

**ASSESSMENT OF GROWTH CHARACTERISTICS
DUE TO POLYCYCLIC AROMATIC HYDROCARBONS
UPTAKE BY TWO DIFFERENT WETLAND PLANTS
(*PHRAGMITES KARKA* AND *VETIVERIA ZIZANIOIDES*)**

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Abstract

Suitable wetland plants species (*Phragmites Karka* and *Vetiveria Zizanioides*) were screened for their tolerance ability to uptake different polycyclic aromatic hydrocarbons (PAHs) concentrations from artificial wastewater. The experiments were carried out in pots planted with 24 plants from *P. Karka* and *V. Zizanioides* exposed to three different concentrations of PAHs for 20 days. The growth characteristics of both plants and uptake of three compounds of PAHs (Phenanthrene, Pyrene and Benzo[a]pyrene) were investigated. The shoot height of *P. Karka* and *V. Zizanioides* were significantly increased upon treatment with PAHs from 75.50 to 82.16 and 73.16 to 102.83 cm respectively. Biomass of *V. Zizanioides* was significantly decreased with the increase in the concentration of PAHs. On the contrary, the biomass of *P. Karka* was significantly increased with relatively high concentrations. The relative growth rate, shoot concentration factor and the translocation factor of *P. Karka* was higher than *V. Zizanioides*. Under all conditions, the highest PAHs uptake was by *P. Karka*, and uptake was increased gradually from the lower molecular weight compounds to the high molecular weight compounds. The conducted experiments in this study provide clear evidence of the ability of *P. Karka* and *V. Zizanioides* plants to grow in the environment with different concentrations of PAHs. Overall, *P. Karka* plant was characterised by a high ability to uptake and accumulate PAHs.

Keywords: Anthropogenic PAHs, PAHs uptake mechanisms, Phytoremediation, Testing growth, Wetland plants tolerance.

1. Introduction

Constructed wetlands (CW) have been successfully used as alternative methods to remove many toxic chemicals from contaminated soils and wastewaters such as polycyclic aromatic hydrocarbons (PAHs) [1-3]. Wetland plants are an integral part of those systems and many researchers report that these plants have a positive role in removing contaminants through an advanced interaction with water body media and microorganism [4-6].

The utilization of phytoremediation can amend the complex organic and toxic pollutants to non-toxic components through the capabilities of endogenous genetic, biochemical and physiological plants [7]. A large body of evidence indicates that CWs with plants are more efficient at removing PAHs compounds compared with unplanted CWs [8-11]. Nevertheless, the effects of plant species on CW performance vary considerably [8, 12, 13].

The average removal of PAHs was 79.2% for the SSFCW (subsurface Flow constructed wetland), planted with *Phragmites australis* and *Arundo donax* [14]. Meudec et al. [15] investigated the bioaccumulation of polycyclic aromatic hydrocarbons from contaminated sediments with 0.2%, 2% and 20% of heavy fuel oil (w/w) in *Salicornia* tissue. Another study by Wang et al. [16] recorded that the distribution of PAHs within the plant tissue of *Scirpus* showed that the concentrations of low molecular weight compounds (LMW, 2-4 rings) were ranged from 51.9 to 181.2 ng g⁻¹. The highest concentrations were found in leaves.

The accelerated removal of pyrene and benzo[a]pyrene was observed in *Phragmite australis* rhizosphere sediments with plants, while both compounds continued in sediments without plants and in sediments with sterilized plants, indicating that the accelerated removal was largely due to decomposition by rhizosphere bacteria [17]. Analysis of the accumulation and distribution of sixteen polycyclic aromatic hydrocarbons (PAHs) in the plants of *Phragmits australis* grown on the wetland showed that the sequence of PAHs content in the plants was leaves > stems > roots at 2583, 2198 and 899 µgkg⁻¹ DW, respectively [2].

Constructed wetlands are suitable for PAHs removal generated from industrial effluents at different concentrations, thus, it is essential to select tolerant plant species that are able to produce biomass aboveground and to develop a healthy belowground system [18]. The common factors for plant selection are pollutant resistance, tolerance to environmental conditions, large biomass production, high productivity, low bioaccumulation potential and food conversion potential, and suitability for different soil types [19].

