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The potential of phytoremediation with Salix on pyrite ash from Skutskär paper mill.

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ABSTRACT

Abstract

Soil quality is essential for healthy and resilient ecosystems that can provide plants and animals with necessary nutrients, and soils free from contaminants are crucial for future food production. Yet the development of new methods for remediation of contaminated soil is low prioritised. Current methods, such as excavation, incineration and landfill dumping, are damaging the soil environment and structure. The problem with contaminated sites is global and growing and the need for more sustainable, cheap and less harmful methods for remediation of these sites is therefore alert. One method that has been highlighted lately is phytoremediation where pollutions in water, air or soil are removed with green plants (Pilon-Smiths, 2005). Salix, part of the willow family, is one of the most promising species for the method.

In this study the potential of phytoremediation with Salix was tested on pyrite ash from Skutskärs mill in central Sweden. The ash is a residue from the production of sulphuric acid where pyrite ore (Fe_2S) is burned. Since the ore always is contaminated with other minerals than pure pyrite the ash contains metals such as As, Cd, Co, Cr, Cu, Ni, Pb and Zn. The overall aim of this thesis was to examine the potential of phytoremediation with Salix to clean the contaminated ash. This was done by examining Salix tolerance and accumulation ability in a pot experiment during the summer 2014. Four different clones of Salix were planted in three different substrates, pure pyrite ash (PA), pyrite ash mixed with bark in evenly big parts (PAB), and for control normal S-soil was used (R). The plants height was measured approximately each 10^{th} day to see the growth development. After three months the plants were harvested and separated in leaves, shoots and roots and metal and nutrient analyses of the plant parts were performed.

The results showed that the pyrite ash inhibited the growth of all clones and from the metal analyses it was shown that the metal concentrations in the plants that had grown in one of the pyrite ash substrates (e.g. PA, PAB) were significantly higher than in the reference clones. However, these plants also suffered from deficit of phosphorus and for plants that had grown in the PAB the concentrations of nitrogen were remarkably low. Due to this it cannot be said if the poor growth depended on toxicity of the substrate or lack of nutrients. Possibly it was a result from both. Plants grown in PAB had higher roots mass than plants grown in PA, this indicates that the bark affected the plants positively. However, despite the bigger root mass the plant growth in height was not improved and the plants did not extract metals more effectively.

Results from metal analyses in the reference showed that Salix do have potential for remediation of Zn and Cd. Further studies are recommended on how Salix could work out to stabilise the material and inhibit the metals to leach out. More knowledge is also required for how elements interact with each other and are affected of processes in the soil.

Keywords: phytoremediation, Salix, metals, contaminated soils, pyrite ash, extraction, accumulation, tolerance

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REFERAT

Hälsosamma jordar med god balans av närings- och mineralhalter är avgörande för stabila och välfungerande ekosystem. Det bidrar till att markorganismerna, som står för nedbrytning och mineralisering, kan vara effektiva och i slutändan är rena jordar nödvändiga för en hållbar matproduktion. Trots det går utvecklingen av nya saneringsmetoder långsamt. Aktuella metoder som lagring på deponier och förbränning innebär stora ingrep i markmiljön som kan skada befintliga marksystem och struktur. Problemet med förorenade områden är växande globalt och behovet av hållbara, skonsamma och billiga saneringsmetoder är stort. En sådan metod som fått växande uppmärksamhet under de senaste åren är fytoremediering, där mark, luft och/eller vatten renas med gröna växter (Pilon-Smiths, 2005). En av de mest lovande växterna för ändamålet är Salix som tillhör videsläktet.

I den här uppsatsen undersöks om fytoremediering kan vara ett alternativ till att rena kisaska från Skutskärs bruk. Askan är en restprodukt från förbränning av svavelkis(Fe_2S) som tidigare var ett steg under produktionen av svavelsyra. Den ursprungliga malmen var aldrig ren svavelkis utan alltid förorenad av metaller som As, Cd, Co, Cr, Cu, Ni, Pb and Zn, vilket fick till följd att också kisaskan fick ett högt innehåll av dessa metaller. Det övergripande syftet med uppsatsen var att undersöka potentialen hos Salix för att rena kisaskan. I ett krukväxtförsök undersöktes salix ackumuleringsförmåga och tolerans mot den metallförorenade askan. Fyra kloner av salix (78 198, Gudrun, Jorr och Tora) planterades i tre olika substrat; ren kisaska (PA), en blandning av kisaska och bark i lika stora delar (PAB) och en referensjord(R). Plantorna mättes i höjd var 10e dag och när plantorna skördats skickades rot, skott resp. bladdelar från varje planta till laboratorium för analys av metall och näringsinnehåll.

Resultatet visade att kisaskan påverkade tillväxten negativt för alla kloner. Metallhalterna var signifikant högre i de plantor som vuxit i substraten med kisaska (PA och PAB) än referensplantorna. Plantorna i kisaskesubstraten hade förutom höga metallhalter dessutom anmärkningsvärt låga fosforhalter i sin biomassa. Detta gör det problematiskt att peka ut en huvudorsak till den låga tillväxten, troligtvis är det en kombination av toxiska effekter från metallerna och näringsbrist som ligger bakom. Barken som blandades med kisaskan i ett av substraten (PAB) hade positiv effekt på rotutvecklingen men inga andra förändringar, varken i växtlighet eller metall- eller näringsupptag, kunde påvisas.

De erhållna resultaten från metallanalyserna på referensplantorna visade på god potential hos salix att rena marken ifrån Zn och Cd. För kisaskan från Skutskärs bruk rekommenderas ytterligare studier på Salix stabiliseringsförmåga och potential att hindra metaller från att laka ur materialet. Generellt efterfrågas mer forskning på hur element påverkar och interagerar med varandra i markmiljön.

Nyckelord: fytoremediering, Salix, förorenad mark, kisaska, metaller, extraction, accumulering, tolerans

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POPULÄRVETENSKAPLIG SAMMANFATTNING

Fytoremediering med Salix som metod för att rena kisaskan från Skutskärs bruk ifrån metaller.
Helen Legeby

Förekomsten av förorenade områden är ett växande problem globalt. I Sverige har allt hårdare reglering bidragit till att spridningen av föroreningar på nya områden minskar men stora insatser behövs för tidigare utsläpp. Ca 80 000 områden är identifierade som förorenade eller misstänkt förorenade områden där inventering och eventuella åtgärder är nödvändiga. Utvecklingen av effektiva och billiga saneringsmetoder går alltför långsamt. Den vanligaste metoden, ofta kallad ”dig and dump”, är att gräva bort den förorenade jorden och deponera den på en säkrare plats. En metod som inte är långsiktigt effektiv, är kostsam och många gånger kan skada värdefulla ekosystem. Nya skonsammare metoder är därför efterfrågade både ifrån privat och offentlig sektor.

Skutskärsbruk, ca 10 mil norr o. Stockholm, är ett av Sveriges största pappersbruk. Det startade i mitten på 1800-talet som ett sågverk av två norrmän, Astrup och Sörenssen. För att ta till vara på spillved och andra restprodukter startades under 1890-talet sulfat och sulfit massabruk. 1949 hade metoderna för massatillverkning utvecklats och Skutskärs bruk började producera svavelsyra som behövdes i de nya teknikerna. Framställningen innebar rostning, eller bränning, av svavelkis, ett mineral som framförallt består av järn (Fe) och svavel (S), men också av andra mineraler innehållande andra metaller så som zink, arsenik, bly, koppar, kadmium mfl. Från rostningen av svavelkisen bildades en restprodukt, kisaska, vars innehåll till stor del utgörs av dessa metaller. Materialet var praktiskt som fyllnadsmaterial eftersom ingenting växte i det och det var vanligt att det användes som sådant både inom industriområdet för att skapa nya landområden och utanför i samhället Skutskär t.ex. längs tågrälsar och trädgårdsgångar. Då var kunskapen om askans toxiska egenskaper liten.

Idag har den studerats noggrannare och kunskapen om hälsoriskerna är större. Däremot är mindre forskat kring hantering och åtgärder av det. Huvudproblemet med kisaskan är de höga metallhalterna. Då metaller inte kan brytas ner ackumuleras de i naturen, djur och människor, och uppnår till slut halter som kan skada biologiska processer. När det gäller metallföroreningar är målet med saneringen att få dem att ta så liten volym i anspråk som möjligt och sedan förvara dem på en säker plats eller utvinna enskilda metaller för återanvändning.

Under de senaste två decennierna har fytoremidiering som saneringsmetod fått växande uppmärksamhet från forskare och aktörer inom området. Det är en metod där växter används för att rena förorenade områden. Det finns olika grenar av fytoremediering varav fytoextraktion är en av de mest beprövade. Idén är att låta växten ta upp (extrahera) föroreningarna i sin biomassa som sedan skördas och bränns. Är föroreningarna inorganiska (som metaller) kommer de att finnas kvar i askan efter förbränningen men i en väsentligt mindre och mer lätthanterlig volym än då de var spridda i marken. Fördelen med metoden är att den är skonsam mot markmiljön och de processer som pågår där och att den är relativt billig. Beroende på ämne, markegenskaper och klimat etc. lämpar sig olika växter olika bra. Salix är en videsort som i stor utsträckning används som energigröda i Sverige. Den har hög tillväxt och är tålig vilket gör att den kan växa där andra grödor inte kan. Senare studier har också visat på högt upptag av framförallt kadmium och zink men även av andra metaller och därför är den intressant som växt för fytoextraktion.

I den här uppsatsen undersöks möjligheterna att rena kisaskan från metaller genom fytoextraktion med Salix. I ett krukväxtförsök planterades fyra kloner av Salix (78 198, Gudrun, Jorr och Tora) i tre olika substrat. Det ena var 100 % kisaska, i det andra blandades kisaska och bark i lika stora delar. Det tredje substratet fungerade som referens och var vanlig blomjord. Mer specifikt var syftet att undersöka toleransen hos klonerna och om den skiljde sig dem emellan, samt om/hur väl de kunde extrahera metallerna från kisaskan. Idén med barken vara att den skulle luckra upp den relativt svårgenomträngliga och sandiga askan och därmed underlätta för rotutvecklingen hos plantan.

Resultatet visade på mycket låg tillväxt hos de plantor som växte i de kisaskebaserade substraten. Som förväntat ökade rotmassan hos de plantor som vuxit i barkblandningen, men ingen annan växtparameter (skottvikt, bladvikt eller höjd) påverkades. Totalkoncentrationen av metallerna i kisaskan var generellt mycket hög, långt över normala bakgrundshalter i Sverige, men den mobila/biotillgängliga fraktionen var låg och askan var så gott som inert. Metallupptaget var högre i de plantor som vuxit i kisaskan eller barkblandningen i jämförelse med referensen, men referensjorden innehåller heller inte samma höga koncentrationer. Däremot hade referensen generellt bättre förmåga att omfördela metallerna i sin biomassa, medan det mesta av metallerna stannade i rötterna i de plantor som vuxit i kisaska. Klonerna skiljer sig i sin genuppsättning, men pga av den kraftiga påverkan som kisaskan hade på alla kloner var det svårt att urskilja unika skillnader i deras tolerans och ackumuleringsförmåga. Krukförsöket pågick också under endast en säsong, och det kan uppstå väsentliga skillnader i tillväxt och upptag i kommande säsonger. Klon 78 198 påverkades dock minst i höjdtillväxt, även om det var marginellt, och hade samtidigt högst totalupptag av metaller. Ytterligare studier är dock nödvändiga för att kunna med signifikans peka ut den mest lämpliga klonen. Då tidigare forskning har visat på hög tolerans hos Salix för metallhalter i sin biomassa undersöktes också näringsupptag som möjlig orsak till den dåliga tillväxten. De viktigaste näringsämnena kan sägas vara kväve (N), fosfor (P) och kalium (K). Resultaten ifrån näringsanalyserna visade oväntat att de plantor som vuxit i 100-procentig kisaska hade högst upptag av N, därefter kom referensplantorna och lägst upptag hade de plantor som vuxit i blandningen. P-halten var mycket låg i plantorna från båda kisaskesubstraten. K-halten visade inte på någon större skillnad mellan kisaskesubstraten och referensen.

Slutsatserna ifrån studien är att ingen av klonerna kan växa i kisaskan som den är, om fytoextraktion ska vara möjlig måste askan modifieras på något sätt. Tillväxten avstannade dock inte helt och andra tänkbara tillsatser än bark kan vara mer lämpade som utblandning av den täta askan och som dessutom kan ha positiva effekter på tillväxt och upptag. Många parametrar (pH i marken, metaller, biotillgänglighet, växternas samspel med markorganismer, tillgång till näringsämnen etc.) spelar in för resultatet och det är svårt att peka ut någon som skulle vara tongivande för resultatet. Kunskapen om hur metallerna interagerar med varandra och hur de påverkar växtupptaget är fortfarande liten och mer forskning på hur olika processer och element i jorden samverkar efterfrågas. Vidare skulle det vara intressant att undersöka huruvida Salix kan fungera för fytostabilisering eller fytoimmobilisering istället där växten hindrar metallerna från att laka ur marken.

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1. INTRODUCTION

The problem with contaminated sites is global and growing and the need for more sustainable, cheap and less harmful methods for remediation of these sites is getting more and more alert. Only in Sweden 80 000 sites have been identified as contaminated or high risk of being contaminated. One method that has been highlighted lately is phytoremediation where pollutions in water, air or soil are removed with green plants (Pilon-Smiths, 2005). The idea of using plants for soil improvements is old but the term phytoremediation was introduced in the 80s. Salix, part of the willow family, is one of the most promising species for the method. It was introduced as an energy crop in the 60's because of its high ability to produce biomass. In the end of the 70's researchers found that the species also had high capacity to accumulate both inorganic (e.g. metals) and organic (e.g. PCB, PAH's, benzene, toluene) substances from the soil.

Thus, as an academic subject, phytoremediation is a relatively new technical area with most research from 1990 and onward (Ali, et al., 2013). Current methods for remediation, such as excavation, incineration and landfill dumping, are damaging the soil environment and structure. With phytoremediation the disturbance of structure and of important biochemical systems and biodiversity can be avoided. Sometimes it even has positive effect on these crucial factors for a healthy soil. The method has primarily been tried in the US but the interest for it grows also in Europe (Pilon-Smiths, 2005). Sweden is a pioneer of the technology of phytoremediation with already developed infrastructure and administration in the matter due to a relatively long history of Salix plantations for energy (Dimitriou & Aronsson, 2010).

Most of existing remediation systems involves organic pollutants rather than inorganic pollutants (e.g. metals). In the US the analogy is 80 to 20 % respectively (Pilon-Smiths, 2005). Metals as contaminators are of special concern since they are non-degradable and with time accumulate in the environment. From there they transport to the top of the food chain and affect biogeochemical cycles on the way. The sources of metals can be both natural and from human activity such as industries, mines, transports etc. Since the demand for technical products is increasing around the world, more and more metals transported from deeper soil layers, are found on the surface of the earth. This emphasizes the importance of investigating methods to handle this growing issue.

