British Journal of Applied Science & Technology
4(8): 1285-1295, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Analyses of Species of Phosphorus in Four Selected Streams within Abakaliki, Metropolis, Nigeria

N. O. Omaka[1], I. F. Offor[1*], F. I. Nwabue[1,2], K. Onyekezini[2] and C. C. Mba[2]

[1]Department of Chemistry/Biochemistry, Federal University Ndufu Alike, Ikwo, P.M.B.1010, Abakaliki, Ebonyi State, Nigeria.
[2]Department of Industrial Chemistry, Ebonyi State University, P.M.B.053, Abakaliki, Ebonyi State, Nigeria.

Authors' contributions

This work was carried out in collaboration between five authors. Authors NOO and FIN designed the study. Authors KO, IFO and CCM collected samples from the field, performed the laboratory tests and produced a first draft of the manuscript. Authors NOO, IFO and FIN checked the data for validity, performed the statistical analysis and carried out the analyses of the study. Authors IFO, NOO, KO and CCM further managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

Received 30th October 2013
Accepted 28th December 2013
Published 21st January 2014

ABSTRACT

This work investigates the speciation of phosphorus in four selected streams within Abakaliki, Nigeria using phytase hydrolysis of phytic acid as an indicator. Phytic acid is an important organophosphorus compound that can be hydrolysed by phytases occurring in soil and water. It is the main form in which phosphorus is stored in seeds hence its availability in natural environment like water and soil. Different species of phosphorus exist in nature as a result of the interconversion, hydrolysis and oxidation occurring between organic phosphate molecules. Investigation of these species of phosphorus (PHP, FRP, TDP and DOP) is crucial to the understanding of the biogeochemical processes prevalent in any aquatic ecosystem. Water samples were collected from four streams (Presco campus, Ama, Udele and Iyi-okwu streams) in Abakaliki, Nigeria within a period of 5 days in October, 2012. The samples were digested with 0.04M peroxydisulphate and 0.01M H_2SO_4 at 121ºC for 45 minutes in an autoclave. After

**Corresponding author: E-mail: offorifeanyi@gmail.com;*

digestion, the presence of PHP, FRP, TDP and DOP were investigated in the water samples using a spectrophotometer at wavelength of 820 nm. Results obtained showed varying concentrations of PHP, FRP, TDP and DOP ranging from ±0.0093-0.069 $mgL^{-1}P$, 0.205-0.233 $mgL^{-1}P$, 0.297-0.520 $mgL^{-1}P$ and 0.081-0.127 $mgL^{-1}P$ respectively. Recovery studies using Adenosine-triphosphate (ATP) and the calcium salt of phytic acid (PTA) as model organic phosphorus compounds gave quantitative recoveries ranging from 92-96%. One-way ANOVA showed that sampling location significantly influenced the mean concentration of TDP, FRP and DOP in the affected streams. This technique was effective in digesting both the model compounds and the real water samples. Also, the soluble phytase was effective in hydrolyzing phytic acid and phytase hydrolysable phosphorus (PHP) present in the water samples.

Keywords: Phosphorus; phytase; organo-phosphorus; peroxydisulphate; spectrophotometry; water.

.

1. INTRODUCTION

Natural waters make up less than 0.01% of the earth's total water resources covering approximately 3% of the earth's total land mass [1]. Frequent changes in the spatial and temporal distribution of this relatively small percentage of natural water, coupled with the large geomorphologic variability has resulted in a variety of water bodies ranging from ephemeral pools to large lakes and mighty rivers. A significantly large proportion of the Earth's biodiversity representing nearly all kinds of organisms ranging from microorganisms to mammals inhabit inland waters [1].

Phosphorus is found in natural waters in the form of dissolved inorganic phosphorus, orthophosphate or soluble reactive phosphorus; collectively called total dissolved phosphorus [2]. Phosphorus is also present in water as dissolved organic phosphorus and particulate organic phosphorus, collectively known as total organic phosphorus. A combination of total dissolved phosphorus and total organic phosphorus is known as total phosphorus [2].

