani*	Cu, Zn	Mesocosm	Hamilton, New Zealand	Headley and Tanner 2007; Tanner and Headley 2011
Stormwater (airport runoff)	*Phragmites australis*	Cd, Cr, Cu, Pb, Zn	Field trial	London, UK	Revitt et al. 1997b
Stormwater (road runoff)	*Carex virgata*	Cu, Zn	Field trial	Auckland, New Zealand	Borne et al. 2013, 2014
Stormwater (road runoff)	*Juncus effusus, Carex riparia*	Cd, Ni, Zn	Field trial	Nantes, France	Ladislas et al. 2015
Stormwater (urban runoff)	Mixture of 11 species	Cd, Cu, Pb, Zn	Field trial	Padova, Italy	Zanin et al. 2018
Stormwater (urban runoff)	*Carex apressa*	Al, Cu, Fe, Mn, Zn	Field trial	Queensland, Australia	Schwammberger et al. 2019

Chloride removal applications

Data on chloride removal by FTWs are limited (Table 5). Ijaz et al. (2015) found plants to remove Cl^- in a short-term mesocosm trial with sewage effluents (1 mg Cl^- L^{-1}). Additionally, a mesocosm study compared the ability of four species in an FTW to decrease salinity (1-2 g NaCl L^{-1}) in the presence of several organic pollutants, indicating large differences in phytodesalination capacity among the species (Siahouei et al., 2020). Unfortunately, this trial had a very high plant-to-effluent ratio (1.7-6.3 plants L^{-1} effluent) and a very long exposure time (five weeks), thus the results are unlikely to transfer to field conditions where the volumes are larger and exposure times shorter during precipitation events due to limited pond volume. Neither of the two field trials that measured $Cl^–$ concentration found it to be affected by FTWs (Peterson et al., 2021; Zanin et al., 2018). Additionally, a few studies have evaluated the removal of other pollutants in the presence of saline water, including Huang et al. (2017) who found weak salinity (0.11-0.35 % salinity) to negatively affect the aboveground tissue concentrations of Cu and Zn, but not of Pb and Cr. Inoculation of microbes increased removal of chloride, nutrients and various cations (Gao et al., 2020; Ijaz et al., 2015). Saline water (3%) affected plant growth on FTWs, suggesting a careful plant choice is needed to ensure sufficient treatment performance (Sanicola et al., 2019).

Despite the common use of deicing salt on roads in snowy winters, few studies have been performed in cold climates, and only one study has been conducted on stormwater. As no other stormwater treatment method such as ponds or grass swales can intercept $Cl^–$, any contribution of FTWs to removing $Cl^–$ from stormwater could be useful.

Table 5. Studies of chloride removal by FTWs in mesocosm or field scale. These studies were identified using a structured search in Web of Science database by combining "chloride", "NaCl" or "Cl" and any of the FTW synonyms in Box 1 (e.g., "floating treatment wetland" or "artificial floating bed"). Reviews, microcosm studies, and studies performed with free-floating plants are excluded.

Water type	**Species**	**Chloride concentration**	**Study system**	**Location**	**Reference**
Domestic and industrial wastewater	*Brachiaria mutica*	939, 1150 mg NaCl L^{-1}	Mesocosm	Faisalabad, Pakistan	Ijaz et al. 2015
River water	Mixture of 15 species	50-300 mg NaCl L^{-1}	Field trial	Illinois, USA	Peterson et al. 2021
Stock solution (artificial industrial wastewater)	*Cyperus alternifolius, Amaranthus retroflexus, Closia cristata, Bambusa vulgaris*	1000, 2000 mg NaCl L^{-1}	Mesocosm	Teheran, Iran	Siahouei et al. 2020
Stormwater (urban runoff)	Mixture of 11 species	0.6 mg NaCl L^{-1}	Field trial	Padova, Italy	Zanin et al. 2018

Stormwater applications

FTWs were early recognized as a suitable tool for stormwater treatment (Kerr-Upal et al., 2000). Stormwater often contains medium levels of pollutants, is commonly collected in ponds with varying water depths, is challenging for sediment rooted plants, and is preferably treated outside wastewater plants and at a low to medium cost. The first known use of FTWs for road runoff was for nutrient removal in a stormwater pond (Chang et al., 2012). Since then, several studies have been performed (e.g., Borne 2014, 2013ab; Chang et al. 2013; Khan et al. 2013b; Ladislas et al. 2015; Wang et al. 2014; 2015; White and Cousins 2013; Winston et al. 2013, Ziajahromi, 2020; Xu et al. 2017, Ge 2016). Despite the high number of studies, only five of them have investigated heavy metal removal from stormwater, whereof two dealt with road runoff (Table 4).

Applications in Sweden and other sites with cold climate

The knowledge of how metal removal FTW functions in cold climates is limited since most studies were performed in locations with warm climates (Tables 4 and 5). Although Sweden is a long country with several climate zones, the climate is generally characterized by differences in temperature and light intensity over the year, whereas the precipitation is rather evenly distributed (Persson, 2015). To survive, plants need to be adapted to a cold coastal climate, including snowy winters, and springs with high light intensity combined with occasional cold days.

Cold climates, including snow, ice, and temperatures below 0 °C for extended periods during winter, provide several challenges and likely affect FTW plant performance. Firstly, most of the species used in other FTW studies (Table 4 and 5) are not adapted to a cold climate or may not have the same growth period as in a warm climate, meaning that the results from these studies are difficult to transfer to cold conditions. Secondly, low temperatures and salinity caused by deicing salt used on roads likely decrease heavy metal accumulation by plants (Findlay and Kelly, 2011; Fritioff et al., 2005), but the effect remains to be verified for FTWs. Thirdly, the exposed placement on an FTW may be a challenge even for native species (Barco et al., 2021; Hubbard et al., 2011). FTWs may increase the formation of ice on the pond and on the raft itself as they provide a windshield which decreases water movement (Van de Moortel et al., 2010). The ice may negatively affect the plants as it risks damaging plant tissues and limiting oxygen exchange with the roots (Wang et al., 2015). Fourthly, biological treatment mechanisms, such as plant uptake and microbial processes, are limited or non-existent during winter due to reduced metabolism in plants and microbes (Nsenga Kumwimba et al., 2021). This may limit the usefulness of FTWs in cold climates. However, physical processes such as reduced water speed and trapping of particles on roots, resulting in increased sedimentation, may be efficient also during winter.

A few studies with metal or chloride removal by FTWs in cold climates have been performed. These include Minnesota, Vermont, and Illinois, US (Kiiskila et al., 2019, 2017; Oddsson et al., 2021; Peterson et al., 2021; Tharp et al., 2019), Sudbury, Canada (Gupta et al., 2020), and Khanka Lake, China

(Li and Guo, 2017). Looking more closely at the climate of the sites, clear differences between these and the Swedish climate emerge. All the sites above are characterized by an inland climate with large differences in temperature between summer and winter and thick snow layers in wintertime. Sweden, on the other hand, has mainly a coastal climate with smaller differences between winter and summer, and the snow layer is only constant throughout the winter in northern parts. This results in colder summers and warmer winters compared to the North American and Chinese sites. Consequently, this can have different effects on plant performance including survival. Moreover, only a few of the species in these studies are native to Sweden. Thus, the findings on removal efficacy and plant survival from these studies should be interpreted with caution for Swedish conditions. Nevertheless, the promising findings regarding treatment efficacy and plant survival indicate that FTWs functions in cold climates and that cold winters do not themselves limit the usefulness of FTWs. However, it is necessary to adapt and evaluate FTWs for Swedish conditions to ensure optimal performance. Despite the lack of studies, the first FTW in Sweden was installed in 2011 in the Norra Sörentorp stormwater pond, along the E4 highway north of Stockholm (Fig. 5, page 24) (Emell and Welin, 2015). It consists of a plastic mat, approx. 1 m long and as wide as the pond, anchored at each side in the middle of the pond. The species planted on the FTW were seven wetland plant species. When I visited the FTW ten years later, most species were still there, with the addition of a few others that had spontaneously colonized the raft. For further description of this FTW, see *Box 2: The first FTW in Sweden – field notes*.

The second installation was made in 2013 in lake Rönningesjön, in Täby north of Stockholm (Fig.5) (Dunér and Myhrberg, 2014). It replaced a stormwater treatment plant and consists of 28 small FTWs based on Bio Haven floating islands (Floating Island International, Inc. Shepard, MT). The total area of the FTWs is 150 m^2, and these are placed in a 5000 m^2 shielded part of the lake that acts as a stormwater sedimentation pond. Target pollutants are excessive nutrients, especially P. No formal evaluation of the installation has been performed so far, however, a bachelor thesis found the P removal to be lower than expected (Dunér and Myhrberg, 2014).

A number of others has followed these installations, and FTWs are gaining increasing interest. Installation of FTWs is often included in action plans by municipalities, generally with the aim to improve water quality, promote biodiversity, and aesthetical improvement of the site (Alvbåge, 2022). The water source is typically urban runoff. Moreover, Sweden has over 1 400 stormwater ponds, of which at least 400 mainly treats road runoff (Falk, 2007). These could potentially be retrofitted with FTWs to improve their pollutant removal capacity.

However, efficacy evaluation of the FTWs in Sweden or FTWs treating road runoff in similar climates is still missing. Recently, a study evaluated the use of FTWs for excess N removal from explosives residues from mining activities in northernmost Sweden, finding high growth rates of the locally sourced plants and denitrification as the main removal pathway (Choudhury et al., 2019). Moreover, FTWs deployed in Southern Baltic Sea accumulated N and P, and the plants tolerated the brackish water during April-September when the study was conducted (Karstens et al., 2021).

Figure 5. Floating treatment wetlands in Norra Sörentorp stormwater pond, installed 2011 (top), and lake Rönningesjön, installed 2013 (bottom).

Plant selection

While over 50 species have been evaluated for metal removal and over 30 for chloride removal by FTWs (Tables 4 and 5), little emphasis has been put on determining which species should be selected to optimize pollutant removal. The scope of most studies has been nutrient removal, design, placement, and overall treatment performance (as reviewed by Bi et al., 2019; Colares et al., 2020; Lucke et al., 2019; Pavlineri et al., 2017), while plant selection and removal of heavy metals and chloride were less studied. In general, plant selection seems to be of low priority in the majority of the studies. Plant choice appears to be based on availability, survival, and growth potential rather than removal capacity (e.g., Gupta et al., 2020; Huang et al., 2017; Hubbard et al., 2011b; Siahouei et al., 2020). The reasons might be studies questioning the contribution of plant accumulation in heavy metal treatment by FTWs because they found other removal pathways to be of larger importance (Borne et al., 2014), which may have downplayed the role of plants. The focus on nutrient removal can be explained by its urgent priority in many water bodies, and early FTW studies found positive effects (de Stefani et al., 2011; Hubbard et al., 2011; Van de Moortel et al., 2010). This might have further reduced the interest in plant selection, as N removal is mainly mediated by microbial processes, only indirectly influenced by plants.

