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# **DIVISÃO 3 - USO E MANEJO DO SOLO**

## **Comissão 3.1 - Fertilidade do solo e nutrição de plantas**

### **ACCUMULATION OF PHOSPHORUS FRACTIONS AND CONTAMINATION POTENTIAL IN VINEYARD SOILS IN THE SOUTHERN REGION OF THE STATE OF SANTA CATARINA, BRAZIL<sup>(1)</sup>**

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#### **SUMMARY**

**In vineyards, if phosphate is applied both before planting and at intervals during growth without consideration of technical criteria, the soil P fractions may be increased and their proportions altered. This study was carried out to evaluate the accumulation of P fractions and the parameters of the adsorption isotherm in a sandy Typic Hapludalf soil in vineyards with a history of successive and excessive phosphate fertilization. In December 2010, two vineyards were selected, one 4 and the other 15 years old, in Urussanga, State of Santa Catarina (Brazil). Three trenches were dug in each area and soil was collected from the 0-5, 5-10 and 10-20 cm depth ranges. The soil samples were dried in a forced-air oven, sieved and subjected to chemical analyses, P chemical fractionation and P adsorption isotherms. Excessive phosphate fertilization, before and during cultivation, particularly in the older vineyard and, consequently, with a longer history of phosphate fertilization, increased the inorganic P concentrations to the depth of 20 cm, especially in labile fractions extracted by anion exchange resin and NaHCO<sub>3</sub> in the non-labile fraction,**

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as well as in the non-labile fraction extracted by  $1.0 \text{ mol L}^{-1} \text{ HCl}$ . The application of phosphate fertilizers and the long cultivation period increased the P levels in the organic labile fraction extracted by  $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ , and especially in the moderately labile fraction extracted by  $0.1$  and  $0.5 \text{ mol L}^{-1} \text{ NaOH}$ . Phosphate fertilization of older vineyards, i.e., cultivated for 15 years, increased the amounts of P desorbed in water, indicating a risk of contamination of surface waters and groundwater. The phosphate fertilization before planting, without considering the results of soil analysis, and during cultivation, disregarding the results of soil analysis, leaf analysis and expected yield, led to a reduction in the maximum P adsorption capacity in the 0-5 cm layer of vineyard 2, indicating saturation of part of the reactive particle adsorption sites.

**Index terms:** phosphate fertilization, phosphorus chemical fractionation, phosphorus adsorption isotherms, environmental contamination, *Vitis sp.*

## **RESUMO: ACÚMULO DE FRAÇÕES DE FÓSFORO E POTENCIAL CONTAMINANTE EM SOLOS DE VINHEDOS NA REGIÃO SUL DO ESTADO DE SANTA CATARINA, BRASIL**

*As aplicações de fertilizantes fosfatados na adubação de pré-plantio e de manutenção, sem considerar critérios técnicos, podem causar o acúmulo e a alteração das frações de P em solos cultivados com videira. Este trabalho objetivou quantificar o acúmulo de frações de P em solos com textura arenosa, cultivados com videira e com histórico de aplicações excessivas de fertilizantes fosfatados. Em dezembro de 2010, foram selecionados dois vinhedos, um com quatro e outro com 15 anos de idade, localizados no município de Urussanga, Santa Catarina, Brasil. Foram abertas três trincheiras em cada área e, em seguida, foi coletado solo nas camadas de 0-5, 5-10 e 10-20 cm. O solo foi seco, passado em peneira e submetido a análises de atributos químicos, fracionamento químico de P e isothermas de adsorção. As aplicações excessivas de fertilizantes fosfatados na adubação de pré-plantio e de manutenção em vinhedos, em solos de textura arenosa, aumentaram o teor de P até 20 cm de profundidade, especialmente nas frações lábeis extraídas pela resina trocadora de ânions e  $\text{NaHCO}_3$  e na fração não lábil extraída por  $\text{HCl } 1,0 \text{ mol L}^{-1}$ . A aplicação de fertilizantes fosfatados e o tempo de cultivo da videira influenciaram os teores de P em formas orgânicas na fração lábil extraída por  $\text{NaHCO}_3$   $0,5 \text{ mol L}^{-1}$  e, principalmente, na fração moderadamente lábil extraída pelos extratores  $\text{NaOH } 0,1 \text{ mol L}^{-1}$  e  $0,5 \text{ mol L}^{-1}$ . As adubações fosfatadas nos vinhedos com longo período de cultivo, 15 anos, aumentaram a quantidade de P desorvido em água, indicando risco de contaminação ambiental das águas superficiais e subterrâneas. A adição de fertilizantes fosfatados na adubação de pré-plantio, sem considerar os resultados da análise de solo, da adubação de manutenção, do tecido vegetal e da expectativa de produção, resultou nos valores mais baixos de capacidade máxima de adsorção P na camada de 0-5 cm do vinhedo 2, com longo período de cultivo, indicando a saturação de parte dos sítios de adsorção.*

*Termos de indexação:* adubação fosfatada, fracionamento químico de fósforo, isothermas de adsorção de fósforo, contaminação ambiental, *Vitis sp.*

## **INTRODUCTION**

The State of Santa Catarina has the fifth largest area of grapevine cultivation in Brazil, with approximately 5,000 hectares (IBGE, 2011). In this state, the first vineyards were planted by Italian immigrants in 1873, in soils near the sea, with a sandy texture and predominance of 1:1 clay, low contents of organic matter and acids, and low natural fertility. Currently, before planting vineyards, the soil acidity is usually reduced by liming to increase the water pH to 6.0, and before planting, phosphate and potassium fertilizers are used to increase the P and K contents in the soil. After planting the

vineyard, when soil analysis indicates the need, additional lime is applied to the soil surface during the vine cycle, without incorporation. Also according to need, especially based on the results of leaf analysis and yield expectation (CQFSRS/SC, 2004; Brunetto et al., 2009), N, P and K are applied on the soil surface without incorporation (maintenance fertilization). However, the applied nutrients, especially P, are not always determined by technical criteria and, as a result, a buildup of this element in the upper soil layers and saturation of adsorption sites of the reactive particles in soil may occur over the years (Korboulewsky et al., 2002; Gatiboni et al., 2008).

