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The Phytoextraction of Toxic Metals by Fast Growing Trees in Long Term Field experiment

Master´s thesis

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Declaration

I hereby declare that I have authored this master's thesis carrying the title "The Phytoextraction of Toxic Metals by Fast Growing Trees in Long Term Field experiment" independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I further state that I have not infringed the copyrights of third parties in connection with its creation.

In Prague on 26 April 2021 Duong Phiruny

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The Phytoextraction of Toxic Metals by Fast Growing Trees in Long Term Field experiment

Summary:

Pollution of heavy metals in soil has become main environmental problems. Phytoextraction is the promising tool to use for cleaning up the environment from contaminants. The phytoextraction by fast growing trees was investigated using the long term contaminated site. The objectives of this study were 1) to evaluate the ability of the uptake and accumulation amount of metals from soil by fast growing trees and 2) to compare the accumulation ability of metals between shorter and longer harvesting periods. For this study, rotation periods of 4 years and 2 years of *Salix* and *Populus* clones were set as experimental factor to observe if the rotation length could affect the removal of heavy metals. Two Salix clones (S1- (*S. schwerinii* × *S. viminalis)* × *S. viminalis),* S2 - *S. smithiana*) and two *Populus* clones P1 – (*P. maximowiczii* × *P. nigra,* P2 – *P. nigra)* were be investigated from 2016 and harvested in 2020 for 4 years rotation period as well harvested in 2018, and again in 2020 for two 2 years-rotation periods. Fresh harvested biomass was weighed, dried and weighed again for dry biomass. The grounded samples were used to determine the concent of risk elements. The results showed that 4 years-periods had better performance in term of biomass productions and elements removal. Clone that had the best performance was willow S2 due to its high biomass production and high accumulation of heavy metals. Between poplars P1 clone showed higher ability to accumulate metals, than P2. The highest remediation factors were found by S2 with the RF of Cd was 8.39%, Pb was 0.06% and Zn was 3.23% per four-years period. Rotation length did have crucial impact on the removal of concentration whereas, 4 years-rotation is recommended for the efficient removal of Cd and Zn from medium contaminated soil. Phytoextraction of Pb was not successful due to high soil Pb content and low mobility

Keywords: Phytoextraction, Heavy metals, Willows, Poplars

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1. Introduction

Heavy metals present naturally in soil. However, with the increases of industrialization due to the increases of human consumption, environmental contamination by heavy metals, their contents grow rapidly. Heavy metals contamination in soil is a serious environmental problem due to its possibility to accumulate in plants thus contamination of food chain which cause harmful effect to human health and environment. Heavy metals are different from organic pollutants due to their persistency in the environment. They are non-degradable and can be the threat for the environment in long term. Heavy metals have been introducing to environment through various sources, naturally and via anthropogenic activities includes mining, smelting, application of fertilizers, industrial processes. The toxicity of heavy metals is often determined by their mobility rather than their concentrations thus, their mobility is influenced by many factors such as soil pH, redox potential, soil organic matter and the concentration of heavy metal itself. Upon arising condition, many environmental cleanup techniques have been researched and implemented to access their presence and mobility in soil in order to provide the efficient method to free soil from these harmful contaminants (Tangahu et al., 2011). Recently, phytoremediation is one of the environmentally friendly and cost-effective technologies which is plant being used to extract contaminants from soil by plant root and being store in different plant part. Many studies have been conducted and many plants species have been successfully tested for their ability to take up various heavy metals such as Ni, Cd, Cr, Hg, As and more (Tangahu et al., 2011). Phytoextraction is one of the phytoremediation technologies which hyperaccumulator or accumulator plant species being used for the removal of contaminant from the soil through plant roots and translocate the contaminant to the above ground biomass of the plants which later can be harvested and burned gaining energy and recycling the metal from ash (Agbontalor, 2007). Plants that are suitable for this technology are those species that are tolerant to the high concentration of heavy metals, able to produce large amount of biomass, fast growth and extend root system (Di Lonardo et al., 2011).

2. Literature Review

2.1. Heavy Metals in the Soil

Heavy metals are the metallic chemical elements that are toxic and dangerous in low concentration. Those elements such as, mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb) become among the most toxic. Heavy metals are mostly present in all types of soils, but their concentrations are different. They are neither degradable nor destroyable and they are also known to be carcinogenic. Heavy metals are concerned due to their effects on human health and environment thus, their toxicities are usually influenced by dose, route of exposure, concentration, chemical species, gender and nutritional status of the individual (Tchounwou et al., 2012). They are presented in the environment naturally but through the increases of the anthropogenic activity, the concentration of these heavy metals also increases. Heavy metals are usually present in soil due to the weathering of parental rock and anthropogenic activities such as burning fossil fuels, ore smelting, or coal combustion. He et al., (2015) reported that there are more than 10 million sites of soil pollution were reported worldwide with more than 50% of those sites were contaminated by heavy metals and metalloids while in China the contamination by heavy metal is up to 80%. With this regards, heavy metal has become a global problem that require the concern from government, scientists and public concern. The government regulations have been set up to control the pollution. Regulatory standards of heavy metals for agricultural soil have been created but those standards are different depends on elements and countries (Table 1). Netherlands has seen to allow higher limit for As, Cd, Hg and Zn while Cr and Pb limits are highest in Germany, Cu and Zn limits are highest in USA (He et al., 2015).

Table 1 The regulatory standards of various heavy metals concentration ($mg.kg^{-1}$) in agricultural soils in different countries.

Country	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Australia	20	3	50	100	1	60	300	200
Canada	20	3	250	150	0.8	100	200	500
China	20-40	0.3-0.6	150-300	50-200	0.3-1.0	40-60	80	200-300
Germany	50	5	500	200	5	200	1000	600
Tanzania	1	1	100	200	2	100	200	150
Netherlands	76	13	180	190	36	100	530	720
NZ	17	3	290	$>10^4$	200	N/A	160	N/A
UK	43	1.8	N/A	N/A	26	230	N/A	N/A
USA	0.11	0.48	11	270	1	72	200	1100

2.1.1. Sources of Heavy Metals

Geological Sources

The occurrence of heavy metals in the soil could results from natural sources with the various concentrations. In unpolluted soil, heavy metals likely to originate from lithosphere mainly from the mineral part of the soil which is used to form the rock and minerals for earth’s crust. Through the process of weathering of those parent rock, those elements were introduced into soil profile but this process is rarely toxic (Smiljanić et al., 2019). Due to the disturbance of anthropogenic activities on geochemical cycle, some soil accumulates higher amount of heavy metal which could reach to be contaminated and toxic (Wuana & Okieimen, 2011).

Anthropogenic Sources

Although heavy metals are naturally present in soil, anthropogenic activity could increase the concentration as well. The increases due to soil can accumulate the emissions of heavy metals in the environment from rapid industrial processes especially in the urban and industrial areas. Industrial activities that could poses the risk of increase of the accumulation of heavy metals in soil includes mining, smelting, combustion of fossil fuels, disposal of both municipal and industrial waste. Besides industrial activities, the advancement of agriculture has increased the uses of pesticide and other advance application to secure the food production (Li et al., 2019). The presence of heavy metals which results from anthropogenic activities does not decay and poses the harm to soil environment, ecology and human health which is become the main issue nowadays. There are two types of heavy metals emission through anthropogenic sources, point sources and diffuse or nonpoint sources (Smiljanić et al., 2019).

Point sources are the types of emission of heavy metals through discharge from particular source includes industrial processes, mining, textile, wastewater by products, smelting which mostly industrial related. They can be easier to control (Smiljanić et al., 2019). Types of heavy metals correlates to point sources listed in table below.

Table 2 Types of heavy metals and their sources of releases through former and present industrial activities (Smiljanić et al., 2019)

Heavy metal	Sources of releases
Arsenic (As)	Phosphate fertilizers, mining, smelting, paints, textile, industrial dusts, pharmaceuticals, wastewater, pesticides, gold, lead, copper and nickel smelting, iron and steel production, industrial waste, and combustion of fossil fuel.
Cadmium (Cd)	Mining, application of phosphate fertilizer and pesticide, electronic applications, pigments and paints, industrial and incineration dust and fumes, wastewaters, battery, PVC products
Chromium (Cr)	Mining and metallurgy, plating of metal, rubber, photography, industrial dust and fumes, tanning, leather, chemical and textile industries, fertilizers, paints and pigments
Lead (Pb)	Mining, industrial dust and fumes, addition of lead in gasoline, fossil fuel combustion, solid and industrial waste, waste incineration, paints and pigments, explosive products, ceramics, some PVC products, pesticides, fertilizers, Manufacturing of lead—acid batteries and urban runoff.
Mercury (Hg)	Mining, smelting, Chlor-Alkali, scientific instruments, chemical production, industrial dust and wastewater, combustion of fossil fuel, incineration of solid and municipal waste, application of fertilizers and pesticides, Electrical switches, fluorescent bulbs, Mercury products (batteries, thermometers, mercury amalgam), cellulose, paper and explosive products
Nickel (Ni)	Mining, metal coating, iron-steel production, industrial dust, waste incineration, application of fertilizers, coal burning, Cd-Ni battery, chemical production, food processing industries.
Zinc (Zn)	Mining and metallurgy, Zn coating on iron-steel products, electroplating, fertilizers, Metal waste.
Copper (Co)	Mining and metallurgy, Plating, Rayon, waste from electronic products, application of pesticides, paints and pigments, textile production and explosive products.

The other types of anthropogenic source of pollution is diffuse or nonpoint sources. It is the pollution cause by unspecified sources and variety of activities which is harder to control and mostly results from application of fertilizer, agricultural practice, traffic and landfill. Through the intensive agriculture, in order to secure the production, large amount of fertilizer has been used worldwide. Phosphorous fertilizer usually contains the substituent of heavy metals concentration includes; Cd (cadmium), As (arsenic), Cr (chromium), Pb (lead), Hg (mercury), Ni (nickel), and V (Vanadium) (Smiljanić et al., 2019).

Aside from mineral fertilizers, organic fertilizers such as livestock manure and sewage sludge (biosolid) also contribute to the releases of heavy metals. Due to their rich of nutrients, sewage sludge such has been use in agriculture soil. Sewage sludge is produced by wastewater treatment plants. The application of sewage sludge has been used in many European countries due to its high content of N, P and other nutrients (Kubátová et al., 2016). These materials also contain various heavy metals such as; As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Tl, Sb and more. Heavy metals from wastewater mainly originated from industry, runoff and corrosion (Smiljanić et al., 2019). Table below shows the concentration of heavy metals in sewage sludge (Table 3).

Cd concentration ranged from nd (not detected) in Zhaoqing to 242 $mg.kg^{-1}$ in Beijing, Pb ranged from 9.11 $mg.kg^{-1}$ in Beijing to 255 $mg.kg^{-1}$ in Zhenyang, Cu ranged from 7.73 $mg.kg^{-1}$ in Beijing to 4567 $mg.kg^{-1}$ in Guangzhou, Zn ranged from 79.1 $mg.kg^{-1}$ in Beijing to 1177.62 $mg.kg^{-1}$ in Yangzhou, Cr ranged from 42.9 $mg.kg^{-1}$ in Xiamen to 1312.75 $mg.kg^{-1}$ in Yangzhou and Ni ranged from 46.5 $mg.kg^{-1}$ in Xi'an to 148 $mg.kg^{-1}$ in Guangzhou (Zhang et al., 2017)

Table 3 Concentrations of heavy metals in sewage sludge in cities in China

Sampling Site	Description	Cd	Pb	Cu	Zn	Cr	Ni
Shenyang	Mixed sewage sludge	5.0	255	170	290		
Xiamen	Dehydrated sewage sludge	2.75	22.2	157	397	42.9	83.8
Yangzhou	Dehydrated sewage sludge	2.41	137.94	251.52	1177.62	1312.75	79.68
Beijing	Sewage sludge Compost	242	9.11	7.73	79.1		
Guangzhou	Dehydrated sludge of mixed wastewater	5.99	81.2	4567	785	121	148
Zhaoqing	Dehydrated domestic sewage sludge	nd	17.4	93	509	15.5	51
Xi'an	Dehydrated sewage sludge	10.48	165.5	216.9	1101	772	46.5

Certain livestock manure (poultry, cattle, and swine) has been used also to apply as organic fertilizer to crops and pasture. In livestock productions (pig and poultry), Cu and Zn are added in the diet as growth promoter and As can also be found in the poultry health products. The concentration can be increased through rapid application which lead to the contamination of soil (Smiljanić et al., 2019).

