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Phytoremediation of Pyrene by the Aquatic Plant Azolla pinnata

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ABSTRACT

The current study tested the ability of the aquatic plant Azolla pinnata to remove the pyrene and determine the effect of Pyrene on the pigments (chlorophyll a, b, and carotenoids) and antioxidant parameters (CAT, SOD, MDA and, ROS) in the plant. The plant was exposed to different concentrations of Pyrene (1,5,10,15,25, and 50 mg/l) for 14 days. The results showed that the high removal rate of pyrene reached 99% for 25 and 50 mg/l of pyrene on the 14th day of the experiment, while the lowest removal rate was 42.1% for 1 mg/l on the third day.
The results showed the lowest values of total chlorophyll, chlorophyll a and carotenoids were 0.55, 0.25, and 0.433 µg/g recorded at 50 ppm respectively. While chlorophyll b recorded a low value of 0.32 µg/g at 25 mg/l, the highest concentration of these pigments was registered in the control group. Also, the results showed a gradual increase in antioxidant values compared to the control group, recording the highest values of 0.2873 and 1.04 U/g at 50 ppm and the lowest value of CAT and SOD at 5ppm, compared to the control group. Also, the study recorded a gradual increase in ROS and MDA values with increasing concentrations of pyrene, the highest value was 1.6367 and 14.4433 µmole/g for ROS and MDA at (50ppm), whereas the lowest value was recorded at 5 ppm compared to the control group. Statistical analysis at ($p<0.05$) showed significant differences between all the interactions.

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INTRODUCTION

Pyrene is a polycyclic aromatic hydrocarbon (PAH) consisting of four benzene rings with the chemical formula C16H10 (Ahn *et al.*, 2010). Pyrene is found in the distillate of coal tar; it can be used to make dyes, resins and plastics; it can also be used to make pesticides and plasticizers; pyrene is a carcinogen, mutagen and tumor promoter (Cerniglia, 1993). In addition, the US Environmental Protection Agency (EPA) has listed 16 PAHs, including pyrene (PY) among the 129 priority pollutants (Jin *et al.*, 2007). Several publications have reported that pyrene can be degraded by various plants (Liste and Alexander, 2000). Phytoremediation involves the use of green plants to degrade PAHs in polluted soils, sediments and water (Marmiroli *et al.*, 2006). This method is considered to be environmentally sustainable and cost-effective (Dhir *et al.*, 2009). Aquatic plants can produce significant biomass with efficient bioremediation capabilities, providing a cost-effective and easily manageable solution (Miranda *et al.*, 2014)

Many aquatic plants are suitable phytoremediators for polluted aquatic ecosystems, such as *Azolla pinnata* (Eribo and Kadiri, 2016; Sood *et al.*, 2012). Azolla is an aquatic plant that floats freely on the surface of the water (Kumari *et al.*, 2017). *Azolla pinnata* is characterized by a fast growth rate, low operational cost, effective pollutant absorption and sustainable implementation (Dhir *et al.*, 2009; Gomes *et al.*, 2018; Kollah *et al.*, 2016; Kosesakal, 2018;

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Mostafa *et al.*, 2021). For these reasons, *Azolla* is often used in phytoremediation studies. It can be used to degrade hydrocarbons and heavy metals (Sood *et al.*, 2012). *A. pinnata* was used for the bioremediation of pyrene, a representative 4-ring PAH. Various studies have been done and are still ongoing to use *Azolla* as a phytoremediator(Kaur *et al.*, 2022; Latif *et al.*, 2023).

In another study concluded that 50% of the aromatic hydrocarbon concentration was reduced after 19 days in the *A. pinnata* plant (Cohen *et al.* (2002). While in the study of Miranda *et al.* (2014) that observed *Azolla sp*. removal of high phosphate wastewater resulted in complete removal of NH_4, NO_3 and up to a 93% reduction of PO4. Also in another study reported that the two grass plants (*Festuca arundinacea* and *Pannicum virgatum*) removed about 38% of pyrene in 190 days (Chen *et al.* (2003). In the study of AbdulRada *et al.* (2014) high levels of naphthalene and pyrene were recorded (12.24 ,0.370 ppm) in lettuce plant, while the fluorene level was 1.207 ppm in the radish plant in Baghdad city. While Saleh *et al.* (2017) studied the bioremediation of PAHs by *Zea mays* (a maize plant) in soil ,and the results showed that there was a complete removal of pyrene after eight weeks. According to Kosesakal (2018), *Azolla filiculoides* showed the highest effectiveness in degrading 3-4 ring PAHs when exposed to crude oil concentrations ranging from 0.05% to 0.2%. This suggests that the bioremediation capacity of *A. filiculoides* in removing PAHs is closely related to the level of oil present in the polluted water source.