In water bodies, phosphorus is present in several soluble and particulate forms such as organically bound phosphorus, inorganic phosphates and inorganic orthophosphates [3]. In addition, it is also a biologically active element which cycles through many states in the aquatic ecosystem and its concentration in any given state depends on the degree of metabolic synthesis or decomposition occurring in the aquatic system [4].

Furthermore, phosphorus is also the element most likely to cause a stimulation of plant production, often leading to excessive production and thus, its level in water is often measured as a means of monitoring eutrophication and its associated problems [5].

Phosphorus is an essential nutrient required by plants and animals for growth and development. In the aquatic environment, several species of phosphorus are available, but the activity prevalent in the area could have an overall influence on the concentrations of the various species. The determination of phosphorus species in the environment provides essential data for checking the health of ecosystems, investigating biogeochemical processes and monitoring compliance with legislation [6]. Phosphorus export from both point and nonpoint sources of pollution can bring about increased primary production and eutrophication resulting in seasonal development of toxic algal blooms which can have a

major impact on the global water quality [6-8].Organic phosphorus compounds take part in terrestrial and aquatic biological phosphorus cycles and are distributed in nature. Elemental phosphorus is primarily stored in the form of phytic acid in many plant tissues especially bran and seeds [9].

There are four operationally defined phosphorus fractions commonly used to predict algal biomass and growth rate. These phosphorus fractions commonly measured in natural waters are total phosphorus (TP), total reactive phosphorus (TRP), filterable reactive phosphorus (FRP) and total dissolved phosphorus (TDP) [10].

According to Lambert et al. [10], many studies have been done in the past on procedures for the preservation of water samples for the analysis of filterable or soluble phosphorus. These procedures can be summarized into two groups i.e. those advocating the use of bactericides [11] or those advocating the use of freezing method [12]. However, there is paucity of research on short term changes that can occur in the field before samples are preserved [13-14]. There also seems to be a dearth of research data on the changes that can occur in other commonly measured phosphorus fractions [10,12,15].

Concentration of phosphorus in natural waters fluctuates with changes in physicochemical conditions and biological activities. Phosphorus species are found in dissolved, colloidal and particulate fractions as inorganic and organic compounds and in biotic and abiotic particles [16]. Accurate determination of dissolved organic phosphorus (DOP) is of importance for the study of phosphorus cycling, algal assimilation and terrestrial fluxes towards the sea. These measurements are based on the conversion of complex forms of phosphates by hydrolysis and oxidation of molecules containing P-O-P, C-O-P and C-P bonds [6,16,17].

Dissolved organic phosphorus constitutes a significant portion of the bio-available phosphorus (BAP). It is the difference between total dissolved phosphorus (TDP) and filterable reactive phosphorus (FRP), i.e.

$$DOP = TDP – FRP \quad \text{Eq. (1)}$$

Bio-available phosphorus is the sum of immediate available phosphorus and phosphorus that can be transformed into available forms by naturally occurring processes such as: physical, chemical and biological processes. This fraction of the total phosphorus is important for understanding the biogeochemical processes prevalent in any aquatic ecosystem [6,16-19].

Phytic acid also known as myo-inositol hexakis dihydrogen phosphate is a naturally occurring non-toxic phosphoric ester that is found in cereals, pollens, legumes, seeds and plants as well as aquatic environment [9]. Phytic acid or phytate is also the storage form of phosphorus in all grains and oil seeds averaging 60-90% [20,21]. Phytate is commonly known as a substance known to decrease mineral adsorption; however, it has also been looked at as a possible beneficial vitamin-like substance [22]. Phytic acid is considered an anti-nutritional factor since it chelates magnesium, zinc, calcium and also reacts with proteins, thus, decreasing their bioavailability and important mineral nutrients [23]. In natural waters, it is hydrolyzed by phytases to the less phosphorylated forms namely inositol polyphosphates and orthophosphoric acid [24]. There are two types of phytases which can be distinguished by their position or carbon of attack and these are the plant derived 3-phytases and animal derived 6-phytases [25,26].

