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Article

Influence of the phenological stages of Heliconia psittacorum on the removal of hydrocarbons from contaminated soils

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Abstract: The phytoremediation potential of Heliconia psittacorum was evaluated for removing total petroleum hydrocarbons (TPH) and heavy metals (Cd, Pb, Ni) from highly contaminated soils in the Ecuadorian Amazon. An experiment was set up with 225 plants distributed across three phenological stages: rhizome propagation, growth, and pre-flowering. Survival, plant height, and contaminant levels were measured before and after application. The soil had an initial TPH level of 10,457.69 mg/kg, exceeding the regulatory limit for agricultural use by more than 2,000%. The results indicated that the growth stage achieved the highest TPH removal efficiency (56.43%), followed by pre-flowering (44.30%), while rhizome propagation achieved only 6.01%. No significant removal of Cd or Pb was observed, although Ni showed a reduction only in the growth phase (12.07%). The highest survival and growth rates were observed in pre-flowering, reflecting greater tolerance to contamination stress. It was concluded that H. psittacorum has high phytoremediation potential in advanced phenological phases, making it a viable biotechnological alternative for the ecological recovery of soils affected by oil activity in tropical regions. These findings support the implementation of strategies based on plant phenology to optimize environmental remediation.
Keywords: phytoremediation; soil contamination; hydrocarbons; native species; environmental recovery

1. Introduction

Soil contamination by total petroleum hydrocarbons (TPH) and heavy metals is one of the most persistent and complex environmental problems in regions affected by extractive activities, particularly in tropical ecosystems. TPH have high chemical stability, low bioavailability, and a marked tendency to age in the soil, which hinders their natural degradation and prolongs their negative effects on soil structure, microbial activity, and the functionality of terrestrial ecosystems At the same time, the simultaneous presence of heavy metals such as cadmium (Cd), lead (Pb), and nickel

(Ni) increases residual ecotoxicity and can interfere with the biological processes responsible for the natural attenuation of the contaminant, especially in soils with historical or aged contamination

In Latin America, and particularly in the Ecuadorian Amazon, the magnitude of the problem is especially critical due to the recurrence of oil spills and the limited response capacity of conventional technologies. Concentrations of TPH that far exceed regulatory limits for agricultural use have been reported, leading to accelerated soil degradation, loss of biodiversity, and risks to human health and local livelihoods . These conditions underscore the need to implement environmental remediation strategies that are technically and ecologically viable, sustainable, and adapted to the soil and climate conditions of tropical ecosystems.

In this context, phytoremediation has emerged as a low-environmental-impact in situ remediation alternative, particularly suitable for large areas of contaminated soil where physical-chemical methods are economically unviable or ecologically disruptive . However, current scientific evidence indicates that, in the case of complex organic pollutants such as TPHs, the efficiency of phytoremediation does not depend primarily on the direct absorption of the pollutant by the plant, but rather on rhizodegradation processes, in which the plant acts as a modulator of the rhizospheric environment, stimulating microbial communities with hydrocarbonoclastic capacity through the release of root exudates, soil oxygenation, and root system development .

The rhizosphere is a highly dynamic microenvironment where functional interactions between the plant and soil microorganisms are established, which are decisive for the degradation and stabilization of hydrocarbons. Experimental and field studies have shown that the presence of pollution-tolerant plants significantly increases the abundance and diversity of TPH-degrading bacteria, promoting the mineralization of complex fractions of the pollutant and reducing its residual ecotoxicity. Likewise, it has been documented that both rhizospheric microorganisms and endophytes associated with plants play a key role in the degradation of hydrocarbons, reinforcing the integrated nature of the phytoremediation process .

Within this framework, *Heliconia psittacorum* has been identified as a native tropical species with high potential for phytoremediation applications. Previous research has documented its tolerance to chemical stress conditions, its ability to grow in contaminated soils and waters, and its association with functional microbial communities in the rhizosphere and root system . Available evidence indicates that *H. psittacorum* acts primarily as a selective root accumulator, with limited aerial translocation of metals, and that its contribution to remediation is based on phytostabilization and microbial stimulation mechanisms rather than direct phytoextraction

