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Evaluation of survival, growth, and phytoremediation potential of *Populus* and *Salix* seedlings grown in polluted soils

Mir Md Abdus Salam

School of Forest Sciences
Faculty of Science, Forestry and Technology
University of Eastern Finland

Doctoral dissertation

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Author: Mir Md Abdus Salam

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Dissertation supervisors:
Professor (Emeritus) Paavo Pelkonen
School of Forest Sciences, University of Eastern Finland, Finland

Professor Heli Peltola
School of Forest Sciences, University of Eastern Finland, Finland

Pre-examiners:
Professor Hardi Tullus
Institute of Forestry and Engineering, Estonian University of Life Sciences, Tartu, Estonia

Professor Mirosław Mleczek
Faculty of Forestry and Wood Technology, Department of Chemistry, Poznań University of Life Sciences, Poznań, Poland.

Opponent:
Research Professor Hannu Ilvesniemi
Natural Resources Institute Finland (LUKE), Helsinki, Finland

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ABSTRACT

The aim of this thesis was to evaluate the survival, growth, and phytoremediation potential of *Populus* and *Salix* seedlings grown in polluted soils. More specifically, the following topics were studied: (1) the survival and growth of two European aspen clones and four hybrid aspen clones grown in control soil (pristine), old creosote soil polluted with hydrocarbons, and pristine soil spiked with fresh diesel oil at three different planting densities in a greenhouse over two growing seasons (**Article I**); (2) the survival, growth, and hydrocarbon removal of three European aspen clones and seven hybrid aspen clones grown in hydrocarbon-contaminated soil (including polycyclic aromatic hydrocarbons (PAHs) and total petroleum hydrocarbons (TPHs)) under field conditions over 4 years (**Article II**); and (3) the growth and metal accumulation ability of *Salix psammophila* seedlings with bamboo biochar (BBC) amendment at ratios of 0–7% in soils heavily contaminated by Cd and Zn in a pot experiment over 180 days (**Article III**).

In study I (**Article I**), the survival rates of European aspen and hybrid aspen clone seedlings were 70–100% in control soil, 99% in the old creosote-contaminated soil, and 22–59% in the diesel-contaminated soil across all planting densities. The heights of aspen seedlings were 5–44% and 9–38% lower and the stem dry biomass was 9–93% and 34–63% lower in diesel-contaminated and creosote-contaminated soils, respectively, compared to the control. Low plant density increased survival rates and growth compared to higher density treatments. Of all the clones, hybrid aspen clones 14 and 291 and European aspen clone R3 showed reasonable survival and growth across all treatments. Soil treatment, planting density, and clone type significantly affected survival rate, height, and stem dry biomass ($p < 0.05$).

In study II (**Article II**), the highest survival rates in old creosote-contaminated soils were in clone 291 (72%) among hybrid aspen clones and clone R3 (70%) among European aspen clones. Hybrid aspen clones 14 and 34 had 16–211% greater heights than other hybrid aspen clones. The height of European aspen clone R3 was also 25–35% greater than that of other European aspen clones. However, clone type did not significantly affect seedling survival or height ($p > 0.05$). Among hybrid aspen clones, clone 134 had the largest hydrocarbon removal at a depth of 5–10 cm and clone 191 at a depth of 10–50 cm. Clone 14 also showed potential for removing hydrocarbons at both soil depths. In European aspen clones, clone R2 had the highest hydrocarbon removal at both soil depths. However, all clones showed an ability to remove total PAHs and TPHs from the soil (but $p < 0.05$ only at a soil depth of 5–10 cm). The reduction in hydrocarbon levels in the soil was more prominent at a soil depth of 5–10 cm than at a depth of 10–50 cm. Based on studies I and II, European aspen and hybrid aspen clones can be considered candidates for the remediation of soils polluted with PAHs and TPHs.

In study III (**Article III**), BBC ratios of 1% and 5% resulted in only slight decreases in characteristics, especially height (0.6–1.3%) but also total dry biomass (2–10%), of *S. psammophila* seedlings compared to the control, whereas BBC 3% increased these measurements slightly (2% increase). BBC 7% reduced the height (16%) and total dry biomass (26%) of seedlings compared to the control. BBC amendment increased the accumulation of Cu, Cd, and Zn in different plant tissues, especially Cd and Zn accumulation (23–30% and 13–24%, respectively), in the BBC 3% treatment compared to the control. Based on these findings, *S. psammophila* with BBC amendment can be considered a candidate for phytoremediation. However, metal accumulation in the roots, stems, and leaves was not significantly affected by the BBC 1–7% treatments ($p > 0.05$), except for Pb accumulation in the roots and Cu accumulation in the stem ($p < 0.05$).

Overall, hybrid aspen, European aspen, and *S. psammophila* seedlings showed reasonable survival and growth, photosynthetic activity, efficient hydrocarbon removal from soil and metal accumulation ability both under greenhouse conditions and in a field experiment. Therefore, these species could be used to depollute areas affected by a range of hydrocarbons or Cd and Zn. However, future research should be conducted in the field to verify the abilities of hybrid and European aspens and *S. psammophila* to remediate soil contaminated by hydrocarbons, Cd, or Zn, and such studies should also use different planting densities and soil amendments over longer periods.

Keywords: soil, pollution, bamboo biochar, hydrocarbons, metals, *Populus*, *Salix*

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Joensuu, January 2024

Mir Md Abdus Salam

LIST OF ORIGINAL PUBLICATIONS

I. Salam MMA, Ruhui W, Sinkkonen A, Pappinen A, Pulkkinen P (2022) Effects of contaminated soil on the survival and growth performance of European (*Populus tremula* L.) and hybrid aspen (*Populus tremula* L. × *Populus tremuloides* Michx.) clones based on stand density. Plants 11(15):1970. https://doi.org/10.3390/plants11151970

II. Salam MMA, Mohsin M, Rasheed F, Ramzan M, Zafar Z, Pulkkinen P (2020) Assessment of European and hybrid aspen clones efficiency based on height growth and removal percentage of petroleum hydrocarbons—a field trial. Environmental Science and Pollution Research 27:45555–45567. https://doi.org/10.1007/s11356-020-10453-4

III. Li X, Xiao J, Salam MMA, Ma C, Chen G (2021) Impacts of bamboo biochar on the phytoremediation potential of *Salix psammophila* grown in multi-metals contaminated soil. International Journal of Phytoremediation 23(4):387–399. https://doi.org/10.1080/15226514.2020.1816893

Reprints of **Articles I–III** are published with the kind permission of the journals concerned.

Author's contribution

Mir Md Abdus Salam was responsible for the data analysis and writing and preparation of the original drafts of **Articles I–III**. Pertti Pulkkinen was responsible for resources, planting materials, funding acquisition, conceptualization, methodology, and supervision and participated in the writing (review and editing) of **Articles I** and **II**. Aki Sinkkonen contributed to conceptualization, methodology, and writing (review and editing) and Wen Ruhui to data curation of **Article I**. Ari Pappinen contributed to the writing (review and editing) of **Article I**. Muhammad Mohsin participated in writing and original draft preparation and Fahad Rasheed, Zikria Zafar, and Muhammad Ramzan in the writing (review and editing) of **Article II**. The first author of **Article III,** Xiaogang Li, was responsible for conducting experiments and data collection and contributed to the data analysis, writing, and original draft preparation of **Article III**. Jiang Xiao contributed to the data analysis of **Article III**. Chuanxin Ma contributed to the writing (review and editing) of **Article III**. Guangcai Chen was responsible for supervising, planning, and designing experiments; acquiring funding; and participating in the writing (review and editing) of **Article III**.

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ABBREVIATIONS

P. tremula	*Populus tremula*
P. tremuloides	*Populus tremuloides*
P. deltoides	*Populus deltoides*
P. wettsteinii	*Populus wettsteinii*
A. pyrifolia	*Acacia pyrifolia*
A. stellaticeps	*Acacia stellaticeps*
Q. pagoda	*Quercus pagoda*
Q. texana	*Quercus texana*
S. jiangsuensis	*Salix jiangsuensis*
S. integra	*Salix integra*
S. schwerinii	*Salix schwerinii*
S. triandra	*Salix triandra*
S. rubens	*Salix rubens*
S. psammophila	*Salix psammophila*
S. alba	*Salix alba*
PHC	Petroleum hydrocarbon
PAH	Polycyclic aromatic hydrocarbon
TPH	Total petroleum hydrocarbon
BETX	Benzene, toluene, ethylbenzene and xylenes
PCE	Perchloroethylene
EDTA	Ethylenediaminetetraacetic acid
EDDS	Ethylenediamine-N,N'-disuccinic acid
N	Nitrogen
P	Phosphorus
K	Potassium
Cu	Copper
Cd	Cadmium
Zn	Zinc
Pb	Lead
Cr	Chromium
Ni	Nickel
CO_2	Carbon dioxide
$ZnSO_4$	Zinc sulfate
C/N	Carbon/nitrogen
BBC	Bamboo biochar
CAPF	Contaminated abandoned paddy field
TF	Translocation factor
BCF	Bioconcentration factor
Pn	Photosynthetic rate
Tr	Transpiration rate
US EPA	United states environmental protection agency
USDA	United states department of agriculture
USA	The United States of America
NY	New York
SPSS	Statistical package for the social sciences

DPS	Data processing system
SD	Standard deviation

1 INTRODUCTION

1.1 Background of the study

Soil pollution is a major environmental problem globally. Metals like Zn, Cu, Pb, Cd, and Ni and petroleum hydrocarbons (PHCs), such as benzene, toluene, naphthalene, acenaphthene, acenaphthylene fluorene, and phenanthrene, are primary pollutants that contaminate the soil (Ali and Khan 2018; Jin et al. 2018; Saxena et al. 2020). The United States Environmental Protection Agency (US EPA) has listed metals and PHCs as priority environmental pollutants (Siles and Margesin 2018; Manoj et al. 2020). PHCs are considered main pollutants due to their intractable, resistant, and toxic nature, which can have carcinogenic and mutagenic effects (Siles and Margesin 2018; Guirado et al. 2021). Contamination is caused by land-use changes, industrial discharge, mining activities, disposal of landfill and creosote waste products, sewage sludge applications, oil and chemical accidents during transportation, and a range of other factors (Ali et al. 2013; Rehman et al. 2019; Singh et al. 2019; Yadav et al. 2021). Environmental degradation, loss of trees and habitats, ecosystem disturbances, and a reduction in soil and groundwater quality are all consequences of contamination (Marinescu et al. 2010; Shakoor et al. 2013; Ahmad et al. 2020; Hoang et al. 2020). Contamination can also threaten human health (Lenart-Boroń and Wolny-Koładka 2015; Pan et al. 2018; Ahmad et al. 2020). Therefore, it is crucial to find environmentally friendly and cost-efficient solutions to overcome these problems and to remediate soil (Hoang et al. 2021).

Remediation methods for contaminated soils, such excavation, air and vapor extraction, and chemical treatment, are costly and disruptive (Nadim 2000; Pinto et al. 2016; Khalid et al. 2017; Gitipour et al. 2018; Patel et al. 2020). Recently, phytoremediation, or planting woody species as an alternative soil remediation method for contaminated soils, has been suggested (Haynes 2009; Pandey et al. 2009; Amin et al. 2019). This method uses plants to remove pollutants from soil and water. Its potential has been extensively studied, and it is effective at degrading, extracting, filtrating, volatilizing, and holding pollutants (Fig. 1) (Thangavel and Subhuram 2004; Fasani et al. 2012; Manara et al. 2012; Fasani et al. 2018; Gitipour et al. 2018; Patel et al. 2020; Yadav et al. 2021).

