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FACTORS AFFECTING REMOVAL OF POLYCYCLIC AROMATIC HYDROCARBONS FROM SEAWATER BY DRY BROWN SEAWEED *PADINA PAVONICA*

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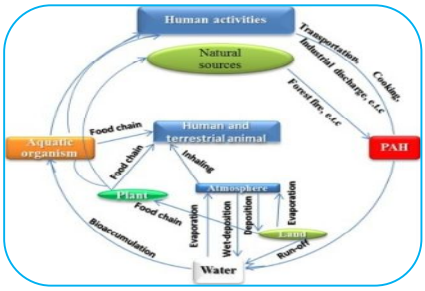
ABSTRACT



Removal of the polycyclic aromatic hydrocarbons (PAHs) from seawater was studied using dead tissue of brown seaweed Padina pavonica under various conditions for ten days. Different PAHs concentrations (25, 50, 75 and 100%) showed that the maximum removal capacity and PAHs removal percentage were recorded at concentration of 50% followed by concentration of 75%. In general, the data showed that within the same time period (10 days), the removal capacity and removal percentage increased with increase of PAHs concentration at 25% and 50%, then they decreased with increase concentration from 75% to 100%. P. pavonica dry biomass showed significant variations (at $P < 0.05$) in all remained concentrations values with changing pH of PAHs. Interestingly, at the same concentration the percentages removal and removal capacities of PAHs were generally lower in high acidic (pH 2) and alkaline (pH 11) solutions, but higher for treatments around neutral (pH 5, 7 and 9). The removal capacitiy and removal percentage of PAHs were the highest for the saltiest solution (36% - the salinity of seawater) and were the lowest at 0% (PAHs adding distilled water). The results indicated that P. pavonica dry biomass achieved the maximum efficiency for PAHs removal at concentration of 50% of PAHs at pH 5 and salinity 36%, which represent the optimum factors. The present study provided valuable information for achieving optimal sorption of PAHs using Padina pavonica as an effective sorbent for removing PAHs in seawater.

KEY WORDS: *PAHs, seaweed- Padina pavonica, sorption.*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are of special concern because they have cytotoxic properties (mutagenesis and carcinogenesis) (IARC, 2010, Harrison *et al*., 2016 and Haiba *et al*., 2019). When PAHs come into contact with humans, the toxic effects are alarming, ranging from cardiovascular events, diabetes, and oxidative stresses to genetic mutations. One of the main ways PAHs come into contact with humans is through the ingestion of contaminated foods resulting from bioaccumulation and biomagnification in the food chain (Alegbeleye *et al*., 2017).

PAHs are highly toxic chemicals for marine environments and can easily enter the food chain through algae. Moreover, PAHs pose a threat to marine life due to their rapid diffusion and accumulation in algae (Duan *et al*., 2015). There are 16 PAHs marked priority pollutants by the US environmental protection agency (U.S. EPA) (Nwaichi *et al*., 2010; Harrison *et al*. 2016 and Qari & Hassan, 2017), each of which consists of between two and six attached benzene rings (Bojes and Pope,

2007 and Urbano *et al.,* 2021). These priority pollutants have been recognized as such due to their potential toxicity to terrestrial and aquatic organisms, including humans (Samanta *et al*., 2002; Bojes & Pope, 2007). Though each PAH has unique physical and chemical properties, in accordance with structure and number of rings, they are typically solid at ambient temperatures and are characterized by high melting and boiling points, low vapour pressures and low solubility in water (particulary those with higher molecular weights) (Douben, 2003). Contamination of PAHs in urban runoffs has been found and later to receiving water bodies (Shinya *et al*., 2000; Aryal *et al*., 2005).

PAHs are ubiquitous in urban areas and distributed widely in marine environments (Qari & Hassan, 2017; Haiba, 2019). They occur naturally (constituents of crude oil) or due to human activities (e.g. petroleum industries, combustion) (Harrison *et al*., 2016). They are of special concern because they have cytotoxic properties (mutagenesis and carcinogenesis) (Harrison *et al.* 2016; Haiba *et al*., 2019). In their thorough review, Torres *et al*. (2010) stated that continuous operative discharges from ships, marine tanker collisions, and refineries discharge high amounts of pollutants in marine ecosystems. PAHs are highly toxic chemicals for marine environments, and they can easily enter the food chain through algae. Moreover, PAHs pose a threat to marine life due to their rapid diffusion and accumulation in algae (Duan *et al*., 2015).

