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Original Article

Phytoextraction of Pb and Cd by *Catalpa bignonioides* in Crude Oil-Contaminated Soil

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ABSTRACT

Soil contamination by heavy metals and petroleum hydrocarbons has become a serious issue that threatens the environment and human health, necessitating effective remediation approaches. This study investigated the phytoextraction potential of *Catalpa bignonioides* seedlings (1 and 2 years old) for lead (Pb) and cadmium (Cd) in soils contaminated with 1% and 2% crude oil. The aim was to assess the capability of plants to extract these heavy metals from polluted soils. Results showed that both crude oil concentrations significantly reduced plant biomass and chlorophyll content compared to the control. Pb and Cd concentrations in contaminated soils were higher than in oil-free soils but remained within the acceptable limits. In the control soil, Pb concentrations were 2.290 mg kg^{-1} (roots) and 1.957 mg·kg^{-1} (shoots), while in 2% crude oil soil, they increased to 2.493 (mg·kg^{-1} in roots) and 2.467 (mg·kg^{-1} in shoots). The highest Cd concentrations 6.535 (mg·kg^{-1} in roots), 2.904 (mg·kg^{-1} in shoots) were found in 2% crude oil-treated plants. TF values for Pb approached or reached 1 in 2-year-old seedlings, while for Cd were consistently below 1. Pb exhibited higher BAF values overall (3.601–4.400), whereas the bioaccumulation factor (BAF) for Cd exceeded 1 only in 2-year-old plants. Overall, older plants removed significantly greater amounts of Pb and Cd, suggesting *Catalpa bignonioides* is a promising candidate for remediating crude oil-polluted soils.

Keywords: Metal uptake, Soil remediation, Petroleum hydrocarbons, Heavy metals, Translocation factor, Bioaccumulation factor.

1 Introduction

Soil is the main environmental matrix that supports life for all living organisms. Due to increase in population and human activities the soil is continuously exposed to various types of pollutants including persistent pollutants, such as polycyclic aromatic hydrocarbons (PAHs) [1].

Hydrocarbon contamination of the soil environment, primarily from petroleum hydrocarbons is becoming more common worldwide. This is because of the world's significant reliance on petroleum as a primary energy source [2]. Petroleum derivatives are frequently seen in urban soils, adjacent to industrial sites and areas associated with crude oil and natural gas extraction. Oil-derived substances trigger various mechanisms that affect living organisms as well as non-living elements within ecosystems once they are introduced. Pollution of the environment with petroleum hydrocarbons is the most common among other pollutant types, and immediately reduces ecosystem functioning which affects human health, given their potential to elicit carcinogenicity, teratogenicity, and mutagenicity [3].

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When soil is polluted with crude oil, certain hydrocarbon compounds toxify soil biota by reducing microbial diversity and affecting plant growth; Due to nutrients non-availability for plants and microorganisms [4].

Petroleum contamination significantly alters soil composition, leading to an accumulation of various pollutants, including, polycyclic aromatic hydrocarbons, volatile organic compounds and heavy metals. Evidence from multiple studies shows that crude oil pollution in soil is closely associated with augmented levels of heavy metals including (Fe, Mn, Zn, Ni, Hg, Pb,Cu and Cd, Cr)[2]. Introduction of toxic metals into the global biosphere has significantly increased due to ongoing emissions from anthropogenic sources such as industries, smelting, sludge dumping, transportation, application of mineral fertilizers, herbicides and pesticides in agricultures, mining and other sources [5]. Heavy metals cannot be degraded or destroyed; they accumulate in the environment which leads to build up in plant, animal and human tissues through the food chain [6].

