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Potential Efficiency of Green Algae Scenedesmus quadricauda In Bio-Remediation Of Polycyclic Aromatic Hydrocarbon Benzo [a] Pyrene (BaP)

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Abstract

Using algae to break down or detoxify dangerous environmental pollutants thereby changing them into a non-hazardous condition is known as bioremediation. Investigating the ability of the green algae *Scenedesmus quadricauda* (Turpin) Brébisson to breakdown a particular polycyclic aromatic hydrocarbon (PAH) known as Benzo [a] pyrene (BaP), Under regulated laboratory circumstances and on BG11 media, the alga was cultivated and exposed to different BaP dosages (0.5, 1, and 1.5 mM). High performance liquid chromatography (HPLC) study helped to ascertain the BaP concentration. Involving as the growth curve, doubling time, photosynthetic pigments, total protein, carbohydrates, and Lipid peroxidation (Malondialdehyde MDA) levels, the research investigated various physiological and biochemical aspects. Furthermore, measured were the levels of catalase (CAT), superoxide dismutase (SOD), and reactive oxygen species (ROS). Whereas the lowest growth rate was 0.00047 on the 15th day at concentration of 1.5 mM, the maximum growth rate (k) recorded was 0.391 on the 7th day at concentration of 0.5 mM. Doubling time also varied from 0.00014 throughout 15th day with 1.5 mM and from 0.1179 throughout 7th day with 0.5 mM BaP. The results showed a definite influence of different quantity of BaP degradation by *S. quadricauda*; the greatest magnitude was 40.13 throughout 15th with 0.5 mg/l, while the lowest magnitude was 0 throughout 1st day with 0.5 Mm. While the min magnitude 0.41µg/ml in 0.5 mM throughout 1st day, the max magnitude of chlorophyll-a was 18.71 (µg/ml) in 1.5 mM throughout 15th day. Whereas the greatest magnitude was 9.19 µg/ml in 1.5 mM throughout 15th day, the lowest magnitude of chlorophyll b was to 0.36 µg/ml in 1.5 mM throughout 1st day. While the min was 0.013 on 1st day with 1mM, the max magnitude of ROS was 0.28 until 15th day with 1.5 mM. With 1mM over 1st day, the carbohydrate showed a max magnitude of 35.13 µm/ml; with 1.5 mM over 15th day, the min magnitude was 12.25(µm/ml). While the min protein content 1.83 µg/ml in 1.5 Mm throughout 8th day, the max protein content was 2.14 µg/ml in 1 mM throughout 8th day, Moreover, SOD fluctuated between 22.22 µg/ml in 0.5 mM throughout 1st day, and 60µg/ml in as the min magnitude throughout 8th day with 1.5 mM. The results show that magnitudes of CAT fluctuated between 13.33µg/ml in 8th and 15th mM throughout 15th day and 73.33µg/ml in 1mM throughout 15th day. MDA showed the largest magnitude—59.92 µmol/l in 1.5 mM over 1st day—while the lowest magnitude, 36.58 µmol/l in 1mM over 15th day.

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Key Words	Bioremediation, Scendesmus qudricauda, HPLC, Benzo [a] pyrene (BaP), BG11 medium
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1. Introduction

In many environmental media, bioremediation is the use of biological systems to either eradicate or reduce pollution (Luka et al. 2018). Studies have shown that it is a safe, effective, cheap, environmentally friendly replacement for sustained remedial action against hazardous and recurring pollutants (Shukla et al. 2010; Singh, 2006). Unwanted effects of pollution and environmental harm follow naturally from the advancement in industry and technology. The main causes of the environmental effects connected with industrial activity are the development of industrial waste. Because of problems with conventional approaches of pollution control (Nandal et al. 2015). Reducing pollutants and sources of pollution in water is of utmost importance given the severe scarcity of fresh water worldwide, and ensuring that these treatments are economical and environmentally friendly makes them a suitable option compared to traditional alternatives such as chemical precipitation and ion exchange, these alternatives used to produce waste that is difficult to dispose of (Tewari et al. 2023). Many sophisticated operations integrating biological, physical, and chemical technologies were developed and used recently to clean polluted regions. In this regard, biological therapy—which uses microorganisms like bacteria, fungi, and algae to break down pollutants such hydrocarbons and convert them into ecologically friendly compounds—is very promising (Amran et al. 2022). Bioremediation is the process of breaking down organic contaminants into water and carbon dioxide or of turning organic pollutants into non-toxic metabolites (Alexander, 1999). Often used for the removal of different aquatic pollutants, microalgae are also very important in bioremediation (Hwang et al. 2016). Specifically employed for the conversion and breakdown of polycyclic aromatic compounds (PAHs) and removal from the environment, green algae are As shown by García de Llasera et al. 2016, they were used to break down enduring toxins like *Scenedesmus* sp.

Organic substances with carbon and hydrogen atoms bound together in a chemical structure called polycyclic aromatic hydrocarbons (PAHs). One may say of them as benzene rings joined in different configurations without any extra atoms or substituents present (Lawal, 2017).

Though there are more than 100 kinds of PAHs, the United States Environmental Protection Agency (US EPA) has ranked 16 polycyclic aromatic hydrocarbons (PAHs) based on their unique characteristics on top of priority (Keith, 2015). Based on their ring count, the molecules listed might be classified as high molecular weight, medium molecular weight, and low molecular weight compounds (Alegbeleye et al. 2017; Pandya and Kumar, 2021). Each of the low molecular weight compounds (LMWC), fluoranthene [Fla], phenanthrene [Phen], anthracene [Ant], fluorene [Flu], acenaphthene [Acp], acenaphthylene [Acpy], and naphthalene [Nap], has 2–3 rings.

Benzo[a]pyrene (BaP) is a major polycyclic aromatic hydrocarbon (PAH) with a high molecular weight. Comprising a polycyclic aromatic hydrocarbon with a chemical formula C20H12 and a molecular weight of 252.3 g/mol, BaP It develops as a side effect of incomplete combustion at temperatures between 300 and 600 degrees centigrade. At 25-degree centigrade BaP has a density of 1.24 g/cm3, a melting point of 179 degree centigrade, and a boiling point of 495 degree centigrade (Liu et al. 2019).

