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# Efficiency of Green Algae *Chlorella vulgaris* in Remediation of Polycyclic Aromatic Hydrocarbon (Anthracene) from Culture Media

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## Abstract:

The fresh water green alga *Chlorella vulgaris* was selected to study its ability to degrade one of polycyclic aromatic hydrocarbon (PAHs)known is anthracene (ANT). ANT is widely used in artificial products such as wood preservatives; insecticides; dye and coating materials. This algae was cultivated in CH-10 medium under constant laboratory conditions and exposed to different concentrations (1, 3, and 5 mg/l) of anthracene for 3, 5, 7, 9 and 15 days, with the concentration of ANT measured by high performance liquid chromatographic analysis (HPLC). The results showed that *C.vulgaris* has high ability to reduce anthracene to 80% at 1mg/Lcon centration after 3 days and 100% after 5 days, while at 3 and 5 mg / L concentration, the highest percentages were 89% and 99%, respectively after 9 days, with the complete removal (100%) was achieved after 15 days. The results indicate that this alga(*C. vulgaris*) has high ability for remediation of ANT and yield not toxic compound to environment

**Key words:** Anthracene, Bioremediation, *Chlorella vulgaris*, Green algae, Polycyclic aromatic hydrocarbon(PAHs).

## Introduction:

Bioremediation involved the use of biological systems for the removal or reduction of pollution from different environmental media (1).It has confirmed to be a safe, effective, low-cost and environmentally friendly alternative for sustainable remediation environmental pollution by hazardous and recalcitrant pollutants( 2,3).Bioremediation is use to convertorganic pollutants into harmless metabolites or to mineralize the pollutants into carbon dioxide and water (4) Microalgae have many roles in bioremediation and are widely used to remove different aquatic pollutants (5). Specifically, green algae is used in transforming and degrading polycyclic aromatic compounds (PAHs) and removing these compounds from the environment (6).They have been used to degraders recalcitrant pollutants such as *Chlorella vulgaris* (7).

PAHs are a significant anthropogenic pollutant in the environment(8, 9), they contain two to eight conjugated aromatic rings (10). They are widespread in water, air, soil, dust, and sediment. Their anthropogenic sources and natural sources in the environment include volcanic eruptions, forest fires, diagnosis and biosynthesis(11, 12).

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PAHs are characterized by their high toxicity and carcinogenic properties, making it a priority pollutant according to the US Environmental Protection Agency (13, 14).Anthracene (ANT) is tricyclic aromatic hydrocarbon causes many problems associated with health and environmental impact (15).It represents one of the most widespread concerns because of its toxicity to biological systems, such as fish and algae (16). It also shows accumulation in the food chain and affects all types of biological life (17, 18).The present study aimed to investigate the ability and efficiency of green alga *Chlorella vulgaris* in remediation of different ANT concentration of from polluted media.

## Materials and Methods:

### Culture and algal growth

The unicellular green microalga *C. vulgaris*(19) was grown on Chu-10(20)for 15 days under constant laboratory conditions at 25 ± 2 ° C and a light system of 16: 8 hours light/ dark cycles(21). This culture transported into 1000 ml of media and incubated for 14 days to increase of algalbiomass (22).

**Experimental Design**

This alga wasexposed to ANT which is dissolved in dimethyl sulfoxide (DMSO) and added to the culture medium to prepare final concentrations of 1, 3 and 5 mg/l which is prepared in 250 ml of liquid culture media.The control mediaused the same concentration of ANT without adding alga under the same culture conditions. Three replicates were used for this experiment. DMSO (0.1% v/v) control sample was prepared for each experiment; this chemical had shown to have no significant effect on *Chlorella vulgaris* (21,22).

All sample treatments (5 ml) were filtrated by Millipore filter ( 0.45 µm) and analyzed by high performance liquid chromatography (HPLC) to follow the % decrease inanthracene concentration in the culture medium after 3 , 5 , 7 , 9 and 15 days.

