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Impact of Phosphorus Sources on Biomass and Pigments Content of *Spirulina platensis*

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ABSTRACT

Spirulina sp. is a photosynthetic blue-green microalga with high-quality bioactive compounds including pigments. This study was designed to investigate various phosphorus sources, such as Na2HPO4 and KH2PO4, addressing their impact on the biomass and pigments of *Spirulina platensis* using different concentrations. The highest biomass obtained was 2.75 and 1.98g at the concentration of 0.5g/ l for each source, respectively, which is lower compared to the control obtained from Zarrouk Medium. While, the results of chlorophyll a, total chlorophyll, and carotenoids were 25.47, 38.14, and 12.04, respectively, with 0.5g/ l KH2PO4 compared with the other source, Na2HPO4. Notably, the pigments tested in Zarrouk medium were the best compared to the other sources of phosphorus. It was concluded that carotenoids were not significantly affected by the change in phosphorus concentration. Interestingly, in this respect, it appeared that increasing the production of chlorophyll a resulted in an enhancement in biomass and total chlorophyll production in *Spirulina*. Chl a and total Chl concentrations were significantly different ($P< 0.05$).

INTRODUCTION

Spirulina, a non-toxic blue-green algae found in marine, freshwater, and alkaline lakes, was approved by the Food and Drug Administration in 1981 as a safe food **(Verne`s *et al.*, 2019)**. *Spirulina*, a highly cultivated microalga is well known for its valuable products **(Ghaeni *et al.*, 2015, Matos *et al.*, 2017; Shao *et al.*, 2019)**. It has nutraceutical benefits owing to its richness in dietary compounds including highly valuable proteins, carbohydrates, vitamins, minerals, polyphenolics, polyunsaturated fats, and bio-pigments as chlorophyll group, carotenoids, and allo-phycocyanin **(Shao *et al.*, 2019; Mohy El-Din, 2020)**. In addition, it includes vitamins, nutritional minerals, and vital amino acids **(Belay, 2008; Sharoba, 2014; Gutiérrez-Salmeán *et al.*, 2015)**. These bioactive compounds can be used in potential applications. **(Chandi & Gill, 2011; Dubini & Antal, 2015; Rizzo *et al.*, 2015)**. It can be used in various industries, such as cosmetics, pharmaceuticals, poultry, plant biological stimulants, foods, animal feed, and

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fertilizers (**Priyadarshani & Rath, 2012; El-Sheekh *et al*., 2014; Suganya *et al*., 2016; Soni *et al*., 2019)**. *Spirulina* has a balanced composition of all essential components, thus represents a complete food containing high quality protein content ranges from 50 to 70 percent of its dry biomass **(Falquet, 1997; Hosseini *et al*. 2013)**. Besides, it contains antioxidants which are essential for protecting the body from the free radicals, such as polyphenolics, flavonoids, and tocopherols **(Kumar *et al*., 2005; El-Baky *et al*., 2008, Chu *et al*., 2010; Michael *et al*., 2018)**.

Carotenoids are responsible for the red and yellow colors visible in nature **(Vonshak *et al*., 1996; Habib *et al*., 2008; Theodore & Georgios, 2013)**. Phosphorus stress impacts the efficiency of chloroplasts and decreases the process of photosynthesis **(Rolead *et al*., 2013)**. Phosphorus is a crucial element in algal growth and cell division, as it is a key component of DNA and RNA-phosphorylated sugar **(Roopnarain *et al*., 2014)**. Phosphorus is involved in algal growth, signal transduction, photosynthesis, and energy transfer through ATP and NADP **(Roopnarain *et al*., 2014; Yaakob *et al*., 2021)**. In microalgae, the fast reprocessing of phosphorus in the natural environments has led to obvious limitations in these organisms **(Alipanah *et al*., 2018)**. Microalgae vary in their phosphorus requirements and can uptake orthophosphate and polyphosphate as macronutrients **(Roopnarain *et al*., 2014; Yaakob *et al*., 2021)**.

MATERIALS AND METHODS

1. Microorganisms

Spirulina paltensis strain was collected from the Hydrobiology Lab, National Institute of Oceanography and Fisheries, Alqanater Elkhairya, Egypt, and cultivated in Zarrouk's medium.

