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Effectiveness of Effective Microorganisms (EM) for Bioremediation of Hydrocarbon Polluted Soils- A Systematic Review

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ABSTRACT
Increasing development continued population growth and heavy demand and dependence on petrochemical products have led to exceptional economic growth and development. However, inevitably this craving for fossil fuels has caused serious environmental issues over recent decades. The eco-toxicity and the potential health implications that petroleum hydrocarbons pose for both environmental and human health have led to increased curiosity in developing environmental biotechnology-based methodologies to detoxify environments impacted by petrogenic compounds. Different approaches have been applied for remediating polluted sites with petroleum derivatives. Bioremediation represents an environmentally sustainable and economical emerging technology for maximizing the metabolism of organic pollutants and minimizing the ecological effects of oil spills. Bioremediation relies on microbial metabolic activities in the presence of optimal ecological factors and necessary nutrients to transform organic pollutants such as petrogenic hydrocarbons. A comprehensive search for original articles in three different databases, (Scopus 18, Springer 68, and Google scholar 58 kinds of literature respectively), was performed. We included in our search documents published from 2010 to 2022. After the screening process and the addition of gray literature publications, we obtained 23 articles for theoretical analysis. Results. The studies were distributed mainly in Asia and part of Africa, without any important temporal variation. They also showed a tendency to describe the use and its potential of effective microorganisms and other microbes for bioremediation of hydrocarbon polluted soils. Besides the mechanisms of enhancing soil physical, chemical, and biological properties, Conclusively, based on the review findings, effective microorganisms can serve as a potential medium for bioremediation of crude oil polluted soils, the literatures reviewed showed a percentage composition of bacteria 16% pseudomonas, 13% bacilli and 10% Rhodobacteria, and fungi 20% Cunninghamellae, 14% Mycorrhiza, Planerchaetechry osporum and 13% Aspergillus spp, Fusarrium spp, Craphium spp and penicillium, the synergy of these bacteria and fungi which has been found to enhance crude oil bioremediation has an advantage over the other bioremediation methods, therefore recommends that, effective microorganisms can effectively serve as a bioremediation medium for hydrocarbon polluted soils, it should be seen as a bio fertilizer which enhance soil structure and nutrient availability to crops for healthy food production and a product for bioremediation and reclaiming the soil and water from hydrocarbon pollution .

Keywords: Effective Microorganisms (EM), Hydrocarbon, Bioremediation

INTRODUCTION

Currently, hydrocarbons represent one of the most significant causes of environmental contamination affecting the soils for agricultural uses. The accelerating growth of the World's population which was estimated to have exceeded 7.954 billion people as of November 2022 (World population Wikipedia, 2022), has a great Threat to the scarcely available land. World Health Organization (WHO) estimates that, by 2029, the world's food production will have to be increased by 50% (Chittora, et al; 2020). Agricultural soil in the world is severely damaged by hydrocarbon contamination and the use of chemical fertilizers band excessive irrigation, which has led to erosion, nutrient depletion, and salinity.

The perception of effective microorganisms (EM) is centered on the inoculation of the substrates to shift the bacterial stability and thus create an enhanced ecology that favors better productivity. Photosynthetic bacteria are the backbone of the EM, working synergistically with other microorganisms to provide the nutritional requirement to the plant and also reduce the disease problem. There are primarily 5 types of microbes used to formulate EM solution. Photosynthetic microbes (Phototrophic bacteria): are independent self-supporting microorganisms. These bacteria synthesize amino acids, nucleic acids, bioactive substances and sugars, substances from secretions of roots, organic matter (carbon) by using sunlight and the heat of soil as sources of energy. Lactic acid bacteria: produces lactic acid from sugars. Food and drinks such as yogurt and pickles have been made by using lactic acid bacteria. However, lactic acid is a strong sterilizer. It suppresses harmful microorganisms and increases the rapid decomposition of organic matter. Yeasts: synthesize antimicrobial and useful substances for plant growth from amino acids and sugars secreted by photosynthetic bacteria, organic matter, and plant roots. *Actinomycetes*: are the structure of which is intermediate to that of bacteria and fungi, produces antimicrobial substances from amino acids secreted by photosynthetic bacteria and organic matter. These antimicrobial substances suppress harmful fungi and bacteria. Fermenting Fungi: such as *Aspergillus* and *Penicillium* decompose organic matter rapidly to produce alcohol, esters, and antimicrobial

substances. These suppress odors and prevent infestation of harmful insects and maggots.

