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Chapter 11

Phytoremediation and Physiological Effects of Mixed Heavy Metals on Poplar Hybrids

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Additional information is available at the end of the chapter

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Abstract

The effects of mixed heavy metals differ not only in different plants but also on the hybrids exposed. In this chapter, we focus on phytoremediation and the physiological effects of mixed heavy metals on four poplar hybrids. According to the results obtained from greenhouse pot experiments with mixed heavy metals, the photosynthetic and transpiration rates were affected by increased heavy metal concentrations. The concentration of heavy metals copper, chromium, cadmium, and zinc in the plant roots, stem and leaves varied with the concentration of mixed heavy metal as well as individual heavy metals. Based on the phytoextraction potential; hybrid 1 (Eco 28) was deduced as the best candidate for phytoremediation in mixed heavy metal contamination treatment. The results obtained are valuable in understanding how specific hybrids respond to mixed heavy metal stress especially when using them as bioindicators for phytoremediation experiments in multi-metal contaminated sites. Selection of new plants along with field trials over extended periods will increase the possibility of further enhancing and establishing phytoremediation technology in the future.

Keywords: phytoremediation, mixed heavy metals, poplar hybrids, physiological effects, phytoextraction potential

1. Introduction

1.1. Phytoremediation

Phytoremediation has gained considerable interest and support in the last decade. This environment-friendly green technology has gained its popularity over the years in terms of its success with other conventional techniques. The specific definitions of phytoremediation are

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various however the basic definition involves growing of plants in a contaminated matrix with the intentions of removing, transforming or stabilizing environmental contaminants (**Figure 1**) [1–4]. The generic term phytoremediation originates from the Greek prefix φυτο "phyto" – plant, attached to the Latin root "remedium" – to correct or remove, restoring balance, or remediating [5, 6].

Various physical, chemical, and biological techniques are available to remediate metal contaminated soils. Classical environmental cleanup methods are known as ex situ methods which are typically expensive and destructive include excavation, thermal treatment, chemical soil washing, soil incineration, volatilization, vitrification, chemical extraction, solidification, and landfills [5, 7] which either detoxifies or destroys the contaminant chemically or physically. As a result, the contaminant undergoes stabilization, solidification, immobilization, incineration or destruction [8]. These methods are not only labor intensive and expensive but also produce a residue rich in heavy metals which require further treatment. Moreover, these physiochemical technologies used for soil remediation render and create irreversible changes to soil properties altering the land usage as a medium for plant growth, as they remove all biological activities along with disturbing the native soil microflora [2, 5].

Hence, phytoremediation has been recognized as a cost-effective and eco-friendly method of remediating heavy metals from contaminated environments. From the five different processes involved in phytoremediation, phytoextraction has been identified as the superior

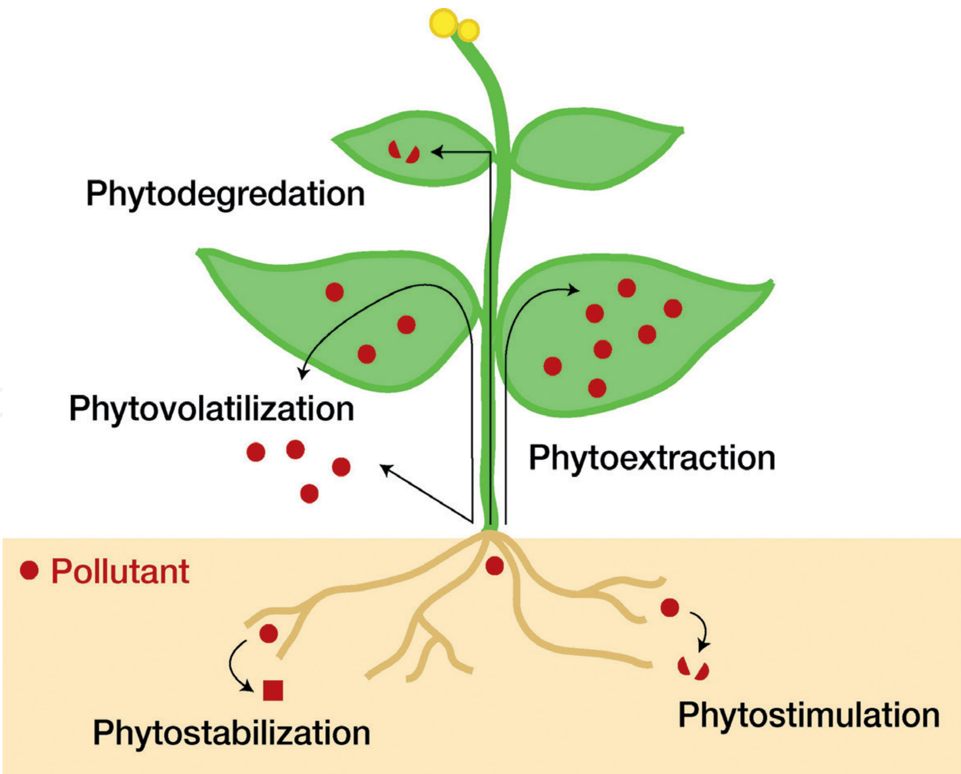

Figure 1. Possible pollutant fates during phytoremediation: the pollutant (represented by red circles) can be stabilized (phytostabilization) or degraded (phytostimulation) in the rhizosphere, sequestered or degraded (phytoextraction, phytodegradation) inside the plant tissue, or volatilized (phytovolatilization) in the air [4].