2. Media

Spirulina paltensis was grown in Zarrouk medium, containing various elements and trace elements in distilled water. The pH was adjusted to 8.2 using a NaOH solution, as per Zarrouk's medium method **(Zarrouk, 1966)**. Two different phosphorus sources (KH_2PO_4 and Na_2HPO_4) with three levels (0.5, 1.0, and 1.5) g/l were used instead of K_2HPO_4 (the standard source of phosphorus) and tested on Zarrouk medium. *Spirulina paltensis* was maintained in 500-ml Erlenmeyer flasks containing 90% medium and 10% subculture. Conical flasks were cleaned and sterilized using a steam autoclave at 121°C for 20min **(Abou-El-Souod *et al*., 2016)**.

3. Growth measurement

a. Biomass yield (g/l)

Wet biomasses of *Spirulina paltensis* were taken at 3-day intervals for an 18-day incubation period. Wet biomasses were dried at 105°C for two hours, and their dry weights were estimated **(AOAC., 2000).**

b.Estimation of pigments

Chlorophyll group (mg/g dw)

Chlorophyll a and total Chlorophyll concentrations measured by spectrophotometry and calculated by using equations assessed in the study of **Seely *et al*. (1972)**.

Total carotenoids (mg/g dw)

Total carotenoids were determined by the spectrophotometric method at 450nm. A standard compound was used for preparing the calibration curve according to the **AQAS (1990)**.

RESULTS AND DISCUSSION

Table 1. Dry biomass yield (g/l) and pigments content (µg/g dry weight) of *Spirulina platensis* under different phosphorus sources

Biochemical compound	KH_2PO_4 concentration			Na_2HPO_4 concentration			Zarrouk medium (control)
	0.5 g L1	1.0 g L1	1.5 gL1	0.5 g L1	1.0 g L1	1.5 gL1	
Dry biomass	2.75	2.52	0.46	1.94	1.89	1.05	2.82
Chl a	25.47	16.87	11.77	25.3	14.71	14.28	26.54
Total Chl	37.04	27.38	19.64	36.26	19.03	13.39	37.23
Carotenoids	12.04	12.54	8.48	8.82	7.19	3.54	12.67

Biomass yield

The dry biomass yield (g/l) of *Spirulina platensis* under different phosphorus sources was compared to the control (Zarrouk medium), as shown in Table (1) and Figs. (1, 2). The results revealed that by using 0.5 g/l KH2PO4, the highest biomass yield was 2.75g compared to 2.82 g obtained from Zarrouk's medium. Upon using Na2HPO4 at levels of 0.5g/ l, the boimass yield was 1.94g compared to the control (2.82g/ l). Therefore, the Zarrouk medium is superior to the two phosphorus sources; however, when considering costs, it is better to use KH2PO4 rather than K2HPO4 in the Zarrouk medium. Additionally, 0.5g of the KH2PO4 compound should be used, while 2.5g is used in Zarrouk. Phosphorus concentration doesn't affect dry biomass, but phosphate limitation is linked to decreased productivity, cell division, and chlorophyll synthesis (**Khozin, 2006; Rocha, 2018)**. Researchers have confirmed that a phosphate level of 0.5g/ l is optimal for improving the biomass yield by *Spirulina platensis* due to a favorable N:P weight ratio. **(Costa *et al*., 2002; Radmann *et al*., 2007)**. Actually, **Bulgakov and Levich (1999)** discovered that the

N:P ratio of 5 to 10 is commonly found in cyanophyta-controlled communities, with optimal values varying between various algae such as *S. platensis* and *Scenedemus obliquus* **(Çelekli *et al.*, 2008)**. The present study found that the blue-green alga produced a higher biomass than that recorded in prior research on *Spirulina* **(Lodi *et al.*, 2005; Soletto *et al.*, 2005; Colla *et al.*, 2007**). The Tukey HSD comparison test revealed significant differences in biomass production at various phosphate concentrations upon increasing the concentrations, causing a decrease in biomass yield (Figs. 1, 2). The maximum ($P< 0.05$) biomass production was recorded at 2.75 & 0.5g/ l phosphorus (Fig. 1**). Raoof *et al.* (2006)** elucidated that, increasing phosphorus levels of 1.0–1.5g/ l in Zarrouk medium reduced the biomass yield of *S. platensis*.

