Review

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Review

Use of Anaerobic Digestate as Soil Amendment for Fungi Inoculation in Soil Remediation: A Systematic Review

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Simple summary

The use of anaerobic digestate from bioenergy production as soil amendment is a promising alternative for plant growth and soil stabilization, nevertheless, the excessive accumulation of nutrients contained in digestate, such as phosphorous and nitrogen, could have negative impacts for the ecosystem. Furthermore, contaminants i.e. from mining or even industrial activities represent a latent risk for animals and humans, if they enter in the food chain. Both problems could be addressed when inoculating anaerobic digestate with fungi, for soil remediation. Through the present review, a systematic search was performed to find the scientific literature published during the past ten years regarding the inoculation of fungi in anaerobic digestate for plant growth and soil remediation. The combined application of anaerobic digestate and fungi contributed to contaminant reduction, improving soil structure. The absence of vegetation at certain phases of remediation may be necessary. Important findings were that biomass source used for anaerobic digestion plays a crucial role, whereas the solely use of cattle manure achieved the best fungi colonization and organic matter degradation. It was suggested that temperature resulted in higher remediation efficiencies. Further research must be done for specific contaminant, fungal strain, and vegetation.

Abstract

The need to research cost-effective alternatives for soil remediation has become an important topic in the recent years. Pollutants like heavy metals or pharmaceuticals are constantly being accumulated in soils, becoming hazardous for the environment, especially if they reach human or animal food-chain. On the other side, the accumulation of anaerobic digestate resulting from the biogas production process, could also represent a latent risk, due to its discharge as biofertilizers, whereas high quantities of nitrogen (N) and phosphorous (P) contained are accumulated. Digestate inoculation by means of fungi represents a cost-effective alternative to treat both, contaminated soils and anaerobic digestate. Through the present systematic review, scientific researches performed during the past ten years were summarized. Focus of this research was to understand the impacts of fungi and anaerobic digestate as soil amendment, for contaminant removal and plant growth. Soil stabilization and remediation showed to have a higher impact than plant growth itself. The origin of the biomass used for anaerobic digestion is very important to determine digestate quality and effect in the soil, being cattle manure more suitable for remediation purposes. Fungal strain, pH, temperature, and existing contaminants must be considered before a successfully practical application takes place.

Keywords: soil amendment; mycorrhizal; fungi; anaerobic digestate; soil remediation; contaminants

1. Introduction

Due to the population growth and human development, the environment is experimenting severe problems including climate change (temperature increase, sea level elevation), soil, water, and air pollution, biodiversity loss, overharvesting, and deforestation, among others. Consequences of environmental damage affect direct or indirect human being. Pollution can lead to public health problems, such as respiratory illnesses or waterborne diseases. Economic impacts in agriculture, livestock farming, fishing, or tourisms has been observed. Problems regarding resource scarcity, food chains affection, as well as environmental disasters are increasing worldwide. To overcome this problematic a multidisciplinary approach should be developed and implemented, whereas the greenhouse gas emissions mitigation, renewable energies use, pollution reduction, as well as natural habitats remediation should be in focus.

Anaerobic digestion (AD) has been widely used for the simultaneous green energy production and waste management. Together with the energy production in form of biogas, anaerobic digestate results after AD process. This digestate is nutrient-rich and can be used as a valuable fertilizer, due to its content of essential nutrients for plant growth. On the other side, mycoremediation refers to the use of fungi for removal or break down of pollutants from soil and water. Fungi, i.e. mycorrhiza or endophytic fungi have the capacity to degrade pollutants like heavy metals or pesticides, so that they are less harmful for the environment. Digestate could be used for a better fungi growth and thus a more efficient pollutant mycoremediation. Few researches have been carried out regarding this topic. This systematic review explores the scientific research done during the last ten years regarding the effect of anaerobic digestate as soil amendment to achieve better or more efficient rates of soil remediation through fungal strains.

1.1. Anaerobic Digestion Process

AD comprises a series of biochemical reactions in which different bacterial consortia break down organic matter into individual components, forming a mixture of gas called biogas [1]. Biogas is described as a byproduct of microbial metabolism, whereas different microorganisms convert organic matter almost entirely into biogas [2,3]. In principle, all kind of organic matter that is degraded under anaerobic conditions is suitable for biogas production, i.e. poultry droppings, agricultural crop wastes, or cattle manure [2]. However, not all organic matter components can be broken down by the same bacterial strains. Biomass with high woody substance are slowly decomposed, due to lignin content. The organic material used to produce biogas is called substrate. The substrate´s chemical composition, in particular carbohydrates, fats, and protein content, is decisive for the amount of biogas produced and its methane content.

As in (CH4: methane, CO2: carbon dioxide, H2: hydrogen) , the process of biogas production is divided in four steps; hydrolysis, acidification (acidogenesis), acetic acid formation (acetogenesis), and methane formation (methanogenesis). During the hydrolysis, the complex compounds of biomass (carbohydrates, proteins, and fats) are broken down into simpler organic compounds. Proteins become amino acids, carbohydrates become sugars, and fats become fatty acids. The hydrolytic bacteria involved in this process release enzymes that biochemically decompose material. In the acidification step, acid-forming bacteria break down the intermediate products in lower fatty acids like acetic, propionic, and butyric acid, as well as carbon dioxide and hydrogen. In addition, small amounts of lactic acid and alcohols are also formed. During the acetogenesis, products derived from acidogenesis are then converted by acetogenic bacteria into biogas precursors such as acetic acid, hydrogen, and carbon dioxide. In this step, water content can negatively affect the process. An excessively high hydrogen content prevents the conversion of the intermediate products for energetic reasons. Products which inhibit methane formation could be formed, such as organic acids, propionic acid, isobutyric acid, isovaleric acid, as well as caproic acid. For this reason, acetogenic bacteria (hydrogen producers) must be in close association with hydrogen-consuming methanogenic archaea, which consume hydrogen together with carbon dioxide in order to form methane, and thus ensure acceptable environmental conditions for acetic acid-producing bacteria. During the methanogenesis, acetic acid, hydrogen, and carbon dioxide are mainly converted into methane by strictly anaerobic methanogenic archaea. Hydrogenotrophic methanogens produce methane from hydrogen and carbon dioxide, whereas the acetoclastic methanogens form methane by acetic acid division. It can be said that around 70 % of methane comes from acetic acid decomposition, and only 30 % from hydrogen utilization [3,4].

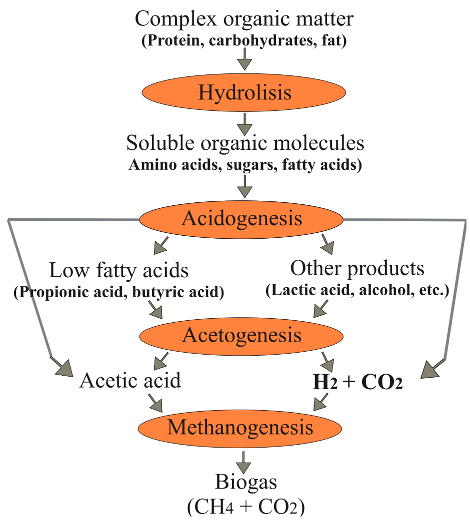

Figure 1. Stages in biogas production (CH_4: methane, CO_2: carbon dioxide, H_2: hydrogen).

The different bacterial strains multiply at different speeds. The so-called doubling time is the time it takes for a bacterial population to double in size [5]. Bacterial strains belonging to the first two steps, hydrolysis and acidogenesis, have a remarkable slower generation time, than methanogenic bacteria. Doubling time of bacteria strains involved during the biogas production is shown in Table 1 [5].