Mostly produced by industrial activities, Benzo[a]pyrene (BaP) results from industries releasing smoke and incomplete combustion of fossil fuels. It also results from household activities such cooking and smoking (Sinha et al. 2005; Sun et al. 2013). Both the United States Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC) have labeled it as a Set 1 carcinogen. It also is a major contaminant in soil, air, and water (Bhatt et al. 2018).In the environment BaP may undergo chemical degradation, photolysis, volatilization, and adsorption. Still, microbial degradation is mostly responsible for its loss (Haritash and Kaushik 2009). Furthermore, affecting all kinds of biological life, it shows the process of accumulation in the food chain (Haritash and Kaushik 2009; Okpishi et al. 2017). Petroleum pollutants are easy to form mucous membranes on the surface of plant roots, obstructing plant root respiration and nutrient absorption, and even causing root rot and plant death in severe cases (Yang et al. 2024).The current investigation aimed to explore the ability of green algae *S.quadricauda* in bioremediation of various Benzo [a] pyrene amount of from contaminated media, and used some physiological and biochemical parameter as tools to bioremediation process.

2. Materials and Methods

2-1 Algal strain

Chlorophyceae species *Scenedesmus quadricauda* originally from the Algae Culture Collection (UTEX) at the University of Texas in Austin, Texas, USA.*S. quadricauda* was cultivated under controlled circumstances with a light intensity of286 μE/m²/s, a light/dark cycle of 16:8 hours, and a temperature of 25±2 degree centigrade (Chia et al. 2013), to prevent grouping and hasten their rate of expansion, the civilizations were also gently moved by hand every day(Selvan et al. 2013).

2-2 Preparation of algal culture Media

The improvement of chlorophyta (Patel et al. 2015) has come from BG-11 medium Autoclave the BG-11 medium at 121 degrees Celsius for 15 minutes to sterilize it. After freezing to try to prevent too strong precipitation, using a pH meter revealed that the pH of the medium changed to around 7.4 using 1N NaOH and 1N HCl solutions. As Gour et al. (2014) report, a 10 ml algal culture is placed in a flask holding 100 ml of BG11 medium and allowed to flourish for 15 days. Following that, this culture is placed in 1000 ml of media and allowed to incubate for two weeks minimum before beginning the experiment, therefore ensuring continuous lab conditions. Photoperiod greatly affects the cell division in asexual reproduction. Constant illumination accentuates this division that occurs all of the light time (Salman et al. 2023). Therefore, the period of light exposure may vary based on the objectives of the farming: a light-dark cycle mirroring the natural solar cycle favors regular and vigorous growth, while continuous lighting stimulates fast development (Sánchez-Bayo, 2020; Allen, 2003).

2-3 Experimental Design

S. quadricauda's culture medium was exposed to different benzo (a) pyrene (0.5, 1, and 1.5) mM dosages for 15 days. The research primarily aims to track daily variations in algae during a fifteen-day period. To determine the time to doubling (G) and the rate of growth (k), the algae are incubated. On the first, eighth-, and fifteenth-days assessments also include chlorophyll a and b, total protein, and many enzymatic responses including reactive oxygen species (ROS), lipid peroxidation MDA, superoxide dismutase (SOD), and catalase (CAT).

2.4. Determination of growth rate and doubling time

Every day the cell count of green algae *S. quadricauda* is determined using a UV-Vis spectrophotometer. This is accomplished by measuring the optical density (OD) at 685 nm, therefore enabling the cell density in cells per millilititer. This measurement clarifies many development stages, lets us estimate the biomass size, and ascertain growth rates. Especially, the formula of Richmond (2004) helps one find the doubling time and growth rate during the exponential development phase**.**

$K = 3.322 * (\log OD_t - \log OD_0) / t$

$G = 0.301 / K$

K: growth rate G: doubling time t: time

OD_0: optical density at the experiment beginning (zero time).

OD_t: optical density after (t) day.

2-5 Estimation of Chlorophyll

A technique detailed in Ref. (Juneja et al. 2013) was used to measure the total chlorophyll concentration. 2 mL sample was taken and then subjected to a centrifugal force of 12,500

revolutions per minute for five minutes. The solid residue left behind from the algae then was mixed with 2 mL of methanol (90%). After an incubation period of twenty hours in a dark atmosphere at a temperature of twenty degrees, the sample was submerged in a water bath at a temperature of 64 ◦C for five minutes. At last, the material was discarded after five minutes of centrifugation at 12,500 rpm. Spectrophotometry at three separate wavelengths— 470 nm, 652 nm, and 665 nm—quantified the filtrate

The chlorophyll content was determined by the following Equations (1) – (2) (Lin et al. 2013):

$$\text{Chlorophyll a (mg/l)} = (12.7 \times A663) - (2.698 \times A645) \quad (1)$$

$$\text{Chlorophyll b (mg/l)} = (22.9 \times A645) - (4.68 \times A663) \quad (2)$$

2-6 Estimation of reactive oxygen species (ROS)

Taking 2 ml specimen, the estimate technique entails two times washing it under Phosphate-buffer solution. The resultant precipitate—which comprises of algal cells—is then combined with two milliliters of perchloric acid (200 micromol). For three minutes, the cells break down under sonication with pauses every twenty-second. The specimen is then rapidly thrown by half an hour of spinning it at 10,000 r/m. The volume is set at 5 ml after the filtrate is mixed using a micro pipette. One 1.5 ml extract then is gathered and combined with 0.1 ml working solution. The working solution consists of 0.28 ml of sulfuric acid, 19.6 mg of ammonium ferrous sulfite, 14.3 mg of xylenol, and 3.64 g of sorbitol. The sample is kept half an hour at 30 degree Celsius under incubation (Salman *et al.* 2023). The sample is next evaluated at 560 nm, and the equation helps one to ascertain the quantity of ROS:

$$\%\text{inhibition ROS} = \triangle\text{uninhibited} / \text{Min}(10)$$

2-7 Antioxidant enzymes:

The process consisted of discarding a 2 ml the specimen at a speed of 4000 – 6000 r/m. After washing the resultant algal precipitate in a buffer solution at pH 7, the discarding process was repeated. The volume was then changed back to 2 ml. The algae cells were then disturbed for three minutes using an ultrasonic gadget called Sonication. Every 20 seconds the model pauses, then is thrown at 10,000 rpm for 30 minutes. The resultant filtrate is then mixed in small quantities and the volume is changed to 5 ml (Bradford, 1976).

