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Carbon Sources and Riverine Algal Biomass: An Experimental Study

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ABSTRACT

A lotic ecosystem is considered a source of carbon dioxide (CO_2) in the atmosphere where it becomes supersaturated with CO_2, which contributes to the global carbon cycle. To enhance our comprehension of the roles of CO_2 in rivers, an outdoor experiment was designed with controlled carbon source inputs to investigate the roles of the dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) in the phytoplankton community. Plastic enclosures were installed in the Tigris River within Baghdad for that goal. Samples were collected on the first day, as well as on the 5th and the 12th days from 14 enclosures. The enclosures were treated by artificial glucose ($C_6H_{12}O_6$) (10, 20, 30mg/ l) as DOC sources, while sodium bicarbonate ($NaHCO_3$) (10, 20, 30µM) was used as a DIC source. The results showed that the concentration of nitrate (NO_3^-) and phosphate (PO_4^{3-}) changed over time and weren't affected by the treatments. On the other hand pH, DOC, and CO_2 concentrations were affected by treatments. Moreover, our results indicated that DOC and DIC treatments had a direct impact on phytoplankton biomass growth via increasing chlorophyll (Chl) concentration. Overall, it was concluded that different carbon sources (DOC and CO_2) could be essential factors that shape river ecosystems function through influencing the base of food webs.

INTRODUCTION

Photosynthetic organisms hold a pivotal role in the earth ecosystems, particularly in aquatic environments. These minuscule organisms serve as the primary producers in aquatic food chains due to their ability to harness sunlight through the process of photosynthesis and convert inorganic compounds into organic matter **(Utami *et al.*, 2021)**. They are not only a primary source of nutrition for a myriad of zooplankton, small fish, and filter-feeding organismsbut also influence the distribution and abundance of higher trophic levels, including larger fish, marine mammals, and seabirds **(Fu *et al.*, 2020)**. For that reason, many researchers are concerned with studying phytoplankton communities in different Iraqi aquatic ecosystems **(Al-Magdamy, 2019; Al-Saeedi & Salman, 2022; Hassan *et al.*, 2023; Jalal, 2023**). Phytoplankton communities are shaped by a complex interplay of environmental factors, including temperature, light availability, nutrient concentrations, dissolved oxygen, and pH. Therefore, several researchers focused on the correlation between environmental variables and phytoplankton communities **(EL-Sheekh *et al*., 2010; Bergström & Karlsson, 2019; Zhang *et al.*, 2019; Xu *et al.*, 2022; Wahhab & Hassan, 2023)**. The study of phytoplankton also extends to the intricate taxonomy and physiology of various species as different groups exhibit a wide range of adaptations to their respective environments, their life cycles, growth rates, and responses to changing conditions, providing critical insights into the dynamics of aquatic ecosystems **(Albueajee *et al.*, 2020)**. **Weiss *et al*. (2018)** reported that different aquatic ecosystems have undergone a deficiency of carbon sources. Phytoplankton are important in global biogeochemical cycles related to carbon release or sequester in an aquatic ecosystem. Environmental changes have a direct impact on phytoplankton communities through increasing carbon sources in aquatic ecosystems **(Beardall *et al.*, 2009)**. Different carbon sources (DOC and DIC) can originate from water within or outside the water body. Autochthonous DOC primarily stems from aquatic plants or algae, whereas allochthonous DOC originates outside the water body, usually coming from soils or terrestrial plants **(Reitsema *et al.*, 2018)**.

Allocthonous DIC, especially CO_2, can enter aquatic ecosystems through atmospheric diffusion, weathering of rocks and minerals, as well as deforestation and land-use changes **(de Araújo *et al.*, 2019; Nisha *et al.*, 2021; Cao *et al.*, 2023)**. In contrast, autochthonous CO_2 is generated within the water body, arising from activities such as respiration and the mineralization of DOC, where DOC is the main indirect source of CO_2 **(Kadhim & Hamdan, 2023)**.

In Iraqi inland aquatic ecosystems, many researchers focused on the study of the influences of environmental variables on phytoplankton communities **(Hassan *et al.*, 2011; Jaffer *et al.*, 2023)**, and others addresse**d** phytoplankton as a biological indicator for the assessment of water quality **(Bakaeva *et al.*, 2021)**. otably, there is a scarcity of studies related to carbon sources and their impacts on Iraqi aquatic ecosystems. Hence, our study focused on the role of carbon sources in aquatic ecosystems by studying their influences on phytoplankton biomass growth.