However, the capability of wetland plants to tolerate and grow in the presence of high concentrations of toxic polycyclic aromatic hydrocarbons is still unclear. The exact way that the eco-physiological properties of the plants relate to the treatment process, which makes them an essential component of the design, is still too vague.

Therefore, this study aims to comparatively investigate the capability of two species of wetland plants namely *Phragmites Karka* (*P. Karka*) and *Vetiveria Zizanioides* (*V. Zizanioides*) to tolerate and uptake the PAHs from artificial wastewater with different concentrations. Based on the previous study both selected plants were reported to have a very high tolerance for many pollutants for examples, aluminium, manganese and a range of heavy metals [20].

2. Materials and methods

2.1. Experimental set-up

In this study, the experiments were carried out at the Environmental Engineering Laboratory, Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia. A total of 24 plants (12 *P. Karka* and 12 *V. Zizanioides* of the same age group 10 days) were planted in plastic pots (0.45 m × 0.15 m × 0.15 m), which were filled with 20:80 (v/v) mixture of soil and sand. The pots for each plant were divided into 4 categories including controls, each containing three replicates ($n = 3$) as shown in Fig. 1. Disease-free plant seedlings of *P. Karka* and *V. Zizanioides* were purchased at the similar growth potential from Wetland, Putra Jaya, Malaysia. Plants are irrigated once a day using tap water for three months followed by exposing to synthetic wastewater of high PAHs concentration for 20 days. Values of PAHs concentrations were 0 (control), 2500, 5000 and 10000 μgL^{-1} .

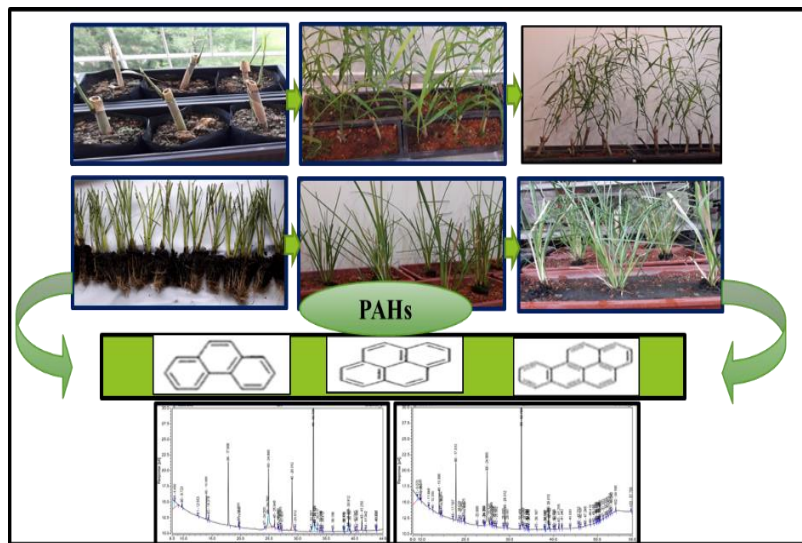

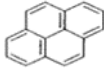
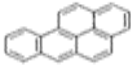


Fig. 1. Capability of *P. Karka* and *V. Zizanioides* in PAHs tolerance and uptakes.

2.2. Preparation of artificial wastewater

Artificial wastewater was prepared by dissolving three compounds of PAHs in acetone, which was then mixed with distilled water. These compounds were used as a model for PAHs pollutants, and have different characteristics, as shown in Table 1. The compounds were purchased from Sigma-Aldrich GmbH (Munich, Germany). The ratio of the three compounds of PAHs in synthetic wastewater was prepared based on reported in the earlier research (Phenanthrene 74.60, Pyrene 17.10, Benzo[a]Pyrene 8.28) [21, 22]. From the ratio of these compounds, three different concentrations of synthetic wastewater (10000, 5000, and 250 μgL^{-1}) were prepared.

Table 1. Characteristics of PAHs compounds used to prepare synthetic wastewater.

