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Estimation of Chlorophyll-a and Pheophytin-a in an East Euphrates Drainage Water

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ORIGINAL STUDY

Estimation of Chlorophyll-a and Pheophytin-a in an East Euphrates Drainage Water/(Al - Haffar) Al - Diwaniya/Iraq

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Abstract

This study was conducted at three sampling sites of Al − Haffar Drainage and was intended to estimate the spatial concentration of chlorophyll-a and pheophytin-a. For 6 months starting in December 2018 and lasting until May 2019. The air and water temperatures, light penetration, pH, electrical conductivity, dissolved oxygen, nitrate, and phosphate levels were measured. The results indicated a distinct spatial and temporal variation in the values of the variables that were analyzed. The third site reported an increase in air temperature and light penetration, while the second and third sites recorded the highest values of pH and electrical conductivity, and the third site also had values of dissolved oxygen that reached 8.9 mg/l. Nitrate concentrations were lowest in the third site (248 g/l), while phosphate concentrations were lowest in the second site. The results of the current study show a significant decrease in the values of chlorophyll-a and pheophytin-a over the last months at all sites.

Keywords: Chlorophyll-a, Pheophytin-a, Al-Haffar, East drainage water, Al-Diwaniyah

1. Introduction

Algae are one of the most crucial elements of aquatic ecosystems and have been used to assess the quality of water. Due to their nutritional requirements, rapid growth, and brief life cycles, the Chlorophyll content of the aquatic environment is greatly influenced by the spatial and temporal variation of algae [1]. Algal photosynthesis creates oxygen, which keeps the water from being anoxic, permits bacteria growth, and operates quickly in an aerobic environment [2,3].

Phytoplankton is considered the basic component of an aquatic food chain and also the source of oxygen and the main primary producer of all types of water bodies [4]. They are the basis of the nutrient cycle in ecosystems and play a major role in maintaining the balance between living organisms and abiotic factors [5]. Phytoplankton is productive organisms and is the basis of the aquatic system, so they are one of the most important components of any aquatic ecosystem. They solve many basic environmental problems by producing useful materials that are compatible with the aquatic ecosystem [6,7]. Due to its sensitivity to changes in the environment, phytoplankton is a good indicator for determining the environmental condition of surface water bodies [8,9]. This has been confirmed by several studies, including [10,11].

The most important pigments produced by microalgae are chlorophyll, carotene, and phycobilin, which are all employed in the food, pharmaceutical, and cosmetics industries [12,13]. One of the beneficial bioactive molecules is chlorophyll, Microalgae biomass can be used to extract this molecule, It was utilized as a natural food coloring for a long time and it is an anti-oxidant in nature [14].

Chlorophyll is a natural food coloring that is more expensive than commercial colorants. There are various forms of chlorophyll in plants, including chlorophyll a, b, and c that do not participate in photosynthesis, When chlorophyll is exposed to

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weak acids, oxygen, or light, it speeds up the oxidation process and produces a large number of degradation compounds [15,16].

Chlorophylls are green pigments with a porphyrin ring around which electrons can freely move. The ring can quickly receive or lose electrons, and thus the ability to give other molecules with energetic electrons This is the basic mechanism through which chlorophyll catches the light, the sun's light energy [17]. Chlorophyll-a is the most abundant natural pigment [18] and is present in all algae, so it is used in estimating the biomass of benthic algae [19].

Green plants have chlorophyll-a, a photosynthetic pigment that absorbs light energy and converts it to carbohydrates from water and carbon dioxide. Chlorophyll is a vital component of the photosynthetic process, which is responsible for green plants' light sustainability [13].

The macro porphyrin ring with four pyrrole rings makes up the framework of the chlorophyll molecule. The methyl group in the ring II of chlorophyll-a is replaced by a formyl group in chlorophyll-b [20]. For several reasons, such as chlorophyll-a role as an indicator to gauge the productivity of algae in lakes and oceans, photosynthesis pigments have piqued the interest of ecologists and hydrologists. In the cells they are contained in, pigments go through additional chemical transformations and ultimately oxidize into colorless substances. Physical, chemical, and biological elements are in charge of controlling these changes [21]. According to Refs. [22,23] the essential elements influencing the maintenance of pigments are the absence of light, low temperatures, and a lack of oxygen. Thus, the aim of the present study was to evaluate Chlorophyll-a, Pheophytin a with physical-chemical variables in an east Euphrates Drainage Water.