In the study of Mostafa et al. (2021) showed that plant *A. pinnata* has the potential to phytoremediation freshwater polluted with low levels (up to 0.5 g/L) of petroleum hydrocarbons (PHs) and the degradation rate of total PHs was 92% in the plant after 7 days of treatment. Al-Baldawi *et al.* (2012) mentioned that the removal of copper (Cu) from polluted water by the floating plant *Azolla sp.* recorded highest removal rates of 100% after 5 days of exposure.

The purpose of the current study is to investigate the efficacy of A. pinnata in removing pyrene from cultured plant media at different concentrations and the effect of pyrene on some antioxidant parameters stimulated in the plant by exposure to this PAH.

MATERIALS AND METHODS

Experimental Design

The floating plant, *A. pinnata*, collected from the Euphrates River (Al-Shamiya region), was exposed to different concentrations of pyrene supplied by Thomas Baker Chemicals/India. Stock solutions of pyrene were prepared at different concentrations of 1, 5, 10, 15, 25 and 50 mg/l by dissolving in a small amount of ethanol and complete to the desired volume then made a diluting it through plant culture medium to obtain these concentrations. The experiment was carried out in the culture room with constant light intensity (3500 lux.) and a photoperiod (16/8 light/dark) with a temperature of 28C˚ and a pH7, during 14 days (Indira *et al.*, 2014) at the College of Science, University of Al-Qadisiyah (Diwanyiah, Iraq). They were planted in glass containers of all the same dimensions (40cm in length, 25cm in width and 25cm in height) filled with 10 liters of water per container. Our experiment was designed in 21 containers (glass tanks) divided into six treatments, and one treatment was planted only without pyrene (control). with three replications for each treatment.

Pyrene determination by HPLC

Aliquots of the sample (5 ml) were taken every 7 days, extracted twice with an equal volume of ethyl acetate and passed through anhydrous sodium sulfate to remove any traces of water. The ethyl acetate extract was then subjected to HPLC analysis (Guo *et al.*, 2021). To determine the residual concentration of pyrene, calibration standards were prepared by dissolving different pyrene concentrations (5-2000 µg/ml) in ethyl acetate. The identification of the concentration of PY was carried out using an HPLC model Waters 600E equipped with an autosampler Waters

717 Plus and a dual wavelength UV detector model Waters 2487 (set at 254 nm). The operating conditions were as follows Column: SUPELCOSIL™ LC-PAH, 15 cm x 4.6 mm, 5µm Injection volume: 2.0 µl. Mobile phase: acetonitrile (A): water (W) isocratic programmer, HPLC grade Water%: 40% Acetonitrile%: 60%. The removal efficiency was calculated according to the equation below:

$$R\% = \frac{C0 - C1}{C0} X100$$

where C_0 and C_1 are pyrene concentrations before and after treatment

Determination of the photosynthetic pigments

Total chlorophyll, chlorophyll a, chlorophyll b, and carotenoids were measured after 14 days according to Jeffrey and Humphrey (1975) A 0.1 g fresh sample was homogenized in 90% acetone, and then the homogenates were centrifuged at 6,000 rpm for 10 min, and the absorbance of the supernatant was evaluated spectrophotometrically at the wavelengths of 663, 645, 664, 647 and 630nm.

Chlorophyll a (µg /g) = (12.7×A663) − (2.698×A645)
Chlorophyll b (µg /g) = (22.9×A645) − (4.68×A663)
Total chlorophyll (µg/g) = chl- a + chl- b = (20.2 × A645) + (8.02×A663)
Carotenoid (µg/g) = 1.67 E664 - 7.60 E647 + 24.52 E630

Detection of Antioxidants parameters

Antioxidant parameters were analyzed using specific methods for each. Catalase (CAT) levels were quantitatively assessed by the methodology outlined by Hadwan and kadhum Ali (2018), Superoxide dismutase (SOD) levels were determined by the procedure detailed by Stephenie *et al.* (2020), Malondialdehyde (MDA) levels were estimated using the method developed by Hegazy (2011), and Reactive Oxygen Species (ROS) levels were measured by the protocol established by Erel (2005). These methods are described below:

Catalase (CAT) Determination

For catalase assay, 50 mg of biomass was homogenized in 2 mL phosphate buffer (0.5 M, pH 7.5), followed by centrifugation at 12,000 rpm for 30 min at 4°C to collect the supernatant. The supernatant was then used to prepare a reaction mixture consisting of 1.6 mL phosphate buffer (pH 7.3), 100 µL EDTA (3 mM), 200 µL H2O2 (0.3%) and 100 µL of the supernatant in a cuvette. Catalase activity in the supernatant was determined by monitoring the reduction of H2O2 by measuring the decrease in absorbance at 240 nm compared to a blank containing the same reaction mixture without H2O2.

$$\text{Catalase Activity of test kU} = \frac{2.303}{t} * log \frac{S^o}{S} \quad (1)$$