type where the plants extracts heavy metals from the contaminated soil [9]. Phytoextraction (also known as phytoaccumulation, phytoabsorption or phytosequestration) is the uptake of contaminants from the soil by plant roots and their translocation and accumulation in the above-ground biomass [8, 10, 11]. In addition, rapid growth with high biomass production, extensive root system, high survival and adaptation to low-quality soil substrates and high tolerance to excessive concentrations of heavy metals are properties exhibited by plants suitable for phytoremediation. In general, species and hybrids of poplar, jatropha, and willow are being exploited for the dual purpose of phytoremediation as well as energy production [4, 12–14]. Generally, plants have the potential to absorb metals from the substrate; however, few are capable of extracting, accumulating, and tolerating high concentrations of heavy metals in their system. The discovery of hyperaccumulator plants which are capable of absorbing heavy metals 50–500 times than normal plants has greatly contributed to the revolutionary advancement of phytoextraction technology [15].

1.2. Heavy metal pollution

Sources of pollution in the environment are widely due to global industrialization. Natural and anthropogenic sources are means through which heavy metals enter the environment. Significant natural sources include weathering of minerals, erosion and volcanic activity whereas anthropogenic sources include mining, smelting, electroplating, use of pesticides and fertilizers along with biosolids in agriculture, sludge dumping, industrial discharge, and atmospheric deposition [16–20]. Phytoremediation technology is applicable to a broad range of contaminants, including metals, radionuclides [21–23], and organic compounds such as chlorinated solvents, polycyclic hydrocarbons, pesticides, explosives, and surfactants [3].

From a chemical point of view, heavy metals are defined as elements with metallic properties and an atomic number of >20 and specific gravity of >5. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb, and Zn [2]. In this chapter the term "heavy metals (HMs)" will be referred to those potentially phytotoxic elements that are a major environmental concern due to their persistence in the environment and their impact on humans via the food chain. Heavy metals have adverse effects on human health and therefore heavy metal contamination deserves special attention [5]. HMs such as As, Cd, Hg, Pb, and Se are non-essential since they do not perform any known plant physiological function [24]. However, Co, Cu, Fe, Mn, Mo, Ni, and Zn are essential elements which are required for normal plant growth and metabolism [24]. These essential HM's at supra-optimal concentrations can lead to poisoning [25]. All essential metals are toxic at high concentrations since they cause oxidative stress due to the free radical formation and disrupt the function of pigments and enzymes by replacing essential metals [8, 26].

2. Poplar hybrids

Populus is a genus of 25–35 species of deciduous flowering plants in the family of Salicaceae, native mostly to the Northern Hemisphere. English names commonly applied include poplar, aspen, and cottonwood. This genus has a large genetic diversity and can grow from 15 to 50 m (49–164 ft.) tall, with trunks of up to 2.5 m (8 ft. 2 inch) in diameter. The genus *Populus* is divided into 6 sections on the basis of leaf and flower. Four different poplar clones were selected based on previous research as well as their response in terms of biomass in the field [27–29]. The responses of these four chosen clones were better with seasonal changes as well. These clones included:

1. Eco 28 – *Populus euramericana guinier*
2. DN 034 – *Populus deltoides* × *P. nigra*
3. TN 074 – *Populus trichocarpa* × *P. nigra*
4. TD 225 – *Populus trichocarpa* × *P. deltoids*

Early phytoremediation studies used hyperaccumulator species [30, 31] which are plants that are able to accumulate unusually high levels of metals in their tissue. Studies conducted on willows and poplars (Salicaceae family) showed that the efficiency of metal extraction is markedly lower compared to hyperaccumulators, even if on a large scale basis the removal of metals from soil could be higher [32]. According to Zacchini et al. [28], Salicaceae plants thrive in a wide range of soil and climatic conditions [33] and also express a good metal tolerance hence making them good candidates for phytoremediation work.

3. Poplar hybrids for phytoremediation

Four poplar hybrids were selected based on their genetic diversity, growth, and wellbeing in the field. Cuttings of approximately 15 cm from the hybrids were planted in 2 L pots filled with sandy loam soil. Two months after sprouting and root stabilization, the plantlets were treated with mixed heavy metals of concentrations ranging from 0 mg L^{-1} as control followed by 5, 50, 100, 200 and 500 mg L^{-1}.

The mixed heavy metals utilized included Chromium III chloride (Cr_3Cl), Copper (II) chloride ($CuCl_2$), Cadmium chloride ($CdCl_2$) and Zinc chloride ($ZnCl_2$). Individual HMs was separately prepared and equal amounts were mixed for each concentration. Each plantlet was treated with 20 ml of mixed HM only once during the 3 month treatment period while the plants were watered regularly. Photosynthesis and transpiration rates were measured using LCi-SD (ADC Bioscientific Ltd., Hoddesdon, UK) portable photosynthesis system before and during the treatment period along with the photosynthetic pigments.

After the 3 month treatment period, the plants were harvested for further heavy metal analysis. Plant leaves, stem, and roots were separated, thoroughly washed with running tap water and finally with distilled water after which morphological characteristics were noted and the plants were prepared for further biochemical and heavy metal analysis. Each concentration constituting of four replicates were analyzed for various parameters and an average was used for statistical analysis. Various plant parts including leaves, stems, and roots were dried at 50°C for 48 hours. These dried plant parts were ground using the 8000 mixer mill (SPEX. SamplePrep).

