ChemSearch Journal 6(1): 1 – 7, June, 2015
Publication of Chemical Society of Nigeria, Kano Chapter

Received: 29/12/2014 Accepted: 04/02/2015
http://dx.doi.org/10.4314/csj.v6i1.1

An Assessment of the Levels of Phosphate in Local and Foreign Detergents in Kano Metropolis, Kano, Nigeria

[1]Audu, A. A. and [2]Yusuf, S.
[1]Department of Pure and Industrial Chemistry, Bayero University, P. M. B. 3011, Kano, Nigeria
[2]Department of Chemistry, Jigawa State University, P. M. B. 048, Kafin Hausa, Nigeria
Email: aaaudu.chm@buk.edu.ng; sayzay37@yahoo.com

ABSTRACT

Concentration of phosphate in some commercial detergents commonly used in homes, laundry services, schools and offices in Kano Metropolis have been determined. The analyses were carried out on both local and foreign detergents using a spectrophotometric method. The analysis was based on the formation of phosphomolybdate with added molybdate ion followed by its reduction with sodium sulphide in aqueous tetraoxosulphate (VI) acid medium. The system obeys Lambert-Beer's law at 715 nm in the concentration range 0.30-12.24 mgdm^{-3} and the data were computed using percentage and t-test. The values obtained for local detergent samples were Bn (0.041%), Om (0.066%), Gn (0.013%), Bb (0.046%), Br (0.046%), Kl (0.035%), Bp (0.053%), Ep (0.010%), Zp (0.025%), Jb(0.014%), and Ar (0.042%) while those of the foreign detergents were Ps (0.005%), Td (0.042 %) ,Lk (0.027%), Ta (0.022%), Dm (0.007%), Bg (0.005%), Bs (0.026%), Kn (0.031%), and Lb (0.006%). The accepted level of phosphate in detergents is 0.5% (MSA, 2010). Thus, the concentration of these detergent samples fall within the set limit. This study has revealed that continuous use of these products could result in an increase in the phosphate levels in laundry discharges into soil, ponds, lakes and rivers. Excessive amount of phosphate has long been implicated in the eutrophication of surface water bodies, as such, to promote lake/river recovery and improve trophic status, it is imperative that phosphate loads in surface waters are reduced.

Keywords: Chelated, Eutrophication, Phosphate, Phosphomolybdate, Synthetic detergent

INTRODUCTION

Excessive use of phosphate as water softeners is criticized by environmentalists, since it contributes to water pollution (Glennie *et al*., 2002). The phosphates in domestic waste water pass through sewage disposal systems into rivers and lakes. They nourish bacteria which grow excessively and deplete the water of dissolved oxygen, thus killing fish. The phosphates may also produce massive overgrowth of water plants. When this crop of plant dies, there will be excessive decay and putrefaction which may also kill the fish (Philip, 2003).

In addition, excessive phosphates are known to accelerate the natural aging of lakes (eutrophication) (Ansar and Khad, 2005). They enter water ways as runoff from agricultural lands as fertilizer and as human and animal waste. The largest source of phosphorus entering the environment is synthetic detergent which contains phosphate compounds to soften water, increase the pH of water, and increase surfactant efficiency (Glennie *et al*., 2002). Detergents comprised 51 % of the phosphorus loading of urban wastewaters except for the Inanda catchment where it comprised 37 % as a result of industries in the Pietermaritzburg area contributing *ca*. 30 % to the loading (Manushani, 1994).

In 1986, Heynike and Wiechers of the Water Research Commission (WRC) undertook an investigation into detergent phosphorus and its impact on eutrophication in the Republic of South Africa. Their study showed that detergents comprised between 35 and 50 % of the total phosphorus loading on domestic wastewaters and presented a significant source of phosphorus to the environment.

In the mid 1960s, many of the nations' rivers and lakes were rapidly turning green and choking with aquatic plant growth. It was later observed that the primary reason for these deleterious changes in water quality was the high levels of phosphorus, one of the several major plant nutrients, found in domestic and municipal sewage effluents. The principal source of effluent phosphorus was from phosphates used in laundry detergents (Conflict Research Consortium, 1994). There was a growing public consensus that in order to save lakes phosphates must be banned from detergents Glennie *et al.* (2002).