Studies on the accumulation of P fractions in soil have mostly used the chemical fractionation technique proposed by Hedley et al. (1982) (Conte et al., 2003; Rheinheimer et al., 2003; Gatiboni et al., 2007, 2008), because this method allows for a more complete diagnosis of P accumulation in soil, not only of the plant-available P. Results of P chemical fractionation show that after long-term successive and excessive phosphate fertilization, a buildup of inorganic and organic P fractions in the soil may occur, with different degrees of binding energy. In general, the buildup is greater in the inorganic fractions (Conte et al., 2003; Boschetti et al., 2009; Santos et al., 2008; Pavinato et al., 2009; Tokura et al., 2011). Phosphorus adsorption occurs initially at the most avid sites and with a greater binding strength, and then the remaining P is redistributed in lower energy fractions (Rheinheimer & Anghinoni, 2001). So, it is to be expected that the greater quantity of phosphate fertilizers applied, the greater will be the saturation of sites with higher binding energy, and consequently, the more P will be available. Continued P applications on the soil surface tend to cause saturation of the adsorption surfaces, which reduces adsorption energy and, consequently, increases desorption and availability (Bolland et al., 1996; Berwanger et al., 2008). Higher P availability to the vines or even to the plants that also grow in the vineyards can be expected, but on the other hand, the potential transfer of the nutrient by the solution drained through the surface soil or by leaching into the soil profile will also be higher, particularly in sandy soils, and this can contaminate surface or underground waters (Gatiboni et al., 2008; Ceretta et al., 2010; Pizzeghello et al., 2011).

Estimates of both P availability and the probability of P transfer can be improved by adding the maximum phosphate adsorption capacity to the results of P chemical fractionation in soil. For this purpose, the adsorbed P and P in the solution are related by adsorption isotherms fitted to the mathematical model proposed by Langmuir (Barrow, 1983; Novais & Smyth, 1999). This model shows a decrease in the P adsorption capacity after P application, especially in the upper layers of soil after successive phosphate fertilization, as in vineyards. Adjustments of the adsorption isotherm by other models, as proposed by Koski-Vähälä & Hartikainen (2001), aggregate the amount of P desorbed in distilled water ( $Q_0$ ), by which the required P concentration in the solution can be calculated for half the maximum adsorption ( $km$ ) and the P equilibrium concentration (Koski-Vähälä & Hartikainen, 2001; Sinaj et al., 2002). This last parameter can be used as an environmental indicator (Berwanger et al., 2008) of the likelihood of P leaching through the soil profile into the groundwater as well as of P loss in runoff to surface waters (Ajmone-Marsan et al., 2006; Gatiboni et al., 2007, 2008; Chien et al., 2011). The purpose of this study was to evaluate the accumulation of P fractions and the contamination

potential of a vineyard soil (sandy Typic Hapludalf), after long-term successive and excessive phosphate fertilization.

## MATERIAL AND METHODS

### Site description

The study was carried out using soil samples from vineyards in Urussanga, in the southern part of the State of Santa Catarina, Brazil. The predominant soil type in the region, which is usually used for growing grapevines, is sandy Typic Hapludalf (Soil Survey Staff, 1999), with a sandy surface texture, granite substrate and predominance of 1:1 clay (Uberti, 2005). The climate in the region, according to the classification proposed by Köppen-Geiger, is humid subtropical (Cfa), with an average annual temperature of 19.4 °C and an average annual rainfall of 1,619 mm.

In December 2010, two vineyards with different ages and consequently, with different sequences of phosphate fertilization were selected: vineyard 1 (latitude 28° 34' 34.2", longitude 49° 16' 07"; 29 m asl) planted in 2006 with 3,333 plants per hectare (1.0 x 3.0 m), and vineyard 2 (latitude 28° 33' 49.5", longitude 49° 15' 50.2"; 24 m asl), planted in 1995, with 3,030 plants per hectare (1.1 x 3.0 m). In both vineyards, the Niagara Rosada variety was grafted onto a Paulsen 1103 rootstock in the trellis training system, in an area of nearly 1.0 ha. Prior to planting the vines, lime was applied on the soil surface of the vineyards to raise the pH in water to 6.0, followed by incorporation. Then, 400 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> was applied on the surface with subsequent incorporation. In addition, as of the second year after planting, when the vines began to produce, maintenance fertilization was required (CQFSRS/SC, 2004); approximately 90 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 60 kg ha<sup>-1</sup> K<sub>2</sub>O were applied on the soil surface without incorporation. Superphosphate and potassium chloride were usually used as sources. Over the years, the weeds in the grapevine rows were periodically controlled with herbicide. Between the vine rows, spontaneous vegetation was maintained, consisting of *Lolium perenne*, *Paspalum notatum* and *Trifolium repens*. This vegetation was periodically mowed and the residues were deposited on the soil surface.

### Collection of soil samples, analysis of soil properties, P chemical fractionation and P adsorption isotherms

Four years after planting vineyard 1, and 15 years after planting vineyard 2, six trenches (0.3 x 0.5 x 0.5 m) were randomly dug in the rows of both vineyards. Soil samples were collected from the 0-5, 5-10 and 10-20 cm layers and later dried in a forced-air oven at 45 °C. Then, the soil was crushed, sieved

(2 mm) and stored in three portions. The first sample portion was analyzed for particle size distribution of the soil constituents by the pipette method (Embrapa, 1997). In addition, the pH values in water were determined (1:1), as well as the exchangeable contents of aluminum (Al), calcium (Ca) and magnesium (Mg) (KCl 1.0 mol L<sup>-1</sup> extractant) and exchangeable K (Mehlich-1 extractant) (Tedesco et al., 1995) (Table 1). Total organic carbon was determined according to the method proposed by Embrapa (1997). In all samples, we also determined the levels of iron extracted by dithionite-citrate-bicarbonate (DCB) (Mehra & Jackson, 1960) and by ammonium oxalate at pH 3.0 (Tedesco et al., 1995). The levels of H+Al were calculated based on the SMP index through the equation proposed by Kaminski et al. (2001) and adopted by CQFSRS/SC (2004) (Table 1).

