TOTAL PHOSPHORUS DETERMINATION IN AQUATIC PLANTS USING AN IGNITION METHOD

DETERMINAÇÃO DE FÓSFORO TOTAL EM PLANTAS AQUÁTICAS UTILIZANDO O MÉTODO DA CALCINAÇÃO

Rinaldo José da Silva Rocha[1,2]
Marcela B. Cunha-Santino[1,3]
Jurity A. M. Milan[4]
Irineu Bianchini Jr.[1,5]

Abstract: In order to gain time and generate less waste in the determination of the phosphorus content in aquatic macrophytes and in its detritus, it is proposed the use of ignition method to replace the digestion procedure of samples. The remaining material from decomposition experiment was dried and submitted to ignition (550 ºC; 2 h). The same samples had their phosphorous contents quantified by colorimetric method that includes previous acid digestion. The phosphorous contents were also measured in the ash derived from ignition. For all samples (n = 104), the ignition method was less efficient, the average of global yield was 14.2%. However, the equivalence between these two procedures was obtained and confirmed by the linear relationship with high r^2 value (0.95).

Keywords: Aquatic macrophytes. Elemental composition. Lajes Reservoir.

Resumo: Para ganhar tempo e gerar menos resíduos na quantificação de fósforo em plantas aquáticas e seus detritos, propõe-se a utilização do método de ignição para substituir o processo de digestão das amostras. Os detritos remanescentes de um experimento decomposição foram secos e submetidos à ignição (550 ºC; 2 h). As mesmas amostras tiveram seus conteúdos de fósforo quantificados pelo método colorimétrico que inclui a prévia digestão ácida. Os conteúdos de fósforo também foram determinados nas cinzas derivadas da ignição. Para todas as amostras (n = 104), o método de ignição foi menos eficaz, a média de rendimento global foi de 14,2%. Contudo, equivalência entre estes dois procedimentos foi obtida e confirmada pela relação linear com valor alto de r^2 (0,95).

Palavras-chave: Macrófitas aquáticas. Composição elementar. Reservatório de Lajes.

1 INTRODUCTION

Phosphorus is a vital element; however, its surplus causes changes in aquatic environments. In the situation of enrichment of water with nutrients (i.e. eutrophication), when nitrogen and phosphorus does not constitute a limiting factor, usually occurs blooms of algae and of the aquatic weeds (Rast et al., 1989). There are other several consequences linked with eutrophication, among which are mentioned: i) anoxia or anaerobic; ii) blooms of cyanobacteria; iii) effects on human health by producing cyanotoxins; iv) high concentrations of organic matter resulting from plant debris; v) restrictions on fishing and recreational activities; vi) changes in biodiversity and alterations in species composition; vii) an increase in the cost for treating water; viii) potential reductions in shipping and transportation capacity; ix) decrease in water transparency; x) changes in the pH variation (Tundisi and Matsumura-Tundisi, 2002; Shaw et al., 2003; Xavier et al., 2005; Hilton et al., 2006; Smith and Schindler, 2009; ANA, 2015). Thus, studies dealing with phosphorus cycling in different compartments (e.g. sediment, debris, water) and organisms (e.g. plankton, aquatic macrophytes) are usually required.

[1] Programa de Pós-Graduação em Ecologia e Recursos Naturais. Universidade Federal de São Carlos (UFSCar).
[2] Biólogo da Light Energia S.A. Gerência de Meio Ambiente. E-mail: rinaldo.rocha@light.com.br
[3] Prof. Adjunto do Departamento de Hidrobiologia. UFSCar. E-mail: cunha_santino@ufscar.br
[4] Bióloga do Departamento de Hidrobiologia. UFSCar. E-mail: jurity@ufscar.br
[5] Prof. Titular do Departamento de Hidrobiologia. UFSCar. E-mail: irineu@ufscar.br