Heavy metal pollution has both direct and indirect impacts on human health [7]. When present above certain thresholds, these elements can negatively impact the productivity of soil, plants and animals, ultimately leading to widespread environmental damage [8]. Among them, cadmium (Cd) is considered highly toxic to all living organisms due to it is high solubility, making its presence in soil particularly hazardous [9]. It is readily taken up by plants and concentrates in consumable tissues, hence creating a hazard for food safety [10]. Due to its high toxicity, cadmium use has declined. Human exposure occurs through inhalation, cigarette smoke, ingestion of contaminated food, and occupational exposure [11]. Cd bioaccumulation in the body leads to acute and chronic toxicity due to biomagnification [12]. Similarly, lead (Pb) is a harmful, non-essential heavy metal that ranks among the most ubiquitously distributed trace-level pollutants in soils and plants. Pb causes a range of harmful effects on living organisms at the morphological, physiological, and biochemical levels. Due to its persistence in natural reservoirs such as soils and water, and continual release from human activities (vehicular traffic emissions and industrial discharges), Pb is now ubiquitously dispersed throughout ecosystems and is commonly detected within the tissues of almost all organisms [13]. Pb accumulates in the upper eight inches of the ground and remains largely immobile, further exacerbating its environmental impact [14]. Various remediation methods, including chemical and physical techniques, are available for addressing heavy metal pollution. While these methods can effectively reduce contamination, they often result in secondary pollution due to their non-eco-friendly nature that can negatively impact biological activity, soil structure, and soil fertility [15]. Additionally, some of these techniques require significant financial investment, making them costly to implement. As a result, there has been growing interest in exploring bioremediation through organic approaches functioning as a more eco-conscious and resource-efficient option. Phytoremediation has emerged as a promising strategy, utilizing plant species to uptake, concentrate and neutralize heavy metals from polluted soils [16]. Phytoremediation is an environmentally friendly method and green technology that uses sunlight as its primary energy source, in situ treatment technology, cost-effective, and aesthetically pleasing, phytoremediation is an appropriate choice [17]. Due to its low-cost and effective heavy metal cleanup technology, phytoremediation has gained popularity recently [18]. Catalpa bignonioides, or Southern catalpa, is a fast-growing deciduous tree native to the southeastern U.S., known for its large leaves and tolerance to pollution. Its broad foliage aids in air purification, while its resilience to heavy metals like Pb and Cd makes it suitable for phytoextraction due to high biomass and metal accumulation in roots and shoots [19,20]. Some local studies in Iraq and Kurdistan have investigated phytoremediation of crude oil contaminated soils using various plants and trees [21-23]. This study was conducted to evaluate the potential of *Catalpa bignonioides* seedlings to phytoremediate lead (Pb) and cadmium (Cd) in soils contaminated with crude oil.

2 Materials and Methods

The current research was implemented during 2023/2024 aimed to evaluate the ability of Catalpa trees grown in Duhok city to uptake Cadmium and Lead from soil polluted by crude oil. Seedlings aged (one and two years) were transferred into plastic containers filled with sandy clay loam soil polluted with 1% and 2% crude oil (weight/ weight), separately planted in pots (25cm diameter X 35cm depth), with EC 0.789 $dS.m^{-1}$, pH 7.8, total organic matter 1.13 %, total Pb 2,29 mg/kg and total Cd 0.73 mg/kg. Concentration of Pb and Cd in crude oil was 1.12 and 0.0029 µg.ml-1 respectively.

A Completely Randomized Block Design (CRBD) was implemented, consisting of a two-factor experiment; the first factor was seedlings' age (one and two years old), while the second factor was the crude oil contaminated soil with 1% and 2% (weight/weight). The design was of three replicates, five plants in each experimental unit.

Treatment means were compared using Duncan's Multiple Range Test at a 5% significance level**.** Pots were irrigated with well water to field capacity, and chlorophyll content in the leaves was measured using a SPAD meter. After 8 months of transplantation, plants were removed from the soil and separated (into roots and shoots). They were carefully washed to remove any deposits and oven-dried at 70°C for 48 hours. Consequently, the total biomass was determined. Shoots and roots were separately milled and stored until heavy metal analysis was performed. 0.5 gram of sample powder was digested with an acid mixture (HNO_3 and $HClO_4$) in the ratio 4 to 1. Subsequently, 50 ml of deionized water is added to dilute the digest solution and is lastly filtered.

Soil samples were digested according to the procedure followed by Jakson [24], soil samples were taken by the completion of the experiment, following the removal of plants from their containers same amount of soil were collected from all pots of each treatment units, mixed well, then air dried and after that crushed and sieved (using 2 mm sieve). 0.5 g of soil samples were digested using a 3-acid mixture of sulfuric acid, nitric acid, and perchloric acid (3:1:1). Lead and cadmium concentration in all samples were analyzed by atomic absorption spectrophotometer AAS.

The translocation factor (TF), bioaccumulation factor (BAF), tolerance index (TI), and removed metal were estimated to determine the phytoextraction efficiency of Catalpa.

The translocation factor (TF) ($\frac{metal\ concentration\ in\ shoots}{metal\ concentration\ in\ roots}$) was calculated to assess the plant's ability to transport the absorbed metals from the root system to the shoot system [25].

The bioaccumulation factor (BAF) ($\frac{metal\ concentration\ in\ shoots}{metal\ concentration\ in\ soil}$). BAF indicates the amount of metal concentrated in above-ground tissues [25].

While the tolerance index (TI) ($\frac{biomas\ of\ polluted\ plants}{biomas\ of\ control\ plants}$) measures the tolerance of plants at increasing levels of lead and cadmium concentration in the soil.