Phytoremediation is an environmentally friendly and efficient solution for cleaning up contaminated sites. Compared to traditional technologies, it does not have harmful impacts on the biological activity, structure, or quality of soil (Pandey et al. 2016; Chi et al. 2017; Liang and Wang 2017; Gitipour et al. 2018; Ziegler-Devin et al. 2019; Patel et al. 2020; Yang et al. 2021). It has been suggested as a sustainable remedial option for soils contaminated with organic and inorganic pollutants (Cameselle et al. 2013; Chirakkara and Reddy 2015a, 2015b; Chirakkara et al. 2016). Additionally, the efficiency of phytoremediation can be improved either by enhancing the ability of plants to take up pollutants or by ameliorating the soil to accelerate the mobility of contaminants (Ahmad et al. 2014; Zeng et al. 2015; Khalid et al. 2017).

The feasibility of combining phytoremediation and biomass production in contaminated soils has also been explored. For example, fast-growing woody plants like *Populus* and *Salix* have been found to be relatively tolerant of pollutant toxicity and capable of accumulating metals and removing PHCs while still producing large amounts of biomass (Mosseler et al. 2014a; Xue et al. 2015; Gkorezis et al. 2016; Zalesny et al. 2016; Masarovičová and Kráľová 2018; Xu et al. 2018; Touati et al. 2019; Wani et al. 2020; Eadha 2022). *Populus* and *Salix* species can grow in polluted soils of poor quality, such as in abandoned farms and old mining sites (e.g. Mosseler et al. 2014b, 2014c, 2017; Wani et al. 2020). *Populus* and *Salix* plants can also tolerate relatively low nutrient levels, unbalanced pH levels, and increased salt levels in soil (Mosseler et al. 2014b, 2014c; Castaño-Díaz et al. 2017). Their use offers both environmental and economic benefits, including improvement of soil quality, ecosystem restoration, and sustainable bioenergy production (Volk et al. 2004; Christersson 2011; González-García et al. 2012; Isebrands et al. 2014; Gkorezis et al. 2016; Asad et al. 2017; Ziegler-Devin et al. 2019; Hepner et al. 2021; Eadha 2022; Kaivapalu et al. 2023). For example, in Finland, it has been estimated that *Salix* biochar production from all marginal lands could offset 7.7% of yearly agricultural greenhouse gas emissions (Leppäkoski et al. 2021).

Recently, *Populus* spp. (e.g. European aspen) have been found to be efficacious in phytoremediation of contaminated sites in North America and eastern Europe (Eadha 2022). They may also help to decrease hotspots of specific contaminants to safe levels over extended time periods (Eadha 2022). For example, Lopez-Echartea et al. (2020) reported that *Populus* spp. can remove PHCs from sandy soils under arctic climate conditions. In many phytoremediation experiments, *Populus* spp. have been utilized to remediate sites polluted with hydrocarbons and metals (Burken and Schnoor 1997; Kelley et al. 2001; Palmroth et al. 2002; Weyens et al. 2009; Andreolli et al. 2013).

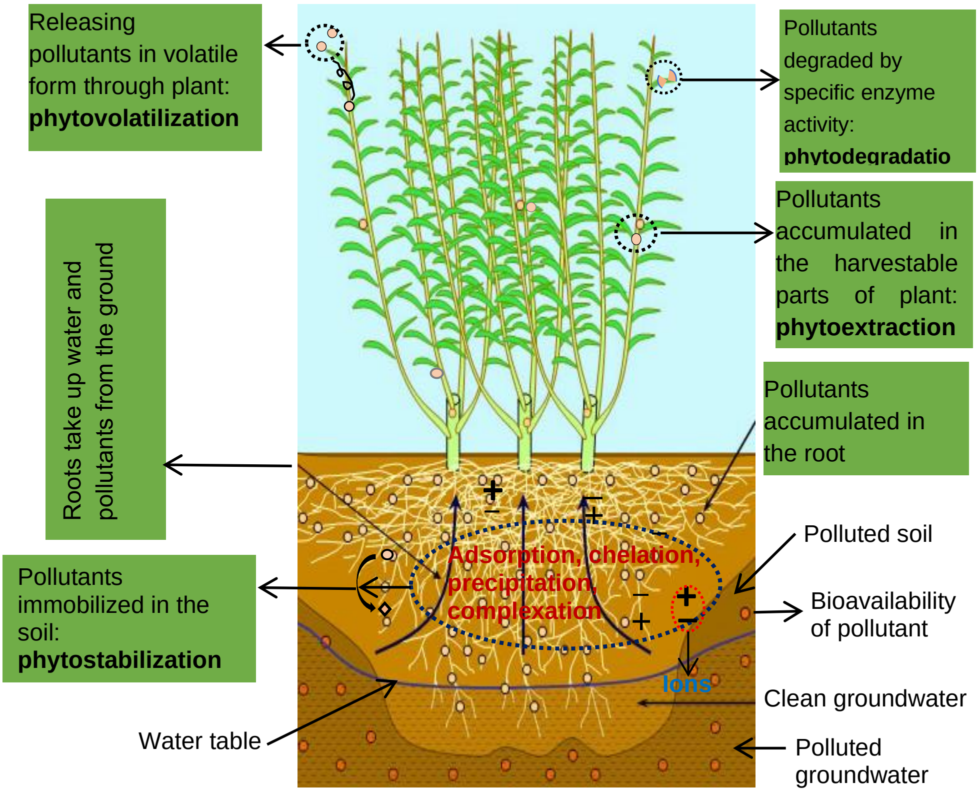


Figure 1. Schematic diagram of the different approaches used in phytoremediation. Adapted from Wang (2004).

Salix psammophila, a tree species found in northern and northwestern China, has also been found to be ideal for phytoremediation due to its abilities to withstand harsh conditions, grow quickly, resist drought, and regenerate well (Zhong et al. 2022; TPD 2023). *Salix* roots contain ligands of low molecular weight, such as phytochelatins and organic acids, that can transport pollutants and aid in their detoxification (Cieśliński et al. 1998; Parisová et al. 2013; Sun et al. 2013; Dresler et al. 2014; Gąsecka et al. 2019). *Salix* has been shown to be able to remove pollutants (Cd, Zn, Pb, and Cu) from soils, treat landfill leachate, and accumulate large amounts of metals in its tissues (Duggan 2005; Jensen et al. 2009; Mleczek et al. 2010; Ziegler-Devin et al. 2019). Additionally, the use of *S. alba* is recommended in plantations to reduce pollutant loads (Bajraktari et al. 2019).

The effectiveness of phytoremediation depends on the use of appropriate plant species (and genotypes) and environmental conditions (Ahmad et al. 2020; Ancona et al. 2021). Plants with high biomass production and pollutant accumulation potential are ideal (Meers et al. 2007; Mleczek et al. 2009; Karp et al. 2011). Different *Salix* cultivars have been found to have varying pollutant uptake efficiencies – some genotypes (e.g. clones) accumulate specific pollutants well while excluding others (Vyslouzilova et al. 2003; Yang et al. 2015b; Cao et al. 2018; Yang et al. 2019). Additionally, the quantities of pollutants that accumulate in the various plant organs have been shown to vary considerably among cultivars and treatments (Mleczek et al. 2010; Yang et al. 2015a, 2015b, 2019).

Efficient photosynthesis is crucial for the survival and growth of plants, as well as for phytoremediation (Na et al. 2014; Pilipović et al. 2019). Parameters such as chlorophyll fluorescence (Fv/Fm), CO_2 uptake, and transpiration rate are essential for assessing plant tolerance to pollutants and selecting suitable plants for phytoremediation (Marwood et al. 2001; Bramley-Alves et al. 2014a; Mohsin et al. 2019; Salam et al. 2019). Pollutant movement from roots to leaves is often facilitated by transpiration, which relies on water availability and soil moisture, which are crucial for photosynthesis and CO_2 assimilation (Cao et al. 2017, 2018; Salam et al. 2019). Stress caused by PHCs can be indicated by reduced photosynthetic efficiency (Nydahl et al. 2015). Thus, it is important to evaluate key photosynthetic parameters of plants in stressful environments.

Planting tree species at a suitable stand density is also vital for rehabilitating degraded and polluted soils (Lewis et al. 2022). Planting density affects the recovery of vegetation in disturbed soils, the soil availability of nutrients for plant growth, and soil erosion (Schoenholtz et al. 2000; Healey and Gara 2003; Lei et al. 2019; Lewis et al. 2022). In a recent pot experiment, high planting density increased the PHC removal rates of plants grown in contaminated soil (Lewis et al. 2022). However, the effects of different planting densities on plant growth and removal of pollutants in PHC-contaminated soils are still unclear.

Adding organic and inorganic chelates can improve pollutant uptake efficiency (Tangahu et al. 2011). Pollutant toxicity can result in decreased plant growth (Sharma and Dubey 2007). Organic fertilizers promote plant growth and biomass production, as they contain primary nutrients like N, P, and K that are needed by plants (Khan et al. 2018). Soil amendments like organic fertilizers, biochar, and biosolids enhance plant chlorophyll and protein synthesis, activate enzymes, and improve soil structure and quality. These processes support plant growth and affect the abilities of plants to remove pollutants from the soil (Meagher 2000; Halim et al. 2003; Luo et al. 2005; Medina et al. 2006; Evangelou et al. 2007; Kim et al. 2010; Mench et al. 2010; Hanus-Fajerska et al. 2012; Zand et al. 2020; Yan et al. 2020; Hoang et al. 2021).

Metals are immobilized and their bioavailability decreases when using a variety of amendments to aid plant establishment in contaminated soils. Biochar amendment is one of the most common methods for immobilizing metals in contaminated soils (Xiao et al. 2023). Wu et al. (2019) also reported increased interest in rehabilitating soils contaminated with metals using biochar produced from agricultural, forest-based, and other cheap raw materials. The use of biochar is interesting, as it has a large surface area, an effective functional group, a significant porosity, a high cation exchange capacity, and the abilities to retain nutrients and resist soil decomposition and degradation (Thomas et al. 2020; Xiao et al. 2020; Wen et al. 2021).

By improving the physicochemical and biological properties of soils, complexation, and precipitation mechanisms, biochar amendment can enhance soil fertility, plant productivity, and the removal of soil metals by plants (He et al. 2019). *Salix* phytoremediation experiments have found that various combinations of materials, including lime and organic matter (Fisher et al. 2006); citric acid and oxalic acid (Mitton et al. 2012); biochar (Nobert et al. 2016; Lebrun et al. 2018); lime and municipal biosolids composted with wood chips (Meiman et al. 2012); and compost, iron grit, and vegetable materials (Lebrun et al. 2020), have improved plant growth and photosynthetic parameters, reduced leaching of metals, and enhanced the mobility and bioavailability of metals and their uptake by the plants. Soil amendments can either make pollutants more available or bind them, but their effectiveness depends on various factors, such as the type and source of amendments, rate of application, and characteristics of plants and soil (Rakotonimaro et al. 2017; Schnackenberg et al. 2022). Plants can also affect the physical, chemical, and microbial features of soil (Bolan et al. 2014). Synthetic chelators like EDTA can persist in soil, causing metal leaching and harm to the environment (Smolińska and Król 2012; Lee and Sung 2014; Yan et al. 2020). Appropriate plant species, with or without soil amendments, depending on the type of soil pollution, may simultaneously offer good survival and growth as well as phytoremediation capacity in polluted sites. However, a better understanding is still needed of the survival, growth, and phytoremediation potential of different woody plant species, like *Populus* and *Salix*, for example, in soils polluted heavily with hydrocarbons and metals.