S^o is the absorbance of the standard tube
S is the absorbance of the test tub

Superoxide dismutase (SOD) Determination

The activity of SOD (Cu-Zn) was determined by a simple and rapid technique based on the ability of the enzyme to inhibit the autoxidation of pyrogallol. In the presence of EDTA at pH 8.2, the autoxidation of pyrogallol reaches 50%. This method is based on the principle of

competition between the autoxidation of pyrogallol by O2-¯ and the dismutation of this radical by SOD

$$\%\ \text{Inhibition of pyrogallol autoxidation} = \frac{\Delta A\ \text{control} - \Delta A\ \text{test}}{\Delta A\ \text{control}} X100\%$$

$$(Cu - Zn)\text{SOD Activity} = \frac{\%\ \text{inhibition of pyrogallol autoxidation}}{50\%}$$

Malondialdehyde (MDA) Determination

Thiobarbituric acid reactive substances (TBARS) were used to assess lipid peroxidation in sera. The TBARS test is a reliable and standardized method for quantifying lipid peroxidation in serum. The MDA-TBA adduct, formed by the reaction of MDA and 1,3-diethyl-2-thiobarbituric acid (DETBA) at high temperatures (90-100°C) under acidic conditions, is typically measured colourimetrically at 530-540 nm or fluorometrically with an excitation wavelength of 515 nm and an emission wavelength of 555 nm. The fluorometric measurement offers much higher sensitivity in detecting this reaction.

$$\text{MDA} = \frac{\text{Absorbance}}{d X \zeta} X D.F$$

d = 1cm, ε =extinction coefficient = 1.56x 105 M-1cm-1
D.F = dilution factor

Reactive Oxygen Species (ROS) Determination

The Total Oxidant Status (TOS) of the sample was determined using a novel method developed by Erel. Oxidants present in the sample oxidise the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by the presence of glycerol molecules in the reaction medium. In an acidic environment, the ferric ion forms a colored complex with xylenol orange. The intensity of the color, which can be measured spectrophotometrically, correlates with the total amount of oxidant molecules in the sample. The test is standardized using hydrogen peroxide and results are expressed in micromolar hydrogen peroxide equivalents per litre (μmol H2O2Eq/L).

Statistical analysis

To evaluate the ability of *A. pinnata* to bioremediate pyrene and the activity of antioxidant enzymes, MDA and ROS in control and treatment groups exposed to different pyrene concentrations, statistical analysis was performed using one and two-way analysis of variance (ANOVA) and least significant differences (LSD) by SPSS V26.

RESULTS AND DISCUSSION

Biodegradation of Pyrene by Azolla pinnata

The results showed a high efficiency of pyrene removal by the *A. pinnata* plant at concentrations of 5, 10, 15, 25 and 50 mg/L. The highest removal rate reached 99.7% for 50 mg/L of pyrene on the 14th day of the experiment (Table 1, Fig. 1). The lowest removal rate was 42.1% for concentrations of 1 mg/L on the third day of the experiment and the highest removal rate of 99% was recorded for concentrations 25 and 50 on the 14th day of the experiment. Statistical analysis showed significant differences between treatments and the control sample with different pyrene concentrations ($P \leq 0.05$).

Table 1. Removal efficiency of pyrene by *Azolla pennata*

Exposure time (days)	Pyrene concentrations mg/l												LSD
	1 ppm		5 ppm		10 ppm		15 ppm		25 ppm		50 ppm		
	R.E. %	Mean±SE	R.E. %	Mean±SE	R.E. %	Mean±SE	R.E. %	Mean±SE	R.E. %	Mean±SE	R.E. %	Mean±SE	
3	42.1	0.5793±0.0015 E a	87.3	0.636±0.0046 D a	91	0.905±0.0031 C a	93.8	0.926±0.0122 C a	94.1	1.4817±0.1379 B a	94.8	2.5913±0.047 A a	0.18
5	72	0.2803±0.0011 E b	96.4	0.181±0.0022 F b	95.7	0.426±0.0052 D b	96.6	0.517±0.0038 C d	97.3	0.6647±0.0086 B b	97.9	1.0387±0.031 A b	0.15
7	72.9	0.2713±0.0022 E b	97.2	0.139±0.0021 F c	96.8	0.3183±0.0035 C c	98.1	0.2843±0.0058 D b	97.8	0.5507±0.0107 B c	98.8	0.6113±0.0067 A c	0.17
14	82.7	0.1727±0.0019 C c	97.9	0.107±0.0027 E d	97.3	0.266±0.0116 A d	98.6	0.213±0.0052 B c	99.4	0.16±0.0081 D d	99.7	0.16±0.0044 D d	0.147
LSD		0.19		0.165		0.19		0.152		0.146		0.116	

Capital and small letters indicate differences in pyrene concentration and days collection