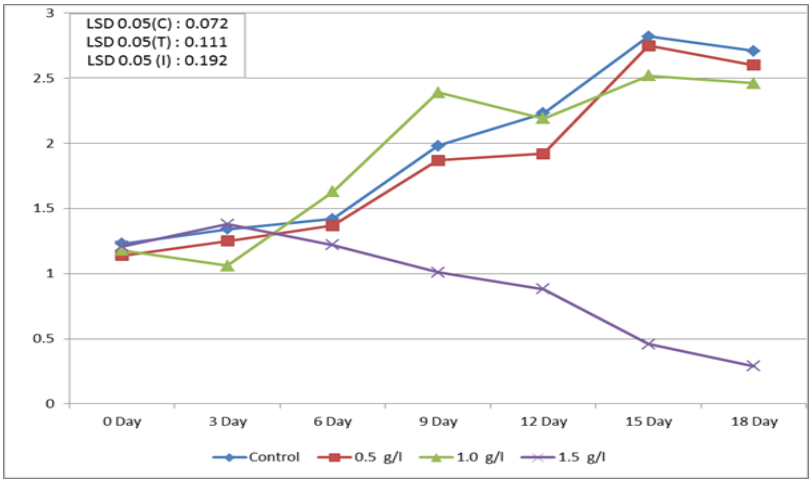

Fig. 1. Growth curves of *Spirulina platensis* dry weight grown on different levels of KH_2PO_4

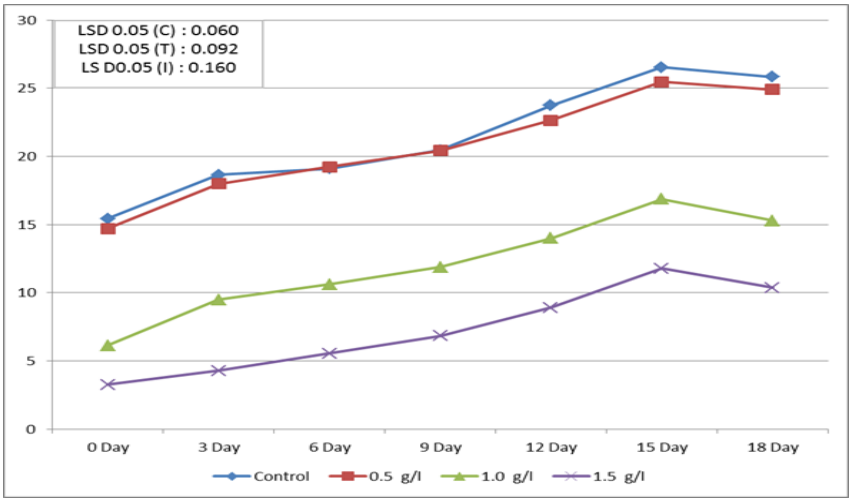

Fig. 2. Growth curves of *Spirulina platensis* dry weight grown on different levels of Na_2HPO_4

Pigments content

Table (1) shows the concentrations of Na2HPO4 addressed for the pigment production of the tested isolate. Chl a and total Chl were significantly higher when Na2HPO4 was used at a concentration of 0.5g/ L, while carotenoids were not significantly affected by the change in Na2HPO4 concentration. Similar findings were reported in the studies of **Pribyl *et al.* (2005)** and **Ontawong *et al.* (2013)**. In this respect, **Ikegaya *et al.* (2008)** reported that, upon increasing the production of Chl a, an increase was detected in the production of total Chl and biomass in *Spirulina* sp. The Chl a and total Chl concentrations were considerably (P<0.05) affected by altering the culture medium with the increase of Na2HPO4. Figs. (3- 10) show chlorophyll a, total chlorophylls, and carotenoids contents affected by phosphorus concentrations of 0.5, 1.0, and 1.5g/ l, respectively, during 18 days. The maximum production of Chl a, total Chl, and carotenoids was obtained by Zarrouk medium at 23.45, 35.08, and 12.01mg/ g, respectively, followed by the low levels of the two phosphorus sources. KH_2PO_4 as a source of phosphorus, recording the highest content of pigments at a level of 0.5g/ l, which followed the control treatment, where the levels of decrease were 25.3, 36.26, and 8.82mg/ g, giving lower values than the control, followed by Na_2HPO_4 at the same level for the previously mentioned pigments, respectively. The study found no significant difference in the pigment content at high phosphorus concentrations (P<0.05). Phosphorus concentration in *Azolla pinnata* blue-green alga reduced chlorophyll content while enhancing astaxanthin content in *Haematococcus pluvialis* green algae **(Brinda *et al.*, 2004; He *et al.*, 2007)**. Previous studies also showed that phosphorus is effective for algae carotenoids and chlorophyll production **(Latas, 1994; Buapet *et al.*, 2008)**. Phosphorus starvation has been reported to stimulate carotenoids and chlorophylls **(Subudhi, 1979; Brinda *et al.*, 2004; He *et al.*, 2007)**.