From the stored soil samples, a second portion was subjected to P chemical fractionation, according to the method proposed by Hedley et al. (1982), with the modifications proposed by Condron & Goh (1989), at a temperature of  $\pm 25$  °C, briefly described as follows: 0.5 g samples of soil were subjected to sequential extraction with anion exchange resin (AER), which extracts the labile P fraction; 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub>, which extracts the labile inorganic P fraction not extracted by the anion exchange resin, plus the labile organic P fractions; 0.1 mol L<sup>-1</sup> NaOH, which extracts the moderately labile inorganic P fraction bound to Fe and Al oxyhydroxides and silicate clays with intermediate binding energy, and the organic P fraction of moderately labile fractions; 1.0 mol L<sup>-1</sup> HCl, which extracts the inorganic P fraction contained in calcium phosphates; and 0.5 mol L<sup>-1</sup> NaOH, which extracts intermediate-labile fractions of inorganic P present mainly in microaggregate and low lability

organic P fractions. After these extractions, the remaining soil was dried in a laboratory oven at 50 °C, and 0.1 g of soil was subjected to digestion with 1 mL H<sub>2</sub>SO<sub>4</sub> 1:1 (water/acid) + 2 mL H<sub>2</sub>O<sub>2</sub> + 1 mL saturated MgCl<sub>2</sub>, which extracts the soil residual inorganic and organic P, called recalcitrant P, as described by Gatiboni (2003). The inorganic P of the alkaline extracts of NaHCO<sub>3</sub> and NaOH was determined according to the method proposed by Dick & Tabatabai (1977). In these alkaline extracts, total P was determined after digestion in an autoclave with 10 mL ammonium persulfate + 1 mL sulfuric acid, and the organic P was obtained by the difference between total P and inorganic P. The P in the acid extracts was determined according to Murphy & Riley (1962). Phosphorus forms of the Hedley fractionation were grouped into geochemical and biological P (Cross & Schlesinger, 1995). Geochemical P was obtained by the sum of the inorganic fractions and the residual P (anion exchange resin + 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub> + 0.1 mol L<sup>-1</sup> NaOH + 0.5 mol L<sup>-1</sup> NaOH + 1.0 mol L<sup>-1</sup> HCl + residual P), and biological P by the sum of the organic fractions (0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub> + 0.1 mol L<sup>-1</sup> NaOH + 0.5 mol L<sup>-1</sup> NaOH).

The parameters of P adsorption isotherms were determined in soil samples from the 0-5 and 10-20 cm layers. One (1.0) g of dried soil was filled in 15 mL tubes with 10 mL of the 1 mmol L<sup>-1</sup> CaCl<sub>2</sub> solution containing 10 P concentration levels (0, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, and 300 mg L<sup>-1</sup>), equivalent to 0, 5, 10, 20, 50, 100, 200, 500, 1,000, 2,000, and 3,000 mg kg<sup>-1</sup> P in the soil, respectively. The tubes were then subjected to continuous end-over-end shaking (16 h at 33 rpm and  $\pm 25$  °C). Next, the samples were centrifuged at 1,350 g for 10 min, and in the extract, the remaining P content in solution was

**Table 1. Chemical and physical properties in different layers of a sandy Typic Hapludalf soil in vineyards**

| Soil property   | Vineyard 1 (4 years old) |         |          | Vineyard 2 (15 years old) |         |          |
|---|--------------------------|---------|----------|---------------------------|---------|----------|
|   | 0-5 cm                   | 5-10 cm | 10-20 cm | 0-5 cm                    | 5-10 cm | 10-20 cm |
| Clay, g kg <sup>-1</sup>                                      | 96.5                     | 111.6   | 104.8    | 88.0                      | 135.0   | 202.1    |
| Sand, g kg <sup>-1</sup>                                      | 623.0                    | 614.0   | 604.1    | 586.0                     | 560.8   | 523.4    |
| Silt, g kg <sup>-1</sup>                                      | 280.7                    | 274.3   | 291.1    | 323.3                     | 303.8   | 274.7    |
| TOC, g kg <sup>-1(1)</sup>                                    | 18.1                     | 14.9    | 15.9     | 26.7                      | 19.4    | 16.6     |
| pH (H <sub>2</sub> O)   | 5.3                      | 5.3     | 5.4      | 6.3                       | 6.4     | 6.2      |
| K <sup>+</sup> , mg kg <sup>-1</sup>                          | 249.9                    | 157.1   | 129.8    | 275.6                     | 237.6   | 257.2    |
| Ca <sup>2+</sup> , cmol <sub>c</sub> kg <sup>-1</sup>         | 3.7                      | 4.1     | 3.8      | 5.5                       | 4.9     | 4.1      |
| Mg <sup>2+</sup> , cmol <sub>c</sub> kg <sup>-1</sup>         | 3.4                      | 3.1     | 2.9      | 3.3                       | 3.2     | 3.2      |
| Al <sup>3+</sup> , cmol <sub>c</sub> kg <sup>-1</sup>         | 0.5                      | 0.6     | 0.7      | 0.0                       | 0.0     | 0.0      |
| H+Al, cmol <sub>c</sub> kg <sup>-1</sup>                      | 5.2                      | 5.0     | 5.1      | 2.5                       | 2.6     | 3.1      |
| CEC <sub>pH 7.0</sub> , cmol <sub>c</sub> kg <sup>-1</sup>    | 12.9                     | 12.6    | 12.1     | 12.0                      | 11.3    | 11.1     |
| CEC <sub>effective</sub> , cmol <sub>c</sub> kg <sup>-1</sup> | 8.2                      | 8.2     | 7.7      | 9.5                       | 8.7     | 8.0      |
| Fe <sub>d</sub> , g kg <sup>-1(2)</sup>                       | 27.1                     | 28.0    | 29.6     | 17.5                      | 21.5    | 27.7     |
| Fe <sub>ox</sub> , g kg <sup>-1(3)</sup>                      | 6.9                      | 6.9     | 7.0      | 2.8                       | 3.1     | 3.3      |