Pollution reflected the entrance of undesired substances into the ecosystem. The Petroleum components must be separated into four fractions: aromatic, saturated, resin and asphaltene fractions analyzed through absorption chromatography (Bijay, et al., 2012). Due to the high energy content hydrocarbons are the world's most commonly used primary energy and fuel resources. The anthropogeneous discharge of hydrocarbons into the environment is strongly connected exorbitant use of Petroleum products, which is extensively widespread all over the world (Winkelmann et al., 2019). The use of bioremediation by using microorganisms has been found many in the literature Das, et al; 2011, Gutiérrez, et al; 2014, Chen, et al; 2015, Agnello, et al; 2016, Feng, et al; 2017. Microbial remediation can be simply defined by employing microorganisms to lower the bioavailability of pollutants (especially organic contaminants) to make them less toxic to the ecosystem. These microorganisms are capable to break down (or metabolize) contaminants by using them as a food source. The introduction of specific competent strains of microorganisms has been widely used in the bioremediation of soil pollution Xu & Zhou (2017). According to Feng, et al; 2017, bioremediation is an environmentally friendly technology for cleaning up or recovering polluted soils.

. Hence, bioremediation can be considered one of the best technologies to deal with petroleum product contaminated soil. However, field data on the effective microorganisms mechanisms for bioremediation are limited and disorganized. Additionally, there is no current review articles focused on describing such mechanisms, which means that the knowledge about the topic is still insufficient, this is because EM is seen as addictive to manure or as a spray directly in the fields which increases the microfauna diversity of the soil. Based on the identified weaknesses/disadvantages or incompleteness of the microbial remediation (Chee Kong and Shih, 2019) in their findings recommended future studies should focus on how to increase the effectiveness of the bioremediation technology that should further reduce environmental stress on the terrestrial and aquatic ecosystems. However, even though a few successful fungal applications were reported (Rodriguez–Rodriguez et al. 2014), normally speaking, fungal bioaugmentation has been much less studied than bacterial-mediated bioremediation processes. In addition, to date, no research have investigated the bioaugmentation with reinoculation of both fungi or fungal–bacterial consortia for bioremediation of soils infected with excessive ranges of crude oil (Rodriguez–Rodriguez et al. 2014).

Therefore, through a systematic review of scientific literature published in the last 10 years, the main objective of this review was to describe the action of various effects of different methods of bioremediation on hydrocarbon contaminated soils and their relation to effective microorganisms.

Materials and Methods

*2.1. Search Strategy. T*he study was carried out following the Prisma statement, it proposes a methodology for systematic reviews. A systematic literature search was performed in Scopus, Springer, and Google scholar databases. The search was performed using the general route [(effectiveness of effective microorganisms in bioremediation, effectiveness of microorganisms in hydrocarbon contaminated soils, and bioremediation of hydrocarbon polluted soils)]. in Scopus, Springer, and Google scholar databases, the time limits “2011 to present” and “limit to 2011–2021,” respectively, were used to cover the scientific literature published between these dates.

In January 2022, the references found, together with the respective abstract, were registered in a Microsoft Office Excel database. Duplicates were eliminated.

Inclusion and Exclusion Criteria. The study included original research articles from the scientific literature of the last 10 years, written in English, which reported the effectiveness of effective microorganisms for bioremediation of hydrocarbon in soil, types of bioremediation, microbes involved in bioremediation, the composition of effective microorganisms (EM). Articles that describe bioremediation in water and phytoremediation were excluded to analyze only the action of bioremediation in the soil in natural and experimentally reproducible conditions. Data were extracted from each publication and tabulated for further analysis.

Reproducibility. The reproducibility of the study was assessed by using the search paths in the databases selected, together with the application of inclusion and exclusion criteria by one reader independently.

