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André Somavilla1\*, Paulo Ivonir Gubiani2, Pedro Bolzan Parisi3

### Abstract

The centrifugation of samples is frequently required in laboratory analysis, whether be it for chemical, physical or biological analysis of soil. The software Centrifuge 1.0 is a simple tool available that enable researchers to quantify the energy applied to samples during the centrifugation process. This study aims to demonstrate the new option of calculus incorporated into the software, such as sedimentation of particles in fluids in the centrifuge and analyses the deviation on outputs caused by variations in the values of the parameters used. In its 2.0 version, the Centrifuge software is more useful in carrying out laboratory techniques, because it can be applied to quantify energy in samples or sedimentation of solids particles in fluids during the centrifugation process. The software Centrifuge 2.0 is freely available and can be obtained with the authors.

Keywords: Sedimentation. Particles size. Centrifugation. Soil Analysis.

## Centrifuge 2.0 - um software útil para separação de partículas do solo e extração da solução do solo

#### Resumo

A centrifugação de amostras é frequentemente necessária em análises laboratoriais, seja em análises químicas, físicas ou biológicas do solo. O software Centrifuge 1.0 é uma ferramenta simples que permite aos pesquisadores quantificar a energia aplicada às amostras durante o processo de centrifugação. Este estudo tem como objetivo demonstrar a nova opção de cálculo incorporada ao software, para cálculo de sedimentação de partículas em fluidos com uso de centrífuga e analisar as incertezas atribuídas aos parâmetros utilizados pelo software. Na versão 2.0, o software Centrifuge é mais útil na execução de técnicas de laboratório, pois pode ser aplicado para quantificar a aplicação de energia em amostras ou para sedimentação de partículas de sólidos em meio fluidos durante o processo de centrifugação. O software Centrifuge 2.0 está disponível gratuitamente e pode ser obtido com os autores.

Palavras-chave: Sedimentação. Tamanho de partícula. Centrifugação. Análises de solo

<sup>&</sup>lt;sup>1</sup>Universidade Federal de Santa Maria. Santa Maria, RS. Brasil.

https://orcid.org/0000-0002-3595-6200 <sup>2</sup>Universidade Federal de Santa Maria. Santa Maria, RS. Brasil.

https://orcid.org/0000-0002-8840-3976

<sup>&</sup>lt;sup>3</sup>Universidade Federal de Santa Maria. Santa Maria, RS. Brasil.

https://orcid.org/0000-0002-7752-3374

<sup>\*</sup>Autor para correspondência: somavillaa@gmail.com

#### Introduction

This study aims to explicit the math principles and the functionality of the new calculus option available in the Centrifuge software (Somavilla *et al.*, 2017). The new version can be used to calculate sedimentation of particles in fluids during the centrifugation process.

The option of introducing this function in the software is due to the fact that the centrifugation of samples is a procedure commonly used in laboratories of soil chemistry, physics and biology in analyzes that sedimention of particles is requerid. However, this procedure is often carried out erroneously, mainly due to the lack of information in traditional analysis methodologies.

Centrifugation of solid samples dispersed in fluids has two main purposes. The first one consists in separating all the solid particles (soil, sediment, etc.) from the solution, for later analysis of the supernatant. The second is to separate distinct sized solid particles (soil, sediment, etc.) dispersed in the solution. In both cases, centrifugation makes it possible to apply force greater than that of gravity on the particles, which speeds up the droop speed and reduces sedimentation time.

Fluid and solid phase separation in chemical soil analysis methodologies is simply described as the centrifugation of the sample at a certain rotation speed. There are methodologies for soil phosphorus fractionation (Mehlich, 1984; Hedley e Stewart, 1982; Hedley *et al.*, 1982; Olsen e Sommers, 1982; Mehlich, 1953), analysis of 31P nuclear magnetic resonance (Vestergren *et al.*, 2012; Rheinheimer, 2002), extraction of Cadmium, Cobalt, Copper, Nickel, Lead, Zinc, Iron and Manganese (Tiesser *et al.*, 1979), soil ammonium (Silva *et al.*, 1966), separation of nematodes from soil (Jenkins, 1964), sequential extraction of heavy metals (Keller eVédy1994), determination of the cation exchange capacity and soil pH, (Gillman, 1979), among others. In the mentioned examples, the authors indicate the speed of centrifuge

spin and the time of centrifugation. This is mainly due to the fact that it is only necessary to sediment every solid particle without the need of fractionate the different particles size.