(<sup>1</sup>) TOC: total organic carbon; (<sup>2</sup>) Fe<sub>d</sub>: iron extracted by dithionite-citrate-bicarbonate; (<sup>3</sup>) Fe<sub>ox</sub>: iron extracted by oxalate.



determined by the Murphy & Riley (1962) method. The sorbed P was estimated by the difference between the amount added and the amount recovered in the equilibrium solution. The data were then adjusted by Langmuir's mathematical model of an adsorption site (Equation 1), as described by Barrow (1983):

$$P_{\text{sorbed}} = (k \text{ MPAC} * P_{\text{solution}}) / (1 + k P_{\text{solution}}) \quad (1)$$

where: *MPAC* is the maximum adsorption capacity of P; *k* is a constant that provides information on the affinity of the adsorbate to the substrate surface, and *P<sub>solution</sub>* is the concentration of P in the equilibrium solution.

The phosphorus equilibrium concentration was obtained by fitting the data to the mathematical model proposed by Koski-Vähälä & Hartikainen (2001). These authors introduced the amount of P desorbed in distilled water (*Q<sub>o</sub>*), which is visually represented by the intersection in the ordinate axis, and concentration of P equilibrium (PEC) by the intersection in axis *x* (Equation 2):

$$P_{\text{sorbed}} = (\text{MPAC} * P_{\text{solution}}) / (km + P_{\text{solution}}) - Q_o \quad (2)$$

where: *MPAC* is the maximum P adsorption capacity; *P<sub>solution</sub>* is the P concentration in the equilibrium solution; *Q<sub>o</sub>* is the amount of P desorbed in distilled water and *km* is the P concentration in the solution that allows half the maximum P adsorption.

### Statistical treatment of the data

The results of P content in different layers of the vineyard soil and the same layer of different vineyard soils were subjected to analysis of variance (SAS, 2003). Phosphorus contents were used as dependent variables, and soil depth and site as independent variables. The results of all analyses were tested for significance by the F test ( $p \leq 0.05$ ). Multiple comparisons for P contents, where the result of analysis of variance was statistically significant, were carried out using Tukey's HSD (Tukey's Honestly Significant Difference) test ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

### Phosphorus fractions

Anion exchange resin (AER) and sodium bicarbonate ( $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ ), which extracts the inorganic P content; and sodium bicarbonate  $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ , which extracts organic P, are the first extractants used in chemical P-fractionation, as proposed by Hedley et al. (1982). These substances extract labile P fractions that can actively contribute to supply the plants with the nutrient but that can also be transferred by runoff from the soil surface into surface waters, or by leaching into the soil profile, thus maximizing groundwater contamination (Graetz & Nair 1995; Gatiboni et al., 2008; Pizzeghello et al.,

2011). In the soil of the 4-year-old vineyard 1, the contents of inorganic P extracted by AER and of inorganic P extracted by  $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$  were highest in the 0-5 cm layer (Table 2).

In vineyard 2, the highest inorganic P contents extracted by AER were found in the 5-10 cm layer, and the highest inorganic P contents extracted by  $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$  in the 0-5 cm layer. However, in all layers, higher inorganic P contents were extracted by AER and inorganic P by  $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$  from soil of vineyard 2 than of vineyard 1. This is because the soil of vineyard 2 was phosphate-fertilized for 15 years, with  $400 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$  before planting and approximately  $90 \text{ kg ha}^{-1} \text{ year}^{-1} \text{ P}_2\text{O}_5$  during cultivation. It is worth noting that if the P contents in the soil before planting vineyard 1 and 2 were considered very low ( $< 7.0 \text{ mg P dm}^{-3}$ , assuming an average clay level of  $< 20\%$  in the 0-20 cm layer - unpublished data) (CQFSRS/SC, 2004), the maximum recommended dosage would have been  $150 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$ , to increase the P content in the soil up to the sufficiency level. This shows that the amounts of P applied to the soil were higher than those required by the crop and, therefore, the inadequacy of the fertilization management. The total P content in the vine leaf blades observed over the years was generally considered normal ( $0.12\text{-}0.40\%$ ) (unpublished data) (CQFSRS/SC, 2004) and, therefore, P application was not recommended; nevertheless, over the years, the vineyards received  $90 \text{ kg ha}^{-1} \text{ year}^{-1} \text{ P}_2\text{O}_5$ , maximizing the problem of excessive fertilization. Therefore, the amount of P fertilized before planting and during cultivation exceeded the soil and vine requirements, and could be one of the reasons for the high levels of inorganic P extracted from the vineyard soils by AER and inorganic P extracted by  $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ , particularly from vineyard 2. The data of inorganic P extracted by AER and inorganic P extracted by  $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$  in the soil agree with data published elsewhere (Oberson et al., 1999; Dobermann et al., 2002; Zhang et al., 2004; Herlihy & McGrath, 2007; Boschetti et al., 2009; Tokura et al., 2011); these studies also used the technique of P chemical fractionation and reported that the level of the two nutrient fractions in the soil would increase with the applied rate of soluble phosphate fertilizers and would tend to be higher than other fractions removed by other extractants during fractionation. This may be attributed to the greater quantity of P applied in relation to the quantity extracted by the plants, causing P accumulation in different fractions, including in labile fractions extracted by AER and inorganic P extracted by  $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ .

In vineyard 2, the inorganic P extracted by AER from the 10-20 cm soil layer was  $116.3 \text{ mg kg}^{-1} \text{ P}$ , which is higher than that of  $18.4 \text{ mg kg}^{-1} \text{ P}$  found in vineyard 1 (Table 2) and, according to CQFSRS/SC (2004), this value is considered very high ( $> 40 \text{ mg dm}^{-3}$ ). The increased P content in the upper and also in deeper layers of the vineyards soils,

especially in vineyard 2, reinforce the idea that the amounts of phosphate fertilizers applied before planting and during cultivation were quite beyond plant demand; this would clearly maximize the risk of the element being removed by surface water runoff and/or leaching, since the soil of the vineyards has a sandy loam texture and low iron oxide content (Table 1), while the crystalline iron oxides are correlated with the soil clay content (Hernández & Meurer 1998; Zago et al., 2012). Nevertheless, the specific surface area of Fe forms with low crystallinity, extracted by ammonium oxalate, is greater; these are therefore more effective in P adsorption than crystallized iron oxides (Johnson & Loeppert, 2006).

In the soil of vineyard 1, the highest organic P contents extracted by 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub> were found in the uppermost soil layer (0-5 cm), according to the distribution of the organic matter in the sampled layers (Table 1), but in the soil of vineyard 2, the highest contents were observed in layers to a depth of 10 cm. Also in vineyard 2, we found the highest organic P contents extracted by 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub> in all layers, which can be attributed to the deposition and subsequent decomposition of plant residues from the above-ground part of species that cover the soil surface, or grow in and between the vine rows, as well as trimmed branches and senescent vine leaves, and even senescent roots of the plant cover and vines

**Table 2. Phosphorus inorganic and organic fractions extracted by: anion exchange resin (AER); 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub>; 0.1 and 0.5 mol L<sup>-1</sup> NaOH; Pi 1.0 HCl and H<sub>2</sub>SO<sub>4</sub> + H<sub>2</sub>O<sub>2</sub> + MgCl<sub>2</sub> (residual P), from a Sandy Typic Hapludalf soil in vineyards**

| Layer  | Inorganic P                                |              |       | Organic P           |              |       |
|--------|--|--------------|-------|---------------------|--------------|-------|
|        | Vineyard 1                                 | Vineyard 2   | CV    | Vineyard 1          | Vineyard 2   | CV    |
|        | 4 years old                                | 15 years old |       | 4 years old         | 15 years old |       |
|        | mg kg <sup>-1</sup>                        |              | %     | mg kg <sup>-1</sup> |              | %     |
|        | AER  |              |       |                     |              |       |
| 0-5    | 56.7 aB                                    | 169.1 aA     | 1.49  |                     |              |       |
| 5-10   | 28.4 bB                                    | 171.0 aA     | 0.81  |                     |              |       |
| 10-20  | 18.4 cB                                    | 116.3 bA     | 0.57  |                     |              |       |
| CV (%) | 1.33                                       | 1.23         |       |                     |              |       |
|        | 0.5 mol L <sup>-1</sup> NaHCO <sub>3</sub> |              |       |                     |              |       |
| 0-5    | 46.6 aB                                    | 94.4 aA      | 8.54  | 29.6 aA             | 46.2 aA      | 7.10  |
| 5-10   | 28.2 bB                                    | 85.5 bA      | 1.37  | 13.6 bB             | 54.0 aA      | 2.74  |
| 10-20  | 20.7 bB                                    | 73.5 cA      | 3.75  | 6.7 cB              | 21.2 bA      | 6.35  |
| CV (%) | 18.95                                      | 2.54         |       | 11.71               | 5.33         |       |
|        | 0.1 mol L <sup>-1</sup> NaOH               |              |       |                     |              |       |
| 0-5    | 111.6 aA                                   | 131.1 aA     | 5.34  | 113.2 aB            | 148.3 bA     | 1.04  |
| 5-10   | 74.6 bB                                    | 114.0 bA     | 0.36  | 100.4 bB            | 197.1 aA     | 2.87  |
| 10-20  | 57.0 cB                                    | 74.6 cA      | 0.89  | 86.4 cB             | 146.5 bA     | 3.36  |
| CV (%) | 6.10                                       | 1.43         |       | 3.85                | 2.06         |       |
|        | 1.0 mol L <sup>-1</sup> HCl                |              |       |                     |              |       |
| 0-5    | 25.3 aB                                    | 167.6 aA     | 2.81  |                     |              |       |
| 5-10   | 19.5 BB                                    | 108.9 bA     | 0.59  |                     |              |       |
| 10-20  | 15.5 cB                                    | 19.5 cA      | 5.51  |                     |              |       |
| CV (%) | 1.73                                       | 2.31         |       |                     |              |       |
|        | 0.5 mol L <sup>-1</sup> NaOH               |              |       |                     |              |       |
| 0-5    | 62.1 aB                                    | 72.8 aA      | 3.78  | 33.4 aB             | 61.1 bA      | 4.32  |
| 5-10   | 46.4 bA                                    | 51.3 bA      | 6.34  | 18.8 abB            | 75.4 aA      | 14.56 |
| 10-20  | 42.8 bA                                    | 45.5 bA      | 10.74 | 7.4 bB              | 64.2 bA      | 8.43  |
| CV (%) | 4.83                                       | 5.34         |       | 12.39               | 2.67         |       |
|        | Residual P                                 |              |       |                     |              |       |
| 0-5    | 137.7 abA                                  | 120.8 aB     | 0.61  |                     |              |       |
| 5-10   | 142.2 aA                                   | 114.6 aB     | 3.45  |                     |              |       |
| 10-20  | 136.1 bA                                   | 109.6 aB     | 0.90  |                     |              |       |
| CV (%) | 1.44                                       | 3.67         |       |                     |              |       |

Means followed by the same lowercase letter in the column and the same capital letter in the line do not differ by the Tukey test ( $\alpha = 5\%$ ).