Skutskärs bruk is a paper mass plant and the area has been an industrial area since the middle of 19th century. In the middle of the 20th century Skutskärs bruk started to produce sulphuric acid. The process involved burning of pyrite ore (FeS_2) which gives a by-product called pyrite ash containing metals such as As, Cd, Cr, Pb, Zn and others that had been parts of the primal ore. The ash was not concerned as harmful rather the opposite, and was used as filling material in the close by society of Skutskär. The production of sulphuric acid ended during the 60's and since then the knowledge concerning toxic effects due to ash deposition has emerged.

1.1 OBJECTIVES AND HYPOTHESIS

The overall aim of this thesis was to examine the potential of phytoremediation with Salix to clean the contaminated material of pyrite ash from the paper mill area of Skutskärs bruk in Sweden. The study describes a pot experiment with four different Salix clones (78 198, Gudrun, Jorr and Tora), and a comparison between the clones capability and tolerance against the pyrite ash is performed. They were planted in three different substrates to examine their tolerance of pyrite ash and uptake of metals and if their potential as metal accumulators differed. The following three hypotheses were to be further investigated:

- The pyrite ash will affect Salix growth negatively because of the high concentration of metals in the ash.
- The plants in the mixed substrate with bark will grow better than the plants planted in pure pyrite ash because of better soil structure.
- The extraction efficiency of the clones will differ depending on clone and compound.

2. BACKGROUND

Even though the interest of the environment and conservation has a long history in Sweden with the start of “The Swedish society for Nature Conservation” already in 1909, it was not until the middle of the 20th century that the contemporary regulations for environmental management took its first steps (Bremle, 2012). Since then laws and regulations within this field have developed step by step. The contemporary environmental politics in Sweden is mainly based on the Environmental Code that was voted for in the parliament in 1999. In the same voting 16 environmental goals were decided to set the direction for Swedish actors concerned (Naturvårdsverket, 2003). Among these goals *Poison free environment* (Swedish: Giftfri miljö) was one of them. More specifically this implies that the quantity of the exposure of chemicals in the daily life should not be toxic neither for humans nor for the biological diversity and also that dispersion of compounds with toxic effects shall be very small. The year for the goal is set to be 2020 (Naturvårdsverket, 2015). An extensive effort to identify and characterise contaminated areas started as a step to fulfil the goal. The responsibility to reach the goal is not upon one actor, authorities, landowner and practitioners have important roles (Miljöbalken 10 kap. 2§). The county administrative board or sometimes the municipality board are regulatory authorities with the role to evaluate and, possibly, demand commitment from the practitioners. Thus there are ongoing investigations of contaminated areas initiated of private and public sector, with the aim to analyse risks but also to conclude necessary action plans.

Roughly there are two types of contaminations, organic and inorganic. The ideal solution for many cases of contaminated soil and the treatment of such, would be if organic pollutants could be destroyed and the volume of inorganic pollutants could be as little as possible and safely stored (Naturvårdsverket, 2003). Although new methods are available, the majority of the remediation ends up with the “dig and dump”-practice and *ex situ* treatments, especially when it comes to metal-contaminated soil (Pulford & Watson, 2003). The advantages with “dig and dump” are that it most often is relatively cheap and quick. There is also a well-developed infrastructure where sites with different permissions adapted for certain pollutants and levels of contamination can handle a wide range of varieties amongst the masses. This makes it easy for actors to follow a certain work process, which often is not the case for remediation *in situ* that normally is coupled to additional investigations and permissions from the authorities (Naturvårdsverket, 2003). However, the method of “dig and dump” does not solve the real issue of cleaning the soil, but only move it to another site and pushed to the future. Other methods that are relatively common include soil washing, incineration and biological cleaning. Soil washing and incineration are most often performed *ex situ*, including economically and environmental costs connected to transportation, more or less long distances (Pilon-Smiths, 2005). Biological cleaning has been proven somewhat successful for organic contaminations but is less effective for inorganic pollutions such as metals (Raskin, et al., 1997). These methods also demand high technology management which makes them expensive and complex (Pulford & Watson, 2003).

The interest for cheaper and more effective solutions is increasing and research of new remediation methods are demanded from both authorities and operators.

2.1 SKUTSKÄR PAPER MASS MILL

The paper mass mill in Skutskär, Sweden, (figure 1) has a long history of activities with more or less damaging effects on the environment.

In the late 19th century the production of sulphate and sulphite masses started at the mill, and became a source of heavy pollutions such as metals, Hg and oil pollutions. This was a development of the lumber mill that had started on the site 1869, with by-products such as charcoal, tar and turpentine, products that also have been deposited in several parts of the area. For example earlier reports have concluded that around 5 tons of Hg have been dumped in the area (Ledin, 2010). In course of time the chemicals and rest products have been reused in the extent that the technical development allowed.

Figure 1. A map over Sweden with Skutskär paper mill where the pyrite ash came from marked in red. The yellow mark is Ultuna (SLU), where the experiment took place.

In 1949 Skutskärs paper mill started the production of sulphuric acid. The sulphuric acid production included burning of pyrite ore which resulted in big amounts of the by-product pyrite ash. The material had excellent properties as filling material and was used as such in the town of Skutskär and along railwails. In the introduction for the annual report in 1952 (Ledin, 2010) the authors have written that,

[...]The waste from the mill has built new land for us in the sea. (My translation)

This reveals two important environmental aspects, first of all that a lot of waste from the industry ended up below the water surface and secondly that a big part of the industrial area is built on it. The production of sulphuric acid ended at 1969 (Ledin, 2010). In 2005 the county administration board in Uppsala started an inventory of the industrial area of Skutskär paper mass mill. The aim was to conduct a survey and to classify the polluted areas. The conclusions from the inventory were that the mill should be classified in group 1, very high risk, and that it should be further investigated (Jansson & Duell, 2005). Since then, several remediation actions have been taken and a lot of the contaminated masses are deposited in landfills.

This thesis examines if phytoremediation could be an alternative method for taking care of the pyrite ash masses. Since the pyrite ash is spread out in the industrial area the outline idea is to, if such remediation would be reality, find a proper site in or close to the area where the ash could be transferred and phytoremediation take place.

2.2 PYRITE ASH

Since the late 19th century the production of sulphuric acid has been a common part of the paper mas industry. The acid is needed in the process and is produced by burning pyrite ore (FeS_2) (Chunxia, et al., 2009). In Sweden there are several mines that have provided the producers with pyrite ore, Skutskärs bruk received most of their ore from the mine in Falun. The burning waste, called pyrite ash contains mainly iron oxides and hematite (Fe_2O_3) in particular, which gives the characteristic red colour (figure 2). Until the 50s this product was actually used as an iron source in the steel industry (Nordback, et al., 2004). In the process of burning, including many physiochemical transformations, other elements from the headstream precipitate and the ash also contains metals such as Cd, Zn, Pb, As, Cu, Cr, Mn, Tl and Ni (Chunxia, et al., 2009). Therefor pyrite ore is never a product of pure iron sulphide but always contaminated with other sulphides such as chalcopyrite ($CuFeS_2$), zincblende (FeZnS), pyrrotite (Fe_9S_{10}) and arsenopyrite (FeAsS) (Nordback, et al., 2004) The fractions of metals differ depending on which mine it comes from and can be reduced by purifying the ore before roasting (Morath, 1960). When working with this kind of contaminations it is important to take into account that no product of pyrite ash is the other alike. Local specific examinations are in general necessary (Nordback, et al., 2004).

To be able to control the potential disturbance of ecosystems and the toxic effects from the trace elements it is important to examine their behaviour during the roasting and in what complexes they end up in the ash (Chunxia, et al., 2009). Until recently the knowledge of pyrite ash and its toxicity has been scarce and for a long time it was used as a filling material next to train rails, underneath buildings and in bays to create new land (Nordback, et al., 2004). For example a large part of Falun, the city next to Falun mine, is built on the residues (Lin & Qvartfort, 1996). Lately though, several studies have shown that the trace metals do transport in the soil and water, and can possibly be a source to relevant damage of the environment and the species that lives there (Chunxia, et al., 2009). Despite this there are relatively few studies made on the environmental risks associated with the wastes (Oliviera, et al., 2012) (Chunxia, et al., 2009).

(Landberg & Greger, 1994)The burning process contains following steps (Morath, 1960):

$$2\ FeS_2 \rightarrow 2FeS + S_2 \quad (1)$$

$$S_2 + 2\ O_2 \rightarrow 2\ SO_2 \quad (2)$$

$$4\ FeS + 7\ O_2 \rightarrow 2Fe_2O_3 + 4\ SO_2 \quad (3)$$

$$3\ FeS + 5O_2 \rightarrow Fe_3O_4 + 3\ SO_2 \quad (4)$$

$$6\ FeS + 4\ SO_2 \rightarrow 2\ Fe_3O_4 + 5\ S_2 \quad (5)$$

Figure 2. The picture shows the point where the pyrite ash for the experiment was collected. The pyrite ash is the reddish material, coloured from its high content of iron.

From the heat one of the two sulphur atoms is eliminated (1) and the precipitated disulphuric form together with the oxygen sulphurdioxide (SO_2) (2). The other product from step one, FeS also reacts with the oxygen to hematite (Fe_2O_3) or magnetite (Fe_3O_4) and sulphurdioxide (3, 4). The last step (5) shows that FeS also can be oxidized through direct reaction with SO_2 (Morath, 1960). The content of trace elements differs due to the content in the headstream but also from properties in the production such as temperature and type of oven. Just like the sulphur the trace metals often appear as oxides. Earlier it was a common belief that many of the metals would appear as stabile sulphides (S^{2-} + Me^{+}). Recent studies have though shown that this is not the case since zinc is the only metal that has been found as a sulphide (ZnS) (Nordback, et al., 2004). Metals share some characteristics but they can also be classified in different ways. One way is to group them into hard and soft acceptors. “Hard” acceptors (or metal ions) (e.g. Al, As, Ca, K, Mg and Na) are more likely to make complexes with oxygen-containing ligands and “soft” (Cd, Cu and Pb) acceptors generally form bonds with sulphur and nitrogen-containing ligands (Appenroth, 2010). On the other hand, to divide the metals into different classifications and by them expect to draw calculations about their toxic behaviour in soil is not advisable. Their behaviour in soil and their affection of the living cells when accumulated is extremely complex and the knowledge about how they interact with each other and how their presents affect these processes is still poor (Appenroth, 2010).

In a report from the Swedish Geotechnical Institute (SGI), Nordback et al. (2004) investigated how the content in pyrite ashes occurred. Their conclusions were that they are mostly bound to sulphate (SO_4^{2-}). It is the most common form for sulphur after the burning and it strongly binds the metal cations, due to its strong negative charge. Also sulphite (SO_3^{2-}) is a possible bound surface for the metals. Both of these sulphur based complexes are acidifying and relatively soluble. Depending on the relationship between surface/volume the main content in the ash, iron oxide, could also be effective in binding cations (Nordback, et al., 2004).

2.3 METALS AS SOIL POLUTIONS

Metals appear naturally in the soils and some of them (e.g. Zn, Cu, Fe and Ni) (2B, chapt 2) are essential for animals and plants living there. However, they turn out to be a problem when they too quickly and in large quantities become accessible to their environment. One important difference between metals and organic substances is that metals are non-degradable. Instead they have different oxidation numbers depending on pH, redox-potential and other soil properties. Associated to these processes they form reactive oxygen species (ROS) which are highly reactive molecules that can disturb ordinary reactions in for example the cell (Zitka, et al., 2013). Because they are non-degradable they accumulate in the environment and they transport from the lower trophic level (microbes, bacteria) to higher trophic level (e.g. animals and humans) and can possibly cause damage in the whole chain (Ali, et al., 2013).

As mentioned above some metals, mainly the more toxic “soft” metals, form bonds to nitrogen containing ligands. This means that they can make complex with all proteins which amino acids are based upon (Appenroth, 2010). Moreover they can replace other substances that are vital for the organism. Several nonessential metals, for example Cd, As, Pb and Hg, can cause cell damage and cell death even in small concentrations (Ali, et al., 2013). Attempts have been made to define when the levels of the elements concentrations do tress pass limits of what green plants generally tolerance. Because of the complexity of a soil the limits do not only depend on the concentrations of the actual element. In fact, factors as pH and DOC (dissolved organic carbon) are of more importance (Alloway, u.d.), which is why it gets nearly impossible to decide values that fit for all soils and soil properties.

2.3.1 Metal occurrence in soil

Metals rarely exist as ions but typically in complexes with other compounds that are present in the soil and the soil water. They bind to both organic and inorganic surfaces and in soil there are mainly three colloidal particles they bind to: *Humus*, *oxides and hydroxides* (mainly Fe-, Mn- and Al- oxides) and *clay minerals*. The metal complexes exist both as solid material and in the soil solution. When bound inside solid material they generally have low mobility and it is only through long processes (years) such as weathering or changing in redox potential that they can be released. Metals on the surface of the solid material are more mobile but the most important source to highly mobile and bio-available metals in soil are the free hydrated ions ($Me+ H_2O^{2+}$) in the soil water (Young, 2013).

Humus

Humus normally exists in the top layer of the ground and thus mainly binds shallow sited metals. It is very heterogeneous due to its origin as old plant and animal decay, but generally it contains C (50-60%), O (30-40%), N(5%) and H (5%). As a potential adsorbing surface for cations, humus is a good candidate. It is negatively charged with a linear increase from relatively weak charge if pH is low and stronger when pH is high. The heterogeneity of the material with a mix of ligands including N, O and S, makes the material suitable for almost all metals with an extra predomination to bind to alkali and alkaline earth-cations. And generally, if humus is available, the biggest fraction of the metals will be held as organic complexes (Young, 2013).

Oxides

Almost all elements in the periodic system form oxides. In soil context though, Fe-, Al- and Mn-oxides are the most discussed and all of them form several different oxides and hydroxides. Oxides do both adsorb and release H^+ ions and become hydrous oxides and it is firstly then they get an electrical charge. This capacity makes them amphoteric, which means they can both be negatively and positively charged on the surface. The charge depends, as for humus material, mainly on the pH-value in the soil environment. At high pH-values the oxides releases H^+-ions and adopt a negative charge, whereas for low pH-values H^+-ions are adsorbed and they get at positive charge.

Clay

The clay content in a soil is an important factor for the level of adsorbed metals mainly for their large surface, for some clays the surface is as big as 600 m^2g^{-1}. Most important and also common are the phyllosilicates which is layers of silica and aluminium (Alloway, u.d.). Clays are a result of weathering minerals. During the process water molecules infiltrate between the layers and the positive ions on the surface is pushed away. Thus the clay gets negative surfaces that can attract the positive metal ions present in the soil (Eriksson, et al., 2011).

