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A Study of Exotic Macrophytes for the Phytoremediation of Flooded Agricultural Land Polluted with Cadmium

Thesis for obtaining a master‘s degree at the University of Natural Resources and Applied Life Sciences Vienna

Submitted by Rodrigo Valencia Cotera

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DECLARATION

I hereby declare that the following work is based on research and experiments done by me, that the thesis is of my own composition, that proper citation was given to the work of others and that it has never been published before to obtain a professional degree.

The thesis work was done in the Institute of Forest Ecology (IFE) belonging to the Department of Forest- and Soil Sciences of the University of Natural Resources and Life Sciences, Vienna (BOKU)

Vienna, Austria January 9, 2017 Rodrigo Valencia Cotera

To my grandmother María Felisa Gómez Prats (10.02.1934 - † 18.03.2016)*

one of the most influential persons in my life.

May her memory always live in our hearts.

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"If I have seen further, it is by standing on the shoulders of giants." Isaac Newton

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ABSTRACT

Cadmium (Cd) is a heavy metal that is extremely toxic for humans and the environment. Cd is released into the environment through industrial use, fertilizers, mining and waste incineration among others. Once Cd reaches the environment it accumulates in the soil and in the sediments of aquatic ecosystems, where it enters the food chain and it is biomagnified as it moves through the trophic levels. The possible use of *Egeria densa* and *Cabomba caroliniana* as cadmium phytoremediation species was investigated. The plants grew 34 days in microsystems composed of a 1.5 L PET bottle with 200g of polluted soil, one plant and 1.1 L of unpolluted tap water. The soil was polluted with cadmium to concentrations of 0, 3, 15, 25 and 50 µg of Cd per gram of soil (µg/g). After the harvest the root and plant weight were recorded; the roots were scanned to obtain root morphology information. The Cd concentrations in the water and in the plants were measured using ICP. The soil properties and cadmium concentration in the soil had the biggest effect on the Cd concentration in the plant. *Egeria densa* showed symptoms of phytotoxicity and adsorbed more Cd but it also leached more Cd into the water. While *Cabomba caroliniana* did not show symptoms of phytotoxicity it adsorbed less Cd and it leached minor amounts into the water. To the extent of this research neither of species showed signs of being particularly useful to be used as Cd phytoremediator in sediments of aquatic ecosystems.

ZUSAMMENFASSUNG

Es wurde die Einsatzmöglichkeit von *Egeria densa* und *Cabomba caroliniana* zur aquatischen - Phytoremediation von Kadmium erforscht. Die Pflanzen wuchsen für 34 Tage in aquatischen Microsytemen, bestehend aus einer 1,5 L PET Flasche, 200 g kadmiumverseuchtem Boden, einer Pflanze und 1,1 L Leitungswasser. Der Boden wurde mit Kadmiumlösungen verschmutzt um Konzentrationen von 0, 3, 15, 25 und 50µg Kadmium (Cd) pro Gramm Boden (µg/g) zu ereichen. Nach der Ernte wurden die Wurzeln- und Sprossbiomassen gewogen. Die Wurzel wurden zudem gescannt um die Wurzelmorphologie zu erhalten. Die Cd Konzentrationen im Boden und im Wasser wurden mit einer ICP-OES gemessen. Die Bodenbeschaffenheit und Kadmiumkonzentrationen im Boden hatten den stärksten Einfluss auf die Kadmiumkonzentrationen in den Pflanzen. *Egeria densa* zeigte Phytotoxizitätsymptome und sie nahm mehr Cd auf aber sie gab auch mehr Cd in das Wasser ab. Im Gegensatz zeigte *Cabomba caroliniana* keine Phytotoxizitätsymptome, nahm jedoch auch weniger Cd auf. Nach den Ergebnissen dieser Fallstudie ist keine der beiden getesteten Pflanzarten besonders gut für die Cd Phytoremediation in aquatischen Ökosystemen geeignet.

KEY WORDS

Agricultural land, *Cabomba caroliniana*, Cadmium, *Egeria densa*, Macrophytes, Phytoremediation, Sediments

INTRODUCTION

Pollutants of aquatic ecosystems

Pollutants enter aquatic ecosystems by run-off, precipitation or point sources. It is estimated that 80% of the water used by humans is directly discharged into waterways without any previous treatment; in developing countries it can reach up to 90% (Corcoran et al., 2010) Water pollution is also a result of other anthropogenic activities such as mining, industrial processes and agriculture (Sood et al., 2012).

Heavy metals are toxic environmental pollutants alike organic pollutants and nutrients such as nitrogen and phosphorous. The majority of these external pollutants accumulate in the sediments of rivers and lakes where they affect the biota and become sources of pollution (Wu et al., 2014). Human activities have mobilized trace metals into the environment through various industrial operations (Khilji and Firdaus-e-Bareen, 2008); these metals have usually very toxic effects for humans and the environment. The sources of heavy metals can vary but they are the most common priority pollutant from urban runoff, that is street and rooftop runoff (Li et al., 2008) other sources include: mining, industry and untreated sewage among other anthropogenic origins (Harguinteguy et al., 2015). Remembering that 1.8 million children die every year of water related disease is enough to understand the magnitude of the problem. (Corcoran et al., 2010)

To reduce pollution and because they are persistent, accumulative and non-biodegradable the European Union law under the Directive (76/464/ EEC) has classified heavy metals on their List 1 of dangerous materials. The discharge of List 1 pollutants had to be eliminated by member states according to this directive (European Union, 1976). Years after, under the Directive (83/513/EEC) the limits of cadmium discharge were set to 0.2 mg of cadmium per liter of discharge for all industrial sectors which used cadmium (European Union, 1983). In other words, discharge water should not exceed a cadmium concentration of 0.2 mg/l.

When polluted discharge water is released into the aquatic ecosystems, water pollutants might stay suspended in the water column, be accumulated by the sediments or adsorbed by living organisms such as plants and fish (Karickhoff et al., 1979 as cited by Trueman and Eber, 2013). In the case of heavy metals, they are usually sink and end up stored in the sediments of the polluted water bodies (Wu et al. 2014; Delmotte et al. 2006; Trueman and Eber, 2013).

Sediments are largely eroded soils that are continuously dispersed and their particles fractioned. The composition of the sediments is highly dependent on the dynamics of the water body. Even in the same water body the sediments might be different from one level to the other, for example in the middle of the river the sediments might be composed largely of sand and the suspended sediment might be mainly clay (Karickhoff et al., 1979).

Sediments of aquatic ecosystems can absorb, accumulate and transform pollutants (Trueman and Eber, 2013). Nutrients, heavy metals and organic contaminants are usually stored in the sediments and can reach very high concentrations (Wu et al., 2014). But this could cause an uneven distribution of pollutants in the sediments as each zone would have a different sorption capacity (Karickhoff et al., 1979). It is important to find new ways of removing pollutants from aquatic ecosystems as sediments are not a sufficient sequestration pool (Trueman and Eber, 2013) for the increasing amount of pollutants.

Sediments are a large pollution source in a water body due to their high accumulation of pollutants (Wu et al., 2014). Transfer of pollutants from the sediments to the overlaying water and from the water to the sediments can be enhanced by two processes: solute transport and particle transport. Enhanced diffusion, bioirrigation and advective irrigation being solute transfer processes and biodifussion, bioadvection, biodeposition and bioresuspension being particle transport processes. Some processes can enhance the accumulation of pollutants in the sediments while others such as bioturbation can increase their mobility (Delmotte et al., 2006).

Cadmium as environmental pollution

Cadmium (Cd) has an atomic weight of 112.411 g/mol, its atomic number is 48 and has a density of 8,65 g/cm3. It has no known metabolic significance to living organisms (Malec et al., 2009) but it is has several industrial applications. It is used as a raw material for the production of nickel-cadmium batteries (62%), pigments and paints (16%), surface coatings and platings (9%), as a plastic stabilizer (9%), non-ferrous alloys (2%) and electro-optics (2%) (Ross, 1994 as cited by Malec et al., 2009) Other sources state that in the year 2000 cadmium was used for electroplating (8%), pigments (12%), stabilizers (4%), alloys (1%) and batteries (75%) with around 1.1196 metric tons produced annually in the whole world during the 2000s (U.S. Department of Health and Human Services, 2014) In the European Union (E.U.) these values might be lower as the European Union has banned batteries and accumulators containing cadmium under the Batteries Directive (2006/66/EC), however, detailed information on the Cd use in the E.U. is scarce.

Cadmium and its components are extremely toxic to humans. It is known to be carcinogenic based on sufficient evidence from epidemiological and mechanistic studies in humans (U.S. Department of Health and Human Services, 2014). The metal usually targets the liver, kidneys, respiratory track and sense of smell and it is classified as a reproductive toxin (IPCS, 1992; Young, 2005; Blom, 1974). Cadmium strongly pollutes aquatic ecosystems, sediments act as the final capturing medium. But its sorption and precipitation are governed by a complicated combination of temperature, pH, sediment composition, oxygen and grain size (Delmotte et al., 2006).

Cadmium is released into the environment by several anthropogenic activities. It pollutes agricultural soil through fertilizer application and it does so in combination with other heavy metals; in particular lead and arsenic (Atafar et al., 2008; Blume et al., 2016). Other sources include metal industries, waste incineration, cement production, power stations (Rai et al., 2003; Blume et al., 2016) and mining activities (Ishii et al., 2015; Solomon and Byrne 2016) more specifically it is usually a byproduct of zinc mining as it occurs as a minor component in zinc ores (IPCS, 1992; Blom, 1974). Between 1988 and 1997 about 48,080 to 288,031 kg of cadmium and 374,213 to 1.8 million kg of cadmium compounds were reportedly released into the environments just in the U.S. (U.S. Department of Health and Human Services, 2014).

In Austria it is released to the air by the burning of fossil fuels and biomass. The emissions also come from the metal industry as the recycling of scrap metal that is coated with cadmium-based

paint volatilizes Cd as scrap metal is melted. The zinc metal industry also releases Cd as it is found in zinc ores. In summary the cadmium emissions in Austria are caused by the industry (41.6%), energy supply (25.6%), small consumer (23.8%), traffic (8.8%), agriculture (0.1%) and others (0.1%) (Umweltbundesamt, 2016).

As it has been previously mentioned, cadmium is a common pollutant found in agricultural land. Humans liberate cadmium into the environment in many different ways but the main causes of cadmium in agricultural land are: irrigating with water coming from zinc mines, use of sewage sludge as fertilizer, phosphate fertilizers, waste incineration and metal industry (Roberts, 2014; Hutchinson & Meema, 1987). Atmospheric deposition also plays a big role in the increase of background concentrations of cadmium in the soil. Waste incineration and metal industry liberate cadmium into the air which eventually is deposited in the soil (Norton et al., 2007; Roberts, 2014; Hutchinson & Meema, 1987). As a general rule it can be said that non-polluted soils have cadmium concentrations between 0.1 to 1.0µg/g (Roberts, 2014; Hutchinson & Meema, 1987) but this concentration can be increased due to phosphate fertilizer application as cadmium occurs naturally in the phosphate rocks. The amount of cadmium added is proportional to the amount and frequency of fertilization (Roberts, 2014).

Once cadmium has reached the soil it can be adsorbed by plants and enter the food chain through food crops (Longanathan et al., 2012; Ishii et al. 2015). Not only is cadmium toxic to humans but once it enters the food chain it is biomagnified as it moves up through the trophic levels (Croteau et al., 2005). Food is just one of many ways by which it might enter the human body. Dietary cadmium is the main source of cadmium for the non-smoking population (IPCS, 1992). Average cadmium concentrations in the food supply of the U.S. are around 2 to 40 ppb and the daily adult intake is approximate 30 µg (U.S. Department of Health and Human Services, 2014). Concentrations of cadmium that are critical for feed range in the 0.5-1µg/g (Blume et al. 2016). Because tobacco is a plant it can accumulate cadmium. For this reason, the smoking population is also exposed to cadmium via tobacco smoke which increases their cadmium intake. A pack of 20 cigarettes has around 2 to 4 µg cadmium which of this amount between 25 to 50% is absorbed by the lungs. An uptake of around 1 to 2 µg of cadmium (IPCS, 1992).

After the Itai-itai disease happening in Japan in the first half of the XX century it was clear that cadmium poisoning was possible by consuming food that had been irrigated with cadmium polluted water. The water used to irrigate the rice fields came from the Jinzu River which had been polluted by the mining companies in the region extracting zinc and other metals (Aoshima, 2016). The Itai-itai disease is the only documented case of cadmium poisoning through dietary cadmium (Hutchinson & Meema, 1987).

Cadmium sorption capacity of the soil

There are different factors that control the bioavailability of cadmium as well as the soil sorption capacity. Among them the pH of the soil has the strongest effect in the cadmium sorption capacity of the soil (Longanathan et al. 2012; Christensen, 1984; Sim et al., 2009); with soils with a pH 6 having a very high affinity for cadmium (Blume et al. 2016, Christensen, 1984). At the

same time, in the pH range of 4 to 7.7 the sorption capacity increases by 3 for every pH increase of 1 (Christensen, 1984).
The organic matter content is also one of the most important factors controlling the uptake of cadmium by plants along with sorption capacity of the soil and amount of acetate-extractable Cd (Blom, 1974). Furthermore, the soil organic matter also has a role in the amount of cadmium soil can adsorb. Many studies have demonstrated that the removal of organic matter causes a reduction in the soil sorption capacity (Zhao et al., 2014). In average the capacity of the soil is reduced by 20% when the organic matter is removed (Lin et al, 2007 as cited by Zhao et al., 2014). In the specific case of cadmium the adsorbed amount increases with the increase in organic carbon (C) (Sim et al., 2009).
In addition to this, the sorption of cadmium and other heavy metals is strongly related to the percentage of clay minerals in the soil (Zachara and Smith, 1994; Spark et al., 1995 as cited by Choi, 2005) with smectite, a clay mineral, having a high affinity for cadmium (Choi, 2005).

Phytoremediation

Phytoremediation is the use of plants for cleaning the environment from organic and inorganic pollutants as they can stabilize the pollution in the soil, extract the pollutants or degrade them (Pilon-Smiths, 2005; Trueman and Eber, 2013). Phytoremediation is a very practical cleanup technology where certain plant species are used to adsorb and remove pollutants from the ecosystem (Trueman and Eber, 2013). In the case of inorganic pollutants, like heavy metals, they cannot be degraded but captured and stored; this is referred to as sequestration. Sequestration is held by harvestable plants which uptake the pollutants and store them in their tissue (Pilon-Smiths, 2005). Plants take up heavy metals and normally the concentrations of heavy metals are higher in the roots and leaves than in the stems, fruits and seeds (Wahid et al., 2009; Blume et al., 2016).
Phytoremediation is an attractive approach because it requires no energy input rather than solar energy and in comparison with other methods such as soil excavation, washing or incineration it is, on average, a tenfold cheaper (Glass, 1999; Trueman and Eber, 2013). At the same time, conventional processes including chemical and mechanical methods are not only less cost-effective but less eco-friendly than phytoremediation and have the disadvantage of producing toxic sludge which is difficult to dispose of or to treat (Ahluwalia and Goyal, 2007; Trueman and Eber, 2013). Phytoremediation has also limitations such as the extent of the plant‘s root system in relation to the depth of the contaminant, the time needed to develop a root system, the pollutant concentration in the medium and the tolerance of the plants to the different concentrations of the pollutant (Pivetz, 2001).
As phytoremediation is a relatively new technology most of the recent research is focused on determining the most appropriate species based on the pollutant, the environmental conditions and the capabilities of the plant. The desired attributes in a plant to be a useful phytoremediator are: tolerance to high environmental concentrations, accumulation of high amounts, ability to bioconcentrate the metal at low concentrations, high biomass and phytoaccumulation in different nutrient levels (Khilji and Firdaus-e-Bareen, 2008).

Heavy metals can be removed from the water and sediments of aquatic ecosystems using traditional methods. The effectiveness however, depends on many factors such as the concentration and the target heavy metal. Some processes such as chemical precipitation can remove zinc and cadmium but will fail to completely remove lead or mercury (Newete and Byrne, 2016).

Previous studies have demonstrated the capacity of macrophytes to take up heavy metals from the sediments and water and therefore have a potential for phytoremediation (Rai et al., 2003; Mishra and Tripathi, 2008; Wu et al., 2014) for example, *Hydrocotyle umbellata*, *Lemna minor* and *Eichornia crassipes* (Khilji and Firdaus-e-Bareen, 2008; Mishra and Tripathi, 2008). Macrophytes can enrich heavy metals in their tissues by three different patterns: (1) restricting the entrance of heavy metals and thus they attach to the cell wall, (2) metals are adsorbed but stored in the roots and do not move into the stems and leaves and (3) by accumulating them in all parts of the plant, a behavior typical from hyperaccumulators (Mishra and Tripathi, 2008).

Some particular plant species can accumulate high amounts of heavy metals beyond the normal ranges, they are called hyperaccumulators. They can also survive in environments that are phytotoxic for other plant species. Hyperaccumulators are ideally the best option for phytoremediation.

There are four features that a species has to have in order to be called a hyperaccumulator:

1. The concentration per dry weight in the shoots should be 10,000 µg/g (1% of the dry weight) for zinc and manganese, >1,000 µg/g (0.1% of the dry weight) for arsenic, lead, copper, cobalt, nickel and chromium, 100 µg/g (0.01% of the dry weight) for cadmium and 1 µg/g (0.0001% of the dry weight) for gold (Agunbidae et al., 2009; Abu Bakar et al., 2013).
2. Translocation property: the concentration should be greater in the shoots as in the roots. (Ali et al., 2013)

$$Translocation = \frac{Concentration\ in\ shoots\ (\mu g/g)}{Concentration\ in\ roots\ (\mu g/g)} x100$$

3. Enrichment property or accumulation factor (AF): The concentration in the plant should be greater than in the environment. In other words AF > 1 (Ali et al., 2013).

$$AF = \frac{Concentration\ in\ plant\ (\mu g/g)}{Concentration\ in\ soil\ (\mu g/g)} x100$$

4. Tolerance: the plant should have a high tolerance to toxic contaminants, beyond the normal levels that cause phytotoxicity to other species (Ali et al., 2013).

Phytotoxicity

Phytotoxicity is the capacity of a compound to cause permanent or temporal damage to plants ("PP 1/135 (4) Phytotoxicity assessment", 2014). A great variety of substances can cause phytotoxicity including: heavy metals, herbicides, fungi and plant produced substances among others.

In the case of cadmium it has no beneficial effect to plants and when accumulated affects the growth and development of the plant. Some effects of cadmium phytotoxicity include plant stunting, leaf rolling, chlorosis and necrosis, diminished stomatal conductance and gas exchange, perturbed nutrient status, hormonal imbalance, oxidative stress and plant death (Wahid et al., 2009).

Concentrations that are critical for plant growth range in the 5-10µg/g that means that cadmium contaminated plants do not necessarily show any signs of damage or differ from healthy plants (Blume et al. 2016). For example, high concentrations of cadmium can induce phytotoxicity in land and water plants. This can be detected by monitoring the chlorophyll a and chlorophyll b levels. For example, a high dose of cadmium, that is, 200µM (22.48mg/l) in water solution; affects the macrophyte *Potamogeton pectinatus* by reducing chlorophyll a level in 48.59%, chlorophyll b levels in 37.17% and the total chlorophyll levels in 44.4% in just 96 hours (Rai et al., 2003).

According to da Rosa Corrêa et al. whose research was based on *Lactuca sativa*, *Avena sativa* and *Brassica campestris*, cadmium starts to affect plant development at concentrations above 3mg/kg. (Table 1, 2 & 3)

Table 1. *Effects of different cadmium concentrations in the soil on lettuce (Lactuca sativa) (da Rosa Corrêa et al., 2006)*

Cd Concentration (mg/kg)	0	0.05	0.19	0.39	3.12	6.25	12.5	25	50	100
Biomass fresh weight (mgx10^-2)	2.64	2.65	2.64	2.65	2.53	2.37	2.04	1.88	1.85	1.3
% biomass reduction	0	-0.38	0.00	-0.38	4.17	10.23	22.73	28.79	29.92	50.76

Table 2. *Effects of different cadmium concentrations in the soil on oats (Avena sativa) (da Rosa Corrêa et al., 2006)*

Cd Concentration (mg/kg)	0	0.05	0.19	0.39	3.12	6.25	12.5	25	50	100
Biomass fresh weight (mgx10^-2)	10.7	10.7	10.8	10.6	10.4	10.8	9.5	9.0	7.1	5.8
% biomass reduction	0	0	-0.93	0.93	2.80	-0.93	11.21	15.89	33.64	45.79

Table 3. *Effects of different cadmium concentration in the soil on Chinese cabbage (Brassica campestris var. chinensis) (da Rosa Corrêa et al., 2006)*

Cd Concentration (mg/kg)	0	0.01	0.19	0.39	1.56	3.12	25	50	100
Biomass fresh weight (mgx10^-2)	96.9	97.9	97.3	97	96.2	95.4	95.6	94	92.3
% biomass reduction	0	-1.03	-0.41	-0.10	0.72	1.55	1.34	2.99	4.75

It is very difficult to determine a concentration range of Cd in the sediments in which water plants could live. Not because of the lack of research, but because research of heavy metal phytoremediation with macrophytes almost always takes the water as the medium through which the plants would adsorb the heavy metals. Previous research does indeed provide the cadmium concentrations that are phytotoxic to water plants but they are expressed in µg/l as the polluted medium was water.