(Brunetto et al., 2009, 2011), contributing to P cycling (Martinazzo et al., 2007).

In general, the P inorganic fractions extracted by 0.1 and 0.5 mol L<sup>-1</sup> NaOH represent the inorganic P bound to the oxides and reinforced by silicate clays with intermediate binding energy. Organic P extracted by 0.1 and 0.5 mol L<sup>-1</sup> NaOH represents the organic P of medium lability and organic P physically protected on the internal surface of the microaggregates, respectively (Cross & Schlessinger, 1995; Gatiboni 2003) (Table 2). The highest inorganic and organic P contents extracted by 0.1 mol L<sup>-1</sup> NaOH from the soil of vineyards 1 and 2 were found in the 0-5 cm layer. But the highest inorganic P contents extracted by 0.1 mol L<sup>-1</sup> NaOH in all layers examined were found in vineyard 2, except in the 0-5 cm layer, where the values were identical to vineyard 1. This can be partially attributed to the longer period under cultivation and annual fertilization in vineyard 2, as opposed to vineyard 1. Greater organic P contents extracted by 0.1 mol L<sup>-1</sup> NaOH in all soil layers were found in vineyard 2, compared to the vineyard 1 soil. This may be due to organic matter buildup on the soil surface and in the surface layer, by plant roots and also residues of the above-ground part of some species of plant cover (Boschetti et al., 2009; Brunetto et al., 2009, 2011), as well as with branches and dead leaves fallen from vines, and the renewal of their roots (Brunetto et al., 2009, 2011). The inorganic P content extracted by 0.5 mol L<sup>-1</sup> NaOH in vineyards 1 and 2 tended to be greater in the 0-5 cm layer, but was similar in the same soil layer of both vineyards. On the other hand, the levels of organic P extracted by 0.5 mol L<sup>-1</sup> NaOH in vineyard 2 tended to be similar in all layers, and were higher than those found in vineyard 1. This indicates that in sandy loam soils of vineyard under long-term successive fertilizer applications, organic P will accumulate not only in the upper soil layer but also in the subsoil.

Inorganic P extracted by 1.0 mol L<sup>-1</sup> HCl, as described by Cross & Schlessinger (1995), would preferably extract the Ca-bound fractions. The highest inorganic P contents extracted by 1.0 mol L<sup>-1</sup> HCl were found in the surface soil layer of vineyards 1 and 2 (Table 2). But in all layers, higher contents were found in vineyard 2, which is older and has a longer history of phosphate fertilization, associated with greater exchangeable Ca contents and soil pH. It is worth noting that P, in the case of the inorganic P fraction, extracted by 1.0 mol L<sup>-1</sup> HCl, bound to calcium, is normally derived from primary apatite minerals or from newly formed Ca phosphates. According to Cross & Schlesinger (1995), the contents of P extracted by 1.0 mol L<sup>-1</sup> HCl (1 % of total P) from highly weathered soils are low, and higher (up to 66 % of total P) when extracted from little weathered soils. In moderately weathered soils, as those found in the southern region of Brazil, the occurrence of apatite minerals is however rare. Rheinheimer & Anghinoni (2001) reported that in no-tillage systems in south

Brazil, an increase in P-Ca fractions was observed due to the continuous application of phosphate fertilizers and lime on the soil surface. It is likely that a similar phenomenon occurred in these vineyards, especially in vineyard 2, where the addition of phosphates and lime to the soil surface over the years, as well as during seasonal fertilization, in addition to low P export by grape clusters, contributed to the accumulation of this nutrient in a number of fractions, even allowing the maintenance of part of the Ca-bound P, originating from the phosphate itself or limestone (Table 1) applied to correct soil acidity.

Phosphorus contents extracted by H<sub>2</sub>SO<sub>4</sub> + H<sub>2</sub>O<sub>2</sub> + MgCl<sub>2</sub> (residual P), which is a recalcitrant P fraction inaccessible by the selective chemical extractants in soil and which can contribute to plant nutrition only in cases of extreme lack of the nutrient in soil, were similar in the soil depths of vineyard 1 and 2. However, the highest residual P contents in all soil layers were found in the vineyard 1 soil. This may be due to the greater concentration of iron oxides extracted by dithionite-citrate-bicarbonate and by ammonium oxalate (Table 1) in vineyard 1, and these minerals are known P consumers with high binding energy and low reversibility.

Geochemical P contents were higher in the 0-5 cm soil layer of vineyards 1 and 2 (Table 3). However, the greatest contents were found in all layers of vineyard 2, which may be associated, as already reported, to the longer period of phosphate fertilization. Furthermore, the geochemical P contents in all soil layers of vineyards 1 and 2 were greater than those of biological P (Table 3). In general, organic P fractions

**Table 3. Geochemical, biological and total phosphorus in a sandy Typic Hapludalf soil in vineyards**

| P fraction <sup>(1)</sup> | Layer  | Vineyard 1          | Vineyard 2   | CV   |
|---------------------------|--------|---------------------|--------------|------|
|                           |        | 4 years old         | 15 years old |      |
|                           | cm     | mg kg <sup>-1</sup> |              | %    |
| Geochemical P             | 0-5    | 440.0 aB            | 755.9 aA     | 1.01 |
|                           | 5-10   | 339.2 bB            | 645.4 bA     | 0.83 |
|                           | 10-20  | 290.5 cB            | 438.9 cA     | 0.88 |
|                           | CV (%) | 1.37                | 0.52         |      |
| Biological P              | 0-5    | 176.2 aB            | 245.7 bA     | 2.37 |
|                           | 5-10   | 132.7 bB            | 326.4 aA     | 1.26 |
|                           | 10-20  | 100.5 cB            | 232.0 cA     | 1.85 |
|                           | CV (%) | 4.26                | 1.51         |      |
| Sum of all fractions      | 0-5    | 616.2 aB            | 1001.6 aA    | 1.36 |
|                           | 5-10   | 471.9 bB            | 971.8 bA     | 0.96 |
|                           | 10-20  | 391.0 cB            | 671.0 cA     | 1.04 |
|                           | CV (%) | 1.98                | 0.70         |      |

<sup>(1)</sup> Geochemical P is the sum of inorganic and residual fractions; biological P is the sum of the organic fractions; total P is the sum of the inorganic, organic, and residual fractions. Means followed by the same lowercase letter in the column and the same capital letter in the line do not differ by the Tukey test ( $\alpha = 5\%$ ).