Although the technical aspects are necessary to improve FTW systems, plant choice must not be overlooked as it is of key importance for FTW function. We argue that pollutant removal may increase by selecting plants with high accumulation capacity. Hence, several studies identify the need to improve the knowledge of plant selection and plant performance in varying environmental conditions (e.g., Lane et al., 2016; Saad et al., 2016; Sanicola et al., 2019), especially for cold climates (Nsenga Kumwimba et al., 2021; Zou et al., 2016). I hope this thesis will contribute to increasing the much-needed knowledge.

Box 2: The first FTW in Sweden – field notes

Sweden's probably oldest floating wetland, constructed in 2011 in the stormwater pond Norra Sörentorp at the E4 highway in northern Stockholm, was visited by a representative from the Swedish Transport Administration and me on a summer day in 2020. The purpose was to visually inspect the pond and floating wetland and discuss design and maintenance. We found a well-functioning pond despite a lack of maintenance (Fig. 15).

The lack of maintenance had caused one anchorage point to come loose, and the other was well on its way, but the raft remained in place. The floating wetland was overgrown with mainly *Carex* sp. (probably *C. elata*) with a few additional small *Iris pseudacorus*, *Lythrum salicaria*, and *Phragmites australis* per square meter. Some *Typha latifolia* grew at the edges of the wetland. Only a few small gaps in the vegetation cover exposed the raft material. Compared to the original plantation, only *Juncus conglomeratus* and possibly *C. acuta* had disappeared. In return, *Lycopus europaeus*, *C. pseudocyperus*, *Betula* sp., and *Salix* sp. had colonized the wetland. The roots of the plants were about 20-50 cm long but with various gaps, which means that the water volumes could take an easier route around the root mass instead of through, which reduces the treatment effect.

A thick sediment layer had formed in the upper part of the pond, mainly in the inlet ditch and around the inlet. The water in the lower half of the pond was clear and contained a large population of salamanders (*Salamandridae*). This indicates a clean aquatic environment; otherwise, salamanders do not thrive.

In many ways, this floating wetland appears to be a good example of how a floating wetland should be designed. It is placed in a pond with calm conditions concerning wind, waves, and ice, which supports long durability. The raft is relatively large, and because the raft runs across the pond, all the water will pass underneath. This should result in a good treatment effect. Due to the location in the middle of the pond, larger particulate pollutants have time to settle before they reach the wetland, which is probably good for root health and provides better conditions for plant uptake. The material, a plastic mat, seems to be durable and less sensitive to wear and UV radiation than spun PET (polyethylene terephthalate) plastic.

The disadvantages of this floating wetland include, above all, the lack of harvesting opportunities. The pond lacks contact with the road and requires an approximately 500 m walk, partly through unpaved terrain, to be reached. Harvesting and transporting harvested material is therefore difficult and has not been done. Accumulated metals will thus be released back to the water; some of them will move to the sediment. Although this is a net positive, the effect is smaller than what could have been achieved. Thus, the principal function of the floating wetland will be increased sedimentation of all kinds of pollutants, including heavy metals and P, and the removal of nitrate and nitrite through bacterial denitrification.

The long and narrow FTW is placed across the Norra Sörentorp stormwater pond close to E4 highway in northern Stockholm. Some gaps in the vegetation expose the plastic mat of the raft. Dense root mats have formed beneath the raft.

Research aims

In this thesis, I explored if plants on floating treatment wetlands could function for metal and chloride removal from water. The main objective was to identify plant species that would be best suited for this task. The planned application for these findings is treatment of stormwater originating from roads and cities in Sweden, which resulted in a focus on Swedish plants and climate conditions. The findings could also be useful in other contexts, for other types of polluted water as wastewater, and in other climates. To fill the aforementioned knowledge gaps, I focused on three aims.

- First, I wanted to identify species from the Swedish flora that had high removal capacity of heavy metals (**study I**) and chloride (**study III**). Additionally, I wanted to identify traits in plants connected to high phytoremediation potential to further understand mechanisms and provide tools for identifying additional species (**studies II and III**).

- Second, I wanted to improve the understanding of how environmental factors affect the phytoremediation capacity of plants. More specifically, I wanted to explore how temperature and salinity affect the removal of heavy metals and chloride by plants, common environmental factors in a country such as Sweden with changing seasons and application of road salt during winters (**studies III and IV**). As plants in phytoremediation applications will receive repeated flushes of pollutants, I wanted to explore how previous exposure to pollutants affected the ability of the plants to remove additional doses/flushes (**study V**).

- Third, I wanted to provide some practical insight in the floating wetland's performance under field conditions, to provide information for future large-scale field trials and commercial usage. More specifically, I wanted to measure the growth and metal accumulation of the plants, and test the construction and handling of a low-plastic raft design (**study VI**).

Comments on Materials and Methods

Selection of plant species

We collected specimens from 34 plant species as material for **studies I-III**, aiming to identify species with high removal of heavy metals and chloride. For a list of the species, see **studies I-III**. We based the selection of these species on several criteria:

- All of the species were native to Sweden, to ensure that they would be able to survive Swedish applications, and to prevent the risk of introducing species that could be invasive in nature (Wang and Sample, 2014). Many of the included species have global distribution, which means that the findings from these studies will be applicable also in other parts of the world.
- All of the species were naturally growing in or near water, thereby increasing the chances that they could thrive in hydroponic conditions in experimental setups in greenhouse studies and in floating treatment wetlands. These species are commonly adapted to waterlogged conditions with morphological adaptations, such as aerenchyma to enable transport of oxygen to the roots (Carter et al., 2006; Visser et al., 2000). Throughout this thesis, these plants are called “wetland plants”.
- All of the species were perennial so they would return every year, and not require propagation from seed or planting of new plants. Most perennial species also had a long growing season, thereby increasing the likelihood of efficient pollutant removal during most of the year.
- Most of the species had large biomass, as it promotes high total accumulation per plant even though the tissue concentration may be low (Vymazal, 2016).
- Most species are common in the Swedish flora, and none of them are endangered (The Swedish Species Information Centre, 2020) or classified as invasive according to EU regulation no 1143/2014 (EU, 2014), to not affect the natural flora of Sweden.
- Some of the selected species were halophytes, thus they tolerated saline water (i.e., 80 mM NaCl or above) (Flowers et al., 2021);

the intent was to test if this trait promoted the removal of heavy metals (**study II**) or chloride (**study III**).

- None of the species were free-floating or submerged plants although such species have been shown to accumulate high amounts of heavy metals (Fritioff and Greger, 2003), as they are not suited to grow on FTWs. Likewise, no trees or shrubs were included in the studies as their use would risk catching wind and flipping the FTW. Additionally, no known hyperaccumulator species were included. They can accumulate large concentrations of pollutants in their tissue; however, commonly this occurs at the cost of low biomass production, resulting in low removal capacity per plant (Arthur et al., 2005).

We collected each species at a single site, meaning that we have one ecotype per species. This choice was made for logistic reasons; in order to evaluate many species, we had to limit the time spent sourcing and testing each species. Implications of this is discussed in section *Causes of variation in removal capacity*.

Based on the findings in **study I-III**, we identified three plant species as promising for future applications and used them in **study III-VI**; study III contained both a screening part and an analysis part (Table 6, page 35). These were *Carex pseudocyperus, Carex riparia* and *Phalaris arundinacea* (Fig. 6). All these species have large biomass and large root systems dominated by thin roots, a combination of traits which promotes efficient pollutant removal (**study II**). They had shown efficient removal of all heavy metals (*C. pseudocyperus* and *C. riparia,* **study I**) and chloride (*P. arundinacea,* **study III**). *Carex pseudocyperus* and *C. riparia* are glycophytes and naturally grow in non-saline environments, while the halophyte *P. arundinacea* can grow in environments with both high and low salinity. All three species are easy to grow and propagate, which was important for facilitation of the studies and for future applications.

Figure 6. Carex pseudocyperus (top left), Phalaris arundinacea (top right), Carex riparia (bottom).

Selection of pollutants and concentrations

The pollutants that I focus on in this thesis are the abundant and problematic heavy metals Cd, Cu, Pb and Zn, and the less considered pollutant Cl^-, which nonetheless causes several environmental problems (see section *Introduction*). In stormwater (and other wastewaters), these pollutants exist alongside many other substances as nutrients, organic material, particles, and other organic and inorganic compounds. Moreover, the metals are found in both dissolved and particulate forms. We chose a minimalistic approach for **study I-V** and used only the target pollutants dissolved in deionized water (**study I-III**) or with the addition of nutrient solution (**study III-V**) (Table 6). This enabled a controlled environment, well suited for reproduction of the results, and simpler analysis of samples. However, the results should be verified in the field, which was done in **study VI**.

The concentrations we used aimed to be high enough to produce clear responses that were easy to detect with the analysis methods used, while still being low enough to be environmentally relevant. Many studies uses extremely high concentrations of heavy metals, much higher than found in stormwater, which results in toxic effects on the plants (Fischer et al., 2014; Liang et al., 2019). Nonetheless, the concentrations in our studies exceeded those in natural stormwater, as the metals in our studies were available in dissolved form, which is bioavailable, contrary to natural waters where parts of the metals are in particulate form, which is less available for plant uptake (Revitt and Morrison, 1987; Tanner and Headley, 2011).

Selection of evaluation measures

Phytoremediation performance of the plants in the **studies I-VI** was determined mainly by two measures; removal and accumulation capacity, defined as:

Removal capacity = Remaining pollutant concentration in water of plant treatment / (Initial pollutant concentration in water – Remaining pollutant concentration in water of control treatment) (1)

Accumulation = Concentration of pollutant in tissue after plant exposure – Concentration of pollutant in tissue before plant exposure (2)

Thus, removal capacity includes all processes related to the plants in water (accumulation, adsorption, microbial processes, sedimentation of particles including precipitates (see section *Treatment methods for heavy metals and chloride in FTWs*)), while accumulation only includes the pollutant that has accumulated in the plant tissue, whether in the cell walls or in the symplast. For FTW applications, both removal and accumulation aspects are important. The removal capacity describes the overall effect of the FTW on the water quality, while the accumulation describes the amount of metal that can be removed by harvesting the plants. We did not quantify the contribution of accumulation to the removal capacity, and data on this is limited for FTWs as discussed in section *Accumulation*.