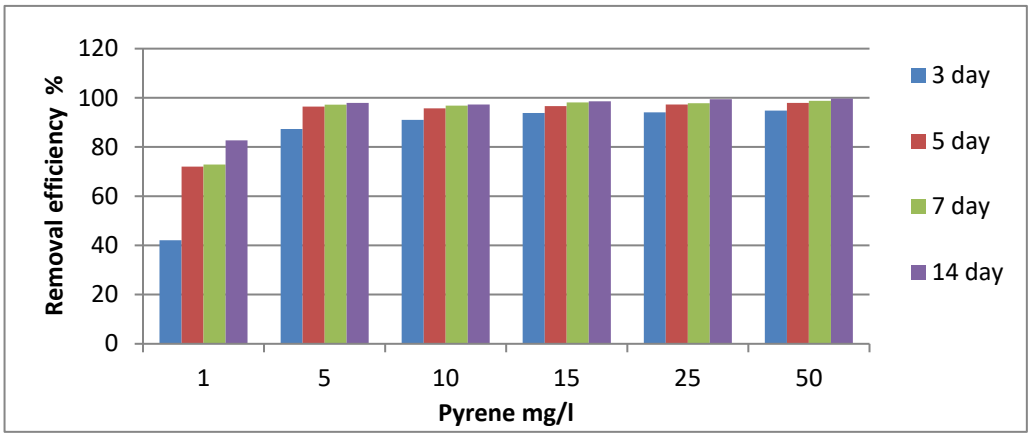

Fig. 1. Removal efficiency of pyrene by *Azolla pinatta*

According to the obtained results, the removal percentage of pyrene by *A. pennata* significantly increased with the increase in exposure days. These results are in agreement with Houshani *et al.* (2019), who showed that pyrene had more tendency to be degraded by the maize plant (Juhasz and Naidu, 2000) and Meudec *et al.* (2006) observed that PAHs with a higher molecular weight and lower water solubility, such as pyrene, are a greater stable and recalcitrant to be removed than PAHs with a lower molecular weight; therefore, with an increasing number of rings, PAH becomes less water-soluble and more lipophilic. Thus, the bioconcentration of PAHs by aquatic plants increases with the partitioning of PAHs between the sediment or water and the plant root system and the movement of PAHs between the root and the shoot (McGlynn and Livingston, 1997).

Furthermore, these findings are consistent with Alwan (2015), who found that the plant *Hydrilla verticillata* has a high accumulation of pyrene as a PAH. They also agree with Salehi-Lisar and Deljoo (2015), who documented the accumulation of fluorene as a PAH in sunflower, wheat and alfalfa plants. In addition, Duxbury *et al.* (1997) demonstrated that the *Lemna gibba* plant has a significant capacity for the uptake and depuration kinetics of three representative anthracene and benzo[a]pyrene phenanthrenes. (Liste and Alexander, 2000) reported that *Ceratophyllum demersum* and *Typha domingensis*, two aquatic plants from the Euphrates River, exhibited a high accumulation of PAHs.

Effect of Pyrene on Photosynthetic Pigment Content

The current results showed a decrease in the content of total chlorophyll, chlorophyll a, chlorophyll b, and carotenoids in the plant with increasing pyrene concentration after 14 days when the plant was exposed to 5, 10, 15, 25, and 50 mg /l of pyrene. The minimum values of total chlorophyll, chlorophyll a and carotenoids were (0.55, 0.25, and 0.433) µg /g recorded at 50 ppm respectively. While chlorophyll b recorded a low value (0.32) µg/g at 25 mg/l, the highest concentration of these pigments was registered in the control group (Table 2, Fig. 2). Statistical analysis at ($p<0.05$) showed that there were significant differences between all the interactions.

The results of this study are consistent with the study of Atta *et al.* (2020) ,who showed that the effect of crude oil on *Eichhornia crassipes* caused a decrease in the content of chlorophyll and carotenoids. They are also consistent with Olaranont *et al.* (2021) who studied the effect of crude oil on the physiological plant *Ipomoea pes-caprae* and reported a decrease in carotenoid and chlorophyll content. Alzurfi *et al.* (2019) showed that the aquatic plant *Hydrilla verticillata* cultured with different concentrations of crude oil for 24 days showed inhibition of chlorophyll

Table 2. Effect of different pyrene concentrations on photosynthesis pigments of *Azolla pennata*

photosynthesis pigments	control	Pyrene concentrations mg/l						LSD
		1 ppm	5 ppm	10 ppm	15 ppm	25 ppm	50ppm	
Chlorophyll a	3.4667±0.23821 A	0.7600±0.07937 B	0.6533±0.07055 C	0.4067±0.01764 D	0.3133±0.00882 E	0.3467±0.02603 E	0.2500±0.02082 F	0.069
Chlorophyll b	2.0400±0.03606 A	1.2933±0.13170 B	0.6000±0.08888 C	0.4300±0.02646 D	0.4500±0.02646 D	0.3200±0.01528 E	0.2533±0.02333 F	0.05
Total chlorophyll	5.9033±0.07860 A	2.1500±0.03786 B	1.1800±0.08963 C	0.8133±0.01764 D	0.7867±0.01453 D	0.6867±0.01453 E	0.5500±0.02309 F	0.064
Carotenoids	2.6267±0.15899 A	1.6367±0.11348 B	0.9000±0.04359 C	0.6200±0.02646 E	0.7007±0.01213 D	0.4800±0.01732 F	0.4333±0.02028 F	0.057