Phytase is the enzyme that degrades (catalyzes or breaks down) phytate in order to release its phosphorus [21]. Phytase was originally proposed as a food additive to reduce phytate content by releasing orthophosphate [27].

This work investigates the different species of phosphorus found in four selected streams within Abakaliki metropolis, Nigeria. The amount of total available phosphorus in each stream was estimated by the hydroxylation ability of the phytase enzyme on phytic acid content of each water sample.

2. METHODOLOGY

2.1 Reagents

Anhydrous Sodium acetate, Acetic acid, Adenosine-5^1-triphosphate (ATP) disodium salt, Sulphuric acid, L-Ascorbic acid, Antimony potassium tartrate (BDH Chemicals Ltd, UK) Phytic acid-calcium salt (PTA-Ca), Potassium phosphate dibasic trihydrate, potassium peroxodisulphate (Sigma Aldrich Chemical Company, USA).

Ammonium molybdate (Merck Chemicals, Germany)

EDTA-disodium salt (Thomas Baker Chemicals, Pvt Ltd., India)

All reagents were of analytical grade and were prepared using deionised water. Except otherwise stated, all reagents were stored at 4°C and brought out from storage at least one hour before use.

2.2 Apparatus

UV-Visible grating spectrophotometer (HANNA, Model 8610) equipped with 4 ml quartz cuvette, pipettes of different volume capacities (1ml, 2ml and 5ml), refrigerated centrifuge and Asatel autoclave digester.

2.3 Sampling and Pretreatment

Water samples were collected from four different locations within Abakaliki metropolis. Two litres of water were collected 300cm below the surface of each stream using a grab sampler. The water samples were stored at 4ºC in precleaned glass bottles. All glass wares, high density polyethylene (HPDE) bottles and containers used were first cleaned with a phosphate free detergent, rinsed three times with deionised water and soaked in 10% (v/v) HCl overnight (12 hours) and finally rinsed three times with deionized water. The dried containers were stored in air tight clean polyethylene bags to prevent contamination by dust [18,23,28]. Location 1 is the stream at Presco Campus which is one of the 3 campuses of Ebonyi State University, Abakaliki. Location 2 is Ama stream situated beneath the bridge along Afikpo Road. Location 3 is the Iyi-udele stream situated along Nna Street while location 4 is the Iyi-okwu stream at Hope high school along Onwe Road. A map of Abakaliki showing the different sampling locations is given in Fig. 1.