1.2 Objectives of the study

The aim of this thesis was to evaluate the survival, growth, and phytoremediation potential of *Populus* and *Salix* seedlings grown in polluted soils. More specifically, the following topics were studied:

(1) the survival and growth of two European aspen clones and four hybrid aspen clones cultivated in control soil (pristine), old creosote soil polluted with hydrocarbons, and pristine soil spiked with fresh diesel oil at three different planting densities in a greenhouse over two growing seasons (**Article I**);

(2) the survival, growth, and hydrocarbon removal abilities of three European aspen clones and seven hybrid aspen clones cultivated in hydrocarbon-contaminated soil (including polycyclic aromatic hydrocarbons (PAHs) and total petroleum hydrocarbons (TPHs)) under field conditions over 4 years (**Article II**); and

(3) the growth and metal accumulation ability of *S. psammophila* plants with bamboo biochar (BBC) amendment at ratios of 0–7% in soils heavily contaminated by Cd and Zn in a pot experiment over 180 days (**Article III**).

2 MATERIALS AND METHODS

2.1 Studies on the growth, survival, and hydrocarbon removal of *Populus* clones grown in polluted soils in a greenhouse (Article I) and a field (Article II)

The first study (**Article I**) was conducted from June 2013 to October 2015 in a plastic greenhouse at the Haapastensyrjä tree-breeding centre located in Southern Finland (60°36′ N, 24°25′ E). In total, 486 micropropagated seedlings of four hybrid aspen (14, 191, 27, and 291) and two European aspen (R3 and R4) clones with a height range of 0.5–15 cm were transplanted into plastic planting pots, at low (one plant per pot), medium (two plants per pot), and high (six plants per pot) densities. These pots were filled either with new, clean, pristine soil (control); old creosote soil polluted with hydrocarbons; or pristine soil spiked with fresh diesel oil (new diesel). The creosote soil and pristine soil used in the pots were collected at 0−50 cm soil depth from a former wood treatment area (~7 ha) located in Somerharju, southeastern Finland (60°92′ N, 27°56′ E, Luumäki) (see Fig. 1 in **Article I**). The pristine soil spiked with fresh diesel oil collected from a local Läyliäinen Neste station served as diesel-contaminated soil. The greenhouse experiment had a randomized factorial block design, consisting of 54 pots (three soils, three planting densities, and six clones), 162 individual plants per block, and a total of 486 trees in the three blocks. However, treatments were not repeated in a block. In the greenhouse, the locations of the pots were varied to avoid the effects of microclimatic variation. Seedlings were grown in a greenhouse during the growing season but were placed in non-heated storage areas from late autumn to early spring in 2013 and 2014.

In autumn 2015, before the seedlings were harvested, three to six healthy and fully developed leaves were taken from each hydrocarbon-contaminated soil treatment for chlorophyll fluorescence (Fv/Fm) measurements using a portable fluorometer (PAM 2100 Walz, Effeltrich, Germany). The height of all seedlings was measured before harvesting. Their stem dry biomass was determined in a laboratory. Based on these measurements, the effects of soil contamination (creosote, diesel, or uncontaminated control), planting density (low, medium, or high), and clone type on the survival, growth, and chlorophyll fluorescence (Fv/Fm) of seedlings were analysed. Statistical analyses in the software R (v 4.1.2; R Core Team 2021) evaluated whether soil contamination treatment, clone type, and/or planting density significantly affected the survival, growth, or Fv/Fm values of seedlings (see the layout of the study in Table 1 and details in the Materials and Methods of **Article I**).

The second study (**Article II**) was conducted from 2013–2017 as a field experiment in an area of ~7–8 ha located in an old wood impregnation plant in Somerharju in southeastern Finland (60°92′ N, 27°56′ E, Luumäki) that became contaminated with hydrocarbons from the use of creosote in wood processing 64–75 years ago. The central part of the study area (~2 ha), where this experiment was established, was the most contaminated area. This study area was first cleared of all plants (small pine and birch trees) except European aspens. Thereafter, in total, 200 sample plots of 20 × 20 m each were established, of which 40 plots with elevated hydrocarbon concentrations in the soil were divided into four blocks (I–IV) (see Fig. 1 in **Article II**). Based on a study by Mukherjee et al. (2014), hydrocarbon concentrations in the soil of these plots ranged from 0.16–714 mg total PAH kg^{-1} (total PAH: sum of 16 priority PAH pollutants defined by the US EPA), 10–580 mg C10–C21 kg^{-1}, 10–1780 mg C22–C40 kg^{-1}, and 20–2350 mg C10–C40 kg^{-1} (**Articles I** and **II**).

Each of these 40 plots with elevated soil concentrations of hydrocarbons was further divided into 400 subplots (each with an area of 1 m^2). In each subplot, micropropagated seedlings of three European aspen clones (R2, R3, and R4) and seven hybrid aspen clones (14, 27, 34, 134, 172, 191, and 291) with a height range of 0.5–15 cm and that were 2 years old in 2012 were planted in May 2013 at a density of 10,000 seedlings ha^{-1}. Two of the three European aspen clones (R3 and R4) and four of the seven hybrid aspen clones (14, 27, 191, and 291) were also used in the greenhouse experiment (**Article I**). The height and survival (%) of seedlings were measured at the end of the experiment in September 2017. Furthermore, five creosote soil samples were randomly taken at depths of 5–10 cm and 10–50 cm from the four corners and the middle of each square plot (as was done in 2011; see Mukherjee et al. 2014) in four blocks to evaluate the percentage of hydrocarbons removed from the soil by European aspen and hybrid aspen clones (**Article II**). A standard analytical method, US EPA 3540C (US EPA 1996), and the protocols described in Liu et al. (2014) were used to determine hydrocarbon concentrations in the soil, using chromatography and mass spectrometry (**Article II**). The hydrocarbon removal percentage was calculated based on the hydrocarbon concentration in the soil before and after the phytoremediation experiment. Statistical analyses using the SPSS statistical package (v 25.0, IBM Corporation, Armonk, NY, USA) and Microsoft Excel 2016 (Redmond, WA, USA) determined whether there were statistically significant differences among clones in seedling height and, additionally, whether the blocks and/or clone types significantly affected hydrocarbon removal (see layout of the study in Table 1 and details in the Materials and Methods of **Article II**). Statistical significance was defined as $p < 0.05$.

2.2 Study on the effects of bamboo biochar amendment on the growth and metal removal of *S. psammophila* seedlings in a pot experiment (Article III)

The third study (**Article III**) was conducted from April–September 2016 in a greenhouse at the Research Institute of Subtropical Forestry, Chinese Academy of Forestry, in Hangzhou, China (29°53′ N, 119°54′ E). In total, 25 one-year-old *S. psammophila* cuttings (~15 cm long, ~0.8 cm diameter) obtained from a local nursery were used for the pot experiment. Cuttings were planted in pots that were filled with either control soil (unamended polluted soil) or polluted soil amended with BBC at ratios of 1, 3, 5, and 7% (BBC/soil, w/w). The study had a randomized design with five replications and five treatments. Soil samples for the pot experiment were collected from an abandoned paddy field in Fuyang district (29°52′ N, 119°54′ E), Hangzhou city, Zhejiang province, China, where wastewater from the $ZnSO_4$ chemical plant polluted the sampled farmland with metals. Five soil samples were randomly taken from the 0.5 × 0.5 × 0.2 m plot area in the contaminated abandoned paddy field (CAPF). The average pH, organic matter, total N, total P, total K, hydrolysable N, available P, and available K values of the soil sampled from the CAPF were 7.3, 91 g kg^{-1}, 4.2 g kg^{-1}, 1.1 g kg^{-1}, 7.0 g kg^{-1}, 332 mg kg^{-1}, 58 mg kg^{-1}, and 132 mg kg^{-1}, respectively (see Fig. 1 in **Article III**). Based on classifications of soil pH and soil texture reported by the USDA (1998), the soil samples were alkaline, and the soil texture was silty loam. The mean levels of Cd, Zn, Cu, and Pb in the soil were 59 mg kg^{-1}, 2108 mg kg^{-1}, 114 mg kg^{-1}, and 212 mg kg^{-1}, respectively (see Table 2 in **Article III**). Five soil treatments with BBC amendment with ratios of 1–7% were used in this study: T0 (control), T1 (BBC 1%), T2 (BBC 3%), T3 (BBC 5%), and T4 (BBC 7%). The BBC was collected from the Yaoshi Charcoal Production

Company in Hangzhou, China. During the experiment, the positions of the pots were varied to prevent effects of microclimatic variation.

At the end of the experiment in September 2016, five healthy and fully developed leaves were taken from seedlings from each treatment grown in the contaminated paddy soils for measurements of photosynthetic and transpiration rates (E, mmol H_2O m^{-2} s^{-1}) and CO_2 uptake (A, μmol CO_2 m^{-2} s^{-1}). Transpiration rate and CO_2 uptake were detected using a portable infrared gas exchange analyser (LI-6400 XT, Lincoln, USA), according to the manufacturer's guidelines. Before harvesting in September 2016, seedling heights were measured. After harvesting, the dry weights of roots, shoots, and leaves of sampled seedlings were measured. Inductively coupled plasma atomic emission spectrometry (Perkin Elmer Optima 8000, Waltham, MA, USA) was used to determine the metal concentrations of the dried samples (leaves, stems, and roots) of seedlings in each soil treatment.

In addition, a bioconcentration factor (BCF) was calculated to evaluate the ability of a plant to remove metals from the soil and accumulate them in its tissues based on metal concentrations of harvested tissue and soil (see Arsenov et al. 2019). Furthermore, a translocation factor (TF) was calculated to evaluate a plant's ability to translocate metals from the roots to the shoot. The TF was estimated based on metal concentrations in the aboveground portions and the roots of the plant (see Xiao et al. 2023). Statistical analyses using Data Processing System software (DPS 13.01, Zhejiang University, Hangzhou, China) determined whether the BBC treatments significantly affected growth, photosynthetic activities, BCF, or TF (see the layout of the study in Table 1 and details in the Materials and Methods of Article III).

Table 1. Study layouts of the different experiments of studies I–III.

