REMOVAL EFFICIENCY OF *ULVA LACTUCA* DRY BIOMASS FOR PETROLEUM CONTAMINATIONS

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ABSTRACT

Petroleum contamination of environments, which results from spillage of crude oil, is always a global concern and an ongoing threat to human health and natural environments because of its high incidence and long-term environmental impact. The aim of this study was to investigate the removal efficiency of the dry green alga *Ulva lactuca* biomass for biosorption of *n*-alkane (light and heavy) and polyaromatic hydrocarbons (PAHs) in water soluble fractions (WSFs) of crude oil from Ras El-Adabya, Suez Gulf at different time intervals (2-10 days). The physicochemical properties of water and crude oil samples were performed. Algae, which was collected from Alexandria (Abu- Qir region) between May and July 2021, was subjected to different WSFs concentrations (25%, 50%, 75%, and 100%) of crude oil by varying exposure time 2, 4, 6, 8, and 10 days. The removal efficiency of *U. lactuca* dry biomass was measured and monitored using gas chromatographic analysis (GC). Results showed that the concentrations of petroleum hydrocarbons (light *n*- alkanes, heavy *n*-alkanes, and PAHs) significantly decreased by increasing the contact time of the biomass to ten days, which recorded its lowest content. Removal capacity and removal percentage of *U. lactuca* for three types of hydrocarbons increased with increasing of contact time till it reached maximum at 10 days. Data showed that removal percentage of light *n*-alkanes, heavy *n*-alkanes and PAHs was best at 25 % of crude oil which reached 31.18 %, 17.88% and 40.57 %, respectively.

Key words: Algae; *Ulva lactuca*; Adsorption; Crude Oil; Water Soluble Fraction; Gass Chromatograpic (GC) Analysis.

INTRODUCTION

Reliability to clean water is considered as one of the biggest human goals in the twenty-first century (Qu *et al.,* 2013). Our water resources' quality is getting worse every day because of the persistent introduction of harmful substances (Huang *et al.,* 2021) and the supply and quality of water are extremely impacted by the increase in global pollution, industrialization, and rapid economic growth (Bhatt *et al.,* 2022). Industrialization,

civilization, agriculture, and other environmental and global changes are the primary causes of water contamination (Haiba, 2019; Ahmad *et al.*, 2021).

Environmental pollution by oil spill was a serious concern in both developed and developing countries. Crude oil with its derivatives has been considered as main pollutants of marine ecosystems (Ławniczak *et al.*, 2020). Crude oil spills are one of the main sources of water pollution causing big environmental damage, economic destruction, and huge losses to marine biodiversity. The risk linked with oil spills increases a lot of policy issues on the safe transport of oil. Well-organized removal of these crude oil spills is a necessity for the protection of the marine environment and life. Both offshore and onshore oil response strategies together with continuous monitoring systems must be applied at the spill site to diminish the destructive impacts on the environment and human health. Currently used remediation technologies are still not effective to completely return the polluted aquatic environment to its normal ecology (Singh *et al.*, 2020). The oil spill pollution causes negative effects on both living organisms and human health. Some of the oil hydrocarbons including persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are carcinogenic and can be toxic even at low concentrations (Karan *et al*., 2011; Almeda *et al*., 2014).

Crude oil is a complex mixture of aliphatic, alicyclic, and aromatic hydrocarbons with a smaller percent of non-hydrocarbons compounds like naphthenic acids, thiols, phenols, sulfur compounds, heterocyclic nitrogen and metalloporphyrin's (Atlas and Bartha,1987). The non- hydrocarbons elements exist as components of complex molecules predominantly hydrocarbons in character, but containing small quantities of chromium, sulfur, nitrogen, oxygen, vanadium, and nickel. The hydrocarbons in crude oil are classified into three types: naphthalene, paraffin, and aromatics (Merian *et al*., 2004). Four major classes of hydrocarbons occur in crude oil depending on how atoms bind together in the molecules: Straight-chain alkanes (*n*-alkanes), cyclo-alkane, Branched alkanes, and Aromatics.

Water soluble fraction (WSF) of crude oil is that small fraction of oil containing components which are completely or reasonably soluble in water (Kavanu, 1964). WSF is

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formed during a long period of oil water contact (Baker, 1970). WSF of crude oil may be toxic to the living organisms in the aquatic environment. This is so because this fraction consists of toxic components such as the polycyclic aromatic hydrocarbons (PAHs), mono-aromatic hydrocarbons like benzene, toluene, ethylbenzene, xylene, phenols, and heterocyclic compounds (Rodrigues *et al.*, 2010). Many organisms can accumulate hydrocarbons in their lipid compartments (Mohammed, 2005; Nwabueze & Agbogidi, 2010). Polycyclic aromatic hydrocarbons, for example naphthalene are toxic, persistent, and carcinogenic (Preuss *et al.*, 2003). The sorption process is one of the easiest, efficient, and cost-effective methods for decreasing oil spill pollutions (Cheu *et al.*, 2016). Among the used sorbents, the most preferable sorbents are the biosorbents which are biodegradable, non- toxic, and low cost (Karan *et al*., 2011).

The biomasses of micro-and macroalgae have been reported as a suitable biosorbent for water decontamination polluted by different contaminants. The algal biomass contains numerous active sites of hydroxyl, carboxyl, and amine in their cell wall structure that are accessible for biosorption (Romera *et al.*, 2007). Lei *et al.* (2002) denoted that the removal of polycyclic aromatic hydrocarbons (PAHs) by *Selenastrum capricornutum* was the most effective while Chung *et al.* (2007) explored the effectiveness of consuming dried *Sargassum hemiphyllum* to eliminate aqueous phenanthrene (PHE). Akl *et al.* (2023) highlighted the role of marine algae in removing pollution resulting from oil spills. In the present study, we used dry marine alga *Ulva sp.* as biosorption of *n*-alkanes and polycyclic aromatic hydrocarbons (PAHs) to detect removal efficiency of this harmful pollution.

MATERIALS AND METHODS

Chemical materials

All chemicals and reagents were purchased from Sigma Aldrich and Merck chemicals. They were at analytical grade and used directly without further pretreatment.

Crude oil and water samples Collection

Crude oil and water samples were collected from the Egyptian Gulf Suez (Ras El-Adabya, Suez port) between May and July 2021(Figure 1). The used seawater samples for

culturing were collected in clean bottles from the same collecting areas. These samples were taken to the laboratory and followed by filtration and kept in the dark till being used.

Figure (1). Ras El-Adabya is located on the western shore of Suez Gulf (10 kilometers south of Suez city)

Algal samples collection, identification, and preparation

Marine algal species was collected from Alexandria (Abu Qir region) between May and July (2021). It is carefully washed several times with natural seawater and then with distilled water (2-3 times) to purify from salt and sand then collected in plastic bags and kept in an ice box at 20°C for further investigation.

Algal species was identified according to Aleem (1993) and Jha *et al.* (2009). The algal samples were air-dried (26–30 °C) with indirect light for 10 days followed by oven-drying for 48 h at 60 °C. Dried seaweed was crushed and powdered with a mixer grinder, and the powdered seaweed samples were stored in an airtight container for future use.

Physicochemical properties of sea water sample

The physicochemical parameters of the water sample were analyzed in Egyptian Petroleum Research Institute (EPRI). Density, specific gravity, pH, electrical conductivity (EC), resistivity, total dissolved solids (TDS), salinity, alkalinity, total hardness, cations (lithium, sodium, ammonium, potassium, magnesium, calcium, strontium and barium) and anions (fluoride, chloride, bromide, nitrate, nitrite, phosphate, sulfate, hydroxide, carbonate and bicarbonate) were analyzed according to Standard Test Methods such as Standard method (SM) and American Society for Testing and Materials, (ASTM).

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Physicochemical properties of crude oil sample

The physicochemical properties of the crude oil were estimated in Egyptian Petroleum Research Institute (EPRI) such as density, specific gravity, American Petroleum Institute gravity (°API gravity), pour point, kinematic viscosity, water content, ash content, flash point, sulfur content, saturated content, aromatic content, resin content and asphaltene content according to Standard Test Methods of ASTM.

Preparation and adsorption for water-soluble fractions (WSFs) of crude oil

Both preparation and adsorption were used according to method described by Boylan & Tripp (1971).