Removed metal (µg. g^{-1} d. wt) by plant ($metal\ concentration * shoot\ biomas$) was calculated to quantify the amount of metal that can be removed from the soil by a single [26].

Plants with TF and BAF greater than one (>1) are suitable for phytoextraction.

The results demonstrate a clear trend in the growth parameters of *Catalpa bignonioides*, (1- and 2- years old) grown in soil contaminated with varying concentrations of crude oil (1% and 2%) compared to controlled plants, presented in Table 1, showing that the largest biomass was found in seedling of 2 years old treated with 2% of Crude oil (21.563 $g.m^{-2}$). While the smallest biomass was (11.232$g.m^{-2}$) found in 1 year old seedlings at 1% crude oil. This difference can be attributed to the greater physiological and morphological resilience of older seedlings, which are generally more tolerant to environmental stressors [27].

Table 1: The effect of crude oil concentrations on roots and shoots biomass of Catalpa seedlings.

Treatments		Roots and shoots biomass
Seedling age	1 year	11.232 b
	2 years	21.477 a
crude oil conc.	0 %	18.252 a
	1 %	16.314 b
	2 %	16.716 b
Age X Oil conc.	1year X 0 %	15.033 b
	1year X 1 %	11.232 c
	1year X 2 %	11.868 c
	2year X 0 %	21.470 a
	2year X 1 %	21.397 a
	2year X 2 %	21.563 a

Means with the same letter are non-significantly different from each other based on Duncan's multiple range test at 0.05 level.

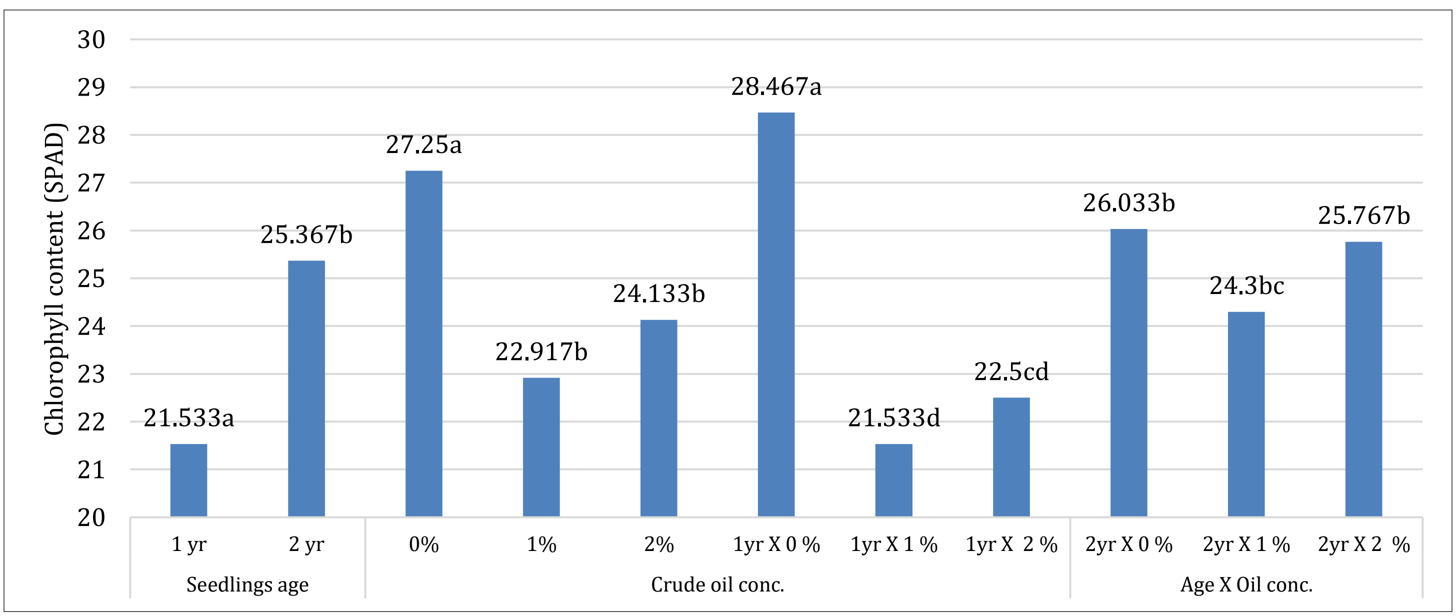

Figure 1: The effect of seedling age, crude oil concentration and their interaction on chlorophyll content (SPAD) in Catalpa seedlings. Means with the same letter are non-significantly different from each other based on Duncan's multiple range test at 0.05 level.

