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Research Article
Bioavailability of Phosphate to Microalgae *Oscillatoria* sp. with Diffusive Gradient in Thin Film (DGT) Technique

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Abstract

Background and Objective: Phosphorus is one of the most important nutrients as a form of phosphate ion in the aquatic environment. The high concentration of phosphate caused the algae bloom. The objective of this study is to determine phosphate uptake by microalgae *Oscillatoria* sp. and influenced of optical density, salinity and concentration of the microalgae. **Material and Methods:** Technique diffusive gradient in thin film was used to measure phosphate concentration in solution and it related to phosphate uptake by microalgae *Oscillatoria* sp. using the spectrophotometric method. **Results:** The phosphate concentration diffused in the DGT unit related to phosphate uptake in microalgae *Oscillatoria* sp. The linear correlation between phosphate in DGT unit and phosphate uptake in *Oscillatoria* sp. with the salinity of 0, 15 and 30 g L^{-1} resulted in R^2 was 0.9820, 0.9449 and 0.9677, respectively. The effect of phosphate concentration by the DGT technique correlated significantly with uptake by *Oscillatoria* sp. The optical density, algae concentration and salinity were contributed to phosphate bioavailability in *Oscillatoria* sp. From this study, it can explore the equation to predict of phosphate uptake by *Oscillatoria* sp. by using $[P_{alga}] = \{(k \times OD)+f) \times \{P_{DGT}]\}$. **Conclusion:** The DGT technique was fairly predictive of bioavailability phosphate to microalgae *Oscillatoria* sp. Based on the phosphate concentration diffused in the DGT device, it can predict the phosphate concentration in algae cells without the destruction of algae cells. This is the new simple method for measuring bioavailability phosphate in the aquatic environment.

Key words: Phosphate bioavailability, salinity, DGT technique, microalgae, *Oscillatoria* sp.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Phosphorus is the primary nutrient causing eutrophication and contributes to toxic algae blooms in the aquatic environment[1,2]. One of the toxic algae found in the aquatic is *Oscillatoria* sp[3]. Bioavailability of phosphate for algae growth must be determined to control the algae bloom. The most bioavailable form of phosphate in solution as the soluble reactive phosphorus[4]. A standard method for measuring bioavailability of phosphate has not been universally established[5]. Several methods have been reported in the literature including, colorimetric method[4], sequential extraction[6] and radiolabeled P-33 isotop[7,8]. The phosphate uptake in microalgae is important to determine because it can control algae bloom in the aquatic system.

The technique of diffusive gradient in thin film (DGT) is a passive sampling device and it measures the time-average concentration of solutes at a deployment time[9]. The DGT device consists of a filter membrane placed between solution and the device, a diffusive film of fixed thickness and a trap binding gel enclosed by a plastic holder[10]. The DGT method has been introduced a method to test the intrinsic mobility and bioavailability contaminant including P[11-13]. In situ measurement of bioavailability phosphate could potentially be used as a method for predicting bioaccumulation by the aquatic organism. The DGT is a passive diffusion device that measures phosphate bioavailability[14]. The DGT technique has correlated well with metal is taken up by biota when uptake is sufficiently fast to be diffusion-limited and it is likely to best mimic bioavailability for uptake by biota[15]. Some of those studies were applied to the bioavailability of metal in soil[11,16] and bioavailability of phosphate in the soil[17-20].

Several studies have shown that DGT measured concentration correlates well with concentration accumulated within the plant[21]. The studied bioavailability of phosphate in aquatic for microalgae has not been reported in the last time. The correlation between the DGT technique and algae uptake for phosphate will be explored in this paper. The objective of this study was to the prediction of bioavailability of phosphate by the DGT technique for uptake algae *Oscillatoria* sp. with different salinity and concentration of the algae.

MATERIALS AND METHODS

Experimental site: Research work was carried out in analytical chemistry laboratory Politeknik AKA Bogor, Indonesia in July-December, 2018.

Material and research tool: The DGT devices were purchase from DGT research. Ltd. It consists of plastic molding, a diffusive gel of polyacrylamide, the binding gel of ferrihydrite and membrane 0.45 μm pore size. The chemicals were $NaNO_3$ (Merck), $NaHPO_4$ (Merck), K_2HPO_4 (Merck), NH_4Cl (Merck), Fe-EDTA (Merck), Mn-EDTA (Merck), Na_2-EDTA (Merck), $ZnSO_4$. 7 H_2O (Merck), $CoSO_4$.7 H_2O (Merck), Na_2MoO_4. 2 H_2O (Merck), $CuSO_4$. 5 H_2O (Merck), H_2SeO_3 (Merck), $MnCl_2$. 4 H_2O (Merck), thiamin-HCl, biotin, vitamin B12. Instrumentation of Spectrophotometer UV-Vis (Perkin Elmer).