1. Preparation

One part of crude oil was mixed with 20 parts of seawater in a glass stoppered bottle. The mixture was stirred by a magnetic stirrer at low speed for 12 hours. The 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 various volumes of the medium yielded lower percentages of oil extract. Four concentrations of fresh WSF were used (25%, 50%, 75% and 100%) of crude oil and prepared by using seawater for dilution to keep all the WSF at the same solution constituents, the only difference is the concentration of the WSF.

2. Adsorption

The biosorption experiment was carried out using algal species as biosorbents. All experiments were conducted in 1 L beakers by adding 3g of dry algal biomass to 1 L of water-soluble fractions of crude oil (WSFs) of a specific concentration. The WSFs concentration is prepared by adequate dilution of its stock solution using seawater. The experiment occurred for 10 days, at different time intervals (2, 4, 6, 8, and 10 days) with aeration. The entire experiment was performed at natural light condition and room temperature (25±2°C) with three replicates.

Determination of *n*-alkanes and polycyclic aromatic hydrocarbons in water-soluble fraction

Analysis of the *n*-alkanes (light and heavy) and polycyclic aromatic hydrocarbons (PAHs) in WSF of crude oil was performed according to (Alkio *et al*., 2005). One liter of WSF from each flask was taken and extracted with dichloromethane (DCM) using a separatory funnel with a Teflon stopcock. Extraction was repeated three times. After the complete separation between the aqueous and organic phases, the organic phase passed through granular anhydrous sodium sulfate mounted in filter paper over the collection flask. The organic extracts were combined in a 100 ml Erlenmeyer flask. The extract was concentrated to about 1 ml using a rotary evaporator where the water bath temperature was maintained at around 40 °C, then, to the final evaporation (for drying) by using a gentle stream of clean and dry nitrogen. The extracted residue was re-solved in n-hexane in a GC vial fitted with a screw cap lined with Teflon, and the final volume of extract was adjusted at 1 ml to inject into Gas chromatograph (GC) (for *n*-alkanes and polycyclic aromatic hydrocarbons (PAHs) detection) at the optimum operating conditions.

Removal efficiency of crude oil by marine alga:

The removal capacity (RC) of crude oil was calculated as:

$$Rc = \frac{V_0 C_0 - V_f C_f}{\mathrm{m}}$$

And the removal percentage (R %) of crude oil is obtained as:

$$R\% = \frac{V_0 C_0 - V_f C_f}{V_0 C_0} x100$$

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

Gas chromatographic analysis (GC):

Normal (light and heavy) alkanes and polycyclic aromatic hydrocarbons (PAHs) in different concentrations of WSFs were analyzed using gas chromatograph equipped with flame ionization detector (FID) and nitrogen as a carrier gas (1 ml min^{-1}) (Model Thermo-

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scientific ISQ, 2009). TG-1MS column (100% dimethyl polydioxanone, 30 m length, 0.32 mm column ID, 0.25 µm film thickness) was used. The instrument was calibrated by injection of the standards component mixture at three different concentrations (1, 3, 5 ng/µl), for *n*-alkanes and PAHs prior to and between the analysis of each sample batch (Alkio *et al.*, 2005).

Both *n*-alkanes and PAHs were analyzed on the same chromatographic conditions of the column temperature program, where the temperature of the injection port was 250°C with spitless injection mode (3 min) then split mode with split ratio 1:10. The column temperature was programmed from 80 to 240°C with an increasing rate of 7°C min^{-1} and then to 300°C with rate of 3°C min^{-1}, and held for 5 min at 300°C. The FID temperature was 300°C.

Statistical analysis

Data obtained from different tests were analyzed by Analysis of Variance using General Linear Model (GLM) procedure within a package program of Statistical Analysis System Institute (1999). Means were separated by using Least Significant Difference (L.S.D) test at a degree of significance ($p \leq 0.05$).

RESULTS

The removal efficiency of marine macroalgae in removing water pollutants has received great attraction in the last decades due to their wide range of applications. In the present study the ability of *U. lactuca* to adsorb crude oil hydrocarbons from WSFs was investigated.

Algal samples identification:

The algae collected were identified as *Ulva lactuca* (Chlorophyta).

Physicochemical properties of sea water sample:

The physicochemical properties of the sea water sample were presented in Tables 1 and 2. Both density and specific gravity were approximately 1.04. The sea water sample was alkaline in nature and conductivity was 8 x 10^{-2} mohs/cm. Resistivity was equal to 0.125

Ohm-m, TDS was 63356.1 ppm, salinity was 58231.8 mg/l, alkalinity was 207.1 mg/l and total hardness was 12027.4 mg/l as shown in Table (1).

Table (1). Physical Properties of the sea water from Egyptian Gulf Suez (Ras El-Adabya, Suez port)

PHYSICAL PROPERTIES	RESULT	STANDARD TEST
Density (g / ml) at 60 F	1.04615	SM 2520 C
Specific gravity at 60 F	1.04719	ASTM D–1429
pH at 25 ℃	8.051	ASTM D–1293
Conductivity (mohs/cm) at 25 ℃ ,100 ^{o}C	8 x 10^{-2}	ASTM D–1125
Resistivity (Ohm-m) at 25 ℃	0.1250	ASTM D–1125
Total Dissolved Solids, TDS (ppm)	63356.1	SM 2540 C
Salinity (mg/l) as NaCl	58231.8	SM 2520 A
Alkalinity (mg/l) as $CaCO_3$	207.1	ASTM D–1067
Total Hardness (mg/l) as $CaCO_3$	12027.4	ASTM D–1126

The most cation abundant in the sea water was sodium (18890 mg / L) and the least abundant was lithium (0.07 mg / L); while the most anion abundant was chloride (35292 mg / L) and the lowest abundant was fluoride (243.25 mg / L). Some cations and anions were not detected as barium, nitrate, nitrite, phosphate, hydroxide, and carbonate as shown in Table (2).

Table (2). Inorganic Chemical Constituents of the sea water from Egyptian Gulf Suez (Ras El-Adabya Suez port).

CATION	MG/L	MEQ/L	ANION	MG/L	MEQ/L
Lithium	0.07	0.010	Fluoride	243.25	12.805
Sodium	18890.00	821.337	Chloride	35292.00	994.176
Ammonium	249.17	13.812	Bromide	1056.81	13.231
Potassium	776.34	19.859	Nitrate	Nil	Nil
Magnesium	2378.98	195.766	Nitrite	Nil	Nil
Calcium	893.36	44.579	Phosphate	Nil	Nil
Strontium	6.29	0.144	Sulfate	3317.29	69.099
Barium	Nil	Nil	Hydroxide	Nil	Nil
			Carbonate	Nil	Nil
			Bicarbonate	252.54	4.139

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Physicochemical properties of crude oil sample

Table (3) showed that physicochemical properties of crude oil sample. °API gravity was approximately 39.3. The saturate content was 60.17 wt. %, Aromatic content was 19.74 wt. %.

Table (3). Physicochemical properties of the crude oil

Physical Properties	**Result**	**Standard Test Method**
Density, (g /cm^3) at 15.56°C	0.8276	ASTM D–4052
Specific gravity, at 15.56°C	0.8285	
°API gravity	39.3	
Pour Point, °C	9	ASTM D–97
Kinematic Viscosity at 40°C, cSt (centi stock)	3.0830	ASTM D–445
Water Content, V/V%	0.01	ASTM D–95
Ash Content, wt. %	0.0829	ASTM D–482
Flash Point, °C	< -20	ASTM D–56
Chemical Properties	**Result**	**Standard Test Method**
Sulfur Content, wt. %	0.284	ASTM D–4294
Saturate Content, wt. %	60.17	-------
Aromatic Content, wt. %	19.74	-------
Resin Content, wt%	19.04	-------
Asphaltene Content, wt. %	1.05	-------

Oil adsorption at concentration of 25 % by *U. lactuca*

The effect of contact times (2,4,6,8, and 10 days) on the oil adsorption [*n*-alkanes (light or heavy) and PAHs] by dry *U. lactuca* at a concentration of 25 % WSFs. Results showed that these algae can be able to adsorb any kind of oil fractions and the best adsorption rate percentage was found after 10 days. The removal percentage was 31.2, 17.9 and 40.6% in the case of light *n*- alkanes, heavy *n*-alkanes and polycyclic aromatic hydrocarbons (PAHs), respectively. It is obvious the ability of *U. lactuca* to adsorb PAHs was more. There was a large significant decrease ($p \leq 0.05$) at each time until reach to 10th day, where the concentration of crude oil [*n*-alkanes (light or heavy) and PAHs] decreased on all as shown in Tables 4, 5 & 6.