Table 1. Doubling time of different bacterial groups.

Bacterial group	Doubling time (hours)
Hydrolitic and acidogenic bacteria	
Bacteriodes	< 24
Clostridien	24 – 36
Acetogenic bacteria	
Syntrophobacter	40 – 60
Syntrophomonas	72 – 132
Methanogenic bacteria	
Methanobacterium	12 – 60
Methanosarcina	120 – 360
Methanococcus/Metanosaeta	240

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Key factors to achieve the well-being of different bacterial strains, and thus efficient anaerobic digestion processes, should be put down in focus. Key factors to consider inside the bioreactor are temperature, oxygen, pH-value, nutrient supply, as well as inhibitory substances. Operating parameters which should be monitored during the AD process are hydraulic retention time or time that a substrate remains in the bioreactor (d), organic matter room load ($kg{\cdot}m^{-3}{\cdot}d^{-1}$), CH_4 productivity ($m_{CH4}{}^3{\cdot}m^{-3}{\cdot}d^{-1}$), CH_4 yield ($m^3{\cdot}t^{-1}$), biomass degradability (%), and mixing of substrate in bioreactor. The composition of biogas can only be influenced to a limited extent by targeted process control. It primarily depends on the composition of the input material [3].

1.2. Biogas Composition

Biogas composition may vary, but in general it contains methane (CH_4), carbon dioxide (CO_2), water (H_2O), hydrogen sulfide (H_2S), nitrogen (N_2), and hydrogen (H_2), as shown in Table 2 [3].

Table 2. Biogas composition.

Component	Concentration
Methane (CH_4)	50 – 75 %
Carbon dioxide (CO_2)	25 – 45 %
Water (H_2O)	2 – 7 % (20 – 40 °C)
Hydrogen sulfide (H_2S)	20 – 20 000 ppm
Nitrogen (N_2)	< 2 %
Oxygen (O_2)	< 2 %
Hydrogen (H_2)	< 1 %

Regarding the quality of the gas mixture, the concentration of hydrogen sulfide (H_2S) plays an important role. H_2S is found in biogas as a trace gas in very small amounts, as is shown in Table 2. Firstly, it should not be too high, since even low concentrations of this gas inhibit the degradation process. Secondly, high H_2S concentrations in biogas lead to corrosion damage in combined heat and power plants and boilers during use [3,6].

The methane content is of primary importance, as it represents the combustible portion of biogas, and thus directly influences its calorific value. The achievable yield of methane is essentially determined by the composition of the substrate used, mainly the proportion of fats, proteins, and carbohydrates. The specific methane yields of the aforementioned groups of substances decrease in the order mentioned. A higher methane yield can be achieved with fats than with carbohydrates [3]. Table 3 shows the specific biogas yield and methane content of the corresponding substance group [3].

Few biogas plants practice mono-digestion, which means the digestion of only one kind of substrate. Most of anaerobic digesters use different crops or seasonal substrates, what guarantees the effectiveness of the AD process, increasing digester loading capacity and methane production. This ameliorates the buffer capacity, lowering pH level within methanogenesis, contributing in a better nutrient balance, i.e., C/N ratio (carbon:nitrogen ratio), among others [6]. Ref. [7] suggested that the hydraulic retention time (HRT) and temperature in bioreactor are interconnected; as temperature increases, HRT decreases. Both parameters are very important for pathogen inactivation.

Table 3. Specific biogas and methane production of each substance group.

	Biogas yield (l/kg_{ODM})	Methane content (%)
Digestible protein	700	71
Digestible fat	1250	68

Digestible carbohydrates	790	50

1.3. Use of Biogas

The produced biogas can directly be used as a cooking fuel, or be injected in a co-generator (CHP) for the simultaneous heat and power generation. Also, CH_4 content in biogas can be enriched to upgrade biogas to biomethane, so that it can be used with minimal modifications as natural gas. Finally, biogas can be used to produce value-added chemicals used in energy or industrial processes [2,4,6,8]. Figure 2, modified from [8], shows the different pathways for biogas utilization.

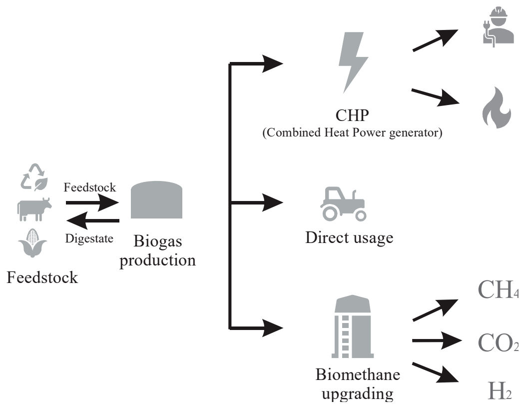

Figure 2. Potential utilization pathways for biogas [8].

Biogas is thus a clean energy source, which contributes to reduction of greenhouse gas emissions. [2] reported that in Switzerland 8 % of renewables energies was produced from biogas. The use of biogas can also diminish the use of firewood for cooking. It was reported by [2] that for 2040 about 200 million people will use biogas as cooking biofuel in Asia and Africa, contributing to the social development of emerging and developing lands [2,6]. In 2018, it was reported that China was the biggest biogas producer worldwide. Around 50 million small scale bioreactors are found, as well as 4000 farm scale and 2500 industrial scale reactors. India is also reported to have many small scale biogas plants, and Europe has an expanding tendency of biogas production [6]. In year 2022, [4] contabilized around 132000 biogas plants worldwide, being 17783 in European countries with an installed capacity of 10.5 GW. Also 700 biogas plants for biomethane upgrading were found, whereas almost 80 % of them were located in Europe. After 2005, the number of biogas plants increased tremendously, especially in Germany, France, Switzerland and Holland [4].

Some biowastes such as sludges, manures, or agricultural residues, are being dispread in agricultural soils as biofertilizers or in open up landfills. The continual disposal of residues in soils can lead to accumulation of both, nutrients such as nitrogen, phosphorous or potassium, or even heavy metals, leading to negative impacts in health of pasture-raised cattle [4]. Besides, the use of biowastes disposal in soils implies a significant contamination source, whereas landfill gases consisting of volatile organic compounds, CH_4, and CO_2 are released in the environment indiscriminately. Landfill gases have a great impact in ozone layer depletion [4].

AD could be successfully used for the optimal biowastes conversion to bioenergy in form of biogas, or even as biofertilizer. After biogas production, a partially stabilized wet suspension of less or even non-degradable materials, remains as residue [4]. The remining residue is called anaerobic digestate, and can be used in agricultural soils as a biofertilizer, guaranteeing necessary nutrients for humus and soil structure maintenance, and thus crop growth [6].

1.4. Anaerobic Digestate

As already pointed out, biogas digestate is reach in beneficial nutrients for plant growth. Nutrient content and properties depend on the biogas plant input material and the operation

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parameters used. Crucial factors affecting digestate quality are bacterial activity, water and nutrient content, C/N-ratio in input material, particle size and concentration, inhibitory or toxic compounds, pH, oxygen presence, microbial composition, as well as reactor temperature, design, and mixing [9–11]. Several authors have reported that biogas digestate is a valuable product, it can be used for plant and animal nutrition, seed germination, irrigation, water obtention, or even for gaining bio-pesticides, phosphate salt, as well as carbon [12–17]. Figure 3 shows the possible uses of biogas digestate according to [7].

