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Bacillus spp. potential for bioremediation of pesticides and their residues in soil and water environments

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“Toda obra, cualquiera que sea, literaria, política, científica, debe estar respaldada por una conducta”

- Miguel Ángel Asturias –

Dedicated:

To my dad who is my pillar and has taught me to do everything with love. To my aunt and my dear sister who are my unconditional support.

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SUMMARY

ABSTRACT

LOPEZ-FLORIÁN, Jhoselyn Lorena, M.S., State University of Santa Cruz, Ilhéus-BA, March, 2021. **Bacillus spp. potential for bioremediation of pesticides and their residues in soil and water environments.** Supervisor: Dr. Leandro Lopes Loguercio. Co-supervisor: Dr. Valter Cruz-Magalhães

The *Bacillus* genus has shown a great potential for use in bioremediation strategies. Their species are aerobic and facultative anaerobic microorganisms that form endospores, which are resistance structures that allow their survival in different environments and in a variety of physical and chemical conditions. In addition, strains of *Bacillus* spp. contain catabolic genes, which can be involved in the degradation of xenobiotic compounds, such as the various types of pesticide residues. The objective of this research was to investigate, by systematic review and metanalysis (SR-M), what is the current knowledge about the *Bacillus* genus and its potential for use in bioremediation strategies of agrochemical pesticide residues in soil and water. Emphasis was given to the diversity of species/strains and to the trends in the types of degradable residues, as well as to genes and proteins involved in xenobiotic metabolization processes. From the searches conducted, 854 studies were obtained from the *Scopus* and *PubMed* databases, of which 144 were included in the systematic review after a series of classification stages. The categories with the largest number of studies were *insecticides* (28.1%), *herbicides* (19.5%), *fungicides* (4.8%) and *nematicides* (0.46%). Bioaccumulation and bioamplification are important consequences of excess of pesticide residues present in the environment. These issues affect not only the terrestrial and aquatic environments, but also non-target organisms through the food chain. That is why processes such as bioremediation are of great importance to decontaminate soil and water from pesticide residues. For this, it is necessary to know appropriate bacteria to work with, their possible metabolic routes, possible residues to be targeted and optimal physico-chemical parameters, such as pH and temperature, required for best possible decontamination effect. In this SR-M, a high number of *Bacillus* species were identified for pesticide biodegradation in soil and water, with 36.41% being ‘unidentified’ *Bacillus.* For the identified species, *B. cereus* (13.04%) was the most studied one, followed by *B. sutilis*

(10.86%) and *B. megaterium* (5.97%). In addition, as shown by metanalytical results, they could degrade highest concentrations/amounts of the pesticides studied, when compared to the other species tested. Despite the high number of *Bacillus* species ´found to be studied in this regard, the amount of research that present genes/proteins involved in these biodegradation processes is not equivalent. Only 11 of the selected studies have covered this type of information. Most of the bacteria tend to use hydrolysis for pesticide degradation, with some of the genes involved being *opd* and *atz*. With this SR-M, it was possible to obtain articles that suggest that *Bacillus* spp. are good candidates for application in bioremediation processes, further increasing the understanding about this genus as a source of potential microbes for the development of eco-friendly decontamination methods for pesticide residues in soil and water. Our results allowed us to gather an array of information on the overall status of research in this area, including countries most involved in *Bacillus*-based remediation studies, most-studied bacterial species, main physico-chemical parameters involved, and genes/proteins related to biodegradation processes. The synthesis of information we provided can certainly be applied towards delineation of future empirical investigation of pesticide residues bioremediation in soil and water, which will have a positive impact on terrestrial and aquatic species, ecosystems services and decrease in carbon emissions.

Key words: pollution, biodegradation, xenobiotic compound, co-metabolic process, remediation.

RESUMO

LOPEZ-FLORIÁN, Jhoselyn Lorena, M.S., Universidade Estadual de Santa Cruz, Ilhéus, março de 2021. **Potencial de Bacillus spp. para bioremediação de pesticidas e seus resíduos em ambientes de solo e água.** Orientador: Dr. Leandro Lopes Loguercio. Co-orientador: Dr. Valter Cruz-Magalhães

O gênero *Bacillus* tem mostrado um grande potencial para uso em estratégias de biorremediação. Suas espécies são microorganismos aeróbicos e anaeróbicos facultativos que formam endósporos, que são estruturas de resistência que permitem a sobrevivência dessas bactérias em diferentes ambientes, e em uma variedade de condições físicas e químicas. Além disso, as cepas de *Bacillus* spp. contêm genes catabólicos, que podem estar envolvidos na degradação de compostos xenobióticos, tais como os vários resíduos de pesticidas. O objetivo desta pesquisa foi investigar através de uma revisão sistemática (RS) qual é o conhecimento atual sobre o gênero *Bacillus* e seu potencial para uso em estratégias de biorremediação de resíduos de agrotóxicos no solo e na água, bem como identificar os potenciais genes com algum envolvimento nessa condição. Foi dada ênfase à diversidade de cepas e a tendências nos tipos de resíduos degradáveis, bem como aos genes e proteínas envolvidos nos processos de metabolização xenobiótica. Das pesquisas realizadas, foram obtidos 854 estudos dos bancos de dados *Scopus* e *PubMed*, dos quais 144 foram incluídos na revisão sistemática após uma série de etapas de classificação dos estudos. As categorias com o maior número de estudos foram inseticidas (28,1%), herbicidas (19,5%), fungicidas (4,8%) e nematicidas (0,46%). Bioacumulação e bioamplificação são importantes conseqüências do excesso de resíduos pesticidas no ambiente. Estes problemas afetam não apenas os ambientes terrestres e aquáticos, mas também organismos não-alvo através da cadeia alimentar. É por isso que processos como a biorremediação são de grande importância no auxílio da descontaminação o solo e a água contaminados com resíduos de agrotóxicos. Para isso é necessário conhecer os microorganismos envolvidos nesse processo, suas possíveis rotas metabólicas, os possíveis resíduos a serem descontaminados, e parâmetros físico-químicos requeridos para melhor descontaminação, tais como pH e temperatura. Nesta RS, foi identificado um alto número de espécies de *Bacillus* para a biodegradação de pesticidas no solo e na água, sendo que 36,41% foram *Bacillus* não identificados em nível de espécie. Para as espécies identificadas, as mais estudadas foram *B. cereus* (13,04%), *B. sutilis* (10,86%) e *B. megaterium* (5,97%). Além disso, essas espécies conseguem degradar concentrações maiores de pesticidas em comparação com as outras espécies relatadas nos estudos. Apesar de haver um grande número de espécies do gênero *Bacillus* sendo estudadas, o número de pesquisas que apresentam genes/proteínas não é equivalente. Apenas 11 dos estudos obtidos mencionaram este tipo de informação. A maioria destas bactérias utiliza a hidrólise para a degradação de pesticidas, sendo *opd* e *atz* alguns dos genes envolvidos. Com esta revisão (RS) foi possível obter artigos que ajudaram a responder as perguntas de pesquisa propostas, entendendo mais sobre *Bacillus* spp. como organismos potenciais para métodos de biorremediação aplicáveis a resíduos de pesticidas em solo e água. Os resultados nos permitiram agrupar informações sobre o status da pesquisa nesta área, os países mais envolvidos nesses estudos de remediação com o gênero *Bacillus*, suas espécies mais estudadas, os parâmetros físico-químicos e genes/proteínas envolvidas no processo de biodegradação. Esta síntese de informações pode ser aplicada em futuros estudos de biorremediação de resíduos de pesticidas no solo e na água, que terão um impacto positivo nas espécies terrestres e aquáticas, nos serviços ecossistêmicos e no decréscimo das emissões de carbono.

Palavras-chave: poluição, biodegradação, compostos xenobióticos, processo co-metabólico, remediação.

1. INTRODUCTION

Pesticides are ‘xenobiotic’ compounds, a word that comes from the Greek “*xeno*” meaning “*strange*”, and “*bio*” meaning “life”. These compounds have the characteristics of being aromatic, which gives them chemical stability. In addition, they are water soluble and some of them can be volatile (ODUKKATHIL; VASUDEVAN, 2013), being essentially classified according to the target organism they affect, i.e, herbicides, nematocides, fungicides, insecticides, etc. They can also be classified by their chemical groups: organochlorines, organophosphates, carbamates, pyrethroids, among others (HAKEEM; AKHTAR; ABDULLAH, 2016). As it is widely acknowledged, there has been an ever increasing accumulation of these compounds in the environment (soils and water), which is a consequence of the intensification of activities related to agriculture and industry (KUMAR et al., 2011; PINGALI, 2012). Although xenobiotic compounds (insecticides, fungicides, herbicides, etc.) can be beneficial for the management of agricultural crops, their improper use can cause harm to humans and other organisms, including aquatic ones (AKTAR; SENGUPTA; CHOWDHURY, 2009). Among these affected organisms, one must also include bacteria, which have significant importance in ecological services, contributing to the carbon cycle, decomposition of organic matter and degradation of xenobiotic compounds; therefore, the types and intensities of interactions they have with hosts, whether animal or plant, are relevant in this context (CAVICCHIOLI et al., 2019).

The increase in the levels of pesticide usage and their residues in the environment tend to lead to a reduction in bacterial populations, thereby affecting the balance of microbial communities; this, on its turn, leads to decreases in the efficiency of conversion of organic matter into biomass. These circumstances cause a higher percentage of carbon emitted into the atmosphere, when compared to what is fixed in the soil, thus increasing the greenhouse effect and altering soil health and productivity (CAVICCHIOLI et al., 2019). In this scenario, the process of bioremediation has been studied as a technique that allows cleaning up contaminated soil and water by microorganisms, plants and others. Bioremediation can be defined as a biological strategy to repair contaminated environments without causing modifications to the properties and biodiversity of soil and water (KUMAR et al., 2011). It is therefore an environmentally friendly technique, usually displaying lower costd when compared to other methods, e.g., landfilling, use of chemical methods, etc. (NEHA; BN; HITESH, 2018).

For this remediation method to be effective, one must (i) know the possible products that will arise from the degradation reaction, (ii) evaluate the degree of resulting toxicity, (iii) assess microorganisms and their catabolic genes with potential to metabolize the target residual compounds, and (iv) optimize the biological activity of the bioremediation agent (ANTIZAR-LADISLAO, 2010). For an efficient microbial-based biodegradation to occur, not only specific aspects of bacterial metabolism are important to understand, but also the conditions of surrounding environmental factors, such as temperature, pH and nutrients available (UQAB; MUDASIR; NAZIR, 2016). Bacteria that display the potential to contribute with biodegradation of residual pesticides belong to *Bacillus* spp. They are aerobic and facultative anaerobic microorganisms that have the ability to form endospores. These characteristics allow strains and isolates of this genus to be tolerant to many environments (soil, water, extreme environments, etc.) with different physical and chemical conditions (RADHAKRISHNAN; HASHEM; ABD ALLAH, 2017; SOLTANI et al., 2019). Furthermore, it has been found that these isolates may contain catabolic genes/enzymes involved in the biodegradation of pesticides and other hydrocarbons (ODUKKATHIL; VASUDEVAN, 2013).

Scientific literature indicates the relevance of the *Bacillus* genus and the genes/proteins of its strains/isolates in the process of biodegradation of xenobiotic compounds and their environment-contaminating residues. This context allows the development of an analytical and integrating study strategy based on a distinct but yet empirical type of scientific methodology known as systematic review (SR). As defined by Moher et al. (2009), SR is a planned review aiming at answering a specific scientific question, in which identification, selection, evaluation and interpretation of relevant and related empirical research is possible. This method also allows the re-collection of empirical data from the selected studies, with the possibility of applying statistical methods for meta-analysis and assessment of the collected data and results (LEENAARS et al., 2012; MOHER et al., 2009). Thus, in this project, we aimed to unravel the existing scientific knowledge about the *Bacillus* genus and the true potential of this taxonomically defined group of microorganisms as a possibly high-efficacy method for bioremediation of pesticide residues in soil and water. By means of a SR and meta-analysis, our objectives were to assess (i) geographical and temporal pattern of studies/publications on this issue in the world, (ii) types of residues studied for their biodegradation with bacteria, (iii) *Bacillus* species showing biodegradation activities, (iv) environmental factors affecting this metabolism, (v) genes and proteins of *Bacillus* spp. involved in processes of bioremediation of pesticide residues in soil and water, and (vi) comparison of *Bacillus*-based bioremediation with other chemical and biological remediation methods. An established research protocol was employed to assure representativeness of the selected primary studies used in this work.

2. LITERATURE REVIEW

2.1. Pesticides – concept and classification

Pesticides are xenobiotic substances, a word that comes from the greek *-xeno* meaning 'strange', and *-bio* meaning 'life'. These compounds have the characteristic of being aromatic, which gives them a reasonable chemical stability. In addition, they are water soluble and some of them can be volatile (KUMAR; NAIN; SINGH, 2017). They can be classified according to the target organism they are supposed to control/affect, such as herbicides, nematocides, fungicides, insecticides, etc. They can also be classified by their chemical groups, such as organochlorines, organophosphates, carbamates, pyrethroids, among others (KUMAR; NAIN; SINGH, 2017). As chemicals, they can also be classified based on their nature as natural or synthetic, with the former being originated from organisms and their products that can have effect on agricultural pests (a system also known as 'biological control'). For instance, pesticides from plant extracts are considered as natural ones, such as alkaloids and phytoalexins. In the synthetics' class, we can otherwise find compounds that include metals like As, Ag, Ta, Pb, Py Hg, and those mentioned above (i.e. organochlorines, organophosphates, etc), which go through chemical engineering and industrial processes of synthesis/manufacturing.