Phosphate level of River Jakara in Kano State, Nigeria, was determined over a period of twelve month. The concentration of phosphate was found be higher than the international maximum permissible limit (Dike *et al*., 2010). High concentrations of nitrates and phosphates lead to eutrophication of water bodies (Taylor *et al*., 1997). Such environmental problems are increasingly occurring on a worldwide basis and now affect marine as well as freshwater ecosystems (Jens *et al*., 2000). As nitrates and phosphates are added to water bodies, they lead to overgrowth of phytoplankton, and this leads to depletion of dissolved oxygen (Wolfe and Patz, 2002). In the last several decades there has been a global increase in harm to fish and other aquatic life (Rabalais, 2002).

Phosphates perform many functions in washing powders and detergent. They soften hard water by binding with calcium ions and magnesium ions. In this way they prevent the lime in water from depositing or settling on the textile fibre. If the water is hard and contains dissolved lime, its ability to dissolve soap decreases and the cleansing powder deteriorates. Moreover phosphates stabilize the alkalinity of the surfactants. They keep the dissolved dirt in the water and prevent it from penetrating back into clothes (Sharma, 2006). The most important advantage of the synthetic detergents is better wetting and cleansing action and no consumption by hard water because of higher solubility of their Ca^{2+} and Mg^{2+} ions (Sharma, 2006).

Phosphates are also known to help peptize and suspended certain types of particulate matter, and aid in killing germs (Duthie, 1972). Phosphates detergents are generally safe to use with minimal toxicity problems. The major drawback is that secondary waste water treatment removes only a small percentage of phosphorus from the effluent (CRC, 1994).

Algae require primarily carbon, nitrogen and phosphorus to grow. Following Leibig's law of the minimum, the rate of algae growth (i.e. productivity) is controlled or limited by the nutrient in least supply relative to demand (Wetzel, 1993). Although algae are about 50% carbons, the relative abundance of dissolved carbon (IV) oxide and carbonates in natural waters rarely makes carbon the limiting nutrient. In most temperate fresh water, phosphorus is the limiting nutrient even though algal cells are less than 1% phosphorus (Wetzel, 1983). Thus, the rate of algal growth is proportional to the supply or input of phosphorus.

Phosphorus, which is an important nutrient, occurs widely in the environment and in the aqueous environment the phosphorus greatly encourages microbial growth which is undesirable. The determination of phosphorus is therefore of importance to chemical analysts and limnologists. Detergents, sewage and fertilizers are known to be the major sources of phosphorus and the level of 0.03 - 0.40mgdm^{-3} of total inorganic phosphate had become the maximum acceptable level in water (Abha and Gupta,1983).

Phosphate is not harmful but is a natural and essential macronutrient for all living organisms. Normally, the phosphate concentration of surface waters is so low that it is a limiting factor for growth of algae and higher plants. Consequently, when excess phosphate is released into the aquatic environment, the resulting over fertilization, leads to increased growth of algae (Ansar and Khad, 2005).

Lakes naturally receive carbon, nitrogen, phosphorus and other algal nutrients from rainfall and runoff. Overtime, as lakes receive more nutrients they naturally become more productive. Eventually lakes will fill in with organic matter, turn into bogs, and develop into dry land. This natural process of nutrient enrichment, or eutrophication, can easily take tens of thousands of years (Congressional Report, 1970).

However, scientists were alarmed by the phosphate levels they found coming from the drainage system, they were significantly higher than those of most agricultural runoff sources. This could potentially cause great harm to nearby surface water systems and the many diverse organisms that inhabit them (Balogh, *et al*., 2006).

Eutrophication is the process by which the nutrients in a water body increase resulting in an increased rate of productivity, generally of phytoplankton and of macrophytes in shallow waters. The algae content increase in the water and the subsequent death of algae bloom leads to consumption of the oxygen dissolved in water, creating hypoxic and at times, near anoxic situation. This excess eutrophication kills aquatic life and causes odour and increase pathogenic organisms (Sharma, 2006). There are many detrimental effects when a lake goes from pristine clear to pea soup green. If the lake serves as a source of drinking water, excessive algal growth clogs intakes, makes filtration more expensive, increases corrosion of pipes, and often cause taste and odour problems (Revelle and Revelle, 1988).

Due to the concomitant secondary processes (organic load of waters, oxygen depletion after the organic bio-mass is microbially degraded), the overall water quality may be considerably reduced. Detergent phosphates released with laundry waste water are quickly converted into orthopshosphate. Thus, the use of sodium triphosphate in detergent came under critical scrutiny although many other phosphate sources exists that contribute to eutrophilication of surface waters (Smulders, 2002).

