



SCIENTIFIC NOTE

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## Inorganic phosphorus fractions extracted by sodium dithionite-citrate-bicarbonate and determined using the malachite green method

### *Fração de fósforo inorgânico extraída por ditionito-citrato-bicarbonato de sódio e determinada pelo método verde de malaquita*

**ABSTRACT:** Phosphorus (P) fractionation is a common tool used in studies on soil P dynamics. However, the P fraction extracted by sodium dithionite-citrate-bicarbonate (DCB), occluded in iron oxides (P-occluded), is difficult to quantify. This study presents an alternative and simpler method for the quantification of the P-occluded fraction extracted by DCB. Samples were collected from a clayey Oxisol at a depth of 0-0.2 m. The extraction method followed the procedure described by Zhang and Kovar and malachite green method was used for determination. The malachite green method produced satisfactory quantification results, and it revealed a pattern that represents extract P concentrations according to the absorbance level. The method is suitable for simple determinations of P occluded in extracts of DCB.

**RESUMO:** O fracionamento de fósforo (P) é uma das principais ferramentas nos estudos sobre a dinâmica de P no solo. Entretanto, a fração de P extraído por ditionito-citrato-bicarbonato de sódio (DCB), ocluído em óxidos de ferro (P-ocluído) tem apresentado problemas para a sua quantificação. O objetivo foi propor método alternativo e mais simples para a quantificação da fração P-ocluído extraído por DCB. As amostras foram coletadas na camada de 0-0,2 m de um Latossolo argiloso. O método de extração seguiu o procedimento descrito por Zhang e Kovar, enquanto que para determinação utilizou-se o método verde de malaquita. Houve satisfatória resposta do método Verde de Malaquita no processo de quantificação, sendo possível estabelecer um padrão que representa a concentração de P dos extratos em função da absorbância. O método é adequado e simples para determinações da fração P-ocluído em extratos de DCB.

## 1 Introduction

The (P) phosphorus fractionation technique serves as an instrument for understanding P forms in soil and their dynamics. Among the main applications of P fractionation, its use for P level monitoring in differently managed soils has been emphasized, as this function allows for more effective uses of phosphorus fertilization (Sugihara et al., 2012).

The inorganic P (Pi) fractionation method presented by Zhang & Kovar (2009) is based on the method described by Kuo (1996) and involves the sequential separation of fractions extracted from  $\text{NH}_4\text{Cl}$  (soluble P);  $\text{NH}_4\text{F}$  (Fe-P);  $\text{NaOH}$  (Al-P);  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 + \text{NaHCO}_3 + \text{Na}_2\text{S}_2\text{O}_4$  (dithionite-citrate-sodium bicarbonate) (P occluded in iron oxide or occluded-P); and  $\text{H}_2\text{SO}_4$  (Ca-P). However, quantifying the occluded-P fraction using Zhang & Kovar (2009) method has proven difficult, as the dithionite-citrate-sodium bicarbonate (DCB) extractor generates interference during the blue staining process necessary for spectrophotometer readings when the molybdenum blue method (Ranno et al., 2007), one of the most commonly used methods due to its efficiency and practicality, is used.

Some researchers have sought alternative ways to quantify via plasma spectroscopy in an attempt to solve the DCB extractor's interference problem (Harrell & Wang, 2007). However, this technique requires access to expensive equipment that is not available in all soil analysis laboratories, unlike spectrophotometers, which are often available.

The malachite green method is colorimetric, is highly sensitive when used for various extracts, and has generated good results for quantifying P in low quantities (Hart & Cornish, 2009). Thus, it may be used as a feasible alternative method in studies on the fractionation and quantification of occluded-P fractions. This method is considered the most sensitive and least prone to interference among the colorimetric methods (Singh & Shukla, 2003). Thus, the malachite green method is straightforward, inexpensive relative to plasma spectroscopy methods, highly sensitive and effective at determining occluded-P fractions extracted via DCB. The goal of this study is to propose an alternative method to the molybdenum blue method for quantifying occluded-P extracted by DCB.

## 2 Materials and Methods

Samples of a dystrophic yellow Oxisol with a clayey texture were analyzed. The samples were collected from the 0 - 0.20 m soil layer of the municipality of Paragominas (02° 51' 54" S and 48° 23' 40" W and altitude of 88 m) in the northeastern state of Pará, Brazil. The soil analysis indicated the following:  $\text{pH}_{\text{water}}$ : 5.3, available P: 5 mg  $\text{kg}^{-1}$  and  $\text{K}^+$ : 3.2 mmol  $\text{kg}^{-1}$ ,  $\text{Ca}^{2+}$ : 31 mmol  $\text{kg}^{-1}$ ,  $\text{Mg}^{2+}$ : 9 mmol  $\text{kg}^{-1}$ , OM: 36 g  $\text{kg}^{-1}$ ,  $\text{Al}^{3+}$ : 2 mmol  $\text{kg}^{-1}$ , H+Al: 69.3 mmol  $\text{kg}^{-1}$  and V%: 38%, determined according to EMBRAPA (2011).

