“Germination and growth of Deschampsia cespitosa and Festuca ovina in arsenic soil”

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ABSTRACT

Arsenic contaminated soil is one of the major arsenic sources for potential harmful ecological impact on living organisms. Phytoremediation using viable plant species is an emerging, biological technology for the removal of toxic contaminants from soil. Although hyperaccumulating plants and plant varieties are known, there is still a need to find new (and preferably local) plant species for phytoremediation.

The aim of this study was to investigate the germination and growth of two grass species common in the northern Fennoscandia with respect to the influences of high concentration arsenic. The study was conducted on Deschampsia cespitosa and Festuca ovina species that were collected from both arsenic contaminated and arsenic free areas at Green Stone Belt area in Kittilä, Central Lapland. Seed germination, seedling growth and cuttings growth in both the arsenic contaminated soil (collected from Kuotko area) and controlled soil (collected from Kallo area) were observed over a period of 8 weeks (2017 to 2018). Seed or cutting origin, soil type and inoculant plant origin, all either contaminated (arsenic) or control, were the studied factors.

Germination and leaf number of Deschampsia cespitosa were significantly affected by contaminated condition originated seed and soil and performance was markedly better in controlled condition. Germination of Festuca ovina was also significantly higher in controlled condition originated seed although the performance was better in contaminated condition soil. Leaf number of Festuca ovina performed better in contaminated condition originated seed.

In cuttings growth, leaf number for Festuca ovina, was higher in controlled condition originated plants than on contaminated condition. Oppositely contaminated condition soil type produced longer leaves. Leaf number and leaf length of Deschampsia cespitosa were not significantly affected by arsenic treatment. Festuca ovina resulted slightly higher shoot biomass in controlled condition than contaminated condition. Root biomass of both Deschampsia cespitosa and Festuca ovina were not affected by arsenic treatment.

The overall results show that Deschampsia cespitosa and Festuca ovina were both affected in the arsenic concentrated soil. However, Deschampsia cespitosa did suffer from high arsenic soil conditions whereas Festuca ovina was as fruitful in arsenic concentrated soil as in control soil in all the greenhouse growth experiments including germination and growth results.
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References
1. Introduction

1.1 Soil pollution and vegetation

Soil is one of the major part of biosphere. Soil is a reservoir of many crucial micro and macro nutrients, minerals and also act as a natural buffer for different chemical transportation (Yaylali-Abanuz, 2011). Wide range of pollutants affect the natural function of soil. Soil contaminants can reach soils in many different ways and by various routes (Edwards, 2002). Soil pollutions occur in different manners including heavy metals contamination, improper discharge of industrial effluents, sewage, fertilizers washes off, litters, landfill leachates etc. Heavy metals in soil have been considered to be a significant pollutant due to its persistence and negative impacts on the environment (Luiza et al., 2018).

Due to its contaminant characteristics, it’s necessary to take the soil pollution seriously and prevent the further contamination as well as try to find efficient and cheap methods for cleaning contaminated soils (Nriagu, 1979). Impacts of soil pollution is diverse because soil ecosystem is complex and diverse (Edwards, 2002). There may be predicted effects from direct acute toxicity to soil organisms to indirect changes in dynamics of soil processes interacting with climate (Edwards and Thompson, 1973). Harmful ecological impacts of soil pollution is apparent in different levels of ecosystem including organism-population level, community level, ecosystem level and landscape level. Plants exposed to these harmful chemicals and heavy metals are suffering from reduced primary and secondary productivity, growth and high mortality. Also community level impact on plant/plant, plant/microbial, or plant/faunal interactions and changes in spatial heterogeneity of plants and soil organisms are concerning issues of soil pollution (Edwards, 2002).

Impacts of soil pollutant on natural ecosystem interact with number of factors including the persistence of the pollutants, the resilience of a system to overcome the effects of a pollutant, the potential for soil organisms to develop resistance to a pollutant, the potential of an ecosystem to utilize alternative organisms accordingly there is also considerable potential for variable bioremediation mechanisms for reducing the pollutant impacts (Edwards, 2002).
1.2 Heavy metals contamination

Heavy metals have metallic properties such as malleability, conductivity, cation stability, and ligand specificity (Raskin et al., 1994). They occur naturally having high atomic weight and density. Their atomic density is 5 times higher than water. Some heavy metals including Co, Cu, Fe, Mn, Mo, Ni, V, and Zn are trace elements for the plants. They are required in very small quantities and excessive amounts have negative effects on the organisms (Chibuike and Obiora, 2014). On the other hand, heavy metal elements like Pb, Cd, Hg, and As do not have any beneficial effect on organisms (Chibuike and Obiora, 2014). Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. The degree of toxicity largely depend on the dose factor, route of exposure and also the physiological and phylogenetic characteristics of the exposed species. They are considered to be poisonous due to their harmful impacts (Tchounwou et al., 2014).

Rapid industrialization due to the expanding population throughout the world is resulting environmental pollution. Contamination of air, water and soil by the growing urbanization and industrialization do have significant negative environmental impacts. Human health deterioration, environmental degradation are some of the common impacts environmental pollution has brings the necessity to address these problems with scientific explanations (Yaylali-Abanuz, 2011).

Soil pollution with heavy metals is more concerning issue than ever. Heavy metals are naturally present in soil but anthropogenic and natural causes increases the concentration of heavy metals to an unacceptable level which is harmful for both plants and animals. Some of the human activities that brings this poisonous element to soil include mining and smelting of metals, burning of fossil fuels, use of fertilizers and pesticides in agriculture, production of batteries and other metal products in industries, sewage sludge, and municipal waste disposal (Chibuike and Obiora, 2014).

Heavy metal contamination of soil along with other organic pollutants do have threatening human impacts. Because the heavy metals penetrates into the water and to the vegetation which they can eventually bio accumulate (Chatterjee and Chatterjee, 2000). In addition, heavy
metals have direct impacts on plant growth, performance, and yield. They cause changes in physiological and biochemical processes including disturbances in light reactions, respiration, photosynthesis, which apparently pose a harmful impacts on the overall ecosystem (Chatterjee and Chatterjee, 2000). In addition some of the direct impacts that high concentration of heavy metals pose on plants includes inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress. They also have some partial effects includes replacement of essential nutrients at cation exchange sites of plants (Tchounwou et al., 1999).

Considering the extent to which soil heavy metal contamination can bring negative effects, decontamination of soil becomes a crucial topic to take further. Different approaches can be considered fruitful including ex-situ and in-situ approaches to improve the soil decontamination (ERNST, W.H.O, 1996).

1.3 Arsenic contamination

Arsenic is a toxic element widely encountered in the environment and in organisms (Tu and Ma 2003). Due to its highly toxic nature and colossal abundance, arsenic (atomic number 33) contamination has become a high environmental concern (Mondol and Suzuki, 2002). The spread of arsenic is worldwide. Countries including Bangladesh, India, China, Taiwan, Vietnam, USA, Argentina, Chile and Mexico are highly exposed to arsenic contamination (Bhattacharya and Ghosh, 2015). In a wider region of Bangladesh and in certain places of India, crop production is hindered due to exposure to arsenic contaminated soil especially paddy soils in Bangladesh contains high Arsenic concentration (Li et al., 2009) and (Saha and Ali, 2007). Most dominant sources of this arsenic in the soil is the use of arsenic contaminated water for irrigation (Alam and Sattar, 2000) and (Ullah, 1998). For instance, arsenic contamination in soil, through industrial activities resulted 1217 mg/kg of arsenic in the soil in Chenzhou, southern China (Liao et al., 2005).

