Adaptability and development of *Populus tremula L.* x *Populus tremuloides Michx*. and Finnish native *Populus Tremula* on polluted soils by PAHs and sodium chloride.

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Summary

The comparison of growth and adaptability between *Populus tremula L.* x *Populus tremuloides Michx.* and the native Finnish seedling *Populus tremula*. To understand the genotype and phenology of the same species for their application on bioremediation as phytoremediation. The experiment was conducted to analyze the development on the species on polluted soils with PAHs and natrium chloride. This is the first experiment conducted in Finland for the better understanding on how to utilize the ecosystems services provided by these species. As well bioremediation and the variety of the processes offered and the possibility on decreasing costs and providing aesthetical values.

Language: English Key words: Bioremediation, phytoremediation, PAHs, natrium chloride, phenology, genotype, biomass, adaptability, devolpment.

Glossary and abbreviations

BTEX benzene, toluene, ethyl benzene, and xylenes.

CO_2 Carbon dioxide

DARTS Decision Aid Remediation Technology Selection

EEA European Environmental Agency

ERA Ecological Risk Assessment

EU European Union

LEO Lines of Evidence

METLA Metsäntutkimuslaitos Finnish Forest Research Institute

PAH Polycyclic Aromatic Hydrocarbons

PBCs Polychloro Biphenyls

SEE South East European

SVE Thermally Enhanced Soil Vapour Extraction

TNT Trinitrotoluene

TPH Total Petroleum Hydrocarbons

UEPA United States of America Environmental Protection Agency

United States of America

WCE Western and Central Europe

WEO Weight of Evidence

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1 Introduction

The importance to understand chemical compounds and the possible negative impacts on human health and to our environment is vital. That is why we need to understand where, when and how this chemicals compounds have been utilized and their half-life. This to be able to tracked them and if it is necessarily prevent further dispersion or their proper disposal and avoid further impacts. One example of these chemicals compounds that was analyzed in this research and their possible bioremediation by phytoremediation are the Polycyclic Aromatic Hydrocarbons (PAHs).

PAHs which has been utilized historically in wood protection and water stopper for constructions in marine, land or fresh waters. This has been utilized broadly in Finland and EU on the protection of crossing timbers and railroad ties, bridges, pier decking's, poles log for homes, fencing and equipment for children grounds. One of the most common PAHs utilized on these is creosote which is a mixture of multiple of thousands chemicals but lesser than 1%. Which this is mainly compose of six compounds PAHs, alkylated PAHs (up to 90%), tar acids/ phenolics; tar bases/ nitrogen containing heterocycles; aromatic amines; sulfur containing heterocycles; and oxygen containing heterocycles including dibenzofurans (WHO, 2004). In the saturation of PAHs to wood products the supererogation of the same may filtrates to the environment. By this means the high probability to find PAHs on different sites from this wood products utilized could persist for decades. In some experiments conducted in different laboratories the research focuses on the ecotoxicological behavior of PAHs on the biota. This means that the high obstruction of movement on high molecular weight compounds are connected to a rapid downwards transportation in low molecular weight compounds, where the specifics in the physicochemical properties are correlated to the variability of soil types and their environmental surroundings (WHO, 2004).

However possible spills and the propagation of chemical compounds are latent by transporting high amounts of chemicals. An estimated rate of 150 accidents reported annually of hazardous products only occurring in Finland. The total amount in 2007 from transported chemicals in Finland was about 79% flammable solutions as fuel, 9% corrosive, 6% gasses, and 4% oxidizing materials or peroxides. Around 95 million tonnes by roadway and 5.6 tonnes by railroad (RIMA, 2013). By this percentage a high probability that there could be more spills and threats to the nature and human health still present. The need to develop technologies which provide achievable solution to stop the propagation and restoration are required.

1.1 Objective

1.2 Aims

There is a need to understand the future applications of bioremediation by poplars trees as phytoremediation just to mention one, where in certain areas pollution exceeds the established thresholds (PAHs). The experiment was based on searching results on growth and then succession of the tested species. By this means the Finnish Forest Research Institute (METLA) has conducted an investigation related to the comprehension on the phenological traits on adaptability and development of two tree species. The test was conducted on the species *P. tremula L. x P. tremuloides Michx* and seedlings *of P. tremula.* These were planted on different polluted pools by low heated oil, (diesel) and pools stressed by natrium chloride. The experiment consisted on the observations, measurements collected based on the phenotypic plasticity and growth on the specific characteristics as (total biomass, stem diameter, length etc.) and later analyzed statistically.

The experiment contemplated hybrid aspens clones due to the phenological results that showed positive traits on an earlier experiments which consisted on the comparison between the hybrid aspen and the local aspen on growth and phenology. The results exposed the variables on growth features as stem volume, height, and basal diameter between the hybrids and non-hybrids aspens (Yi et al., 2001). The understanding on the special features based on this results the faster succession from the hybrids compared to the local aspen could bring new aspects, where the results could bring traits to apply bioremediation by phytoremediation. By this more research should be conducted to analyze the possible reduction of the chemical compounds describe.

1.3 Justification

The high relevance to apply new technologies for the degradation or remediation of contaminated sites by PAHs compounds are of vital importance. After understanding the fate of contaminants on the environment and the toxicokineticts these pose for the biodiversity. Bioremediation could be one suitable solution *in situ* for reducing economical costs, energy and directing the ecosystem services from the biodiversity of certain species to improve it. Bioremediation on this research aims to described and understand the role of certain species for future phytoremediation proposes. The multiple possibilities which this out coming technology in Finland could offers, are many if it is properly addressed. The used of ecosystem services as bioremediations of anthropogenic impacts by PAHs compounds can be tackle by phytoremediation from the research of the species.

2.0 Theoretical framework

The experiment was analyzed quantitatively based on the collection and the observation of specific characteristics. These characteristics were analyzed logically with the help of the previous information researched by the predecessor researchers on the related topics.

The researched conducted on the 17th century by Marcello Malpighi which emerge on the publication "Fluid Flow in Plants''. This publication is one of the innovative theories on bioremediation due to the direct relation on the uptake of water by plants and by so the possibility to utilized it as a form to clean contaminated water by phytoremediation (Kramer and Boyer, 1995).

As well on the publication "Experiments on Plant Hybrids'' by Gregor Mendel on 1865 where the results of his research on cell theory and fertilization suggested how a new organism are originated from the fusion of two cells. The natural order for breeding forms on the dominant and the recessive type to become into a hybrid. There should be some momentary accommodation of the two modifying characters in the hybrid as well as partition process in the aliment of the pollen cells and the egg cells (Olby, 2013). Nowadays plants are designed on laboratories to achieve special characteristics for multiple proposes.

The phenological traits were analyzed in order to know beforehand which species should be cloned this is based on the phenology. This could not be possible without the researched carried by two of the most recognized scientists or civil scientists on the 17th century. Robert Marsham and Carolus Linnaeus due to their work on the systematic recordings on climatic conditions. Marsham on "Indications of spring" in England and Linnaeus on "Philosophia botanica" (NEON, 2014).

3.0 Background

In human history soils have been crucial for the development of any civilization in any period of time the historical need of natural resources and the complexity of the ecosystem services provided by these are root to human's subsistence (Haygarth & Ritz, 2009). Soils are one of the most complex systems in our biosphere therefor soils should be integrated in the management within landscapes. Due to anthropogenic disturbances there are negative impacts which deteriorate soils, primarily suited for food production as well as transformed into urban areas or platforms for construction (Haygarth & Ritz, 2009). Contamination of natural resources as ground water, water surface, soils, air and sediments is the result of our mechanized modern world (R. Boopathy, 2000).

Europe is not an exception facing considerable problems as the loss of top soil due to erosion or construction activities, acidification and contamination. This is also increased by the absence of actions taken by European directives, the lack of soil protection and the scarce research (EEA, 2011). Furthermore the estimation of localities in the EU reaches about 1,5 million contaminated sites which were detected before 2011 (EEA, 2011). On Finnish soils approximately 20,000 sites, with pollutants as petroleum hydrocarbons have been detected (EPA, 2009). The next pie chart represents the amount of pollution caused by each factor.

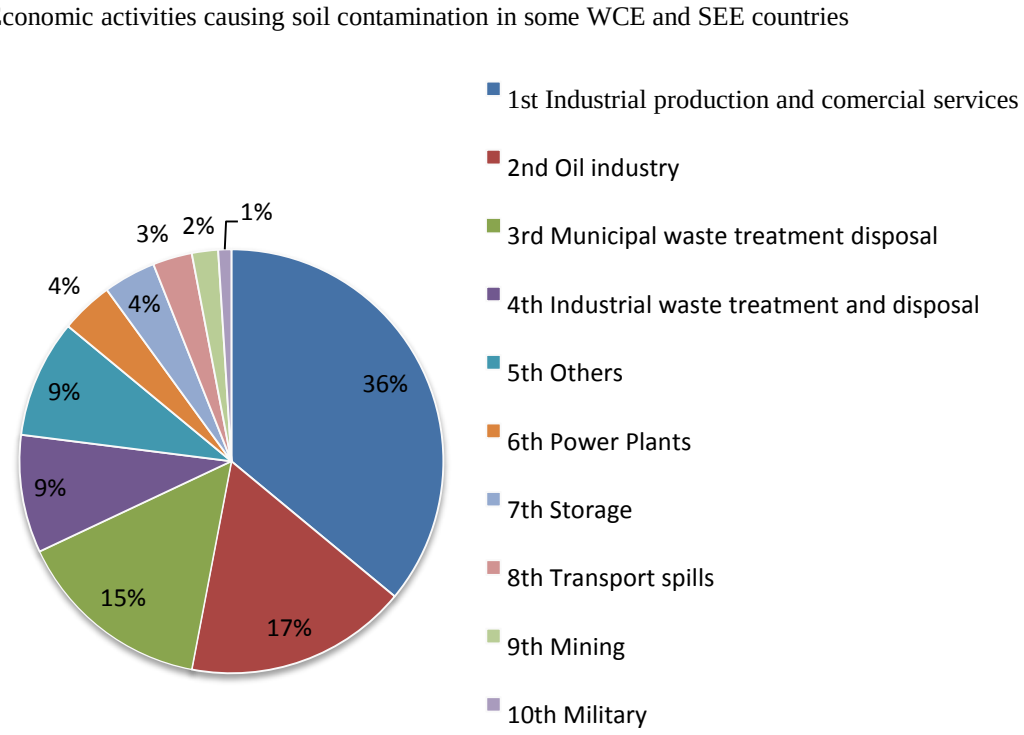


Figure 1 Soil contamination (EEA, 2007).

3.1 Pollution and contamination

However contamination is the existence of a component, where it does not belong, exceeding the established threshold value. Pollution means that the presence of contaminants causes biological harm to a community on species level. This does not mean that all contaminants are pollutants but all pollutants are contaminants. The differences between pollution and contamination cannot be conducted based on chemical research. This because there could be lack of data by implementing only one test for chemicals. The analysis should include toxicity and bioavailability levels (Chapman, 2006). There are different factors that control the fate of different pollutants in contaminated ecosystems. These are localization, persistence, bioconcentration factors, bioaccumulation factors and bioavailability.

By this means the fate of any chemical compound inside any ecosystem is further more intricate by the circumambience of these through soils, air, surface waters and onwards the food chain. Toxicokinectic models are beneficial in order to forebode the fate of chemicals in species level. Therefor more complex models are demanded to estimate the fate of the entire ecosystem (Walker, et al., 2012). The integration of different methodologies is important to obtain reliable results as Lines of Evidence (LOE), the results on toxicity following key species and Weight of Evidence (WOE). This kind of research contributes for two specific kinds of data; definitive assumptions concerning pollution and complementary data, which is required to determine a holistic conclusion. By this variable factors are taken into consideration as sewerage inputs, sediments or environmental niches, which can be impacted by different pollutants.

A precisely conducted WOE integrates primary observations levels on an ecological risk assessment (ERA), which requires to be traced if crucial fluctuations are raised during the ERA process, which demands to be answered (Chapman, 2006).

If the concentration is lower (<) than the contaminant-specific threshold value there is no need for further requirements. When the concentration is higher or equal (≥) to the threshold value the extent of contamination and evaluation of risk has to be quantified pose on (Mikkonen, 2011).

- Surrounding environments (possible spreading)
- Population risk (human risks)
- Nature (ecological and biological risk)

4.0 Remediation

Remediation is the process or method applied to extract or retain poisonous or hazardous materials from an area (EPA, 2009). The contamination by variable pollutants in different ecosystems demands specific remediation techniques (R. Boopathy, 2000). The pollutants are incorporated directly to the environment due to different incidental spills, for example during transportation, emanation from waste disposal, or from storage areas. By this means the requirements to experiment with multiple methods of remediation that could be successfully implemented for the faster and adjustable extent of the according physical conditions.