Preparation of *Oscillatoria* sp.: Microalgae *Oscillatoria* sp. obtained from an Indonesian culture collection, LIPI Cibinong. It was grown at 25°C and pH adjusted to 8. The medium for alga growth consist of 200 mg $NaNO_3$, 1.4 mg $NaHPO_4$, 5 mg K_2HPO_4, 2.68 mg NH_4Cl, 5.2 mg Fe-EDTA, 0.332 mg Mn-EDTA, 37.2 mg Na_2-EDTA, 0.023 mg $ZnSO_4$.7 H_2O, 0.014 mg $CoSO_4$. 7 H_2O, 0.0073 mg Na_2MoO_4. 2 H_2O, 0.0025 mg $CuSO_4$.5 H_2O, 0.0017 mg H_2SeO_3, 0.2 mg tiamin-HCl, 0.0015 mg biotin, 0.0015 mg vitamin B12 dan 0.18 mg $MnCl_2$.4 H_2O in 1 L of seawater. The algae were harvest after 14 days of growth.

Phosphate uptake by *Oscillatoria* sp.: Uptake of phosphate by *Oscillatoria* sp. was done with different algae concentrations. This experiment was carried out with different of alga dosage, initial phosphate concentration dan salinity of medium. The *Oscillatoria* sp. was 0.25, 0.5, 0.75 and 1.0 g in 250 mL of solution. The phosphate concentrations in the range of 0.2-1.0 mg L^{-1} with the salinity of 15 and 30 g L^{-1}. *Oscillatoria* sp. was incubated in medium for 24 h. After 24 h the solution was centrifugated and phosphate in the supernatant was measured by spectrophotometric with the molybdenum blue method.

Phosphate accumulation using in situ DGT method: The principle of the DGT technique is the diffusion of analyte into a diffusive layer and accumulation of it in the binding gel. The concentration of analyte was determined after eluted it from the binding gel and phosphate dissolved was determined using the spectrophotometric method. The DGT device with the binding layer containing ferrihydrite, the diffusive layer consisted of a polyacrylamide hydrogel and protective of the cellulose nitrate filter membrane. Standard piston-type DGT holder with a 2 cm diameter exposure window purchased from DGT Research Ltd. This device consists of a plastic base, polyacrylamide hydrogel and ferrihydrite as a binding gel. A binding gel was placed on the bottom of the holder and was covered in order by a diffusive gel and cellulose nitrate filter

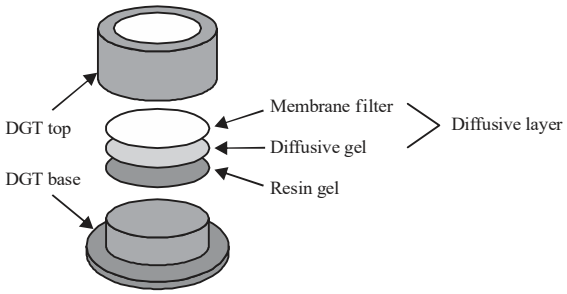

Fig. 1: Components of DGT device
Source: Yao *et al.*[22]

membrane. The DGT device was deployed in 250 mL of phosphate solution with a concentration of 0.2-1 mg L^{-1} for 24 h. The phosphate was eluted from binding gel using H_2SO_4 0.5 M. The DGT response was studied by the salinity of NaCl 0, 15 and 30 g L^{-1}. The phosphate concentration was measured by spectrophotometric with the molybdenum blue method.

Parameter measured: The DGT measured mass (M) of phosphate in the binding gel was calculated using the equation[22]:

$$M = Ce\ (V_{gel}+V_{acid}) \quad (1)$$

where, M is the mass of phosphate accumulated, Ce is the concentration of phosphate in acid, V_{gel} is the volume of gel and V_{acid} is volume acid for elution.

The phosphate concentration in DGT device was calculated in the equation[22]:

$$C_{DGT} = \frac{M\Delta g}{DtA} \quad (2)$$

where, C_{DGT} is the phosphate diffused to the DGT unit, M is the accumulated of phosphate (μg), A is the area exposure (cm^2) after a given time (t). The Δg was the thickness of gel membrane (cm) and D is the diffusion coefficient ($cm^2\ sec^{-1}$)[22]. The scheme of the DGT device can be seen in Fig. 1.

Statistical analysis: Experiments and analysis were conducted triplicate and represented as Mean±SD. Data were evaluated using the excel program and represented the linearity of the curve.

RESULTS

Relationship between algae concentration and optical density: Application of DGT technique to the prediction of phosphate bioavailability using phosphate concentration in a