For example, *Lemna minor*, a commonly used test plant experienced a reduction of 26% in chlorophyll-a and 9,4% in chlorophyll-b after 96h of exposure at concentrations of 5,0 mg/l and the antioxidant system was disrupted at concentrations of just 0,5 mg/l (Hou et al. 2007) These values cannot be extrapolated to sediments and this left little to no room to work with sediment polluted with heavy metals. For that matter this research was based on the phytotoxic concentrations for land plants.

Plants adsorbing pollutants is just the first step of the phytoremediation process. Nutrients and pollutants go through a yearly cycle as macrophytes uptake them during the summer season and release them in the winter as the biomass decays (Jackson, 1998). For this reason it is important to have the available technology to harvest and dispose of the biomass in order to avoid the pollutants being liberated into the water body again.

Few options are available to dispose of the phytoremediation plants once the phytoremediation process is done. Some possible options for the safe disposal of plant biomass are briquetting, incineration, gasification and mine waste storage facilities (Newete and Byrne, 2016).

Some other solutions suggest using the hyperaccumulators as sources of raw material. The heavy metals can be extracted and reused thus closing the cycle and transforming the phytoremediation process into a ~~"p~~hytomining" process.

Invasive species and phytoremediation

In the past, biogeographic barrier such as mountain ranges, cascades and oceans limited the habitat of aquatic plants and prevented they movement beyond their established area. This created unique regional faunas (Rahel, 2007). Nevertheless, humans have created pathways for aquatic biota to move beyond their established environment. Some of these pathways are construction of canals, ship ballast water and intentional release, just to name a few (Rahel, 2007).

The spread of non-native species beyond their natural habitat brings severe consequences to the environment and subsequently to humans. Noxious species can potentially alter the aquatic ecosystems and fisheries in undesirable ways (Rahel, 2007; Gallardo et al., 2015; Jackson et al., 2014) as they generally cause a decrease in diversity and abundance of the local species (Gallardo et al., 2015).

Another negative consequence is the homogenization of habitats, this happens when a species comes and dominates the habitat of a local species (Rahel, 2007). The introduced species then becomes a cosmopolitan species; in other words, widespread throughout the world.

Invasive macrophytes affect aquatic communities through biomass production, photosynthesis, decomposition and substrate stabilization (Schultz and Dibble, 2011). Through biomass production macrophytes cause an increase in allopathic chemicals and a decrease in light penetration. Through photosynthesis they increase the pH and the dissolved oxygen. When they decompose they increase detritus and reduce the amount of dissolved oxygen. They positively stabilize the substrates as they increase sedimentation and reduce turbidity (Schultz and Dibble, 2011). Introduced macrophytes compete with local macrophytes, positively alter the environment for phytoplankton, and negatively alter the habitat for benthic organisms and fish (Gallardo et al., 2015).

However, even with so many negative impacts invasive macrophytes might be used for phytoremediation. Little attention has been given to the possible use of invasive plants in phytoremediation, but pollution itself could cause the ecosystems to decay and leaving them more vulnerable to invasion by non-native species. This change in species domination might leave non-native species as the only effective phytoremediators under those conditions (Trueman and Eber, 2013). In addition, invasive and non-native species might offer advantages that the local varieties might not, such as rapid growth, better tolerance to pollution, better adaptation to the environment and ease to harvest.

One example is *Eichhornia crassipes* (water hyacinth), a free-floating and mobile macrophyte. It is an introduced pest in many countries and is considered one of the most invasive aquatic plants in the world. It causes severe economic losses as it affects: navigation, agriculture, public safety, water quality and recreation (EPPO, 2009). Still, *Eichhornia crassipes* has rhizofiltration and phytoextraction capacities. It can accumulate heavy metals in its roots and shoot in high degree and consequently it can be used for phytoremediation (Agunbiade et al., 2009; Newete and Byrne, 2016; Mishra and Tripathi, 2008). It has also showed a tolerance to high concentrations of cadmium; up to 100mg/L (Li, 2015). An advantage is that it is easy to harvest as it is a free-floating macrophyte and grows in thick mats on the water surface.

A second example of this is *Potamogeton illinoensis* a macrophyte with the capacity to adsorb organic contaminants. Organic contaminants such as endocrine disrupting chemicals (EDCs) are usually found in water systems and are very difficult to remove. EDCs are pollutants that disrupt the endocrine system and mimic hormones such as Bisphenol-A (Trueman and Eber, 2013). *Potamogeton illinoensis* a macrophyte that is considered invasive in Illinois, U.S.A. was compared by Trueman & Erber to the local species *Potamogeton crispus*; the invasive species could accumulate an average of 66% higher level of estrogenic compounds, 94% more

Bisphenol-A, 76% more estrone, 55% more 17 β-estradiol and 31% more 17 α-ethynylestradiol than the native species, partially because the invasive species is 72% larger than the local species (Trueman & Erber, 2013).

These examples indicate that while exotic and invasive species alter the environment in a negative way they may also have a positive impact on the ecosystem through phytoremediation. In some cases they can be even more effective than the local existing varieties or species as they can adapt better, grow faster and adsorb more pollutants or specific pollutants that the local species cannot.

Phytoremediation with *Egeria densa* and *Cabomba caroliniana*

Several studies have demonstrated the potential of *Egeria densa* to be used as an aquatic phytoremediator of heavy metals.

For example, *Egeria densa* when compared to *Cabomba piauhyensis* and *Hydrilla verticillata* under hydroponic conditions it has the highest arsenic removal efficiency with a 92.5% and *H. verticillata* having a 84.5% and *C. piauhyensis* a 55.8% It also proved to be the best species to remove zinc as it presented a removal efficiency of 93.7% compared to 92.3% of *H. verticillata* and 87.4% of *C. piauhyensis*. Lastly, it proved to be the worst species for aluminum phytoremediation as it had a removal efficiency of only 30.3% compared to 83.8% of *C. piauhyensis* and 59.1% of *H. verticillata* (Abu Bakar et al., 2013). Abu Bakar et al. also demonstrated that *Egeria densa* can have dry-weight concentrations 195.95 μg/g for arsenic, 441,38 μg/g for zinc and 66,86 μg/g for aluminum.

Harguiteguy et al. (2015) found that when *Egeria densa* was exposed to different concentrations in the range of 0 to 10mg/l for nickel, 0 to 15mg/l for lead and 0 to 20mg/l for zinc under hydroponic conditions, the maximum accumulations occurred at the highest concentrations. At the same time an increased accumulation of the metals was observed as the concentrations in the water rose. Table 4 shows a summary of several other phytoremediation studies with *Egeria densa* and the concentrations of heavy metals achieved.

Table 4. *Accumulation of heavy metals by E. densa according to several different studies. *non-living biomass*

Study	Metal concentration (dry weight)
Abu Bakar et al, 2013	Al 66.86 μg/g As 195.95 μg/g Zn 441.38 μg/g
Pietrobelli et al, 2009 *	**Cd 70.25 mg/g** Cu 45.42 mg/g Zn 30.40 mg/g
Molisani et al., 2006	Hg 177 ng/g
Harguiteguy et al, 2015	Pb 2302.5 μg/g Zn 1083.6 μg/g

In the case of *Cabomba caroliniana* no studies were found or known of which used this macrophyte as a possible phytoremediator.

OBJECTIVES AND HYPOTHESES

The research project compared the efficiency of two different macrophytes for the phytoremediation of flooded soil which was previously polluted with cadmium with the intention of creating floodplains to mitigate flood risks, remediate agricultural land and clean sediments of lakes and rivers. *Egeria densa* and *Cabomba caroliniana* were proposed as both of them are exotic species in Austria. They are both considered neo-biota in Austria and listed by the Lebensministerium in the Aquatic Neo-Biota 2013. *Cabomba caroliniana* is also considered a potentially invasive species in Austria (Lebensministerium 2013).

The main objectives of this research were:

1. Measure the amount of cadmium adsorbed by the plants to determine if they are effective for phytoremediation under the given conditions.
2. Monitor the levels of cadmium in the water to detect if the plant mobilizes the cadmium from the soil into the water during the growing process and thus increasing the hazard.
3. Compare if the soil composition had any effect on the cadmium adsorption.

In addition to the objectives of the research, several hypotheses were formulated.

The hypotheses were:

1. Both plants will adsorb cadmium to some degree.
2. The sandier the soil the higher the cadmium concentration will be in the plant.
3. Phytotoxicity will affect the plants at some point.
4. Cadmium will not leach from the soil but remain absorbed into the soil.
5. The cadmium concentration will be higher in the plants than in the water.

MATERIALS AND METHODS

Species

Two different exotic macrophyte species were used for the experiment: *Egeria densa* and *Cabomba caroliniana*.

Egeria densa

Egeria densa also known as Brazilian waterweed, Brazilian elodea or Anacharis (Hara et al., 2015) is a submerged and rooted aquatic plant. It original ecosystem included regions of Brazil, Argentina and Uruguay but now it is present in all continents except Antarctica (Yarrow et al., 2009). It is a very common aquarium plant and can be bought in aquarium shops. It is considered a weed in many countries as it competes with native plants and blocks water flow and water turbines causing economic loss and environmental damage (Cabrera Walsh et al., 2012; Yarrow et al., 2009). Its optimum relative growth temperature is 20.7°C in culture and its optimum temperature of net photosynthesis at 35°C (Haramoto & Ikusima, 1988) In Vienna can be found in the *Alte Donau* (old Danube) (Lebensministerium, 2013).

Egeria densa is usually rooted between 1 to 2 m below the water table (Yarrow et al., 2009) but can survive as non-rooted fragments that float and move through the water column. It‘s stems reach up to 3m in length and 1 to 3 mm in diameter and has internodes between 2.5 to 24 mm long. Branches develop from internodes in between 0 to 15 internodes. The leaves are 3 cm long and up to 5 mm wide. *Egeria* grows in very long strains until it reaches the surface where it forms dense mats. Flowers of *Egeria* grow up to 3 cm over the water surface and when submerged they close and trap air inside to keep them dry (Cook & Urmi-König, 1984 as cited by Yarrow et al., 2009).

It has also positive effects on the ecosystems as it prevents re-suspension of sediments and controls the growth of phytoplankton as it has the ability to remove nutrients from the water column (Yarrow et al., 2009).

Cabomba caroliniana

Cabomba caroliniana is a submersed macrophyte native to South and North America which is considered a serious pest. It has submersed and floating leaves. Submersed leaves are divided and fan-shaped and floating ones are long and lean usually 1-3 mm wide and 20 mm long (Øgaard, 1991). It is found in shallow waters and littoral zones, between 1 to 3 meters but can also grow in deeper waters. Its optimal ecosystem consists of warm temperatures which are between 13 and 27°C, slightly acidic water with a between pH 4 and 6 and humid climates. Still, this species can survive below freezing temperatures and can survive under ice during the winter with the broken fragments regrowing in the spring (Hogsden et al., 2007).

It spreads rapidly through fragments and has a very high resistance to desiccation it can last between 3h and up to 42h outside the water (Bickel, 2014) which explains its rapid spread by humans into Australia, Asia and Europe. *Cabomba caroliniana* is also classified as neo-biota in Austria and as potentially invasive by the Lebensministerium (Lebensministerium, 2013).

This plant is also a common aquarium plant and can also be easily acquired in aquarium shops, even in countries like Austria where it is considered potentially invasive. Commercial trade is, as explained by McCracken et al., 2013, the main cause of this species‘ introduction into other countries.

Plants origin

The plants were obtained through the Fressnapf Handels GmbH aquarium located in Albert-Schweitzer-Gasse 7, 1140 Vienna, Austria. The aquarium had direct contact to Dennerle GmbH a German wholesale company specialized in water plants. The plants were already acclimatized to grow ex situ and were all of the approximate same size. This gave the advantage of having standardized plants which would develop at the same rate and that 100 specimens could be available at the same time.

Before performing the experiment it was necessary to test that the experiment set-up. A pre-experiment was performed to determine the stability of the microsystems. The pre-experiment‘s objective was to detect any change in the microsystems that could affect the experiment such as algae growth, water turbidity or any other condition that could affect the macrophytes‘ development (Annex 1).

Soil properties

Two soils were used for the experiment. The first soil was obtained from the fields of the BOKU campus in Tulln an der Donau (48°19'01.6"N 16°04'03.5"E; Figure 1) The second soil was obtained from the Donau Insel-Handelskai (48°14'34.1"N 16°23'30.2"E; Figure 2) Once collected both soils underwent the same processing. They were sieved to 2 mm, autoclaved at 121°C for 15 minutes and finally oven-dried at 105°C overnight before being used.

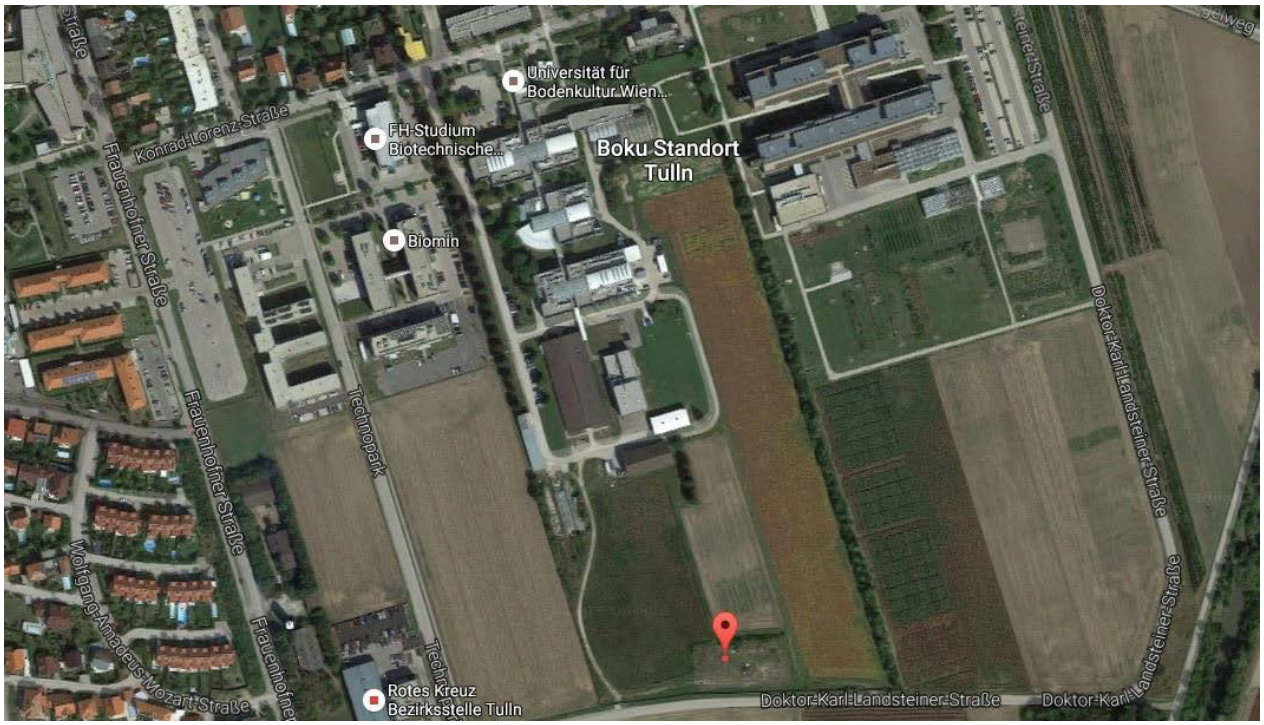

Figure 1. *Satellite image of the BOKU campus in the city of Tulln an der Donau with the soil extraction site marked by a red arrow.*

Figure 2. *Satellite image of the Danube Island in city of Vienna near the subway station Handelskai and with the soil extraction site marked by a red arrow.*

Grain size distribution

The soils were then analyzed with the help of Dr. Karin Wriessnig of the Institute of Applied Geology (IAG) of the BOKU. She followed the method described below to obtain the grain size.

Approximately 50g air dried soil samples are mixed step by step with c. 200ml 10% H2O2 to disperse the sample and to destroy organic matter. After 2-5 days, when additional peroxide shows no effect, the samples are put into a water bath to 95°C. After 2 days the rest of the peroxide in the glass has been destroyed and the sample is ready for sieving.

Water content: c. 20g are dried in the oven at 105°C and weighed to calculate a correction factor air dry/oven dry.

The sample is sieved with a vibrating sieve with mesh sizes 2000µm, 630µm, 200µm, 63µm and 20µm. The grains on each sieve are dried at 105°C and weighed. Particles smaller 20µm that pass through the sieve are collected in a glass and put into the waterbath to reduce the volume of the suspension. The thickened sample is mixed well on a magnetic stirrer and 50ml are pipetted out and mixed with 5ml 0,5% Na-polyphosphate to prevent coagulation. After ultrasonic treatment the sample is measured in the Sedigraph (micromeritics SediGraph III Atlanta, U.S.A.). The particles settle according to Stokes‘ Law, the settling velocity depends on particle density, viscosity of the liquid, temperature and particle size.

From the results of sieving and sedimentation analysis we calculate the percentage of the grain size classes and the sum curve.

Soil pH and nitrogen and carbon content

The pH of the soil was also measured. For the measurement 5 ml of soil were placed in test tubes. Two test tubes for the Donau Insel soil and two test tubes for the Tulln soil were prepared. Two of the test tubes, one for each soil, were given 25 ml of 0.01 Mol $CaCl_2$ and the other two were given 25 ml of deionized water and shaken for 30 sec. each. They were left for 24 h to rest. Afterwards, they were shaken again for 30 sec. and then the pH was measured.

The soil nitrogen and carbon content was measured by the lab-technicians in the laboratory of the Institute of Forest Ecology of the BOKU. The carbon content could then be used to calculate the % of organic matter in the soil:

$$\% \text{ Organic matter} = (\%\text{Carbon})(1.724)$$

Soil sorption capacity

For the experiment the soils were polluted using a $CdCl_2$ water solution. It was thought that if the concentration of cadmium was too high then it could leach from the soil into the water. For this reason, it was necessary to first study the soil‘s characteristics and behavior to determine the concentrations of absorbed and bioavailable cadmium after it was polluted.

Cadmium sorption into the soil is a very fast process. During the first 10 minutes after exposition 95% of the cadmium is adsorbed into the soil and equilibrium is reached after just 1 hour (Christensen, 1984).

The total amount of a nutrient or pollutant in the soil is not equal to the bioavailable amount as a fraction of the ions will be adsorbed into the soil. The capacity of the soils to absorb ions depends mainly on the characteristics of its particles and their specific superficial area (m^2/g). On the other side, the bioavailability is dependent on the concentration, the relationship between the nutrients and contaminants in the soil solution, the replenishment rate and the mobilizing ability of the plants. These parameters are described using an adsorption isotherm. Plants are only capable of absorbing pollutants present as ions (Blume et al., 2016).

The sorption capacity of the soil was tested. Both soils were sieved to 2 mm and autoclaved at 121°C for 15 minutes. Afterwards it was left to dry overnight in the oven at 105°C. It was then observed that the soil could hold up to 700 µl of water per every 2 g of soil. Based on this, cadmium solutions were prepared to deliver 160, 80, 40, 20, 10, 5 and 0 µg of cadmium per gram of soil for every 700 µl of solution.

Once the soil was ready, 50ml test tubes where prepared with 2 grams of soil. The soil was then polluted using the seven cadmium solutions mentioned above. Two replicas for every concentration were prepared in case one was spilled or more measurements were needed. The tubes were then left 2 hours at room temperature to allow the cadmium to sorb into the soil. It was left 2h to make sure that the cadmium was completely adsorbed even when 1h is enough for complete sorption (Christensen, 1984).

After two hours the tubes were filled with 20 ml of water to achieve a 1 to 10 ratio of soil to water and placed for 7 h in the rotation shaker at 22 rpm. When the shaking was done the tubes were placed in a flat surface and left overnight to sediment.
The next morning the tubes were centrifuged at 3,500 rpm. The solutions were then filtered using 292 grade filters. This clear solution free of sediments was then analyzed for Cd with the aid of inductively coupled plasma (ICP) machine PerkinElmer® Optima 8300 (Waltham, U.S.A.).