The industry and the governments around the globe have understood the multiple risks of the complex chemical mixtures as polycyclic aromatic hydrocarbons (PAHs), heavy metals, total petroleum hydrocarbons (TPH), and polychloro biphenyls (PBCs) and more compounds, which pose damage to the environment and human health (Riser-Roberts, 1992). Taking the pollutants into a matrix often is not efficient enough. Also enhancing the density could boost the amount of transformable and bioavailable fragments. The implication of any remediation procedure should be placed after analyzing that there are possible risks to health, distribution or ecology. It also varies from the specifics of the natural sites conditions and proposes where the contamination characteristics and boundaries are including higher or lower crucial demarcations (Enact, 2013).

There are different challenges concerning concentrations of different pollutants on soil types and in order to prevent further dispersion suitable and affordable remediation techniques are required. Remediation methods are directly related to multiple factors which are legislative frameworks and requirements, pollutants, location-condition, quantities of material disposal, soil conditions, humidity content and areas proposes. Therefor different remediation solutions could be applied *in-situ* (on site) and *ex-situ* (off site) (Enact, 2013). On both *ex-situ* and *in-situ* the different techniques have been integrated into one group named physico-chemical, other groups are related to the type of treatment as physical, chemical, electrical. (EUGRIS, 2005). Treatment methods could be separated for surface and soil remediation as well as for groundwater remediation. Another classification results in the consideration of biological, chemical and physical processes including their techniques within categories (Hamby, 1996).

4.1 Bioremediation

The biological process, which utilizes microorganisms to decrease or nullify the concentrations of pollutants or hazardous compounds in a contaminated area, is called bioremediation (R. Boopathy, 2000). Bioremediation is one of the newer techniques and could be utilized to clean-up ground water, sediments, grounds, lagoons, sewage and streams. Bioremediation often could be applied on diverse heterogeneous landscapes where the contaminant is available within the soil particles, diffused in soil liquids and in the soil atmosphere. Due to these intricacies outstanding bioremediation is related to a multidisciplinary advance including variable disciplines as engineering, microbiology, ecology, geology and chemistry. Bioremediation is also recognized, *in situ* or *ex situ*. The relation with microbes in order to complete a successful bioremediation includes the different techniques, which are related to the bioaugmentation of microbes for a specific site including the abiotic factors to enable degradation (Held & Dörr, 2000). The figure 2 explain the biodegradation by microbes.

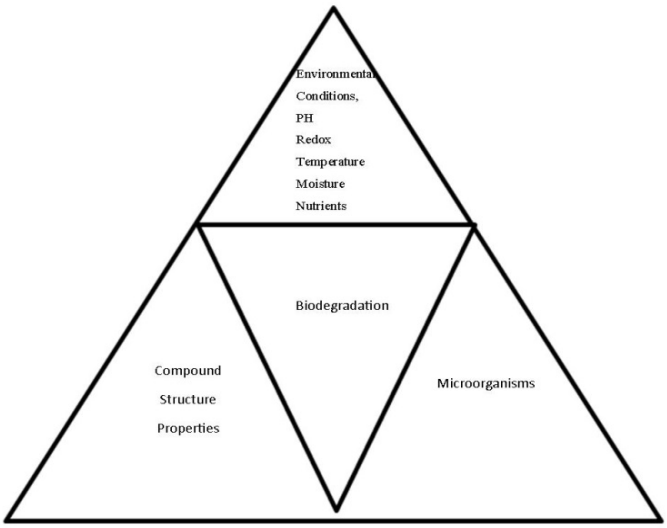


Figure 2 represents the biodegradation triangle to comprehend the microbial degradation of any synthetic organic or natural compound, starting from abiotic and biotic factors and structures, and physicochemical characteristics of the compounds (Suthersan, 1999).

However bioremediation provides diverse benefits compare to conventional techniques as landfilling or incineration. Bioremediation can be applied on site offering less disruption and decreasing the expenses, as it eradicates the waste, excludes long term arrearage and it has better public acceptance. This could be achieved as well with chemical-physical treatment techniques. In specific cases some chemicals are not able to be biodegrade, like heavy metals, radionuclides and certain chlorinated compounds. The microbial metabolism of some pollutants may create toxic metabolites (Hoeppel & Hinchee, 1994). These as well can be biotransformed into compounds decreasing their toxicity and transportability, where the microorganisms in charge of these processes could deteriorate important molecular sites (Tsang et al., 1994). Moreover bioremediation is an intensive process which should be analysed based on the specific environment (Hoeppel & Hinchee, 1994).

Bioremediation is actually a generic term for different technologies ranging from nutrient addition and aeration of waste-containing soils to the use of bioreactors by highly content or very specific conditions of microbial strains. However the aim of bioremediation possibilities is the same: the capability of microorganism to biodegrade via their metabolic cycle and of environmental compounds. The concept of biodegradation clarifies that the materials should be mineralized by aerobic biodegradation, during which an organic compound is converted to carbon dioxide, water and inorganic ions (if the material contents are sulphur, bromine, chlorine). In the process of anaerobic biodegradation the compound is

diminished to methane, inorganic ions and could be hydrogen sulphide under certain conditions (Strauss, 1997).

4.2 Phytoremediation

The term phytoremediation means (phyto = plant and remediation= correct evil). Phytoremediation is the term given to the process of different plants on the proceeding from ecological pollutants. Plants work as photovoltaic mechanical specialist, which treats different environmental systems by taking up soluble water contaminants straight from the root system (Pilon-Smits & Freeman, 2006). The entrenched of techniques that utilized plants to restore contaminated sites (EURODEMO, 2009). Phytoremediation is an *in situ* method for decontamination of soils; as well a low cost method where there are no other lower cost effective, most suitable technology or non-integration with other remediation methods. Profound rooted grasses, trees, aquatic plants all these could have interaction with the phytoremediation area. Phytoremediation have been experimented to degrade: BTEX, TPH, PAH, 2, 4, 6,-trinitrotoluene-TNT, hexahyro-1, 3, 5-trinitro-1, 3, 5-triazine, etc (Schnoor, 2000).

Phytoremediation can be systematizing by the pollutant fate degradation, extraction, containment or as an integration of these. Phytoremediation also could be classified based on the diverse processes involved (EUGRIS, 2003). The different methodologies including extraction from soil or groundwater, pollutants, amount of pollutant in plant tissue, degradation of pollutants by multiple biotic and abiotic processes; volatilization or transpiration of volatile compounds from plants to the atmosphere; immobilization of pollutants in the root area; hydraulic control of contaminated groundwater (plume control), and run off, erosion and irruption by flora convers; as well the introduction of similar micro fauna to increase the process of biodegradation on the contaminated site(EPA, 2000). These processes are express in the table I and in the figure 3 and 4.

Table I phytoremediation process (UNIDO, 2008).

Rhizofiltration	Method which utilizes the plant roots in the isolate of pollutants
Phytoextraction	Method integrating the complete structure of the plant in the uptake of pollutants from the ground
Phytotransformation	Suitable to water and soil including the degradation of pollutants by means of the plant metabolism
Phyto-stimulation or plant assisted	Utilized for water and soil which interact boosting the microbial to accomplish biodegradation on the root zone (rhizosphere)
Phytostabilization	Process in which the plant decreases the movement and trespassing of latent contamination in soil
Phytovolatization	Transpiration across plants to the atmosphere

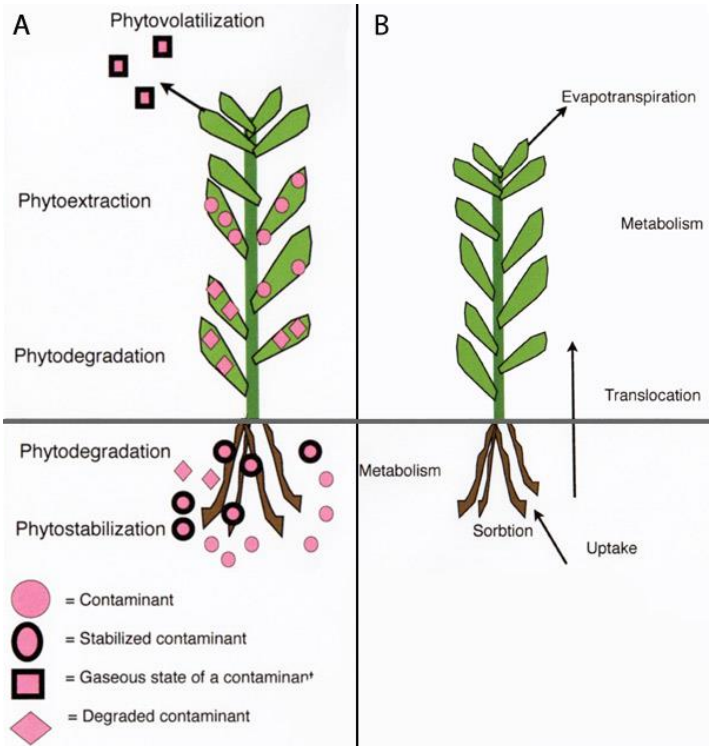


Figure 3 Schematic model of different phytoremediation technologies involving removal and containment of contaminants; (B) physiological processes that take place in plants during phytoremediation (Nature Education, 2011).

By this means the pollutants are binded, to the soil and not bioavailable then incapacitated and removed by any means of transport. The reduction from risks to humans could be achieved by modifying the pollutants to non-hazardous compounds, where the contamination is non-available (EPA, 2000). The next figure represents the processes.

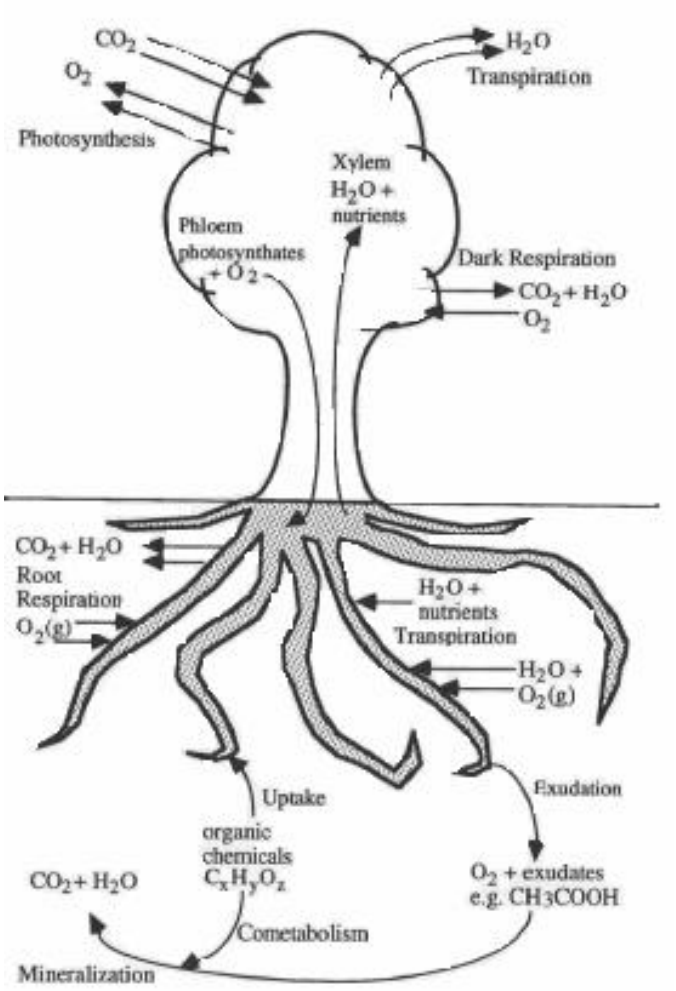


Figure 4. Phytoremediation process (Schnoor, 2000).

Over all some species which have been utilized for phytoremediation or could be utilized most likely are deep rooted plants e.g. poplars (*Populus*), alfalfa (*Medicago sativa*), and Indian mustard (*Brassica juncea*), sunflower (*Helianthus annuus*), to encounter, mitigate and retain pollutants located many meters into the subsurface (Cunningham et al., 1997). Growth rate on plants are limited by phytoremediation meaning that long term vision is required to be able to be achievable (Nellessen & Fletcher, 1993).

4.3 Remediation analysis

In order to develop the best technology applied to the specific characteristics of the pollutants and soil types it is require to understand which kind of tools are compulsory for the suitable practices. Due to the complexity on costs and time consuming for soil remediation operations including the variety of establish and rising soil remediation technologies.

There is the need to apply the most optimal solution for remediation when this is closely related to human decision makers understanding the possible options assessed and the available technologies with their opportunities. For this multiple methodologies there have been developed as one example of remediation analysis (DARTS) Decision Aid Remediation Technology Selection. These methodologies are aiming to search the most suitable selection of available technical, economic, social, legal and environmental criteria, as well *in situ* or *ex situ* remediation technologies for each specific environmental remediation case (UNIDO, 2008).