2.2. Pesticides in agriculture

The increase in human population on the planet has certainly influenced the ever growing demand for the food production. In this context, and considering the predominating global economic and historical circumstances, as well as the dominating scientific paradigms of that time (middle 20^{th} century, post-war era), the development and use of pesticides appeared as necessary, due to the supposed benefits of reduction in agricultural losses achieved by the control of pests and diseases (BHATTACHARJEE et al., 2014). Moreover, the immediate results in pest control they provide were very attractive, and consideration of their potential impact on the environment were clearly neglected or mistakenly handled by general policies of the countries in that regard

(PINGALI et al., 2010). Today, it is predicted that the population will grow up to 9.1 billion people by 2050, so the demand for food will continue to increase at a faster rate, while the amount/availability of natural resources such as soil and water, two of the most important factors for agriculture, are already severely limited (HINGURAO; NERURKAR, 2018). By 1940, the *DDT* (dichloro-diphenyl-trichloroethane) began to be used as insecticide and became one of the most widely used pesticide in the world, with the heaviest known impact on the environment and non-target organisms (HINGURAO; NERURKAR, 2018). Then, the *Endosulfan*, a pesticide classified as persistent organic pollutant (POP), was introduced in 1950 (DURAISAMY; MUTHUSAMY; BALAKRISHNAN, 2018). In 1952, pesticides' production began in India, a country where they continue, thus far, to be most widely used and produced (KWAK; RHEE; SHIN, 2013). In 1965, the pesticide *Chlorpyrifos* took over the market, and even though it was considered one of the least toxic, the large environmental accumulation of its residues has been showing relevant effects on plants, animals and humans (GEED et al., 2017). In 2018, Asia was the continent with the highest percentage of total use of pesticides in agriculture (52.4%), followed by America with 32.3%, Europe with 11.6%, Africa with 2% and Oceania with 1.7%. With respect to countries, China has used a total of 1,763,000 tons in agriculture, followed by the United States with 407,779 tons and Brazil with 377,176 tons (FAOSTAT, 2020). Although some pesticides have been restricted in many parts of the world, due to enforcement of policies to protect health and environment (PINGALI, 2012), some countries still use them in large-scales, because they are agriculture-dependent and/or their pesticide regulation/enforcement is not well structured (DAVIES, 2003; SINGH; KAUR; SINGH, 2012). For example, India has approximately 70% of its population depending on agriculture, so that organophosphates are examples of relevant pesticides used on a large scale (SINGH; KAUR; SINGH, 2012).

2.3. Effects of pesticides on environment and human health

As it is widely acknowledged, there has been an increase in the levels of these pollutant compounds in the environment (soils and water), which is a consequence of the intensification of agricultural and industrial activities in the last six to seven decades (WIJEKOON; YAPA, 2018). Although pesticides can be beneficial for pest treatments, their inappropriate use can cause harm to humans and other organisms, including aquatic ones (DOBRZANSKI et al., 2018). Children, elderly people, and agricultural workers who handle pesticides are those at highest risk. According to the World Health Organization, there are about 3,000,000 cases of poisoning per year in developing countries (DOBRZANSKI et al., 2018). The processes of ‘bioaccumulation’ and ‘bioamplification’ are further consequences from the indiscriminate use of pesticides, which intensify pollution and damage to the environment and living beings. The former is a process in which the pesticides and their residues accumulate in the fatty tissues of the organisms. Although some of these compounds can be properly discarded by humans, most that have reached the environment can yet accumulate in a variety of species, and so, can cause damages of many types, either directly to those species, or to humans that may consume those organisms (CHOJNACKA; MIKULEWICZ, 2014, WANG, 2016). With regards to the bioamplification process, it occurs as a direct result from the bioaccumulation of residues and undesired compounds in organisms. In this case, a pesticide’s amplification in the environment comes from its movement through the trophic chain, when smaller organisms containing the residues in their tissues are fed to larger ones and so on (HAKEEM; AKHTAR; ABDULLAH, 2016).

In addition to having direct and toxic effects on humans, animals and plants, pesticides can also change physicochemical characteristics of soil and water, such as pH and temperature, which are two of the most important variables related to the speed and efficiency of their degradation (ACHARYA et al., 2014; WIJEKOON; YAPA, 2018) These variations can act as factors of selective pressure on organisms present in soil and water that are contaminated with those xenobiotic compounds (DOBRZANSKI et al., 2018). As a consequence of the increase in pesticides and their residues in the environment, bacterial communities have decreased, showing a negative effect on the balance of microorganisms (AKTAR; SENGUPTA; CHOWDHURY, 2009; ONWONA-KWAKYE et al., 2020). Under these circumstances, the efficiency of general biogeochemical cycles, and so, conversion of organic matter into biomass can be negatively affected. Hence, these conditions can be one of the relevant causes of higher percentages of carbon going to the atmosphere, rather than being fixed in the soil, which can thereby increase the greenhouse effect, and so, affect soil health and productivity (LIU et al., 2012).

2.4. Microbial-based biodegradation of pesticides and the *Bacillus* genus

Bacteria are important for various ecological services, such as contribution to the carbon cycle, decomposition of organic matter and degradation of xenobiotic compounds, in addition to their relevant interactions with a variety of animal or plant hosts (AKBAR; SULTAN; KERTESZ, 2015). One category of bacteria that has the potential to contribute to the biodegradation of xenobiotic pesticides are the species and strains belonging in the *Bacillus* genus. They are aerobic and facultative anaerobic microorganisms that have the ability to form endospores. These characteristics allow strains and isolates of this genus to be tolerant to many distinct environments (soil, water, extreme environments, etc.), with different physical and chemical conditions (LIU et al., 2012). *Bacillus* spp. have also the ability to work in consortia with other gram-negative microbes such as *Pseudomonas* (SALUNKHE et al., 2013), which allow a higher percentage or complete degradation of xenobiotic compounds such as the pesticides. In addition, it was found that these two genera may contain catabolic genes/enzymes involved in the biodegradation of pesticides and other hydrocarbons (HINGURAO; NERURKAR, 2018). Most of the genes found in bacteria encode phosphotriesterases and carboxylases, which are mostly involved in the hydrolysis of an array of aromatic compounds. The action of pesticides metabolization by bacteria is reasonably expected, since these compounds are indeed a rich source of carbon, phosphorus or nitrogen, depending on the type of pesticide applied (LIU et al., 2015). It is worth mentioning that the plasmid-mediated horizontal transfer of genes among bacteria allow them to adapt and resist to contaminated environments (SMALLA; JECHALKE; TOP, 2015). Nevertheless, despite this gene exchange possibility, there are certain catabolic genes that are found in bacterial chromosomes, which turn them less mobile (JUHAS et al., 2009; MARTINEZ et al., 2001). Finally, it is noteworthy that there are yet other genes which, in spite of being transferred through plasmids, are not useful for the degradation of certain pesticides (HOGAN et al., 1997).

Silva Moretto et al. (2019) have studied plasmids related to the biodegradation of the herbicide *Atrazine* and concluded that the microorganism containing the catabolic plasmid for the degradation of this pesticide has its origin in the *Bacillus* genus. This result was obtained from places that had either reports of this herbicide’s usage or from others where this compound was not applied (BHATTACHARJEE et al., 2014). Besides having an effect on soil, pesticides become a great threat to water niches such as corals: they cause bleaching and death in these organisms, due to the decrease of the bacterial community with which they maintain a symbiosis (CAVICCHIOLI et al, 2019). Studies have found species of the *Bacillus* genus with the ability to degrade pesticides in that type of niche. Therefore, aquatic environments and related ecological niches have been the focus of research for bacteria degrading xenobiotic compounds, as well as for the study of their associated genes, proteins and metabolic pathways (SABDONO; RADJASA, 2008).

Bacillus is one of the preferred group of bacteria that interact with plants, which prefer to host *Bacillus* spp., due to the properties of their enzymes that benefit the plant's resistance to xenobiotic compounds, such as the pesticides. It has been reported that *Bacillus* spp. influence the growth process of many plant parts, such as roots (SINGH; SINGLA, 2019). Considering the importance of the carbon (C), phosphorus (P) and nitrogen (N) cycles, and that P is always a limiting nutrient for plants in most agricultural settings, it is worth mentioning that strains/isolates from *Bacillus* can not only solubilize phosphates taken from soil, so that the plant can absorb this element, but also fix N_2 and reduce the amount of C that goes to the atmosphere. This latter condition can obviously contribute to amelioration of C emissions, and so, the effects on climate change (ARORA, 2020; CORRALES RAMÍREZ et al., 2017; WIJEKOON; YAPA, 2018).

2.5. Soil and water bioremediation

Bioremediation is a methodological approach that allows the cleaning of contaminated soil and water through application of microbes, plants and others organisms, which have a built capacity of transforming/degrading xenobiotic contaminants during their growth (WANG, et al., 2022). Therefore, one can define bioremediation as a biological strategy for the restoration of polluted environments, without causing modifications on the basic properties of the soil, water and biodiversity (KUMAR; NAIN; SINGH, 2017). Therefore, it can be seen as an eco-friendly technique, usually showing lower costs when compared to other methods, e.g., landfill, use of chemical remediation, etc. (KUMAR; NAIN; SINGH, 2017).

For this technique to be effective, one must address the following aspects: (i) to know possible products that can arise from the xenobiotic degradation, evaluating their degree of toxicity; (ii) to evaluate the microorganisms that could have catabolic genes with metabolic potential for the removal/hydrolysis of the target compounds; and (iii) to assess the conditions and factors that lead to optimization of the biological activity of the chosen microbes (BHATTACHARJEE et al., 2014). For efficient biodegradation to occur, it is important to consider not only the specific aspects of the bacterial metabolism, but also the surrounding environmental conditions, such as temperature, pH and nutrients available to the microorganism (KUMAR; NAIN; SINGH, 2017). These will allow a proper bacterial growth, in addition to having an optimal/acceptable degradation rate for the decomposition of the target pesticide(s). Another relevant aspect to consider is the type of contaminated soil, since depending on its porosity and humidity, the pesticide can be even more strongly absorbed by the environment rather than degraded by the bacteria applied (ODUKKATHIL; VASUDEVAN, 2013).

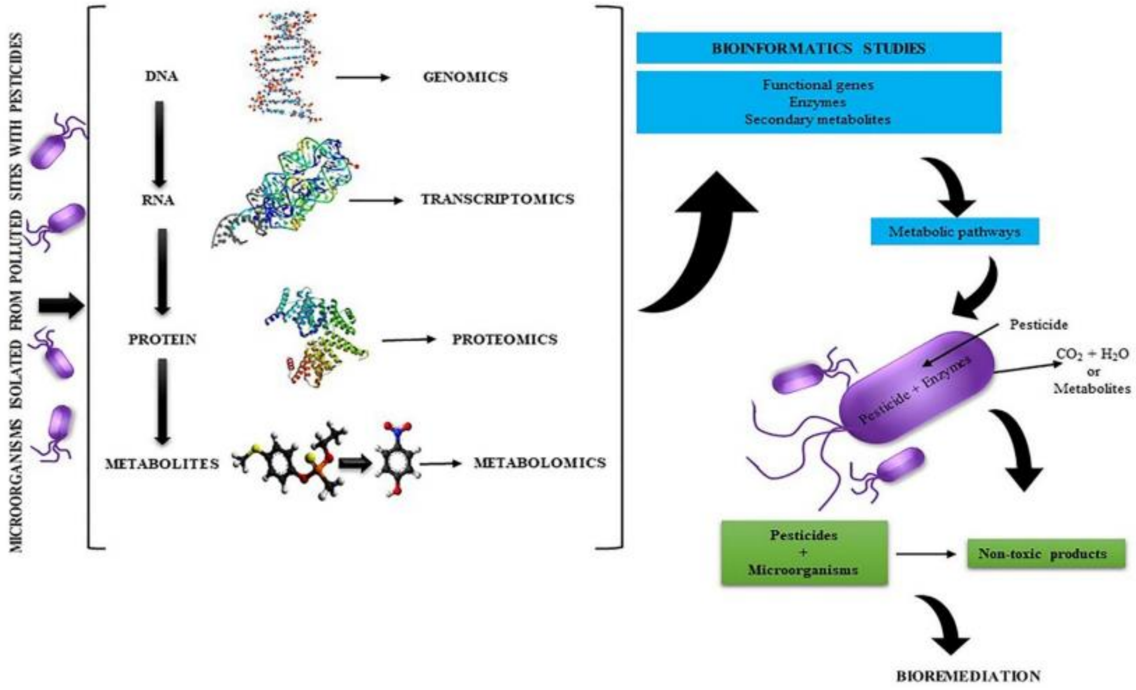
There are several types of techniques for bioremediation both *in situ* and *ex situ*. ‘Biosparging’ and ‘Bioventing’ are particular examples, which are based on inducing aeration and, if necessary, addition of nutrients required by the bacteria (AZUBUIKE, CHIKERE & OKPOKWASILI, 2016); while the former is more related to the saturated (phreatic) zone, the latter is related to the vadose zone (between the surface and the underground water table). In addition to those methods, one can deploy ‘bioreactors’, which allow degradation of xenobiotics under controlled growth conditions for the degrading microbe (ANTIZAR-LADISLAO, 2010). Their use will depend on the cost, since for the *in situ* techniques, procedures involving soil excavations are required (KUMAR et al., 2011).

Bioremediation has also some drawbacks. The main and most worrying one is related to the products that can be generated from the degradation performed by bacteria, since some of them can be even more toxic than the compound itself (ODUKKATHIL; VASUDEVAN, 2013). It is a process that tends to take more time than others, starting with the fact that the investigation on the degrading organism(s) is of utmost importance; knowing the environmental conditions, genes and proteins involved in the degradation process of the target xenobiotic compound(s) (KUMAR et al., 2011; SINGH, 2008) are a relevant part of the process of developing a bioremediation procedure.