Egypt is witnessing rapid and tumultuous industrial and demographic growth (Hassan *et al*., 2018). Alexandria is the second large city in Egypt, and it has about 40% of Egyptian industrial activities (El Maghraby& Hassan, 2017 and Haiba, 2019).

At present, bioremediation is often the most suitable method for remediation of organic pollutants such as PAHs from especially petroleum hydrocarbons, because it is cost effective (Prince & Clark, 2004, Perelo, 2010, Das & Chandran, 2011, Megharaj *et al*., 2011, Parkash & Irfan, 2011, Jain & Bajpai, 2012 and Varjani & Upasani, 2017). Brown *et al*. (2017) concluded that bioremediation can be a viable mechanism for treating soils contaminated with petroleum hydrocarbons. Algae play an important role in the bioaccumulation of several harmful compounds (Kaur & Bhatnagar, 2002, Todd *et al*., 2002, Murray *et al*., 2003, Lei *et al*., 2002, 2007, Bopp & Lettieri, 2007 and Vidyashankar & Ravishankar, 2016). Morever, Integrated green algal technology for bioremediation was repoted in Sivakumar *et al.* (2012). These bioremediation abilities of algae are helpful for environmental clean-up (Lim *et al*., 2010, Ellis *et al*., 2012 and Chekroun *et al*., 2014).

Algae cannot synthesize PAHs, but they are known to be a good accumulator of PAHs (Haiba *et al*., 2019). Algae have three different ways to remove Polyaromatic hydrocarbons from the environment; 1) adsorption of PAHs on the surface of algal cells depending upon the active groups present on that sufaces, 2) accumulation of PAHs within the algal cells and 3) transformation of PAHs which depending upon the enzymatic actions. The third method of removal is considered the effective one due to get rid of PAHs toxcicity (El-Sheekh *et al.*, 2012).

The phytoremediation of PAHs has restricted success because of the high toxicity of this session of contaminants (Dhankher *et al*., 2012). The accumulation and biodegradation of two characteristic polycyclic aromatic hydrocarbons (PAHs), phenanthrene (PHE) and fluoranthene (FLA), by the diatoms was studied by Hong *et al*. (2008), using two algal species *Skeletonema costatum* and *Nitzschia* sp. The scientists reported that the accumulation and degradation abilities of *Nitzschia* sp. were higher than those of *S. costatum*. Degradation of FLA by the two algal species was slower, demonstrating that FLA was a more intractable PAH compound. The microalgal species also showed equivalent or greater efficiency in the elimination of the PHE-FLA mixture compared with PHE or FLA alone. Tomar *et al*. (2022) studied the impact of polycyclic aromatic hydrocarbons on photosynthetic and biochemical functions and its bioremediation by *Chlorella vulgaris*.

The efficiency for eliminating metallic ions and polar organics in wastewaters by the marine brown seaweed, *Sargassum*, has long been documented (Chan *et al*., 2005). *Sargassum* has other attractive characteristics, such as qualified ease of collection, rich in worldwide coastal areas, stable quality. Chung *et al*. (2006) explored also the effectiveness of consuming dried *Sargassum hemiphyllum* to eliminate aqueous Phenanthrene (PHE). Various factors as temperature, shaking rate and initial PHE concentration that may affect the uptake of PHE were also investigated.

Focusing on the adsorptive capacity and the uptake mechanism, the ability of *Padina pavonica* for dye removal was investigated. Dye removal percentage increased as the initial dye concentration increased with the maximum removal percentage of 73.2% (Fakhry, 2013). The removal of Cu(II) and Pb(II) ions from aqueous solutions using the brown algae *Padina pavonica* J.V. Lamouroux 1816 was studied, and the effects of solution pH, the contact time, and the initial metal concentration on biosorption were investigated (Ozudogru, 2013).