Capital letters indicate differences in pyrene concentration

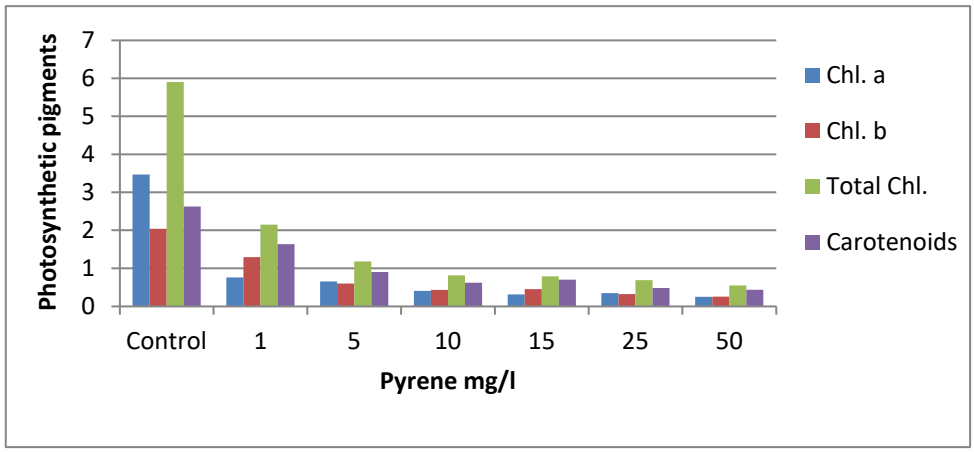

Fig. 2. Means total chlorophyll, chl-a, chl-b and carotenoids µg/g of *A. pinnata* after 14 days of exposed to different concentration of pyrene with control.

a, b, carotenoids and protein content. Xun *et al.* (2015) observed that phenanthrene and pyrene decreased the photosynthetic capacity of *Lemma minor*, which may be a protective response to limit the production of Reactive Oxygen Species (ROS) in chloroplasts. PAHs inhibit the enzymes needed for chlorophyll synthesis, and by inhibiting the photosynthetic process in chloroplasts as well as the formation of ROS (Reactive Oxygen Species) they affect photosynthesis and explain the effect of the photosystem II on complex external factors (Romero *et al.*, 2011).

Effect of pyrene on Antioxidant Enzymes (CAT, SOD), MDA and ROS Contents

The current study showed a gradual increase in antioxidant parameters values of the plant compared to the control treatment, recording the highest values (0.2873 and 1.04 U/g) at 50 ppm and the lowest value of CAT and SOD (0.2393 and 0.95 U/g) at the concentration of 5ppm, compared to the control group (0.238 and 0.9233 U/g) for CAT and SOD, respectively. Also, the study recorded a gradual increase in ROS and MDA values with increasing concentrations of pyrene, the highest value was (1.6367 and 14.4433 µmole/g) for ROS and MDA, respectively, at (50ppm) of pyrene, whereas the lowest value was (0.9067 and 13.2267 µmole/g) at 5 ppm compared to the control group (0.8633 and 13.15 µmole/g) for MDA and ROS, respectively (Table 3, Fig. 3). Statistical analysis at ($p<0.05$) showed that there were significant differences between all the interactions.

The results are consistent with Song *et al.* (2011) who found a significant increase in CAT, SOD, MDA and ROS and activity with increased PAH concentrations in the leaves and roots of

Table 3. Effect of different pyrene concentrations on Antioxidant parameters of *Azolla Pinatta*

Antioxidants	Control	Pyrene concentrations mg/l						LSD
		1 ppm	5 ppm	10 ppm	15 ppm	25 ppm	50ppm	
CAT U/g	0.238±0.00173 A	0.2393±0.00713 A	0.2537±0.00837 A	0.25±0.02082 A	0.2783±0.00982 A	0.273±0.01007 A	0.2873±0.01217 A	0.009
SOD U/g	0.9233±0.01453 B	0.95±0.01155 B	0.8467±0.08413 C	0.8867±0.05239 C	0.94±0.02646 B	1.1±0.10536 A	1.04±0.01528 A	0.042
ROS µmole/g	13.15±0.1193 D	13.2267±0.1507 D	13.8±0.10017 D	14.07±0.02082 C	14.2933±0.19462 B	14.4167±0.11319 B	14.4433±0.11977 A	0.142
MDA µmole/g	0.8633±0.01764 E	0.9067±0.01202 E	0.9133±0.01856 E	1.29±0.00577 D	1.3733±0.01202 C	1.44±0.02082 B	1.6367±0.02028 A	0.016