The physical and chemical properties including the levels of heavy metals were determined using the aqua regia method. Soil heavy metal analysis involved, a collection of the soil samples which were air-dried and sieved and digested using the aqua regia method. Soil samples 1.5 g were digested and the samples were analyzed for various HMs by ICP – AES analysis (iCAP 7000 Series ICP spectrometer, Thermo Fisher Scientific, Waltham, MA, USA).

The biological accumulation coefficient (BAC) was calculated as the ratio of heavy metal in the shoot to that in soil given in Eq. 1 [34]. Biotranslocation coefficient was determined as a ratio of heavy metals in plant shoot to that in plant root given in Eq. 2 [35, 36]. Eq. (3) shows the bioconcentration factor (BCF), calculated as metal concentration ratio in plant roots to soil; Eq. (4) shows the concentration index (CI), calculated as the concentration of heavy metals in the treated plant to control [35, 36].

These factors were used to determine the phytoextraction potential of the studied plants. Shoot in the equations refers to stem + leaves and HM for heavy metal.

$$BAC = HM_{shoot}/HM_{soil} \tag{1}$$

$$BTC = HM_{shoot}/HM_{root} \tag{2}$$

$$BCF = HM_{root}/HM_{soil} \tag{3}$$

$$CI = HM_{treated\ plant}/HM_{normal\ plant} \tag{4}$$

Data are expressed as mean ± standard error of mean (SEM and statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Tests using SPSS Version 21 (IBM Corp., USA). Statistical significance was accepted at $P < 0.05$.

3.1. Effects of heavy metals on photosynthesis and transpiration

Exposure of poplar hybrids to mixed HMs under greenhouse conditions showed an increase in the rate of photosynthesis with increased HM concentrations (**Table 1**).

The highest rate of photosynthesis was observed in Hybrid 1, 14.54 μmol m^{-2} s^{-1} at 500 mg L^{-1} mixed HMs. This was observed as the highest photosynthetic rate among all four hybrids. Fluctuations in hybrid 2 photosynthetic rates were observed across all concentrations with the lowest of 7.75 μmol m^{-2} s^{-1} at 5 mg L^{-1} and highest of 12.60 μmol m^{-2} s^{-1} at 200 mg L^{-1} HM concentration. The lowest photosynthetic rate of 2.61 μmol m^{-2} s^{-1} at 200 mg L^{-1}, increased 5 times to 10.08 at 500 mg L^{-1} was observed in hybrid 3. Hybrid 4, on the other hand, had a significantly higher photosynthesis rate of 8.20 μmol m^{-2} s^{-1} at 5 mg L^{-1} which decreased to 6.84 and 7.23 at 50 and 100 mg L^{-1} respectively. A clear pattern can be observed in terms of photosynthesis rate for all 4 poplar hybrids, the photosynthesis rate increases with increasing mixed HM concentrations with the majority being significant around 200–500 mg L^{-1} mixed heavy metal concentrations. This increase in the photosynthetic rate at high HM concentrations could be due to the

Heavy metal concentrations (mg L^{-1})	Photosynthesis rate – A (μmol m^{-2} s^{-1})			
	Hybrid 1 (Eco 28)	Hybrid 2 (DN 034)	Hybrid 3 (TN 074)	Hybrid 4 (TD 225)
0	10.21 ± 0.20^{bc}	10.12 ± 0.26^{b}	9.05 ± 0.34^{ab}	9.05 ± 0.17^{a}
5	8.82 ± 1.24^{c}	7.75 ± 0.26^{c}	9.72 ± 0.27^{a}	8.20 ± 0.54^{ab}
50	11.32 ± 0.18^{b}	11.43 ± 0.52^{ab}	7.11 ± 0.42^{c}	6.84 ± 0.63^{b}
100	8.56 ± 0.23^{c}	10.21 ± 0.93^{b}	8.12 ± 0.46^{bc}	7.23 ± 0.50^{b}
200	8.71 ± 0.21^{c}	12.60 ± 0.52^{a}	2.61 ± 0.50^{d}	8.88 ± 0.53^{a}
500	14.54 ± 0.70^{a}	11.73 ± 0.11^{a}	10.08 ± 0.20^{a}	7.70 ± 0.56^{ab}

Mean values ± SEM, (n = 10).

Means within columns followed by the same letters are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

Values significantly different from control (0).

Table 1. Photosynthesis rates of four poplar hybrids treated with mixed heavy metals (Cd, Cr, Cu, and Zn) under greenhouse conditions.

ability of the hybrids to tolerate high concentration of HMs. This can be based on previous studies on higher plants and trees [37] and effects of chromium stress on photosynthesis [38] are due to carbon dioxide fixation, electron transport, photophosphorylation and enzyme activities [38] whereas cadmium stress leads to Fe(II) deficiency which seriously affected photosynthesis [39]. Hence, if the photosynthesis rates did not decrease this would mean that either the plant is capable of surviving in an environment with high HMs or due to competition with other heavy metals and other environmental factors limited heavy metal are absorption and translocation.

Variations in transpiration rates were observed across all hybrids, the highest value coincided with 50 mg L^{-1} for hybrid 1 at 11.23 mmol m^{-2} s^{-1} followed by 500 mg L^{-1} at 8.89 mmol m^{-2} s^{-1} (**Table 2**).