Chemical analyses of biomass are utilized in several environmental studies and are essential to describe the interactions between the Earth compartments (i.e. atmosphere, ocean, lithosphere) and the biosphere. In the ecological and geochemistry studies, the decomposition experiments are conducted in order to discuss the turnover rates of elements into the ecosystems (Schlesinger, 1997). For these experiments, the litter bag technique is one of the main ones (Swift et al., 1979). This method consists of putting the same target organic resource (e.g. leaves, roots, branches, and animal) into the bags (with known mesh) and incubating them within the soil or aquatic ecosystem (e.g. lake, river); Swift et al., (1979); Bärlocher (2005). Over time, the litter bags are recovered to evaluate the biomass loss in order to describe the decomposition kinetics derived from the predominant environmental conditions (e.g. temperature, oxygen availability, moisture, redox potential, pH, nutrients concentrations, type of organisms; Silva el al., 2011) and to verify the changes of resources (Flindt and Lillebø, 2005). Owing to the inorganic compounds inclusion into the litter bags (i.e. colloids, sand, clay, gravel) during the experiment time, to describe the organic decay it is necessary to establish the ash content of organic resource and its detritus in order to present “ash free” results (i.e. organic matter basis); Bärlocher (2005). Thus, in this context, to explain the accurate kinetics of decay of organic matter or target elements (e.g. P, N, S, and C) the ash determinations for these detritus samples are necessary. On the other hand, several chemical analyses include the acid digestion (usually with perchloric acid mixtures) as extraction procedure, before the use the determination methods. Colorimetric determination of total phosphorus in plant samples includes a preliminary acid digestion to convert the phosphorus of tissues into orthophosphate (Allen et al., 1974). Thus, in this study it is proposed the use of ignition method to substitute the digestion procedure, in order to simplify the analytical procedures involved with the total phosphorus quantification. Based on this proposed replacement, it is possible to use the ashes produced in the calcination for the phosphorus determination, thereby eliminating the need to carry out the acid digestion of the samples, gaining time and generating less waste.

2 MATERIAL AND METHODS

2.1 GENERAL EXPERIMENTAL PROCEDURES

Lajes Reservoir is oligo-mesotrophic, clear water (4.0 ≥ Secchi disc depth ≤ 6.5 m) with slightly alkaline on the surface and slightly acid near the sediment (Guarino et al., 2005). The maximum water level is located at elevation 416 m, where area = 32 km^2, volume = 475 hm^3; average depth = 15 m; maximum depth = 45 m. The average concentration of dissolved oxygen varies between 0.01 (in the bottom) and 7.68 $mg.L^{-1}$ (at the surface); the mean pH is 7.87, and turbidity 0.9 NTU (Rocha et al., 2003; Guarino et al., 2005). The litter bags were incubated (2.0 m of depth) nearby the dam (22° 42' 02" S and 43° 52' 54" W), in January 2010.

2.2 PLANT MATERIAL

For litter bags preparation, five species of aquatic macrophytes were harvested (*Brachiaria subquadripara* (Trin.) Hitchc., *Eichhornia crassipes* (Mart.) Solms, *Pistia stratiotes* Linnaeus, *Sagittaria montevidensis* Cham. and Schltdl., *Salvinia auriculata* Aubl.). Then, in laboratory, the plants were washed with running water, and dried at 50 °C, until constant mass. For each species were prepared 30 litter bags (20 × 35 cm, mesh = 200 µm) containing 20.00 g (dry mass basis) of plant fragments. For analysis of mass loss during decomposition were considered the following sampling days: 0, 1, 3, 5, 10, 20, 30, 60, 90 and 120. On these days, for each species, 3 litter bags were taken to the laboratory; the detritus were dried at 50 °C until constant weight, and the masses were estimated gravimetrically (Bärlocher, 2005; Silva et al., 2011).

2.3 TOTAL PHOSPHORUS DETERMINATION

For each aquatic plant (zero-day sample) and its remaining detritus (recovered from litter bags) samples, two total phosphorus determinations were made (by colorimetric method); one sample was prepared from the acid digestion method and the other by using the ignition method.

The total phosphorous content of each treatment was compared and the frequency distribution of the yields was considered. The yields were calculated considering the value obtained by acid digestion method as 100%, according to Equation 1. These parameters were submitted to the Shapiro-Wilk normality test (Shapiro and Wilk, 1965), to a significant level of 0.05.

$$Yield = \frac{[P]_{IG} \times 100}{[P]_{AD}} \quad (1),$$

where: $[P]_{IG}$ = phosphorus content in plant material or its detritus calculated according ignition procedure (mg g^{-1}); $[P]_{AD}$ = phosphorus content in plant material or its detritus calculated according acid digestion (mg g^{-1}).