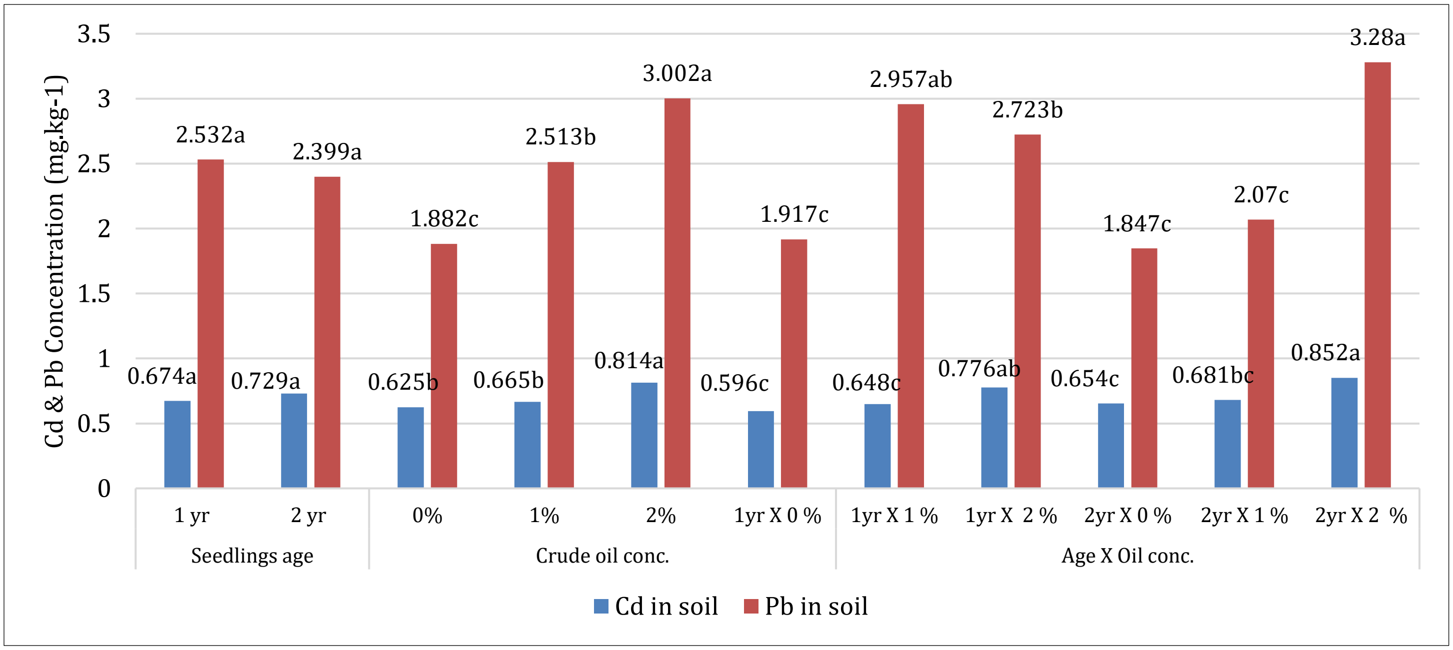

Figure 2: Effect of seedling age, crude oil concentration, and their interaction on cadmium (Cd) and lead (Pb) concentrations in soil ($mg \cdot kg^{-1}$). Means with the same letter are non-significantly different from each other based on Duncan's multiple range test at 0.05 level.

It appears from Figure 1, below that the chlorophyll content was highest among the control plants (28.467 SPAD), but significantly declined as the intensity of Petroleum contamination increased.

The greatest chlorophyll content was recorded in the control treatment with no crude oil contamination (0%), as uncontaminated soil provides optimal nutrients for chlorophyll synthesis. In contrast, 1-year-old seedlings showed the lowest chlorophyll content under 1% crude oil (21.533), likely due to their underdeveloped root systems, reduced nutrient uptake, and higher sensitivity to hydrocarbon toxicity, which disrupts chloroplast function [28]. Interestingly, in 2-year-old seedlings, chlorophyll levels increased again under oil contamination, suggesting greater resilience due to a more developed root system, improved stress tolerance, and potential detoxification mechanisms. This indicates that older seedlings can better withstand crude oil stress and maintain chlorophyll production compared to younger ones [29].