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Table (4). Values of light *n*-alkanes (µg/l) in WSFs at 25 % crude oil concentrations after using dry *U. lactuca* as adsorbent at different periods of time.

INITIAL VALUE OF LIGHT *N*-ALKANES (MG/L)		**FINAL VALUE AFTER THE INCUBATION TIME (DAYS)**				
		2nd day	**4th day**	**6th day**	**8th day**	**10th day**
C10	4.55 ± 0.33	4.22± 0.71	3.75± 0.17	3.04±0.01	2.79±0.31	1.80±0.54
C11	3.62± 0.21	2.40±0.30	1.62±0.14	0.75± 0.05	0.64±0.54	0.52±0.21
C12	12.22± 0.41	9.25±0.60	8.12± 0.11	7.44±0.09	6.39± 0.42	6.01±0.31
C13	11.84± 0.65	10.56±0.40	9.32±0.19	8.12±0.03	7.63±0.13	6.66±0.64
C14	13.20± 0.21	12.40±0.80	11.48± 0.16	10.32± 0.12	9.45±0.07	8.88±0.47
C15	8.33±0.11	7.77±0.40	6.32±0.13	5.66± 0.16	4.01±0.05	3.26±0.44
C16	20.73± 0.16	20.43±0.60	19.15± 0.18	18.48± 0.17	17.70±0.01	16.12±0.33
C17	12.41± 0.22	11.50±0.12	10.80± 0.12	9.60±0.11	8.81± 0.12	7.95±0.15
Pristane	24.70± 0.34	23.82±0.74	22.70± 0.15	21.10±0.15	20.24± 0.54	19.18± 0.07
C18	27.66± 0.61	27.35± 0.63	26.91±0.01	25.30± 0.08	24.60± 0.74	23.56± 0.67
Phytane	22.25± 0.43	22.06±0.64	21.90± 0.05	20.14± 0.04	19.22± 0.35	18.70± 0.81
C19	10.32± 0.63	9.15±0.41	8.03±0.09	7.55±0.07	6.45± 0.04	5.03± 0.09
C20	17.77± 0.41	16.48±0.34	15.25± 0.07	14.05±0.24	13.40±0.06	12.80±0.20
Σ light *n*-alkanes	**189.60^{a}± 4.7**	**177.39^{b}±6.7**	**165.35^{c}±1.6**	**151.55^{d}±1.3**	**141.33^{e}±3.4**	**130.47^{f}±4.93**
Adsorption rate % (Removal percentage %)		6.40	12.80	20.10	25.50	**31.20**

Note: a, b, c, d, e and f are labels to indicate significant differences between groups.

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Table (5). Values of heavy *n*-alkanes (µg/l) in WSFs at 25 % crude oil concentrations after using dry *U. lactuca* as adsorbent at different periods of time.

INITIAL VALUE OF HEAVY N-ALKANES (MG/ L)		**FINAL VALUE AFTER THE INCUBATION TIME (DAYS)**				
		2nd day	**4th day**	**6th day**	**8th day**	**10th day**
C21	16.27± 0.50	16.20± 0.39	15.38±0.29	15.32± 0.29	14.31±0.25	14.12±0.18
C22	17.33±0.52	17.15±0.42	16.88± 0.35	16.77± 0.28	15.81± 0.36	15.77± 0.22
C23	15.66±0.67	15.40±0.55	14.91±0.38	14.89± 0.31	14.71± 0.26	14.69±0.19
C24	14.30±0.53	14.15±0.45	13.61±0.32	13.55± 0.25	13.00± 0.21	12.93±0.25
C25	11.92±0.49	10.54±0.42	10.03±0.41	9.91±0.25	9.32± 0.16	9.24±0.19
C26	11.15±0.52	10.38± 0.48	9.91±0.41	9.82± 0.18	9.03± 0.32	8.94±0.16
C27	7.15±0.62	7.01± 0.48	6.01±0.55	5.99± 0.36	5.96± 0.18	5.47±0.14
C28	17.02±0.55	16.91± 0.38	15.23±0.28	14.89± 0.22	14.80± 0.32	14.20± 0.15
C29	16.00±0.52	15.95±0.53	14.61±0.29	14.55± 0.18	14.28± 0.15	14.21±0.26
C30	4.88±0.21	4.61±0.33	3.38±0.43	2.34±0.51	1.90± 0.36	1.30±0.15
C31	16.44±0.48	15.83±0.38	14.90±0.14	14.87± 0.12	14.71± 0.09	13.64±0.05
C32	5.57±0.33	5.37±0.15	4.03± 0.16	3.94± 0.12	3.80± 0.12	3.61± 0.01
C33	5.63±0.33	5.22± 0.16	5.19± 0.15	5.12± 0.11	5.00± 0.09	4.91±0.07
C34	4.20±0.19	3.85± 0.15	3.16± 0.11	2.11± 0.16	1.91± 0.07	1.55± 0.02
C35	10.00± 0.32	9.77± 0.21	9.18± 0.09	8.15± 0.07	7.93± 0.11	7.89±0.05
C36	6.20±0.18	5.72± 0.14	5.36± 0.12	5.30± 0.11	5.16± 0.08	5.11±0.02
Σ Heavy *n*-alkanes	**179.72^{a}±6.96**	**174.06^{a}±5.6**	**161.77^{b}±4.5**	**157.52^{bc}±3.5**	**$151.63c^{d}$±3.1**	**147.58^{d}± 2.1**
Adsorption rate % (Removal percentage %)		3.10	10.00	12.40	15.60	**17.90**

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Table (6): Values of PAHs (µg/l) in WSFs at 25 % crude oil concentrations after using dry *U. lactuca* as adsorbent at different periods of time.

INITIAL VALUE OF PAHS (MG/L)		**FINAL VALUE AFTER THE INCUBATION TIME (DAYS)**				
		2nd day	**4th day**	**6th day**	**8th day**	**10th day**
Naphthalene (Nap)	0.76±0.01	0.69± 0.02	0.66±0.02	0.64±0.01	0.61±0.02	0.55±0.01
Acenaphthylene (Acy)	0.94±0.02	0.92±0.01	0.89±0.09	0.86±0.02	0.74±0.01	0.65±0.02
Acenaphthene (Ace)	0.63±0.01	0.61±0.07	0.59±0.01	0.56±0.06	0.51±0.07	0.49±0.05
Fluorene (Flr)	0.99±0.05	0.97±0.06	0.95±0.06	0.93±0.02	0.80±0.04	0.76±0.01
Phenanthrene (Phn)	0.63±0.03	0.62±0.07	0.59±0.01	0.56±0.03	0.52±0.02	0.48±0.04
Anthracene (Ant)	0.98±0.06	0.96±0.09	0.94± 0.05	0.92±0.01	0.83±0.04	0.78±0.02
Fluoranthene (Fla)	2.01±0.08	2.00±0.02	1.89±0.04	1.80±0.04	1.00±0.04	0.90±0.05
Pyrene (Pyr)	1.21±0.09	1.19±0.04	1.01±0.09	0.90±0.06	0.82±0.06	0.77±0.03
Benz[a]anthracene (BaA)	0.87±0.04	0.85±0.03	0.79±0.07	0.71±0.07	0.64±0.02	0.56±0.04
Chrysene (Chy)	1.99±0.08	1.97±0.07	1.42±0.01	1.39±0.06	1.30±0.06	1.22± 0.06
Benzo[b]fluoranthene (BbF)	2.38±0.07	2.00±0.06	1.93±0.03	1.90±0.07	1.81±0.03	1.50±0.01
Benzo[a]pyrene (BaP)	1.06±0.03	1.00±0.04	0.90±0.09	0.82±0.03	0.71±0.02	0.64±0.06
Indenol[1,2,3cd] pyrene (Inp)	2.52±0.04	2.41±0.07	2.14±0.03	1.01±0.07	0.90± 0.01	0.81±0.04
Dibenz[a,h]anthracene (DibA)	0.98±0.06	0.97±0.05	0.94±0.02	0.83±0.09	0.72±0.03	0.60±0.07
Benzo[ghi]perylene (Bghip)	0.78±0.03	0.76±0.07	0.66±0.07	0.57±0.04	0.48±0.04	0.42±0.03
Σ PAHs	**18.73^{a}±0.7**	**17.92^{a}±0.8**	**16.30^{b}±0.7**	**14.40^{c}±0.7**	**12.39^{d}±0.6**	**11.13^{e}±0.6**
Adsorption rate % (Removal percentage %)		4.30	13.00	23.10	33.90	**40.60**

Note: Each result represented the mean of triplicate experiments ± SD (n=3), Means within the same row with the same superscript letters are not significantly different at $p \leq 0.05$.