2.1.2. Cadmium

Cadmium has proton number of 48 and is located at the end of the second row of transition elements, has atomic weight of 112.4, density 8.65 g cm^{-3}, melting point 320.9°C, and boiling point 765°C. Cadmium (Cd) is one of the most toxic elements in the environment such as in air, water, and soil. In soil, its presence results from anthropogenic activities and natural sources and become one of the hazardous elements in soil environment due to its toxicity even with low concentration. This element does not play any role in growth and evolution of human, plant and animal. Cadmium concentrations are different depends on soil (low concentration in uncontaminated soil and high concentration in contaminated soil). Generally, the content of Cadmium presents in sedimentary rock 0.3 $mg.kg^{-1}$ and in sandy soil 0.53 $mg.kg^{-1}$. Although cadmium does not contribute to any metabolic function in plant, it does accumulate in plant roots, shoots and other parts. Cd accumulation in vegetables and edible plants parts are the main exposure of Cd to human health (Khan et al., 2017). Cadmium in the environment mostly presents along with sulfate ores of zinc, can be form during the production of zinc industry and has been increased in the environment. The increase of Cd concentration in the environment is mainly due to smelting of ore, wastewater by product and overuse of fertilizer (Xu et al., 2020).

The presence of cadmium in soil can be from natural sources and anthropogenic activities. Naturally, the presence of cadmium in the atmosphere due to volcano eruption, forest fire and wind erosion. However, rock weathering is also responsible for cadmium content in the environment. Mafic and ultramafic rocks have high amount of cadmium (Hurst, 1995).

The Fig. 1 shows the naturally presented concentration of Cd in soil in various countries. (A) From natural/geogenic sources, (B) Site that impacted by mining, (C) Urban soils, (D) Soil irrigated by wastewater. In A, the content of Cd in Jamica is the higher compare to other countries which is around 200 $mg.kg^{-1}$, in B, the content of Cd is highest in Tunisia which is around 16 mg.kg^{-1}, in C, Cd content is highest in Pakistan which is 6 mg.kg-1 and in D, the content of Cd is highest in India which is around 19 mg.kg^{-1} (Khan et al., 2017).

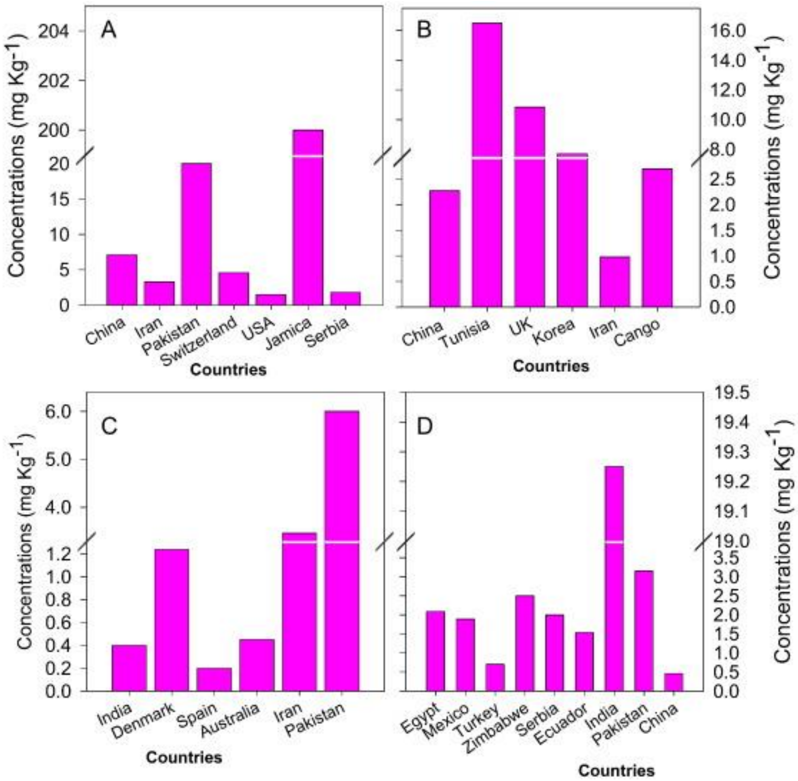

Figure 1 Concentration of Cd in soils of different countries. (A) From natural/geogenic sources, (B) Site that impacted by mining, (C) Urban soils, (D) Soil irrigated by wastewater.

In 2009, the total emission of cadmium in China was estimated to be 743 metric tons while industrial process contributed 57% of the total emission (Cheng et al., 2014). Beside industrial process, the uses of phosphorus fertilizer also contribute to the increases of cadmium concentration. Phosphorus fertilizers often contain considerate amount of Cd which will be later introduced to agricultural soil. Application of manure also contribute to the Cd concentration in agriculture soil. In addition, the uses of sewage sludge, industrial and urban waste also poses the risk of Cd in soil. These materials usually contain various heavy metals include Cd (Khan et al., 2017).

2.1.3.Lead

Lead is a metal belongs to group IV and period 6 of the periodic table with atomic mass 207.2, proton number 82, density 11.4 g cm^{-3}, melting point 327.4°C, and boiling point 1725°C (Wuana & Okieimen, 2011). Lead (Pb) contamination has become global concern due to its accumulation in food chain and groundwater. Lead is known as toxic to environment due to its persistence in the environment for long period of time (1000 to 3000 years). Lead presents in soil in the range between 10 $mg.kg^{-1}$ to 50 $mg.kg^{-1}$. Lead in soil solution is able to move from upper to lower horizon which could polluted also the groundwater. In soil, Pb often presents in various ions which characterized by its mobility, bioavailability and toxicity. Pb is usually present in soil in the form of Pb (II) (ionic lead), oxides and hydroxides, and lead-metal oxyanion complexes. The predominant form of Pb are phosphates, carbonates (at pH>6), hydroxides, oxides, sulfides, and pyromorphites. Those are known to be the most stable and insoluble forms of lead in soil (Rigoletto et al., 2020).

2.1.4. Zinc

Zinc belongs to group IIB, period 4, proton number 30, atomic mass 65.4, density 7.14 g cm^{-3}, melting point 419.5°C, and boiling point 906°C. Zinc is one of the essential elements for plants, human and animals in term of growth and development. Zinc content in uncontaminated soil is influenced by rock weathering chemical composition of parent rock. The content of Zn in rock vary depends on rock types. In magmatic rock is between 40 to 120 $mg.kg^{-1}$ while in sedimentary rock ranges from 20 to 80 $mg.kg^{-1}$, in sandstone is between 15 to 30 $mg.kg^{-1}$ and in limestone and dolomite is from 10 to 25 $mg.kg^{-1}$ (Noulas et al., 2018).

Zinc in undisturbed agricultural soils ranges from 10 to 300 $mg.kg^{-1}$. Sandy soil holds lowest value of Zn content while highest is in organic soils. Even though Zn is an essential element, but it can be toxic with excess amount (Tóth et al., 2016). The excess amount of Zn can be caused by natural weathering and anthropogenic activity as its described in table 2. Zn can interrupt the soil activity by its negative effects on the activity of microorganisms and earthworms, and the breakdown of organic matter (Tóth et al., 2016).

2.1.5. Mobility and Bioavailability of Heavy Metals

Characterization of bioavailability, leachability and toxicity is very important because the hazards of these elements are defined by their mobility rather than total concentration in soil. To understand their bioavailability, knowledge about their distribution in soil fraction is very important. As I have mentioned, metals are part of natural soil component, their presents are most likely to be in mineral fraction which is an important component of clay minerals, iron and manganese oxide which have significant influences on soil chemistry. The bioavailability, leachability and toxicity of heavy metals in soil environment are controlled by chemical reactions includes, redox reaction, sorption/desorption, complexation and dissociation (Violante et al., 2010). Some metals are less mobile, and some are more mobile in soil and their movement usually happen through soil profile to aquifer or via the uptake by plant roots. Heavy metals are more toxic when they are more mobile because they can be easily taken up by plant and also moved to ground water. The bioavailability of metal in soils most likely depends on the partition of the metals between the solid and solution phases (Rieuwerts et al., 1998).

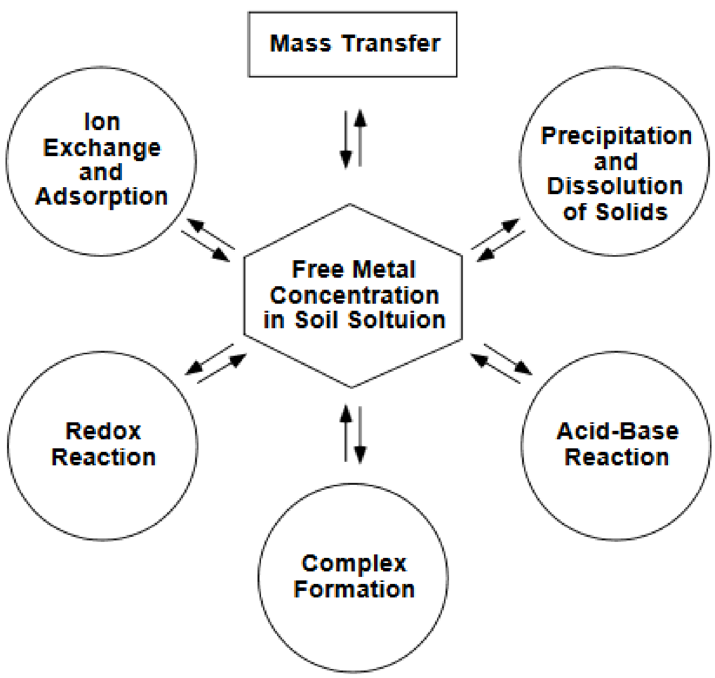

Figure 2 Principal control of free trace elements concentration in soil (Mattigod et al., 1981)

Sorption and Desorption

Sorption is the process in which the compound is being removed from solution to solid phase while in contrast, desorption is when ion or molecule from solid phase is being released to solution (Sposito, 2008). In sorption-desorption process, organic and inorganic ligand control the solution concentration thus bioavailability, leachability and toxicity of heavy metal in soil. Some elements are present as solution in soil in both cationic and anionic form but predominantly cationic. Heavy metals in soil form soluble complex with organic (aromatic, and amino acids and soluble constituents of fulvic acids) and inorganic ligand (SO_4^{2-}, Cl^-, OH^-, PO_4^{3-}, NO_3^- and CO_3^{2-}). Soil components that are involved in sorption of heavy metals includes, humic substances, phyllosilicates, carbonates, and other minerals includes Fe, Mn, Al, Ti oxide. Generally, high sorption in soil result lower concentration of heavy metals in soil solution (Violante et al., 2010).

Precipitation and dissolution

Precipitation is the process of heavy metal to leave their solution state to form solid phase with other chemical agent (phosphate, carbonate, and sulfate) in soil. Formation of solid phase occur when heavy metals are compatible with the element of clay mineral and thus able to replace the element through clay mineral. For example, the replacement of Cd with Ca in $CaCO_3$, this reaction happens because Ca and Cd have the same ironic and most likely to happen only when Cd concentration is high (Rieuwerts et al., 1998). In contrast, dissolution is the transformation of metal from precipitation to solution which allow plants and other organisms to take them up because they are more mobile in solution form (Misra et al., 2009).

Redox reaction

Reduction of heavy metals in soil may happened chemically as well biologically. Redox reaction plays an important role in heavy metal transformation. Redox reactions, both biotic and abiotic are responsible for controlling oxidation state of some heavy metals includes Pb, Cr, Se, Co, As, Ni and Co and also their mobilities, leachability and toxicity. For example, Cu (II) reduce to Cu (I) by reaction of Fe^{2+} or hydrogen sulfide (H_2S). Aside from iron, microorganisms in soil also play important role in redox reaction. For some highly toxic metals, microbes in soil reduce their toxicity via detoxification pathway (e.g. Cr, Hg, U) (Violante et al., 2010). Redox reaction of iron also control mobility and toxicity of Cd in rhizosphere by oxidizing to Fe plaque and absorb

Cd concentration in rhizosphere thus it limit the concentration being taken by plant and decrease the availability of Cd (Zhang et al., 2012).

Factors influence mobility and bioavailability of heavy metals in soil:

- **Soil pH:** soil pH influences the mobility. The mobility of heavy metal is lower in alkaline soil solution and thus high in acidic soil solution.
- **Soil organic matter:** dissolved organic matter interacts with the clay minerals which lead to the limit the access for microorganisms. SOM of the clay fraction could stabilize the effect of the metals and decrease their bioavailability (Quenea et al., 2009).
- **Soil texture:** soil texture affects sorption. Soil particle size influence the solubility of metal in soil. For example, Cd is more soluble and easily available for plant in sandy soil than in clay soil. Pb is also more available in clay fraction but also in sandy fraction (Rieuwerts et al., 1998).
- **Soil moisture and temperature:** soil moisture and temperature could increase the microorganism in soil which influence redox reaction. For example, the reduction of $Cr^{(VI)}$ by Cr-reduction-bacteria is increase when soil temperature increases.
- **Bioavailability of heavy metals:** Concentration and speciation also influence the reaction. E. g. Se reduction increases with the increase of concentration.