Results of two heavy metals in soil Figure 2, indicated that Cd levels were generally unaffected by seedlings ages of one and two years, respectively. However, crude oil contamination increased Cd accumulation, with the highest concentration at 2% oil (0.814 a) compared to the control (0.625 b). Notably, significant results observed regarding their interaction with the highest value (0.852). This suggests that crude oil enhances Cd retention in the soil, likely due to increased organic matter binding or altered soil pH affecting metal solubility, similar to the mechanisms described by Bolan [30]. Specifically, the reduced availability of Cd may result from its precipitation as $CdCO_3$, a form that is unavailable to plants a phenomenon commonly observed in calcareous soils such as Dukan soil. Additionally, Cd may become bound to functional groups present in organic matter, further reducing its availability.

This observation aligns with studies [31] which identified increased cadmium (Cd) concentrations in soils contaminated with crude oil in the Niger Delta region of Nigeria. They reported a clear link between crude oil activities and heavy metal pollution, supporting the idea that Crude petroleum acts as a source of Cadmium accumulation. The higher Cd levels in 2-year-old seedlings grown in 2% crude oil (0.852 a) could be attributed to the more developed root systems of mature plants, potentially facilitating greater metal absorption. This notion is supported by studies that found that plant roots can significantly influence the distribution and bioavailability of heavy metals in contaminated soils [27].

Pb accumulation was also unaffected by seedling age, but crude oil contamination significantly increased Pb levels, with the highest concentration at 2% oil (3.002 a) and the lowest in the control (1.882). The current findings were supported by others who found the concentration of lead in oil contaminated sites was 43% higher than it was in uncontaminated sites [32]. This rise in Pb content under oil stress is likely due to decreased microbial degradation of Pb-bound organic matter or reduced leaching. The presence of crude oil can increase the bioavailability of lead by affecting soil structure, porosity, and microbial activity, which can all influence how lead interacts with the soil and its components (Adesina & Adelasoye, 2014). Notably, 2-year-old seedlings in 2% crude oil showed the highest Pb levels (3.280 a), indicating that older plants may influence Pb retention in the rhizosphere, possibly due to root exudates altering Pb mobility.
Generally, crude oil-contaminated soil did not elevate Pb and Cd concentrations beyond safe limits (25-500) and (3-6) $mg.kg^{-1}$ soil