Table 6. *Summary of objectives, conditions, and analysis methods for the studies included in the thesis. Note that* ***study III*** *has been divided in two parts for the sake of clarity.* ***Study III a*** *includes the screening of species for chloride removal and* ***study III b*** *includes follow-up tests on chloride tolerance and accumulation. CP = Carex pseudocyperus, CR = Carex riparia, PA = Phalaris arundinacea.*

Study	**I**	**II**	**III a**	**III b**	**IV**	**V**	**IV**
Main objective	Screening of metal removers	Traits related to metal removal	Screening and traits of chloride removers	Salinity tolerance and accumulation	Effect of temperature and salinity	Effect of alternating solution concentration	Field study
Species	34 species	34 species	34 species	CP, CP, PA	CP, CP, PA	CP	CP, CP, PA
Environment	Greenhouse	Greenhouse	Greenhouse	Greenhouse	Greenhouse	Greenhouse	Field
Cd µg L^{-1}	1.2	1.2	1.2	-	1.2	0-112	Ambient
Cu µg L^{-1}	68.5	68.5	68.5	-	68.5	1-127	Ambient
Pb µg L^{-1}	78.4	78.4	78.4	-	78.4	0-1036	Ambient
Zn µg L^{-1}	559	559	559	-	559	2-1962	Ambient
Cl^{-} mg L^{-1}	50	50	50	0-15 000	0, 60, 600	-	Ambient
Temperature °C	22	22	22	15	5, 15, 25	15	Ambient
Duration	5 d	5 d	5 d	28 d	5 d	5+5 d	12 weeks
Analysis	Water	Water	Water	Plant parts	Water	Plant parts	Plant parts

Selection of experiment environments

We performed **study I-V** in a greenhouse and **study VI** in the field (Table 6, page 35). Greenhouses allow a controlled and constant environment over time, where all plants species could be evaluated in identical conditions as in **study I-III and V** or with differences in just a specific environmental factor, namely, temperature in **study IV** (Brisson and Chazarenc, 2009). Field conditions provide a more complex situation with changing weather conditions and interactions with other biotic and abiotic factors. Ultimately, field trials are often necessary as they provide insights that are difficult to achieve in other ways, but yield fewer possibilities to identify individual factors that need to be adjusted to increase the efficacy of the treatment. We therefore performed **study VI** in the field to evaluate the survival, growth, and heavy metal accumulation of plants during field conditions, and practical aspects of the raft construction.

By comparing many species side by side under identical conditions in a controlled environment, we overcame the problem with comparing species evaluated in different studies with different settings as described by Brisson and Chazarenc (2009). However, for practical reasons we had to limit the number of replicates to three and could analyze only one environmental condition in **study I** and in the screening part of **study III** (Table 5). Furthermore, we only investigated the removal of ions over a short term and in a synthetic stormwater. We chose a number of species that performed well and tested them further in several environmental conditions in the accumulation and tolerance part of **study III** and **studies IV-VI**.

The differences in experimental conditions might explain the differences between the outcome of **study I** conducted in the lab, and **study VI** conducted in the field. It is important to remember that the field trial is not the “correct answer”, as it covered warm weather and did not include chloride or lower metal concentrations in the water for all metals except Cu. Other studies comparing lab and field performances of removal capacity have found both high and low resemblance between the two environments (Ladislas et al., 2015, 2013; Watson et al., 2003). Regardless, it is important to perform screenings to investigate a wide range of species, but due to practical limitations, such studies are often shorter and not run under field conditions.

Selection of sample analysis methods

We consistently used atomic absorption spectrometry (AAS) for heavy metal analysis and ion chromatography (IC) for chloride analysis in the thesis.

The AAS detects the absorption of specific wavelengths of light by atomized substances. The atomization can be achieved in several ways, most commonly by heating with a flame (known as flame AAS, or FAAS) which is predominantly used for higher concentrations, or in a graphite tube (graphite tube AAS, or GFAAS) for low concentrations. In all studies in this thesis, we used FAAS for Zn and GFAAS for Cd, Cu and Pb. Prior to AAS analysis, we digested plant samples with HNO_3 and H_2O_2 at high temperature in a Speedwave microwave oven (Berghof, Germany) and filtered water samples through 0.45 µm filters, a standard method for separation of dissolved and particulate metals (Berggren Kleja et al., 2006; Viklander et al., 2019). To increase the precision and avoid interference from other substances in the sample (matrix effects), we used standard additions for all samples. Since pH and Cl^- concentration can affect the chemical speciation of metals and hence the detection, we added HNO_3 and Cl^- to all water samples to level out differences. Lastly, reference material NJV 94-4 (*Phalaris arundinacea*), was used for verification of plant material analysis.

The chloride content of water and plant samples was analyzed with IC according to ISO 10304-1:2009. In IC, the liquid samples are pumped through an anion-exchange column under high pressure. The column separates anions due to their different affinity to the column matrix, causing the anions to travel through the column at different velocities. A conductivity meter detects the amount and type of anions, and the results are converted to concentrations using a standard/reference curve. We used two different setups for IC analysis, an older manually operated setup for **study III** and a modern automated IC setup for **study IV**. However, both utilized the same type of pre-column and ion-exchanged column, and thus should provide identical results.

Selection of statistical methods

We calculated the correlations between plant traits and heavy metal removal (**study II**) and chloride removal (**study III**) differently in the two studies. Since many trait parameters had a non-normal distribution, Spearman rank correlation tests were used in **study II**. In **study III**, we log-transformed the data to meet the assumptions of normality, and tested with Pearson correlation tests. I recalculated the correlations from **study II** with the correlation from **study III** (Appendix 1) finding only minor differences that did not affect the conclusions of **study II**. We did not develop a model for chloride removal as we did for metal removal in **study II**, as there was high co-dependence between the traits.

Results and Discussion

The overall purpose of the thesis is to determine if floating treatment wetlands could be useful for metal and chloride removal from water under Swedish conditions. Currently, Swedish stormwater contains large volumes of heavy metals and chloride, which can cause several environmental issues, including being toxic to biota at elevated concentrations (Ejhed et al., 2010; Lindgren, 2001; Lundmark et al., 2007).

In the six studies in this thesis, we demonstrate the capacity of wetland plants to remove heavy metals and chloride under various conditions, an ability that can be utilized for the remediation of polluted waters. This is a summary of the studies:

- In **study I**, we show how heavy metal removal differs between plant species and duration of exposure.
- In **study II**, we further investigated the connection between plant traits and heavy metal removal capacity, identifying several traits connected to high removal capacity.
- In **study III**, we studied the removal of chloride, a pollutant much less studied from a phytoremediation perspective, identifying species with high removal capacity and high tolerance towards chloride, and traits connected to high chloride removal capacity.
- In **study IV**, we demonstrate how temperature and salinity, which commonly have seasonal variation in polluted waters such as road runoff, can affect heavy metal removal by plants.
- In **study V**, we studied the effect of solution concentration changes on plant accumulation of heavy metals,
- Lastly, in **study VI** we investigated some field aspects of plant accumulation in a pilot field trial.

The results presented in this thesis further advances our knowledge of the importance of plant choice for phytoremediation of polluted waters, and how the surrounding environment affects the remediation capacity of plants.

Variation in heavy metal and chloride removal capacity of plants (Aim 1)

Plants are directly or indirectly involved in most removal processes of FTWs, and the removal capacity differs between species (Headley and Tanner, 2012; Ladislas et al., 2015). As most of the studies on FTWs and rhizofiltration have been conducted in warmer climates (Tables 4 and 5), very few of the species used in those studies can grow in the Swedish climate. Besides being adapted to the Swedish climate, to be useful on an FTW the plants must also effectively support the removal processes on FTWs. Therefore, to create useful FTWs for Swedish conditions, the first step is to identify several candidate species.

Species for rhizofiltration

Among the 34 plant species we compared in the short-term **studies I and III** (see section *Comments on materials and method regarding species selection*), we found large differences between species in metal and chloride removal capacity. Most of the investigated plant species caused a significant decrease in the concentration of all heavy metals in the water, but the removal rate differed between species and some species only had a minor effect on the concentrations (**study I**). Many species effectively removed all four investigated metals (Cd, Cu, Pb, and Zn) after only a short time, but some species only efficiently removed one or a few of the metals. Almost all species were able to remove almost all Pb, which has a short retention time in water and tightly binds to organic material such as plant roots and other surfaces (Bradl, 2004; Jamla et al., 2021). The differences in Cl^- removal capacity between species were smaller, although a few species had a markedly higher removal capacity than the rest (**study III**).

Based on the rate of removal and total removal capacity, **study I** identified *C. pseudocyperus* and *C. riparia* as the most suitable species for further studies of heavy metal removal capacity, and **study III** identified *P. arundinacea* for further studies of chloride removal capacity. *Glyceria maxima* also had high chloride removal capacity and quickly removed all investigated metals except Cd (**studies I and III**), but was not selected for further studies as it was difficult to propagate in the greenhouse and easily can become invasive in nature (Clarke et al., 2004). The variation in removal capacity between species, and between pollutants, suggests that a careful selection of plant species could promote a high removal capacity of FTWs.

The fate of the pollutants was not investigated in the screening **studies I and III**, but the removal likely consisted of accumulation in plant tissues, precipitation, and adsorption to root surfaces that were in contact with the polluted water. In a field environment, i.e., a stormwater pond or a lake, plants promote microbe-mediated metal sulfide formation and sedimentation, further increasing the removal capacity (Borne et al., 2014; Gupta et al., 2020).

Our studies focused on plants that naturally grow in a cold climate, and could thus provide phytoremediation data for species that have not been studied before for this purpose. One such species is *Carex pseudocyperus,* which turned out to be an efficient heavy metal remover (**study I**). Other species that were included have been evaluated in multiple studies as *Phragmites australis* and *Typha latifolia* (Tables 4 and 5), which have a global distribution and are recommended for use on FTWs due to their high metal removal capacity (Shahid et al., 2020). By including these species, we could benchmark others against them, finding that many species performed better than those two did. A possible explanation for this is the low amount of fine roots *P. australis* and *T. latifolia* formed in hydroponic culture, a trait which **Study II** identified as an indicator trait of low removal since it indicates a small area for adsorption and absorption of pollutants. Other low-removers were *Iris pseudacorus*, *Lysimachia vulgaris,* and *Eriophorum angustifolium*. As expected, no hyperaccumulator species were identified in our screening. These generally are rare and small plants (Arthur et al. 2005), and thus were not included in the screening as we mainly included common and large plants, as discussed in section *Comments on Materials and Methods*.

Studies I and III focused on the *relative differences* between species in terms of metal and chloride removal, rather than their *absolute* removal. Our reasoning was that the removal capacity in a short-term lab experiment is generally higher than under field conditions (Pavlineri et al., 2017). For this reason, we did not calculate the estimated removal per area or biomass under theoretical field conditions for metals based on **study I**, as this type of calculations easily overestimates the FTW removal capacity. Rather, we waited for data from **study VI** (estimated removal based on these figures is discussed in section *Theoretical removal efficacy of FTWs in cold coastal climate*). However, we calculated the estimated removal of Cl^- per FTW area based on **study III**, as no chloride was present in the water during **study VI**. Needless to say, our estimate that a 500 m^2 FTW could remove 7 kg Cl^- during a month of needs to be verified in the field.