**HPLC analysis of Anthracene.**

The samples were quantifiedwith HPLC instrument model (SYKAM) made in Germany.

The standardspure ANT(from Sigma–Aldrich Chemical Co., USA) and samples (50 µl) were injected into the HPLC system after they were filtered by Millipore filter 0.45µm and the time taken for a sample to pass through the system is recorded as its retention time.

The concentration of residual anthracene was determined by the following formula:

$$\text{C sample} = \frac{\text{C standerd} * \text{A sample}}{\text{A satanderd}}$$

C: concentration, A: area

Statistical Analysis of fall treatments were performed by SPSS program(version 23) (23).

## Results and Discussion:

**Bioremediation of Anthracene**

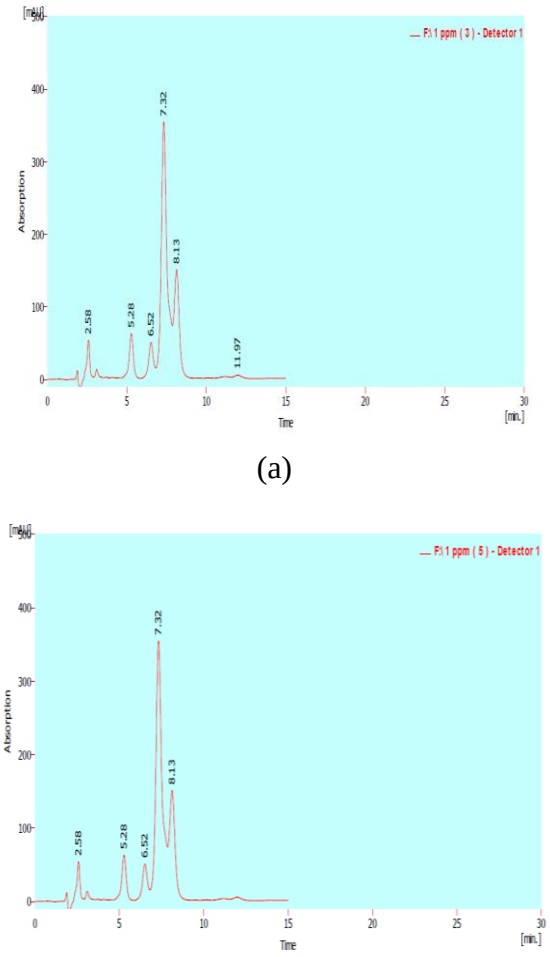
The results showed that *C.vulgaris* had high ability to removed anthracene after 3, 5, 7, 9 and 15 days of treatments because *Chlorella vulgaris* is identified as tolerating and effectively degrading polycyclic aromatic hydrocarbons that may be toxic in the environment and converting these compounds into non-toxic form(24). Moreover Kumar et al.(25) revealed that PAHs interacted in alga with cytochrome P450 monooxygenase CYP active sites through intermolecularhydrogen bonding, hydrophobic bonding π–πinteractions and van der wails interactions making algae have the abilityto remediate PAHs more efficiently than other microorganisms. The results observed significant differences ($p \leq 0.05$) between treatments and residual concentration.

Fig.(1) Showed that anthracene degradation occurred at a concentration of 1mg/l when compared with controls. The concentration reached to 0.2 mg at three days and the removal percentage was 80%.After 5 days the removal percentage was 100%, while concentration of ANT in control was 0.99, 0.4, 0.1 and the removal percentage was 10%, 60%, 90% after 3, 5, 7 days, respectively as seen in table (1). These results in comparison with other studies (24) on the same alga, showedcompleted degradation of acenaphthene with 1.25 mg/l at 4th day, where the removal percentage was 100% and the concentration of fluoranthene decreased from an initial 1.5 mg /l to 0.3 mg /l after a remediation period of four days. Also, El-Sheekh et al.(26) showed highest percentage of degradation of anthracene by *Elysiaviridis*after 7 days was 92.28%.