However, the phytoremediation response of *Heliconia psittacorum*, like that of other plant species, is not constant or uniform, but varies depending on the physiological state and phenological stage of the plant. Changes in biomass allocation, root system development, metabolic activity, and rhizosphere exudate production throughout the plant life cycle can substantially modify plant-microorganism interaction and, consequently, the efficiency of contaminant removal processes

Several studies have shown that intermediate and advanced phenological stages tend to exhibit greater phytoremediation efficiency, associated with greater structural development and more stable physiological systems, while early growth stages show greater vulnerability to chemical stress and a lower capacity for rhizospheric interaction . Despite this evidence, most phytoremediation studies continue to evaluate plant performance statically, without explicitly considering the influence of the phenological stage on the removal of complex organic contaminants in soils

There is limited understanding of how the different phenological stages of *Heliconia psittacorum* influence its phytoremediation efficiency in highly TPH-contaminated soils in tropical environments. This knowledge gap restricts the optimization of phytoremediation strategies based on native species and limits their effective application in ecological restoration programs in the Amazon. In this context, the present study evaluates the influence of the phenological stage of *Heliconia psittacorum* on the removal of total petroleum hydrocarbons and heavy metals from contaminated soils, with the aim of providing a scientific basis for the design of more efficient environmental remediation strategies adapted to degraded tropical ecosystems.

2. Materials and Methods

The experiment lasted a total of 40 days, during which parameters of survival, plant growth, and the efficiency of total petroleum hydrocarbon and heavy metal removal from the soil were evaluated. The research was conducted using an experimental, quantitative, and explanatory approach, structured in four phases: (i) initial determination of contaminants (TPH and heavy metals) in the soil; (ii) implementation of treatments with Heliconia psittacorum in three phenological stages (rhizome propagation, vegetative growth, and pre-flowering); (iii) evaluation of plant survival and height in *Heliconia psittacorum* according to the phenological stage; and (iv) physical-chemical analysis of the soil after treatment to determine the efficiency of contaminant removal.

The research was conducted in the province of Orellana, Ecuador: the contaminated soil sample was obtained at Hacienda Jungal in the parish of San Luis de Armenia (Figure 1 A), the rhizomes of *H. psittacorum* were collected in the parish of Tres de Noviembre (Figure 1B), the greenhouse was in the vicinity of the Escuela Superior Politécnica de Chimborazo – Campus Norte, and the contaminant analyses were carried out in the LABSU laboratory, Figure 1C.

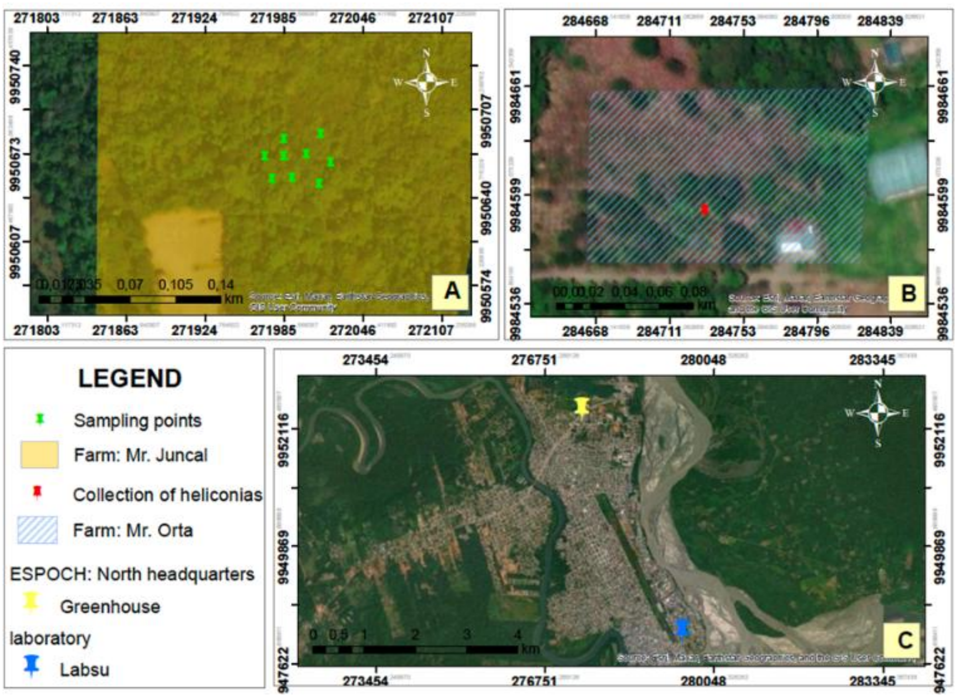

Figure 1. Geographic location of soil sampling, rhizome collection, greenhouse, and laboratory for conducting the study.

