

**A REVIEW OF PROCEDURES FOR
EVALUATION OF BIOAVAILABLE
PHOSPHORUS IN PARTICULATE MATERIALS**

by

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MANAGEMENT PERSPECTIVE

Phosphorus control programs instituted in the early 70's have resulted in lower P concentrations in the waters of Lake Erie and Ontario. Improved trophic conditions are now indicated by shifts in algal species populations. Eutrophication-related problems, however, still exist in local nearshore areas.

It has been well documented that particulate materials such as sediments and eroded soils contribute significantly to total phosphorus loading. Thus, evaluation of bioavailable phosphorus from these sources is of paramount importance.

The objective of this report is to summarize the information on the use of chemical extractions for estimating particulate phosphorus available for aquatic plant growth. In particular, the utility of the citrate-dithionite-bicarbonate (CDB) extraction for measuring the bioavailable particulate phosphorus is evaluated. Based on the compiled information, the selection of a suitable procedure for the estimation of bioavailable P can be made.

ABSTRACT

Chemical extraction procedures used for the evaluation of bioavailable particulate phosphorus are examined and evaluated. In particular, the utility of the citrate-dithionite-bicarbonate (CDB) extraction in measuring the bioavailable phosphorus in particulate materials is assessed.

The CDB-extractable P is a reasonably good predictor of bioavailable phosphorus, although it represents the maximum amount of P which would be released, e.g. over a long period of time under anoxic conditions. The NaOH-extractable P represents a more labile fraction of particulate P, which would be available to the biota in oxic waters over the short term.

PERSPECTIVE - GESTION

Les programmes de contrôle du phosphore mis en oeuvre au début des années 1970 se sont soldés par une réduction des concentrations de phosphore dans les eaux des lacs Érié et Ontario. Les modifications constatées dans la population de certaines espèces d'algues indiquent que les conditions trophiques sont maintenant améliorées. Toutefois, des problèmes reliés à l'eutrophisation se manifestent encore localement dans certaines zones côtières.

Il est prouvé que des particules, notamment les sédiments et les particules de sol érodé, contribuent de façon significative à la charge totale en phosphore. L'évaluation de la biodisponibilité du phosphore provenant de ces sources s'avère donc d'une importance capitale.

Ce rapport vise donc à présenter un résumé des renseignements qui existent sur le recours à des procédés d'extraction pour estimer la quantité de phosphore à l'état de particules disponibles pour favoriser la croissance des plantes aquatiques. L'étude porte plus particulièrement sur l'évaluation de l'utilité de l'extraction par citrate-dithionite-bicarbonate (CDB) lorsque l'on veut mesurer la biodisponibilité du phosphore dans les particules. Une synthèse de ces renseignements permet de sélectionner une méthode appropriée pour estimer la biodisponibilité du phosphore.

RÉSUMÉ

On procède à l'examen et à la pondération des méthodes d'extraction chimique qui servent à évaluer la biodisponibilité du phosphore à l'état de particules. L'étude porte plus particulièrement sur l'extraction au citrate-dithionite- bicarbonate (CDB) comme méthode de mesure de la biodisponibilité du phosphore de certaines particules.

Le phosphore (P) extractible par la méthode au CDB constitue un indice relativement fiable de sa biodisponibilité, quoiqu'il représente la quantité maximale du phosphore qui pourrait se libérer sans oxygène pendant une longue période. Par contre, le phosphore (P) extractible par la méthode au NaOH constitue une fraction plus instable de phosphore à l'état de particules, c'est-à-dire qu'il serait disponible à court terme pour le biotope dans des conditions d'eau contenant de l'oxygène.

INTRODUCTION

There is a considerable body of research addressing the topic of biological availability of phosphorus in freshwater ecosystems. The topic is important, since it has an impact on future water quality management strategies.

The purpose of this report is to examine and evaluate the utility of the citrate-dithionite-bicarbonate (CDB) extraction for measuring bioavailable particulate phosphorus.