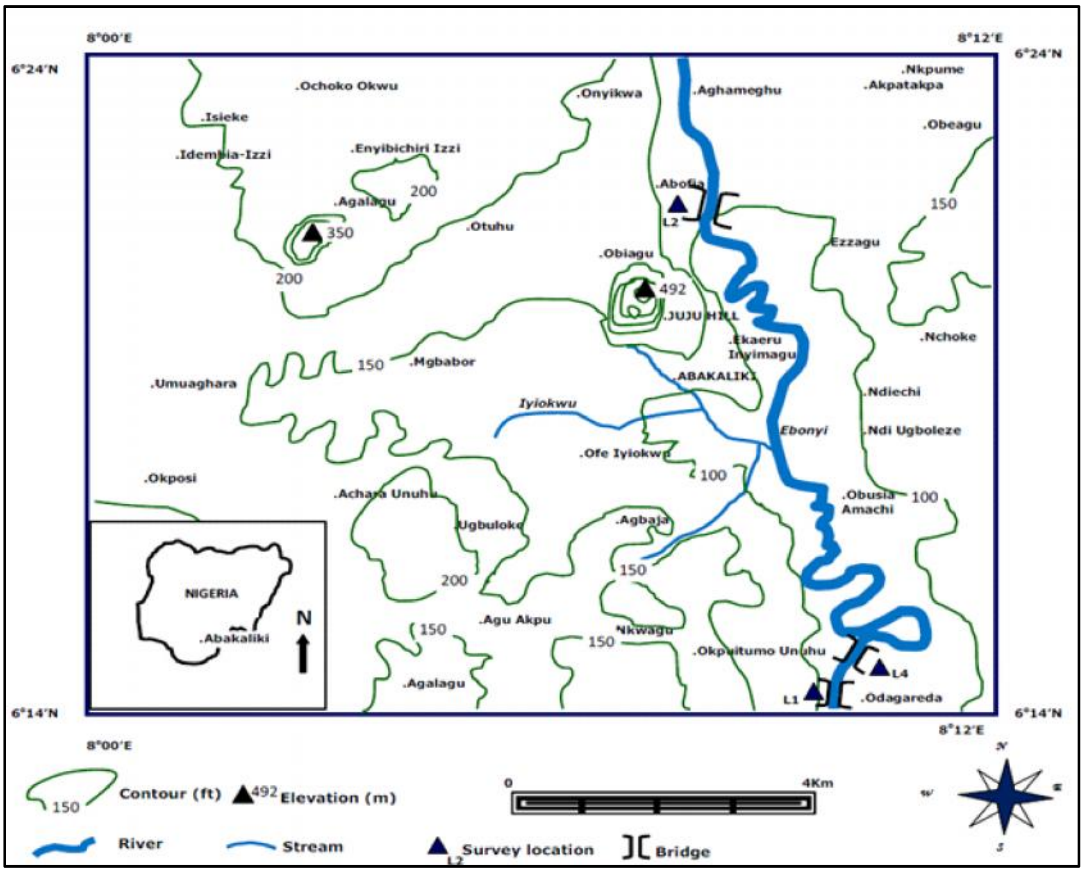

Fig. 1. MAP of Abakaliki showing the study areas

The water samples after collection were filtered in-situ using 0.4µm filter paper (cellulose acetate type) and brought in water coolers containing ice blocks to the laboratory where they were stored at 4ºC until the time for analysis. Important physico-chemical parameters that will aid in the interpretation of results like DO (dissolved oxygen) and TDS (total dissolved solid) were measured in-situ using probes [18,28].

2.4 Digestion of the Water Samples

About 10ml of each water sample was digested by adding 15ml of 0.004M peroxo disulphate spiked with 0.01M H_2SO_4. Final volume of the container was 50ml (reagents plus the filtered water). As a control, two samples, one with 0.10 mg PTA as P containing 0.08 mgL^{-1} P and the other with 0.10 mg ATP as P containing 0.10 mgL^{-1} P were each used for spiking the samples to be digested. Digestion reagents were also added and made up to the 50ml mark of the digestion bottle. All the samples were digested in the autoclave at 121ºC for 45 minutes [18]. After digestion, all the samples were cooled at room temperature and pH adjusted to 7.0 -7.5.

2.5 Measurement of the Phosphorus Fractions

TDP and FRP fractions were analyzed on 20ml of each sample, filtered through a 0.45 µm membrane filter (Millipore, HA) after digestion. DOP fraction was gotten as the difference between TDP and FRP fractions (see Equation 1). About 3 replicates of each phosphorus fraction were determined. The digestion reagent used was prepared by using 0.004M peroxodisulphate spiked with0.01M H_2SO_4. Final volume of the container was 50ml.

2.6 Spectrophotometric Determination of Phosphorus as Orthophosphate

To determine phosphorus as orthophosphate, a clean test tube containing 12.4ml of the water sample was gotten and antimony tartrate was added as a catalyst. Then, acidified phosphomolybdate was added and it was subsequently catalyzed by the antimony tartrate. Furthermore, 1.8ml molybdate reagent and 0.8ml ascorbic acid containing 0.0001M EDTA were also added to the test tube [23,29]. The test tube was allowed to stand for 30 seconds after which it was subjected to spectrophotometric analysis in order to determine the amount of orthophosphate present in the water sample. Before use, the spectrophotometer was allowed to attenuate for 30 minutes and deionised water used to run the blank. Similar procedure was employed for the determination of FRP, TDP, and PHP.