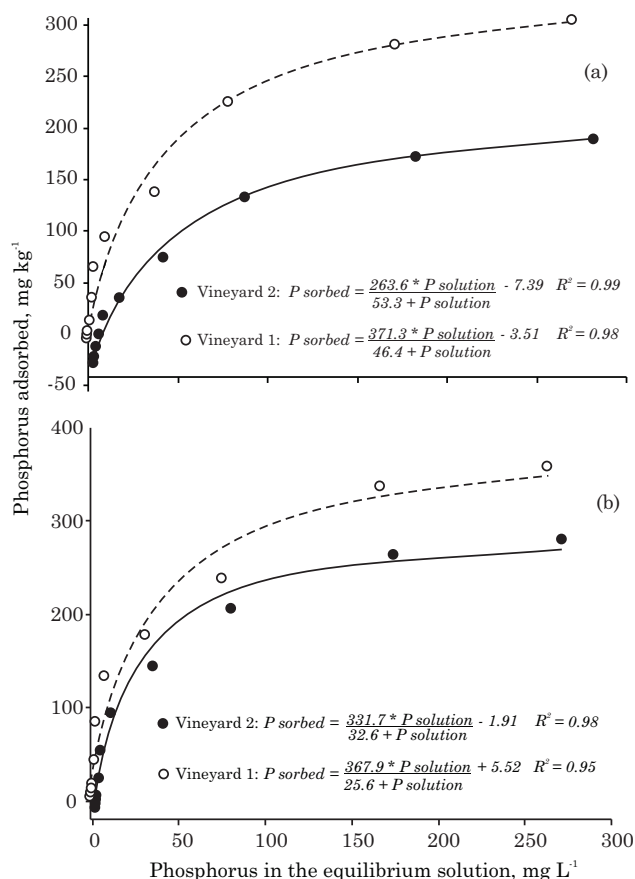
are more important in soils with low P availability, where the low inorganic P content in the soil solution triggers an organic P mineralization process (Gatiboni et al., 2008), while in case of accumulation of inorganic P, the organic P fraction is used to a lesser extent. The biological P content in vineyard 1 was highest in the 0-5 cm layer, where a greater concentration of total organic carbon was also found (Table 1). In contrast, in vineyard 2, the highest biological P contents were observed in the 5-10 cm layer. The highest contents of the sum of concentrations of all P fractions were observed in the 0-5 cm layer in the soils of both vineyards (Table 3). But, taking into account all layers, higher contents were found in the soil of vineyard 2, than of vineyard 1, similarly to the values of geochemical P and biological P.

### Parameters of phosphorus adsorption isotherms

Phosphate fertilization before planting and during cultivation in vineyards 1 and 2, and, as a consequence, the adsorption of part of this P by the functional groups of the reactive particles in the 0-5 and 10-20 cm layers, change the maximum phosphate adsorption capacity (MPCA) of the P adsorption isotherm in soil (Figure 1). The MPCA values, a parameter adjusted by the Langmuir equation, were 369 and 284 mg kg<sup>-1</sup>, respectively, in the 0-5 cm layer of vineyards 1 and 2, and differed statistically. In vineyard 1 we found a higher MPCA value, compared to vineyard 2 (Table 2), indicating that the soil is less P-saturated in vineyard 1 than in vineyard 2. The lower MPCA values in vineyard 2 can be attributed to the longer period of P application, which favored occupation of the P adsorption sites, particularly those located in the reactive inorganic soil particles (Nwoke et al., 2003). These MPCA values are consistent with this type of soil, which has a small quantity of phosphate adsorption sites, due to the low clay concentration (Table 1) and also the presence of 1:1 clay minerals (Rheinheimer et al., 2003; Uberti, 2005). In contrast, the MPCA values in the 10-20 cm layer in vineyards 1 and 2 were 367 and 334 mg kg<sup>-1</sup>, respectively (Table 2). It is worth noting that in the 0-5 cm layer, we found lower MPCA values than in the 10-20 cm layer, both in vineyard 1 and 2, which could be explained by the lower clay content in the surface soil (Table 1) and lower quantities of adsorbed phosphate (Rheinheimer et al., 2003), since in periodic maintenance fertilization, the phosphate fertilizers were applied on the soil surface, without incorporation, which could cause the occupation of the adsorption sites over the years (Parfitt, 1978; Barrow et al., 1998). Furthermore, the higher MPCA values in both soil layers of vineyard 1 may also be associated with the higher Fe oxide contents extracted by dithionite-citrate-bicarbonate and by ammonium oxalate (Table 1).