Analysis of Results. The data extracted and analyzed were related to the taxonomic classification of effective microorganisms, the mechanisms that intervene in soil hydrocarbon remediation, and the respective microbes involved in bioremediation, gathered were employed.

Results

After using the search protocol indicated, a total of 144 articles were obtained (Scopus 18, Springer 68, and Google scholar 58). Once 38 duplicates were deleted at the Microsoft Excel database, 106 publications were evaluated based on their title and abstract content; 54 of them were discarded since they did not meet the inclusion criteria. A total of 52 articles were analyzed; 35 of them met the exclusion criteria, the search protocol resulted thus in a total of articles selected for systematic review (Figure 1).

Three articles classified as a thesis or a dissertation were added exhaustively for a total of 20 papers obtained, following the same inclusion and exclusion criteria. This gray literature was found in Google Scholar (Table 1, Figure 1).

Distribution of Studies according to the Country of Origin and Publication Year.

The systematic search of the scientific literature published between 2010 and 2021 resulted in a total of 29 articles, carried out in 12 different countries, among which India stands out with 5 publications, China, Australia, and Nigeria stand out with 2 publications respectively, followed by Iraq, Canada, Japan, Mexico, Norway, Malaysia, Libya, Ethiopia and Romania with 1 publication. The high number of publications in these countries is probably due to knowledge of organic farming which involves the use of effective microorganisms Shahbandeh, (2022)

3.2. Research Objectives. Although all literature analyzed was determined by the inclusion and exclusion criteria established and is based on the same research premise, their diverse research objectives can be classified into four subgroups according to certain general features (Table 1). Simultaneously, the objectives can be divided into two groups. the first one is the processes of bioremediation of hydrocarbon contaminated soils which is the most predominant study analyzed in this systematic review, the second group, contains the efficacy of microbes in bioremediation through different methodologies

Discussion

Effective microorganisms used for bioremediation. Effective Microorganisms (EM) is a mixed culture of beneficial microorganisms that can be applied as inoculants to shift the microbial diversity of soils and plants in ways that can improve soil quality, and the growth, yield, and quality of crops. The microorganisms comprising EM are neither exotic nor engineered types but are naturally-occurring species that have been isolated from natural environments worldwide and selected for their specific beneficial effects and compatibility in mixed cultures (Higa, 2013). Degradation of an natural compound through indigenous

microbes with none synthetic enhancement is called an "intrinsic bioremediation" and that is one of the first-class remedial moves for soil contamination. Generally, biodegradation method mineralization of natural components to the soluble inorganic compounds or to convert natural components to different soluble natural compounds. The microbes present in the soil first recognize the oil and its constituent by biosurfactants and bio emulsifiers, and then they attach themselves and use the hydrocarbon present in the petroleum as a source of energy and carbon Bijay, et al: 2012]. The low solubility and adsorption of high molecular weight hydrocarbons limit their availability to microorganisms. The addition of biosurfactants enhances the solubility and removal of these contaminants, improving oil biodegradation rates [http://www.pollutionissues.com/, 2011]. Microorganisms produce enzymes in the presence of carbon sources which are responsible for attacking the hydrocarbon molecules. Many different enzymes and metabolic pathways are involved to degrade hydrocarbons contained in petroleum. But lack of an appropriate enzyme will either prevents attack or will act as a barrier to complete hydrocarbon degradation