**Table1. The residual concentration of anthracene in 1mg/l after exposure time compared with control**

| Exposure time (days) | control | | Concentration of anthracene1mg/l | |
|---|---|---|---|---|
| | Residual Concentration mg/l | Removal Efficiency (%) | Residual Concentration mg/l | Removal Efficiency (%) |
| **3** | 0.99 | 10% | 0.2 | 80% |
| **5** | 0.4 | 60% | - | 100% |
| **7** | 0.1 | 90% | - | - |
| **9** | - | - | - | - |
| **15** | - | - | - | - |

(-)not detectable




(a)

(b)

**Figure 1. HPLC Chromatogram of *C.vulgaris* at 1 mg\l anthracene after 3 days (A), after 5 days (B)**

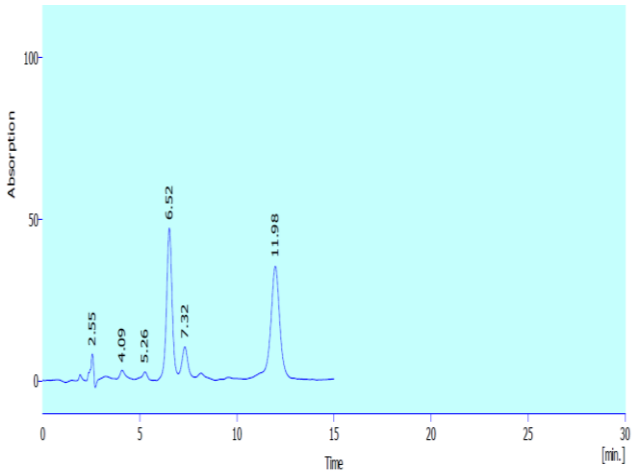
Anthracene was reduced from an initial value of about 3 mg/l to 1.2, which corresponds to 60% the lowest removal efficiency after three days of treatment (Table 1,Fig.2A). whereas after 5 and 7 days, anthracene concentration was reduced to 0.5 and 0.2 mg/l, which was equivalent to a removal efficiency of approximately 83and 93%, respectively. Compared with control, concentrations of anthracene were 2.77, 1.5, 0.42 and the removal percentage 7%, 50%, 86% after 3, 5 and 7 days, respectively (Table 2, Fig.2B, D).

Fig.(2C) showed the greatest reduction of residual concentration and removal efficiency of anthracene after nine days of treatment (0.05 mg/l and 98%, respectively). The anthracene disappeared completely after fifteen days when compared with control (Table 2, Fig. 2E) and these results were in agreement with the study of (27) where *Anabaena fertilissima* performed higher biodegradation of ANT showing a sharp reduction in 2.5 mg/L on the 16$^{th}$ day.

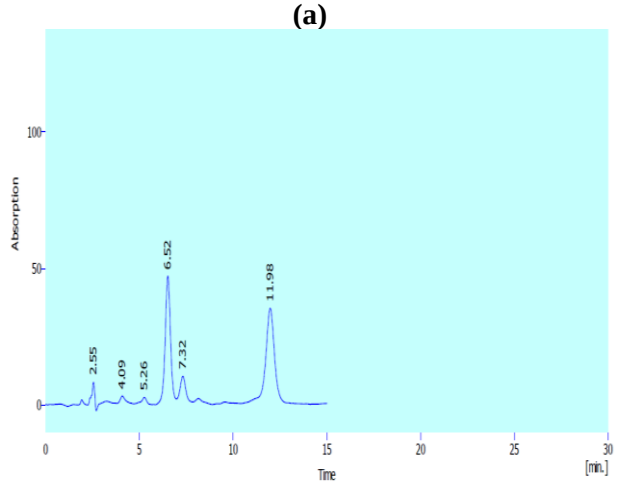
**Table2. The residual concentration of anthracene in3mg/l after exposure time compared with control**

| Exposure time (days) | control | | concentration of anthracene3mg/l | |
|---|---|---|---|---|
| | Residual Concentration mg/l | Removal Efficiency (%) | Residual Concentration mg/l | Removal Efficiency (%) |
| **3** | 2.77 | 7% | 1.2 | 60 |
| **5** | 1.5 | 50% | 0.5 | 83 |
| **7** | 0,42 | 86% | 0.2 | 93 |
| **9** | 0.2 | 93% | 0.05 | 98 |
| **15** | - | - | | - |