Depending on the metal it prefers to bind to different surfaces, and that affects its mobility and bioavailability since the extractability between the surfaces also differs. In table 1, important metals for the thesis and their soil properties are listed.

Table 1. Metals and their properties in soil.

Element	Occurance in soil	Toxikologic effects	Ox no.	Avarage topsoil level in Sweden (mg/Kg)	Mobility
As	If pH<8 arsenic (As) binds to primaraly Al- and Feoxides if such are available. They also occurs in hard soluable sulphides.	Negative effects on reproduction, skinproblems, carcinogenic.	As(V), As (III)	4	low
Cd	Cadmium (Cd) forms firstly complexes with humus, if pH is high it partly also binds to Fe-oxides.	Mainly toxic for animals since it replaces Zn in some cellular processes. Carcinogenic.	II	0.23	low
Co	Cobolts (Co) forms strong bindings to humus but also to Mn-oxides, especially if pH is high. Soluability increases with lower pH.	Essential element for plants and animals. Toxicological effects supposly from the ability to replace Zn in cellular processes. Carcinogenic.	II	-	low
Cu	Cupper (Cu) forms very strong bindings to humus, but also to oxides when pH<4. Transport is du mostly through solved humuscomplexes.	Essentail element for plants and animals. If toxic levels Cu can cause damages in kidney, liver, immune system. Very reactive but not carcinogene.	II, I	15	low
Cr	In low pH (<6) Crome (Cr) is adsorbed to Al- and Fe-oxides. In anaerob environments with low pH it is strongly bound to humus, if pH is higher and oxygen access is good, Cr is relatively soluable.	Crome is essential but has toxicological effects mainly on the lungs. Carcinogenic.	Cr (VI), Cr(III)	20	high
Ni	Nickel (Ni) forms complexes with humus, but binds also to oxides, especially if pH is high.	If the concentrations is too high Ni has toxicological effects on animals primaraly because it inhibit the uptake of Zn. Carcinogenic. 10-15% of the human population is allergic.	II	-	low
Pb	Lead (Pb) makes strong bindings to humus and oxides even when pH is low(<4). If pH is high and there is big attendence of Pb it is likely that $PaCO_3$ falls out. Transport is primaraly through humus and oxides.	Can be damaging for nervous system and inlectual ability. It could also lead to high blood pressure, and hart disease. Children are especially sensitive.	II	17	low
Zn	Zink (Zn) binds primaraly to humus but also partly to oxides if pH is high. Solubility increases with decreasing pH.	Essential element for plants and animals. Small toxical effect.	II	59	low

3. PHYTOREMEDIATION

Because of its simple construction and cheap management phytoremediation has gained attention as an alternative method among relevant actors and government agencies the recent years (Greger & Landberg, 2006). In phytoremediation soil, water or gaseous substrates are cleaned with plants and their associated microbes (Pilon-Smiths, 2005). This thesis will only discuss remediation of *soil*.

The idea to extract or stabilise pollutants in the soil with plants is very old and it's difficult to point out a specific source to the development of the techniques. Anyhow, recent studies have shown remarkable results which have promoted the development of this remediation method (Raskin, et al., 1997) (Även 4A). The management is, as mentioned above, relatively simple and cheap. The low cost is due to low technological- and *in situ* management and big parts of the sanitation period the plants "take care of themselves". In some cases it is necessary to transfer the contaminated masses to be able to apply the method and this will result in higher costs. The cleansed masses can be returned to where they originally were found (Susarla, et al., 2002), or be used as filling material somewhere else. Another cost effective aspect is that phytoremediation is solar driven which gives the method a big economically advantages before others (Pilon-Smiths, 2005). This makes it a "green" and CO_2- neutral process. Generally it is also appreciated by the public because of the aesthetically expression (Ali, et al., 2013).

There are, of course, also disadvantages of the method. Phytoremediation often implies long periods for cleaning to adequate levels. The biological processes make the method slow, and if the site demands an acute and fast sanitation then phytoremediation will not be an accurate alternative. Also the pollutants have to be bioavailable and not located too deep in the ground for the roots to reach them. For herbaceous species the root depth is normally up to 0.5 meters, meanwhile some tree species could reach 3 meters below the ground (Pilon-Smiths, 2005). In Sweden the soil pollutants are mostly shallower than that since the groundwater level generally is near the ground surface. When it comes to depth of filling material, for example the pyrite ash in Skutskär, it is highly varied and the choice of method and species must be considered. Moreover the method is site specific which means that it is crucial to find a plant that the climate, toxicity level and soil characteristics at the site allow to grow. This makes it hard to find a general process that will work everywhere for all types of pollutants, which is an advantage for more conventional methods as "dig-and-dump" and incineration (Pilon-Smiths, 2005).

Several subsets of phytoremediation have been identified. The most important are:

Phytoextraction- where the plants extract the pollutants into their biomass.

Phytostabilisation- where the plants stabilize the pollutants through root secretion or changing the soil structure.

Phytodegradation – where the plants degrade the pollutants and make them less harmful.

Rhizofiltration- where the plant roots by sorption, mainly of metals, keep contaminants from leaching into the water.

(Salt, et al., 1998)

In this thesis phytoextraction is in focus.

3.1 PHYTOEXTRACTION AND UPTAKE OF METALS AND NUTRIENTS IN PLANTS

The short explanation of phytoextraction is that the plants remove pollutants from the soil and can thereafter be harvested and burned. The ash which contains the non-degradable contaminants has a considerable smaller volume than the original material and can be stored more safely. Phytoextraction is the most promising method among phytoremediation techniques for sanitation of metals.

Not all plants are suitable for phytoextraction. Greger and Landberg (1999) suggest some characteristics that are especially important for plants to be used as phytoextractors. They should be able to accumulate high levels of metals, partly to defuse these compounds but also to let the nutrient elements still be present in the soil. Another important factor for the remediation to be effective is the ability to translocate the metals from the roots to the aerial parts of the plant and that the biomass production is high (Greger & Landberg, 2006). Efficiency in translocation depends on many factors, such as morphological characters and physic chemical properties of the soil. The presents of metals and how they interact with each other will also affect the accumulation and translocation in the plant. Depending on metals their interaction can both prevent and promote the plant up take (Mleczek, et al., 2010).

The roots play a major part for the plant uptake and translocation. They stand for all uptakes of nutrients and minerals the plant need for growth except for carbon (Raskin, et al., 1997). With the microbes and other soil organisms, the roots create a mutualistic relationship in what usually is called mycorrhizae takes action in the soil. In this exchange, the root produces enzymes that the microorganisms need for degradation and metabolism. Through the soil organisms' degradation and mineralisation, nutrients and minerals become available for the plant roots. In healthy soils the biodiversity of the microbes is enormous and this is the reason why almost all organic compounds can be degraded, since the microbes have different preferences (Eriksson, et al., 2011). All these actions and thus also the uptake, occur in the rizosphere, an area about 5mm wide around the roots (Kennedy, 2005). Thus a good root development is important for the plants potential to reach necessary nutrients and minerals.

There are mainly two ways for the metals to enter the plant, either through passive transport with the soil water or through active transport where the compounds is passing the cell walls of the roots (Yoon, et al., 2006). The cell walls contain negative groups and attract therefore the positive metal ions that are transferred into the plant (Pilon-Smiths, 2005).

3.2 NUTRIENTS AND THEIR ROLE FOR PLANT GROWTH

Nutrients are, similar to metals, almost always bound to complexes in the soil and soil water, they exist seldom as free ions. Also similar to metals many of the nutrients form positive charged ions and bind to negatively charged surfaces in soil as humus and clay. By secret acids (H^+) the plant roots make the nutrients available. Deficiency of nutrients effects plant growth, leaf colour and texture of the plants etc. These nutrients are called macronutrients opposed to micronutrients that includes the necessary metals (chap 2). Macronutrients are other elements in the periodic system. In table 1 normal values of the nutrients concentrations in plant tissue are listed.

Nitrogen (N) – is together with phosphorus and potassium the nutrients that often is deficient and therefore is one of the most important fertilizers. N is an important component in proteins, amino acids and for the plants metabolism. It cannot be replaced by any other element which makes it crucial for the plant and deficient of it is discovered quickly.

Phosphorus (P) – similarly to N, P cannot be replaced and it is noticed early if deficiency would appear. It also takes part in important processes in the cell and limitations of phosphorus inhibit the growth and the plants get very small.

Potassium (K) – K does not occur in organic forms but only as components in minerals and is dissolved through weathering. It is very mobile and has an important role to transport other nutrients and minerals in the plant.

Calcium (Ca) – Ca is important for stability in the plant since it is a central component in processes that build up the cell walls. Unbalance in the substrate between the elements is more often the reason for calcium deficiency in the plant than actual lack of the element itself in the soil.

Magnesium (Mg) – Mg does, similarly to K occur in inorganic forms and is released through weathering. One important role of Mg is its participation in the photosynthesis. It is also an important carrier of proteins and nucleic acids and by deficiency small dots of accumulations of complexes can be seen on the leaves.

Sulphur (S) – S is most commonly bound as complexes with sulphate that is relatively soluble. It is an important component for several proteins, amino acids, vitamins etc. Abundance of S can be stored in large quantities as sulphate until it is needed.

Table 2. Normal values for nutrient concentrations in plant tissues.

Nutrient:	Normal concentrations in plants (%)
N-tot	1-5
P	0.1-0.5
K	1-6
Ca	0.1-5
Mg	0.1-0.5
S	0.1-1.5

3.3 PLANTS FOR REMEDIATION

Some plants (e.g. Allyssum Bertolonii, Pteris vittat, Alyssum lesbiacum) (Pilon-Smiths, 2005) have been identified as hyper accumulators which mean they can accumulate high levels of contaminants. Especially metals as Ni, Zn and Cu have shown to be effectively accumulated with levels of 1-5 percent of the dry weight (Raskin, et al., 1997). The definition of a hyper accumulator is that the accumulation degree is > 0.1% for Cu, Co, Cr, Ni or Pb in its plant tissue (> 1% for Mn and Zn) (Yoon, et al., 2006), and > 0.01% for Cd). However, these plants are normally herbaceous species with small biomass productivity and the total amount of pollutants extracted from the soil is little (Susarla, et al., 2002). Some species of trees also accumulate high amount of metals even though their concentration never reaches the high accumulators levels. But due to their larger biomass the total amount extracted metals can be larger than the amounts of the high accumulators.

It has been discussed whether it is better to use a hyper accumulator with less biomass production than species that accumulate far less but with better growth so that in the end the total amount extracted is the same. Researchers as Chaney (1997) promote this procedure since it is easier to handle a small amount of biomass. On the other hand, if it is possible to make use of the biomass as an energy source, it seems that the species with higher biomass production but inferior accumulation is a preferable choice. This is also in accordance with the study of Dimitriou and Aronsson (2010) where it was concluded that a higher biomass production increases the evapotranspiration and thus decreases the risk of leaching. Again, it is important to have good knowledge about the soil properties and characteristics of the actual pollutants (Dimitriou & Aronsson, 2010), and also what the main target with the remediation is. Another important aspect in this context is that plants with higher biomass production (e.g. trees) have a wide genome which means that they are better prepared for acclimation (Pulford & Watson, 2003).

3.4 SALIX

In recent studies Salix has proven to be an effective accumulator of both organic and inorganic compounds. The species belongs to the family of willow and has in the latest decades grown in popularity as a good option for renewable energy source because of its high biomass productivity. The yearly biomass production in the south of Sweden is approximately 8.0 ton/ha, about twice as much as for fir (Christersson, 2013). In comparison with many other trees, willow (e.g. Salix) has a quick start with high biomass production in early life. This opens up for a short harvest cycle (3-4 years) and remediation to tolerable levels in a short period of time. Most Salix species do regrow after harvest why there are no costs for replantation. If the harvest in addition is used for energy, more energy will be produced per unit time (Christersson, 2013).

Salix easily adapts to new environmental conditions and has been observed colonising contaminated sites which indicates a general high tolerance (Pulford & Watson, 2003) (Miroslaw Mleczek, 2009). Their capability of adaption is proven to be gradually. As an example a site with high contamination of Cd that was too toxic for the species in the beginning could after some time be colonised by Salix (Klang-westin & Ericsson, 2003). Moreover, it has high resistivity to diseases and dottiness that can be due to the soil (Miroslaw Mleczek, 2009). Economically, it is cheap to cultivate and has low intense technical management (Pulford & Watson, 2003). There are around 200 species (e.g. S. viminalis, S. dasyclados, S. Schwerinii) and 400 clones (e.g. Gudrun, Jorr, Tora, 78 198). Their characteristics differ not only between species but also from clone to clone, not the least when it comes to capability to accumulate metals. In a study from 1999 (Greger and Landberg) examined the accumulation of Cd, Cu and Zn. They found no correlation between uptake and tolerance and likewise no correlation between uptake and translocation in the plant. In addition it could not be shown that some clone were better than another in accumulation aspect. Thus one clone accumulated Cd but not Cu whereas another clone could have the opposite relation. However, there was no difference within the clone groups, even though they were taken from different sites with different kinds of soils their accumulation level is constant (Greger & Landberg, 1999). Cd and Zn and their interaction with Salix, are the two most examined metals and many studies have shown that Salix has good accumulation capability of these two ((Mleczek, et al., 2010) (Fischerová, et al., 2005) (Vyslouzilova, et al., 2003)).

For some metals (Cd, Zn and Cu) Salix has the best growth in soils with small concentrations, whereas it is negatively affected when the concentrations are too big or too small. Greger and Lundberg could in their study from 1994 show that the limitations differed among the clones. For Cd, Cu and Zn the maximum level for the clones survival ranged between 3-10 µM, 1-7 µM and 50-100 µM respectively (Greger & Landberg, 1994). These limits and values point out indications of optimum concentrations, but since all experiments have their own unique set up with their own well defined environments they cannot be used as reference values for any other experiment. Another study that illuminates the toxic effects from the essential element Zn is the study from 2003 by Vyslouzilova et al. In their experiment they compared how Zn and Pb (not essential) affected the yield and accumulation of seven salix clones. They found that high content of Zn reduced the yield more than corresponding content of Pb, and further that the clones accumulated both Cd and As in larger extend in soils with high concentration of Zn than high concentration of Pb. Due to the toxic effect from the Zn content the reduced biomass resulted in lower total uptake despite higher accumulation efficiency.

In a recent study (Tingwey, et al., 2014) *Salix viminalis* was planted in soil with different concentrations of Cu to observe the tolerance and accumulation. The study showed that the level of Cu uptake (mg Kg^{-1} DW) increased with increased concentrations in the soil. The soil available Cu level followed the same trend. The results also showed that *Salix viminalis* had no vegetative growth in soils with concentrations above 250 mg Cu Kg^{-1} (Tingwey, et al., 2014).