The sources of arsenic are diverse. Arsenic is being produced both naturally and anthropogenically (Tu and Ma 2003). Commonly arsenic is found in the earth’s crust (approx. 3 mg As kg⁻¹) (Cullen and Reimer, 1989). Arsenic contamination in soils has become a major concern to government and industry as more arsenic related human health problems are surfacing worldwide (EPA, 2001).
Arsenic is toxic mineral that occurs in environment and in organisms. Both natural and anthropogenic activities are responsible for the entrance of arsenic into the aquatic and terrestrial environment (Tu and Ma 2003). Anthropogenic activities, in particular in the form of coal combustion, using wood preservatives and arsenic based pesticides act as a triggering factor in upraising the soil arsenic concentration (Tu and Ma 2005). Arsenic is a commonly produced waste product generated from the mining of metals, in particular tin, but also with many other metals (Francosconi et al., 2002).

Natural sources of arsenic occurs due to several natural phenomena such as volcanic eruptions and soil erosion, wind erosion, low temperature volatilization from soils, marine aerosols and anthropogenic activities (ATSDR, 2000) and (Cullen and Reimer, 1989). Industrially arsenic is used as raw elements to produce different agricultural substances such as insecticides, herbicides, fungicides, algaecides, sheep dips, wood preservatives, and dye stuffs. Their use in veterinary medicine for the eradication of tapeworms in sheep and cattle have also been widespread (Tchounwou et al., 1999)

Arsenic contamination is solely associated with the chemical forms and oxidation states of arsenic. Other factors including physical state, gas, solution, or powder particle size, the rate of absorption into cells, the rate of elimination, and the nature of chemical substituents in the toxic compound also exacerbate the arsenic toxicities (Gontijo and Bittencourt, 2005). There are a number of forms of arsenic occur in earth, few major inorganic forms of arsenic includes the trivalent arsenite and the pentavalent arsenate. The organic forms are the methylated metabolites – monomethylarsionic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsine oxide (ATSDR, 2000). Among the naturally occurring form of arsenic, arsenite and arsenate are considered to be the most toxic forms of species found in the environment. Arsenate uncoupled oxidative phosphorylation, and some organisms including marine algae which are constantly exposed to arsenate in seawater, have biochemical processes to intracellularly convert arsenate to harmless organoarsenic compounds. This way arsenate believed to elicit its toxic effect (Edmonds and Francesconi, 1987) and (Edmonds et al., 1997). High level of arsenic concentration within soil and water can cause toxicity to plants and animals. Plants with high concentration of arsenic lead to transfer the arsenic to the food chain
and pose potential risk to human and other animals including chronic effects like skin, bladder, lung, and prostate cancer. Non-cancer effects of ingesting arsenic at low levels include cardiovascular disease, diabetes, and anemia (Liu et al., 2012). What forms of arsenic are accumulated by hyperaccumulators is important to determine to predict their suitability to remediate arsenic contaminated soils (Francesconi et al., 2002).

1.4 Phytoremediation and arsenic accumulating plants

It has become a necessity to determine the detrimental effects of heavy metals on plants subjected to toxic metal contamination (Brown, 1995). There are plenty of approaches available to remove the heavy metals contamination from the soil. Already ranges of investigations are conducted in heavy metal tolerance in plants (Brown and Brinkmann, 1992). But phytoremediation is the practical biological technology due to its inexpensive and easy implementation (Luisa et al., 2018). In the recent years, anthropogenic activities including industrial effluent, improper dumping of heavy metal rich materials have accelerated the soil contamination to a large extent and brought the phytoremediation in demand (Maria et al., 2017). Phytoremediation, involves the use of plants to metabolize or concentrate chemical compounds in restoring polluted soil areas (Napoles and Abalos, 2008). Certain plants can accumulate heavy metal in their roots and shoots. These traits has led to the development of phytoremediation (Raskin et al., 1994).

Selecting the feasible phytoremediator is a major challenge. Plant’s rate of biomass production and its ability to accumulate the contaminant is the major factor in determining the potentiality of the phytoremediator (Reeves and Beker, 2000). Other factors including plant’s tolerance to arsenic and lifecycle have been evaluated for possible arsenic phytoremediation species to identify (Francesconi et al., 2002). Hyperaccumulators are often described as different level of biomass plants. There are certain cases of evidence that many hyperaccumulators are low biomass plants including *Thlaspi caerulescens*, which typically produces 2-5 t ha\(^{-1}\) of shoot dry matter (McGrath et al., 2002). However, there are also fast growing hyperaccumulators that can produce a large biomass. For instance are the *Alyssum bertolonii* and *Berkeleya coddii*, which produced 9 and 22 t ha\(^{-1}\) of shoot dry matter (Robinson et al., 1997). For Successful phytoextraction hyperaccumulation of metals or metalloids is crucial. Root uptake, xylem loading and vacuolar transport contributes to hyperaccumulation of heavy metals. However
more knowledge about molecular mechanism and practical use of hyperaccumulating plants need further research (McGrath and Zhao, 2003).

Ernst, W.H.O, 1996, stated in his paper that phytoremediation can only be a naturally designed economical technique to clean for slightly contaminated soil. Revegetation is possible in the contaminated soils only if high tolerant heavy metal plants are being used. Different plants are identified as soil arsenic accumulators and most of the plants contain inorganic arsenic species. Thus plant based phytoremediation technologies are emerging for the natural removal of toxic arsenic from the soil. Measuring the concentrations of arsenic in plant provide substantial information for the potential role of plants in translocating toxic arsenic biologically (Zhang et al. 2002).

Arsenic contamination of soil deserves to get a cost efficient biological solution in form of hyperaccumulation, ex-situ bioleaching, biostimulation (Wang and Zhao, 2009). But it was not very widely known about the arsenic uptake plants recently. Microbial studies benefited to execute arsenic uptake mechanisms (Bhattacharjee and Rosen 2007). Plants can occur on heavy metal contaminated soil due to possible evolution of specific heavy metal tolerant ecotypes (Garrside and Mcneilly, 1974). One study conducted by Ma et al., 2001 has drawn attention of many researchers to focus on the evolution of many phytoremediation potential of various arsenic hyperaccumulators. The physiology of the ecotypes are different from the non-tolerant plants. These tolerant plants can uptake and accumulate toxic ion within the plant tissues (Garrside and Mcneilly, 1974). Species like in Escherichia coli, yeast and humans, some aquaglyceroporins, a subfamily of the aquaporin superfamily grow with large pores that allow passage of neutral molecules such as glycerol, can transport arsenite (Zhao et al., 2009).