2.6. OMICs for bioremediation

As indicated above, for a bioremediation process to get developed, the xenobiotic-biodegrading bacteria must be studied. Because some of them cannot be cultivated in the laboratory, some molecular techniques such as 16S rRNA gene sequencing become of great importance for the identification of bacteria in the sample (RODRÍGUEZ et al*.,* 2020). However, despite being a widely used technique for identification, it does not provide further information beyond a possible phylogenetic comparison among the members of the sampled microbial community. Moreover, as already mentioned above, knowing characteristics such as metabolic products and pathways, genes and proteins involved in the process is very important. Today, these issues can be addressed in a first-tier and exploratory manner by using the omics’ tools: genomics, metagenomics, transcriptomics, proteomics, metabolomics, etc. These approaches can help providing more detailed information about the microbial community’s structure and functional characteristics, as well as about the genome and metabolism of the selected microbial organisms (Fig. 1) (LOVLEY, 2003; RODRÍGUEZ et al., 2020).

Simpler physiological or genetic analyses are not usually enough to allow understanding of the complexity of microbial community involved in the degradation of pesticides (HUANG et al., 2018; YU et al., 2015). For instance, the presence of certain genes does not necessarily mean that they will be expressed, or that the amount of mRNAs/proteins generated will be high enough to provide acceptable rates of xenobiotics degradation (RODRÍGUEZ et al., 2020). A more complete picture of the system to be employed in bioremediation can be achieved by coupling physiological analysis with the information provided by DNA/RNA microarrays, proteomics (to analyze the levels and types of expressed proteins) and metabolomics (to analyze metabolites and the metabolic pathways involved). The omics can help to have a clearer and more specific/efficient process of bioremediation in the *in situ* and *ex situ* techniques. It is worth mentioning that bioinformatics is also an important area in the omics' analysis process, since it allows modeling and simulating metabolic pathways, as well as knowing the most efficient routes for the degradation of a given compound. This also allows the analysis of genomes, proteins and the generation of databases that can be of further use for future assessments and studies (LOVLEY, 2003; RODRÍGUEZ et al., 2020).



Source: RODRÍGUEZ et al., 2020

Figure 1: Analysis of bacterial isolates with omics for retrieval of valuable information for bioremediation technique. Microorganisms isolated from soil and water can be studied from OMICs. From these bioinformatics studies we obtain information necessary to know genes/proteins, secondary products, among others. Important when applying a microorganism in the bioremediation method.

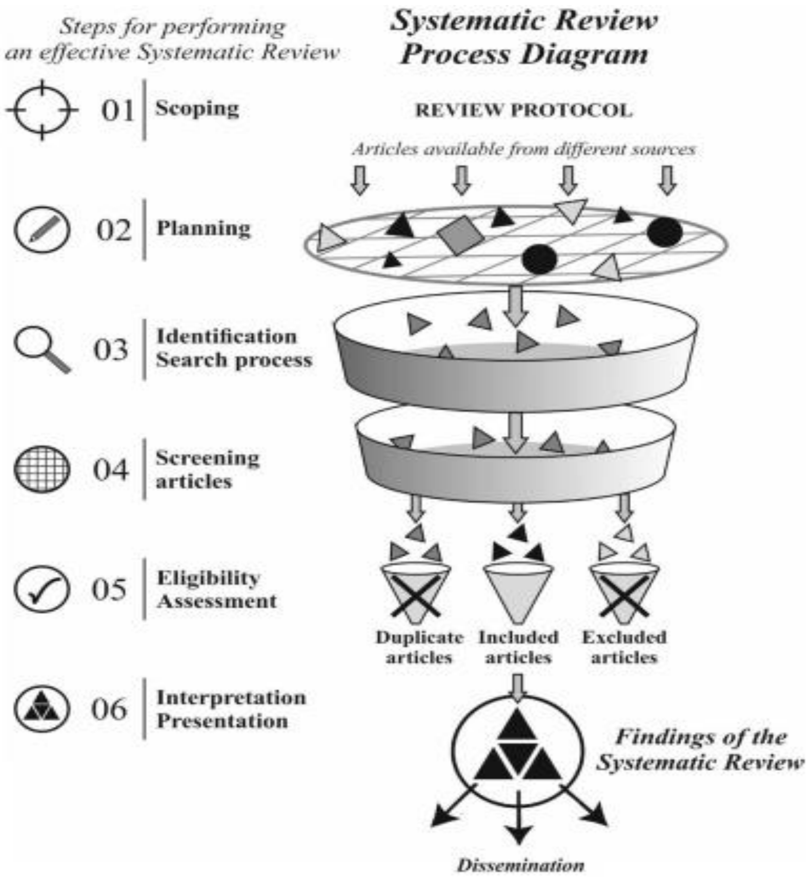
2.7. Systematic Review in agricultural/environmental sciences

The art of combining information from different sources began in 1904 by a British statistician, Karl Pearson. The aim was to test the effectiveness of vaccines to prevent the spread of typhoid fever in soldiers (O'ROURKE, 2007). Later, in 1930, W. Cochran and F. Yates have worked together to describe approaches that are very similar to the fixed-effect model and the random-effect models used in statistical meta-analyses. In 1977, G. Glass and M. Smith managed to standardize and place the measurements of different experiments on the same scale, opening the avenue for multivariate analyses. Meta-analysis began to take place in medicine and social sciences, but it was not until the 1990's for the method to be fully developed and employed, mainly in the areas of health, medicine, ecology, evolution and biological conservation. It was in 1995 that Arnquist and Wooster published a small introduction of meta-analysis for ecologists in the "*Trends in Ecology and evolution*" (GUREVITCH et al., 2018; O'ROURKE, 2007).

In spite of this potential, many ecologists over time declared that the studies they carried out were too heterogeneous to combine them with this type of research and statistics in a meaningful way. Nevertheless, several scientists further developed this research method as a "remote sensing tool", which allowed them to generalize the results of individual studies to have a wider knowledge and to allow them to predict and solve urgent problems for which direct and primary empirical data were difficult to obtain (GUREVITCH et al., 2018; O'ROURKE, 2007). Moher and colleagues (2009) have defined a systematic review (SR) as a planned and directed search in the specialized literature to answer a specific question, allowing the researchers to identify, select and evaluate primary studies and investigations of relevance to that question. Morever, a SR also allows one to collect empirical data obtained from those studies selected for the investigation, turning it also possible to include statistical methods for meta-analyses of data and results (WIJEKOON; YAPA, 2018). A SR can not only identify gaps in current scientific knowledge but also generating new lines of study, with formulation of hypotheses that were probably never studied in primary material or that cannot be carried out due to external limitations and unsolved problems (WILT; FINK, 2007).

Even considering the current ease of access to literature databases and articles retrieval through the internet, a SR face some issues to be addressed and solved. We can start with an initial selection of studies where, in many cases, the necessary information is not presented in the abstract or title and, therefore, their relevance become uncertain. In this case, specific parts of the studies should be reviewed to sort out their relevance. Another common issue to tackle is the high number of the so called 'predatory free access' journals, which fill the web with lower quality, scarcely reviewed studies, which can also be retrieved through the search strings developed for any given SR (BRAMER et al., 2018). Under these conditions, the number of related studies can be inflated after the search, thereby creating a misleading idea of the current depth of information on a given subject. Another aspect to deal with is that there are lots of studies that one must pay for, in order to have access to them; although this can be circumvented by writing to the authors to request reprints. Nevertheless, such an approach is time-consuming and is frequently unsuccessful. All these issues can cause a delay in the review process and decrease the amount of information that can be relevant to the research (GUREVITCH et al., 2018).

A particular systematic review in which the world of SRs and meta-analysis in agro-science was assessed has concluded that about one third of this type of study is in the area of agriculture (KOUTSOS; MENEXES; DORDAS, 2019). This can be justified by a little knowledge that scientists have about the potential of this research method, by the existence of heterogeneity among the retrieved studies, and by the time and effort required to make a systematic review in comparison to a classical literature review. Nevertheless, this method of study and analysis can be promising in agro-science for summarizing information and having clearer conclusions based on already published scientific evidence (KOUTSOS; MENEXES; DORDAS, 2019).



Source: KOUTSOS; MENEXES; DORDAS, 2019

Figure 2: Schematic view of a systematic review steps.

3. METHODOLOGY

In order to carry out this SR, the research problem was defined and the questions were structured. Based on this, a protocol established by the research team was followed (Table 1), in which we have three major steps: planning, execution and summarization. Two databases were used to search for primary studies: *PubMed* and *Scopus*. Together with the research group, an analysis was made and the group of keywords (strings) that would be used for this SR were decided upon. The general keywords and Boolean operators used for all the strings were: “Bacillus” AND “bioremediation” OR “phytoremediation” OR “degradation”. Moreover, five keywords were added, each forming a separate string: “pesticides”, “insecticides”, “fungicides”, “nematicides” and “herbicides”. Finally, these 5 strings were combined with the two keywords “soil” and “water”, thus forming a total of 10 strings (per database)(Table 1).

From applying this search scheme, a number of articles was obtained from each database; a preliminary identification of the articles was done, with the duplicate studies being eliminated (Fig. 3). Afterwards, the first and second selection were performed, with the same inclusion and exclusion criteria being used for both selections. In the first selection, the analysis was done on title and abstracts (when non-article types of publications were removed – Table 1) and, for the second selection, the assessments were performed in the full article text (Fig. 3). In order to manage the information and the whole process, including duplicate studies' detection and removal, the software StArt® (*State of the Art systematic review*) v 3.3 Beta 03 (by the Federal University of São Carlos – UFSCar, Brazil) was used. After these stages were completed, the data were extracted from the finally selected articles that composed the assessment database to allow answering the research questions. The management of collected data was done in regular spreadsheets (Microsoft Excel®); general information from the article and specific data such as *Bacillus* species, pesticides, concentrations, genes and proteins, among others (Table 1), were recorded. The BioEstat® software (AYRES et al, 2007) was used for the meta-analyses, which were based on a fixed-effect model, with the data collected on the percentage of pesticides degradation being logarithmic transformed (ln) prior to the analyses.

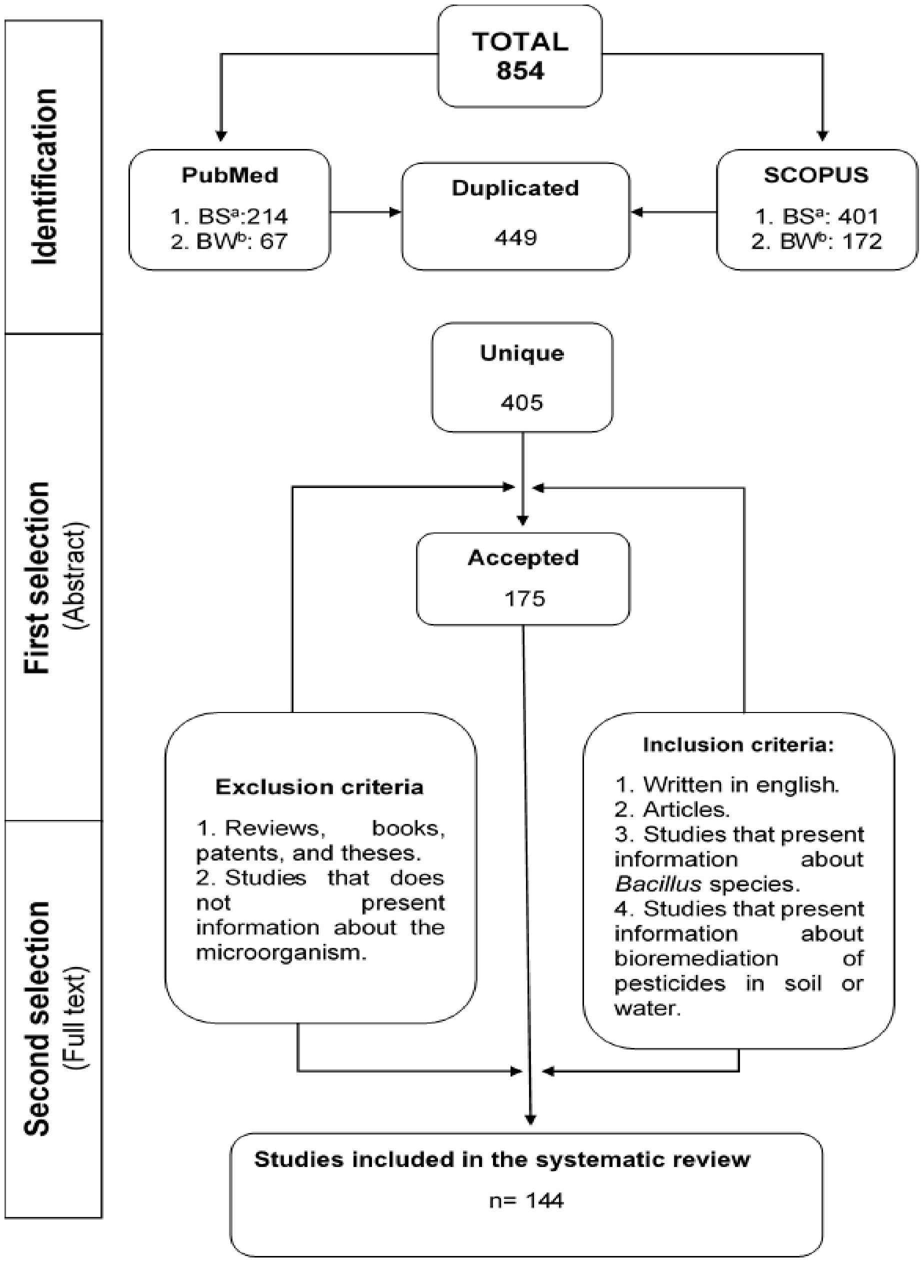


Figure 3: Flow chart for selection of studies for the Systematic Review. The identification phase shows the studies that were retrieved in *PubMed* and *Scopus*, using the corresponding search strings. The first selection (2^{nd} phase) shows the accepted studies classified according to the information presented in the 'Abstracts' of the retrieved articles. Finally, in the second selection (3^{rd} phase), the articles and data were assessed and collected based on the full text, thereby obtaining the information included in this systematic review.