As discussed above present metals in the substrate affect the plant accumulation of soil elements. It is shown that both Cu and Zn result in higher translocation factor for Pb. However, when it comes to Cu vs. Zn, the first reduce the accumulation of the latter whilst Zn increases the uptake of Cu (Mleczek, et al., 2010).

4. MATERIAL AND METHODS

A pot experiment with four different clones of Salix was performed in Ultuna, Sweden, from the 6th of May to the 25th of July 2014. The purpose was to examine the potential of Salix to remediate the pyrite ash from metals. To do this, two main aspects were to be explored specifically. Firstly to see if the clones of Salix could grow in the very contaminated soil; this would tell something about the clones' tolerance for the pyrite ash. The other important aspect to examine was their capability to actually clean the soil. This was done by studying their capability to extract and accumulate the metals present in the soil. The selected clones were 78 198, Gudrun, Jorr and Tora, all relatively well established and studied by other researchers. They were planted in three different substrates whereof one was reference. The other two were, 100 percent pyrite ash, and a mix of pyrite ash and bark in equally big parts. The bark as an additive was chosen with the basic idea that it would loosen up the relatively hard packed pyrite ash, and help the growth of roots. Skutskärs papper mill have big amounts of bark residues from the production why the bark also in reuse aspect was considered as a good choice for additive. During the experiment the length of the shoots was measured continuously and after 11 weeks the plants were harvested. They were analysed for metals part by part not only to find their capability to accumulate but also to find out how well they could transport the elements in their own biomass.

4.1 LOCATION FOR THE EXPERIMENT

Ultuna (59°49´N, 17°39´E) is situated in central Sweden near the eastcoast (figure 1). The climate is temperate with an average temperature in February of -4 degrees Celcius, and in july 18 degrees Celcius. The average precipitation in the area is around 500 mm/year (SMHI, 2014). The experiment was performed outside with controlled watering initially. After four weeks the head part of the water came from the natural precipitation, with tapped water during very dry periods.

4.2 THE PYRITE ASH

The pyrite ash in the project was collected from Skutskär pulp mill in Älvkarleby municipality, Sweden, in April 2014. The location from where it was taken was paved and located west of the round sediment tank near the shoreline (figure 3). The site for the sample point was chosen because the ash visually seemed very homogenous. This was highly desirable for the project since it gives the possibility to exclude variations in the result because of heterogeneity in the material (figure 4). Still lumps of different coloured material were found, and these were taken out (figure 5). The ash was sandy and compact, with a reddish colour. The bark was non-processed with its natural humidity kept and could also be collected from the industrial area. Everything arrived to SLU, Ultuna the 5th of May 2014 where the experiment took place.

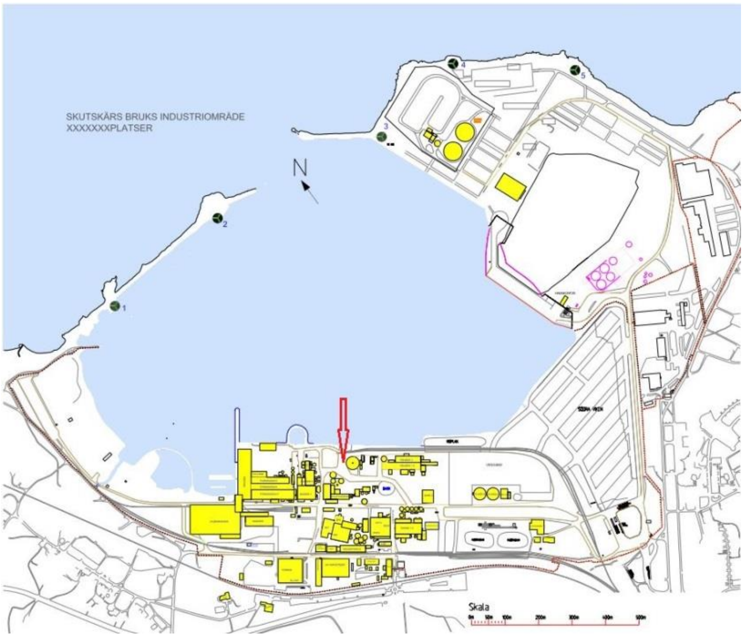


Figure 3. Skutskär paper mass mill with the site for the sample marked with a red arrow.

Figure 4. The pyrite ash when it arrived to Ultuna.

Figure 5. Lump from other material found in the pyrite ash.

4.3 EXPERIMENTAL SET UP

Three different treatments with different substrates where prepared during the 6th and 7th of May of 2014.

A. The first treatment was with hundred percent pyrite ashes, and is in the following referred to as PA.
B. The second treatment was with pyrite ash and bark mixed in equally big portions (PAB).
C. The third treatment, the reference with regular plant S-soil (R).

The biggest parts in the bark was sorted out with a sieve so only parts less than approximately 1 cm^2 was used in the experiment, thereafter it was accurately mixed with the ash into a homogenous mass. The pots used in the experiment were 5.5-liters big, with a diameter of 19.5 cm and 18.5 cm in height. They were filled with the substrates with approximately five centimetres margin and weighted before the cuttings were planted.

All Salix cuttings were from one year old shoots and collected from the experimental sites of the Department of Crop Producton Ecology, Swedish University of Agricultural Sciences, in Ultuna. The chosen clones are all of known provenance and come from three different species; *Salix viminalis*, *Salix schweiriini* and *Salix dsycaldos*. The two first are narrowed-leaved whereas the latter has broader leaves. In the following a short description of each chosen clone can be read:

78 198 – is a *S. viminalis* and is a common reference clone in Sweden.

Gudrun – S. dasyclados is hardy against frost and resistant for some pests. It is broad leafed and therefore force out weeds effectively.

Jorr – similarly to 78 198, Jorr is a *S. viminalis*. It is a quick starter and has continued high productivity. Jorr is resistant against fungi but can be sensitive to frost.

Tora – is a hybrid between *S. schweiriini* and *S. viminalis* and is a standard clone in Sweden when it comes to energy crops mainly due to its high productivity. It has also very low rate of infestation of pests.

Before planted all cuttings were measured in length and weight. The pots were placed in drip trays outside in an area protected with grids which prevents for example birds or other animals to reach them. By placing them outside the plants were exposed to the Swedish climate and gave indications of how well the clones managed in these latitudes. Each treatment involved five replicates (totally 60 plants).

A week after planting, 3.5 grams of fertilizer were given to all plants (200kg/ha), including the reference (Christersson, 2013).

During the first five weeks the plants were watered with tap water every Monday, Wednesday and Friday to make sure they did not suffer from drought. To saturate the pots, 4 dl were given to each plant. In week three a layer of green algae was observed in the pots, in particular on the reference soil. This could be an indication of too much water, and the amount was halved to 2 dl per pot. In week five all plants had established and the controlled irrigation was terminated. From then plants were only given water in very hot and dry periods and the trays were also emptied during periods with heavy rain since the drainage was not working as in a natural condition. The intention was to examine how they managed the natural climate with the natural temperature and precipitation.

From the beginning of week four the plants were measured approximately every 10th day until the end of the experiments. The shoot length was measured with a measuring stick from where they started on the cutting to where the top leaves began.

From 25th to 27th of July the plants were cut down. With a pruning shears the shoots were cut from the stem and the leaves were separated from them by hand. Both wet weight and length were measured for shoots and leaves. Due to budget limitations in the project it was not possible to analyse all roots. By taking the roots from the plant with the longest shoots in each treatment and for each clone type, a selection of twelve root samples was made. The roots were thoroughly washed from soil with taped water.

It was important to interrupt the experiment at the same day and therefore the cutting of leaves and shootings for all plants was realised first, and thereafter the selected roots was washed from the soil. All three parts were packaged in paper bags one by one and dried in 65 degrees Celsius until their weight no longer decreased.

4.4 ANALYSES AND CALCULATIONS

All plant parts and the pyrite ash were send to laboratories for analyses of metals and nutrient content.

4.4.1 Nutritional and metal analyses of the substrates (PA, PAB and R)

The nutritional analyses were performed by Agrilab AB. The nitrogen was analysed with the Swedish standard SS-ISO13878, and the results for total-P, Ca, K, Mg, Na and S with the standard analyses SS 0283 11.

The methods for analysing metal concentrations in soil respectively ash differ to some extent. Soil is normally analysed with weaker acids than ashes, as the compounds in ashes generally are harder bound to the material. The pyrite ash in this project was defined as soil since it was taken from the ground. This simplified the comparison since the same method could be used for all substrates. ALS Scandinavia uses following steps in the analyses. Before detection the sample is dried in 50°C and dissolved with HNO_3 and H_2O_2. The detection method used is Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

4.4.2 Nutritional and metal analyses of the plant parts

The amount of dry material was not sufficient to divide it and send it to different labs for analysis (nutrients and metals) as there is a minimum weight required for the results to be significant. Therefore, shoot and leaf material from each plant were divided in equally big parts and mixed together clone wise. These collective samples were then sent to the lab for nutritional analysis according to the same procedure as in section 4.4.1. The rest of the material from the clones were analysed for metals, plant by plant. The metal analyses for the plant parts by ALS Scandinavia are made through the standard ICP-SFMS.

4.4.3 Leaching analyses

To determine the toxicity of the contaminants in a soil it is seldom enough to only analyse the total concentration. It is also necessary to examine how much of the total concentration that could leach out and thus become bioavailable. There are several leaching tests, for example column-test, shaking (=batch) tests and biological tests. The principle behind them all is basically that a solid mass is run through with pure water, or water with more or less strong acids. The water face affects the properties, primarily pH, in the soil and some of the contaminants will let go and become mobile. This indicates risks for transportation of the contaminants to adjacent waters or other sensitive environments and higher bioavailability.

The leaching analyses were done by the European and US-standard SS-EN 12457-3. A shaking test in two steps with L/S 2 (2 litres Liquid/kg Solid sample) initially shaken for 6 hours, thereafter the volume of water is gradually increasing until it reaches L/S 10 and shaken for 18 hours. In total, the sample is shaken for 24 hours. The final result is a worst case leaching behaviour over a certain period of time. L/S10 reflects the maximum leachable quantity for a period between 60 to 100 years.

4.4.4 Bio concentration factor (BCF) and Translocation factor (TF)

Bio concentration factor (BCF) indicates how well the plants extract the metals from the soil. It is calculated according to:

$$BCF = C_{harvested\ tissue}/C_{soil} \qquad (6)$$

Where $C_{harvested\ tissue}$ is the total metal concentration in the biomass from the plant (roots, leaves and shoots), and C_{soil} is the metal concentration in the soil the plant grows in.

Translocation factor (TF) specifies the efficiency in the plant to transport a substance from the roots were the intake is to the aerial parts (e.g. shoots and leaves). It is calculated according to:

$$TF = C_{aerial parts}/C_{root} \qquad (7)$$

Here the numerator, $C_{aerial\ parts}$, is the total metal concentration in the parts above ground, thus only leaves and shoots but not roots, and C_{roots} is the metal concentration in the root mass.

Through those measurements it is possible to quantify the plants' efficiency in uptake and translocation and get an indicator of how well the specie will do as a phytoextractor. For the specie to be suitable for the method both BCF and TF should be greater than 1 (Ali, et al., 2013).

4.4.5 Effect from the pyrite ash on the clones

One of the main aims in the study was to answer the question whether there are differences in accumulation capability and tolerance between the four different clones. One way to do this is to quantify the affection on the clones from the pyrite ash treatment. For this the ratio between the reference and PA growth was calculated as following:

$$\frac{weight_{PA,\ clone\ x}}{weight_{R,\ clone\ x}} \qquad (8)$$

$$\frac{height_{PA,\ clone\ x}}{height_{R,\ clone\ x}} \qquad (9)$$

If the ratio between the weight/height in the plants that have grown in the pyrite ash and the reference is 1 or close to 1 the clone is slightly affected. If it is <1 the plant has been negatively affected from the treatment. If the ratio >1 the plant has been positively affected from the treatment.

5. RESULTS

In the following chapter the results from the study is presented.

5.1 PYRITE ASH

Analyses of the pyrite ash showed high content of As, Cd, Cu, Pb and Zn which all exceeded both background values (Naturvårdsverket, 2009) and limit values defined by the Swedish environmental protecting agency (Naturvårdsverket, 2009). Levels of three of the metals, Cr, Ni, and Co were not higher than the limit values though Co exceeded the background value. Compared with the metal concentrations in the reference soil Cr and Ni were the only metals where the levels had the same magnitude as in PA and PAB. All the other metal concentrations were considerably smaller in the reference soil. The bark had some dilutive effect for As, Cd, Cu, Pb and Zn, whereas the concentrations of Co, Cr and Ni showed no significant differences between the two pyrite based substrates (PA and PAB).

In figure 6 the total metal content in each treatment is shown. The analyses were executed after the experiment was finished. Since the uptake in proportion to total content was small the levels were estimated to be comparable. The red and green lines are limit and background values resp. for each metal.