Sequential P extraction was performed following the method described by Zhang & Kovar (2009) based on differences in solubility of the inorganic P forms as revealed by several extractors. Soluble-P was extracted from 1 mol  $\text{L}^{-1}$   $\text{NH}_4\text{Cl}$ , Al-P was extracted from 0.5 mol  $\text{L}^{-1}$   $\text{NH}_4\text{F}$ , Fe-P was extracted from 0.1 mol  $\text{L}^{-1}$   $\text{NaOH}$ , and occluded-P was extracted from dithionite-citrate-sodium bicarbonate (DCB) composed of 0.3 mol  $\text{L}^{-1}$   $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ , 1 mol  $\text{L}^{-1}$   $\text{NaHCO}_3$ , and  $\text{Na}_2\text{S}_2\text{O}_4$ .

Soluble-P, Fe-P, Al-P, and Ca-P fraction extracts can be quantified using the phosphomolybdate method according to Murphy & Riley (1962). In contrast, unlike other fractions, the occluded-P fraction cannot be determined using the phosphomolybdate method, as the extraction solution interferes with the method. This fraction was thus obtained using the malachite green method as described by Rao et al. (1997).

The following procedure was used to prepare the malachite green study solution: Solutions 1 and 2 (described below) were added to a 1-L flask, and the volume was reached through the addition of water and then stored in a dark flask. Solution 1 was prepared with 36.18 g of ammonium heptamolybdate tetrahydrate ( $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ ) dissolved in 250 mL of water and 476 mL of 5 mol  $\text{L}^{-1}$   $\text{H}_2\text{SO}_4$  and 0.229 g of malachite green oxalate. Solution 2 was prepared with 1 g of polyvinyl alcohol (PM = 72,000 g  $\text{mol}^{-1}$ ) in 100 mL of water, which was heated slightly to facilitate dissolution.

As the method employed is highly sensitive, the samples were diluted so that proportions of the extract and study solution used for the original methods were altered, and thus, the volumes used were as follows: 0.5 mL of extract + 8.5 mL of water + 1.0 mL of study solution (total volume: 10 mL). Addition and recovery tests were performed beforehand to confirm the method's effectiveness at quantifying phosphorus under the conditions cited. The absorbance data were subjected to a linear regression analysis as a function of P levels in the DCB solution.

## 3 Results and Discussion

The molybdenum blue method did not efficiently quantify the occluded-P fraction extracted by DCB. Because extracts do not require blue staining to be quantified using a spectrophotometer, it proves infeasible for this type of analysis. This occurs because dithionite and citrate can interfere with occluded-P fraction quantification. The presence of excess dithionite results in sulfur precipitation as the molybdic acid reagent is added, and thus, it is necessary to oxidize the medium for four hours to eliminate dithionite interference, as proposed by Weaver et al. (1968) in determining silicon in DCB extracts and by Weaver (1974) in determining P in DCB extracts. However, in our study, exposing the extracts to air did not eliminate the extract's interference.

This dithionite interference occurs in the presence of excess citrate concentrations in relation to those of molybdate (Zhang & Kovar, 2009). According to Zhang & Kovar (2009), adding 3 mL of 5% ammonium molybdate solution to each sample can solve the problem. This is necessary to offset the effects of excess citrate, which forms highly stable complexes in the presence of molybdate. Similarly, phosphomolybdate complexes are prevented from forming (Killefer & Linz, 1952). This procedure also did not generate results that could be quantified using a spectrophotometer.

It is likely that the four-hour extract air-exposure period for oxidation purposes is insufficient for soils under the conditions cited and considering dithionite concentrations used in the method described by Zhang & Kovar (2009) (1,000 mg). According to Weaver et al. (1968), dithionite interference elimination procedures were tested at levels ranging from 3 to 500 mg. Thus, at a dithionite concentration of 1,000 mg,

it is expected that a longer duration would be necessary for the oxidation process to sufficiently eliminate the interference.

When the malachite green method was employed based on methods described by Rao et al. (1997), green staining processes occurred, and these processes can be quantified using a spectrophotometer. However, under the expected conditions of the original methods, no standard staining processes can be directly related to phosphorus concentrations in the samples. It is assumed that the method's high sensitivity resulted in highly intense staining and that it was not possible to differentiate between the samples, thus exceeding the linearity limits within which the Lambert-Beer law is valid. In this case, P levels in a solution cannot be quantified using a spectrophotometer unless they are reduced via dilution (Marczenko, 1986). Thus, extract and study solution proportions used in the original methods were altered with dilutions until the interference was eliminated. The following final volumes were used: 0.5 mL of extract + 8.5 mL of water + 1.0 mL of study solution (total volume of 10 mL).

The pH constituted another adjusted factor. A pH level higher than 1.8 is necessary for green staining to develop using this method. In preparing the study solution, sulfuric acid was added in high proportions. In adding 2 mL of study solution for every 10 mL of total volume, as the original methods suggest, a loss of sensitivity occurred, as the pH level fell below 1.8. Adjusting the extraction solution, water, and study solution proportions and correcting the pH level made it possible to obtain the staining standard directly related to the solution's P levels. The standard curve was prepared according to Table 1.