Biologically Available P in Freshwater Ecosystems

Phosphorus is present in aquatic environments as dissolved phosphorus and particulate P. Table 1 lists the forms of phosphorus in aquatic environments and their availabilities to the biota.

The immediately bioavailable forms of phosphorus in aqueous systems are the dissolved inorganic phosphates (DRP) (Logan et al. 1979, Expert Committee on Engineering and Technological Aspects of Great Lakes Water Quality 1980; Sonzogni et al. 1982). Other forms of P may become available through conversion to inorganic P (Sonzogni et al. 1982) on depletion of soluble orthophosphate. For instance, particulate P which represents a major reservoir of P to organisms, may be converted to available forms through the processes of dissolution and desorption.

TABLE 1 Summary of P forms, their biological availabilities and methods for their analyses (modified from Sonzogni et al. (1982)

Phosphorus Fraction and Form	Bioavailability	Method of Analysis
	<u>Dissolved</u>	
DRP: H_2PO_4^- , and HPO_4^{2-}	Directly and immediately available.	Inorganic phosphate in filtrate (Strickland and Parsons, 1968; APHA 1980); dissolved inorganic phosphate is defined as the phosphate not retained by a 0.45 μm filter.
DCP ¹ : compounds with P-O-P bonds and P-O-C bonds	Converted to DRP through fairly rapid hydrolysis.	Inorganic phosphate analysis following acid hydrolysis of filtrate (APHA 1980)
	<u>Particulate</u>	
NAIP ² : P adsorbed on metal hydroxides (Fe, Al), Fe- and Al-P minerals, non-apatite Ca-P minerals	Partially available through dissolution or desorption of phosphate when DRP concentration is low due to dilution, biological uptake, or chemical immobilization (see Table 2).	Sequential extraction with a CDB reagent followed by 1N NaOH or by other equivalent procedures (see Table 2) (Williams et al. 1976a, 1976b).
AIP ³ : Ca-P mineral	Essentially unavailable because of insolubility of apatites.	HCl extraction following NAIP extraction (Williams et al. 1976a, 1976b).
Organic P: nucleic acids, phospholipids, inositol phosphates, other	Converted to DRP through biological mineralization; may be rapid for substantial fraction of fresh plant tissue or animal wastes; slow for soil and sediment organic P.	Difference between total particulate P and inorganic particulate P (Sommers et al. 1972).
Condensed P	Released from plant tissues at senescence and hydrolyzed to DRP; small fraction compared with organic P.	Usually included as organic P except for fraction hydrolyzed during inorganic P extraction; can be estimated by selective hydrolysis (Clesceri and Lee, 1965).

¹ Dissolved condensed phosphates; ² non-apatite inorganic P; ³apatite inorganic P.

Procedures for Estimating Biologically Available Particulate Phosphorus

Much of the information regarding assessment of bioavailable particulate phosphorus has been summarized by Lee et al. 1980, by Expert Committee on Engineering and Technological Aspects of Great Lakes Water Quality 1980 and by Sonzogni et al. 1982.

At present, extraction procedures and bioassay techniques are utilized to estimate biologically available P in particulate fractions. Chemical extraction procedures are generally less costly and time consuming, consequently they are frequently chosen for estimation of bioavailable P in solids. Despite these already mentioned advantages, selectivity can be a major problem of the chemical extraction procedures. Table 2 lists some of the commonly utilized extraction procedures for evaluation of phosphorus forms in particulate samples. Table 3 shows that much of the work concerning bioavailable particulate phosphorus was carried out on erosional materials, marine and lake sediments, urban runoff and wastewater particulates.