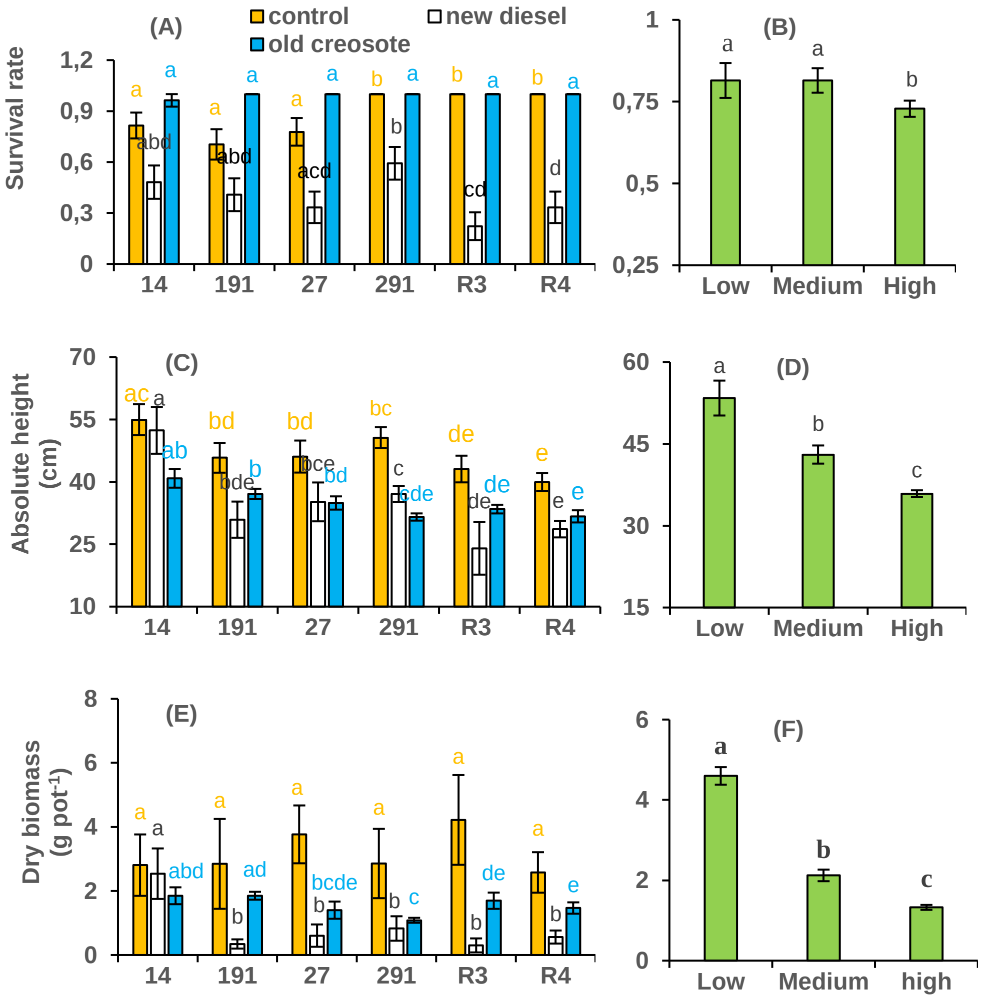
Study layout	**Study I**	**Study II**	**Study III**
Study duration	2 years, 4 months (June 2013–October 2015)	2013–2017	180 days (April–September 2016)
Study location	Greenhouse experiment at Haapastensyrjä tree-breeding centre, Finland	Field experiment in Luumäki, Finland	Greenhouse experiment at the Research Institute of Subtropical Forestry, Chinese Academy of Forestry, in Hangzhou, China
Species	2 European aspen and 4 hybrid aspen clones	3 European aspen and 7 hybrid aspen clones	*S. psammophila* (cuttings)
Pollution treatments	Control, old creosote-contaminated, and fresh diesel-contaminated soils	Old creosote-contaminated soil	Soil contaminated with metals (Cd, Zn, Cu, and Pb)
Other treatments/factors	3 planting densities		Bamboo biochar amendment at ratios of 0, 1, 3, 5 and 7%
Variables measured/ analysed	Survival, height, stem dry biomass, and chlorophyll fluorescence (Fv/Fm, in leaves) of seedlings	Survival, height, and hydrocarbon removal efficiency at different soil depths	Height, dry biomass, photosynthesis and transpiration rates of leaves, metal accumulation of seedlings, and TF and BCF values

TF = metal concentration in the shoot/metal concentration in the root; BCF = metal concentration in the shoot/metal concentration in the soil

3 RESULTS

3.1 Survival and growth of *Populus* seedlings in control, old creosote-contaminated, and fresh diesel-contaminated soils in a greenhouse (Study I)

The survival rates of seedlings of the four hybrid and two European aspen clones under different planting density treatments ranged from 70–100% in control soils, 22–59% in fresh diesel-contaminated soils, and 99% in old creosote-contaminated soils (Fig. 2A). The survival rate was 10% lower at high planting density than at medium and low densities (Fig. 2B). Among hybrid aspen clones, clone 29l had the highest survival rate (86%) across all soil and planting density treatments, followed by clones 14 (75%), 191 (70%), and 27 (70%). European aspen clone R4 had a slightly higher survival rate (78%) than clone R3 (74%). Soil treatment, planting density, and clone type significantly affected the survival rate ($p < 0.05$).



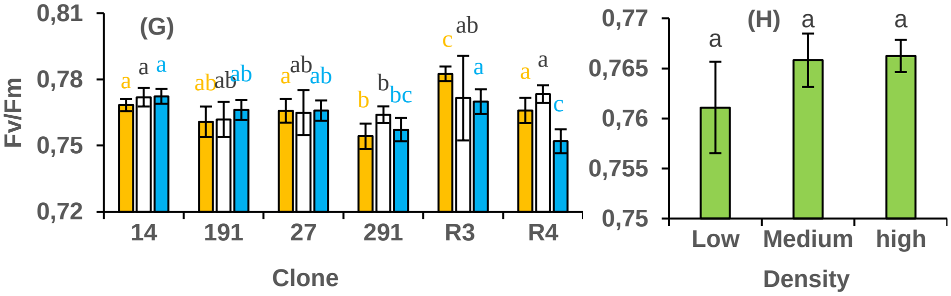


Figure 2. Left: Average (*n* = 3) survival rate (A), height (C), stem dry biomass (E), and Fv/Fm values (G) of various clones grown in different soil treatments. Right: Average (*n* = 3) survival rate (B), height (D), stem dry biomass (F), and chlorophyll fluorescence (Fv/Fm) values (H) of various clones grown at different planting densities. Error bars indicate ± standard error. The same lowercase letters indicate that there were no statistically significant differences between means of clones within the same soil treatment or between density treatments ($p > 0.05$).

Seedlings grown at medium and high planting densities had 19–33% and 53–71% lower heights and stem dry biomass, respectively, compared to seedlings grown at low planting density (Figs. 2 D and F). Among hybrid aspen clones, clone 14 had 20–25% greater height and 24–51% larger stem dry biomass than other clones across all soil and planting density treatments. Among European aspen clones, clone R3 had 6% greater height and 35% greater stem dry biomass than clone R4. In the old creosote-contaminated soil treatment, stem dry biomass was larger in all clones except clone 14 compared to the diesel-contaminated soil treatment (Fig. 2E). Soil treatment, planting density, and clone type significantly affected the height and stem dry biomass of seedlings ($p < 0.05$).

Hybrid aspen clones had slightly higher chlorophyll fluorescence (Fv/Fm) values in contaminated soils compared to control soil. The European aspen clone R4 had a higher Fv/Fm value in the fresh diesel-contaminated soil than in other soil treatments. In contrast, clone R3 had a higher Fv/Fm value in control soil (Fig. 2G). Among hybrid aspen clones, clone 14 had the highest Fv/Fm value, as did clone R3 among European aspen clones, across all treatments. Clone type significantly affected the Fv/Fm value ($p < 0.05$), unlike soil treatment or planting density (see Table 2 and Section 3.5 of Article I).

The heights of seedlings of hybrid and European aspen clones across all planting density treatments were 5–44% and 19–38% lower in fresh diesel-contaminated and old creosote-contaminated soils, respectively, compared to the control (Fig. 2C). The stem dry biomass was 9–93% and 34–63% lower in diesel-contaminated and old creosote-contaminated soils, respectively, compared to the control (Fig. 2E).

3.2 Survival and growth of *Populus* seedlings in old creosote-contaminated soil in a field (Study II)

Among the seven studied hybrid aspen clones, clone 291 had the highest survival rate (72%) in old creosote-contaminated soil.

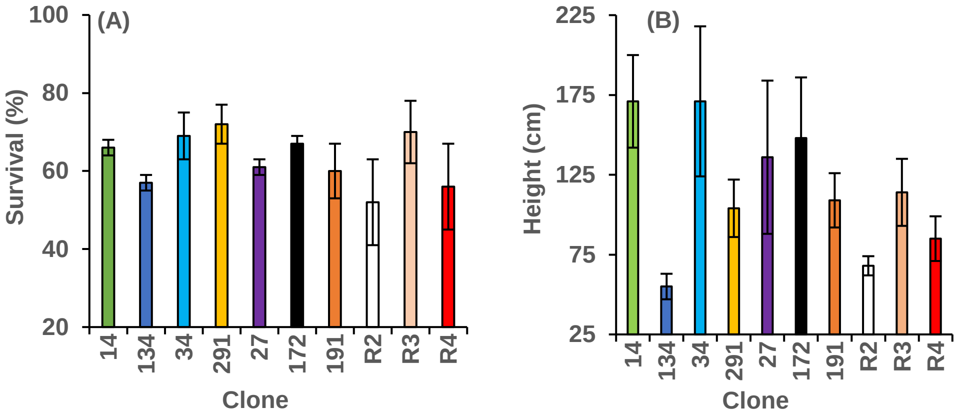


Figure 3. Mean (*n* = 4) survival percentage (A) and height (B) of *Populus* plants from blocks I–IV. Error bars represent ± standard error.

Clone R3 had the highest survival rate (70%) among the three European aspen clones studied (Fig. 3A). Clones 14 and 34 had the greatest height (171 cm) among hybrid aspen clones, at 16–211% greater than heights of the other clones. Additionally, the heights of clone R3 were 25–35% greater compared to the other European aspen clones, R2 and R4 (Fig. 3B). However, clone type (and block) did not significantly affect seedling survival or height ($p >$ 0.05).

3.3 Growth of *S. psammophila* seedlings with bamboo biochar amendment in soils contaminated with metals (Study III)

Treatments BBC 1% (T1) and BBC 5% (T3) decreased the height of *S. psammophila* seedlings only slightly (0.6–1.3%) compared to the control. In contrast, BBC 3% (T2) increased height slightly, by 2% (Fig. 4A).

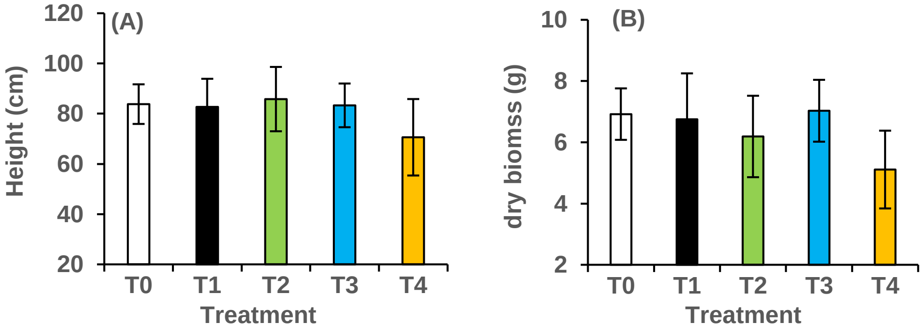


Figure 4. Effects of bamboo biochar-amended soil treatments (T0: BBC 0%, T1: BBC 1%, T2: BBC 3%, T3: BBC 5%, and T4: BBC 7%) on absolute growth in height (cm) (A) and dry biomass production (g; the sum of biomass of roots, leaves, and shoots) (B) of *S. psammophila* plants. Error bars represent standard deviation (mean ± SD, *n* = 5).