2-8 Estimation of Carbohydrates

Following a phosphate-buffer solution wash and sonication-assisted breakdown, 2 mL sample of the material was removed and aerobically dried. Then, using distilled water, the resultant solution was diluted to a final volume of 5 mL. 1 ml sample of the substance was then mixed with 1 millilitre of phenol (5%), five millilitre of sulphuric acid (96%), The mixture was then swirled ten minutes nonstop. The sample then spent thirty minutes submerged in a water bath

set at 30–35 ◦C. After that, it was evaluated at 490 nm and matched the known glucose standard curve produced by dissolving 100 mg of glucose in 100 ml of distilled water (Herbert, 1971).

2-9 Estimation of total protein

Several methods—including centrifugation, dialysis, chromatography, precipitation, and ultrafiltration—have been used to separate and concentrate microalgal proteins (Salman et al. 2023). Based on the updated method by Ermis et al. (2020), based on the original procedure by Lowry et al. (1951), the total protein content was determined. The operation included mixing 2 mL of Biuret solution with 0.5 mL of the already produced extract (2–4). The liquid was then thoroughly stirred for thirty minutes using a preheater set to 30 degrees centigrade. The sample was then evaluated at 555 nm and matched with the reference solution depending on the Bovine Serum Albumin Protein level ranging from 0 to 0.1 ml. Dissolving 0.1 g of Bovine Serum Albumin in 100 mL of buffer solution produced a quantity of 100 µg/l, the reference solution.

2-10 Estimation of Superoxide dismutase (SOD)

The estimate calls for mixing two milliliters of Tris-buffer with one hundred microliters of the extract (2–4). The first reading (△A0) is then obtained by measuring the absorbance at 420 nm after five minutes. After adding 0.2 ml of Pyrogallol solution, the absorbance is once more measured at 420 nm following five minutes to get the second reading (△A1). △A1 is computed utilizing the formula by Zhao (2017) at hand.

SOD activity (µ/mL) = $[(\triangle_{A0} - \triangle_{A1}/\triangle_{A0})/50\%] \times$ Volume of sample

2-11 Estimation of catalase (CAT)

The estimate calls for blending one millilititer of hydrogen peroxide with one hundred microliter of the extract (2-4). The combination is then ascertained at 240 nm of wavelength. Ten minutes later, the measurement is taken once again after hydrogen peroxide addition. Apply Frary et al. (2010) formula to get the catalyst's quantity.

CAT= $[(\triangle Abs_{240}/Min_{10}) \times (Reaction\ volume_{100})]/0.001$

2-12 Lipid peroxidation (Malondialdehyde MDA)

The quantification of lipid peroxidation was conducted using the Thiobarbituric acid method to measure the quantity of Malondialdehyde (MDA), as described by Aust (1985) and Burtis and Ashwood (1999). 100 µl specimens were combined with 1000 µl of TCA (20%) and 1000 µl of TBA (0.6%). Tubes are agitated by vortexing. The sample was heated in a water bath at a temp of 100 degree centigrade for a duration of 15 min. Afterward, it was allowed to drop down to room temp. The sample was then subjected to centrifugation at a speed of 4000 revolutions per minute (4 degree centigrade) for 15 min. Finally, the absorbance of the sample was measured at a wavelength of 532 nm, using a blank as a reference. The findings were quantified in terms of millimoles per milligram of protein (mmol/mg protein).

Amount of Malondialdehyde (MDA) =Absorbtion at 532nm / E × b

Whereas:

E= Extinction coefficient (153mmol/cm),

b = light bath (1cm)

2-13 Estimation of Benzo (a) Pyrene

Briefly after a 7-day incubation period, the cultures were filtered, and the B(a)P compound was extracted from the specimens using an ultrasonic bath using a solvent combination of acetonitrile and dichloromethane (at a ratio of 3:1 v/v). A Dionex UltiMate 3000 system and a fluorescence detector were used in high-performance liquid chromatography (HPLC) to determine the B(a)P amount. Benzo(a)pyrene set the excitation wavelength (λex%) at 250 nm and the emission wavelength (λem%) at 400 nm. Chromatography with a gradient under the use of a mobile phase comprising a combination of water and acetonitrile was the separation method used. Thermo Scientific HYPERSIL GOLD C18 PAH column with dimensions of 250 x 4.6 mm and particle size of 5 µm was the chromatographic column utilized.Made by Merc, the solvents used in the tests were of high-performance liquid chromatography (HPLC) grade. Calibrating curves with magnitudes ranging from 0.1 to 10 ng·cm−3 were generated using the Sigma-Aldrich benzo(a)pirene standard at 1000 µg/mL. The standard solvent used in construction of the calibration curves was methanol. The method demonstrated a linearity higher than 0.999%.Still, the coefficient of variance revealed a level of accuracy below 15%. With a signal-to-noise proportion of 10 for a specimen with a very low amount of B(a)P, approaching to the detection limit, the limit of quantification (LoQ) for the method was found. Measuring the LoQ magnitude, we found 0.01 ng cm^{-3}. The correlation between the recovery rate and the reference material (SRM2585) was 83%. Once used to find B(a)P in the atmosphere, this approach Nine strains in all were exposed to various dosages of B(a)P (7.8, 15, 78, 312, and 624 ng L−1) and temperatures (10, 15, 20, 25, and 30 degree centigrade) in Wiśniewska et al. (2023). The experiment also included blank specimens.

2-14 Analysis of statistics

For every treatment, the experimental data in this work came from minimum of three replicas. SPSS version 26 was used for statistical investigation. The data were expressed as mean and standard deviation. The relevance of variances was evaluated using an analysis of variance (ANOVA). If the related p-magnitudes were less than 0.05, variations were judged noteworthy.

Findings and Discussion

1. Growth Rate and Doubling time

Several studies have demonstrated the effect of exposure to PAHs on algal growth rate and doubling time. This study showed that different amounts of benzo-a-pyrene inhibited the

growth rate, while increasing the doubling time to a long period slowed cell division. There is a significant variance ($p \leq 0.05$) between the various treatments of Benzo [a] pyrene (0.5,1, 1.5) mM, the present study demonstrates, the maximum growth rate (k) at the amount of (0.5) mM was 0.391 throughout 7th day, while the minimum growth rate was 0.00047 at the amount (1.5) mM throughout 15th day, The shorter doubling time (G) was 0.00014 during 15th day with (1.5)mM, while the longest value 0.1179 during 7th day with (0.5) mM benzo (a) pyrene (Fig. 1,2).The inhibitory impact of toxicity could be attributed to the extremely lipophilic characteristics of Benzo [a] pyrene , which enables it to easily enter cells via the plasma membrane and rapidly incorporate into all biological lipoprotein membranes (Miller & Ramos, 2001). It induces alterations in the permeability and structure of membranes and disrupts crucial metabolic activities, such as photosynthesis, respiration, and transmembrane transport (Sikkema et al. 1994; Tripuranthakam et al. 1999; Aksmann & Tukaj, 2004).Calow & Sibly (1990) proposed that various forms of toxic stress cause metabolic alterations in organisms, depleting their energy reserves and negatively impacting their growth and biochemical composition. This finding was reported by Echeveste et al. (2010), who noted a reduction in algae growth when exposed to high amounts of PAHs.