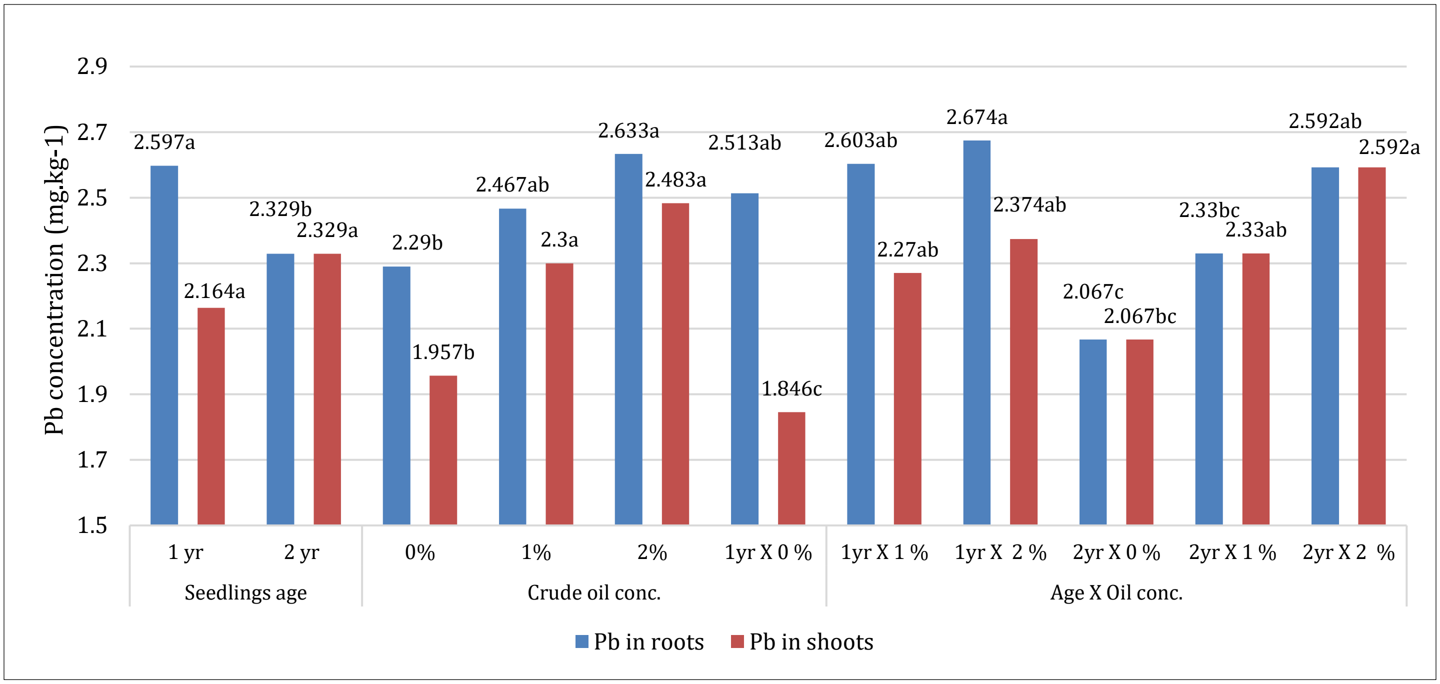

Figure 3: Effect of seedling age, crude oil concentration, and their interaction on lead (Pb) concentrations in root and shoot ($mg \cdot kg^{-1}$D.Wt). Means with the same letter are non-significantly different from each other based on Duncan's multiple range test at 0.05 level.

respectively recommended by WHO/FAO in agricultural soils [33].

Pb accumulation Figure 3, varied with seedling age and crude oil contamination. One-year-old seedlings had higher Pb in roots (2.597 mg/kg) than two-year-olds (2.329 mg/kg). This aligns with studies showing younger plants accumulate more heavy metals due to higher metabolic activity and root surface area [34]. Pb levels increased with oil pollution, peaking at 2% contamination (2.633 mg/kg in roots, 2.483 mg/kg in shoots), consistent with research indicating that crude oil alters soil properties, increasing heavy metal bioavailability [35]. The highest Pb accumulation occurred in 1-year-old seedlings at 2% oil (2.674 mg/kg roots), while the lowest was in 2-year-olds in uncontaminated soil (2.067 mg/kg). Pb in shoots was highest in 2-year-old seedlings at 2% oil (2.592 mg/kg). A comprehensive review notes that roots generally accumulate significantly more Pb than shoots due to restricted translocation, and younger plants tend to uptake higher Pb levels than mature ones [36]. Other supporting findings indicate that contaminated soils can enhance metal uptake and incorporation into plant tissues [37]. These results highlight how both oil pollution and plant age influence Pb uptake and translocation. This may be due to several physiological and anatomical factors. Younger roots have more actively dividing cells and root hairs, increasing Pb uptake through higher permeability and surface area [38]. Young plants also show higher metabolic activity and nutrient demand, absorbing Pb via pathways meant for essential elements like calcium [39]. In contrast, older seedlings develop thicker root barriers and stronger detoxification systems, such as phytochelatin production and vacuolar sequestration, which reduce Pb uptake [40].

Cd concentrations in shoots Figure 4, were comparable between 1-year-old (2.757 mg/kg) and 2-year-old seedlings (2.688 mg/kg). Cd levels in shoots increased from 2.511 mg/kg in the control to 2.753 mg/kg and 2.904 mg/kg at 1% and 2% oil concentrations, respectively. These results indicate that crude oil contamination elevates Cd accumulation in both roots and shoots, while seedling age primarily influences Cd translocation to shoots. The interaction between seedling age and oil concentration revealed that 1-year-old seedlings at 2% oil had the highest root Cd levels (6.560 mg/kg), whereas 2-year-old seedlings at 0% oil had the lowest (4.527 mg/kg). In shoots, the highest Cd concentration was observed in 2-year-old seedlings at 2% oil (2.941 mg/kg), and the lowest in 2-year-old seedlings at 0% oil (2.467 mg/kg). These findings suggest that crude oil pollution significantly enhances Cd uptake in Catalpa seedlings, with minimal influence from seedling age. This increase is consistent with findings indicating that crude oil pollutants can modify soil properties, leading to greater heavy metal absorption by plants. The current findings align with previous research [32]. However, contrasting results were reported [41], where tested plants exhibited the highest Cd accumulation in soil at 50 mL crude oil, while the lowest Cd concentrations were found at 200 mL contamination. These differences may be attributed to species-specific variations in Cd uptake.