Multi criteria analysis is required to be performed in order to analyse the proximate assets of the remediation options and chose from a variety of them, the most effective for site clean-up application. The differences maybe demanding, when there are multiple and more conflictive assets, where the decision maker is required to specify aims relative balancing for the atypical criteria. Approximate evaluating is utilized to search the most suitable answer. The balancing can be modifying to appraise sensitive solutions or to express variable solutions (UNIDO, 2008).

The criteria and grading process utilized in remediation techniques execution evaluation of database at DARTS are distributed as a diagram process, allowing a clearly and allocating the optimal model of remediation. The assessment of criteria concluded and integrated scheme of phases which allows the progress of analysis of benefits and risks correlated to a favourable remediation assessment (UNIDO, 2008). The next table shows one example of the information which is needed to take into consideration for the analysis of the possible remediation technique.

Table II Represents the assets which DARTS is taking in consideration (UNIDO, 2008).

Criteria	Issue
Applicability	
	Pollutant class
General Applicability	Soil class
	Profundity of pollutant
Site-Specific Applicability	Pollutant concentration amount
	Minimum activable amount
	Decontaminated matrix quality
Performance Assessment	Security
	Evolution status
General Assessment	Accuracy and Sustenance
	Data requirements
	Standalone character
	Public acceptability
Time-Cost Assessment	Clean-up time required
	Overall cost

4.4 *Ex situ* remediation

Ex situ remediation methods are alternatives corrections to contaminated settings where soil or water is displaced from the initial position and treated on the affected area or off site by different techniques (EUGRIS, 2005). *Ex situ* remediation involved methods as bio piling, land farming, process by bioreactors, onward thermal, chemical and physical mechanisms. Off site remediation is not only a technique but more over include the expenses which are related not only with remediation processes, as well as the excavation, transportation of soil and the technology required and other techniques that could be developed. This kind of remediation techniques are high on economic terms and machinery (Koning, et al., 2000). Nevertheless *ex situ* remediation could prevent the further dispersion from the pollutants to another environment. As well accede homogenization of the contaminated soil on previous treatment and assure the monitoring from the soils to reach the acceptable levels on an earlier period of time (UNIDO, 2008).

4.5 *In situ* remediation

In situ (on site) remediation methods aim of extracting, reducing, chemically transforming, controlling or compressing pollutants within soil or groundwater without displacing the matrices from the terrain to control the contamination on the area, without transporting it to other place (EUGRIS, 2005). However *in situ* treatments are often utilized where the equipment is limited due to the negative effects on the nearest areas (CETS, 1993). Also *in situ* remediation processes could be grouped into different classes based on their treatment operation: physic-chemical, thermal, electrical and biological. Some of the processes have been categorized into an only group named physic-chemical this is because the complexity from the composition of different pollutants on the soil. By this the diversity of pollutants called ‘‘cocktail’’ is required the application of multiple remediation processes or treatments to decrease the density of pollutants to acceptable levels (EUGRIS, 2005). As in figure 5 it is summarize.

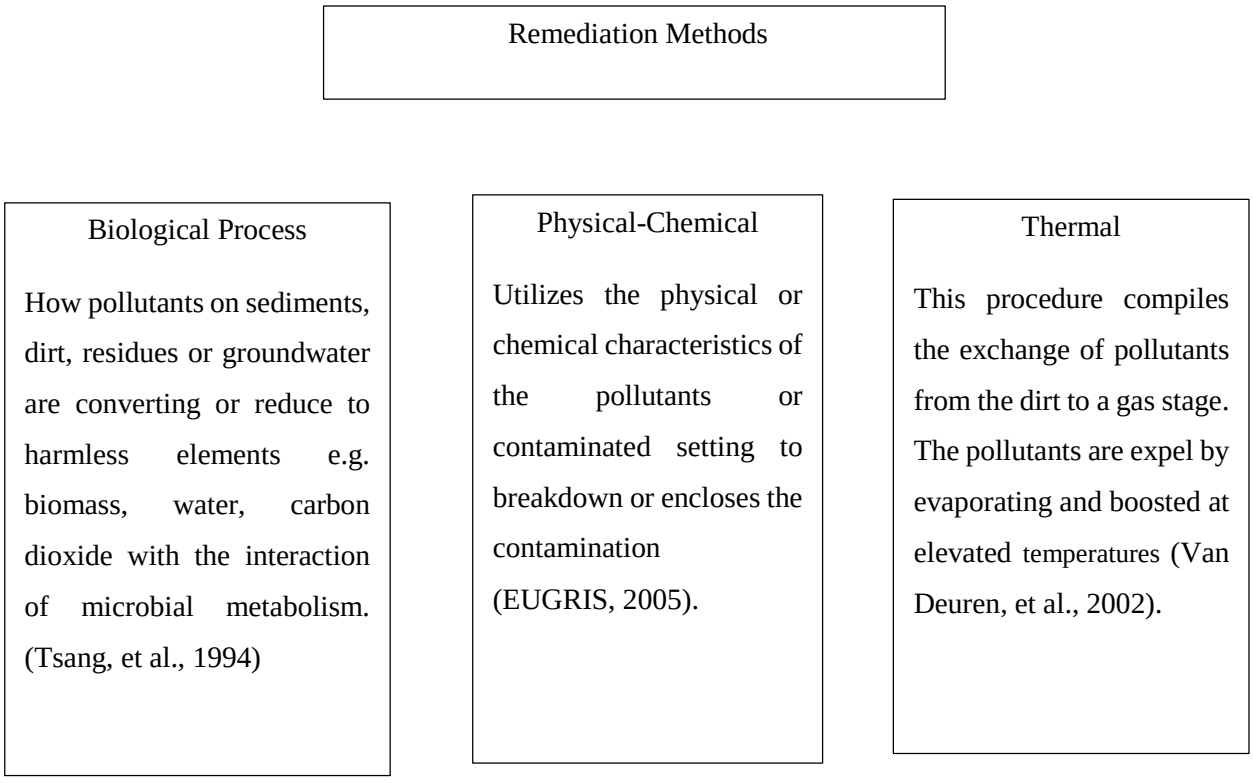


Figure 5 represents the remediation technologies summary

4.6 Soil remediation techniques

In this chapter there are different tables explaining the variable technologies available.

Table III Soil biological treatments in situ and ex situ charts (UNIDO, 2008).

Biological treatment	
Insitu	*Ex situ*
Bioventing	Biopiles
Phytoremediation	Bioreactor
Land farming	Composting
Enhanced bioremediation	Land farming
Natural attenuation	

Table IV Physico-chemical treatments in situ and ex situ techniques (UNIDO, 2008).

Physico-chemical treatment	
In situ	*Ex situ*
Electroreclamation	Dehalogenation
Lasagna Process	Solar detoxification
Soil flushing	Soil washing
Fracturing	Chemical extraction
Polymer	Separation
Soil vapour extraction	
Solidification/stabilization	
Chemical reduction/oxidation	
Contaminent barriers	

Table V Thermal treatments in situ and ex situ techniques (UNIDO, 2008).

Thermal treatment	
In situ	*Ex situ*
	Open burning
Enhanced thermal SVE	Incineration
	Plasma arc process
	Pyrolysis
	Thermal desorption
	Hot gas decontamination
Vitrification	

5.0 Petroleum and derives

Petroleum is a vast variety of thousands of conglomerates and it can be separated into four major sets: alkanes, aromatics, resins, and asphaltenes. In overview the alkane division is the highest to be biodegraded but on the other side the resins and asphaltenes are insusceptible to biological degradation. The aromatic compounds certainly the PAHs are on the transitional biodegradability although are highly due their toxicity and bioaccumulation (Wrenn & Venosa, 1996).

5.1 Aerosols

Mostly aerosols tend to have climate and human health effects and rather that 90% come from anthropogenic derivate and the 10% from natural sources which are not comparable (Kiehl & Rodhe, 1995). This could be related to negative aspects on human health as cardiovascular problems, asthma or respiratory illnesses and death (WHO, 2006). The PAHs metabolites which are created after the uncompleted burning of organic material and then expelled to the atmosphere, where these compounds are currently in different gases and particles remarkably volatile or lighter in the atmosphere. PAHs containing 2 or 3 molecular rings which are on the basis of gas stage where the bigger compounds with aromatic rings are attached to the particles in the atmosphere (Seinfeld & Pandis, 1998). Metabolites of PAH can react with DNA generating cancer, and the remediation of PAHs is a tough action due to the chemical composition of the same tending to reduce bioavailability and in worst scenarios on older hazardous compounds (ATSDRc, 1990).

5.2 PAHs

Polycyclic aromatic hydrocarbons or polynuclear aromatic hydrocarbons have diverse rings in their molecular structure. Including often endow compounds as anthracene, naphthalene and more conglomerated compounds as benzo (a) pyrene, pyrene. For this the biodegradation of PAHs is related on the intricacy of the chemical structure and the expanse of enzymatic adjustment. Usual PAHs which consist of two or three rings as anthracene, naphathalene and phenanthrene are debased at certain rates when O_2 is present. Four rings compounds as chrysene, pyrene and pentacyclic are heterogeneity, highly resistant to degradation and are contemplated recalcitrant (Mckenna, 1979).

These are the factors that can influence the degradation of PAHs under anaerobic and aerobic conditions:

- Solubility
- Amount of fused rings
- Type of replacement
- Number of exchange - placement of replacement -Nature of the atoms in heterocyclic compounds

These factors are mixed into unique criterion specified as a structure-biodegradable relationship generalized concerning structure decomposition connected to aerobic environments, this do not apply to anaerobic environments (Alexander, 1994). Aerobic biodegradation of the two and three rings on PAHs is realized by the diversity and quantity of soil bacteria. As the amount of combined rings and the intricacy of the supplanted groups multiply, the reciprocal degree of degradation minimize. The consequence of alkyl substituents becomes more difficult to predict (Cookson, 1994). Figure 6 show the chemical molecules of some PAHs compounds

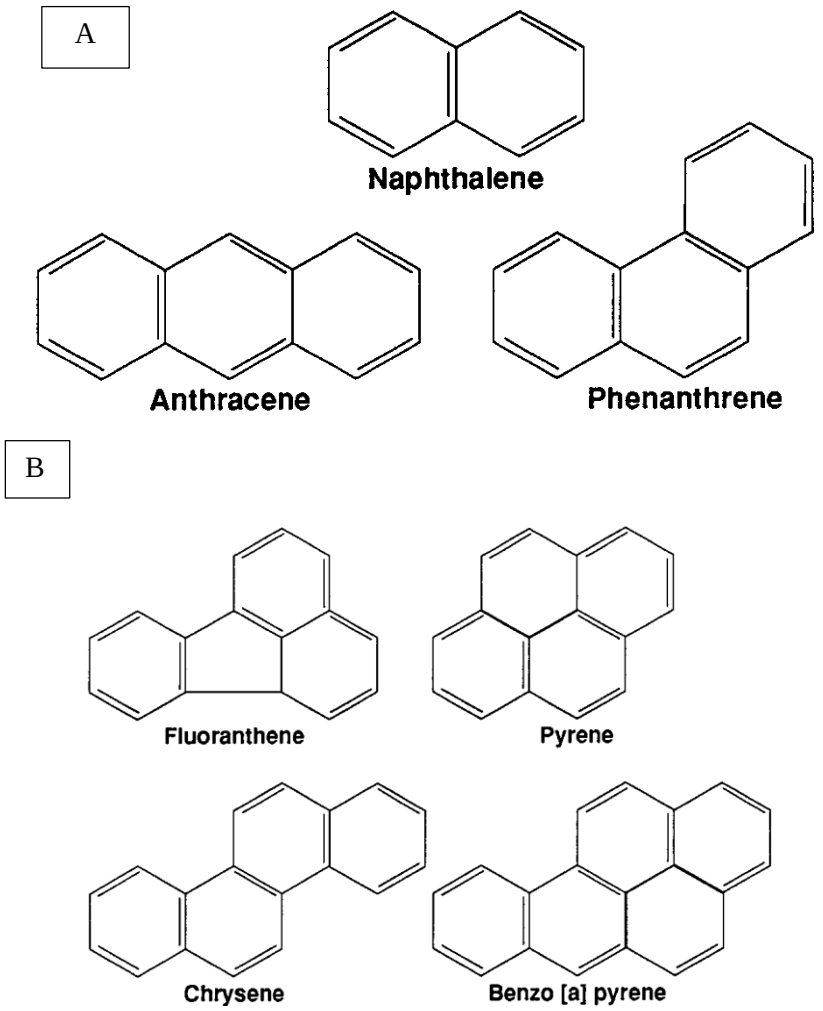


Figure 6 shows the molecular structure from PAHs. A stands for the faster degradable and B for slowly degradable or persistent PAH (Suthersan, 1999).