Experiment set-up

As this research is based on the uptake of heavy metals flooded agricultural land, it was decided to base the range on the concentrations of cadmium that cause phytotoxicity to land plants. For this reasons the limits established by the European Union were taken as a base. The European law limits cadmium on agricultural soils to 1-3 µg/g (European Union, 1986). This gave the first two pieces of the puzzle: 0 µg/g for the control and 3 µg/g for the first step.
According to da Rosa Corrêa et al. whose research was based on *Lactuca sativa*, *Avena sativa* and *Brassica campestris*, cadmium starts to affect plant development at concentrations above 3mg/kg. As the concentrations that affect the most sensitive organism should be used as a guide and not the ones affecting the hardiest organism; *Lactuca sativa* and *Avena sativa* were used as a guide (Table 1, 2 & 3).
As mentioned above, concentrations that could give valuable results and at the same would not kill the plants were preferred. For that reason 100 mg/kg was discarded as this concentration causes a 50.7% reduction in biomass in *Lactuca sativa* (Table 1) and it would imply a high risk of causing phytotoxicity to *Egeria densa* and *Cabomba caroliniana*.
This then set up the highest limit to 50 mg/kg as a loss of 30% in biomass was acceptable. This left 0, 3 mg/kg as lower limits and 50 mg/kg as the highest limit. Two more values could be selected in between and 15 and 25mg/kg were chosen as they are 5 times and ca. 8 times higher than the limit values established by the European law. As a conclusion the Cd concentrations were set to 0, 3, 15, 25 and 50 mg/kg (µg/g).
To build the microsystem transparent 1.5 liter polyethylene terephthalate (PET) bottles were used. The top of the bottles was cut so they could be filled with soil and to facilitate the planting. Every container was then filled with 200 g of unpolluted soil, a plant and 1.1 liter of unpolluted tap water.
One week before the experiment began, two specimens of *Egeria densa* and two specimens of *Cabomba caroliniana* were placed in microsystems with unpolluted soil and placed in the same greenhouse that was going to be used for the experiment to observe if they survived under those conditions. Intense direct sun radiation and the greenhouse effect elevated the temperature of the water above 40°C which killed the four plants and caused excessive evaporation. It was then concluded that direct sunlight should be reduced using a shade cloth, which could help keep the temperatures below 30°C and reduce evaporation to a minimum.
The soils were then polluted with cadmium solutions. The stock solution was prepared with $CdCl_2$ and Milli-Q water to a concentration of 1000 mg/l. The behavior of the prepared soils was

observed and it was concluded that 200 g of soil could hold 85 ml of water before being saturated.

The diluted solutions were then prepared to deliver 3, 15, 25 and 50 µg/g in 8 5ml of solution. Non-polluted soil was used as a control. This gave a total of five different cadmium concentrations: 0, 3, 15, 25 and 50 µg/g.

Every cadmium concentration was added to 20 bottles: five with Donau Insel soil and *Egeria densa*, five with Donau Insel soil and *Cabomba caroliniana*, five with Tulln soil and *Egeria densa* and five with Tulln soil and *Cabomba caroliniana*; adding up to a total of 100 microsystems (Table 5).

In the end the 100 microsystems consisted of cut 1.5 liter polyethylene terephthalate (PET) bottles containing 200 g of cadmium-polluted soil, 1.1 liter of tap water and one plant. The microsystems were then randomly bound in groups of four to avoid being tilted and so they could be easily moved around to ensure they all received enough light.

The microsystems were placed in the foil tunnel of the BOKU campus in Tulln an der Donau (48°19'05.1"N 16°03'58.1"E) on the 14.07.2015. They were placed on a table and covered with shade cloth.

Table 5. *Configuration of the different microsystemswith 5 replicates each*

Cd Concentration (µg/g)	**Soil**	**Species**
0	Tulln	*Cabomba caroliniana*
3	Tulln	*Cabomba caroliniana*
15	Tulln	*Cabomba caroliniana*
25	Tulln	*Cabomba caroliniana*
50	Tulln	*Cabomba caroliniana*
0	Tulln	*Egeria densa*
3	Tulln	*Egeria densa*
15	Tulln	*Egeria densa*
25	Tulln	*Egeria densa*
50	Tulln	*Egeria densa*
0	Donau Insel	*Cabomba caroliniana*
3	Donau Insel	*Cabomba caroliniana*
15	Donau Insel	*Cabomba caroliniana*
25	Donau Insel	*Cabomba caroliniana*
50	Donau Insel	*Cabomba caroliniana*
0	Donau Insel	*Egeria densa*
3	Donau Insel	*Egeria densa*
15	Donau Insel	*Egeria densa*
25	Donau Insel	*Egeria densa*
50	Donau Insel	*Egeria densa*

Figure 3. *Microsystems used for the experiment with Egeria densa on the left and Cabomba caroliniana on the right.*

The plants were then kept for 34 days under those conditions. The experiment started on the 14.07.2016 and the plants were harvested on the 18.08.2016. The water was refilled every two days, or as needed, to compensate for evaporation losses and to maintain the same water level. A visual inspection of the plants was done every time the water was refilled.

Harvesting and Processing

During harvest, first a sample of 50ml of water was taken from every microsystem and stored in plastic test tubes. Afterwards, the water was poured out into 60l containers to properly dispose of the cadmium polluted water. The plant was pulled out gently, this could be done as the water-sutured soil was still very runny and the soil offered no resistance.

The plants were first visually inspected for damage and then placed in resealable polyethylene bags to maintain the humidity and keep the temperature low during transportation. As a final step the soil was also packed in resealable low-density polyethylene bags to transport to the lab and kept for disposal.

Once in the lab the plants were stored in a cool room at 4°C and later processed in a series of stages. Once a stage was started there was no interruption to avoid different conditions that could lead to discrepancies in the results.
Each specimen was washed with tap water to remove any adhering soil. When the plant was clean the roots were cut. The roots where then separated and stored in petri dishes so they could be scanned later on. The plant material was then stored in paper bags, labeled and then dried in an oven (65°C, 48h).
The second stage was scanning the roots. The petri dishes containing the roots were filled with water so the roots would float and then scanned using a Epson® Expression 10000XL scanner. The images were stored and processed using the PC program WinRHIZO Pro (Regent Instruments 20012, Quebec, Canada).
Subsequently, the dried plant material was weighted. The weight of every plant was recorded to compare any differences in the biomass and find if there was any relationship between the plant weight and the cadmium concentration in the sediment. The dead specimens will not be used as they turned into sludge, sank to the bottom and mixed with polluted soil.
Finally, the dried above-ground plant material was milled using a ball mill until powdered. Each specimen was individually milled and the powder stored in paper bags. This step was crucial as the powdered material was then digested using acid to be able to examine the cadmium concentrations.
The dried roots were weighted and recorded. It was during this step that it was noted that the root material was not enough to get an ICP reading as the minimum amount of dry material is 60 mg. The roots rarely weighted more than 25 mg and on average they weighted 11.95 mg

Cadmium analysis
First, the urease inhibition test developed by Wittekindt et al. (Wittekindt et al., 1996) was tested to measure the cadmium concentrations in the plants after the experiment and also to measure the amounts of cadmium leached from the soil into the water. This method however, had errors and the detection limits were above the detection limits needed for this research (Annex 2). The samples would then be analyzed using a inductively coupled plasma optical emission spectrometry (ICP-OES).
Because the project had a limited budget, a decision was made to pool the samples and if the results were promising more measurements could be done afterwards on the individual samples. In order to pool the results 5 mg of milled plant material were taken from every replicate of the same species, soil and concentration to make one sample. This allowed reducing the number of measurements from 100 to 20.
The same pooling procedure was done with the water samples of every group. The water samples were also analyzed to determine if the cadmium had leached into the water either from the soil or mobilized by the plants.
The milled material and the water samples were sent to the laboratory of the Lab of Forest Ecology of the BOKU. The samples were then digested and analyzed in the ICP with the help of Mr. Marcel Hirsch.

Statistics

Once the ICP results were obtained they were analyzed with SPSS version 21 for Mac OS. One-way, two-way and three-way ANOVAS were performed for the above soil biomass weight, root weight and root morphology. Soil type, plant species and cadmium concentration in the soil were used as the three factors.

Correlations were also made between the above ground biomass weight, root weight, cadmium concentration in the water, cadmium concentration in the plants and cadmium concentration in the soils for the general experiment and for each species.

RESULTS

Soil properties

The soil texture was different between the two soils. The greatest difference being the clay content as the soil from Tulln has more clay than the soil from Donau Insel. The second difference is the sand and silt contents with the soil from Donau Insel being the sandiest soil and the soil from Tulln being the siltier (Table 6).

Table 6. *Soil texture of both soils as obtained from the soil analysis showing sand, silt and clay fractions.*

Sample	Tulln	Donau Insel
% sand	30.7	35.1
% silt	42.4	48.5
% clay	27.0	16.4

With the soil fraction available it was possible to classify the soils using the soil texture triangle. The soil form Tulln is a clay loam soil and the Donau Insel soil is a loam soil (Figure 4).

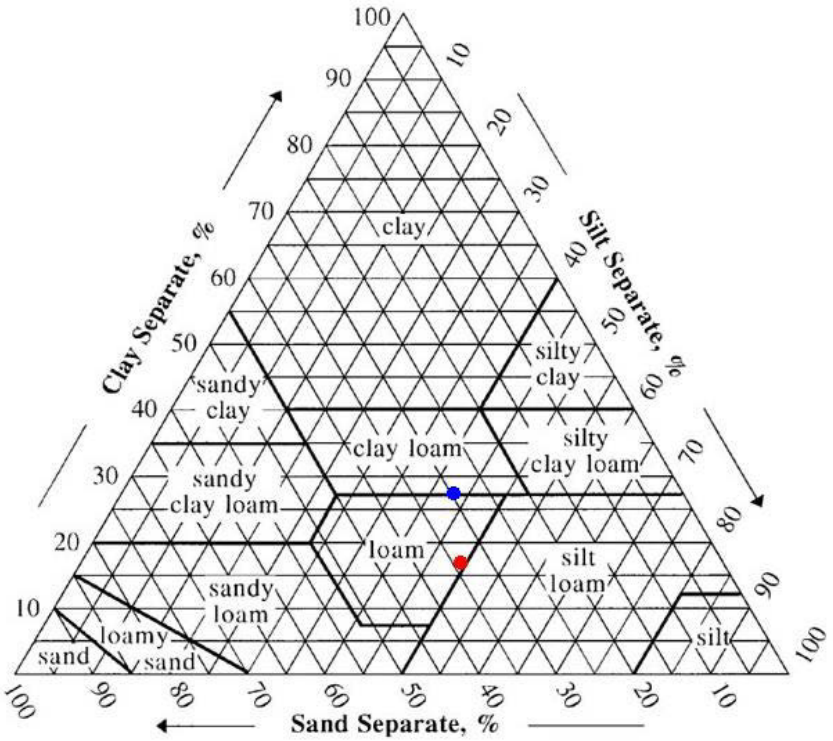

Figure 4. *Soil texture triangle with the blue dot denoting Tulln soil and the red dot the Donau Insel soil ("Soil Texture Calculator | NRCS Soils", 2016)*

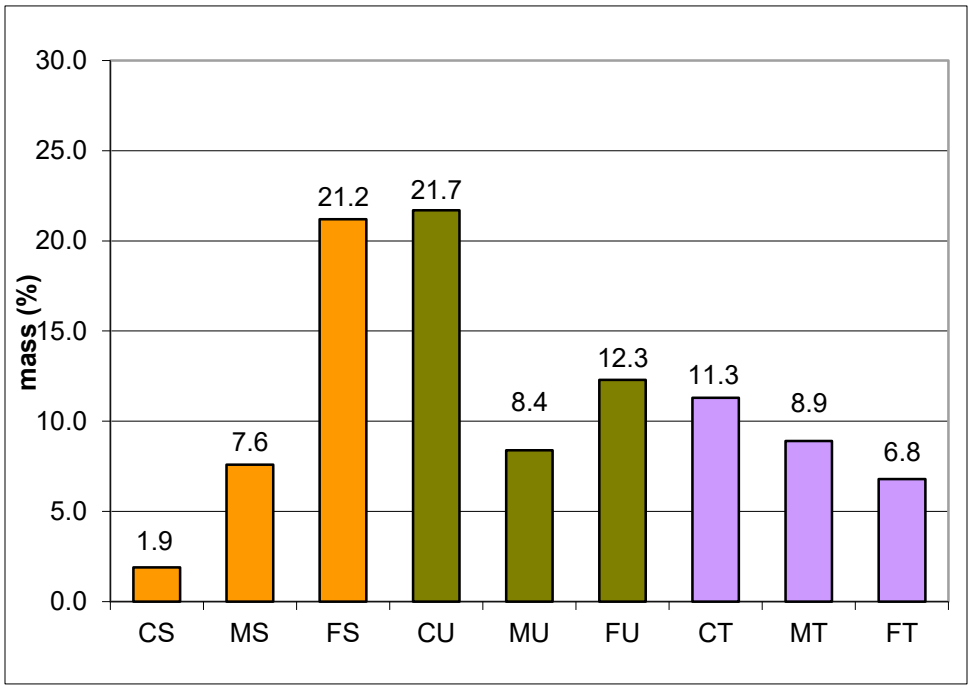

Figure 5. *Tulln soil grain size classes and mass percentage. This soil is low in coarse sand (CS) and medium sand (MS) but very high in fine sand (FS) and corarse silt (CU) with the levlels of medium silt (MU), fine silt (FU), coarse clay (CT), medium clay (MT) and fine clay (FT) decrease almost linearly. Where the particle size for every class is CS <2000 - 630 µm, MS < 630 - 200µm, FS < 200 - 63 µm, CU < 63 - 20 µm, MU < 20 - 6,3 µm, FU < 6.3 - 2 µm, CT < 2 - 0.63 µm, MT < 0.63 - 02 µm, FT < 0.2 µm*

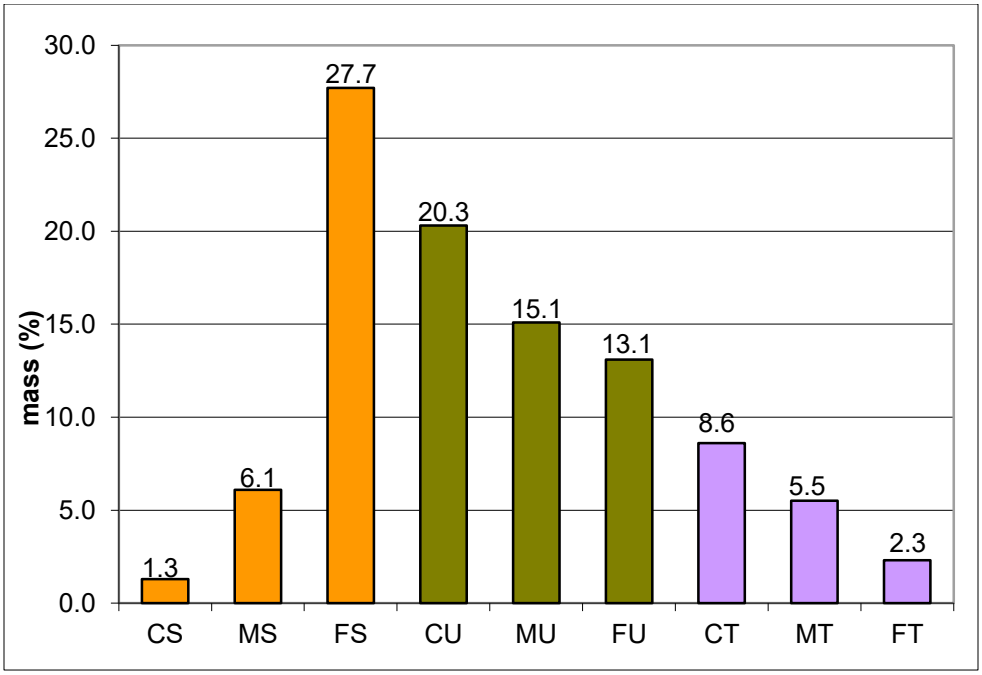

Figure 6. *Donau Insel soil grain size classes and mass percentage. This soil is also low in coarse sand (CS) and medium sand (MS) but very high in fine sand (FS) after which the percentage of corarse silt (CU), medium silt (MU), fine silt (FU), coarse clay (CT), medium clay (MT) and fine clay (FT) decrease agian almost linearly. Where the particle size for every class is CS <2000 - 630 µm, MS < 630 - 200µm, FS < 200 - 63 µm, CU < 63 - 20 µm, MU < 20 - 6,3 µm, FU < 6.3 - 2 µm, CT < 2 - 0.63 µm, MT < 0.63 - 02 µm, FT < 0.2 µm*

Soil pH and nitrogen and carbon content

The soil pH was higher for Tulln soil as for Donau Insel soil. The Tulln soil had a pH 8.34 in H_20 and pH 7.55 in $CaCl_2$ and the Donau Insel soil had a pH 7.94 in H_20 and pH 7.37 in $CaCl_2$

As of the nitrogen and carbon content the Tulln soil had 0.27% nitrogen and 6.11% carbon while the Donau Insel soil had 0.14% nitrogen and 3.37% carbon. With the carbon content it was possible to determine the % organic matter as: % Organic matter = (%Carbon)(1.724). Therefore, the Tulln soil had 10.53% organic matter while the Donau Insel soil had 5.81% organic matter.

Soil sorption capacity

The results showed that the amount of cadmium extracted by water had a direct relation to the amount applied in the soil. (Figure 7) After the water extraction ca. 99,9% of the cadmium applied to the Tulln soil and ca. 99.8% of the cadmium applied to the Donau Insel soil remained in the soils (Figure 8). The Tulln soil had a significant higher Cd sorption than the Donau Insel soil. However, the cadmium concentration did not affect the cadmium absorbed/extracted (Table 7). A table with the details can be found in Annex 3.

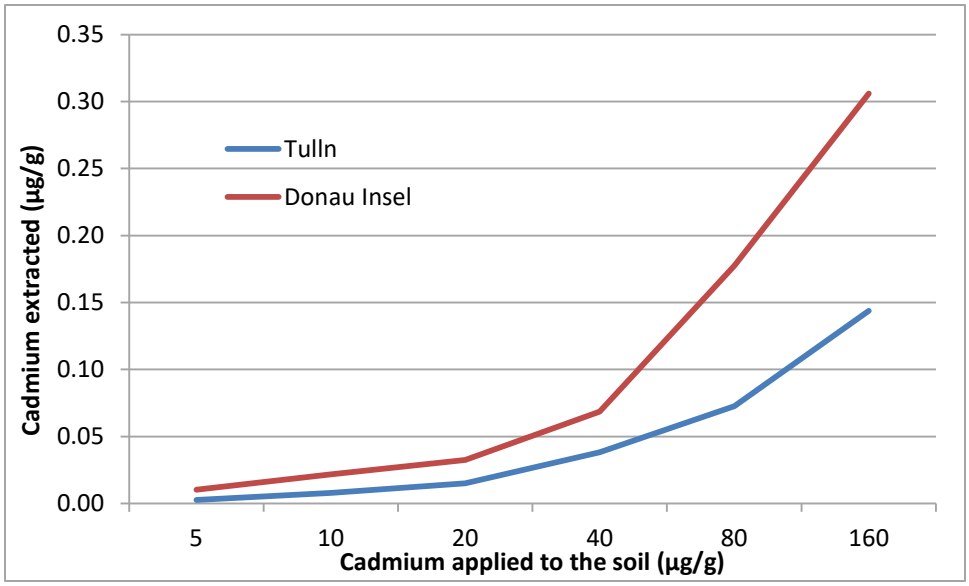

Figure 7. *Relation between the cadmium applied to the soil and the cadmium extracted with the water extraction method. The Donau Insel soil retained less cadmium than the Tulln soil.*

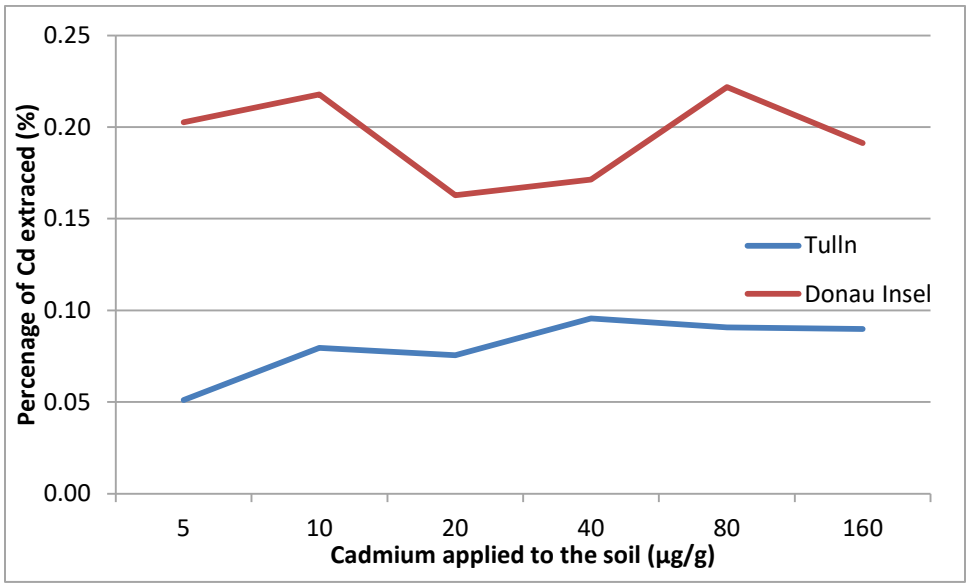

Figure 8. *Comparison between the percentage of cadmium extracted from both soils with the water extraction method. The Tulln soil retained in average 99,9% of the applied cadmium while the Donau Insel soil retained in average 99,8% of the cadmium applied.*

Table 7. *Two-way ANOVA without replication to determine if the cadmium concentration and the soil type have any effect on the amount of cadmium absorbed by the soils.*

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Cd concentrations	0.001899	5	0.00038	0.822023	0.582519	5.050329
Soil type	0.039137	1	0.039137	84.72571	**0.000254**	6.607891
Error	0.00231	5	0.000462			
Total	0.043345	11				

Visual observations of the plants

During the harvest it was noted that the color of the plants changed as the cadmium concentrations increased. This phenomenon was only observed in *Egeria densa* and not in *Cabomba caroliniana* and it was more noticeable in the *Egeria densa* growing in Donau Insel soil. In the case of *Cabomba caroliniana* none of the specimens presented any visible damage.