However, even in these cases, the standardization not only of the spin speed of the centrifuge, but mainly of the energy applied to the sample is extremally necessary. Without this standardization, different equipment can apply different energy with the same spin speed, mainly due to variations in the distance of the spin axis. This can lead to insufficient energy application and, consequently, to faulty separation of the sample phases. The correct amount of energy to be applied in samples centrifuged using different equipment can be estimated by the software Centrifuge 1.0. The Centrifuge 1.0 is freely available and described by Somavilla *et al.* (2017) for the extraction of soil solution.

In the specific cases where it is desired to separate fractions of solid particles of different diameters or density by centrifugation, as for example for separation of sand, silt and clay (Fernández-Ugalde *et al.*, 2013), fractions of the clay size (Laird *et al.*, 1991) or soil nanoparticles (Bakshi *et al.*, 2014), the accuracy regarding the energy applied to the sample must be much greater. In order to guarantee correct energy application new calculus option of sedimentation of solid particles in fluids was added to Centrifuge 1.0 software (Somavilla *et al.*, 2017), resulting in the Centrifuge 2.0 version.

### Software Development

Version 1.0 of the Centrifuge software was restructured. Its user interface became more dynamic and intuitive and new functionality were incorporated (Figure 1). In addition, in the initial window a basic image was introduced, with the definition of some parameters used in the calculation procedures available in the software, which facilitates the understanding by the user.





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In the initial interface, the user has access to the two main Software functions available in the "Menu" section: "Particle sedimentation" and "Energy applied to sample". In each the screens (Figure 2) the user can access the item that represents their need and perform the calculation from a series of parameters. As an example, it is possible to visualize in Figure 3 the interface for quantification of the particle sedimentation time.

Figure 2 – Command tabs for the estimation of particle sedimentation (a) and quantification of energy applied to sample (b)

Centrifuge 2.0 -		×	Ocentrifuge 2.0	- 0	$\times$
Menu •			Menu •		
Particles sedimentation Getting		Energy Estimate of	applied to sample		
Time			G - Force		
Particle size			Getting Spin movement	Getting Spin movement	
Depth			Spin radius	Tension	
Spin movement			G - Force		
	a	)		b)	ł

Centrifuge 2.0 calculates the sedimentation of particles in a fluid using Stokes's law, described by Hathaway (1956) and Bortoluzzi e Poleto (2013) and expressed implicitly as:

$$T - \frac{n_i \log_{10} \left(\frac{R_1}{R_2}\right)}{3.81 r^2 N^2 (D_p - D_f)} = 0$$
 (Eq. 1)

Where T is the total time (s);  $n_i$  is the viscosity (P);  $R_1$  and  $R_2$  are the initial and final distance of particle from rotation axis, respectively (cm); r is de radius of the

particle (cm); N is the angular velocity (RPS); Dp and Df are the density of the particle and de fluid, respectively (g cm<sup>-3</sup>).

In Figure 3 it is possible to observe an example of the software being used to calculate the duration of centrifugation for the sedimentation of particles with diameter greater than 0.002 mm. This example corresponds to the separation of silt and clay fractions, commonly performed in soil particle size analysis.

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Figure 3 - Quantification of the time required for sedimentation of solid particles in fluid medium

Ocentrifuge 2.0

Centrifugation time							
Depth (cm)	7						
Particle size (mm)	0.002						
Viscosity of fluid (N s m $^{\text{-2}}$ )	0.001						
Particle density (kg m <sup>-3</sup> )	2650						
Fhuid density (kg m <sup>-3</sup> )	1000						
Internal spin radius (cm)	5						
RPM	500						
Spin movement Hertz							
🗌 V (m s	-1)						
The time is 14.52 min.							
Run Help	Exit						

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The example shown in Figure 3 was also used to evaluate the magnitude of the deviations on the outputs of equation (1) caused by changing the values of the equation parameters. The analysis was performed from the individual alteration of the equation parameters to quantify the time, particle size, spin movement and depth. Each parameter varied by 1, 2 and 3% for more and less than its default value (Figure 3). The results were expressed as percentage error in relation to the value obtained using the standard parameter values. Although this analysis evaluates the sensitivity of equation (1) to its parameters, the term error was introduced to give error connotation in the outputs if the change in parameters is caused by an error in the choice of values.

Among the analyzed parameters, the most important ones which cause greater error if values are erroneously introduced in the equation (1) are: spin movement and particle size (with error up to 10%) and particle density (with error up to 7.14%) in the depth (Figure 4). In general, the spin movement is the parameter that requires greater accuracy, because errors in its value can result in large errors in the fractionation of particles.

