Fronteiras: Journal of Social, Technological and Environmental Science
http://periodicos.unievangelica.edu.br/fronteiras/
ISSN 2238-8869

Article

Extractor Solutions Improve the Extraction/Quantification of Microbial Load by Conventional Cultivation Techniques

Adriana Barboza Alves[1], Alan Rodrigues Teixeira Machado[2], Osania Emerenciano Ferreira[3]

[1] Master from the Postgraduate Program in Environmental Sciences, University of the State of Minas Gerais , University of the State of Minas Gerais, University Analyst. ORCID: 0000-0003-2202-1622. E-mail: adriana.alves@uemg.br

[2] PhD in Chemistry from the Federal University of Minas Gerais, Adjunct Professor I at the State University of Minas Gerais. ORCID: 0000-0002-4803-4600. E-mail: alan.machado@uemg.br

[3] PhD in Agricultural Microbiology from the State University Paulista Júlio de Mesquita Filho, Adjunct Professor I at the State University of Minas Gerais. ORCID: 0000-0003-1590-5550. E-mail: osania.ferreira@uemg.br

ABSTRACT

Limitations in quantifying microorganisms in conventional soil microbiological analyses cause experimental errors. There is a difficulty in performing the correct dilution of the sample, which is influenced by the specific granulometric characteristics of each soil. This study aimed to evaluate different combinations of extracting soil solutions. Clayey loam and sandy loam are the two types of soil evaluated according to current humidity, pH, and granulometric analysis. For the quantification of the Colony Forming Units (CFU g^{-1}) of the soil, extracting solutions of H_2O (distilled water), NaCl (sodium chloride), $Na_4P_2O_7$ (sodium pyrophosphate), and $C_{64}H_{124}O_{26}$ (Polysorbate Tween-80) were used for analyses. The method of in-depth serial dilution was used in this study to quantify the microorganisms. The means of each microbiological feature were examined and compared using the Tukey test with a 5% chance of being correct. The granulometric characteristics of the soil affected the microbial quantification. The solution of H_2O + $Na_4P_2O_7$ + $C_{64}H_{124}O_{26}$ allowed greater quantification for sandy loam soils, and for clayey loam soil, the solution of H_2O + $Na_4P_2O_7$ was the most efficient.

Keywords: counting; dilution; granulometry; microorganisms.

RESUMO

Limitações na quantificação de microrganismos em análises convencionais de solos ocasionam erros experimentais. Existe uma dificuldade em se fazer a correta diluição da amostra, que é influenciada pelas características granulométricas específicas de cada solo. Esse estudo teve como objetivo avaliar diferentes combinações de soluções extratoras de solos. Avaliaram-se dois tipos de solo, um francoargiloso e outro francoarenoso. Nestes determinou-se o pH, a umidade atual, as análises granulométricas. Para a quantificação das unidades formadoras de colônia (UFC.g^{-1}) do solo, utilizou-se as soluções extratoras de H_2O (água destilada), NaCl (cloreto de sódio), $Na_4P_2O_7$ (pirofosfato de sódio) e $C_{64}H_{124}O_{26}$ (polisorbato Tween-80). A contagem de microrganismos foi realizada através do método de diluição seriada em profundidade. As médias de cada atributo microbiológico foram comparadas entre si através do teste de Tukey 5% de probabilidade. As características granulométricas do solo afetaram a quantificação microbiana. A solução de H_2O + $Na_4P_2O_7$ + $C_{64}H_{124}O_{26}$ possibilitou maior quantificação para solos francoarenosos e para solo francoargiloso, a solução de H_2O + $Na_4P_2O_7$ foi a mais eficiente.

Palavras-chave: contagem; diluição; granulometria; microrganismos.

Submissão: 05/10/2023 Aceite: 07/07/2025 Publicação: 04/09/2025

v.14, n.3, 2025 • p. 30-41. • DOI http://dx.doi.org/10.21664/2238-8869.2025v14i3.7101

Introduction

Only 1% of soil microorganisms are known, according to estimates. The cultivation and quantification of these microorganisms by conventional techniques are limiting because these living beings need to have all the physical and chemical conditions necessary for their growth, being difficult to optimize these conditions in the laboratory (Stursa et al. 2009). Another factor that can affect this quantification is the adequate dilution and homogenization of the sample, which is essential for developing new methodologies for its study, which allow quantifying the maximum possible of these microorganisms.