3. RESULTS AND DISCUSSION

Selected physico-chemical parameters of water samples collected from different streams within Abakaliki metropolis are shown in Table 1. The results of the physic-chemical parameters were presented as Mean±Standard deviation to represent triplicate measurements.

Table 1. Selected Physico-chemical Parameters (DO, TDS, TDP, FRP and DOP) of water samples from different streams within Abakaliki Metropolis, Nigeria

Location	DO (mg/L)	TDS (mg/L)	TDP($mgL^{-1}P$)	FRP($mgL^{-1}P$)	DOP($mgL^{-1}P$)
1	7.6	60	0.360±0.65	0.233±0.84	0.127±1.42
2	8.0	14	0.305.±1.25	0.223±0.79	0.081±0.75
3	10.9	68	0.520±1.33	0.233±1.52	0.119±1.28
4	10.1	26	0.297±2.05	0.205±1.05	0.092± 0.69

LEGEND: DO=Dissolved oxygen, TDS=Total dissolved solids, TDP=Total dissolved phosphorus, FRP= Filterable reactive phosphorus, DOP= Dissolved organic phosphorus
DOP = TDP – FRP
1=Stream at Presco Campus; 2=Ama stream at Afikpo Road; 3=Iyi-udele stream at Nna Street; 4=Iyi-okwu Stream at Hope High School.

The Dissolved Oxygen (DO) values recorded in this study ranged from 7.6-10.9 in streams 1 and 3 respectively whereas the Total dissolved solids (TDS) of the 4 streams investigated ranged from 27-68mg/L in streams 4 and 3 respectively.

Streams 1 and 3 recorded relatively higher phosphate levels (evidenced by their high TDP and FRP values of 0.360±0.65 $mgL^{-1}P$; 0.233.±0.84 $mgL^{-1}P$ and 0.520±1.25 $mgL^{-1}P$; 0.2233±0.79 $mgL^{-1}P$ respectively) than streams 2 and 4 (TDP and FRP values of 0.305±1.33 $mgL^{-1}P$; 0.223±1.52 $mgL^{-1}P$ and 0.297±2.05 $mgL^{-1}P$; 0.205±1.05 $mgL^{-1}P$ respectively). This trend can be attributed to the greater amount of wastes emanating from the residential

homes, retail shops, restaurants and car wash services located within reasonable distance to streams 1 and 3 as opposed to streams 2 and 4.

In addition, Table 1 also show that the phosphate concentration in this study (TDP fraction) ranged from its lowest value (0.297mg/L) in stream 4 to its highest value (0.520mg/L) in stream 3. This trend was relatively higher than the mean range of phosphates (0.03-0.05mg/L) in different stream tributaries of Ebonyi River [30].The range of phosphate (as TDP) concentration recorded in this study (0.297-0.520mg/L) was however lower than that of the mean phosphate level (0.460-0.520mg/L) of streams and spring waters of Ebonyi South Senatorial Zone of Ebonyi State [31].

Factors such as changes in pH and temperature, varying rate of evaporation, degree of domestic activities (bathing, cloth washing, dish washing etc.) involving use of phosphate soaps and detergents, runoffs from agricultural lands where orthophosphates have been applied as fertilizers, organic wastes from plant and animal wastes and food residue may have contributed to the differences noticed in the phosphate concentrations among the 4 streams investigated [32,33].

The United States Environmental Protection Agency (EPA) recommends that total phosphates concentration (as phosphorus) should not exceed 0.1mg/L in streams not discharging directly into reservoirs and also not to exceed 0.05mg/L in streams discharging directly into reservoirs. To prevent algal growths, EPA also recommends that total phosphates as phosphorus concentrations in water should not exceed 25µg/L within a lake or reservoir [34]. In view of this, we can conclude that in all 4 streams investigated in this study, the total phosphate concentration exceeded the permissible EPA limit of 0.1mg/L for streams not discharging directly into reservoirs. Furthermore, comparing the mean phosphate concentration in this study (0.371mg/LTDP fraction) with the maximum permissible limit for phosphates in natural waters (5 mg/L) set by the defunct Federal Environmental Protection Agency (FEPA) in Nigeria; we can conclude that it is still within the 1991 FEPA limit for phosphates in natural water [35].