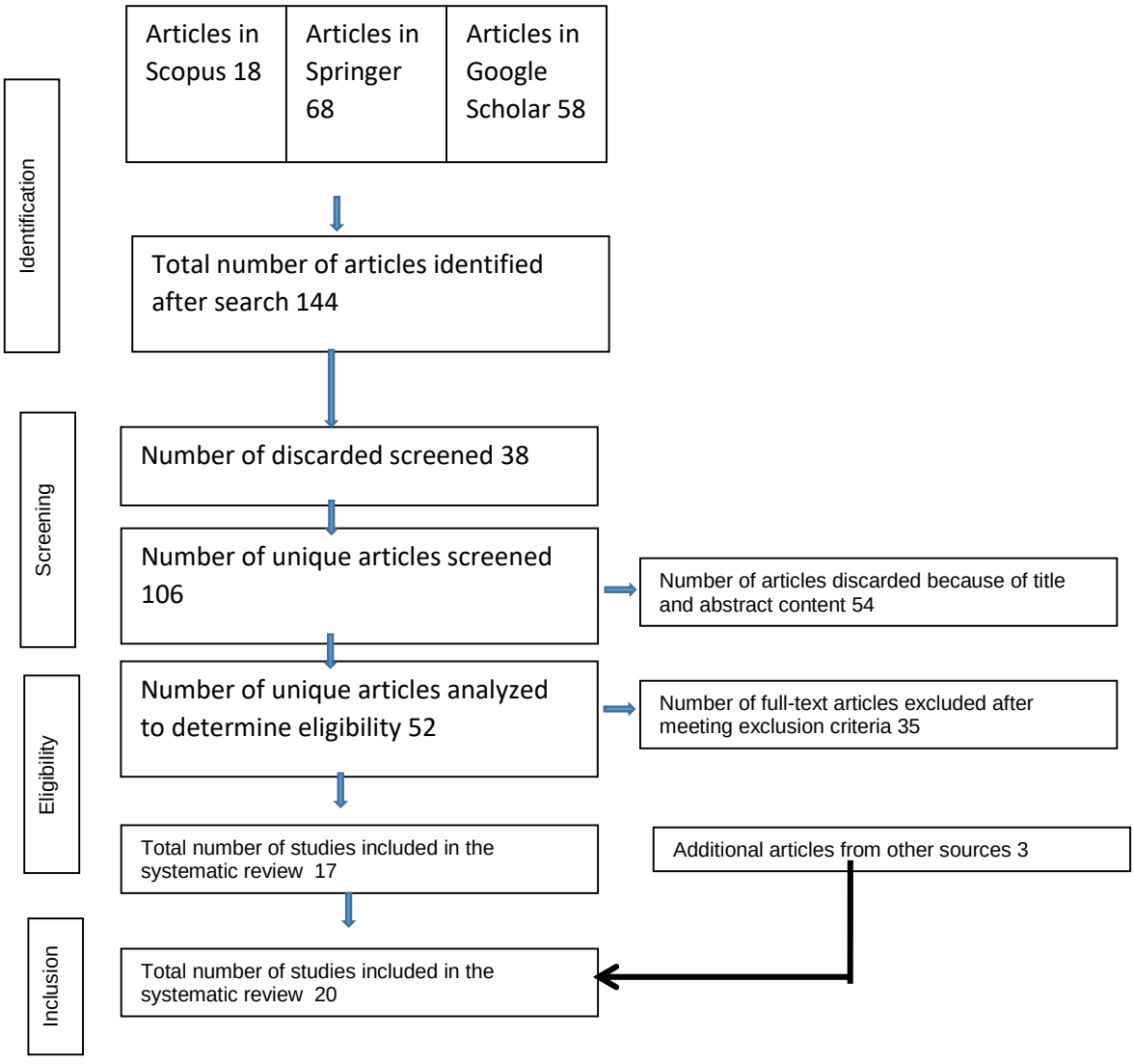

Figure 1: Flowchart representing the search protocol used for the systematic review

Table 1: Research Objectives

Research objectives	Reference
To determine the process of bioremediation of hydrocarbon contaminated soils	Bijay et al; 2012. Mandalaywala & Ratna Trivedi; 2016, Dobos & Carmen, 2011
To identify bacterial treads responsible for bioremediation of hydrocarbon contaminated soils	Chee &Shih ; 2019
Advantages and weaknesses of microbial bioremediation	Rasheed et al, 2016