Microorganisms provide several ecosystem services, according to (Pires 2016), the world population depends directly on microorganisms, when related to nutrient recycling and supply for agriculture and livestock. Of Brazil the soils at the Cerrado biome are responsible for a large part of this agricultural productivity. Due to their formation of origin and the high weathering process, these soils are naturally acidic, with low cations and high aluminum concentration, limiting agropastoral systems (Fageria e Stone 1999).

Among the physical indicators that evaluate soil quality are texture, structure, density, particle aggregation, resistance to penetration, hydraulic conductivity, porosity and water infiltration rate (Reichardt e Timm 2012). Thus, the sandy texture in soils allows greater hydraulic conduction capacity due to the presence of macropores, in addition to providing lower water adsorption force, when compared to clayey soils (Brady e Weil 2013). Texture is a physical indicator of the soil, which relates proportions of sand, silt and clay; This relationship determines soil resistance, water dynamics and ecological processes, which are nutrient cycling and ionic exchange (Stefanosk et al. 2013).

Modifications of previously natural environments in areas of large agricultural production have caused great soil degradation, increasing compaction, reducing biological activity and, consequently, causing loss of nutrients and organic matter through erosion processes (Moraes et al. 2012; Boldaji e Keller 2016). Knowing the physico-chemical and biological characteristics of Cerrado soils is extremely important to attribute the type of management used to improve productivity adequately.

The most varied extraction methods can be the reason for contradictory results, with different conclusions in identical experiments regarding the analysis of soil microbiota. Paz-Lima et al. (2016) used aqueous extracts in different soil and water ratios to quantify the microbiota in different soil managements. Varela (2012) also uses this same extraction technique to analyze microorganisms in soils degraded by coal mining. Bueno et al. (2018) quantified the high formation of colony forming units (CFUs) in soils of agroforestry areas after extraction of samples in saline solution (0.85% NaCl). Some works use solutions with sodium pyrophosphate and tween 80, such as (Santos e Batista 2015), who evaluated the presence of bacteria in soils with domestic and industrial solid waste; (Moraes 2016) used a 0.1% pyrophosphate soil extractor solution to quantify total bacteria in soils planted with corn and inoculated with growth-promoting bacteria. The use of biological indicators, promoters of growth and development of cultures, has been adopted in monitoring programs, which evaluate the alterations in the soil and its quality (Silveira 2011). The quantification of the microbial load is an essential tool to assess and quantify biodiversity in terrestrial environments.

The definition of solid, liquid, and gaseous soil phases is fundamental in the characterization of physical, chemical, and biological attributes, especially in the choice of technique to be used, for example, to quantify microbial load. The solid phase (sand, silt, and clay) has a more significant characteristic of resistance to changes in the initial phase. The liquid and gas phases vary, depending on the management used (Souza et al. 2013). For the authors, knowledge of the liquid phase, the soil solution, is fundamental because it is in this phase that the availability of nutrients for plants, the processes of environmental contamination, and the characterization and quantification of excess salts in the soil.

The microbial load is also determined qualitatively and quantitatively. The literature is limited in terms of comparisons of effective techniques for the greatest possible extraction from the soil, when it comes to microbiological analyses. For an efficient evaluation, it is necessary to use the ideal extraction solution, that is, one that has chemical affinity with the texture of the analyzed soil. The study hypotheses were: (i) Different combinations of extracting solutions have different dilution capacities; (ii) The texture of the soils, in this case, sandy and clayey, influences the interaction of the molecules of the applied extracting solutions. This work evaluates the efficiency of three methodologies used to extract microorganisms from soils in different combinations in a Cerrado soil samples in Frutal (MG).

Materials and Methods

Areas of study

The experiment was carried out in two agricultural areas in the Cerrado, Fazenda Marmeleiro and Fazenda Mata da Chuva, both located in the municipality of Frutal, MG. The soils are of the red-yellow latosol type and are subject to sugarcane planting. The samples were taken and analyzed during the dry month of July 2020.

Collection method

Samples were collected at a 0-25 cm depth in four property plots, totaling eight different samples. The collected soils were placed in sterile bags and taken to the laboratory for analysis. Then, were homogenized by agricultural fund, reducing to two samples in total.

Physicochemical analyses of soils

The analyzes of pH and current humidity were carried out, according to (Teixeira et al. 2017). Then, the sample was prepared, obtaining the air-dried fine earth (ADFE) and granulometric analysis, with the mass determination of the soils' fractions. All of the analyses were carried out, in a laboratory, in triplicate.