(-)not detectable




**(a)**

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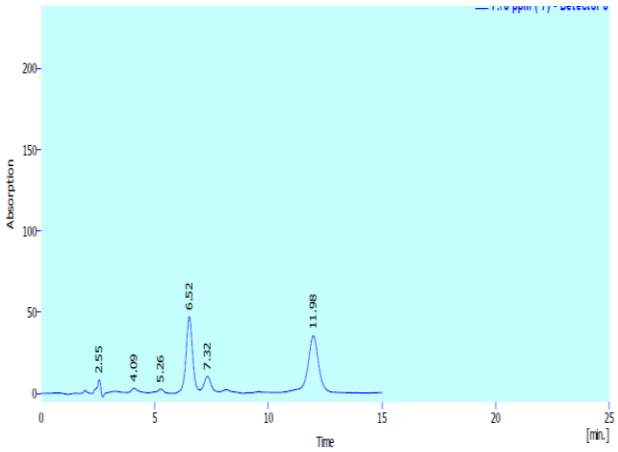
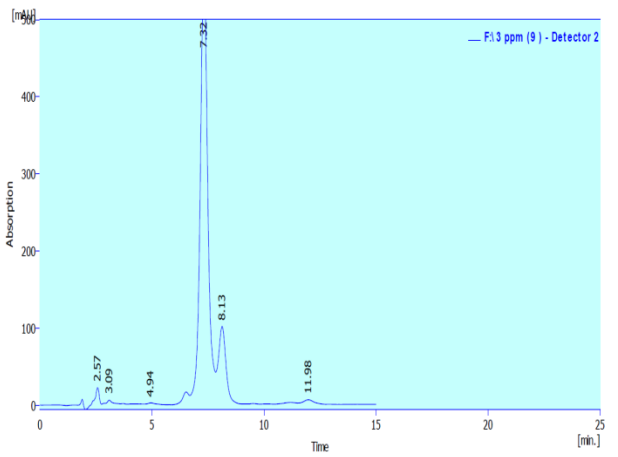
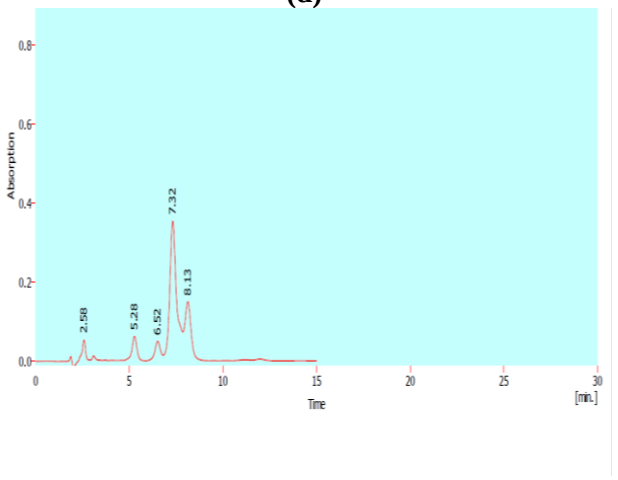



**(c)**




**(d)**




**(e)**

**Figure 2. HPLC Chromatogram of *C.vulgaris* at (3 mg\l) anthracene after 3 days (A), after 5 days (B), after 7 days (C), after 9 days (D), after 15 days (E)**