Compound name	Phenanthrene	Pyrene	Benzo[a]Pyrene
Compound abbreviation	PHE	PYR	Bap
Benzene rings	3	4	5
Formula	C ₁₄ H ₁₀	C ₁₆ H ₁₂	C ₂₀ H ₁₂
Water solubility (mgL ⁻¹ at 25 °C)	1.18	0.12	0.0038
Molecular weight (gmol ⁻¹)	178	202	252
Boiling point (°C)	339-340	360-404	493-496
<i>K_{ow}</i>	4.46	4.88	6.35
Purity of compounds (%)	98	98	≥ 96
Chemical structure			

2.3. Measurement of growth parameters

2.3.1. Plant height

The heights of two plants (*P. Karka* and *V. Zizanioides*) were measured from the ground to the top of the plant using a scale for every three days of the experimental period.

2.3.2. Plant weight

When the experiments were completed, plants were harvested. The roots were gently washed and thoroughly separated from the soil, sand and other detritus using the sieve method and were dried on paper towels. Harvested plants from the pots were grouped in envelopes. Fresh shoots and roots of the two types of plants were weighted immediately after harvesting. Plant components were labelled and dried in the oven at 70 °C and weighted after they were completely dried. The dry root biomass and shoots were recorded in g plant⁻¹.

2.3.3. Relative growth rate

The relative growth rate (RGR) was based on total dry biomass and it had been calculated using Eq. (1) [23].

$$RGR = \frac{[In(w_2) - In(w_1)]}{(t_2 - t_1)} \quad (1)$$

where W_1 and W_2 are the primary and final dry weights, respectively, for a complete plant sample and t_1 and t_2 are the treatment times in days.

2.4. Extraction of PAHs from plants

The uptake and subcellular distribution of PAHs in different parts of plants were investigated. In this study, the procedure reported by the previous researcher [24] to extract PAHs from the plants was employed. The plant was dried at room temperature, ground, homogenized and sieved through a sieve no. 200 μm. Samples of 1 g from different parts of plants were extracted by ultra-sonication in

1:1 (v/v) resolution, of mixed dichloromethane and acetone. The solution was then decanted, collected and regenerated. The sample solution was sonicated for 1 hour, and the solution was decanted, assembled and regenerated again. This procedure was repeated three times. The solution was then concentrated with a vacuum rotary evaporator at 40 °C. After the solvent was removed, the residue obtained was dissolved in 2 mL of acetone and filtered through 0.45 mm polytetrafluoroethylene (PTFE) filters and injected for Gas Chromatography-Flame Ionization Detector GC-FID analysis.

2.5. Analysis of PAHs by GC-FID

Samples extracts (2 µL) were examined by utilizing Gas Chromatography (model Hewlett-Packard (HP) 6890) equipped with the HP5973 mass selective detector and a DB-5 capillary column (30 mm × 0.25 mm × 0.25 m).

The separation was attained consistently with the subsequent program: the beginning oven temperature was 80 °C held for one min, and raised to 275 °C at 15 °C min⁻¹, held for one min; then to 285 °C at 10 °C min⁻¹ was retained for one min; then raised to 295 to 295 °C at 5 °C min⁻¹, held for one min. Nitrogen was used as carrier gas (1.5 ml min⁻¹) and make-up gas (35 ml min⁻¹). A 1.0l aliquot was injected from the extract into the split mode. The injector was controlled at 250 °C and also the detector at 300 °C [25].

2.6. Shoot and root concentration factor

The shoot and root concentration factor (SCF - RCF) was used to determine the translocation of PAHs contaminants in the plants, as described in Eq. (2) [26].

$$SCF \text{ or } RCF = \frac{C_{Shoot} \text{ or } C_{Root}}{C_{Solution}} \quad (2)$$

where, C_{shoot} and C_{root} are the PAHs concentration (µg L⁻¹) in the shoot and the root respectively.