The brown alga *Padina boryana* used to determine polyromantic hydrocarbons (PAHs) bioaccumulation at a Jeddah City seashore. PAHs were measured in the coastal water and in algal tissues using gas chromatography mass spectrometry (GC-MS). The high concentrations of PAHs in algal tissues demonstrated the utility of using *Padina boryana* as a biomonitor of PAH contamination and bioavailability in the coastal waters (Qari and Hassan, 2017).

MATERIALS AND METHODS

Algal species

Fresh samples of *Padina pavonica* L Thivy in Taylor were collected in May from the coastal zone of Abu-Qir, Mediterranean Sea, Alexandria, Egypt. The alga grows on submerged rocks up to 50 cm depth. It has flattened fan-shaped thallus up to 10 cm. The thallus is calcified with concentric bands of hairs. After harvesting, whole alga was extensively washed several times with natural sea water to remove any attached sand then the rhizoidal portions were removed to avoide microbial contamination. The algal materials were conveyed to the laboratory in plastic bages filled with sea water and dried in oven at 50°C overnight. Subsequently, it was sieved and stored in desiccator before use. The adsorption of PAHs by *P. pavonica* algal biomass was investigated by using a series of 1000 ml beakers, which were prepared containing 1000 ml known concentration (50%). Adsorbent dry biomass (3 gm) was added to each beaker for 10 days. Aerator was used for agitation and room temperature was (29± 2°C). The biomasses were removed and the petroleum hydrocarbons concentration in the PAHs were measured.

Sea water

The used water samples for culturing were collected in clean bottles from the same collecting areas. These samples were taken to the laboratory, filtered and kept in dark till being used.

Preparation of PAHs of crude petroleum oil

PAHs crude petroleum oil was prepared by using the modified Boylan and Tripp method (1971) as follows: One part of crude oil was mixed with 20 parts of seawater in a glass stoppered bottle. The mixture is stirred by a magnetic stirrer at low speed for 12 hour. This mixture was then transferred to a separation funnel and allowed to stand for 4 hours. The aqueous phase was then drained and the remaining non-soluble fractions were discarded. The aqueous phase was designated as 100% oil extract. Dilution of this stock solution with volumes of the medium yielded lower percentages of oil extract, using Egyptian crude petroleum oil from Amria company and prepared by using seawater for dilution.

Determination of the concentration of polycyclic aromatic hydrocarbons remained in seawater

PAHs remaining in the incubated seawater were measured, for these measurements, 1liter seawater from each flask was taken and extracted with dichloromethane (DCM). The extracts were rotary evaporated down to 1ml, then analysed by Gas Chromatography-Mass Spectrometer (GC-MS) (thermo-scientific ISQ, 2009) and helium as carrier gas at 1 ml min^{-1}. TG-1MS column (100% dimethyl polysiloxane, fused silica 30 m, 0.32 mm item diameter (i.d.) and 0.25 µm film thickness) was used. The injection port temperature was 250°C with splitless injection mode (3 min), then split mode with split ratio 1:100. Column temperature was programmed from 80 to 240 at 7°C min^{-1} and then to 300°C at 3°C min^{-1} and held for 5 min at 300°C (Alkio *et al.,* 2005). A series of blanks was also conducted following the procedures described above.

In addition, three affected factors were studied, including modification in pH (pH 2-11), ionic strength (0.0-0.6M NaCl) and initial concentrations of PAHs of crude oil (25-100%) of the tested solution.

Removal efficiency of PAHs by algae

The removal capacity (RC) of PAHs is calculated as:

$$RC = \frac{V_oC_o - V_f C_f}{m}$$

While the removal percentage (%R) of PAHs is obtained as:

$$\%R = \frac{V_oC_o - V_f C_f}{V_oC_o} X\ 100\%$$

Where V_o and C_o are the initial volume and concentration of solution, V_f and C_f are the final volume and concentration of PAHs and m is the mass of alga used (Chung *et al*., 2007).