BBC 1% and BBC 3% reduced the total dry biomass of seedlings by 2–10% and BBC 5% increased it by 2% compared to the control (Fig. 4B). Compared to BBC 1–5%, BBC 7% reduced the height (16%) and total dry biomass (26%) of seedlings compared to the control (Fig. 4). The photosynthetic rate (Pn) of seedlings increased (2–29%) during the earlier cultivation stage (before 90 days) with BBC 1–5 %, in contrast to BBC 7%. However, after 90 days, Pn was similar in all BBC treatments. The changes in transpiration rate (Tr) were similar to those of Pn (see Fig. 3 in **Article III**). However, height, total dry biomass, Pn, and Tr were not significantly affected by metal contamination treatment ($p > 0.05$).

3.4 Removal of hydrocarbons by *Populus* seedlings in old creosote-contaminated soils in a field (Study II)

The greatest quantities of PAHs at a soil depth of 5–10 cm were removed by clones 134 (58%), 191 (55%), and 14 (54%) among the hybrid aspen clones. In contrast, at a soil depth of 10–50 cm, the greatest quantities of PAHs were removed by clone 191 (61%), while clones 134 and 14 removed 3–6%. The greatest quantities of C10–C21 at a soil depth of 5–10 cm were removed by clones 134 (66%), 14 (64%), and 291 (41%) (Table 3 in Appendix 1). At a soil depth of 10–50 cm, the greatest quantities of C10–C21 were removed by clones 191 (56%) and 172 (36%). The greatest quantities of C22–C40 at a soil depth of 5–10 cm were removed by clone 134 (81%), followed by clones 34, 191, 291, and 14 (60–71%). In contrast, the greatest quantities of C22–C40 at a soil depth of 10–50 cm were removed by clones 191 and 14 (81%). The greatest quantities of C10–C40 at a soil depth of 5–10 were removed by clone 134 (79%), followed by clones 172 (60%) and 191 (68%). Clone 14 removed the greatest quantity of C10–C40 (72%) at a soil depth of 10–50 cm. European aspen clone R2 removed more PAHs, C10–C21, C22–C40, and C10–C40 than clones R3 and R4 at soil depths of 5–10 cm (~81%) and 10–50 cm (69–89%) (Table 3 in Appendix 1). All clones showed the potential to remove total PAHs and TPHs from the soil (however, this effect was significant ($p < 0.05$) only at a soil depth of 5–10 cm) (see Fig. 3 in **Article II**).

3.5 Metal accumulation in *S. psammophila* seedlings with bamboo biochar amendment in soils contaminated with metals (Study III)

BBC amendment increased the accumulation of Cu, Cd, and Zn in plant tissues of *S. psammophila* seedlings, especially Cd and Zn accumulation with BBC 3% (Fig. 5). Overall, the highest levels of Cd and Zn were found in the leaves, whereas the highest levels of Cu and Pb were found in the roots (Fig. 5).

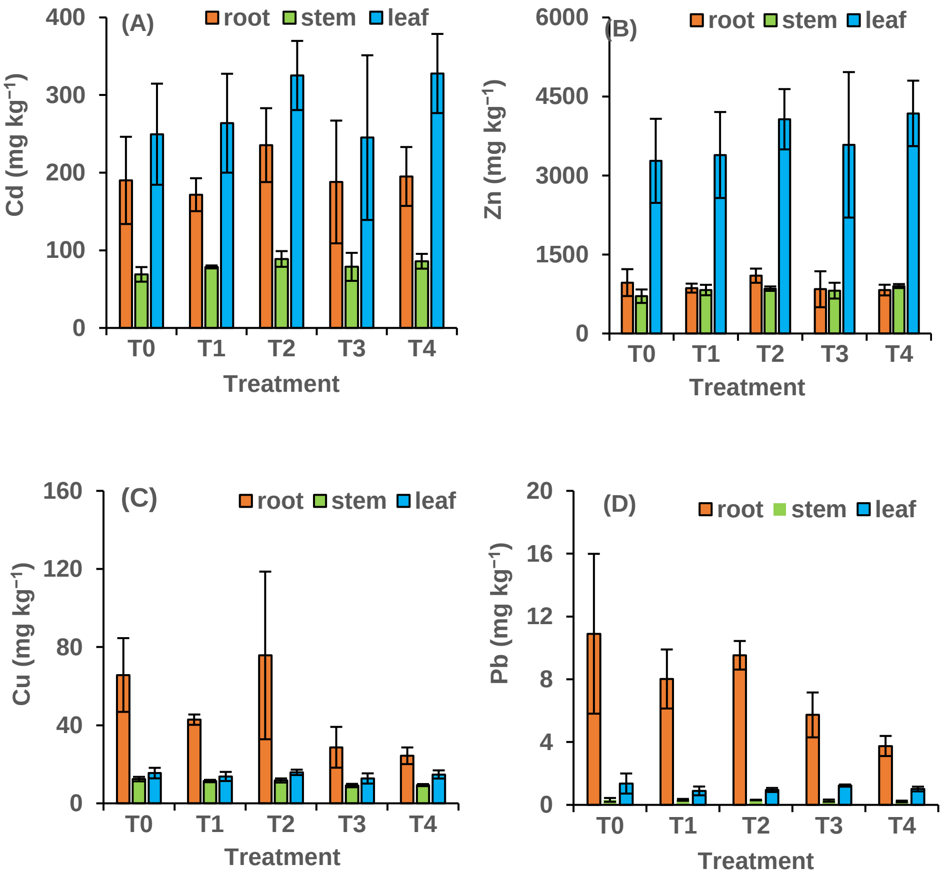


Figure 5. Mean (*n* = 5 for each treatment) Cd (A), Zn (B), Cu (C), and Pb (D) concentrations (mg kg^{-1}) in different tissues of plants grown in contaminated soil amended with bamboo biochar (BBC). Error bars represent ± standard deviation. T0: BBC 0%, T1: BBC 1%, T2: BBC 3%, T3: BBC 5%, and T4: BBC 7%.

In the roots, BBC 3% increased Cd (24%), Zn (13%), and Cu (15%) accumulation the most compared to the control. In the stems, BBC 1–7% increased Cd and Zn accumulation by 13–28% compared to the control. Cd accumulation was highest with BBC 3% compared with BBC 1%, BBC 5–7%, and Zn accumulation was highest with BBC 7% compared with BBC 1–5%. With BBC 3% and BBC 7%, Cd accumulation in leaves was 30% higher than in the control. Additionally, with BBC 3% and BBC 7%, Zn accumulation was 24–27% higher than in the control. However, metal accumulation in the roots, stems, and leaves was not significantly affected by the BBC 1–7% treatments ($p > 0.05$), except for Pb accumulation in the roots and Cu accumulation in the stem ($p < 0.05$).

BBC 3% increased the total content of Cd (38%), Zn (35%), Cu (60%), and Pb (16%) compared to controls (T0) at the whole plant level. In contrast, BBC 1%, BBC 5%, and BBC 7% decreased these measurements (1–23% Cd, 3–18% Zn, 20–49% Cu, and 18–39% Pb) compared to controls (Table 2).

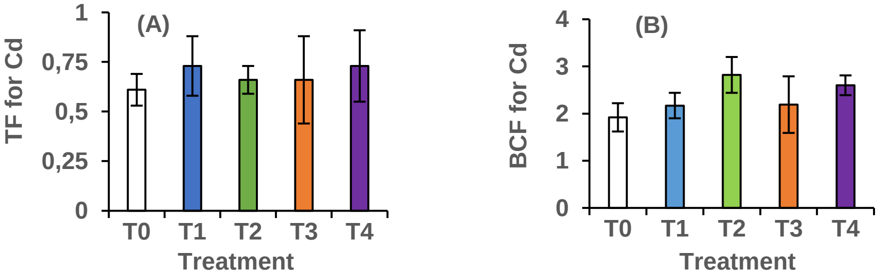
Table 2. Metal uptake by plants grown in contaminated soils amended with bamboo biochar (*n* = 5). Data are presented as mean ± standard deviation. Dissimilar letters between soil treatments indicate significant differences ($p < 0.05$). T0: BBC 0%, T1: BBC 1%, T2: BBC 3%, T3: BBC 5%, and T4: BBC 7%.

Plant tissue	Tr	Cu	Zn	Cd	Pb
		μg plant^{-1}			
Roots	T0	58 ± 6.5ab	858.8 ± 117.2ab	168.8 ± 29.6ab	9.6 ± 3.6a
	T1	43.7 ± 10.2ab	871.5 ± 183.6ab	173.2 ± 35.7ab	8.3 ± 3.3a
	T2	79.4 ± 55.1a	1088.8 ± 277.6a	238.4 ± 86.8a	9.5 ± 2.6a
	T3	26.9 ± 5.3b	792.1 ± 241.1ab	184.6 ± 74.9ab	5.5 ± 0.9ab
	T4	17 ± 2.3b	579.1 ± 75.9b	135.9 ± 14.6b	2.6 ± 0.3ab
Aboveground parts	T0	72.4 ± 7.9ab	7614.4 ± 241.3a	679.2 ± 23.1a	6.6 ± 1.4a
	T1	59.9 ± 8.4ab	7371.7 ± 1113.2a	666.5 ± 79.6a	5 ± 1.6a
	T2	86.7 ± 33.1a	10013.4 ± 2601.1a	920.2 ± 261.7a	5.9 ± 2.1a
	T3	58 ± 2.1ab	8339.5 ± 2369.3a	705.5 ± 218.1a	5.9 ± 2.1a
	T4	50.6 ± 10b	7840.8 ± 1935.6a	712.4 ± 173.9a	4.4 ± 0.5a
Total	T0	130.5 ± 3.9ab	8473.3 ± 301.1a	848. ± 36.8a	16.2 ± 3.7a
	T1	103.6 ± 15.6bc	8243.2 ± 1134.3a	839.7 ± 85.6a	13.3 ± 2.7a
	T2	166.2 ± 67.9a	11102.2 ± 2858.9a	1158.5 ± 348.3a	15.5 ± 4.2a
	T3	84.9 ± 3.2bc	9131.6 ± 2601.3a	890.1 ± 292.3a	11.4 ± 1.6ab
	T4	67.6 ± 12.3c	8419.9 ± 2002.6a	848.3 ± 188a	6.9 ± 0.8b

Tr: treatment

Additionally, in the shoots (aboveground), BBC 3% increased the total content of Cd (+ 38%), Zn (+ 36%), Cu (+ 45%), and Pb (+ 18%) compared to controls. In contrast, BBC 1%, BBC 5%, and BBC 7% reduced the content of Cd (− 1–24%), Zn (− 3–17%), Cu (− 13–33%), and Pb (− 1–26%) (Table 2). In the roots, BBC 3% also resulted in the greatest content of Cd, Zn, Cu, and Pb. With the other treatments, these content measurements decreased compared to controls, except for BBC 1%, which led to a slight increase in Cd and Zn content (1–3%) compared to controls (Table 2). Soil treatments significantly affected only the total Cu content and total Pb content at the whole plant level ($p < 0.05$).