2.1.6. Effect of Heavy Metals on Soil Microorganism

Heavy metal pollution in soil is considered as one of the main environmental problem. Heavy metals effect soil biology especially, microorganism community. Heavy metal has metallic properties such as ductility, malleability, conductivity, cation stability, and ligand specificity (Singh & Kalamdhad, 2011). Heavy metals affect microbial process by their toxicity and decrease their microbial population. Soil microorganisms are very important for maintaining the quality of soil and participate in formation of soil organic matter, decompose hazardous substances and also play an important role in biochemical cycle of soil. Heavy metal interferes with enzymatic activity of microorganism. In serious contaminated area, heavy metal inhibits microbial activity which has serious impact on soil environment and ecosystem (Xie et al., 2016).

Table 4 Activity of heavy metals in soil and their effects on soil enzymatic activities

Heavy metal	Activity in soil	Reference
Cd	Reducing microbial population and activity, interfere with microbial respiration and decrease activity of soil enzyme (protease, urease, alkaline phosphatase and arylsulfatase)	(Mahmood et al., 2018; Shi & Ma, 2017)
Pb	Decrease activity soil enzyme (urease, catalase, invertase and acid phosphatase)	(Singh & Kalamdhad, 2011)
Cr	Reduce microbial population, affect microbial cell and metabolism	(Singh & Kalamdhad, 2011)
As	Influence the proliferation of specific microorganism results in decrease the diversity of tolerant species, inhibit phosphatase and sulfatase.	(Singh & Kalamdhad, 2011; Turpeinen et al., 2004)
Zn	Inhibition of toxicity to cellular activities and growth of microbes	(Babich & Stotzky, 1978)

2.2. Heavy Metal Uptake Mechanism by Plants

The accumulation of heavy metals in plant usually undergo many processes those include; heavy metals mobilization, root uptake, xylem loading, transportation from root to shoot, compartmentation of cellular and sequestration (Yan et al., 2020). The uptake of heavy metals is usually being done by plant roots. Plant root has ability to release root exudate that is being used for solubility of microelement to support their system (Liphadzi & Kirkham, 2005). Plant can also pose the ability to translocate and store these materials. The evapotranspiration process allow water that are evaporating from plant leaves to absorb nutrient and other element from soil transfer them into roots and later from roots to shoots. The uptake of those elements occurs in two pathways, passive diffusion and active transport.

Upon entering root cell, heavy metals are able to form various complexes with other chelators such as organic acids. These complexes include carbonate, sulfate and phosphate precipitates later are immobilized through extracellular space and intracellular space (vacuoles). The sequestration of these heavy metals occurs in vacuole and later may being transported to xylem stream or stele through root symplasm and can be translocated to shoot through xylem vessels. Active and passive transports allow these heavy metals to distribute into leaves, cellular compartments and plant vacuole where they are being sequestrated (Yan et al., 2020).

2.2.1.Factors Affect the Uptake Mechanism

The uptake mechanism of heavy metals is affected by many factors:

- Plant species: The ability to take up heavy metal is likely to depend of the plant species. Different plant species able to uptake and accumulate different amount of heavy metal and their biomass production is also different (Tangahu et al., 2011).
- Soil properties: pH, addition of fertilizer and chelate, are also the factor that influence the uptake. The uptake of some metals like Pb is influenced by pH, organic matter and the content of phosphorus in soil (Tangahu et al., 2011b).
- Root zone: As we know that plant roots produce root exudates to solubilize the metal in root zone, the better root distribution in soil profile, the better the uptake of metals (Keller, 2003).
- Climate: Temperature affects the root development. The increase of soil temperature influences the growth of root due to the improvement of metabolic activity of root cells. In contrast, lower soil temperature results in reduced tissue nutrient concentrations which leads to poor root growth (Onwuka, 2018).
- Addition of chelating agent: Addition of chelating agent, micronutrients, and stimulation of microbial activity in soil leads to the increases of bioavailability of heavy metal in soil. In some phytoremediation methods, synthetic chelating agent such as NTA and EDTA are used to improve the phytoextraction of heavy metal in contaminated soil (Tangahu et al., 2011).
- Bio availability of heavy metal: The availability of heavy metal in root zone is also the factor affects the uptake. High accumulation can be done only at the site contain the high concentration of heavy metal in soluble form (Sheoran et al., 2016).

2.2.2.Effects of Heavy Metals on Plant Development

Heavy metal poses toxicity to plant. Macro and micronutrients are essential for plant growth and development. Although some metals (Co, Mo, Zn and Ni) are microelements, with the exceed concentration could be harmful for plant. Cd, Pb and As may also have positive impact on plant at low concentration (Alloway Brian, 2013). In adverse effect, the high accumulation of heavy metal from contaminated soil can cause negative impact on plant. When being accumulated in plant tissue, heavy metals produce toxic effects in plant which results in interference of growth and development, seed germination, root elongation, photosynthesis, chlorosis, and other negative impact. Table 5 below shows the toxicity of heavy metal element to plant and plant parts (Fazal Ur Rehman Shah, Nasir Ahmad, Khan Rass Masood, Jose R. Peralta-Videa, 2010).

Table 5 Toxicity of heavy metal on plant

Heavy metal	Toxicity in plant	Reference
Cd	Inhibit plant growth and production by causing both biotic and abiotic stress, reduce chlorophyll content and reduce photosynthesis.	(Shanmugaraj et al., 2018)
Pb	Inhibit seed germination, root elongation, plant growth, seed development, transpiration, inhibit chlorophyll, water and protein content.	(Pourrut et al., 2011)
Cr	Inhibit root elongation, germination of seed, growth in stem and leaves and effect on total dry mass production and yield, reduction of photosynthesis and water retention.	(Shanker et al., 2005)
As	Interfere with plant growth and development, causing reactive oxygen species induce lipid peroxidation which leads to plant death	(Farooq et al., 2016)
Zn	Causing root blunt, thickening and restraint on root cell division and elongation reduce flower production, inhibited Fe translocation in young leaves which lead to curling, rolling and death of leaf tip and chlorosis.	(Rout. R.G., 2003)

2.3. Phytoremediation of Heavy Metals

As the pollution of heavy metal in soil exceeds, many remediation techniques of heavy metal have been developed. The term of phytoremediation refers to the uses of plants (trees, shrubs, grasses, and aquatic plants) to remove, degrade or isolate the contaminants from environment (Ansari et al., 2016). There are several technologies within phytoremediation such as, phytoextraction, phytostabilisation, phytovolatilization and phytofiltration.

- Phytoextraction: Contaminants are taken up and translocated by plant roots to above ground part of plant (shoot) which can be harvested (Tangahu et al., 2011).
- Phytostabilisation: This is a technology to prevent the migration and movement of heavy metals in soil through erosion or deflation. The certain plant species are used to immobilize these contaminants in soil by adsorption into roots or precipitate in root zone (Bolan et al., 2011).
- Phytovolatilization: In this technology, plants are used to take up contaminants from soil, convert these elements to less toxic volatile form and release into atmosphere through plant transpiration process via leaves (Yan et al., 2020).
- Phytofiltration: Contaminants are taken up by roots of plants from water or contaminated aqueous environment (Ansari et al., 2016).

2.3.1. Phytoextraction of Heavy Metals

Phytoextraction is one of the sub-processes of phytoremediation by which is metal being removed from soil and accumulated in plant tissues. This method is simple and eco-friendly, therefore is in the center of interest when we talk about cleaning up the soil environment. In phytoextraction, heavy metals are removed from soil by plants that accumulated, utilized, transported, and concentrated those contaminants in their shoots. The criteria for plants to be used for phytoextraction are; (1) tolerance to heavy metal even in high concentration, (2) high biomass production, (3) effective for accumulation of heavy metal in plant parts that are easy to harvest (Anoopkumar et al., 2020).

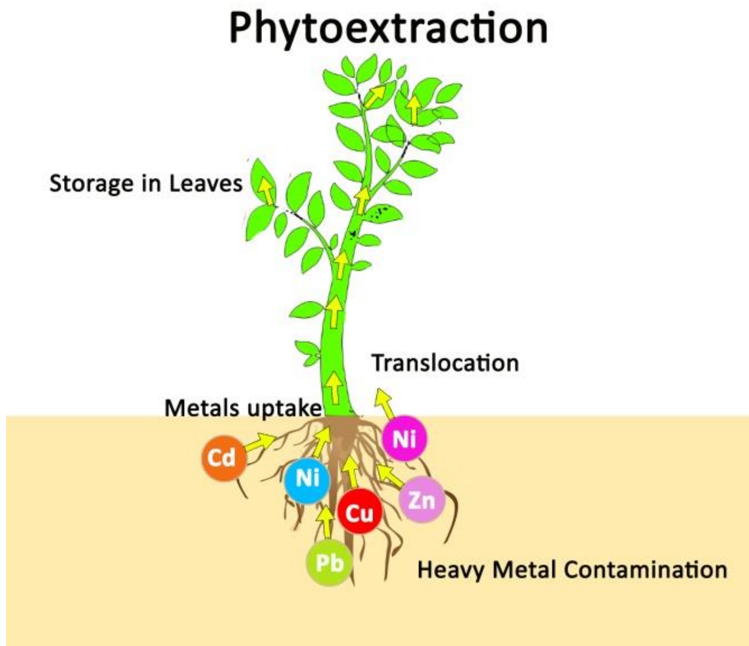

Figure 3 Phytoextraction of heavy metals. The heavy metals such as Cd, Ni, Co, Pb and Zn in soil are transferred to the plant shoots via roots (Anoopkumar et al., 2020).

2.3.2.The Effectiveness of Phytoextraction

The efficiency of phytoextraction depends on many factors includes soil type, site location, type of trees and their characteristic such as the ability to accumulate heavy metals and the density of their root system. Several studies have been conducted to experiments on tree species and their ability to accumulation. Edible plants are reported not to be suitable for phytoextraction due to the risks in food chain. In contrast, ornament plant (*Calendula officinalis* L., *Tegetes erecta* L., *Celocia cristata* L., and *Chrysanthemum indicum* L.), fast growing trees (willow, poplar) are more suitable for this process (Asgari Lajayer et al., 2019 and Kubátová, et al., 2018).

Plant acts either as “accumulator” or “excluder”. The role of plant as accumulator is being able to take up the toxic element and being survive despite their concentration in plant tissue. This function also able to transform toxic element or biodegrade into inert form and store in their tissue (Jan & Parry, 2016). Whereas the role of excluder is the plant that is tolerant to heavy metal which happen mostly when plant can survive in the contaminated soil. They most likely adopt the tolerance by permit the toxicity in root only (Baker, 1981).

Hyperaccumulator is the type of plant that has ability of take up particular metal or metalloid up to thousand ppm (Tangahu et al., 2011). Heavy metals that are accumulated by hyperaccumulators do not retain in the roots, but they are translocated to shoots and later being accumulated in above ground organs e.g. leaves. They can concentrate heavy metals up to 100 to 1000 times higher that nonaccumulators while showing no symptom of phytotoxicity (Rascio & Navari-Izzo, 2011). According to Global Database of hyperaccumulators species in 2017, there are 721 species of hyperaccumulator those includes 523 species for nickel, 53 species for copper, 42 species for cobalt, one specie for chromium, 42 species for manganese, 20 species for zinc, two species for rare earth elements, 41 species for selenium, two species for thallium, seven species for cadmium, five species for arsenic, and eight species for lead and some species can hyperaccumulate more than one element (Reeves et al., 2018).

The highest concentration of Cd was found in *Phytolacca Americana,* As was found in Pteris vittata, Pb was found in Pteris vittata, Zn was found in *Thlaspi caerulescens* and Ni was found in *Psychotria douarrei* (Table 6) (Yan et al., 2020).