None of the plants growing in the soil with no cadmium (0µg/g) presented any visible damage as the whole plant kept a green color and the features of the plant were not altered.

The plants growing in 3 µg/g, did not present any observable damage for the exception of one *Egeria densa* growing in Donau Insel soil which died. This does not mean that the rest of the plants were not damaged or poisoned but it was imperceptible to the naked eye.

After the threshold of 15 µg/g the *Egeria densa* started to present perceptible damage. The plants started yellowing and the damage increased as the concentration of cadmium increased. The yellowing started at the tips and moved downwards. In the case of the plants growing in soil

polluted with 15µg/g the tips and up to half of the plant was yellow. The plants growing in 25 µg/g presented more damage, in some specimens more than half of the plant was yellow. Lastly, the plants growing in Donau Insel soil with 50 µg/g presented the highest damage as almost 75% of the plant was yellow in the surviving specimens and many died at this concentration turning completely brown or decomposing into sludge.

Plant biomass

The dry weight of the above-ground biomass (Figure 9) and the dry weight of the roots (Figure 10) were measured separately at the end of the experiment to determine if the cadmium had any effect on the biomass of the plant and the root development.

Figure 9 shows the average above-ground biomass weight. In general the dry weights show no appreciable pattern and seem to be completely random. There is a clear and significant difference (p-value < 0.001) between the biomass of both species as *Cabomba caroliniana* weights around half of *Egeria densa*. However, neither the soil type (p-value = 0.112) nor the cadmium concentration in the soil (p-value = 0.873) had a significant effect on the dry weight of the above-ground biomass and no interaction was found between this factors (data not shown).

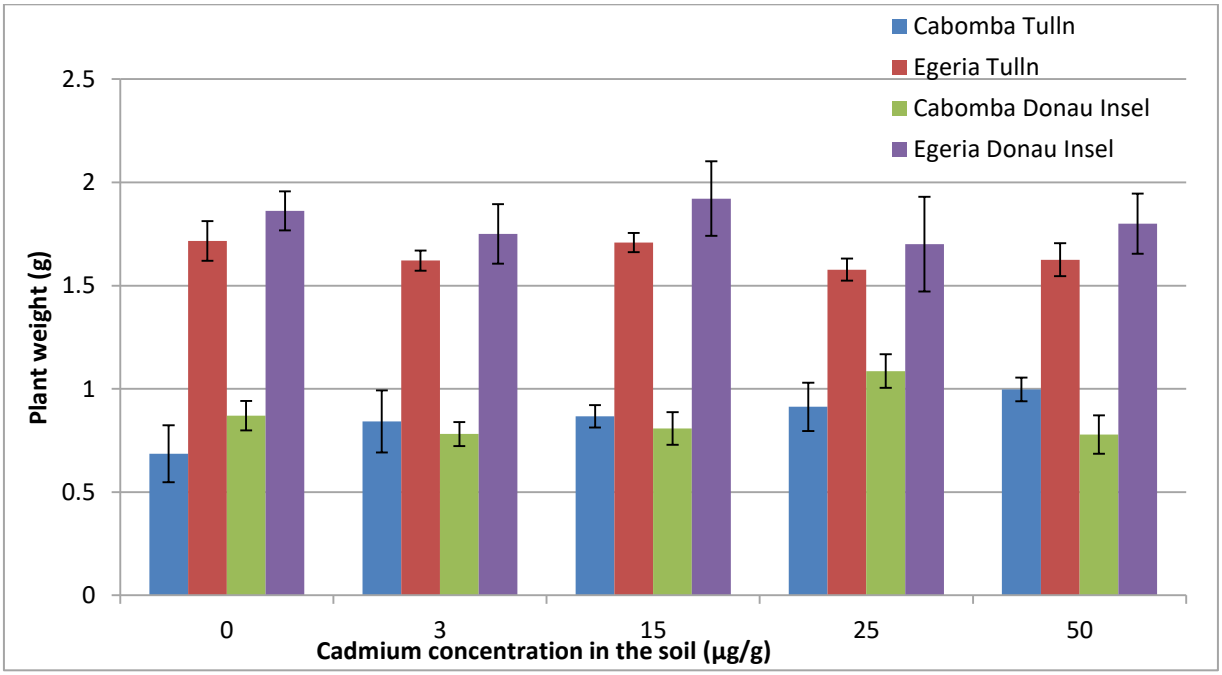

Figure 9. *Dry weight in grams of the plant material without roots. The weights of the plants is completely random and did not follow any percibable pattern for the exeption of Cabomba caroliniana growing in Tulln soil. Also there was nosignificant difference between the weights of any configuration.*

At the same time the only correlation found for all cases is between the above ground plant biomass and the plant roots (ρ = .814 significant at 0.01). This is the only correlation found for the soils as for Tulln soil the correlation exist between shoot weight and root weight (ρ = .945 significant at 0.01) and also for Donau Insel soil (ρ = .742 significant at 0.05). However no correlations between shoot weight and any other variable were found in the specific cases.

Figure 14 shows the root dry weight. Once again *Cabomba caroliniana* has a significantly smaller root biomass (p-value < 0.001) than *Egeria densa.* For all plants there was a correlation (ρ = .814 significant at 0.01) between the root weight and the plant weight.
The soil had no significant effect on the root weight (p-value = 0.428) but the concentration of cadmium in the soil did affect significantly the development of the rooting system in both species (p-value = 0.007)
The plant species and the cadmium concentration in the soil (p-value = 0.012) and between the soil type, the plant species and the Cd concentration in the soil (p-value = 0.037) that affect the root dry weight in both species. There was however, a significant interactions between the soil type and the cadmium concentration in the soil (p-value = 0.006).

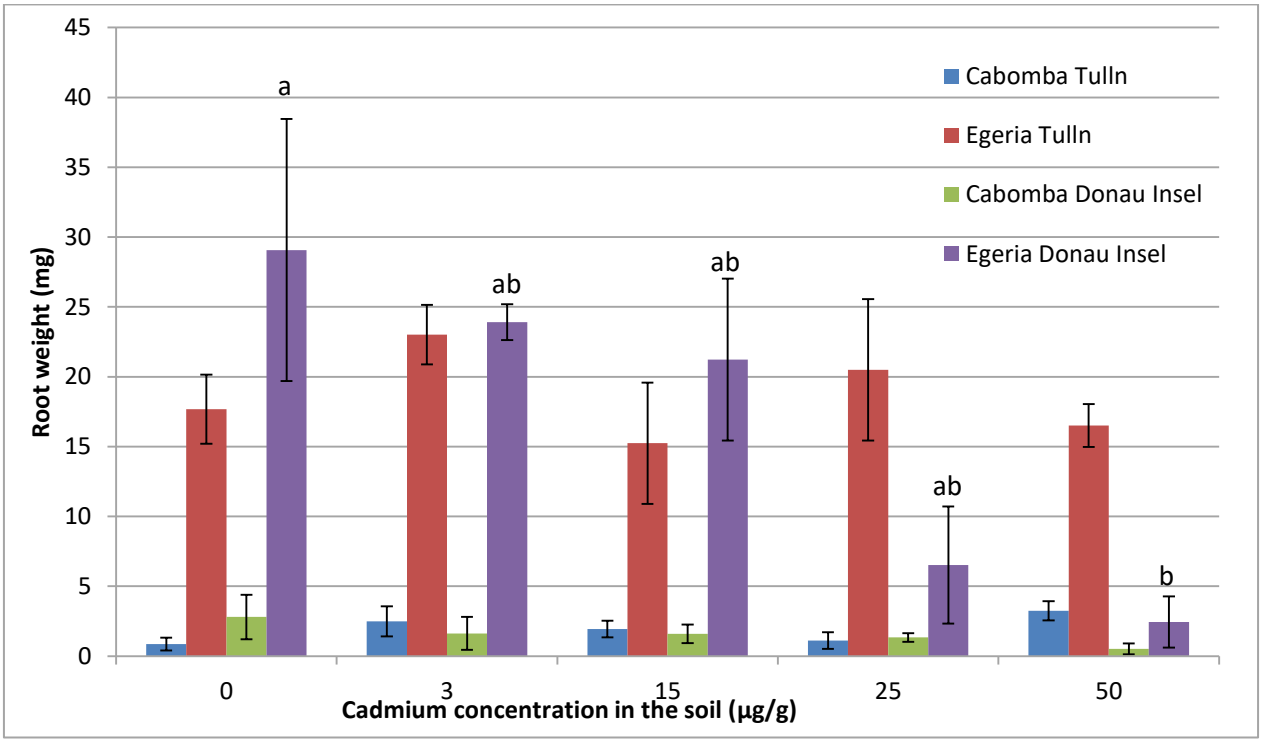

Figure 10. *Root weight in micrograms of the roots. The plants growing in Tulln soil did not follow any pattern but in the case of the plants frowing in Donau Insel soilthe weight the rooting system decreased with the increase of the cadmium concentration in the soil. The were only significantdifferences between the root weights of Egeria densa growing in Donau Insel soil. Post-hoc analysis was performed to find significant differences in the data.*

In the one-way ANOVA for *Cabomba caroliniana* when both cases are compared (Tulln and Donau Insel soil) the root dry weight of was not significantly affected by neither the cadmium concentration in the soil (p-value = 0.890) nor the soil type (p-value = 0.517) and no correlation was found between the root weight and other factors.
For *Egeria densa* the cadmium concentration in the soil did had a significant effect (p-value = 0.011) and correlated (ρ = -.701 significant at 0.05) to the root dry weight but the soil type did not (p-value = 0.496). There was also a significant interaction between the cadmium concentration in the soil and the soil type (p-value = 0.019) that affected *Egeria's* dry root weight. There was also

a correlation between the root weight and the Cd concentration in the plant (ρ = -.730 significant at 0.05) as well as with the Cd concentration in the water (ρ = -.780 significant at 0.01).

The plants growing in the Donau Insel soil showed a traceable trend. Both species *Cabomba caroliniana* (Figure 11) and *Egeria densa* (Figure 12) and have a decrease in their root dry weight as the concentration of cadmium increases in the soil. But only the trend followed by *Egeria densa* in Donau Insel soil is statistically significant (p-value = 0.008)

For *Cabomba caroliniana* and *Egeria densa* growing in Tulln soil no correlation was found between the root weight and any other variable. For *Cabomba caroliniana* in Donau Insel (Figure 11) soil the root weight correlated with the Cd concentration in the plant (ρ = -.909 significant at 0.05) and the Cd concentration in the soil (ρ = .938 significant at 0.01). For *Egeria densa* in Donau Insel soil (Figure 12) the root weight correlated only with the Cd concentration in the soil (ρ = -.936 significant at 0.05).

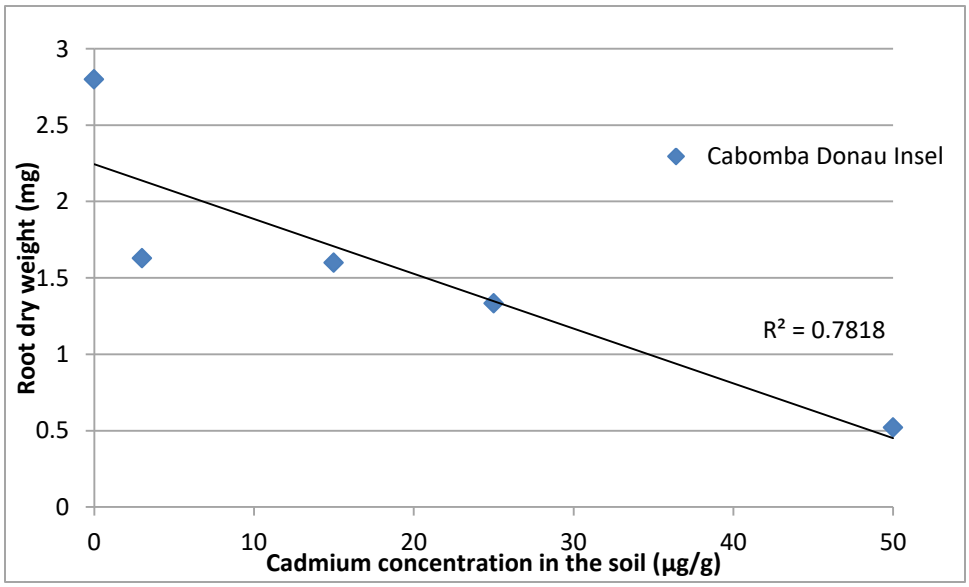

Figure 11. *Dry root weigh of Cabomba caroliniana growing in Donau Insel soil. The root weight decreases as the concentration of cadmium in the soil increases.*

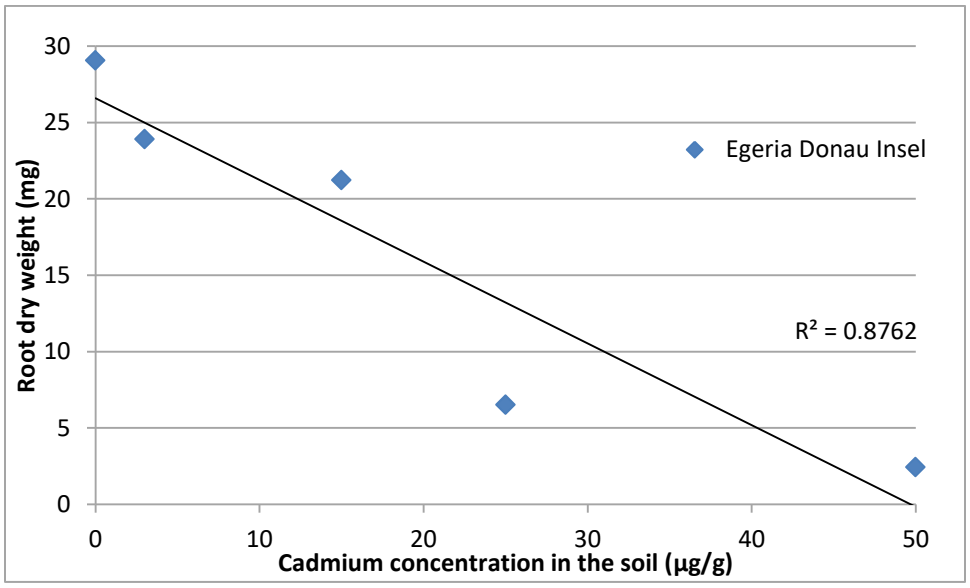

Figure 12. *Dry root weigh of Egeria densa growing in Donau Insel soil. The root weight decreases as the concentration of cadmium in the soil increases.*

At the same time the scanned roots gave extra information about the rooting systems. (Figure 13) In the case of the plants growing in Tulln soil there is no appreciable trend as both species follow an irregular pattern. In the case of Donau Insel soil however, the root length of both species followed a decreasing trend that is statistically significant for *Egeria densa* as well as for *Cabomba caroliniana.* (P-value = 0.004)

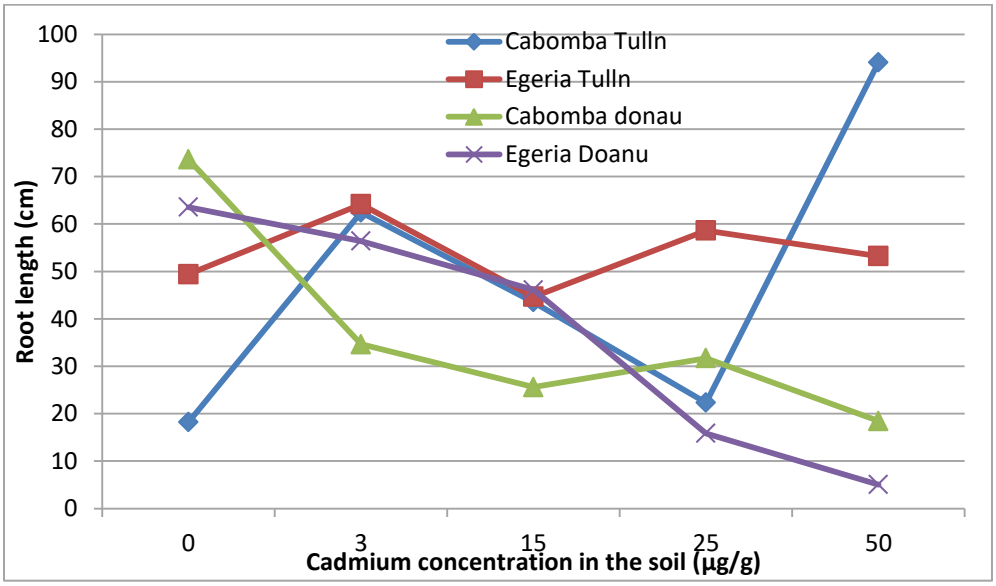

Figure 13. *Average root length compared to the cadmium concentration in the soil. In the case of Donau Insel soil the root length for both species was reduced as the concentration of cadmium increased.*

Plant Cd concentration

After 34 days all plants contained cadmium to some degree (Figure 14). There was an increase in the cadmium concentration in the above-ground biomass as the concentration in the soil

increased. There was a general correlation between the Cd concentration in the plants and the Cd concentration in the soil (ρ = .782 significant at 0.01) and also with the Cd concentration in the water (ρ = .870 significant at 0.01) (Figure 18).
Both species showed increment in cadmium concentrations but the species *Egeria densa* adsorbed more cadmium than *Cabomba caroliniana*. There was also a difference between the soils as the plants growing in Donau Insel soil adsorbed more cadmium than the plants growing in Tulln soil.
For *Cabomba caroliniana* there was a correlation between the Cd concentration in the plant and the Cd concentration in the soil (ρ = .811 significant at 0.01). For *Egeria densa* the same correlations were found (ρ = .884 significant at 0.01) and (ρ = .891 significant at 0.01) respectively the same as a correlation with the root weight (ρ = -.730 significant at 0.05).

A detailed table with the results can be found Annex 7.

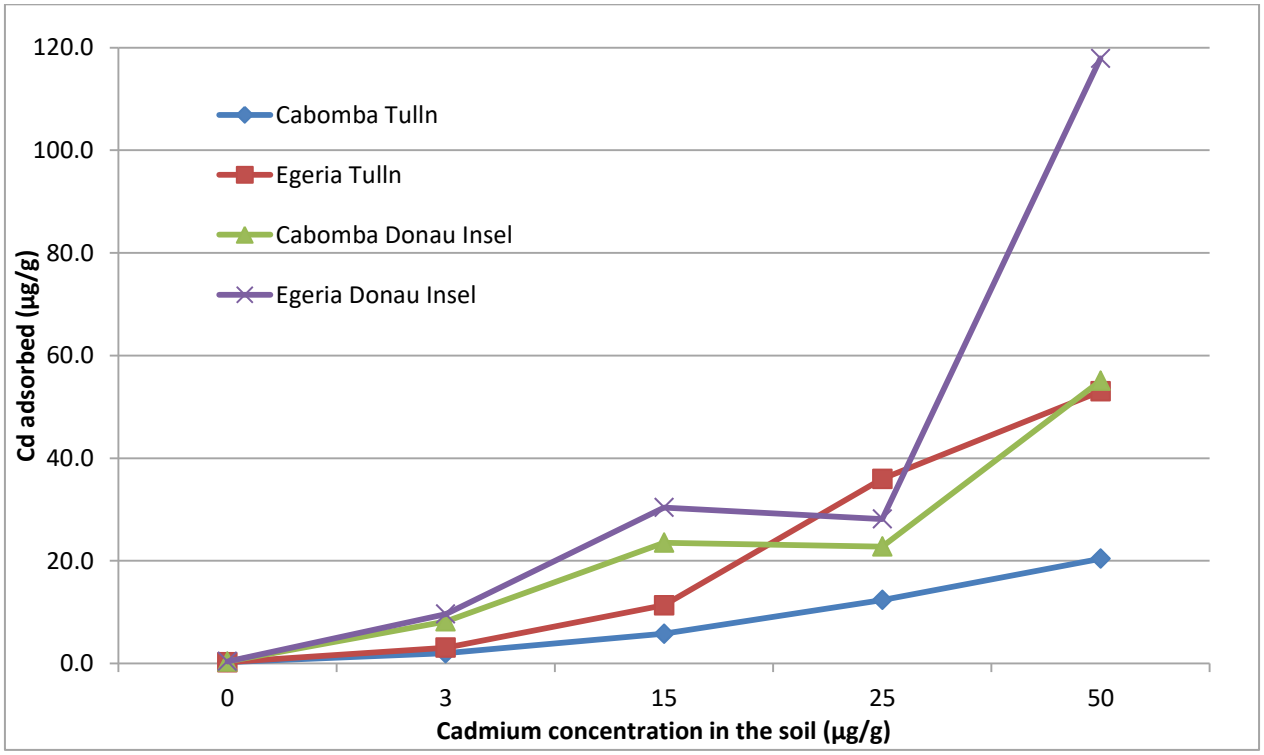

Figure 14. *Cadmium adsorption by all the different arrangements. Plants growing in Donau Insel soil adsorbed more cadmium than the plants growing in Tulln soil. At the same time there is a difference between the species as Egeria densa adsorbed more Cd than Cabomba caroliniana.*

Table 8. *The different configuration showing the amount of cadmium removed and the accumulation factor*