A higher TF value indicates a greater ability of plants to absorb heavy metals and translocate them within their tissues [42]. From table 2, crude oil pollution increased the translocation factor (TF)

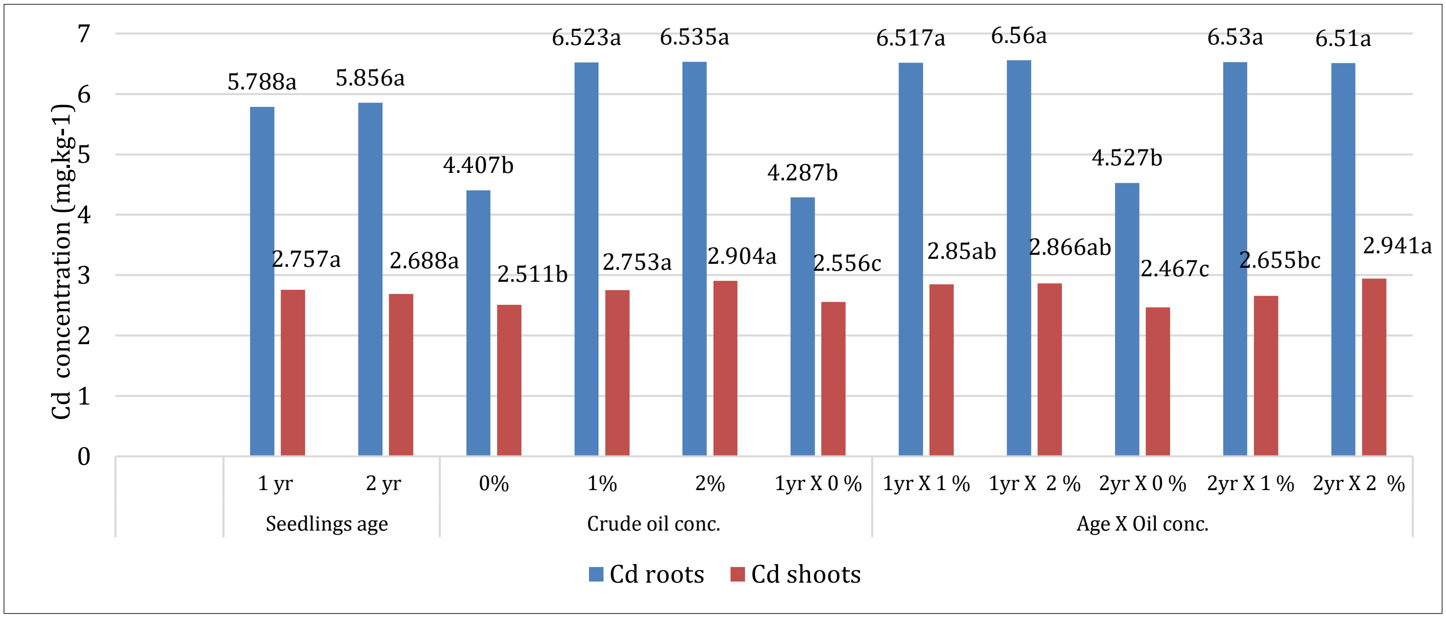

Figure 4: Effect of seedling age, crude oil concentration, and their interaction on cadmium (Cd) concentrations in root and shoot ($mg \cdot kg^{-1}$D.Wt). Means with the same letter are non-significantly different from each other based on Duncan's multiple range test at 0.05 level.

for Pb, rising from 0.867 in uncontaminated soil to 0.944 at 2% oil concentration, while the TF for Cd decreased from 0.572 to 0.444. This reduction indicates that crude oil contamination may hinder metal movement from roots to shoots, likely due to changes in soil composition or root function [43]. Typically, TF values above 1 suggest greater metal accumulation in shoots than in roots. Several factors influence heavy metal absorption and distribution within plants, including soil type, pH, organic matter content, calcium carbonate ($CaCO_3$) levels, plant species, chemical forms of the metals, irrigation water source, and climatic conditions. In particular, high soil $CaCO_3$ content can reduce metal availability by increasing pH and causing metals to precipitate or bind to soil particles, limiting their uptake and movement within plants [44]. These findings indicate that seedling age has a greater effect on Pb mobility than Cd, while crude oil contamination further restricts metal translocation, possibly due to modifications in soil aeration and metal-binding capacity.

A Translocation factor (TF) value below 1 implies that the amount of both Pb and Cd retained in the roots exceeds the amount transported to the shoots. This is attributed to the root system's capacity to restrict the movement of Cd to the aboveground parts of the plant, a phenomenon typically observed only when they present in high concentrations [45]. According to Suchkovaa et al. (2010), the Catalpa plant cannot be classified as a Pb hyperaccumulator, as no TF values greater than 1 were recorded However, TF values exceeding 1 were observed in some cases for Pb, particularly in two-year-old plants. Lead TF values that were close to or equal to 1 indicate that Pb is nearly equally distributed between roots and shoots, which is a sign of efficient internal transport [46].