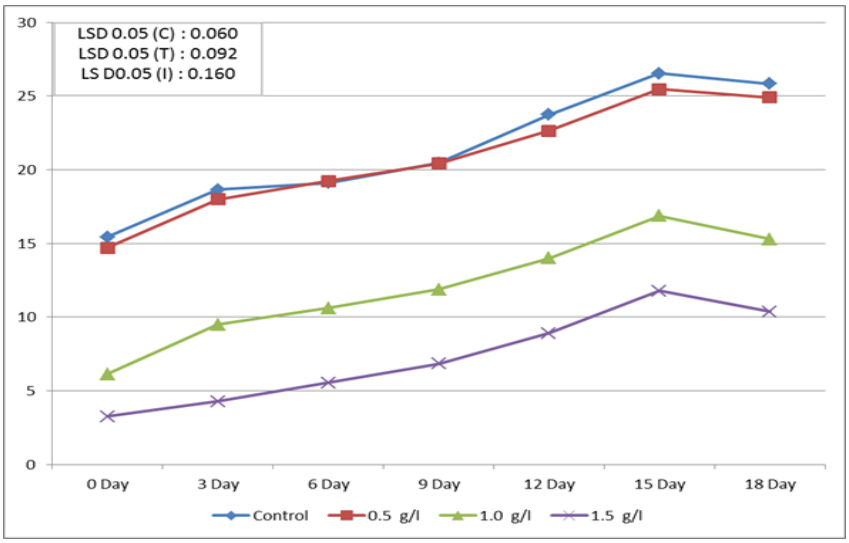

Fig. 3. Chl a content of *Spirulina platensis* in different concentration of KH_2PO_4

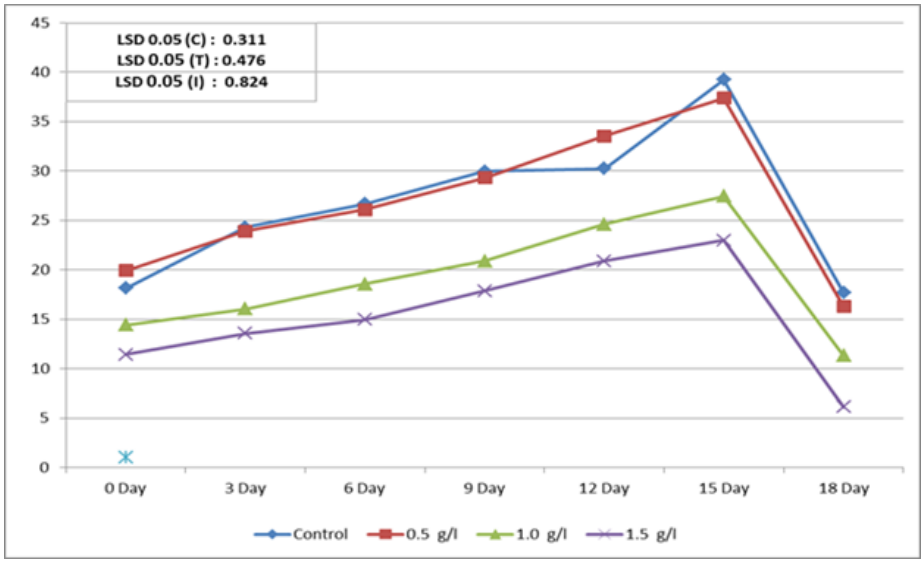

Fig.4. Total chlorophyll content of *Spirulina platensis* in different concentrations of KH_2PO_4