2.1. Phase 1: Initial characterization of contaminants

El muestreo del suelo se llevó a cabo utilizando una pala desinfectada con hipoclorito de sodio al 0,5 %. Se recogieron nueve muestras compuestas de 20 kg a una profundidad de 0 a 30 cm. Estas se homogeneizaron mediante mezcla en zanja y división manual en cuartos para obtener submuestras de 0,5 a 1 kg [22]. Las muestras se almacenaron en bolsas de polietileno de alta densidad etiquetadas, se transportaron en cajas térmicas y se mantuvieron a 4 °C durante todo el traslado, de conformidad con la norma NTE INEN 2266 .

The TPH concentration was determined using ISO 16703 / EPA 8015 [24,25], and heavy metals (Cd, Pb, Ni) using EPA 3050B / EPA 6010C / ISO 11047 .Additionally, prior to establishing the experimental treatments, physicochemical parameters of the soil were determined, including pH, electrical conductivity, organic matter, and exchangeable cations, to establish a baseline for evaluating edaphic changes after the phytoremediation process. The analytical results obtained were interpreted in relation to the regulatory limits established for agricultural soils in Ecuador, as presented in Table 1 (Limits for TPH and heavy metals in Ecuador), which provides the threshold values used as reference criteria for assessing contamination levels and environmental risk.

Table 1. Limits for TPH and heavy metals in Ecuador.

Parameter	**Unit**	**Limit value (agricultural use)**
TPH	(mg.kg-1)	50
Cd	(mg.kg-1)	1
Pb	(mg.kg-1)	50
Ni	(mg.kg-1)	50

2.2. Phase 2: Implementation of treatments

A completely randomized design (CRD) was implemented, consisting of three treatments corresponding to the phenological stages of Heliconia psittacorum, with 25 plants per replicate. Each treatment included three replicates, resulting in a total of nine experimental units and 225 plants under study. The structure of treatments, number of replicates, and plant distribution are detailed in Table 2, which summarizes the experimental configuration applied in this phase.

Table 2. Treatments under study and replicates.

Treatment	Description	Replications	Number of plants
Propagation by stem	Propagated plants	3	75
Growth	Plants in the growth stage	3	75
Pre-flowering	Plants beginning to flower	3	75
Experimental units		9	2

Prior to the establishment of treatments, the contaminated soil was homogenized and conditioned with an organic amendment (composted manure) to improve substrate structure, enhance microbial activity, and facilitate nutrient availability. This preconditioning step was essential to ensure adequate plant establishment and uniform growth conditions across experimental units, minimizing variability associated with soil heterogeneity. Furthermore, the incorporation of organic matter contributes to improving physicochemical properties such as cation exchange capacity and moisture retention, which are critical factors in phytoremediation processes.

2.2.1. Technical considerations

The experimental treatments were established considering three phenological stages of Heliconia psittacorum: (i) propagation by rhizome, corresponding to the initial phase of vegetative establishment; (ii) vegetative growth, characterized by active development of the aerial and root systems; and (iii) pre-flowering, defined by plants with advanced structural development prior to flowering.

During the experiment, the survival and vegetative growth of *Heliconia psittacorum* were evaluated periodically. Measurements were taken every five days over a total period of 40 days, considering the three established phenological stages. Polyethylene bags of different sizes were used according to the phenological stage: ½ pound (4×8 cm) for propagation by stem, 2 pounds (6×10 cm) for growth, and 3 pounds (7×11 cm) for pre-flowering. Irrigation was adjusted to each phase (180 mL, 500 mL, and 800 mL per plant, respectively), applied three times per week. The substrate consisted of 180 kg of contaminated soil mixed homogeneously with 5% organic amendment (composted manure).

Young rhizomes (≤3 months, 30 g, 5–10 cm) were propagated, disinfected, and planted 3 cm deep in contaminated soil. During growth, seedlings (~40 cm, healthy) were transplanted after 37 days of cultivation into contaminated soil. Before flowering, plants measuring 50–60 cm and with ≥5 leaves, previously cultivated for 5 months, were transferred to contaminated soil, avoiding disturbance of the primary and secondary roots.