With a few exceptions (Canviro Consultants Ltd. 1983), most of the studies indicate a close correlation between NaOH-extractable P and available P estimated by bioassay techniques. Sagher et al. (1975) showed that 60-80% of NaOH-extractable P was removed by Selenastrum during incubation studies. Logan et al. (1979b) concluded that NaOH extraction estimates the amount of P, in particulate

TABLE 2
**Summary of some commonly utilized procedures for extraction of phosphorus forms (from Canviro
 Consultants 1984)**

Method	Description	Reference	Forms of Phosphorus Measured
NTA	Undried particulate sample subjected to three successive extractions with 0.01 M nitrilo-triacetic acid (NTA), combined and analyzed for ortho-P.	Golterman (1973)	Calcium and iron associated inorganic phosphorus
Anion Exchange	Particulate material contacted with ion exchange resin for 24 hrs. Resin sieved out of sample and ortho-P eluted from resin with acid solution and analyzed.	Cowen (1974) Schroeder (1976) Cowen & Lee (1976) Armonstrong <u>et al.</u> (1979)	Exchangeable phosphorus in equilibrium with soluble ortho phosphate
NaOH	Particulate sample (dried or undried) extracted for 16 hrs with 0.1 N NaOH -1.0 M NaCl solution (soil/solution ratio 1:1000). Particulate separated by centrifugation and solution analyzed for ortho-P.	Sagher (1976)	Aluminum and iron associated inorganic phosphorus.
Citrate-Dithionite-Bicarbonate (CDB)	Particulate sample (freeze dried) is contacted with citrate-dithionite-bicarbonate reagent at 85°C for 15 minutes. Particulate separated by centrifugation and solution analyzed for ortho-P.	Williams <u>et al.</u> (1976a)	Non-apatite inorganic phosphorus. Felt to be more rigorous extraction than NaOH procedure.

TABLE 2 Summary of some commonly utilized procedures for extraction of phosphorus forms (from Canviro
Consultants 1984) cont'd.

Method	Description	Reference	Forms of Phosphorus Measured
HCl	Particulate sample removed from CDB + NaOH extraction contacted with 1 N HCl for 18 hrs. particulate separated by centrifugation and solution analyzed for ortho-P.	Mayer and Williams (1981)	Apatite inorganic phosphorus,
Total Extractable Phosphorus	Particulate sample subjected to successive extraction in HCl for 16 hrs, 1 NaOH for 4 hrs and 1 N NaOH for 16 hrs at 90°C. Separated extract analyzed for total-P.	Mehta (1954) Mayer and Williams (1981)	Organic and non-organic phosphorus. Does not include certain inert phosphorus forms.
Organic Phosphorus	Separated extract from total extractable P solution analysed for ortho-P. Difference between total-P and ortho P assumed to be organic-P.	Mehta et al. Mayer and Williams (1981)	Organic phosphorus.
Total Phosphorus	Particulate sample subjected to rigorous digestive (perchloric acid, Persulfate, sulfuric-nitric acids or others). Digested solution analysed for ortho-P.	Logan et al. (1979) and several others	All forms of phosphorus including most inert and/or resistant forms.

TABLE 3. Summary of studies on available particulate phosphorus from diffuse sources (Canviro Consultants, 1983)

Particulate Source	Chemical Fraction Considered Available	Bioassay Technique	Bioavailability Correlation	Reference
Marine Sediments	NTA	<u>Scenedesmus</u>		Golterman (1973)
Urban Runoff	NaOH or Anion Exchange	<u>Selenastrum</u>	Available P = Soluble Reactive P + 0.2 PTP	Cowen (1974) Cowen & Lee (1976)
Soils, Soil Runoff	NaOH	2 and 30-day assays with <u>Selenastrum</u>	66-94% of NaOH Particulate Inorganic P	Sagher et al. (1975)
Lake Sediment	NaOH	28-day assay with <u>Selenastrum</u>	74% of NaOH Extractable P	Sagher et al. (1975) ¹
Soils	Aluminum Saturated-Cation Exchange			Huettl et al. (1979)
Lake Sediment	Exchangeable Inorganic-P	12-wks with macrophyte <u>M. spicatum</u>	13-17% of Particulate Total P	Li et al. (1974)
Bluff Erosion, Lake Sediments & Tributaries		12-18 days with <u>Scenedesmus</u>	NaOH Extractable Inorganic P	William et al. (1980)