Usually the plants which are expected to be adapted in the arsenic contaminated soil show rapid growth, lower water demands and greater varieties of environmental conditions (Luisa et al., 2018). Phytoremediation, the use of plants for environmental restoration, has been proposed as a cost effective alternative technology to remediate arsenic contaminated soils (Lasat, 2002). It preserves the top soil and reduces the amount of hazardous materials generated during cleanup (Raskin and Ensley, 2000). Plant biomass production and plant elemental uptake are two key factors for successful application of phytoremediation (Reeves and Baker, 2000).
1.5 Soil conductivity and pH

The soil pH and EC test very effective source to determine the nature of soil and soluble salts status in the soil. Measuring of pH and electrical conductivity (EC) parameters is important because it will provide valuable information for assessing soil condition for plant growth, nutrient cycling and biological activity (Kadam, 2016). pH and EC are major indicators for the Soil and crop management practices (Rawls, 1997).

Electrical conductivity levels allow to determine the amount of water and water soluble nutrients available in the soil. Soil EC has direct impacts on biochemical processes including respiration, residue decomposition, nitrification, and denitrification and Soil microorganism activity (Adviento-Borbe et al., 2006). Soil pH (acidity) is negative algorithm of hydrogen ion concentrations. It controls the behavior of metals and many other soil processes. In acid soil (pH<7) heavy metal cations are most mobile. Plants grow in more acidic soil are exposed to uptake more heavy metals. Adding lime is one method to reduce the bioavailability of metals (Oliver, 1997).
1.6 Aims of the study

Soil arsenic (As) levels are generally low in Finland. However, in some parts of Finland, especially in the northern Finland like NW Lapland and in Pirkanmaa area, there are high levels of arsenic in soil.

The overall objective of this research was to investigate the performance of two grass species common in Fennoscandia and also in arsenic soils with respect to the influences of high concentration arsenic. Specifically, this study will investigate the following question:

How arsenic contamination of the soil, plant population origin in the arsenic soil site, or inoculant plant origin in the arsenic site affect a) the seed germination and seedling growth and b) growth of cuttings of Deschampsia cespitosa and Festuca ovina.
2. Material and methods

2.1 Site description

Two sites of high arsenic content soil (As sites) and two sites of low arsenic content soil (Control sites) from Green Stone Belt area in Kittilä, Central Lapland, Finland were chosen for plant and soil collection (Fig. 1, Table 1). High arsenic (As) sites were located in Kuotko, northern Kittilä. Distance between As sites were 1.3 km. Both sites were old gravel pits/quarries with bare soil and rocks. Area is known for high arsenic contents in soil (Närhi et al. 2013). Control sites were located in Kallo, western Kittilä, c. 70 km to south west from As sites. Control sites are old gravel pits with bare soil and rocks.

![Map showing collection sites in Kittilä, Central Lapland, Finland.](image)

*Figure 1. Collection sites in Kittilä, Central Lapland, Finland.*
Table 1. Coordinates (WGS84) of the collection sites in Kittilä, Central Lapland, Finland, number of whole plant individuals collected in 2017 and number of grass individuals sampled for seed in 2016 and 2017.

<table>
<thead>
<tr>
<th>Site</th>
<th>Coordinate Lat.</th>
<th>Coordinate Lon.</th>
<th>Plant species</th>
<th>Number of whole plants 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>As1 (Kuotko)</td>
<td>68° 1' 55,264&quot;</td>
<td>25° 24' 34,592&quot;</td>
<td>D. cespitosa</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F. ovina</td>
<td>5</td>
</tr>
<tr>
<td>As2 (Kuotko)</td>
<td>68° 1' 26,852&quot;</td>
<td>25° 25' 57,975&quot;</td>
<td>D. cespitosa</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F. ovina</td>
<td>5</td>
</tr>
<tr>
<td>Control1 (Kallo)</td>
<td>67° 28' 30,092&quot;</td>
<td>24° 34' 38,330&quot;</td>
<td>D. cespitosa</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F. ovina</td>
<td>5</td>
</tr>
<tr>
<td>Control2 (Kallo)</td>
<td>67° 29' 11,870&quot;</td>
<td>24° 37' 46,872&quot;</td>
<td>D. cespitosa</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F. ovina</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>D. cespitosa</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F. ovina</td>
<td>20</td>
</tr>
</tbody>
</table>

2.2 Sampling

Grass sampling

Five *Deschampsia cespitosa* individuals and five *Festuca ovina* individuals were collected as whole (plant with roots and adjacent soil) from each site (total of 20 individuals/species) in late September/early October 2017. Plants were chosen randomly and the minimum distance between individual plants was 1 m. Plants were planted to pots with original soil and they were kept in cold storage (+4-6°C) for two weeks with lights provided and further in green house for 8 week before establishment of the experiment.
Seed sampling

Seeds of Deschampsia caespitosa individuals and Festuca ovina individuals were collected in autumn 2016 and in autumn 2017 from the collection sites. Number of plant individuals sampled in each site are presented in Table 1. Plants were chosen randomly for seed collection and the minimum distance between individual plants sampled was 0.5 m. Seeds were air dried in room temperature of 48 hours and then stored in +4°C before establishment of the experiment.

Soil sampling

Soil for experiment was collected from the same sites where plants and seeds were collected in autumn 2017. All soil was taken from the recently (less than five years ago) excavated gravel pits from the top soil, depth of 0-20 cm. Soils of As sites were sieved, pooled and carefully mixed before use in the experiment. Soils from control sites were handled similarly and pooled before the experiment.

2.3 Methods

To study the effects of soil arsenic on the growth of grasses, two grass samples including Tufted hair grass (Deschampsia caespitosa) and Sheep fescue (Festuca ovina) were collected from both the areas with arsenic contaminated soil and controlled soil area. Soil, whole plants and seed samples were also collected from the same areas. Effects of arsenic contamination, plant origin (previous exposure on arsenic) and inoculation of root microbes on germination of seeds and growth of seedlings and grass cuttings of these two grass species were tested in two greenhouse experiments.

2.4 Study species

2.4.1 Festuca ovina

The genus of Festuca is widely distributed in Europe, Asia, North America and northern Africa (Robert et al, 2013). In Iran, particularly eighteen perennial and annual species of Poa genus grow. Both Festuca ovina and Festuca arundinacea naturally grow in different areas of Europe with the altitude of 750 to 2900 m and they used for grazing the sheep and goats (Rechinger, 1970).
Festuca genus has a quite great value as livestock material. They are used for grazing and forage conservation. Study conducted by Brown and Brinkmann, 1992 found out that Festuca ovina leaf samples contain higher amount of zinc (Zn) and lead (Pb) than some other plants.

Figure 2. Deschampsia cespitosa and Festuca ovina in the field (Source: Piippa Wäli)

2.4.2 Deschampsia cespitosa

Deschampsia cespitosa, is widespread perennial flowering grass species. Flowering occurs from May to September and seeds mature from late June to late September depending on location can be found in different arctic and temperate regions including whole Finland.

2.5 Greenhouse growth experimental design

2.5.1. Preparation and storage of seeds, plants and soil material

All the seed, plant and soil samples were kept in the laboratory of Botanical Garden. Before planting focal plants onto experimental pots in greenhouse, all the focal plants were stored on water bottle for one week in room temperature. By that time all the plants had grown small sized roots in the water bottle before the final experiment started.
To prepare the germination experiment, 40 pots were set on a bench in the greenhouse where *Deschampsia cespitosa* and *Festuca ovina* seeds were sown.

