FACTORS THAT MAY ALTER SOIL MICROBIAL BIOMASS: A REVIEW

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ABSTRACT - Although the application of agro-industrial effluents on soil is an important practice of environmental management in the industry, this practice can cause impacts on soil microbiology due to the nutrient load and other components present in this waste. Thus, the objective of this study was to evaluate and select relevant articles, involving studies of factors that may influence the amount of biomass in the soil, comparing methodology and progress in studies with this theme. For this, articles indexed in three electronic databases were used: Web of Science, Scopus and Scielo. From these databases, 18 articles related to the topic were selected, all published between 2005 and 2018. For the total of selected scientific studies, 11 have methodologies related to the scope of the study and 7 reinforce the theoretical basis in the elaboration and complementation of the study. It was observed that the main articles were published in the English language, mainly in studies related to the Scopus and Web of Science bases and the most recurrent methods were titrimetric and spectrophotogrammetric methods. It is also concluded that the majority of the studies analyzed relate to microbial biomass alteration of the soil under influence of effluent disposal, as well as a variation of these communities as a function of soil cover.

Keywords: wastewater, soil microbial analysis, soil microbiology.

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RESUMO - Embora a aplicação de efluentes agroindustriais no solo seja uma prática importante da gestão ambiental nas indústrias, esta prática pode causar impactos no solo, devido à carga de nutrientes e outros componentes presentes nestes resíduos, como o excesso de matéria orgânica. Assim, o objetivo deste trabalho foi avaliar trabalhos relacionados a aplicação desses resíduos, envolvendo estudos de fatores que possam influenciar a quantidade de biomassa no solo, comparando a metodologia e o progresso nos estudos com essa temática. Para tanto, foram utilizados artigos indexados em três bases de dados eletrônicas: Web of Science, Scopus e Scielo. Destas bases de dados, foram selecionados 18 artigos relacionados ao tema, todos publicados entre 2005 e 2018. Para o total de estudos científicos selecionados, 11 possuem metodologias relacionadas ao escopo do trabalho e 7 reforçam a base teórica na elaboração e complementação do estudo. Observou-se que os principais artigos foram publicados na língua inglesa, principalmente em estudos relacionados às bases Scopus e Web of Science e os métodos mais recorrentes foram os métodos titrimétricos e espectrofotogramétricos. A maioria dos trabalhos analisados relata alteração biomassa microbiana do solo sob influência de disposição de efluentes, bem como uma variação destas comunidades em função da cobertura do solo.

Palavras-chave: águas residuárias, análise microbiana do solo, microbiologia do solo.

INTRODUCTION

The global human demographic increase, in addition to changes in the eating habits of the world population, imposed an increase in demand for resources linked to agricultural systems for food production, making clear the increase in world demand for natural resources. (BUAINAIN et al., 2016). For a sustainable balance between agro-industrial activities, the disposal and the reuse of their wastes, requires a balanced management in land use, where these effluents will be discharged. Thus, given the proper management of these residues, it is possible to evaluate the effects of the interaction between industry and the environment through the analysis of microbial biomass, due to the sensitivity of soil microorganisms. (CHAVARRIA et al., 2018; MERCANTE et al., 2008).

Microbial biomass is an important part of the living soil community and is directly linked to the mineralization of organic matter and nutrient cycling. However, it can be intensely affected by soil quality through the application of fertilizers and industrial waste

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(MEMOLI et al., 2018; KUMAR et al., 2018; SHI et al., 2018; ZHANG et al., 2108).

As the excess of nutrients contained in industrial effluents influences on soil and plants toxicity, they promote degradation and bioaccumulation processes in the soil microbiology, directly impacting in the reduction or increase of microbial biomass according to the characteristic of the residue (BASHIR et al., 2018; MAPANDA et al., 2005; SINGH e GUPTA, 2018).

Microorganisms play an important role in the planet's biogeochemical cycles and provide an infinity of ecosystem services for nature. Some of these living beings are excellent biological indicators, used in technical water treatment systems, both waste and potable, as well as in waste management and bioenergy generation. (BORER et al., 2018; PALOMO et al., 2016).

From the data collection, a statistical evaluation is essential to obtain comparative results. Statistical tools are commonly applied to data related to soil bioindicators, clearly showing the results achieved with the study. Among the statistical tools or software used, some stand out, such as Excel, Infosat and SPSS 19.0 software (MOGHIMIAN et al., 2017; WEI et al., 2018; ZHANG et al., 2108).