The TF values for Cd, Zn, Cu, and Pb of *S. psammophila* plants were also generally higher with BBC treatments compared to the control (BBC 0%), except for Pb with BBC 3% (Fig. 6). BBC 1–7% also resulted in higher Cd, Zn, and Cu BCF values compared to the control. Among all treatments, BBC 3% resulted in the highest BCF values for Cd (2.82), Zn (0.84), and Cu (0.15), but not for Pb (Fig. 6). However, there were no statistically significant effects of soil treatment on TF or BCF values for metals ($p > 0.05$), except for TF for Pb ($p < 0.05$).



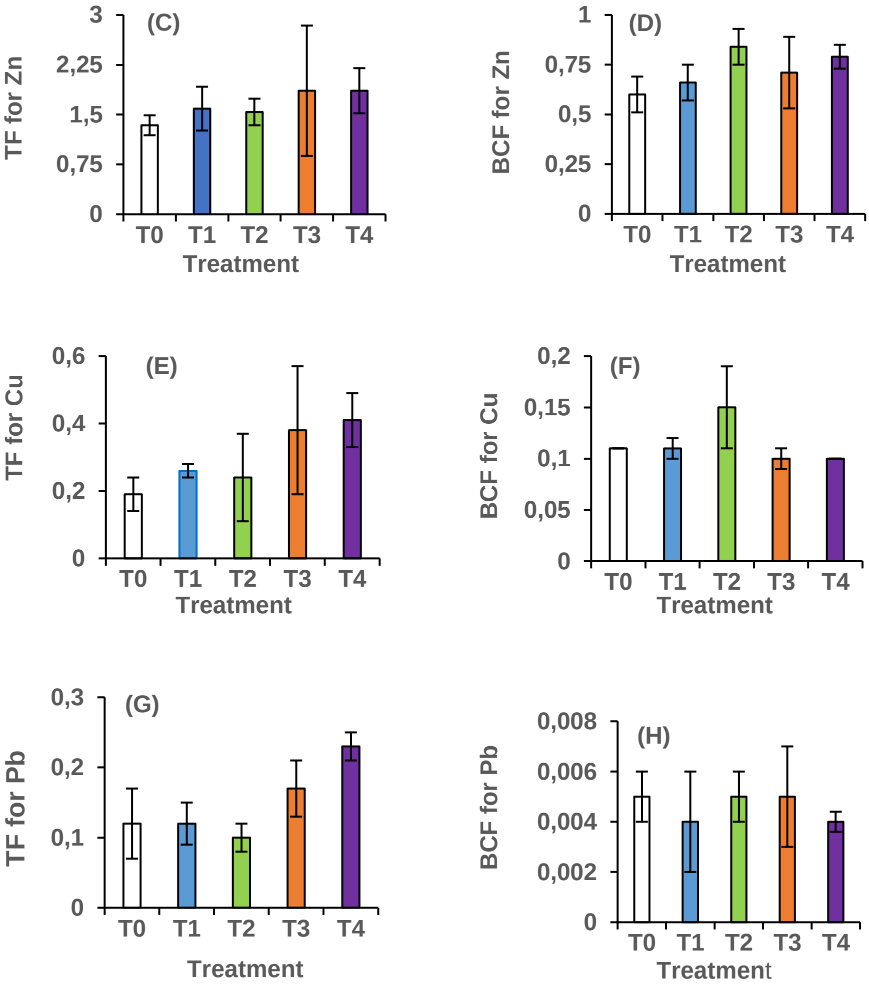


Figure 6. Left: Mean (*n* = 5, for each treatment) translocation factors (TF) for Cd (A), Zn (C), Cu (E) and Pb (G). Right: Mean (*n* = 5, for each treatment) bioconcentration factors (BCF) for Cd (B), Zn (D), Cu (F) and Pb (H) for *S. psammophila*. Error bar indicates ± standard deviation. Legends: T0: BBC 0%, T1: BBC 1%, T2: BBC 3%, T3: BBC 5%, T4: BBC 7%.

4 DISCUSSION

4.1 Survival, growth, and hydrocarbon removal potential of *Populus* seedlings (Articles I and II)

Overall, the survival rates of European aspen and hybrid aspen clone seedlings across all planting densities in the greenhouse experiment (**Article I**) were 70–100% in control soil, 99% in the creosote-contaminated soil, and 22–59% in the diesel-contaminated soil. Additionally, seedling heights were 5–44% and 9–38% lower in diesel-contaminated and old creosote-contaminated soils, respectively, compared to the control. Furthermore, their stem dry biomasses were 9–93% and 34–63% lower, respectively. The survival rate and growth were also greater at lower planting density compared to higher density treatments. Among all aspen clones, hybrid aspen clones 14 and 291 and European aspen clone R3 had reasonable survival and growth across all treatments. The survival rate, height, and stem dry biomass were all significantly affected by soil treatment, planting density, and clone type ($p < 0.05$).

In the field experiment (Article II), the highest survival rates across all hybrid aspen and European aspen clones were observed in hybrid aspen clone 291 (72%) and European aspen clone R3 (70%) in old creosote-contaminated soils. The greatest heights were observed in hybrid aspen clones 14 and 34, which were 16–211% greater than in the other hybrid aspen clones. European aspen clone R3 had also 25–35% greater height than other European aspen clones. Unlike in the greenhouse experiment, clone type did not significantly affect seedling survival or height in the field experiment ($p > 0.05$). The clonal variation observed in the survival rates of aspens in both the greenhouse and field experiments (Articles I and II) might be due to different growing conditions and growth periods. Overall, a high survival rate in polluted soils may be explained by the plant's strong tolerance and defence mechanisms in the presence of effective non-enzymatic and enzymatic antioxidants under stress, as was reported by McIntosh (2014).

Additionally, in a study by Yan (2012), hybrid clones of *Populus* and *Salix* survived at a rate of 67% when they were grown in soil that was highly contaminated with TPHs (250 000 mg kg^{-1}) and PAHs (4100 mg kg^{-1}). Phytoremediation field and lab experiments demonstrated that hybrid *Populus* plants survived in contaminated soil with a high concentration of TPHs (> 20 000 mg kg^{-1}). A higher survival rate was observed in the field than in the laboratory (Yan 2012). An area with BETX-contaminated groundwater had a 100% survival rate for trees (*Populus, Salix,* and *Acer*) (Yan 2012). Over 93% of the hybrid *Populus* trees survived in soils contaminated with PCE, 1,1,1-trichloroethane, toluene, and xylenes (Yan 2012). Additionally, in a pot experiment, woody plant species (i.e. *Acacia inaequilatera*, *A. pyrifolia*, *A. stellaticeps*, and *Banksia seminuda*) survived in soil contaminated with TPHs (4370–7500 mg kg^{-1}) until the end of the experiment (Hoang et al. 2021).

Fresh diesel oil-contaminated soil possibly has high toxicity because of the presence of toxic fractions with low boiling points, such as the range C10–C19, and low biodegradability, which restrict plant growth and development (Brils et al. 2002; Trindade et al. 2005; Jonker et al. 2006; Zhang et al. 2007). A similar result was observed in this study: survival was low in fresh diesel oil-contaminated soil in Populus in the greenhouse experiment (Article I). In contrast, the creosote-contaminated soil treatment resulted in robust plant survival (99%), which indicates that hybrid aspen and European aspen seedlings have high resistance to aged PAH-contaminated soil. Additionally, Hoang et al. (2021) reported that woody plant species significantly suppressed height and biomass growth when cultivated in TPH-contaminated soil, as found in the present study (**Article I**).

Several studies (e.g. Wang et al. 2013; Odukoya et al. 2019; Bashir et al. 2020; Ukalska-Jaruga et al. 2020) have shown that PAH toxicity affects the physicochemical and nutrient properties, water availability, and soil microbe population of soil, which leads to reductions in nutrient and water uptake from the soil and in photosynthesis, which further inhibit plant growth. Aged hydrocarbon-contaminated soil are more degradable and bioavailable and less toxic and facilitate plant growth and development (Nydahl, et al. 2015; Khan et al. 2018; Haider et al. 2021). Interestingly, in diesel-contaminated soil, aspen growth was higher in this study (**Article I**) than in plants exposed to aged creosote-contaminated soil. However, this result might have several explanations. First, plants may have received more nutrients released from the oil and soil that stimulated their growth and photosynthesis (see, e.g., Adieze et al. 2012). Second, plant growth was measured at the end of the growing period (after 2 years). With a longer measurement period, the toxicity of hydrocarbons may be reduced to a greater extent due to the weathering process and breakdown of hydrocarbons by microbes such as bacteria (see, e.g., Odukoya et al. 2019), which might promote plant growth. Plants compete more with each other for growth-regulating factors, such as light, water, and nutrients, when planting densities are increased, resulting in abiotic stress and higher interplant competition, which leads to decreased survival and growth rates, as reported by Ciampitti and Vyn (2011), Rossini et al. (2011), and Zhang et al. (2021), for example. When plants are densely cultivated, they may also suffer low-N stress during certain growth phases, leading to lower survival and growth (Mi et al. 2016). According to Meng and Chi (2015), low-density treatments led to the highest rates of survival and robust growth, followed by medium-density and high-density treatments; these results are consistent with those of the greenhouse experiment of the present study (**Article I**). Besides different growing conditions, plant morphology (e.g. roots), physiology (e.g. root exudates), and microbial interactions in the rhizosphere differ among species; therefore, some plants are more resistant to hydrocarbons and stress, and vice versa (see, e.g., Sharonova and Breus 2012). In this study, both in the greenhouse and field experiments (**Articles I** and **II**), plant growth varied among the clones, which may be due to their differences in physiological and chemical properties and adaptability under stress (see, e.g., Greger 2005; Salam et al. 2019; Landberg and Greger 2022). A different growth environment and study duration may also be contributing factors (see, e.g., Salam et al. 2019).

In the greenhouse experiment (**Article I**), all studied hybrid aspen and European aspen clones also showed sufficient photosynthesis efficiency, as Fv/Fm (chlorophyll fluorescence) values were 0.75–0.78 across all treatments. Across all soil and density treatments, clone 14 had the highest Fv/Fm value, while the European aspen clone R3 had higher Fv/Fm values than clone R4. Overall, an Fv/Fm value < 0.75 indicates that a tree has low photosynthesis efficiency (Bęś et al. 2019; Salam et al. 2019). Contamination can interfere with internal processes in plants and thereby decrease their fitness, as noted by Nebeská et al. (2021). In previous phytoremediation experiments, *Populus* plants had Fv/Fm values of 0.72–0.76 when grown in diesel-contaminated soil (Pajević et al. 2009). Another phytoremediation experiment conducted on Scots pine and European beech in diesel-contaminated soils showed Fv/Fm values from 0.57–0.71 (Bęś et al. 2019). Higher Fv/Fm values result in good photosynthesis efficiency and growth (Salam et al. 2019). According to Pajević et al. (2009), distinct soil treatments can affect morphological and ecological features of clones and species in various ways. The variation in the Fv/Fm values of the plants could also be due to different plant species, growth conditions, soil treatments, and exposure times.