Table 1: Protocol for formulation of questions, hypotheses, strings and possible results of the systematic review and meta-analysis.

General information	
Description	Pesticides are chemical compounds used for pest control in agriculture, and tend to be stable, water soluble, and volatile. The inappropriate use of these compounds affects the environment, affecting non-target organisms such as humans, aquatic and terrestrial animals, and plants. It is important, therefore, to look for methodologies that help decrease or eliminate the residues of these agrochemicals. One of the techniques being investigated for this purpose is *bioremediation*, which can be employed to clean up contaminated soil and water using different organisms such as bacteria, fungi, algae, etc. Bacteria of the *Bacillus* genus have specific characteristics that present potential for use in bioremediation strategies. These bacteria can be aerobic and facultative anaerobic, and have the characteristic of forming endospores that allow survival in different environments, and in varying physical and chemical conditions. Furthermore, they possess catabolic genes that can act in the degradation of xenobiotic compounds such as the pesticides. The study of isolates and bacterial species of this genus are important to find potential agents for bioremediation of pesticide residues in the environment.
Objetives	To review literature studies on bioremediation of pesticides and their residues in soil and water involving *Bacillus* spp., in order to: 1. Analyze species of *Bacillus* that have strains with potential for bioremediation of pesticide residues in soil and water. 2. Identify genes and proteins of *Bacillus* spp. that are functional for the process of bioremediation of these residues. 3. Identify environmental factors involved in the behavior of *Bacillus* spp. in relation to bioremediation processes. 4. Verify the variables used to measure the effects of degradation or removal of wastes in the environment. 5. To evaluate the results of applying *Bacillus* spp. in the bioremediation of agrochemical residues in soil and water in comparison with other remediation methods (chemical and biological).
Research questions	
Questions	1. Could *Bacillus* spp. display microorganisms with potential to bioremediate pesticide compounds in soil and water? 2. Which genes are involved in the biodegradation process of pesticide residues in the *Bacillus* genus? 3. For what types of pesticide residues could bioremediation with *Bacillus* spp. be useful? 4. What environmental factors are important for the bioremediation process to be most efficient? 5. What variables need to be assessed to allow the degradation effect of pesticide residues to be evaluated? 6. How much impact would the use of these microorganisms have for the environment when applied in bioremediation processes? 7. Can bioremediation applying *Bacillus* spp. be an effective and efficient method when compared to other methods (chemical and biological)?

Population	Members of the *Bacillus* genus and types of xenobiotic pesticide residues in soil and water.
Intervention	Strains and species of *Bacillus* spp. described in the literature with the ability to metabolize pesticide residues in soil and water.
Comparisons	1. Among strains and species of *Bacillus* spp. for bioremediation of agrochemical residues in soil and water. 2. Among genes and proteins that are involved in bioremediation of different soil and water environments. 3. Between different levels of environmental factors that may affect the benefits of *Bacillus* strains and species applied in bioremediation processes. 4. Among the degradation effects of pesticide residues in the environment. 5. Between remediation methods (chemical and biological) and bioremediation with *Bacillus* strains and species.
Hypotheses	1. The genus *Bacillus* contains strains and species viable for bioremediation of xenobiotic pesticides in soil and water. 2. *Bacillus* spp. contain catabolic genes to metabolize different types of pesticide residues in soil and water. 3. Temperature, pH and humidity are relevant factors in the behavior of *Bacillus* strains and species in the bioremediation of pesticide residues in soil and water. 4. Bioremediation with *Bacillus* spp. has a low level of impact to the environment. 5. Bioremediation using *Bacillus* spp. is a methodology with a high percentage of efficiency and effectiveness compared to other methodologies.
Expected result	From the application of the methodology here described, we expected to improve the knowledge on *Bacillus* spp. suitable for bioremediation of pesticide residues in soil and water. In addition, we hope to expand the information on genes and proteins that are essential for the biodegradation process, which can be studied on genetically modified microorganisms. Finally, to have data that increase the knowledge of the impact of bioremediation with *Bacillus* spp. and the rate of biodegradation that could be achieved in different soil and water environments, by taking into account the different environmental factors that affect the behavior of the microorganisms in xenobiotics degradation.
Type of study	Primary sources of empirical studies such as scientific articles.
Studies Identification	
Key words	*Bacillus,* bioremediation, phytoremediation, degradation, pesticides, herbicides, nematicides, fungicides, soil, water,
Strings	A total of 10 search strings were established, being applied on both databases (with their sintaxes being specific for each database). All strings contained '*Bacillus*' AND 'bioremediation' OR 'phtoremediation' OR 'degradation' keywords (kw) and boolean oparators. To assemble each of the 10 different strings, these general kw were combined with 'soil' and with 'water' kw (2 separate strings); finally, these 2 strings were combined with each of the 5 other kw, i.e., 'pesticides', 'herbicides', 'insecticides', 'fungicides' and 'nematicides', thus forming the 10 strings in total.
Selection criteria	1. Articles in English

	2. Articles that include *Bacillus*.
Search engines	1. *Scopus* 2. *PubMed*
Search strategy	1. Combinations of proposed keywords (strings). 2. Use the search engines that have a sufficient amount of articles to answer the research questions. 3. Apply inclusion and exclusion criteria to classify the articles obtained in the two-step searches.
Selection and evaluation of studies	
Inclusion and exclusion criteria for selection of studies	*Inclusion*: - English language. - Document type: Article. - Articles focusing on bioremediation of pesticides in soil and water. - Articles containing information on *Bacillus* species. *Exclusion*: - Reviews, patents, thesis or books. - Articles that do not contain the *Bacillus* microorganism.
Strategy for initial study selection	Title, abstract, and keywords related to the proposed theme.
Strategy for final selection of studies	To make the selection of articles, they must contain all the inclusion criteria. And based on the reading afterwards, evaluate if the information contained is helpful to complete the study.
Synthesis of the data and presentation of the results	
Data Extraction Strategy	For data extraction, the abstract of the article will be read, to serve as an indicator of the usefulness of the research information retrieved. Then, the microorganisms that have been studied and the *Bacillus* species will be reviewed. Furthermore, the proteins and genes mentioned will be analyzed. Finally, the degradation rate of these microorganisms will be read and extracted, and the countries where the studies are being done will be evaluated.
Data Summarization Strategy	Graph, tables, figures, forest plots.
Publication Strategy	1. High impact journal. 2. Journal related to biotechnology, microorganisms and environment.

4. RESULTS

The search of this review was performed in the *Scopus* and *PubMed* databases, considering their wide scientific literature coverage and the high number of journals with quality peer-review provided (Fig. 3). According to the appropriate strings developed for each database (Appendix 1), we have included, for both databases, 10 different combinations of the keywords, being 'Soil' or 'Water' with each one of the five words describing the types of residues (Tables 1 and 2). The 10 different strings were the result of an exhaustive prior analysis of the searches' return in each database, in which the best possible relationship between number of publications and connection with our study was assessed. After the search strategy was finally defined, a total of 854 articles has returned within our area of interest, with 573 being from *Scopus* and 281 from *PubMed*; these numbers included duplicated studies, as more than two of the keywords' strings could be found in a same paper, and same articles can be found in both databases. The total number of articles retrieved, discriminated by the keywords combinations in each database, is shown in Table 2. Among the four categories of pesticides used as keywords in combination with 'soil' and 'water', the studies with the word 'Insecticides' appeared in the highest proportion (28.1%), followed by those with the words 'Herbicides' (19.5%), 'Fungicides' (4.8%) and 'Nematicides' (0.46%). Overall, the amount of studies for bioremediation of the four types of pesticides in water was ~2.4 x less than those for soil; water-related papers from the *Scopus* database represented 20.1%, and from the *PubMed*, 7.8% of the total number of retrieved studies. Therefore, the studies retrieved for bioremediation in soil represented ~2/3 of total, with 46.9% of them coming from *Scopus* and 25.1% from *PubMed* (Table 2). After the step of elimination of duplicate studies, a total of 405 unique papers remained (Fig. 3), i.e., 47.5% of the studies retrieved by the strings proceeded forward in the review process. After applying the inclusion/exclusion criteria (Table 1) in the assessment of the unique Abstracts, 175 papers were selected (first-stage selection); then, after applying the same criteria in the full-text of the Abstract-based selected articles, a total of 144 papers remained, so being the final number of studies used in this review (Fig. 3).

Table 2: Total[1] number of retrieved studies by the 10 strings on the search for pesticide bioremediation in soil and water with *Bacillus* spp..

Key Word	*Soil*		*Water*		*Total*
	Scopus	*PubMed*	*Scopus*	*PubMed*	
Pesticides	178	103	86	35	402
Fungicides	22	3	13	3	41
Herbicides	87	43	25	12	167
Insecticides	113	64	46	17	240
Nematocides	1	1	2	0	4
Total	401	214	172	67	**854**

[1] The table presents the total number of articles obtained by the searching strings, which combined the words 'Soil' and 'Water' with each of the five 'keywords' (kw) indicated on the left column, in each database. These numbers, therefore, include duplicated studies that were later removed in a pre-selection phase, using the StArt® software.

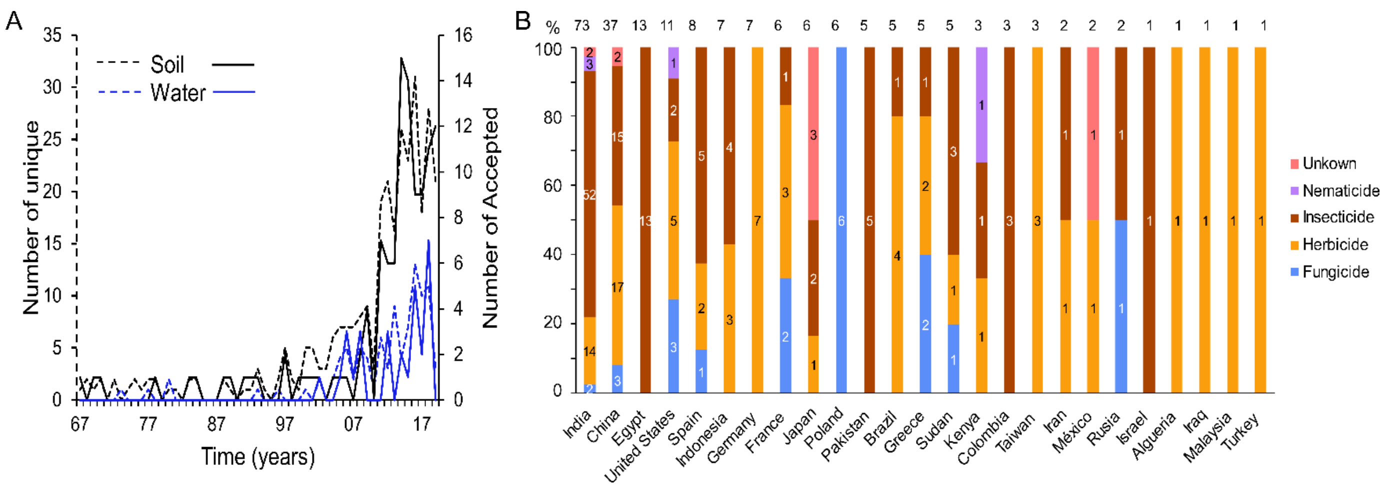


Figure 4: Temporal and geographical pattern of publications on *Bacillus* spp. and bioremediation of pesticide residues. (**A**) Amount of bioremediation studies in soil and water with *Bacillus* spp. per year. The scale on the left indicate the total number of articles retrieved by the strings, after removal of the duplicates studies (dotted lines); on the right, it is shown the number of finally accepted studies included in the systematic review (solid lines). (**B**) Number and proportion of pesticides groups in different countries. The total number of studies found per country is shown on the top of each multicolored vertical bars. The number within each colored bar in the graphic shows the individual number of studies for each pesticide group indicated in the legend on the right side; the group represented by "unknown" includes studies of biodegradation of residues, intermediates or compounds that are used in the formulation of the pesticide, but that was not defined as any of the other four types.

Aiming at assessing the time and places where the empirical research on bioremediation of pesticide residues by *Bacillus* spp. has been developed, we plotted the finally accepted numbers of articles retrieved from our search protocol; in addition, the total amount of studies per country, distributed among the types of pesticide residues is also shown (Fig. 4). For both environments (soil and water), a steadier increase in the publications began from around the year 2000, with a peak by 2014-2016. The number of studies on biodegradation of pesticides in soil was always higher than those for water and showed a higher increase rate by the year 2010, showing ~2.5 x more studies to date (Fig. 4A). The quantitation patterns of soil studies in relation to water was essentially the same for both the unique and selected number of articles retrieved (Fig. 4A), indicating an important consistency between the search and selection procedures. About half of the studies on biodegradation of the different pesticide groups in soil and water were from India and China, with for the former being twice as much as for the latter (Fig. 4B). The total number of 219studies/mentions of the pesticide groups for all countries is higher than the total number of selected articles in our database (146), because certain studies have included more than one type of residue/xenobiotic in their analyses. In terms of the xenobiotic compounds evaluated, the most frequently mentioned was 'insecticides' (51.9%), followed by 'herbicides' (32%). Despite that 'nematicide' was the least mentioned pesticide, it is worth clarifying that many studies related to nematicide and fungicide were included in the insecticide's research work, i.e., the same compound was used for two different targets. This also occurred with 'acaricides' (data not shown), a keyword that was not included in the search strings. The countries in which this type of xenobiotic compound was mentioned are India (13), Indonesia (1), China (2), Colombia (1), Egypt (1), Pakistan (1) and Sudan (1). The pesticides known as *endosulfan*, *monocrotophos* and *malathion* appeared with a higher frequency in the research works classified as insecticide/acaricide.