Causes of variation in removal capacity

Studies that identify attributes of highly performing plants have been requested as their results would advance the selection process and provide an increased understanding of which features affect removal capacity (Brisson and Chazarenc, 2009). We succeeded in identifying some easily measured attributes that indicates a high removal capacity of metals and chloride. According to **studies II and III** and Appendix 1, the cause of the variation between species in removal capacity is connected to differences in size, morphology, and transpiration capacity**.** The highest degree of correlation was found between pollutant removal capacity and fine root biomass, aboveground biomass, and total biomass. Based on these findings, we hope that suitable species in other parts of the world with a different range of native species thereby can easily be identified and evaluated.

It is possible that additional high performers could be identified among the original 34 species with if they were exposed to other conditions. Additionally, we might have overlooked some suitable species by not including them in them in the screening studies (**studies I and III**). However, based on the clear correlation between traits and performance (**studies II and III**), such species would likely have the traits determined in these studies.

As noted in section *Selection of plant species*, we examined one ecotype per species. Potentially, this means that the selected ecotype could be non-representative in its growth, removal capacity, and other aspects studied in this thesis. Genetic differences have been detected that affect Cl^- removal capacity in *P. arundinacea* (Maeda et al., 2006), and in metal removal capacity of several species (Balafrej et al., 2020; Lange et al., 2017; Tian et al., 2017). However, as the species removal capacity in this thesis was mainly connected to morphological traits such as fine root and total biomass **(Studies II, III),** the general removal pattern would likely be the same even if other ecotypes were used.

There seem to be two approaches towards genetic differences in plants used for phytoremediation. On one hand, the use of native plants is highly encouraged (e.g. Wang et al. 2015; Guittonny-Philippe et al. 2015; Tharp et al. 2019). These are often collected in the field, and the genetic variation is not considered. On the other hand, other areas of phytoremediation research are highly interested in genetic variation of plants in phytoremediation capacity, as it can provide insights in the phytoremediation mechanisms on a cellular level (Rai, 2008; Yan et al., 2020). These differences could be used for genetic modification of species to develop plants with increased phytoremediation capacity. The studies in this thesis follow the first approach, as genetic variation of the plants was not studied.

According to **study II**, plants that efficiently removed one metal were likely to also remove other metals efficiently. Additionally, positive relationships were found between the removal capacity of Cl^- after 5 days of exposure and the removal capacity of Cu, Pb, and Zn after 0.5 h exposure (**studies I and III**; Fig. 7). These data suggest that the same species can be utilized for metal and chloride removal, but the speed of removal differs. This is likely due to the large differences in concentrations between the pollutants, as the chloride concentration was several magnitudes higher than the metal concentrations.

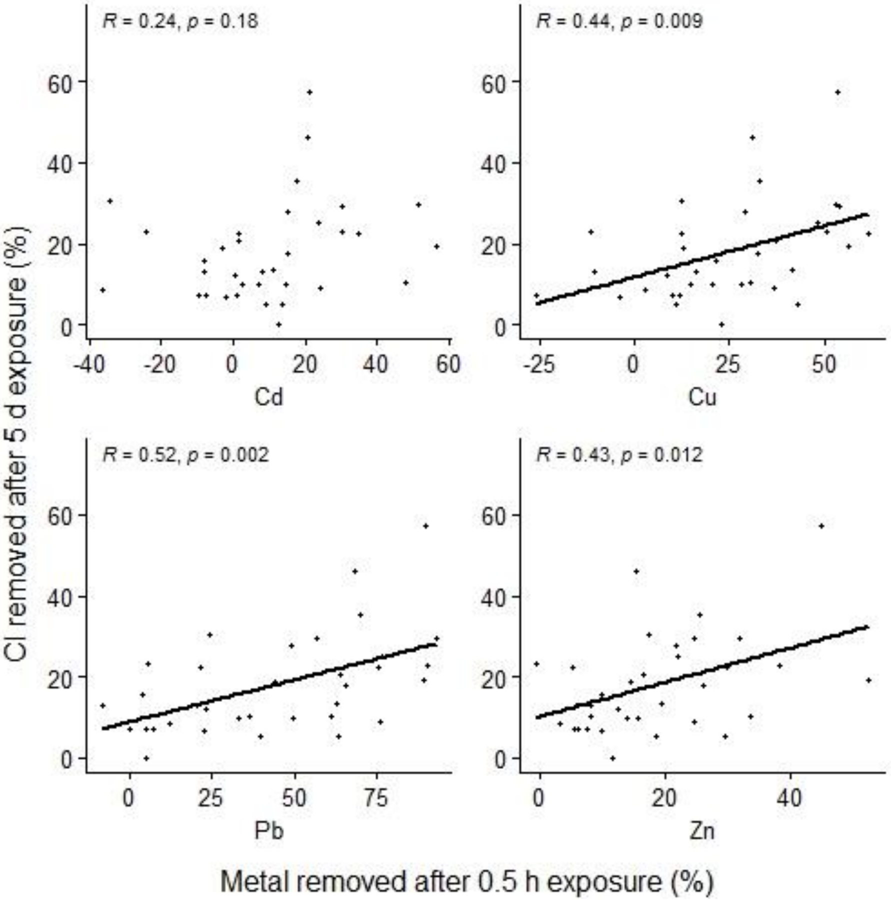

Figure 7. Correlations between metal removal capacity after 0.5 h of exposure and Cl^- *removal capacity after 5 days of exposure. Each point represents the average value for a species, where n = 3. Regression lines represent significant Pearson correlations at p<0.05. Data is collected from* ***studies I and III****.*

We also investigated the tissue concentration of chloride and heavy metals, finding that the accumulation in roots and shoots differed between species (**studies III and VI**). The highest tissue concentration of Cl^- was found in shoots of *C. riparia* and *P. arundinacea* (**study III**). This seems to be a result of their tolerance to Cl^-, which allowed them to maintain transpiration and thus Cl^- transport to aboveground tissue, in combination with apparently weak exclusion mechanisms which otherwise would have restricted root-to-shoot translocation (Teakle and Tyerman, 2010). The highest root tissue concentrations of Cd, Cu, and Zn were found in *Phalaris arundinacea* (**study VI**). The Pb root concentrations, as well as shoot tissue concentrations of all metals were more similar between species but still displayed some differences. These differences demonstrate that species-specific variation exists besides the above-mentioned factors. This variation may be caused by differences in accumulation, excretion, and exclusion mechanisms (Jamla et al., 2021; Sruthi et al., 2017). The impact and relative importance of these factors on removal capacity in the species analysed in **studies I-VI** remains to be investigated.

The shoots, consisting mainly of leaf tissue, had Zn concentrations below the toxicity limit except for *P. arundinacea* which had 2 % and 10 % more Zn compared to the toxicity threshold for leaf tissue, at the stormwater pond sites Silverdal and Lilla Essingen, respectively (Broadley et al. 2011, **study VI**). The shoot tissue concentration of Cu was generally twice the toxicity limit for leaf tissue for most species. No plants demonstrated any visible signs of metal toxicity symptoms (chlorosis, necrosis) in any of the studies, but as no control treatments without metals were used, toxicity symptoms as decreased growth may have gone unnoticed. Nevertheless, this indicates that the studied species had sufficient tolerance to heavy metals to be suitable for stormwater treatment applications.

Agreement with plant decision tools for other phytoremediation methods

A few other studies have screened plant species and developed decision tools for plant choice, but focused on other applications (i.e., with regard to remediation systems, species, and pollution sources). Despite the differences, the identified plant traits are often similar to those we identified in **studies II and III** (e.g., Read et al. 2009; Lai et al. 2012; Guittonny-Philippe et al. 2015; Gao et al. 2015).

For constructed wetlands, the attributes connected to a high removal capacity of nutrients, as N and P, are various root parameters similar to the traits we identified in **studies II and III**. These include root biomass, the biomass of roots <1 mm diameter, root activity, root number, and root length (Choudhury et al., 2022; Gao et al., 2015; Lai et al., 2012). Additionally, a number of shoot traits connected to removal capacity have been identified, such as above-ground biomass, photosynthetic rate, and aerial height (Choudhury et al., 2022; Gao et al., 2015; Guittonny-Philippe et al., 2015; Lai et al., 2012). Similarly, in biofilters, extensive root systems, including root length and root biomass promote the removal of nutrients (Payne et al., 2018; Read et al., 2009). Metal removal in biofilters is more affected by filter material characteristics than by the plants in the system. Nevertheless, weak correlation exist between removal of Cu and Zn, and the plant attributes leaf area, leaf biomass, shoot height, root length: root biomass ratio, and percent fine root biomass (Read et al., 2009).

Altogether, these identified plant attributes connected to pollution removal suggest that regardless of pollutant, treatment system, and investigated species, large plants with extensive root systems of thin roots and shoots with large leaf areas are high-performing. The large root area exposes the plant to the pollutant, thus supporting rhizosphere activity and pollutant uptake, whereas the leaf biomass promotes growth, water uptake, and oxygen transport to the roots. This indicates that the plants from **studies I-VI** are likely to also remove nutrients from the water at the same time as they remove metals and chloride. However, the same species can have different morphology depending on nutrient availability, the toxicity of pollutants, duration of the study, water velocity, genotype, plant age, raft construction, and climate conditions (Chen et al., 2016; Hadad et al., 2018; Weiss et al., 2014). As an example, *Carex acutiformis* developed 1.74 ± 0.12 m long roots in one study, but only 0.12-0.15 m long roots in another study (Castro-Castellon et al., 2016; Van de Moortel et al., 2010). These differences in growth were possibly caused by differences in the nutrient load between the studies, as lower nutrient availability generally results in longer roots (Chen et al., 2016; Weiss et al., 2014). Thus, it is important to ensure that these desired plant traits actually develop in the conditions at the planned site of the FTW (or other) system.

Summary and conclusion

Overall, we conclude that there is potential for FTWs for chloride and metal removal from polluted water in the Swedish climate, as we have identified Swedish wetland plants that can remove metals and chloride from water. However, there is a clear variation between heavy metal and chloride removal capacity between species, showing that if plant -mediated removal should be maximized, species must be selected with care.

Impact of external factors on heavy metal and chloride removal (Aim 2)

A major question regarding the usefulness of FTWs in Sweden is how they perform under changing conditions, to understand how the efficacy can vary over seasons and between sites. In order to quantify this, the next step after identifying several candidate species is to determine how external factors affect plant removal capacity and growth.

External factors affecting stormwater quality

The environment in which plants live is rarely static, but changes with the season and weather conditions. Moreover, for plants in an FTW or other phytoremediation treatment system for polluted water, the effects of variations in the incoming polluted water are added. The amounts and composition of pollutants in the stormwater are affected by the season, changes in activity in the catchment area, and time since the last precipitation event.

Under cold winter conditions, the concentrations of many heavy metals increase (Behbahani et al., 2021; Viklander et al., 2019). In addition, salt from deicing salt will often be present in the stormwater, increasing the proportion of dissolved metal forms. In summer, the heavy metal levels and chloride levels are usually lower. However, if a long time has passed since the last precipitation event, more pollutants have accumulated on the surfaces that the stormwater passes, and what reaches the FTW can be expected to contain high levels of pollutants (Huber et al., 2016).