Oil adsorption at concentration of 50 % by *U. lactuca*

Tables 7, 8 & 9 represented the effect of contact times (2, 4, 6, 8 and 10 days) on the oil adsorption [*n*-alkanes (light or heavy) and PAHs] by dry *U. lactuca* at a concentration of 50 % WSFs. The removal percentage was 21.0, 9.7 and 39.3% in the case of light *n*- alkanes, heavy *n*-alkanes, and PAHs, respectively. It is obvious the ability of *U. lactuca* to adsorption was decreased by increased crude oil concentrations.

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Table (7). Values of light *n*-alkanes (µg/l) in WSFs at 50 % crude oil concentrations after using dry *U. lactuca* as adsorbent at different periods of time.

INITIAL VALUE OF LIGHT *N*-ALKANES (MG/ L)		**FINAL VALUE AFTER THE INCUBATION TIME (DAYS)**				
		2nd day	**4th day**	**6th day**	**8th day**	**10th day**
C10	10.80± 0.33	9.80± 0.71	8.00± 0.17	6.37± 0.01	5.00± 0.31	3.00± 0.54
C11	8.30± 0.21	7.30± 0.30	6.20± 0.14	4.00± 0.05	3.12± 0.64	1.80± 0.21
C12	24.89± 0.41	23.70±0.60	22.00±0.11	21.00±0.09	19.00± 0.42	17.35± 0.31
C13	24.00± 0.65	23.90±0.40	23.00± 0.19	21.19± 0.03	19.61± 0.13	18.53±0.64
C14	27.90±0.21	26.40± 0.80	25.10± 0.16	24.16±0.12	23.73±0.07	22.00± 0.47
C15	14.90± 0.11	13.85± 0.40	12.90±0.13	11.00± 0.16	10.25±0.05	8.63± 0.44
C16	38.80± 0.16	37.30± 0.60	36.00± 0.18	35.01±0.17	34.00± 0.01	31.90± 0.33
C17	22.10± 0.22	21.00±0.12	19.98±0.12	18.90± 0.11	17.12±0.12	16.00± 0.15
Pristane	56.00± 0.34	55.40± 0.74	55.10±0.15	54.80± 0.15	52.01±0.54	51.30± 0.07
C18	56.80±0.610	56.20± 0.63	55.00±0.01	54.90±0.08	51.00± 0.74	50.09± 0.67
Phytane	36.99± 0.43	35.66± 0.64	35.60±0.05	34.30±0.04	33.80± 0.35	31.10± 0.81
C19	20.19± 0.63	20.00±0.41	19.40± 0.09	18.20± 0.07	16.90± 0.04	14.01± 0.09
C20	37.88± 0.41	37.60± 0.34	36.32± 0.07	35.00± 0.24	34.86±0.06	34.00± 0.20
Σ light *n*-alkanes	**379.55^{a}±4.8**	**368.11^{b}±6.7**	**354.60^{c}±1.6**	**338.83^{d}±1.3**	**320.40^{e}±3.5**	**299.71^{f}± 4.93**
Adsorption rate % (Removal percentage %)		3.00	6.60	10.70	15.60	**21.00**

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Table (8). Values of heavy *n*-alkanes (µg/l) in WSFs at 50 % crude oil concentrations after using dry *U. lactuca* as adsorbent at different periods of time.

INITIAL VALUE OF HEAVY *N*-ALKANES (MG/L)		**FINAL VALUE AFTER THE INCUBATION TIME (DAYS)**				
		2nd day	**4th day**	**6th day**	**8th day**	**10th day**
C21	32.95 ± 0.43	31.94 ±0.04	31.90 ±0.46	29.99 ±0.65	29.80 ±0.09	29.77 ±0.01
C22	33.29±0.34	33.28 ±0.63	32.28 ±0.07	31.00 ±0.41	30.03 ±0.68	29.73 ±0.19
C23	31.60±0.25	31.40 ±0.14	31.00 ±0.35	30.28 ±0.34	29.05 ±0.58	29.00 ±0.45
C24	27.35±0.62	27.32 ±0.06	26.300±0.06	24.11 ±0.08	24.00 ±0.74	23.98 ±0.61
C25	23.86±0.41	22.84 ±0.32	21.25 ±0.05	20.25 ±0.63	20.00 ±0.10	19.79 ±0.05
C26	21.63± 0.70	21.60 ±0.19	21.40 ±0.25	21.21 ±0.04	20.03 ±0.07	19.00 ±.31
C27	14.99±0.05	14.96 ±0.17	14.90 ± 0.07	14.87 ±0.81	14.80 ±0.03	14.79 ±0.04
C28	35.13±0.03	34.11 ±0.05	34.90 ±0.04	34.80 ±0.06	33.06 ±0.39	33.00 ± 0.11
C29	31.35±0.54	31.32 ±0.41	31.29 ±0.55	31.01 ±0.61	30.59 ±0.83	30.5 ± 0.18
C30	8.54±0.61	8.44 ±0.01	8.38 ±0.31	8.30 ±0,.37	8.13 ±.0.15	8.10 ± 0.50
C31	33.19±0.45	33.16 ±0.02	33.10 ±0.17	33.60 ±0.46	32.40 ±0.12	32.10 ± 0.36
C32	11.45±0.53	11.42±0.33	11.33 ±0.02	11.22 ±0.54	10.60 ±0.04	9.80 ±0.41
C33	11.00±0.05	10.98±0.61	10.92 ±0.48	10.89 ±0.60	10.22 ±0.61	9.94 ±0.06
C34	10.00 ±0.01	9.98 ±0.03	8.96 ±0.37	8.91 ±0.64	8.37 ±0.57	7.32 ±0.36
C35	21.00±0.06	20.89±0.14	19.81 ±0.61	18.40 ±0.15	18.20 ±0.05	17.18 ±0.21
C36	11.88± 0.07	11.86 ±0.07	10.78 ±0.14	10.66 ±0.03	10.22 ± 0.30	10.19 ±0.07
Σ heavy *n*-alkanes	**359.21^{a}±5.2**	**355.50^{ab} ±3.2**	**348.50^{b}±4.0**	**339.50^{c} ±6.42**	**329.50^{d} ±5.4**	**324.21^{d} ±3.92**
Adsorption rate % (Removal percentage %)		1.00	3.00	5.50	8.30	**9.70**

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Table (9). Values of PAHs (µg/l) in WSFs at 50 % crude oil concentrations after using dry *U. lactuca* as adsorbent at different periods of time.

INITIAL VALUE OF PAHS (MG/L)		FINAL VALUE AFTER THE INCUBATION TIME (DAYS)				
		2nd day	**4th day**	**6th day**	**8thday**	**10th day**
Nap	1.44 ± 0.06	1.43±0.04	1.39±0.03	1.20±0.06	1.00±0.01	0.90±0.01
Acy	3.36± 0.05	3.35±0.03	3.30±0.05	3.19±0.01	3.00±0.06	2.80±0.01
Ace	0.89±0.02	0.89±0.09	0.76±0.01	0.66±0.02	0.50±0.01	0.30±0.01
Flr	4.82± 0.04	4.81±0.03	4.77±0.06	4.14±0.07	4.00±0.05	3.83±0.03
Phn	0.89± 0.01	0.87±0.02	0.80±0.04	0.71±0.03	0.68±0.04	0.51±0.02
Ant	0.98±0.03	0.96±0.04	0.91±0.02	0.86±0.01	0.71±0.08	0.66±0.07
Fla	4.40±0.01	4.30±0.01	4.00±0.09	3.75±0.09	3.15±0.08	3.00±0.06
Pyr	6.00± 0.07	5.99±0.02	5.69±0.08	5.51±0.02	4.26±0.04	4.00±0.05
BaA	5.66±0.06	5.65±0.05	5.61±0.07	5.50±0.07	4.12±0.09	3.01±0.04
Chy	6.08±0.03	5.00±0.04	4.39±0.02	3.63±0.05	3.51±0.04	2.47±0.03
BbF	6.55±0.06	6.54±0.01	6.00±0.05	4.93±0.06	4.05±0.07	3.01±0.08
BaP	2.89± 0.04	2.87±0.01	2.80±0.07	2.71±0.04	2.50±0.06	2.09±0.09
Inp	8.09±0.01	7.41±0.08	6.21±0.08	5.14±0.04	5.60±0.06	5.00±0.03
DibA	2.88± 0.04	2.87±0.03	2.40±0.06	2.22±0.06	2.14±0.04	2.01±0.08
Bghip	1.38± 0.04	1.37±0.01	1.28±0.08	1.16±0.02	1.09±0.02	0.57±0.02
Σ PAHs	**56.31^{a}± 0.57**	**54.31^{b}±0.51**	**50.31^{c}±0.81**	**45.31^{d}±0.65**	**40.31^{e}±0.75**	**34.16^{f}±0.63**
Adsorption rate % (Removal percentage %)		3.6	10.7	19.5	28.4	**39.3**

Note: Each result represented the mean of triplicate experiments ± SD (n=3), Means within the same row with the same superscript letters are not significantly different at $p \leq 0.05$.