Figure 4 – Error analyses of time (a), particle size (b), depth (c) and spin movement (d)



Where: S-particle size; h-depth; ni-viscosity of fluid; Dp and Df-particle and fluid density, respectively; ri-internal spin radius; RPM-spin movement; T-time.

Alterations in the parameters related to the fluid (viscosity and density) resulted, respectively, in errors of 4.3 and 2.9% in the depth. Both the viscosity and the density of the fluid are changed by changing the fluid temperature. Thus, it is important to control fluid temperature during the centrifugation process.

The sedimentation of particles by centrifugation can be performed in different fluids. As standards for viscosity and fluid density parameters, the software provides values for pure water at 20°C. However, these parameters can be changed in order to make than proper for the particular analyses conditions.

## Conclusion

In version 2.0 Centrifuge software has a greater applicability in laboratory techniques and can be used for quantification of applied energy in soil samples and for sedimentation of solid particles in fluid.

The spin movement and the particle diameter are the parameters which should receive most attention from the user due to the possibility of causing greater output errors.

#### References

Bakshi, S., He, Z., Harris, W. G. 2014. A new method for separation, characterization, and quantification of natural nanoparticles from soils. Journal of Nanoparticle Rresearch, 16: 2261.

Bortoluzzi, E. C. ; Poleto C. 2013. Metodologias para estudos de sedimentos: ênfase na proporção e na natureza mineralógica das partículas. 2. ed. Porto Alegre, ABRH.

Fernández-Ugaldi, O., Barré, P., Hubert, F., Virto, I., Girardin, C., Ferrage, E., Caner, L., Chenu, C. 2013. Clay mineralogy differs qualitatively in aggregate-size classes: clay-mineral-based evidence for aggregate hierarchy in temperate soils. European Journal of Soil Science, 64: 410 – 422.

Gillman G. P. 1979. A proposed method or the measurement of exchange properties of high-weathered soils. Soil Research, 17: 129-141.

Hathaway, J. C. 1956. Procedure for clay mineral analyses used in the sedimentary petrology laboratory of the U.S. geological survey. Clay Minerals Bulletin, 3: 8-13.

Hedley M.J., Stewart J. W. B. 1982. Method to measure microbial phosphate in soils. Soil Biology and Biochemistry, 14: 377-385.

Hedley, M. J., Stewart, J. W. B., Chauhan, B. S. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Science Society of America Journal, 46: 970-976.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter, Charleston, 48: 692.

Keller C. ; Védy J. C. 1994. Distribution of cadmium fractions in two forest soils. Journal of Environmental Quality, 23: 987-999.

Laird, D. A., Barak, P., Nater, E. A., Dowdy, R. H. 1991. Chemistry of smectitic and illitic phases in interstratified soil smectite. Soil Science Society of America Journal, 55: 1499–1504.

Mehlich, A. 1953. Determination of P, Ca, Mg, K, Na and NH4 by North Carolina Soil Testing Laboratoris. North Carolina Soil Test Division.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Communications in Soil Science And Plant Analysis, 15: 1409-1416.

Olsen, S. R.; Sommers, L. E. 1982. Phosphorus. In: Page, A. L.; Miller, R.H., Keeney, Q.R. (Eds.) Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. 2. ed. SSSA. Madison, 403-430.

Rheinheimer, D. S., Anghinoni, I., Flores, A. F. 2002. Organic and inorganic phosphorus as characterized by phosphorus-31 nuclear magnectic resonance in subtropical soils under management systems. Communications in Soil Science And Plant Analysis, 33: 1853-1871.

Silva, J. A. ; Bremner, J. M. 1966. Determination and isotope-ratio analysis of different forms of nitrogen in soils. 5. Fixed ammonium. Soil Science Society of America Journal, 30: 587-594.

Somavilla, A., Dessbesell, A., Rheinheimer, D. S. 2017. Centrifugation methodology to extract soil solution. Scientia Agraria, 18: 44-47.

Tessier, A., Campbell, P.G. C., Bisson, M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry, 51: 844–850.

Vestergren, J., Vincent, A. G., Jansson, M., Persson, P., Ilstedt, U., Gröbner, G., Giesler, R., Schleucher, J. 2012. High-resolution characterization of organic phosphorus in soil extracts using 2D 1H-31P NMR correlation spectroscopy. Environmental Science & Technology, 46: 3950–3956.