

Review

Soil symbionts in alleviation of heavy metals-laden sewage sludge-impacts in legumes: implications for phytoremediation

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Abstract

Increasing urbanization and industrialization are releasing a considerable proportion of solid wastes into the environment globally. A large number of addition of solid wastes including sewage sludge contributes to the agricultural soils with organic matter and several nutrients. However, sewage sludge has been the major source of large amounts of heavy metals in agricultural soils. Therefore, the impacts of the heavy metals-laden sewage sludge on the health of agricultural soil and plant/crops are obvious. Notably, soil microorganisms particularly arbuscular mycorrhizal (AM) fungi and root-nodule bacterium *Rhizobium* are two root symbionts widely reported to alleviate the impact of heavy metal-laden sewage sludge and promote plant growth and productivity. Integration of AM fungi with *Rhizobium* has been found promising especially in leguminous crops, where the net influence of this combination is supposed to be very high. This paper aims to: (i) briefly introduce the representative leguminous crops chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medik.); (ii) overview the microbial symbiosis in legumes; (iii) highlight the major sources of the heavy metal-contamination; (iv) enlighten major constituents of sewage sludge, status of heavy metals in sewage sludge, and the main impacts of sewage sludge on the health of both soil and plants; (v) discuss the major insights into the interaction of microbes with heavy metals, with implications of the outcomes for the phytoremediation; and also to (vi) mention the major aspects, to date least explored in the current context.

Keywords Heavy metals · Sewage sludge · *Rhizobium* · Arbuscular mycorrhizal fungi · Phytoremediation

1 Introduction

Legumes or pulses (Family: Fabaceae) are second to cereals in terms of their consumption as a fundamental staple food, and are widely grown crops and are primarily sold as dry goods worldwide [1]. The pulses are adequately rich in protein, carbohydrates (including dietary fiber), fatty acids, minerals and vitamins; hence form a significant portion of the human diet [2]. Additionally, these legumes are also known for their ability to fix atmospheric nitrogen, enrich soil nitrogen (N) and increase crop growth and yield. The tripartite relationship of the family leguminosae (Fabaceae) members with arbuscular mycorrhizal (AM) fungi and *Rhizobium* helps in the uptake of nutrients by plants, improves their growth and restores the unbalanced ecosystem [3]. In crop rotation strategy, the pulse crops help in controlling weeds and plant diseases and improve soil texture and fertility. Appropriate sources of plant nutrition and superior crop management practices result in greater yield of pulses. Phosphorus (P) is one of the essential elements for the proper growth and yield of pulses. The pulses fix the atmospheric-N through *Rhizobium* and P helps in nodule development and N₂-fixing

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efficiencies of *Rhizobium*. On association with suitable *Rhizobium* strain, pulses enhance their natural ability to solubilize rock phosphate and thus absorb more P from the soil on additional symbiotic association with AM fungi that also helps in solubilization of P [4]. Symbiotic rhizobial strains resistant to heavy metals have been found in legumes growing in contaminated sites like mine deposits and serpentine soils [5, 6]. Thus, association of pulses, *Rhizobium* and AM fungi form a tripartite symbiotic association. The application of sewage sludge for agricultural use is encouraged as part of their disposal management due to the presence of majority of elements essential for plant growth. But, presence of some toxic heavy metals in this solid waste and their uptake by plants pose a serious threat to the entire agroecosystem. Therefore, in this review, phytoremediation is discussed which is a technique used by plants for removing toxic substances from the sewage sludge amended soil. Due to their propensity to colonize marginal lands and nutrient deficient soils, legumes have been suggested as useful species for phytoremediation [7]. Common legumes having interaction with AM fungi and *Rhizobium* and which can be used as an implication for phytoremediation to remove heavy metals present in sewage sludge and are also widely used in our diet are mainly chickpea and lentil crops which are discussed in this review.

Chickpea (*Cicer arietinum* L.), often known as Bengal gram is a popular pulse crop in the human diet. It is a rabi crop that requires cool climatic conditions to thrive and a warmer temperature to mature [8]. Chickpea grows well on sandy and loamy soils with adequate drainage. It is a popular pulse crop cultivated and consumed worldwide, particularly in Afro-Asian countries. It is a valuable source of carbohydrates and protein, with higher protein content than other pulses. Owing to availability of high levels of all necessary amino acids (except for S-containing ones), chickpea has a number of health benefits. The dietary intake of legumes in addition to grains reduce the risk of cardiovascular disease, type 2 diabetes, gastrointestinal illnesses and several malignancies [9]. Thus, one of the main aims of chickpea breeders is to boost output by either improving the genetic makeup of cultivars or reducing biotic and abiotic stressors of the plant [10]. **Lentil (*Lens culinaris* Medik.)** is a self-pollinating cool-season legume. As a pulse crop, it is cultivated in subtropical and warm temperate regions, and at high altitudes in the tropics. Many research reports in recent years have revealed that pulses, particularly lentils, may offer several health benefits besides meeting basic nutrient requirements for humans [11]. Lentil is high in bioactive phytochemicals like recoverable and non-soluble phenolics, flavonoids, tocopherols, saponins, phytic acid and phytosterols linked to the health advantages of lentil consumption [12]. However, various biotic and abiotic variables such as drought, salinity, excessive temperature and mineral deficiencies limit lentil yield. Various agronomic approaches, including microbial symbiosis and organic fertilizer application in lentil are being investigated to increase the nutritional demands modern agricultural areas [13].