ANT concentration (5mg/l) were reduced to(2.9mg/l) after three days equating to minimum removal efficiency 62% (Fig.3A). After 5, 7 and 9 days of treatment, its removal efficiency was90 , 93 and 99 % which equates to 1.6, 0.6 and 0.2 mg/l, respectively (Table 3, Fig. 3). The data presented in (Fig. 3E) results showed ANT completed degradation after fifteen days of treatment compared with the control. These study agreement with Patel et al. (27) who reported that *Anabaena fertilissima*can degraded anthracene and pyrene after 16 days of treatment by 46.3% at 5.0 mg/L and 33.79% at 3.0 mg/L. this is similar to Raghukumar et al. (28) who reported that the blue green algae *Oscillatoria salina*, *Plectonema terebrans* and *Aphanocapsa* sp. could degrade of ANT by 90.6%, 62.7%, and 41.9%, respectively, after 10 days of treatment. Hong et al. (29) observed high ability of *Nitzschia* sp. for accumulation and degradation off luoranthene (FLA), this result was agree with other study (30,31).

**Table3. The residual concentration of anthracene in 5mg/l after exposure timecompared with control**

| Exposure time (days) | Concentration of anthracene | | | |
|---|---|---|---|---|
| | 5mg/l control | | 5mg/l | |
| | Residual Concentration mg/l | Removal Efficiency (%) | Residual Concentration mg/l | Removal Efficiency (%) |
| **3** | 2.9 | 42 | 1.88 | 62% |
| **5** | 1.6 | 68 | 0.46 | 90% |
| **7** | 0.6 | 88 | 0.34 | 93% |
| **9** | 0.2 | 96 | 0.04 | 99% |
| **15** | 0.01 | 99 | - | - |