6.0 Tree diversity on Finnish forest

In Finland the amount of native trees species is rather low 4 main conifers and 27 broadleaved trees order scrubs or small trees and some of the broadleaves species have a reduce distribution area. Some areas the predominant specie could be only pine as in northern region. Broadleaves often dominate on mixed stands were there are specific characteristics as rich mineral grounds, uplands with grass vegetation forest. But slowly the transformation in Finnish forest has being notorious since the early 1950s the division of pines stands incremented as the consequences from regenerating areas with the same. As this the notable transformation of the reduction of zones from predominant deciduous forest by partly in southern Finland (Mmfi, 2011). The next pie chart shows the distribution in percentage of the trees species.

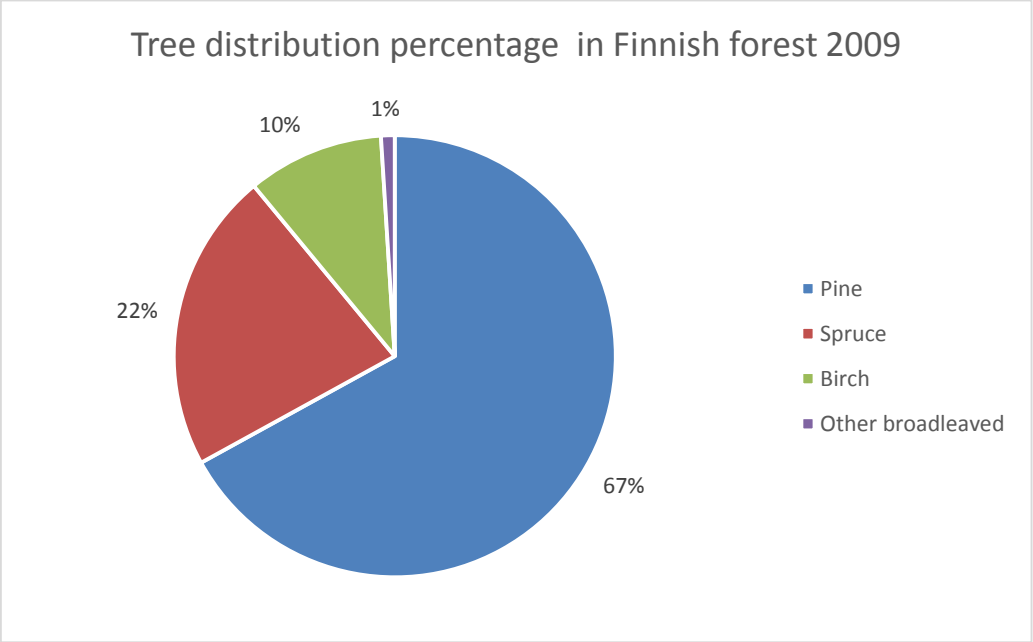


Figure VII represent the percentage of trees species located in Finland (METLA, 2011).

6.1 Hybrid poplars and characteristics for phytoremediation

Human interaction to obtain benefits from different trees species has modify and transformed them, one result is the cross between European aspen and the North American (*P. tremuloides*). Hybrid aspen can develop faster on a period of time compare to their relatives the European aspen where in Fennoscandia it is proved that can reach heights of 20 meters in about 25 years (Hynynen and Karlsson, 2002). Propagation of aspen can be both sexually and asexually (Eckenwalder, 1996), rather to reach prosperous sexual reproduction is low due to the crop of capable seeds (Bärring, 1988, Worrell 1995).

Populus is division of deciduous flourishing tree among 20-35 species which are distributed uneven around the globe and especially in the Northern regions of the world. This order has been divided under three brought groups along with poplars, aspens and cotton woods. Black poplars or cotton woods are situated at temperate areas as North America, Europe and Western Asia. Some of this relevant species of black poplars are *P. fremontii, P. nigra, P. deltoides, P. canadensis*. Second broad category of aspens which is named as white poplar is present to circumpolar subarctic (Yadav et al., 2010). The next species inhabits on cool temperate climate and the southern mountains regions. The species are *P. tremula, P. adenopoda, P. alba, P. canescens, P. davidiana, P.grandidentata, P. sieboldii* and *P. tremuloides*. The large scale group of balsam poplars inhabits at cool temperate regions of North America and Asia which gathers multiple species essentially as *P. angustifolia, P. balsamifera, P. cathayana, P. koreana, P. laurifolia, P. maximowiczii, P. simonii, P. trichocarpa, P. tristis, P. ussuriensis*, and *P. yunnanensis.* There is one group which comes as the order of the Mexicans poplars, subtropical poplars and bigleaf poplars (Yadav et al., 2010). Poplars are valuable for their hardwood tree and as well-known specie for their characteristics based on the deep root system for the process on phytoremediation. It is as well-known for the action on decreasing hazardous substances in the environment due to the remarkably adaptability on the process of photosynthesis (Soudek et al., 2004). Poplar cultivations can have a faster growth progression about 90.6 Mg ha^{-1} on course of 5 to 8 years (Das & Chaturvedi, 2005). The specific and extensive root setup of poplar provide effective uptake of pollutants in the water. In superposition the green canopy fixes and retains carbon by its exclusive approximation of photosynthesis. Therefor decreases atmospheric CO_2 chemically by electron shift and physically cutting down CO_2 amounts on the environment. Poplar have different phases as a decontamination actor were their leafage could be grouped and incinerated. On the other side the polluted biomass could be in incinerated on the specifics on the pollution and then treated related to the pollution compounds, by this decreasing the levels of the contamination. The poplar harvested wood can be utilized by paper industry as an important raw material, pulp and high quality fibers (Stettler et al., 1996). As well for matchsticks (Diet & Schnoor, 2001).

6.2 Hybrid aspen phenology

The crossbreed between European aspen (*P. tremula L.*) and the North American trembling aspen (*P. tremuloides Michx.)* has demonstrated better development on Finnish soils (Beuker, 1989) which started at 1950s the plantation of hybrid aspens (METLA, 2011). It is a spread specie in Finland, usually growing in a variety of stands including birch, spruce and pine. Latest investigations in wood thread permit to integrate small fibres into a rich variety with coniferous in high standards for papermaking in Finnish industry related to forestry which is interested to profit by aspen in the production of short fibres. The propagation to plant certain clones of hybrid aspen has grown. The crossbreed between European aspen (*P. tremula L.*) and the North American trembling aspen (*P. tremuloides Michx.)* has demonstrated better development on Finnish soils (Beuker, 1989). Genetics advance from aspen crossbreeding schemes expose in the USA (Einshphar, 1984) and Europe (Melchoir, 1985). The difference between progenies of interspecific hybrids which growth faster than progenies of intraspecific crosses (Yu, et al., 2001).

Aspen account for a vast genetic resources that it can be utilized through specific interspecific breeding, hybridization or cloning (Li, 1995). One of the dynamic strengths on hybrid poplar is the vigour which has characterized the breeding between poplars (Larsen, 1970). The augmentation is a process on the results that vitality is reflected on the next factors as water and nutrient efficacy, carbon allotment patterns and shoot which are correlated to increase phenology. This attributes can modify the performance of *Populus* on phenology foil, photosynthetic ability and stomatal morphology (Michel et al., 1990). It has been proved that interspecific aspen hybrids developed rapidly than intraspecific hybrids at earlier stages (juvenile). The biographers attribute this to greater internode number and length as well foil amount. The volume from the sprout of hybrids *P. tremula L. x P. tremuloides Michx.* could be the outcome of the late shoot which accord to the length period of height growth this by the heterosis of the poplars (Li et al., 1998).

6.3 Propagation of hybrid aspen

The most crucial form of reproduction on hybrid aspen it is clonal diversity. The effective peculiarity on the bloomed clones besides rooted assisted were range of clones for large-scale propagation. The importance to search clones where the high amount of divisions for each log plant can be acquaint. For clone propagation standards on aspen were rate of growth and fibre attributes, as well capability to regenerate and efficacy are of high importance. It is valuable to acquire clones which multiple cuttings per log plant can be taken for extensive production propagation (Stenvall, 2006).

Stem, root cuttings and micropropagation are some of the techniques that can be utilized to reproduce aspen. Micropropagation have diverse methods as: organs, embryos, single cells or protoplasts can be artificially grown in vitro (Bonga, 1985). Often micropropagation tend to be technologically challenging, requiring much work, high technology facilities, and very expensive (Vasil, 1994). Any how micropropagation is secure and effective method to be utilized for aspen reproduction (Winton 1971, Ahuja 1983, Ahuja 1984). Cutting propagation is other method which is often applied on commercial plant manufacture (Hartmann et al., 2002). The plant it is cut into a smaller parts where it is possible to regenerate into an entire plant. This cuttings belongs to the roots or stems this is depending on the desirable specie and propagation conditions (Mahlstede and Haber 1957, Hartmann et al., 2002). Different species of *Populus* can be propagated by hardwood cuttings but this is more complicated, instead leafy softwood could be utilized. For European aspen and the closest related *Populus*, roots cuttings technique can be apply for their propagation (Hartmann et al., 2002). Root cuttings technique consist on taking apart different portions of the root system of one hybrid aspen were the ability to bring forth new shoots and roots. This in order to be able to provide efficient rooting which are essential to utilize roots not longer than one centimeter in diameter (Stenvall, 2006).

7.0 Materials and Methods

The methodology followed on the experiment settings aim was to understand the adaptability and development of the seedlings planted on the different environments with their specific stressors. By this the measurements will be followed by statistical analysis to understand the relation between the pools treatments and the species adaptability possibilities on each location.

7.1 Experimental settings

The experiment was conducted on the Finnish Forest Research Institute (METLA) Haapastensyrjä located at the (60°37'4.92"N 24°26'6.91"E WSG 84). The field test was a setup on August 2009 with 20 aspen seedlings (reproduced by root propagation) divided on 15 hybrid aspens (clones: KHL, 14, 134, 172, 191, 23, 27, 287, 291, 294, 34, 444, 457, 476, 9) and 5 European aspens clones (R2, R3, R4, R7, R8). The division of the clones was selected by prior experiments based on their phenology, growth, propagation and they were collected based on field tests as nurseries or field result tests. There were 8 pools with sandy till soil that have been treated with different stressors. The sizes of the pools were (3m x 11m x 40 cm deep). The first four pools were polluted by diesel or low heated oil and (the soil from a polluted site was utilized as well) in a concentration of 0.8% this PAHs. The second two pools started with a concentration of 3,5% of common salt utilized for food (Natrium chloride) and started to increase

the amount of salt on the year 2010 and to .5610g*pool. The last two pools were utilized as control without any pollutants. The division was done at first of 4 pools (2 oil, 1salt, & 1control) were established inside a greenhouse, mimic warmer climate conditions as global warming. The other 4 pools (2 oils, 1 salted, & 1control) were settled outside the greenhouse without any disruptions. By this 5 replicates seedlings were cultivated per clone in each pool inside the greenhouse and outside. Giving a total of 800 seedlings planted with a starting average height of 32cm each individual. We need to remark that there were not any fertilized or pesticides applied to any of the trees inside the pools. The pools of inside the greenhouse were watered once per week or more if the case there were warmer days (100 L for the 4 pools). As well in the polluted pools containing oil there were deliberately some isolated spaces without any tree. This was to understand the possibility of decreasing the oil by evaporation or due to the trees in symbiosis with the present bacteria. The next figures show how the experiment was design and constructed.