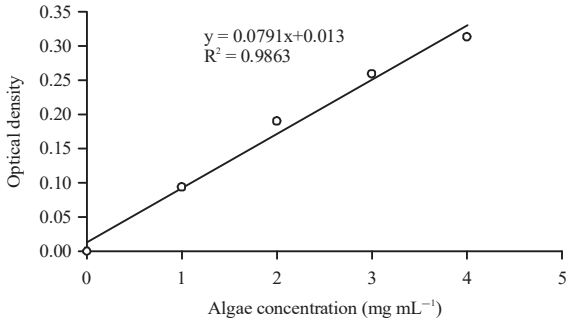

Fig. 2: Correlation between algae concentration and optical density

Table 1: Correlation between salinity and phosphate accumulation

Salinity (g L^{-1})	Equation	R^2
0	y = 9.7429x+0.2952	0.9887
15	y = 11.501x-0.4157	0.9883
30	y = 9.8629x+0.4352	0.9916

range of 0.2-1.0 mg L^{-1} in 250 mL of solution. The correlation between algae concentration and optical density was shown in Fig. 2. The optical density for *Oscillatoria* sp. in different concentration showed the concentration of 1 mg mL^{-1} (0.094±0.013), 2 mg mL^{-1} (0.190±0.018), 3 mg L^{-1} (0.259±0.017) and 4 mg L^{-1} (0.313±0.018). Based on Fig. 2 was resulted in the linear correlation between the increase of concentration and optical density with R^2 was 0.9863. Increased algae concentration caused an increase in optical density.

Phosphate accumulated in DGT unit: The capability of the DGT device to the uptake of phosphate in different salinity was shown in Fig. 3 and Table 1. Based on Fig. 3, it can be seen that the ability of the DGT device to accumulate phosphate in the binding gel in different salinity shows a linear correlation. In generally, salinity does not affect the absorption ability of the DGT device. From the experiment result, it can be stated that the DGT device can be used to determine phosphate with different salinity. The result showed a linear correlation with $R^2 = 0.9887$ for the salinity of 0 g L^{-1}, $R^2 = 0.9833$ for the salinity of 15 g L^{-1} and $R^2 = 0.9916$ for the salinity of 30 g L^{-1}. The ability of the DGT device to accumulate phosphate can be used to predict phosphate uptake by *Oscillatoria* sp. at different salinity conditions.

Relationship between phosphate diffused in DGT unit and phosphate in algae cell: The phosphate concentration diffused in the DGT unit related to phosphate concentration in algae cell. It showed in Fig. 4. There is a linear correlation between the concentration of phosphate in the DGT device and phosphate in *Oscillatoria* sp. The *Oscillatoria* sp.