First to prepare the set up for greenhouse growth experiment, both the *Deschampsia cespitosa* and *Festuca ovina* seeds were prepared for germination. Substantial amount of cutted shoots and inoculant (rooted plants) were prepared as a preliminary work for the final greenhouse experiment.

2.5.2. Experiment 1: Germination

To prepare the germination experiment, 20 pots were set on a bench in the greenhouse where 10 pots were dedicated for *Deschampsia cespitosa* and another 10 pots for *Festuca ovina*. 10 seeds were placed on each experimental soil pot for both *Deschampsia cespitosa* and *Festuca ovina*.

2.5.3. Experiment 2: Growth experiment

First to prepare the set up for greenhouse growth experiment, both the *Deschampsia cespitosa* and *Festuca ovina* plants were prepared for planting. Substantial amount of cuttings and inoculant (rooted plants) were prepared as a preliminary work for the final greenhouse experiment. 150 pots for a full factorial combination of, plant, soil and inoculant (rooted plant) designed for the growth experiment. Each treatment was replicated 10 times. In each pot, two cuttings of each species, one inoculant (rooted plant) and 6 seeds were planted consecutively (the data of the germination of these seeds in these pots is not included in this thesis). The
pots were arranged in 8 trays and placed fully randomly on a greenhouse bench. Artificial lighting was supplied. The soil in each pot was carefully watered with watering jar.

2.5.4. Growth experimental measurements

Eight weekly inventory data collection was performed after transplanting of plants in the experimental pots. At each inventory, leaf number and largest leaf length were measured. The data were recorded on notebook each week. After 8 weeks of consecutive inventory data collection, all the 160 pots were harvested. After measuring plant height, leaf number of two focal plants and one inoculant (rooted plant) from each pot were harvested. Finally, after measuring largest leaf’s height, counted the total leaf number, all the plants were cutted at 2 cm above the soil. Then the harvested plants were rinsed thoroughly. Roots and shoots of each focal plant was separated and collected in paper bag in most of the cases. But in some cases, the roots of the inoculant (rooted plant) were hard to separate. In that case, the roots of the inoculant (rooted plant) companion plant were included with the focal plant root as they could not be separated. Such roots were collected in the paper bags as mixed root. Then all the bags containing shoot or root material were oven dried at 65 degree for 72 hours for biomass analysis. At the harvesting, inoculant (rooted plant) roots and nodules were recovered, focal plants root and shoot were collected in different paper bags.

2.5.5. Measuring germination and seedling growth

Germination data were collected daily for about two weeks. The data were recorded on notebook. When the seed pots stopped to germinate anymore and each pot reached the final number of germination, the final data were recorded. In this thesis only the final germination is reported.

Then from all the 20 pots, maximum 5 seedlings were left to grow. At the final inventory, final leaf numbers of each seedling was measured and the seedlings were dried in 60 °C for biomass analysis (the biomass data is not included in this thesis).
2.5.6 Measuring plant biomass

To measure biomass, focal plants shoots, roots, inoculant (rooted plant) roots were separately dried in the oven at 60°C temperature for 24 hours. Then the dried samples were weighed. Data were converted to excel for further analysis. After conducting the greenhouse growth experiment, biomass data were collected in respect to three factors including the origin of the focal plants, type of soil (controlled or contaminated) and the origin of inoculant (rooted plant) which had been used for microbial inoculation for new unrooted grass cuttings.

2.5.7 Measuring grain size of soil

This test is performed to determine the percentage of different grain sizes contained within each soil samples. Sieve analysis is performed to determine the degree of coarse distribution of the soils.

Two types of soil samples were considered such as arsenic contaminated soil (Kuotko) and controlled soil (Kallo) from Kittilä. Particularly arsenic contaminated soil and controlled soil from the plant growth experimental pot were used. Sieves set were assembled according to the mesh size of the sieves. The larger sieves was placed on the top. Carefully poured the soil samples into the top sieve. Then gently shacked the top sieve and collected the retained soil and weigh them with a digital weighing meter. Then the same procedures were followed for the other sieves. Finally all the retained soils from each sieve were measured with the same digital weighing meter.
2.5.8 Grain size analysis

Recorded the weight of the used two types of dried soil samples. Calculated the percentage of each retained soil on each sieve by dividing the weight retained on each sieve by the original sample mass for both Kuotko and Kallo soil samples.

**Kuotko (contaminated condition) soil**

Total soil sample mass: 176.2 gm

1. Mass retained on top sieve: 36.8 gm
2. Second largest grain: 40.8 gm
3. Third largest grain: 78.9 gm
4. Fourth grain: 19.7 gm

**Kallo (controlled condition) soil**

Total soil sample mass: 221.5 gm

1. Mass retained on top sieve: 72.7 gm
2. Second largest grain: 45.0 gm
3. Third largest grain: 43.8 gm
4. Fourth grain: 50.0 gm

**Table 2. The percent retained calculation of both Kuotko and Kallo soil:**

<table>
<thead>
<tr>
<th>Grain size mass</th>
<th>% retained Kuotko Soil=( Mass retained/Total mass) X 100</th>
<th>% retained Kallo Soil=( Mass retained/Total mass) X 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass retained on top sieve</td>
<td>20.8</td>
<td>32.8</td>
</tr>
<tr>
<td>Second largest grain</td>
<td>23.2</td>
<td>20.3</td>
</tr>
<tr>
<td>Third largest grain</td>
<td>44.7</td>
<td>19.8</td>
</tr>
<tr>
<td>Fourth grain</td>
<td>11.1</td>
<td>27.1</td>
</tr>
</tbody>
</table>
2.6. Soil pH and electrical conductivity (EC)

Special apparatus

A partial sample from 42 pots were used in the analysis. To investigate the pH and EC of 42 soil samples from growth experimental pots were taken and analysis was performed using pH and EC meter, plastic beaker with lids, soil scissor, and measuring glass tube. In addition soil material from the start of the experiment was analyzed.

The pH was measured as follows: Soil scoop 40 ml and 50 mL of pure water (Distilled water) to obtain a 2:1 water-to-soil ratio. 40 gm of soil from 42 soil samples were used to measure pH and EC. 50 ml distilled water were used to prepare the soil solution.

![Image of pH and EC meters]

*Figure 5. Soil EC and pH Analysis*

2.6.1 Electrical conductivity (EC) of soil samples

PROCEDURE

First placed 40ml of soil with a soil scissor in a plastic cup. Then 50 mL of pure water was added, stirred, and allowed to shake for an hour. Without stirring the sample, the sample was kept for one hour taking out of the shaker. The conductivity meter was calibrated by rinsing the electrode with the same distilled water used for making the soil sample solution. After that the electrical conductivity (EC) of each of the soil sample solution in the plastic funnel tube was measured. Then the interior and exterior of the probe with pure water between every samples
was rinsed. Any excess water from the exterior of the probe by blotting with a tissue was removed. Finally all meter readings as displayed were recorded.

2.6.2 pH of soil samples

PROCEDURE

First placed 40ml of soil with a soil scissor in a plastic cup. Then 50 mL of pure water was added, stirred, and allowed to shake for an hour. Without stirring the sample, the sample was kept for one hour taking out of the shaker. The pH meter was calibrated by rinsing the electrode to the buffer liquid (pH 7). Then another rinsing was done to the buffer liquid (pH 4). After that pH of each of the soil sample solution in the plastic funnel tube was measured. Then the interior and exterior of the probe with pure water between every samples was rinsed. Any excess water from the exterior of the probe by blotting with a tissue was removed. Finally all meter readings as displayed were recorded.