Thus, several data and scientific information have been collected from several past researches and this review is drafted based on the scientific reports obtain from several sources including Science direct, Web of science etc. to interest the interdisciplinary scientific research. This review aims to provide a brief overview of the representative leguminous crops and enlighten the microbial symbiosis in them; it also emphasize the main sources of heavy metal contamination particularly sewage sludge and to highlight its major constituents, the status of heavy metals in sewage sludge, and the main effects of sewage sludge on soil and plant health. The review also discusses the key findings regarding the interaction between microbes and heavy metals, strategies for phytoremediation and its future prospects in agriculture sector.

2 Symbiosis of microbial organisms in leguminous crops

Benefits of dual inoculation of nitrogen fixing bacteria (*Rhizobium*) and symbiotic fungus (AM fungi) have been studied by a number of workers. Amirnia et al. [14] studied that nitrogen-fixing soil bacteria along with AM fungi improve seed yield and quality of lentil crop; Havugimana et al. [15] also studied the role of dual inoculation of *Rhizobium* and AM fungi on pulse crops production; Khan et al. [16] and Yaseen et al. [17] also reported the influence of AM fungi and *Rhizobium* inoculation on the growth and quality of pulse crop. The dual inoculation of legumes with *Rhizobium* and AM fungi help plants in combating their nutritional deficiency [18], and in turn, helps in improving plant biomass [19] and yield [20] (Fig. 1).

2.1 *Rhizobium*

Nitrogen-fixing root nodulating bacteria (*Rhizobium*) are free living in soil and root regions of both leguminous and non-leguminous plants. *Rhizobium* tends to form a symbiotic alliance specifically with the roots of leguminous crops and form root nodules. As root nodules turn older after a period of N₂-fixation and tissue starts decaying, these motile *Rhizobium* forms get released into the soil. The release of rhizobia from nodules is crucial for the evolution of N₂-fixing