Soil pH analysis

The soil was collected (10 g), weighed, and transferred to a 50 mL beaker aiming to measure the hydrogenic potential (pH). Then, 25 mL of distilled water was added to the soil using a beaker, and, with the aid of an individual glass rod, homogenization was necessary for approximately 1 minute. The mixture was left to rest for one hour, and the pH of the solution could then be measured by immersing the combined electrode (water and KCl at 3 mol L^{-1}).

Soil humidity analysis

Humidity determination was defined using porcelain capsules, where 30 g of moist soil were weighed on a precision analytical balance and placed in a kiln at 105 ºC for 24 h. After removing the kiln, the capsules were directed to within the desiccator with silica indicating the humidity until they reached room temperature. After cooling, the capsules containing the dry soils were weighed again on the same analytical precision balance. The following equation can calculate the current gravimetric humidity (Kg Kg^{-1}).

$$H_g = \left(\frac{a - b}{b}\right)$$

wherein:
Hg = gravimetric humidity (Kg Kg^{-1})
a = Wet sample mass (Kg)
b = Dry sample mass (Kg)

Obtaining the ADFE (Air-Dried Fine Earth)

The samples were manually broken up and spread on 180 g/M^2 kraft paper trays and placed in a dry and ventilated place, exposed to the sun until complete desiccation.

Granulometric analysis

First, the residual humidity of the soil was measured, according to (Teixeira et al. 2017). For that, 10 g of ADFE were weighed in previously dried and tared porcelain capsules and subjected to a temperature of 105 ºC for 24 h. Calculations for determination of residual humidity and soil mass correction factor were estimated specified below:

$$U_r = \left(\frac{a-b}{b}\right) \qquad f = \frac{a}{b}$$

wherein:

Ur = Residual humidity (kg kg^{-1})

f = Factor used to correct soil mass, used for dry soil mass in laboratory determinations using ADFE (Factor "f")

a = Air-dried sample mass (g)

b = Mass of the sample dried in a kiln at 105 ºC (g)

For granulometric analysis, 20 g of ADFE were necessary from each sample. The soils were transferred to 250 mL Erlenmeyer with a glass funnel, using 100 mL of distilled water for this transfer. Then, 10 mL of 1 mol L^{-1} sodium hydroxide (NaOH) solution was prepared and added to the mixture. All Erlenmeyer flasks were adjusted and placed on a shaker table rotated at 50 rpm for 16 h. After the rotational termination, the samples passed through 0.053 mm mesh sieves with the aid of funnels to the 1000 mL beakers. All retained material was washed with distilled water, taking care to avoid splashing, always observing the volume limit of the beakers. After washing the material retained on the sieves, the volume of 1 L in each beaker was total and completed using distilled water. The sands retained in the sieves were transferred to previously dried and tared beakers, subjected to drying in a kiln for 24 h at 105 ºC. After drying and cooling, the coarse sand and fine sand fractions were examined and determined, submitting the total sand to a new 0.212 mm sieve. The retained and weighed fraction constituted the coarse sand fraction. Fine sand passed through the sieve, was also weighed, and the value of its mass was obtained and recorded.

The pipette method was used and implemented in this assay to determine silt and clay (Teixeira et al. 2017). The control test was realized and prepared in another 1000 mL beaker, adding 10 mL of 1 mol L^{-1} NaOH and completing the volume with distilled water. With a volumetric pipette, 25 mL of the solution was collected and transferred to a 50 mL beaker, previously dried and tared. The solution was dried in a kiln for 24 h at 105 ºC. After drying and cooling, the beaker mass was obtained and measured again. All solutions containing the test tubes, including the control test, were subjected to suspension temperature measurement using a thermometer. Then, all suspensions were shaken vigorously for 1 min with glass rods, immediately collecting 25 mL of each supernatant solution. Likewise, the collected solutions were separated and placed in 50 mL beakers, previously dried and tared, and placed in a kiln for 24 h at 105 ºC. The silt fraction settling time was waited, following the temperature of the solution, as described (Table 1).

Table 1. Sedimentation time calculated for the silt fraction (0,002 mm < Ø < 0,05 mm) in the function of the temperature of the suspension for a depth of 5 cm and soils with an average particle density of 2.65 kg dm^{-3}, in a determination, carried out at sea level

	Sedimentation time at 5 cm	
Temperature (ºC)	Hours	Minutes
15	4	23
16	4	16
17	4	10
18	4	4
19	3	58
20	3	52
21	3	46
22	3	41
23	3	36
24	3	31
25	3	26
26	3	21
27	3	17
28	3	13
29	3	9
30	3	5

Source: Teixeira *et al.* (2017, p. 95).