Oil adsorption at concentration of 75 % by *U. lactuca*

Tables 10,11 & 12 represented the effect of contact times (2, 4, 6, 8 and 10 days) on the oil adsorption [*n*-alkanes (light or heavy) and PAHs] by dry *U. lactuca* at a concentration of 75 % WSFs. Results show that the best adsorption rate percentage of *U. lactuca* was found after 10 days. The removal percentage was 19.1, 9.0 and 25.1% in the case of light *n*-alkanes, heavy *n*-alkanes, and PAHs, respectively. A similar effect of contact time on the adsorption functions of *U. lactuca* towards light *n*-alkanes was observed. An inverse relationship appears between the concentration of crude oil and the ability of *U. lactuca* to absorb it.

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Table (10). Values of light *n*-alkanes (µg/l) in WSFs at 75 % crude oil concentrations after using dry *U. lactuca* as adsorbent at different periods of time.

INITIAL VALUE OF LIGHT *N*-ALKANES (MG/L)		**FINAL VALUE AFTER THE INCUBATION TIME (DAYS)**				
		2nd day	**4th day**	**6th day**	**8th day**	**10th day**
C10	16.66± 0.33	15.00±0.71	14.00±0.17	13.00±0.01	12.6±0.31	10.60±0.54
C11	12.30 ± 0.21	12.00±0.30	11.00±0.14	9.30±0.05	8.20±0.64	6.00±0.21
C12	36.98 ± 0.41	35.60±0.60	34.00±0.11	32.00±0.09	30.70±0.42	28.00±0.31
C13	38.00±0.65	37.00±0.40	36.00±0.19	34.00±0.03	32.00±0.13	30.00± 0.64
C14	46.60 ± 0.21	45.00±0.80	44.00±0.16	42.00±0.12	40.00±0.07	38.00± 0.47
C15	34.00 ±0.11	32.00±0.40	31.00±0.13	30.00±0.16	29.00±0.05	26.00±0.44
C16	58.66±0.16	57.00±0.60	56.00±0.18	55.00±0.17	53.00±0.01	51.00±0.33
C17	45.10± 0.22	44.00±0.12	43.00±0.12	43.00±0.11	41.00±0.12	39.00±0.15
Pristane	576.80± 0.34	76.00± 0.74	73.00±0.15	72.30±0.15	69.00±0.54	65.18±0.07
C18	87.50± 0.61	87.00±0.63	85.00±0.01	84.00±0.08	81.00±0.74	77.88± 0.67
Phytane	66.00 ± 0.43	65.00±0.64	63.00±0.05	60.00±0.04	57.00±0.35	53.00±0.81
C19	33.00± 0.63	33.00±0.41	31.00±0.09	29.00±0.07	26.00±0.04	24.00±0.09
C20	18.00± 0.41	17.00±0.34	16.60±0.07	14.00± 0.24	13.00±0.06	12.00±0.20
Σ light *n*-alkanes	**569.60^{a}± 4.7**	**555.60^{b}±6.7**	**537.60^{c}±1.6**	**517.60^{d}±1.3**	**492.50^{e}±3.5**	**460.66^{f}± 4.93**
Adsorption rate % (Removal percentage %)		2.50	5.60	9.10	13.50	**19.10**

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Table (11). Values of heavy *n*-alkanes (µg/l) in WSFs at 75 % crude oil concentrations after using dry *U. lactuca* as adsorbent at different periods of time.

INITIAL VALUE OF HEAVY *N*-ALKANES (MG/L)		**FINAL VALUE AFTER THE INCUBATION TIME (DAYS)**				
		2nd day	**4th day**	**6th day**	**8th day**	**10th day**
C21	47.25± 0.43	45.92±0.04	44.01±0.46	43.00±0.65	42.40±0.09	41.89±0.01
C22	50.30±0.34	49.00±0.63	48.22±0.07	48.00±0.41	47.09±0.68	46.80±0.19
C23	51.00± 0.25	50.94±0.14	50.91±0.35	49.00±0.34	48.30±0.58	48.19±0.45
C24	46.00± 0.62	45.90± 0.06	45.88±0.06	44.01±0.08	43.15±0.74	42.60±0.61
C25	37.11± 0.41	37.09± 0.32	37.00± 0.05	36.27±0.63	36.20±0.10	35.12±0.05
C26	32.15± 0.70	32.07±0.19	31.94±0.25	30.90±0.04	29.10±0.07	28.00±0.31
C27	20.99± 0.05	20.89±0.17	19.82±0.07	19.44±0.81	19.00±0.03	17.26±0.04
C28	53.01± 0.03	53.00±0.05	52.15±0.04	51.60±0.06	50.60±0.39	48.26±0.11
C29	46.06± 0.54	45.01±0.41	44.96±0.55	44.90±0.61	43.96±0.83	43.06±0.18
C30	13.58± 0.61	13.51±0.01	13.45±0.31	13.15±0.37	12.01± 0.15	11.66±0.50
C31	48.84± 0.45	47.80±0.02	46.00±0.17	45.80± 0.46	44.91±0.12	44.82±0.36
C32	15.20±0.53	15.00±0.33	14.88±0.02	14.25±0.54	13.81±0.04	12.11±0.41
C33	12.56± 0.05	12.54±0.61	12.41±0.48	12.06±0.60	11.90±0.61	10.96±0.06
C34	11.56± 0.01	11.53±0.03	11.47±0.37	11.03±0.64	10.38±0.57	10.00±0.36
C35	32.76±0.06	32.61±0.14	32.56±0.61	32.41±0.15	31.16±0.05	30.30±0.21
C36	19.00± 0.07	18.56±0.07	17.71±0.14	17.55±0.03	17.49±0.30	17.34±0.07
Σ heavy *n*-alkanes	**537.37^{a}±5.2**	**$531.37a^{b}$±3.2**	**523.37^{b}±4.0**	**513.37^{c}±6.4**	**501.46^{d}±5.4**	**488.37^{e}±3.9**
Adsorption rate % (Removal percentage %)		1.10	2.60	4.50	6.70	**9.10**

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Table (12): Values of PAHs (µg/l) in WSFs at 75 % crude oil concentrations after using dry *U. lactuca* as adsorbent at different periods of time.

INITIAL VALUE OF PAHS (MG/L)		**FINAL VALUE AFTER THE INCUBATION TIME (DAYS)**				
		2nd day	**4th day**	**6th day**	**8th day**	**10th day**
Nap	3.74±0.06	3.73±0.04	3.61±0.03	3.59±0.06	2.51±0.01	2.39±0.01
Acy	2.89±0.05	2.89±0.03	2.70±0.05	2.69±0.01	2.61±0.06	2.40±0.01
Ace	1.60±0.02	1.50±0.09	1.30±0.01	1.10±0.02	1.08±0.01	0.80±0.01
Flr	3.82±0.04	3.81±0.03	3.70±0.06	3.60±0.07	3.59±0.05	3.12±0.03
Phn	0.96±0.01	0.95±0.02	0.90±0.04	0.88±0.03	0.87±0.04	0.77±0.02
Ant	1.12±0.03	1.12±0.04	1.60±0.02	1.30±0.01	1.10±0.08	1.00±0.07
Fla	7.08±0.01	6.07±0.01	5.00±0.09	4.30±0.09	4.12±0.08	4.00±0.06
Pyr	8.20±0.07	7.89±0.02	7.19±0.08	7.80±0.02	7.30±0.04	6.30±0.05
BaA	3.90±0.06	3.80±0.05	3.60±0.07	3.20±0.07	3.00±0.09	2.96±0.04
Chy	8.20±0.03	8.10±0.04	8.00±0.02	7.20±0.05	6.40±0.04	6.33±0.03
BbF	7.90±0.06	7.70±0.01	7.50±0.05	7.30±0.06	7.00±0.07	6.48±0.08
BaP	4.10±0.04	4.00±0.01	3.90±0.07	3.20±0.04	3.00±0.06	2.96±0.09
Inp	10.50±0.01	10.48±0.08	10.08±0.08	10.00±0.04	9.88±0.06	8.30±0.03
DibA	5.32±0.04	5.31±0.03	5.29±0.06	5.25±0.06	5.23±0.04	4.90±0.08
Bghip	3.88±0.04	3.86±0.01	3.84±0.08	3.80±0.02	3.60±0.02	2.10±0.02
Σ PAHs	**73.21^{a}±0.57**	**71.21^{b}±0.51**	**68.21^{c}±0.81**	**65.21^{d}±0.65**	**61.29^{e}±0.75**	**54.81^{f}±0.63**
Adsorption rate % (Removal percentage %)		2.70	6.80	11.00	16.30	**25.10**

Note: Each result represented the mean of triplicate experiments ± SD (n=3), Means within the same row with the same superscript letters are not significantly different at $p \leq 0.05$.