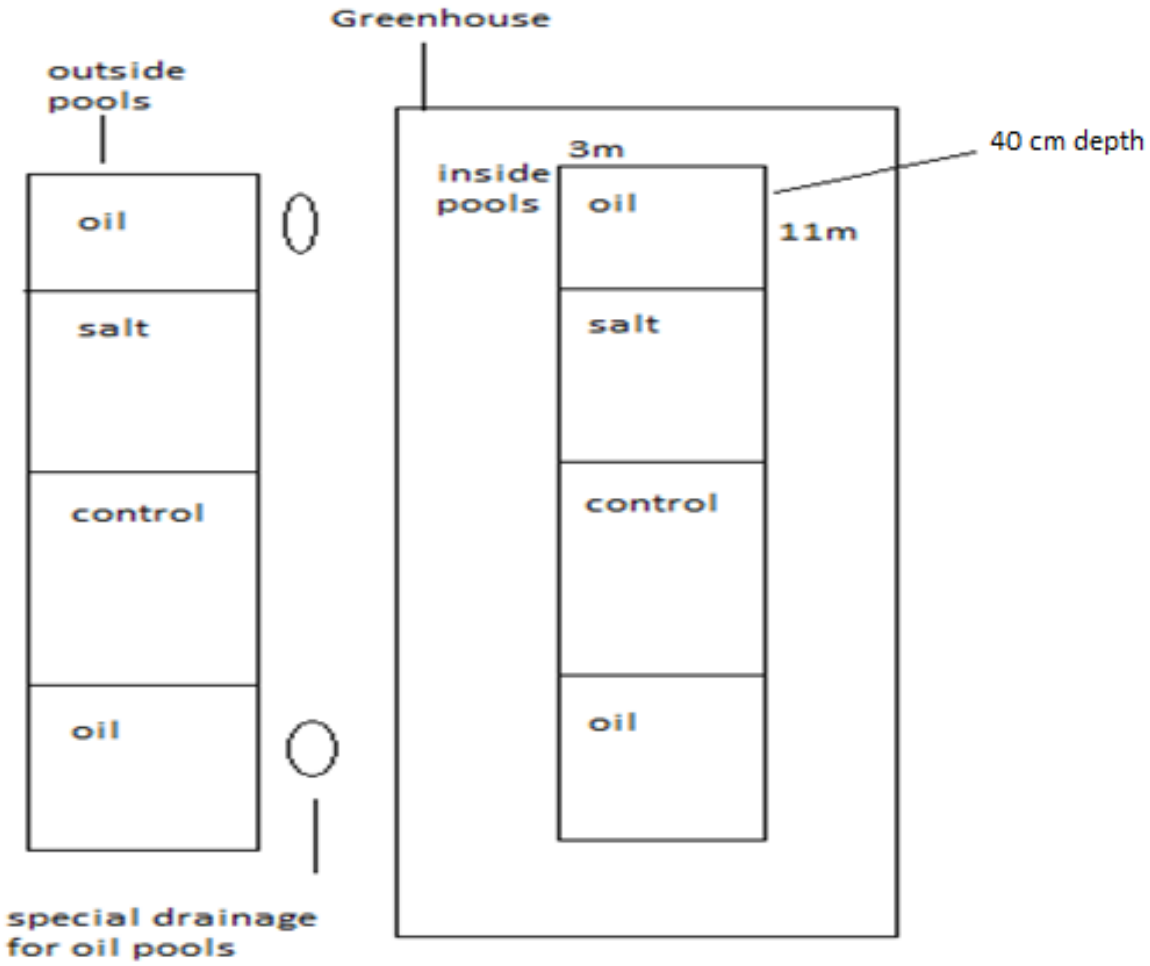


Figure.8 represents how was designed and settled the pools for the experiment on phytoremediation inside the greenhouse and outside.

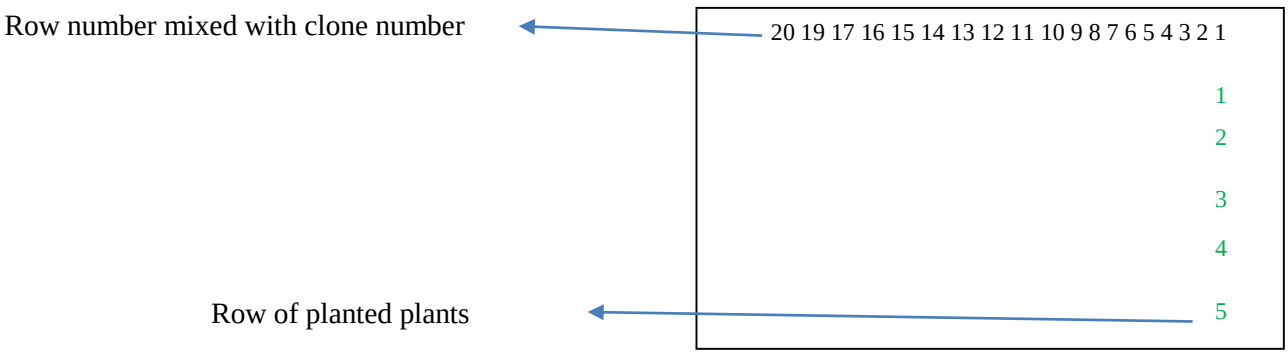


Figure 9 demonstrates in which order the trees for the experiment were planted and the rows with the descriptions differing the order planted but following the same idea in each pool.

The numbers on colour green represents the planted plants and the **black** numbers represent the row in combination with the clone given number e.g. row 1 represents the first row and the clone number is 134.

The next table describes how was settled the trial experiment on the pools with the native species including the amount of clones planted and the hybrids clones for the bioremediation experiment. The table express on the left side the number of the clone planted and the in the right side the columns and the amount of clones planted in each row. By this the total amount planted was of 800 seedlings on the 8 pools with the different treatments in each one.

VI Table of clones settled on the pools for the phytoremediation experiment and the ramets found in each pool (METLA, 2011).

Clones planted	Amount of individuals in each row							
	1	2	3	4	5	6	7	8
134	5	5	5	0	5	5	5	0
14	5	5	5	10	5	5	5	10
172	5	5	5	0	5	5	5	0
191	5	5	5	10	5	5	5	10
23	5	5	5	10	5	5	5	10
27	5	5	5	0	5	5	5	0
287	5	5	5	0	5	5	5	0
291	5	5	5	10	5	5	5	10
294	5	5	5	0	5	5	5	0
34	5	5	5	0	5	5	5	0
444	5	5	5	0	5	5	5	0
457	5	5	5	0	5	5	5	0
476	5	5	5	10	5	5	5	10
9	5	5	5	0	5	5	5	0
KHL	5	5	5	0	5	5	5	0
R2	5	5	5	10	5	5	5	10
R3	5	5	5	10	5	5	5	10
R4	5	5	5	10	5	5	5	10
R7	5	5	5	10	5	5	5	10
R8	5	5	5	10	5	5	5	10

7.2 Measurement

The adaptability and development for the hybrid *P. tremula L. x P. tremuloides Michx*. and native *P. tremula* on 8 different pools were analyzed by taking in consideration multiple measurements. The data collected started at the end of April to the 5th of September 2011 (104 days). The measures of all the trees was gathered 2 or 3 times every month following the same time periods as previous weeks from the collected dates. Measuring each one of the trees from soil to the last bud was conducted with the same tools and procedures. This was for the accurate comprehension of the development of the clones or their variances on growth between clones and the treatments. On different pools as 1,2,3,5 and 6 the plants were to short (20cm) and in these pools all leaves were counted. In the case of other pools there were not sufficient personnel to count and measure all leaves, instead was chosen a branch of the plant and measure all the leaves. The branch length was measured as well, considering the mean size of the tree branch and all the branches were counted. As well there was one harvest on every second tree (one tree/row so one fifth of the trees) on the pools numbers 2, 3, 4, 6, 7 and 8. For this a special root shovel in order to get same volume was utilized, taking the tree including what it could be taken of the root system and multiple measurements were taken as fast as possible. The trees were washed with cold and hot water to obtain the peat away. Photographs were taken of the root system and it was not possible to collect all the peat off due to the roots system attached to it. The trees were measured again after a drying process on the oven for 11 to 16 days at temperature of 38° to 40° Celsius degrees straight after were dry the measurements took in place. The measurements criteria is showed on the table number VII. There could be some analytical bias on the statistical results due to the collection on the measurements which could be affected based on the rotation on the personnel, whom participated on the gathering and filling the measurements on the excel sheets.

Table VII explain what measurements were taken into consideration and the equipment utilized to obtain the data and for the statistical analysis (METLA, 2010).

Variable	Explanation
Location	Inside or Outside
Treatment	Oil_1; Oil_2 ; Control ; Salt
Pool	Number of the pool (1 to 8)
Row	Number of the row
Clone	Number of the clone
n_plant	Number of the plant in a row (1 to 5). All the plants in the same row have the same clone number.
Running number	
Harvest date	Number of the day we dug the tree out of the pool
Processing date (wet measurements)	Number of the day we did the first measurements (wet measurements)
planting depth (cm)	Distance from the root up to the border between soil and surface (measured on the stem)
total stem height (cm)	Distance from root to the last bud
Stem diameter (cm)	Measured with an electronic caliper
root wet weight (g)	
stem with branches wet weight (g)	
stem without branches wet weight (g)	
total branch wet weight (g)	
root dry weight (g)	root weight after drying in the oven
stem dry weight (g)	stem weight after drying in the oven
total branch dry weight (g)	branch weight after drying in the oven
weight loss root (g)	root wet weight (g) - root dry weight (g)
Weight loss stem (g)	stem without branches wet weight (g) - stem dry weight (g)
Nr of the day we put them in the oven	First day of drying
Nr of the day of we measured dry weight	Last day of drying
Number of days in the oven	Last day of drying - First day of drying
Note	Remark
note (depth of roots taken starting from the main root)	
Number of roots in the planting depth	
between or at	To clarify if the distance you are giving is the distances of all the roots or the first and the last
value 1 (cm)	Distance from the main root to the root number 1
Value 2 (cm)	Distance from the main root to the root number 2
Value 3 (cm)	Distance from the main root to the root number 3

7.3 Statistical analysis

The considerable information that was only accounted for the analysis was given by the researchers in charge of the experiment. The data can be found at the research unit of Haapastensyrjä from the Finnish Forest Research Institute. The analysed pools account for (inside oil, inside salt, inside control and outside oil, outside control, outside salt). The statistical analysis aim, was to examine and recognize the variances on survival, development (if happened), adaptability and the fittest clones in every pool within treatment, where understanding the behavior of the clones on the polluted sites by PAHs compounds it is require. Therefore if positive results were observed the application to development of a methodology for bioremediation could be proposed. This methodology could be applied on future bioremediation sites with similar characteristics. The parameters followed for the statistical analysis of the data collected it is presented on table VIII and the total parameters collected can be located under the appendix on materials and methods. The assayed data collected was systematized in order of relevance for the analysis of total biomass following specific variables and with the program (IBM, SPSS Statistics Inc. 2013). The analysis conducted was heterogeneity, multiple comparisons, A nova, A nova variable analysis and at last Univariate Analysis of Variance including Post Hoc Test and Tukey test.

Table VIII represents the data assetes collected for the measurements which were taken into consideration for the statically analysis conducted (METLA,2010).

Pools: Inside oil, inside control, inside salt and out side oil, outside control, outside salt

Total stem height (cm) From root to last bud	Stem diameter (cm)	Root dry weight (g)	Stem dry weight (g)	Total branch dry weight (g)	Stem +Branch dry weight (g)

8.0 Results

8.1 Adaptability

To be able to understand the behaviour on the species analysed and determine if there was adaptability or not into the different pool treatments and locations, the need to reduce the variables from the data parameters was important. The statically analysis parameters are showed on the table VIII and it was followed to identify the possible significant variables on the clones. The analysed data corresponds to the correlation within pool treatments and location, where the response on survival, adaptability and growth were positive. This could be observed on the development gained during the past three years since the experiment started. The data collected and subsequently tested brought results on the amount of alive clones and the successional development. Some of the main variables were height and stem diameter. Thereby the criteria of the data for the statistical analysis was selected based on the total biomass taking in consideration the specific variables of treatment (oil, salted, control), location (inside or outside) and hybrid clones (*P. tremuloides Michx. x P. tremula L.)* and native clones *(P. tremula).* The figure 10 compares the clones on height from each location and treatment between (*P. tremuloides Michx. x P. tremula L.*) and the native *P. tremula* clones.

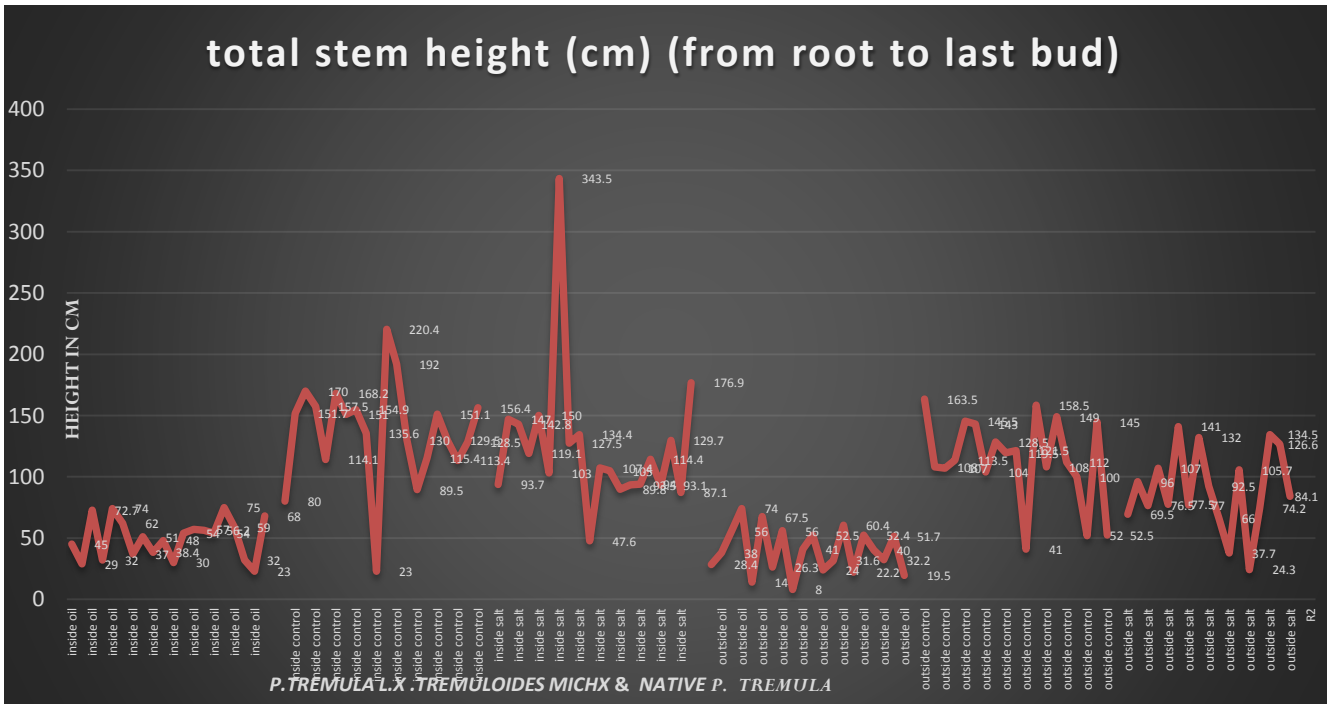


Figure 10 graphic shows the differences on development between treatments, location on (P. tremula L. x P. tremuloides Michx & P. tremula) clones the graphic was made on excel.