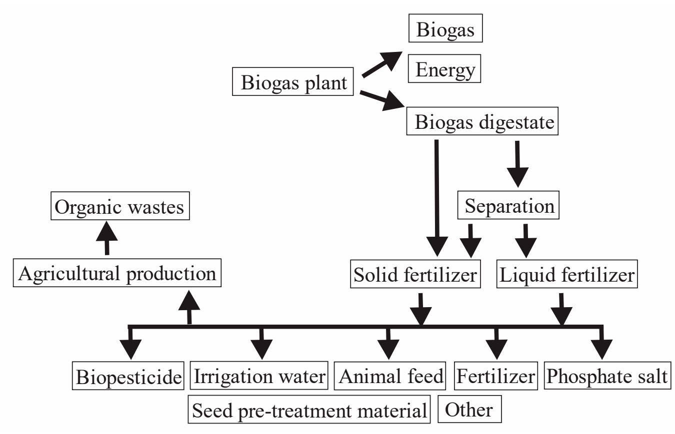

Figure 3. Possible use of biogas digestate [7].

When using digestate as biofertilizer, the biostability of the digestate should be taken in consideration, that means that digestate should not include pathogens or a high organic matter content, so that it should not be hazardous for living things. The main factor influencing the digestate biostability is the bioreactor temperature. After AD, mass content decreased by 90 – 95 %. Besides, when comparing digestate with cattle manure, pathogenic populations of *E. coli*, and *Salmonella sp.* were present in manure, whereas digestate run at mesophilic temperatures showed almost no pathogens [18]. The only disadvantage of sanitization is the loss of N in ammonia, what can be overcome by adding ammonium sulfate in digestate [7].

As already pointed out, AD substrate affects directly the biogas production yield as well as the digestate characteristics. When digesting urban residues, the amount of heavy metals in digestate, i.e. Cu, Zn, Cr, Cd, Pb, As, Ba, Ni, Co, Mn, Pt, and Sb, could be found in toxic concentrations, so that its use as biofertilizer is not recommended. According to [19], dry matter, organic matter, pH, N, P, Cd, Cu, Ni, Pb, Zn, Hg, and Cr, should be analyzed in anaerobic digestate at least every six months. Limit values of trace elements (TE) in anaerobic digestate for agricultural use as fertilizer are limited to six elements showed in Table 4.

Table 4. Limit values permitted in anaerobic digestate for its agricultural use [19].

Trace element in anaerobic digestate	Limit values in mg·kg^{-1} (dry matter)
Cd	20 - 40
Cu	1000 – 1750
Hg	16 – 25
Ni	300 – 400
Pb	750 – 1200
Zn	2500 - 4000

To avoid this problematic, risk might be minimized reducing contaminant factors through denitrification applications and membrane filtration [7]. Furthermore, remediation techniques could be carried out to diminish the concentration of heavy metals in soils.

1.5. Mycoremediation

Mycoremediation is a remediation method whereby fungi are used to degrade or remove polluted ecosystems. Mycorrhiza refer to the mutualistic associations of plant roots and mycorrhizal fungi. Mycorrhizal fungal hyphae, which makes up the body of a mycorrhiza fungus, is fixed to plant roots and soil particles building filaments which tolerate contaminants such as heavy metals, leading to plant adaptation to nutriment diminishment, as well as temperature or pH modifications [20]. Mycorrhizal fungi are found in roots of many plants. These symbiotic associations benefit the host plants, whereas water or soil nutrients uptake is enhanced, particularly P and N contents. Furthermore, mycorrhiza fungi collaborate with other soil microorganisms enhancing nutrient absorption [21,22]. The structure and function of mycorrhizal associations are wide, there are arbuscular mycorrhiza (AM), ectomycorrhiza (EcM), ectendomycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza, ericoid, and orchid mycorrhiza. Amongst them, AM and EcM are the most common, moreover AM fungi has an economical and ecological importance. AM fungi are used as biofertilizers and cannot be cultivated without a host plant. The intracellular hyphal network of EcM shows more extensive associations than AMF [21]. AMF could either increase heavy metal (HM) uptake and transport from roots to shoot, or stabilize HM in roots and shoots, diminishing uptake. Some authors report that AMF could accumulate HMs in a nontoxic form within the roots of the plant and in the extracellular mycelia [21].

1.5.1. Effects of HM in Soils

HMs presence in soils could be hazardous for plants and consumers, when found over safe and tolerable limits. High concentrations of HM show a negative effect on microorganisms and microbial processes, leading to plant growth reduction. HM could land in soils by different ways, i.e. fossil fuels, mining and smelting, municipal wastes, and use of fertilizers or pesticides [23–25]. Furthermore, some metals like Zn, Cu, Mn, Ni, and Co are essential for plant growth and are considered micronutrients, other metals such as Cd, Pb, or Hg does not show any biological function [25]. An excessive concentration of HM can induce to metabolic disruptions in plants, altering cellular activities and affecting nutrient uptake, plant development, or even inducing reactive oxygen species production. This could be reflected in poor plant growth, turgor stem pressure reduction, leaf chlorosis, seed germination drop, and senescence [23]. Metals could be found in soils in different forms such as free ions, soluble complexes, exchangeable ions, precipitated or insoluble oxides, carbonates and hydroxides, or even as silicate materials. The toxic effects of metals depend on their ability to be transferred from the soil to living organisms, so called bioavailability, and it depends on physico-chemical and biological factors [25].

1.5.2. Mycorrhiza for Metal Remediation

As already pointed out, mycorremediation refers to degrading or removing contaminants from the environment, using fungi cultivated in a host plant. It is an economic and effective alternative to reduce concentrations of soil contaminants. Metal-tolerance plants are being used to extract HM from soils, translocating and accumulating into shoots, leaves, and other structures. When colonizing these plants with mycorrhizal fungi, greater plant growth, protective mechanisms and buffer capacity for abiotic stress can be achieved. Several studies have shown an increase of metal uptake in plants inoculated with AMF, especially regarding As, Cr, Cd, Pb, Zn, Mn, Cu, Al, and Co. Thereby, an increased metal content in roots, shoots, and fronds was found, so that phytoextraction could easily take place [21,23].

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In soils heavily contaminated with HMs, plant colonization through mycorrhiza has been detected. Several authors report high levels of mycorrhizal colonization in agricultural soils polluted with heavy metals [25].

Plant AMF tolerance may take place due to different factors. On the one hand, a higher nutrient (especially P) uptake lead to higher plant growth, and thus higher biomass availability for metal distribution within the plant. On the other hand, AMF-assisted plants have the capacity to bioaccumulate metals, hindering their translocation to shoots and roots [23,26,27].

Mycorremediation could be used as a low-cost alternative for soil remediation, showing beneficial effects on plant growth, metal attenuation, and productivity.

1.5.3. Use of Anaerobic Digestate in Mycoremediation

As already mentioned, anaerobic digestate is the substance remaining after anaerobic digestion to biogas and biomethane production. Thus, anaerobic digestate is rich in nutrients and can be used as biofertilizer or even as soil amendment. Digestate has the potential to ameliorate biological, chemical and physical soil properties, i.e. by pH-adjustment or soil aggregation and boosting, influencing soil microbiota and enzymatic activity [28].

In the rhizosphere, a nutrient interchange between plants and microorganisms takes place. In any case, microorganisms composition depends on the soil management techniques [28]. Anaerobic digestate could be applied in soils where mycorrhiza colonize plant roots, promoting a better soil nutrient adsorption, especially regarding P and N. [12] reported that AM fungi has the capacity to change soil characteristics, whereby AMF filamentous hyphae causes that plants reach water and nutrients more extensively in the soil, reducing the irrigation needs and the use of chemical fertilizers. It has been proved that the combination of anaerobic digestate with mycorrhiza has beneficial impacts for soil nutrient and organic carbon contents, resulting in a more efficient plant growth [29]. Furthermore, the application of biogas digestate derive in a higher fungi or mycorrhizal root colonization, augmenting symbiotic benefits for plant development, even in polluted soils whereas plants can adapt better to stress [12,19,28,30–34].