The highest transpiration rate at 9.99 mmol m^{-2} s^{-1} at 200 mg L^{-1} followed by 9.61 at 50 mg L^{-}1 was observed for hybrid 2. Significantly different transpiration rates for control were observed at low mixed heavy metal concentrations of 5 and 100 mg L^{-1}. The transpiration rates of hybrid 3 were most significant at all concentration with the highest 10.87 mmol m^{-2} s^{-1} at 50 mg L^{-1} followed by 10.14 mmol m^{-2} s^{-1} at 100 mg L^{-1} and the lowest of 3.42 mmol m^{-2} s^{-1} at 200 mg L^{-1}. Hybrid 4 had the lowest transpiration rates at 50 mg L^{-1} (3.55 mmol m^{-2} s^{-1}) and the highest at 200 mg L^{-1} (8.14 mmol m^{-2} s^{-1}). No specific relationships were observed for transpiration rates across the different mixed HM concentrations in hybrid 4 however, the rates observed across all 4 hybrids of poplar significant. However, the differences varied for each hybrid; generally, a decrease in transpiration rate was observed with increase in mixed HM concentrations. According to Carlson et al., gas exchange measurements are often used to detect the most sensitive site of action [40]. In case of the studied hybrids even though the photosynthetic rate at high HM concentrations increased, the decrease in transpiration rate clearly suggests that the plants were stressed in certain ways. A more detailed study would help deduce the specific areas affected by the HMs.

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Heavy metal concentrations (mg L^{-1})	Transpiration rate – E (mmol m^{-2} s^{-1})			
	Hybrid 1 (Eco 28)	Hybrid 2 (DN 034)	Hybrid 3 (TN 074)	Hybrid 4 (TD 225)
0	7.45 ± 0.24^{c}	10.17 ± 0.41^{a}	8.29 ± 0.39^{b}	5.99 ± 0.16^{c}
5	5.96 ± 0.70^{d}	8.64 ± 0.52^{bc}	9.96 ± 0.1^{b}	6.38 ± 0.15^{bc}
50	11.23 ± 0.05^{a}	9.61 ± 0.26^{ab}	10.87 ± 0.44^{a}	3.55 ± 0.53^{d}
100	7.84 ± 0.28^{c}	8.77 ± 0.78^{abc}	10.14 ± 0.72^{a}	6.24 ± 0.61^{c}
200	5.30 ± 0.11^{d}	9.99 ± 0.38^{ab}	3.42 ± 0.53^{c}	8.14 ± 0.34^{ab}
500	8.89 ± 0.13^{b}	7.64 ± 0.1^{c}	9.53 ± 0.28^{ab}	7.52 ± 0.47^{a}

Mean values ± SEM, (n = 10).

Means within columns followed by the same letters are not significantly different at P < 0.05 according to Duncan's multiple range tests.

Values significantly different from control (0).

Table 2. Transpiration rates of four poplar hybrids treated with mixed heavy metals (Cd, Cr, Cu, and Zn) under greenhouse conditions.

Heavy metal stress alters many physiological and metabolic processes in plants. Based on this, the data presented in this chapter demonstrates that mixed HM exposure leads to a significant decrease in photosynthetic pigments in poplar hybrids. Chlorophyll content often measured to assess the impact of environmental stress, since changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity [41]. In the present study, the photosynthetic rates decreased for all poplar hybrids across all HM concentrations except hybrid 1 (Eco 28). Decline in photosynthetic rates has been exhibited in other plants, due to a reduction in photosynthetic pigments by the HMs. In various plants, HMs such as Hg, Cu, Cr, Cd, and Zn have been found to decrease chlorophyll contents [42]. This decline in photosynthetic pigments is most probably due to the inhibition of the reductive steps in the biosynthetic pathways due to the high redox potential of many HMs. In addition, protochlorophyllide reductase the key enzyme, involved in the reduction of protochlorophyll to chlorophyll is well known to be inhibited HMs [43]. Various authors have reported a similar decrease in chlorophyll content under heavy metal stress in cyanobacteria, unicellular chlorophytes (*Chlorella*), gymnosperms such as *Picea abies* and angiosperms, such as *Zea mays*, *Quercus palustrus* and *Acer rubrum*, sunflower as well as almond [44–46]. There are few reports that show an enhancement of pigments after exposure to heavy metals [47].

Various studies have also been conducted on the effects of single heavy metals on different plant species. The effects of Cd and Pb on *Brassica juncea L.* exhibited a decline in growth, chlorophyll content and carotenoids, however, Cd was found to be more detrimental than Pb [48]. According to the study on physiological effects of Cd and Cu on peas (*Pisum sativum*), photosynthetic pigments and photosynthesis rates declined at all concentrations of Cd and only at high Cu concentrations [49].

The effects of mixed heavy metals, which compete with each other in the soil-water medium could be one of the reasons for the contradictory segments of the data. Root uptake and levels of accumulation in leaves vary depending on the hybrid, hence an overall expected decline in hybrids 2, 3 and 4. Whereas hybrid 1 had the highest photosynthetic rate, decreases for 5, 50 and 100 mg L^{-1} were observed. However, a slight increase which was lower than 0 mg L^{-1} was observed at 500 mg L^{-1} HM concentration. Hybrid 1 The significant increase in photosynthetic and transpiration rates in hybrid 1 is also supported by the increase in photosynthetic pigments. The overall BAC of HMs in hybrid 1 would be a contributing factor in understanding how hybrid 1 responds to high concentrations of mixed HMs. This highlights for a better understanding on the form of heavy metal ions in the soil solution and their interaction with the plant roots and eventually their absorption into the system and translocation to above ground parts.

Essential heavy metals (Cu and Zn) are constituents of many enzymes and proteins and are required for normal plant growth and development. However, greater concentrations of any HMs either essential or non-essential can lead to toxic symptoms and growth inhibition in most plants. Overall, a decrease in plant photosynthetic efficiency can be partly responsible for the decrease in plant growth and biomass production.