2.7. Translocation factor

The transfer of PAHs from root to shoot was measured by the Translocation Factor (TF) [26], which is described by the following formula.

$$TF = \frac{C_{Shoot}}{C_{Root}} \quad (3)$$

2.8. Statistical analysis

All statistical tests were performed using SPSS version 20. A two-way analysis of variance (ANOVA) was applied to determine the significance of two types of wetland plants to tolerate and uptake the PAHs from artificial wastewater with different concentrations of PAHs on height, biomass, RGR and investigate the SCF, RCF, and TF.

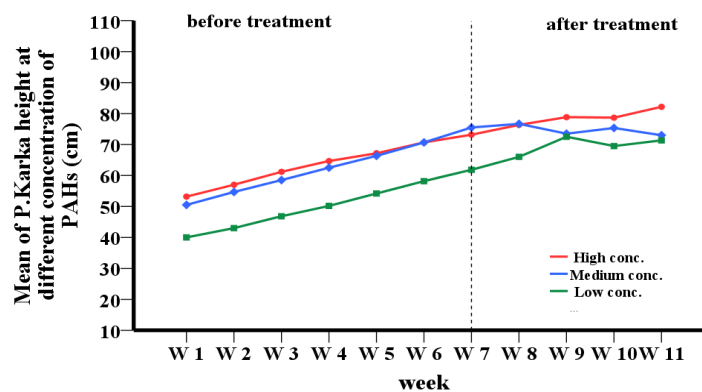
The result was expressed as the mean value of the triplicate results, the standard deviation for triplicate and the confidence limit being 95%.

3. Results and Discussion

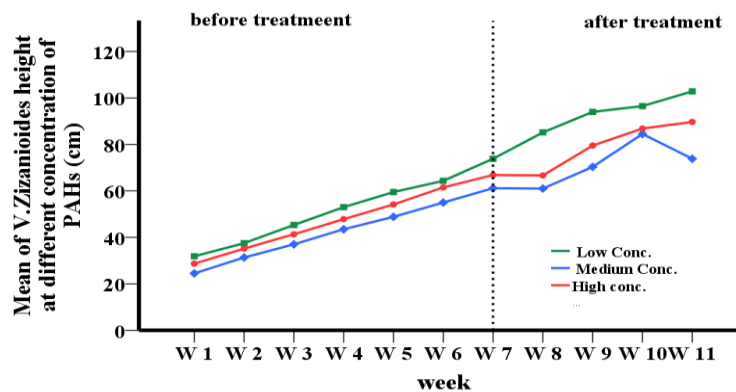
The selection of suitable wetland plants is a key aspect for the treatment of wastewater contaminated with PAHs in constructed wetlands, where the plants used should be capable to stand up the changes and potentially toxic effects of wastewater [27, 28]. The growth and tolerance capability for PAHs uptake for two wetland plants were tested throughout the period of the experiment (three months without treatment and twenty days were treated at different concentrations of PAHs ($2500, 5000$ and $10000 \mu\text{gL}^{-1}$)).

3.1. Effect of different concentrations of PAHs on plants height

During the treatment period, the shoot heights of *P. Karka* and *V. Zizanioides* were monitored to determine the effect of different concentrations of PAHs and this was compared with control plants. The shoot heights of the plants were observed to increase from 75.50 to 82.17 and 73.16 to 102.83 cm, respectively as shown in Fig. 2.



(a) Height of *P. Karka*.



(b) Height of *V. Zizanioides*.

Fig. 2. Average height of *P. Karka* and *V. Zizanioides* before and after treatment at different concentrations of PAHs over the 11 weeks.

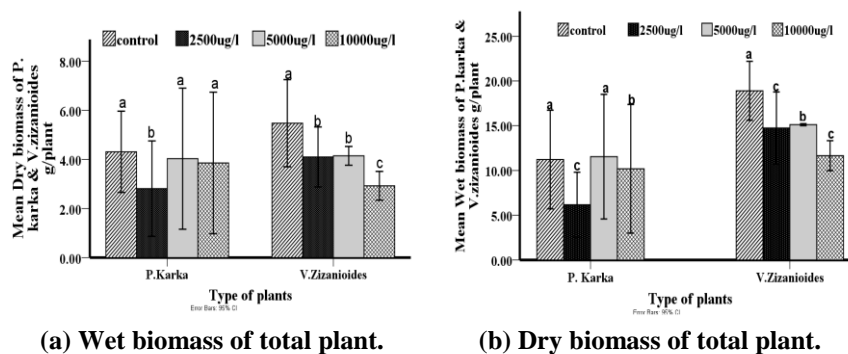
The negative effect of PAHs on the *P. Karka* growth was evident where a significant ($p < 0.05$) decrease in plant height was observed in the low and medium conditions of treatment than control. The peak height of *V. Zizanioides* that has been grown in all conditions of PAH treated wastewater was reduced. This decrease in plant height was highest in PAHs medium and high transaction conditions.