Species	Soil	Cd in soil (µg/g)	Total Cd adsorbed (µg)	% of Cd Removed	Accumulation Factor
Cabomba caroliniana	Tulln	0	0.18	0	0
	Tulln	3	1.67	0.24	0.57
	Tulln	15	4.88	0.16	0.37
	Tulln	25	11.28	0.22	0.48
	Tulln	50	20.35	0.20	0.40
Egeria densa	Tulln	0	0.39	0	0
	Tulln	3	4.99	0.77	0.95
	Tulln	15	19.34	0.63	0.74
	Tulln	25	55.27	1.10	1.43
	Tulln	50	87.70	0.87	1.06
Cabomba caroliniana	Donau Insel	0	0.33	0	0
	Donau Insel	3	6.39	1.02	2.60
	Donau Insel	15	19.02	0.62	1.54
	Donau Insel	25	24.72	0.49	0.90
	Donau Insel	50	42.90	0.43	1.09
Egeria densa	Donau Insel	0	0.73	0	0
	Donau Insel	3	16.86	2.70	3.08
	Donau Insel	15	58.89	1.94	2.00
	Donau Insel	25	47.86	0.94	1.11
	Donau Insel	50	212.21	2.12	2.35

Survival rate and phytotoxicity

Out of 100 plants involved in the experiment only seven perished. The seven plants that died all belonged to the same treatment: *Egeria densa* growing in Donau Insel soil. The survival rate for the other treatments was 100%. (Figure 15)

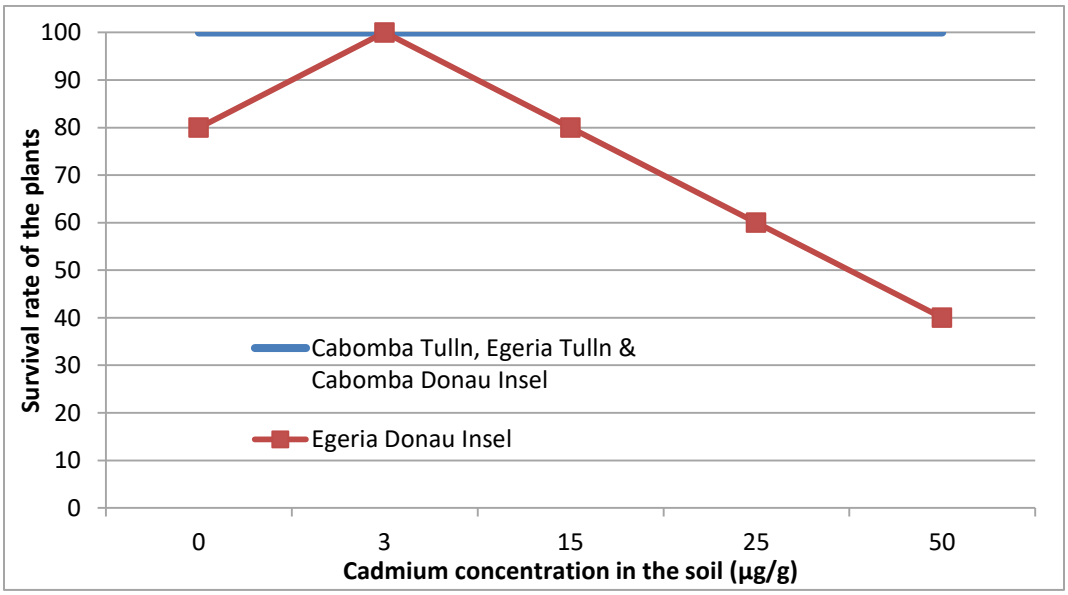

Figure 15. *Survival rate of the plants after 34 days growing in polluted soils with different cadmium concentrations.*

Water concentration

The cadmium levels in the water were measured after the experiment was complete to detect any cadmium that might have leached from the soil into the water.

The percentage of cadmium mobilized into the water at all times stayed below 0.12%. (Figure 16) There is a perceivable difference in the amounts leached by the different soils with the Donau Insel soil leaching more cadmium as the Tulln soil. At the same time there is a difference between the cadmium leached between both species growing in Donau Insel soil.

There is a correlation between the cadmium concentration in the water and the cadmium in the soil (ρ = .497 significant at 0.05) this means that the cadmium concentration in the water rises with the increase in the Cd concentration in in the soil.

Both species growing in Tulln soil followed almost the same trend as very low values of cadmium were leached into the water. The highest quantity of cadmium was leached by *Egeria densa* in Donau Insel soil as the concentration in the water rose to 8.0 µg/l (Figure 17).

In the case of *Cabomba caroliniana* and when both soils are taken into account the concentration of cadmium in the water only correlates to the cadmium concentration in the plant (ρ = .805 significant at 0.01) but there was no correlation with the Cd concentration in the soil.

In the case of *Egeria densa* and taking both soils into account there was a negative correlation between the Cd concentration in the water and the root weight (ρ = -.780 significant at 0.01) and two positive correlations to the Cd concentration in the soil (ρ = .638 significant at 0.05) and the Cd concentration in the plant (ρ = .891 significant at 0.01). This means that as the Cd concentration in the water increases the root weight decreases and as the Cd concentration in the soil and in the plants rises so does the Cd concentration in the water.

For the Tulln soil there was no correlation between the Cd concentration in the water and any other variable. But in the case of Donau Insel soil the behavior repeats as there was a correlation between the Cd concentration in the water and the concentration in the plants (ρ = .938

significant at 0.01) and the Cd concentration in the soil (ρ = .724 significant at 0.05). This means that in the case of Donau Insel soil the concentrations in the water rise with the increase of Cd concentrations in the plants and the soil.

For *Cabomba caroliniana* growing in Tulln soil there was no correlations between the Cd concentration in the water and any other factor. For *Egeria densa* growing in Tulln soil there was again a correlation between the Cd concentration in the water ant the Cd concentration in the plant (ρ = .919 significant at 0.05) and the Cd concentration in the soil (ρ = .938 significant at 0.01).

For the plants growing in Donau Insel soil there was a correlation for *Cabomba caroliniana* between the Cd concentration in the water and the Cd concentration in the soil (ρ = .879 significant at 0.05). For *Egeria densa* the Cd concentration in the water correlates with the Cd concentration in the plants (ρ = .980 significant at 0.01) and with the Cd concentration in the soil (ρ = .956 significant at 0.05)

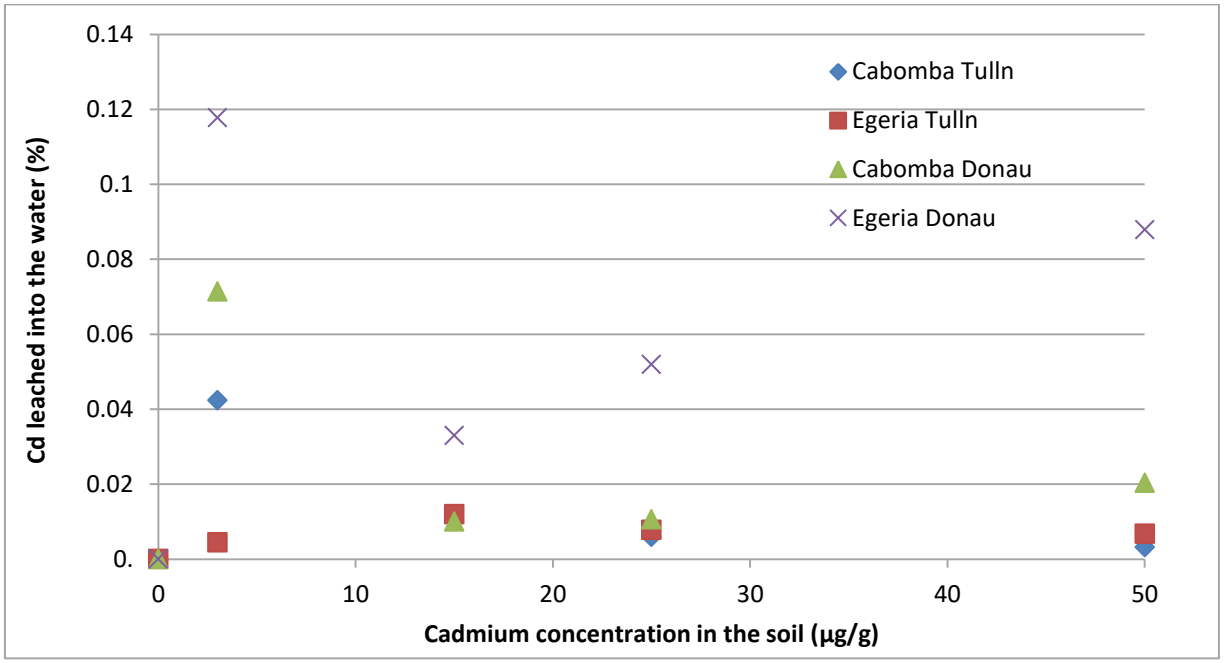

Figure 16. *Comparison between the percentages of cadmium leached into the water from the soil and the cadmium concentration in the soil. The highest percentages of cadmium leached come from Egeria densa growing in the Donau Insel soil.*

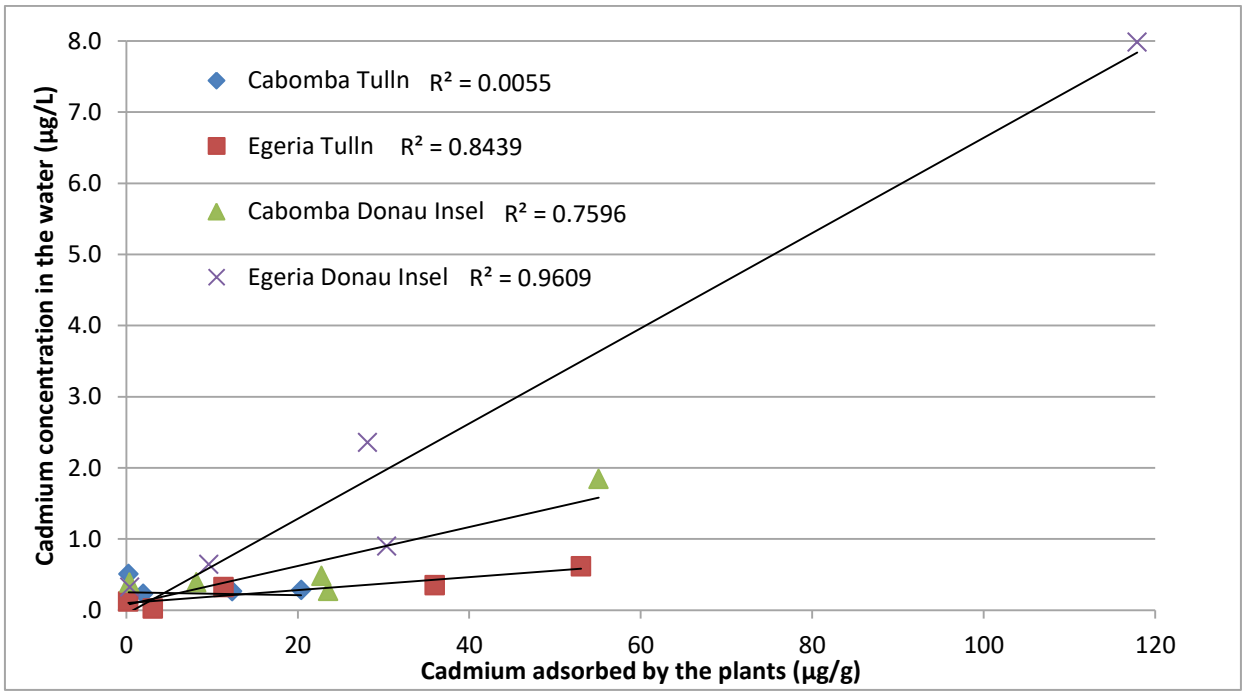

Figure 17. *Relation between the cadmium adsorbed by the plants and the cadmium leached into the water. Three behaviors are highly significant (Egeria densa in Tulln soil, Cabomba caroliniana in Donau Insel soil and Egeria densa in Donau Insel soil)*

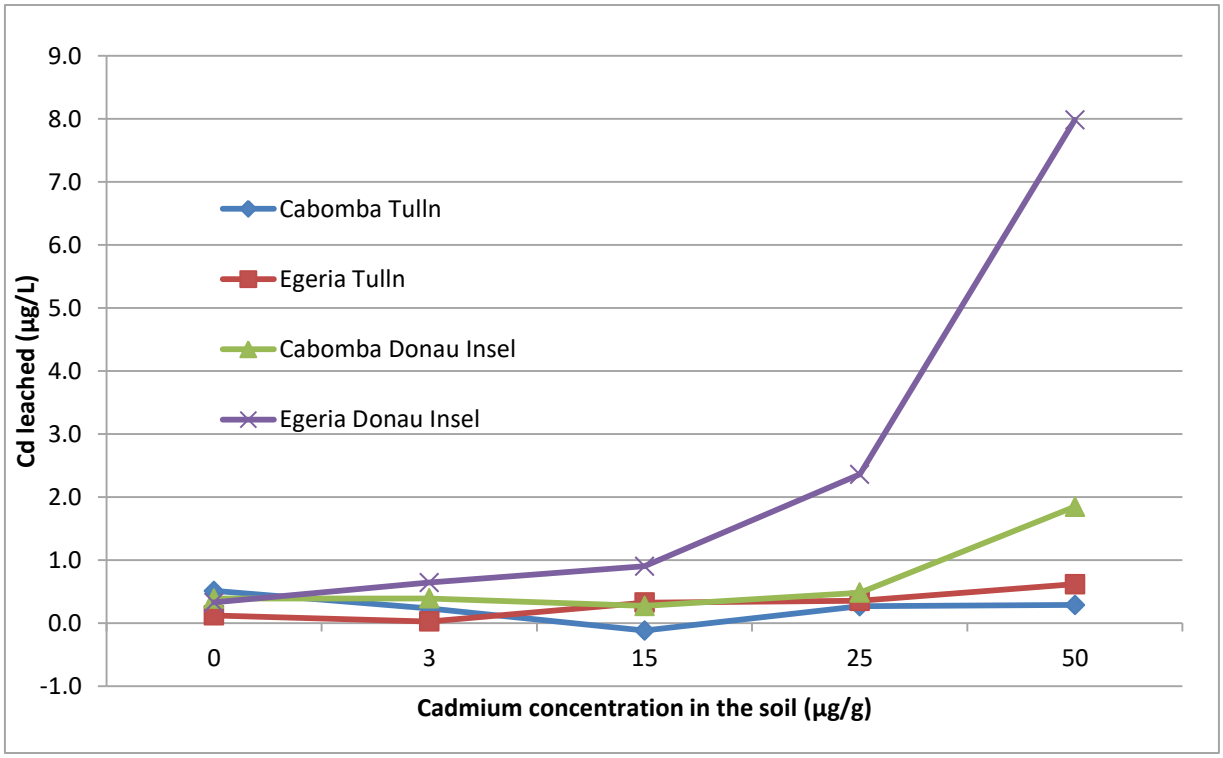

Figure 18. *Cadmium leached into the water from the soil. Cadmium leached from the Donau Insel soil but almost no cadmium was leached from the Tulln soil.*

There was also relationship between the survival rate of the plants and the amount of cadmium leached into the water. (Figure 19) The treatment with the lowest survival rate was also the treatment were the cadmium was leached into the water the most. With the lowest survival rate (40%) leaching 8.0µg/l

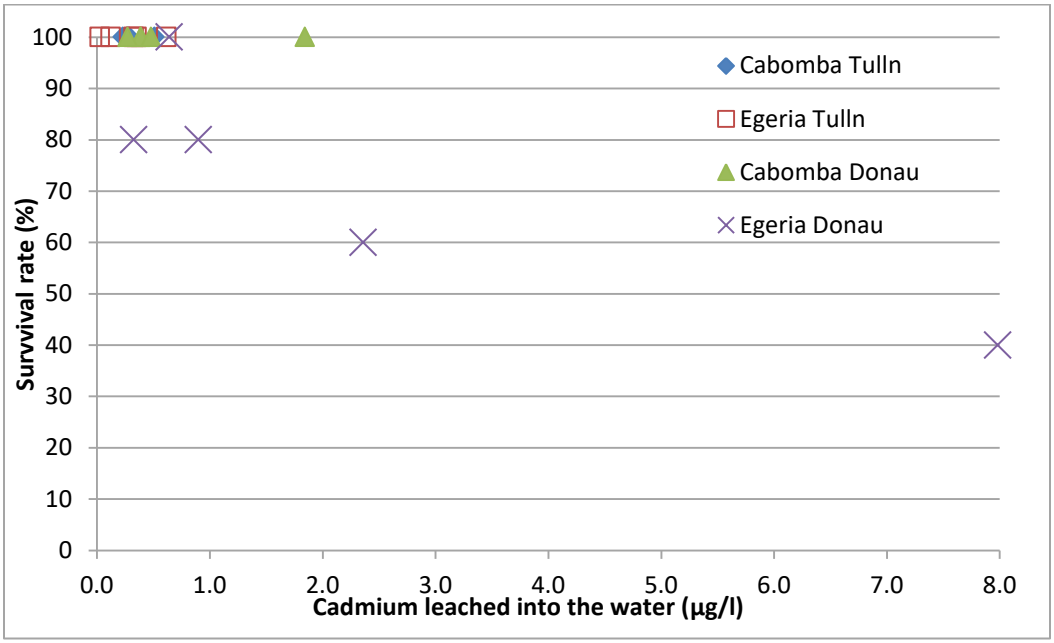

Figure 19. *Relation between the cadmium leached into the water and the survival rate of the plants. Egeria densa in Donau Insel soil was the configuration with the lowest survival rate and also the highest volume of cadmium leached into the water.*

DISCUSSION

What is the effect of Cd in the plant biomass allocation?

No correlation between the above-ground plant biomass and any other factor was found; for the exception of *Cabomba caroliniana* growing in Tulln soil. There the plant biomass increased as the concentration of cadmium increased in the plant and the soil. This result contradicts current knowledge; as cadmium is toxic to living organism and it does not normally promote development or growth (Malec et al., 2009). This suggests that this result might have happened by chance as the plants‘ size and weight was not the same at the beginning of the experiment.

Because the fresh weight of the plants was not recorded neither before nor after the experiment it was impossible to determine if the plants grew during the experiment. This data could have been used to determine if cadmium reduced the plant development to any degree. But it is known that Cd inhibits growth and causes growth reduction in plants (da Rosa Corrêa et al., 2006; Ladislas et al., 2011) which might have happened during the experiment.

Similar to the shoot there is a significant difference between the root weights of both species. The roots of *Cabomba caroliniana* are thin like human hairs while the roots from *Egeria densa* are thicker. This is also why the root weight correlates with the plant weight.

The root development was indeed affected by the cadmium levels and the soil type. In the Tulln soil were cadmium was less bioavailable the rooting system development was not affected. In the Donau Insel soil there are traceable trends that suggest that cadmium has actually an effect in the root development, as the higher the concentration in the soil the smaller the rooting system. This negative effect of Cd in root development has already been observed before (De Salvatore et al., 2008; Walter et al., 2006).

There is less evidence that suggests that the reduction of the rooting system had any effect on the cadmium adsorption, as in only one case there was a correlation. So, apparently the reduction in the rooting systems did not affect the ability of the plants to adsorb cadmium from the soil as the plants with the smallest rooting systems were the ones that adsorbed the most cadmium from the medium. This could be explained as the higher the concentration of Cd in the medium the higher the Cd concentration in the roots will be; which in turn increases the translocation factor (He et at., 2007). At the same time high Cd concentrations in the medium reduce root elongation (Chen et al., 2003).

Is there a significant difference in cadmium adsorption and phytotoxicity between species?

Egeria densa adsorbed more cadmium in both soils and thus it can be said that is it a more efficient phytoremediator than *Cabomba caroliniana*. At the same time there was no statistical difference between the means of the cadmium adsorbed between the two species. Besides this, the differences in the cadmium adsorbance are noticeable at high concentrations of 15, 25 and 50 µg/g which under our experiment conditions were are already phytotoxic for *Egeria densa*.

As for the behavior of the plants, there was an increase in the cadmium concentration in the plants as the concentration in the soil increases. (Malec et al., 2009; He et at., 2007). This suggests that the concentration adsorbed is highly proportional to the concentration in the soil for both plants and thus the statistical correlation. This relates to previous evidence as it has been

observed that the sediment heavy metal concentration is the main factor affecting the heavy metal concentration in submerged macrophytes (Jackson, 1998; Ladislas et al. 2011). This questions the paradigm that rooted macrophytes only adsorb heavy metals dissolved in the water column and adds evidence to say that rooted macrophytes actually adsorb the metals from the sediments the same way they adsorb nutrients such as phosphorous (P) and nitrogen (N) (Best and Mantai, 1978; Carignan and Kalff, 1979, 1980; Barko and Smart, 1981as cited by Jackson, 1998)

The behavior of *Egeria densa* is compatible to what was found by previous studies: the highest concentrations of heavy metals in the plant are found in the plants growing in soil with the highest heavy metal concentrations and the concentration in the plant increases as the concentration in the medium increases. (Malec et al., 2009; Harguiteguy et al, 2015; Jackson, 1998; Ladislas et al. 2011)

In the case of *Cabomba caroliniana* none of the specimens presented any visible damage which might suggest a higher tolerance to environments heavily polluted with cadmium as concentrations of 50µg/g did not seem to affect the plant at all. On the other hand *Egeria densa* showed already symptoms of phytotoxicity at concentrations of 15µg/g and it showed heavy symptoms of phytotoxicity and plant death at 50µg/g.