After the sedimentation time of the silt fraction, 25 mL were removed from the supernatant solutions in triplicate so that it did not go deeper than 5 cm in the beaker. The aliquots were separated, transferred to clean beakers, previously dried and tared, and taken to a drying kiln at 105 ºC for 24 hours. After cooling in a desiccator, the beakers were immediately prepared and weighed using a balance with an accuracy of 0.0001 g. All masses, previously weighed at the balance, were reserved for analysis. Calculations for different parameters were estimated and obtained according to the equations below (Equations 1-5).

1 - Equation of the sum of the mass of fractions

$$Sm = \left(\frac{m_{fs} \times f}{m_i}\right) + \left(\frac{(m_{ts} - m_{fs}) \times f}{m_i}\right) + \left(\frac{(m_{sc} - m_c) \times f}{m_i \times R_v}\right) + \frac{(m_c - m_{control}) \times f}{m_i \times R_v}$$

Wherein:

S_m = Sum of the mass of fractions (g)

m_{fs} = Fine sand mass (g)

m_{ts} = Total sand mass (g)

m_{sc} = Silt + clay mass (g), dried in a kiln

m_c = Clay mass (g), dried in a kiln

$m_{control}$ = Control mass (g), dried in a kiln

m_i = initial sample mass (g)

R_v = Ratio of pipetted volume to the total volume of the beaker

f = humidity correction factor for initial mass

2 - Equation of the fine sand concentration.

$$T_{fs} = \left(\frac{m_{fs} \times f}{m_i}\right) \times \left(\frac{1000}{S_m}\right)$$

Wherein:
T_{fs} = Fine sand concentration (g kg^{-1})
m_i = Initial sample mass (g)
m_{fs} = Fine sand mass (g)
f = humidity correction factor for initial mass
S_m = Sum of the mass of fractions (g)

3 - Equation of the coarse sand concentration.

$$T_{cs} = \left(\frac{(m_{ts} - m_{fs}) \times f}{m_i}\right) \times \left(\frac{1000}{S_m}\right)$$

Wherein:
Tcs = Concentration of coarse sand (g kg^{-1})
m_i = Initial sample mass (g)
m_{ts} = Total sand mass (g)
m_{fs} = fine sand mass (g)
f = humidity correction factor for the initial mass
S_m = Sum of the mass of fractions (g)

4 - Equation of the silt concentration.

$$T_s = \left(\frac{(m_{sc} - m_c) \times f}{m_i \times R_v}\right) \times \left(\frac{1000}{S_m}\right)$$

Wherein:
T_s = Silt concentration (g kg^{-1})
m_i = Initial sample mass (g)
m_{sc} = Silt + clay mass (g), dried in a kiln
m_c = Clay mass (g), dried in a kiln
R_v = Ratio of pipetted volume to the total volume of the beaker
f = humidity correction factor for initial mass
S_m = Sum of the mass of fractions (g)

5 - Equation of the clay concentration.

$$T_c = \left(\frac{(m_c - m_{control}) \times f}{m_i \times R_v}\right) \times \left(\frac{1000}{S_m}\right)$$

Wherein:
T_c = Clay concentration (g kg^{-1})
m_i = Initial sample mass (g)
m_c = Clay mass (g), dried in a kiln

$m_{control}$ = Control mass (g), dried in a kiln
R_v = Ratio of pipetted volume to the total volume of the beaker
f = Humidity correction factor for initial mass
S_m = Sum of the mass of fractions (g)

Soil extraction methods

Three methodologies studied were evaluated, using three different substances, which are sodium chloride, sodium pyrophosphate and Tween 80. These elements were diluted in water in different combinations. 10% water and soil solution (Paz-Lima et al. 2016); 0.9% NaCl solution (Bueno et al. 2018); 0.1% pyrophosphate solution + 0.1% tween (Santos e Batista 2015). The experimental design consisted of four replications six treatments (Table 2).

Table 2 - Treatments used as soil extractor solutions for microbiological analysis

Treatments		Concentrations
1	H2O	100%
2	H2O + NaCl	0.9% NaCl
3	H2O + Na4P2O7	0.1% Na4P2O7
4	H2O + C64H124O26	0.1% C64H124O26
5	H2O + NaCl + C64H124O26	0.9% NaCl + 0.1% C64H124O26
6	H2O + Na4P2O7 + C64H124O26	0.1% Na4P2O7 + 0.1% C64H124O26

*H_2O = Distilled water; NaCl = sodium chloride; $Na_4P_2O_7$ = Sodium pyrophosphate; $C_{64}H_{124}O_{26}$ = Tween 80. Source: Prepared by the author, 2023.