Statistical analysis

Statistical analyses were performed using employed SPSS version 10.0 for testing significant of differences between treatments at the 0.05 probability level (p = 0.05). Bioremediation experiments were tested with analysis of variance (ANOVA- one way) (Duncan, 1957).

RESULTS

Factors affecting efficiency of *P. pavonica* dry biomass on PAHs removal

These factors included initial concentration of PAHs of crude petroleum oil (25%, 50%, 75% and 100%), modification in pH (pH 2, 5, 7 and 11) and salinity (0.0%, 18% and 36%).

Effect of initial concentration:

In order to investigate the effect of various PAHs initial concentrations on the removal of PAHs, a series of concentrations of 25%, 50%, 75% and 100% were prepared. Adsorbent dry biomass (3g) was added to 1000 ml of each PAHs solutions. The mixtures were constantly shaken for the desired time (ten days) and then filtered. Each remained seawater measured for PAHs.

Adsorption of PAHs by *P. pavonica* dry biomass with varying its concentration after ten days was shown in Table (1). PAHs concentration remained in seawater decreased from 24.30 to 9.40 and 8.18 µg/l at concentrations of 25% and 50%, respectively, then these remained compounds increased to 11.77 and 12.59 µg/l at concentrations of 75% and 100%, respectively, compared to their value in low concentrtions. The decrements and increments of PAHs due to different concentrations of PAHs was significant.

It was observed that the maximum removal capacity for PAHs were 5.37 µg/g dry matter with removal percentage 66.33% at concentration 50% (Table 2). It must be mentioned that concentration of 50% was considered the best one for most removal efficiency of PAHs followed in decreasing order by concentrations of 25% and 75%. Accordingly, concentration of 50% was selected for further adsorption experiments.

Table 1: Effect of concentration of polycyclic aromatic hydrocarbons of petroleum crude oil on the removal of polycyclic aromatic hydrocarbons (µg/l) using *Padina pavonica* dry biomass for 10 days.

Concentration of Polycyclic aromatic hydrocarbons (µg/l)					
PAHs	**Initial conc.**	**25%**	**50%**	**75%**	**100%**
Naphthalene	9.43 ±0.38	3.54±0.22	3.22 ±0.18	4.32 ±0.36	6.22 ±0.24
Acenaphthane	2.47 ±0.29	1.06±0.25	0.61 ±0.16	1.49 ±0.22	1.63 ±0.22
Flurene	0.51 ±0.12	0.23±0.04	0.26 ±0.05	0.34 ±0.04	0.36 ±0.08
Phenanthrene	0.81 ±0.16	0.30±0.04	0.32 ±0.07	0.35 ±0.08	0.40 ±0.05
Benzo (a)	2.85 ±0.25	0.79±0.18	0.76 ±0.12	1.04 ±0.14	1.27 ±0.25
Chrysene	1.27 ±0.26	0.74±0.15	0.62 ±0.11	0.68 ±0.12	0.80 ±0.16
Benzo (a)	0.88 ±0.15	0.38±0.08	0.31 ±0.03	0.42 ±0.09	0.62 ±0.11
Indeno (1, 2, 3 cd) Pyrene	2.44 ±0.21	0.96±0.15	0.81 ±0.12	1.23 ±0.18	1.35 ±0.16
Dibenzo (a, h) anthracene	0.47 ±0.07	0.24±0.02	0.26 ±0.04	0.32 ±0.02	0.36 ±0.04
Pyrene	3.17 ±0.21	1.16±0.18	1.01 ±0.11	1.58 ±0.16	1.81 ±0.21
∑ PAHs	**24.30^{a}±11.42**	**9.40^{b}±7.78**	**8.18^{b}±3.74**	**11.77^{a}±4.55**	**12.59^{a}±5.91**

Different superscript letters within column indicates significant differences at $P < 0.05$ according to one-way ANOVA.