The sensitivity of *Egeria densa* to cadmium is a disadvantage as it means that even when this species can adsorb more cadmium it will have to be harvested before phytotoxicity causes damage to the plant as it will decay and liberate the cadmium into the water. This process would be similar to the nutrient spike experienced in the winter when macrophytes die and cause a rise of C, P and N in the surrounding water (Jackson, 1998). If this species is chosen to be used as a phytoremediator it should not grow for long periods of time in heavily polluted cadmium sediments to avoid phytotoxicity that can lead to the release of cadmium into the water body.

Are the species hyperaccumulators?

It is now possible to analyze the available information determine if both species behave as hyperaccumulators. Hyperaccumulators are the ideal phytoremediator as they can tolerate high concentrations of pollutants and adsorb high levels of pollutants and store them. (Agunbidae et al., 2009; Abu Bakar et al., 2013; Ali et al., 2013) As previously mentioned hyperaccumulators have four unique characteristics:

1. They should accumulate at least 100µg of cadmium per gram of dry weight. In other words they need to have concentrations of 100µg/g or higher. (Agunbidae et al., 2009; Abu Bakar et al., 2013)
2. Translocation of the pollutant. This means the concentration in the shoots should be greater than in the roots. (Ali et al., 2013)
3. The concentration in the plant should be higher than in the environment. That is measured with the Accumulation Factor (AF) obtained by dividing the concentration in the plant by the concentration in the soil and should be greater than one. (Ali et al., 2013)
4. Have a tolerance to cadmium levels that induce phytotoxicity to other plants. (Ali et al., 2013)

From all the essays only the *Egeria densa* growing in Donau Insel soil and a concentration of 50µg/g exceeded a concentration in the plant higher that 100 µg/g. The fact that only one arrangement surpassed the minimum does not give much evidence to support that *Egeria densa* is a hyperaccumulator nor has any other found study suggested this. Few evidence was found but other studies have found dry weight Cd concentrations of 2.1 – 3.4 µg/g (Mudrock and Capabianco, 1979 as cited by Malec et al., 2009) and 0.79 µg/g (Desy et al.,2002 as cited by Malec et al., 2009) in *Egeria densa* growing in flowing rivers and 70.25 mg/g for dead biomass (Pietrobelli et al, 2009).

In addition to this, the rooting systems were too small to get the minimum 20 mg needed to do an ICP measurement. Thus, it was impossible to obtain cadmium concentrations in the roots with the available equipment; which is also an important piece of information to determine the translocation factor (TF) to detect a hyperaccumulator.

On the other hand, the accumulation factor (AF) (Table 8) gave positive results for *Egeria densa* under all of the concentrations and for both soils. The concentrations in the plants exceeded at all times the concentration of cadmium from the soils they were growing on. On the other side for *Cabomba caroliniana* the results were inconsistent as the AF was less than one in the Tulln soil and higher than one in the Donau Insel soil, meaning that in only one case it reached a concentration in the plant greater than the concentration in the environment. These AF are slightly higher than average AFs found in rooted macrophytes growing in Cd polluted sediments (Jackson, 1998)

At the same time, even when the uptake of cadmium was positive by both species in all scenarios it is important to point out that the total Cd uptake was in the range of 0,64 to 2,81% for *Egeria densa* and 0,16 to 1,07% for *Cabomba caroliniana.*

Even when the plants adsorbed relatively high amounts of cadmium and followed a trending patter the results and the lack of information, such as the TF, do not give us enough evidence to classify them as hyperaccumulators. For this reason this study cannot confirm that *Egeria densa* and *Cabomba caroliniana* are cadmium hyperaccumulators.

Table 9. *Comparison between species for hyperaccumulation of cadmium*

	Cabomba caroliniana	***Egeria densa***
Accumulation of 100µg/g	No	Plausible
Translocation of cadmium	Unknown	Unknown
Accumulation factor (AF)	Inconsistent	Yes
High tolerance to cadmium	Yes	No

Water concentration

After the water extraction was performed it was clear that the soil could retain almost 100% of the cadmium applied to it. So, if there was any cadmium leaching from the soil into the water

during the experiment it would have had to be mobilized by the plant. This is the main reason why the cadmium levels in the water were measured after the experiment was complete.
The percentage of cadmium mobilized into the water at all times stays below the 0.12% which correlates with the results from the water extraction as the soils held at all times 99.8% or more of the cadmium applied to pollute it. There is a slight difference again with the amounts leached by the different soils with the Donau Insel soil leaching more cadmium as the Tulln soil, which once again relates with the results from the water extraction.
The highest quantity of cadmium was leached by *Egeria densa* in Donau Insel soil. The concentration in the water rose to 8,0µg/l. A possible explanation to this increase in the cadmium leached into the water might be due to the decaying plants. The scenario with the highest level of cadmium also has the lowest survival rate. As the cadmium concentrations in the Donau Insel soil increased *Egeria densa* showed heavy symptoms of phytotoxicity and the cadmium released into the water might have come from the leaves and stems of the dying plants. This is expected as around 25 to 30% of the Cd stored in the macrophyte tissue is lost into the water during the plant decay (Jackson 1998).
The general trend is that the cadmium leached into the water increases as the concentration of cadmium in the plants and in the soil rises. So, at higher concentrations more cadmium will be mobilized into the water. This is bad news for a possible phytoremediator as the objective is to retain the cadmium in the plant material and not to mobilize it into the water. But this can be explained as Cd causes chlorophyll reduction (Rai et al., 2003; Hou et al. 2007, Malec et al., 2009) which in turn causes plant decay (Wahid et al., 2009; Pivetz, 2001) and release of Cd into the surrounding water (Jackson, 1998).
Still the concentrations mobilized into the water are very low and they are not toxic to *Lemna minor* a plant that is commonly used as a test plant for heavy metal phytotoxicity. *Lemna minor* can tolerate concentrations of 4 mg/l without showing any symptoms of phytotoxicity (Khellaf & Zerdaoui, 2009) so this concentration should not be a toxic concentration neither for *Egeria densa* nor *Cabomba caroliniana.* This is confirmed for *Egeria densa* as Cd concentrations of 33.71 mg/l cause a 15% reduction in chlorophyll a after 7 days without the plants showing any signs of necrosis (Malec et al., 2009).

Soil sorption capacity

There was an evident difference between the cadmium absorption capacities of both soils. The soil from Tulln had a higher sorption capacity as in average 153.70% more cadmium was extracted from the Donau Insel soil. But by percent, this difference is not too great as the Tulln soil retained 99.9% of the cadmium while the Donau Insel soil retained 99.8% after the water extraction. The variance in soil sorption capacity can be attributed to three main differences between the soils:
The soil composition with the Tulln soil having 27% clay compared to just 16.4% of the Donau Insel soil. Usually, the sorption of cadmium and other heavy metals is strongly related to the percentage of clay minerals in the soil (Zachara and Smith, 1994; Spark et al., 1995 as cited by Choi, 2005). The difference in clay content will explain why cadmium was more difficult to

extract from the Tulln soil. In contrast, the composition of clay is what makes the difference in the bioavailability of cadmium. Donau Insel soil had a significantly less clay but this clay was mainly composed of smectite; a clay mineral with a high affinity for cadmium (Choi, 2005).
The pH, and once again the Tulln soil had a higher pH than the Donau Insel soil. It is important to remember that pH is one of the most crucial factors to determine the soil sorption capacity (Longanathan et al. 2012; Christensen, 1984; Sim et al., 2009). The higher the pH the higher is the capacity of the soil to adsorb and hold cadmium (Blume et al. 2016, Christensen, 1984). For that matter, Tulln soil has a higher sorption capacity and Cd would be less bioavailable.
The organic matter content, and for a third time, the Tulln soil exceeds the Donau Insel soil. The organic matter also plays an important role in the sorption capacity (Blom, 1974) and a soil with organic matter will have a higher sorption capacity than a soil without organic matter (Lin et al, 2007 as cited by Zhao et al., 2014). In the specific case of cadmium the adsorbed amount increases with the increase in organic carbon regardless of the absorbent type (Sim et al., 2009).
The difference in the soil sorption capacity and the soil characteristics (composition, pH and organic matter content) might have played a very important role in controlling how much cadmium was adsorbed by the plants, as the plants growing in Donau Insel soil adsorbed significantly more than the plants growing in Tulln soil. The evidence appears to tell us that the cadmium in Donau Insel was mainly bioavailable and the cadmium in Tulln soil was not.

CONCLUSION

A clear difference between the amounts of cadmium adsorbed by both species and the soil properties (soil composition, pH and organic matter content) influenced how much cadmium was bioavailable. Higher clay contents, higher pH and higher organic matter made the cadmium less bioavailable thus making the Donau Insel soil more phytotoxic in comparison to the Tulln soil. This explains why plants growing in Tulln soil had lower cadmium concentrations and no perceivable phytotoxicity effects such as changes in the root morphology and mass, plant discoloration and/or plant death.

Based on the collected evidence it difficult to conclude which species is more suitable for phytoremediation in aquatic ecosystems. However, *Cabomba caroliniana* has the advantages of high tolerance to cadmium without showing symptoms of phytotoxicity and it almost does not mobilize cadmium into the water. The disadvantages however, are that it did neither accumulate high levels of cadmium nor achieved a consistent accumulation factor. At the same time due to its lower biomass the amount of cadmium that it can store in its tissue is smaller than the amount *Egeria densa* can store.

In contrast, *Egeria densa* can store higher amounts of cadmium, has an accumulation factor that is consistent and greater than 1 and can reach high adsorption concentrations of up to 117.90µg/g. However, symptoms of phytotoxicity occur at Cd concentrations of 15µg/g and the cadmium it mobilizes increases as the phytotoxicity increases. So the high amounts adsorbed from the soil are mobilized into the water as the plant dies thus reducing the duration in which this plant can be left in polluted environments.

Therefore, with the available evidence it is not possible to know if the species are hyperaccumulators and at the same time they did not show any particular characteristics that make them exceptionally good for phytoremediation in aquatic ecosystems. While both species in fact did adsorb cadmium more studies are needed to reject the use of these plants as phytoremediators and/or to define under which conditions and timespans this macrophytes might be the best option for cadmium phytoremediation from flooded sediments.

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ANNEXES

Annex 1: Pre-Experiment to determine the stability of the microsystems

The aim of this experiment was to observe any possible algae growth in the microsystems or any other condition caused by the soil and water mixture that could affect the development of the aquatic macrophytes; and if this was the case, to take the adequate precautionary measures before the experiment.

It reproduced two scenarios: sterilized and not sterilized system. The sterilized environment intention was to reduce the algae growth as much as possible.

The test started on the 18.04.2016 and lasted until the 18.05.2016, or 56 days before the experiment.

As a first step, non-sieved soil, the same intended to be used in the experiment, was weighted and separated into two 405g batches. One batch of 405g was sterilized by autoclaving at 121°C for 15 minutes and then spent 45 more minutes in the autoclave until it had cooled down. The other 405g did not undergo any kind of sterilization process.

Afterwards eight PET cups with a volume of 400ml were prepared. Four of these plastic cups were first rinsed with tap water and then sterilized with acetone. The other four cups were just rinsed with tap water but not sterilized

The four sterilized cups were filled first with 100g of the autoclaved soil each and afterwards the other four non-sterilized cups were filled with 100g of non-sterilized soil each. The filling of the cups was done with a metal spoon rinsed with tap water and the sterilized with acetone.

As a final step the eight cups were filled with 200ml of tap water; which gave a total weight of 313g per cup. All the cups were then covered with a plastic foil to avoid evaporation and placed at the window of the laboratory of the Department of Forest Ecology (Waldökologie) of the BOKU.

Figure 20. *The eight cups at the first day of the experiment*

After 7 days (25.04.2016) no algae growth was observed in the microsystems and the water was still translucent. There was no water loss as the cups weighted the same as in the beginning.

At day 9 (27.04.2016) a white-brown sludge forming on top of the soil was observed. There was no significant difference between the sterilized and non-sterilized systems as the sludge formed in all of the eight (8) systems and the water was still transparent. The sludge was most possibly sedimentation of soil particles suspended when the cups were filled with water.

At day 14 (02.05.2016) the first clear differences between the systems were observed. Strings of green algae grew and bubbles formed at the bottom of the non-sterilized systems. The bubbles stayed at the bottom of the system, some suspended a centimeter above the soil and some floated to the surface. It‘s believed that the bubbles of gas formed due to organic matter decomposition under anaerobic conditions (anaerobic digestion). The sterilized systems remained with no apparent change, no algae growth and no bubbles formed.

At day 16 (04.05.2016) the amount of bubbles had decrease significantly maybe due to 2 days of cloudy skies and the systems lack of sunlight. The algae were present in the same amounts. There was still no observable change in the sterilized systems.

At day 30 (18.05.2016) the microsystems had not changed significantly compared to day 16. In the non-sterilized systems the algae growth had slightly increased and some bubbles were still present at the bottom, but effervescence was no longer observed. The sterilized microsystem remained unchanged: no algae growth neither gas formation was observed during or after the 4 weeks.

The pH of the water was measured at day 30. The measurement was done using a Hanna HI98128 pH meter with an accuracy of ±0,05 pH. The instrument was rinsed with deionized water (18,2 MΩ) after each measurement to avoid cross-contamination.
It was suspected that the water in the non-sterilized microsystems would acidify due to the anaerobic digestion that occurred in the soil. This was refuted by the measurements as no pH was below 7. The pH levels in all the microsystems were still within the acceptable range of 4,5 to 9,5 to allow plant growth (Wetzel 2001) as none of them surpassed a pH = 8,50
The pH of the tap water used (Vienna, Austria) to fill the microsystems is usually in the range of pH=7,83 this means that the sterilized systems acidified on average pH=0,23 and the non-sterilized systems alkalinized on average pH=0,58 (Table 10).

Table 10. *Results from pre-experiment 1*

Non-sterilized			**Sterilized**		
Replicate	pH	Temp.	Replicate	pH	Temp.
1	8,41	23,1 °C	1	7,49	23,3 °C
2	8,34	23,6 °C	2	7,34	23,0 °C
3	8,50	23,4 °C	3	7,61	23,4 °C
4	8,40	23,7 °C	4	7,95	23,2 °C

To prove if there was really a significant difference in the pH of the two systems a single-factor ANOVA was performed (Table 11)

Table 11. *Single-factor ANOVA for soil sterilization and non-sterilization*

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Processes	1,32845	1	1,32845	37,01277	0,000897	5,987378
Within Processes	0,21535	6	0,035892			
Total	1,5438	7				

The soil sterilization has indeed a significant effect on the final pH of the water after the experiment. For these reasons and after 30 days of observation *it was concluded that for the experiment the soil had to be autoclaved beforehand to avoid algae blooms and alterations in the microsystems* that could lead to plant death, changes in the cadmium sorption and mobilization, and acidification of the water among other possible complications.

Figure 21. *Comparison between non-sterilized soil (left) and sterilized soil (right)*

Annex 2: Urease Inhibition Test

The method was studied to verify if it was a feasible way to measure the levels of cadmium in the plants and the water during and after the experiment. It presented several advantages as it is has an extremely low cost compared to ICP measurement, it is fast so a high volume of samples can be analyzed in a short time and can be easily performed with basic lab equipment.

The method is based on the hydrolysis of urea into ammonia. Urease is the enzyme that catalyzes this reaction and its activity can be determined by the amount of urea hydrolyzed, some substances such as heavy metals strongly inhibit the enzyme (Wittekindt et al., 1996). In order to measure the amount of inhibition substance present in the medium, a solution with a defined amount of urea and urease is prepared. The final amount of ammonia is measured; if the final amount of ammonia is reduced then inhibitors are present (Wittekindt et al., 1996).

$$(NH_2)_2CO + H_2O \xrightarrow{urease} NH_3 + H_2NCOOH \rightarrow 2NH_{3(gas)} + CO_{2(gas)}$$
$$NH_{3(gas)} + H_2O \rightarrow NH_4^+ + OH^-$$

(Tisdale, 1985)

The methodology developed by Wittekindt et al. in their paper *A Microtiter-Plate Urease Inhibition Assay-Sensitive, Rapid and Cost-Effective Screening for Heavy Metals in Water*. The solutions were prepared according to the specifications given in the paper and the process followed with detail.

Preparation of materials

First 10ml of urease stock solution with a concentration of 2.5g/l was prepared. Urease from Jack Beans was used to obtain a concentration of 200,000 U/l. The solution was then separated in 200 µl Eppendorf tubes and stored at -20°C

Next the pH-12 buffer was prepared using 30g of trisodiumphosphate, 30g of sodiumcitrate and 3g of Ethylenediaminetetraacetic acid (EDTA) then Milli-Q water was added ad 1L solution.

The urea solution was prepared at 50% (weight/volume) using 12.5g of urea dissolved in 25 ml of Milli-Q water.

Acetate buffer (0.2M, pH = 5.0) was prepared using sodium acetate

Reagent-A was also prepared by dissolving 6g of phenol in 70mL of the pH-12 buffer previously prepared. Afterwards 0.02g of sodium nitroprusside was added to the solution. In the end pH-12 buffer was added until the total solution was 100ml.

Reagent-B was prepared by mixing 16g of sodiumhydroxide and 7.0ml of sodiumhypochloride and adding Milli-Q water until the total volume of the solution reached 1L.

Then the ammonia test solution was prepared by adding 10mg of ammonia to 1L of Milli-Q water resulting in a concentration of 10mg/l

To finish with the solutions a cadmium solution with a concentration of 1000mg/l was prepared using CdCl2. Cadmium chloride was used because it is soluble in water and acetone. (U.S. Department of Health and Human Services, 2014) The CdCl2 used was 98% pure and it is composed of 61.32% cadmium and 38.68% chloride, then 416.02mg were added to 250ml to achieve the 1000mg/l concentration.

Cadmium stock solution
CdCl2 = 183.314g/mol and 98% pure Cd = 112.414 g/mol Cl = 35.45g/mol

$$\%Cd = \frac{112.414 g/mol}{183.314\ g/mol} x100 = 61.32\%$$

$$\%Cl = \frac{(\frac{35.45g}{mol})(2)}{183.314\ g/mol} x100 = 38.68\%$$

$$Amount\ of\ CdCl2 = \frac{1000mg\ of\ Cd\ per\ Liter}{(.6132)(.98)} = 1664.07mg\ of\ CdCl2\ per\ Liter$$

To 250ml of Milli-Q water then we add ¼ of that amount (416.02mg of CdCl2) to obtain a 1000mg/l concentration.

Preparation of enzymes and samples

Urease test solution was prepared approximately 15 minutes before starting by adding 8.0µl to 50ml of Milli-Q water resulting in a concentration of 0.4µg/l and not 0.4mg/l as stated in the paper. This error in the paper caused a lot of confusion in the process and delayed the use of the method as the error has to be found before the method could be used.

Diluent solution was prepared by using 5.0ml of urea-solution, 2.0ml of acetate buffer and then adding Milli-Q water until the total volume reached 250ml of solution.

Microplate essay

To perform the essay the 96 hole plate was divided into zones as shown in Figure 22. The rows A to D were used for the ammonia calibration concentration test and the rows E to H for the inhibition test. The ammonia background concentration test would be then used to relate the wave length obtained in the inhibition test with the actual ammonia concentration and thus be able to calculate the amount of inhibition caused by the test concentrations.

100µl of diluent solution was given to all wells from rows A to H and lanes 1 to 11. Afterwards 200µl of ammonia stock solution were given to the wells in rows A to D in lane 12 and 200µl of cadmium stock solution were given to the wells in rows E to H in lane 12.

After this, with the aid of a multichannel pipette, logarithmic dilution on the basis of 2 was performed by transferring 100µl of the stock solutions from right to left; starting at lane 12 and finishing at lane 3.

The wells in rows A to D and lanes 1 and 2 were used as reagent blacks; this means no ammonia was added. In the case of wells rows E to H lanes 1 and 2 they were used as the negative control for the inhibition test. In other words, no cadmium was added to obtain 0% inhibition or full hydrolysis of urea into ammonia.

Because no urease was going to be needed in the zone of the background concentration, the volume was substituted with 50µl of Milli-Q water in each well of lanes 1 to 12 and rows A to D.

The reaction was then started by adding 50µl of urease test solution to all wells in lanes 1 to 12 rows E to H and the plate was then incubated at 25°C for 15 minutes to allow the reaction to take place.

After the incubation period the reaction had to be stopped. This was done by adding 60µl of Reagent-A and then 90µl of Reagent-B to all wells. Then, the microtiter was again incubated for 1 hour at room temperature to allow the color to develop.

After the hour had gone by the microplate was read with the aid of a BioRad xMark™ Microplate Absorbance Spectrophotometer at 630µl.