Action Mechanisms of Bioremediation

Hydrocarbons are insoluble in water, thus to consume hydrocarbon derivatives, microorganisms need to emulsify it first in the solution or medium. For this, they are known to produce surface-active agents i.e., biosurfactants thus rendering the hydrocarbons susceptible to biodegradation [Panda & Panda (2013)]. The microbes present in the soil first recognize the oil and its constituent by biosurfactants and bio emulsifiers, and then they attach themselves and use the hydrocarbon present in the petroleum as a source of energy and carbon [APHA & AAWI (2012)]. The low solubility and adsorption of high molecular weight hydrocarbons limit their availability to microorganisms. The addition of biosurfactants enhances the solubility and removal of these contaminants, improving oil biodegradation rates [http://www.pollutionissues.com/Na-Ph/Petroleum.html, (2011)]. Microorganisms produce enzymes in the presence of carbon sources which are responsible for attacking the hydrocarbon molecules. (Bijay, et al; (2012). In presence of oxygen complete degradation of oil takes place. If large quantities of oil are present, the oxygen in the soil will be depleted very fast causing the anaerobic condition.

Anaerobic bacteria will use other electron acceptors like nitrate, iron, or sulfate, but the energy yield for the bacteria is less than oxygen used as the electron acceptor Braddock, et al; (2019). The lower energy yield by anaerobic bacteria results in lower degradation and hence a longer period is required for remediation

Fungal PAH Degradation Like bacteria, the preliminary step of fungal PAH metabolism includes the creation of atmospheric oxygen to the aromatic nucleus. Non-ligninolytic fungi have a tendency to make use of cytochrome P-450 monooxygenase enzymes to comprise oxygen, ensuing in arene oxide intermediates. These intermediates can both go through similarly metabolism with the aid of using epoxide hydrolase to shape trans-dihydrodiols or go through non-enzymatic

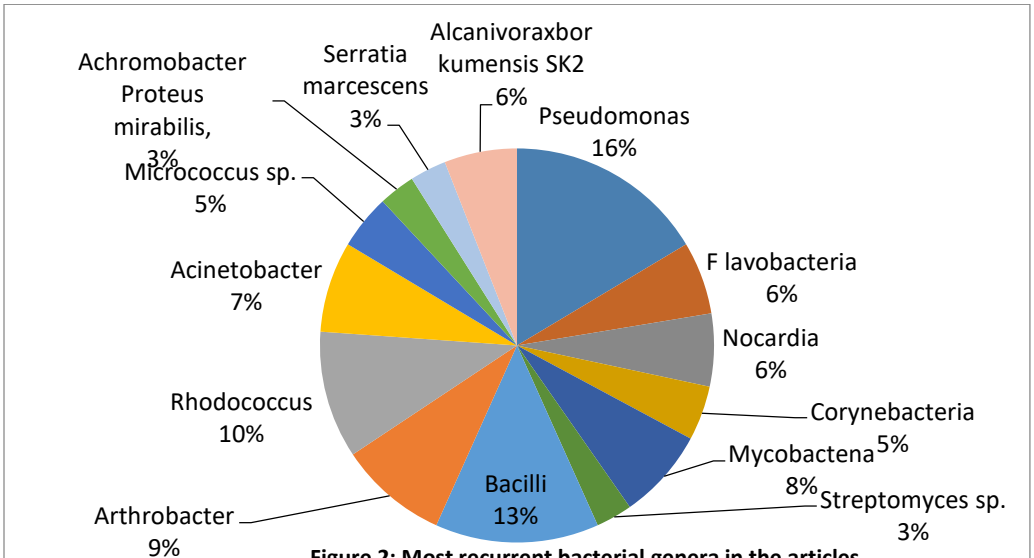

Figure 2: Most recurrent bacterial genera in the articles reviewed uesd for bioremediation of hydrocarbon. Source: own elaboration

Bacterias that can degrade petroleum products are- *Pseudomonas, Aeromonas, Moraxella, Beijerinckia, F lavobacteria, chrobacteria, Nocardia ,Corynebacteria, Atinetobacter, Mycobactena, ,Modococci , Streptomyces, Bacilli, Arthrobacter,Aeromonas, Cyanobacteria etc.* . (Bijay, et al: 2012). Another xenobiotic that pollutes the surroundings is oil aggregate or crude soil. This form of xenobiotic also causes severe environmental harm while oil waste is launched into the surroundings. Bioremediation of this xenobiotic via microbes, which include Aspergillus terreus, A. sulphureus, Nocardia spp., Pseudomonas spp., Rhodococcus rhodochrous, and Streptomyces sp. (Sorkhoh et al. 2019), Acinetobacter calcoaceticum, lavobacterium sp., and Pseudomonas aeruginosa (Mandri and Lin 2010), Bacillus cereus (Kebria et al. 2019), Bacillus sp., and Enterobacter sp. (Zhang et al. 2010), Micrococcus sp. Pseudomonas sp. (Nikhil et al. 2013), **Effective microbial consortium**