The removal capacitiy and removal percentage of PAHs using *P. pavonica* dry biomass were listed in Table (2). Interestingly, the removal capacitiy of PAHs was the highest (5.35 µg/g dry matter) for the saltiest solutions (36%) and was the lowest (3.65 µg/g dry matter) for 0% (Crude petroleum oil adding distilled water). It was noticed that the highest removal percentage of PAHs was recorded at 36%, which achieved 66.13%, (Table 2). The results indicated that *P. pavonica* dry biomass achieved the maximum efficiency for PAHs removal at concentration of 50% of WSFs of crude petroleum oil at pH 5 and salinity 36%, which represent the optimum factors. Table (2) showed that the removal capacitiy PAHs was the maximum value (5.58 µg/g dry matter) at pH5 after ten days. In the same trend, this alga dry biomass achieved highest percentage removal (73.66%) after ten days at pH 5. Interestingly, at the same concentration the percentage removal and removal capacitiy of PAHs were generally lower in very acidic (pH 2) and alkaline (pH 11) solutions, but higher for treatments around neutral (pH 5, 7 and 9).

Table 2: Removal efficiency of Polycyclic aromatic hydrocarbons by *P. pavonica* dry biomass under influence from different factors for 10 days.

(A) PAHs concentration (%)	Removal capacity (µg/l)	Removal percentage
25	4.17	51.56
50	5.37	66.33
75	4.96	61.31
100	3.16	39.01
(B) pH		
2	4.68	44.55
5	5.58	73.66
7	5.24	69.33
9	5.33	71.77
11	4.76	45.72
(C) Salinity (%)		
0	3.65	45.10
18	4.22	52.09
36	5.35	66.13

Effect of different pH:

At the same concentration (50%), the pH series span from acidic (pH 2) through neutral (pH 7) to alkaline (pH 11) conditions. Interestingly, *P. pavonica* dry biomass showed significant variations (at $P < 0.05$) in the all remained concentrations values with changing of pH of PAHs (Table 3). Remained concentrations of PAHs in the solution recorded its minimum values 7.54 µg/l at pH 5.

Table 3: Effect of pH on the removal of polycyclic aromatic hydrocarbons (µg/l) using *P. pavonica* dry biomass for 10 days.

Polycyclic aromatic hydrocarbons conc. (µg/l)						
	pH					
PAHs	Initial conc.	2	5	7	9	11
Naphthalene	9.43 ±0.41	3.03 ±0.29	2.59±0.18	2.37±0.31	2.95±0.35	3.07±0.24
Acenaphthane	2.47 ±0.32	1.18 ±0.29	0.95±0.08	0.99±0.09	0.39±0.05	1.16±0.21
Flurene	0.51 ±0.12	0.32 ±0.07	0.24±0.05	0.23±0.08	0.20±0.02	0.38±0.12
Phenanthrene	0.81 ±0.18	0.34 ±0.08	0.29±0.04	0.34±0.04	0.33±0.05	0.52±0.09
Benzo (a) anthracene	2.85 ±0.26	0.84 ±0.21	0.60±0.12	0.62±0.15	0.62±0.15	0.82±0.37
Chrysene	1.27 ±0.33	0.68 ±0.12	0.37±0.07	0.51±0.05	0.50±0.09	0.52±0.11
Benzo (a) flurencene	0.88 ±0.15	0.47 ±0.09	0.22±0.05	0.24±0.05	0.21±0.02	0.42±0.09
Indeno (1,2,3,cd)	2.44 ±0.22	1.37 ±0.18	1.03±0.04	1.24±0.09	1.21±0.08	1.30±0.18
Dibenzo (a,h) anthracene	0.47 ±0.05	0.33 ±0.05	0.20±0.04	0.21±0.01	0.24±0.04	0.27±0.07
Pyrene	3.17±0.31	1.64±0.21	1.44±0.22	1.46±0.18	1.48±0.18	1.62±0.33
∑ PAHs	**24.30^{a}±11.26**	**10.25^{b}±4.55**	**7.54^{b}±1.43**	**8.57^{b}±2.91**	**8.30^{b}±2.18**	**10.01^{b}±3.02**

Different superscript letters within column indicates significant differences at P < 0.05 according to one-way ANOVA.