2.7 Data and statistical analysis

The data analysis were conducted separately for both plants. For germination experiment, total germination and leaf number as a biomass estimate were the response variables and the explanatory variables were the origin of seed material (arsenic Kuotko sites and control Kallo sites) and the type of the soils (arsenic Kuotko soil and control Kallo soil).

For growth experiment with cuttings, leaf number and length, shoot biomass and root biomass were analyzed. Plant origin (arsenic Kuotko sites or control Kallo sites), companion inoculant (rooted plant) origin (arsenic Kuotko sites or control Kallo sites) and type of soil (arsenic Kuotko soil or control Kallo soil) were used as explanatory factors. Soil pH and electrical conductivity were analyzed using electrical meter. All analyses were performed with R program.
3. Results

3.1. Germination and seedling growth experiment

Germination of *Deschampsia cespitosa* was markedly inhibited by the arsenic treatment (Fig. 6, Table 3). Germination was higher in case of Kallo (controlled condition) originated seeds than Kuotko (contaminated condition) originated seeds. Germination, where the seeds were sown in the soil originated from Kuotko (arsenic contaminated) area was lower than in the Kallo (controlled condition) soil factor though the variability was fairly high (Fig. 6, Table 3).

![Germination Experiment Diagram](image)

*Figure 6 – Total germination of Deschampsia cespitosa and Festuca ovina in relation to seed origin (arsenic Kuotko sites and control Kallo sites) and soil type (arsenic Kuotko sites and control Kallo sites) in the greenhouse germination experiment.*

For *Festuca ovina*, total germination showed a statistically significant impact, in response to "seed origin"- explanatory variable (Fig. 6, Table 3). Kallo (Controlled condition) originated *Festuca ovina* seeds germinated better than Kuotko (contaminated condition) originated seeds in greenhouse experiment. Whereas quite oppositely, the contaminated (Kuotko condition) soil type condition resulted higher germination rate than Kallo (controlled condition) in case of
*Festuca ovina* though the result was not significant. There was also evidence of delayed germination at few of the treatment levels (data not shown).

Table 3—Statistical results of two-way ANOVA testing for the impacts of seed origin and soil type on total germination as response variable of *Deschampsia cespitosa* and *Festuca ovina*.

<table>
<thead>
<tr>
<th>Deschampsia cespitosa</th>
<th>Festuca ovina</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total germination</strong></td>
<td><strong>Total germination</strong></td>
</tr>
<tr>
<td><strong>Variables</strong></td>
<td><strong>Df</strong></td>
</tr>
<tr>
<td>Origin</td>
<td>1</td>
</tr>
<tr>
<td>Soil</td>
<td>1</td>
</tr>
<tr>
<td>Origin *: Soil</td>
<td>1</td>
</tr>
</tbody>
</table>

However, it can be noticed that the contamination with arsenic in the form of focal plant origin and soil type, affected the leaf number for *Deschampsia cespitosa*. Leaf number was significantly higher in Kallo (controlled condition) originated seeds than Kuotko (contaminated condition) (Fig. 7, Table 4).

Also the soil type had an impact on the leaf number of *Deschampsia cespitosa* seedlings: the seedlings grown in Kuotko soil had lower leaf number (Fig. 7, Table 4). Opposite to that, seed origin or the soil type had no significant impact on the seedling leaf number of *Festuca ovina* although the leaf number was marginally impacted and performed better in Kuotko (contaminated condition) than Kallo (controlled condition) in response to origin of the seeds variable (Fig. 7, Table 4)
Figure 7 – Leaf number of Deschampsia cespitosa and Festuca ovina in relation to seed origin (arsenic Kuotko sites and control Kallo sites) and soil type (arsenic Kuotko sites and control Kallo sites) in the greenhouse germination experiment.

Table 4 – Statistical results of two-way ANOVA testing for the impacts of seed origin and soil type on seedling growth experiment as response variable of Deschampsia cespitosa and Festuca ovina.

<table>
<thead>
<tr>
<th></th>
<th>Deschampsia cespitosa</th>
<th></th>
<th>Festuca ovina</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf Number</td>
<td>Leaf number</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Variables</strong></td>
<td>Df</td>
<td>F value</td>
<td>Pr(&gt;F)</td>
<td>Df</td>
</tr>
<tr>
<td>Origin</td>
<td>1</td>
<td>4.734</td>
<td>0.0449 *</td>
<td>1</td>
</tr>
<tr>
<td>Soil</td>
<td>1</td>
<td>6.575</td>
<td>0.0208 *</td>
<td>1</td>
</tr>
<tr>
<td>Origin*Soil</td>
<td>1</td>
<td>2.180</td>
<td>0.1593</td>
<td>1</td>
</tr>
</tbody>
</table>
3.2 Growth of grass cuttings in growth experiment

3.2.1 Leaf number

Focal plant origin, soil type or the inoculant (rooted plant) origin did not affect the leaf number of *Deschampsia cespitosa* (Fig. 8, Table 5). In addition, there was a statistically significant interaction between plant origin and inoculant (rooted plant) (*P* = 0.019 *, Table 5).

*Figure 8—Leaf number of *Deschampsia cespitosa* and *Festuca ovina* cuttings in relation to plant origin, soil type and inoculant (rooted plant) origin in the greenhouse growth experiment.*

For *Festuca ovina*, the focal plant origin had significant impact on the leaf number: the amount of leaves was higher in Kallo (controlled condition) originated plant than in Kuotko (contaminated condition) arsenic-soil originated plants (Fig. 8, Table 5). The other studies factors did not have an impact on the leaf number of *Festuca ovina* (Fig. 8, Table 5).
Table 5—Statistical results of three-way ANOVA testing for the impacts of plant origin, soil type and inoculant (rooted plant) origin on leaf number as response variable of *Deschampsia cespitosa* and *Festuca ovina* cuttings at the end of the experiment.

|                  | *Deschampsia cespitosa* |                  |  | *Festuca ovina* |                  |  |
|------------------|-------------------------|------------------|  |------------------|------------------|  |
|                  | Leaf number             |                  |  | Leaf number      |                  |  |
| Variables        | Df | F value | Pr(>F) | Df | F value | Pr(>F) |  |
| Origin           | 1  | 0.249   | 0.620 | 1  | 9.668  | 0.00295 ** |  |
| Soil             | 1  | 0.058   | 0.810 | 1  | 1.828  | 0.18184 |  |
| Rooted           | 1  | 2.667   | 0.107 | 1  | 1.708  | 0.19660 |  |
| Origin:Soil      | 1  | 0.004   | 0.947 | 1  | 1.170  | 0.28410 |  |
| Origin:Rooted    | 1  | 5.758   | 0.019 *| 1  | 0.343  | 0.56034 |  |
| Soil:Rooted      | 1  | 0.289   | 0.593 | 1  | 1.170  | 0.28410 |  |

3.2.2. Leaf length

In case of *Deschampsia cespitosa*, plant origin, soil type and inoculant (rooted plant) origin had no statistically significant impact on leaf length (Table 6).