The values of the constant related to the affinity of the adsorbate to the substrate surface (*k*) of the

0-5 cm layer tended to be higher in the soil of vineyard 1 (0.0290 mg L<sup>-1</sup>), compared to the soil of vineyard 2 (0.0086 mg L<sup>-1</sup>) (Table 2). The *k* values in the 10-20 cm layer in vineyards 1 and 2 were 0.0796 and 0.0258 mg L<sup>-1</sup>, respectively. The lower *k* values in the 0-5 cm layer of vineyards 1 and 2, compared to those found in the 10-20 cm layer, may be associated with the occupation of the adsorption sites of the reactive particles by phosphate ions (Bolland et al., 1996; Nwoke et al., 2003). The higher mean values for *k* in the 10-20 cm layer indicate that the P adsorption sites in this layer are much more avid, which may be expected because of the lower quantity of the element in the deeper layers, as diagnosed during P chemical fractionation, especially by the labile and moderately labile fractions (Figure 2). In the parameters of the adsorption isotherm adjusted by the equation proposed by Koski-Vähälä & Hartikainen (2001), the MPCA values in the 0-5 cm soil layer were 371 and 264 mg kg<sup>-1</sup>, in vineyards 1 and 2, respectively, virtually identical to those obtained through the Langmuir model. No difference was observed in the highest value of P concentration in the solution that allows for half the P maximum adsorption (*km*)



**Figure 1.** Relation between the adsorbed P and P in the equilibrium solution in the 0-5 cm (a) and 10-20 cm (b) layers in a Sandy Typic Hapludalf soil in vineyards, and equations fitted to the Langmuir model.

**Table 4. Parameters related to phosphorus adsorption isotherms in the 0-5 and 10-20 cm layers in a Sandy Typic Hapludalf soil in vineyards**

| Vineyard   | Parameter fitted by |                         |                                     |                          |                          |                    |
|------------|---------------------|-------------------------|-------------------------------------|--------------------------|--------------------------|--------------------|
|            | Langmuir equation   |                         | Koski-Vähälä & Hartikainen equation |                          |                          |                    |
|            | MPAC <sup>(1)</sup> | <i>k</i> <sup>(2)</sup> | MPAC                                | <i>km</i> <sup>(3)</sup> | <i>Qo</i> <sup>(4)</sup> | PEC <sup>(5)</sup> |
|            | mg kg <sup>-1</sup> | L mg <sup>-1</sup>      | mg kg <sup>-1</sup>                 |                          | mg L <sup>-1</sup>       |                    |
|            | 0-5 cm              |                         |                                     |                          |                          |                    |
| Vineyard 1 | 369                 | 0.0290                  | 371                                 | 46.4 <sup>ns</sup>       | 3.51                     | 0.70 bA *          |
| Vineyard 2 | 284                 | 0.0086                  | 264                                 | 53.3                     | 7.39                     | 1.78 aA            |
| Average    |                     | 0.0188 B *              |                                     |                          | 5.45 A **                | -                  |
|            | 10-20 cm            |                         |                                     |                          |                          |                    |
| Vineyard 1 | 367                 | 0.0796                  | 368                                 | 25.6                     | -5.52                    | -0.17 bA           |
| Vineyard 2 | 334                 | 0.0258                  | 332                                 | 32.6                     | 1.91                     | 0.33 aB            |
| Average    |                     | 0.0527 A                |                                     |                          | -1.80 B                  | -                  |
|            | Mean                |                         |                                     |                          |                          |                    |
| Vineyard 1 | 368 a **            | 0.0543 a *              | 370 a *                             |                          | -1.01 b **               | -                  |
| Vineyard 2 | 309 b               | 0.0172 b                | 298 b                               |                          | 4.65 a                   | -                  |
| CV (%)     | 14.46               | 18.30                   | 11.98                               | 28.40                    | 34.39                    | 14.28              |

<sup>(1)</sup> Maximum phosphate adsorption capacity; <sup>(2)</sup> Constant that can provide information on the affinity of adsorbate to the substrate surface; <sup>(3)</sup> Phosphorus concentration in the solution that allows half of maximum P adsorption; <sup>(4)</sup> Quantity of P desorbed in water; <sup>(5)</sup> Concentration of P equilibrium; <sup>ns</sup>: not significant; \* and \*\*: significant at 5 and 1 %, respectively. Means followed by the same lowercase letter in the column and at the same depth, and capital letter in the same treatment but at different depths do not differ by the Tukey test ( $\alpha = 5\%$ ).

between the two layers in either vineyard. However, the highest P concentrations desorbed in water (*Qo*) were found in the 0-5 cm soil layer, and the soil of vineyard 2 showed higher levels when compared to vineyard 1, both in the 0-5 cm and in the 10-20 cm layers. The P equilibrium concentration (PEC) was highest in the 0-5 and 10-20 cm layers of the soil of vineyard 2. The concentration of P desorbed in water (*Qo*) indicates the quantity of P desorbed in water after equilibrium. Thus, the soil of vineyard 2 has greater potential for P release into water resources. This may be related to the greater application of P to the vineyard 2 soil, likely due to the periodic maintenance fertilizations (90 kg ha<sup>-1</sup> year<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>).

Phosphorus equilibrium concentration is an important theoretical property in studies of soil pollution potential because it indicates that in the case of transfer of P-rich soil (sediment) by erosion into aquatic environments, the element can potentially be released into the waters whenever the concentration of water-soluble P in the aquatic environment is lower than the phosphorus equilibrium concentration. Thus, the 0-5 cm soil layer of vineyard 2 has a greater potential for sediment contamination than vineyard 1.

## CONCLUSIONS

1. Excessive application of phosphate fertilizers before planting and during cultivation (maintenance fertilization), particularly in the older vineyard with

a, consequently, longer history of phosphate fertilization, increased the inorganic phosphorus contents to the depth of 20 cm, especially in labile fractions extracted by anion exchange resin, and NaHCO<sub>3</sub> in the labile fraction, as well as in the non-labile fraction extracted by 1.0 mol L<sup>-1</sup> HCl. Vineyard 2 had increased phosphorus contents in the organic labile fraction extracted by 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub>, and especially in the moderately labile fraction extracted by 0.1 and 0.5 mol L<sup>-1</sup> NaOH.

2. Phosphate fertilization of the older vineyard (15 years of cultivation), increased the amounts of phosphorus desorbed in water, indicating the risk of contamination of surface waters and groundwater.

3. The lower values of maximum capacity of phosphorus adsorption in the surface soil layer (0-5 cm) of vineyard 2 indicated saturation of part of the reactive particle adsorption sites.

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