2. Materials and methods

2.1. Study area

The East Euphrates drainage (Al-Haffar) is an extension of the eastern Shamiya drainage, starting from the Al-Kifl district in the province of Babil and then joining the Diwaniyah governorate at the Al-Mahanawia district. It then continues on its track to Al-Shamiya district, deviates to Al-Shanafiya district, goes to Al Samawa governorate, reaches Dhi Qar governorate, and finishes there. The drain receives water from numerous agricultural regions that it passes through (Water Resources in Al-Shamiya district).

The study was conducted at three different locations. The first location is close to the village of Al Abtan on the road that connects Al Diwaniyah governorate to Al Najaf governorate, the second site is situated roughly 5 km to the south of the first site. While the third site is situated around 11 km to the south of the first site (Fig. 1).

2.2. Collection of water samples for physical-chemical analyzes

Field samples were collected for six months (from December 2018 to May 2019) and water samples were collected for physical and chemical analyzes in polyethylene containers of 2 L capacity and from 30 cm deep underwater in the morning. The temperature of water and Air, Electrical conductivity, and *pH*, have been measured in situ by conductivity meter, and pH meter. Dissolved Oxygen dissolved oxygen was measured using the Azide Modification of the Winkler Method, As shown in Ref. [24]. Furthermore, the cadmium reduction method was

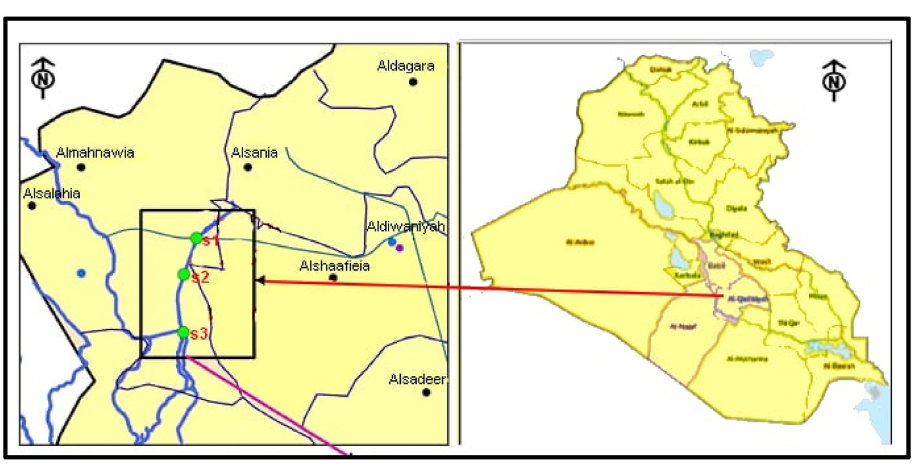


Fig. 1. The study sites.

applied according to Ref. [25] to measure the nitrate concentration. For assessing phosphate concentrations, the mixed solution method described In [24].

2.3. Chlorophyll-a and Pheophytin-a estimation

The sample (1000 ml) was filtered for the assessing chlorophyll-a and Pheophytin-a using the Millipore and filter paper with a diameter of 0.45 μm and a radius of (47) mm, (2) milliliters of magnesium carbonate solution (1 %) were added before the filtration process was complete to make the medium basal. After drying, the paper was ground using 8 ml of 90 % acetone gradually. After this step was finished, the samples were stored in the dark at a low temperature (4C) for 18−20 h. Then the sample is concentrated using a centrifuge for 15 min at a speed of 3000 rpm, after which the volume of the concentrated liquid is completed with acetone and the absorbance is read using acetone as blank at wavelengths (665) and (750) nm. A drop of (2N) HCl acid was added to the extract after the readings were completed, and it was left for (10) minutes before the absorbance also was measured at the previous wavelengths [26]. Lorenzen's equations shown in Ref. [27]were used to calculate the concentrations of chlorophyll-a and Pheophytin-a as below:

$$\mu g\ Chlorophyll\ a\ per\ sample = 11.9[2.43(Db - Da)] \quad (1)$$

$$\mu g\ pheophytin\ a\ per\ sample = 11.9\ (V/l)(1.7\ Da) - Chlorophyll\ a \quad (2)$$