MATERIALS AND METHODS

The experiment desecration

To elucidate the roles of various carbon sources and their impact on phytoplankton biomass, we conducted an experiment mirroring the environmental conditions of the Tigris River. The experiment commenced on April 5, 2023 and ran for a duration of 12 days. Clear plastic enclosures were fixed with special bases at the water surface (Fig. 1) and filled each with seven liters of river water. The enclosures were treated with organic (glucose; 10, 20, and 30mg/ L) and inorganic carbon (sodium bicarbonate; 10, 20, and 30µM). Glucose was used as an indirect CO_2 source (DOC), while sodium bicarbonate ($NaHO_3$) was considered as a direct CO_2 source. These materials were added in triplicates for each concentration compared to the control.

(a) (b)

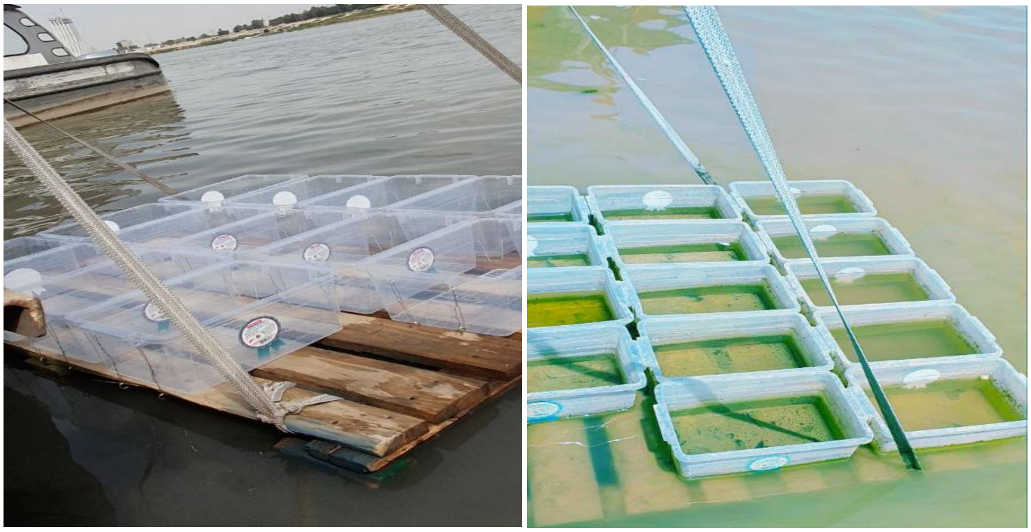

Fig. 1. Photos of *in situ* experiment showing samples collection: **(a)** Before filling out water in the enclosures, **(b)** during the treatment period.

Field measurements

Field measurements adhering to standard methods were conducted for various parameters **(APHA, 2005)**. Al-Hanan portable pocket were used in field measurement of water temperature (WT) and pH. Initially, the device underwent calibration before being used. Subsequently, the probe was immersed in water, and the recorded result was noted once the reading was stabilized.

Estimating NO_3 levels following procedure **(APHA, 1998)** involved taking a 50ml water sample, adding 1ml of HCl (1N), and measuring an absorbance at 220 and 275nm with a spectrophotometer. The results were expressed in milligrams per liter µg/ l.

For PO_4 measurement, the ascorbic acid method was employed **(Lind, 1979)**, in which 8ml of a compound solution was added to a 50ml filtered sample, forming a blue complex solution. The optical absorption of this solution at 860nm wavelength was measured, and results were expressed in µg/ l.

CO_2 concentrations were measured following the method which was approved by **Golterman (1978)** and adopted by **Hadi (1981)**. This involved titrating 100ml of water sample with 0.2N sodium carbonate until pH reached 8.4, then titrating another 100ml with 0.1N hydrochloric acid until pH equaled 4.2. The total CO_2 was calculated using the equation (1):

$$X = ((A + B) * 4.4) \qquad (1)$$

Where, A is the volume of titration with 0.2N Na_2CO_3, and B is the volume of titration with 0.1N HCl. The final CO_2 value was obtained by multiplying X by 10 and expressed in mg/ L.