2.4 DIGESTION METHOD

To make the plants digestions it was used the modified perchloric acid method (Allen et al., 1974): 10 ml of concentrated HNO_3 and 5 ml of concentrated H_2SO_4 were added to 100 mg of dry plant material in a 100 ml test-tube. The samples were boiled until clear on a hot plate (ca. 300 °C for 4 h). After cooling, the sample was diluted to 100 ml in a volumetric flask, and an aliquot was withdrawn for orthophosphate determination by the ascorbic acid reduction colorimetric method (Murphy, 1962; APHA, AWWA and WEF, 1998). Blanks and standards were treated as samples.

2.5 IGNITION METHOD

Dry plant materials (500 mg) were ignited in a muffle furnace in a porcelain crucible (550 °C for 2 h); Wetzel and Likens (1991). After cooling, the residues were washed into a 100 ml Erlenmeyer flasks with 10 mL 1N HCl. The samples were diluted to 100 ml in volumetric flasks, and orthophosphate were determined as in the acid digestion method (Murphy, 1962; APHA, AWWA and WEF, 1998). Standards and blanks were not ignited.

3 RESULTS AND DISCUSSION

Considering the acid digestion as reference, the selected aquatic plants presented the following content of total phosphorus: *S. auriculata*: 0.04%; *E. crassipes*: 0.13%; *S. montevidensis*: 0.20%; *P. stratiotes*: 0.12% and *B. subquadripara*: 0.05%. These values were lower than the average (0.26%) reported in a compilation that considered 35 measures (Bianchini Jr. and Cunha-Santino, 2008). However, these values are into the range of phosphorous concentration presented by tissues of wetland plants (0.01-0.82%), and the phosphorous content in plant tissues can also be greater when its availability increases (Gopal and Ghosh, 2009). Phosphorus uptake by macrophytes is maximum during the growing season, followed by a decrease or even cessation in the fall/winter. Typically, uptake rates of phosphorus by many aquatic macrophytes are highest during early-spring growth, before maximum growth rate is attained. This early uptake and storage of nutrients provides a competitive edge for growth-limiting nutrients during the periods of maximum demand. Phosphorus concentrations are higher in young plants, but as the biomass increases, tissue phosphorus concentration decreases (Reddy and DeLaune, 2008). Because of their high biomass yield, nutrient recovery rate and high protein, starch and flavonoid content, aquatic plants, such as duckweeds, have received increasing attention for nutrient recovery from wastewater (Zhao et al., 2015).

For all kind of samples (different species of aquatic plants and detritus), the ignition method was less efficient; the average yields of P content varied among 12.5 (*P. stratiotes*) and 16.8% (*S. montevidensis*); Figure 1A. Except to the set of samples constituted by *B. subquadripara*, the yields presented normal distribution and the total phosphorus content yield did not change considering original plant tissues and its detritus (Table 1). If taken in account all samples (n = 104) the average of global yield was 14.2% (± 2.86; standard deviation) and also presented normal distribution (Figure 1B). These results were similar to verified for sediments samples, where the acid digestion was better extractor; however, the recovery was better (Andersen, 1975), probably by the inclusion of one more step to process (i.e. after calcinations, the residue (ash) was washed into a 100 mL flask with 25 mL 1N HCl and boiled for 15 min on a hot plate). Concerning the effect of the time for which the ignited residue is boiled with HCI an experiment with sediment was made. Recoveries of 97, 98, 98, 99 and 100% were obtained after 1, 2, 5, 15 and 30 min of boiling (Andersen, 1975). The quantitative equivalence between these two procedures (acid digestion and ignition) can be stressed by the linear relationship (Figure 1C) with high r^2 value (0.95). In this case, the slope is the proportional value between these two procedures.