Where sodium triphosphate is used as builder in household detergents it contributes to up to 50% of soluble phosphorus in municipal wastewater, therefore a reduction in the use of phosphate based detergents should have a positive impact on the eutrophication of surface water bodies (Glennie et al. 2002). As a consequence of the partial removal of phosphate in sewage treatment plants and the input of phosphates by other sources (human excretion, food industry, agricultural fertilizers), the share of detergent phosphates in surface waters was estimated to be about 40%. This balance showed already that the reduction of phosphates in detergent is an important but not the sole factor in solving the eutrophication problem of surface waters (Finch and Smith, 1981).

The most comprehensive approach involves chemical elimination of phosphates in the sewage treatment plant (tertiary treatment), removing the total phosphorus content of the wastewater. This approach is realized in some countries to a greater or lesser extent. Nevertheless, phosphate reduction in detergents, i.e. at its source, provides immediate relief to receiving waters and, ultimately, also to the coastal areas of the seas, which are increasingly confronted with eutrophication problems (Smulder, 2002). The legislative pressure towards phosphate reduction in detergents accompanied by the availability of suitable substitutes has resulted in a noticeable quality improvement of a number of surface waters today (Gerike, et al. 1989). Phosphate balances of the river Rhine in 1979 and 1989 showed that the measured phosphorus load reduction of about 28400tons/annum within this period corresponded very well with the expected phosphorus reduction due to the use of phosphate –reduced or free detergents (26700tons/annum) (Gerike, et al. 1991). The aim of this research is to determine and compare the levels of phosphates in detergent locally produced with those of foreign origin and relate same to the world accepted standards.

MATERIALS AND METHODS

In the preparation of reagents, chemicals of analytical grade purity and distilled water were used. Visible spectrophotometer cell model 7400 with 1cm matched quartz cells and a digital balance readable 0.001 was used.

All glass-wares as well as plastic containers including crucibles and pipettes were thoroughly washed using a detergent solution followed by rinsing in tap water and distilled water. The cleaned glass-wares were finally dried in an oven overnight at 30^0C.

Sampling

Twenty (20) different brands of powdered detergent samples were purchased from retail outlets, stores and open market in Kano, Northern Nigeria. The representative samples that were purchased and used for the purpose of this work include: Bp(Polythene), Bn(Carton), Jb(Polythene), Zp(Polythene), Ep(Polythene), Br (Polythene), Kl (Polythene), Gn(Polythene), Om(Polythene), Bb(Polythene), Ar (Polythene), Dm (Carton), Ps(Carton), Ta(Carton), Bs(Carton), Td (Carton), Bg(Polythene), Lk(Polythene), Kn(Polythene), Lb(Carton).

Sample Preparation

5g of each of the powdered sample was weighed into porcelain crucibles and placed in a muffle furnace set at 350˚C for half an hour; fume was released and the obtained ash was transferred into a $150cm^3$ beaker. It was dissolved with $50cm^3$ water and the solution was made acidic by adding 28 cm^3 of 5 $moldm^{-3}$ tetraoxosulphate (VI) acid (assay 98%, spec. Gravity 1.84). It was then heated on a water bath at 99.9˚C for about 10 minutes to expel the hydrogen sulphide and alkyl nitrite. The resulting solution was filtered into a $100cm^3$ volumetric flask and diluted to the mark with water. The solution was used for its phosphate content analysis (Mahadevaiah, et. al. 2007).

Determination of Phosphate

A series of ten $10cm^3$ volumetric flasks were labelled. To each flask was added $0.5cm^3$ of 5.9 x 10^{-3}M ammonium molybdate (specific gravity, 2.36), $3cm^3$ of 0.125M tetraoxosulphate (VI) acid and aliquots of disodium hydrogen phosphate (specific gravity, 1.7) which ranged from 0.30-12 $mgdm^{-3}$ (i.e. 0.10, 0.20, 0.30, 0.50, 1.00, 2.00, 2.50, 3.00, 3.50 and $4.00cm^3$.) were added. Finally, to each flask, $1cm^3$ of 6.4 x 10^{-3}M sodium sulphide (specific gravity, 1.86) solution was added as a reducing agent. Each solution was allowed to set at room temperature for about 20 minutes to enable full colour development. The absorbance values of the standard solutions were measured at 715nm which was used in preparing the working (calibration) curve (Mahadevaiah, 2007).