Laboratory measurements

The water samples were transferred directly to the laboratory for analysis immediately after collection. For DOC measuring, we obtained a water sample and adjusted the pH to 2.0 by adding H_2SO_4 to eliminate particulate organic carbon. The filtered sample, using a 0.45 Millipore filter in the field, was then transported to the laboratory for chemical oxygen demand (COD) determination. In the lab, a digestion solution comprising distilled water, potassium dichromate, sulfuric acid, and mercuric sulfate was prepared. An acid reagent containing H_2SO_4 and silver sulfate was also prepared. Combining these solutions with 2ml of the sample, we subjected the mixture to 120 minutes of digestion at 150°C in a tube. The result was read on a color meter and expressed as mg/ l. The COD result was later input into the plutocalc water program, selecting total organic carbon (TOC), which is equivalent to DOC since it was initially filtered as described in **Williams *et al.* (2010)**.

To determine phytoplankton biomass, we followed the method outlined in **Vollenweider (1969)**. This involved filtering 1000ml of the water sample through a 0.45µm pore size filter paper using a vacuum. The filtered material was wrapped in cellophane paper and allowed to dry at 20°C. Subsequently, it was soaked in 6ml of 90% acetone and ground in a dark environment using a grinding bowl. The resulting extract was transferred to a test tube, and the grinding container was rinsed with 2ml of acetone, which was then added to the test tube. The sealed tube was kept in the dark at 4°C for 18-20 hours, with periodic shaking. After 24 hours, the extract was shaken again and concentrated by centrifugation at 3000 cycles for 30 minutes. The concentrated liquid was transferred to a test tube, and the volume was adjusted to 10ml with acetone. Readings were taken at wavelengths of 665 and 750. Following the addition of 2ml of 2N HCL, readings were repeated at the same wavelengths.

The Chl concentrations were determined using a specific equation (2):

$$11.9\ [2.43\ (Db - Da)]\ (V/L) \qquad (2)$$

Where, Da = The optical density of the Chl extract after the addition of acid

Db = Optical density of Chl extract before adding the acid.

V = The volume of acetone utilized in the extraction process.

L = Photocell length (cm). The results are expressed in µg/ l.

Statistics analysis

For statistical analysis, repeated measured ANOVA was used to estimate the differences of variables with time and with different treatment concentrations.

RESULTS AND DISCUSSION

The findings revealed that parameters such as WT, NO_3, and PO_4, exhibited temporal changes without detecting any interaction effects between treatments and time, while remaining unaffected by DOC and DIC treatments (Suppl. 1, 2), (Fig. 2). Conversely, pH, DOC, and CO_2 were influenced by DOC and DIC treatments and exhibited changes over time (Tables 1, 2 & Fig.2).

Table 1. The statistical outcomes of the repeated measures ANOVA (influence of DOC treatment on physical and chemical variables)

Factor	Stat.value	DOC input	Time	DOC * time
Water temperature	F	$0.0001_{(3,23)}$	$8616.5_{(2,23)}$	$1.83_{(6,23)}$
	P	1	<0.0001	0.17
pH	F	$83.95_{(3,23)}$	$326.71_{(2,23)}$	$0.80_{(6,23)}$
	P	<0.0001	<0.0001	0.58
NO_3^{-1}	F	$1.17_{(3,23)}$	$9.52_{(2,23)}$	$1.54_{(6,23)}$
	P	0.06	0.003	0.24
PO_4^{-3}	F	$2.98_{(3,23)}$	$9.67_{(2,23)}$	$1.85_{(6,23)}$
	P	0.07	0.003	0.16
DOC	F	$349.97_{(3,23)}$	$904.43_{(2,23)}$	$66.27_{(6,23)}$
	P	<0.0001	<0.0001	<0.0001

Table 2. The statistical outcomes of the repeated measures ANOVA (influence of DIC treatment on physical and chemical variables)

Factor	Stat.value	DIC input	Time	DIC * time
Water temperature	F	$0.33_{(3,23)}$	$6543.37_{(2,23)}$	$0.70_{(6,23)}$
	P	0.08	<0.0001	0.64
pH	F	$83.95_{(3,23)}$	$326.71_{(2,23)}$	$0.80_{(6,23)}$
	P	<0.0001	<0.0001	0.58
NO_3^{-1}	F	$1.44_{(3,23)}$	$19.95_{(2,23)}$	$0.86_{(6,23)}$
	P	0.07	<0.0001	0.54
PO_4^{-3}	F	$2.73_{(3,23)}$	$9.96_{(2,23)}$	$2.39_{(6,23)}$
	P	0.08	0.009	0.09
DIC	F	$102.61_{(3,23)}$	$175.23_{(2,23)}$	$1.85_{(6,23)}$
	P	<0.0001	<0.0001	0.17