Throughout the experiment, environmental and soil variables were monitored daily: air temperature (25–30°C) was controlled with an Extech 445 815 digital thermohygrometer, relative humidity (80–90%) with a Testo 608-H1 hygrometer, and light exposure (12 h.day-1) was recorded using a PCE-174 lux meter. Soil temperature was measured with a Hanna HI 145 penetration thermometer, while soil moisture (60–70%) was determined with a Decagon GS3 sensor.

2.3. Phase 3: Evaluation of survival and height

The survival rate of *H. psittacorum* in TPH-contaminated soil was evaluated by counting the number of living plants at the beginning and end of the experiment (40-day period), defined as those with at least one intact green leaf. The survival rate was calculated by comparing the number of living plants at the end to the beginning of the trial using the formula:

$$\%\,Efficiency = \left(\frac{Number\ of\ live\ plants\ at\ the\ end}{Number\ of\ live\ plants\ at\ the\ start}\right) * 100 \qquad (1)$$

Height was measured from the base of the soil to the tip of the longest leaf using a caliper for the rhizome propagation treatment and a flexometer for the other treatments every five days for 40 days. These values were recorded for subsequent statistical analysis.

2.4. Phase 4: Analysis of contaminant removal efficiency

At the end of the experiment, soil samples were taken from each experimental unit for post-treatment determination of total petroleum hydrocarbons (TPH) and heavy metals (Cd, Pb, and Ni), applying the same analytical methods used in the initial characterization. The removal efficiency (RE) of each contaminant was calculated as the percentage reduction in initial concentration relative to final concentration, according to equation (2). The data obtained were subjected to statistical analysis using InfoStat software (version 29-09-2020, National University of Córdoba, Argentina) to compare the different treatments.

For survival, repeated measures ANOVA was used or, in case of non-compliance with assumptions, Friedman ANOVA, followed by post hoc tests (Tukey at 5%). For contaminant removal efficiency, ANOVA or Kruskal-Wallis was used according to the statistical assumptions with Tukey at 5%. Contaminant removal efficiency was evaluated using the formula:

$$Eficiencia\ (\%) = \frac{Concentración_{initial} - Concentración_{end}}{Concentración_{initial}} * 100 \qquad (2)$$

3. Results and Discussion

3.1. Characterization of contaminants

The soil had an initial total petroleum hydrocarbon (TPH) concentration of 10,457.69 mg/kg, a value that far exceeded the maximum permissible limit for agricultural use in Ecuador (50 mg/kg), indicating severe contamination. The detailed results of contaminant characterization are presented in Table 3. This level of contamination is characteristic of soils impacted by oil activities and confirms the need to apply specialized and environmentally compatible remediation strategies.

Table 3. Values resulting from the characterization of contaminants in the soil.

Parameter	**Unit**	**Value obtained**	**Limit value (agricultural use)**
TPH	(mg.kg-1)	10 457.69	50
Cd	(mg.kg-1)	<1.50	1
Pb	(mg.kg-1)	13.56	50
Ni	(mg.kg-1)	16.30	50

About heavy metals, the initial concentrations of cadmium (< 1.50 mg/kg), lead (13.56 mg/kg), and nickel (16.30 mg/kg) were found to be below the limit values established for agricultural soils. However, even at moderate concentrations, these elements can influence the bioavailability of organic contaminants and the physiological response of plants involved in phytoremediation processes, particularly in soils with historical contamination.

The high concentration of TPH observed suggests low bioavailability of the contaminant and limited natural degradation by soil microbiota, conditions commonly associated with the hydrophobic nature of hydrocarbons and the aging of the contaminant in the soil, which reduces its microbial accessibility . In this context, the plant-rhizosphere interaction plays a fundamental role in stimulating the degradation, transformation, and stabilization of residual hydrocarbons.

3.2. Survival of Heliconia psittacorum

Marked differences in the survival of Heliconia psittacorum were observed between the phenological stages evaluated. The rhizome propagation stage had the lowest survival rate, with 14.67% of plants alive, while survival increased considerably in the growth stage (89.33%) and reached its maximum value in pre-flowering (98.67%), as shown in Figure 2 (Survival of H. psittacorum plants by stage).