Since plants cannot move from adverse conditions, they need to be able to adapt to survive. These adaptations can in turn affect their effectiveness in phytoremediation. To investigate how some of the tested species react to changes in the stormwater, we exposed them to different levels of salt (**study III**), salt in combination with different temperatures (**study IV**), and varying heavy metal levels (**study V**).

Effect of salinity

The levels of Cl^- in stormwater can result in toxic symptoms for plants, but the tolerance differs between species (Teakle and Tyerman, 2010). To study if Cl^- levels could harm the species we had selected for further studies (*C. pseudocyperus, C. riparia,* and *P. arundinacea*), ultimately intended for FTWs in saline water, we investigated the Cl^- tolerance in **study III**. All plants were all able to tolerate 50 mg Cl^- L^{-1} which corresponds to the yearly average Cl^- concentration in stormwater ponds (Alm et al., 2010). The concentration corresponding to elevated levels of Cl^- in stormwater ponds during winter (Semadeni-Davies, 2006), 500 mg Cl^- L^{-1}, resulted in decreased growth after 1-4 weeks, dependent on the species, but no mortality. Levels of 5 000 mg Cl^-

L^{-1} and above reduced both growth and survival (**study III**). However, chloride concentrations of 500 mg Cl^{-} L^{-1} or above are commonly only present for a few hours up to a few days in stormwater ponds, connected to rain or snowmelt events during winter, and thus in combination with low temperatures (Semadeni-Davies, 2006; Westerlund and Viklander, 2008). Since the root uptake of Cl^{-} is slower at low temperatures (Cram, 1983), it may protect the plants from accumulating toxic Cl^{-} concentrations during short salinity peaks.

Moreover, salinity is known to decrease metal accumulation, but its effects differ between plant species and between metals (Fritioff et al., 2005). For the species we studied, salinity had a negative effect on Cd and Pb removal but did not affect the removal of Cu, Zn, and Cl^{-} (**study IV**) (Fig. 8). In the longer term, growth reduction would occur due to the toxic effects of the salt (**study III**), which reduces the accumulation due to fewer uptake and adsorption sites. An FTW that purifies water that contains road salt can thus be expected to show a lower removal of some of those metals as long as salinity is elevated. The overall removal capacity ability can also be impaired as Na^{+} from the road salt increases the mobilization of Cd and Zn from the sediment, and thus further increases the heavy metal content in the water (Du Laing et al., 2008; Greger et al., 1995).

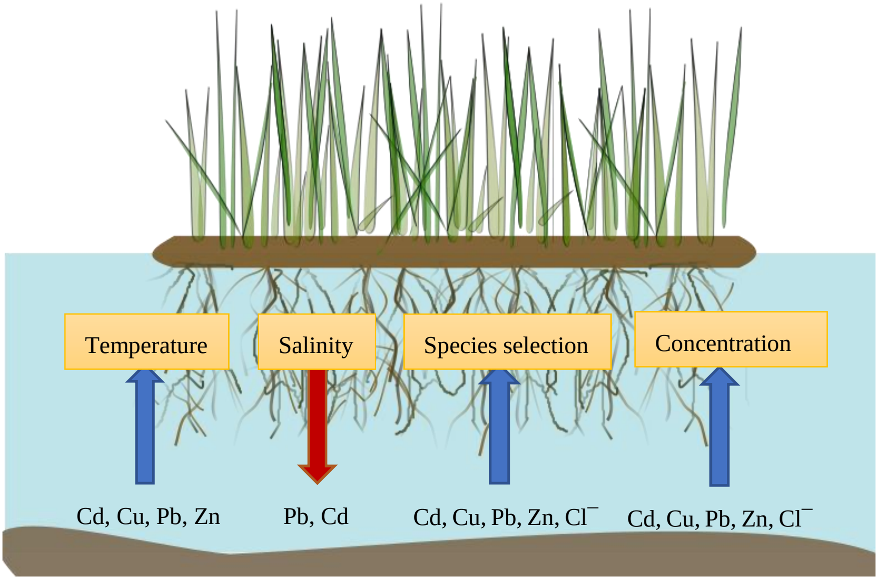

Figure 8. The external factors temperature, species selection, and concentration in the solution increase the removal of metals and chloride, whereas increased salinity decreases removal of Pb and Cd.

Interestingly, plant species with high Cl^{-} tolerance generally showed low heavy metal accumulation, and vice versa, for the species we studied. *Carex riparia* had the highest Cl^{-} tolerance (**study III**) but showed lower heavy metal accumulation compared to *C. pseudocyperus* and *P. arundinacea* (**study IV**). The latter species removed more metal, but displayed toxicity symptoms from 50 mg Cl^- L^{-1} upwards. For *C. pseudocyperus*, the removal of Cu and Pb was higher than for the other species independent of salinity and temperature and was likely connected to its higher root mass, which promotes the removal of these metals which are primarily stored in plant roots (**studies V and VI**).

Phalaris arundinacea showed low Cl^- tolerance and higher removal of Cl^- , Cd, and Zn compared with the other species (**studies III and IV**), potentially caused by differences in Cl^- tolerance mechanisms between the species. The tolerance towards high Cl^- levels in the tissue of halophytes such as *P. arundinacea* can also result in effective protection against the negative effects of metals (Manousaki and Kalogerakis, 2011). This results in a maintained metabolic rate, including accumulation of Cl^- and metal in tissues, also at increased Cl^- and metal concentrations. However, the low Cl^- tolerance in **study III** for *P. arundinacea* was surprising as it is a halophyte that has been shown to tolerate 10 000 mg Cl^- L^{-1} (Prasser and Zedler, 2010). This might be caused by either use of different ecotypes between studies, or the low nutrient content of the solution, a feature that generally promotes Cl^- toxicity, especially for plants with high nutrient demand such as *P. arundinacea* (Haneklaus et al., 2018; Maeda et al., 2006; Usťak et al., 2019). For an FTW designated for saline conditions, using *P. arundinacea* and *C. pseudocyperus* would be preferable due to their higher metal accumulation. However, if Cl^- concentrations commonly exceed 500 mg Cl^- L^{-1}, *C. riparia* would be preferred as growth otherwise would be limited, which would decrease long-term total accumulation of heavy metals in plants.

Effect of temperature

The temperature can also affect the accumulation of metals. High water and air temperatures allow better removal of metals compared to low temperatures (**study IV**) (Fig. 8, page 47). Removal increased with increasing temperature from 5 °C to 15 °C but was similar between 15 °C and 25 °C. Thus, there seems to be threshold somewhere between 5 °C and 15 °C. The range of 5-15 °C is important because it includes the average temperature of many spring and autumn months (approx. March-April to September-November dependent on location in Sweden) (SMHI, n.d.). Further research with higher resolution should investigate if the threshold temperature is closer to 5 °C or 15 °C and if it differs between species. This could affect the species selection on FTWs, which should be tailored to fit the site-specific conditions.

Temperatures below 5 °C were not included in **study IV**, but are common during winters in Sweden. At temperatures below 5 °C, the removal capacity is likely to decrease further. The metabolism of plants and biofilms decreases and ultimately stops. This effect of decreasing temperatures decreases the release of oxygen through the roots, which slows down biofilm removal processes and active uptake by plants of substances such as metal and chloride ions (Brunham and Bendell, 2011; Nsenga Kumwimba et al., 2021). Plant adsorption and increased sedimentation are influenced by temperature to a lower extent, as these are physical processes (Brunham and Bendell, 2011). Nevertheless, reduced efficacy of FTWs can be expected at low temperatures.

Several measures have been evaluated to improve N and P removal of FTWs in cold conditions (as reviewed by Nsenga Kumwimba et al. 2021). Of these, increased retention time, selection of cold-tolerant plants, mechanical aeration, bioaugmentation with cold-tolerant microbes, and addition of absorbing filter materials to the raft might be useful for increased metal and chloride removal during winters. Additionally, to support adsorption and sedimentation processes, it is important to select plants with large root systems to create as much contact between the plants and the water as possible. Moreover, a high amount of aboveground biomass will favor a milder microclimate and result in higher survival (Vymazal, 2011). This stands in contrast to shoot harvest in the autumn, which can result in maximum removal (Wang et al., 2014; Zheng et al., 2015).

Temperature effects on survival

The low temperatures of winter may affect the survival of the plants on the FTW, jeopardizing future use. In a few studies, ice encasement of the rafts was observed, which risks damaging plant tissues and limiting oxygen transport to the roots (as reviewed by Wang et al. 2015; Nsenga Kumwimba et al. 2021). The ice has had varying impacts on plant survival dependent on species and location, further underlining the importance of appropriate plant selection. *Pontederia cordata*, used on FTWs with good results in the southeast U.S., did not survive winter in the northeast U.S. (Chang et al., 2013; Tharp et al., 2019). *Iris pseudacorus* survived ice encasement in Virginia, U.S., but suffered great losses during winter in northern Italy (Barco et al., 2021; Wang et al., 2015). However, high survival in cold conditions is possible. Plants of *T. latifolia*, *C. lacustris*, and *J. canadensis* survived two winters on FTWs in Sudbury, Canada, where the average winter air temperature is -10.3 °C, i.e., lower than in most of Sweden (Gupta et al., 2020; Persson, 2015). Moreover, an FTW field and mesocosm trial focused on N removal in northernmost Sweden with six native species (of which *C. rostrata*, *C. palustre*, *E. agustifolia* were included in **studies I-III**) found high survival and dense stands of up to 8 kg aboveground biomass m^{-2} after two years of operation (Choudhury et al., 2019).

None of the species of **studies IV-VI** was studied for winter survival on FTWs. However, I found *C. pseudocyperus* growing on the first FTW installment in Sweden; it had not been included in the FTW plantation but it had spread there naturally (see *Box 2: The first FTW in Sweden – field notes*). It is impossible to know how many seasons it had been growing there, but this observation indicates that *C. pseudocyperus* is adapted for survival on FTWs. Another aspect of plant suitability for winter climates is the start of the growth period. Of the species we have studied, *C. riparia* has an exceptionally early growth start, as indicated by the vigorous shoots breaking through the ice in March (Fig. 9). It also has a long growth period as it remains green until the end of October.

Figure 9. The long growing season of Carex riparia. Shoots break through the ice in March (left) and remain green in the end of October (right). Pictures taken at Flemingsbergsviken, where the specimens were collected, on March 20, 2022 (left), and October 25, 2020 (right).