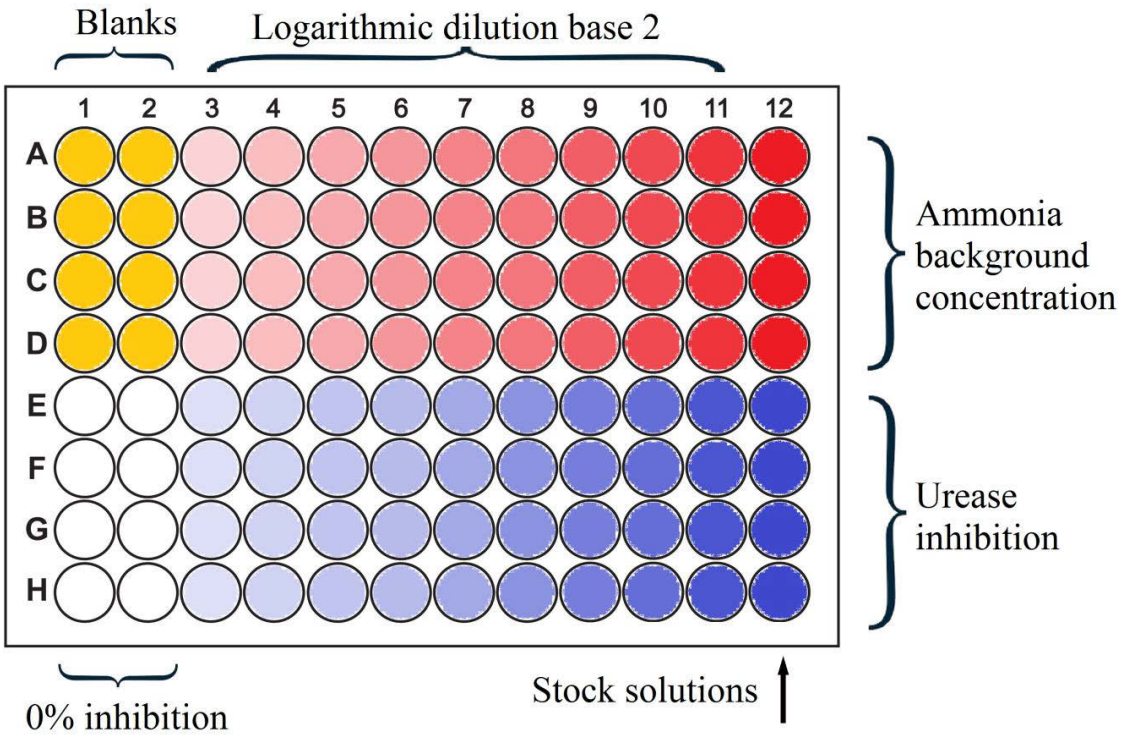

Figure 22. *Microtiter essayshowing the highest concentrations of ammoinia in red and the cadmium stock solution in blue. The fading color indicates the dilution.*

The method was tested for two weeks and failed to obtain any valuable results. The results were curves were no valuable information could be obtained (Figure 23). The values of the different cadmium concentrations exceeded the values of the wells with 0% inhibition and followed no predictable path. It was also impossible to relate the values of the spectometry due to the fact that one reflection value was given by three or sometimes four different cadmium concentrations.

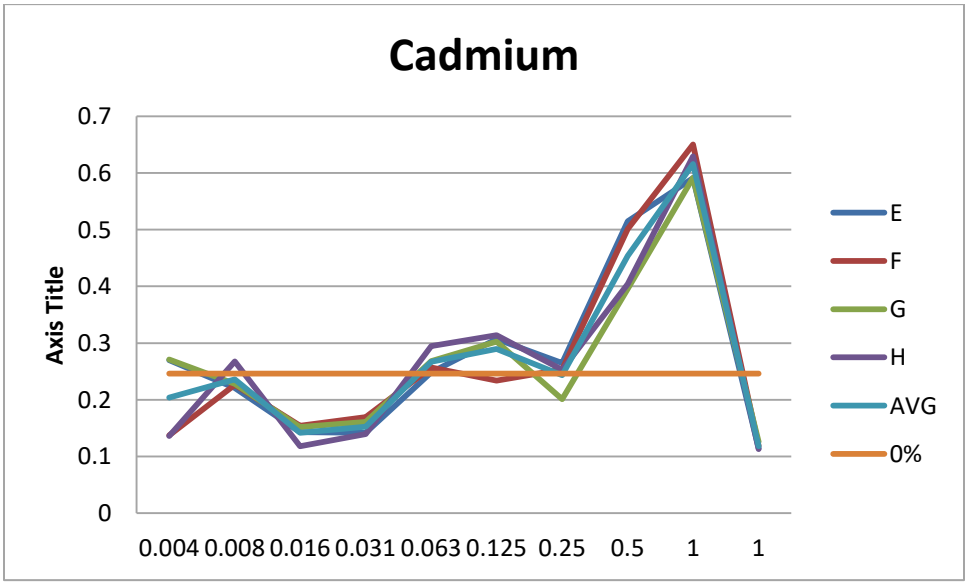

Figure 23. *A typical curve obtained during the first two weeks of the esay.*

For this reason it was concluded that the method had an error. Most probably an error in the units that lead to an error in the concentrations of the solutions. To detect the error several test were carried of to play with the different variables and compare its results.
The first step to detect the error was to review te paper to verify that the recipe was followed accordingly. The urea solution was re-done as it was the cheepest to remake and the method was retryed but it lead to no results. It was then observed that the urease solution might not be actually working as many of the wells staid transparent. This meant that no reaction was taking place.
The paper stated that 10ml of urease stock solution with a concentration of 2,5 g/l were prepared and then separated in 20µl alicots. Afterwards the solution was diluted by adding 8,0µl to 50ml resulting in a concentration of 0,4mg/l this prooved to be false as:

$$\frac{2{,}5\ \text{g}}{\text{L}} \times \frac{\text{L}}{1000\ \text{ml}} \times 10\ \text{ml} = 0{,}025\ \text{g}\ (25\ \mu\text{g})$$

$$\frac{25\mu\text{g}}{10\ \text{ml}} = 2{,}5\mu\text{g/ml}$$

$$\frac{2{,}5\mu\text{g}}{\text{ml}} \times 0{,}008\ \text{ml} = 0{,}02\ \mu\text{g}$$

$$\frac{0{,}02\ \mu\text{g}}{50\ \text{ml}} = 0{,}0004\ \mu\text{g/ml}$$

$$\frac{0{,}0004\mu\text{g}}{\text{ml}} \times \frac{1000\ \text{ml}}{\text{L}} = 0{,}4\ \mu\text{g/L}$$

This gave another hint to suspect of the urease stock solution. At the same time it was noticed that the method (Wittekindt et al, 1995) used urease from jack beans with 88 U/mg protein and MW 480.000. The urease available had a concentration of 15 to 50 U/mg this meant that the urease solution that was being used was also around 5,87 times weeker that needed. A new urease stock solution was prepared to match the one of the method (88.000 U/l)
To deteect the error a comparison was made which played with three variables: urease concentration, strength of the solution and the concentration of cadmium. Two runs were made, the first one using a initial cadmium concentration of 1 mg/l and the second with a concentration of 1000 mg/l
The comparisons were done in a microplate with the lanes A and B having a urease concentration of 0,4µg/l using the old stock (37.500 U/l), lanes C and D having a urease concentration of 0,4mg/l using the old stock (37.500 U/l), lanes E and F having a urease concentration of 0,4µg/l

using the new stock (88.000 U/l) and finally lanes G and H having a urease concentration of 0,4mg/l using the new stock (88.000 U/l)

After the first comparison was made it was clear that urease concentrations in the range of mg/l were not only wrong but gave no valueable information at all. In the tests were concentrations of urease in the range of µg/l were used it was noticed that a slight curve was formed below 0,5 mg/l.

The second test only confirmed this when it was observed that the esay done with the new stock and using a concentration of 0,4µg/l followed a tracable path (Figure 24) that started at a high inhibition when the cadmium concentration was 1000 mg/l and tended thoughwards a smaller inhibition when the cadmium concentration was lowered. This indicated that the method was working.

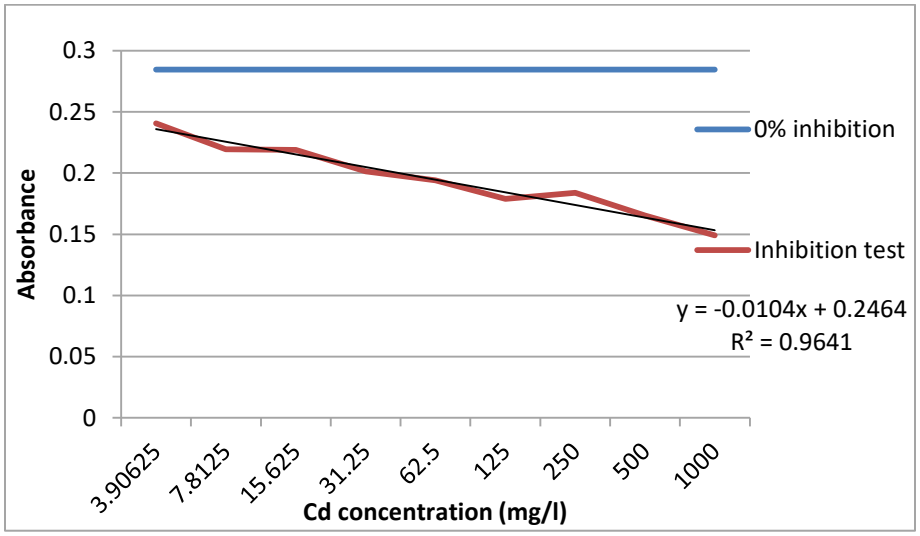

Figure 24. *Second comparison gave results with the new stock (0,4 ug/l)and cadmium concentration of 1000mg/l*

After obtaining results that could be traced a third essay was made to test the method under this conditions. The new urease stock solution was used (88U/mg) diluted to 0,4µg/l and the cadmium concentration at 1000mg/l. This time all the lanes were prepared the same way and an average of the results was made and graphed. (Figure 25)

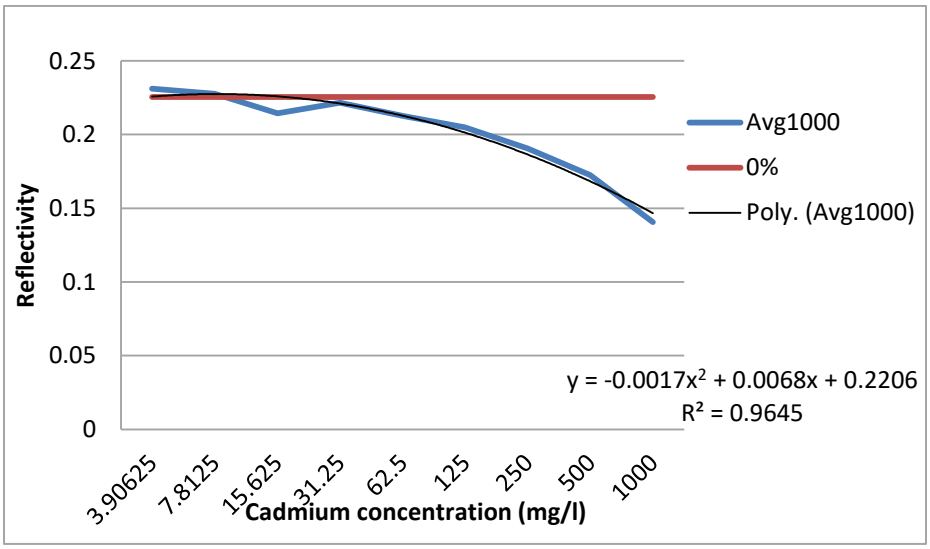

Figure 25. *Results from the urease inhibition test working*

The test gave results that relate to the results found by Wittekindt et al. According to their study the method has a detection limit for cadmium of ca. 20mg/l which correlates with the graph and values of the essay as below 15,625 the values are almost the same and is difficult to distinguish. The urease inhibition test (UIT) was no longer used because the cadmium concentration in the water was below the detection limits of the UIT.

Annex 3: Results from the soil sorption capacity test

Table 12. *ICP measurement of water-extracted cadmium for Tulln soil.*

Soil concentration (µg/g)	Cd detected by ICP (µg/l)	µg per 20ml	µg per 10ml	% extracted	% in soil
0	0	0	0	0	0
5	0.2558	0.005	0.003	0.051	99.95
10	0.7949	0.016	0.008	0.079	99.92
20	1.5115	0.030	0.015	0.076	99.92
40	3.8206	0.076	0.038	0.096	99.90
80	7.2560	0.145	0.073	0.091	99.91
160	14.3808	0.288	0.144	0.090	99.91

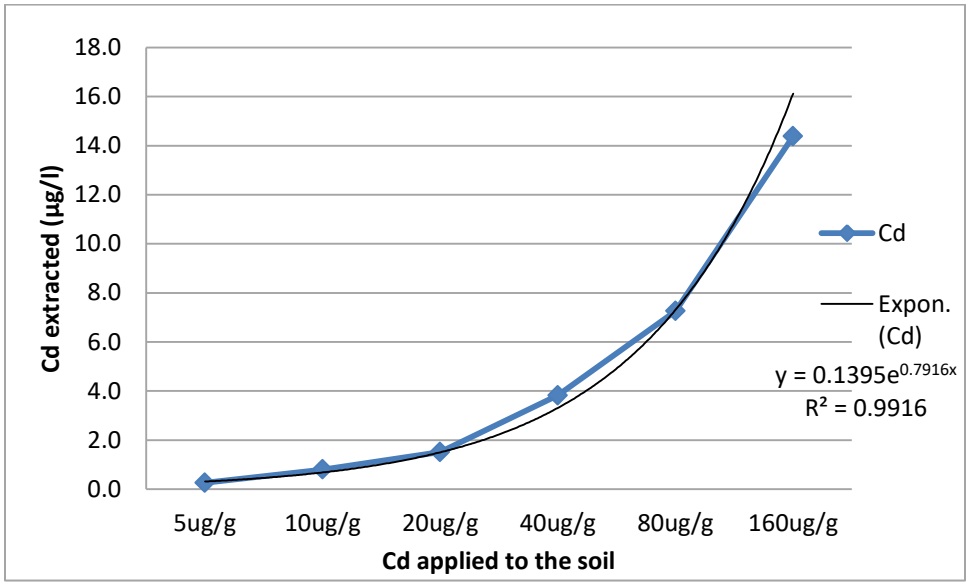

Figure 26. *Relation of cadmium extracted in µg/l to the cadmium applied to the soil for the Tulln soil.*

Table 13. *ICP measurement of water-extracted cadmium for Donau Insel soil.*

Soil concentration (µg/g)	Cd detected by ICP (µg/l)	µg per 20ml	µg per 10ml	% extracted	% in soil
0	0	0	0	0	0
5	1.0134	0.020	0.010	0.203	99.80
10	2.1772	0.044	0.022	0.218	99.78
20	3.2553	0.065	0.033	0.163	99.84
40	6.8570	0.137	0.069	0.171	99.83
80	17.7485	0.355	0.177	0.222	99.78
160	30.5891	0.612	0.306	0.191	99.81

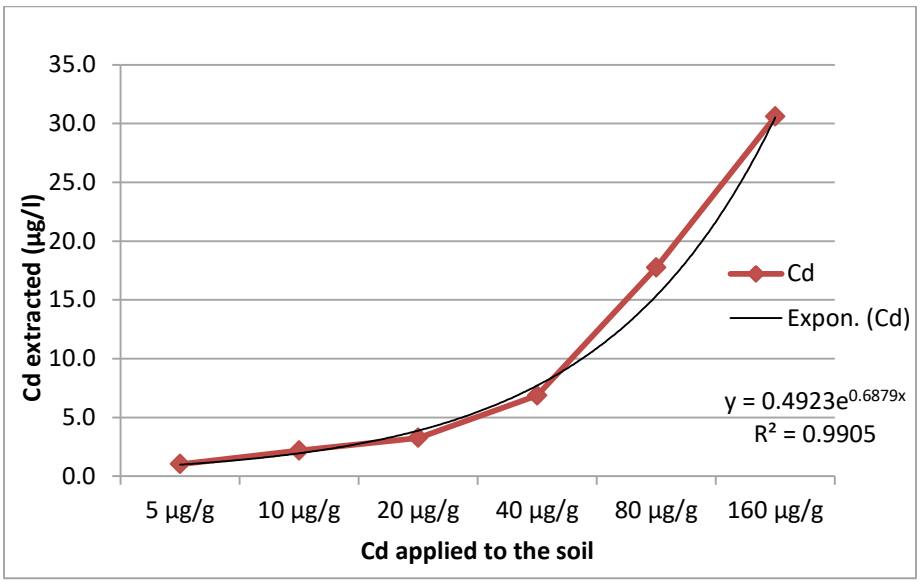

Figure 27. *Relation of cadmium extracted in µg/l to the cadmium applied in to the soil in µg/g for the Donau Insel soil.*

Table 14. *Differences in the soil sorption capacities of both soils used for the experiment*

Soil concentration (µg/g)	Extracted from Tulln (µg/l)	Extracted from Donau Insel (µg/l)	Difference rate	Difference %
5	0.256	1.013	3.96	296.15
10	0.795	2.177	2.74	173.90
20	1.511	3.255	2.15	115.37
40	3.821	6.857	1.79	79.48
80	7.256	17.749	2.45	144.61
160	14.381	30.589	2.13	112.71
		Average	2.54	153.70

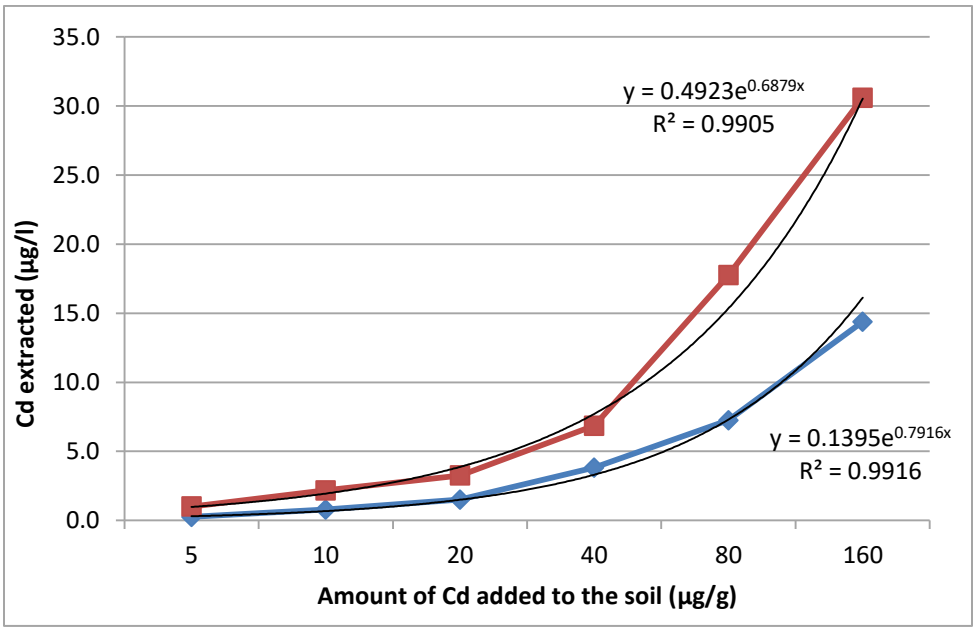

Figure 28. *Relation of cadmium extracted in µg/l to the cadmium applied in to the soil in µg/g for the both soils. The red line being Doanu Insel and blue line is Tulln*

Annex 4: Soil properties

Table 15. *Grain size percentage for the different soils used.*

	Tulln	**Donau Insel**
2000.0	100.1	100.0
630.0	98.2	98.7
200.0	90.6	92.6
63.0	69.4	64.9
20.0	47.7	44.6
6.3	39.3	29.5
2.0	27.0	16.4
0.6	15.7	7.8
0.2	6.8	2.3
CS	1.9	1.3
MS	7.6	6.1
FS	21.2	27.7
CU	21.7	20.3
MU	8.4	15.1
FU	12.3	13.1
CT	11.3	8.6
MT	8.9	5.5
FT	6.8	2.3

Table 16. *Detailed description of the different soil fractions acronyms used for the detailed soil fraction description.*

CS	coarse sand	< 2000 - 630 µm
MS	medium sand	< 630 - 200µm
FS	fine sand	< 200 - 63 µm
CU	coarse silt	< 63 - 20 µm
MU	medium silt	< 20 - 6.3 µm
FU	fine silt	< 6.3 - 2 µm
CT	coarse clay	< 2 - 0.63 µm
MT	medium clay	< 0.63 - 02 µm
FT	fine clay	< 0.2 µm