3. Results and discussion

3.1. Physical-chemical properties

One of the main environmental factors is heat. It affects crucial processes and is necessary for the dissolution of elements and gases in the aquatic environment and to its importance for the existence of living things as well as its availability. The results show that there is a difference in air and water temperatures between the months Tables (1)−(3) explain that the third site had the highest air temperature, which was 36 °C while the first site had the coolest air, with a low of 11 °C, which may be attributed to the different sampling times during the day [28]. There is also a tiny difference across the study sites, which may be caused by their proximity to one another [5]. rightly observed that the water's temperature is influenced by the flow rate and the amount of sunlight exposure. Light penetration is related to suspended matter content, dissolved substance residue, and water purity [29]. The third site had the highest light penetration, measuring 60 cm, according to the results. The lowest degree of light penetration that the three sites measured in December were 20 cm. The strong light penetration could be a result of the low water level and lack of materials. According to the results of this study, 8 is the highest pH value in the second and third site in December however its decrease is based on an increase in carbon dioxide's solubility. May be due to increasing pH values when carbon dioxide levels rise as a result of photosynthesis [30].

Organic pollutants increase electrical conductivity because they carry electrical charges [31] salts and dissolved particles also can cause that [32]. The lowest level of water in the drainage may be the reason for the highest value of electrical conductivity, which is 4345 μs/cm, according to the data in Tables 1−3. It demonstrated that either there was a negative association between electrical conductivity and water level or that the draining activities carried out by washing the lands along the drainage increased the salt content of the water [33]. As oxygen enters water bodies through the gas exchange process or the photosynthesis process, it shows the health status and cleaning of such water bodies [32]. The third site recorded the highest dissolved oxygen value of 8.9 mg/l, while the first site recorded the lowest value of 7.5 mg/l. The increase in dissolved oxygen values is caused by phytoplankton While the rise in contaminants declines [30,32]. Most aquatic systems are rich in nitrates and phosphates, such enrichment may be natural due to agricultural

Table 1. Chemical-Physical analyzes of Site 1.

Factors	December	January	February	March	April	May
Air Temperature (°C)	17	11	14	26	25	30
Water Temperature (°C)	13	12	15	18	22	28
Light Penetration (cm)	20	40	45	47	49	49
pH	7.5	7.9	7.8	7.9	7.6	7.7
Electrical Conductivity (μs/cm)	3185	3198	3188	3195	3192	3220
Dissolved Oxygen (mg/l)	7.78	8.07	7.72	7.89	7.61	7.51
Nitrate (μg/l)	320	382	299	250	298	315
Phosphate (μg/l)	1.20	1.26	1.19	2.20	2.22	2.22

Table 2. Chemical-Physical analyzes of Site 2.

Factors	December	January	February	March	April	May
Air Temperature (°C)	18.5	15	22	29	30	35
Water Temperature (°C)	14	12.5	16	22	24	28
Light Penetration (cm)	20	33	50	48	38	41
pH	8	7.9	7.6	7.7	7.5	7.5
Electrical Conductivity (μs/cm)	3630	3750	4136	4282	4094	3170
Dissolved Oxygen (mg/l)	7.9	8.01	8.7	7.9	7.91	7.88
Nitrate (μg/l)	320	373	295	250	285	315
Phosphate (μg/l)	1.9	0.91	0.99	1.26	2.21	2.20

Table 3. Chemical-Physical analyzes of Site 3.

Factors	December	January	February	March	April	May
Air Temperature (°C)	19	13	22	29	30	36
Water Temperature (°C)	14	14	16	20	23	25
Light Penetration (cm)	20	28	38	40	58	60
pH	8	7.9	7.4	7.5	7.6	7.3
Electrical Conductivity (μs/cm)	3867	4065	3945	4345	3978	3900
Dissolved Oxygen (mg/l)	8.9	8.03	8	8.14	8.06	7.97
Nitrate (μg/l)	323	345	325	300	248	259
Phosphate (μg/l)	1.25	0.99	1.22	1.22	1.35	1.78

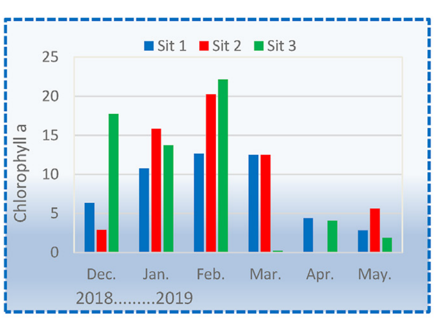
runoff or a consequence of contamination by domestic and industrial wastewaters [34]. The water body is disturbed by wind, which reduces light and increases nutrients. This means that the depth and size of the ecosystem affect the productivity of the algae [23]. It is evident from the results that nitrate and phosphate levels increased in the first site, which recorded the highest value of nitrates, which was 382 μg/l, and the highest level of phosphates, which was 2.22 μg/l. In comparison, the third site recorded the lowest value of nitrates, which was 248 μg/l, and the second site had the lowest level of phosphates, which was 0.91 μg/l. The increase in nitrate concentration may be caused by high oxygen levels, which enhance the conversion of nitrites to nitrates, or by rainfall which dissolves nitrate oxides present in the atmosphere by the nitrification process [30]. Rainfall also increased phosphate drift into the water bodies [35], While algae reduced the amount of phosphate and nitrate in the water by feeding on them also the adsorbing phosphate on clay particles may reduce it [36].