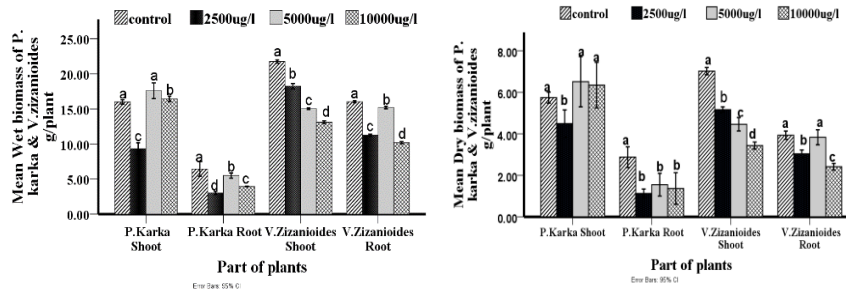
The behaviour of *P. Karka* and *V. Zizanioides* in coping and growth with different concentrations of PAHs in synthetic wastewater was completely different. Treatments having high concentration showed a positive influence on *P. Karka* height while giving a negative effect on *V. Zizanioides* height as compared to control plants. Huebner et al. [29] and Xia et al. [30] observed a similar trend.

Meanwhile, biomass response varied significantly ($p < 0.05$) among species as shown in Fig. 3.

At the same conditions of treatment, *P. Karka* recorded higher dry biomass in the shoot compared with *V. Zizanioides* [29] suggested that Phragmits greatly increased its production of shoots, especially in the first 10 days of treatment. The shoot biomass of *V. Zizanioides* decreased gradually with increased concentration of PAHs. Nisa et al. [31] reported that the biomass of *V. Zizanioides* grown in all PAH contaminated soils was decreased and this decline in plant biomass was the highest in maximum contamination treatments. On the other hand, *V. Zizanioides* had greater root biomass under different PAHs concentrations than *P. Karka*, this finding is in agreement with the results obtained by Jelani et al. [32] who reported that the adding of PAHs significantly ($p < 0.05$) increase biomass in plants cultivated in polluted soil.

The results of the relative growth rate (RGR) test were identical with the ANOVA analysis for the total biomass of both plants under different concentrations of PAHs. The RGR in the shoot system of *P. Karka* was greater at 5000 and 10000 μgL^{-1} of PAHs concentrations than that in control plant and 2500 μgL^{-1} of PAHs. Whereas, the RGR in the root system of *P. Karka* at different concentrations of PAHs was significantly ($p < 0.05$) lower than in the control. On the other hand, the RGR of *V. Zizanioides* in the shoot and root systems was significantly ($p < 0.05$) lower at different concentrations of PAHs than in the control and at 5000 and 10000 μgL^{-1} of PAHs concentrations showed no significant difference as shown in Fig. 4.



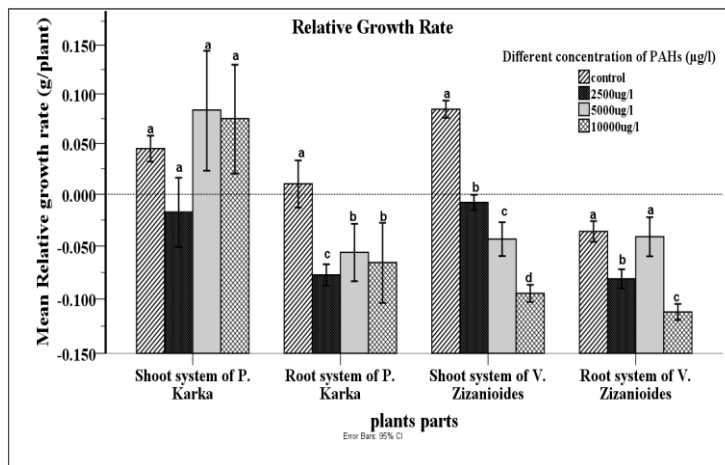


(c) Wet biomass at different parts of plants.