Table 6 Heavy metal and their concentrations in specific types of plants species which were grown in different environments

Heavy metal	Plant species	Maximum concentration in plant ($mg.kg^{-1}$)
Cd	*Phytolacca Americana*	10,700
	Sedum alfredii	9,000
	Prosopis laevigata	8,176
	Arabis gemmifera	5,600
As	*Pteris vittata*	8,331
	Pteris ryukyuensis	3,647
	Pteris quadriaurita	2,900

	Corrigiola telephiifolia	2,110
Pb	*Medicago sativa*	43,300
	Brassica juncea	10,300
	Brassica nigra	9,400
	Thlaspi rotundifolium	8,200
Zn	*Thlaspi caerulescens*	51,600
	Eleocharis acicularis	11,200
	Thlaspi calaminare	10,000
	Deschampsia cespitosa	3,614
Ni	*Psychotria douarrei*	47,500
	Phyllanthus serpentinus	38,100
	Alyssum markgrafii	19,100
	Alyssum corsicum	18,100

2.4. Accumulation of Metals in Willow and Poplar Biomass

Fast growing trees are known for their biomass production and good accumulation of pollutants. Fast growing trees have been used in phytoremediation due to their rapid growth, deep root system, high biomass production and their abilities to accumulate contaminant. Phytoextraction by Salicaceas species, poplar (*Populus sp.*) and willow (*Salix sp.*) has been recognized as efficient tool to clean up contaminated site due to their large biomass production (Pajević et al. 2016). Although, compare to other hyperaccumulator species, willow and poplar take up lesser amount of heavy metals but they could provide economic benefits due to their biomass production. The research of Kubátová et al. (2018) showed a Salix clone, *S. smithiana* could produce biomass yield up to 14.55 $t.ha^{-1}$/year. The same research also shows *Populus* clone, *P. maximowiczii* ×*P. nigra* could produce biomass yield up to 14.55 $t.ha^{-1}$/year .

Willow and poplar are reported to be able to accumulate high amounts of Cd and Zn. However, the metal accumulation ability depends on clones, growth performance, root density and period of harvest some clones that accumulate low amount of these metals (Greger & Landberg, 1999). Kacálková et al., (2015) shows that Cd and Zn concentrate much more in leaves follow by twigs and roots. *Populus nigra x maximowiczii* accumulate Cd 3.03 $mg.kg^{-1}$ and Zn 561 $mg.kg^{-1}$ in leaves. However, there are also some studies found that Zn concentrates highest in root than in other parts of poplar. The study of Fernàndez et al., (2012) shows that Zn is mainly accumulate in roots (40 000 mg $Zn.kg^{-1}$) in *Populus deltoides x maximowiczii* and *P. x canadiensis euramericana.* The research of Kubátová et al., (2018) shows 2 *Salix* clones (*S. schwerinii* × *S. viminalis)* × *S. viminalis and S. smithiana*) accumulated Cd 58.1 $mg.kg^{-1}$ in leaves. In the same study also shows that Pb concentrate slightly more in branch in *Populus* clone (*P. maximowiczii* × *P. nigra*) which is 30.6 $mg.kg^{-1}$.

The accumulation of heavy metals by fast growing trees can be improved by many factors as following:

Application of sewage sludge: the application of sewage sludge could increase the mobility of heavy metals in soil which make them easily be taken up by plants. The experiment of Kubátová et al. (2016) shows that the removal of Cd, Zn and Pb from harvesting the shoot of *Salix* clone (*S. schwerinii* × *S. viminalis*) × *S. viminalis*) that were treated with sewage sludge is higher than in the control treatment.

Age of trees: many studies have confirmed that the accumulation of heavy metals varied with the age of trees. Metal's accumulation is higher in trees during their early growth stage due to their high nutrient uptake. The study of Kubátová et al. (2018) shows that Cd, Pb and Zn concentrations in Salix clone (*S. smithiana*) are highest during the first 4 years than 6 years of planting.

Seasonal variation: season affects concentration of metals in trees. The study of Kubátová et al. (2018) shows that the concentrations of Cd, Pb and Zn in 2 Populus clones (*Populus deltoides x maximowiczii and P. x canadiensis*) and 2 Salix clone (*S. schwerinii* × *S. viminalis*) × *S. viminalis* and *S. smithiana*) are higher during summer harvest compared to winter harvest due to the higher phytoextraction efficiency in summer.

3. Scientific Hypothesis and Objectives

3.1. Hypothesis

We expect that fast growing trees will be able to take up sufficient amounts of metals from the soil and accumulate them in the above ground biomass. The accumulation will differ according to metals and trees species.

3.2. Objective

The aim of this study is 1) to evaluate the ability to uptake and accumulate amounts of metals from soil by fast growing tree and 2) to compare the ability to accumulate metals between tree species.

4. Material and Method

4.1. Study Site and Field Experiment

The experimental field was established in April 2008 on multi-risk-element contaminated agricultural soil (49°42'24"N, 13°58'32"E), mostly by Zn, Cd and Pb. The site is near the town of Příbram, 58 km south of Prague, Czech Republic. The site was previously known to be long term mining and smelting site of lead ore and was contaminated by the emission from smelter nearby. The elevation of the site is 500 m above sea level, with a mean annual precipitation of 700 mm and a mean annual temperature of 6.5 ℃.

Two *Salix* clones ((*S. schwerinii* × *S. viminalis)* × *S. viminalis* and *S. smithiana*) and two *Populus* clones *((P. maximowiczii* × *P. nigra) and P. nigra)* were grown in the contaminated site (hereafter donate S1, S2, P1 and P2). Those homogeneous 20-cm long cuttings of clones were used in this experiment. The experimental site comprises total of 32 rows, 8 plots with 4 rows and each row contain 1 clone. Each row has the area of 7.5m x 1.3m while clones were planted 0.25m away from each other. Whole plots were arranged randomly with four replications. Each plot has four sub-plots, corresponding to two Salix *clones* and two *Populus* clones.

The soil condition of the experimental site is weakly acidic Haplic Cambisol. The cation exchange capacity of 166 $mmol_{H+}kg^{-1}$, C_{org} of 4.1%, C/N ratio of 9. The soil had mean pH_{H2O} of 5.66 and pH_{KCl} of 5.27. Pseudo-total (aqua regia-soluble) mean (± standard error) concentrations of REs in the soil (0–20 cm) were as follows: 7.3 ± 0.22 mg Cd/kg, 218 ± 5.9 mg $Zn.kg^{-1}$ and 1370 ± 33 mg $Pb.kg^{-1}$ (n = 50).

The first harvest was done in 2012 after 4 years of planting. 2 years after, the second harvest was carried out in February 2014 and the third harvest were carried out in February 2016 which is also 2 years after the last harvest, in February of 2016 the harvest after 4 years was done as well as published in the study of Kubátová et al. (2018). Our experiment started after harvest of 2016, the results present the harvest at 2020, for 4 years harvest period and results 2 years harvest period were carried out in February 2018 and again after 2 years in February 2020.

4.2. Harvest of Plant Material

The fresh plant material (branches) was cut 20 cm to determine total concentration of risk elements. After harvested, fresh samples were weighed for fresh matter at the field. Later, the branches were dried at 60 ℃ and weighed for dry matter.

4.3. Laboratory Analysis

The dried biomass samples were ground using stainless steel Retsch friction mill (Retsch, Haan, Germany; particle size 0–1 mm). The total concentrations of elements (Cd, Pb, and Zn) in branches' biomass were analyzed using inductively coupled plasma with optical emission spectroscopy (ICP-OES; Agilent 720, Agilent Technologies Inc., USA). The samples were decomposed using dry ashing procedure.

Dry ashing procedure (dry decomposition)

- The dry sample is inserted onto a cold hot plate, starting with a temperature of 170 °C
- One after the temperature was increased to 230 °C, one hour later to 290 °C, and after one hour to 380 °C. This temperature was set for one hour and then the hot plate was turned off.
- The samples were transferred to a cold muffle furnace, at 350 °C. The temperature was increased to 400 °C after 30 min, then increased to 450 °C and after 30 min and increased 500 °C after 30 min
- Samples were left at 500 °C temperature for 16 h

4.4. Remediation Factor (RF)

The potentials of trees phytoextraction were expressed as remediation factor (RF) which refers to the proportion of elements removed by harvested biomass from the total contents of elements at the site was calculated as:

$$RF\ (\%) = \frac{C_{plant} DM_{Plant}}{C^{soil} W^{soil}}$$

Where:

C_{plant} is the concentration of metals in plant dry biomass (g.t^{-1})

DM_{plant} is dry matter of plant biomass yield (t)

C_{soil} is the total concentration of metals in soil (g.t^{-1})

W_{soil} is the amount of soil in top horizon (t.ha^{-1})

This formula was modified according to (Komárek et al., 2008)

4.5. Statistical Analysis

The data was analyzed using SPSS version 25 (IBM, SPSS, USA). Data were checked for homogeneity of variance and normality by using Levene and Shapiro–Wilk tests. Collected data did not meet assumptions for the use of analysis of variance (ANOVA) and were necessary evaluated by the nonparametric Kruskal–Wallis test (Kubátová et al., 2016)

5. Results

5.1. Concentration of Elements in Willow and Poplar Clones

The results from table 7 showed the concentrations of Cd in S1 clone. The highest in the order 2020_{2y}>2018_{2y}>2020_{4y} whereas for S2, the Cd concentrations were highest in the order 2020_{2year}>2020_{4y}>2018_{2y}. There were statistically different between 2020_{4y} and other harvest periods. In P1, the concentrations of Cd were highest in the order 2018_{2y}>2018_{2y}>2020_{4y} while P2 ranked in the order 2020_{4year}>2018_{2y}>2020_{2y}. Among all clones both *Salix* clones (S1 and S2) had highest concentration of Cd ranged from 18.28 mg.kg^{-1} to 20.95 mg.kg-1 and the highest value was in S1 of 2020_{2y}. In contrast, both *Populu*s clones had lower Cd accumulation which ranged between 9.22 mg.kg^{-1} to 12.5 mg.kg^{-1} and the lowest was in P1 in 2020_{4y}.

Pb concentrations in S1, S2 and P1 were highest in the order 2020_{4y}>2018_{2y}>2020_{2y} while P2 value were in the order 2020_{4year}>2018_{2y}>2020_{2y}. Among all clones, P1 had the highest concentrations of Pb in all harvest periods which ranged from 30.01 mg.kg^{-1} to 31.97 mg.kg^{-1}. Meanwhile the lowest Pb content was found in P2 in 2018_{2y} with the value of 17.7 mg.kg^{-1}. The concentration of Pb in both Salix clones ranged between 18.3 mg.kg^{-1} to 24.41 mg.kg^{-1}.

The concentrations of Zn for S1, P1 and P2 were highest in the order 2020_{2year}>2018_{2y}>2020_{4y} while S2 were highest the order 2020_{2year}>2020_{4y}>2018_{2y}. Amongst all clones, S1 had the highest concentration of Zn ranged from 215.19 mg.kg^{-1} to 249.54 mg.kg^{-1}. In contrast, the lowest concentrations were in P1 which was 106.24 mg.kg^{-1} in 2020_{4y}. The concentrations of Zn in S2 were between 190.22 mg.kg-1 to 217.22 mg.kg-1 and in P2 were between 107.19 mg.kg^{-1} to 121.64 mg.kg^{-1}.

Table 7 Mean (± SE) concentration of Cd, Pb and Zn in branches of *Salix* (S1- (*S. schwerinii* × *S. viminalis)* × *S. viminalis)*, S2 - *S. smithiana*) and *Populus* P1 – (*P. maximowiczii* × *P. nigra, P2 – P. nigra*) in 20182y, 20202y and 20204y harvest.

Elements	Harvest Year$_{period}$	Clones			
		S1	S2	P1	P2
Cd (mg.kg^{-1})	2018$_{2y}$	20.45±0.74^{ACa}	18.7±1.41^{Ab}	10.39±0.5^{Aa}	12.5±0.86^{Ab}
	2020$_{2y}$	20.95±0.73^{ABbd}	20.66±2.32^{ABac}	10.99±1.18^{ABcd}	10.13±0.61^{ABab}
	2020$_{4y}$	18.28±1.06 ^{BCbd}	17.6±0.98 ^{BCac}	9.22±1.01^{BCab}	11±1.45^{BCcd}
Pb (mg.kg^{-1})	2018$_{2y}$	18.3±0.9^{Ba}	18.23±2.32^{Bb}	30.07±1.31^{Babc}	17.7±0.99^{Bc}
	2020$_{2y}$	20.25±1.14^{ABc}	21.2±4.16^{Aa}	30.01±1.91^{Aab}	18.09±1.39^{Ab}
	2020$_{4y}$	21.38±1.27^{ABb}	24.41±1.8^{ABc}	31.97±2.15^{ABab}	19.78±1.51^{ABa}
Zn (mg.kg^{-1})	2018$_{2y}$	228.9±9.42^{Bab}	190.22±13.89^{Bb}	120.89±5.68^{Ba}	113.6±4.95^{Bb}
	2020$_{2y}$	249.54±8.67^{Abd}	217.22±21.97^{Aac}	120.63±8.15^{Bab}	121.64±7.55^{Bcd}
	2020^{4y}	215.19±15.13^{ABbd}	212.21±13.09^{ABac}	106.24±6.17^{ABcd}	107.19±14.18^{ABal}

Difference between clones and harvest periods were evaluated using the Kruskal-Wallis test at $P \leq 0.05$. Clones with the same capital letter were not significantly different. In each clones, harvest periods with the same capital letters were not significantly different. Within harvest period, clones with same lowercase were not significantly different.