In addition, soil type and inoculant (rooted plant) had a significant interaction (Table 6). For *Festuca ovina*, plant origin was statistically significant; plants from Kuotko (arsenic contaminated condition) has longer leaves (Fig. 9, Table 6).
Figure 9—Leaf length of *Deschampsia cespitosa* and *Festuca ovina* cuttings in relation to plant origin, soil type and inoculant (rooted plant) origin in the greenhouse growth experiment.

Table 6—Statistical results of three-way ANOVA testing for the impacts of plant origin, soil type and inoculant (rooted plant) origin on leaf length as response variable of *Deschampsia cespitosa* and *Festuca ovina* cuttings at the end of the experiment.

<table>
<thead>
<tr>
<th></th>
<th><em>Deschampsia cespitosa</em></th>
<th></th>
<th><em>Festuca ovina</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf length</td>
<td>Leaf length</td>
<td></td>
</tr>
<tr>
<td>Variables</td>
<td>Df</td>
<td>F value</td>
<td>Pr(&gt;F)</td>
</tr>
<tr>
<td>Origin</td>
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<td>2.851</td>
<td>0.0956</td>
</tr>
<tr>
<td>Soil</td>
<td>1</td>
<td>0.289</td>
<td>0.5925</td>
</tr>
<tr>
<td>Rooted</td>
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<td>0.6320</td>
</tr>
<tr>
<td>Origin:Soil</td>
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<td>0.064</td>
<td>0.8017</td>
</tr>
<tr>
<td>Origin:Rooted</td>
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<td>0.015</td>
<td>0.9039</td>
</tr>
<tr>
<td>Soil:Rooted</td>
<td>1</td>
<td>5.128</td>
<td>0.0265 *</td>
</tr>
</tbody>
</table>
3.2.3. Shoot biomass

For *Deschampsia cespitosa*, the focal plant origin had significant impact on the shoot biomass: the shoot biomass was higher in Kuotko (contaminated condition) than Kallo (controlled condition) ($P = 0.0369^*$, Fig. 10, Table 7). The other studies factors did not have an impact on the shoot biomass of *Deschampsia cespitosa* (Fig. 10, Table 7). In addition, there was also a significant interaction between plant origin and inoculant (rooted plant) ($P = 0.0391^*$, Table, 7).

Focal plant origin, soil type or the inoculant (rooted plant) origin did not affect the shoot biomass of *Festuca ovina* (Fig. 10, Table 7). However, the origin of focal plant had a marginally significant impact ($P=0.0587$, Table 7) on shoot biomass. *Festuca ovina* resulted slightly higher shoot mass in Kallo (controlled condition) than Kuotko (contaminated condition).

*Figure 10*– Dry weight of shoots of *Deschampsia cespitosa* and *Festuca ovina* in relation to plant origin, soil type and inoculant (rooted plant) origin in the greenhouse growth experiment.
Table 7—Statistical results of three-way ANOVA testing for the impacts of plant origin, soil type and inoculant (rooted plant) origin on shoot weight as response variable of *Deschampsia cespitosa* and *Festuca ovina* cuttings at the end of the experiment.

<table>
<thead>
<tr>
<th></th>
<th><em>Deschampsia cespitosa</em></th>
<th></th>
<th><em>Festuca ovina</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Shoot weight</strong></td>
<td><strong>Shoot weight</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Variables</strong></td>
<td><strong>Df</strong></td>
<td><strong>F value</strong></td>
<td><strong>Pr(&gt;F)</strong></td>
</tr>
<tr>
<td>Origin</td>
<td>1</td>
<td>4.522</td>
<td>0.0369 *</td>
</tr>
<tr>
<td>Soil</td>
<td>1</td>
<td>0.005</td>
<td>0.9456</td>
</tr>
<tr>
<td>Rooted</td>
<td>1</td>
<td>2.692</td>
<td>0.1052</td>
</tr>
<tr>
<td>Origin:Soil</td>
<td>1</td>
<td>4.417</td>
<td>0.1119</td>
</tr>
<tr>
<td>Origin:Rooted</td>
<td>1</td>
<td>4.417</td>
<td>0.0391 *</td>
</tr>
<tr>
<td>Soil:Rooted</td>
<td>1</td>
<td>0.853</td>
<td>0.3589</td>
</tr>
<tr>
<td>Origin:Soil:Rooted</td>
<td>1</td>
<td>0.815</td>
<td>0.3698</td>
</tr>
</tbody>
</table>

3.2.4. Root biomass

Focal plant origin, soil type or the inoculant (rooted plant) origin did not affect the root biomass of *Deschampsia cespitosa* (Fig. 11, Table 8). However, root biomass was slightly higher in Kallo (controlled condition) than Kuotko (contaminated condition) for all the three explanatory variables (Fig. 11). In addition, *Deschampsia cespitosa* had marginally significant impact (P = 0.0558, Table 8) between origin and inoculant (rooted plant).

Focal plant origin, soil type or the inoculant plant origin did not affect the root biomass of *Festuca ovina* (Fig. 11 Table 8).
Figure 11 – Dry weight of roots of Deschampsia cespitosa and Festuca ovina in relation to plant origin, soil type and inoculant (rooted plant) origin in the greenhouse growth experiment.

Table 8 – Statistical results of three-way ANOVA testing for the impacts of plant origin, soil type and inoculant (rooted plant) origin on root weight as response variable of Deschampsia cespitosa and Festuca ovina cuttings at the end of the experiment.
3.3. Soil pH and electrical conductivity (EC) in soil after the experiment

After the experiment soil pH ranged from 4.6 to 5.3. Electrical conductivity varied greatly according to the arsenic treatments in soils after the experiment. EC ranged from 33 to 250 mS/cm. For Deschampsia cespitosa, there was a statistically significant impact of soil type both on pH and EC: pH was lower and EC was higher in Kuotko (arsenic contaminated condition) soil (Fig. 12, Fig 13, Table 9, and Table 10). In addition, the plant origin had also significant impact on pH after the experiment for Deschampsia cespitosa: pH was higher in Kuotko (arsenic contaminated condition) soil (Fig. 12, Table 9).

For Festuca ovina, there was a statistically significant impact of soil type on pH (Fig. 12): pH was lower in Kuotko (arsenic contaminated condition) soil (Fig. 12, Table. 9). Soil type and inoculant (rooted plant) had a non-significant impact on EC for Festuca ovina (Table 10). There was also a marginally significant impact of plant origin on EC in Festuca ovina (Fig. 13, Table 10).

Figure 12 – pH of Deschampsia cespitosa and Festuca ovina in relation to plant origin, soil type and inoculant (rooted plant) origin.
**Figure 13**—Electrical conductivity of *Deschampsia cespitosa* and *Festuca ovina* in relation to plant origin, soil type and inoculant (rooted plant) origin.

Table 9—Statistical results of three way ANOVA testing for the impact plant origin, soil type and inoculant (rooted plant) origin on the pH of *Deschampsia cespitosa* and *Festuca ovina* growth pots at the end of the experiment.