(d) Dry biomass at different parts of plants.

*Note: Letters a, b and c represent the statistically significant differences on effect three different concentrations of PAHs on the biomass of plant parts ($p < 0.05$).

Fig. 3. Total wet and dry biomass of different parts of two wetland plants *P. Karka* and *V. Zizanioides* of after 20 days exposure of PAHs at different concentrations (2500, 5000 and 10000 $\mu\text{g/L}^{-1}$).



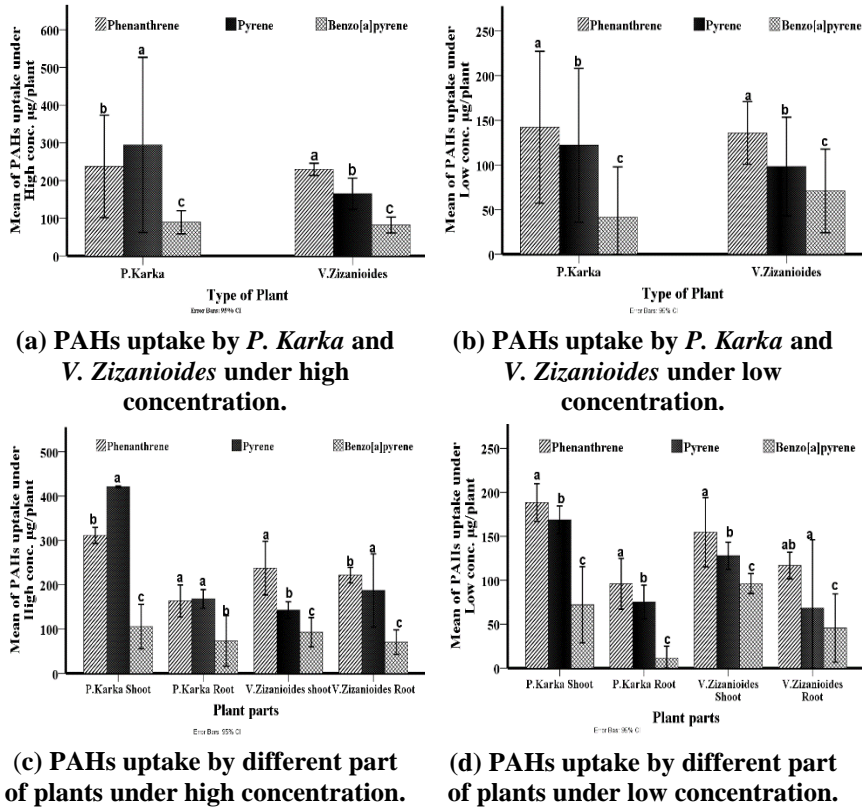
*Note: Letters a, b and c represent the statistically significant differences on effect three different concentrations of PAHs on the Relative growth rate of plant parts ($p < 0.05$).

Fig. 4. Relative growth rate (RGR) of species influenced by three compounds of PAHs after 20 days of exposure.

3.2. PAH uptake by native *P. Karka* and *V. Zizanioides* plants

Trends can clearly be seen in the patterns of PAHs uptake by different parts of plants under high and low concentrations, and these were illustrated in Figs. 5 (a) to (d). Evidently, the PAHs uptake by both plants at treatment with a high concentration was significantly ($p < 0.05$) higher than in case of treatment at low concentrations, which was in the total plant of *P. Karka* under high and low concentration (238, 295, and 90 $\mu\text{g/plant}$) and (142, 122 and 42 $\mu\text{g/plant}$) respectively. As well as was in the total plant of *V. Zizanioides* under high concentration (230, 166 and 82 $\mu\text{g/plant}$) and low concentration (136, 98 and 71 $\mu\text{g/plant}$).