5.2. Total Biomass Yield

Figure 4 described the comparison of total dry biomass between 2018$_{2y}$ and 2020$_{2y}$ and 2018$_{2y}$ + 2020$_{2y}$ and 2020$_{4y}$ of all 4 clones. Dry biomass of all clones were highest in 2020$_{4y}$ compared to other harvest periods and compared to 2018$_{2y}$ + 2020$_{2y}$. The highest value was in clone S2 in all harvest period with the value of 89.63 t.ha^{-1} in 2020$_{4y}$ and between 16.26 t.ha^{-1} to 30.78 t.ha^{-1} in 2018$_{2y}$+2020$_{2y}$. The lowest biomass production can be observed in P2 with the value ranged from 12.27 t.ha^{-1} in 2020$_{4y}$ and between 3.06 t.ha^{-1} to 3.71 t.ha^{-1} in 2018$_{2y}$+2020$_{2y}$. P1 and S1 value ranged between 12.27 t.ha^{-1} 54.31 t.ha^{-1} in 2020$_{4y}$ and between 6.7 t.ha^{-1} to 17.43 t.ha^{-1} in 2018$_{2y}$+2020$_{2y}$.

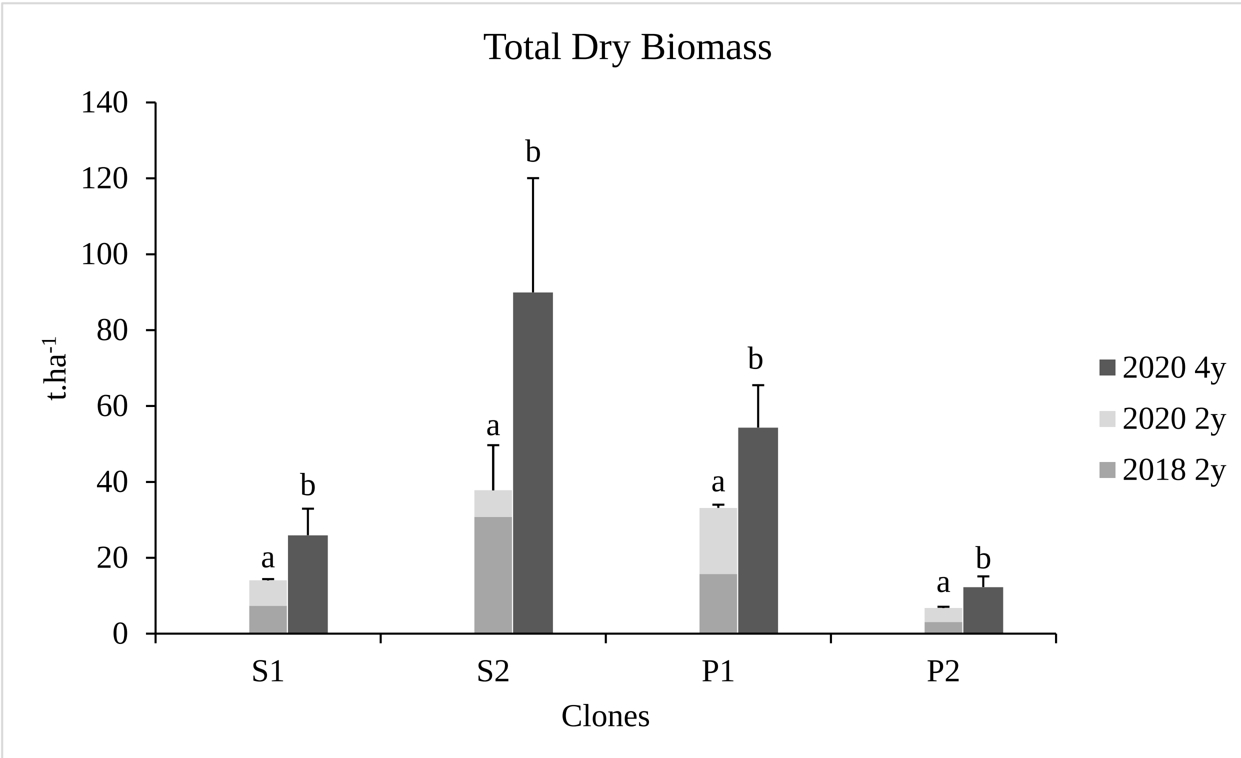

Figure 4 The mean (± SE) total dry biomass yield of Salix clones (S1 and S2) and Populus clones (P1 and P2). The difference between harvest periods were evaluated using the Kruskal-Wallis test at $P \leq 0.05$. In each clone, periods with the same lowercase were not significantly different.

Figure 5 dedicated the comparison of annual dry biomass yield between 2018_{2y}, 2020_{2y} and 2020_{4y} amongst all clones. The results showed that annual biomass production were in 2020_{4y} and S2 had the highest biomass productions compared to all clones. There were significant different between 2020_{4y} and other harvest periods. The highest value ranked in descending order 2020_{4y} $>2018_{2y}>2020_{2y}$ for both *Salix* clones. Whereas, in the *Populus* clones, the value ranged in descending order $2020_{4y} >2020_{2y}>2018_{2y}$. In 2020_{4y}, the highest value was observed in S2 clone (22.48 $t.ha^{-1}$) and the lowest was in P2 (3.06 $t.ha^{-1}$). S1 and P1 ranged from 6.48 $t.ha^{-1}$ to 13.47 $t.ha^{-1}$. In 2020_{2y}, the highest value was observed in P1 clone with the value of 8.71 $t.ha^{-1}$ and the lowest was in P2 with the value 1.85 $t.ha^{-1}$. Both *Salix* clones ranged from 3.35 $t.ha^{-1}$ to 3.5 $t.ha^{-1}$.

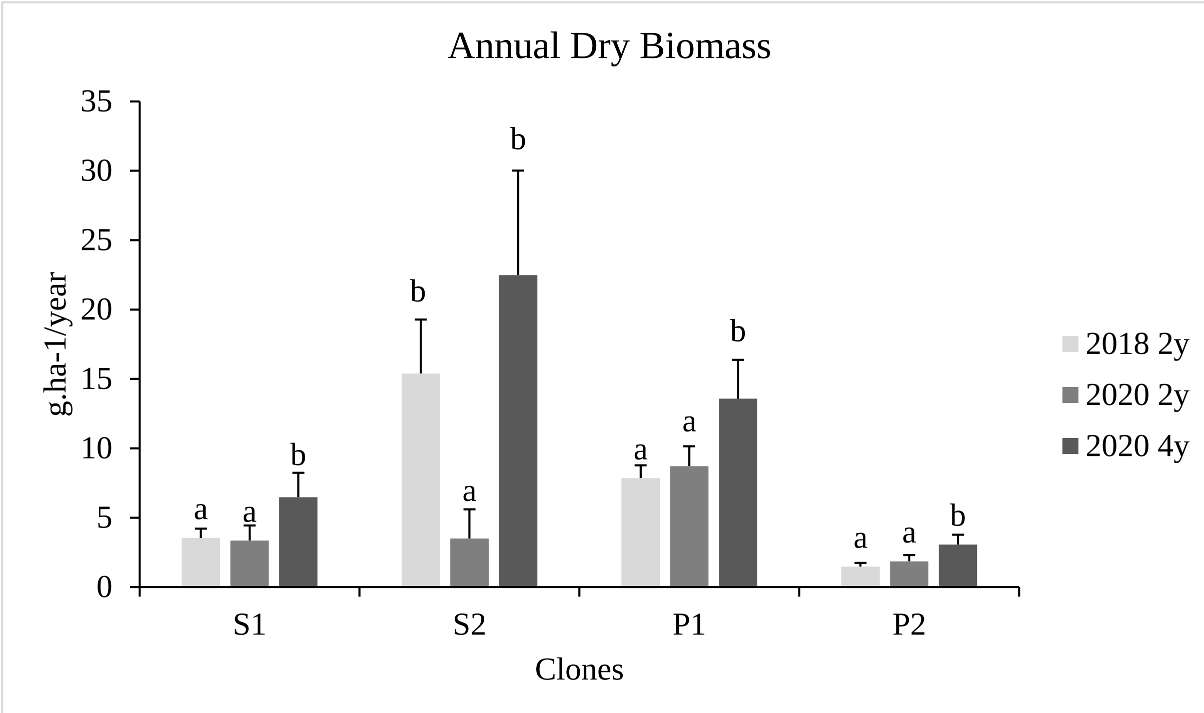

Figure 5 The mean (± SE) annual dry biomass yield of Salix clones (S1 and S2) and Populus clones (P1 and P2).The differences between harvest periods were evaluated using the Kruskal-Wallis test at $P \leq 0.05$. In each clone, harvest periods with the same lowercase were not significantly different.

5.3. Removal of Risk Elements by Harvested Biomass

In Figure 6 dedicated to the comparison of total removal of Cd between 2018_{2y},+ 2020_{2y} and 2020_{4y} of all 4 clones. In each period, there were statistically significant differences at all clones. Cd removals by all clones were highest in four years harvest 2020_{4y} compared to two short harvest periods 2018_{2y} + 2020_{2y}. P2 was significantly different from all clones in 2018_{2y}+2020_{2y}. The highest values were found in clone S2 in all harvest period with the value of 1654.09 $t.ha^{-1}$ in 2020_{4y} and ranged from 93.44 $g.ha^{-1}$ to 859.14 $g.ha^{-1}$ in 2020_{2y}+2018_{2y}. The lowest Cd removal was in P2 with the value ranged from 148.47 $g.ha^{-1}$ in 2020_{4y} to between 26.24 $g.ha^{-1}$ to 34.89 $g.ha^{-1}$ in 2018_{2y}+2020_{2y}. P1 and S1 value ranged between 452.07 $g.ha^{-1}$ to 487.76 $g.ha^{-1}$ in 2020_{4y} and between 143.8 $g.ha^{-1}$ to 186.53 $g.ha^{-1}$ in 2018_{2y}+2020_{2y}.

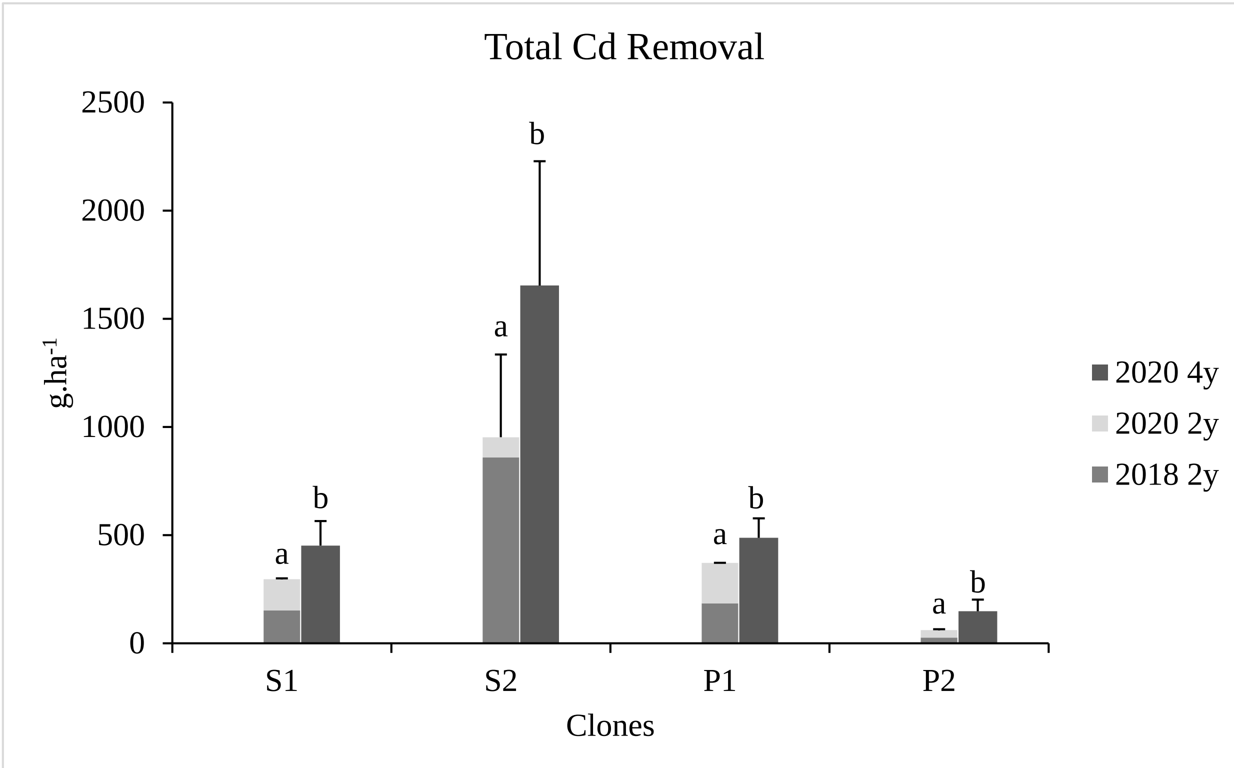

Figure 6 The mean (± SE) Total Cd removal by harvested biomass of *Salix* clones (S1 and S2) and *Populus* clones (P1 and P2). The difference between harvest periods were evaluated using the Kruskal-Wallis test at $P \leq 0.05$. In each clones, harvest periods with the same lowercase were not significantly different.