1.6. Fundamental Framework

Further studies should be carried out regarding the influence of digestate on soils inoculated with mycorrhiza, in order to understand changes in structure and composition of mycorrhiza, as well as soil nutrient accessibility and mycorremediation potential.

The present paper comprehends a systematic review related to the scientific research done the past 10 years regarding the use of anaerobic digestate for fungi cultivation and mycorrhizal colonization for soil remediation. The effects of digestate application and fungi colonization in soil nutrients/contaminants behavior, microbial community, as well as plant growth and organic matter removal, are discussed.

2. Materials and Methods

In order to enhance reliability, transparency, and integrity of the report, PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines were followed for the development of the present review.

2.1. Articles Selection

An initial search was carried out for English-language scientific articles published between years 2015 and 2025 in databases SCOPUS, SCIENCE DIRECT, PUBMED and on GOOGLE SCHOLAR using Boolean operators AND and OR. Different combination of terms were used with the Boolean connectors, corresponding to each used platform, as shown in Table 5.

In total, 119 articles were initially found, whereupon the inclusion and exclusion criteria were stablished.

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Table 5. Results of article selection.

Database	Terms combination with Boolean operators	Number of articles found
SCOPUS	(biogas AND digestate AND mycorrhiza AND metal AND remediation) OR (biogas AND digestate AND mycoremediation) OR (biogas AND slurry AND mycorrhiza AND metal AND remediation) OR (biogas AND slurry AND mycoremediation) AND PUBYEAR > 2014 AND PUBYEAR < 2026 AND (LIMIT-TO (DOCTYPE, "ar"))	59
GOOGLE SCHOLAR	(anaerobic digestate + mycorrhiza + metal remediation) OR (biogas digestate + mycoremediation) OR (biogas slurry + mycorrhiza + metal + remediation) OR (biogas + slurry + mycoremediation)	43
SCIENCE DIRECT	(anaerobic AND digestate AND mycorrhiza AND metal AND remediation) OR (biogas AND digestate AND mycoremediation)	17
PUBMED	(anaerobic AND digestate AND mycorrhiza AND metal AND remediation) OR (biogas AND digestate AND mycoremediation)	0

2.1.1. Inclusion Criteria

In order to include the articles for the systematic review, only empirical research within years 2015 and 2025 were included. Only articles with scientific experimental techniques were considered.

2.1.2. Exclusion Criteria

Review articles, chapters, manuals or books were excluded. Articles were also excluded, when the main topic was not related neither to anaerobic digestate and its use for fungal growth, nor to the use of anaerobic digestate as soil amendment for plant growth or for soil remediation.

2.1.3. Systematic Search

Duplicates generated due to the use of different databases were excluded, so that five duplicates out of 119 articles were found. A total of 114 articles were screened, from which 54 articles were excluded due to the publication type, at this point only empirical research was considered, leaving only 60 papers. From these 60 papers, 33 of them were not retrieved, because they contained neither fungi, nor digestate information, instead they described other microorganisms, bacteria, or biochar use for soil remediation. From the remaining 27 papers, 18 were excluded because they considered soil remediation either only with fungi or only with digestate, but they did not consider the simultaneous application of both. At the end, only nine research articles were left, including four articles regarding fungi growth in anaerobic digestate and five papers about the effect of mycorrhizal fungi and digestate application for plant growth or soil remediation. Figure 4 summarizes the PRISMA flow diagram resulted after every stage of the article's selection process.

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The use of anaerobic digester for fungal growth, for both improvement of plant development as well as soil remediation, has not been widely researched, although there is existing literature demonstrating that digestate can be used as soil amendment for mycoremediation, whereas fungi cultivation in anaerobic digestate could give a reference of how fungi development takes place under substrates derived from anaerobic digestion. When considering the publication years of the articles accepted, between 2015 and 2020 only two articles were published, whereas the last five years, seven articles regarding the topic were found. This fact represents the necessity to find new methods for soil remediation.

The results of the systematic search are presented below beginning with mycoremediation, and the countries contributing to this research regarding the use of fungi for soil remediation, and the most commonly techniques used.

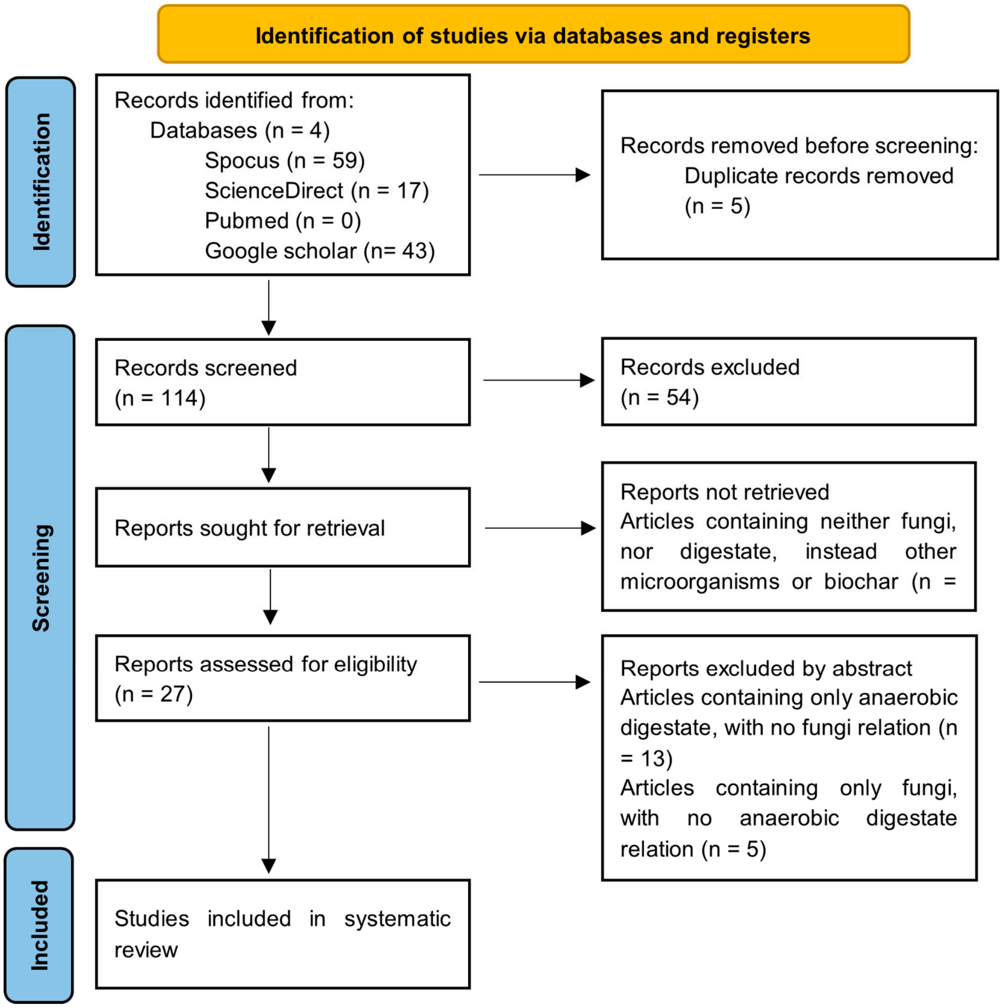

Figure 4. Four different stages of the article's selection process.