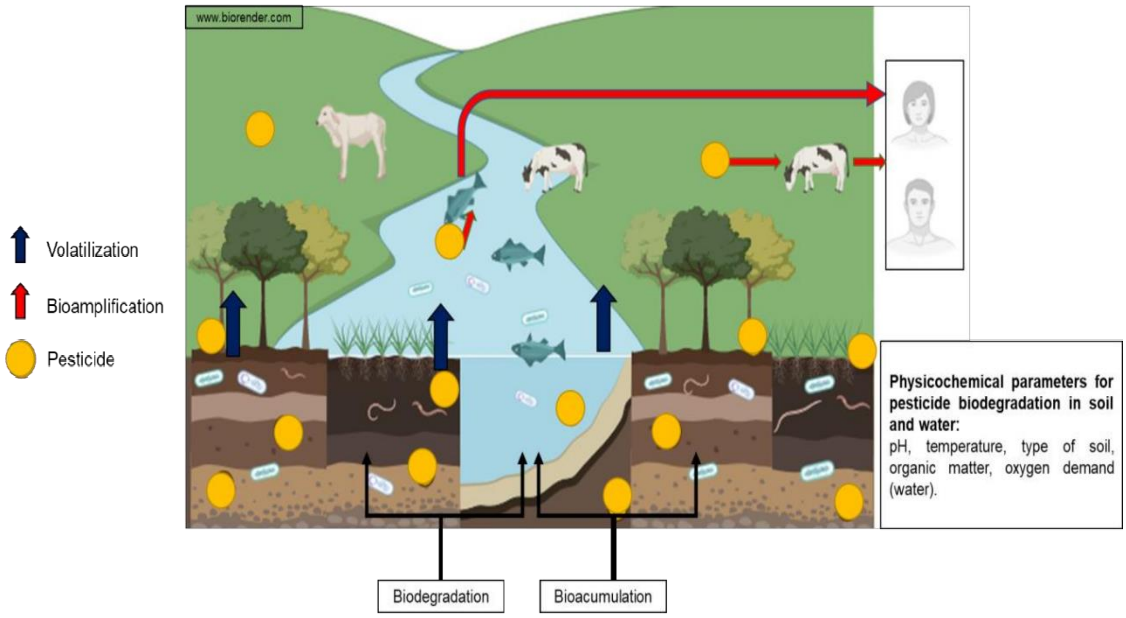


Figure 5: Pesticides movements/dissipation in soil and water environments. An array of physicochemical variables/parameters showing prominence in the studies on pesticide degradation in soil and water (usually analyzed *in vitro*) are indicated in the right-hand box. The blue arrow represents the *volatilization* of the pesticide from the soil or water to the atmosphere, while the red arrow represents the process of *bioamplification*, and the yellow circle any type of pesticide that can be used in agriculture. In soil are earthworms that help with pH regulation. Although there can be *bioaccumulation* of pesticides and their residues in the soil and water resources such as lakes, rivers, groundwater, and wastewater, the bacteria and other microbes present in soil and water help with the biodegradation of pesticides' residues through different metabolic pathways

Based on data and information collected from the selected studies, two major environmental effects with harming potential were frequently mentioned, as a result of the pollution caused by the high levels of xenobiotics accumulation. An analytical scheme was drawn to allow an integrated assessment of the environmental behavior of pesticides, including physicochemical variables involved (Fig. 5). Because the pesticides have been used in excess throughout the world, they have a direct consequence in the environment, affecting animal and human health (AKTAR; SENGUPTA; CHOWDHURY, 2009; REMBISCHEVSKI; CALDAS, 2018; VERMA; JAISWAL; SAGAR, 2014). One of these two environmental effects, as a direct consequence of the xenobiotics entrance in the food chain, is the *bioaccumulation* of pesticides and their residues, even when and where biodegradation processes take place (HAKEEM; AKHTAR; ABDULLAH, 2016). This effect results from a common feature of the pesticides, i.e., their easy volatilization that allows a wide dispersion in water and soil surfaces (HASSAAN; EL NEMR, 2020a; KUMAR et al., 2018). The second effect is a consequence of the fact that the xenobiotic residues can remain within tissues and organs of crops and other organisms that are part of the food chain, so that they can lead to another environmentally relevant phenomenon called *bioamplification* (Fig. 5). For both cases, the results are an indirect raise in the levels of chemical pollution in the environment (HAKEEM; AKHTAR; ABDULLAH, 2016), thereby reinforcing the need of widespread application of bioremediation strategies.

Under these circumstances, we can point to the relevance found for the existence of different layers of soil, which can contain different amounts of oxygen and nutrients, as well as various species of aerobic and anaerobic bacteria. These bacteria can be found both in water and in soil, and they can contain a variety of metabolic pathways which allow the degradation of a wide range of xenobiotic compounds, including the various types of pesticides (ANTIZAR-LADISLAO, 2010; SINGH, 2008; WOŁEJKO et al., 2020). In addition, these bacteria play a role in the N and C fixation cycles in soils. The results from our literature assessment indicated that, for the biodegradation process, it is important to evaluate physicochemical parameters such as pH, temperature, soil type, organic matter and oxygen demand (mainly in aquatic environments). Temperature and pH have shown to be the most relevant parameters to observe, as a great number of studies have addressed these factors (DOOLOTKELDIEVA; KONURBAEVA; BOBUSHEVA, 2018a). They can have a strong influence in the capacity of bacteria to degrade xenobiotic compounds of interest, which, in turn, depends on the enzymatic reactions that take place in different metabolic pathways (BHATTACHARJEE et al., 2014; FARHAN et al., 2014; KHAN et al., 2016). Together, all these aspects can alter the biodegradation rates of the pesticide residues of concern.

Our review of the selected articles also revealed that knowing the bacterial genes, enzymes and other components of metabolism that are involved in pesticide compounds metabolization is a major challenge to be overcome for the establishment of useful bacteria-mediated bioremediation methods. Moreover, it is also important to understand how those genetic and molecular elements interact with physicochemical parameters, especially pH and temperature (JAISWAL; SINGH; SHUKLA, 2019; RODRÍGUEZ et al., 2020; ZHU et al., 2019), and how bacteria can survive to low oxygen demands (ACEVES-DIEZ; ESTRADA-CASTAÑEDA; CASTAÑEDA-SANDOVAL, 2015; KUMAR; PHILIP, 2006). These aspects are all related to the fact that bacteria need to adapt and perform well to the environment to be remedied, beyond their characteristics of being studied/produced in laboratory (CYCOŃ; MROZIK; PIOTROWSKA-SEGET, 2017; KUMAR et al., 2011). Finally, in order to successfully apply bacteria-mediated remediation strategies, it is important to understand which will be the new substances to be generated from the degrading metabolism, as they must be non-toxic or less toxic than the initial xenobiotic compound (KUMAR et al., 2018).

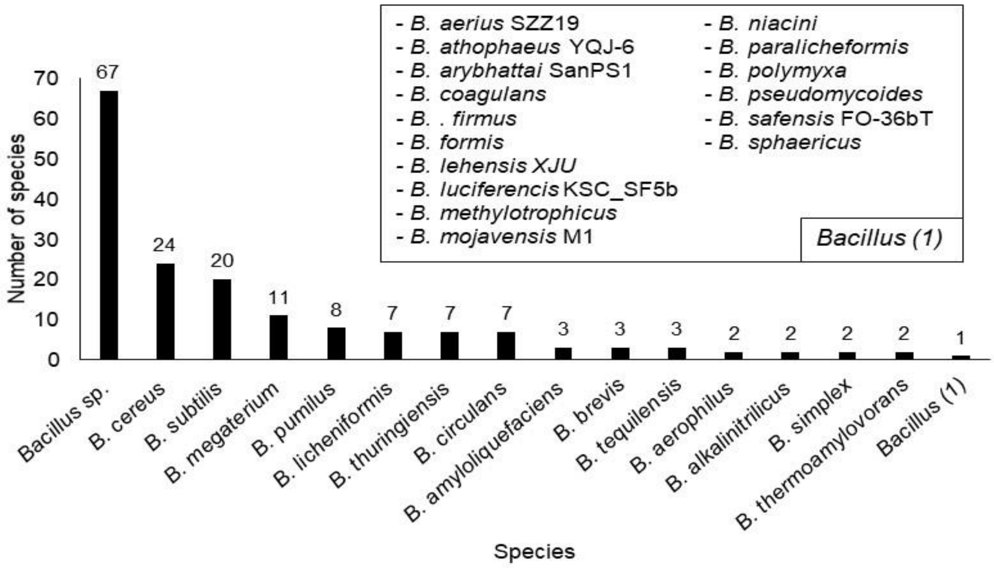


Figure 6: Species/strains of *Bacillus* with biodegradation activities found in the finally selected studies. The number of "species" in the graph represents the data pooled out from different strains found in the articles. The "*Bacillus (1)"* label and the box it indicates represent all the species found that contained only a single strain under study. The "*Bacillus* sp." label represents all the strains that were not identified up to the species level. A total of 184 strains/isolates were identified in the assessed studies.

For analysis of the degradative microorganisms operating in the environment and their bioremediation potential, the first step was to identify isolates/strains showing capacity to metabolize the different types of pesticides and/or their residues. A total of 184 strains of *Bacillus* capable of pesticides' biodegradation in soil and water were found within the studies included in this review (Fig. 6). The results indicated that the label of unidentified *Bacillus* species ('*Bacillus* sp.') showed the highest number of strains addressed, being 36.41% of all studied. Among the strains identified beyond the genus level, a total of 30 *Bacillus* species were found (Fig. 6). Nevertheless, only three species, i.e., *B. cereus*, *B. subtilis,* and *B. megaterium*, comprised ~30% of the strains present in the studies, corresponding to 13.04%, 10.86%, and 5.98% of total, respectively. About

53% of all the species identified appeared as being represented by a single strain ('Bacillus (1)' label and box – Fig. 6).

All these *Bacillus* strains were also investigated concerning the range of pesticide residues concentrations within which they were found to operate (Table 3). Likely due to being the most recurrent species found in the experiments, *B. cereus, B. subtilis* and *B. megaterium* strains showed the highest levels of maximum pesticide residues concentration against which they were tested, e.g., 6, 50 and 5 x 10^3 mg L^{-1}, respectively (Table 3). Those strains where tested at these pesticide levels against *glyphosate*, *chlorpyrifos* and a mix of pesticides, respectively (ARABET et al., 2014; FAN et al., 2012; VIDYA LAKSHMI; KUMAR; KHANNA, 2008). On the other hand, the lowest level of maximum concentration found was 50 mg L^{-1} for strains of three species (*B. amyloliquefaciens, B. brevis* and *B. aerophilus*), although there were only few experiments found for these strains in the selected database of papers (Table 3). Concerning the minimum concentrations of residues found in the studies, these varied from 50 to less than 0.1 mg L^{-1}; a strain in the *Bacillus (1)* group (Fig. 6) showed the lowest value observed in the studies (Table 3), corresponding to the *Bacillus aerius* SZZ19 strain, which was tested for the *diuron* herbicide (HANAPIAH et al., 2018). For unidentified *Bacillus* sp., the minimum concentration of 9 mg L^{-1} and the maximum of 20 x 10^3 mg L^{-1} were found within the 71 experiments assessed, all corresponding to tests with the same pesticide, i.e., *metolachlor* (KANISSERY et al., 2018; LI et al., 2018).

Table 3: Range of pesticide residues' concentrations[1] that different *Bacillus* species were found to operate.

Bacillus	**No. of experiments** [2]	**Pesticide residues' concentration** (mg L^{-1})	
		Min.	**Max**.
cereus	24	0.1	6000
subtilis	20	5	50000
megaterium	10	20	5000
pumilus	8	3,77	400
licheniformis	7	50	500
thuringiensis	7	1	750
circulans	7	5	5000
amyloliquefaciens	3	30	50
brevis	3	5	50
tequilensis	3	50	200
aerophilus	2	50	50
alkalinitrilicus	2	50	100
simplex	2	50	750
thermoamylovorans	2	*nd* [3]	*nd*
sp.	71	9	20000
Bacillus (1)	16	4.3×10^{-4}	400

[1] The concentrations of residues depended on the species and type of pesticide under study in each article/experiment. The 'max.' and 'min.' values were defined by simply annotating the lowest and highest concentrations found for the strains/experiments included in each group of species indicated in the left column.

[2] The indicated 'number of experiments' represent the number of strains tested per article. These strains might be subjected to different treatments within the same article, depending on the aim and methodologies of the studies.

[3] '*nd*': not determined.