The comparison of annual Cd removal between 2018_{2y}, 2020_{2y} and 2020_{4y} amongst all clones were displayed in figure 7. In each harvest period, there were statistically significant differences among clones. The annual removal of Cd was highest in 2020_{4y} for S1, P1 and P2 while S2 is highest in 2018_{2y}. S2 also had the highest removal of Cd compared to other clones. There were significant different between 2020_{2y} and other harvest periods. The highest value ranked in descending order 2020_{4y} >2020_{2y}>2018_{2y} for both *Populus* clones. Whereas, in the S1 decrease in the order 2020_{4y} >2018_{2y}>2020_{2y}. S1 ranked in descending order 2018_{2y} >2020_{4y}>2020_{2y}. In 2020_{4y}, the highest value was observed in S2 clone (413.52 g.ha^{-1}/year) and the lowest was in P2 (37.11 g.ha^{-1}/year). S1 and P1 ranged from 113.01 g.ha^{-1}/year to 121.94 g.ha^{-1}/year. In 2020_{2y}, the highest value was observed in P1 clone with the value of 93.26 g.ha^{-1}/year and the lowest was in P2 with the value 17.44 g.ha^{-1}/year. Both *Salix* clones ranged from 46.71 g.ha^{-1}/year to 71.9 g.ha^{-1}/year. In 2018_{2y}, the highest value was observed in S2 clone with the value of 429.59 g.ha^{-1}/year and the lowest was in P2 with the value 13.12 g.ha^{-1}/year. P1 and S1 clones ranged from 76.19 g.ha^{-1}/year to 92.53 g.ha^{-1}/year.

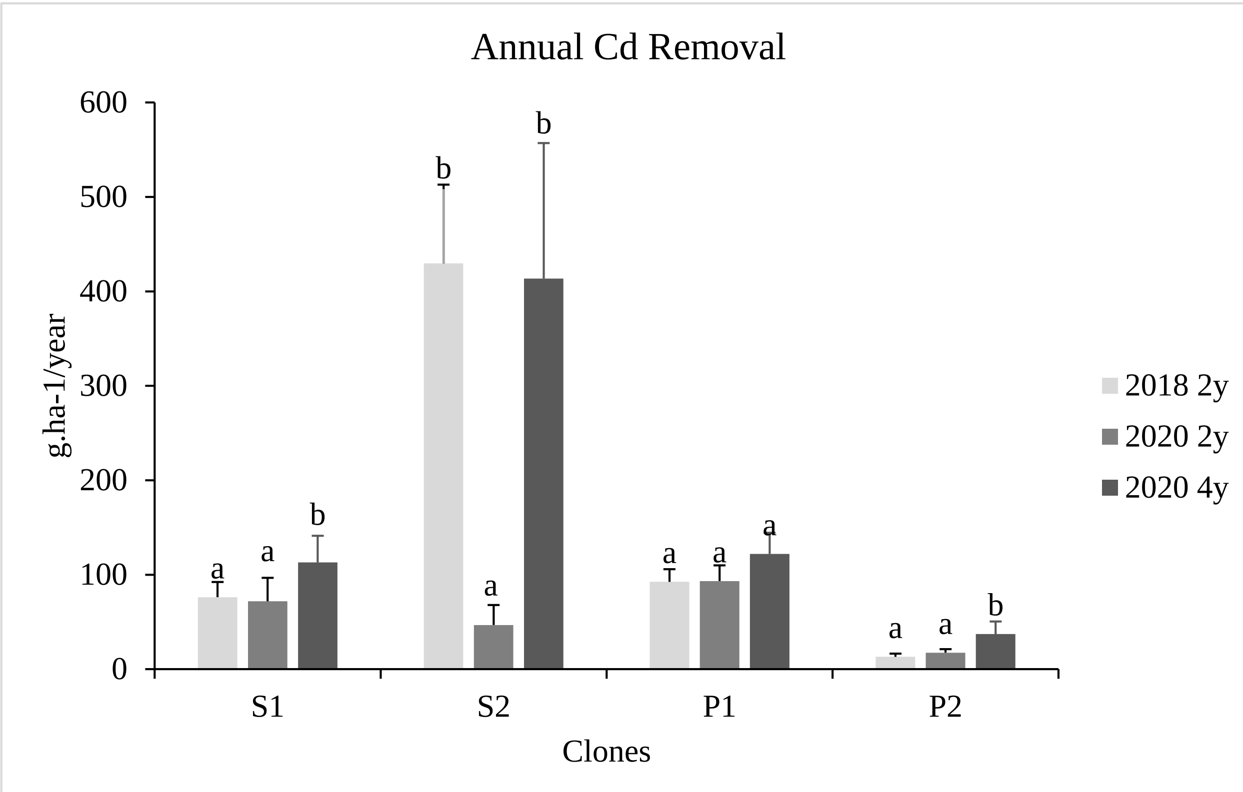

Figure 7 The mean (± SE) annual Cd removal by harvested biomass of *Salix* clones (S1 and S2) and *Populus* clones (P1 and P2). The difference between harvest period were evaluated using the Kruskal-Wallis test at P ≤ 0.05. In clones, harvest period with the same lowercase were not significantly different.

The comparison of total removal of Pb between 2018_{2y}, + 2020_{2y} and 2020_{4y} of all 4 clones was shown in figure 8. In each period, there were statistically significant differences among clones. Pb removal in all clones were highest in 2020_{4y} compared to other harvest periods and compared to 2018_{2y} + 2020_{2y}. There were significantly different between all harvest periods. The highest value was in clone S2 in all harvest period with the value of 2306.49 g.ha^{-1} in 2020_{4y} and ranged from 102.74 g.ha^{-1} to 735.05 g.ha^{-1} in 2018_{2y}+2020_{2y}. The lowest Pb removal was in P2 with the value ranged from 249.31 g.ha^{-1} in 2020_{4y} to between 42.64 g.ha^{-1} to 66.1 g.ha^{-1} in 2018_{2y}+2020_{2y}.

S1 and P1 value ranged between 495.42 g.ha^{-1} to 1563.5 g.ha^{-1} in 2020_{4y} and between 124.75 g.ha^{-1} to 554.05 g.ha^{-1} in 2018_{2y}+2020_{2y}.

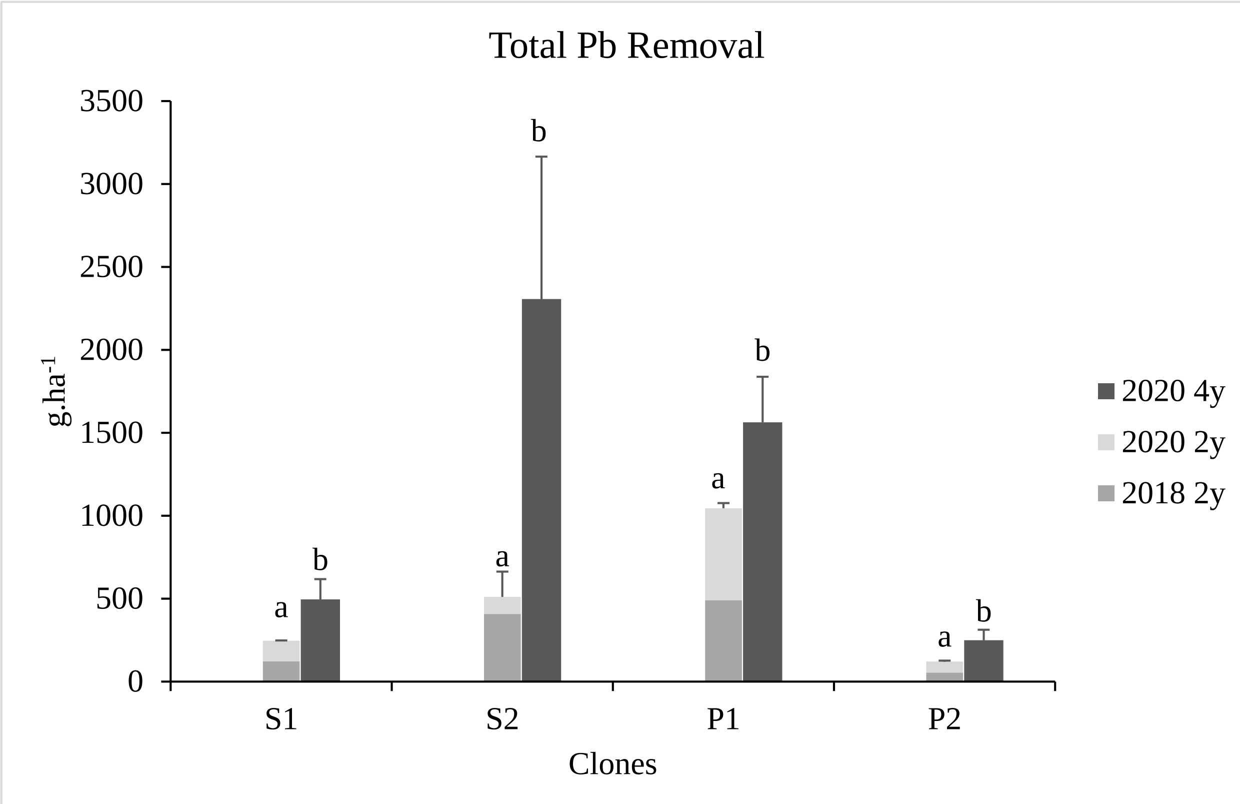

Figure 8 The mean (± SE) Total Pb removal by harvested biomass of *Salix* clones (S1 and S2) and *Populus* clones (P1 and P2). The difference between harvest period were evaluated using the Kruskal-Wallis test at $P \leq 0.05$. In each clones, harvest period with the same lowercase in the same harvest period were not significantly different.

Figure 9 showed the comparison of annual Pb removal between 2018_{2y}, 2020_{2y} and 2020_{4y} amongst all clones. In each period, there were statistically significant different amongst clones. The annual removal of Pb was highest in 2020_{4y} in all clones. S2 also had the highest removal of Pb compared to other clones. There were significant different between 2020_{4y} and other harvest periods. The highest value ranked in descending order 2020_{4y} >2020_{2y}>2018_{2y} for all clones except S2 which decrease in the order 2020_{4y} >2018_{2y}>2020_{2y}. In 2020_{4y}, the highest value was observed in S2 clone (2306.49 g.ha^{-1}/year) and the lowest was in P2 (249.31 g.ha^{-1}/year). S1 and P1 ranged from 49542 g.ha^{-1}/year to 1563.5 g.ha^{-1}/year. In 2020$_{2y}$, the highest value was observed in P1 clone with the value of 554.04 g.ha^{-1}/year and the lowest was in P2 with the value 66.1 g.ha^{-1}/year. Both *Salix* clones ranged from 102.73 g.ha^{-1}/year to 124,74 g.ha^{-1}/year. In 2018$_{2y}$, the highest value was observed in S2 clone with the value of 367.52 g.ha^{-1}/year and the lowest was in P2 with the value 21.32 g.ha^{-1}/year. P1 and S1 clones ranged from 59.7 g.ha^{-1}/year to 211.87 g.ha^{-1}/year.