The objective of this systematic review was to present, analyze and discuss studies that evaluated the microbial biomass of soils submitted to the application of effluents, the evaluation methods used, as well as the number of studies on the theme over the years and the journals they carried out the dissemination of these studies, thus, seeking to verify the advances in the area.

MATERIAL AND METHODS
Search Methodology

Initially, a preliminary assessment of the topic was carried out on the Scopus platform. Graphs were obtained from the website showing data on the variation in the number of studies on the chosen themes in a given period of time, the number of publications made by several countries, and the volume of articles produced in each research center within those countries. After this step, the methodology proposed by Kitchenham et al. (2009) for the selection of articles was adopted, and a research protocol and definition of the electronic search was formulated in the indexed databases. The point that guides this research is: which methodology is more suitable for quantifying the microbial biomass carbon?

For strategic research purposes, a definition was created according to the research source, language of the articles, types of documents, year of publication and keywords. For the search sources, three main ones were defined, as shown in Table 1.

TABLE 1 - Demonstration of the name and web pages of the bases surveyed.

Search source	Web page
Scopus	https://www.scopus.com/
Web of Science	https://www.webofscience.org/
Scielo	http://www.scielo.org/

Source: the authors.

The Scopus and the Web of Science search bases were chosen because they are recognized as solid and reliable international bases, favoring research and the number of articles related to the topic, as well as the reach of keywords. The Scielo platform was included because it is the largest national knowledge base and presents a larger number of articles in Portuguese. The languages used in the searches were Portuguese and English, justifying the first as the official language of the country of origin of the article and the second because it is the universal scientific language, which appears in the vast majority of abstracts and scientific studies published in all languages. Table 2 describes the keywords used in the searches.

TABLE 2 - List of words used in the search in Portuguese and English.

Keywords	
Português	Inglês
Biomassa Microbiana	Microbial Biomass
Microbiologia do Solo	Soil Microbiology
Análise Microbiana do Solo	Soil Microbial Analysis
Águas Residuais	Wastewater

Source: the authors.

In the search platforms (Table 1), "or" or "and" were used as operators for keywords shown in Table 2. The results obtained with the insertion of the operators were added, resulting in the total of articles for each platform. Such mechanisms allow to inform the search system a link between the words, thus improving the search, in addition to better targeting it.

The inclusion of articles was established based on the criteria:

a) use of microbial biomass as a bioindicator,

b) articles available in their entirety and with a clear methodology and

c) journals indexed in the scientific production levels of the Graduate Programs in Brazil (Qualis Capes).

The exclusion of the criteria was established based on the criteria:

a) bibliographic review articles and

b) theses, monographs, annals or dissertations.

Initially, articles were screened by title. The next step consists of reading the abstracts. The studies selected according to the inclusion criteria, from their referring abstracts, were read in full, and inserted as elements of analysis of this study. After the selection, the remaining articles were qualified through Qualis Capes, a system used to classify the production of articles published in scientific journals from the scientific production of graduate programs. Subsequently, a table was created showing the respective titles and the classification obtained in Qualis Capes. The data were tabulated and transformed into spreadsheets and graphs with the aid of the Microsoft® Excel program.

Analysis of the theme

For documents referring to a specific period, there was an increase in the number of publications on the themes over the years, with a drop in 2018 (Figure 1A). In some countries, notably the number of studies was greater compared to other places. This can be clearly demonstrated in Figure 1B, in which the United States and China have a much higher volume of published studies in the area, thus pointing to an immense field of possible studies in countries like Brazil that appeared in the tenth place in publication in these areas. However, it is necessary to consider political and economic changes in these countries in the last decades and the influence of industry on the researched topic.