Among all hybrid aspen clones in the field experiment (**Article II**), clone 134 removed the greatest quantity of hydrocarbons at a depth of 5–10 cm and clone 191 at a depth of 10–50 cm. In addition, clone 14 showed potential for removing hydrocarbons at both soil depths. In European aspen clones, clone R2 removed the greatest quantity of hydrocarbons at both soil depths. Clonal variation in rates of hydrocarbon removal from the soil was evident. However, all clones showed the potential to remove total PAHs and TPHs from the soil, especially at a soil depth of 5–10 cm ($p < 0.05$ only at a depth of 5–10 cm). Based on the present study's findings in the greenhouse and field experiments (**Articles I** and **II**), European aspen and hybrid aspen clones can be considered candidates for the remediation of soils polluted with PAHs and TPHs. This is because they showed 1) reasonable survival and growth (**Articles I** and **II**, also photosynthetic activity in Article **I**), 2) high rates of hydrocarbon removal from soil (**Article II**), and 3) the capacity to adapt in soils polluted with a wide range of hydrocarbons (i.e. 0.16–714 mg total PAH kg^{-1}, 10–580 mg C10–C21 kg^{-1}, 10–1780 mg C22–C40 kg^{-1}, and 20–2350 mg C10–C40 kg^{-1}).

In the field experiment (**Article II**), hydrocarbon concentration levels (PAHs, C10–C21, C22–C40, and C10–C40) in the soil in 2017 were substantially lower than those in 2011 at depths of 5–10 cm and 10–50 cm. Soil hydrocarbon levels were more effectively reduced at a depth of 5–10 cm than at 10–50 cm. Hydrocarbon removal was greatest in the topsoil surface layer (5–10 cm deep), maybe because this layer might contain the largest quantity of hydrocarbons and because more roots of young seedlings are located at this depth. The clones studied reduced soil hydrocarbon levels to a large extent, which is likely also due to the phytodegradation process, in which enzymes within plant cells degrade or mineralize hydrocarbons efficiently (Da Cunha et al. 2012). A field experiment conducted in Finland found that *P. tremula* × *P. tremuloides* seedlings removed 53% of PHCs from sandy soil after 1 year of monitoring, whereas 78% of PAHs were removed after 3 years of monitoring (Lopez-Echartea et al. 2020). In another field study with *P. nigra* seedlings, the concentration of PHCs decreased from ~1150 mg kg^{-1} to ~200 mg kg^{-1} after 1 year of treatment in which soils were supplemented with horse manure (Doni et al. 2012). A growth chamber experiment using *P. deltoides* × *P. wettsteinii* seedlings in boreal soil with an initial diesel concentration of 5000 mg kg^{-1} resulted in diesel fuel being removed from the soil by seedlings (Lopez-Echartea et al. 2020). Yan (2012) reported that, after 7 years of monitoring, the hybrid *Populus* showed removal rates of 64% PAHs and 74% naphthalene from the soil. Da Cunha et al. (2012) also reported that *S. triandra* and *S. rubens* are efficacious for the remediation of hydrocarbon-polluted soils. In their phytoremediation experiment, *S. triandra* and *S. rubens* reduced pyrene concentrations in the soil from ~23 μg kg^{-1} to 0.1 μg kg^{-1} (or below detectable levels), benzo[k]fluoranthene concentrations from ~29 μg kg^{-1} to below detectable levels, chrysene concentrations from ~126 μg kg^{-1} to below detectable levels, and benzo[a]pyrene concentrations from ~4 μg kg^{-1} to below detectable levels. Variability in hydrocarbon concentrations, clone types, photodegradation rates, types and natures of pollutants, plant adaptability to stress, physiological and biochemical processes, the bioavailability of hydrocarbons to plants, and types of hydrocarbon degraders may all contribute to this discrepancy, i.e. variation in hydrocarbon removal rates among clones (see, e.g., Yan 2012; Khan et al. 2018; Salam et al. 2019; Lopez-Echartea et al. 2020; Haider et al. 2021; Hoang et al. 2021). Phytoremediation of hydrocarbons by different species of plants, including closely related genotypes, can vary significantly, as reported by Ikeura et al. (2016) and Dagher et al. (2019).

According to Lopez-Echartea et al. (2020), the reason for good hydrocarbon removal rates in PAH-contaminated Finnish boreal soil where *P. tremula* × *P. tremuloides* seedlings were grown in a phytoremediation experiment could be the presence of active PHC-degrading bacteria, such as *Pseudomonas*, *Burkholderia, Rhizobacter, Sphingomonas, Thermomonas*, and *Flavobacterium*. Similarly, Mukherjee et al. (2013, 2015) detected active PHC-degrading bacteria in the families Burkholderiaceae, Acetobacteraceae, Pseudomonadaceae, and Sphingomonadaceae in boreal soils where *Populus* plants were cultivated. These hydrocarbon-degrading bacteria and plants might play important roles in removing hydrocarbons from soil. Plant roots might provide favourable growth conditions for microbes that aid in detoxifying hydrocarbons (Pajuelo et al. 2011). The organic acids from root exudates might affect hydrocarbon bioavailability and stimulate the microbial growth and activity in the soil that facilitated the biodegradation of hydrocarbons reported by Yan (2012).

4.2 Growth and metal accumulation of *Salix* seedlings with different bamboo biochar treatments (Article III)

In the greenhouse experiment (**Article III**), BBC 1% and 5% did not strongly affect the height (decrease of 0.6–1.3%) or total dry biomass (decrease of 2–10%) of *S. psammophila* seedlings compared to the control. This was also the case for BBC 3% (2% increase). However, BBC 7% reduced the height (16%) and total dry biomass (26%) of seedlings compared to the control. Across all BBC treatments of *Salix* seedlings, adding BBC to soil did not significantly improve plant growth or photosynthesis, although it slightly increased photosynthetic and transpiration rates, especially in treatments T1–T3 (BBC 1–5%) (**Article III**). However, the application of BBC at 3% proved to be the most effective in terms of growth and photosynthesis.

The effects of metal toxicity and nutrient deficiencies may contribute to delayed plant growth and development (Kuzovkina et al. 2004; Wang et al. 2016; Tőzsér et al. 2017). In the present study (**Article III**), as a result of the elevated C/N ratios due to the high doses of BBC (BBC 7%), amended soil facilitated a favourable environment for the growth of soil microbes, which compete against plants for protein and nitrogen, resulting in a decrease in plant growth and photosynthesis. In addition, the release of toxic hydrocarbons from BBC might stunt plant growth and photosynthesis, according to Li et al. (2022a). Another pot experiment conducted by Li et al. (2022a) found that adding BBC to soil collected from the same study site used in the present study slightly affected *S. psammophila* plant growth and photosynthesis, which is consistent with the findings of this study (**Article III**). Ali et al. (2017) reported that plant growth was enhanced when 2.5% BBC (BBC/soil, w/w) was added to soils contaminated with Cd, Zn, Cu, and Pb, whereas 5% BBC decreased plant growth but accelerated the rate of photosynthesis. It is possible that the increased plant growth and photosynthetic rates observed in the BBC-amended soil treatments are the result of the soil being enriched with nutrients and organic matter (Salam et al. 2019).

Elevated levels of organic matter and essential nutrients, such as available K (see **Article III**), may reduce metal-induced oxidative stress and enhance plant growth and photosynthetic rates under pollutant stress (Najeeb et al. 2011; Ehsan et al. 2014; Arsenov et al. 2017; Arsenov et al. 2019; Frédette et al. 2019; Arsenov et al. 2020). In addition to aiding in the transportation of water and nutrients through the xylem, K may also be responsible for activating enzymes that reduce metal toxicity and that together enhance growth, tolerance, and photosynthesis (Wang et al. 2019). Rakotonimaro et al. (2017) reported that plant growth was enhanced in contaminated mine soils supplemented with a combination of 5% dry weight of compost (sewage sludge) and lime. By adding poultry and eucalyptus biochar to Cd-contaminated soil, total biomass production was also increased (Lu et al. 2016). According to Mench et al. (2006), the utilization of inorganic soil amendments containing phosphate fertilizers exhibited positive effects on plant growth by metal sorption or chemical alteration. These results suggest that the effect of BBC on *Salix* growth may be a complex result of the combined action of soil type, soil fertility, different application rates of BBC, and plant species (Salam et al. 2019; Li et al. 2022a; Xiao et al. 2023). According to Zulkernain et al. (2023), many other factors can affect the biomass of plants, including the type and quantity of chelators, species, contamination levels, and timing and rates of chelators. The growth of

Salix plants also differs according to soil amendments and doses, growing periods, metal concentration levels, types of contaminants, soil characteristics, growth conditions, and species, as found by Mohsin et al. (2016), Wang et al. (2016), and Salam et al. (2019).

BBC amendment increased the accumulation of Cu, Cd, and Zn in different *S. psammophila* tissues, especially Cd and Zn accumulation (23–30% and 13–24%, respectively) in the BBC 3% treatment compared to the control in this study (**Article III**). Based on these findings, *S. psammophila* with BBC amendment can be considered a candidate for phytoremediation. However, metal accumulation in the roots, stems, and leaves was not significantly affected by BBC 1–7% treatments ($p > 0.05$), except for Pb accumulation in the roots and Cu accumulation in the stem ($p < 0.05$).

In this study (**Article III**), Cd and Zn accumulation in *S. psammophila* was higher in leaves, followed by roots and stems. This might be due to high translocation potentials within plants (Bedell et al. 2009). However, the quantities of Cu and Pb in roots were higher than in stems and leaves across all treatments (**Article III**), indicating that high concentrations of Cu and Pb were retained in the roots of the plants. These results are consistent with those of Xiao et al. (2023), who reported that, in a pot experiment, Cd was mainly found in the leaves of *Salix* cultivated in CAPF soil, but Cu and Pb were primarily found in the roots. Additionally, in similar CAPF soil, *Quercus texana* leaves and *Q. pagoda* leaves contained higher levels of Cd than their roots. Among other *Quercus* species, Cd and Zn concentrations appeared to be in chronological order: roots > leaves > stems (Li et al. 2022b). Across all treatments, Mleczek et al. (2018) reported that the greatest quantities of Cr, Cu, Ni, and Zn were found in the roots, followed by the shoots and finally the leaves of *Salix*. Pb mainly accumulated in the roots of *Salix* in the studies of Zhivotovsky et al. (2011) and Wang et al. (2021). Similarly, Padoan et al. (2020) concluded that *Salix* clones accumulated the highest amount of Cu in stems. Metal accumulation varied within the different tissues of the plant and between treatments (Li et al. 2022b; Xiao et al. 2023). The results of this study (**Article III**) are also consistent with those reported by Rattan et al. (2005) and Irshad et al. (2015). According to Bouazizi et al. (2010), the higher accumulation of metal in roots results from a tolerance mechanism acquired by the plant that diminishes the effect of metal stress. As was observed in this study (**Article III**) for Cu and Pb accumulation, many metal-tolerant woody species restrict the translocation of metals to the shoot. This could be due to the presence of exclusion mechanisms probably meant to protect photosynthesis from toxic levels of metals (Borišev et al. 2009; Zhivotovsky et al. 2011). Roots possess a substantial attraction towards metals, but this affinity is limited only to the surface of the roots (Sahi et al. 2007).