3.2. Chlorophyll-a and Phaeophytin-a

Chlorophyll is the basis of the ecosystem's energy. Plants, algae, and photosynthetic bacteria all contain this green pigment. It is essential to the process of photosynthesis because it converts CO_2 into carbohydrates, which are then converted into proteins, lipids, nucleic acids, and organic compounds. It also absorbs and converts solar energy into chemical energy [37].

Environmental variables, time, and the succession of species all have an impact on the pigment content of living algal communities. Changes in the percentage of pigments follow changes in the nutritional status [21,38]. While [39−41] pointed out that the chlorophyll-a concentrations are dependent upon many factors, such as water temperature, light level, nutrients, and the algal biomass and growth rate.

[42] refer that the quantity of algae present in the water directly relates to the concentration of Chlorophyll-a, Therefore, chlorophyll-a is mostly too



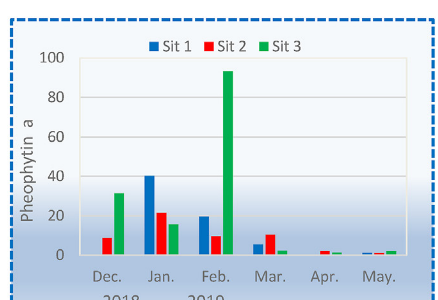
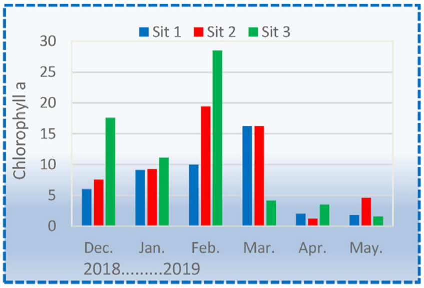


Fig. 2. Chlorophyll-a Pheophytin-a at three study sites at a wavelength of 665 nm.



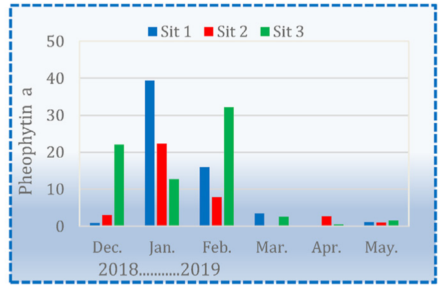


Fig. 3. Chlorophyll-a Pheophytin-a at three study sites at a wavelength of 750 nm.

responsible for the variance in impedance for various algae species concentrations in water. This indirect assessment of chlorophyll-a provided a successful determination of the species of algae [1] (see Fig. 2).

Fig. 3 show that there is a correlation between the levels of nitrate and chlorophyll-a The lack of chlorophyll-a in some months of the study may be caused by a lack of nutrients as [43] mentioned that the speed of algal blooms is slow during times when nitrates are low or in the presence of signs of food stress. (Increase in C: N and C: Chl.), The effect of nitrate deficit on the growth of the overall algal population results in an increase in the ratio of carotenoids to chlorophyll [21].

The most significant elements influencing the degradation of pigments, either directly or indirectly, include temperature, light intensity, and oxygen. These factors do this by influencing invertebrate communities and microorganisms that feed communities of algae [3,21,43].

Most grazing experiments and environmental pigment samples derived from grazed phytoplankton contained pheophytin a [44] because. These pigments are typically present in the grazers' feces and digestive tracts [45]. Moreover, Fig. 3 show that pheophytin-a values increased during the first three months of this study. The observed pigment patterns might be a result of rapid phytoplankton growth with grazing, which releases nutrients that support growth. Chlorophyll-a derivative would then be produced as a result of cell disruption brought on by grazing, and the concurrent rise in pheopigments (both pheophytin a and pheophorbide a) and decrease in Chlorophyll-a would be the result of herbivores ingesting algal cells. The confusion on grazing and declining physiological conditions, as well as the lack of additional data about zooplankton, make further interpreting of pigment patterns complicated [43].

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