Bacillus genus and its species are abundant in the environment, especially in polluted sites (SALUNKHE et al., 2013), such as those contaminated with pesticides. However, despite an important amount of research on the potential application of this bacterium for bioremediation process (Table 2, Fig. 3), the amount of proteins and genes found in the selected studies was not as proportional to the number of species analyzed as we might expect (Fig. 6, Table 3). Only 11 strains from 9 species of all *Bacillus* spp. found in this study were related to identification and characterization of genes/proteins/enzymes associated with degradation processes (Table 4). Most of the strains/species of *Bacillus* observed in the assessed articles use hydrolytic pathways for pesticide/residues degradation in soil and water. Among the genes reported as being involved in such biodegradation, dioxygenase (reductase) activities were found in three reports, and membrane transport and ribosomal protection functions in one report each (Table 4). The *B. subtilis* species was found to be able of concomitantly hydrolyzing distinct xenobiotic compounds through two different genes (although these activities were found in different strains): the *opd* gene can metabolize organophosphates, whereas the *atz* is able to transform atrazine into cyanuric acid (Table 4).

Table 4: Genes and proteins involved in the metabolic pathway of pesticides degradation, reported in different strains/species of *Bacillus*.

Species	Ref.	Gene	Protein	Major function
Bacillus amyloliquefaciens YH-5	(LI; CHI; GE, 2019)	*hy-1*	Hydrolase HY-1	Hydrolysis of Carbendazim.
Bacillus cereus	(JIANG et al., 2019)	*ampA*	Arylamidase	Catalyze the amine bond.
		carboxylesterase gene	Carboxylesterase	Hydrolysis of carboxylic ester bonds.
		mpd	Methyl parathion hydrolase	–
		opdA	Organophosphate hydrolase	
		opdE		
		ophB		
Bacillus cereus MCASO2	(DURAISAMY; MUTHUSAMY; BALAKRISHNAN, 2018)	*oph*		Hydrolysis of Chlorpyrifos
Bacillus sp. JF8	(SHIMURA et al., 1999)	*bph*	Dyoxygenases	Methyl-byphenyl degradation pathway
Bacillus megaterium Mes11	(BARDOT et al., 2015)	operon gnt	PhaB - Polyhydroxyalkanoate (PHA)-Specific Acetoacetyl CoA Reductase	short-chain dehydrogenase/reductase
Bacillus polymyxa	(DOOLOTKELDIEVA; KONURBAEVA; BOBUSHEVA, 2018)	P450 genes	NA	Catalyze the degradation pathway of Aldrin.
Bacillus sp.	(HOGAN et al., 1997)	*tfdA*	2,4 dichlorophenoxyacetic acid/α-ketoglutarate dioxygenase	–
	(FANG et al., 2016)	*tet39*	–	Major facilitator superfamily of transporters
		tetA		
		tetC		
		tetL		
		tetM		Ribosomal protection protein (RPP)
Bacillus pumilus W1	(JIANG et al., 2019)	*opdA*	Organophosphate hydrolase	Hydrolysis of organophosphorus pesticides
Bacillus sp. Za	(ZHANG et al., 2018)	*lacE*	LacE(esterase)	Lactofen-hydrolyzing esterase
Bacilus subtilis	(ACHARYA et al., 2014)	*opd*	Organophosphate hydrolase	Hydrolysis of monocrotophos
Bacillus subtilis HB-6	(WANG et al., 2014)	*atzB*	Hydroxiatrazine hydrolase	Transform atrazine into cyanuric acid
		atzC	N-isopropylammelide isopropylamino hydrolase	
		trzN	TrzN	

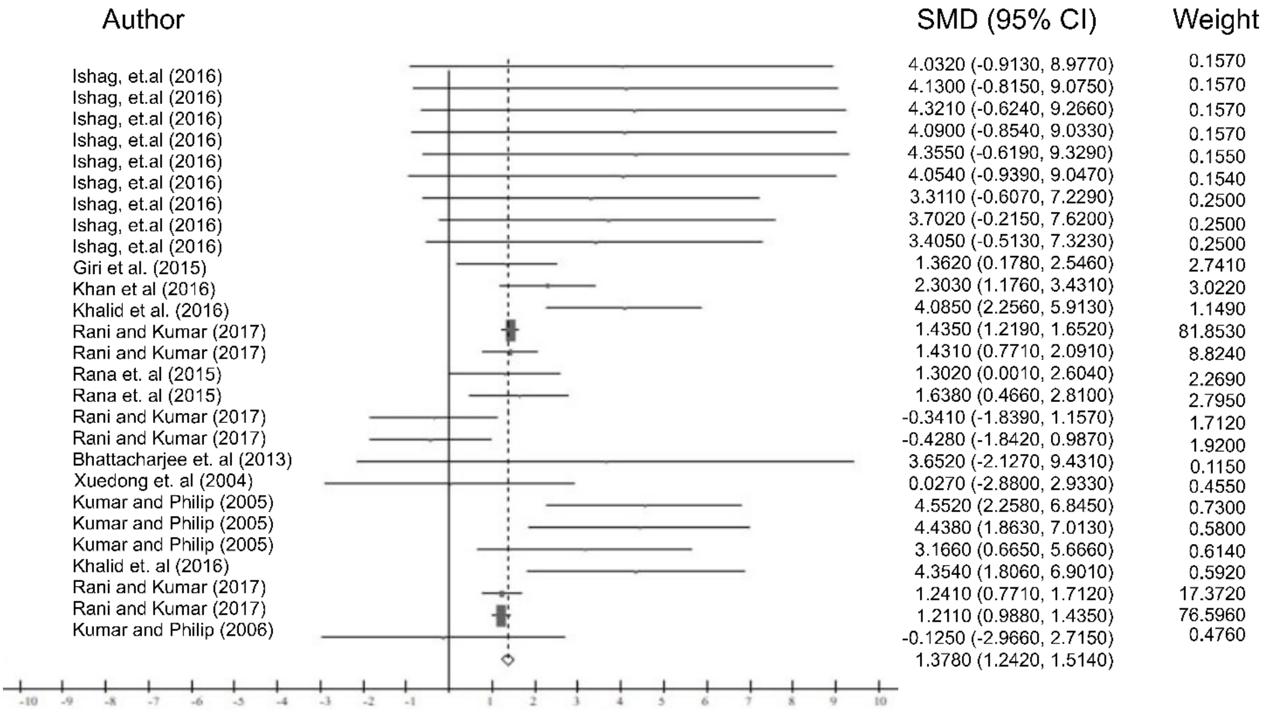


Figure 7: Meta-analysis of pesticide residues degradation with *Bacillus* spp. Forest plot comparing various pairwise experiments (studies) containing treatments 'with' *vs* 'without' the presence of a *Bacillus* strain. The combined mean effect (open diamond at the bottom) of all studies indicated a positive significant effect of *Bacillus* treatment in the increase of pesticide degradation rates. The authors' column shows the information on the studies used in the analysis. The columns on the right side shows the standard mean differences (SMD) of the studies with the respective 95%-confidence intervals of upper and lower limits, and the relative weight of each study for the metanalytical combined effect. The percentage values of increased degradation by *Bacillus* application in all studies were logarithmic transformed (ln) prior to the analysis. A fixed-effect model for continuous data were employed; all studies presented the same sample size (3 replicates).

With the aim of quantifying the effect of increase in degradation rates of pesticides by applying *Bacillus* strains among the selected reviewed articles, a meta-analytical approach was employed in 27 studies (experiments), using logarithmic-transformed percentage data (ln) and a fixed-effect model (Fig. 7). In this analysis, the pairwise comparisons of each experiment included treatments *with* and *without* application of *Bacillus* spp. strains, either as a single bacterium or in consortia with other bacteria (in some of the treatments without *Bacillus*, other bacterial species were employed). Despite a large within-study variability for most experiments (studies), almost all of them pointed towards a mean effect of increasing degradation rates of pesticides by application of *Bacillus*. The combined effect of all the pairwise experiments tested yielded a meta-analytical mean of 1.378 in favor of *Bacillus*-applied treatments, with 95%-confidence interval of values staying between 1.242 to 1.514 (Fig. 7). Considering that only two studies showed remarkable weights of 77 and 82 points on the final meta-analytical outcome, the analysis was repeated without these two studies, to verify whether these two experiments would be biasing the tendency observed. The results with the 25 remaining experiments did not alter either the positive combined effect in favor of *Bacillus*-application treatments, or the statistical significance of the meta-analytical mean (not shown).

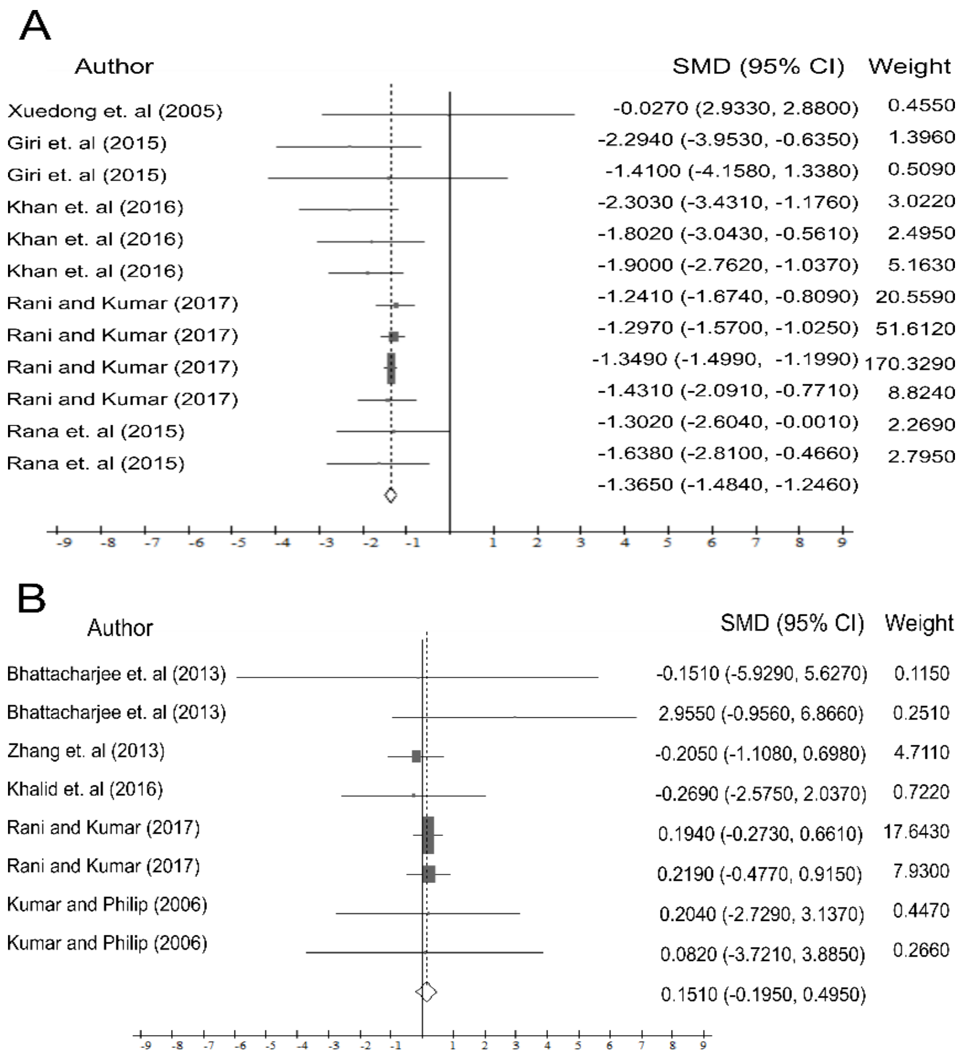


Figure 8: Meta-analyses of pesticide residues degradation with *Bacillus* spp treatments (forest plots) based on sub-groups of pairwise treatments comparisons. **(A)** Pairwise comparisons among those experiments (studies) in which the treatments without *Bacillus* have not added anything on the control treatments beyond the culture media used. **(B)** Pairwise comparisons of treatments with presence of *Bacillus* strains either as a single treatment or as in consortium with other bacteria. For all remaining information, see legend of Figure 7.

In the attempt to assess in further details the positive effects detected for *Bacillus* treatments in the improvement of pesticides degradation rates, the selected studies were divided into two types of comparisons. In one type, applications of a single *Bacillus* strain were compared to control treatments in which some level of pesticide degradation was detected (those experiments in which controls showed no pesticide degradation were excluded); in the other type, *Bacillus* treatments were compared pairwisely, with each strain being applied as a single treatment or in consortia with other bacteria (Fig. 8). Again, a fixed-effect model was employed, using logarithmic-transformed percentage data (ln). Not unexpectedly, since these studies are a subset of those shown in Fig. 7, the results confirmed the positive effect of *Bacillus* treatments in accelerating the degradation rates of pesticides (Fig. 8A; minus signals are simply indicating an inverted display of the compared data in the spreadsheet of the statistical software employed). The combined effect was of -1.365 in favor of *Bacillus*, with 95%-confidence intervals ranging from -1.484 to -1.246. Based on the 12 studies of this subset, one experiment in the study by Rani and Kumar (2017) showed the highest relative weight in the analysis, contributing alone with 36.8 % of the combined effect (Fig. 8A). A re-analysis without this particular study have not change the magnitude of the effect observed (not shown).

Finally, we were interested in verifying whether single applications of *Bacillus* strains would differ from their application in consortia with other bacteria; hence, a subset of studies we found comparing these treatments were assessed (Fig. 8B) in the same general conditions of the previous analyses. The results from the eight experiments composing this subset of studies showed that there was no statistical difference between the treatments of *Bacillus* applied alone or in consortium with other bacteria (gram – or +) as the meta-analytical combined effect of 0.151 showed the values of its 95%-confidence interval on both sides of the *zero* effect line (i.e., CI = -0.195, 0.495). Again, an experiment in the study of Rani and Kumar (2017) showed more than half of the relative weights on the combined effect of the meta-analysis (55 %). However, its absence have not changed the trend observed (not shown).