Figure 2. Survival of *H. psittacorum* plants by stage.

The low survival rate recorded during the rhizome propagation phase can be explained by the poor development of the root system and the limited initial physiological capacity of the plants to tolerate chemical stress induced by high concentrations of TPH. In this phase, reduced production of root exudates restricts the stimulation of beneficial microbial communities, decreasing the ability to adapt to the contaminated environment, as has been reported in phytoremediation studies in early stages of plant development

In contrast, the high survival rate observed in the growth and pre-flowering stages indicates greater physiological tolerance to hydrocarbon stress, associated with a more developed root system and a more stable metabolic response. Previous studies have shown that plants in advanced phenological stages have a greater capacity to activate antioxidant and cell protection mechanisms, which increases their survival in contaminated soils .

3.3. Variability in plant height

The height growth of *Heliconia psittacorum* showed differential behavior depending on the phenological stage. During rhizome propagation, the plants showed limited growth, reaching an average final height of 5.58 cm on day 40. During the growth stage, a sustained increase was observed, with values rising from 8.06 cm to 13.47 cm, while pre- flowering showed the highest heights throughout the experimental period, increasing from 39.46 cm to 43.03 cm (Figure 5).

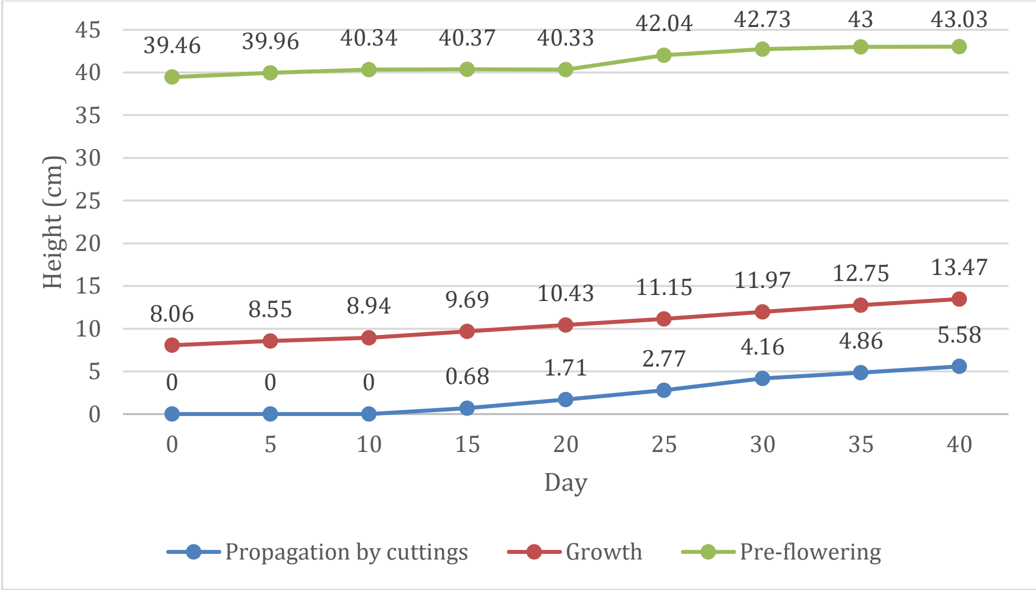

Figure 3. Variability in the height of *H. psittacorum* plants over 40 days.

The low growth observed during rhizome propagation is associated with limited initial metabolic activity and poor root system development, which restricts water and nutrient absorption and reduces the plant's ability to cope with chemical stress conditions. This behavior has been previously reported in young plants exposed to persistent organic pollutants .

In contrast, the sustained growth observed during the growth and pre-flowering stages can be attributed to increased photosynthetic activity, greater root development, and increased release of organic exudates into the rhizosphere, factors that favor both plant growth and the stimulation of microbial processes associated with hydrocarbon rhizodegradation

3.4. Efficiency in contaminant removal

The efficiency of total petroleum hydrocarbon (TPH) removal varied significantly between the phenological stages evaluated. The highest efficiency was recorded during the growth stage (56.43%), followed by pre-flowering (44.30%), while rhizome propagation showed minimal removal (6.01%), demonstrating that the phenological state of *Heliconia psittacorum* directly influences its phytoremediation performance (Table 4).