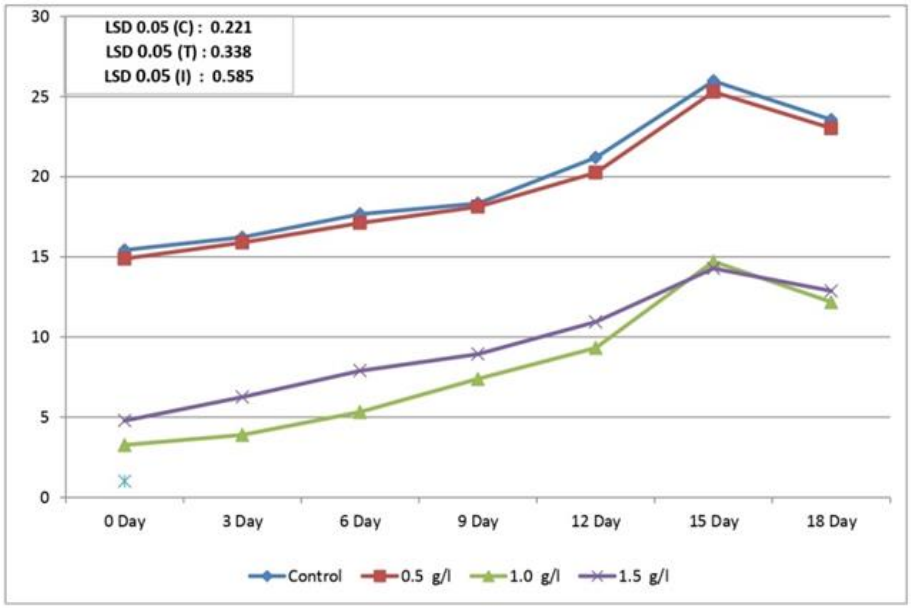

Fig.5.Chl a content of *Spirulina platensis* in different concentration of Na_2HPO_4.

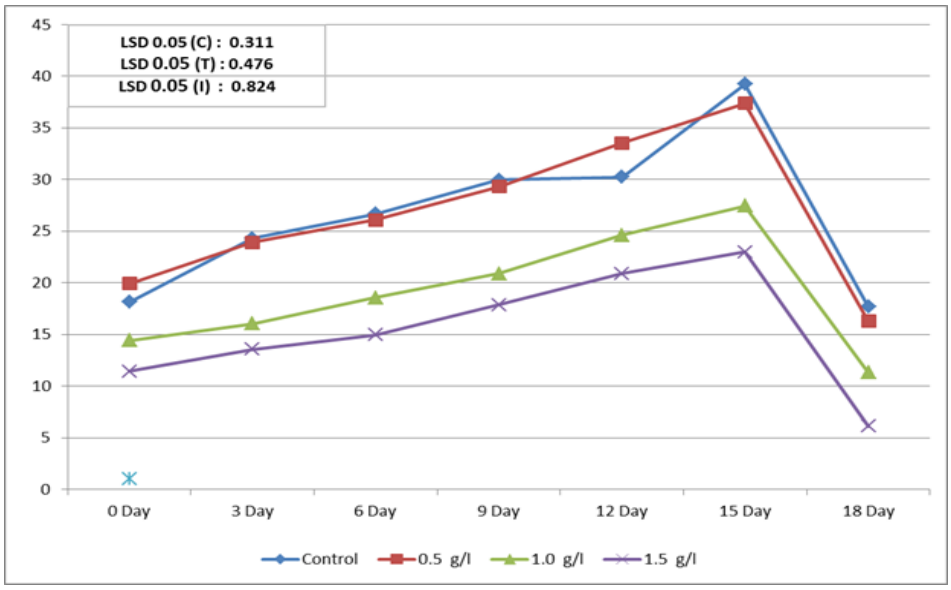

Fig. 6. Total chlorophyll content of *Spirulina platensis* in different concentrations of Na_2HPO_4