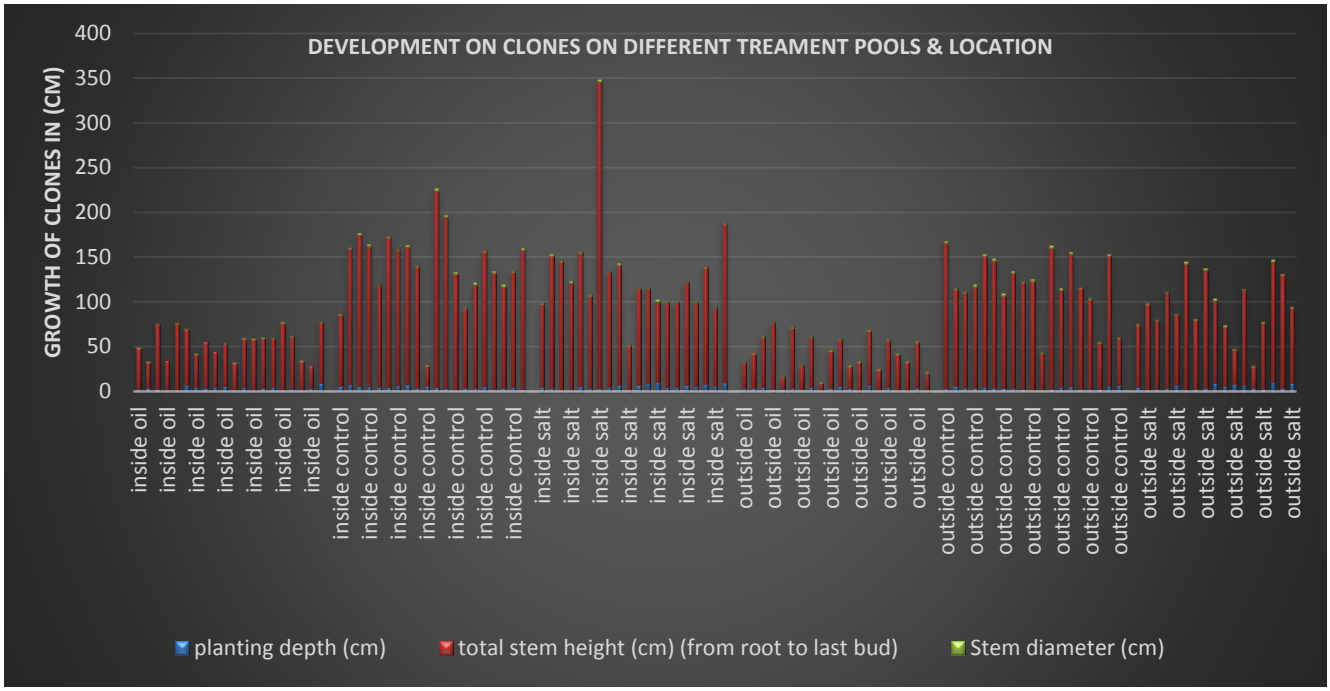
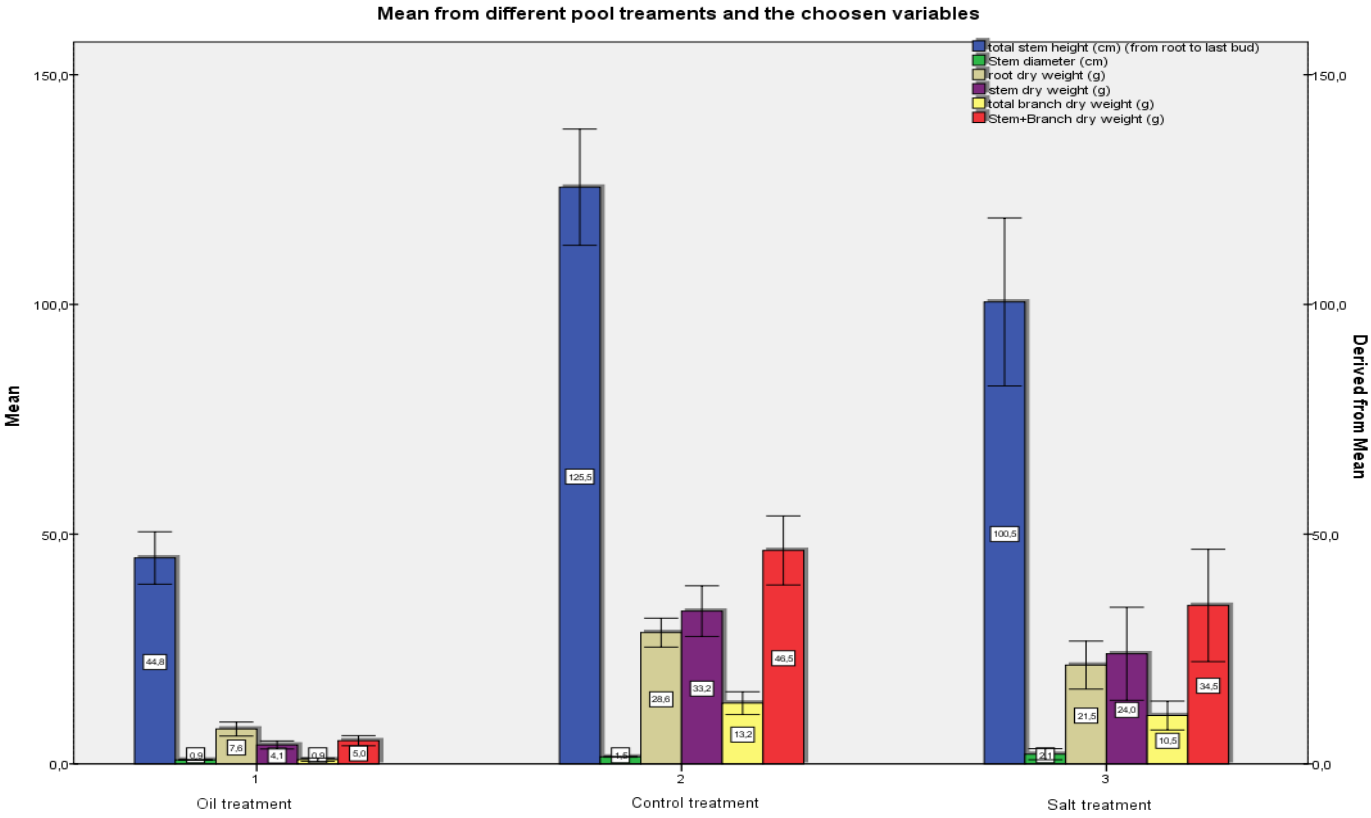


Figure 11 compares the development gained on the different clones during the 3 years since the experiment started on the different pool treatments and locations. (Excel sheet)

8.2 Growth

Results of variance denotes that on every Treatment*Location the interaction did not explain significantly the variation of the measured trait. But never the less treatment has the same response inside greenhouse and outside. There were considerable variables especially on location and treatment thus, this means that treatment explained significantly the growth variation. The results on significant effects considering the increase on total stem height (cm) (from root to last bud) which results on positive growth related to the soil treatment. Some with considerable influences was root weight (cm) where treatment has a clear influence on the root system for possible phytoremediation. By this means a possible solution for this kind of compounds found on different type of soils. Stem dry weight has as clear weight by treatment so the results show that Stem + branch dry weight (g) were affected by treatment reflecting the possible relation between the treatment and the species. In general this meant that the treatment has a clear relationship on the characteristics on the species which are going to be described on multiple comparisons. The results from univariate analysis can be found under appendix results. Figure 12 demonstrates that there was influence on the clones on location and treatment, which this means that there were some positive results on growth. Further research could bring new results to be able to determine more accurate results on the decreasing of possible chemicals compounds where poplars can be apply for bioremediation.



The figure 12 represents the graphs on the results obtain from the statically analysis which demonstrates that the highest trees were developed on the control pools (inside and outside), the second group on height was on the salted treatment (inside and outside) and the last group to be developed height was on the location with oil (inside and outside) (SPSS, 2013).

8.3 Multiple comparisons

The results of the multiple Univarable analysis included Post Hoc and Tukey test have showed that there are some minor variations between treatments 2 (control) and 3 (salt). Thus 1 (oil) and 2 (control). As well 3 (salt) and 1 (oil) differs on the characteristics analyzed. The tables were taken from the results on the statistically analysis conducted with the program (IBM, SPSS Statistics Inc. 2013).

Table IX showed the results on multiple comparisons between root dry weights in (g) between treatments (SPSS, 2013).

Multiple Comparisons

Dependent Variable: root dry weight (g)

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-20,98*	2,195	,000	-26,20	-15,77
	3	-13,90*	2,195	,000	-19,12	-8,69
2	1	20,98*	2,195	,000	15,77	26,20
	3	7,08*	2,195	,005	1,86	12,30
3	1	13,90*	2,195	,000	8,69	19,12
	2	-7,08*	2,195	,005	-12,30	-1,86

Based on observed means.

The error term is Mean Square(Error) = 96,401.

*. The mean difference is significant at the 0,05 level.

Table IX shows the significant variances on root dry weight (g) between the different treatments. 1-.(oil), 2.-(control) and 3.- (salt). Meaning that the oil pool does not permit the development from the root system growth as same as in the 2.-(control) pool and 3.-(salt) pool.

The table X represents stem diameter (cm) differences between treatments.

Multiple Comparisons

Dependent Variable: Stem diameter (cm)

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-,6714	,45726	,310	-1,7580	,4153
	3	-1,2489*	,45726	,020	-2,3356	-,1622
2	1	,6714	,45726	,310	-,4153	1,7580
	3	-,5775	,45726	,419	-1,6642	,5091
3	1	1,2489*	,45726	,020	,1622	2,3356
	2	,5775	,45726	,419	-,5091	1,6642

Based on observed means.

The error term is Mean Square(Error) = 4,182.

*. The mean difference is significant at the 0,05 level.

The table X represents there were significant variables on the stem diameter in (cm) on the treatments 1-. (oil), and 3.- (salt), where it did not let develop the same diameter comparing to 2.- (control pool) .

Table XI demonstrates the results on multiple comparisons on stem dry weight (g) between treatments (SPSS, 2013).

Multiple Comparisons

Dependent Variable: stem dry weight (g)

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-29,120000*	4,3789814	,000	-39,526466	-18,713534
	3	-19,851250*	4,3789814	,000	-30,257716	-9,444784
2	1	29,120000*	4,3789814	,000	18,713534	39,526466
	3	9,268750	4,3789814	,091	-1,137716	19,675216
3	1	19,851250*	4,3789814	,000	9,444784	30,257716
	2	-9,268750	4,3789814	,091	-19,675216	1,137716

Based on observed means.

The error term is Mean Square(Error) = 383,510.

*. The mean difference is significant at the 0,05 level.

The variable analysis on table XI represents the differences on stem dry weight (g) among the different treatments. 1-.(oil), 2.-(control) and 3.- (salt). This significantive changes represents the evolution which the results on total dry weight is higher on the treatments 2.-(control) and 3.-(salt) compare to the lesser growth on the treatment 1.-(oil) on the total dry weight analysis.

Table XII shows the results on multiple comparisons on total branch dry weight (g) between treatments (SPSS, 2013).