<table>
<thead>
<tr>
<th></th>
<th><strong>Deschampsia cespitosa</strong></th>
<th></th>
<th><strong>Festuca ovina</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td></td>
<td>pH</td>
<td></td>
</tr>
<tr>
<td><strong>Variables</strong></td>
<td>Df</td>
<td>F value</td>
<td>Pr(&gt;F)</td>
<td>Df</td>
</tr>
<tr>
<td>Origin</td>
<td>1</td>
<td>4.814</td>
<td>0.04865*</td>
<td>1</td>
</tr>
<tr>
<td>Soil</td>
<td>1</td>
<td>11.517</td>
<td>0.00533***</td>
<td>1</td>
</tr>
<tr>
<td>Rooted</td>
<td>1</td>
<td>0.515</td>
<td>0.48656</td>
<td>1</td>
</tr>
<tr>
<td>Origin:Soil</td>
<td>1</td>
<td>1.721</td>
<td>0.21406</td>
<td>1</td>
</tr>
<tr>
<td>Origin:Rooted</td>
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<td>0.029</td>
<td>0.86787</td>
<td>1</td>
</tr>
<tr>
<td>Soil:Rooted</td>
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<td>0.173</td>
<td>0.68477</td>
<td>1</td>
</tr>
<tr>
<td>Origin:Soil:Rooted</td>
<td>12</td>
<td>1.358</td>
<td>0.26658</td>
<td>12</td>
</tr>
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</table>
Table 10 – Statistical results of three way ANOVA testing for the impact plant origin, soil type and inoculant (rooted plant) origin on the EC of *Deschampsia cespitosa* and *Festuca ovina* growth pots at the end of the experiment.

<table>
<thead>
<tr>
<th>Variables</th>
<th>EC (Deschampsia cespitosa)</th>
<th>Pr(&gt;F)</th>
<th>EC (Festuca ovina)</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Origin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.034</td>
<td>0.857</td>
<td>1</td>
<td>4.639</td>
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<td><strong>Soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.17307</td>
<td>5.37e-10***</td>
<td>1</td>
<td>64.242</td>
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<tr>
<td><strong>Rooted</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.019</td>
<td>0.894</td>
<td>1</td>
<td>0.020</td>
</tr>
<tr>
<td><strong>Origin:Soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.617</td>
<td>0.447</td>
<td>1</td>
<td>2.298</td>
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<tr>
<td><strong>Origin:Rooted</strong></td>
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<tr>
<td>1</td>
<td>0.021</td>
<td>0.888</td>
<td>1</td>
<td>0.114</td>
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<td><strong>Soil:Rooted</strong></td>
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<td>1</td>
<td>0.213</td>
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<tr>
<td>12</td>
<td>0.003</td>
<td>0.959</td>
<td>12</td>
<td>0.795</td>
</tr>
</tbody>
</table>

*Note: *** indicates a highly significant result.*
4. Discussion

Plant development is crucial in the establishment of new individuals in plant community where seed germination and seedling growth are critical stages. Environmental variability including soil moisture and soil nutrients have marked impact on plant germination (Fay and Shultz, 2009).

Metal resistance studies are emerging now. Vegetative response to root length, shoot height, root and shoot biomass and total biomass are being used widely to understand the potential level of metal resistance of plants (Abedin and Meharg, 2002a).

This study evaluated the relationship between soil available and plant available arsenic, and their effect on plant growth and germination in (*Dechampsia cespitosa* and *Festuca ovina*). First the study focused on addressing a number of query including the seed germination and seedling growth performance and survival differences between Kuotko (contaminated condition) and Kallo (controlled condition) site for these two grass species. Second the focal plants cuttings survival and performance differences between contaminated and controlled condition were investigated in this study.

4.1. Effects of controlled and contaminated arsenic condition on germination and seedling growth of *Dechampsia cespitosa* and *Festuca ovina*

*Festuca ovina* showed better tolerance to arsenic as it appeared to perform equally (in terms of leaf number) or potentially even better (germination) in arsenic treatments (Kuotko condition) than in control treatments (Kallo condition) which is quite opposite to *Dechampsia cespitosa*.

Arsenic contamination reduced *Dechampsia cespitosa* germination rate and seedling growth. It germinated significantly weaker in the arsenic contaminated soil in comparison to the controlled soil type. *Festuca ovina* on the other hand appeared to perform better in germination and seedling growth in Kuotko (contaminated condition).

Most existing studies including the study conducted by Petersen et al., showed that higher arsenic concentrations in plants have usually been found to cause higher phytotoxicity, in particular growth inhibition (Peterson et al., 1981). Also Liebig (1966) observed in his study
that abnormal amount of arsenic contamination soil can be directly attributed to the lower germination rate and reduction of seedling viability. This publication supports the germination results in this study. In contrast, in case of Festuca ovina germination of seeds and seedling biomass were not affected by arsenic contamination of growth substrate. This suggests that the seeds of Festuca ovina had greater tolerance of arsenic than Deschampsia cespitosa.

Study conducted by Gulz et al., (2005) showed that arsenic uptake and biomass production plant species in a greenhouse experiment were not inhibited by the arsenic treatment and the growth continued unaffected. The results showed by Aldana et al., (2013), showed that seed germination of Festuca rubra was not significantly affected by arsenic (As) levels lower than 25 mg L⁻¹, indicating that this grass has a relatively high tolerance of arsenic (As) at the germination stage that supports the finding of the this study for Festuca ovina.

4.2. Effects of controlled and contaminated arsenic condition on growth (leaf number, leaf length, shoot biomass and root biomass) performance of Deschampsia cespitosa and Festuca ovina cuttings

Deschampsia cespitosa, growth experiment using (leaf number, leaf length, and root weight growth estimates) showed no statistically significant results. The study conducted by Grant and Dobbs, (1997) showed that the growth of the focal plant reduced in relation to the arsenic treatments but there was also no significant correlation found.

All though the result was insignificant still it seemed to maintain consistency of biomass production in Kallo (controlled condition) than Kuotko (contaminated condition) in most of the response variables in respect to all the explanatory variables for Deschampsia cespitosa. On the other hand oppositely shoot biomass was significantly better in Kuotko (contaminated condition) soil, although the growth consistency was observed in Kallo (controlled condition) soil. This outcome contrasts many other studies. The study conducted by Chun-xi et al., (2006) showed that the length and biomass of root and shoot reduced by 56.53%, 40.19%, 25% or 20.62%, respectively, at the highest arsenic concentration. Subsequent growth of the grasses was reduced in the contaminated condition in - Deschampsia cespitosa showing the clear response to the Kuotko (contaminated condition) treatment all though in most of the cases the correlation was non-significant.
*Festuca ovina* appeared to be potentially better adapted to arsenic especially in terms of cuttings establishment. Growth data (Leaf number and leaf length) of *Festuca ovina* showed to be significantly affected by the arsenic concentration of the original site where the plants were collected and provided a significant result in both cases of response variables (leaf number and length). *Festuca ovina* produced statistically significantly more leaves and heavier shoots when originated from Kallo (controlled condition) soil although the growth consistency was observed in Kuotko (contaminated condition) with no such fluctuations. On the other hand, leaf length was higher in plants originating from Kuotko (arsenic contaminated condition) soil than in plants originating from Kallo (controlled condition) soils. In many studies plant species have been shown to exhibit a higher degree of tolerance in greenhouse experiment where the plants have originated from the arsenic contaminated site than growing on an uncontaminated site (Quaghebeur and Rengel, 2003).