As can be seen in Table 2, recovery studies were performed using Adenosine-triphosphate (ATP) and the calcium salt of phytic acid (PTA) as model compounds thus, giving quantitative recoveries in the range of 92–96%.

Table 2. Recovery studies using Adenosine-triphosphate (ATP) and the calcium salt of Phytic acid (PTA) as Model Organic Phosphorus Compounds

Samples after autoclave digestion (mgL^{-1} P)	**Percentage Recovery**
0.1 mg ATP as P + 0.10	92
0.1mg PTA as P + 0.10	93
Samples after phytase incubation	**Percentage Recovery**
0.2mg PTA as P + 0.08 (mgL^{-1} P)	
0.2mg PTA as P + 0.10 (mgL^{-1} P)	96
0.2mg PTA as P + 0.08 (mgL^{-1} P)	92

Table 3 shows the results obtained during the incubation of water samples (PHP fraction) from 4 streams within Abakaliki metropolis at 37ºC for 40 minutes (batch A) and 32ºC for 7 days (batch B).

Table 3. Incubation of water samples (PHP fraction) from 4 streams within Abakaliki metropolis at 37ºC for 40 minutes (Batch A) and 32ºC for 7 days (Batch B)

Location	PHP (Batch A) $mgL^{-1}P$	PHP (Batch B) $mgL^{-1}P$
1	0.00	-0.0093
2	0.044	0.077
3	0.014	0.036
4	0.047	0.069

LEGEND: PHP=Phytase hydrolysable phosphorus
1=Stream at Presco Campus; 2=Ama stream at Afikpo Road; 3=Iyi-udele stream at Nna Street; 4=Iyi-okwu Stream at Hope High School.

In Table 3, the relatively high values of PHP in streams 2 and 4 (Ama and Iyi-okwu streams) is an indication that these streams are located close to shrubs and flower gardens, hence leading to increased eutrophication and subsequent PHP abundance in the concerned streams. This observation is in tandem with a similar research carried out by Nagashima et al. [24]. On the other hand, the negative values for PHP in stream 1 (Presco campus stream) implies that the availability of PHP was limited by heavy metal inhibition. Both batches (batch A and B) were effective during incubation but the effects of increased temperature and duration of exposure resulted in faster rate of hydrolysis of the available phosphorus by the enzyme phytase. A similar observation were made by Omaka et al. [26] when they used enzymatic flow injection technique to determine phytase hydrolysable phosphorus (PHP) in natural waters using immobilized 3-phytase.

The mean phosphate concentration of 0.371mg/L recorded in this study exceeded the WHO limit (0.1mg/L) for phosphate in drinking water [31], thus, making water from these 4 streams being investigated to be unsafe for drinking water purposes as a result of their high phosphate (PO_4^{3-}) content levels.

In Table 4, One-way ANOVA was employed to find the significant differences between the levels of TDP, FRP and DOP concentrations in the selected streams being investigated. The significance was set at $P<0.05$. Statistical analysis of data was carried out using SPSS 16.0 for Windows (SPSS Inc., Polar Engineering and Consulting, 2007).

Table 4. One-way ANOVA for four selected streams within Abakaliki, Nigeria

	Sum of squares	df	Mean squares	F	Sig.
Between groups	0.002	1	0.002	0.037	0.879
Within groups	0.045	1	0.045		
Total	0.047	2			

The significant value of the F test in the ANOVA Table (Table 4) is 0.879, thus rejecting the null hypothesis that average concentrations of TDP, FRP and DOP are equal across the different streams studied. Thus, the results of the One-way ANOVA showed that sampling areas (stream location) significantly influenced the mean concentrations levels of TDP, FRP and DOP in the affected streams.