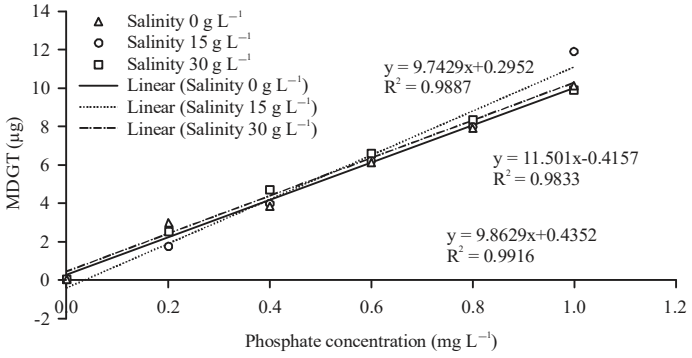

Fig. 3: Effect of salinity on phosphate accumulation in DGT device

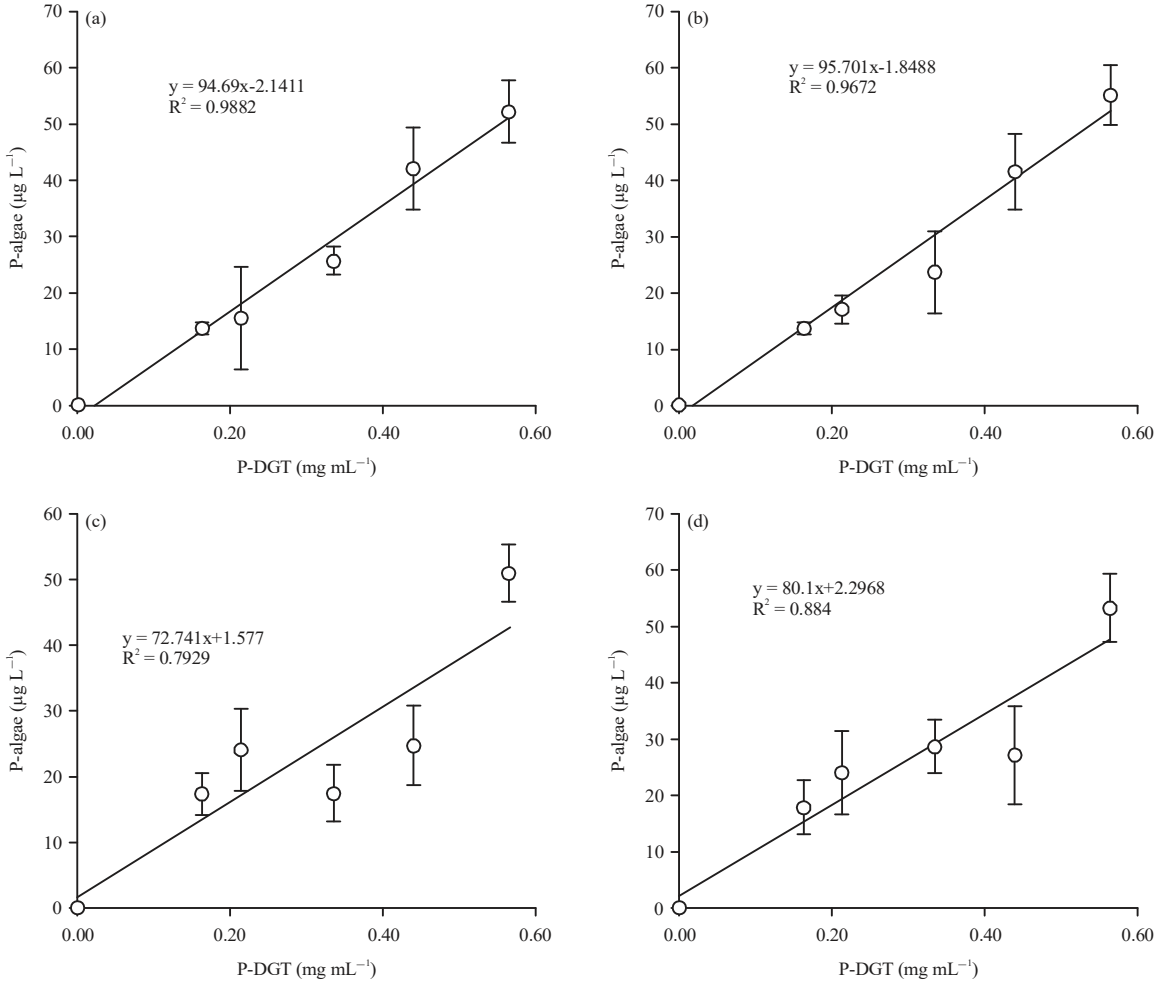

Fig. 4(a-d): Correlation between P-alga vs. P-DGT with different concentration of *Oscillatoria* sp. (a) 1 mg mL^{-1} (b) 2 mg mL^{-1} (c) 3 mg mL^{-1} and (d) 4 mg mL^{-1}

concentration of 1 and 2 mg mL^{-1} with R^2 = 0.9820 and 0.9672. At the algae concentrations of 3 and 4 mg mL^{-1}, the correlation values decreased to 0.7929 and 0.884. Based on the result, the DGT technique using ferrihydrite as the binding gel can be used to predict phosphate bioavailability in *Oscillatoria* sp. with R^2 = 0.9820.