3.2. Phytoextraction potential of poplar hybrids

The biological accumulation coefficient (BAC), biological translocation coefficient (BTC), bioconcentration factor (BCF) and metal accumulation or concentration index (CI) are given in **Tables 3–6** respectively.

3.2.1. Biological accumulation coefficient (BAC)

Individual HMs showed variations in BAC in the studied hybrids. Copper and chromium BAC values were below 1.0 across all treatments in hybrid 1. However, Cd BAC values were at a high of 24.01 at 5 mg L^{-1} followed by 15.81 at 100 mg L^{-1}, decreasing by almost half to 7.50 and 8.13 for 200 and 500 mg L^{-1} respectively. BAC values for Zn were in the range of 2–3 with the highest significant value noted at 50 mg L^{-1}. The BAC values for hybrids 2, 3 and 4, Cu and Cr were all less than 1, whereas Cd and Zn were higher especially at 50 mg L^{-1} decreasing gradually to 500 mg L^{-1}. BAC values for hybrid 3 were higher for Cd and Zn.

3.2.2. Biological translocation coefficient (BTC)

Copper BTC were less than 1.0 for all hybrids across all HM concentrations. Chromium values fluctuated between 0.06 and 1.94. No significant differences were observed at higher heavy metal treatment concentrations. For Eco 28, 50 and 10 mg L^{-1} heavy metal treatment concentrations had BTC values >1.0, whereas for hybrid 2 and 3 the BTC values were greater than 1 at 200 mg L^{-1}. Cadmium BTC values greater than one ranging up to 3.0 for lower heavy metal concentrations only. The highest values were observed at 5 mg L^{-1} for hybrids 3 and 4. Zinc, on the other hand, had the highest BTC for all hybrids.

3.2.3. Bio-coefficient factor (BCF)

BCF values for Cu and Cr for all 4 hybrids were less than 1.0 across all treatments. However, hybrid 1 Zn BCF was slightly above 1.0, with the highest value of 1.21 at 100 mg L^{-1}. For hybrid 2 at 5 mg L^{-1} and hybrid 4 at 5, 50, and 200 mg L^{-1} the BCF value was greater than 1.0.

Hybrid	HM conc. ($mg\ L^{-1}$)	BAC			
		Cu	Cd	Cr	Zn
Eco 28	0	0.10 ± 0.10^{b}	0.23 ± 0.23^{c}	0.83 ± 0.27^{a}	2.38 ± 0.35^{ab}
	5	0.12 ± 0.10^{ab}	nd	0.78 ± 0.19^{a}	2.73 ± 0.16^{ab}
	50	0.17 ± 0.25^{a}	24.01 ± 3.00^{a}	0.18 ± 0.44^{a}	3.16 ± 0.11^{b}
	100	0.13 ± 0.01^{ab}	15.81 ± 5.28^{ab}	0.14 ± 0.44^{a}	2.90 ± 0.15^{ab}
	200	0.12 ± 0.01^{ab}	7.50 ± 1.22^{bc}	0.12 ± 0.36^{a}	2.78 ± 0.26^{ab}
	500	0.11 ± 0.62^{b}	8.13 ± 3.73^{bc}	0.98 ± 0.43^{a}	2.12 ± 0.57^{b}
DN 034	0	0.15 ± 0.10^{a}	nd	0.22 ± 0.12^{a}	1.08 ± 0.19^{a}
	5	0.12 ± 0.01^{ab}	nd	0.09 ± 0.11^{a}	3.51 ± 0.22^{a}
	50	0.88 ± 0.19^{b}	27.72 ± 10.23^{a}	0.85 ± 0.27^{a}	3.10 ± 0.95^{a}
	100	0.96 ± 0.20^{b}	8.59 ± 2.08^{b}	0.10 ± 0.28^{a}	2.40 ± 0.71^{a}
	200	0.12 ± 0.01^{ab}	14.55 ± 6.12^{ab}	0.14 ± 0.38^{a}	3.11 ± 0.11^{a}
	500	0.12 ± 0.01^{ab}	6.52 ± 1.18^{b}	0.09 ± 0.20^{a}	3.18 ± 0.24^{a}
TN 074	0	0.15 ± 0.01^{a}	nd	0.58 ± 0.01^{a}	4.20 ± 0.35^{a}
	5	0.14 ± 0.13^{a}	nd	0.11 ± 0.06^{a}	4.81 ± 0.28^{a}
	50	0.13 ± 0.16^{a}	58.26 ± 26.00^{a}	0.13 ± 0.56^{a}	3.81 ± 0.13^{a}
	100	0.15 ± 0.17^{a}	17.30 ± 2.60^{b}	0.53 ± 0.01^{a}	4.34 ± 0.25^{a}
	200	0.14 ± 0.64^{a}	6.95 ± 1.16^{b}	0.23 ± 0.15^{a}	3.46 ± 0.87^{a}
	500	0.16 ± 0.02^{a}	11.08 ± 4.41^{b}	0.68 ± 0.21^{a}	4.89 ± 0.45^{a}
TD 225	0	0.09 ± 0.02^{ab}	nd	0.13 ± 0.04^{a}	2.25 ± 0.60^{a}
	5	0.12 ± 0.02^{ab}	nd	0.10 ± 0.02^{a}	2.42 ± 0.50^{a}
	50	0.15 ± 0.18^{a}	15.10 ± 2.49^{a}	0.19 ± 0.09^{a}	2.83 ± 0.27^{a}
	100	0.98 ± 0.15^{ab}	10.06 ± 1.46^{ab}	0.06 ± 0.01^{a}	1.93 ± 0.42^{a}
	200	0.07 ± 0.02^{b}	8.90 ± 4.28^{ab}	0.12 ± 0.05^{a}	1.48 ± 0.54^{a}
	500	0.93 ± 0.02^{ab}	6.81 ± 2.96^{bc}	0.15 ± 0.04^{a}	1.71 ± 0.49^{a}

Values are Mean ± SE, (n = 4). nd – not detected.
For each metal in each treatment values followed by the same letters are not significantly different at $P \le 0.05$ according to Duncan's multiple range test.