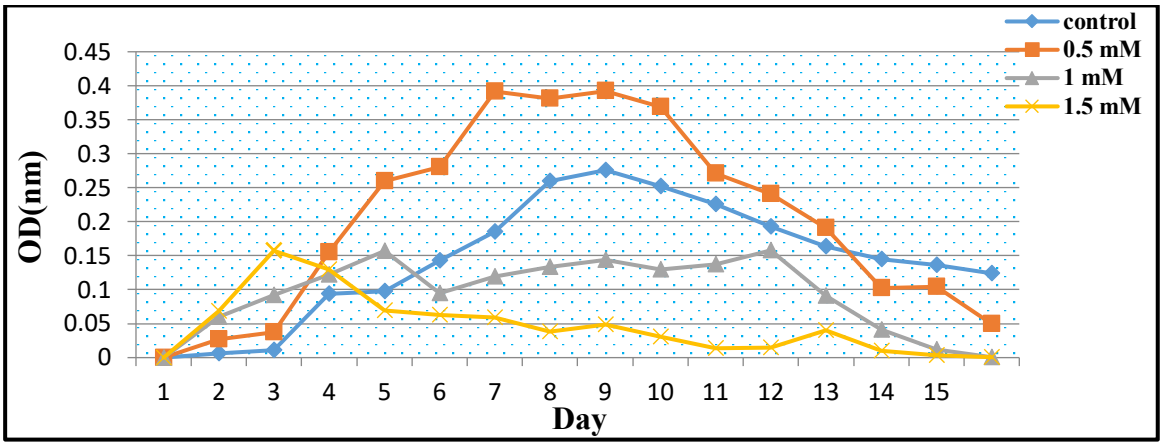

Figure1: Growth rate of *S. quadricauda* at concentration of benzo (a) pyrene.

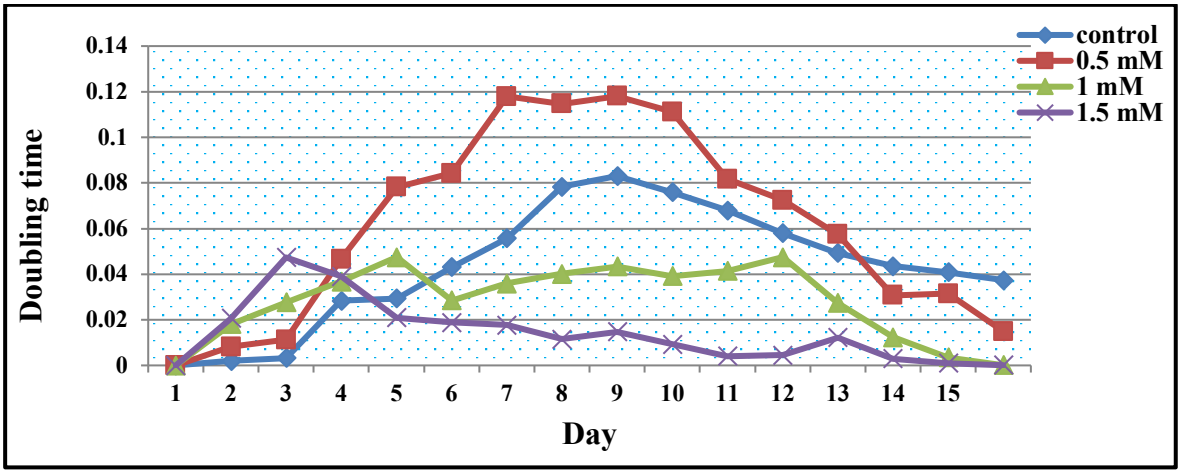

Figure 2: Doubling time of *S. quadricauda* at various concentration of benzo (a) pyrene.

2. Algeal pigments

In the study, benzo [a] pyrene treatments at various concentration led to reduce the chlorophyll a and b in *S.qudricauda* , The results show chlorophyll a in cell *S. quadricauda* treatment with different concentration of Benzo [a] pyrene decrease to 0.41 ± (µg/ml) in 0.5 mM during 1st day, while the high value was 18.71± (µg/ml) in 1.5 mM during 15th day, The results show chlorophyll b decrease to 0.36 ± (µg/ml) in 1.5 mM during 1st day, while the high value was 9.19 ± (µg/ml) in 1.5 mM during 15th day compared to control (Fig 3,4) The synthesis of pigments may be inhibited by the lipophilic nature of aromatic hydrocarbons, which can alter the permeability and fluidity of the cell membrane. This alteration could disrupt the lipid bilayer and reduce energy transduction, affecting the activity of membrane-associated proteins (Heipieper et al. 1995). MOHAPATRA & Schiewer (2000) suggested that the interaction of toxicants with the membrane and dispersion of lipoprotein membranes are responsible for changes in behavior and pigment content in *Synechocystis.* Mostafan & Helling (2002) proposed that the decrease in chlorophyll, carotenoid, and phycobiliprotein contents might be a result of direct inhibition of pigment synthesis by the organic compound or accelerated degradation of pigments since increased reactive oxygen species (ROS) and degradation of the thylakoid membrane by polycyclic aromatic hydrocarbons (PAHs).