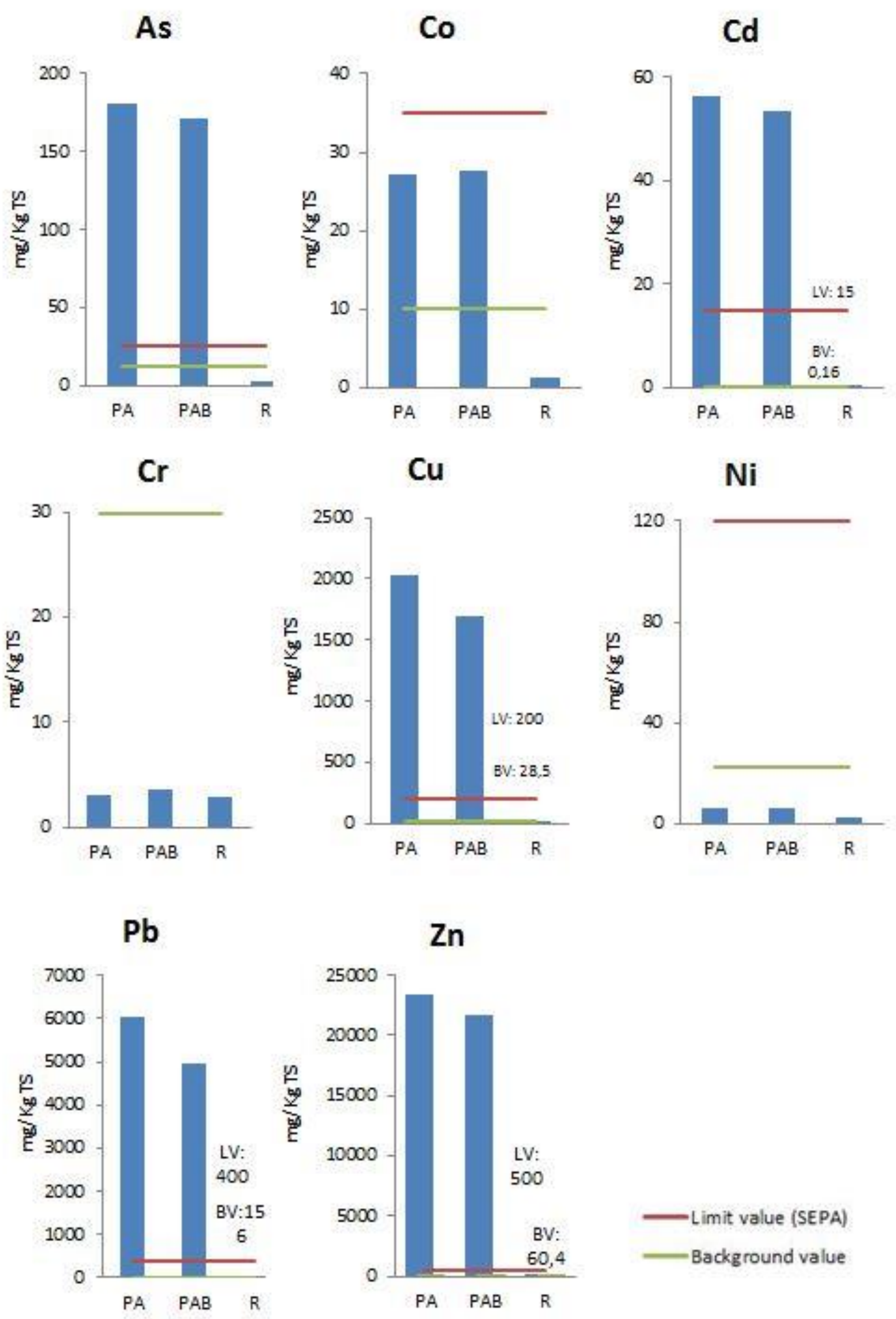


Figure 6. Total metal concentrations in all treatments (PA, PAB and R). The red line is the limit value for less sensitive land use **(Naturvårdsverket, 2009)** and the green line marks the background values in Sweden **(Naturvårdsverket, 2009)**.

5.2 GROWTH

During the first 4-5 weeks there was no visible difference between the clones in the different treatments. In week 5 the leaves in PA and PAB started to turn yellow and the growth was clearly inhibited. There were also distinct differences of bushiness between the reference plants and plants from the other two substrates. The reference plants almost crawled outside their pots at the end of the experiment, whereas the plants in PA and PAB seemed small and weak. By the end of June (approx. week 8) some of the plants in PA and PAB started to lose their leaves (Figure 1). Variations between the clones was not observed. Some plants were infested by aphids but generally the extent of infections was small.

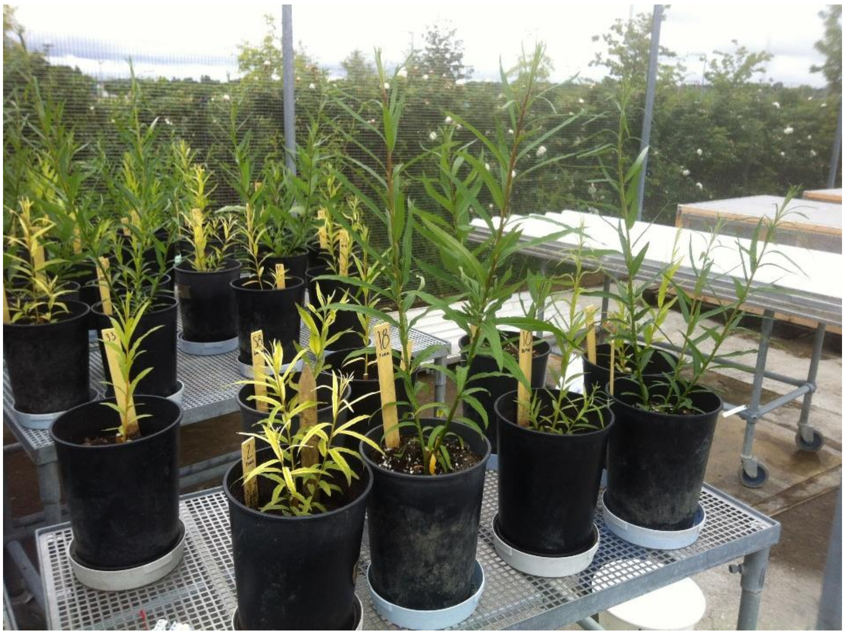


Figure 7 Picture from the experiment in week 7. Plants grown in pyriteash substrates to the left in the picture have clearly been affected in growth compared to the reference plant in the middle (no 18).

5.2.1 Weight

The dry weight (DW) measurements are shown in figures 8-11. The values for shoots and leaves are average values with n=5. Since the root values were taken from the one plant with highest measured height, n=1 and there were no standard deviation. All plant parts had significantly lower values in PA and PAB compared to the reference. However, the differences between PA and PAB were not significant except for the growth of roots where the plants in PAB had a stronger root development than the plants in PA.

The weight in each treatment had nearly the same magnitude for all clones. The reference showed differences especially for Tora, that had higher root mass, and Gudrun that developed higher leaf mass than the other clones. Jorr was the clone with the highest shoot mass. In total weight Tora had highest values of all clones in the reference, whereas Jorr showed the highest total mass in PAB. The results from total weight in PA did not show any significant differences between the clones.

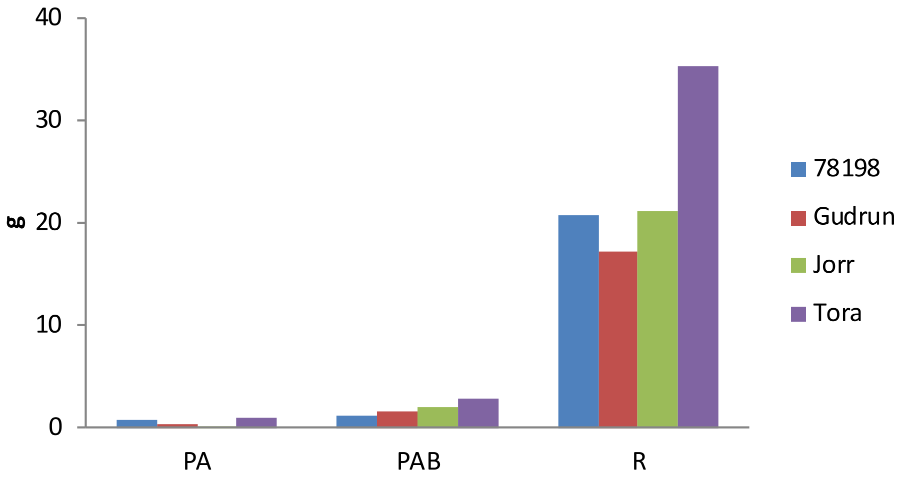


Figure 8. Root weight (g DW) in all treatments (PA, PAB and R), n=1, for all four clones.

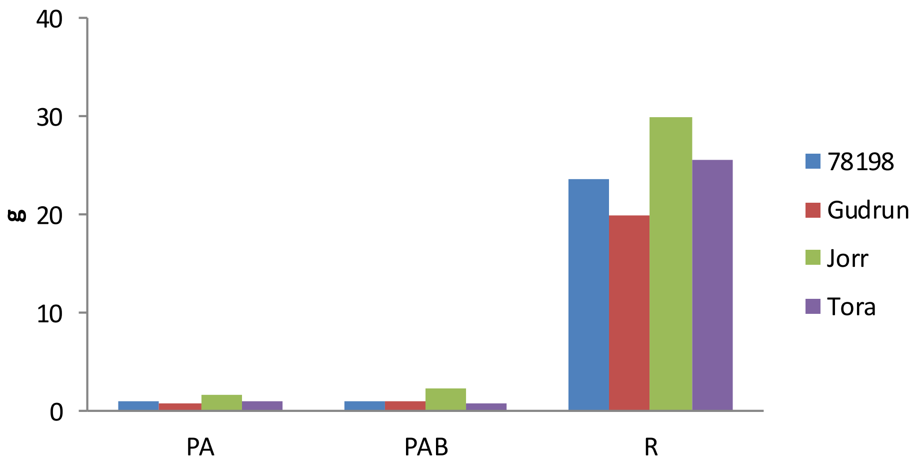


Figure 9. Shoot weight (g DW) in all treatments (PA, PAB and R), n=5, for all four clones.

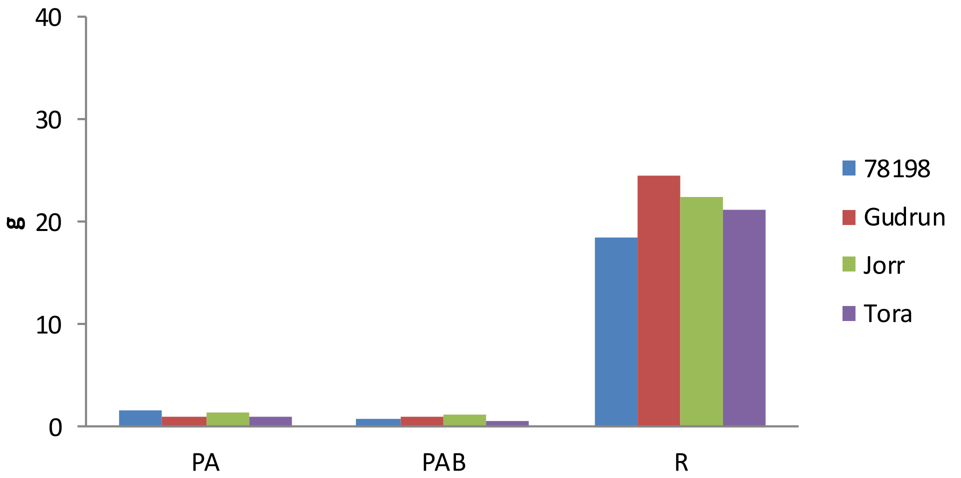


Figure 10. Leaf weight (g) in all treatments for all four clones.

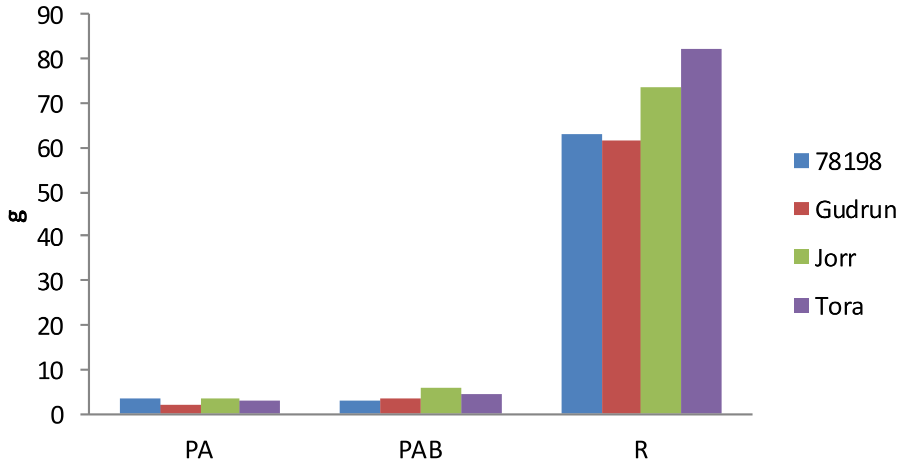


Figure 11. Total plant weight (g) in all treatments (PA, PAB and R) for all four clones.

5.2.2 Height

Also the results from the height measurement show the inhibition in the pyrite ash substrates (figures 11-14). The values of the two first measurement occasions show small differences among the three treatments, but the gap increased in the measurements thereafter. The height in the reference has a steep slope whereas the slope of PA and PAB levels away from the second or third measurement. Thereafter it had weak height development.

For all clones, the height in PA was greater than the height in PAB, though the differences for Gudrun were very small. Jorr had the biggest height in the final measurement, and Gudrun had the lowest. The differences were notable and could be seen also in the other two treatments.

For all treatments the clones had following order Jorr>78 198>Tora>Gudrun in the aspect of greatest actual value in height.

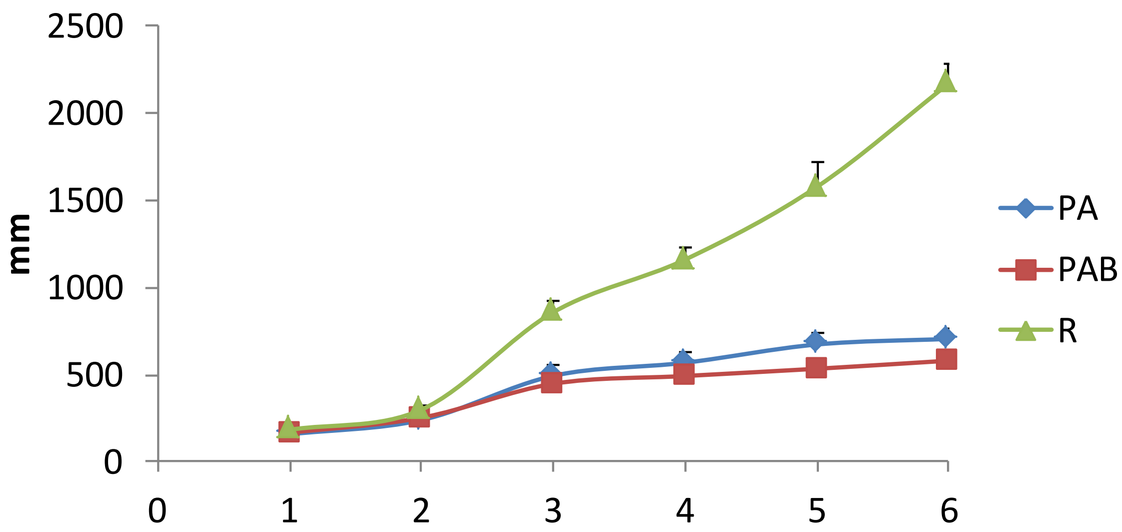


Figure 12. The measurement of height for clone 78198.