In this study **(Article III**), the addition of BBC to the soil led to an increase in Cd, Zn, and Cu accumulation, as well as higher TF and BCF values, in *Salix*. BBC also increased soil pH levels, essential nutrients, and organic matter (**Article III**), while reducing pollutant toxicity through adsorption. This promoted plant growth and physiological parameters during the growth phase. The complexation and precipitation of metals, as well as the movement of metals and nutrients from the soil to the roots and from the roots to the shoot, may have been influenced by these growth and physiological parameters (see, e.g., Anjum et al. 2016, 2017; Ali et al. 2017; Li et al. 2022a). In various previous studies, soil pH and texture, nutrient levels, cation exchange capacity, and organic matter content were found to contribute to increased translocation of metals from soil to plants (e.g. Anjum et al. 2017; Gallego et al. 2012; Gordon 2015).

In phytoremediation experiments, metal and nutrient accumulation in plants has been shown to be significantly improved when the soil was supplemented with EDTA and EDDS (Meers et al. 2005, 2007, 2008), humic substances (Karaca et al. 2018), or biodegradable and environmentally friendly organic acid (Ding et al. 2014; Mleczek et al. 2018) or inoculated with growth-promoting rhizobacteria and fungi (Gordon 2015). Tai et al. (2018) reported that metal accumulation was significantly enhanced in plant tissues when EDTA and organic acid were added to the soil. In this study (**Article III**), total metal content in the whole plant increased when plants were supplemented with BBC. This may be due to elevated metal concentrations and the greater amount of dry biomass in the BBC-amended soil treatments. Compared to soil that had not been amended, Mohsin (2016) found that lime and wood ash significantly increased the total amount of Cu and Zn in plants. A low dose of BBC added to soil resulted in poor physiochemical properties of the soil, but a high dose of BBC resulted in an increased soil C/N ratio and the release of toxic metals. This adversely affects plant growth, physiological activities, and metal accumulation efficiency (see, e.g., Li et al. 2022a), resulting in reduced metal content in the plant. Ali et al. (2017) observed that BBC-amended soil reduced Cd, Zn, Cu, and Pb uptake by plants grown in mine-contaminated soils. Further, as BBC quantities were increased in the mine soil of Tongguan, the TF and BCF values for cultivated plants decreased, along with the uptake of Cd, Zn, Cu, and Pb by the plants (Ali et al. 2017).

Plants with TF and BCF values > 1 have been considered highly efficient for the remediation of contaminated soils (Arsenov et al. 2019, 2020). Moreover, TF and BCF values > 1 indicate that the plant is a hyperaccumulator (Antoniadis et al. 2017; Usman et al. 2019). Based on this study (**Article III**), *S. psammophila* may be a suitable plant for Zn accumulation in contaminated soils amended with BBC due to the higher TF value for Zn (TF > 1) than for other metals. Lower TF (TF < 1) values observed for Cd, Cu, and Pb may indicate that *S. psammophila* plants are suitable for the phytostabilization of Cd and Cu. This is because they mostly accumulate those elements in the roots, and plants excluded Cd, Cu, and Pb (TF < 1; Hussain et al. 2017). In this study (**Article III**), *S. psammophila* also showed lower Pb content in the BBC-amended soil. This could be because plants restrict metal translocation from the soil to the roots and from the roots to the aboveground tissues under metal stress. Additionally, metal mostly accumulated in the roots when the plants were grown in CAPF soil. Root mechanisms may slow the translocation of metals from belowground to aboveground plant tissues (Wieshammer et al. 2007).

In this study (**Article III**), a strong absorption for Cd (1 < BCF < 10, Nematollahi et al. 2020), intermediate absorption for Zn and Cu (0.1 < BCF < 1), and weak absorption for Pb (0.01 < BCF < 0.1) were found for *S. psammophila* grown in CAPF soil. In a pot experiment by Xiao et al. (2023), a high TF value for Cd (TF > 1) and a low TF value for Cu and Pb (TF < 1) were found for *S. jiangsuensis* '172' (SJ-172) grown in CAPF soil amended with bone biochar. The bone biochar-amended soil showed higher total metal content in the plant than unamended soil (Xiao et al. 2023). Adding BBC to soil accelerated Cd and Zn accumulation by *S. psammophila* plants, while Cu and Pb accumulation was slowed. As a result, increased TF values for Cd and Zn (TF > 1) were found for *S. psammophila* (**Article III**). The addition of BBC to CAPF soil also increased the total Cd and Zn content in the whole plant in a study by Li et al. (2022a).

The TF values of *Q. texana* and *Q. pagoda* plants for Cd were > 1 and for Zn were < 1 when plants were grown in CAPF soil in the field. Arsenov et al. (2017) reported that, when the soil was supplemented with citric acid, TF values in *Salix* increased by 30–37% compared to unamended soil treatments. This was because the addition of citric acid considerably improved the mobilization and translocation of metals around the plant. Similarly, Lehmann et al. (2003) reported that the addition of biochar increased plant accumulation of Zn and Cu, whereas Goliński et al. (2015) reported that *Salix* was an effective Zn accumulator (TF > 1).

Shi et al. (2017) noted that *S. integra* showed high TF values for Zn (1.42–2.18). Salam et al. (2019) also noted that *S. schwerinii* is a potential accumulator of Cu, Zn, and Ni in soils amended with lime and N10O. In a phytoremediation experiment, *Salix* genotypes were potential accumulators of Cd, Zn, and Cu (Yang et al. 2021). These cultivars also showed the best Zn phytoextraction potential. These differences (variation in metal accumulation in the different plant tissues and variation in TF and BCF values) may be due to species variability, metal behaviour in the plants, and exposure times and have also been reported by Arsenov et al. (2017, 2019, 2020).

5 CONCLUSIONS

Based on this work (**Articles I–III**), both *Populus* and *S. psammophila* seedlings have a high potential for survival, growth, and hydrocarbon (*Populus*) reduction from soil or metal (*Salix*) accumulation. However, *Populus* clones showed various responses in terms of resistance to pollutant stress, growth, and hydrocarbon reduction capacity under different soil treatments and growth conditions. Based on both the greenhouse and field experiment data, the hybrid aspen clones 14, 291, 34, 134, and 191, as well as the European aspen clones R2 and R3, are the most suitable options for phytoremediation. These clones displayed the abilities to withstand hydrocarbon stress, exhibit acceptable growth, and effectively remove hydrocarbons from contaminated soils. Specifically, clones 14, 291, R2, and R3 may be the most effective for remediating soil contaminated with hydrocarbons. Similarly, *Salix* cultivars exhibited diverse responses in growth and metal accumulation capacity with BBC-amended soil treatments. To further test the potential of these species for phytoremediation, we could evaluate their capabilities in soils contaminated either with hydrocarbons or metals along with BBC amendment. Some *Populus* clones and *Salix* cultivars could also be useful for assessing plant–soil interactions in many disturbed sites across the world, especially in areas affected by industrial activities that require restoration. However, unsuitable soil conditions, such as unbalanced pH levels, low nutrient levels, and elevated metal toxicity levels, can hinder plant-based remediation. Nutrient and pH levels are essential for regulating plant growth, which is the main requirement for efficient phytoremediation. Therefore, improving nutrient and pH levels through soil amendments like BBC could be an effective solution for remediating disturbed sites worldwide. Using varying planting densities is a viable method for investigating the potential of phytoremediation for the remediation of sites that are polluted with metals and hydrocarbons.

The findings of this work confirm that the selection of *Populus* clones and *Salix* cultivars for phytoremediation should focus especially on biomass production; genetic variations can be utilized to enhance the effectiveness of phytoremediation to remediate contaminated soil. Nonetheless, it is worth noting that the *Populus* and *Salix* plants in the greenhouse experiments (**Articles I** and **III**) were exposed to contaminated soil for a relatively brief period and grown under controlled environmental conditions. The plants in the field experiment (**Article II**) were also exposed for a limited duration. Therefore, it is a challenge to evaluate the phytoremediation potential of young *Populus* clones and *Salix* cultivars under these conditions. In boreal regions, it is also a challenge to implement efficient phytoremediation experiments due to the cold climate, slow plant growth, and low biodegradation rate. During the greenhouse experiment, different plant densities were also tested (**Article I**). To thoroughly test the phytoremediation capabilities of *Populus* clones and

Salix cultivars with different planting densities, further experiments must be conducted in the field. These experiments should use a range of *Populus* clones and *Salix* cultivars, as well as different soil amendments and plant densities. The tests should be conducted over 10 years for *Populus* and 4 years for *Salix* to provide comprehensive results on the survival and growth of the plants and the rates of removal of hydrocarbons and metals from the soil by the plants. This thesis presents a practicable demonstration of proficient clonal efficiency and soil amendment that can improve the phytoremediation capability of *Salix* and *Populus* and indicates the method's potential as a feasible, cost-efficient, and innovative approach for *in situ* remediations of soils contaminated by multiple metals and hydrocarbons. Other suitable *Populus* clones and *Salix* cultivars and fast-growing woody plants, such as birch, in combination with inorganic and biodegradable organic chelates, could also be used to assess plant growth capacity and plant–soil interactions to explore the phytoremediation efficiency of plants grown in soils contaminated with metals and hydrocarbons.

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APPENDIX 1

Table 3. Removal percentages of hydrocarbons by *Populus* seedlings grown in the field.

Clones	Pollutants (mg kg^{-1})	Soil depth at 5–10 cm		Reduction %	Soil depth at 10–50 cm		Reduction %
		year			year		
		2011	2017		2011	2017	
34	PAHs	22	18	15	2	4	nr
	C10–C21	30	33	nr	13	20	nr
	C22–C40	159	64	60	25	22	12
	C10–C40	189	95	50	40	42	nr
291	PAHs	297	201	32	119	159	nr
	C10–C21	246	146	41	167	182	nr
	C22–C40	991	325	67	509	400	21
	C10–C40	1237	453	63	675	600	11
27	PAHs	26	131	nr	2	3	nr
	C10–C21	49	63	nr	15	20	nr
	C22–C40	210	120	43	21	20	2
	C10–C40	258	180	30	32	40	nr
191	PAHs	202	90	55	32	12	61
	C10–C21	95	113	nr	48	21	56
	C22–C40	452	157	65	185	35	81
	C10–C40	253	268	nr	165	53	68
172	PAHs	161	190	nr	30	42	nr
	C10–C21	152	180	nr	75	48	36
	C22–C40	455	280	38	324	110	66
	C10–C40	606	474	22	399	160	60
134	PAHs	259	108	58	220	213	3
	C10–C21	266	90	66	116	210	nr
	C22–C40	1135	212	81	430	345	20
	C10–C40	1400	300	79	545	570	nr
14	PAHs	334	155	54	256	240	6
	C10–C21	278	101	64	343	165	52
	C22–C40	727	210	71	764	146	81
	C10–C40	1005	315	69	1105	310	72
R2	PAHs	244	45	82	74	8	89
	C10–C21	300	60	80	170	52	69
	C22–C40	980	185	81	579	90	84
	C10–C40	1280	241	81	749	137	82
R3	PAHs	253	448	nr	123	196	nr
	C10–C21	357	450	nr	610	207	66
	C22–C40	1016	660	35	554	353	36
	C10–C40	1373	1113	19	1165	565	52
R4	PAHs	132	137	nr	101	159	nr
	C10–C21	123	160	nr	96	195	nr
	C22–C40	709	340	52	330	368	nr
	C10–C40	675	500	26	303	558	nr

nr = no reduction.