Effect of species and interaction effects

Contrary to our expectations, we found no interaction effects between salinity and temperature on metal accumulation in **study IV**, unlike Fritioff et al. (2005) and Bastos et al. (2019). However, it was clear that the choice of species plays a major role under these conditions, in many cases greater than the effect of salt and temperature (Fig. 8). Interactions between species and temperature also occurred, i.e., the species reacted differently to the differences in salinity and temperature. For example, *P. arundinacea* removed more Cd and Zn than *C. pseudocyperus* and *C. riparia*, but only under certain conditions. *Phalaris arundinacea* showed higher removal of Cd at higher salinity and a higher removal of Cd and Zn at higher temperatures compared with the other species. Chloride removal was higher for *P. arundinacea* than for the other two species, corroborating the findings of **study III**. *Carex pseudocyperus* removed more Cu and Pb than *P. arundinacea* and *C. riparia*, probably due to its larger root mass, which is not directly affected by either salt or temperature. To better adapt the species selection at FTWs to the expected conditions, it is important to know how temperature and amount of pollution, including the use of road salt, vary over the year at the site.

Effects of changing metal concentrations

The concentration of the stormwater changes over time, which can be expected to affect the metal removal capacity of plants in phytoremediation systems. We tested how *C. pseudocyperus* reacted to switching between high and low levels of heavy metals in stormwater (**study V**), and found the changes to have a substantial effect on the concentration of the substances in the plant (Figs. 8, 10). If the plant was first exposed to a low concentration followed by a higher concentration, the tissue concentration increased during the second exposure. Conversely, If a highly concentrated solution was followed by a lower concentrated solution, the plant tissue concentration decreased due to some of the accumulated metal leaking into the water again. However, the tissue concentration was still higher than for plants exposed to solutions with low concentrations during both the first and second exposure. The mechanisms behind these changes in tissue concentration are the transmembrane transport – inward and outward – of small water-soluble substances, such as metal ions, between two cells, as well as between cells and the apoplast or the surrounding water (White, 2011). The system strives towards an equilibrium; thus, when the metal concentration of the surrounding solution decreases, metals will move from the plant to the solution. However, some of the metal becomes tightly bound to cell walls or sequestered within vacuoles as a detoxification mechanism for excessive metals, or translocated to the shoot, or used in metabolic processes (Küpper and Andresen, 2016). These processes result in a net accumulation of metal for plants.

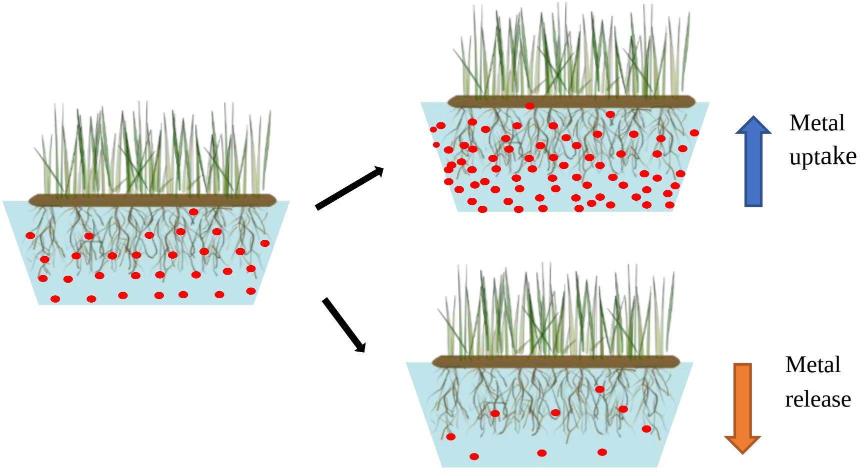

Figure 10. When plants that had been pre-exposed to a metal (red dots) solution were transferred to a solution with higher metal concentration, the metal accumulation in plant tissues, especially roots, continued. If the second solution had a lower metal concentration, already accumulated metals were released from the plants.

There was no metal concentration saturating the uptake by the roots of the plants. As long as the metal concentration was higher than before, root metals concentrations increased. For the shoot parts, the concentration of Cu did not exceed 90 µg g DW^{-1}, even though the concentration in the solution increased. This limits the usefulness of *C. pseudocyperus* in phytoremediation of Cu, especially if only the shoot parts are harvested. However, the highest concentrations we used in this experiment far exceed the concentrations in stormwater, especially for Cd and Pb (Tables 1 and 6).

Study V included only one species, and it is impossible to tell if the other species in this thesis would have performed in the same way. Similar studies show varying results. Nyquist and Greger (2007) used similar concentrations and duration of exposure but found no leakage of Cu and Zn, only of Cd, for the submerged species *Elodea canadensis*. Landberg et al. (2011) found that previous exposure limited further uptake of Cd but not Zn by the tree species *Salix viminalis*. Moreover, both Cd and Zn leaked when the surrounding concentration was lowered, but the Cd leakage was higher for some clones, probably as a tolerance strategy. Sricoth et al. (2018) did not alter the concentration of the solution but performed repeated dosing over 15 days of high concentrations of Cd and Zn and found a decrease in removal capacity, suggesting a saturation of the tissues for all six wetland plant species they studied. Similarly, Weiss et al. (2014) used lower concentrations and saw a decline in root tissue accumulation of *Scirpus validus* after 30 days for Cd, Cu, Pb, and Zn. However, the plants will grow during a long exposure, which may counteract plant tissue saturation by dilution and thereby increase their uptake and storage capacity. If the conditions at the site are harmful for the plants, this may reduce growth or affect morphology in such a way that uptake is restricted (e.g., by lignification of root tissue caused by salinity and nutrient deficiency) (Barcia-Piedras et al., 2019; Cheng et al., 2012). Once again, this highlights the importance of selecting species adapted to the conditions of the site.

Summary and conclusion

Overall, we conclude that external factors affect the removal capacity of plants. We confirmed that temperature, salinity, and metal concentration influenced metal and chloride removal capacity, but the extent differs between species. It will be important to select species that have high removal in the intended environment, such as *P. arundinacea*, to maximize the efficacy of FTWs.

Field performance (Aim 3)

Besides identifying plant species with high removal capacity and determining the effect of typical environmental factors on this capacity, other aspects – growth, survival, and long-term performance – must be considered to determine if FTWs would be a suitable tool in Swedish conditions. These aspects are best evaluated in a field trial.

Species performance in field

To replicate field conditions in the lab is difficult as these conditions are very complex. To supplement the experiments we had performed on isolated parts of the plants' performance in a controlled environment, we finished by testing the plants' performance on floating wetlands in two stormwater ponds in **study VI** (Fig. 11,12, pages 54-55). We built two small rafts of PVC pipes, chicken wire, coconut fiber mat, and placed plants of the species *C. pseudocyperus*, *C. riparia,* and *P. arundinacea* tested in **studies I-V** in them. The experiment lasted for 12 weeks during the later part of summer.

The results of **study VI** showed that the FTW plants grew rapidly and accumulated significant amounts of heavy metals. *Phalaris arundinacea* showed the strongest growth; its root mass increased ten times at one of the ponds, but all species analyzed increased their biomass by at least 45%, and all plants survived. The metal concentrations in plants were in the order Zn > Cu > Pb > Cd. Roots generally had higher metal concentrations than the shoots, and the total accumulation correlated with plant biomass. The accumulation of metals differed between the ponds, which was only partly explained by differences in the pond water metal concentrations. Large differences in shading between the sites, with Lilla Essingen pond being shaded most of the day while Silverdal pond was exposed to sun, could have influenced growth and uptake.

The performance of the species differed somewhat between our studies, as discussed In Comments on materials and methods. The longer field **study VI** found *P. arundinacea* to both have the strongest growth and to accumulate more Cd, Cu and Zn than the *Carex* species did, whereas the shorter greenhouse **study I** found *P. arundinacea* to have only average efficiency since its metal removal was slower than in several other species. *Carex pseudocyperus* and *C. riparia* showed efficient and quick removal of all four investigated metals in **study I** but had often lower tissue concentrations than *P. arundinacea* in the field in **study VI**. Likely, the better performance of *P. arundinacea* can be attributed to its extensive root system, thinner roots, and strong growth compared to the other species; factors that both support high tissue accumulation and high total accumulation according to **study II** (Fig. 13, page 56). **Study II** measured the mass of thin roots per plant by determining the weight of the roots with a diameter of 1 mm or less, but a more detailed analysis

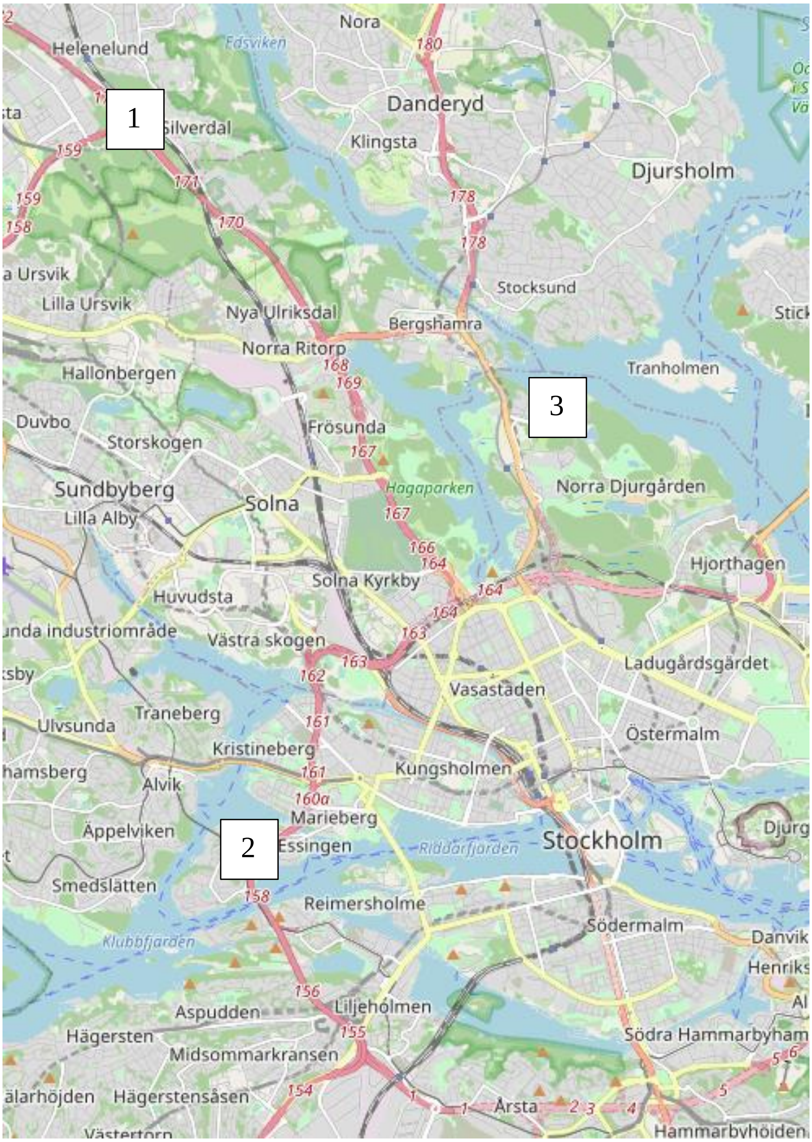

Figure 11. Map of Stockholm showing the placement of Silverdal stormwater pond (1), Lilla Essingen stormwater pond (2), and Stockholm University (3). *© OpenStreetMap contributors, used under Open Data Commons Open Database License.*

would have been preferable. The 0.3 mm diameter of *P. arundinacea* roots corresponds to approx. ten times more surface area than the 1 mm diameter roots of *C. pseudocyperus*, with *C. riparia* somewhere in between. Interestingly, two of the species used in **study VI**, *C. riparia* and *P. arundinacea*, had already been evaluated in FTWs for metal removal from stormwater (Ladislas et al., 2015; Zanin et al., 2018), but compared to the earlier findings we had generally higher metal accumulation in the plants. These differences are likely caused by the lower metal concentrations in the pond water of the previous studies. This further highlights the effects of pollution load on the outcome of FTW treatments, as discussed by Pavlineri et al. (2017). To the best of our knowledge, the metal accumulation properties of *C. pseudocyperus* have not been studied before, despite its distribution on all continents except South America and Antarctica (POWO, 2022). This means that we have identified a new species with high metal removal capacity that can be useful for remediation activities.