Table 3. Biological accumulation coefficient (BAC) of Cu, Cd, Cr and Zn for the four poplar hybrids under greenhouse conditions.

3.2.4. Concentration index (CI)

The CI values were highest for the heavy metal Cd (**Table 6**) and increased with increasing heavy metal treatment concentrations across all studied hybrids. However, the highest CI was observed in hybrid 1 at 200 $mg\ L^{-1}$ and 500 $mg\ L^{-1}$ followed by hybrids 4, 3, and 2. Copper and chromium CI values were similar, with no significant differences between the two hybrids, whereas CI values for Zn were less than 1.0.

Hybrid	HM conc. (mg L^{-1})	BTC			
		Cu	Cd	Cr	Zn
Eco 28	0	0.35 ± 0.58^{ab}	3.40 ± 0.65^{a}	0.32 ± 0.72^{b}	2.44 ± 0.54^{a}
	5	0.28 ± 0.38^{b}	1.96 ± 0.44^{b}	0.79 ± 0.24^{b}	2.54 ± 0.45^{a}
	50	0.49 ± 0.10^{a}	1.25 ± 0.06^{bc}	1.94 ± 0.73^{a}	2.81 ± 0.31^{a}
	100	0.31 ± 0.56^{b}	1.30 ± 0.74^{bc}	1.19 ± 0.49^{ab}	2.42 ± 0.15^{a}
	200	0.26 ± 0.16^{b}	1.01 ± 0.26^{bc}	0.64 ± 1.44^{b}	2.76 ± 0.45^{a}
	500	0.90 ± 0.40 ^{c}	0.43 ± 0.19^{c}	0.28 ± 1.67^{b}	1.83 ± 1.01^{a}
DN 034	0	0.60 ± 0.32^{ab}	1.75 ± 0.60^{ab}	3.65 ± 1.79^{a}	5.25 ± 0.18^{a}
	5	0.49 ± 0.40^{bc}	2.61 ± 0.42^{a}	0.83 ± 0.27^{b}	5.20 ± 0.75^{a}
	50	0.35 ± 0.10^{c}	1.53 ± 0.70^{ab}	1.07 ± 0.56^{b}	4.28 ± 1.73^{a}
	100	0.33 ± 0.69^{c}	1.08 ± 0.26^{b}	0.78 ± 0.33^{b}	2.70 ± 0.70^{a}
	200	0.35 ± 0.50^{c}	1.08 ± 0.25^{b}	1.12 ± 0.33^{b}	3.93 ± 0.83^{a}
	500	0.30 ± 0.31^{c}	0.74 ± 0.70^{b}	0.69 ± 0.16^{b}	3.82 ± 0.22^{a}
TN 074	0	0.70 ± 0.85^{a}	4.82 ± 0.60^{a}	0.47 ± 0.18^{a}	5.97 ± 1.02^{c}
	5	0.57 ± 0.92^{ab}	2.86 ± 0.56^{b}	1.51 ± 2.64^{a}	4.47 ± 0.17^{c}
	50	0.33 ± 0.23^{bc}	1.43 ± 0.65^{c}	0.44 ± 0.30^{a}	12.80 ± 10.46^{c}
	100	0.50 ± 0.96^{ab}	1.18 ± 0.30^{c}	0.50 ± 0.46^{a}	115.97 ± 10.19^{a}
	200	0.35 ± 0.15^{bc}	0.60 ± 0.32^{c}	1.17 ± 0.83^{a}	47.53 ± 9.36^{b}
	500	0.13 ± 0.16^{c}	1.80 ± 0.32^{c}	0.06 ± 0.04^{a}	11.75 ± 10.62^{c}
TD 225	0	0.31 ± 0.0 4^{a}	2.53 ± 0.78^{a}	1.73 ± 0.85^{a}	3.23 ± 1.32^{a}
	5	0.29 ± 0.52^{a}	2.60 ± 0.93^{a}	1.12 ± 0.33^{a}	2.26 ± 0.43^{a}
	50	0.36 ± 0.58^{a}	2.55 ± 0.79^{a}	1.32 ± 0.77^{a}	2.83 ± 0.38^{a}
	100	0.23 ± 0.03^{ab}	0.88 ± 0.24^{ab}	0.55 ± 0.18^{a}	2.87 ± 0.85^{a}
	200	0.11 ± 0.24^{bc}	0.38 ± 0.07^{b}	0.57 ± 0.27^{a}	1.23 ± 0.38^{a}
	500	0.07 ± 0.03^{c}	0.47 ± 0.20^{b}	0.59 ± 0.27^{a}	0.99 ± 0.37^{a}

Values are Mean ± SE, (n = 4). nd – not detected.

For each metal in each treatment values followed by the same letters are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 4. Biological translocation coefficient (BTC) of Cu, Cd, Cr and Zn for the four poplar hybrids under greenhouse conditions.