Table 4. Efficiency in the removal of contaminants by stages of *H. psittacorum.*

Parameter	Propagation by rhizome (%)	Growth (%)	Pre-flowering (%)
TPH	6.01	56.43	44.30
Cd	0.00	0.00	0.00
No	↑24.51	12.07	↑2.09
Pb	7.98	7.98	7.98

*The upward arrow corresponds to an increase in the parameter rather than removal.

The highest removal observed during the growth stage can be explained by intense rhizospheric activity, associated with high production of root exudates that stimulate hydrocarbon-degrading microbial communities. This mechanism, known as rhizodegradation, has been widely documented as the main process responsible for TPH removal in phytoremediation systems . Likewise, the contribution of phytodegradation processes in more bioavailable fractions of the contaminant cannot be ruled out.

About heavy metals, no significant changes were observed in cadmium or lead concentrations, suggesting low bioavailability of these elements and limited phytoextraction capacity under the experimental conditions. Table 4 shows a decrease in nickel only during the growth stage (12.07%), a pattern that has been associated with greater metabolic activity and root absorption capacity in intermediate phenological stages .

The lower removal efficiency observed during the pre-flowering stage could be attributed to a metabolic reallocation of resources towards the formation of reproductive organs, which reduces the energy available for rhizospheric interaction and contaminant degradation processes. This phenomenon is consistent with the phenological competition for resources reported in recent studies, where reproductive phases show lower phytoremediation efficiency compared to stages of active vegetative growth. Consistent with this, research on other phytoremediation species has shown that periods of increased plant growth are associated with greater degradation of total petroleum hydrocarbons .

About heavy metals, no significant variations in Cd and Pb concentrations were observed among the treatments evaluated, suggesting low bioavailability of these elements in the soil and limited phytoextraction capacity under the experimental conditions, in agreement with what has been previously reported for soils contaminated with aged hydrocarbons . The slight decrease observed in Pb could be associated with passive phytostabilization processes, such as rhizospheric adsorption or root chelation by phenolic compounds exuded by the plant; however, the removal percentages were lower than those reported for species with greater phytoextraction capacity such as Typha capensis and Vetiveria zizanioides

Overall, the results confirm that *Heliconia psittacorum* has high potential for the removal of total petroleum hydrocarbons, especially when established in intermediate and advanced phenological stages, while its capacity for heavy metal removal is limited and dependent on the bioavailability of the contaminant, in line with previous studies on tropical species used in phytoremediation .

4. Conclusions

The present study demonstrated that the phytoremediation efficiency of *Heliconia psittacorum* in soils contaminated with total petroleum hydrocarbons is significantly influenced by the phenological stage of the plant. The results show that phytoremediation performance is not constant throughout the plant development cycle but depends on the physiological state and rhizospheric interaction capacity associated with each phenological phase.

The vegetative growth stage was identified as the most favorable condition for TPH removal, attributable to greater root system development, high metabolic activity, and greater stimulation of rhizospheric processes associated with microbial rhizodegradation. In contrast, rhizome propagation showed limited phytoremediation efficiency, associated with the physiological immaturity of the plants and a lower capacity for chemical stress tolerance. Pre-flowering, although showing high plant survival, showed a reduction in removal efficiency, probably related to metabolic reallocation towards reproductive structures.

Regarding heavy metals, *Heliconia psittacorum* showed limited capacity for phytoextraction of cadmium and lead, suggesting that its main mechanism of action against these contaminants corresponds to passive phytostabilization processes rather than active removal. The behavior of nickel was dependent on the phenological stage, with a greater reduction during the growth phase, reinforcing the importance of considering phenological dynamics in the evaluation of metal remediation.

Overall, the findings confirm that *Heliconia psittacorum* is a plant species with high potential for phytoremediation applications aimed primarily at removing hydrocarbons from tropical soils, especially when established in intermediate phenological stages. This study provides relevant scientific evidence on the role of plant phenology in optimizing phytoremediation strategies and provides a technical basis for the design of environmental restoration programs adapted to Amazonian ecosystems and regions with similar soil and climate conditions

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The following abbreviations are used in this manuscript:

TPH	Total Petroleum Hydrocarbons
Cd	Cadmium
Pb	Lead
Ni	Nickel
RE	Removal Efficiency
EPA	Environmental Protection Agency
ISO	International Organization for Standardization
ANOVA	Analysis of Variance

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