The list of academic research centers represented in Figure 1C, shows a great difference in the volume of studies. The *Chinese Academy of Sciences* research center represents a large part of the published studies, followed by the *Ministry of Education China* research center. This is possibly a reflection of investments in science and technology in areas linked to soil microbiology in that country.
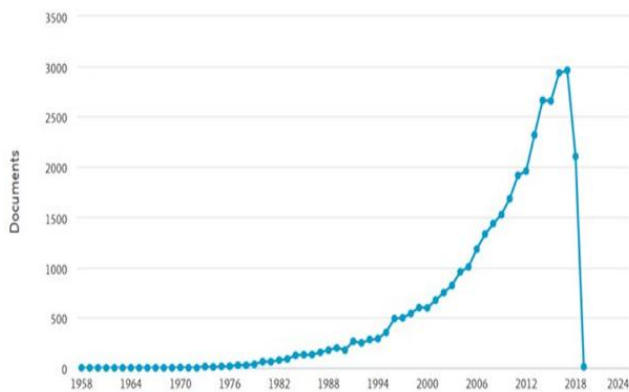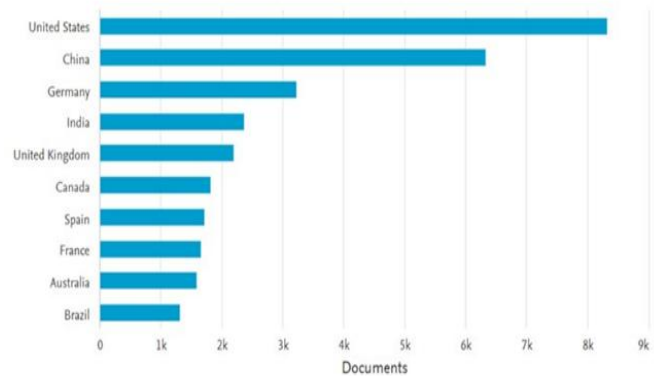
(A)   (B)
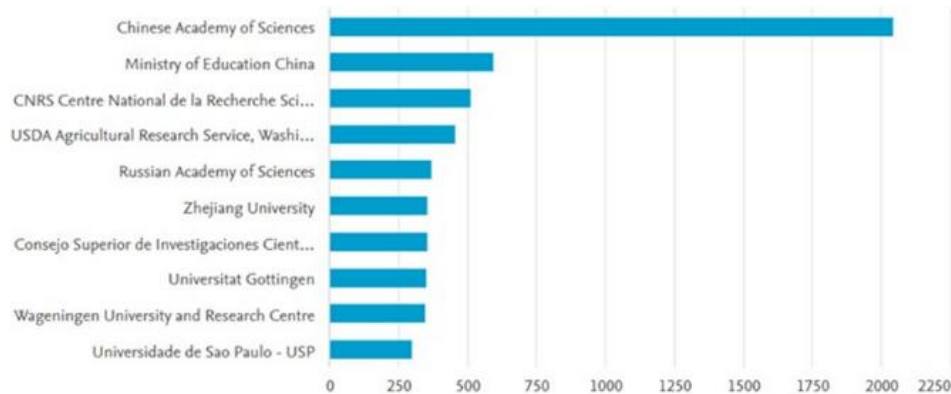
(C)

FIGURE 1 - Documents by year (A), documents by country or territory (B) and documents by research center (C). Source: Scopus (2018).

Most of the articles found, a total of 87% were published in English, the language used worldwide in most scientific productions, only 13% for Portuguese language. There are even incentives from part of some countries to increase the level of knowledge of their inhabitants in relation to this language. With the aim of scientific production with a view to the global dissemination of knowledge, the progression of publications in English is increasing. However, there are still many publications in different languages, despite this fact limiting the globalization of knowledge, it is also important for the local dissemination of knowledge in developing countries,

where most of the inhabitants do not have mastery of the English language and feel great difficulties in access and understanding of these contents published in English.

It was noticed that the Scielo platform groups studies in three languages, English, Spanish and Portuguese. Among the three platforms surveyed, Scielo was the one that most returned articles in Portuguese, probably because it started in Brazil (PACKER et al., 2014), although today it is extended to several countries in South America, North America and Europe. In the graph in Figure 2A, it was found that the Web of Science platform returned 592 articles from the total surveyed and the Scopus database returned 946 papers. The Scielo platform returned 487 articles, 237 of which were published in Portuguese. From the total number of articles found, those in which the title was related to the research topic were selected, resulting in just over 100 articles. Of this amount, 57 were selected from the abstract for further reading, culminating in the selection of 18 papers.

The Scopus and Web of Science platforms presented a better number of reputable material. However, a negative point of these is the restricted access to their information, restricting part of its content to researchers linked to public institutions, or as an alternative, the payment of the material. Obeying the inclusion criteria for the articles found, their classification was evaluated according to Qualis Capes. This resulted in 50% of articles A1, 22% in articles A2, with 22% also articles B1 and 6% articles classified as B2. The chosen studies are listed in Table 3.