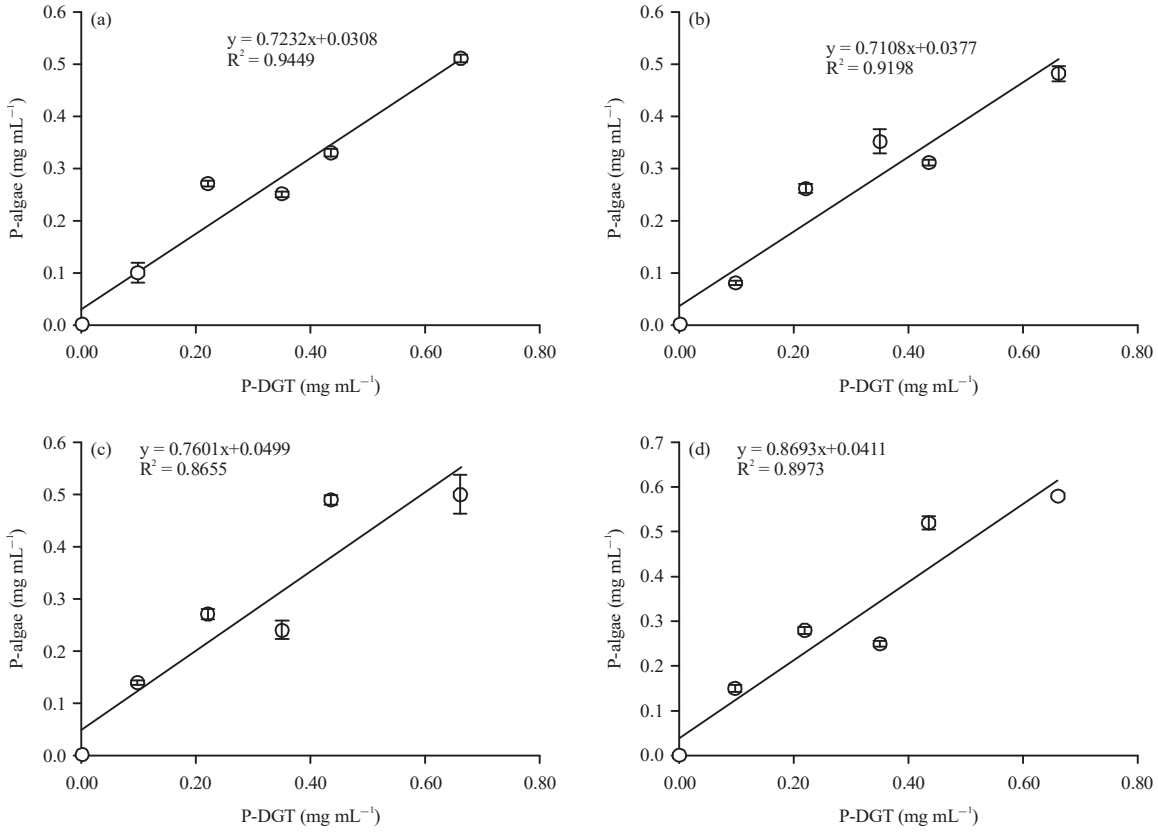

Fig. 5(a-d): Correlation between P-alga vs. P-DGT with different concentration of *Oscillatoria* sp. at salinity of 15 g L^{-1} (a) 1 mg mL^{-1}, (b) 2 mg mL^{-1}, (c) 3 mg mL^{-1} and (d) 4 mg mL^{-1}

Based on Fig. 5, it can be seen that the relationship between phosphate concentration diffuses into DGT with phosphate concentration uptake by *Oscillatoria* sp. with a salinity of 15 g L^{-1}. There is a linear correlation between phosphate in the DGT device and phosphate in *Oscillatoria* sp. The algae concentration of 1 and 2 mg mL^{-1} showed correlation value of 0.9449 and 0.9198. The concentration of *Oscillatoria* sp. at 3 and 4 mg mL^{-1} caused the decreased of correlation to 0.8655 and 0.8973. The DGT technique provides a very good correlation for the detection of bioavailability of phosphate in *Oscillatoria* sp. with 15 g L^{-1} salinity.

Figure 6 shows the relationship between phosphate concentrations that diffuse into DGT device and phosphate concentrations was absorbed by *Oscillatoria* sp. with the salinity of 30 g L^{-1}. There is a linear correlation between phosphate in the DGT device and phosphate in the *Oscillatoria* sp. The concentration of 1 and 2 mg mL^{-1} gives a correlation value of 0.9677 and 0.9181. The concentration of *Oscillatoria* sp. at 3 and 4 mg mL^{-1} shows the correlation value decreased to 0.8562 and 0.8946.

Prediction of phosphate uptake by DGT unit: The DGT technique can be used to predict phosphate available in algae *Oscillatoria* sp. The result was showed in Fig. 7. The salinity of the solution was 30 g L^{-1} which is seawater salinity inoculated *Oscillatoria* sp. algae habitat. The DGT technique can predict the bioavailability of phosphate through p-available by algae *Oscillatoria* sp. There is a linear correlation between algae density and the ratio [P-algae]/[P-DGT] with the correlation coefficient r = 0.9409 (R^2 = 0.8854) with the regression equation y = 0.971x+0.7457. The greater the optical density, the higher the ratio of phosphate concentrations uptake by algae to phosphate diffuse in the DGT unit. Based on the experiment, the phosphate concentration absorbed by the algae *Oscillatoria* sp. can be calculated based on the equation:

$$\frac{[P_{algae}]}{[P_{DGT}]} = k \times \text{Optical density} \quad (3)$$

$$\frac{[P_{algae}]}{[P_{DGT}]} = 0.971 \times \text{Optical density} \quad (4)$$