Statistical Analysis

Results were expressed using standard deviation, bar chart and t-test.

RESULTS AND DISCUSSION

The concentrations of phosphate determined were greater than those analysed by Kathlia, et al. (2013) whose concentration of phosphate in bar, liquid, and powder detergents varied as $0.181mg/dm^3$, $0.172mg/dm^3$ and $0.172mg/dm^3$, respectively. However, these values are lower than those determined by Mahadevaiah, *et al*. (2007) which were found to be 19.5, 25.2 and 18.0 mg/dm^3. Also Samjhana, *et al.* (2013) determined the level of phosphate in detergents and they obtained the following results 63.2, 20.3, 21.1, 34.5, 59.2, 21.9, 19.9, and 75.3 mg/dm^3. These results show a great discrepancy from those we obtained in this study.

The phosphate levels in the locally used detergents ranged between 0.0100 ± 0.001 - 0.0662 ± 0.001 and those of foreign detergents ranged between 0.0047±0.001 - 0.0305±0.000. A critical examination of Tables 1 and 2 revealed that local detergents like Om, Bb, Br, Bp, and Ar with percentage phosphate content corresponding to 0.0662%, 0.0462%, 0.0460%, 0.0529% and 0.0415% respectively have a higher phosphate content than the foreign ones like Ps, Td, Lk, Ta, Dm, Bg, Bs, Kn, and Lb having percentage phosphate content corresponding to 0.00473%, 0.0423%, 0.0272%, 0.0218%, 0.00680%, 0.00491%, 0.0255%, 0.0305% and 0.0064% respectively. However, Ar of local brand with percentage phosphate content as 0.0415% almost has the same concentration of phosphate as Td of the foreign product with percentage composition of phosphate as 0.0423%. In the local detergents analysed Ep has the lowest concentration of 0.010% with a standard deviation of ±0.000. It was observed that Ps of foreign origin with phosphate content of 0.0047% ±0.001 has the least phosphate concentration of all the detergents analysed.

In addition, the mean standard deviation of the local detergents were ±0.001, ±0.002 and ±0.003 whereas those of the foreign detergents was ±0.001. This shows that the mean deviation of the foreign detergents agree among themselves more than those of the local detergents. This means that the foreign companies are keeping to the set standards.

It was observed that the mean percentage phosphate in the local and in the foreign detergents analysed were 0.039% and 0.019% respectively indicating that local detergents contained approximately twice phosphate as builder than foreign detergents. Since the washing capacities of the foreign and local detergents are similar it indicates that foreign detergents are using other builders in addition to phosphates.

After computing the t-test for both the local and foreign detergents analysed, it was observed that at 5% probability and 18 degrees of freedom, the value is 2.101 and the calculated t-value is 2.378. This indicates that the calculated t-value (2.378) is greater than the table t-value, thus one can conclude that there is a significant difference between the concentration of phosphate in the local and in the foreign detergents analysed.

The present study indicated that detergent used locally contain higher levels of phosphate in comparison to the ones used in other parts of the world. Thus, higher amount of of phosphate are therefore released into the sewers, lakes, ponds and rivers, via laundry services, hand washings, kitchen utensils washings etc, by the use of local detergents when compared to the contribution of phosphate from foreign detergents.

Many countries in Europe, for example, Germany, the Scandinavian countries, Italy, Austria, the Netherlands and Switzerland have banned the use of phosphate in detergents (Smulders, 2002). The European Union set an overall limit of 0.5% by weight for the content of phosphorus in household laundry detergents (Malta Standard Authority, 2010). The U.S. and Canada also set 0.5% limit for phosphorus in automatic dishwashing detergents. On comparing this set limit for phosphates with the mean percentage phosphate contents in the local and foreign detergents as contained in Tables 1 and 2, it was observed that, the concentrations of both foreign and local fall within the set limit. The levels of phosphates in these detergents could be due to compliance with standards sets by regulatory bodies which apparently signifies that, the local and foreign detergents analysed are safe for use in the environment.