Excessive discharge of anthropic waste into the soil is one of the causes of environmental pollution in the world. A possible alternative in the reduction of heavy metals and in the nutritional enrichment of the soil is biochar. It is a coal obtained from partial biomass pyrolysis, submitted to specific temperature conditions and little or no oxygen availability (STOCKMANN, 2011; SOHI et al., 2009). The use of biochar from rice straw, rice husk and corn straw were the object of study for Bashir et al. (2018), in order to reduce the mobility of heavy metals in the soil and analyze the behavior of microbial biomass. Using fumigation-extraction method the author finds that microbial biomass was significantly influenced by the use of different types of biochar, increasing from 12 to 99, 52 and 87 mg kg^{-1}, in addition to decreasing the bioavailability of cadmium (Cd). Therefore, the use of biochar in moist soil can influence the increase in the nutritional status of the soil, in addition to the microbiological dynamics and the decomposition of organic matter.

Microbial biomass is sensitive to changes in the environment, especially in cultivated areas. Mercante et al. (2008), evaluated the cultivation of cassava in different management systems and what is the influence on microbial biomass. It was verified through the fumigation-extraction method that the microbial biomass content varied between the systems used. In the conventional system the C-BMS decreases, whereas for the system maintained with the vegetation cover reflected a greater

accumulation of the C-BMS. This is due to the maintenance of plant residues, which promote an increase in microbial activity. After obtaining the data, the statistical analyzes were processed using the Statistica software version 5.0 (StatSoft, 2005).

Other studies have analyzed the anthropic effects on soil microorganisms, through the assessment of microbial biomass. In a study by Kumar et al. (2018), which evaluated soil microbiology through microbial biomass in an area with rice plantation, submitted to fertilization with varied formulations between organic and inorganic fertilizer over a period of 47 years, found a difference between the areas fertilized with different fertilizers. The author describes that the area in which the inorganic fertilizer was used, has critically altered the soil microbiology, due to the excess of N, suggesting a change in the application of balanced fertilizers.

Analyzing soil bioindicators Almeida et al. (2016) and Shi et al. (2018), evaluated the response of soil microorganisms to leachate applications from dairy in pasture areas and irrigated sugarcane plantations. The studies took place at different times of the year. Shi et al. (2018), applied the fertilizer 4 times a year in different plots, with concentrations of 0, 400 and 800 kg in the treatment area, while the control area did not receive treatment. According to the author, the activity of microbial biomass analyzed using the fumigation-extraction technique increased with the application of leachate, whereas for the untreated portion there was a greater impact on soil microbiology, mainly fungal reduction..

Almeida et al., (2016) selected 4 sugarcane areas in chronosequence with 5, 7, 8 and 9 years of cultivation. He also selected an area of native vegetation as a witness. In all crops, conventional soil tillage was used. In fertilization, NPK formulations were used, with variations over the years during planting, complementing the fertilization with N and K through fertirrigation. The results showed that in crops with irrigated sugar cane, keeping only 20% of the straw on the soil surface shows better soil quality after the first harvest. Therefore, the increase in soil microbial biomass is linked to the deposition of straw over the years, showing rates similar to those found in native areas.

Weed and irrigation control practices aim to maintain good productivity in orchard areas. However, the impact caused by these practices is often not considered. A study was developed by Zhang et al. (2018) to control weeds with chemical herbicides and peach irrigation (*Prunus persica* L., Contender cv. on Guardian rootstock). Four systems were implemented in the experimental area, including a control area without weed and irrigation control, weed and irrigation control, herbicide and irrigation weed control, and herbicide and no irrigation weed control. The results showed a significant impact on the size of soil microbial communities.

The microbial biomass evaluated by the fumigation-extraction method was significantly higher in the area without control of invasive plants in relation to

weed control systems and in irrigated than non-irrigated systems. Microbial biomass in treatments without weed control was on average 18.5% and 42.5% higher than the weed control system. This indicates that long-term control of weeds through herbicides reduces soil fertility, decreasing organic C concentrations and nutrient retention (ZHAN, et al., 2018).

It was noticed that the functional potential of soil ecosystems can be analyzed according to the activity and abundance of the microbial community compared to the fundamental properties of the soil. Describing the dynamics of the microbial portion, the physical-chemical properties of the soil have traditionally been used. However, it is necessary that other methods and analyzes are inserted, such as the quantification of microbial biomass. Due to the great importance that soil microbial communities have in the functioning of the ecosystem, it is essential to evaluate and monitor its relationship with soil quality (MEMOLI et al., 2018).

TABLE 3 - List of selected articles, according to the published year and the Qualis/CAPES note.