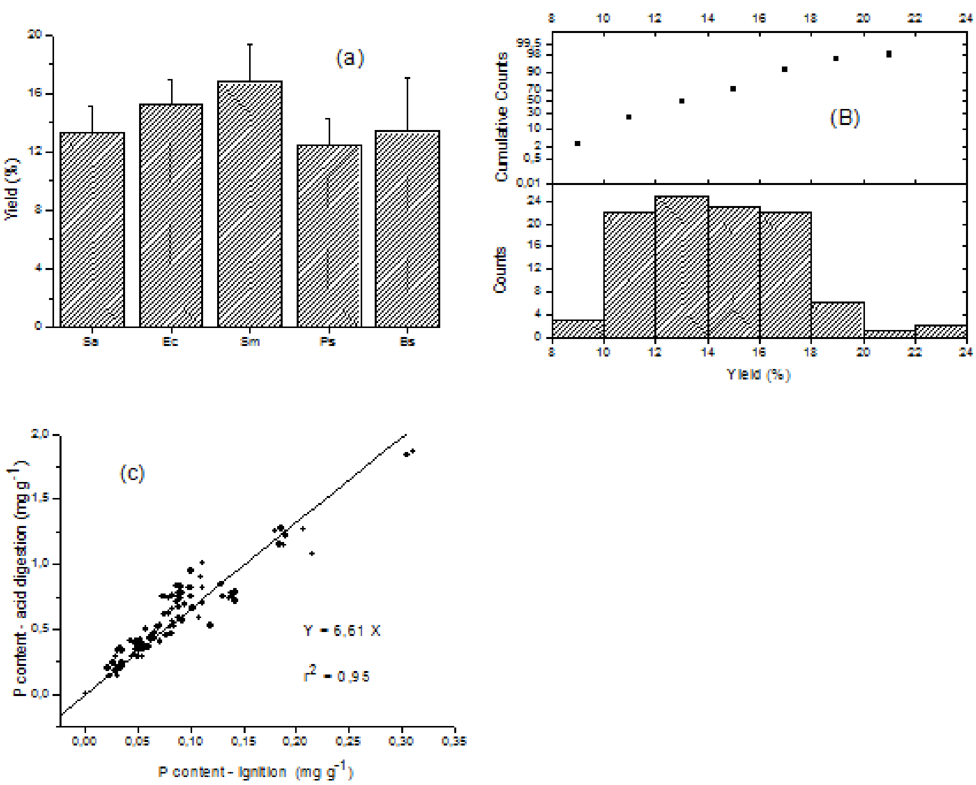

Figure 1. Average yield from ignition method in relation to acid digestion (a); where: Sa = *Salvinia auriculata*; Ec = *Eichhornia crassipes*; Sm = *Sagittaria montevidensis*; Ps = *Pistia stratiotes*; Bs = *Brachiaria subquadripara*; the bars corresponding the standard deviation. Histogram of frequencies and probabilities for all yield data (b), and the relationship between ignition method and acid digestion (c).

Table 1. Maximum (Max) and minimum (Min) values of yields; parameters (W, P) of Shapiro-Wilk test and the number of samples (n).

Plant material and its detritus	n	Max (%)	Min (%)	W	P value	Decision
Salvinia auriculata	21	17.3	10.2	0.974	0.805	normal
Eichhornia crassipes	20	18.7	12.6	0.968	0.709	normal
Sagittaria montevidensis	21	22.5	12.2	0.976	0.840	normal
Pistia stratiotes	21	16.3	10.3	0.918	0.076	normal
Brachiaria subquadripara	21	22.9	9.1	0.881	0.013	not normal
All samples	104	22.9	9.1	0.965	0.054	normal

4 CONCLUSIONS

It can be concluded that a determination of total phosphorus in plant material from ash (ignition method) gives a lower value than the mixed acid digestion method. The reproducibility of the two methods is similar. The main advantage of the ignition method is the convenient destruction by ignition in a furnace. Moreover, the loss on ignition is determined during many plant and plant detritus investigations; hence the residue from this determination can be used for the total P determination simply by dissolving the ash in 1N HCl and diluting to a known volume. A comparison of results obtained with the acid digestion method is recommended before widespread application of the technique.

ACKNOWLEDGEMENTS

This study was supported by the Program of Research and Technological Development of the Brazilian Electric Sector (Agreement: National Agency of Electric Energy (ANEEL), Light Energy and UNESP; ANEEL proc. nº: PD-5161-0005/2010-P&D 05/10) and by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior) for the fellowship provided to R. J. S. Rocha. We are also indebted to Lígia B. R. Bianchini (Mackenzie University) for her critical proof reading of the manuscript.

REFERENCES

ALLEN, S.E., GRIMSHAW, M., PARKINSON, J.A. & QUARMBY, C. Chemical Analysis of Ecological Materials. Oxford: Blackwell, 1974.

ANA - Agência Nacional das Águas. Indicadores de Qualidade - Índice do Estado Trófico. 2015. Available from <http://portalpnqa.ana.gov.br/indicadores-estado-trofico.aspx>.

ANDERSEN, J.M. An ignition method for determination of total phosphorus in lake sediments. Water Res., 10(4): 329-331, 1975.

APHA, AWWA & WEF Standard Methods for the Examination of Water and Wastewater. 20. ed. Maryland: APHA, 1998.