The absorption, accumulation, and translocation of Cu, Cr, Cd, and Zn on the four studied poplar hybrids depend on the plant and soil environment which determines the availability of HMs. BTC and BAC values greater than 1 in addition to metal concentrations suggested by Baker and Brooks [15] would qualify a plant as a hyperaccumulator [36]. In the case of the studied hybrids, all four show translocation and accumulation potential for Cd and Zn

Hybrid	HM conc. (mg L^{-1})	BCF			
		Cu	Cd	Cr	Zn
Eco 28	0	0.32 ± 0.66[a]	0.58 ± 0.56[b]	0.25 ± 0.58[a]	1.18 ± 0.37[a]
	5	0.45 ± 0.67[a]	nd	0.10 ± 0.01[b]	1.16 ± 0.57[a]
	50	0.30 ± 0.10[a]	13.44 ± 4.80[a]	0.08 ± 0.28[b]	1.12 ± 0.90[a]
	100	0.46 ± 0.78[a]	12.45 ± 1.90[a]	0.13 ± 0.13[b]	1.21 ± 0.89[a]
	200	0.47 ± 0.15[a]	8.27 ± 1.43[a]	0.18 ± 0.15[ab]	1.06 ± 0.98[a]
	500	0.58 ± 0.19[a]	6.99 ± 3.00[ab]	0.17 ± 0.06[ab]	0.62 ± 0.21[a]
DN 034	0	0.24 ± 0.22[c]	nd	0.58 ± 0.01[b]	0.78 ± 0.48[a]
	5	0.25 ± 0.01[c]	nd	0.15 ± 0.49[ab]	0.71 ± 0.84[a]
	50	0.28 ± 0.12[bc]	34.18 ± 15.58[a]	0.12 ± 0.31[ab]	0.86 ± 0.11[a]
	100	0.28 ± 0.12[bc]	8.06 ± 0.81[b]	0.20 ± 0.70[a]	0.83 ± 0.15[a]
	200	0.35 ± 0.32[ab]	12.18 ± 3.74[b]	0.15 ± 0.33[ab]	0.93 ± 0.23[a]
	500	0.42 ± 0.24[a]	8.62 ± 1.03[b]	0.14 ± 0.01[ab]	0.83 ± 0.06[a]
TN 074	0	0.22 ± 0.05[a]	nd	0.33 ± 0.42[a]	0.75 ± 0.11[ab]
	5	0.25 ± 0.06[a]	nd	0.47 ± 0.25[a]	1.08 ± 0.80[a]
	50	0.23 ± 0.16[a]	12.68 ± 8.20[ab]	0.20 ± 0.98[a]	0.57 ± 0.31[c]
	100	0.32 ± 0.53[a]	14.69 ± 2.08 [a]	0.11 ± 0.15[a]	0.04 ± 0.04[b]
	200	0.38 ± 0.74[a]	8.68 ± 2.26[ab]	0.20 ± 0.05[a]	0.06 ± 0.01[a]
	500	0.32 ± 0.38[a]	11.08 ± 8.44[ab]	0.17 ± 0.10[a]	0.38 ± 0.34[bc]
TD 225	0	0.28 ± 0.04[c]	nd	0.10 ± 0.02[b]	0.81 ± 0.14[a]
	5	0.40 ± 0.01[bc]	nd	0.11 ± 0.01[b]	1.05 ± 0.16[a]
	50	0.42 ± 0.02[bc]	8.48 ± 3.05[bc]	0.19 ± 0.03[ab]	1.03 ± 0.12[a]
	100	0.43 ± 0.01[bc]	14.72 ± 5.49[ab]	0.11 ± 0.02[b]	0.75 ± 0.09[a]
	200	0.62 ± 0.15[ab]	19.74 ± 6.48[a]	0.22 ± 0.02[a]	1.16 ± 0.07[a]
	500	0.55 ± 0.18[bc]	4.68 ± 1.71[bc]	0.18 ± 0.06[ab]	0.72 ± 0.25[a]

Values are Mean ± SE, (n = 4). nd – not detected.

For each metal in each treatment values followed by the same letters are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 5. Bio-coefficient factor (BCF) of Cu, Cd, Cr and Zn for the four poplar hybrids under greenhouse conditions.

whereas Cr translocation values are only significant for hybrid 1. Copper, on the other hand, has BTC and BAC values lower than 1 which is also supported by the heavy metal concentrations in the tissue (dry weight). The highest BCF (ratio of metal concentrations in the roots to that in soil) was for Cd for all hybrids and only for Zn in hybrid 1, which indicates the ability of the plant to accumulate targeted HMs from the soil medium. Phytoextraction efficiency is