Author	Qualis/ CAPES	Title	Origin of the study	Methodology used
Chavarria et al. (2018)	A1	Response of soil microbial communities to agroecological versus conventional systems of extensive agriculture	Argentina	fumigation-extraction
Kumar et al. (2018)	A1	Continuous application of inorganic and organic fertilizers over 47 years in paddy soil alters the bacterial community structure and its influence on rice production	India	fumigation-extraction
Mapanda et al. (2005)	A1	The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe	Zimbabwe	
Memoli et al. (2018)	A1	Soil elements fraction affect phytotoxicity, microbial biomass and activity in volcanic areas	Italy	
Palomo et al. (2016)	A1	Metagenomic analysis of rapid gravity sand filter microbial communities suggest novel physiology of *Nitrospira* spp.	Denmark	
Singh e Gupta (2018)	A1	Soil microbial biomass: a key soil driver in management of ecosystem functioning	India	
Colman e Schimel (2013)	A1	Drivers of microbial respiration and net N mineralization at the continental scale	USA	
Zhang et al. (2018)	A1	Irrigation and weed control alter soil microbiology and nutrient availability in North Carolina Sandhill peach orchards	USA	fumigation-extraction
Borer et al. (2018)	A1	Spatial organization of bacterial populations in response to oxygen and carbon counter-gradients in pore networks	Switzerland	
Oren et al. (2018)	A2	Chloroform fumigation extraction for measuring soil microbial biomass: The validity of using samples approaching water saturation	Israel	fumigation-extraction
Wei et al. (2018)	A2	Soil microbial biomass, phosphatase and their relationships with phosphorus turnover under mixed inorganic and organic nitrogen addition in a Larix gmelinii plantation	China	fumigation-extraction
Moghimian (2018)	A2	Impacts of changes in land use/cover on soil microbial and enzyme activities.	Iran	fumigation-extraction
Shi et al. (2018)	A2	Soil microbial biomass, activity, and community composition as affected by dairy manure slurry applications in grassland production	Canada	fumigation-extraction
Mercante et al. (2008)	B1	Biomassa microbiana, em um Argissolo Vermelho, em diferentes coberturas vegetais, em área cultivada com mandioca	Brazil	fumigation-extraction
Soares et al. (2016)	B1	Soil organic matter fractions under second-rotation eucalyptus plantations in eastern Rio Grande do Sul	Brazil	

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Continuation of Table 3 - List of selected articles…

Almeida et al. (2016)	B1	Indicadores de qualidade do solo em cultivos irrigados de cana-de-açúcar	Brazil	irradiation-extraction
Bashir et al. (2018)	B1	Efficiency of C3 and C4 plant derived-biochar for Cd mobility, nutrient cycling and microbial biomass in contaminated soil	China	fumigation-extraction
Knupp e Ferreira (2011)	B2	Eficiência da quantificação do carbono da biomassa microbiana por espectrofotometria comparada ao método titrimétrico	Brazil	fumigation-extraction/ spectrophotometry

Source: the authors.

The theme that involves soil microbiology is very wide, this imposes difficulties in choosing and separating articles that may really be linked to the scope of the study, since soil microbiology involves other areas of knowledge with different studies, such as for example, taxonomy and decomposition of organic matter. Initially the searches were performed with keywords used in a generalized way, however with the range of the studies found, a limitation was necessary, consisting of the targeting of the keywords only to the theme of the study.

It was noticed that, based on the articles read, that there are different methodologies for the extraction and quantification of microbial biomass, this can vary according to the study proposal and local conditions to develop the research. One of the methods used to extract carbon from microbial biomass was fumigation with chloroform. This methodology is commonly applied at soil moisture levels of 40-50% of the water holding capacity. Another method used was irradiation-extraction, which uses for the elimination of microorganisms, a microwave oven, with a frequency of 2,450 MHz and energy at 900 W for 180 s (ALMEIDA et al., 2016; SOARES et al., 2017). For the quantification of carbon from microbial biomass (ALMEIDA et al., 2016), Equation 1 was used:

$$Cmic = \frac{(CI - CNI)}{Kc} \qquad \text{(Equation 1)}$$

Where:
Cmic = carbon from microbial biomass ($\mu g\ g^{-1}$),
CI = carbon of the irradiated sample,
CNI = carbon from the non-irradiated sample and
Kc = 0.33 (correction factor for Cmic).

In the study by Chavarria et al. (2018), with a view to assessing the response of soil microbial communities to agroecological systems, in comparison with conventional systems of extensive agriculture, the carbon of microbial biomass was determined using the chloroform inoculation technique. For statistical analysis of the data, Microsoft Office Excel® and INFOSTAT software for Windows were used (INFOSTAT, 2008).