The uptake of phenanthrene by both plants almost the same under two concentrations and the uptake of Pyrene in *P. Karka* was under two concentrations higher than *V. Zizanioides*. While Benzo[a] Pyrene was higher in *P. Karka* under high concentration compared to another plant and quite the opposite was under the low concentrations.



*Note: Letters a, b, and c represent statistically significant differences on the uptake of three different compounds of PAHs in plant parts (p < 0.05).

Fig. 5. Uptake of phenanthrene, pyrene and benzo [a] pyrene by *P. Karka* and *V. Zizanioides* during 20 days treatment with two different concentrations (2500 and 10000 µg/L⁻¹).

On the other hand, under the high concentration of treatment, the *P. Karka* shoot system recorded a high PAHs uptake than the root system and the same occurred in the shoot and root system of *V. Zizanioides* under the same treatment condition. Wang et al. [33] observed a similar trend. In contrast, the root system of *V. Zizanioides* recorded high uptake compared to the root system in *P. Karka*. A study by Nisa et al. [31] and Yu et al. [34] found that the concentration of phenanthrene, pyrene, and benzo[a] pyrene in *V. Zizanioides* was higher in roots as compared to shoots, as the root accumulated more PAHs. Sun et al [35] indicated that the transfer of BAP appears to be weak from root to shoot due to the low solubility of BAP in water. Whereas, the plant of *V. Zizanioides* recorded the biggest height, the richest biomass, and the uptake ability was apparently lower

than the *P. Karka* plant. This is attributed to the initial growth of the *V. Zizanioides* roots, which are quickly spread in a horizontal pattern from a mat [36]. The degradation of PAHs might result primarily from the capacity of plant roots to bring oxygen to the sediments, thereby stimulating bacterial metabolism [17].

In this study, distributions of three compounds of PAHs represent different ranges of molecular weight and contain 3, 4 and 5 rings (Table 1). The low and medium molecular weights with 3 and 4 rings showed that the highest uptake by both plants was in accordance with the results by previous researchers [2, 37]. It is believed that the movement of organic materials within the plant depends on the size and weight of the PAH molecule.

Therefore, an increase in the molecular weight of the compound would result in a decrease in mobility. It is noteworthy that the three different molecular weight compounds showed very similar uptakes by *P. Karka*. However, this did not apply to *V. Zizanioides*, which showed a slightly less uptake of PAHs than *P. Karka*.

The *P. Karka* plants demonstrated a high tolerance, uptake, and accumulation of PAHs that increased with increasing PAHs concentration in synthetic wastewater and found in agreement with the findings of Meudec et al [15], which reported that PAH bioaccumulation in plants depended on exposure duration and pollution degree.

3.3. Bioconcentration and translocation factors

The shoot concentration factor (SCF) and root concentration factor (RCF), defined as the fractionated concentration ratios of PAHs within plant tissues versus the wastewater, were used to assess plant accumulation capacity. The effect of PAHs on the SCFs and RCFs is shown in Tables 2 and 3.

The SCFs of *P. Karka* were significantly ($p < 0.05$) higher than the SCFs of *V. Zizanioides* at high and low -PAH concentration (10000, 2500 μgL^{-1}), and at the same concentration, the RCF of *P. Karka* was significantly lower than the RCF of *V. Zizanioides*. There was no significant difference in the RCF of both plants at low concentrations of treatment.

A similar study by Gao et al [38] on plants from the same vetiveria family, found that the RCFs for the uptake of PAHs were much higher than the SCFs. However, at steady state, the RCFs were approximately two orders of magnitude higher than the corresponding SCFs, when the initial concentrations of phenanthrene and pyrene, were 0.52 and 0.12 mgL^{-1} respectively.