TABLE 3. Summary of studies on available particulate phosphorus from diffuse sources (Canviro Consultants, 1983) cont'd

Particulate Source	Chemical Fraction Considered Available	Bioassay Technique	Bioavailability Correlation	Reference
Great Lakes Tributary Sediments	NaOH	None	14-37% of Particulate Total P	Armstrong et al. (1979)
Great Lakes Shoreline Soils	HCl	None	43% of Particulate Total P	Monteith & Sonzogni (1976)
Great Lakes Tributary Sediments	Non-Apatite Inorganic Phosphorus	None	20-40% of Particulate Total P	Thomas (unpublished)
South African River Sediments	Inorganic Phosphorus	<u>Selenastrum</u>	4-98% of Inorganic-P	Grobler and Davies (1979)
Black Creek, Indiana Sediments	NH ₄ F-Extractable P	<u>Selenastrum</u>	21% of Particulate Total P	Dorich et al. (1980)
Great Lakes Tributary Sediments	Non-Apatite Inorganic-P	<u>Selenastrum</u>	22% of Particulate Total P	Depinto et al. (1981)
Sediment for Lake Kasumigaura (Japan)	Iron Bound-P NaOH	<u>Selenastrum</u>	90% of Fe-P under anaerobic conditions	Hosomi et al. (1982)

materials that can be taken up by algae under aerobic and anaerobic conditions over the short term. Logan et al. (1979a) considered this fraction of particulate P labile. The estimation of labile P is important for particles which remain in the photic zone for a relatively short period of time (e.g. eroded soil particles, resuspended sediments).

Logan et al. (1979a; 1979b) considered non-apatite inorganic phosphorus (NAI-P) to be a measure of maximum available P, i.e. P that would be available over a long period of time under anaerobic conditions. NAI-P is the sum of inorganic P determined by sequential CDB and NaOH extraction.

The procedure used for evaluation of NAI-P (Williams et al. 1976a, 1976b) is essentially a modification of the method originally designed for soils (Chang and Jackson 1957). The fractionation analysis includes an extraction step utilizing the CDB reagent. The CDB treatment has been widely accepted in selective and sequential soil analytical extraction schemes, thus its adaptation for the environmental analyses of sediments and other particulate materials entering the aquatic systems is not surprising.

In terrestrial soils and benthic sediments, CDB-extractable P corresponds to reductant soluble P, which is thought to originate in matrices of crystalline oxides and hydroxides of Fe (Bauwin and Tyner 1957). This fraction would also include P associated with amorphous Fe and Al oxides and other phosphate mineral phases that are readily soluble in water such as brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) and struvite

($MgNH_4PO_4 \cdot 6H_2O$) (Williams et al. 1980a). When the fractionation scheme of Williams et al. (1976a) was tested on a large suite of minerals (Williams et al. 1980a), it was found that the CDB extractant removed, besides P from Fe phosphates, a significant portion of P (66-76%) from fish bone, although the fish bone was identified as poorly crystallized hydroxyapatite. The results of Williams et al. (1980a) emphasize the influence of the degree of crystallinity on the dissolution of mineral phases. Furthermore, some investigators (Psenner et al. 1984) suggested that at higher temperatures (60° - $85^\circ C$) the CDB extractant causes the release and hydrolysis of phosphates from cells of organisms. Indeed, our laboratory results indicate that ~19% of total P was extracted from laboratory cultured algae Chlorella, containing 9.1 mg g^{-1} of phosphorus. There is little doubt that cultured algae which have taken up "luxury" phosphorus and stored it as loosely bound P (Forsee et al. 1971) will contain more readily extractable P than algae in systems where nutrients may be in lesser supply. Nevertheless, in aquatic systems, the contribution of biogenic P from particulate matter with high amounts of cellular material (algae, bacteria, etc.) to CDB-extractable P can not be overlooked.