3. Results and Discussion

From the total of 119 articles found at the first steps of the systematic research, only nine articles, which means 8 %, deal with the inoculation or cultivation of fungi in anaerobic digestate, looking forward to understand their existing symbiosis between plant growth and/or metal remediation. In order to understand the influence of anaerobic digestate in fungi, articles regarding two main topics were included. On the one hand, five articles were considered regarding the inoculation of fungi in anaerobic digestate in order to evaluate plant growth or pollutant removal. On the other hand, four

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articles referring to fungi cultivation in anaerobic digestate and its influence in contaminant removal were included.

As already pointed out, anthropogenic activities trigger the accumulation of pollutants in soils, whereas the increasingly accretion of these elements become hazardous for the environment and human health. To overcome this problematic, biological, chemical, and physical methods of metal remediation have been developed, nevertheless many of these technologies are expensive and can indeed affect soil structure and microbiome. Phytoremediation stands for a cost effective and environmentally friendly method, through which plants and microbiome interact in order to remove, immobilize, or degrade heavy metals in soils. Metal tolerant plants are grown in contaminated soils, so that metal spread can be diminished and the soil surfaced can be stabilized. This strategy is called phytostabilization and has the disadvantage to be slow, due to the time span required for plant growth. Also, the biomass accumulating HMs represent a disposal problem. To overcome this problematic, sowing metal tolerant plants inoculated with microbiota resistant to HM toxicity, has been introduced in the past years, being defined as bioaugmentation-assisted phytoremediation. Microbiota used for this purpose is plant growth promoting rhizobacteria (PGPR) or arbuscular mycorrhiza fungi (AMF) [34].

Ref. [28] reported that AMF provide P and other nutrients to plants receiving carbohydrates in exchange, and protecting them from drought and pathogens. AMF has shown the ability to change the bacterial community composition, furthermore, AMF hyphae has been pointed out to capture different bacterial strains, which otherwise would affect plant nutrient uptake and growth. [19] reported that during colonization, fungi mycelium decomposes growing substrate secreting enzymes which have the capacity of breaking down organic matter into simpler compounds. Besides, fungi can accumulate trace elements, or even dissolve metals through root exudates.

On the other hand, the demand of food production increases as the worldwide population grows. This intensifies the necessity to supply agricultural soil with organic or inorganic fertilizers. Soil fertilization, even as a forest management practice, contributes in soil fertility preservation, enhancing microbial populations and enzymatic activity. However, a continuously soil fertilization with inorganic N and P fertilizers could enhance N and P accumulation resulting in negative ecological effects such as soil acidification, eutrophication, and biodiversity loss [28].

Ever since the anaerobic digestion for biogas production has been widely used in the past years, the resulting digestate has been pointed out as an efficient and low-cost organic soil fertilizer. Anaerobic digestate can be used as soil amendment and could improve the physical, chemical, and biological soil properties, neutralizing soil pH, boosting soil´s total N, and augmenting both, soil microbial community composition, and enzymatic activities [28].

Biogas digestate is rich in organic P and N nutrients, it is supposed to contribute to plant growth and AMF colonization. Nevertheless, some authors suggested that using anaerobic digestate could affect AMF species richness and diversity [28]. The effects of anaerobic digestate as soil amendment on AMF communities has not been widely studied. Information should be investigated regarding the influence of organic fertilizers in symbiotic fungus, especially focusing in how rhizosphere structure and microbiome composition influence soil nutrient availability, plant growth, and even pollutant removal.

In order to have a deeper understanding on the mechanisms of fungi colonization in anaerobic digestate, research articles regarding fungi cultivation in anaerobic digestate were included in the systematic review.

Tables 6 and 7 show a summary of the scientific literature resulted from the systematic review. Table 6 focuses on the articles regarding fungi cultivation in anaerobic digestate and Table 7 on the digestate as soil amendment for plant growth and pollutant removal.

Table 6. Fungi investigated in different countries for fungi cultivation.

Year	Country	Fungi	Results obtained	Reference
2024	Poland	*Agaricus bisporus* and *Agaricus subrufescens*	• 17 out of 45 elements accumulated in fungi • K, Ca, Mg, and Na mostly absorbed by both fungi • Ca, K, Mg, Na, Cr, Fe, and Zn diminished after every harvest • Co, Mn, Mo, Ni, Se, and Si content increased after every harvest • *A. bisporus* absorbed more Na *A. subrufescens* absorbed more B, Cu, and Zn	[19]
2024	Norway	*Agaricus bisporus* and *Agaricus subrufescens*	• Antibiotics were not accumulated • *A. subrufescens* showed a higher efficiency in contaminant removal, and lower contaminants uptake • *A. subrufescens* is grown at higher temperatures • Anticonvulsant was uptaken in low quantities by *A. bisporus* • Half-live dissipation lower for *A. subrufescens* than *A. bisporus*	[31]
2023	Norway	*Agaricus bisporus* and *Agaricus subrufescens*	• Lower accumulation of Per- and polyfluoroalkyl substances (PFAS) in fungi through digestate	[32]
2019	USA	*Pleurotus ostreatus* (non-mycorrhiza)	• Digestate showed more abundance in B, Cu, TN, and Zn • Digestate from manure + organic wastes --> more accumulation of Ca, Fe, K, Mg, Mn, and Na --> lower fungi colonization, due to higher conductivity through wastes	[33]

Table 7. Fungi investigated in different countries as soil amendment with anaerobic digestate.

Year	Country	Fungi	Results obtained	Reference
2024	China	AMF (*Claroideoglomus etunicatum*)	• Digestate resulted in: · Plant growth · N and P increase · Organic matter decrease • AMF no significant effect on soil microbial diversity	[12]

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2023	Portugal	AMF (*Rhizophagus irregularis*)	• Digestate resulted in: Plant yield increase by 9 % in contaminated soils Cd (0.77 %) and Zn (0.13 %) removal Removed biomass successfully used for AD	[34]
2022	China	AMF (not strain specified)	• Highest digestate concentration --> highest AMF diversity • AMF identified: *Glomerales* and *Paraglomerales*, • Lower amounts: *Glomus* and *Paraglomus*, which abundance increased significantly with digestate	[28]
2022	France	AMF (*Funneliformis mosseae*)	• Digestate resulted in: - Plant growth - Zn and Cd immobilization - Cd plant uptake improvement - Decrease in Cd accumulation in shoots - Increase in microbial biomass, soil quality and health • AMF inoculation itself did not resulted in plant growth	[30]
2020	Ireland	AMF (not strain specified)	• Three amendments compared • Anaerobic digestate more abundance of bacterial strains and more mycorrhizal colonization, but lower plant growth	[35]

The effects of digestate application on soils has been analyzed through the present study. Effect of the biomass source for the digestate obtention, the fungal strains, organic matter removal and plant growth, as well as the microbial communities formed and fungi colonization are summarized in the following subchapters. These results confirm the necessity of studying further the topic, for the successfully remediation of polluted sites. A deeper analysis of the pollutants to be removed, the available fungal strains, and the digestate conditions need to be tested in the specific required cases.

3.1. Biomass Source for Anaerobic Digestion

Biomass source utilized during the process of anaerobic digestion is a key factor for nutrient availability. From the articles considered, only four articles used digestate from the solely anaerobic digestion of cattle manure [12,28,32,35]. Table 8 shows the results of different biomass source used in the digestate. [19] and [31] used digestate from co-digesting cattle manure and food waste, and only [33] compared the effect of using only cattle manure anaerobically digested and the co-digestion of cattle manure with food wastes. Edible fungus has been successfully grown in anaerobic digestate, due to the C, P, and N contents, as well as nutrient availability. [33] compared fungi growth in anaerobic digestate from only manure and from manure and food wastes. The second one resulted in a higher nutrient availability, whereas a higher content of K, Ca, Fe, Mg, Mn, and Na was found. Nevertheless, mycelium colonization and fungi yield were lower, due to the relatively food waste salt content and thus higher conductivity. On the other side, TN, B, Cu, and Zn were higher in digestate from only manure.