Oil adsorption at concentration of 100 % by *U. lactuca*

Tables 13, 14 & 15 represented the effect of contact times (2, 4, 6, 8 and 10 days) on the oil adsorption [*n*-alkanes (light or heavy) and PAHs] by dry *U. lactuca* at a concentration of 100 % WSFs. Results showed that the effect of contact time had a great influence on the adsorption functions of *U. lactuca* as in other concentrations. *U. lactuca* was able to adsorb any kind of oil fractions and the best adsorption rate percentage was found after 10 days. The removal percentage was 12.10, 5.6 and 19.0% in the case of light *n*-alkanes, heavy *n*-alkanes, and PAHs, respectively. The ability of *U. lactuca* to adsorption decreased by increased crude oil concentrations.

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Table (13). Values of light *n*-alkanes (µg/l) in WSFs at 100 % crude oil concentrations after using dry *U. lactuca* as adsorbent at different periods of time

INITIAL VALUE OF LIGHT *N*-ALKANES (MG/L)		**FINAL VALUE AFTER THE INCUBATION TIME (DAYS)**				
		2nd day	**4th day**	**6th day**	**8th day**	**10th day**
C10	18.80±0.33	17.12±0.71	16.26±0.17	15.22±0.01	14.89±0.31	13.85±0.54
C11	22.73±0.21	22.12±0.30	21.87±0.14	20.01±0.05	19.57±0.64	17.90±0.21
C12	45.00±0.41	44.01±0.60	43.90±0.11	42.00±0.09	41.00±0.42	37.60±0.31
C13	45.63±0.65	45.13±0.40	44.00±0.19	43.30±0.03	42.00±0.13	39.16±0.64
C14	54.12±0.21	54.00±0.80	52.00±0.16	51.60±0.12	50.49±0.07	47.97±0.47
C15	30.12± 0.11	29.09±0.40	28.60±0.13	27.52±0.16	25.56±0.05	24.00±0.44
C16	78.80± 0.16	78.00±0.60	76.00±0.18	75.00±0.17	73.19±0.01	72.60±0.33
C17	48.74± 0.22	48.00±0.12	47.15±0.12	47.00±0.11	46.00±0.12	45.30±0.15
Pristane	100.80±0.34	99.66±0.74	97.12±0.15	96.01±0.15	95.00±0.54	93.60±0.07
C18	111.36± 0.61	110.67±0.63	109.00±0.01	108.9±0.08	106.45±0.74	103.00±0.67
Phytane	80.20± 0.43	79.00±0.64	78.30±0.05	74.24±0.04	71.00± 0.35	69.15±0.81
C19	43.30± 0.63	43.00±0.41	42.60±0.09	39.20±0.07	36.59± 0.04	34.89±0.09
C20	79.16± 0.41	78.60±0.34	76.60±0.07	73.40±0.24	71.66±0.06	68.04±0.20
Σ light *n*-alkanes	**758.76^{a}± 4.7**	**748.40^{b}±6.7**	**733.40^{c}±1.6**	**713.40^{d}±1.3**	**693.40^{e}±3.5**	**667.06^{f}±4.93**
Adsorption rate % (Removal percentage %)		1.40	3.30	6.00	8.60	**12.10**

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Table (14). Values of heavy *n*- alkanes (µg/l) in WSFs at 100 % crude oil concentrations after using dry *U. lactuca* as adsorbent at different periods of time.

INITIAL VALUE OF HEAVY *N*-ALKANES (MG/L)		**FINAL VALUE AFTER THE INCUBATION TIME (DAYS)**				
		2nd day	**4th day**	**6th day**	**8th day**	**10th day**
C21	55.50 ± 0.43	54.30±0.04	53.90± 0.46	53.80±0.65	53.00±0.09	52.60±0.01
C22	60.03± 0.34	59.00±0.63	58.88± 0.07	58.79±0.41	58.00±0.68	57.40±0.19
C23	72.90± 0.25	71.88±0.14	71.70±0.35	71.69±0.34	71.00±0.58	70.55±0.45
C24	66.12±0.62	65.10± 0.06	65.00± 0.06	64.10±0.08	62.00±0.74	61.81±0.61
C25	44.36±0.41	44.30± 0.32	43.89± 0.05	42.48±0.63	42.40± 0.10	42.22±0.05
C26	50.72± 0.70	50.68±0.19	49.76±0.25	48.41±0.04	48.00±0.07	47.16±0.31
C27	28.00± 0.05	27.94±0.17	27.06±0.07	27.00±0.81	26.00± 0.03	25.60±0.04
C28	68.52±0.03	68.50±0.05	68.00±0.04	67.00±0.06	66.00± 0.39	65.80±0.11
C29	70.90±0.54	70.87±0.41	70.81±0.55	70.72±0.61	70.00±0.83	69.17±0.18
C30	18.98±0.61	18.95±0.01	18.00±0.31	17.85±0.37	17.78±0.15	17.65±0.50
C31	12.03±0.45	12.00±0.02	11.66±0.17	11.61±0.46	11.44±0.12	11.21±0.36
C32	14.77± 0.53	14.75±0.33	14.70±0.02	14.53±0.54	13.44±0.04	13.39±0.41
C33	26.00±0.05	25.80±0.61	25.77±0.48	25.60±0.60	25.00±0.61	24.33±0.06
C34	61.00± 0.01	60.91±0.03	60.00±0.37	59.00±0.64	58.00±0.57	57.68±0.36
C35	38.33± 0.06	38.21±0.14	38.00±0.61	37.55±0.15	36.00±0.05	35.81±0.21
C36	29.97±0.07	29.94±0.07	29.00±0.14	27.00±0.03	26.00±0.30	25.45±0.07
Σ heavy *n*-alkanes	**718.13^{a}±5.2**	**713.13^{ab}±3.2**	**706.13^{b}±4.0**	**697.13^{c}±6.4**	**684.06^{d}±5.4**	**677.83^{d}±3.9**
Adsorption rate % (Removal percentage %)		0.70	1.70	3.00	4.70	**5.60**

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Table (15): Values of PAHs (µg/l) in WSFs at 100 % crude oil concentrations after using dry *U. lactuca* as adsorbent at different periods of time.