(-) not detectable

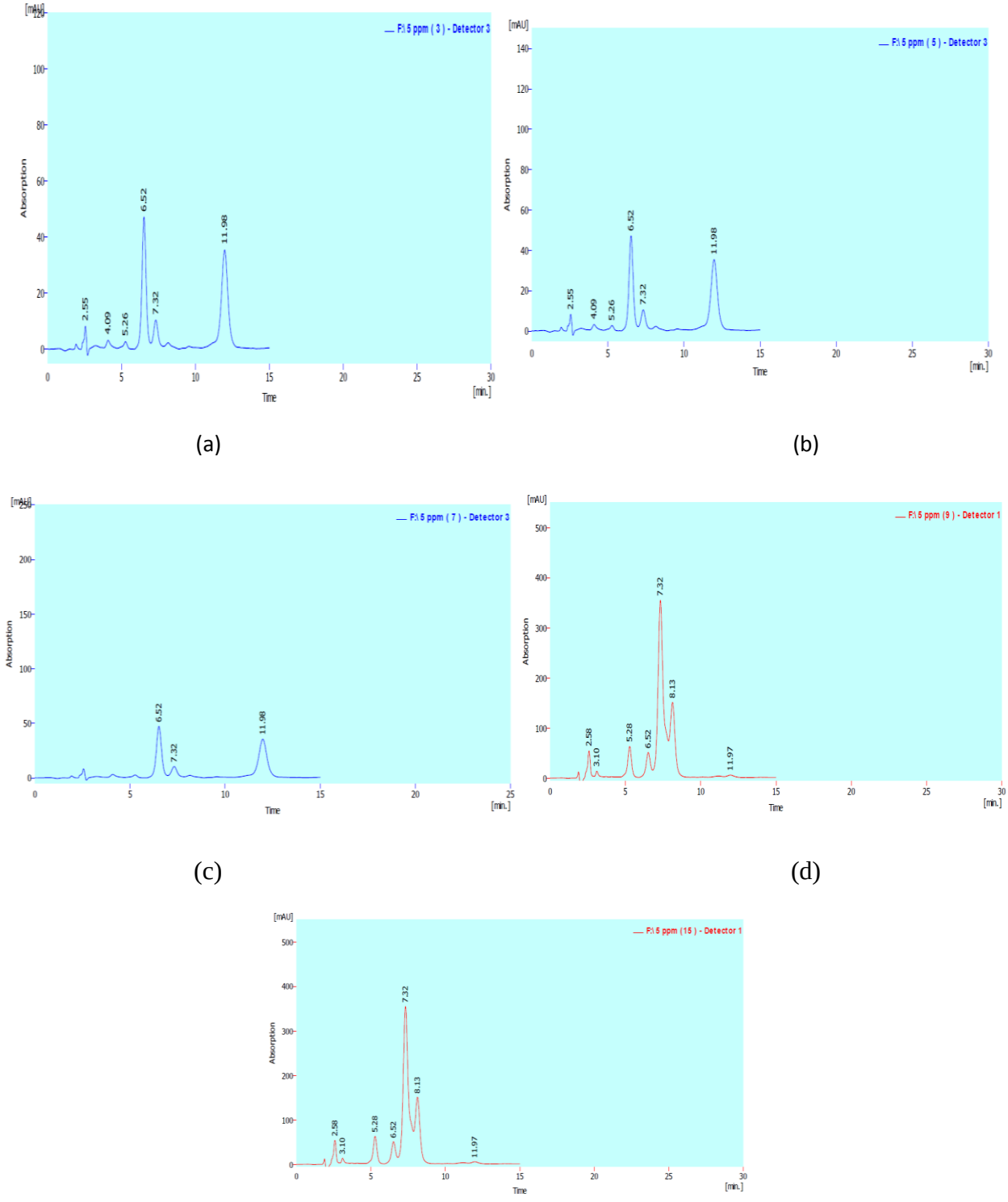



**Figure 3.HPLC Chromatogram of *C.vulgaris* at 5 mg\l anthracene after 3 days (A), after 5 days (B), after 7 days (C), after 9 days (D), after 15 days (E)**

## Conclusions:

The green alga *C.vulgaris*can be used in bioremediation of Anthracene which is one of polycyclic aromatic hydrocarbon(PAHs)from polluted water due to safety compounds produced from alga degradation and the results showed that alga ability to ANT removal is high at low concentration and the pollutant is completely removed after short period**.**

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## Conflicts of Interest: None.

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# كفاءة الطحلب الاخضر*Chlorella vulgaris* في معالجة المركب الهايدروكاربوني الاروماتي (الانثراسين) من الوسط الزرعي

**سراب عظيم جودة** **ميسون مهدي صالح** **جاسم محمد سلمان**

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**الخلاصة:**

تم اختيار احد انواع الطحالب الخضراء (*Chlorella vulgaris* ) لدراسة قابليتها لتحليل احد المركبات الهيدروكربونية الاروماتيةمتعددةالانوية المستعملة في المنتجات الصناعية كالأصباغ والمبيدات وطلاء الاخشاب وغيرها وهو الانثراسينوالذي يكون له تاثيرا سام للنظم الحياتية واحتمالية تأثيراته السرطانية والتطفيرية وحدوث تشوهات خلقية. تم زراعة الطحلب على وسط CH-10 تحت ظروف مختبرية ثابتة، اذ عرض الطحلب لتركيزات من الاتثراسين (1و3و5) ملغرام /لتر ودرست قابليتهعلى تحليل المركب وازالته بعد مرور (3, 5, 7, 9, 15) يوما وقيست التركيزات المتبقية باستعمال جهاز كروموتوكرافيا السائل فائق الأداء(HPLC). اظهرت النتائج ان قابليةالطحلب العالية على ازالة مركب الانثراسن بنسبة 80% عند تركيز 1 ملغم / لتربعد مرور ثلاثة ايام وكانت الازالة 100%بعد مرور 5 ايام اما بالنسبة للتركيزين(5,3) ملغم /لتر فكانت اعلى نسبة ازالة هي 98% ,99 على التوالي بعد مرور تسعة ايام وكانت الازالة كاملة بعد مرور خمسة عشره يوما. وقد بينت الدراسة امكانية الطحلب العالية على تحليل المركب حيويا وانتاج موادصديقة للبيئة.

**الكلمات المفتاحية:** المعالجة الحياتية، المركبات الهيدروكربونية الاروماتية، الانثراسين، الطحالب الخضراء، *Chlorella vulgaris*.