Multiple Comparisons

Dependent Variable: total branch dry weight (g)

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-12,293750*	1,3687429	,000	-15,546510	-9,040990
	3	-9,595000*	1,3687429	,000	-12,847760	-6,342240
2	1	12,293750*	1,3687429	,000	9,040990	15,546510
	3	2,698750	1,3687429	,124	-,554010	5,951510
3	1	9,595000*	1,3687429	,000	6,342240	12,847760
	2	-2,698750	1,3687429	,124	-5,951510	,554010

Based on observed means.

The error term is Mean Square(Error) = 37,469.

*. The mean difference is significant at the 0,05 level.

Table XII correspond to the variable analysis on the total branch dry weight (g) which has differences among the treatment number 1.- (oil). The results showed that the weight on the treatments 2.- (control) and 3.-(salt) the hybrid and non hybrid clones weight after been dryed were higher than in pool treatment 1.- (oil). By this the results showed that in pools 1.- (oil) has not develop the same weight on the total dry branches (g) than in the 3.- (salted) pools or 2.- (control pools).

Table XIII represents the results on multiple comparisons on stem + branch dry weight (g) between treatments (SPSS, 2013).

Multiple Comparisons

Dependent Variable: Stem+Branch dry weight (g)

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-41,413750*	5,2525606	,000	-53,896241	-28,931259
	3	-29,446250*	5,2525606	,000	-41,928741	-16,963759
2	1	41,413750*	5,2525606	,000	28,931259	53,896241
	3	11,967500	5,2525606	,063	-,514991	24,449991
3	1	29,446250*	5,2525606	,000	16,963759	41,928741
	2	-11,967500	5,2525606	,063	-24,449991	,514991

Based on observed means.

The error term is Mean Square(Error) = 551,788.

*. The mean difference is significant at the 0,05 level.

Table XII correspond to the variable analysis on Stem + Branch dry weight (g) which has differences among the treatment number 1.- (oil). The results showed that the weight on the treatments 2.- (control) and 3.-(salt) the hybrid and non hybrid clones weight after been dryed were higher than in pool treatment 1.- (oil). By this the results showed that in pools 1.- (oil) has not develop Stem + Branch dry weight (g) similar than in the 3.- (salted) pools or 2.- (control pools).

Discussion

This study was settled to understand the relation between the hybrid aspen (*P. tremula L. x P. tremuloides Michx.*) and the native aspen (*P. tremula*) testing adaptability and comparing the growth on the different scenarios where two pollutants were added. These spread on different pools with (soil taken from a real spill of a fuel tanker that felt down in a ridge area) PAHs and salt (natrium chloride). One approach was to recreated climate change (inside greenhouse) and facing problems as high contents of salt i.e. due to the intensive agriculture from other regions of the world. The analysis results on adaptability and development from the species (*P. tremula L. x P. tremuloides Michx.*) and (*P. tremula*) brought new possibilities to understand the phenology of the species to cope on different conditions and the reaction to these environments. As well the possible spectrum to introduce bioremediation and the utilization from biomass to generate energy, pulp fibre for paper industry or just esthetical proposes (Soudek et al., 2004). These ecosystem services expressed as; phytoextraction, phytotransformation, phyto-stimulation or (plant assisted), phytostabilization and phytovolatization (UNIDO, 2008). In general phytoremediation is conducted by solar induction on the specie as a systematic extraction of pollutants, from soil, water and air (Doty & Strand, 2008). The development from the species on the polluted pools were positive. By this means the out coming for possibilities to develop a future methodology that can clean up sites with similar characteristics. Expecting that the species could develop: stem height, broad root system, stem diameter, multiple branches and foliage. This to the results obtained on the experiment with a direct correlation with the proportionally amount of pollutants including biotic and abiotic factors in each possible case.

The results represented the highest growth on certain clones in the pools of control and salted. First hypothesis: was that the experiment settings were not accurate on the procedure on planting the trees or that the membrane broke where the trees were planted. Second hypothesis: could be that the root system reached the nutrients outside the pool and therefor the growth. Third hypothesis is about the observations conducted on summer periods that there were some ants nests under certain trees, where mutualism express the role on sharing specifics benefits among species and are pervasive, most of the times ecologically prevalent and essentially transcendental at all levels of biological organization (Boucher et al., 1982). The connexion between ants-aphids may not conform mutualistic unions. The aphid offer ants with carbohydrates as so named honeydew, this bio product is rich aliment of plant sap (Way, 1963). Honey dew structure is a relevant intervene ant–*homopteran* mutualisms due to the trisaccharide melezitose which is highly valuable in this synergy. The honeydew of three *Chaitophorus* aphids (*Homoptera*: *Aphididae*) breeds on two species of *Populus* (*Salicaceae*) and often is tended by ants. These aphids *Chaitophorus populeti*, *C. populialbae*, are higher on content of melezitose rather than *C. tremulae* and the first two tend to be reared on *Populus tremula* than on *P. alba* (Fischer &

Shingleton, 2001). Although the adaptability on the oil and salted was high and development differs between clones and pools. Being clear that the lesser growth was on oil, so there for possible increase of clones should be planted to provide an efficient biodegradation of the compounds as to the response for the extraction of the same.

Conclusion

The adaptability of the (*P. tremula L. x P. tremuloides Michx.*) and the native *P. tremula* was high. Including the growth on the different pools and clones, where on control treatments was the highest followed by salted treatments and the lesser growth occurred on the oil treatments. This is the result of the phenology of the hybrids and non-hybrids clones and the capability to adapt and development under the specifics of biotic and abiotic factors. There was only influence to those pools inside the greenhouse analysing the possible behaviours or effects on the phenology of the clones in case of climate change including the plasticity of the same under polluted soils with the PHAs and natrium chloride. The outside pools response were positive to the natural climatological conditions. This as well represents a positive growth and adaptability to the before mentioned stressors on soil. Another remarkable aspect was the possibility of the mutualism relation that could be developed between the poplars and the ecological factors on the area. This could be a clue for the development of the species or one of multiple possible factors which altered the faster growth and height of some trees taking in consideration the positive inputs for bioremediation by phytoremediation. However further research should be conducted to understand the behaviour of the different clones and the uptake from PAHs and chloride natrium soils. This to understand the ecotoxicology kinetics of the species and the proper use to uptake the pollutants on a contaminated site.

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Appendix

Methods and Materials

Table of all the data collected utilized for the statistical analysis.

Location	treatment	pool	row	clone	n_plant	Running number	Harvest date	Processing date (wet measurements)	planting depth (cm)	total stem height (cm) (from root to last bud)	Stem diameter (mm)	Stem diameter (cm)	root wet weight (g)	stem with branches wet weight (g)	stem without branches wet weight (g)	total branch wet weight (g)	root dry weight (g)	stem dry weight (g)	total branch dry weight (g)	Stem+Branch dry weight (g)	weight loss root (g)	Weight loss stem without branches (g)	Weight loss branches (g)	Nr of the day of we measured dry weight	Nr of the day we put them in the oven	Number of days the trees stayed in the oven	note	note (depth of roots taken starting from the main root)	Number of roots in the planting depth	between or at	value 1 (cm)	Value 2 (cm)	Value 3 (cm)
inside	oil	2	6	23	2	86	298	305	7.5	62	9.40	0.94	24.15	13.15	11.65	1.4	6.25	5.95	0.80	6.75	17.9	5.7	0.6	339	321	18							
inside	oil	2	7	9	2	87	298	305	5	37	5.02	0.502	2.15	3	2.9	0.1	0.6	1.85	0.00	1.85	1.55	1.05	0.1	339	321	18		3 roots at 1,5	3	at	1.5		
inside	oil	2	14	14	2	94	298	305	3.5	56.2	7.68	0.768	15.3	8.05	7.6	0.4	5.2	3.90	0.20	4.10	10.1	3.7	0.2	310	294	16		4 roots between 2 and 3 cm	4	between	2	3	
inside	oil	2	15	KHL	2	95	298	305	5.5	54	10.80	1.08	61.7	14.05	12.45	1.65	14.25	6.40	0.85	7.25	47.45	6.05	0.8	339	321	18							
inside	oil	2	17	27	2	97	298	305	3	59	9.34	0.934	18.4	10.45	9.65	0.75	5.2	4.85	0.45	5.30	13.2	4.8	0.3	339	321	18		1 root found at 4.5 cm above the start of the root area	1	at	4.5		
inside	control	3	1	KHL	2	161	298	300	5.9	80	1.511	1.511	46.15	62.45	39.35	23.05	19.4	22.25	12.65	34.90	26.75	17.1	10.4	310	294	16							
inside	control	3	8	27	2	168	298	300	6.9	154.9	1.934	1.934	96.6	130.05	84.7	45.25	37.4	44.30	24.45	68.75	59.2	40.4	20.8	310	294	16							
inside	control	3	11	14	2	171	298	299	4.2	220.4	1.852	1.852	107	215.75	172.7	42.25	42.5	89.15	25.30	114.45	64.5	83.55	16.95	310	294	16	no branch no stem						
inside	control	3	15	9	2	175	298	300	4.2	115.4	1.577	1.577	85.3	83.25	54.45	28.55	34.35	31.10	15.25	46.35	50.95	23.35	13.3	310	294	16							
inside	control	3	20	23	2	180	298	300	2.3	156.4	1.639	1.639	66	90.8	65.1	25.7	25.55	34.65	13.95	48.60	40.45	30.45	11.75	310	294	16							
inside	salt	4	1	23	2	241	298	299	4.8	93.7	1.483	1.483	59.65	41.65	15.95	25.65	17.8	8.50	13.35	21.85	41.85	7.45	12.3	339	321	18	nch but it s not dete	2 roots at 1 And 2,5 cm	2	at	1	2.5	
inside	salt	4	7	14	2	247	298	298	3	343.5	2.111	2.111	244.45	443.9	396	47.75	73.25	194.50	24.95	219.45	171.2	201.5	22.8	339	321	18		2roots at 1,4 and 1,6 cm	2		1.4		
inside	salt	4	13	14	2	253	298	299	10.7	89.8	1.855	1.855	72.9	119.6	60.1	59.4	27.15	31.95	32.00	63.95	45.75	28.15	27.4	310	294	16		0	0				
inside	salt	4	16	23	2	256	298	298	6.8	114.4	1.329	1.329	35.95	34.25	23.85	9.8	10.15	12.80	5.35	18.15	25.8	11.05	4.45	339	321	18							
outside	oil	6	6	23	2	406	293	294	3.9	67.5	1.155	1.155	172.5	27.2	22.35	4.8	21.8	11.90	2.60	14.50	150.7	10.45	2.2	321	310	11							
outside	oil	6	11	9	2	411	293	294	6	52.5	0.851	0.851	20.25	7	6.65	0.35	4.1	3.40	0.20	3.60	16.15	3.25	0.15	321	310	11							
outside	oil	6	14	27	2	414	293	294	7.5	60.4	0.94	0.94	31.85	11.2	9.6	1.6	7.9	5.20	0.90	6.10	23.95	4.4	0.7	321	310	11		2 roots between 1,7 and 2 cm	2	between	1.7	2	
outside	oil	6	15	14	2	415	293	294	2.1	22.2	0.783	0.783	17.4	5.25	4.55	0.65	4.1	2.30	0.40	2.70	13.3	2.25	0.25	321	310	11							
outside	oil	6	16	KHL	2	416	293	294	4.9	52.4	1.521	1.521	42.8	17.1	15.4	1.8	10.55	8.10	1.20	9.30	32.25	7.3	0.6	321	310	11							
outside	control	7	1	14	2	481	292	293	3	163.5	1.8	1.8	125.2	185.5	132.4	53	40.7	69.00	27.55	96.55	84.5	63.4	25.45	310	294	16							
outside	control	7	4	KHL	2	484	292	293	3.7	113.5	1.92	1.92	92.9	90.8	54.1	36.6	35.4	31.75	21.00	52.75	57.5	22.35	15.6	321	310	11							
outside	control	7	9	9	2	489	292	292	2.5	119.5	1.66	1.66	61.95	90.5	65.65	24.5	22.55	35.15	12.70	47.85	39.4	30.5	11.8	321	310	11		3 ROOTS between 1,5 and 3	3	between	1.5	3	
outside	control	7	15	23	2	495	292	292	3.2	112	1.467	1.467	53.15	75.95	51.3	24.45	25.05	27.85	12.75	40.60	28.1	23.45	11.7	321	310	11							
outside	control	7	19	27	2	499	292	293	6.5	145	1.814	1.814	109.4	188.9	120.2	68.3	38.3	58.70	34.70	93.40	71.1	61.5	33.6	321	310	11							
outside	salt	8	2	14	2	562	293	297	1.7	96	1.598	1.598	140.6	95.3	61.75	33.3	39.45	31.80	16.90	48.70	101.15	44.85	1.5	339	321	18		none					
outside	salt	8	9	23	2	569	293	294	4	132	1.751	1.751	109.6	139.5	101.3	37.65	37.4	52.50	18.60	71.10	72.2	48.8	19.05	321	310	11							
outside	salt	8	12	23	2	572	293	297	9.3	92.5	16.56	16.56	70.4	89.25	56.4	32.8	22.9	28.80	15.80	44.60	47.5	40.6	4	339	321	18		none					
outside	salt	8	18	14	2	578	293	294	11	134.5	19.62	19.62	172.15	224.1	144.75	79.15	72.05	75.65	42.05	117.70	100.1	69.1	37.1	310	294	16		none					

Results

Univariate Analysis of Variance

The next table represents the statically results conducted with the program SPSS on multiple variations analysis between treatment and location.