INITIAL VALUE OF PAHS (MG/L)		FINAL VALUE AFTER THE INCUBATION TIME (DAYS)				
		2nd day	**4th day**	**6th day**	**8th day**	**10th day**
Nap	0.87 ±0.06	0.86 ±0.04	0.81 ± 0.03	0.80 ± 0.06	0.60 ± 0.01	0.50 ±0.01
Acy	0.97±0.05	0.96 ± 0.03	0.95 ± 0.05	0.94± 0.01	0.80 ± 0.06	0.70 ±0.01
Ace	0.90±0.02	0.90 ± 0.09	0.89 ±0.01	0.87 ± 0.02	0.80 ± 0.01	0.70 ±0.01
Flr	1.92±0.04	1.92 ± 0.03	1.69 ±0.06	1.26 ± 0.07	1.00 ± 0.05	0.80 ±0.03
Phn	0.66 ±0.01	0.66 ±0.02	0.65 ±0.04	0.63 ± 0.03	0.55 ± 0.04	0.45 ±0.02
Ant	0.65 ±0.03	0.65 ±0.04	0.64 ±0.02	0.62 ± 0.01	0.60 ± 0.08	0.55 ±0.07
Fla	3.75±0.01	3.62 ±0.01	3.61±0.09	3.60 ± 0.09	3.50 ± 0.08	3.46 ±0.06
Pyr	2.10±0.07	2.00 ±0.02	1.90 ±0.08	1.80 ± 0.02	1.22 ± 0.04	1.16 ±0.05
BaA	2.88±0.06	2.88 ± 0.05	2.87 ± 0.07	2.86 ± 0.07	2.81 ± 0.09	2.77 ±0.04
Chy	4.98±0.03	4.98 ±0.04	4.97 ± 0.02	4.95 ± 0.05	4.90 ±0.04	4.70 ± 0.03
BbF	5.77±0.06	5.66 ±0.01	4.65 ±0.05	4.64 ± 0.06	4.61 ±0.07	4.51 ±0.08
BaP	2.88±0.04	2.88 ±0.01	2.87 ±0.07	2.83 ± 0.04	2.82 ±0.06	2.52 ±0.09
Inp	5.36±0.01	5.36 ± 0.08	5.35 ±0.08	4.65 ±0.04	4.64 ±0.06	4.34 ±0.03
DibA	2.90±0.04	2.90 ±0.03	2.90 ±0.06	2.70 ±0.06	2.66 ±0.04	2.57 ±0.08
Bghip	0.94±0.04	0.90 ±0.01	0.89 ± 0.08	0.89 ±0.02	0.83 ±0.02	0.700 ±0.02
Σ PAHs	**37.53[a]± 0.57**	**37.13[a] ±0.51**	**35.64[b] ±0.81**	**34.04[c] ±0.65**	**32.34[d]±0.75**	**30.43[e] ±0.63**
Adsorption rate % (Removal percentage %)		1.10	5.00	9.30	13.80	**19.00**

Note: Each result represented the mean of triplicate experiments ± SD (n=3), Means within the same row with the same superscript letters are not significantly different at $p \leq 0.05$.

Removal efficiency of dry *U. lactuca* for WSFs of crude oil

The removal capacity refers to the efficiency of removal for a unit weight of the biosorbent 1 gram of dry matter. Table 16 and Figure 2 showed the removal efficiency of the crude petroleum oil by dry *U. lactuca*. In general, the removal capacity and the removal percentage of total light *n*-alkanes, heavy *n*-alkanes and PAHs increased with increasing of contact time. The highest removal efficiency was achieved to the maximum after 10 days for all hydrocarbons for all four concentrations of WSFs.

The removal capacity by the algae was 19.71, 10.71 and 2.53 µg/g for total light *n*-alkanes, heavy *n*-alkanes, and PAHs, respectively with removal percentages of 31.18, 21.04

,19.12%. It reached the optimum value at a concentration of 25%. The best removal percentages for different hydrocarbon groups were for PAHs.

The removal percentage started to decrease for total light *n*-alkanes, and heavy *n*-alkanes from 25% with values of 31.20 and 17.90% till reached 100% with values of 12.10 and 5.60%. While the removal percentage of total PAHs decreased from the conc. at 25% with a value of 40.57% to reach the conc. at 50% with a value of 39.3%, 75% with a value of 25.1% and decreased finally to the conc.100% with a value of 19.0 %.

Petroleum hydrocarbon adsorption by this algal species increased with an increase in contact time at all concentrations of WSFs reach maximum at 10 days. *U. lactuca* can adsorb PAHs (removal percentage 40.57 %) better than light *n*- alkenes (removal percentage 31.18 %) and heavy *n*-alkenes (removal percentage 17.88 %).

Table (16). Removal capacity and removal percentage at different concentrations of petroleum hydrocarbons (WSFs) using dry *U. lactuca*

HYDROCARBONS	WSFS CONCENTRATIONS %	TIME (DAYS)									
		2nd		4th		6th		8th		10th	
		Removal capacity (µg/g)	Removal percentage (%)	Removal capacity (µg/g)	Removal percentage (%)	Removal capacity (µg/g)	Removal percentage (%)	Removal capacity (µg/g)	Removal percentage (%)	Removal capacity (µg/g)	Removal percentage (%)
Total light *n*-alkanes	25	4.07	6.43	8.08	12.79	12.68	20.06	16.09	25.45	19.71	**31.18**
	50	3.81	3.01	8.31	6.57	13.57	10.73	19.71	15.58	26.61	21.04
	75	4.66	2.45	10.66	5.61	17.33	9.12	25.70	13.53	36.31	19.12
	100	3.45	1.37	8.45	3.34	15.12	5.97	21.78	8.61	30.57	12.08
Total heavy *n*-alkanes	25	1.89	3.15	5.98	9.98	7.40	12.35	9.36	15.62	10.71	**17.88**
	50	1.24	1.03	3.57	2.98	6.57	5.48	9.90	8.27	11.66	9.74
	75	2.00	1.11	4.77	2.60	8.00	4.46	11.97	6.68	16.33	9.11
	100	1.77	0.69	4.00	1.67	7.00	2.92	11.36	4.74	13.43	5.61
Total PAHs	25	0.27	4.32	0.81	12.97	1.44	23.11	2.11	33.85	2.53	**40.57**
	50	0.77	3.55	2.00	10.65	3.77	19.53	5.33	28.41	7.48	39.33
	75	0.77	2.73	1.66	6.82	2.77	10.92	3.97	16.28	6.13	25.13
	100	0.13	1.06	0.63	5.03	1.16	9.29	1.73	13.82	2.36	19.00
		Removal Capacity (µg/g)				**Removal Percentage (%)**					

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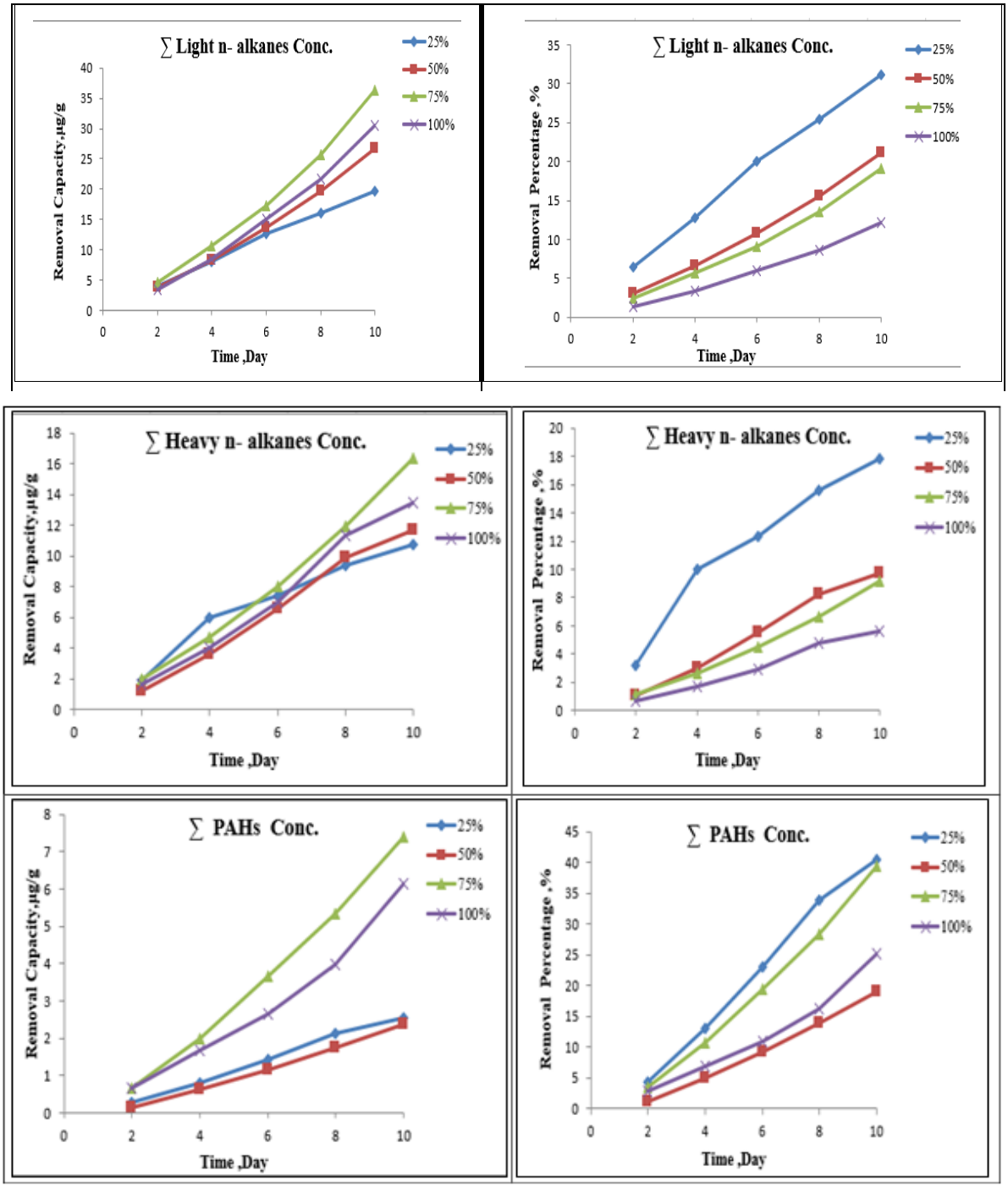