Ref. [33] reported that when cultivating fungi in anaerobic digestate, C/N/P ratios varied according to digestate concentrations. C/N ratios of 44:1 to 55:1 resulted in the most effective growth yield. The most successfully N/P ratio was reported 4:1 to 12:1. N/P ratio was affected when increasing digestate concentration. These facts suggest that digestate source and concentration should be adjusted for an efficient fungi and plant growth, as well as remediation rates.

Table 8. List of articles with the different biomass source for anaerobic digestate.

Biomass source for anaerobic digester	Parameters measured	Results	Reference
Anaerobically digested cattle manure	• Organic matter • Total nitrogen (TN) • Nitrate nitrogen • Ammonia nitrogen • Available phosphorous (P)	• Higher plant growth at digestate application of 13,216.67 kg/hm^2 • Almost all parameters content increased with increasingly digestate application • Only soil organic matter decreased	[12]
Anaerobically digested cattle manure	• Fungi identification • Total carbon • Total nitrogen • Nitrate • Phosphorous (P) • Microbial biomass C • Phosphatase • Nitrate reductase	• Highest digestate concentration showed the highest Shannon diversity • 36.2–42.7% of AMF diversity was *Glomerales* • *Glomus* and *Paraglomus* abundance with digestate application • P and nitrate nitrogen increased with digestate	[28]
Anaerobically digested cattle manure	• Per- and polyfluoroalkyl substances (PFAS)	Low accumulation of PFAS in fungi	[32]
Anaerobically digested cattle manure	• Soil microbiota • Available Phosphorus (P) • Sulphur (S)	Digestate → higher abundance of nematodes, bacterial strains, mycorrhizal colonization, enzymes, and available P	[35]
Anaerobically digested cattle manure and food waste	45 elements: • Major essential elements: Ca, K, Mg, and Na • Trace elements: B, Co, Cr, Cu (copper), Fe, Mn, Mo, Ni, Se, Si, and Zn	• Decrease of Al, As, B, Ca, Cd, Cr, Fe, K, Mg, Mn, Ni, Pb, and Zn • Ni, As, and Cd in fruit bodies do not pose a threat to human health	[19]

	• Trace elements with detrimental health effect: As, Ba, Be, Cd, Hg, Pb, and Tl • Nutritionally nonessential elements: Al, Bi, Ce, Ge, Ir, Li, Nd, Os, Pr, Pt, Rb, Sb, Sn, Sr, Ta, Te, Ti, U, V, W, and Zr	• At the end, substrate rich in, Mg, Mn, Na, Si and Zn (added value) • Fungi valuable source of Cu, K, Na, Se, and Zn	
Anaerobically digested cattle manure and food waste	• Sulfonamide antibiotic • Fluoroquinolone antibiotic • Anticonvulsant carbamazepine	• No antibiotics accumulated in fungi • Anticonvulsant accumulated in small amounts by *A. bisporus*	[31]
Anaerobically digested cattle manure and food waste	• Total solids, N, C, As, P, Ca, K, Mg, Al, B, Cu, Fe, Mn, Na, Zn, Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, and Zn	• Total N, B, Cu, and Zn higher in only manure • Inorganic N, P, K, Ca, Fe, Mg, Mn, and Na higher in manure + food wastes • Lower fungi colonization and yield inhibition with manure + organic wastes	[33]
Anaerobically digested sludge from water treatment	• Cd, Cr, Cu, Ni, Pb, and Zn • Total organic carbon • N • Available Ca, K, Mg, and P	• Digestate → increase in P and Mg • Plant growth • Increase microbial biomass • Cd and Zn immobilization in soil • Reduced Cd plant uptake • Decrease in Cd and Zn bioavailability • Significant decrease in the Pb without AMF • Increase in Cd, Zn, and Pb bioavailability without digestate • AMF decrease Pb bioavailability • Lower bioavailability of Cd, Cu, and Zn	[30]
Biomass resulted from phytoremediation with AMF	Cd and Zn	• Cd and Zn reduced with AMF • Methane produced 183 and 178 mL of $CH_4 \cdot g^{-1}{}_{VS}$	[34]

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3.2. Fungal Strains Researched

Nutrient availability depends greatly on the fungi species. Primarily, *A. bisporus* and *A. subrufescens* were studied for antibiotics, anticonvulsant as well as PFAS removal [31,32]. [19] identified different fungi strains and found a higher degradability in *Pleurotus ostreatus*, *Lentinula edodes*, and *Pleurotus eryngii*. From the articles considered for this systematic review, AMF such as *Funneliformis mosseae*, *Rhizophagus irregularis*, or *Claroideoglomus etunicatum*, were successfully inoculated in anaerobic digestate [12,30,34]. Table 9 lists the articles selected through the systematic research, describing the fungi strain, plant tested, as well as effects in plant growth and pollutant/nutrient behavior.

Specific fungal strains result in different growth rates. [19] reported a higher degradative ability as well as growth rate in anaerobic digestate for *Pleurotus ostreatus*, *Lentinula edodes*, as well as *Pleurotus eryngii*. This difference depends on both, fungi strain and substrate characteristics, such as C/N:ratio, pH, and contaminants content. This author reported that the concentration of trace elements in anaerobic digestate affect directly the concentration of trace elements in the fungi. Although anaerobic digestate represents an adequate nutrient source, nutrient availability depends greatly on the fungi species. *Agaricus bisporus* provide significant amounts of K and Na, while *Agaricus subrufescens* is an important source of Cu and Zn, indicating the specific fungi capacity to bioaccumulate such elements. [19] suggested that Si content could result in augmentation of microbial interaction, whereas pathogens plant colonization could be hindered.

Table 9. List of articles with fungi strain, plant tested, as well as effects in plant growth and pollutant/nutrient behavior.

Fungi	Plant	Effects in plant growth	Effects in pollutant/nutrient removed	Reference
Agaricus bisporus and *Agaricus subrufescens*	N/A	• No significant difference between both fungi growth • Spent mushroom compost resulted in higher fungi yields	• Decrease in content of Al, As, B, Ca, Cd, Cr, Fe, K, Mg, Mn, Ni, Pb, and Zn	[19]
Agaricus bisporus and *Agaricus subrufescens*	N/A	• Not mentioned	• Antibiotics were not accumulated • Anticonvulsant was removed faster by *Agaricus subrufescens* but lower uptake	[31]
Agaricus bisporus and *Agaricus subrufescens*	N/A	• *Agaricus subrufescens* 11 % increase in biomass yield after first harvest and 34 % decrease after second harvest • *Agaricus bisporus* 66 % biomass decrease after first harvest and 38 % after second harvest	• Low accumulation of PFAS in fungi	[32]