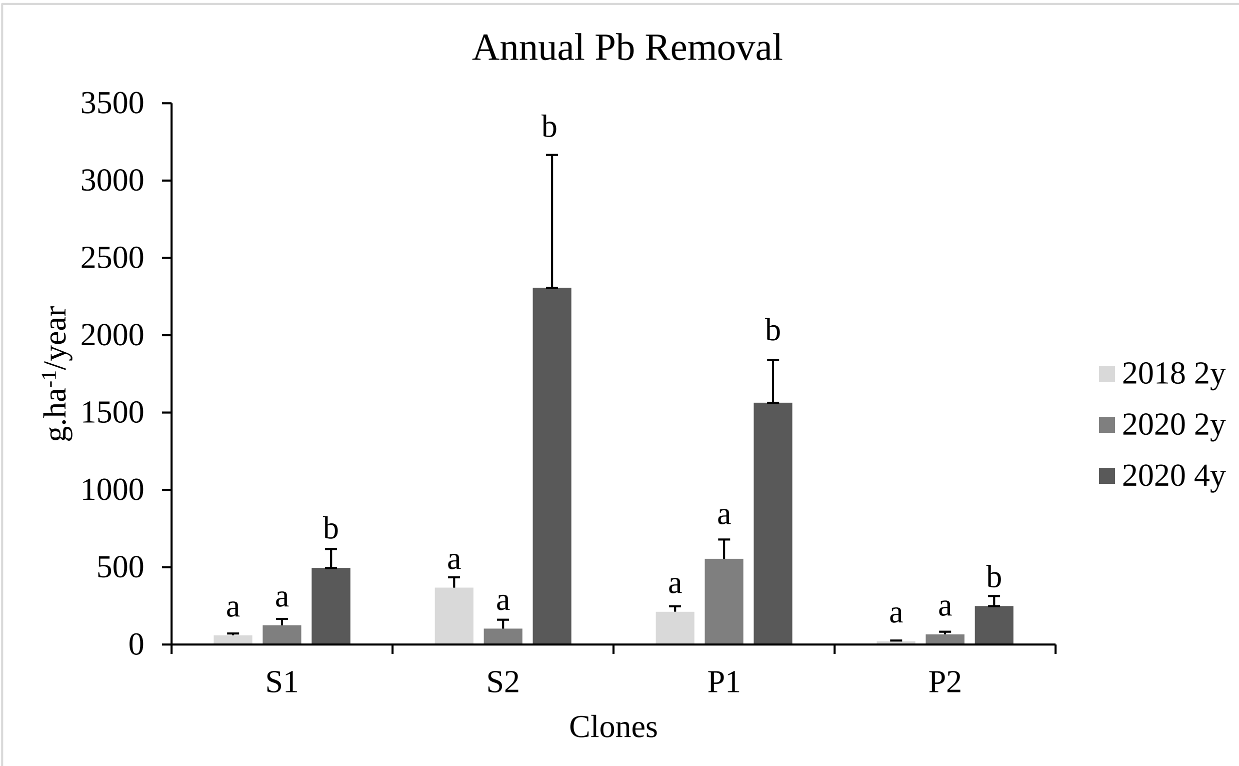

Figure 9 The mean (± SE) annual Pb removal by harvested biomass of *Salix* clones (S1 and S2) and *Populus* clones (P1 and P2). The difference between harvest periods were evaluated using the Kruskal-Wallis test at $P \leq 0.05$. In each clones, harvest periods with the same lowercase were not significantly different.

Figure 10 showed the comparison of total removal of Zn between 2018$_{2y}$, 2020$_{2y}$ and 2020$_{4y}$ and 2018$_{2y}$ + 2020$_{2y}$ and 2020$_{4y}$ of all 4 clones. In each period, there were statistically significant differences in all clones. In each clone, there were also significant different in all periods. between all clone Zn removal in all clones were highest in 2020$_{4y}$ compared to other harvest periods and compared to 2018$_{2y}$ + 2020$_{2y}$. The highest value was in clone S2 in all harvest period with the value of 19060.61 g.ha^{-1} in 2020$_{4y}$ and ranged from 1017.7 g.ha^{-1} to 9116.49 g.ha^{-1} in 2018$_{2y}$+2020$_{2y}$.

The lowest removal of Zn was seen in P2 with the value ranged from 1502.13 g.ha^{-1} in 2020_{4y} to between 268.21 g.ha^{-1} to 417.78 g.ha^{-1} in 2018_{2y}+2020_{2y}. P1 and S1 value ranged between 5382.62 g.ha^{-1} to 5550.39 g.ha^{-1} in 2020_{4y} and between 1634.86 g.ha^{-1} to 2169.6 g.ha^{-1} in 2018_{2y}+2020_{2y}.

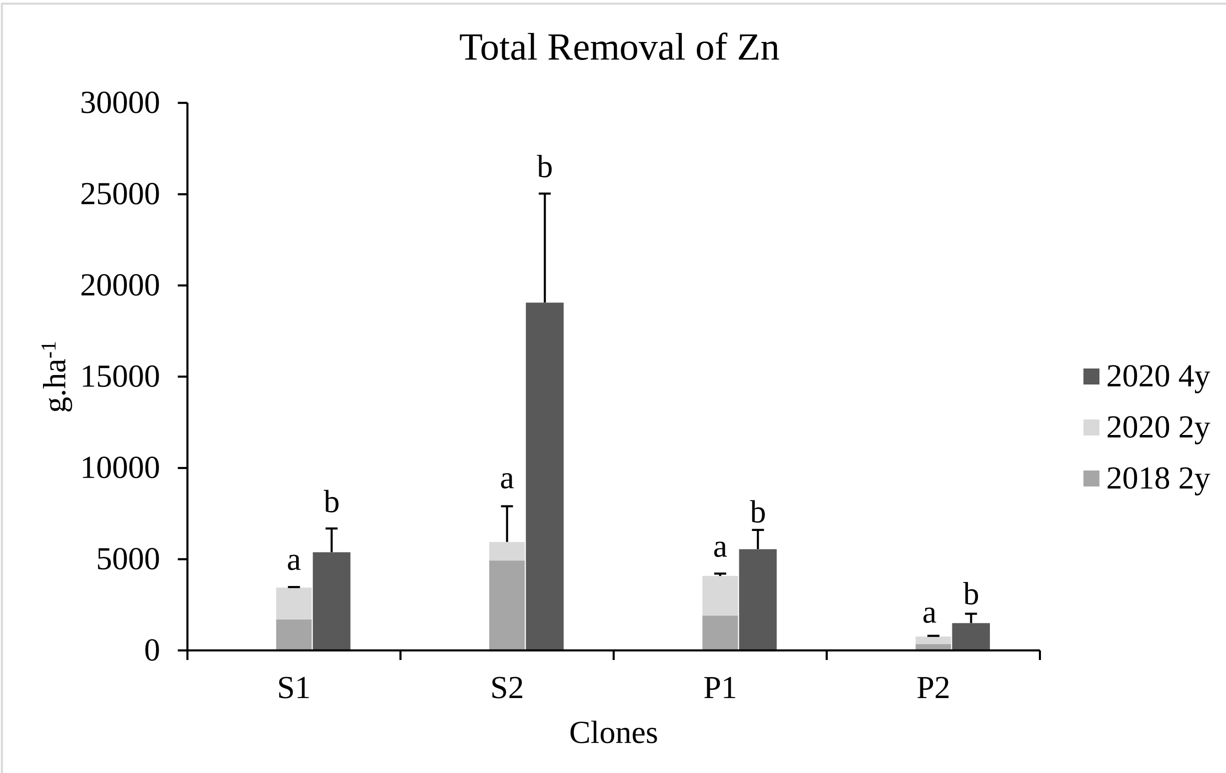

Figure 10 The mean (± SE) Total Zn removal by harvested biomass of *Salix* clones (S1 and S2) and *Populus* clones (P1 and P2). The difference between harvest period were evaluated using the Kruskal-Wallis test at $P \leq 0.05$. In each clones, harvest periods with the same lowercase in the were not significantly different.

Figure 11 dedicated the comparison of annual Zn removal between 2018_{2y}, 2020_{2y} and 2020_{4y} in all clones. In each period, there were statistically significant differences in all clones. The annual removal of Zn was highest in 2020_{4y} in all clones. S2 had the highest removal of Zn compared to 3 other clones in all periods except in 2020_{2y} which P2 had highest Zn removal. There were significant different between 2020_{4y} and other harvest periods. The highest value ranked in descending order 2020_{4y} >2020_{2y}>2018_{2y} for all clones. In 2020_{4y}, the highest value was observed in S2 clone with the value 4765.15 g.ha^{-1}/year and the lowest was in P2 with the value 375.53 g.ha^{-}

^{1}/year. S1 and P1 ranged from 1345.67 g.ha^{-1}/year to 1387.59 g.ha^{-1}/year. In 2020$_{2y}$, the highest value was observed in P1 clone with the value of 277.02 g.ha^{-1}/year and the lowest was in P2 with the value 33.05 g.ha^{-1}/year. Both *Salix* clones ranged from 51.36 g.ha^{-1}/year to 62.37 g.ha^{-1}/year. In 2018$_{2y}$, the highest value was observed in S2 clone with the value of 4558.24 g.ha^{-1}/year and the lowest was in P2 with the value 134.1 g.ha^{-1}/year. S1 and P1 clones ranged from 817.43 g.ha^{-1}/year to 821.72 g.ha^{-1}/year.

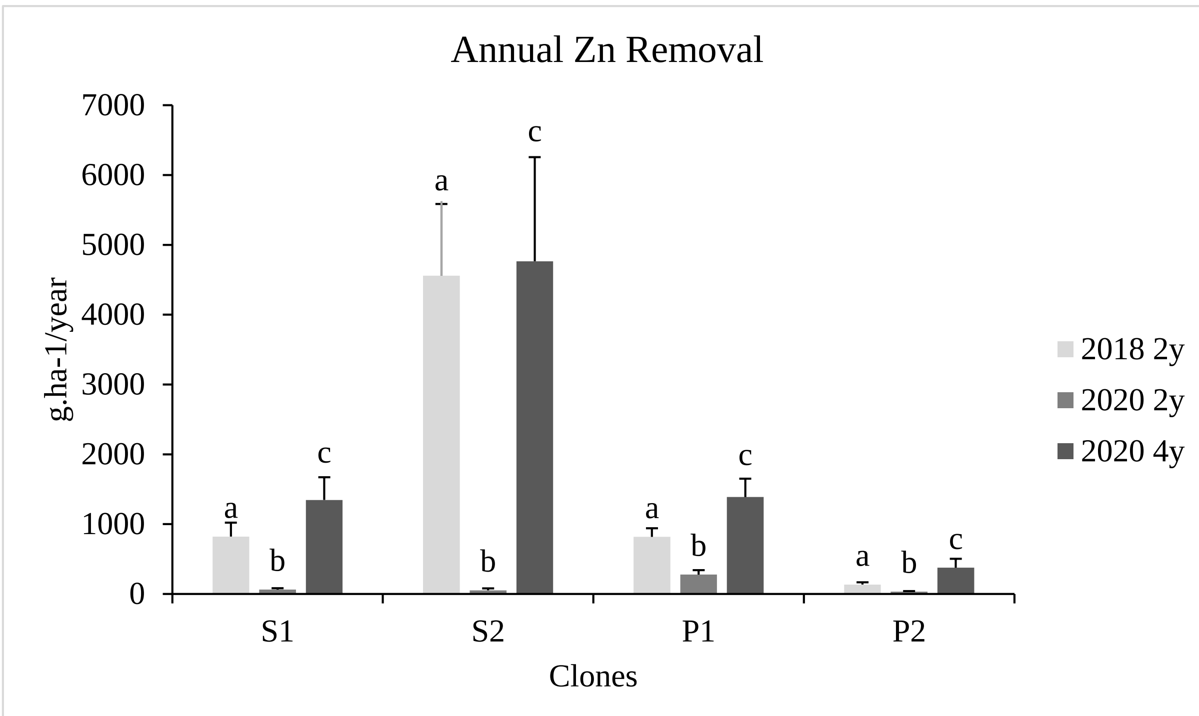

Figure 11 The mean (± SE) annual Zn removal by harvested biomass of *Salix* clones (S1 and S2) and *Populus* clones (P1 and P2). The difference between clones and harvest period were evaluated using the Kruskal-Wallis test at P ≤ 0.05. In each clones, harvest periods, were not significantly different.

5.3. Remediation Factor (RF)

Table 8 is dedicated to remediation factor (RF) of all 4 clones in 2 different harvest periods (2018_{2y}+ 2020_{2y} and 2020_{4y}). There were significantly different between all harvest periods in all elements. RF of Cd in S2, P1 and P2 were highest in the order 2020_{4y}>2018_{2y}>2020_{2y}. The highest RF Cd were in 2020_{4y} compared to harvest periods in 2018_{2y}+2020_{2y}. S2 had the total highest RF of Cd in 2020_{4y} and 2018_{2y}+2020_{2y} (8.39% and 4.82%, respectively) while lowest was in P2 2018_{2y}+2020_{2y} and 2020_{4y} (0.28% and 0.65%, respectively). The RF of Cd in S1 and P1 ranged from 0.29% to 2.47% in 2020_{4y} and 1.54% to 1.87% in 2018_{2y}+2020_{2y}.