Figure (2). Removal capacity and removal percentage at different concentrations of petroleum hydrocarbons (WSFs) using dry *U. lactuca.*

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During the last two decades, wide attention has been paid to the management of environmental contamination caused by petrochemicals. Some methods have been developed for the elimination of such substances as precipitation, evaporation, ion exchange, etc. However, these methods have numerous disadvantages. Algae can be used to decrease the amount of toxic chemicals required to clean and purify water. Algae either accumulate, adsorb, or metabolize these toxic elements into substantial levels (Ibraheem, 2010; Henriques *et al.,* 2017)**.** This study highlights the alternative biological agent richly present in nature as algae for the elimination of such toxic substances from the surroundings.

Adsorption of hydrocarbons by the algal biomass cell wall is a passive process driven by chemical breakdown 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).

Crude oil constituent's emulsification naturally in water occurs because of the presence of surface-active substances, which is related to the energy state of water. So, crude oil may exist in various forms and fractions in water, one of them is aqueous extract (Tukaj, 1987).

The present results show that the dry alga *U. lactuca* can be used in the removal of light *n*-alkanes (from C10 to C 20), heavy *n*-alkanes (from C21 to C36) (Neşer *et al*., 2012) and PAHs from WSFs. This result was consistent with that of Tam *et al*. (2002) who found that dry algae could adsorb hydrocarbons easily. Similarly, Voskoboinikov *et al*. (2021) found that *Fucus vesiculosus* dry algal biomass could absorb hydrocarbons. Algal biomass has removed hydrocarbons in water treatment facilities (Ahmad *et al.,* 2022; Ghodrati *et al.,* 2022).

Higher initial concentrations of sorbate (crude oil) resulted in a higher probability of collision, thus higher uptake by the biomass (Noeline *et al*., 2005; Chung *et al*. 2007). These findings did not agree with the present results which proved that as the concentrations of crude oil increased the removal percentages decreased.

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The sorption process is limited by the sorbent capacity in which the number of sorption sites is a determinant parameter. At lower initial oil concentrations, more amounts of oil compounds could have interacted with the binding sites (Chowdhury *et al.,* 2009). Whereas at higher concentrations, the adsorption percentage was reduced or became constant due to the saturation of sorbents (Lim & Huang, 2007). Although oil compounds with lower viscosity such as crude oil penetrate more deeply within the pores, they are easily released from the sorbents during adsorption, so the oil adsorption is reduced after saturation of the sorbents (Teas *et al.*, 2001).

It is clear from the present results that hydrocarbon adsorption of the selected algal species *U. lactuca* increased with the increase of contact time until 10 days. These results were in harmony with Gamila & Ibrahim (2004) who found that *Scenedesmus obliquus* showed high removal percentage of crude oil which increased with the exposure time. A similar trend was also recorded by Razavi *et al.* (2015) who found that the removal of oil samples by sorbents was rapid, occurring in the first 5–10 min. Oil uptake may be attributed to the presence of vacant voids on the sorbents surface. However, after a lapse of time, less adsorption sites were available to be occupied (Ibrahim *et al*., 2010).

The adsorption of light *n*-alkanes, heavy *n*-alkanes, and PAHs by *U. lactuca* dry biomass at different time intervals of 2,4,6,8 and10 days at concentration of 25% increased with the increase of contact time and reached the maximum at 10 days, where the algae achieved the removal percentages of 31.18%, 17.88%, and 40.57% for light *n*-alkanes , heavy *n*-alkane and PAHs, respectively. This result agrees with those obtained by El-Shoubaky & Mohammad (2016) who reported that gasoline bioaccumulation differed significantly with increasing time of exposure using the two algal species *Ulva lactuca* and *Enteromorpha clathrate*. Akl *et al.,* (2023) found that for all three selected algae *Ulva intestinalis* (green alga), *Sargassum latifolium* (brown alga), and *Corallina officinalis* (red alga), hydrocarbon adsorption increased with the increase of contact time.

U. lactuca dry biomass achieved removal percentages of PAHs > heavy *n*-alkanes > light *n*-alkanes after 10 days, these results agree with those of Akl *et al.,* (2023) who used

Ulva intestinalis, with similar trend observed after 15 days. The observed decrease of light *n*-alkanes, heavy *n*-alkanes, and PAHs in the WSFs by the *U. lactuca* is largely due to the adsorption of these compounds to the algal surface. This suggestion was supported by some previous studies, which documented that most hydrophobic organic compounds (HOCs) tend to bioconcentrate (adsorbed) on algae (Du *et al.*, 2022).

Akl (2017) found that *Ulva pertusa* takes up PAHs from surrounding seawater and gathers at varying levels according to duration of exposure. This agrees with the results of the present study as *U. lactuca* dry biomass adsorbed available PAHs from WSF according to different duration of exposure. The best removal percentages were at 10 days for light *n*-alkanes, heavy *n*-alkanes, and PAHs.

CONCLUSION

In the present study, the results proved that dry *U. lactuca* has a high capacity to adsorb WSFs of crude oil from Ras El-Adabya, Suez Gulf at conc. 25%. The most effective adsorption of oil by *U. lactuca* (dry biomass) was at 10 days and at conc. of 25%. It was concluded that the best removal percentage of PAHs was 41%.

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كفاءة الكتلة الحيوية الجافة لطحلب أولفا لاكتيوكا في إزالة الملوثات البترولية

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المستخلص

يشكل التلوث النفطي للبيئة الناتج من تسريب الزيت الخام قلقا عالميا وتهديدا مستمرا لصحة الأنسان والبيئة نتيجة ارتفاع معدل حدوثه وتأثيره البيئي على المدى الطويل. تهدف هذه الدراسة إلى تقييم كفاءة الكتلة الحيوية الجافة لطحلب أولفا لادمصاص الأجزاء الذائبة من زيت البترول (الهيدروكربونات البترولية) للتخلص منها من منطقة رأس الأدبية بخليج السويس مع تغيير زمن التعرض (التلامس) (2–10 أيام). وقد أجريت التحاليل الفيزيوكيميائية لكلا من عينات المياه والبترول. تم جمع الطحلب من منطقة أبو قير بالإسكندرية في الفترة من مايو الى يوليو 2021 وتعريضها لتركيزات مختلفة من الأجزاء الذائبة لزيت البترول الخام (25% و50% و75% و100%) مع تغيير وقت التلامس (2.4.6.8.10أيام). تم تعيين قدرة طحلب الأولفا على الإزالة باستخدام جهاز كروماتوغرافيا الغاز. أظهرت النتائج أن

تراكيز الهيدروكربونات البترولية (الهيدروكربونات الخفيفة– الهيدروكربونات الثقيلة – الهيدروكربونات العطرية عديدة الحلقات) انخفضت بشكل ملحوظ بزيادة زمن تلامس الكتلة الحيوية الجافة للطحلب إلى 10 أيام والذى سجل أقل نسبة منهم. زادت كل من سعة الحمل ونسبة الإزالة للطحلب للهيدروكربونات البترولية الثلاثة بزيادة زمن التعرض حتى وصلت إلى الحد الأقصى عند 10 أيام. وصلت أقصى كفاءة ادمصاص للهيدروكربونات الخفيفة– الهيدروكربونات الثقيلة – الهيدروكربونات العطرية عديدة الحلقات عند تركيز 25% للزيت 31.81% و17.88% و40.57% بالترتيب.

الكلمات المفتاحية: الطحالب –أولفا لاكتيوكا – ادمصاص – زيت خام – الأجزاء الذائبة بالماء – تحليل كروماتوغرافيا الغاز