Pleurotus ostreatus (non-mycorrhiza)	N/A	• Lower fungi colonization and yield inhibition with manure + organic wastes • Only manure produces 16 times more than control and more than twice than manure + organic wastes	• Manure + organic wastes more abundance of Ca, Fe, K, Mg, Mn, and Na	[33]
AMF (*Claroideoglomus etunicatum*)	Hybrid *Pennisetum*	• Plant growth achieved peak values @ 13,216.67 kg/hm² digestate and 13,733.33 kg/hm² (with AMF)	• Total nitrogen, nitrate nitrogen, ammonia nitrogen, and available phosphorus in increased • Soil organic matter decreased	[12]
AMF (*Rhizophagus irregularis*)	Maize (*Zea mays*)	• Increase in plant yield ca. 9 %	• Cd (0.77 %) and Zn (0.13 %) removal	[34]
AMF (not strain specified)	Poplar	• Plant growth was suggested but not measured	• At the beginning C (4 %), N (11 %), P (91 %) increased with digestate concentration • No removal reported	[28]
AMF (*Funneliformis mosseae*)	Coriander (*Coriandrum sativum L.)*	• Digestate without AMF increased 100 % plant growth • But with AMF only 50 % plant growth • AMF inoculation no significant plant growth	• Pb decrease with digestate • Cd, Zn, and Pb bioavailability increased through coriander with no digestate • Cu bioavailability increased 50 % without AMF • Pb bioavailability decreased with AMF • Pb bioavailability decreased with digestate • Cd, Cu, and Zn increased bioavailability with AMF and digestate	[30]

AMF (not strain specified)	Ryegrass (*Lolium perenne*)	• Lower plant growth with digestate • Total grass dry matter lower	• Digestate compared to inorganic fertilizers: Higher phosphate and sulphate Lower nitrate • Lower uptake P, S, N, K, Ca, Zn, B, and Mg • P concertation was higher	[35]

3.3. Effect on Pollutants/Nutrients Analyzed

In the selected articles, not only heavy metals were considered as pollution source. The idea was to gather a wider approach of the mechanisms involved between fungi and anaerobic digestate, for plant growth and contaminants removal. Table 9 shows the results of the articles describing the fungi strain, plant tested, as well as effects in plant growth and pollutant/nutrient behavior. [32] tested the uptake of per- and polyfluoroalkyl substances (PFAS) in fungi cultivation, resulting in a lower accumulation PFAS in fungi through digestate. The capacity to remove two antibiotics and one anticonvulsant was tested by [31], showing that antibiotics were not accumulated in fungi. Different chemical elements were analyzed for their biodegradation, i.e. Ca, Co, Cr, Fe, K, Mg, Mn, Mo, Na, Ni, Se , Si, and Zn.

3.3.1. Polyfluoroalkyl Substances (PFAS) and Antibiotics

The material remaining after fungi harvesting has been used for degrading polycyclic aromatic hydrocarbons (PAHs) and other persistent organic pollutants, as well as for heavy metal biosorption, or even as biological control for nematodes pests in soils **[27,36]**. [32] tested the uptake of per- and polyfluoroalkyl substances (PFAS) in fungi cultivation. PFAS are used in many industrial and consumer products and are persistent in the environment, being accumulated in the human body. From the research, *A. bisporus* revealed a higher PFAS concentration, in comparison to *A. subrufescens*. Ultra-short chain PFAS showed much greater accumulation compared to long chain PFAS. Also, temperature has a direct influence in the degradation rate of organic matter, whereas digestate composition may become different, influencing pollutant sorption and bioavailability. Meanwhile, short-chained acids are less affected by pH changes, than long-chained.

Ref. [31] compared antibiotics removal in two different fungi, *A. subrufescens* and *A. bisporus*. In general, ciprofloxacin removal rate was by 83–90% and carbamazepine by 57–97%, even though the antibiotic content was low. *A. subrufescens* showed a higher efficiency in contaminant removal. This fungus is considered tropical and was cultivated at higher temperatures. Authors did not report further conditions about the exact growing temperature, but suggested that this fact could had influenced the higher removal rate. Contaminant concentrations in *A. subrufescens* was lower, even when the initial concentration was the same. From the fungi cultivation was observed that organic acids resulted in a higher organic matter degradation with a pH decrease. Control test, with no fungi cultivation, showed a lower organic matter degradation with an increased pH.

3.3.2. Pollutant/Nutrients Analyzed

Table 9 lists the pollutants/nutrient analyzed in the selected research articles. [19] reported Zn as antagonist of some metals such as Cd, Pb, and Ni. Zn presence in fungi hinder high concentrations of other toxic metals. Cd was mycelium age specific, the older the mycelium, the lower Cd accumulation.

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In a study from [12] anaerobic digestate increased the total nitrogen, nitrate nitrogen ammonia nitrogen, as well as available phosphorus content in *Pennisetum* soil, reducing soil organic matter.

The behavior of phosphate and sulphate content was compared using inorganic fertilizer and digestate as organic fertilizer [35]. It was reported that the content of both micronutrients was higher in the anaerobic digestate. Nitrate content was nevertheless lower in digestate, in comparison to inorganic fertilizer, whereas uptake of P, S, N, K, Ca, Zn, B, and Mg was higher. The organic fertilizer resulted in higher pH and available P concentration, but a lower total grass dry matter. Bacteria utilizing sulfonate- and phosphonate were up to five times more abundant in the organic fertilizer. Phytate-utilizing and calcium phosphate-utilizing bacteria were not significantly different between treatments.

Ref. [30] reported a significant decreased of Pb, when inoculating coriander with AMF using anaerobic digestate as soil amendment, resulting in a higher Pb extractability. Trace elements concentration did not change for soil vegetation. A higher bioavailability of Cd, Pb, and Zn was shown when no amendment was applied in the soil. When the vegetated soil was inoculated with AMF, bioavailability of this elements decreased. By non-vegetated soil was inoculated with AMF, Cd, Zn, and Cu bioavailability increased. Cd was the predominantly accumulated element in the coriander shoots.

Ref. [30] demonstrated a decrease in Cd and Zn extractability, also known as bioavailability, as well as in the Pb and Cd accumulation in coriander shoots. It was pointed out, that aromatic plants such as coriander, release metabolites in the rhizosphere to manage nutrient bioavailability and metal stress. The mechanisms involved are related to the organic acids content, which are released as exudates influencing TE availability forming complexes with metal ions or modifying soil characteristics. Several studies have shown that organic amendments increase the immobilization of metals and metalloids. This immobilization results from different processes, i.e. adsorption onto mineral surfaces, formation of stable compounds and organic ligands, surface precipitation, or even ion exchange. These processes were suggested but not deeply researched in previous studies. [37] suggested that applying composted anaerobic digestate could minimize TE mobility and thus toxicity, resulting from the amount of dissolved organic matter. The study from [30] showed an increased total and organic C, P, and Mg contents when applying composted anaerobic digestate, improving soil TE immobilization and plants nutrition, resulting in coriander shoot growth. However, same author reported no significant effect in shoot growth, when inoculating with AMF. Mechanisms related to TE immobilization by AMF inoculation are related to glomalin-related soil proteins production in mycorrhizosphere, TE gathered in fungal structures, such as vacuoles or fungal vesicles in mycorrhizal roots, as well as TE adsorption by extraradical hyphae [38]. Due to the high P and N content in anaerobic digestate, an increased available P in soil has been reported [28].

Further results suggest that anaerobic digestate has a greater impact on the diversity of fungal communities in rhizospheric soil, than in bacterial communities. According to [28], Nitrogen content was not significantly increased after anaerobic digestion application, but nitrate nitrogen was.

3.4. Effect on Plant Growth and Organic Matter Removal

Anaerobic digestate could be used as organic amendment for soil inoculation through mycorrhizal fungus, hence soil quality and health improvement can take place. Table 9 summarizes the plant growth obtained from the articles selected for this systematic review. [30] reported that using anaerobic sludge in soil inoculated with AMF *Funneliformis mosseae* resulted in a better growth of coriander as well as Cd and Zn immobilization in soil and thus lower Cd plant uptake.

In a further study from [30] it was reported that *F. mosseae* (AMF) inoculation neither promoted plant growth, nor root colonization. Anaerobic digestate could improve physical characteristics in soils, such as air permeability, water retention, stability, aggregates, and resistance to soil erosion, releasing nutrients slowly for a long-term plant growth [12].