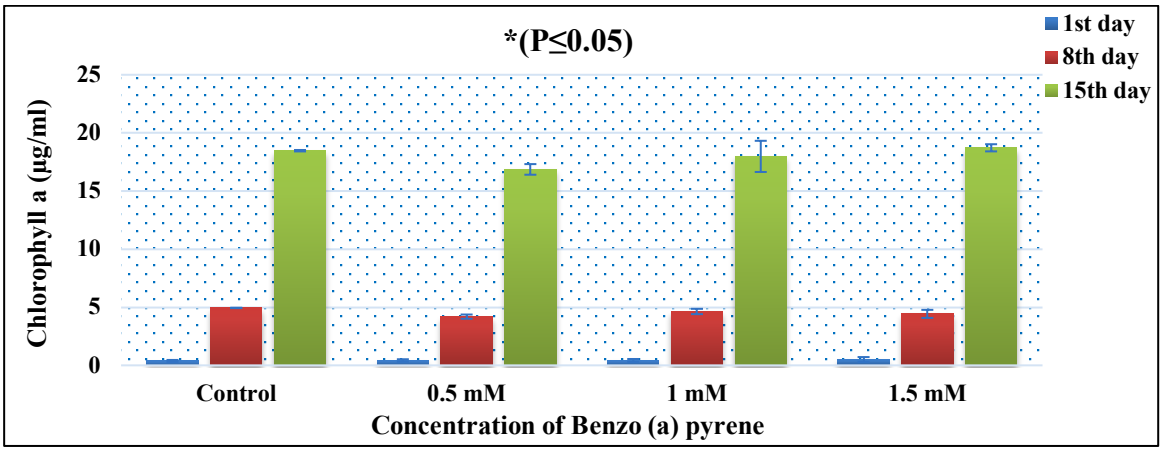

Figure 3: Chlorophyll an amount of *S. quadricauda* at various concentrations of Benzo [a] pyrene .

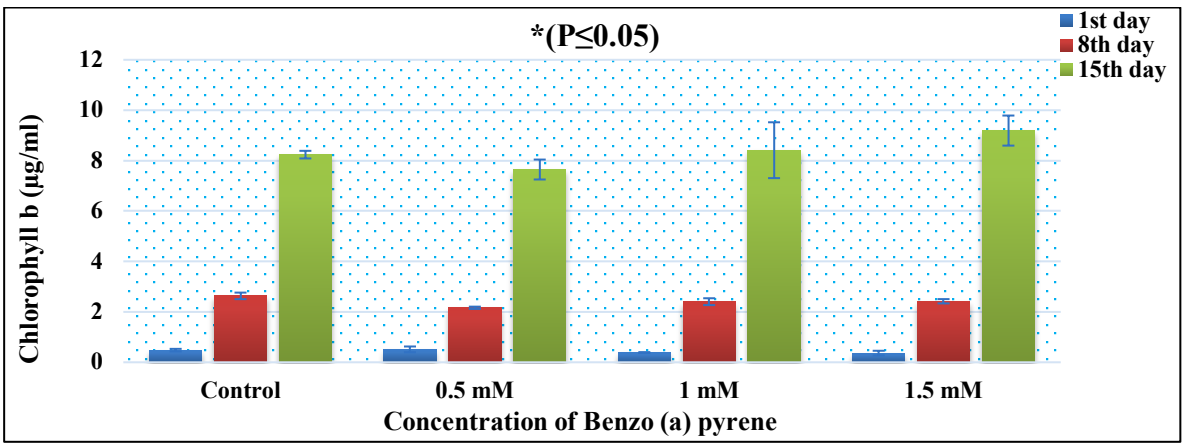

Figure 4: Chlorophyll b amount of *S. quadricauda* at various concentrations of Benzo [a] pyrene .

3. Reactive oxygen species (ROS)

Benzo [a] pyrene was induced oxidative stress for cell algae in treated cultures leading high amount of ROS. where the highest value of ROS is 0.028 % throughout 15th day with 1.5 mM, of Benzo [a] pyrene compared to control (Fig.5). A notable disparity was observed between the treatments and control culture. The hydrophobic feature of Benzo [a] pyrene leads to the overproduction of ROS, which in turn causes alterations in the structure of biomembranes, resulting in an elevated permeability (Sikkema et al.1994; McCann & Solomon, 2000). As a result, significant disruptions in the electron transport chains and/or the process of phosphorylation uncoupling may occur (Aksmann &Tukaj, 2008; Aksmann et al. 2011). An abundance of intracellular ROS can result in the oxidation of macromolecules, such as lipids, proteins, and DNA bases (Meewes et al. 2001). This oxidative process can lead to damage in cell membranes, mitochondria, and chloroplasts, ultimately inhibiting photosynthesis, physiological activities, and cell growth. In response to the assault of ROS, cells have created enzyme systems that act as antioxidant defense mechanisms to eliminate ROS (Takáčová et al. 2014).

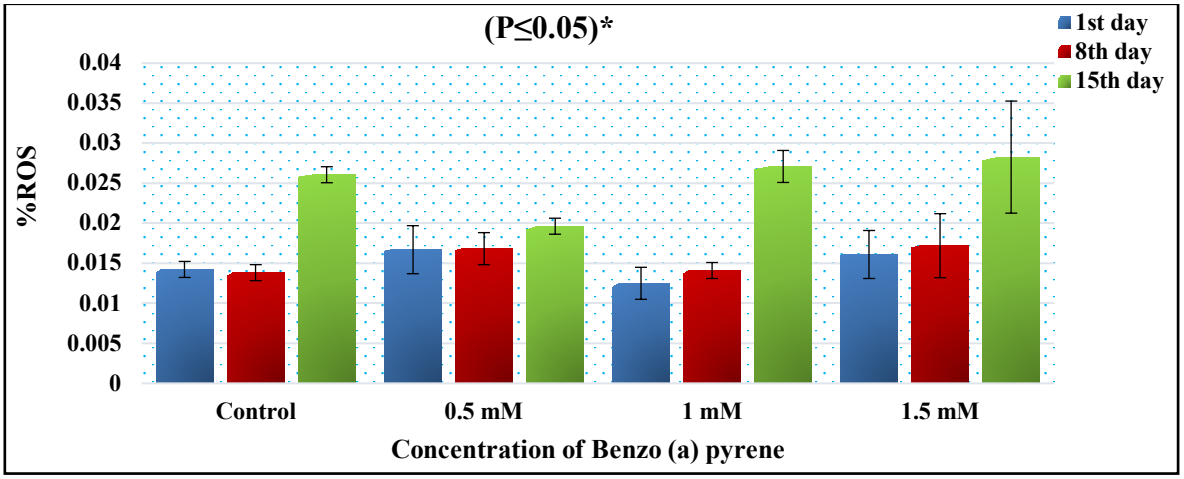

Figure 5: ROS amount of *S. quadricauda* at various concentration of Benzo [a] pyrene .