Capital letters indicate differences in pyrene concentration

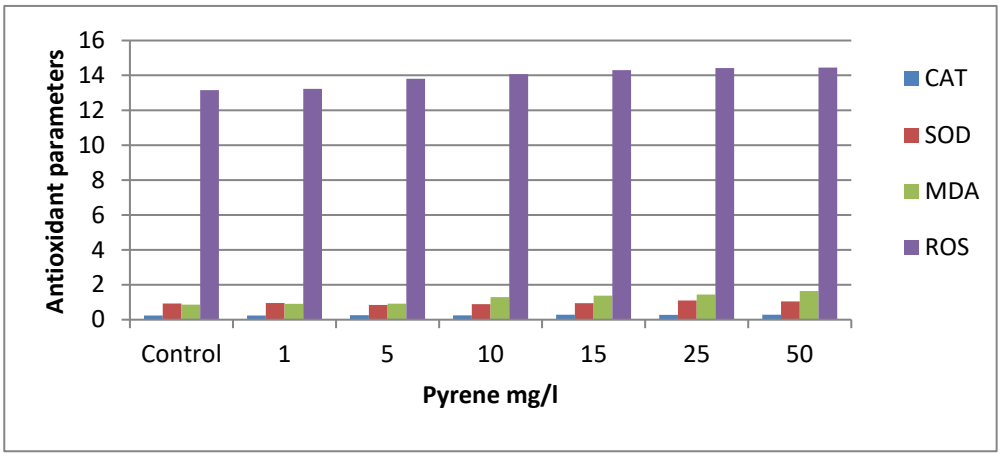

Fig. 3. Means (CAT, SOD U/g) and (MDA, ROS µmole/g) of *A. pinnata* after 14 days of exposure to different concentrations of pyrene compared to the control group

Kandelia candel. They are also in agreement with Arikan *et al.* (2023) who found that exposing Lactuca sativa plants to fluorine increased CAT and SOD activity by 80% and MDA by 40%.

Houshani *et al.* (2019) observed the toxicity of pyrene and phenanthrene on aquatic maize plants, which resulted in a significant reduction in shoot and root length. The increased CAT, SOD and MDA activities in the studied plants may indicate oxidative stress induced by cyclic compounds, leading to ROS production and subsequent increase in antioxidant enzyme activities, which enhance the scavenging ability of H2O2, inhibit ROS accumulation and protect plants from lipid peroxidation and oxidative damage, as highlighted by Hasanuzzaman *et al.* (2012). The increased activities of these antioxidant enzymes may be due to the up-regulation of genes encoding these enzymes, as suggested by González *et al.* (2020). Li *et al.* (2024) subjected the plant *Salix viminalis* to phenanthrene stress to study changes in its roots; they found that CAT, SOD and MDA levels increased significantly in response to an increase in ROS levels, explaining the correlation between them as they represent the defense system in plants and play an important role in detoxifying ROS.

CONCLUSIONS

A. Pinnata proved to be a valuable asset for the bioremediation of PAH compounds such as pyrene, exhibiting an impressive removal efficiency of 99.7%. In addition, the plant showed reduced photosynthetic pigment levels and increased antioxidant levels when exposed to high concentrations of pyrene, as evidenced by changes in chlorophyll content, CAT, SOD, MDA and ROS, suggesting that the plant has a degree of tolerance to this harmful compound, enabling it to withstand and effectively degrade pyrene. this study demonstrated that this plant has a powerful mechanism for bioremediating pyrene and reducing its harmful effects in aquatic environments.

REFERENCES

AbdulRada, N. J., Hussain, K. I., & Ali, A. A. (2014). Separation and determination of poly aromatic hydrocarbons in vegetables samples in Baghdad city using HPLC Technique. *Ibn Al-Haitham Journal For Pure and Applied Sciences*, *27*(1).

Ahn, C. K., Woo, S. H., & Park, J. M. (2010). Surface solubilization of phenanthrene by surfactant sorbed on soils with different organic matter contents. *Journal of hazardous materials*, *177*(1-3),