4. CONCLUSION

The technique applied was effective in digesting both the model compounds and the water samples from selected streams. Also the soluble phytase was effective in hydrolyzing phytic

acid and phytase hydrolysable phosphorus (PHP) present in the water samples. Recovery study gave quantitative results of ranging from 92-96%. Future studies are planned in order to investigate the effect of seasonal variation on the concentrations of TP, TDP, and DOP in selected streams and rivers in Ebonyi state and to also determine the ion(s) that caused the inhibitory effect and thereafter apply the method for the determination of phytic acid in seeds, flowers and fruits. A collaborative study is also currently ongoing to isolate and characterize phytases prevalent within our immediate locality.

ACKNOWLEDGMENTS

The authors are grateful to the Biotech Research Laboratory, Ebonyi State University Abakaliki for the use of some of their facilities. In addition, one of the authors (Dr O. N. Omaka) is highly grateful to Prof. Paul Worsfold of the University of Plymouth UK for the provision of the reagents and the Royal Society of Chemistry (RSC) England for the purchase of a UV-VIS Spec via a research grant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Revenga C, Kura Y. Status and trends of Biodiversity of inland water ecosystems: CBD Technical Series No. 11. Montreal: Secretariat of the Convention on Biological Diversity. 2003;120.
2. Meybeck M. Carbon, Nitrogen and Phosphorus transport by World Rivers. American Journal of Science. 1982;282:401-450.
3. Lind TO. Handbook of common methods in limnology 2nd ed. London: Mosby Company. 1979;232.
4. Adeniji HA. A pre-impounded fishery limnological study of river Nigeria in the proposed Jebba area. Kainji Lake Research Annual Report. 1983;45: 50.
5. Cole CA. Textbook of Limnology. Saint Louis, Arizona: The C.V. Mosby Company. 1983;401.
6. Mckelvie ID**.** Separation, preconcentration and speciation of organic phosphorus in environmental samples. In: Turner BI, Emmanuel F and Darren SB, Editors. Organic phosphorus in the environment. Cambridge UK: CABI Publishing. 2005;1-20.
7. Adeyemo OK, Adedokun OA, Yusuf RK, Abeleye EA. Seasonal changes in physico-chemical parameters and nutrient load of river sediments in Ibadan city, Nigeria. Global Nest Journal. 2008;10(3):326-336.
8. Mckelvie ID. Phosphate. In: Nollet LML and De Gelder LSP, editors. Handbook of Water Analysis. 2nd ed. New York: Marcel Dekker Inc. 2007:273-284.
9. Bilyeu KD, Zeng P, Coello P, Zhang ZJ, Krishnan HB, Bailey A, et al. Quantitative Conversion of phytate to inorganic phosphorus in soyabean seeds expressing a bacterial phytase 1. Plant Physiology. 2008;146(2):468-477.
10. Lambert D, Maher W, Hogg I. Changes in Phosphorus fractions during storage of Lake water. Water Research. 1992;26(5):645-648.
11. Klingaman ED, Nelson DW. Evaluation of methods for preserving the levels of soluble inorganic Phosphorus and Nitrogen in unfiltered water samples. Journal of Environmental Quality. 1976;1:42-46.