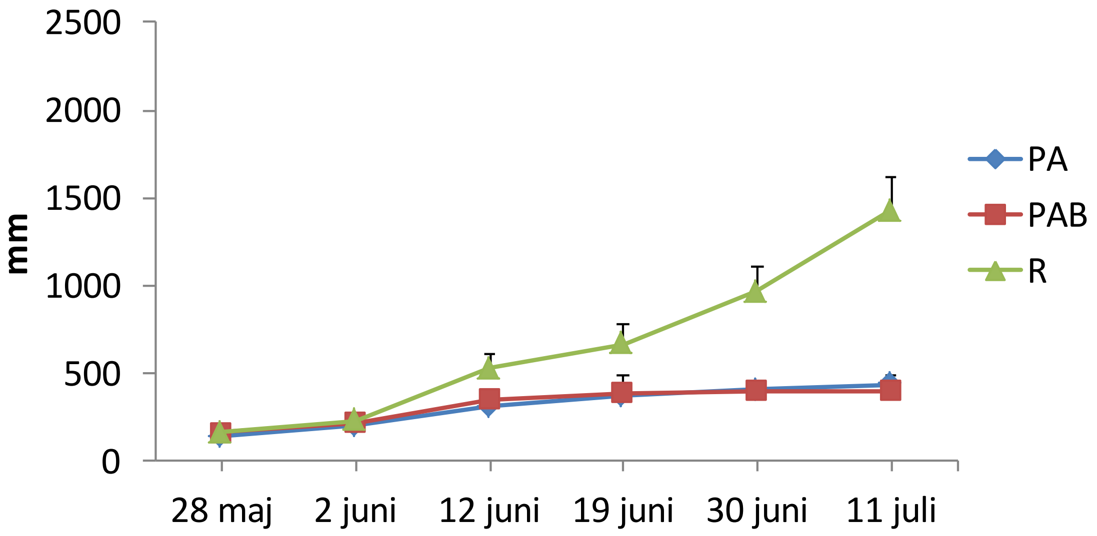


Figure 13. The measurement of height for clone Gudrun.

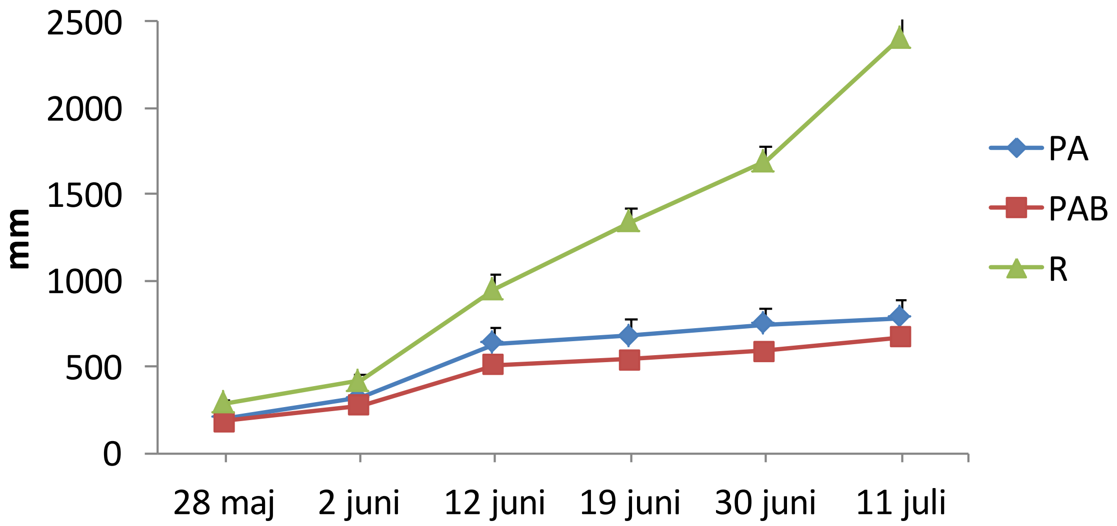


Figure 14. The measurement of height for clone Jorr.

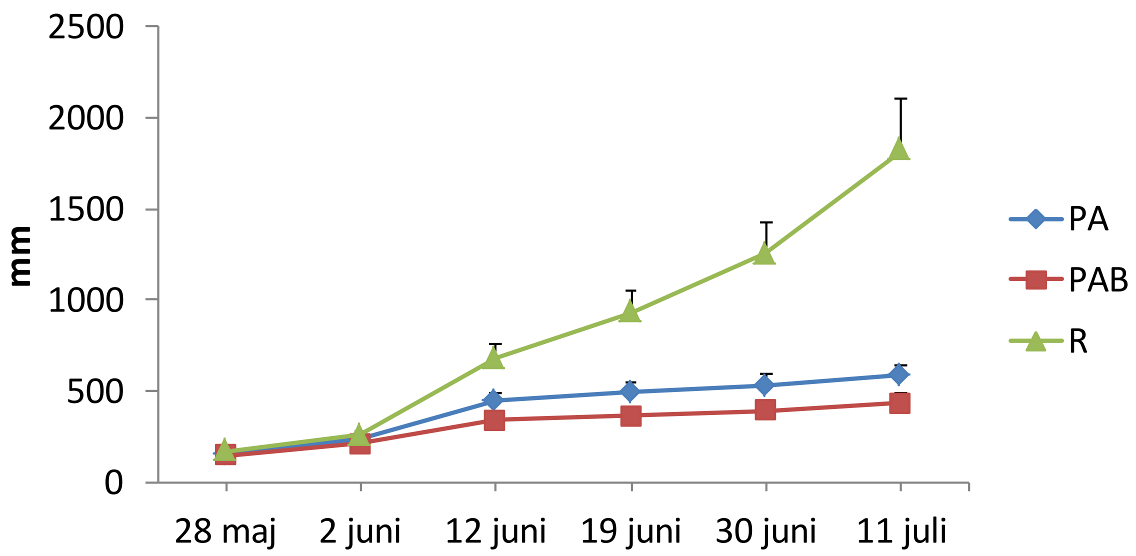


Figure 15. The measurement of height for clone Tora.

The effect (see section 4.5.5.) from the pyrite ash on the *clone height* was very much the same for all clones. This is shown in table 3 with values of the ratio between PA and R. Differences in the ratio on *total weight* showed to be greater, even though all clone ratios were small (<<1). Clone 78 198 had the highest ratio and Gudrun and Tora had the lowest. Jorr was in between.

Table 3. The effect of the pyrite ash on the four clones.

	Height	Weight
78198	0.3249	0.0536
Gudrun	0.3060	0.0348
Jorr	0.3251	0.0458
Tora	0.3214	0.0343

5.3 ACCUMULATION OF METALS

The figures 16-23 show the average metal concentrations (mg Kg^{-1} DW) for the three plants with highest growth for each clone (n=3). For roots, only *the* highest plant in each treatment and clone was selected and sent to analyses, n=1. The analyses showed higher metal content in the plants that grew in the pyrite ash based substrates (PA and PAB). This was most evident in the root analyses which were also the plant part with highest metal concentrations for all clones and all treatments. Except for Cd and Zn the accumulation in roots were much more intense, whereas the concentrations of these two metals were more evenly spread between all three plant parts. Unfortunately the root sample of Gudrun was damaged and could not be analysed. Least differences between pyrite ash substrates and R was seen for Cr and Ni.

In shoots and leaves the differences between the three treatments were not as significant, though it occurred also in these parts. This could especially be seen in the levels of Cd, Pb and Zn. Cu was the only metal where the accumulation decreased with concentration (the concentration of Cu was lower in PAB than in PA, figure 6) for all clones, plant parts and treatments. As shown in figure 6 As, Pb, Zn and to some extent Cd were all in lower concentrations in the diluted PAB this did not, however, show in the metal analyses of plants biomass.

When studying the reference analyses the accumulation levels were generally the same for all clones for Cd, Cu (except for leaf concentration in Jorr) Ni, Pb, Zn and also to some extend for As and Co. Whereas the concentration of Cr was more differentiated especially in shoots, where Jorr was the only clone that extracted above the detection limit. In roots, Jorr and Tora generally had higher metal concentrations in PAB than in PA. For Jorr this was true for all metals except for Cd and Cr. In the case of Tora it was true for all except Cd, Co and Cr, and in Ni where the levels were the same in the both treatments. Moreover, clone 78 198 had the opposite relation with generally higher concentrations in PA than in PAB (in roots).

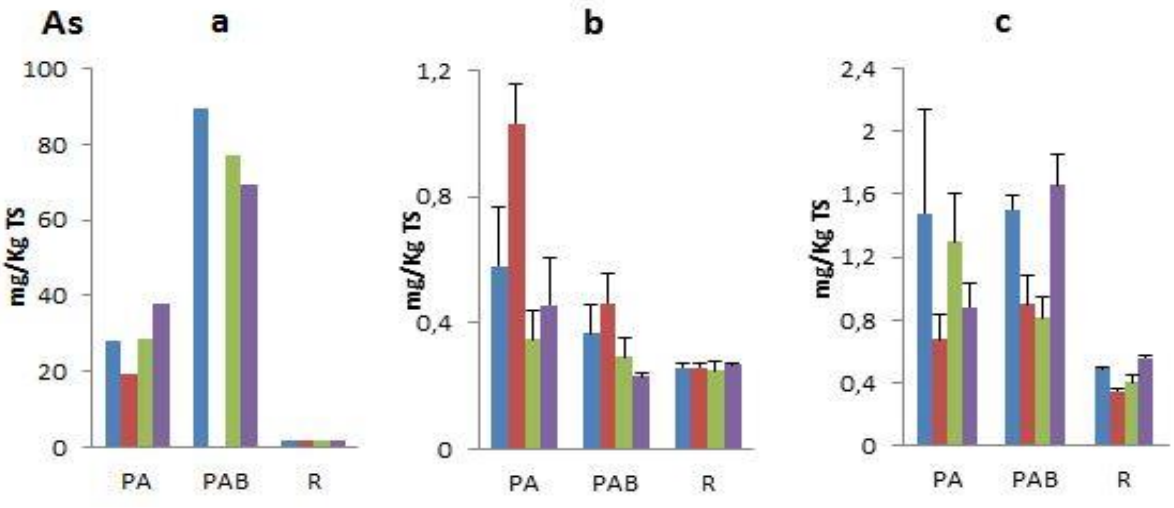


Figure 16. The concentrations of As in a= roots, b= shoots and c=leaves in the four clones in the three different treatments (PA, PAB and R).

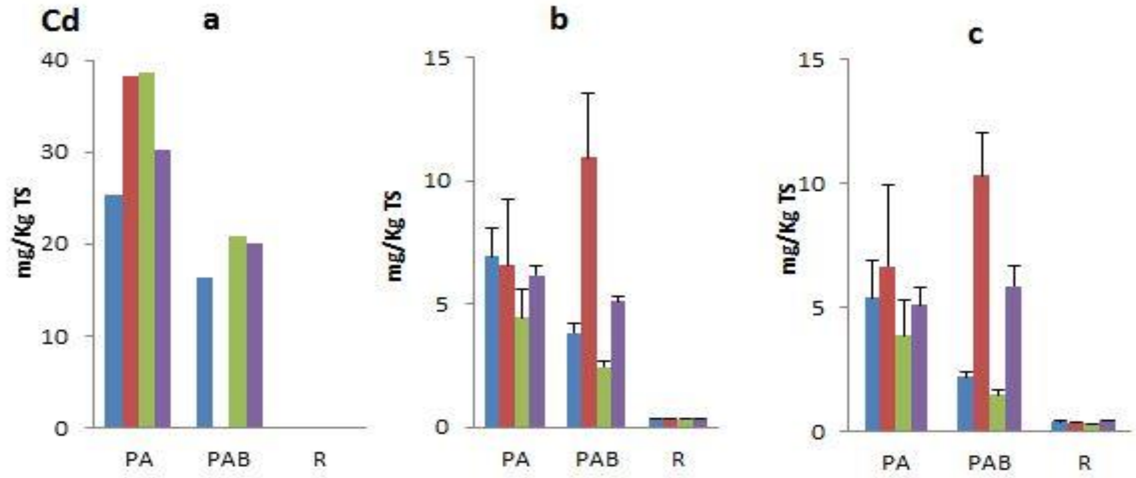
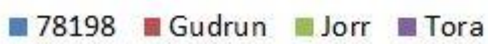


Figure 17. The concentrations of Cd in a= roots, b= shoots and c=leaves in the four clones in the three different treatments (PA, PAB and R).



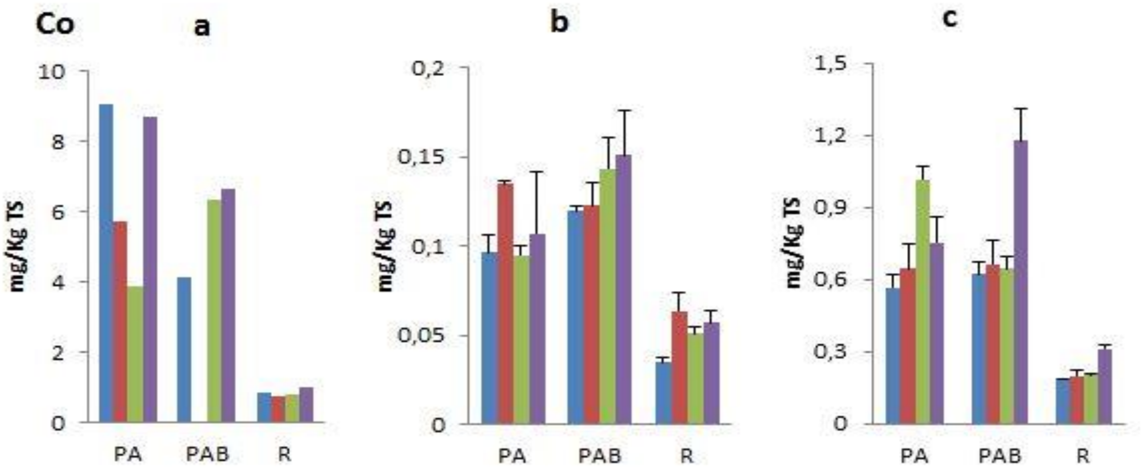


Figure 18. The concentrations of Co in a= roots, b= shoots and c=leaves in the four clones in the three different treatments (PA, PAB and R).

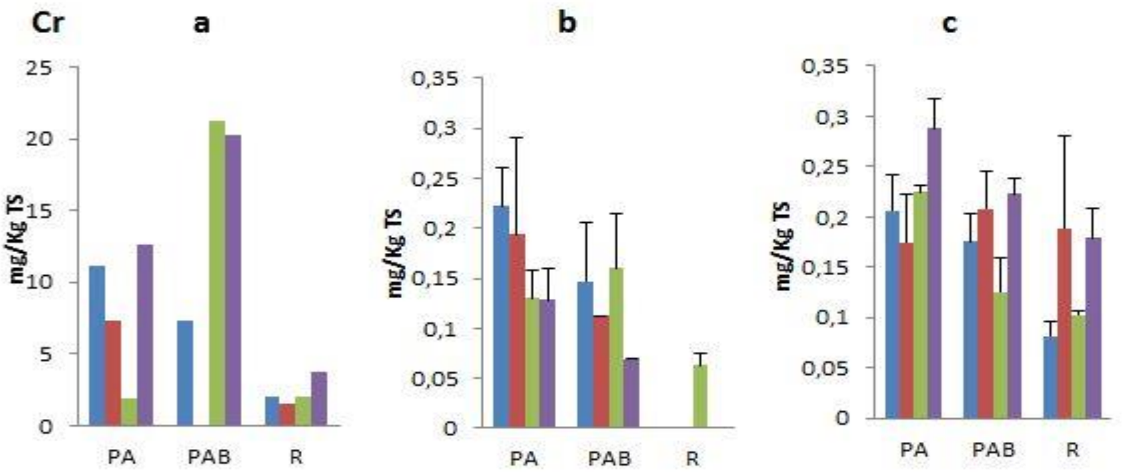


Figure 19. The concentrations of Cr in a= roots, b= shoots and c=leaves in the four clones in the three different treatments (PA, PAB and R).