$$[P_{algae}] = k \times \text{Optical density} \times [P_{DGT}] \quad (5)$$

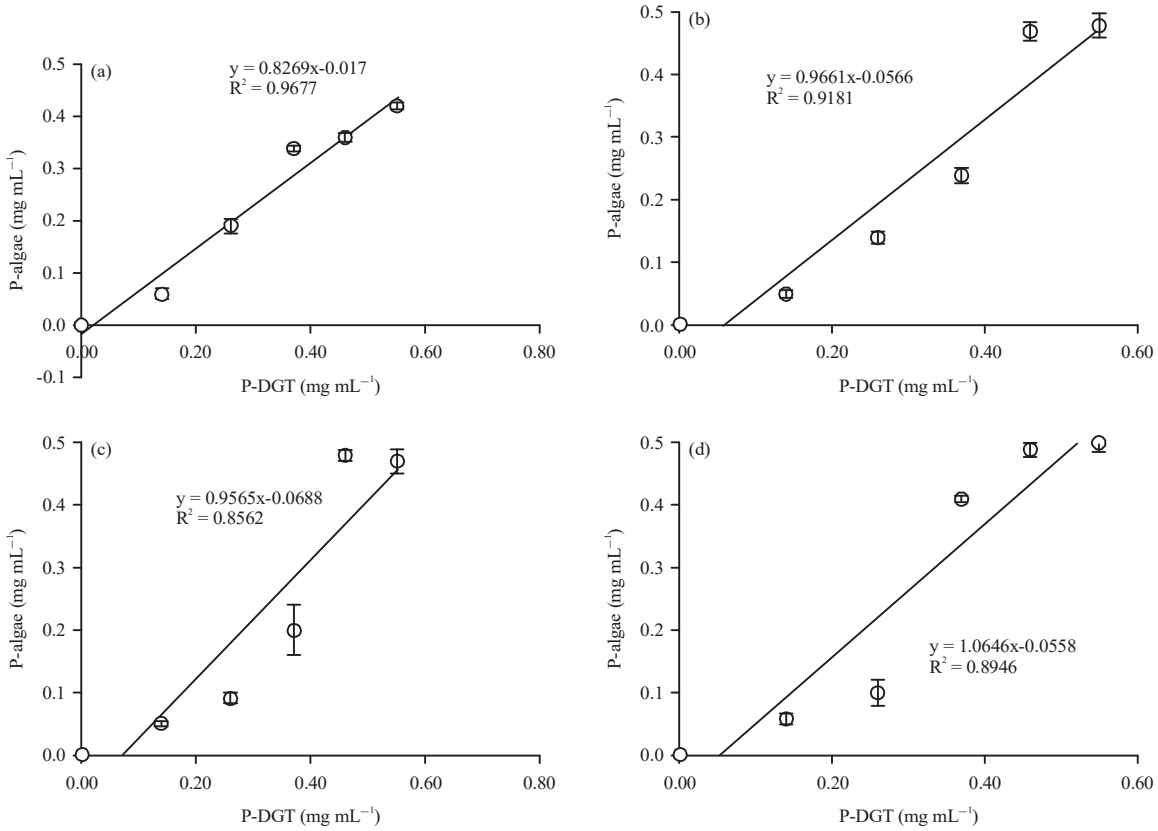

Fig. 6(a-d): Correlation between P-alga vs P-DGT with different concentration of *Oscillatoria* sp. with salinity of 30 g L^{-1}, (a) 1 mg mL^{-1}, (b) 2 mg mL^{-1}, (c) 3 mg mL^{-1} and (d) 4 mg mL^{-1}

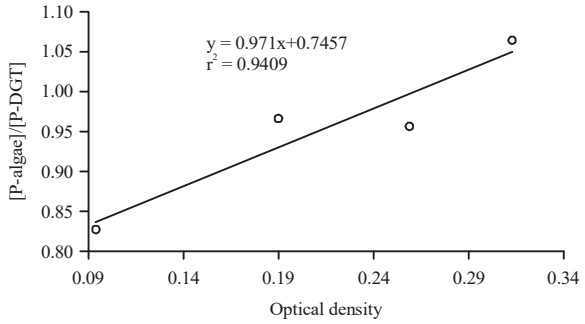

Fig. 7: Correlation between optical density with ratio of phosphate in algae with phosphate in DGT device

Based on the equation, it can be estimated the amount of phosphate uptake by the algae a constant value of k = 0.971. The final equation obtained as follows:

$$\left[P_{algae}\right] = \{(k \times OD) + f) \times [P_{DGT}]\} \quad (6)$$

Where:

$[P_{algae}]$ = Phosphate concentration was uptake by *Oscillatoria* sp. (mg L^{-1})

$[P_{DGT}]$ = Phosphate concentration diffuses to DGT device (mg L^{-1})

OD = Optical density

K = Constant = 0. 971

f = Correction factor = 0.7457

DISCUSSION

Bioavailability of phosphate in microalgae *Oscillatoria* sp. was observed by phosphate uptake in solution. Phosphate uptake was determined by the difference in initial concentration and final concentration in the medium after incubation for 24 h. The phosphate uptake by algae cell was influenced by the optical density, algae dosage and salinity. This research developed the DGT technique to predicted phosphate uptake in the medium for algae cells. Based on the experiment showed a good correlation between P-DGT and P-algae in algae concentration of 1 and 2 mg with the equation of y = 94.69x-2.1411 and y = 95.701x-1.8488, respectively. The concentration of algae in 3 and 4 mg L^{-1} resulted equation of y = 72.741x+1.577 and y = 80.1x+2.2968. The increased salinity caused the decrease of phosphate uptake by *Oscillatoria* sp. and gives the different equations.