Effect of salinity:

At the same concentration (50%) and pH (5) the concentrations of remained PAHs after adsorption by *P. pavonica* dry biomass decreased significantly (at P < 0.05) with increasing salinity of PAHs (Table 4), which record the lowest values (7.34 µg/l), at 36% (salinity of seawater).

Table 4: Effect of salinity on the removal of polycyclic aromatic hydrocarbons (µg/l) using *P. pavonica* dry biomass for 10 days.

Polycyclic aromatic hydrocarbons conc. (µg/l)				
PAHs	**Salinity (%)**			
	Initial conc.	0.0	18	36
Naphthalene	9.43 ±0.41	4.32 ± 0.32	4.21 ± 0.16	3.08 ± 0.33
Acenaphthane	2.47 ±0.32	2.03 ± 0.43	1.88 ± 0.11	0.52 ± 0.21
Flurene	0.51 ±0.12	0.36 ± 0.04	0.31 ± 0.02	0.24 ± 0.08
Phenanthrene	0.81 ±0.18	0.35 ± 0.02	0.32 ± 0.04	0.30 ± 0.14
Benzo (a) anthracene	2.85 ±0.26	1.42 ± 0.16	1.44 ± 0.09	0.68 ± 0.33
Chrysene	1.27 ±0.33	0.55 ± 0.07	0.48 ± 0.05	0.41 ± 0.31
Benzo (a) flurencene	0.88 ±0.15	0.40 ± 0.02	0.31 ± 0.02	0.31 ± 0.09
Indeno (1,2,3,cd) Pyrene	2.44 ±0.22	1.10 ± 0.16	1.12 ± 0.08	0.54 ± 0.19
Dibenzo (a,h) anthracene	0.47 ±0.05	0.31 ± 0.02	0.22 ± 0.04	0.23 ± 0.07
Pyrene	3.17±0.31	2.48 ± 0.07	1.35 ± 0.08	1.04 ± 0.24
∑ PAHs	**24.30^{a}±11.22**	**13.34^{ab}±6.82**	**11.64^{b}±4.14**	**7.34^{b}±2.06**

Different superscript letters within column indicates significant differences at P < 0.05 according to one-way ANOVA.

DISCUSSION

The possible fates of PAHs in the environment include volatilization, photooxidation, chemical oxidation, bioaccumulation, adsorption to soil particles, leaching and microbial degradation (Cerniglia, 1992). The concentration of PAHs in the environment differs widely, depending on the level of industrial development and pollution with petroleum products (Wild and Jones, 1995). Many PAHs are considered among the most harmful contaminants with recognized toxicity towards both terrestrial and aquatic organisms (Burgess *et al*., 2003). Direct contact, inhalation and absorption of soluble PAH pollutants, can cause acute toxicity and carcinogenesis in organisms (Singh *et al*., 2007).

Acute toxicity is associated with fewer-ringed PAHs, while those with high molecular weights and low solubility are typically considered carcinogenic (Bojes and Pope, 2007). The most recognized carcinogenic PAH is benzo(a)pyrene (BaP), which can induce mutations by binding directly to DNA and forming adducts (Leung *et al*., 2007 and Singh *et al*., 2007). In addition to inducing carcinogenesis other PAHs can interact with the physiological mechanisms of an organism, causing oxidative stress (Torres *et al*., 2008). photosynthetic rates (Pn), chlorophyll fluorescence (Fv/Fm), and total chlorophyll in *Ulva lactuca*, were significantly reduced with increasing PAHs concentrations (El Maghraby and Hassan, 2021).

Common background PAH concentrations in coastal marine systems range from tens to hundreds of parts per billion and are dominated by low molecular weight PAHs (3- rings) (Burgess *et al*., 2003 and Latimer & Zheng, 2003). The higher molecular weight PAHs tend to have a more

recalcitrant nature which ensures their deposit and accumulation in marine sediments (Kanaly & Harayama, 2000 and Burgess *et al*., 2003). Aquatic organisms typically achieve uptake and the external environment control the rate of diffusion and tissue concentration (Meador, 2003). Though it is reasonable to assume hydrophobicity of individual PAHs would affect their uptake rate, little variability has been shown between contaminants of varied hydrophobic properties and their uptake rate (Bender *et al*., 1988). PAH availability and specific organism physiology are the two key variables that influence uptake in any marine environment (Meador, 2003). In aquatic systems algae can assimilate PAH contaminants rapidly, removing them from sediments and water column (Greenberg, 2003).