The Translocation Factor (TF) in *P. Karka* was greater at the high concentration treatments and decreased significantly in low concentration treatments and at both condition, the value of TF was higher than 1. This indicates that *P. Karka* was able to translocate PAH from the roots to the shoots. By contrast, the TF value in *V. Zizanioides*

was low at high concentration treatments and increased at low concentration treatments and the value of TF in most case is close to one. That signalize that *V. Zizanioides* had a weak capacity to transfer PAHs from the roots to the shoots. While, the translocation and accumulation of PAHs with low molecular weight in the plants was better, due to their higher solubility [39].

Table 2. Concentrations of PAHs in *Phragmites karka* and *Vetiveria Zizanioides* tissues, shoot and root concentration factors (SCFs and RCFs) and root to shoot transfer factors (TFs) at high condition of treatment (10000 µg L⁻¹).

PAHs	Phenanthrene	Pyrene	Benzo[a]pyrene
<i>P. Karka</i>			
- SCF	0.036±0.004 ^b	0.037±0.004 ^b	0.009±0.004 ^a
- RCF	0.007±0.002 ^a	0.009±0.002 ^a	0.005±0.002 ^a
- TF	5.348±1.350 ^a	4.507±1.350 ^a	2.355±1.350 ^a
<i>V. Zizanioides</i>			
- SCF	0.024 ±0.002 ^b	0.012±0.002 ^b	0.009±0.002 ^a
- RCF	0.021 ±0.001 ^b	0.012±0.002 ^b	0.007±0.001 ^a
- TF	1.152±0.118 ^a	0.573±0.118 ^a	1.242±0.118 ^a

Table 3. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in *P. Karka* and *V. Zizanioides* tissues, shoot and root concentration factors (SCFs and RCFs) and root to shoot transfer factors (TFs) at low condition of treatment (2500 µg L⁻¹).

PAHs	Phenanthrene	Pyrene	Benzo[a]pyrene
<i>P. Karka</i>			
- SCF	0.100±0.023 ^a	0.080±0.023 ^a	0.025±0.023 ^a
- RCF	0.036±0.002 ^b	0.030±0.002 ^b	0.004±0.002 ^a
- TF	2.832±0.891 ^a	2.622±0.891 ^a	6.44±0.891 ^a
<i>V. Zizanioides</i>			
- SCF	0.034±0.018 ^a	0.05±0.018 ^a	0.024±0.018 ^a
- RCF	0.036±0.018 ^a	0.050±0.018 ^a	0.024±0.018 ^a
- TF	0.904±0.717 ^a	1.829±0.717 ^a	1.721±0.717 ^a

*Note: Values (means± SE) followed by the same letter within columns are not significantly different according to the least significant difference test ($p < 0.05$).

4. Conclusions

Plant selection is of the highest importance for maximizing the removal of PAHs in CWs. Wetland plant species that are capable to tolerate and grow in the presence of high concentrations of toxic polycyclic aromatic hydrocarbons could be a good candidate for use in CW for treatment. The results of this study found that exposure of *P. Karka* and *V. Zizanioides* to different concentrations of PAHs did not show obvious toxicity symptoms. While the *V. Zizanioides* showed a large wet and dry biomass and greater height, *P. Karka* plants were characterised by the ability to uptake and accumulate larger quantities of PAHs. Therefore, PAHs uptake by the plants was depending on the PAHs concentration in the wastewater, and the ability of the plant uptake. The outcome of our experience provides a basis for the selection of wetland plants used in the treatment of industrial effluents.

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Nomenclatures

B[a]P	Benzo[a]Pyrene
C_{Root}	Concentration of PAH in Root, $\mu\text{g/g}$
C_{Shoot}	Concentration of PAH in shoot, $\mu\text{g/g}$
$C_{Solution}$	Concentration of PAH in solution, $\mu\text{g/L}^{-1}$
\ln	Natural Logarithm
t_1	Time one, day
t_2	Time two, day
W_1	Weight at time one, g
W_2	Weight at time two, g

Abbreviations

CW	Constructed Wetland
GC	Gas Chromatography
Phe	Phenanthrene
Pyr	Pyrene
RCF	Root Concentration Factor
RGR	Relative Growth Rate
SE	Standard Error
TF	Translocation Factor

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