The DGT device deployed in phosphate solution in beaker glass without algae *Oscillatoria* sp. and other beaker incubated *Oscillatoria* sp. in the medium. The phosphate concentration diffused in the DGT unit is mimic and related to the phosphate uptake in *Oscillatoria* sp. The study of the phosphate bioavailability has been studied by other researchers. The bioavailability of phosphate in tomato plant[23] with R^2 = 0.93, the bioavailability of wheat[24] with the correlation of 0.75 and bioavailability of phosphate in corn[25] with the correlation of 0.90. The DGT technique has been used for the prediction of bioavailability metal in soil. The DGT technique has been used to predict bioavailability of Cu in *Lactuca sative*[5] with R^2 of 0.87, Uptake of methyl mercury in *Oryza sativa*[26] with R^2 = 0.853, Mn in *Horedeum vulgare*[27] with R^2 = 0.60. The DGT technique can predict the bioavailability of *Oscillatoria* sp. in medium with a high salinity of 30 g L^{-1}. The higher phosphate concentration diffuses into the DGT device, the higher of phosphate uptake by *Oscillatoria* sp. A good correlation between phosphate diffusion in DGT and phosphate uptake by algae *Oscillatoria* sp. is shown in a medium with a low optical density value. If the optical density increases, the correlation value decreases due to the awareness of competition between algae to absorb phosphate in the medium. By comparing the correlation between phosphate in the DGT device and uptake of the algae *Oscillatoria* sp. showed a very good correlation. The information obtained could predict the occurrence of algae blooms in the waters. DGT technique can be used to predict phosphate bioavailability by *Oscillatoria* sp. algae in medium with low salinity (0 g L^{-1}), medium salinity (15 g L^{-1}) and high salinity (30 g L^{-1}) with linear correlation. The phosphate bioavailability of *Oscillatoria* sp. algae is greater in areas with high salinity. These good correlations may reflect the dependence of free on the concentration of total dissolved phosphate concentration for some condition[28]. Bioavailability phosphate present in biomass of aquatic macrophytes and phytoplankton such as microalgae, its relationship can be seen with eutrophication. It explores by evaluation quantity of P in aqueous[29]. The information on phosphate quantities was adsorbed by the microalgae would be important. The phosphate accumulated in the DGT device is a fast way to detect phosphate bioavailability in microalgae to early detection of microalgae blooming in the aquatic environment. This method can be applied to the monitoring of blooming algae in lake and seawater. This study limited in phosphate inorganic bioavailable and further information should be studying in organic phosphate form.

CONCLUSION

The DGT technique has been successfully used to predict phosphate bioavailability on the uptake of *Oscillatoria* sp. algae with different optical density and salinity levels. The higher concentration from *Oscillatoria* sp., the greater phosphate accumulated in these algae cells. This experiment is carried out at different salinity of 0, 15 and 30 g L^{-1}. A good linear correlation is shown between phosphate uptake by *Oscillatoria* sp. to phosphate concentrations diffuse on DGT devices.

SIGNIFICANCE STATEMENT

The scope of the present research is to determine phosphate in a solution using a diffusive gradient in thin film related to phosphate uptake by *Oscillatoria* sp. The correlation of phosphate diffuses in the DGT unit with phosphate in *Oscillatoria* sp. indicated the DGT technique can be used to predict bioavailability phosphate. This study will help the research to predict of phosphate uptake by microalgae as early detection. By using this indicator, we can prevent algae blooming in the aquatic environment.

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REFERENCES

1. Seppala, J., P. Ylostalo, S. Kaitala, S. Hallfors, M. Raateoja and P. Maunula, 2007. Ship-of-opportunity based phycocyanin fluorescence monitoring of the filamentous cyanobacteria bloom dynamics in the Baltic Sea. Estuarine Coastal Shelf Sci., 73: 489-500.
2. Ralph, P.J., D. Tomasko, K. Moore, S. Seddon and C.M. Macinnis-Ng, 2007. Human Impacts on Seagrasses: Eutrophication, Sedimentation and Contamination. In: Seagrasses: Biology, Ecology and Conservation, Larkum, A.W.D., R.J. Orth and C.M. Duarte (Eds.). Springer, Dordrecht, The Netherlands, ISBN-13: 9781402029837, pp: 567-593.
3. El Herry, S., A. Fathalli, A.J.B. Rejeb and N. Bouaicha, 2008. Seasonal occurrence and toxicity of *Microcystis* spp. and *Oscillatoria tenuis* in the Lebna Dam, Tunisia. Water Res., 42: 1263-1273.