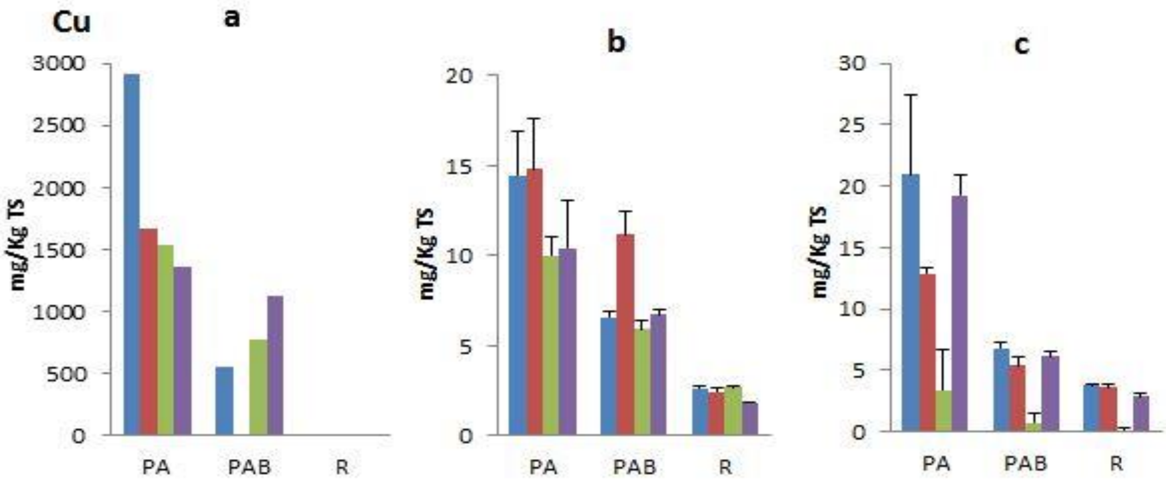
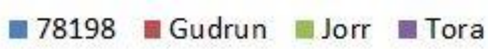


Figure 20. The concentrations of Cu in a= roots, b= shoots and c=leaves in the four clones in the three different treatments (PA, PAB and R).



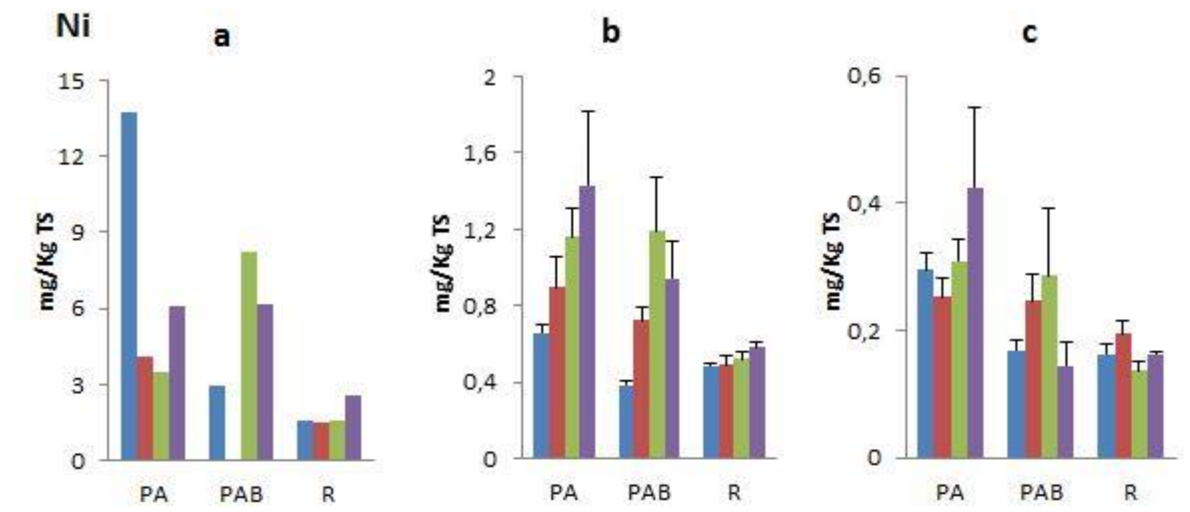


Figure 21. The concentrations of Ni in a= roots, b= shoots and c=leaves in the four clones in the three different treatments (PA, PAB and R).

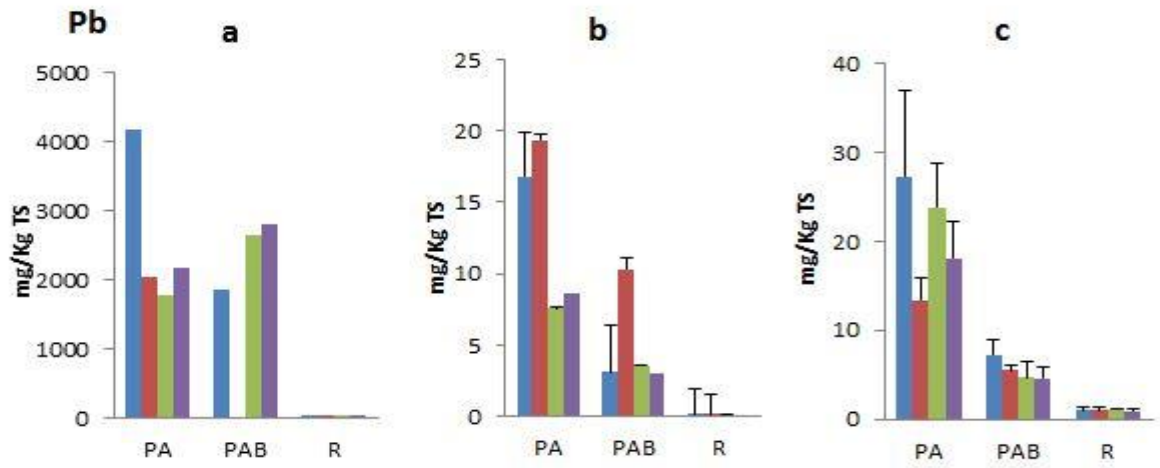


Figure 22. The concentrations of Pb in a= roots, b= shoots and c=leaves in the four clones in the three different treatments (PA, PAB and R).

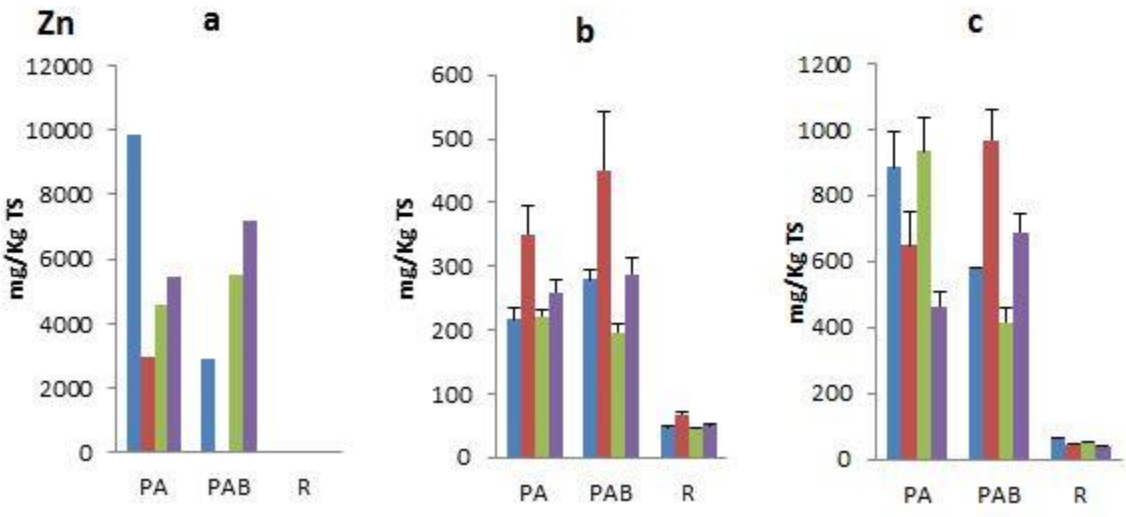
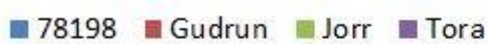


Figure 23. The concentrations of Zn in a= roots, b= shoots and c=leaves in the four clones in the three different treatments (PA, PAB and R).



5.4 BCF AND TF

BCF (bio concentration factor) (eq.1) values could only be calculated for the plants in PA since this was the only substrate analysed for metals. Thus table 4 shows BCF values for all clones in PA, n=1. Clone 78 198 showed BCF above 1 for Cr, Cu, and Ni, Jorr had no values above 1 but was close in the case of Co (0, 94) and to some extent Cd (0,77). In the case of Tora, it exceeded 1 for Cr and close to 1 for Ni (0, 91), and Gudrun exceeded the BCF value for Co and Cr. All clones had low values for As and Zn.

Table 4. The BCF (bio concentration factor) values for the four clones in PA.

Clone	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
78198	0.15	0.51	0.25	2.69	1.19	2.05	0.83	0.50
Jorr	0.15	0.77	0.94	0.47	0.62	0.52	0.35	0.23
Tora	0.20	0.60	0.24	3.07	0.55	0.91	0.43	0.28
Gudrun	0.10	0.77	1.39	1.78	0.68	0.61	0.41	0.15

The TF (translocation factor) (eq. 2) (table 5) was generally lower in the plants grown in PA and PAB than in R. Highest TF did the plants have in R for Cd and Zn where values well above 1 were found. Lowest were the values for Cr and for the rest of the metals the values were approximately the same and low (0,125 – 0,525). None of the TF values for PA and PAB exceeded 1, closest was Tora in PAB for Cd with a TF = 0,274, and 78 198 in PA for Cd with TF = 0,244.

Table 5. The TF (translocation factor) values for the four clones in all treatments (PA, PAB and R).

Treatm.	Clone	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
PA	78198	0.037	0.244	0.037	0.019	0.006	0.035	0.005	0.056
PA	Gudrun	0.044	0.172	0.068	0.025	0.008	0.141	0.008	0.170
PA	Jorr	0.029	0.108	0.143	0.091	0.009	0.212	0.009	0.125
PA	Tora	0.018	0.187	0.050	0.016	0.011	0.151	0.006	0.066
PAB	78198	0.010	0.186	0.090	0.022	0.012	0.093	0.003	0.146
PAB	Gudrun	-	-	-	-	-	-	-	-
PAB	Jorr	0.007	0.094	0.062	0.007	0.008	0.090	0.002	0.055
PAB	Tora	0.014	0.274	0.100	0.007	0.006	0.088	0.001	0.068
R	78198	0.201	2.259	0.125	0.021	0.234	0.205	0.218	1.826
R	Gudrun	0.162	2.818	0.173	0.061	0.215	0.233	0.147	1.597
R	Jorr	0.184	2.654	0.155	0.042	0.216	0.213	0.525	1.756
R	Tora	0.243	4.363	0.182	0.024	0.183	0.147	0.473	2.325

5.5 TOTAL CONCENTRATION

Table 6 below show the total concentrations (mg KG^{-1} DW) in the plants, all parts (roots, shoots, leaves) included. The values are averages of three plants for each treatment and clone, n=3. Highest values for all metals could be found in the plants grown in pyrite ash treatments, whereas the reference plants did extract notably less.

When comparing PA and PAB no general trends can be seen that was valid for all clones except that all extracted more As in PAB then in PA. Clone 78 198 had generally higher concentrations in the biomass grown in PA (except in the case of As). For Jorr the biomass grown in PAB had higher concentration of metals than biomass grown in PA except for Cd and Cu. Tora had higher concentration of Cd, Co, Cu and Ni in the biomass that had grown in PA and more Cr, Pb and Zn (and As) in biomass grown in PAB.

Table 6. In the table the total concentration (mg KG-1 DW) is listed for the four clones and for all treatments (PA, PAB and R)

Clone	Treatm.	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
78198	PA	10.1	12.5	3.2	3.8	983.7	4.9	1410.5	3659.0
Gudrun	PA	7.1	17.2	2.2	2.6	565.8	1.7	692.1	1314.7
Jorr	PA	10.1	15.6	1.7	0.8	519.7	1.6	603.8	1918.0
Tora	PA	13.1	13.8	3.2	4.4	461.9	2.7	730.1	2061.8
78198	PAB	30.5	7.5	1.6	2.6	190.4	1.2	621.9	1264.0
Gudrun	PAB	-	-	-	-	-	-	-	-
Jorr	PAB	26.0	8.3	2.4	7.2	261.4	3.2	885.1	2047.2
Tora	PAB	23.7	10.3	2.7	6.9	381.0	2.4	942.6	2728.6
78198	R	0.9	0.3	0.4	0.7	6.7	0.7	1.4	47.1
Gudrun	R	0.8	0.3	0.3	0.6	6.7	0.7	1.8	49.3
Jorr	R	0.8	0.3	0.4	0.7	7.8	0.7	0.8	41.0
Tora	R	0.8	0.3	0.5	1.3	5.9	1.1	0.7	35.6

5.6 TOTAL AMOUNT EXTRACTED

In table 7 total metal removals are shown. The clone with highest removal (highest extraction for most metals) was found to be different for all treatments. In PA clone 78 198 was most successful in total removal, whereas Jorr had highest level in PAB and Tora in R.

Table 7. Total uptake of metals (mg) in plant biomass in all four clones.

Clone	Treatm.	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
78198	PA	0,034	0,042	0,011	0,013	3,319	0,017	4,759	12,345
Gudrun	PA	0,015	0,037	0,005	0,006	1,217	0,004	1,489	2,829
Jorr	PA	0,034	0,053	0,006	0,003	1,756	0,006	2,040	6,479
Tora	PA	0,037	0,039	0,009	0,012	1,304	0,008	2,062	5,823
78198	PAB	0,097	0,024	0,005	0,008	0,606	0,004	1,979	4,022
Gudrun	PAB	0,050	0,027	0,005	0,014	0,505	0,006	1,708	4,455
Jorr	PAB	0,135	0,046	0,014	0,040	2,197	0,015	5,438	15,040
Tora	PAB	0,103	0,045	0,012	0,030	1,659	0,011	4,106	11,886
78198	R	0,055	0,019	0,023	0,043	0,422	0,047	0,086	2,965
Gudrun	R	0,051	0,018	0,021	0,036	0,416	0,044	0,110	3,048
Jorr	R	0,060	0,020	0,026	0,052	0,573	0,054	0,056	3,022
Tora	R	0,069	0,025	0,038	0,108	0,482	0,090	0,058	2,928

5.6 LEACHING ANALYSES

The results from the shaking test (table 7) indicate that the mobility in the pyrite ash is small for all metals. Most of the compounds do not exceed the European Union criteria for *inert waste* (EU, 2003) (Appendix A). The concentration levels of Cd, Cu, S and Zn leached out are higher but stay within the limits for next level, *non-hazardous* waste (Appendix A.). Measured pH is 7.3 and 7.5 resp.