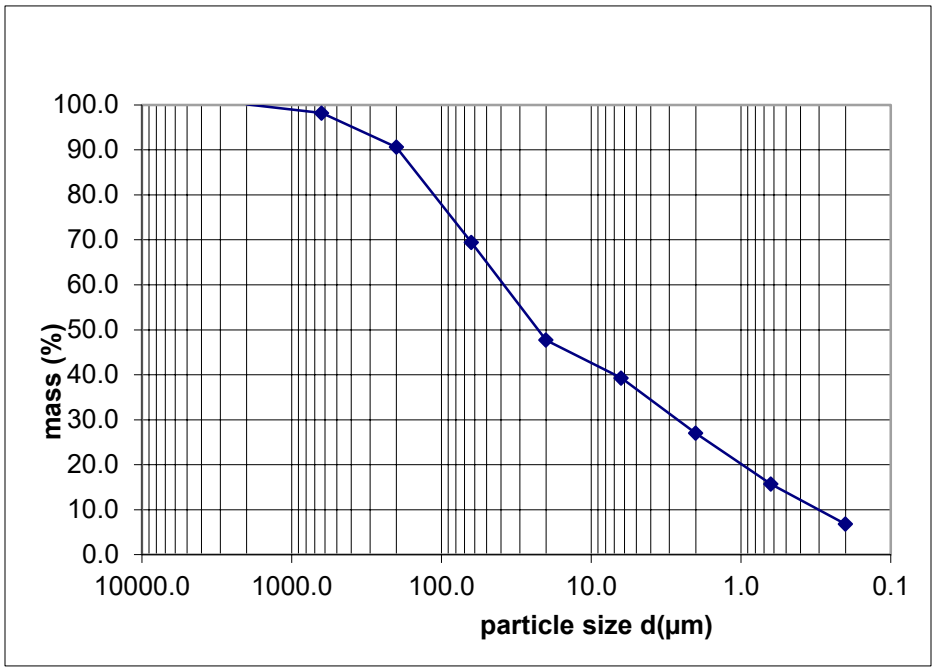

Figure 29. *Tulln soil grain size cumulative sum*

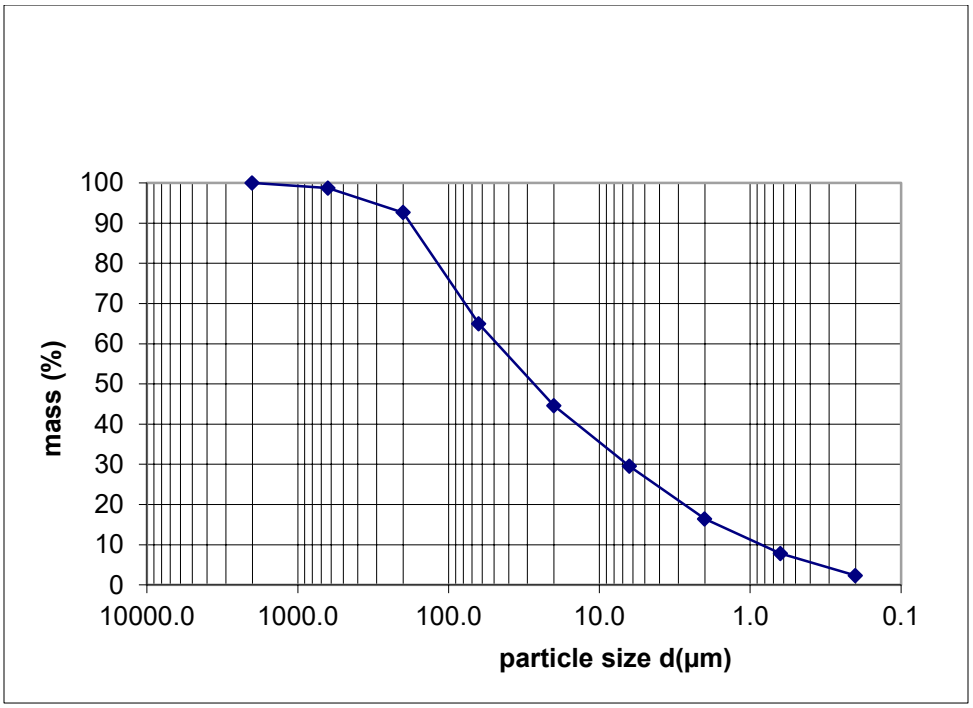

Figure 30. *Donau Insel soil grain size cumulative sum*

Annex 5: Water concentration

Table 17. *Cadmium leached into the water by all the different configurations (pooled results)*

Species	Soil	Soil concentration (µg/g)	Total Cd in soil (µg)	Leached (µg/l)	Total leached (µg)	Percent leached (%)
Cabomba caroliniana	Tulln	0	0	0.509	0.560	NA
		3	600	0.231	0.254	0.042
		15	3000	-0.120	-0.132	-0.004
		25	5000	0.267	0.293	0.006
		50	10000	0.286	0.314	0.003
Egeria densa	Tulln	0	0	0.122	0.134	NA
		3	600	0.024	0.027	0.004
		15	3000	0.325	0.358	0.012
		25	5000	0.352	0.388	0.008
		50	10000	0.616	0.677	0.007
Cabomba caroliniana	Donau Insel	0	0	0.389	0.428	NA
		3	600	0.389	0.428	0.071
		15	3000	0.272	0.299	0.010
		25	5000	0.481	0.529	0.011
		50	10000	1.845	2.029	0.020
Egeria densa	Donau Insel	0	0	0.326	0.359	NA
		3	600	0.642	0.707	0.118
		15	3000	0.900	0.990	0.033
		25	5000	2.359	2.595	0.052
		50	10000	7.984	8.783	0.088

Annex 6: Plant weight, root weight and root morphology.

Table 18. *Weight in grams of dry plant material (roots not included). A code was given to every microsystem to avoid confusing the samples. Every configuration had 5 replicates hence the numbering from 1 to 5.*

Cabomba / Tulln		Egeria / Tulln		Cabomba / Donau Insel		Egeria / Handelsaki		
Code	**Weight (g)**	**Code**	**Weight (g)**	**Code**	**Weight (g)**	**Code**	**Weight (g)**	
A1	0.5382	F1	1.4946	K1	0.8510	P1	1.7354	
A2	0.9633	F2	1.9738	K2	0.6727	P2	1.8431	
A3	0.2340	F3	1.7394	K3	0.8402	P3	1.7780	
A4	0.7333	F4	1.5066	K4	0.8684	P4	1.7245	
A5	0.9580	F5	1.8672	K5	1.1169	P5	2.2295	Dead
B1	1.2025	G1	1.6827	L1	0.8784	Q1	1.1968	
B2	0.8465	G2	1.7486	L2	0.7091	Q2	1.9472	
B3	0.2967	G3	1.6015	L3	0.9488	Q3	1.9816	
B4	0.8741	G4	1.4552	L4	0.7364	Q4	1.7599	
B5	0.9930	G5	1.6194	L5	0.6333	Q5	1.8666	
C1	0.9447	H1	1.6524	M1	0.6329	R1	2.5783	Dead
C2	0.9500	H2	1.7836	M2	0.7320	R2	1.6010	
C3	0.9583	H3	1.5662	M3	0.7125	R3	1.6006	
C4	0.6910	H4	1.8262	M4	1.0840	R4	1.8182	
C5	0.7911	H5	1.7126	M5	0.8781	R5	2.0094	
D1	0.9211	I1	1.5900	N1	1.2080	S1	1.9341	
D2	0.9467	I2	1.5934	N2	0.8919	S2	2.4400	Dead
D3	0.5533	I3	1.5599	N3	0.8826	S3	1.5971	
D4	1.2900	I4	1.4040	N4	1.2300	S4	1.4342	Dead
D5	0.8533	I5	1.7389	N5	1.2170	S5	1.0973	
E1	1.0387	J1	1.7554	O1	0.8949	T1	1.9735	
E2	0.9798	J2	1.8384	O2	0.8171	T2	1.7281	
E3	1.0309	J3	1.6262	O3	1.0400	T3	1.9024	Dead
E4	1.1436	J4	1.3950	O4	0.6180	T4	2.1195	Dead
E5	0.7939	J5	1.5122	O5	0.5240	T5	1.2760	Dead

***Table 19**. Weight in mg of roots. NR= no rooting system was developed. A code was given to every microsystem to avoid confusing the samples. Every configuration had 5 replicates hence the numbering from 1 to 5.*

Cabomba / Tulln		Egeria / Tulln		Cabomba / Donau Insel		Egeria / Donau Insel		
Code	**Weight (mg)**	**Code**	**Weight (mg)**	**Code**	**Weight (mg)**	**Code**	**Weight (mg)**	
A1	N.R.	F1	16.75	K1	3.11	P1	47.36	
A2	0.39	F2	20.09	K2	0.31	P2	14.35	
A3	N.R.	F3	12.92	K3	8.87	P3	46.23	
A4	2.07	F4	12.72	K4	0.91	P4	37.42	
A5	1.85	F5	25.96	K5	0.80	P5	N.R.	Dead
B1	6.35	G1	21.15	L1	1.30	Q1	20.84	
B2	0.54	G2	22.31	L2	0.56	Q2	25.40	
B3	0.40	G3	30.58	L3	6.28	Q3	23.11	
B4	2.30	G4	17.56	L4	N.R.	Q4	28.08	
B5	2.83	G5	23.48	L5	N.R.	Q5	22.15	
C1	2.53	H1	N.R.	M1	N.R.	R1	N.R.	Dead
C2	N.R.	H2	25.16	M2	4.02	R2	19.95	
C3	1.73	H3	12.01	M3	0.92	R3	24.95	
C4	1.82	H4	19.41	M4	1.43	R4	34.48	
C5	3.60	H5	19.61	M5	1.63	R5	26.76	
D1	N.R.	I1	24.15	N1	1.98	S1	22.65	
D2	3.24	I2	1.03	N2	1.05	S2	N.R.	Dead
D3	1.26	I3	26.70	N3	2.12	S3	5.99	
D4	N.R.	I4	29.48	N4	0.56	S4	N.R.	Dead
D5	1.03	I5	21.12	N5	0.96	S5	3.98	
E1	2.20	J1	14.06	O1	0.61	T1	9.50	
E2	4.06	J2	18.93	O2	N.R.	T2	2.70	
E3	5.53	J3	21.05	O3	1.99	T3	N.R.	Dead
E4	2.54	J4	12.83	O4	N.R.	T4	N.R.	Dead
E5	1.89	J5	15.68	O5	N.R.	T5	N.R.	Dead

Table 20. *Root characteristics obtained after scanning the roots and processing the images*.

Species	Soil	Cd in the soil (µg/g)	Avg. length (cm)	Avg. surf area (cm2)	Avg. diam (mm)	Avg. vol. (cm3)
Cabomba	Tulln	0	18.2361	1.40062	0.14534	0.0086
Cabomba	Tulln	3	62.42688	5.07326	0.25654	0.0328
Cabomba	Tulln	15	43.49184	3.18246	0.1859	0.0186
Cabomba	Tulln	25	22.3343	1.59762	0.13616	0.0092
Cabomba	Tulln	50	94.07112	7.36686	0.24658	0.046
Egeria	Tulln	0	49.39894	10.35032	0.65894	0.1732
Egeria	Tulln	3	64.17034	13.96268	0.69364	0.2424
Egeria	Tulln	15	44.64436	9.53162	0.53946	0.1622
Egeria	Tulln	25	58.63784	13.32602	0.71322	0.242
Egeria	Tulln	50	53.22464	11.1815	0.66874	0.1872
Cabomba	Donau Insel	0	73.59224	5.76384	0.25174	0.0362
Cabomba	Donau Insel	3	34.62714	2.986	0.15716	0.0206
Cabomba	Donau Insel	15	25.58544	1.94896	0.1938	0.0116
Cabomba	Donau Insel	25	31.68592	2.47458	0.24708	0.0154
Cabomba	Donau Insel	50	18.44768	1.47658	0.10282	0.0094
Egeria	Donau Insel	0	63.54994	14.03354	0.55328	0.247
Egeria	Donau Insel	3	56.40938	12.28876	0.6957	0.2136
Egeria	Donau Insel	15	46.10558	9.84254	0.5432	0.1678
Egeria	Donau Insel	25	15.84122	3.11958	0.35718	0.0492
Egeria	Donau Insel	50	5.04166	0.9933	0.24744	0.0156

Annex 7: Plant concentration

Table 21. *Cadmium adsorbed by all the different configurations (pooled results)*

Plant	Soil	Cd µg/g	Code	Cd adsorbed
Cabomba caroliniana	Tulln	0	A	0,269 µg/g
	Tulln	3	B	1,984 µg/g
	Tulln	15	C	5,803 µg/g
	Tulln	25	D	12,353 µg/g
	Tulln	50	E	20,405 µg/g
Egeria densa	Tulln	0	F	0,233 µg/g
	Tulln	3	G	3,079 µg/g
	Tulln	15	H	11,332 µg/g
	Tulln	25	I	35,964 µg/g
	Tulln	50	J	53,029 µg/g
Cabomba caroliniana	Donau Insel	0	K	0,380 µg/g
	Donau Insel	3	L	8,185 µg/g
	Donau Insel	15	M	23,539 µg/g
	Donau Insel	25	N	22,767 µg/g
	Donau Insel	50	O	55,087 µg/g
Egeria densa	Donau Insel	0	P	0,393 µg/g
	Donau Insel	3	Q	9,633 µg/g
	Donau Insel	15	R	30,377 µg/g
	Donau Insel	25	S	28,146 µg/g
	Donau Insel	50	T	117,904 µg/g

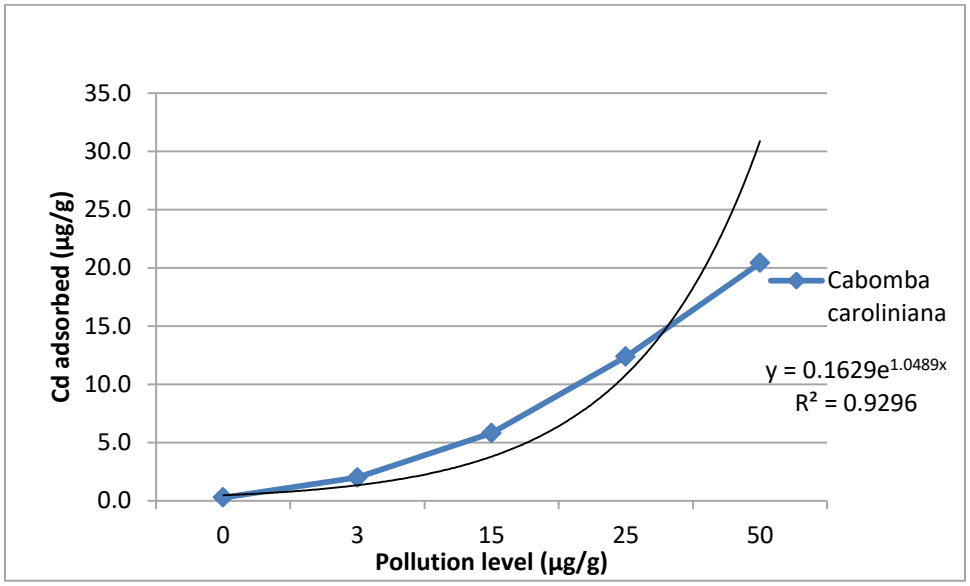

Figure 31. *Cadmium adsorption by Cabomba caroliniana growing in Tulln soil.*

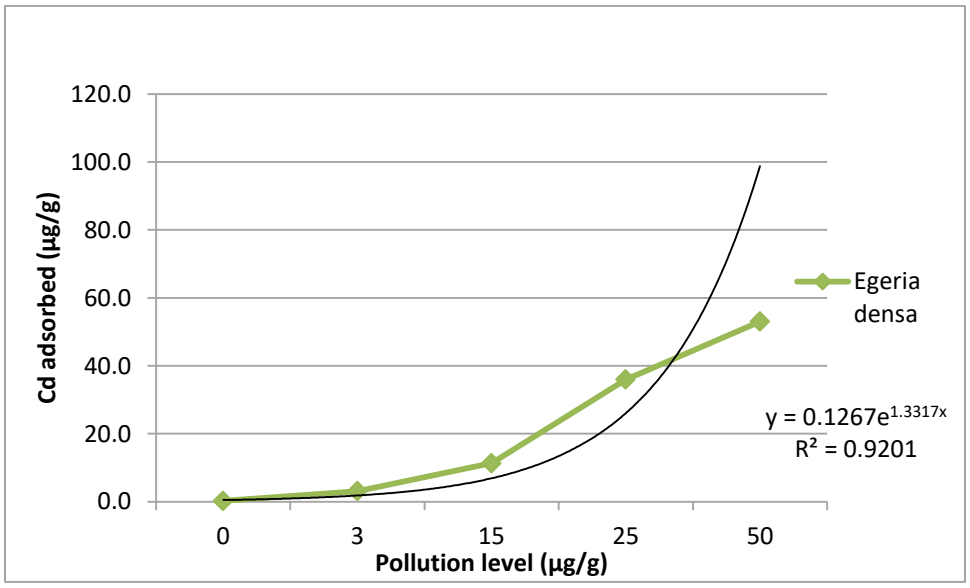

Figure 32. *Cadmium adsorption by Egeria densa growing in Tulln soil.*

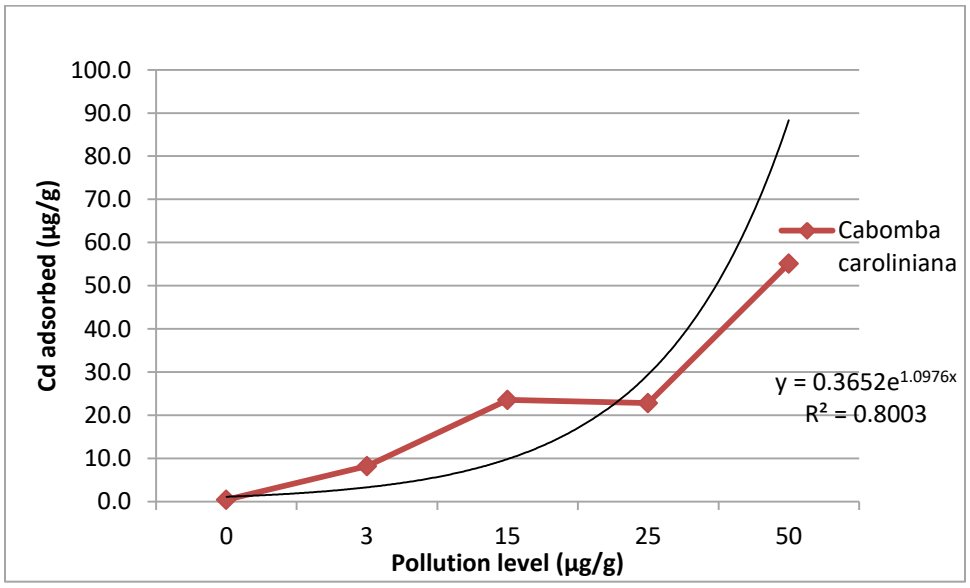

Figure 33. *Cadmium adsorption by Cabomba caroliniana growing in Donau Insel soil*

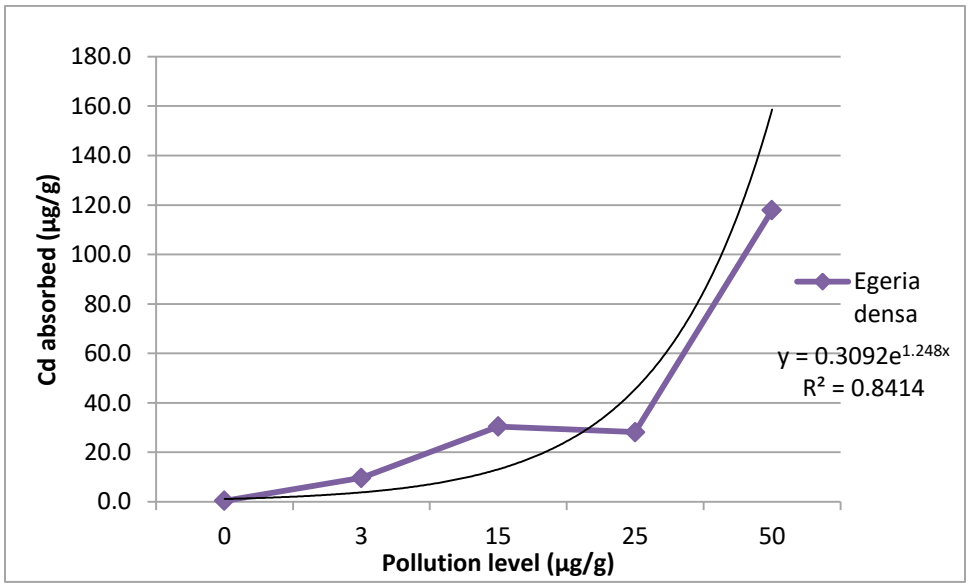

Figure 34. *Cadmium adsorption by Cabomba caroliniana growing in Donau Insel soil*

Annex 8: Experiment Record

14.07.2016 The experiment started when microsystems were completed with the planting of the 100 plants and they were filled with water.

19.07.2016 After five days in the microsystems the plants showed no signs of phytotoxicity. The water temperature was below 30°C and photosynthesis was observed as both species were bubbling. On this date the plants were moved around for the first time to avoid some pots getting more light than others.

21.07.2016 All the specimens were alive and making photosynthesis. None of them showed apparent signs of phytotoxicity. The water was refilled to compensate for evaporation.

25.07.2016 Some of the microsystems had algae growth. In three of the algae was so much that it completely covered the water surface. The cause of the algae bloom it is not known as the soil was autoclaved. Algae might have entered the microsystem through the tap water or inoculation through soil carried by wind.

01.08.2016 almost all of the microsystems had algae growth to some degree. The algae growth was very think in 5 or 6 microsystems of which they all had 25 or 50 µg/g cadmium. It might be an effect of the plants dying.

18.08.2016 Harvest day. What was thought it was algae was instead dead biomass that separated from the plants after the cadmium uptake caused phytotoxicity to some degree.

Annex 9: Photos of the experiment set up

Figure 35. *The PETE bottles with the soil at the greenhouse of the BOKU campus in Tulln an der Donau*

Figure 36. *The foil tunnel in the BOKU campus in Tulln an der Donau*

Figure 37. *Microsystems in the foil tunnel*

Figure 38. *The rooting system of Egeria densa on the harvest day*

Figure 39. *Egeria densa showing symptoms of phytotoxicity with yellow tips*

Figure 40. *Egeria densa showing heavy simptoms of phytotoxicity at 50ug/g. Only two plants survived out of five.*