4. Li, B. and M.T. Brett, 2013. The influence of dissolved phosphorus molecular form on recalcitrance and bioavailability. Environ. Pollut., 182: 37-44.
5. Agbenin, J.O. and G. Welp, 2012. Bioavailability of copper, cadmium, zinc and lead in tropical savanna soils assessed by diffusive gradient in thin films (DGT) and ion exchange resin membranes. Environ. Monit. Assess., 184: 2275-2284.
6. Andrieux-Loyer, F., X. Philippon, G. Bally, R. Kerouel, A. Youenou and J. Le Grand, 2008. Phosphorus dynamics and bioavailability in sediments of the Penze Estuary (NW France): In relation to annual P-fluxes and occurrences of *Alexandrium minutum*. Biogeochemistry, 88: 213-231.
7. Baken, S., S. Nawara, C. van Moorleghem and E. Smolders, 2014. Iron colloids reduce the bioavailability of phosphorus to the green alga *Raphidocelis subcapitata*. Water Res., 59: 198-206.
8. Van Moorleghem, C., N. De Schutter, E. Smolders and R. Merckx, 2013. Bioavailability of organic phosphorus to *Pseudokirchneriella subcapitata* as affected by phosphorus starvation: An isotope dilution study. Water Res., 47: 3047-3056.
9. Ding, S., Y. Wang, L. Zhang, L. Xu, M. Gong and C. Zhang, 2016. New holder configurations for use in the diffusive gradients in thin films (DGT) technique. RSC Adv., 6: 88143-88156.
10. Kalkhajeh, Y.K., H. Sorensen, B. Huang, D.X. Guan and J. Luo *et al*., 2018. DGT technique to assess P mobilization from greenhouse vegetable soils in China: A novel approach. Sci. Total Environ., 630: 331-339.
11. Chapman, E.E.V., G. Dave and J.D. Murimboh, 2012. Bioavailability as a factor in risk assessment of metal-contaminated soil. Water Air Soil Pollut., 223: 2907-2922.
12. Zhang, Y., S. Mason, A. McNeill and M.J. McLaughlin, 2013. Optimization of the diffusive gradients in thin films (DGT) method for simultaneous assay of potassium and plant-available phosphorus in soils. Talanta, 113: 123-129.
13. Degryse, F., E. Smolders, H. Zhang and W. Davison, 2009. Predicting availability of mineral elements to plants with the DGT technique: A review of experimental data and interpretation by modelling. Environ. Chem., 6: 198-218.
14. Philipps, R.R., X. Xu, R.B. Bringolf and G.L. Mills, 2019. Evaluation of the DGT technique for predicting uptake of metal mixtures by fathead minnow (*Pimephales promelas*) and yellow lampmussel (*Lampsilis cariosa*). Environ. Toxicol. Chem., 38: 61-70.
15. Zhang, H. and W. Davison, 2015. Use of diffusive gradients in thin-films for studies of chemical speciation and bioavailability. Environ. Chem., 12: 85-101.
16. Docekalova, H., P. Skarpa and B. Docekal, 2015. Diffusive gradient in thin films technique for assessment of cadmium and copper bioaccessibility to radish (*Raphanus sativus*). Talanta, 134: 153-157.
17. Santner, J., M. Mannel, L.D. Burrell, C. Hoefer, A. Kreuzeder and W.W. Wenzel, 2015. Phosphorus uptake by *Zea mays* L. is quantitatively predicted by infinite sink extraction of soil P. Plant Soil, 386: 371-383.
18. Shafqat, M.N. and G.M. Pierzynski, 2014. The Freundlich adsorption isotherm constants and prediction of phosphorus bioavailability as affected by different phosphorus sources in two Kansas soils. Chemosphere, 99: 72-80.
19. Six, L., E. Smolders and R. Merckx, 2013. The performance of DGT versus conventional soil phosphorus tests in tropical soils-maize and rice responses to P application. Plant Soil, 366: 49-66.
20. Tandy, S., S. Mundus, J. Yngvesson, T.C. de Bang, E. Lombi, J.K. Schjorring and S. Husted, 2011. The use of DGT for prediction of plant available copper, zinc and phosphorus in agricultural soils. Plant Soil, 346: 167-180.
21. Nowack, B., S. Koehler and R. Schulin, 2004. Use of diffusive gradients in thin films (DGT) in undisturbed field soils. Environ. Sci. Technol., 38: 1133-1138.
22. Yao, Y., Q. Sun, C. Wang, P.F. Wang, L.Z. Miao and S.M. Ding, 2016. The combination of DGT technique and traditional chemical methods for evaluation of cadmium bioavailability in contaminated soils with organic amendment. Int. J. Environ. Res. Public Health, Vol. 13, No. 6. 10.3390/ijerph13060595.
23. Menzies, N.W., B. Kusumo and P.W. Moody, 2005. Assessment of P availability in heavily fertilized soils using the diffusive gradient in thin films (DGT) technique. Plant Soil, 269: 1-9.
24. Mason, S.D., M.J. McLaughlin, C. Johnston and A. McNeill, 2013. Soil test measures of available P (Colwell, resin and DGT) compared with plant P uptake using isotope dilution. Plant Soil, 373: 711-722.
25. Heidari, S., A. Reyhanitabar and S. Oustan, 2016. The comparison of Olsen, DMT-HFO and DGT methods for assessment of plant available phosphorus in soils. Int. J. Adv. Sci. Eng. Inform. Technol., 6: 27-34.
26. Liu, J., X. Feng, G. Qiu, C.W. Anderson and H. Yao, 2012. Prediction of methyl mercury uptake by rice plants (*Oryza sativa* L.) using the diffusive gradient in thin films technique. Environ. Sci. Technol., 46: 11013-11020.
27. Mundus, S., E. Lombi, P.E. Holm, H. Zhang and S. Husted, 2012. Assessing the plant availability of manganese in soils using Diffusive Gradients in Thin films (DGT). Geoderma, 183: 92-99.
28. Nguyen, L. and J. Sukias, 2002. Phosphorus fractions and retention in drainage ditch sediments receiving surface runoff and subsurface drainage from agricultural catchments in the North Island, New Zealand. Agric. Ecosyst. Environ., 92: 49-69.
29. Feng, W., F. Wu, Z. He, F. Song and Y. Zhu *et al*., 2018. Simulated bioavailability of phosphorus from aquatic macrophytes and phytoplankton by aqueous suspension and incubation with alkaline phosphatase. Sci. Total Environ., 616: 1431-1439.