799-806.
Al-Baldawi, I. A., Abdullah, S. R. S., Suja, F., Anuar, N., & Idris, M. (2012). Preliminary test of hydrocarbon exposure on Azolla pinnata in phytoremediation process. Int. Conf. Environ. Energy Biotechnol. IPCBEE,
Alwan, S. W. (2015). The Bioaccumulation and Toxic Effect of Pyrene and Phenanthrene in Hydrilla verticillata (LF) Royal. *journal of kerbala university*, *11*(2), 113-121.
Alzurfi, S. K. L., Alasedi, K. K., & Abdulraheem, N. I. (2019). Effect Different Concentrations of Crude Oil on the Pigment content and protein content of Hydrilla verticillata Plant. *Iraqi Journal of Science*, 2141-2148.
Arikan, B., Yildiztugay, E., & Ozfidan-Konakci, C. (2023). Responses of salicylic acid encapsulation on growth, photosynthetic attributes and ROS scavenging system in Lactuca sativa exposed to polycyclic aromatic hydrocarbon pollution. *Plant Physiology and Biochemistry*, *203*, 108026.
Atta, A. M., Mohamed, N. H., Hegazy, A. K., Moustafa, Y. M., Mohamed, R. R., Safwat, G., & Diab, A. A. (2020). Green technology for remediation of water polluted with petroleum crude oil: Using of Eichhornia crassipes (Mart.) Solms combined with magnetic nanoparticles capped with myrrh resources of Saudi Arabia. *Nanomaterials*, *10*(2), 262.
Cerniglia, C. E. (1993). Biodegradation of polycyclic aromatic hydrocarbons. *Current opinion in biotechnology*, *4*(3), 331-338.
Chen, Y.-C., Banks, M. K., & Schwab, A. P. (2003). Pyrene degradation in the rhizosphere of tall fescue (Festuca arundinacea) and switchgrass (Panicum virgatum L.). *Environmental science & technology*, *37*(24), 5778-5782.
Cohen, M. F., Williams, J., & Yamasaki, H. (2002). Biodegradation of diesel fuel by an Azolla-derived bacterial consortium. *Journal of Environmental Science and Health, Part A*, *37*(9), 1593-1606.
Dhir, B., Sharmila, P., & Saradhi, P. P. (2009). Potential of aquatic macrophytes for removing contaminants from the environment. *Critical Reviews in Environmental Science and Technology*, *39*(9), 754-781.
Duxbury, C. L., Dixon, D. G., & Greenberg, B. M. (1997). Effects of simulated solar radiation on the bioaccumulation of polycyclic aromatic hydrocarbons by the duckweed Lemna gibba. *Environmental Toxicology and Chemistry: An International Journal*, *16*(8), 1739-1748.
Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical biochemistry*, *38*(12), 1103-1111.
Eribo, O., & Kadiri, M. (2016). Growth performance and phytoremediation ability of Azolla pinnata in produced water. *Journal of Applied Sciences and Environmental Management*, *20*(4), 1053-1057.
Gomes, M. P., de Brito, J. C. M., Carneiro, M. M. L. C., da Cunha, M. R. R., Garcia, Q. S., & Figueredo, C. C. (2018). Responses of the nitrogen-fixing aquatic fern Azolla to water contaminated with ciprofloxacin: Impacts on biofertilization. *Environmental Pollution*, *232*, 293-299.
González, A., Espinoza, D., Vidal, C., & Moenne, A. (2020). Benzopyrene induces oxidative stress and increases expression and activities of antioxidant enzymes, and CYP450 and GST metabolizing enzymes in Ulva lactuca (Chlorophyta). *Planta*, *252*, 1-13.
Guo, X., Zhang, W., Gu, J., Chen, F., & Yang, Q. (2021). The determination of the level, source, and risk of polycyclic aromatic hydrocarbon content in traditional Chinese medicines using a QuEChERS based extraction and HPLC-UV-FLD analysis. *Journal of Liquid Chromatography & Related Technologies*, *44*(3-4), 210-219.
Hadwan, M. H., & kadhum Ali, S. (2018). New spectrophotometric assay for assessments of catalase activity in biological samples. *Analytical biochemistry*, *542*, 29-33.
Hasanuzzaman, M., Hossain, M. A., da Silva, J. A. T., & Fujita, M. (2012). Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. *Crop stress and its management: perspectives and strategies*, 261-315.
Hegazy, H. G. (2011). Ameliorative effects of ginger and α-lipoic acid on oxidative stress and inflammation in senile female rats. *Afr J Pharm Pharmacol*, *5*(8), 1096-1105.
Houshani, M., Salehi-Lisar, S. Y., Motafakkerazad, R., & Movafeghi, A. (2019). Uptake and distribution of phenanthrene and pyrene in roots and shoots of maize (Zea mays L.). *Environmental Science and Pollution Research*, *26*, 9938-9944.
Indira, D., Reddy, K., Suresh, J., Naidu, V., & Ravi, A. (2014). Optimum conditions for culturing of Azolla (Azolla pinnata). *Int. J. Adv. Res. Biol. Sci*, *1*, 87-89.
Jeffrey, S. t., & Humphrey, G. (1975). New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochemie und physiologie der*