5. DISCUSSION

Pesticides have become one of the most problematic xenobiotic compounds for the environment and its organisms, including humans, due to the residues left in soils and water that generate side effects and serious illness (AKTAR; SENGUPTA; CHOWDHURY, 2009; ODUKKATHIL; VASUDEVAN, 2013). The most affected group are the children, the elderly and people who work in agriculture and are constantly in contact with pesticides (HASSAAN; EL NEMR, 2020b). Xenobiotic compounds such as pesticides and their residues can change temperature and pH in soils and water (BHATTACHARJEE et al., 2014), which has an impact on macro- and microorganisms. This condition affects an array of metabolic processes, photosynthesis, growth (DELCOUR; SPANOGHE; UYTTENDAELE, 2015; LIU et al., 2014) and the environment, with important effects on climate change, since the role of microbes on C and N cycles are important to regulate the greenhouse effect (DELCOUR; SPANOGHE; UYTTENDAELE, 2015; PINGALI, 2012; PINGALI et al., 2010). Despite this scenario, the use of pesticides has been kept in constant or increasing rates, and in high concentrations, likely due to the ever increasing rates in human population in recent decades, which proportionally increase the demand for food production, which in turn, is linked to the impact on natural resources such as soil and water. It is important to emphasize that the quality and quantity of these mentioned resources substantially decrease with the increase in pollution, and will deteriorate even further with the effects of climate change (GADABU, 2020; PINGALI, 2012; POPP; PETŐ; NAGY, 2013). All these aspects point to a view that finding methods that can reduce the levels of these compounds in soil and water is a key scientific challenge faced today. In order to contribute to this issue, a systematic review (SR) was carried out in this study to assess the current knowledge on biodegradation of xenobiotic pesticides in soil and water mediated by *Bacillus* spp..

A SR can be seen as a hybrid research technique that is based on the collection and analysis of published empirical data (WILT; FINK, 2007). The results of a SR provide a consistent understanding of trends and gaps of any given topic, contributing to generation of further relevant research questions, decision-making processes or problemsolving approaches (KOUTSOS; MENEXES; DORDAS, 2019). Two important databases currently available, *PubMed* and *Scopus*, allowed us to obtain representative scientific information on the problem posed, since they contain a large amount of peer-reviewed journals (Table 1). Considering the large amount of publications accessible in these databases, it was important to devise a more efficient search strategy, to narrow down the number of retrieved studies specifically related to the research topic. The results indicated that consolidating the 10 different keywords combinations into separate strings, applied to both databases, was more efficient than devising single but complex strings covering all keywords (Table 2). A fairly specific number of studies were returned, with a large proportion of those being directly related to the area of study (Fig. 3). The rank of studies covering the four types of pesticides obtained (i.e., Insecticide > Herbicide > Fungicide > Nematicide; Table 1), essentially confirmed literature indications that the production of pesticide increase ~11% each year (at least until the outbreak of the Covid-19 pandemic), where the most common ones were the first three in that order (BAĆMAGA; WYSZKOWSKA; KUCHARSKI, 2017). The organophosphate insecticides have been reported as the most used type of pesticide, with a special highlight of applications in some parts of the world, such as in India (SINGH; KAUR; SINGH, 2012).

Previous reports have mentioned that *Bacillus* spp. microorganisms are capable of degrading an array of xenobiotic compounds, such as the pesticides' active principles (ARORA, 2020; CHANDRASHEKAR et al., 2017; WIJEKOON; YAPA, 2018), as well as aromatic compounds present in these chemicals, which are recalcitrant to degradation and tend to become persistent residues in the environment (DOOLOTKELDIEVA; KONURBAEVA; BOBUSHEVA, 2018c; KRISHNA; PHILIP, 2009, 2011; SHARMA; SINGH; GUPTA, 2014). The number of studies per year was assessed to evaluate the trends in *Bacillus*-mediated remediation research, and to have a notion on how representative our systematic sampling of articles was (Fig. 4A). Studies on biodegradation of pesticides and their residues in soil and water began around 1967, a time in which a large number of pesticides groups had been already developed. Therefore, it was not unexpected that, by that time, a considerable amount of pesticide residues had already contaminated and accumulated soil and water bodies. These conditions have been posing a harm not only to the natural and anthropogenic environmental systems (pollution issues), but also to the consumption of agricultural products themselves, as the pesticides residues can be incorporated systemically (by bioaccumulation and bioamplification) in animals, grains, fruits and vegetables, so creating a chronic poisoning effect to humans (HASSAAN; EL NEMR, 2020a; HUANG et al., 2018). Interestingly, the trend observed in Fig. 4A seems to correlate well to the exponential increase in human population of the turning of 20th to 21st century, which naturally raised the demand for food, which in turn pressed towards increase of agrochemicals applications. Hence, this would nicely explain the observed increase in the biodegradation studies in both water and soil shown in Fig. 4A.

Although there are many types of pesticides whose usage have been restricted in various parts of the world, there are some like *Proturon* that have yet been used in India, especially in the 2009–10 years (GIRI et al., 2017a). In addition, the access to, and the price of certain pesticides, such as glyphosate (DIAS; ROCHA; SOARES, 2019), have become very accessible to anyone, without a corresponding regulatory enforcement for purchase and application limitations (HÉBERT; FUGÈRE; GONZALEZ, 2019). In the 2000's years, direct investments in agriculture have temporarilly declined, which affected food prices in 2008 (PINGALI, 2012). For this reason, investments in agricultural research, as well as on its derived aspects (such as impacts on the environment) have increased, a condition that has been reflected in the curve pattern represented in Fig. 4A (with the highest peak seen between 2014-2016, both for water and soil studies. Considering that the human population on earth will be approximately 8.9 million by the year 2050, food production will need to increase, causing a further impact on natural resources, such as water and soil (BURNEY; DAVIS; LOBELL, 2010; KUMAR; NAIN; SINGH, 2017). In this scenario, it is expected that 2.7 times more pesticides than in 2000 will be applied (PINGALI et al., 2010; WOŁEJKO et al., 2020), so that the development of remediation strategies (mostly based on microorganisms) in order to remove all or most of the pesticide residues will be certainly required (DURAISAMY; MUTHUSAMY; BALAKRISHNAN, 2018; KHAN et al., 2016). Hence, it is likely that the growth (or stabilization) of the curve in the graph of Fig. 4A will continue.,

An assessment of the countries from which studies of *Bacillus*-mediated pesticide biodegradation in soil and water have occurred provides a glance on where the amounts and intensity of pesticide usage, their environmental and health impacts, and the research needs can converge. The main country observed in this sense was India (Fig. 4B), likely due to the fact that it shows a large territorial area dedicated to agriculture, and where a high percentage of population (60-70%) depends heavily on this activity (BHADBHADE; SARNAIK; KANEKAR, 2002). It is worth mentioning that, despite the fact that there are pesticides that have been restricted or banned in various countries, India continues to use these xenobiotic compounds; in this context, contamination by residues such as *Lindane* and *Parathion* acquires scientific and public relevance (KRISHNA; PHILIP, 2011). The second country observed with high number of articles selected was China, which is registered as one of the largest consumers of agricultural chemicals, being responsible for ~30% of their use worldwide (WU et al., 2018). This country has a large production of rice, wheat and corn and, therefore, the use of pesticides in these areas is very intense. Interestingly, although relying mostly on chemical pest control, crop yields are not among the highest in the world, ranking around the world average (WEHMEYER; DE GUIA; CONNOR, 2020). Some authors suggest an approximately inverse relationship between the levels of pesticides usage and gross domestic product (GDP) per capita; The rationale is that if GDP is higher, the percentage of pesticide use decreases, likely due to an improved economic capacity to invest in technology and modern agricultural methods, such as integrated pest management (WU et al., 2018).

However, despite such a GDP-pesticide use relationship, poor regulatory frameworks of commercialization and application of agrochemicals can also affect their overuse. This can be observed, for instance, in Brazil and Central America where there is a not well-established pesticide use policy (specially in Brazil where this regulatory scenario has deteriorated even further in the last years; DIAS; ROCHA; SOARES, 2019; DAMASIO, 2019; RUETER; DAMASCENO, 2022), where prices are very accessible to anyone who wants to use them, and where there is a lack of safety control for the application of these xenobiotic compounds, causing pressure on the public health system due to acute and chronic intoxication cases (BRAVO et al., 2011; REMBISCHEVSKI; CALDAS, 2018). Only in 2010-2011, 936 thousand tons of pesticides were used in Brazil; This condition has been steadily increasing since then, with the use of transgenic seeds, being 45% of pesticides applied as herbicides, 14% as fungicides and 12% as insecticides (RIGOTTO; VASCONCELOS; ROCHA, 2014; RUETER; DAMASCENO, 2022). In terms of Central America, the largest imports of pesticides go to Guatemala and Costa Rica, with both countries having pesticide formulation companies for local use. In the period of 2000-2004, 16 pesticides that are classified as hazardous were still being used in these areas (BRAVO et al., 2011). An important aspect we must take into account is that there is no easy or fluid communication between small farmers and the agronomy extension experts. Under these conditions, the above mentioned combination of easy access to, and low prices for the pesticide products, inefficient use policies, and lack of safety regulations, all allow an increase in their use rates, and so, the levels of residues they leave in soils and water. An approach of helping small farmers to learn about new technologies and to become familiar to scientific methods can aid in the reduction of the excessive use of pesticides (BURNEY; DAVIS; LOBELL, 2010; PINGALI, 2012). At the same time, this can also help protecting the health of agricultural workers and their families, as well as important environmental resources, such as soil and water (GADABU, 2020; KUMAR et al., 2011).

Agrochemical and biological pesticides are useful and sometimes necessary for pest and diseases control in conventional agricultural areas, but, as seen above, problems arise from their uncontrolled and excessive use, and from the xenobiotic residues they generate. However, the very nature of the current pesticides' application technologies turns their use into a difficult challenge to overcome. It is worth mentioning that a number of pesticide compounds/formulations can be highly volatile, so that only ~1% of their applied active principles come into contact with the target organisms, whereas the remaining 99% is simply free to reach water, soil and non-target organisms (Fig. 5) (GANGIREDDYGARI et al., 2017). Therefore, integratively understanding the variations in pesticides' metabolisms within involved organisms, both target and non-target ones, can help generating less environmentally aggressive xenobiotic compounds, as well as more reasonable and effective application and remediation strategies (SHARMA; SINGH; GUPTA, 2014).

Residual pesticides in the soil and water tend to bioaccumulate and cause bioamplification (Fig. 5). This corresponds to the amount of pesticide that accumulates in animals' body (mostly in adipose tissues), which can increase its concentration from a small organism to a larger one through the food chain (e.g., humans) (HAKEEM; AKHTAR; ABDULLAH, 2016). Depending on the magnitude of this process, it can cause severe damage in animals and humans. A 2005's study showed that such bioamplified pesticides have led to reproductive deficiencies and liver and kidney malfunctioning in men (BELAL et al., 2018). The accumulation of pesticides in the environment can occur in different levels of soil, groundwater, rivers, lakes, etc., thus reaching fruits and vegetables, and so, bioamplifying in animals and humans through the food chain (AKTAR; SENGUPTA; CHOWDHURY, 2009; HASSAAN; EL NEMR, 2020b). Considering these circumstances, the naturally found bacteria with versatile metabolic capabilities, such as *Bacillus* spp. (JUÁREZ-RAMÍREZ et al., 2015; TANG; YOU, 2012), can display the specific ability to degrade those pesticide-derived xenobiotic compounds (including aromatic ones), thus offering excellent opportunities for development of bioremediation strategies (CAI et al., 2013; IBEKWE; PAPIERNIK; YANG, 2004; SHIMURA et al., 1999).

In order to carry out this metabolic process, the bacterial physiology requires certain conditions of physicochemical factors such as pH, temperature, humidity, dissolved oxygen in water, among others (BHATTACHARJEE et al., 2014; CASTAÑÓN-GONZÁLEZ et al., 2016). The first two factors above are the most relevant, since they are more directly involved in modulation of bacterial growth, and so, in the increase or decrease of metabolization rate of xenobiotic compound (BHATTACHARJEE et al., 2014; ISHAG et al., 2016). An important point to highlight is that these parameters are also affected by the levels of pesticides' residues in soil and water, therefore interfering in the structure of the bacterial community present in the area (DUTTA et al., 2016; GEED et al., 2017). Nevertheless, microorganisms manage to adapt to these contaminated environments, for which there are two important events: the first is the interaction that exists with other organisms, such as earthworms, which have the ability to regulate the soil pH, so being important when carrying out remediation processes (TANG et al., 2012; ZHU et al., 2019). The second event is the ability of bacteria to change and share genetic material among themselves; this helps with adaptation to environments with large amounts of xenobiotic residues due to incorporation and expression of genes conferring resistance to those compounds (HAN et al., 2014). In addition, the possibility of bacteria to work in consortium, do allow greater rates of pesticides' degradation (Fig. 5) (BHATTACHARJEE et al., 2014). *Bacillus* spp. are well known to work nicely in consortium with other gram-negative bacteria, such as *Pseudomonas* (GIRI et al., 2017b; YOUNESS et al., 2018). Therefore, studies with this type of bacteria could be promising in order to have a better biodegradation performance of pesticides and their residues.