*Festuca ovina*, biomass data (dry root weight) showed no significant results with very less variance among the three explanatory variables (focal plant origin, soil type and inoculant plant origin). The study conducted by Xiao-ke et al., (2012), showed that dry matter weight did not differ significantly between the arsenic level difference and the control.

4.3. pH and electrical conductivity (EC) values in the soil after the experiment

Different environmental factors including pH and redox potential and oxidation state, speciation affect the arsenic availability in the geological source (Janga et al., 2016).

The soil analysis result depicts that pH value appeared to be lower in Kuotko (arsenic contaminated condition) soil than that of Kallo (controlled condition) soil for both *Dechampsia cespitosa* and *Festuca ovina* species. According to the study conducted by Fullados et al., (2004), within a 5.0 – 8.0 pH range As(V) were found to decrease as pH became basic which supports this study. In this study, for both *Dechampsia cespitosa* and *Festuca ovina*, soil pH ranged from 4.6 to 5.3 which is acidic (pH<7) condition. Zandsalimi et al, (2011) found that pH was neutral in areas A and B where the arsenic concentration was comparatively higher whereas while it was alkaline in area C with lower arsenic contamination in soil.

EC in response to soil type resulted significantly higher in Kuotko (contaminated condition) than that of Kallo in case of *Dechampsia cespitosa* whereas *Festuca ovina* EC did not respond
to the soil type variable. According to the study conducted by Azin et al., (2008), concentrations of As tend to be lower in shallow aquifers underlying sandy soils with high conductivity soils that contrasts the results of this study. Therefore, pH and Electrical conductivity interacted oppositely in response to soil type variable.

**4.4 Differences in the performance of *Deschampsia cespitosa* and *Festuca ovina***

Therefore, the overall results depicts that *Deschampsia cespitosa* noticeably affected by arsenic concentration. Germination resulted significantly low in case of arsenic contamination. Decrease in Seedling germination by all the three factors: soil type, amendment rate, and soil arsenic source was observed by Quazi et al., 2011. Although growth biomass insignificantly interacted with arsenic in case of *Deschampsia cespitosa* but the results were negative in most of the cases that supports previously conducted many studies. Various studies have reported a decrease in root and shoot length with an increase in arsenic concentration. Walsh and Keeney, 1975 concluded in their study that when the arsenic level increases, the chances of a phytotoxic response by the desired crop also increases. Also study conducted by Quazi et al., 2011 on the effects of arsenical pesticide on rice growth found a relevant results. They found a significant negative correlation between arsenic concentration and root length, shoot height, biomass, germination and plant arsenic uptake.

The overall *Festuca ovina* growth resulted to perform noticeably better in Kuotko (contaminated condition) instead of getting affected by the arsenic concentrated soil and origin. Porter & Peterson, 1975 in their study found that the arsenic level is within the range of values reported for a Variety of plants growing at high arsenic sites.

Over all, arsenic contamination of soil affected on germination and seedling growth of *Deschampsia cespitosa*, but not on growth of transplanted cuttings, which were strongly affected by previous experience with arsenic, the arsenic concentration of the original site where they collected. In *Festuca ovina* only previous experience affected negatively on the performance, both in transplanted cuttings and via maternal effect on seed germination and seedling growth.
4.5. Improvements and future studies

While a greenhouse experiment can never emulate the natural conditions in which the study species lives completely, it is a way to keep out unwanted and unplanned disturbances (Gibson 2002). Investigating the structure and dynamics of plant growth require to study the interaction between plants (Gibson et al., 1997).

Studies on species performance and survival rate on arsenic contaminated soil should take into account other factors also. Other factors also influences the growth of grasses in soil. Variation in nutrient reduces the growth rate. All the necessary nutrient of both the controlled and contaminated soil could be analyzed before the greenhouse growth experiments begin or use standard growth substrate and add only the arsenic on the factor levels needed.

This study has still a lot of scopes to improve. Future improvements on this experiment can be done by improving experimental unit changing from greenhouse experiments to field experiment, having more replicates and comparing Deschampsia cespitosa and Festuca ovina with other individuals of the sample species from different areas of Finland.
5. Conclusions and implications for conservation

Soil contamination with arsenic has occurred in many areas of the world due to the regulatory activities of mining, use of arsenical herbicides, insecticides and wood preservatives, and irrigation with arsenic contaminated groundwater and runoff from arsenic contaminated sites. Excessive uptake of arsenic by crop plants may present a food safety problem (Zhu et al., 2008).

It is good that several hyperaccumulating plants have been discovered and opens a door for phytoremediation of arsenic-contaminated soils (Ma et al., 2001). Inhibitory effect of contaminated soil varies to some extent within the plant species examined (Sharifi et al., 2007).

To evaluate the association between seed/seedling success, plant growth success and soil arsenic variability, this study was conducted addressing few study questions. The higher germination was observed in Kallo (controlled condition) related plants either for plant origin or soil used in the experiments for Deschampsia cespitosa. Germination and seedling establishments of Festuca ovina was not affected by soil arsenic content in the greenhouse which proves Festuca ovina to be a potential phytoremediation species. For total germination and seedling establishment, Deschampsia cespitosa showed to be more sensitive species to the arsenic contamination. Also Festuca ovina seeds germination appeared to be affected by arsenic contamination.

Above and underground growth data (leaf number, leaf length and shoot weight, root weight) of both the focal plants Deschampsia cespitosa and Festuca ovina were not significantly affected by the arsenic contaminated focal plant origin, soil type and inoculant (rooted plant) origin. Although the result was insignificant in relation to most of the explanatory variables exposed to, Festuca ovina leaf number and leaf length were increased by arsenic contamination significantly. On the other hand Deschampsia cespitosa shoot biomass was significantly decreased by arsenic contamination whereas shoot biomass of Festuca ovina also produced marginally significantly results. Root biomass of both Deschampsia cespitosa and Festuca ovina were not affected by arsenic contamination.

In summary, this study demonstrates that Festuca ovina is more tolerant grass species in arsenic soil between the two grass species examined. Although the experiment appears to trigger species specific responses both species showed significant differences according to the origin of plant material, the previous exposure to arsenic. Overall, these results suggest that
arsenic concentration will impact seed germination, seedling growth and plant growth more strongly in *Deschampsia cespitosa* than *Festuca ovina*. According to this research *Festuca ovina* might have slightly promising properties for bioremediation than *Deschampsia cespitosa*.

**Implications**

Of the two species of grasses tested the germination and growth in this study, *Festuca ovina* showed that for ecological rehabilitation of arsenic contamination, and it had the highest survival rate, the largest cover and biomass in contaminated condition and the best phytoremediation efficiency.

The results of this study could be used in a future study on the potentiality of phytoremediation of *Deschampsia cespitosa* and *Festuca ovina* grass species. Since according to this study *Festuca ovina* has a slight advantage in high arsenic soil, any transplant of *Festuca ovina* to these sites with arsenic contaminated soil should be accompanied by the removal of arsenic naturally. Further studies can also be undertaken to address the mechanisms of arsenic uptake and transformation. Germination and growth responses to arsenic-contaminated soil variability affect may be explained further under more different species.
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