Nutrient availability is a key factor for an efficient plant and microbial community growth. The use of anaerobic digestate could guarantee the application of essential micronutrients such as N, P,

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S, C/N ratio, or even heavy metals i.e. Cd, Cr, Cu, Fe, P, or Zn. [30] reported that the application of anaerobic digestate for coriander cultivation under AMF inoculation play a crucial role for restoring soil functionality. Soil processes, such as formation and decomposition of organic matter, as well as cycles of respiration and nutrition, have been impacted by microbial communities. Also soil composition and structure can be regulated by AMF.

Fungi shows advantages over plant production, due to the fact that they can be grown in processed substrates, i.e. composted, pasteurized, or even sterilized media. In the process, some toxic compounds in the substrate could be reduced. [19] reported that fungi mycelium grows within the substrate decomposing material, whereas colonization takes place through secreted enzymes breaking down complex organic matter into simpler components. [39] demonstrated that fungi root exudates dissolve metals from substrate in mycelium zone, influencing mineral adsorption.

It has been reported by [33] that saprophytic whiterot fungi produce extracellular enzymes that degrade cellulose, hemicellulose, and lignin biopolymers, what could enhance the organic matter removal, or even soil remediation grade.

3.5. Effect on Microbial Community

Several authors (**[28,30,35]**) have demonstrated that the application of soil amendment in form on anaerobic digestate result in an enrichment of microbial communities in soil. [28] documented a significant increase of AMF *Glomus* and *Paraglomus*, when using anaerobic digestate. When applying anaerobic digestate in soil, more organic matter needs to be decomposed, thus a wider variety of microorganisms is needed. It has been also demonstrated that different concentrations of anaerobic digestate promote the growth of different fungi. *Glomus* showed a higher abundance when applying increased concentrations or anaerobic digestate, while *Paraglomus* growth was higher with smaller anaerobic digestate concentration.

Ref. [35] reported significantly higher bacteria feeders, nematode abundance and root colonization in mycorrhiza arbuscules, hyphae and vesicles, using anaerobic digestate as fertilizer compared to the use of inorganic fertilizer. Nematode abundance and bacteria feeders showed a higher prevalence of bacteria feeding nematodes from the *Cephalobidae* and *Rhabditidae* families. [30] reported a significant increase in the total microbial biomass using anaerobic digestate as soil amendment. Soil metabolic potential as well as functional richness were low in non-amended soils, nevertheless this value increased when growing coriander in these soils. Dehydrogenase activity was though not altered in non-amended soils. *Phosphomonoesterase* activity was not affected whether by amendment nor by coriander.

The application of organic amendments has demonstrated the enrichment of microbial communities in soils, whereas plant associated microorganisms reduce TE uptake by plants. This could be a reason why [30] reported a significant decrease in extractable Cd and Zn, while Pb and Cd were accumulated in coriander shoots. Furthermore, rhizosphere microorganisms have the capacity to regulate plant uptake and TE bioavailability through mechanisms of oxidation, reduction, complexation, immobilization, adsorption, or dissolution [40].

After AMF addition, [12] reported an improvement in richness and diversity of fungi in hybrid *Pennisetum* soil, however AMF effect was reduced after application of anaerobic digestate. It was suggested, that the different application concentrations could affect the separation of bacterial and fungal diversity. The addition of AMF increased the abundance of *Acidea*, reducing *Plectosphaerella* abundance, when no digestate was applied. When digestate was used, no difference of both species was found, whether with or without AMF.

Ref. [28] reported that the application of high concentrated anaerobic digestate resulted in a significant enrichment of species richness and Shannon diversity of AMF in rhizosphere of poplar plantations. The lower concentration of anaerobic digestate tested showed a decreased in AMF diversity. The abundance of the dominant genera *Glomus* and *Paraglomus* increased significantly. Also, soil fertility and the presence of more soil microorganisms increased with the application of digestate, due to the fact that organic fertilizers provide a higher amount of organic matter to be decomposed.

4. Conclusions

The findings on the use of anaerobic digestates as soil conditioner inoculated with fungi underline their long-term ecological potential for the remediation of polluted soils. This strategy is proving to be a promising and cost-effective tool for improving soil functionality and stability, rather than directly promoting plant growth.

The origin of the biomass used for anaerobic digestion plays a crucial role in determining the quality of the digestate. If the digestate is derived exclusively from cattle manure, its application to fungi inoculated soils will result in increased organic matter decomposition and mycelial colonization, as well as lower salinity and electrical conductivity. In contrast, the addition of mixed organic waste can increase the concentrations of TE and HM, which can impair the development of the microbial community and limit remediation efficiencies.

While the fungal strain selected for inoculation is a key factor, it must be evaluated in conjunction with other variables such as digestate composition, application concentration, pH, temperature, and contaminant type. Although authors specify that pH and temperature in particular affects the degradation of organic matter and removal of contaminants, no further analysis were reported regarding both parameters. Temperate conditions resulted to be more efficient than colder temperatures.

The combined application of fungi and digestate contributed to the reduction of Pb content and its increased bioavailability, while improving soil pH and availability of phosphorus, phosphates, and sulfates as soil nutrients. Aromatic plants such as coriander promote metal complexation and immobilization through their root exudates and the release of organic acids, especially when applied together with anaerobic digestate.

Interestingly, to improve the bioavailability of certain heavy metals, i.e. Cd, Zn, Cu, the absence of vegetation during certain phases of remediation may be necessary — an aspect that warrants further investigation.

Overall, the use of anaerobic digestate, especially from cattle manure, promotes microbial diversity and improves the processes associated with soil remediation and stabilization. However, the benefits to plant growth are less consistent and appear to be secondary to the effects on soil health. Future research should focus on pollutant-specific remediation strategies using digestate from manure, ideally in combination with tropical fungal strains to maximize effectiveness under different environmental conditions.

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Abbreviations

AD	Anaerobic digestion
Al	Aluminum
AM	Arbuscular mycorrhiza
As	Arsenic
B	Boron
Ba	Barium
C	Carbon
Ca	Calcium
Cd	Cadmium
Ce	Cerium
CH_4	Methane
CHP	Combined heat power generator
C/N	Carbon/nitrogen
C/N/P	Carbon/nitrogen/phosphorous
Co	Cobalt
CO_2	Carbon dioxide
Cr	Chromium
Cu	Cupper
EcM	Ectomycorrhiza
Fe	Iron
Ge	Germanium
H_2	Hydrogen
Hg	Mercury
H_2O	Water
HM	Heavy metal
HRT	Hydraulic retention time
H_2S	Hydrogen sulfide
Ir	Iridium
K	Potassium
Li	Lithium
Mn	Manganese
Mo	Molybdenum
Mg	Magnesium
n	Number
N	Nitrogen
Na	Sodium
Ni	Nickel
Nd	Neodymium
N/P	Nitrogen/phosphorous
O2	Oxygen
ODM	Organic dry matter
Os	Osmium
P	Phosphorous
Pb	Lead
PAHs	Polycyclic aromatic hydrocarbons
PFAS	Per- and polyfluoroalkyl substances
PGPR	Promoting rhizobacteria
Pr	Praseodymium
Pt	Platinum
Rb	Rubidium
S	Sulphur

Sb	Antimony
Se	Selenium
Sn	Tin
Sr	Strontium
Si	Silicon
Ta	Tantalium
Te	Tellurium
TE	Trace elements
Ti	Titanium
Tl	Thallium
TN	Total nitrogen
U	Uranium
V	Vanadium
W	Tungsten
Zr	Zirconium
Zn	Zinc

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