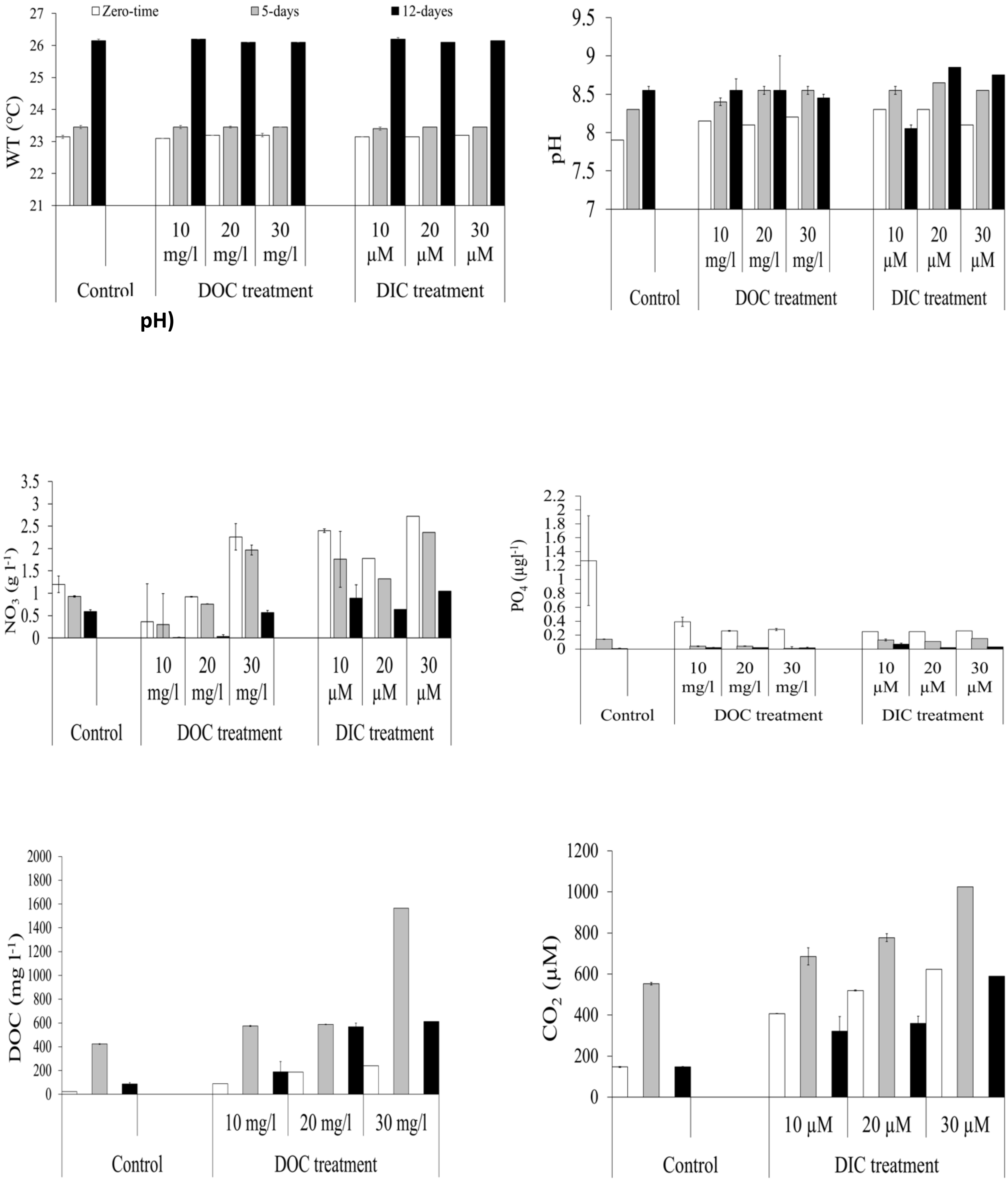

Fig. 2. Physicochemical parameters variations in the experiments of DOC and DIC treatments addition

The stability observed in water temperature and nutrient levels, contrasted with the alterations in DOC and CO_2 concentrations in response to treatments, can be attributed to the additives containing both organic and inorganic carbon. Their impact primarily increased the availability of DOC and CO_2.