PAH contamination has similarities with that of heavy metals as both are anthropogenic contaminants, existing in low background concentrations and present in higher concentrations at biologically productive coastal sites associated with urban and industrial centres (Pinto *et al*., 2003). The petroleum derived PAH products constitute up to 20% of crude oil wastes regulary discharged into the coastal marine environment and, as PAHs are lipophilic, they easily penetrate *Ulva* thullus, resulting in cellular disruption (Lobban and Harrison, 1997). This metabolic disruption is commonly observed as a reduction in photosynthetic rate and growth of *Ulva*, similar to that observed during exposure to heavy metal contaminants (Zambrano & Carballeira, 1999; Han *et al*., 2007& 2009 and Lage-Yusty *et al*., 2009). However, because photosynthesis is also reduced by oil directly coating *Ulva* thullus, reducing CO_2 diffusion and light penetration, photosynthetic rate alone is not a sufficient indicator of hydrocarbon induced stress (Zambrano & Carballeira, 1999). The production of reactive oxygen species (ROS) follow on oxidative stress and accordingly damage to lipids, proteins and DNA as a outcome of PAH exposure has been explored in higher plants (including *Arabidopsis*) (Alkio *et al*., 2005 and Paskova *et al*., 2006), in the aquatic plant *Lemna gibba* (duckweed) and in a marine diatom (Wang *et al*., 2008).

Adsorption of organic compounds by the cell wall of the alga is mainly a passive process driven by chemical partitioning into the hydrophobic biomass. Because a metabolic reaction is not involved in this accumulation, adsorption is usually the same for living and dead cells (Liebe & Fock, 1992). Brown macroalgae registered the highest removal capacities for petroleum hydrocarbons (Flores-Chaparro *et al*., 2017).

The current results of adsorption of PAHs by *P. pavonica* dry biomass showed that remained concentrations of PAHs in the solution were changed significantly (at $P < 0.05$), with changing concentrations. It was observed that the maximum removal percentage for PAHs was 66.33% with removal capacity 5.37 µg/g dry matter at concentration of 50%. It must be mentioned that concentration of 50% of PAHs was considered the best one to remove by *P. pavonica* followed in decreasing order by concentrations of 25% and 75% (Table 2). Chung *et al*. (2007) demonstrated that higher initial concentrations of sorbate resulted in a higher probability of collision, thus higher uptake by the biomass. This relationship was in consistent with our results and other studies such as Noeline *et al*. (2005) and Kumar *et al*. (2011). It is noteworthy to mention that the relationship between removal capacity and removal percentage was in direct proportional and was noticeable under the effects employed by different salinities and initial PAHs concentrations (Chung *et al*., 2007).

In this study the pH series span ranged from acidic (pH 2) through neutral (pH 7) to alkaline (pH 11) conditions. Interestingly, the remained concentration of PAHs varied significantly (at $P < 0.05$) with different pHs after incubation of *P. pavonica* dry biomass for ten days. Our results show that the removal capacitiy of PAHs was the maximum value (5.58 µg/g dry matter) at pH5 after ten days by using 50% crude oil concentration. In the same trend, this alga dry biomass achieved highest percentage removal (73.97%) after ten days at pH 5. Interestingly, at the same concentration (50%), the percentage removal and removal capacitiy of PAHs were generally lower in very acidic (pH 2) and alkaline (pH 11) solutions, but higher for treatments around neutral (pH 5, 7 and 9) (Table 3).