4. Carbohydrate

Carbohydrate is synthesized by photosynthetic organisms like algae and necessary to obtaind energy. All Environmental toxicants have effects on the content of carbohydrate in algae by reduced carbohydrate synthesis, perhaps stress also increase respiration which then used up the carbohydrates that was synthesized previously (Mustafa, 2013). The carbohydrate amount of 12.25 µm/ml is recorded at 1.5 mM on the 15th day. The amount of Benzo [a] pyrene is 15.52 µm/ml and 16.30 µm/ml for 0.5 mM and 1 mM, respectively. The greatest amount of 35.13 µm/ml is seen on the 1st day with 1 mM (Fig.6). Both Nagajyoti, et al. (2009) and Abdul Razak (1985) demonstrated that the decrease in carbs may be attributed to the suppression of Ribulose

Bisphosphate carboxylase (RUBP) activity, leading to a fall in carbohydrate levels. RUBP carboxylase is the predominant enzyme in photosynthesis and plays a crucial role in assimilating carbohydrates and regulating carbohydrate metabolism. It is involved in important metabolic processes including fixation of carbon and glycolysis (Barsanti and Gualtieri, 2006). The toxicant can hinder the process of glucose oxidation metabolism, which is necessary for obtaining ATP and reducing metabolic activity inside the cell (Greenhaff et al. 2004). From the findings of this study, the treatment of *S.qudricauda* with various Benzo [a] pyrene concentration caused significant decrease in total carbohydrate content with increasing concentrations of Benzo [a] pyrene.

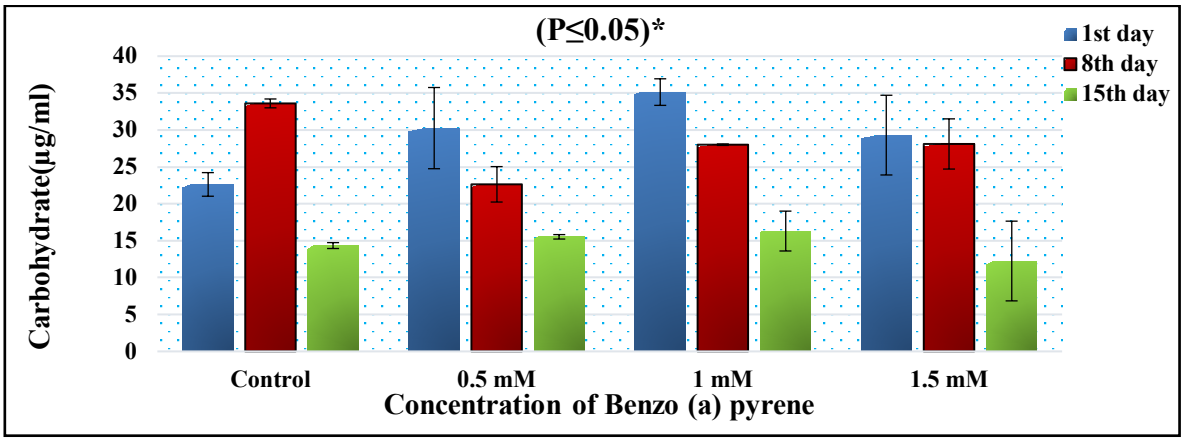

Figure 6: carbohydrate amount of *S. quadricauda* at various concentration of Benzo [a] pyrene .

5. Total protein

Benzo [a] pyrene stress has effect on the content of proteins in all treatments, it causes decrease in total protein with increasing amounts of Benzo [a] pyrene . The max protein content 2.14µg/ml throughout 8th day is record at 1mM , while the min protein content 1.83 mM is recorded at 1.5mM amount throughout 8th day (Fig.7). The reduction in protein synthesis can be attributed to the inhibition of enzymes and structural proteins necessary for the organism's growth (Kapoor & Arora 1996). Additionally, Carfagna et al. (2013) suggest that the decrease in protein content may also be caused by a shortage of carbon skeleton resulting from a low photosynthetic rate. Sub-lethal doses of PAHs may disrupt cell division by inhibiting DNA synthesis, lowering the proportion of cells entering mitosis, and subsequently limiting population expansion and protein production (Carfagna et al. 2013; Chia et al. 2015).

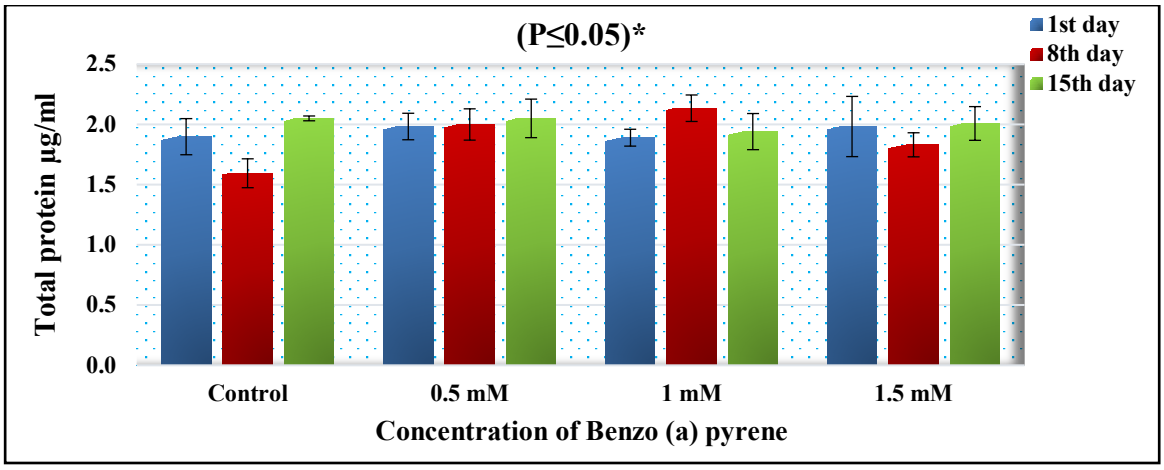

Figure 7: Total protein amount of *S. quadricauda* at various concentrations of Benzo [a] pyrene.

6.Superoxide dismutase (SOD)

In the present study, it can be observed clear effect of various Benzo [a] pyrene amount in the activity of the enzyme (SOD) in alga *S.qudricauda* and indicate to the significant variance ($p \leq 0.05$) between the treatments and control. There were increasing in content SOD at Benzo [a] pyrene 60 µg/ml in amount 1.5 mM throughout 8th day, compared with control (26.66µg/ml). where the lowest magnitude at (0.5 mM) was 22.22 µg/ml throughout 1st day (Fig.8). When the quantity of reactive oxygen species (ROS) in microalgal cells becomes very high, the cells' capacity to eliminate ROS by creating sufficient amounts of oxidative response kinases, such as SOD, is compromised. Prior research by Papadimitriou and Loumbourdis (2002) indicated that some species may counteract oxidative stress by boosting the synthesis of SOD to remove excess ROS. SOD is a crucial defensive mechanism that regulates the levels of free radicals. It is recognized as the primary defense against ROS in live cells. The elevated level of SOD activity leads to the increased breakdown of harmful ROS, such as the superoxide radical (O^-_2), into a less toxic ROS, such as hydrogen peroxide (H_2O_2). This hydrogen peroxide may then be further reduced by the heightened activity of glutathione reductase (GR), as seen in the study by Samanta et al. (2019). However, the decreased functioning of SOD in the presence of high amounts of PAHs is probably due to the excessive accumulation of H_2O_2(Binark et al. 2000).