Microbiological analysis of soils

For counting total microorganisms, 10 g of each rhizospheric soil sample were weighted and added to 250 mL Erlenmeyer containing 90 mL of each extracting solution. The mixtures were adjusted and rotated at 300 rpm for 30 min. After homogenization, serial dilutions were necessary, in which 1,0 mL of the sample was diluted and homogenized, mistuning to 9.0 mL of each corresponding solution. The same technique was meticulously followed at the laboratory until the necessary concentration was confirmed. Thus, the concentrations used in the experiment are from 10^{-4} to 10^{-7}. In quadruplicate, 0.1 mL aliquots of each concentration were incubated at kiln by pour plate in nutrient agar medium. The plates containing the inoculum were kept in BOD (Biochemical Oxygen Demand) at a temperature of 28 ºC for 72 hours. After this period, the colony counter counted the number of existing colonies with 6X magnification. The plates evaluated and counted had between 30 and 300 UFC's, necessarily. The quantitative calculation of colony-forming units was carried out according to (number of colonies found x number of dilutions used x 10).

Statistical analysis

The number of colony-forming units (CFU g^{-1}) in soil was the dependent variable for evaluating the efficiency of extracting solutions. Thus, the results obtained from the microbiological count were associated with a greater or lesser degree of dilution capacity of the extracting solutions. The averages of each microbiological attribute were obtained and compared using the Tukey test at 5% probability.

Results and discussions

The averages were determined and obtained from the results in triplicate from the pH and humidity analyses (Table 3). Environmental factors, such as pH and granulometry, which interfered with the number of

bacteria, influenced the growth of bacterial communities. When it came to pH, Mata da Chuva Farm had the highest pH, the lowest humidity, and the highest number of bacteria (Table 3/Graph 1). Most soil bacteria tend to grow more at neutral pH, between (6.5 to 7.5) (Sudharhsan et al. 2007). The seasonal variation of the bacterial community can be affected by soil humidity (Butenschoen et al. 2011). In this work, the humidity did not affect the number of bacteria.

Table 3 - Results of the averages of pH and current humidity, with analysis of the standard deviation, of the soils of Cerrado

	Marmeleiro Farm	**Mata da Chuva Farm**
pH analysis	5.78 ± 0.62	6.20 ± 0.08
Current humidity analysis (kg kg^{-1})	0.096 ± 0.026	0.18 ± 0.0096

Source: Prepared by the author, 2023.

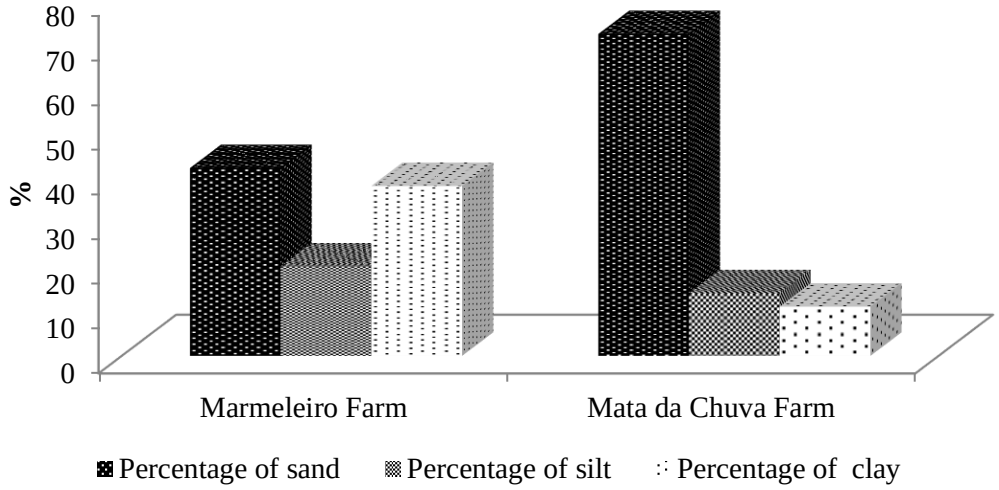
Regarding the granulometric analysis, the highest percentage of sand was determined for Mata da Chuva Farm and also where the highest values of microbial load were observed and obtained in the solution of H_2O + $Na_4P_2O_7$ + $C_{64}H_{124}O_{26}$; Marmeleiro Farm showed a clay loam soil, and the highest microbial load values were obtained and observed in the H_2O + $Na_4P_2O_7$ solution (Table 3/Graph 1). These results evidence the importance of the correct use of the extracting solution, being important standardization of the method taking into account the granulometric characteristics of the soil. The granulometric analysis passed through the average calculation is then submitted to the calculations described in the methodology. The results of all equations can be seen and described in (Table 4).