The highest RF of Pb were in 2020_{4y} compared to all harvest periods and in 2018_{2y}+2020_{2y}. S2 had the highest RF of Pb in 2020_{4y} (0.06%) while the lowest was in P2 (0.005%). P1 had highest RF of Pb in 2018_{2y}+2020_{2y} (0.024%) while lowest was also in P2 (0.002%). The RF of Pb in S1 and P1 ranged from 0.01% to 0.04% in 2020_{4y}. In 2018_{2y}+2020_{2y} both *Salix* clones and RF between 0.006% to 0.012%.

RF of Zn were highest in 2020_{4y} compared to all harvest periods and in 2018_{2y}+2020_{2y}. S2 had the highest RF of Pb in 2020_{4y} and in 2018_{2y}+2020_{2y} with the value of 3.23 % and 1.71%, accordingly whereas the lowest in 2020_{4y} and in 2018_{2y}+2020_{2y} were in P2 with the value of 0.22% and 0.33%, accordingly. The RF of Zn in S1 and P1 ranged from 0.91% to 0.94% in 2020_{4y} and in 2018_{2y}+2020_{2y} between 0.56% to 0.91%.

Table 8 Remediation factor (RF%) indicates the proportion of elements (Cd, Pb and Zn) removed by harvested biomass (S1, S2, P1 and P2) in 20182y, 20202y and 20204y harvest.

Elements	Harvest	Clones			
	$\text{Year}_{\text{period}}$	S1	S2	P1	P2
Cd (%)	2018_{2y}	0.77^{Aab}	4.35^{Ab}	0.93^{Aab}	0.11^{Ac}
	2020_{2y}	0.72^{Bbc}	0.47^{Bcd}	0.94^{Aad}	0.16^{Aab}
	2018_{2y}+2020_{2y}	1.54^{Dab}	4.82^{Dac}	1.87^{Dbc}	0.28^{Dad}
	2020_{4y}	2.29^{Cab}	8.39^{Cac}	2.47^{Cbc}	0.65^{Ca}
Pb (%)	2018_{2y}	0.003^{Aa}	0.01^{Aa}	0.01^{Aa}	0.001^{Aa}
	2020_{2y}	0.003^{Aab}	0.002^{Ac}	0.014^{Abc}	0.001^{Aa}

	$2018_{2y}+2020_{2y}$	0.006^{Cab}	0.012^{Cbc}	0.024^{Cc}	0.002^{Ca}
	2020_{4y}	0.01^{Ba}	0.06^{Bb}	0.04^{Bb}	0.005^{Ba}
Zn (%)	2018_{2y}	0.27^{Aa}	1.54^{Ab}	0.55^{Aab}	0.27^{Ac}
	2020_{2y}	0.29^{Abcd}	0.17^{Aac}	0.36^{Ad}	0.06^{Aab}
	$2018_{2y}+2020_{2y}$	0.56^{Cabc}	$1,71^{Cbd}$	0.91^{Ccd}	0.33^{Ca}
	2020_{4y}	0.91^{Bab}	3.23^{Bc}	0.94^{Bbc}	0.22^{Ba}

Differences between clones and harvest periods were evaluated using the Kruskal-Wallis test at $P \leq 0.05$. Clones with the same capital letter for were not significantly different. In each harvest period, clone with the same lowercase were not significantly different.

6. Discussion

6.1. Concentration of Elements

The results of this study showed that *Salix* clones had higher the concentration of Cd and Zn than *Populus* clones; yet, the concentration of Pb was higher in *Populus* clones than in *Salix* clones (Table 7). Kacálková et al (2015) also showed that *Salix* clones had higher concentration of Cd and Zn than *Populus* in her study. Rutten et al. (2011) supported that *Populus* clones had higher accumulation of Pb compared to other tested *Salix* clones, for example *S. viminalis*. The study of Fischerová et al. (2006) showed that *P. nigra* accumulated higher concentration of Pb than in *Salix* clones which meant clones could inherent their properties.

The concentrations of elements (Cd, Pb, Zn) in this study were higher compared to other studies. For instance, *Salix* accumulated Cd up to 18.26 $mg.kg^{-1}$, Zn up to 215.19 $mg.kg^{-1}$, and Pb up to 24.41 $mg.kg^{-1}$ in the 4 years-period while in 2 years-period the highest of Cd, Pb, and Zn in *Salix* clones were 20.95 $mg.kg^{-1}$, 20.25 $mg.kg^{-1}$ and 249.54 $mg.kg^{-1}$, accordingly. In *Populus* clones the highest concentration of Cd, Pb and Zn in 2 years-period were 10.99 $mg.kg^{-1}$, 31.7 $mg.kg^{-1}$ and 107.29 $mg.kg^{-1}$, accordingly. In addition, in *Populus* clones the highest concentration of Cd, Pb and Zn in 4 years-period of Cd, Pb and Zn were 11 $mg.kg^{-1}$, 31.97 $mg.kg^{-1}$ and 107.19 $mg.kg^{-1}$, accordingly. In contrary, Mleczek et al. (2009) reported that the concentrations of Cd, Pb, Zn were 2.29 $mg.kg^{-1}$, 107.24 $mg.kg^{-1}$, and 3.88 $mg.kg^{-1}$, accordingly, in *Salix* clones in their 2 years-period. Furthermore, Padoan et al. (2019) also found that the highest Zn concentration was 380.6 $mg.kg^{-1}$, while Pb was 8.75 $mg.kg^{-1}$ in *Salix* clones in the 2 years-period. The concentrations of Cd and Zn in Populus found in the study of Kacálková et al. (2009) were 0.76 $mg.kg^{-1}$ and 73.9 $mg.kg^{-1}$, respectively.

Therefore, we can conclude that he high accumulation of elements in our study was due to high concentration of Cd (7.3 $mg.kg^{-1}$), Pb (1368 $mg.kg^{-1}$) and Zn (218 $mg.kg^{-1}$) in soil. Cataldo & Wildung (1978) also proved that the accumulation of elements in plants was influenced by the concentration of elements in soil (Cataldo & Wildung, 1978). In addition, clone's properties and soil type also affect the accumulation of risk elements in plant. S2 clone showed better accumulation of Cd and Zn on Cambisol and the concentration of Cd, Pb, and Zn were found higher in *S. smithiana Willd* compared to other tested *Salix* clones (Tlustoš, 2007).

6.2. Dry Biomass Yield

The biomass yield increased with the increase of rotation years. Based on results from figure 4, 4 years-rotation has higher biomass than 2+2 years-rotation in all clones. The research of Weih (2007) showed that, for *Populus* clones, the highest biomass production was in their 4 years-rotation whereas, *Salix* Clones, the highest biomass yield was in 3 years-rotation. In addition, Ruttens et al. (2011) also showed that the highest biomass yield of *Salix* clones was higher in 3 years-rotation compared to 2 years-rotation. The research of Kubátová (2018a), also showed that for both *Salix* and *Populus* clones, highest biomass yield is 4 years-period compared to 2 years-rotation. The same study also stated that the results of 2+2 were lower compared to 4 years-period which same as stated in the same research (Kubátová, 2018a).

Annually, all clones had highest biomass in 4 years-rotation periods compared to 2 years. S2 achieved 22.48 t.ha^{-1}/year after 4 years-periods. This result was higher than the research of Willebrand et al. (2013) which studied on 6 *Salix* clones. The highest biomass in 4 years-period were between 8 t.ha^{-1}/year to 14 t.ha^{-1}/year while 2 years-periods, biomass yield were so much lower. Furthermore, the study of Weger et al. (2016) showed that S2 had yield of 19 t.ha^{-1}/year after 3 years rotation. In addition, Kulig et al. (2019) had tested 22 *Salix* clones which provided yield between 6.6 t.ha^{-1}/year to 23 t.ha^{-1}/year during their 4 years-rotation. However, the opposite study was found in Szczukowski et al. (2005) which showed that *Salix triandra* had higher yield in their 2 years-rotation compared to 4 years-rotation while other *Salix* clones had higher yield in their 4 years-rotation. This reason makes *Salix triandra* was not suitable for growing in arable land especially for 4 years-rotation periods. Populus clones in our study had annual biomass yield between 3.06 to 13.57 t.ha^{-1}/year. The study of Dillen et al. (2013) reported that annual biomass yield of their most-productive *Populus* clones ranged from 8.5 t.ha^{-1}/year to 10.5 t.ha^{-1}/year. Landgraf et al. (2020) observed that their most productive clones of *Populus* had annual biomass yield average 10 t.ha^{-1}/year. The study of Labrecque and Teodorescu (2005). showed that P1 clone could produce 18.05 t.ha^{-1}/year.

6.2. Risk Element Removal

The results of the removal of Cd, Pb and Zn 4 years-period were higher than both 2 years-period. The removal of elements by clones were shown in the descending order S2>P1>S1>P2. Both studies of Kubátová (2018a) and Kubátová (2018b) showed the same results where S2 had the highest removal of all elements. In addition, the study of Kacálková et al. (2015) showed that the removal of Cd, Pb, and Zn was seen highest in *Salix smithiana* (S2) compared to other clones.

Our study had the highest removal of Cd, Pb and Zn in the 4 years harvest period 1654.09 $g.ha^{-1}$, 2306.49 $g.ha^{-1}$ and 19060.61 $g.ha^{-1}$, respectively. Whereas, the highest in 2 years periods for Cd, Pb and Zn were 859.19 $g.ha^{-1}$, 735.15 $g.ha^{-1}$ and 9116.49 $g.ha^{-1}$, accordingly. The highest removal in the study of Kubátová (2018a) showed the highest removal of elements were around Cd (1300 $g.ha^{-1}$), Pb (1000 $g.ha^{-1}$) and Zn (15000 $g.ha^{-1}$) per plant by S2 in their 4 years-period. Another study of Kubátová et al. (2016) showed the removal (per plant) of Cd (158 $g.ha^{-1}$), Pb (429 $g.ha^{-1}$) and Zn (1720 $g.ha^{-1}$) by *Populus* clones in their 2 years-period.

The high removal of elements in our study linked to the biomass production of clones. The results showed that the biomass productions of all clones decreased with the order S2>P1>S1>P2 while removal of all 3 elements decreased with the same order. This conclusion was in line with the study of Zárubová et al. (2015) which also stated that the longer rotations, the better biomass production which lead to better removal of risk elements.

6.3. Remediation Factor (RF)

The RF was higher with the rotation periods due to their biomass productions. The results of table 8 showed that the 4 years-period had higher RF of all elements compared to 2+2 years-period. The same results were also found in the research of Kubátová (2018a). The RF of Cd, Pband Zn of S2 in 4 years-period were 8.39%, 0.06% and 3.23%. These results higher than the research of Kubátová (2018a) which showed that the RF of Cd was 6.39%, Pb was 0.04% and Zn was 2.55% in their 4 years-period. The highest RF of elements in the study of Zárubová et al. (2015) were Cd (0.95%), Pb (0.003%), and Zn (0.15%) in S2. All RF in the same research of S1, P1 and P2 also showed lower values compare to our research.

The high RF in our study was mainly due to the high biomass yield of the clones which lead to high removal of elements from soil thus, lead to high RF.

7. Conclusions

The phytoextraction precise field experiment set up on the long-term contaminated site with two willows and two poplars clone showed that rotation periods did affect the biomass yield. The results of all 4 clones (S1- (*S. schwerinii* × *S. viminalis)* × *S. viminalis)*, S2 - *S. smithiana*) and *Populus* P1 – (*P. maximowiczii* × *P. nigra, P2* – *P. nigra*) harvested ones within 4 years t period produced significantly better biomass yield compared to 2+2-years periods. Clone S2 had highest biomass yield compared to other clones. In 4 years period it could produce biomass yield up to 89.93 $t.ha^{-1}$ per plant whereas, P2 produced the lowest yield up to 12.27 $t.ha^{-1}$. Biomass yield had major impact on the removals of all elements by harvested branches. Amongst all clones, S2 had better removal of Cd (1654 $g.ha^{-1}$), Pb (2306.49 $g.ha^{-1}$) and Zn (19060.61 $g.ha^{-}$1) per plant in their 4 years period. In contrast, the lowest removals were in P2 which the removal of Cd (148.37 $g.ha^{-1}$), Pb (249.31 $g.ha^{-1}$) and Zn (1502.13 $g.ha^{-}$1). Furthermore, S2 also had highest RFs which were Cd (6.39%), Pb (0.06%) and Zn (2.55%) in their 4 years- period while P2 had highest RF of Cd (0.65%), Pb (0.005%) and Zn (0.22%). In addition, second best performed to S2, was *Populus* clone (P1) with relatively high removal and high RF of Cd and Zn elements. This showed that clones with different parameters had different performances in metals removal, biomass productions and higher RF.

With this aspect, S2 (*S. smithiana*) would be a promising clone for phytoextraction of Cd and Zn due to its high biomass productions and high accumulation of studied metals. Furthermore, 4-years rotation is recommended for phytoextraction on long term contaminated soil.

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