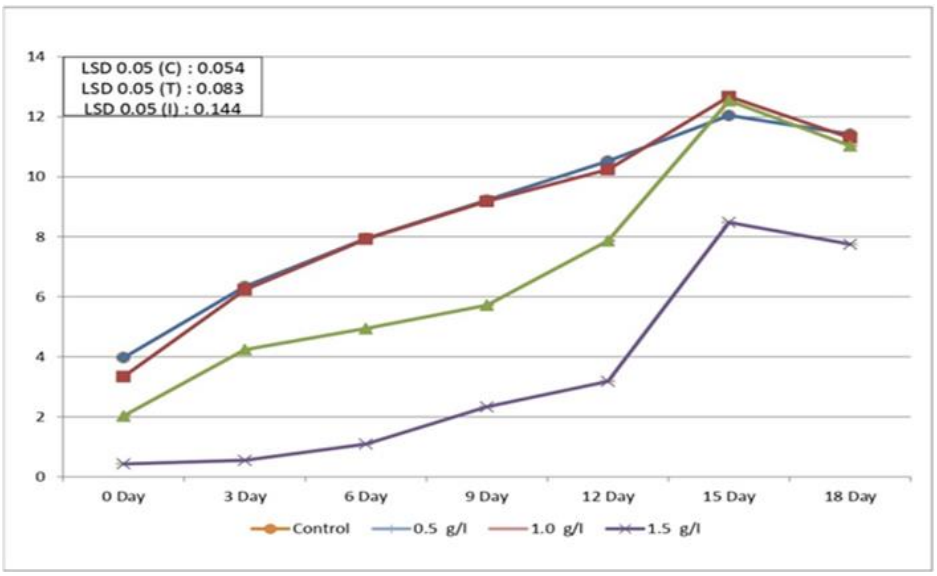

Fig.7. Carotenoids content of *Spirulina platensis* at three levels of KH_2PO_4.

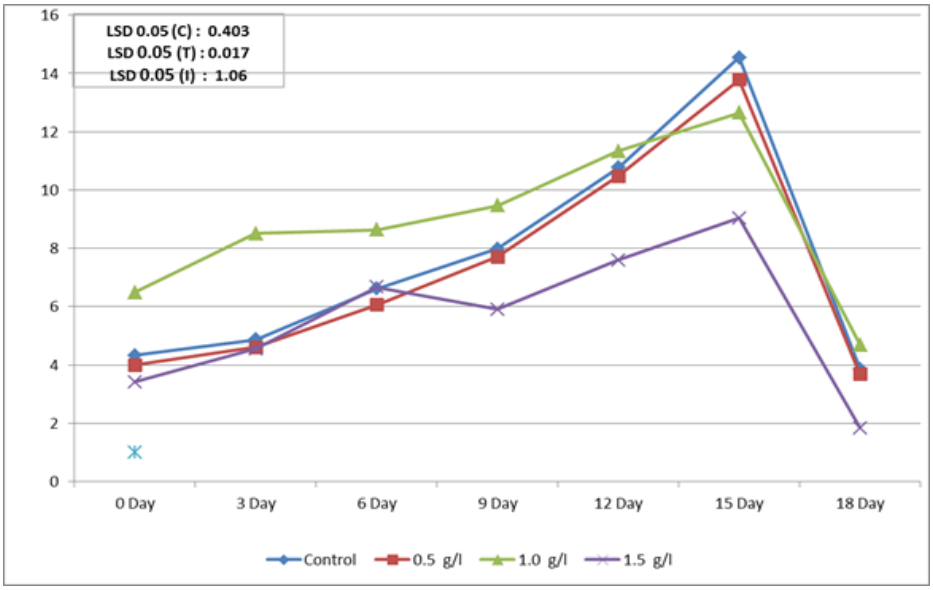

Fig. 8. Carotenoids content of *Spirulina platensis* at three levels of Na_2HPO_4

CONCLUSION

The study examined the impact of various phosphorus sources, cultivating modified Zarrouk medium, and the incubation period of 18 days on *S. platensis* biomass and pigment production. According to the study's results, 0.5g/ l of phosphate generated the greatest amount of biomass and pigments. The statistical analysis revealed that the cultivation period and phosphate concentrations were significant factors in the growth process. The phosphorus concentration (1.0–1.5 g/L) is considered sufficient to decrease total chlorophyll, carotenoids, and biomass yield by *S. platensis*. The results showed that S. platensis has the potential to produce biomass and pigments, depending on the optimum phosphorus concentration.

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