Table 4 - Means of granulometric attributes of the two types of Cerrado soils submitted to a sugarcane plantation

Granulometric attributes	**Marmeleiro Farm**	**Mata da Chuva Farm**
Residual humidity (kg kg^{-1})	0.0014	0.0012
m_i = Initial sample mass (g)	20	20
m_{ts} = Total sand mass (g)	7.8883	14.5605
m_{fs} = Fine sand mass (g)	7,03	12,57
$m_{control}$ = Control mass (g), dried in a kiln	0.0119	0.0119
m_{sc} = Silt + clay mass (g), dried in a kiln	0.2836	0.1305
m_c = Clay mass (g), dried in a kiln	0.1890	0.0645
R_v = Ratio of pipetted volume to the total volume of the beaker	0.025	0.025
f = Humidity correction factor for initial mass	1.0129	1.0057
S_m = Sum of the mass of fractions (g)	0.9499	0.9707
T_{fs} = Fine sand concentration (g kg^{-1})	374.78	651.18
T_{cs} = Concentration of coarse sand (g kg^{-1})	45.79	103.12
T_s = Silt concentration (g kg^{-1})	201.70	136.80
T_c = Clay concentration (g kg^{-1})	377.73	108.99

Source: Prepared by the author, 2023.

The fractions of the forming particles of each soil were measured and obtained using the table 4 findings, as shown in (Graph 1).



Graph 1 - Percentage of sand, silt, and clay fractions in two soils submitted to sugarcane plantation in the Cerrado of Minas Gerais. Source: Prepared by the author, 2023.

Soils were classified and designated as clay loam (Marmeleiro Farm) and sandy loam (Mata da Chuva Farm) according to the Minas Gerais (MG) triangle of soil textural classes (IBGE Pedology Technical Manual 2015). Table 5 shows the results of counting microorganisms subjected to the Tukey test.

Water is a natural extractor and is often used to extract carbon from soil microbial biomass in microbial ecology (Haney et al. 1999). In this work, the extracting solution composed only of distilled water (treatment 1) showed the lowest efficiency, with the value for CFU g^{-1} equal to $2.50.10^4$ for both soil samples (Table 5). (Paz-Lima et al. 2016) found values higher than 7.10^6 CFU g^{-1} in horticultural soils, using only distilled water as an extractor solution. Lower values were reported by (Silva et al. 2010) using the same extractor solution in tropical forest areas, which determined the average count of the order of 10^3 CFU g^{-1} for fungi and 10^5 CFU g^{-1} for bacteria per gram of soil. However, it is essential to note that the counts of CFU g^{-1} levels are observed and influenced by soil conditions and should not be analyzed and compared directly.

The addition of NaCl to water (treatment 2) did not lead to a statistically significant difference between treatments (1 and 2) for the values obtained for CFU g^{-1} (Table 5). (Bueno et al. 2018), evaluating microbiological indicators in agroforestry systems, found aerobic bacteria in 10^9 CFU g^{-1} of soil in their work, using 0.85% NaCl as an extracting solution. These results do not agree with the data of this work since this type of solution has a low capacity to dilute the material to be analyzed, with insufficient levels of quantification of microbial biomas.

Table 5 - Mean values of colony forming units (10^4 CFU g^{-1}) after 72 hours of incubation in Nutrient Agar medium, using different extractor solutions

Treatments		Marmeleiro Farm	Mata da Chuva Farm
1	H_2O	2.50 c	2.500 b
2	H_2O + NaCl	2.50 c	20.00 b
3	H_2O + $Na_4P_2O_7$	177.50 a	77.50 b
4	H_2O + $C_{64}H_{124}O_{26}$	12.50 c	20.00 b
5	H_2O + NaCl + $C_{64}H_{124}O_{26}$	107.50 b	10.00 b
6	H_2O + $Na_4P_2O_7$ + $C_{64}H_{124}O_{26}$	20.00 c	427.50 a

*Values followed by the same letter do not differ, by Tukey's test probability at 5%. Source: Prepared by the author, 2023.

Based on the mineralogy of the two soil samples studied, it is not surprising that the extraction with the aqueous solution of $Na_4P_2O_7$ 0.1 % m/m (treatment 3) revealed a statistically higher value of CFUs for the sample extracted from the loamy loam soil (Tab. 5). These findings are confirmed and justified since the extraction of microbial biomass with this solution can be attributed to the ability of $Na_4P_2O_7$ to chelate with metallic ions, Ca^{2+} and trivalent, present in the soil (Gem et al. 1996).