The advantages of employing mixed cultures as opposed to pure cultures in bioremediation have been well documented. The degradative capacity of any microbial consortium is not necessarily the result of merely adding together the capacities of the individual strains forming the association. It could be attributed to the effects of synergistic interactions among members of the association Mandalaywala & Trivedi R (2016]. The most important groups of microorganisms involved in the biodegradation of hydrocarbons are bacteria (*Achromobacter* sp., *Acinetobacter* sp., *Alcaligenes* sp., *Arthrobacter* sp., *Bacillus* sp., *Brevibacterium* sp., *Corynebacterium* sp., *Flavobacterium* sp., *Micrococcus* sp., *Mycobacterium* sp., *Nocardia* sp., *Pseudomonas* sp., *Spirillum* sp., *Serratia* sp., *Rhodococcus* sp., *Vibrio* sp.) and fungi (*Aspergillus* sp., *Cephalosporium* sp., *Cladosporium sp., Fusarium* sp., *Graphium* sp., *Geotrichum* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Trichoderma sp.*). (Dobos & Carmen (2011).)

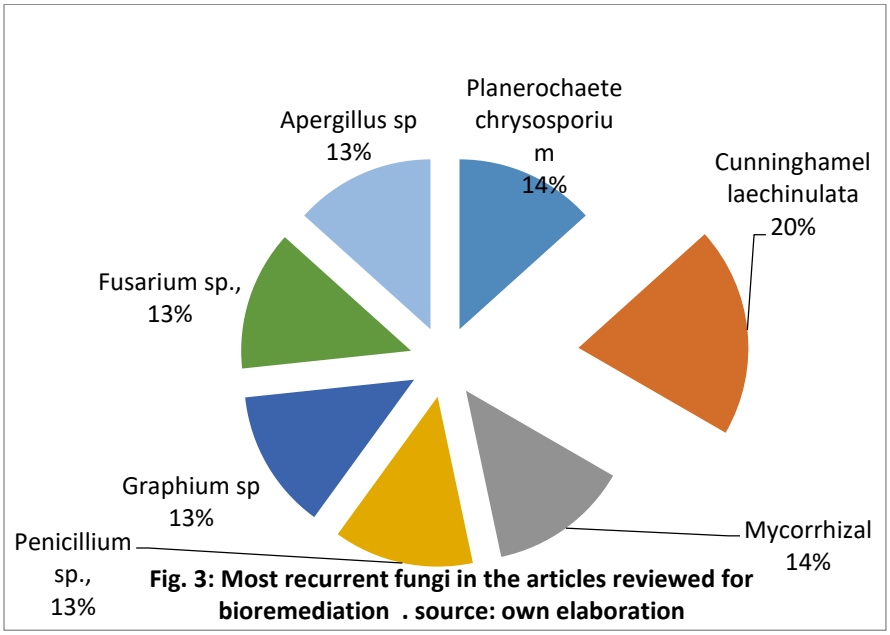

Fig. 3: Most recurrent fungi in the articles reviewed for bioremediation . source: own elaboration