Studies on wastewater particulates (Canviro Consultants Ltd. 1983) have shown that the CDB + NaOH extractable-P and CDB extractable P alone were good predictors of bioavailable particulate P

(BAPP), estimated by algal assay procedures. In the same study the following regression equations were obtained for BAPP as the dependent variable:

$$\text{BAPP} = 971 + 0.55 (\text{CDB} + \text{NaOH}) \quad r = 0.75$$

$$\text{BAPP} = 1473 + 0.51 (\text{TEXP}) \quad r = 0.77$$

$$\text{CDB} = 2886 + 0.82 (\text{TEXP}) \quad r = 0.93$$

The parameters CDB, NaOH and TEXP represent CDB extractable, NaOH extractable and total extractable phosphorus, respectively.

Several different studies on sedimentary materials (Williams et al. 1980b; Mayer 1984) indicate that BAPP estimated by 0.1 N NaOH/1.0 M NaCl accounts, for ~70% of NAI-P (sum of CDB-P + NaOH-P). For bottom sediments, NaOH-P and NAI-P are well correlated with Eh-sensitive ferric oxides determined by Mossbauer spectroscopy (Manning et al. 1981; 1982; 1984), indicating their close association.

Allan and Williams (1978) considered the one-step CDB extraction valuable for rapid evaluation of bioavailable P in sediments, although they recognized the possibility of slight overestimation of bioavailable P by this method.

Recently, Lucotte and d'Anglejan (1985) tested the specificity and effectiveness of several extraction methods using suspended particulate matter from an estuary, reference materials and two single-phase minerals (goethite and hydroxyfluoroapatite). They found procedures employing CDB reagent more reproducible and more specific in extracting iron and associated phosphorus than other extraction

procedures. The reagents used in other investigated extraction procedures included: 0.2 M NH_4 oxalate/0.2 M oxalic acid; 0.25 M hydroxylamine hydrochloride in 25% acetic acid; 0.01 N calcium nitrilotriacetic acid; and 1N sodium acetate/0.2 N sodium tartrate. Lucotte and d'Anglejan found that oxalate reagent and hydroxylamine hydrochloride dissolved some apatite P. In the aquatic environment, apatite-P is generally considered to be biologically inert. A similar observation was reported by Williams et al. (1971c) and Mayer (1984). In general, the CDB-P values are in close agreement with oxalate extractable-P values corrected for apatite phosphorus (Williams et al. 1971c; Mayer 1984). The plot of the two sets of values showed a linear relationship with a slope of about 1 (Mayer 1984).

Krom and Berner (1981) used H_2S gas to estimate the labile inorganic P, associated with iron oxides that are rapidly released upon burial of sediment. The reducing action of H_2S breaks down the iron oxides and dissolves associated orthophosphate. The above method resembles more closely the conditions in natural sedimentary environment, thus a comparison of the results obtained by this method and CDB extraction, complemented with Mössbauer spectroscopy would provide valuable information on the amount of phosphorus associated with reducible iron compounds.

SUMMARY AND CONCLUSIONS

The CDB extraction appears to be an effective method for estimation of maximum bioavailable P in particulate materials. The method is relatively simple and reproducible. The coefficient of variation was 5%. Because the reagent is sufficiently powerful to remove Fe and Al oxides almost completely, its action is easily understood. The obvious disadvantage of the method is the over-estimation of short-term bioavailable P, however, in most applications this may not be a serious concern. Whether this fraction of particulate phosphorus actually becomes available depends on factors such as Eh, pH and physical agitation.

The action of 0.1 N NaOH, on the other hand, is not fully known. In the case of adsorption of P on Fe and Al oxides, and clay minerals increased competition from OH⁻ ions for adsorption sites causes release of phosphate. The method is rapid and inexpensive, but reproducibility may be a problem. How the method reflects anoxic release of Fe and P is open to question.

In conclusion, it can be stated that both the NaOH and the CDB-extractable P are reasonably good predictors of bioavailable particulate P. The latter parameter may be, however, a more suitable indicator of bioavailability in environments where the redox processes control the phosphorus chemistry.

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