Univariate Analysis of Variance Tests Between Subjects Effects			
Dependent variable	Source	F	Sig.
Total stem height (cm) From root to last bud	Treat	45,526	,000
	Location	14,622	,000
	Treat*Location	2,67	,077
Stem diameter (cm)	Treat	3,332	0,39
	Location	2,284	,134
	Treat*Location	1,109	,334
Root dry weight (g)	Treat	35,786	,000
	Location	3,162	,078
	Treat*Location	,472	,653
Stem dry weight (g)	Treat	20,070	,000
	Location	0,80	,778
	Treat*Location	,669	,514
Total branch dry weight (g)	Treat	32,114	,000
	Location	3,085	,082
	Treat*Location	1,072	,346
Stem +Branch dry weight (g)	Treat	26,519	,000
	Location	0,66	,797
	Treat*Location	,649	,524
a. R Squared=,493 (Adjusted R Squared=,471)			

Multiple comparisons

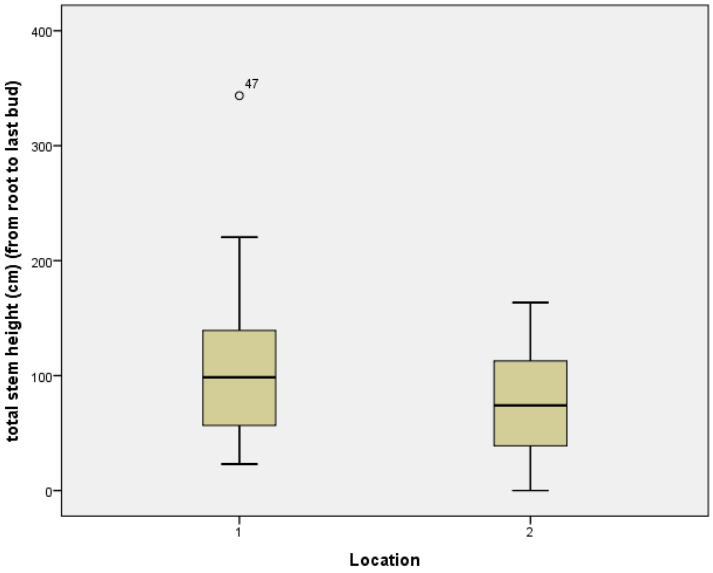
The next are tables and graphs belong to the results on multiple comparisons conducted on the different species on the treatments 1 (oil), 2 (control), 3 (salt). The analysis was done without separating locations inside and outside. The statistical analysis was done with the program IBM SPSS Statistics Inc. 2013.

The following figures represents the interactions between the pool treatments and the hybrid clones and native species.

Total stem height (cm) (from root to last bud)

Multiple Comparisons							
Tukey HSD							
Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
total stem height (cm) (from root to last bud)	1	2	-80,705*	9,263	,000	-102,69	-58,72
		3	-55,715*	9,263	,000	-77,70	-33,73
	2	1	80,705*	9,263	,000	58,72	102,69
		3	24,990*	9,263	,022	3,00	46,98
	3	1	55,715*	9,263	,000	33,73	77,70
		2	-24,990*	9,263	,022	-46,98	-3,00

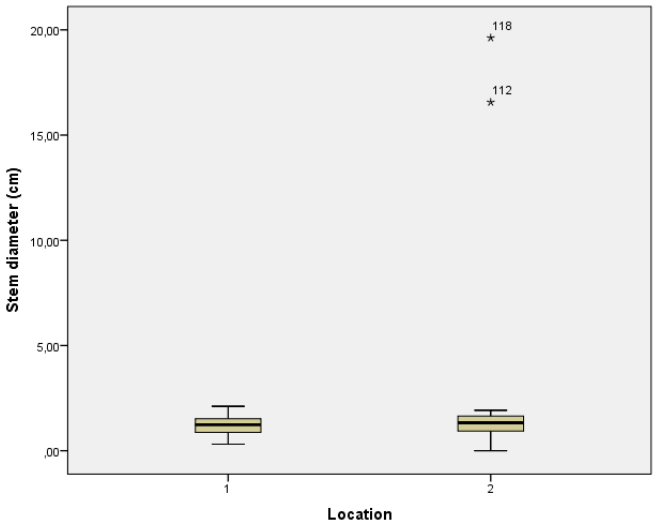
*. The mean difference is significant at the 0.05 level



Stem diameter (cm)

Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Stem diameter (cm)	1	2	-,67137	,48736	,356	-1,8283	,4856
		3	-1,24890*	,48736	,031	-2,4058	-,0920
	2	1	,67137	,48736	,356	-,4856	1,8283
		3	-,57753	,48736	,464	-1,7345	,5794
	3	1	1,24890*	,48736	,031	,0920	2,4058
		2	,57753	,48736	,464	-,5794	1,7345

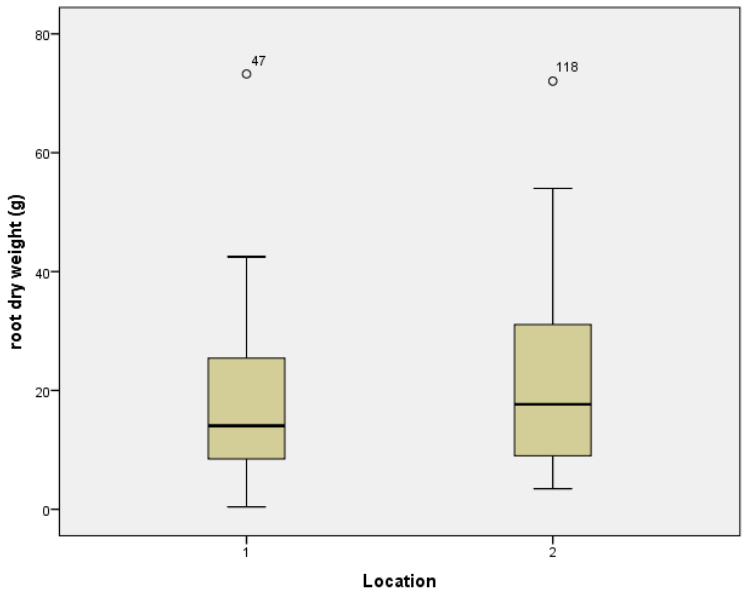
*. The mean difference is significant at the 0.05 level



Root dry weight (g)

Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
root dry weight (g)	1	2	-20,984*	2,535	,000	-27,00	-14,97
		3	-13,902*	2,535	,000	-19,92	-7,89
	2	1	20,984*	2,535	,000	14,97	27,00
		3	7,081*	2,535	,017	1,06	13,10
	3	1	13,902*	2,535	,000	7,89	19,92
		2	-7,081*	2,535	,017	-13,10	-1,06

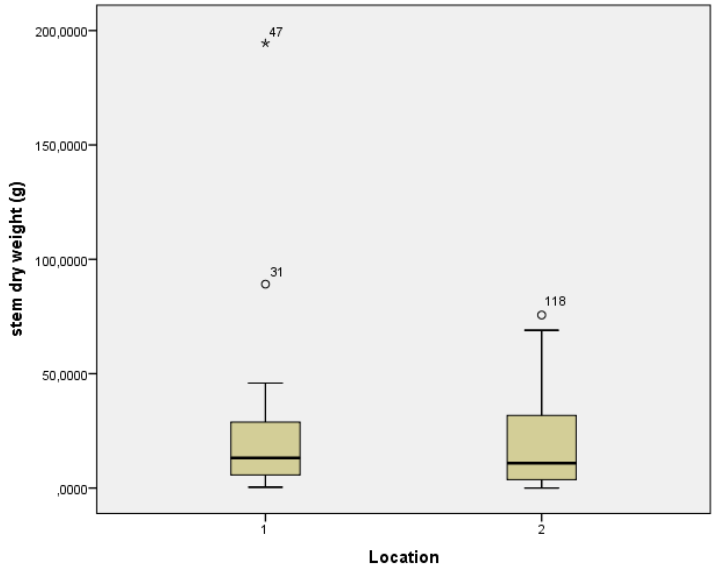
*. The mean difference is significant at the 0.05 level



Dry stem weight (g)

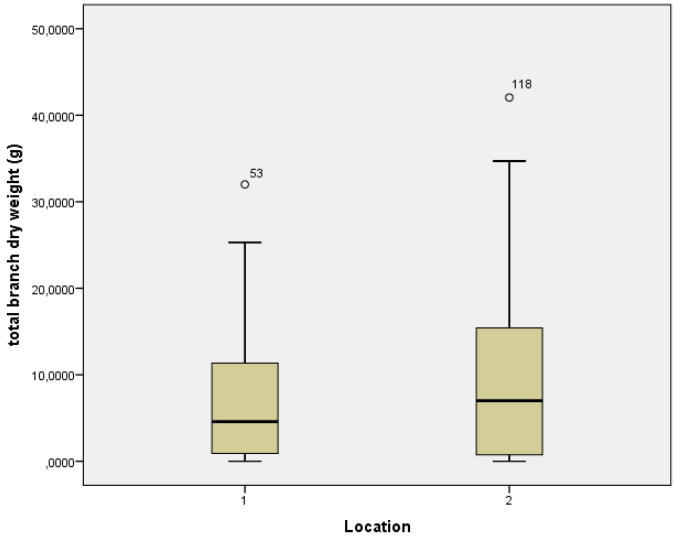
Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
dry stem weight (g)	1	2	-29,1200000*	4,6644468	,000	-40,192983	-18,047017
		3	-19,8512500*	4,6644468	,000	-30,924233	-8,778267
	2	1	29,1200000*	4,6644468	,000	18,047017	40,192983
		3	9,2687500	4,6644468	,120	-1,804233	20,341733
	3	1	19,8512500*	4,6644468	,000	8,778267	30,924233
		2	-9,2687500	4,6644468	,120	-20,341733	1,804233

*. The mean difference is significant at the 0.05 level



Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
total branch dry weight (g)	1	2	-12,2937500*	1,6277183	,000	-16,157809	-8,429691
		3	-9,5950000*	1,6277183	,000	-13,459059	-5,730941
	2	1	12,2937500*	1,6277183	,000	8,429691	16,157809
		3	2,6987500	1,6277183	,226	-1,165309	6,562809
	3	1	9,5950000*	1,6277183	,000	5,730941	13,459059
		2	-2,6987500	1,6277183	,226	-6,562809	1,165309

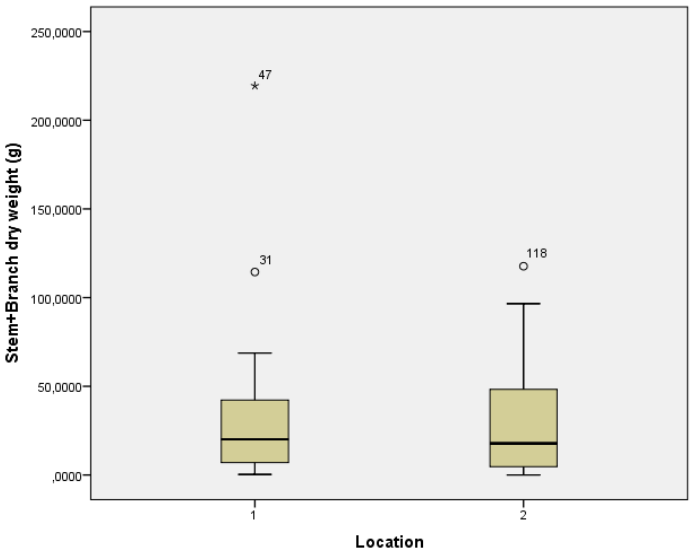
*. The mean difference is significant at the 0.05 level



Stem+Branch dry weight (g)

Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Stem+Branch dry weight (g)	1	2	-41,4137500*	5,8118855	,000	-55,210651	-27,616849
		3	-29,4462500*	5,8118855	,000	-43,243151	-15,649349
	2	1	41,4137500*	5,8118855	,000	27,616849	55,210651
		3	11,9675000	5,8118855	,103	-1,829401	25,764401
	3	1	29,4462500*	5,8118855	,000	15,649349	43,243151
		2	-11,9675000	5,8118855	,103	-25,764401	1,829401

*. The mean difference is significant at the 0.05 level


250,0000
200,0000
150,0000
100,0000
50,0000
,0000
Stem+Branch dry weight (g)
47
31
118
1
2
Location