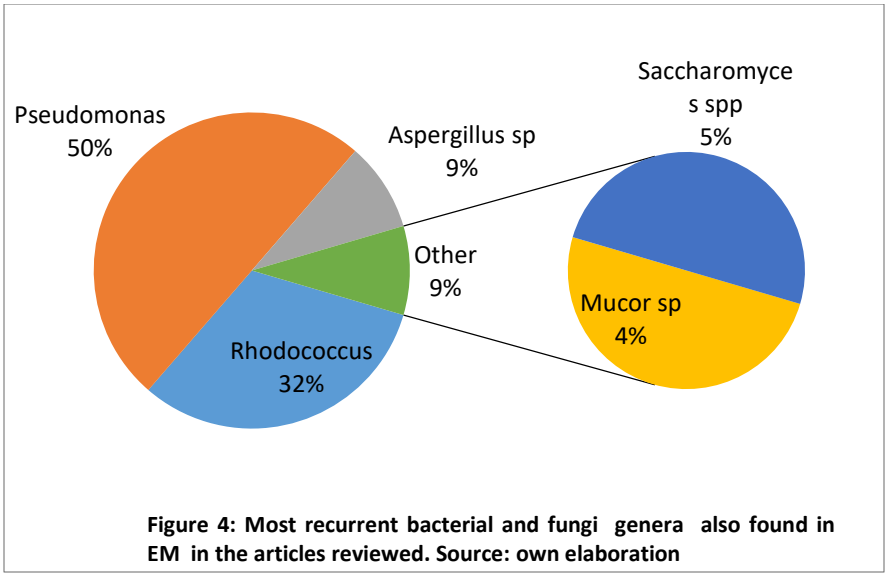

Figure 4: Most recurrent bacterial and fungi genera also found in EM in the articles reviewed. Source: own elaboration

The importance of effective microorganisms can be summarized in five (5) theories as described by Rasheed et al, 2016.

1. Disease Suppressive Soil Theory The term disease-suppressive soil refers back to the organic manner of suppressing the prevalence of plant diseases. Three examples of disease-suppressive soil are: (1) the pathogen fails to grow to be established, (2) the pathogen is present however fails to motive disease, and (3) the pathogen causes disease however declines with monoculture. 2. Organic Energy Theory In the traditional theory, natural substances brought to soil go through decomposition with the aid of using microorganisms, and minerals (vitamins) are released and become available for uptake with the aid of using plants. 3. Inorganic Nutrient Solubilizing Theory Soil microorganisms are essential in decomposing natural substances and recycling their vitamins for uptake with the aid of using plants. Soil productiveness generally decreases as soil organic matter decreases. When this happens, the overall soil microbial populace and its biodiversity additionally have a tendency to decrease. 4. Balanced Population of Soil Microorganisms Theory The stability in population and variety among dangerous and useful microorganisms will determine the soil microbiological equilibrium; and whether or not the soil surroundings is favorable or negative to the increase and health of plants. 5. Photosynthetic and Nitrogen Fixing Theory When EM is applied to soil or plant leaf surfaces, the populations of photosynthetic micro organism and nitrogen-fixing micro organism increase dramatically. This phenomenon is related to the growth of more vigorous plants, better plant yields, and improved crop quality as compared with no EM treatment.

Conclusion

Based on the findings of the reviewed articles, effective microorganisms can be said to have the potential bacteria and fungi responsible for the bioremediation of hydrocarbon in soil. The most occurring bacteria such as pseudomonas, bacilli, and Rhodobacteria spp. which comprises 16%, 13%, and 10% respectively (Fig. 2) responsible for hydrocarbon degradation in soil are components of effective microorganisms, some fungi that have the potential for bioremediation as reviewed from the articles are also found as a component of effective microorganisms such as Aspergillus, Saccharomyces spp. and Mucor spp. (Fig. 3) Hence, effective microorganisms will be effective when used for hydrocarbon bioremediation because it will enhance the microbial population of only healthy microbes, it will not only remediate the soil but will also control the soil pathogens, enhance soil physical, chemical, and biological properties, it will improve soil fertility and crop productivity, it will speed up the rate of bioremediation processes because of its component of bacteria and fungi combination, it has no toxic effect because they are not chemical and hence will awaken the inherent indigenous beneficial microorganisms in the soil after bioremediation.

Gaps in literature

1. No recent literature on the use of effective microorganisms for bioremediation of hydrocarbons in soil
2. Few or no literature is available on the combined culture of bacteria and fungi for bioremediation of hydrocarbon in soil
3. Most literature on effective microorganisms under the period of review is based on agricultural productivity and not on environmental hydrocarbon control of soil.

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