Table 1: Percentage Phosphate Content Determined in Local Detergent Sample

S/N	Sample	Phosphate mgdm^{-3}	Phosphate in % / Std Deviation
1.	Bn	4.12	0.0412$\pm$0.001
2.	Om	6.62	0.0662$\pm$0.001
3.	Gn	1.30	0.0130$\pm$0.000
4.	Bb	4.26	0.0462$\pm$0.001
5.	Br	4.60	0.0460$\pm$0.001
6.	Kl	3.54.	0.0354$\pm$0.002
7.	Bp	5.29	0.0529$\pm$0.003
8.	Ep	1.00	0.0252+0.002
9.	Zp	2.52	0.0252$\pm$0.002
10.	Jb	1.35	0.0135$\pm$0.001
11.	Ar	4.15	0.0415$\pm$0.001

Table 2 :Percentage Phosphate Content Determined in Foreign Detergent Samples.

S/N	Sample	Phosphate mgdm^{-3}	Phosphate in % / Std Deviation
1.	Ps	0.473	0.0047$\pm$0.001
2.	Td	4.230	0.0423$\pm$0.001
3.	Lk	2.720	0.0272+0.001
4.	Ta	2.180	0.0218$\pm$0.001
5.	Dm	0.680	0.0068$\pm$0.000
6.	Bg	0.491	0.0049$\pm$0.000
7.	Bs	2.550	0.0255$\pm$0.000
8.	Kn	3.050	0.0305$\pm$0.000
9.	Lb	0.640	0.0064$\pm$0.000

Analysed results of phosphate in commercially available synthetic detergent powders (Table 3) shows that, the Phosphate concentration in detergents ranged between 0.473 to 6.62mgdm^{-3}. Detergent brands Om and Lb results showed that maximum concentration of 6.62 mg/dm^{-3} were recorded for the local brand Om while a minimum of 0.473mg/dm^{3} was recorded for the foreign detergent Ps. There was a negligible concentration of Phosphate recorded in Ps, Dm, Bg and Lb. Out of the 20 detergents tested , 9 were recorded between 1.00- 3.55mg/ dm^{3} of phosphate concentration while 7 have concentration between 4.00-6.62mg/dm^{3}.

Table 3: Percentage Phosphate Conten. Determined in Local and Foreign Detergent Samples.

S/N	Sample	Phosphate $mgdm^{-3}$	Phosphate in % / Std Deviation
1.	Bn	4.12	0.0412±0.001
2.	Om	6.62	0.0662±0.001
3.	Gn	1.30	0.0130±0.000
4.	Bb	4.26	0.0462±0.001
5.	Br	4.60	0.0460+0.001
6.	Kl	3.54	0.0354±0.002
7.	Bp	5.29	0.0529±0.003
8.	Ep	1.00	0.0252±0.002
9.	Zp	2.52	0.0252±0.002
10.	Jb	1.35	0.0135±0.001
11.	Ar	4.15	0.0415±0.001
12.	Ps	0.473	0.0047+0.001
13.	Td	4.230	0.0423±0.001
14.	Lk	2.720	0.0272±0.001
15.	Ta	2.180	0.0218±0.001
16.	Dm	0.680	0.0068±0.000
17.	Bg	0.491	0.0049+0.000
18.	Bs	2.550	0.0255±0.000
19.	Kn	3.050	0.0305±0.000
20.	Lb	0.640	0.0064+0.000

CONCLUSION

The results indicated that local detergents contain higher percentage of phosphate than the foreign ones. Also it revealed that there is a significant variation in terms of the phosphate levels between the local and foreign detergents. Furthermore, the study has revealed that continuous use of these products could result in an increase in the phosphate levels in laundry discharges into soil, ponds, lakes and rivers which may leads to the eutrophication of surface water bodies. Therefore, to promote lake/ river recovery and improve trophic status, it is imperative that phosphorus loads entering surface waters are reduced

REFERENCES

Abha, C. and Gupta, V. K. (1983). “Spetrophotometric Determination of Phosphate in Polluted Water by Solvent Extraction of Molybdenum Blue” *Analyst, Volume* 108. Pp 1141- 1144.

Ansar, A. and Khad, F**.** (2005). Eutrophication: An Ecological Vision. The Botanical Review, *71*(4), 449-82.

Balogh, J., Fausey, N., Harmel, R., Hughes, K., and King, K. (2006). Nitrate-nitrogen and dissolved reactive phosphorus in subsurface drainage from managed turfgrass. *Journal of Soil and Water Conservation*, *61*(1), 31-41.