In an evaluation of corn development (Moraes 2016), using soil extractor solution $Na_4P_2O_7$ 0.1 % m/m, quantified total bacteria in 10^6 in soils inoculated with *Azospirillum brasilense*. The results recorded for the aqueous solution of tween 80 0.1% w/w (treatment 4) revealed a low efficiency in both soil samples, with CFU g^{-1} values statistically equal to those recorded for treatments 1 and 2 for both soil samples (Tab. 5). Tween 80 sorption in soils considerably impacts organic matter solubilization capacity, i.e., sorption reduces surfactant levels for microbial biomass extraction. Furthermore, clay minerals dominate the sorption of tween 80 even at low concentrations (Kang e Jeong 2015).

According to same authors, anionic surfactants reduce the adsorption of non-anionic surfactants; in the case of this work, $Na_4P_2O_7$ limits the adsorption of tween 80 in soils, mainly clayey. This fact agrees with the results obtained in (treatment 6), in which sandy textured soils have greater porosity and a better contact surface, which favored the reactions of the extracting solution with the sand grains, allowing a greater quantification of microbial biomass. (Santos e Batista 2015) used soil extractor solution containing (0.1% $Na_4P_2O_7$ + 0.1% tween 80). The authors describe their results as contradictory to the other works cited since the highest count of CFUs was determined in dry and not rainy seasons, as mentioned by most authors. According to this work, (Lanza et al. 2004) showed a greater development of the fungus *Metharizium anisopliae* in soils with sandy-clay texture. According to the authors, clayey textures reduce porosity and increase water retention, making aeration and nutritional exploration of the soil by the microorganism difficult.

Conclusions

Soil granulometric characteristics affect microbial quantification in cerrado environments in the Minas Gerais triangle. It is essential to standardize extractor solutions to improve the efficiency of traditional methods of counting microorganisms. In this work, the solution of H_2O + $Na_4P_2O_7$ + $C_{64}H_{124}O_{26}$ is the one that allowed greater quantification for sandy loam soils, with up to 75% of sand; for clayey loam soils, with a higher percentage of clay, the H_2O + $Na_4P_2O_7$ solution is the most efficient.

References

Boldaji MN, Keller T 2016. Degree of soil compactness is highly correlated with the soil physical quality index S. *Soil and Tillage Research* 159:41-46. DOI: https://doi.org/10.1016/j.still.2016.01.010

Brady NC, Weil RR 2013. *Elementos da natureza e propriedades dos solos*. 3. Ed, Editora UFV, Porto Alegre, 716 pp. ISBN: 9788565837743

Bueno PAA, Oliveira VMT de, Gualdi BL, Silveira PHN, Pereira RG, Freitas CES de, Bueno R de O, Sekine ES, Schwarcz KD 2018. Indicadores microbiológicos de qualidade do solo em recuperação de um sistema agroflorestal. *Acta Brasiliensis* 2(2):40-44. DOI: https://doi.org/10.22571/2526-433896

Butenschoen O, Scheu S, Eisenhauer N 2011. Interactive effects of warming, soil humidity and plant diversity on litter decomposition and microbial activity. *Soil Biology and Biochemistry* 43(9):1902-1907. DOI: https://doi.org/10.1016/j.soilbio.2011.05.011

Fageria NK, Stone LF 1999. *Manejo da acidez dos solos de Cerrado e de várzea do Brasil*. Santo Antônio de Goiás: Embrapa Arroz e Feijão, 42p. (Embrapa Arroz e Feijão. Documentos, 92). ISSN 1516-7518.

Gem S, Vaive JE, MacLaurin AI 1996. Analytical aspects of the application of sodium pyrophosphate reagent in the specific extraction of the labile organic component of humus and soils. *Journal of Geochemical Exploration* 56(1):23–36. DOI: https://doi.org/10.1016/0375-6742(95)00046-1

Haney RL, Franzluebbers AJ, Hons FM, Zuberer DA 1999. Soil C extracted with water or K_2SO_4: pH effect on the determination of microbial biomass. *Canadian Journal of Soil Science* 79(4):529–533. DOI: https://doi.org/10.4141/S99-011

Kang S, Jeong HY 2015. Sorption of a nonionic surfactant Tween 80 by minerals and soils. *Journal of Hazardous Materials* 284:143–150. DOI: https://doi.org/10.1016/j.jhazmat.2014.11.010

IBGE – Instituto Brasileiro de Geografia e Estatística 2015. *Manual técnico de pedologia: Guia prático de campo.* Rio de Janeiro.