BÄRLOCHER, F. Leaf mass loss estimated by litter bag technique. In: GRAÇA, M.A.S, BÄRLOCHER, F. & GESSER, M.O. (eds.). Methods to Study Litter Decomposition: a Practical Guide. Dordrecht: Springer, 2005. P.37-50.

BIANCHINI Jr., I. & CUNHA-SANTINO, M.B. As rotas de liberação do carbono dos detritos de macrófitas aquáticas. Oecol. Bras., 12(1): 20-29, 2008.

FLINDT, M.R. & LILLEBØ, A.I. Determination of total nitrogen and phosphorus in leaf litter. In: GRAÇA, M.A.S, BÄRLOCHER, F. & GESSER, M.O. (eds.). Methods to Study Litter Decomposition: a Practical Guide. Dordrecht: Springer, 2005. P.53-59.

GOPAL, B. & GHOSH, D. Natural wetlands. In: JØRGENSEN, S.E. (ed.). Applications in Ecological Engineering. Elsevier: Amsterdam, 2009. P.44-55.

GUARINO, A.W.S., BRANCO, C.W.C., DINIZ, G.P. & ROCHA, R. Características limnológicas de um reservatório tropical antigo (Represa Ribeirão das Lajes, RJ, Brasil). Acta Limnol. Bras., 17(2): 129-141, 2005.

HILTON, J., O'HARE, M., BOWES, M.J. & JONES, J.I. How green is my river? A new paradigm of eutrophication in rivers. Sci. Total Environ., 365(1-3): 66-83, 2006.

MURPHY, J. & RILEY, J.P. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta, 27(1): 31-36, 1962.

RAST, W., HOLLAND, M. & RYDING, S. Eutrophication management framework for the policy-maker. France: UNESCO, 1989.

REDDY, K.R. & DELAUNE, R.D. Biogeochemistry of Wetlands: Science and Applications. Florida: CRC Press, 2008.

ROCHA, R.J.S., MELLO, R.B., GUARINO, A.S. & BRANCO, C.W.C. Dinâmica de variáveis físicas e químicas em um reservatório tropical (Reservatório de Lajes, RJ). In: CONGRESSO BRASILEIRO DE LIMNOLOGIA, IX, 2003, Juiz de Fora. Anais... Juiz de Fora: SBL/UFJF, 2003. P.496.

SCHLESINGER, W.H. Biogeochemistry an Analysis of Global Change. 2. ed. New York: Academic Press, 1997.

SHAPIRO, S.S. & WILK, M.B. An analysis of variance test for normality (complete samples), Biometrika, 52(3-4): 591-611, 1965.

SHAW, G.R., MOORE, D.P. & GARNETT C. Eutrophication and algal bloom. In: SABLJIC, A. (ed.). Environmental and Ecological Chemistry. University of Wisconsin, 2003. P.298-326.

SILVA, D.S., CUNHA-SANTINO, M.B., MARQUES, E.E. & BIANCHINI Jr., I. Decomposition of aquatic macrophytes: bioassays versus in situ experiments. Hydrobiologia, 665(1): 219-227, 2011.

SMITH, V.H. & SCHINDLER, D.W. Eutrophication science: where do we go from here? Trends Ecol. Evol., 24(4): 201-207, 2009.

SWIFT, M.J., HEAL, O.W. & ANDERSON, J.M. Decomposition in Terrestrial Ecosystems. Oxford: Blackwell, 1979.

TUNDISI, J.G. & MATSUMURA-TUNDISI, T. Lagos e Reservatórios. Qualidade da água: O impacto da eutrofização. São Carlos, SP: IIE, 2002.

WETZEL, R.G. & LIKENS, G.E. Limnological Analyses. 2. ed. New York: Springer-Verlag, 1991.

XAVIER, C.F., DIAS, L.N. & BRUNKOW, R. Eutrofização. In: ANDREOLI, C.V. & CARNEIRO, C. (eds.). Gestão Integrada de Mananciais de Abastecimento Eutrofizados. Curitiba: Sanepar, 2005. P273-302.

ZHAO, Y., FANG, Y., JIN, Y., HUANG, J., BAO, S., FU, T., HE, Z., WANG, F., WANG, M. & ZHAO, H. Pilot-scale comparison of four duckweed strains from different genera for potential application in nutrient recovery from wastewater and valuable biomass production. Plant Biol., 17(Suppl. 1): 82-90, 2015.