Hybrid	HM Conc. (mg L^{-1})	CI			
		Cu	Cd	Cr	Zn
Eco 28	0				
	5	1.04 ± 0.13^{b}	2.88 ± 0.49^{d}	0.55 ± 0.48^{a}	0.89 ± 0.06^{a}
	50	0.78 ± 0.18^{b}	8.92 ± 1.66^{c}	0.82 ± 1.63^{a}	0.93 ± 0.07^{a}
	100	1.02 ± 0.14^{b}	17.29 ± 2.11^{b}	0.81 ± 0.10^{a}	0.97 ± 0.06^{a}
	200	1.20 ± 0.32^{b}	31.13 ± 1.62^{a}	0.96 ± 0.15^{a}	0.91 ± 0.05^{a}
	500	1.79 ± 0.73^{a}	33.65 ± 3.28^{a}	1.05 ± 0.31^{a}	0.78 ± 0.06^{a}
DN 034	0				
	5	1.02 ± 0.03^{b}	1.33 ± 0.03^{d}	1.78 ± 0.35^{a}	0.85 ± 0.04^{a}
	50	1.02 ± 0.10^{b}	5.00 ± 0.66^{cd}	1.30 ± 0.25^{a}	0.77 ± 0.16^{a}
	100	1.11 ± 0.04^{b}	6.89 ± 1.39^{c}	1.95 ± 0.54^{a}	0.82 ± 0.12^{a}
	200	1.25 ± 0.70^{b}	16.49 ± 2.33^{b}	1.97 ± 0.35^{a}	0.86 ± 0.04^{a}
	500	1.50 ± 1.48^{a}	29.62 ± 2.21^{a}	1.72 ± 0.17^{a}	0.83 ± 0.05^{a}
TN 074	0				
	5	1.14 ± 0.10^{b}	1.42 ± 0.24^{c}	3.95 ± 1.23^{a}	1.23 ± 0.18^{a}
	50	1.21 ± 0.85^{b}	4.60 ± 0.54^{c}	2.12 ± 0.66^{a}	0.86 ± 0.72^{b}
	100	1.30 ± 0.71^{b}	12.53 ± 0.89^{bc}	0.94 ± 0.11^{a}	0.87 ± 0.44^{b}
	200	1.55 ± 0.18^{b}	20.49 ± 3.25^{ab}	3.07 ± 1.09^{a}	0.94 ± 0.50^{b}
	500	2.39 ± 0.30^{a}	33.17 ± 8.13^{a}	2.20 ± 0.27^{a}	1.23 ± 0.11^{a}
TD 225	0				
	5	1.39 ± 0.03^{b}	2.94 ± 0.53^{d}	0.89 ± 0.15^{c}	1.52 ± 0.02^{a}
	50	1.49 ± 0.03^{b}	8.30 ± 1.01^{c}	1.85 ± 0.43^{a}	1.43 ± 0.12^{ab}
	100	1.47 ± 0.06^{b}	12.58 ± 1.56^{c}	0.67 ± 0.40^{c}	0.96 ± 0.13^{b}
	200	1.65 ± 0.18^{b}	25.98 ± 1.99^{b}	1.63 ± 0.33^{ab}	0.96 ± 0.21^{b}
	500	2.40 ± 0.19^{a}	35.69 ± 2.87^{a}	1.96 ± 0.20^{a}	1.03 ± 0.09^{b}

Values are Mean ± SE, (n = 4). nd – not detected.
For each metal in each treatment values followed by the same letters are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 6. Coefficient index (CI) of Cu, Cd, Cr and Zn for the four poplar hybrids under greenhouse conditions.

related to both plant metal concentration and dry matter yield hence, the ideal plant to remedy a contaminated site should be high yielding with the ability to tolerate and accumulate target contaminants [50].

Selection of poplar hybrids for phytoremediation was based on their general growth and performance. According to the data, the response of the hybrids to the different HMs and concentrations varied. The bioavailability of metals in trees and subsequent metal accumulation in its tissues can vary hugely depending on the metal contamination source and site

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conditions [51]. Based on the mean HM contents in the hybrids, the concentration of Zn is higher in leaves. The highest Zn content of 425.49 mg kg^{-1} dry weight for hybrid 3 followed by 309.52 mg kg^{-1} for hybrid 2 was observed (results not shown). Overall, Cu and Cr were neither well accumulated nor translocated in all hybrids based on the values of BAC, BTC, and BCF. On the other hand, Zn was the only HM that was accumulated in high concentrations especially in the leaves of all hybrids across all concentrations. The highest BAC values were for hybrid 3 at 500 mg L^{-1} whereas BTC values for hybrid 3 Zn were also the highest compared to all other clones, stating clearly the efficient accumulation as well as translocation ability of the hybrid. The BCF values for Cd and Zn for hybrid one were >1 for all concentrations except 500 mg L^{-1} Zn. Based on previous research [51–56] and the results presented in this chapter we can conclude that trees differ in their ability to absorb heavy metals from the soil. The translocation ability from the root to the shoots also vary under different conditions.

4. Conclusion and future prospects

In conclusion based on the physiochemical parameters analyzed, the individual heavy metal contents in each hybrid along with the phytoextraction potential indices, it can be deduced that hybrid 1 (Eco 28) can be selected as a suitable candidate for phytoremediation work with a focus on Cd and Zn phytoextraction capabilities. Hybrid 3 (TN 074) also shows potential as a phytoremediator, however, the studied physiochemical parameters were severely affected by exposure to high concentrations of mixed HMs. This also indicates that subsequent studies are needed to determine the potential of using hybrid 3 as a candidate for phytoremediation of mixed heavy metal contaminated sites. Phytoextraction has been advocated as an effective, eco-friendly and cost-effective technology for the remediation of soils contaminated with heavy metals. The success of phytoextraction depends on several factors which include the concentration of heavy metals in the soil, bioavailability of heavy metals for uptake, and the capability of the plant to absorb and accumulate metals in their tissues. The existing flora diversity needs to be exploited to screen out new and effective hyperaccumulators. In addition to these extensive field-based research for extended durations are also required to better understand heavy metal uptake and accumulation.

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Conflict of interest

No potential conflict of interest was reported by the authors.

Acronyms and abbreviations

A	photosynthesis rate
BAC	bioaccumulation coefficient
BCF	bio-coefficient factor
BTC	biotranslocation coefficient
Chl *a*	chlorophyll a
Chl *b*	chlorophyll b
CI	concentration index
E	transpiration rate

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