After the alterations were made, satisfactory results were obtained for the P concentration, proven by the linear relationship between P concentrations in the DCB extract and absorbance, with an  $R^2$  value of 0.99 (Figure 1). Dilutions generated based on the proportion of samples are critical to malachite green method determinations, as they alter the method's sensitivity by changing the ion-study solution ratio, as observed by Sabarudin et al. (2003), who used a mixed solution of molybdate and malachite green to determine very low P levels in water.

In addition to obtaining the standard curve for P concentrations in the solution, the method needed to be evaluated to test the magnitude of the extraction solution's interference and also whether the modified proportions altered the method's sensitivity.

The method was evaluated via addition and recovery, as described below:

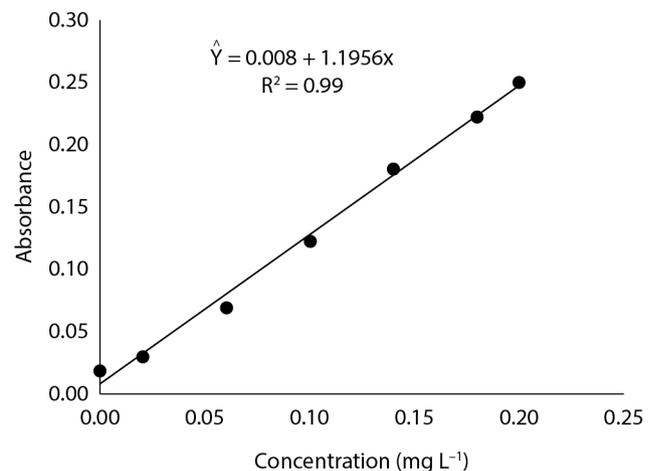
A1: 0.5 mL of sample of an unknown concentration (Abs = 0.026); A2: 0.5 mL of sample of an unknown concentration + 0.06 mg L<sup>-1</sup> (Abs = 0.096); A3: 0.5 mL of sample of an unknown concentration + 0.10 mg L<sup>-1</sup> (Abs = 0.152); A4: 0.5 mL of sample of an unknown concentration + 0.14 mg L<sup>-1</sup> (Abs = 0.212).

The linear absorbance response with P levels was found to be satisfactory, with  $R^2 = 0.99$  (Figure 2). Based on the close relationship between P concentrations and absorbance, the method was considered adequate in quantifying P in the occluded-P fraction extracted by DCB, and the interference was eliminated. Thus, the proposed method may be used in P fractionation analyses in which the extractor interferes in the process.

**Table 1.** Standard curve with a final volume of 10 mL.

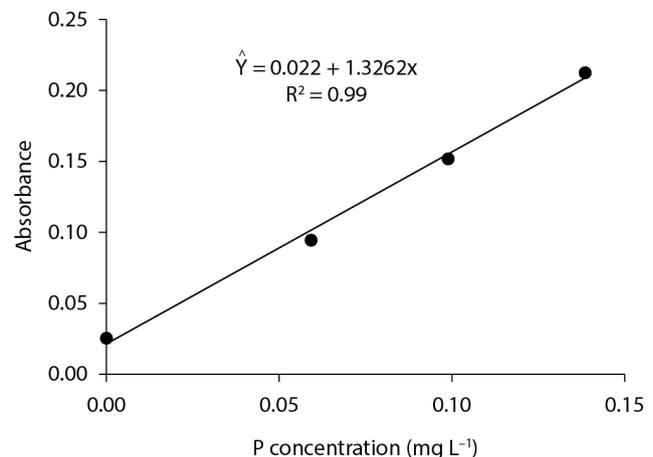
**Tabela 1.** Curva padrão com volume final de 10 mL.

	Stock solution (2 mg L <sup>-1</sup> )	Final concentration (mg L <sup>-1</sup> )
P1	0	0
P2	0.3	0.06
P3	0.5	0.10
P4	0.7	0.14
P5	0.9	0.18
P6	1.0	0.20



**Figure 1.** Standard curve for phosphorus concentrations in the dithionite-citrate-sodium bicarbonate extract obtained using the malachite green method.

**Figura 1.** Curva padrão para a concentração de fósforo em extrato ditionito-citrato-bicarbonato de sódio obtida pelo método de verde de malaquita.



**Figure 2.** The addition and recovery of phosphorus for a sample of dithionite-citrate-sodium bicarbonate extract obtained using the malachite green method.

**Figura 2.** Adição e recuperação de fósforo em amostra de extrato ditionito-citrato-bicarbonato de sódio obtida pelo método de verde de malaquita.

## 4 Conclusions

The molybdenum blue method did not facilitate blue staining in extracts of the occluded-P fraction extracted via DCB, which are conditions required for spectrophotometer quantification. The malachite green method efficiently quantified P extracted by dithionite-citrate-sodium bicarbonate and can be considered a feasible alternative method for determining this P fraction in soils.

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