12. Nelson DW, Romkens MJM. Freezing as a method of preserving runoff (water) samples for analysis for soluble phosphate. Journal of Environmental Quality. 1972;1:323-324.
13. Pichet P, Jamati K, Goulden PD. Preservation du contenu en O-phosphate D' Echantillions Deau Du fleuve Saint-Laurent. Water Research. 1979;13:1187-1191. French.
14. Boyd CE, Tucker L. Determination of filterable orthophosphate in water from fish ponds. Transactions of the American Fisheries Society. 1980;109:314-318.
15. Jenkins D. The differentiation analysis and preservation of Nitrogen and Phosphorus forms in Natural water. In: Gould RF, editor. Trace Inorganics in Water: Advances in Chemistry Series. Washington DC: American Chemical Society.1968;73:265-281.
16. Clesceri LS, Greenberg AE, Trussell RR. Standard methods for the examination of water and waste water. 17th ed. Washington DC: American Public Health Association. 1989;1-175.
17. Aminot A, Kerouel R. An automated photo-oxidation method for the determination of dissolved organic phosphorus in marine and fresh water. Marine Chemistry. 2001;76(1-2):113-126.
18. Worsfold PJ, Gimbert LJ, Mankasingh U, Omaka ON, Hanrahan G, Gardolinski PCFC, et al. Sampling, sample treatment and quality assurance issues for the determination of phosphorus species in natural waters and soils. Talanta. 2005;66(2):273-293.
19. Hosomi M, Sudo R. Simultaneous determination of total nitrogen and total phosphorus in freshwater samples using persulphate digestion. International Journal of Environmental Studies. 1986;27(3-4):267-275.
20. Cosgrove DJ. Inositol Phosphates: Their Chemistry, Biochemistry and Physiology. 2nd ed. Amsterdam: Elsevier Scientific Publishing. 1980;197.
21. Jacela JY, DeRouchy JM, Dritz SS, Tokach MD, Goodband RD, Nelssen JL, et al. Feed additives for swine: Fact sheets as high dietary levels of Copper and Zinc for young Pigs and Phytase. Journal of Swine Health Production. 2010;18(2):87-91.
22. Okazaki Y and Katayama T. Reassessment of the nutritional function of phytic acid with special reference to myoinositol function. Journal of Japanese Society of Nutrition and Food Science. 2005;58(3):151-156.
23. Omaka ON. Flow-injection techniques for investigating the biogeochemistry of nutrients in natural waters. PhD Thesis. England, UK: University of Plymouth. 2006;92-140.
24. Nagashima T, Tange T, Anazawa H. Dephosphorylation of Phytate by using the *Aspergillus niger* phytase with a high affinity for phytate. Applied and Environmental Microbiology. 1999;65(10):4682-4684.
25. International Union of Biochemistry. Enzyme Nomenclature. Orlando, Florida: Academic Press Inc.1984;646.
26. Omaka ON, Keith-Roach M, Mckelvie ID, Worsfold PJ. Enzymatic flow-injection determination of phytase-hydrolysable phosphorus in natural waters using immobilized 3-phytase. International Journal of Environmental Analytical Chemistry. 2008;88(2):91-101.
27. Konietzny U, Greiner R. Molecular and Catalytic properties of Phytate-degrading enzymes (phytases). International Journal of Food Science and Technology. 2002;37:791-812.
28. Gardolinski PCFC, Hanrahan G, Achterberg EP, Gledhill M, Tappin AD, House WA, et al. Comparison of sample storage protocols for the determination of nutrients in natural waters. Water Research. 2001;35:3670-3678.
29. Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural water. Analytica Chimica Acta. 1962;27:31-36.

30. Ude EF, Ugwu LLC, Mgbenka BO, Nwani CD. Trends in Nitrate-Nitrogen, Nitrite-Nitrogen and Phosphorus concentration in Ebonyi River, Nigeria. Continental Journal of Fisheries and Aquatic Science. 2011;5(1):1-7.
31. Afiukwa JN, Eboatu AN. Analysis of Spring Water Quality in Ebonyi South Zone and Its Health Impact. American Journal of Scientific and Industrial Research. 2013;4(2):231-237.
32. Edward JB and Ugwumba AAA. Development Trends and Evaluation of Egbe Reservoir Water Nutrient Status in Ekiti State, Nigeria. Journal of Life Sciences. 2012;4(1):7-16.
33. Dike NI, Oniye SJ, Ajibola VO, Ezealor AU. Nitrate and Phosphate levels in River Jakara, Kano State, Nigeria. Science World Journal. 2010;5(3):23-27.
34. United States Environmental Protection Agency (EPA). Quality Criteria for Water. Washington DC: Office of Water; 1986.
35. Federal Environmental Protection Agency (FEPA). Interim Guidelines and Standards for Industrialized effluents, Gaseous emissions and Noise limitations. Abuja, Nigeria: FEPA Publication. 1991;28.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=408&id=5&aid=3419