Figure 12. Placement of the FTWs in Silverdal stormwater pond (left) and Lilla Essingen stormwater pond (right).

Based on the findings of **study VI** that found *P. arundinacea* to have the highest growth and highest accumulation of Cd, Cu and Zn, resulting in the highest accumulation of most metals compared to *C. riparia* and *C. pseudocyperus* during the studied period, we recommend this species for future FTW use. For this reason, I will use it as an example species for the following theoretical efficacy calculations. However, as we did not determine the relationship between removal capacity and accumulation for our species, potentially any of them could have been efficient promoters of the other mechanisms in removal capacity besides accumulation (i.e. adsorption, microbial processes, precipitation, and sedimentation), and thus have had a greater effect on the water quality than *P. arundinacea*. Nevertheless, most removal capacity mechanisms are linked to an extensive root system, and as *P. arundinacea* had by far the largest root mass, it would seem likely that this species also had the greatest removal capacity of our three selected species in the field. It should be noted that the experiment started in the beginning of July, and that growth and uptake during spring and early summer was not included. Theoretically, the *Carex* species could have had a higher growth or uptake during this period, resulting in a higher pollutant accumulation.

Figure 13. The extensive root system with thin roots of Phalaris arundinacea (left) and outliers of Carex riparia (right) on the FTWs in ***Study VI****. Photos: Mikaela Boltenstern*

Theoretical removal efficacy of FTWs in cold coastal climate

A smaller pilot study like **study VI** is a good basis for a larger field trial, as we were able to compare the growth, survival, and accumulation of the different species in the field, and thus ensure that the species selected for a large field trial are the most suitable for such an environment. However, the effect of the FTWs on the water quality could not be determined due to the limited size of the FTWs, comparatively short duration, and the methodology for water sampling (random sampling).

Some theoretical estimates on FTWs removal capacity can be made based on **study VI** and literature. Given the accumulation of metals found in **study VI** and with a biomass of 3.4 kg DW m^{-2} (Hubbard et al., 2011; Olguín et al., 2017), an FTW planted with *P. arundinacea* could accumulate up to 3.4 mg Cd, 501 mg Cu, 32 mg Pb, and 10 050 mg Zn per m^{2} during twelve weeks.

The metal loads in stormwater ponds exceeds this, but the pond itself removes between 40-90 % of the inlet metal load by sedimentation (Aldheimer, 2006; Alm et al., 2010; Andersson et al., 2012). This means that the metal load of concern for FTW removal is lower. Based on the metal load received by the Lilla Essingen stormwater pond used in **study VI** (Aldheimer, 2006), plants on an FTW covering 100% of the pond surface would accumulate 262 % of the remaining Cd, 150 % of the Cu, only 36 % of the Pb and 386 % of the Zn. As these calculations are based on plant accumulation alone, which is estimated to represent a minor part of the total metal removal by FTWs (Borne et al., 2014; Tanner and Headley, 2011), the total treatment effect of an FTW would likely be higher. On the other hand, 100 % removal will not be achieved, as the removal capacity decreases with decreasing concentration (Bi et al., 2019). Regardless, the calculated metal removal demonstrates the usefulness of FTWs as a complementary tool for metal removal in stormwater ponds. **Study III**, performed in microcosm settings, indicates that up to 14 mg Cl^- per m^2 could accumulate in a month, given 1.4 kg plant material per m^2. Even if this figure has to be verified in the field, as ponds do not retain any Cl^- (Barbier et al., 2018), even minor removal of Cl^- by FTWs would be a bonus.

The FTWs would not only remove metals, but they would also simultaneously remove other pollutants. An FTW planted with 23% *P. arundinacea* decreased the concentration of soluble manganese, dissolved inorganic nitrogen and nitrites, and at the same time reduced phytoplankton and increased zooplankton abundance (Castro-Castellon et al., 2016). *Phalaris arundinacea* has also demonstrated high biomass production, root length of 1.5 m, and long-term survival on FTWs in Italy and the UK (Castro-Castellon et al., 2016; Zanin et al., 2018). Other benefits of FTWs include providing habitats for insects, birds, amphibians, snails, and spiders (Strosnider et al., 2017), erosion control (Hoeger, 1988; Nakamura et al., 1999), and aesthetics (Borne et al., 2015; Peterson et al., 2021). We did not study any other benefits for the FTW in **study VI**, but mallards (*Anas platyrhynchos*) rested on the raft shortly after deployment in the Lilla Essingen stormwater pond.

The *P. arundinacea*-planted FTWs hypothesized in this thesis would not only be a suitable tool for ponds receiving stormwater runoff. In addition, applications in other polluted water sources could be of interest, such as domestic and industrial wastewater, lakes, or rivers, as these waters often contain similar pollutants. Even treatment of acid mine drainage which has a low pH and high sulphate concentrations, could be considered after the plant survival and accumulation have been tested in small-scale studies.

Size, placement and raft materials

The efficacy of FTWs depends on their size in relation to the area of the water body (Chang et al., 2012; Keizer-Vlek et al., 2014; McAndrew and Ahn, 2017). A small FTW as in **study VI** will only be exposed to a small share of the total volume, and additionally, the water will flow around it instead of passing through the root mat because of its hydraulic properties. To ensure high removal, the FTW should cover the majority of the surface and it should be placed in the water body in such a way that all water has to pass through it (Khan et al., 2013; Lucke et al., 2019; McAndrew and Ahn, 2017).

The raft construction of **study VI** was relatively cheap (approx. 1 000 SEK m^{-2}), accessible (only material from a hardware store was needed except for the plant material), quick to assemble (approx. 0.5 days), durable, and lightweight (approx. 4-5 kg m^{-2}) (Fig. 14). The plants and the coconut mat could be removed from the frame, which could be reused. For the construction of larger FTWs, several modules can easily be linked together.

Sunlight and mechanical wear, for example from ice, degrade the plastic material of rafts and release microplastic into the water. Since microplastic is a growing environmental problem, we argue that an FTW used to treat water should not simultaneously contaminate it. Compared to most commercial FTWs, the raft in **study VI** contained only a limited amount of plastic. Only PVC drainage pipes, which are durable and with little exposed surface compared to for example, Biohaven rafts (Stewart et al., 2008), provided buoyancy. The reason we used a small amount of plastic was due the facts that plastic can provide buoyancy, is easy to handle, and is cheap. A master thesis later evaluated plastic-free raft constructions, finding spruce branches, bamboo, and expanded clay to be suitable materials (Fig. 14) (Boltenstern, 2020). The buoyancy of the rafts was lower than that of the PE-framed rafts used in **study VI**, and the buoyancy of the spruce raft decreased over 1.5 months of deployment in water. A recent study utilized the buoyancy of reed stems by enveloping them in metal wire and coconut coir nets, but the buoyancy was limited and the margins of the FTWs was below the water surface (Karstens et al., 2021). While it still supported the plants, these results suggest that this type of construction is suitable for rafts in applications where the whole raft is harvested at the end of the season, or for use with plants that become self-buoyant due to air-filled spaces in the roots and entrapment of gases generated in metabolic processes (Hogg and Wein, 1988).

Figure 14. Low-plastic raft construction of ***study V*** *and non-plastic raft constructions by Boltenstern (2020). Photos middle and right: Mikaela Boltenstern*

Harvest and maintenance

The metals and chloride removed by the FTW from the water will end up in two places, either in the FTW (i.e. in and on plants, litter, biofilm, and raft material) or in the sediment of the pond (Borne et al., 2014; Van de Moortel et al., 2012, 2011, 2010). Harvest of plant material will remove accumulated pollutants (e.g. plant extraction) but may limit further pollutant uptake if too much plant material is removed as this can reduce survival, plant health, exposure area, and storage volume.

Commonly, only aboveground parts are harvested for practical reasons. However, plants take up ions selectively and can limit their translocation to the shoot, as a way of protecting the essential but sensitive photosynthesis process from damage. It was evident in **studies V and VI** that the plants in our study limited root-to-shoot translocation, as the metal concentration was generally lower in shoot than root tissue. Thus, for the maximum removal of metals, root harvest or whole plant harvest followed by replacement should be considered. Even the whole raft can be "harvested", which also would remove pollutants in the litter or adsorbed to the raft surfaces. The shoot tissue concentration of metals and chloride also increased as the concentration in the solution increased (**studies III and V**). A high tissue concentration could on the one hand damage the plants eventually and thus decrease their removal capacity, but on the other hand, also result in making harvesting easier as a larger part of the accumulated metals can be collected by harvesting the shoots only. The amount of metal accumulated in plant tissues varies over the year and often reaches its maximum before or during senescence, calling for careful timing of the harvest (Bragato et al., 2009; Garcia Chance et al., 2019).

Post-harvest management of the harvested plant parts should preferably both prevent the release of the accumulated metals back to nature and get some use from the biomass. Suggested uses include using the biomass as an energy source through pyrolysis or gasification, or as a biomaterial (Liu and Tran, 2021; Quilliam et al., 2015; Yeh et al., 2015). Utilizing the plant material for food, feed, or landfill should be avoided when the plant material contains high levels of heavy metals.

If the plant material of the FTW is not harvested regularly, it will decrease the removal efficacy of the FTWs. Some of the accumulated metal will be released into the water by leakage from plant tissues (Nsenga Kumwimba et al., 2021). From there, it can again be accumulated by plants or biofilm, precipitate, or sediment. However, some of the released metal will not be removed again, but follow the effluent. As treatment efficacy and pollutant load are seasonal, this could theoretically result in higher outflow than inflow of pollutants. The best way to prevent this is regular harvests of plant tissues or by removal and replacement of the whole FTW.