Various inorganic and organic contaminants can be absorbed by plants, storing them in different plant tissues in the case of petroleum-related pollutants [47]. The bioaccumulation factor (BAF) for Pb and Cd in Table 2, varied with seedling age and crude oil contamination. Cd accumulation was significantly higher in 2-year-old seedlings (1.018) compared to 1-year-old seedlings (0.868), while Pb accumulation followed the opposite trend, with 1-year-old seedlings (4.133) accumulating more Pb than 2-year-old seedlings (3.729). Crude oil contamination reduced Cd bioaccumulation, with BAF values decreasing from 1.041 in uncontaminated soil to 0.836 at 2% oil. Conversely, Pb bioaccumulation remained high in uncontaminated (4.036) and 1% oil-treated soils (4.156) but declined at 2% oil (3.601). These results suggest that crude oil contamination reduces Cd accumulation while having a lesser impact on Pb uptake, potentially due to differences in metal mobility, plant uptake mechanisms, and soil conditions under oil stress. BAF is a key indicator of a plant's potential for phytoremediation, showing its capability to accumulate considerable quantities of elements, including metals and metalloids. BAF values greater than 1 for the transfer of metals from soil to shoots suggest a strong potential for phytoextraction in these plant species [48].

Throughout the current study, the amount of Pb and Cd measured levels removed by Catalpa seedlings Table 2, varied based on seedling age and crude oil contamination. Older seedlings (2 years) exhibited significantly higher removal of both Pb (27.715 mg/kg) and Cd (74.951 mg/kg) compared to younger seedlings (1 year), which removed 13.661 mg/kg of Pb and 38.946 mg/kg of Cd. Crude oil contamination influenced metal removal, with Pb removal increasing from 19.602 mg/kg in uncontaminated soil to 23.001 mg/kg at 2% oil, while Cd removal rose from 48.986 mg/kg to 67.001 mg/kg. The highest Lead Pb and Cadmium Cd removal occurred within 2-year-old seedlings at 2% oil (31.299 mg/kg and 91.793 mg/kg, respectively). These findings suggest that older seedlings are more efficient in heavy metal uptake, likely due to larger biomass and more developed root systems.

Table 2: Translocation, Bioaccumulation Factor, and Removal of Pb and Cd by Catalpa Grown in Crude Oil-Polluted Soil.

Treatments		TF Pb	TF Cd	BAF Pb	BAF Cd	Removed Pb	Removed Cd
Seedling age	1 year	0.834 b	0.490 a	0.868 b	4.133 a	13.661 b	38.946 b
	2 years	0.999 a	0.468 a	1.018 a	3.729 b	27.715 a	74.951 a
Crude oil conc.	0 %	0.867 b	0.572 a	1.041 a	4.036 a	19.602 b	48.986 c
	1 %	0.939 ab	0.422 b	0.952 b	4.156 a	20.184 b	54.984 b
	2 %	0.944 a	0.444 b	0.836 c	3.601 b	23.001 a	67.001 a
Age X Oil conc.	1year X 0 %	0.735 c	0.597 a	0.963 b	4.296 ab	14.064 c	35.933 e
	1year X 1 %	0.878 b	0.437 c	0.770 c	4.400 a	13.661 c	38.946 de
	1year X 2 %	0.890 ab	0.437 c	0.872 bc	3.705 c	14.702 c	42.208 d
	2years X 0 %	0.999 a	0.546 b	1.118 a	3.775 bc	25.140 b	62.039 c
	2years X 1 %	1.000 a	0.407 c	1.134 a	3.913 abc	26.706 b	71.021 b
	2years X 2 %	0.998 a	0.452 c	0.801 c	3.498 c	31.299 a	91.793 a

Means with the same letter are non-significantly different from each other based on Duncan's multiple range test at 0.05 level.

Moreover, crude oil contamination appears to enhance metal retention in plants, possibly by altering metal bioavailability in the soil, a phenomenon previously observed in petroleum-contaminated environments [49].

Conclusion and Recommendations

Based on the results of the present study *Catalpa bignonioides* can grow in crude oil-contaminated soils without exceeding WHO/FAO limits for Pb and Cd, making it a viable candidate for phytoremediation. Metal uptake was significantly influenced by both seedling age and oil concentration. Older (2-year-old) seedlings showed greater biomass, higher chlorophyll content, and removed significantly more Cd and Pb than younger plants, highlighting the importance of plant maturity in remediation efficiency.

Although *Catalpa bignonioides* is not a hyperaccumulator, it showed strong potential for phytoextraction, particularly for cadmium, due to higher bioaccumulation and translocation values. These findings support its use in phytoremediation programs targeting moderately polluted oil-affected soils, especially where cadmium contamination is predominant. Further research is recommended to assess long-term performance under diverse environmental conditions.

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Authors Contribution

All authors were equally involved in every phase of the project.

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