Table 8. The concentrations (mg/Kg DS) for the metals after leaching test with L/S 2 and L/S 10. Also pH values are shown.

	As	Cd	Cr	Cu	Ni	Pb	Zn	pH
L/S 2	0.00522	0.053	<0.001	1.3	0.00568	0.0714	3.2	7.3
L/S 10	0.0137	0.195	<0.005	1.22	0.0104	0.444	11.2	7.5

5.7 NUTRITION ANALYSES

The nutrient uptake in the plants is presented in table 8 (n=1), the results represents the total uptake of all plant parts and are shown in percent. Plants of all clones have extracted notably lower levels of P in both PA and PAB in comparison to reference plants. In the case of N-tot, plants grown in PAB had the lowest uptake and plants grown in PA the highest. The reference plants (R) were in between for all clones. There are no standard deviations since these analyses were done on collective samples. A comparison between clones is therefore difficult to make.

For Ca, Mg and S the levels are approximately the same for all clones and treatments. Tora stands out with higher accumulation of Ca and S in PA.

Table 9. Total nutrient uptake from all treatments in all clones, presented in percent (%) (n=1).

Clone	Treatm.	N-tot (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
78198	PA	2.01	0.07	1.58	1.32	0.27	0.71
Gudrun	PA	2.16	0.08	1.32	1.41	0.25	0.66
Jorr	PA	2.10	0.07	1.52	1.36	0.26	0.70
Tora	PA	2.34	0.08	1.63	2.80	0.26	1.45
78198	PAB	0.68	0.07	1.42	0.94	0.15	0.50
Gudrun	PAB	0.54	0.04	1.40	0.70	0.11	0.24
Jorr	PAB	0.76	0.07	1.43	1.09	0.17	0.57
Tora	PAB	0.77	0.07	1.30	1.51	0.20	0.64
78198	R	1.64	0.24	1.48	0.91	0.23	0.38
Gudrun	R	1.68	0.22	1.34	0.99	0.24	0.30
Jorr	R	1.57	0.26	1.46	0.85	0.22	0.31
Tora	R	1.69	0.22	1.29	1.00	0.23	0.34

6. DISCUSSION

6.1 GROWTH

The result shows that all clones are affected by the pyrite ash. Their growth is clearly inhibited and the yellow or very light green leaves indicate stress. Two possible reasons to the lack of growth are that the substrates in PA and PAB are either too toxic from metals and that the plants simply are poisoned, or that they lack of necessary nutrients for the plants.

The dilutive effect from the bark was smaller than expected, despite that half of the substrate in PAB was bark the levels of metals did not decrease considerably which the result in figure 6 points out. However, the root mass in PAB grew bigger than in PA and this was expected since the pyrite ash is a compact material and the presents of the bark probably made it easier for the roots to grow due to aeration and loosen up structure. Most interesting with the mixture bark/pyrite ash is though that it did not seem to affect any other growth parameters. The height in PAB is even lower than in PA despite the stronger root development. The conclusion is that bigger root mass did not, in this soil, give higher potential for growth. If the plants in PAB had extracted significantly more metals due to higher root mass, toxic effects could have been pointed out as a reason. To some extend this was true for Jorr and Tora that had higher concentrations of several metals (at least in their roots) in the plants grown in PAB. However, clone 78 198 had higher values of metals in plants grown in PA but still showed a decrease in height just like the other two. This indicates either that the metals were not, or at least not the only reason for the inhibit growth in height, or that the clones differ in their reaction of high metal concentrations in the substrate. Since the clones show big similarities in both growth and accumulation in the reference the latter statement would point out quite remarkable differences in the genome of the clones and most presumable are that, besides the high content of metals, there are other reasons for the lack of growth.

One such reason could be the amount of nutrients available for the plants. It is found that despite the greater root mass the uptake of N-tot was decreased in PAB, moreover the highest levels of N-tot were found in plants grown in PA compared to both PAB and R. This was not expected. The reason for the low N-tot concentration in PAB plants could have its explanation in the C:N ratio. The bark is a great source of carbon and there is a possibility that the microbes in the PAB could use this to grow in number and by then increase the competition of the N in the soil, which lowers the availability of N for the plants.

Both pyrite substrates had low extraction of P and this could be a reason for lack of growth. It does not matter how high other levels of nutrients are, if there is lack of P the growth is going to be inhibited. This is also confirmed by the result of the references extraction of P which is much more efficient than in PA and PAB and also had a successful growth.

However it can be concluded that Ca-, Mg- *nor* S- halts in biomass were reason for the lack of growth of the plants in PA and PAB since the reference had the same concentrations in their biomass.

6.2 ACCUMULATION FACTOR AND EXTRACTION

Even though the total metal concentrations in the ash were high it does not revile much of the actual toxicity of the material. One should keep in mind that the results from the leaching analyses indicated a more or less inert material, which could be due to the high pH values (7.3). When sulphates are involved the pH is expected to be low and high amount of the metal content appear as free ions. One example is the study on pyrite ash from Falun, which had a pH bellow 3. (Lin & Qvartfort, 1996). Results from that study showed a considerably higher leachability for metals than the ash from Skutskär. The metals are therefore most probable not in ionic form but rather in organically-complexed form or bound to oxides (e.g. SO_{4-}) with small mobility.

Still it can be concluded that the metals do have certain mobility due to the significantly higher concentrations in the biomasses of the plants growing in the pyrite ash based substrates than in the reference. This confirms the newer studies on pyrite ash that refute the earlier belief that the metals mostly are bound in non-soluble sulphides (Nordback, et al., 2004).

The total concentrations in the pyrite ash based substrates (PA, PAB) were low for Cr, Ni and they had approximately the same level as in the reference soil which indicates that the content of these metals were not the reason for the scarce growth in PA and PAB. Concentrations of As, Cd, Cu, Pb and Zn were all far above the limit- and background values and all of these could be conceivable reasons for the poor growth. The concentration of Cu in plant biomass decrease with decreasing levels in the substrate. This could not be shown for any other of the metals and confirms the results from Tingwey et al. 2014. The correlation between concentrations in substrate and biomass could be useful for future planning of phytoremediation for Cu-contaminated soils.

It is difficult to point out certain trends among the clones and their uptake of metals, especially divided in plant parts. An interesting result from the study is that the accumulated levels of metals in leaves are not negligible and that harvest of leaves is an important aspect if remediation the soil from metals is the purpose. This was also observed by Vyslouzilová et al. 2003.

It can be concluded that clone 78 198 has the highest metal concentrations of Co, Cu, Ni, Pb and Zn in PA in its biomass. Tora has highest concentrations of As and Cr and Gudrun was the most successful extractor of Cd. Since 78 198 also had a relatively high biomass it was also the most efficient clone in total uptake, except for Cd and As where Jorr and Tora resp. were more efficient. This could indicate that, for pyrite ash from Skutskärs papper mill, clone 78 198 has the best phytoextraction potential in aspect of accumulation of all clones. The results are though too vague to make any certain conclusions.

6.3 CLONE CHARACTERISTICS AND POTENTIAL FOR PHYTOREMEDIATION

By studying the reference the clones' unique characteristics become visible and this offers a possibility to analyse their suitability for phytoextraction. The variations that are seen in the reference can be interpreted as the variations in each clones genome. The clones have similar characteristics in many aspects, though they do differ in their priorities in growth. This was most evident for Gudrun that had broader leaves and higher leaf mass than the other clones and for Tora that had the highest root mass. In height Jorr was the clone with highest final values in all treatments and Gudrun had the lowest. Because of the short term experiment it is not possible to say if these characteristics will last when the plants are more established in following seasons. This emphasises the need for field studies lasting for several seasons to identify long term properties.

The ratio between the PA and R (table 3) shows that Gudrun and Tora were the most affected clones from the pyrite ash in weight since their biomass had the biggest changes. Jorr was less affected and least affection did the pyrite ash have on 78198. Still the ratio is small for all clones and they were all clearly affected. In height, the difference between the ratios is smaller and conclusions about differences in clone tolerance cannot be drawn. As concluded earlier the effects on the growth were striking and therefor it is difficult to point out any clone that should be preferable to remediate the pyrite ash.

Despite that the reference soil had much lower concentration of metals than the substrates in PA and PAB, the translocation factor (TF) was higher for all metals. This shows firstly that all clones have ability to transport metals, but that it is strongly reduced for the plants in pyrite ash substrates (PA, PAB). The results of TF in the reference show on larger values than 1 for both Cd and Zn. This indicates that all tested clones have the potential to remediate these elements from soil which is in accordance to earlier studies (Fischerová, et al., 2005, Mleczek, et al., 2010, Vyslouzilova, et al., 2003 and Stoltz & Greger, 2002) where the phytoextraction potential of these elements with different clones of Salix have been examined.

The experiment was, however, very short and it is not certain that the differences that could be seen will be the same in season two. Over all though, the results show that potential for growth is approximately the same for all clones, especially when the short period of time for the experiment is considered.

In the aspects of extraction the clones in the reference soil showed small differences for many metals, whereas they were bigger in PA and PAB. The reason for this demands though more research in how compounds interact with each other and how a changed soil environment in certain parameters affects others. As and Zn proved to be hardest for the clones to extract from the soil with BCF-values close to zero. The high content of Zn could explain the low uptake of As in accordance to the study by Vyslouzilova et al. 2003. It could also be an important explanation to the generally bad growth, and confirm earlier studies where high content of Zn in the substrate proved to have toxic effects on salix (Vyslouzilova, et al., 2003, Greger & Landberg, 1994). Because of the wide perspective in this study, comparisons like this could be uncertain when the reference studies often have more narrow perspectives with fewer parameters looked at. Clone 78 198 had a BCF-value (only calculated for PA) greater than 1 for three metals, Cr, Cu and Ni and this clone could thus be a good option for phytoremediation if these metals are the issue. On the other hand the TF for these metals were low in the clone. Consequently these metals will stay in the roots and not be translocated to the aerial parts which obstruct the management since the roots also have to be dug up to reach the metals. If the bad translocation would be due to lack of nutrients this problem might be solved with nutrients added to the pyrite ash. More nutrients could though affect the competition of sites in the soil for cations and possibly increase the concentration of free metal ions and their mobility.

As concluded, the complexity of the soil and how its compounds interact in certain conditions is difficult to anticipate. There have been done few studies that examine more general issues like this. Of course one of the reasons for this is that the results are not as clear as in more simplified studies where two or maximum three parameters are compared, from where it is possible to make conclusions of the effects from certain parameters. Though it is necessary to make broader studies (e.g. field studies) since it is the only way to explore the interaction between the parameters and true complexity in soil. The ash doubtless had very high content of metals and this is probably a great factor for the poor growth and, for most metals, the week extraction. Even though this study cannot point out the exact reason it does point out potential in salix clones for both accumulation and translocation of these metals. A first step could there for bee to use salix clones to stabilise or immobilise (phytostabilisation/phytoimmobilisation) the metals, and from there create a more favourable environment for them to grow and thus increase the potential for phytoextraction.

Another interesting area to explore further would be an alternative additive than bark to the pyrite ash. The bark did have certain positive effect but not enough to increase the tolerance and accumulation ability to a wider extent. Perhaps another additive with diverse composition could improve the substrate so it would be possible to distinguish more differences between the clones and increase their remediation potential.

7. CONCLUSIONS

- The pyrite ash did affect all clones negatively in both height and weight. It is, however, difficult to say the exact cause to the inhibition of growth.
- The pyrite ash proved to be a very inert material and despite the high total content of metals it cannot be concluded that these were the only reasons for the inhibition in growth. Another possible reason is lack of nutrients
- The clones in the substrate with bark mixed did have a better growth in the sense of root mass. However, this did not affect the height in any positive matter since all clone heights were the lowest in PAB. The bark affected the extraction of As in all clones, and clone 78 198 decreased its extraction of all other metals in the substrate with the bark and pyrite ash mixed. No other trends of metal accumulation could be seen.
- The bark had significant negative effect on all clones uptake of N.
- The P-halt in the plants biomass was significantly lower in both pyrite ash substrates than in the reference.
- The clones did not show any clear trends in their extractions of metals. Some clone was better for some certain metal while another was more efficient for others. Though all clones had high efficiency in uptake and translocation factor of Cd and Zn. Jorr and Tora had the highest amounts of total metal uptake in PAB and 78 198 in PA.
- The extent of the inhibition of growth was too big to be able to distinguish any clear differences and/or correlations between clone growth/extraction and tolerance. It is necessary to make the substrate less harmful to be able to clear out variations in tolerance.
- The pyrite ash as a material is difficult to analyse due to its complex composition of many compounds. There are lack of knowledge how so called "cocktails", mixes of many metals and other pollutions, interacts in soil and more research in this area is required.
- The pyrite ash cannot be remediated from the tested clones of Salix as it is, it needs to be dilutive or modified somehow.
- Clones of Salix could work as a stabiliser/immobiliser instead of an extractor of the pyrite ash and that way prevent further leach out. This should be further investigated.

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APPENDIX A

Leaching limit values for inert and non-hazardous waste resp. established by the European Union, Directive 1999/31/EC.

Table 10. Limit values for waste accetable at landfills for inert waste established by the European Union.

Component	L/S = 2 l/kg mg/kg drysubstance	L/S = 10 l/kg mg/kg drysubstance
As	0.1	0.5
Ba	7	20
Cd	0.03	0.04
Cr total	0.2	0.5
Cu	0.9	2
Hg	0.003	0.01
Mo	0.3	0.5
Ni	0.2	0.4
Pb	0.2	0.5
Sb	0.02	0.06
Se	0.06	0.1
Zn	2	4
Chloride	550	800
Fluoride	4	10
Sulphate	560 (*)	1 000 (*)
Phenol index	0.5	1
DOC (**)	240	500
TDS (***)	2 500	4 000

Table 11. Limit values for waste accetable at landfills for non hazardous waste established by the European Union.

Components	L/S = 2 l/kg mg/kg drysubstance	L/S = 10 l/kg mg/kg drysubstance
As	0.4	2
Ba	30	100
Cd	0.6	1
Cr total	4	10
Cu	25	50
Hg	.05	.2
Mo	5	10
Ni	5	10
Pb	5	10
Sb	0.2	0.7
Se	0.3	0.5
Zn	25	50
Chloride	10 000	15 000
Fluoride	60	150
Sulphate	10 000	20 000
DOC (*)	380	800
TDS (**)	40 000	60 000