Furthermore, our results demonstrated that pH varied with the added concentration of DIC. This variation can be attributed to the dissolved high amount of CO_2 in water, forming carbonic acid (H_2CO_3) through the reaction ($CO_2 + H_2O \rightarrow H_2CO_3$). Subsequently, carbonic acid dissociates into hydrogen ions (H+) and carbonate ions (CO_3^{-2}) ($H_2CO_3 \rightarrow 2H^+ + CO_3^{-2}$), leading to a decrease in pH levels **(Liu & Han, 2021)**.

The findings indicated temporal changes in phytoplankton biomass, coupled with direct effects of the treatments on their biomass (Tables 3, 4), as illustrated in Fig. (3). Notably, elevated DOC concentrations led to an increase in phytoplankton Chl concentration, and consequently, a rise in phytoplankton biomass. This effect can be attributed to glucose being a potent DOC source **(Brailsford *et al.*, 2019)**, and serving as an essential indirect source of CO_2, contributing to the elevation of Chl concentration and subsequently enhancing phytoplankton biomass **(Ali *et al*., 2018; Hamdan *et al.*, 2018; Hamdan *et al.*, 2021)**.

Table 3. Statistical results of repeated measured ANOVA (influence of DOC treatment on Phytoplankton)

Factor	Stat.value	DOC input	Time	DOC * time
Phytoplankton	F	$10.01_{(3,23)}$	$259.34_{(2,23)}$	$5.15_{(6,23)}$
	P	0.001	<0.0001	0.007

Table 4. Statistical results of repeated measured ANOVA (influence of DIC treatment on Phytoplankton)

Factor	Stat.value	DIC input	Time	DIC * time
Phytoplankton	F	$34.53_{(3,23)}$	$396.10_{(2,23)}$	$20.31_{(6,23)}$
	P	<0.0001***	<0.0001***	<0.0001***

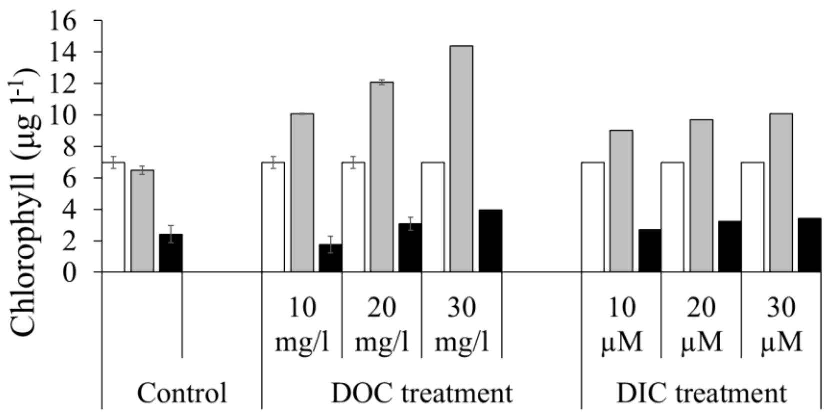

Fig. 3. Chlorophyll-a concentrations in different treatments of DOC and DIC addition

Furthermore, the study revealed a positive impact of inorganic carbon on phytoplankton biomass. Higher concentrations of sodium bicarbonate led to an increase in Chl content, as sodium carbonate serves as a commendable source of CO_2. Given that CO_2 is a crucial substrate for the photosynthetic enzyme, elevated CO_2 levels can stimulate the growth of phytoplankton and consequently enhance their biomass **(Ma & Wang, 2021, Hamdan *et al.*, 2022)**.

CONCLUSION

Overall, direct and indirect sources of CO_2 can play a pivotal role in shaping the aquatic environment by the direct impact of DOC and DIC treatments in stimulating phytoplankton biomass growth by rising Chl concentrations. Given the significant impact of carbon sources on phytoplankton biomass, we recommend maintaining ongoing monitoring of carbon levels, pH, and phytoplankton dynamics in aquatic ecosystems. This will provide insights into long-term trends and help detect any potential shifts in the community composition. Therefore, it is important to pay attention to investigating the impact of DOC and CO_2 on the benthic community to have a clearer picture of the carbon sources role in lotic ecosystems.

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