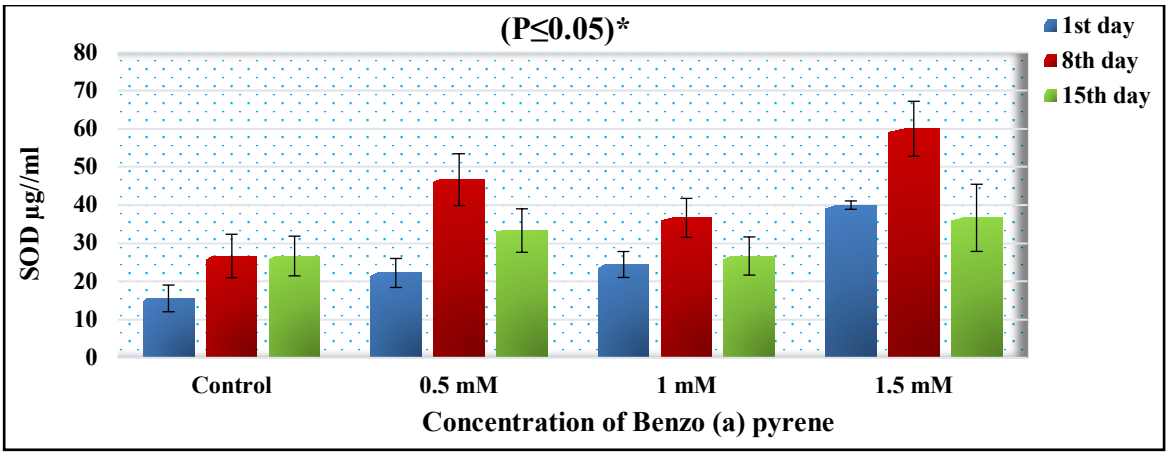

Figure 8: SOD amount of *S. quadricauda* at various concentrationof Benzo [a] pyrene.

7. Catalase (CAT)

In the current research, it can be observed clear effect of various Benzo [a] pyrene amount in the activity of the enzyme (CAT) in alga *S.qudricauda* and indicate to the significant variance ($p \leq 0.05$) between the control and treatments. There were increasing in content catalase at Benzo [a] pyrene 73.33 µg/ml in concentration 1 mM during 15th day, compared with control (10µg/ml). where the lowest magnitude at (1 mM) was 13.33 µg/ml throughout 8th and 15th day (Fig.9). Like other living creatures, algae create several ROS as a result of oxidative metabolism, such as H_2O_2,which may be harmful at high quantities (Torres et al. 2010). The formation of these ROS is sluggish under typical circumstances; nevertheless, contamination, especially PAHs, enhances their creation (Binark et al. 2000; Torres et al. 2010). CAT is an important antioxidant enzyme that plays a crucial role in regulating the redox state inside cells. Its primary function is to protect cells from damage caused by peroxidation. This has been demonstrated in studies conducted by Cheng et al. (2016) and Hassan et al. (2017, 2018).The enzyme catalase (CAT) may mitigate the harmful effects of hydrogen peroxide (H_2O_2) by facilitating its transformation into water (H_2O) (Torres et al. 2010). The excessive generation of ROS may diminish the functioning of CAT, leading to oxidative stress (El Maghraby & Hassan, 2021).

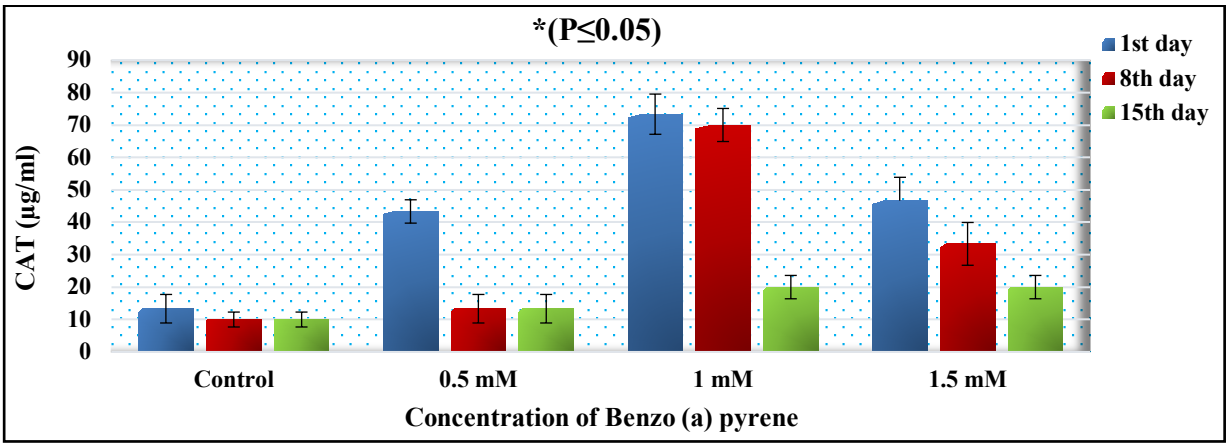

Figure 9: CAT amount of *S. quadricauda* at various amount of Benzo [a] pyrene .

8.Lipid peroxidation (Malondialdehyde MDA)

Lipids, being crucial constituents of the membrane, are the main target of ROS and result in lipid peroxidation by the extraction of hydrogen from the unsaturated fatty acid chain. Thus, lipid peroxidation plays a crucial role in causing cellular damage in living organisms experiencing oxidative stress. Lipid peroxidation generates many harmful compounds, including malondialdehyde (MDA) and aldehydes, from polyunsaturated fatty acids. MDA is a marker for oxidative damage. Algae possess chloroplasts that include a complex network of membranes rich in polyunsaturated fatty acids. These fatty acids are particularly susceptible to peroxidation (Garg and Manchanda, 2009; Halliwell and Chirico, 1993).