Lanza LM, Monteiro AC, Malheiros EB 2004. População de *Metarhizium anisopliae* em diferentes tipos e graus de compactação do solo. *Ciência Rural* 34(6). DOI: https://doi.org/10.1590/S0103-84782004000600014

Moraes C 2016. *Azospirillum brasilense e um isolado solubilizador de fósforo em milho*. 2016. Tese de doutorado, Universidade Estadual Paulista, Jaboticabal, 80 pp.

Moraes MT, Debiasi H, Franchini JC, Silva VR 2012. Correction of resistance to penetration by pedofunctions and a reference soil water content. *Revista Brasileira Ciência do Solo* 36(6):1395-1406. DOI: https://doi.org/10.1590/S0100-06832012000600004

Paz-Lima LM, Silva FC da, Santana I de J, Martins RD, Lemes NM, Rietjens AR 2016. Quantificação da microbiota e diversidade ecológica da meso e macrofauna do solo sob diferentes usos no município de Urutaí (região Sudeste Goiano). *Multi-Science Journal*, 1(4):12-18. DOI: https://doi.org/10.33837/msj.v1i4.112

Pires ECC 2016. *Microbiologia do ciclo do nitrogênio em solos do Cerrado*. Tese de doutorado, Universidade de Brasília, Distrito Federal, 126 pp.

Reichardt K, Timm LC 2012. *Solo, planta e atmosfera: conceitos, processos e aplicações*. 2. Ed, Editora Manole Saúde, Barueri-SP, 524 pp. ISBN-13: 978-8520433393

Santos APA, Batista SB 2015. Isolamento e caracterização fenotípica de micro-organismos isolados de solo poluído por resíduos sólidos do bairro carrapicho município de várzea grande–Mato Grosso. *Connection Line: Revista eletrônica do Univag*. n.12, DOI: https://doi.org/10.18312/1980-7341.n12.2015.198

Silva RB, Moura QL de, Rodrigues H, Barreto P, Nunes HGGC, Rodrigues R, Santos S, Ruivo M de LP 2010. Estudo das colônias de fungos e bactérias em solos de floresta tropical associada à variação das chuvas da região. In: *Congresso Brasileiro de Meteorologia.*

Silveira AO 2011. *Avaliação de metodologias para o monitoramento da qualidade do solo.* Tese de doutorado, Universidade Federal do Rio Grande do Sul, Porto Alegre, 102 pp.

Souza ER. de, Melo HF de, Almeida BG de, Melo DVM de 2013. Comparação de métodos de extração da solução do solo. *Revista Brasileira de Engenharia Agrícola e Ambiental* 17(5):510-517. DOI: https://doi.org/10.1590/S1415-43662013000500007.

Stefanoski DC, Santos GG, Marchão RL, Petter FA, Pacheco LP 2013. Uso e manejo do solo e seus impactos sobre a qualidade física. *Revista Brasileira de Engenharia Agrícola e Ambiental*, Campina Grande 17(12):1301-1309. DOI: https://doi.org/10.1590/S1415-43662013001200008

Štursa P, Uhlík O, Kurzawová V, Koubek J, Ionescu M, Strohalm M, Lovecká P, Macek T, Macková M 2009. Approaches for diversity analysis of cultivable and non-cultivable bacteria in real soil. *Plant Soil Environ* 55(9):389-396. DOI: https://doi.org/10.17221/136/2009-PSE

Sudharhsan S, Senthilkumar S, Ranjith K 2007. Physical and nutritional factors affecting the production of amylase from species of *Bacillus* isolated from spoiled food waste. *African Journal Biotechnology* 6(4):430-435. DOI: https://doi.org/10.5897/AJB2007.000-2025

Teixeira PC, Donagemma GK, Fontana A, Teixeira WG 2017. *Manual de métodos de análises de solo*. Brasília, DF: EMBRAPA.

Varela RB. *Caracterização da comunidade microbiana do solo de áreas em reabilitação pela atividade carbonífera no município de Treviso, Santa Catarina*. [monografia da internet]. Bacharelado em Ciências Biológicas - Universidade do Extremo Sul Catarinense – UNESC, Criciúma, Santa Catarina, 57 pp. [2012]. Disponível:http://repositorio.unesc.net/bitstream/1/1424/1/Roger%20Bitencourt%20Varela.pdf