In the study realized by Wei et al. (2018), part of the study was to analyze the impact caused by the deposition of atmospheric nitrogen on soil microbial biomass, the extraction-fumigation method was used to quantify microbial biomass. The results were positive according to the methodology used, based on the statistical tests of Shapiro-Wilks. Duncan's test at the level of P =

0.05 and one-way ANOVA were used to analyze the effect of adding N on soil parameters, also represented by graphs.

In general, the activities of microorganisms, through their enzymatic actions, have been recognized as excellent indicators of soil quality, but can be affected according to the types of cover and land use (PALOMO et al., 2016; BORER et al., 2018; MOGHIMIAN et al., 2017).

A study by Moghimian et al. (2017), aimed to determine the impact on soil microbiology in different areas, virgin natural forest, degraded natural forest, alder plantation, redwood plantation, improved fallow, garden areas on properties in Northern Iran. One of the parameters used was the quantification of carbon (C) from microbial biomass. The method employed was extraction-fumigation and presented carbon values of microbial biomass almost twice as high under redwood plantation and natural virgin forest than in improved fallow and garden areas.

For statistical analysis, the author used some tests, such as the normality of variables, which was verified by the Kolmogorov-Smirnov's test. Levene's test was used to verify the homogeneity of the variances. One-way analysis of variance (ANOVA) was used to compare data on soil properties among land uses and land cover. Duncan's test was also used to test differences at 5% probability of error. At the end the data were presented in graphs and tables, as well as the other studies mentioned.

The technique of extracting biomass from the soil by CL fumigation-extraction proved to be more present in the researched studies, despite being more laborious and costly (KUMAR et al., 2018) than the irradiation-extraction technique, which is mentioned in 1 study, possibly because most of the studies were carried out in countries such as the USA, China and India, which in general have greater availability of equipment for their researches. Only 4 Brazilian studies were found, due to searches in the bases in Portuguese language, since the others, even if from different countries are edited in English and no study was performed in the Brazilian territory, in addition to the 4 previously mentioned. In a study by Knupp and Ferreira (2011), which compared the fumigation-extraction method (titrimetry) and the spectrophotometric method to extract carbon from microbial biomass, similar results were found between the two methodologies. According to the authors, the volume of waste has been reduced by up to ten times compared to titrimetry. There was a 42% reduction in the concentration

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of potassium dichromate and about 20% in the time of execution of the analysis. Based on the results obtained, the use of spectrophotometry proved to be advantageous from an environmental point of view, in addition to similar statistical results.

In a study by Oren et al. (2018), the fumigation-extraction method was tested to validate the method in samples with moisture content above 50%, since the value commonly used is between 40 and 50%. The claim, according to the author, is that moisture levels above the recommended content could affect the spread of fumigated steam in soil samples. Samples from two different areas with differentiated long-term fertilization were used, resulting in 4 treatments with different levels of organic matter. In the results, the author demonstrates that, despite the humidity, the degradation of microbial cells was between 40% and 90%, refuting the proposal to reduce the fumigation efficiency in samples with high moisture content, thus validating the applicability of the method in wet samples.

CONCLUSION

The systematic review of research in the field of soil microbiology is an efficient method capable of providing an overview of the factors that affect its existence and survival. In addition, the systematic review culminates in advances on methodology and research developed within the proposed theme.

It is also concluded that the search bases or research platforms presented a vast material, with hundreds of articles linked to the general topics of microbiology, soils and wastewater. Of the 18 selected articles, 11 of them present methodologies related to the scope of the study and 7 complemented the theoretical basis in the elaboration of the study. It was observed that the majority of the articles were published in the English language through the Scopus and Web of Science platforms, probably due to their greater coverage in a worldwide context, unlike the Scielo database, which is a little more restricted when compared to the first two ones in the same context.

The large number of articles found in the field of soil microbiology is the result of technological advances, which make available a high number of free material over the internet, mainly within universities. However, it is necessary to emphasize that not only the access to studies, but also the increase in research related to the area, which seeks to resolve and better understand the interaction of mankind with the complex soil structures, contributing to this growth and the supply of scientific studies.

Regarding microbial biomass, it can be concluded that most of the studies analyzed report a change in the microbiological dynamics of the soil under the influence of effluent disposal, as well as a variation of these communities depending on the soil cover. In addition, compared to the methods used for quantification, the most recurrent in the scope of this assessment were the titrimetric and spectrophotogrammetric methods.

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