Other maintenance actions that need to be planned for are the dredging of sediment (including post-dredging management to ensure that the metals do not leak from the sediment), regular control of anchorage of the rafts, and planting new specimens to fill gaps formed by dead plants. The latter can potentially be avoided by selecting plants that are adapted to the site, and that can fill gaps themselves with outliers or seed plants. Given the plant evaluated in this thesis, *P. arundinacea* easily colonized gaps in the raft, whereas the outliers of *C. riparia* were not able to penetrate the coconut mat and establish as new plants (**study VI**). *Carex pseudocyperus* spreads with outliers that form close to the shoot base. In **study IV**, this was part of the increase in plant size during the experiment, but no new separate *C. pseudocyperus* plants colonized gaps on the raft.

Summary and conclusion

Overall, we conclude that it is possible to accumulate heavy metals from stormwater ponds with FTWs in the Swedish climate/cold coastal climate. The removal capacity is affected by the species, the pollution concentration, site conditions, and which plant part is harvested. The outcome of this study is a first indication of how FTWs can work for heavy metal removal in the Swedish climate.

Conclusions

Floating treatment wetlands have shown potential for treatment of polluted waters, but it has been unclear if the mechanisms could function in a cold climate; furthermore, only limited data on chloride and metal removal of FTWs were available. In this thesis, I demonstrated the capacity of wetland plants to remove heavy metals and chloride under various conditions, an ability that can be utilized for the remediation of polluted waters with FTWs.

The main contributions of this thesis:

- There are several Swedish wetland plant species that remove metals and chloride from water.
- There are large variations between heavy metal and chloride removal capacity between plant species. These differences are connected to morphological differences of the plants.
- The external factors salinity, temperature, and pollution load affect the removal capacity of plants. Some of these factors affect plant species to various extents.
- The uptake capacity of plants first studied in laboratory environment is confirmed under field conditions. Furthermore, field experiments also demonstrate that FTWs can be built without plastic, to reduce the risk of microplastic pollution.

These contributions further advance our knowledge of the importance of plant selection for phytoremediation of polluted waters. They also teach us how the surrounding environment affects the remediation capacity of the plants. Based on these findings, we conclude that there is potential for FTWs for chloride and metal removal from polluted water in the Swedish climate. It will be important to select species, such as *P. arundinacea,* expected to have high removal capacity in the intended environment. Overall, a well-thought-out species selection adapted to the conditions at the site (pollution profile, climate) will improve the system's ability to remove pollutants.

Although this study was focused on finding plants for floating wetlands for stormwater treatment in Sweden, we have covered many aspects that apply to other plant species, and are relevant when for phytoremediation treatment of water in other systems. Overall, we believe that this gives a good picture of the plants' removal capacity under many different aspects, which is necessary to use plant-based water treatments optimally.

Future perspectives

Long-term field performance and management

The obvious next step would be to evaluate the ability of FTWs to remove metals and chloride in Sweden in a larger long-term field study. As some authors have struggled with measuring the effects of FTWs in field conditions due to unexpected low pollution loads, the field study should be set in highly polluted water and have FTWs covering a large part of the water surface. To better understand the removal dynamic, the study should monitor the water quality prior to the installation and include a similar control pond without an FTW, as suggested by Lucke et al. (2019). Moreover, the study should sample plants, sediment, and water to understand the distribution between these removal pathways. The water quality in the inlet and outlet should be continuously measured with flow-proportional sampling, which is a more reliable measuring technique than random samples as the water quality can change rapidly (Andersson et al., 2012; Billberger and Svenson, 2006; Viklander et al., 2019). Preferably, the study should also evaluate the removal of other pollutants to provide a complete understanding of the FTW function.

Harvest aspects are another important aspect of metal and chloride removal, preferably to be studied during the proposed field trial. **Study III, V, and VI** show that most metal and chloride are accumulated below the FTW in the roots, but the present knowledge of root harvests of FTWs is low. It would be interesting to study regrowth and removal capacity in relation to harvest intervals, timing, and percentage of the roots harvested. On FTWs with multiple species, it would be interesting to study competition within and between species, especially connected to harvest. Moreover, the study could preferably include some practical aspects as evaluating a plastic-free or low-plastic raft construction, which we briefly touched on in **study VI** and a master thesis (Boltenstern, 2020).

It is important to ensure that FTWs meet the needs of the users also when it comes to practical and financial aspects. Therefore, I propose that the suggested field trial should involve stakeholders such as municipalities, planners, and contractors responsible for construction and maintenance, and the results of the study should be evaluated with current and future criteria for water quality in mind.

Biofilm - composition and contribution

Many studies have identified the biofilms formed on the roots of the plants of FTWs as an important removal pathway (Bi et al., 2019; Headley and Tanner, 2012; Pavlineri et al., 2017; Sanicola et al., 2019). Surprisingly, relatively few studies have studied their development, composition, and contribution to the overall removal capacity of FTWs. Moreover, studies on biofilm processes on FTWs in cold climates have so far only been conducted in waters with very different composition than stormwater (i.e., AMD and industrial wastewater (Choudhury et al., 2019; Gupta et al., 2020)). I would find it very interesting to study the composition of FTW biofilms in Sweden and differences between sites and plant species. As it has been demonstrated that the heavy metal content of biofilms has a linear relationship with the concentration of the surrounding water (Laderriere et al., 2021), the focus for heavy metal removal of biofilms would likely be focused on which plants develop the largest biofilm mass per root surface, and how the biofilms contribute to plant metal or chloride uptake. Also for other pollutants, especially N and organic pollutants, the composition of the biofilms and their remediation effect would be interesting to study.

Samples of the biofilms formed on the roots of the plants in **study VI** were taken, but they remain to be analyzed. Additionally, the FTWs already installed at various locations in Sweden for over ten years could be used for sampling. Findings from such a study could yield an increase in understanding of removal dynamics and plant selection and maybe result in recommending inoculation of roots with microbes to increase the FTW efficacy.

Validation of identified plant traits and ecotypes

Studies II and III identified several traits connected to the removal efficacy of plants. As suggested by Guitonny-Philippe et al. (2015), these traits should be validated as indicators of removal capacity by testing a new set of species. Additionally, it would be interesting to validate these traits by evaluating them under different environmental conditions.

Since only one ecotype of each species was used, a minor study comparing removal capacity of different ecotypes of *C. pseudocyperus*, *C. riparia*, and *P. arundinacea* should be made. If the study would show large variation between the ecotypes, propagation of the best performing ecotypes could be of interest for commercial FTW applications.

Practical implications

The studies of this thesis represent applied research, with the practical objective to provide increased knowledge on FTW function to enable use in Sweden for the treatment of polluted water. Although I would recommend performing additional studies, listed above, to further develop and understand the function of FTWs for metal and chloride removal in a cold climate, some conclusions on how FTWs in Sweden should be designed can already be drawn:

Plant selection. Regardless of pollutant, site, or treatment system, species with large biomass and extensive root systems of thin roots should be used (see section *Agreement with plant decision tools for other phytoremediation methods*). Many FTWs currently include species with small root systems with low surface area (e.g., *Phragmites, Typha,* and *Iris*). By replacing them with species with larger root systems but still high aboveground biomass, the removal of many types of pollutants can likely increase. Since the plants have different strengths under different conditions, we recommend using a mixture of species to provide a stable treatment effect under varying conditions. If only one species can be selected, we recommend *P. arundinacea* for Swedish use. It provides high removal in most conditions and has a high growth and biomass according to **studies I-IV and VI**, but its removal rate is comparatively low according to **study I**; it has somewhat lower removal of Cd and Pb according to **study IV** and low Cl^- tolerance in eutrophic conditions according to **study III**.

Site selection. **Studies III and V** show that the accumulation of pollutants in plants increases with a higher pollutant load. Thus, the FTWs would be most useful in waters with high pollutant load, such as stormwater ponds with water from heavily traveled roads. Moreover, the site must provide access to the FTW for maintenance, like harvest, replacing dead plants, or sediment dredging. As the removal efficacy likely changes between seasons due to differences in temperature, salinity, and pollution load as demonstrated in **studies III-V**, FTWs could be combined with other treatment methods if it is important to reach a certain removal capacity under all conditions.

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Appendix

Correlations between plant traits and metal removal from **study II** recalculated with Pearson correlations instead of Spearman rank correlations (Table A1 and A2). If a correlation changed from non-significant to significant when Pearson was used, it is marked in green. If a correlation changed from significant to non-significant when Pearson was used, it is marked in red. $p<0.05$ was used for both correlation calculations.

Table A1. Recalculation of correlation between measured parameters and metal removal in percent (Table 2 in ***study II****).*

Parameter	**Removed metal % after 0.5 h**				**Removed metal % after 119 h**			
	Cd	**Cu**	**Pb**	**Zn**	**Cd**	**Cu**	**Pb**	**Zn**
Coarse roots (g DW)	n.s.	n.s.	n.s.	n.s.	0.49	n.s.	n.s.	0.53
Fine roots (g DW)	0.59	0.63	0.71	0.73	0.48	0.42	n.s.	0.57
Rhizomes (g DW)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.52
Leaves (g DW)	0.56	0.61	0.69	0.7	0.59	0.51	n.s.	0.58
Stem (g DW)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.66
Belowground parts (g DW)	0.46	0.47	0.54	0.62	0.46	0.55	0.36	0.61
Aboveground parts (g DW)	0.53	0.64	0.73	0.70	0.7	0.57	n.s.	0.75
Total biomass (g DW)	0.56	0.61	0.70	0.72	0.64	0.61	n.s.	0.74
Fresh weight start (g FW)	0.47	0.57	0.70	0.70	-	-	-	-
Fresh weight end (g FW)	-	-	-	-	0.73	0.62	n.s.	0.82
Root:shoot ratio (DW)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*Table A2. Recalculation of correlation between measured parameters and metal removal per fine rot biomass (Table 3 in **study II**).*

Parameters	**Net uptake ug/g fine root DW at 0.5h**				**Net uptake ug/g fine root DW at 119h**			
	Cd	**Cu**	**Pb**	**Zn**	**Cd**	**Cu**	**Pb**	**Zn**
Coarse roots (g DW)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fine roots (g DW)	n.s.	-0.55	-0.36	-0.68	-0.75	-0.87	-0.89	-0.82
Rhizomes (g DW)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Leaves (g DW)	n.s.	-0.52	n.s.	-0.41	-0.47	-0.74	-0.69	-0.61
Stem (g DW)	n.s.	n.s.	n.s.	n.s.	n.s.	-0.52	n.s.	n.s.
Belowground parts (g DW)	n.s.	-0.41	n.s.	n.s.	-0.36	-0.61	-0.59	-0.44
Aboveground parts (g DW)	0.37	-0.48	n.s.	-0.35	-0.36	-0.70	-0.66	-0.52
Total biomass (g DW)	0.39	-0.49	n.s.	-0.35	-0.39	-0.72	-0.68	-0.53
Fresh weight start (g FW)	n.s.	-0.50	n.s.	n.s.	-	-	-	-
Fresh weight end (g FW)	-	-	-	-	-0.37	-0.72	-0.68	-0.53
Root:shoot ratio (DW)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.