The bioremediation processes take advantage of the natural metabolization of pesticides and other xenobiotic compounds by microorganisms, thus, contaminated soils and water can be restored (CYCOŃ; MROZIK; PIOTROWSKA-SEGET, 2017; ODUKKATHIL; VASUDEVAN, 2013; SINGH, 2008). Bioremediation has been considered as advantageous when compared to other chemical and physical remediation processes, due to lower costs and environmental friendliness (JAISWAL et al., 2019; UQAB; MUDASIR; NAZIR, 2016). Nevertheless, some kmowledge limitations have to be overcome such as the understanding of the metabolization process, the interactive response between bacteria, the secondary products that can be formed and their toxicity, the physicochemical parameters of the environment and nutrients necessary for optimal growth of the bacteria, etc. In this review, we studied the *Bacillus* species that could be part of degradation processes of different groups of pesticides (Fig. 6) trying to acknowledge what is already known in this sense.

The *Bacillus* genus has the characteristic of forming spores, which allow it to survive in extreme and contaminated environments (DUTTA et al., 2016; NASUTION et al., 2018). Moreover, its open pangenomic constitution and plasticity (ALCARAZ et al., 2010) allow the genus to broaden its adaptive capacity; for instance, the *Bacillus* sp. JF8 strain (Fig. 6, within *Bacillus* group) has acquired the trait of being a thermophilic bacterium, and several strains display the ability to produce phosphotriesterases and carboxylesterases that are involved in hydrolytic pathways, including those based upon aromatic compounds as carbon sources (SHIMURA et al., 1999). This latter trait is especially important for bioremediation, since most of the pesticide groups have aromatic compounds in their chemical structure, which are recalcitrant to degradation and, therefore, long-lasting in the environment (CAI et al., 2013; IBEKWE; PAPIERNIK; YANG, 2004; SHIMURA et al., 1999). Usually, reductive dechlorination is a anaerobic bioremediation process applied for some aromatic compounds, in which bacteria uses the chlorinated compounds as electron acceptors molecules to aid in their respiration, generating organic molecules, water and chloride (POPEK, 2018); for example, this is a procedure used for *lindane* degradation, where this reaction allows the resulting benzene to be better mineralized by bacteria (KRISHNA; PHILIP, 2009).

In addition to the fact that *Bacillus* spp. can be found in various types of pesticide-contaminated soils, some strains are also found in aquatic niches, such as coral reefs. Considering that corals develop symbiotic relations with bacteria, fungi and viruses (SABDONO; RADJASA, 2008), it would not be unexpected to find bacteria showing the ability to degrade xenobiotic compounds, as it is well-known that sediments of these ecosystems can be full of pesticide residues (GADABU, 2020). This was confirmed from our findings in this review, such as the example of the pesticide-degrading *Bacillus* sp. SP8 strain, which was isolated from corals off the coast of India (Fig 6). This suggests that there is a large reservoir in the aquatic world to be explored, in the search for enzymes that may be useful in pesticides degradation/remediation. The different temperature and pH that can be found in marine environments suggest specific physicochemical characteristics and adapted physiologies to the bacterium and its enzymes (DI DONATO et al., 2018). As it can be seen in Fig. 6, we have a large number of pesticide-degrading *Bacillus* species; however, for many of them we have not been able to fully characterize the metabolic pathways' steps of such degradation processes, so the lack of knowledge on their secondary generated products yet remains as a limitation. This is an important factor to consider, since the toxicity of this secondary compound may be greater than the initial pesticide residue (SHIMURA et al., 1999; ZHANG et al., 2012)

Bacillus spp. are microorganisms that plants tend to “prefer” to harbor as rhizobacteria/endophytes, because they can help in plant growth during abiotic and biotic stresses, and under conditions of high pesticide contamination (CORRALES RAMÍREZ et al., 2017; RADHAKRISHNAN; HASHEM; ABD ALLAH, 2017). Strains present in the soil can convert phosphorus (P) and nitrogen (N) into simpler forms so they can be absorbed by the plant. P is an essential element for central cellular processes, such as phospholipids, ATP and nucleic acid metabolisms, whereas N is required for the processing of proteins and amino acids. An important event in which *Bacillus* spp. have shown to participate is in helping plants to tolerate drought (RADHAKRISHNAN; HASHEM; ABD ALLAH, 2017). The conditions of global warming and climate changes are expected to cause increased salinity to soils, in addition to the water deficits; it has been shown that *Bacillus* can be present and beneficial in both cases, ameliorating the stress effects and increasing photosynthetic gains (EL-BESTAWY et al., 2013; SHRIVASTAVA; KUMAR, 2015). These benefits are likely due to certain *Bacillus*' biological activities such as the synthesis of lipopeptides that may be involved in systemic resistance in plants (RANI et al., 2019). In this genus, there are also the synthesis of enzymes that can be released and help to eliminate the formation of ROS in the plant cells, especially in the presence of heavy metals such as Zn and Cu (RADHAKRISHNAN; HASHEM; ABD ALLAH, 2017). A study showed that *Bacillus* could be present both in soil and the plant and, in both cases, degradation of 3,4DCA could be achieved, so that this type of bacteria can protect plants from the toxicity of pesticides such as *Diuron* or *Endosulfan* (RANI et al., 2019; SINGH; SINGLA, 2019)

In this study, we were able to collect data that showed us how much pesticide different species of the genus can tolerate (Table 3). The different concentrations that were tested have likely being due to preliminary information available for each experimental system, although each species/strain naturally has its own time of growth and adaptation to the exposure of these compounds (GEED et al., 2017; RODRÍGUEZ et al., 2020). Generally, each bacterium has different pathways to metabolize different pesticides and use them as different sources of electron acceptors or carbon sources; for example, strains of *B. cereus* use glyphosate as a source of carbon and phosphorus (LIU et al., 2015; WIJEKOON; YAPA, 2018). We could notice from Table 3 that the three main bacterial species from the *Bacillus* genus tested for pesticides/xenobiotics degradation were *B. cereus*, *B. subtilis* and *B. megaterium*. The first two have important roles in the N cycle, while the *B. megaterium* strains have the characteristic of being thermostable, which allows them to have a better metabolic activity (CORRALES RAMÍREZ et al., 2017). We can also mention the relevance of *B. subtilis* for plants, as it has been reported that strains from this species appeared to help increasing the length of plant roots and leaves, besides improving fruit yield. Overall, the studies included in this review showed that increasing pesticide concentration in the bacterial media caused reduced rates of pesticide degradation (Table 3). Due to this effect, the authors opted to add another carbon source (such as glucose or dextrose) to increase growth in the initial stages, which has caused higher degradation yields. under anaerobic conditions, the carbon source used was glucose, because it also serves as an electron donor (GIRI et al., 2017a; JAGNOW; HAIDER; ELLWARDT, 1977; TANG; YOU, 2012).

Associated genes and proteins are important elements for the understanding of pesticides' metabolisms in *Bacillus*. As it could be summarized in Table 4, most of the genes found in this study are involved with hydrolytic processes. This is certainly due to the fact that bacterial adaptation for xenobiotics' degradation requires a first metabolic transformation, in which the initial pesticide compound change into a less toxic product, with the characteristic of being more water soluble. For this first step to occur, oxidation, reduction or hydrolytic reactions are present. An important feature of general hydrolysis is the catalytic process performed by hydrolases that can be carried out in the absence of redox cofactors, so being appropriate target proteins/genes for studies on pesticide metabolization ability (LUSHCHAK et al., 2018; VERMA; JAISWAL; SAGAR, 2014). As it can be observed in Table 4, genes encoding hydrolases such as *opdA* and *oph* are in charge of specifically degrading organophosphate compounds (GIRI et al., 2017). The *opdA* gene is reported to be found in plasmids, although this was not found in the study included in this review; therefore, this gene could also be found in the chromosome (ALI et al., 2012). As further examples, the *atzA* and *trzN* genes encode hydrolytic enzymes involved in the degradation of *atrazine* (WANG et al., 2014). The *atzA* is the first enzyme to be used, but proteins encoded from *atzA* through *atzF* are also needed. This gene was isolated from a *Pseudomonas* sp. plasmid pADP1 (MARTINEZ et al., 2001). The *atzA* can be interchanged with the *trzN* gene, but due to a difficulty it presents in expressing itself in heterologous organisms, *atzA* is the most commonly used gene for expression (SCOTT et al., 2008). Other genes, e.g., *tfdA*, are found in a relevant group of bacterial species, such as those from *Bacillus* and *Halomonas* sp. (HAN et al., 2014; HOGAN et al., 1997). There are also P450 genes that are important for organophosphates' detoxification; although they show a wide range of substrates and the characteristic of enantiospecific hydrolyzing activities, they yet require redox cofactors. This is usually a limitation when it comes to the process of bacterial biodegradation of pesticides (GADABU, 2020; SINGH; SINGLA, 2019; URLACHER; LUTZ-WAHL; SCHMID, 2004)

At last, it is important to mention that, based on the methodology of our systematic review, no specific work was found that was directly comparing the efficiency/effectiveness between *Bacillus* application and other type of remediation processes (i.e., use of chemical, composts-forming or natural degradation methods). Hence, we were limited to comparisons between *Bacillus* treatments and no application (controls) or comparison between single or *in consortium* applications of *Bacillus* strains. Overall, meta-analyses showed that the treatment with strains *Bacillus* spp. for the biodegradation of pesticides and residues in soil and water is significantly favorable (Figs. 7 and 8). The results suggest that the application of *Bacillus* allows accelerating the degradation of pesticide residues in soil and water, and that the use of its strains, so far, showed no difference in efficiency when used alone or in consortium with other bacteria. Therefore, in terms of defining bioremediation strategies for environments polluted with xenobiotics, the choice between single or combined species applications can be made based on the higher capacity of degrading different types of compounds that the use of bacterial consortia can provide.

We wish to point out that in this systematic review we not only highlighted the importance of soil and water remediation but also the role of microorganisms in this process. Above all, through a qualitative and quantitative analysis, we highlighted the role played by the *Bacillus* genus as a potential remedy for environments contaminated with pesticides. In addition, it is also worth mentioning the role that OMIC's tools have in the identification and analysis of genes, proteins, secondary metabolites and metabolic pathways (JOHNSON, IVANISEVIC & SIUZDAK, 2016); their high-throughput outcomes allow us to advance in the practical understanding of the potential of *Bacillus* species to act as bioremediators in a diverse range of environments contaminated with pesticides.

6. CONCLUSIONS

- Our systematic review was representative of the scientific literature concerning the use of the *Bacillus* genus in bioremediation processes of pesticide residues in the soil and water environments.
- In this sense, our analysis showed that a raise in the number of studies published about this issue was noticed after the first 5 years of this century, with India and China being the countries leading this type of research worldwide.
- There were more studies directed to the soil than to aquatic environments, with residues from herbicides and insecticides being the main xenobiotic compounds studied.
- The physico-chemical variables most studied in pesticides' residues biodegradation by *Bacillus* spp. are temperature, pH, oxygen demand in water, and organic matter (quantitative), as well as type of soil (categorical).
- Among the 30 *Bacillus* species identified in the selected studies, only three of them together (*B. cereus*, *B. subtilis* and *B. megaterium*) comprised 30% of all 184 strains found in the selected studies; about 36% of all strains were not identified up to species level.
- The number of studies reporting genes/proteins involved in the biodegradation metabolism of pesticide residues was relatively scarce, with 14 different types of genes being found, most of them related to hydrolytic enzymes.
- Despite that no study was found directly comparing *Bacillus* strains with other specific type of degradation process (chemical or otherwise), metanalyses indicated these microorganisms have statistically significant degradation potential of pesticide residues, alone or *in consortium* with other bacteria, when compared with controls.

Putting everything into perspective, our results and study suggest that further studies of catabolic genes, proteins involved, and metabolic pathways are needed. This will allow the development of more effective and efficient bioremediation processes. Generating more systematic review studies in the area of agriculture and climate change seems necessary to make more accurate decisions that can positively impact on the improvement of aspects related to environment, society and the economy. The deficit in pesticide control is a phenomenon that will continue to affect human activities, despite the search for other ways to control pests/pathogens and reduce the use of chemically-derived xenobiotics as the main source of pesticides. Therefore, pesticide regulation and education on how to handle and protect people should be a major initiative of large companies, small farmers and governments. We should take into account new technologies for study and promote new ideas to generate future pesticides, such as biopesticides based on RNAi; In this sense, OMIC's tools can give us extensive information on bacterial physiologies, genetic make-ups and metabolic arsenal akin to xenobiotics degradation processes. *Bacillus* spp. are indeed good candidates for biotechnological development towards bioremediation methods. This genus contains a variety of species that can resist to different physicochemical conditions, besides producing proteins and harboring genes that allow them to show high levels of pesticide biodegradation. As suggested by the studies analyzed in this review and meta-analysis, despite the lack of statistically significant differences, consortia of *Bacillus* spp. with other bacteria tend to be more favorable than the use of a single microorganism, as the joint degradation of secondary products can be achieved.

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8. APPENDIX

Appendix 1: strings used for search in PubMed and SCOPUS.

Strings
“bacillus” and “bioremediation” or “phytoremediation” or “degradation” and “pesticides” and “soil”
"bacillus” and "bioremediation” or “phytoremediation” or “degradation" and "herbicides" and "soil"
"bacillus" and "bioremediation or phytoremediation or degradation" and "insecticides" and "soil"
"bacillus" and "bioremediation or phytoremediation or degradation" and "fungicides" and "soil"
“Nematicide” and “bioremediation”
"bacillus" and "bioremediation or phytoremediation or degradation" and "pesticides" and "water"
"bacillus" or "bioremediation or phytoremediation or degradation" and "herbicides" and "water"
"bacillus" and "bioremediation or phytoremediation or degradation" and "insecticides" and "water"
"bacillus" and "bioremediation or phytoremediation or degradation" and "fungicides" and "water"
"bacillus" and "bioremediation or phytoremediation or degradation" and "nematocides" and "water"