CONFLICT RESEARCH CONSORTIUM. *Working Paper 94-54 February 1994*

Congressional report HR 91-1004 April 14, (1970). “Phosphates in Detergents and the Eutrophication of America’s Waters’’ Committee on Government Operations. P 3.

Dike, N. I., Oniye, S. J., Ajibola, V. O., and Ezealor, A. U. (2010). Nitrate and Phosphate Levels in River Jakara, Kano State, Nigeria, Science World Journal Vol. 5 (No. 3) pp 23-27.

Duthie, J.R. (1972): “Detergents: Nutrient considerations and total assessment. In: Nutrients and Eutrophication: The Limiting- Nutrient Controversy.” (G.E. Likens, ed.) Limnology and Oceanography, Special Symposium, No.1:205-216.

Finch, J. A. and Smith, G. W. (1981). Anionic Surfactants, Marcel Dekker, New York. PP 98-99

Gerike, P.; Winkler, K. and Schneider, W. (1991) Tenside Surfactant Detergent. 28 pp 86-89.

Gerike, P.; Winkler, K. and Jakob, W. (1989).Tenside Surfactant Detergent. 26 pp 270-275.

Glennie, E. B., Littlejohn, C., Gendebien, A., Hayes, A., Palfrey, R., Sivil D. and Wright, K. (2002). Phosphates and Alternative Detergent Builders – Final Report pp 6-74, EU Environment Directorate

Heynike J J C & H N S Wiechers, (1986). Detergent Phosphates in South Africa: Impact on Eutrophication and Implications of Banning. Unpublished Report to the Water Research Commission, Pretoria.

Jens, S., Per, L., Rutger, R., Per, J., Mats, O. and Dag, M. (2000). Eutrophication and Contaminants in Aquatic Ecosystems. Ambio; 29 (4-5): 184-194.

Kathlia, D. C. and Carlo, A. T. (2013). Spectrophotometric Determination of Phosphate in Detergent, Volume No. 2 Issue No. 3, Published by Carlo Aguas Tayag.

Mahadevaiah, M. S., Yogendra, K., Mansur, S. A., Galil, M. S., Suresha, M. A. S, and Nagendrappa, G. (2007). A Simple Spectrophotometric Determination of Phosphate in Sugarcane Juice, Water and Detergent Samples, *E-Journal of Chemistry*, 4(4):467-473.

Malta Standard Authority :(2010). Second Floor, Evans Building Merchants Street Valletta VLT03 Malta. P 3.

Manushan,i P. (1994). Detergent Phosphorus in South Africa: Impact on Eutrophication with Specific Reference to the Umgeni Catchment, University of Natal Durban, pp 5.1-5.2

Philip, M. (2003) Advanced Chemistry (Physical and Industrial) Punlished in South Asia by Foundation Books 4764/2A, 23 Ansari Road pp. 168.

Rabalais, N. N. (2002). Nitrogen in Aquatic Ecosystems, *Royal Swedish Academy of Sciences, Ambio* 31(2): 102 – 112.

Revelle, P. and Revelle, C. (1988). The Environmental Issues and Choices for Society. 3rd edition Jones and Bartlett Publisher, Boston pp 749.

Samjhana, P. and Megh, R. P. (2013). Spectrophotometric Determination of Phosphate in Sugarcane Juice, Fertilizer, Detergent and Water Samples by Molybdenum Blue Method Scientific World; 11 (11):58-62.

Sharma, B.K. (2006): Industrial Chemistry (including chemical engineering 5th ed. Published by Goel Publishing House a unit of Krishna Prakashan Media (P) Ltd, pp1252-1279.

Smulders, E**.** (2002). Laundry Detergent. Wiley-VCH Verlay GMbH Weinheim, Germany PP 172.

Taylor, D. J., Green, N. P.O. and Stout, G.W. (1997)**.** Biological Science Cambridge. Cambridge University Press, United Kingdom 984pp.

WetZel, R. G. (1993). Humic compounds from wetlands: Complexation, inactivation, and reactivation of surface-bound and extracellular enzymes. Int. Ver. Theor. Angew. Limnol. Verh. 25: 122-l 28.

WetZel, R.G. (1983). Limnology. 2nd edition Saunders College Publishing, Philadelphia 767. Tests: A Critical Review: Aquatic Toxicology. 5: 1-25

Wolfe, A. H. and Patz, J. A. (2002). Reactive nitrogen and human health: Acute and long term implications. Ambio: A journal of the human environment; 23 (2), 120-125.