The findings study demonstrates a significant variance ($p \leq 0.05$) in the content of the MDA for the alga *S.qudricauda* after treatment, the content of MDA decreased at various amount Benzo [a] pyrene compared with control (34.14 µmol/l), the minimum value are 36.58, 39.02 and 42.50 µmol/l at concentration 1, 0.5 and 1.5 mM respectively, throughout 15th day (Fig.10). This may be due to stimulated antioxidant defense in algae was efficient in eliminating ROS, and inhibiting the algal cells from oxidative damage (Zhang et al. 2018).

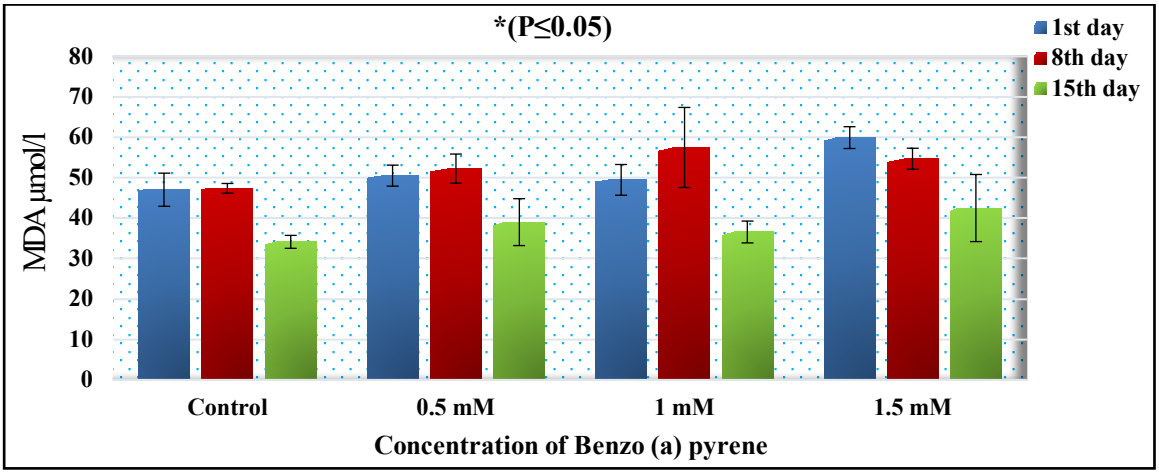

Figure10: MDA amount of *S. quadricauda* at various amount of Benzo [a] pyrene.

9. Bioremediation of Benzo [a] pyrene

Researchers have extensively investigated the capacity of certain types of algae to break down PAH (polycyclic aromatic hydrocarbon) compounds found in water due to their toxicity and carcinogenic properties. In a study conducted by Kumar et al. (2018), it was discovered that PAHs interact with cytochrome P450 monooxygenase CYP in the active sites of algae through intermolecular hydrogen bonding, hydrophobic bonding π–π interactions, and van der Waals interactions. These interactions enable algae to remediate PAHs more effectively than other microorganisms. The results showed the effect of different amounts of benzo[a]pyrene on the degradation of *S. quadricauda*, the highest value was 40.13 throughout 15th with 0.5 mM, while

the lowest value was 0 throughout 1st day with 0.5 and 1 mM (Fig.11), in the present study demonstrated *S.qudeicauda* have high ability to removed Benzo [a] pyrene after 1, 8 and 15 days of treatments. Ei-Sheekh et al. (2012) suggest that the chemical structure of the molecule and the physiological metabolism of the algae appear to determine how polycyclic and heterocyclic aromatic compounds break down under them. Apart from being ingested by cells or absorbed on the wall, the results of the study imply that algae broke down the compound's high removal efficiencies by several processes, including a reduction, oxidation, and the activation of enzymes including cytochrome P450, diphenol oxidase, and Peroxidase, Detoxifying B [a] P and breaking down these toxic chemicals depend critically on these enzyme systems (Takáčová et al. 2014).

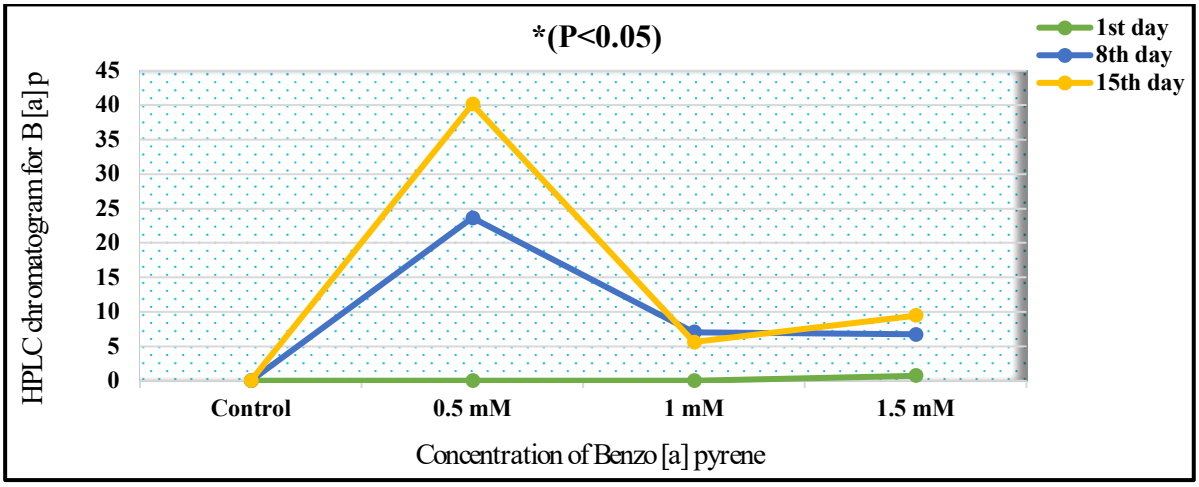

Figure11: HPLC Chromatogram for Benzo [a] pyrene standard at various concentration.

Conclusions:

Benzo [a] pyrene, a form of polycyclic aromatic hydrocarbon (PAH), may be efficiently removed from polluted water by use of the green alga *S.qudricauda* in the process of bioremediation. This results from harmless substance being produced all along the algal breakdown. The results of the investigation show that the alga shows great ability for BaP removal at low concentrations and that the pollution is cleared in a short time span. Algae showed biological reaction in some results of exposure to different biological parameters with different amount of B[a]P such as decreased amount, total protein, chlorophyll a and b, increased oxidative stress ROS and carbohydrates and enzyme activity, SOD, MDA and catalase.

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