pflanzen, *167*(2), 191-194.
Jin, D., Jiang, X., Jing, X., & Ou, Z. (2007). Effects of concentration, head group, and structure of surfactants on the degradation of phenanthrene. *Journal of hazardous materials*, *144*(1-2), 215-221.
Juhasz, A. L., & Naidu, R. (2000). Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo [a] pyrene. *International biodeterioration & biodegradation*, *45*(1-2), 57-88.
Kaur, G., Sandhu, P. K., & Kaushal, S. (2022). Pros and Cons of Phytoremediation. *CORVETTE PRESS*, 170.
Kollah, B., Patra, A. K., & Mohanty, S. R. (2016). Aquatic microphylla Azolla: a perspective paradigm for sustainable agriculture, environment and global climate change. *Environmental Science and Pollution Research*, *23*, 4358-4369.
Kosesakal, T. (2018). Assessment of the biodegradation capacity of Azolla on polycyclic aromatic hydrocarbons in crude oil. *Global Nest J*, *20*(3), 27-32.
Kumari, R., Ojha, M., Saini, V., & Sharma, S. (2017). Effect of Azolla supplementation on proximate composition and digestibility of Labeo rohita (Ham.) fingerlings. *Journal of Entomology and Zoology*, *5*, 715-718.
Latif, A., Abbas, A., Iqbal, J., Azeem, M., Asghar, W., Ullah, R., Bilal, M., Arsalan, M., Khan, M., & Latif, R. (2023). Remediation of environmental contaminants through phytotechnology. *Water, Air, & Soil Pollution*, *234*(3), 139.
Li, X., Liu, J., Chen, F., Cheng, Y., Wang, Y., Li, A., Zhai, F., & Sun, Z. (2024). Phytotoxity of polycyclic aromatic hydrocarbons to Salix Viminalis L. *Pak. J. Bot*, *56*(2), 703-710.
Liste, H.-H., & Alexander, M. (2000). Plant-promoted pyrene degradation in soil. *Chemosphere*, *40*(1), 7-10.
Marmiroli, N., Marmiroli, M., & Maestri, E. (2006). Phytoremediation and phytotechnologies: a review for the present and the future. *Soil and water pollution monitoring, protection and remediation*, 403-416.
McGlynn, S. E., & Livingston, R. J. (1997). The distribution of polynuclear aromatic hydrocarbons between aquatic plants and sediments. *International journal of quantum chemistry*, *64*(3), 271-283.
Meudec, A., Dussauze, J., Deslandes, E., & Poupart, N. (2006). Evidence for bioaccumulation of PAHs within internal shoot tissues by a halophytic plant artificially exposed to petroleum-polluted sediments. *Chemosphere*, *65*(3), 474-481.
Miranda, A., Muradov, N., Gujar, A., Stevenson, T., Nugegoda, D., Ball, A., & Mouradov, A. (2014). Application of aquatic plants for the treatment of selenium-rich mining wastewater and production of renewable fuels and petrochemicals. *Journal of Sustainable Bioenergy Systems*, *4*(1), 97-112.
Mostafa, A. A., Hafez, R. M., Hegazy, A. K., Fattah, A. M. A.-E., Mohamed, N. H., Mustafa, Y. M., Gobouri, A. A., & Azab, E. (2021). Variations of structural and functional traits of Azolla pinnata R. Br. in response to crude oil pollution in arid regions. *Sustainability*, *13*(4), 2142.
Olaranont, Y., Stewart, A. B., & Traiperm, P. (2021). Effects of crude oil on plant growth and leaf anatomical structures in a common coastal plant. *International Journal of Phytoremediation*, *23*(2), 162-170.
Romero, M., Martin-Cuadrado, A.-B., Roca-Rivada, A., Cabello, A. M., & Otero, A. (2011). Quorum quenching in cultivable bacteria from dense marine coastal microbial communities. *FEMS microbiology ecology*, *75*(2), 205-217.
Saleh, M. M., Salman, J. M., & Almamoori, A. M. (2017). Bioremediation of polycyclic aromatic hydrocarbons by using Zea mays and inoculating with bacteria (Pseudomonas aeruginosa) and fungi (Penicillium expansum). *Mesopotamia Environmental Journal (mesop. environ. j) ISSN: 2410-2598*, *3*(3), 10-25.
Salehi-Lisar, S. Y., & Deljoo, S. (2015). The physiological effect of fluorene on Triticum aestivum, Medicago sativa, and Helianthus annus. *Cogent Food & Agriculture*, *1*(1), 1020189.
Song, H., Wang, Y.-S., Sun, C.-C., Wu, M.-L., Peng, Y.-L., Deng, C., & Li, Q. P. (2011). Effects of polycyclic aromatic hydrocarbons exposure on antioxidant system activities and proline content in Kandelia candel. *Oceanological and hydrobiological studies*, *40*, 9-18.
Sood, A., Uniyal, P. L., Prasanna, R., & Ahluwalia, A. S. (2012). Phytoremediation potential of aquatic macrophyte, Azolla. *Ambio*, *41*, 122-137.
Stephenie, S., Chang, Y. P., Gnanasekaran, A., Esa, N. M., & Gnanaraj, C. (2020). An insight on superoxide dismutase (SOD) from plants for mammalian health enhancement. *Journal of Functional Foods*,

68, 103917.

Xun, F., Xie, B., Liu, S., & Guo, C. (2015). Effect of plant growth-promoting bacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) inoculation on oats in saline-alkali soil contaminated by petroleum to enhance phytoremediation. *Environmental Science and Pollution Research*, *22*, 598-608.