In these results in this concern is going in harmony with those of Chung *et al*. (2007). The removal capacities of aqueous phenanthrene (PHE) by brown seaweed *Sargassum hemiphyllum* dry biomass were generally lower in very acidic (pH 2) and alkaline (pH 11) solution, but higher for treatments around neutral (pH 5, 7 and 9), *Sargassum* biomass was very resisting to the alkaline

environment. There was significant decline at $P < 0.05$ in the removal capacities at pH 2 and 11. The uptake of polycyclic aromatic compounds (PAHs) by tissue cultures of the marine alga *Acrosiphonia coalita* occurred very quickly and was affected by changes in seawater pH. The fast rate of uptake was in agreement with other studies from the literature. An examination of the uptake kinetics of phenanthrene accumulation by marine diatoms revealed that short term steady state was achieved within 30 minutes (Fan and Reinfelder, 2003), and the uptake of phenanthrene by dead tissues of the brown seaweed *Sargassum hemiphyllum* reached equilibrium within 24 hours (Chung *et al.,* 2007). The reasons why pH affected uptake are unclear (Chung *et al.,* 2007). It is possible that the change in pH affected binding of PAH to the algal cell constituents. However, PAH compounds are nonionic, so their physical properties should not be greatly affected by pH change. On the other hand, the nature of the organic material present in the algal cell is complex. For example, polysaccharides and proteins are found in algal cell walls. These components can have sulfate, carboxyl, and phosphate groups, which can be charged (Lobban and Harrison, 1997). A change in pH could affect the affinity of PAH to these compounds. Other experiments have shown a relationship between the uptake of PAH by algae and pH. However, an increase in pH usually caused a decrease in the adsorption capacity. For example, the removal of phenanthrene by dead tissue of the *S. hemiphyllum* was decreased under constant alkalinity compared to removal at neutral pH levels (Chung *et al*., 2007).

Although sorption of oil is unlikely to occur through ion exchange, the spontaneous protonation and deprotonation of surface functional groups may still affect sorption. Hydrocarbon and oil droplets often carry negative charge in aqueous solution (Djerdjev and Beattie, 2008). Thus, negative charge on the biosorbent surface at high pH may delay sorption through electrostatic repulsion and this may have an adverse influence on both absorption and adsorption processes. Moreover, pH can also impacts the surface charge on sorbates (Davis *et al*., 2003 and Ozer *et al*. 2006).

The removal capacitiy & removal percentage of PAHs using *P. pavonica* dry biomass were listed in Table (2). Interestingly, the removal capacitiy of PAHs was the highest (5.35 µg/g dry matter) for the saltiest solution (36%) and was the lowest (3.65 µg/g dry matter) for 0% (Crude petroleum oil adding distilled water). It was noticed that the highest removal percentage of PAHs was recorded at 36%, which achieved 66.13%, (Table 2). The results indicated that *P. pavonica* dry biomass achieved the maximum efficiency for PAHs removal at concentration of 50% of WSFs of crude petroleum oil at pH 5 and salinity 36%, which represent the optimum factors.

The above results were in consistence with those of Chung *et al*. (2007) who reported that ionic content in the aqueous solution induced profound effects on the sorption performance of the aqueous phenanthrene (PHE). A removal capacity of sorbate was the lowest (400 µg g^{1}) for the saltiest solution (1M NaCl). It is expected that the elevated electrolyte in the solution interacts more strongly with water molecules than PHE-water interaction, thus reducing the solubility of the aqueous PHE or even dissolved organic matter-PHE interaction (salting out) and forcing the PHE to adhere on other surfaces such as dry biomass of *Sargassum*, glass flask or escape to vapour phase. Their and our results ruled out the increased sorption of PHE by *Sargassum*. Ionic species can interact strongly with *Sargassum* as it is known to be a strong sorbent for heavy metals such as Pb and Cd (Martins *et al*., 2006), the biomass may sorb and accumulate the ionic species such that the ionic charge is higher than the ambient environment. However, whether these interactions are significant in controlling the sorption behaviours of PHE are still unknown. Hoffman *et al*. (1984) demonstrated that urban runoff is a major source of PAHs discharging to coastal areas. Typical salinity of seawater is roughly equivalent to 36% and the finding of a weaker sorption capacity of PHE by *Sargassum* under a low salinity suggested that a decrease in the sorption of medium hydrophobic organic compounds (HOCs) by the organism may happen in coastal areas receiving urban discharges.

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