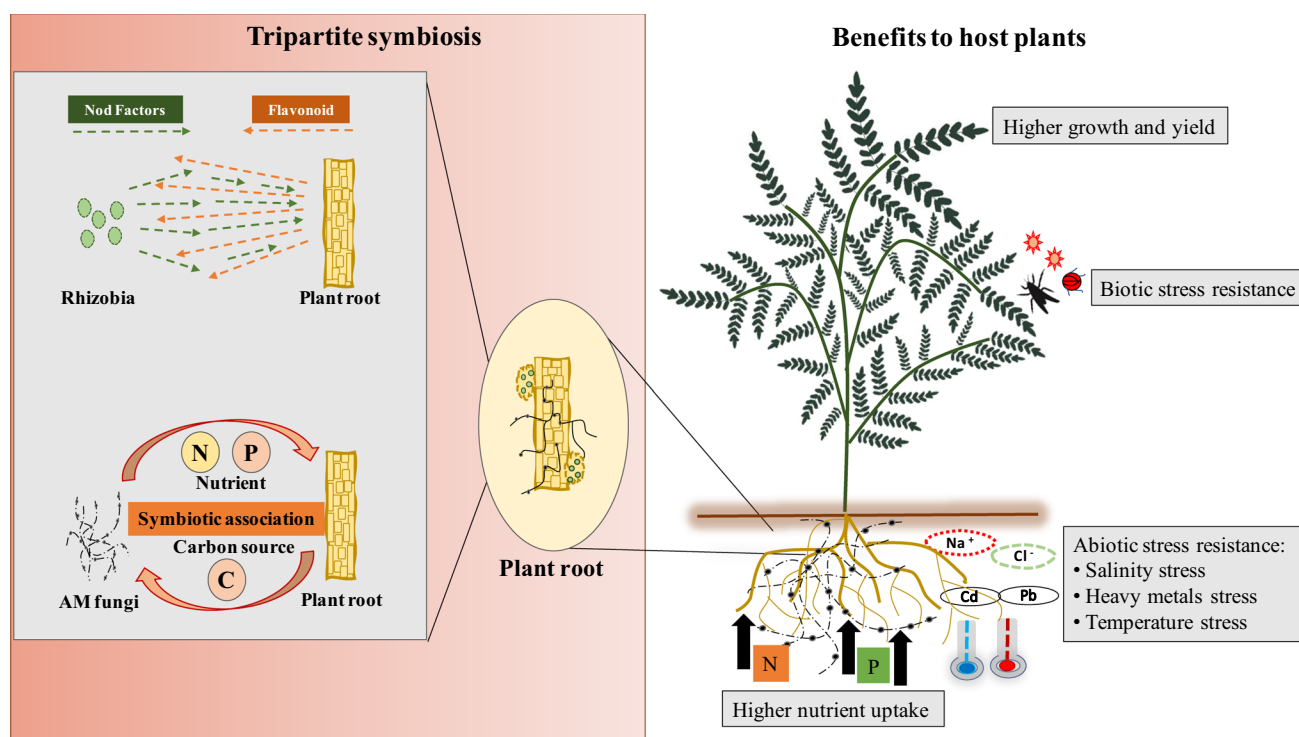


Fig. 1 Schematic representation of the tripartite symbiotic relationship between leguminous crop, *Rhizobium* and AM fungi

bacteria [21]. They act as an inoculum for the subsequent crops of a particular species of the legume [22]. After seed germination, the roots develop all the way through the soil and the loss of organic material supplies the driving force for the vigorous expansion of the microbial population around the root, known as the rhizosphere effect [23]. Saprotrophs stimulates microbial action. Mutualistic biotrophic symbiosis develops between rhizobia and legumes and mycorrhizal fungi may also interact with the host and resulting in more beneficial tripartite symbiosis [24].

Biological N₂-fixation is a complex process demanding the association of the bacteria (*Rhizobium*) and the host plant species. *Rhizobium* infests the root hairs of legume crops and results in the development of nodules, a site for fixing free air nitrogen which is present as a N₂ molecule, where two N atoms are bonded through a triple bond. It requires a huge amount of energy to reduce it to ammonia (NH₃), a form available to the plants with the help of the nitrogenase enzyme [25]. These bacteria occur in a variety of soil types and vary in their number, nodulation and N₂-fixing ability [26]. These bacteria improve pulse production and minimize the application of N₂ based fertilizers. To ensure an adequate rhizobial population, the legumes seed be inoculated with an efficient rhizobial strain. It helps in improving nodulation, N₂-fixation as well as growth and yield of pulses [27]. The efficient germination of seeds and their growth into a mature plant is determined by how well the prevailing soil conditions are for the plant. The plants in interactions with the rhizospheric microorganisms influence these beneficial factors [28]. Root nodulating symbiotic *Rhizobium* bacteria effectively regulates plant growth either naturally [29] or in stressed conditions, whether it be biotic stress such as soil pathogens [30] or abiotic stress like salinity, drought [22, 31], nutrients deficiency and heavy metals [22, 32]. Heavy metal toxicity affects the growth of host plants through adverse effects on population of beneficial microbes, causing nutrient deficiency or damaging the plant growth either directly or indirectly. Therefore, the association of root nodulating bacteria is important for leguminous crops for maintaining N-uptake and growth of the host plant, which can be halted by the presence of toxic heavy metals in soil.

2.2 Arbuscular mycorrhizal (AM) fungi

Arbuscular mycorrhizal (AM) fungi form a symbiotic association with more than 80% of terrestrial plants. The AM fungi are distributed worldwide in a varied range of climatic conditions. The host of AM fungi includes a variety of plant groups belonging to angiosperms, gymnosperms, pteridophytes and even bryophytes [33]. AMF associations are often reported

in submerged aquatic plants [34] in addition to some floating plant species [35]. The AM fungal association has been reported in a number of plants species including wheat, maize, beans, legumes, tomatoes, potatoes, soybeans, banana, mango, apples, oranges, grapes, castor, tobacco, tea, coffee, cocoa, sugarcane, asparagus, rubber, cardamom, pepper and millets [36]. AM fungal symbionts intake photosynthetic carbon from the root cortex cells of their hosts for the growth of intracellular arbuscules and intercellular hyphae in the apoplasts of the root cortex cells [37]. Through their specialized mycelium, AM fungi derives P, N and other minerals from a large soil volume and transfer them through the root system of host to their aboveground parts with the help of arbuscules [38].

The hyphae of AM fungi associated with host plants expand the surface area of the roots and improve uptake of nutrients by absorbing from a larger soil volume [39]. The AM fungi expand their extra radical hyphae several centimeters deep into the soil, and not only take hold of large amounts of nutrients but also absorb several undesired heavy metals present in the soil [40]. However, the efficacy of the nutrients uptake differs with species of AM fungi and their host plants [13, 41]. The increased uptake and flow of minerals like Ca, Cu, Fe, K, Mg, Mn, N, P, S and Zn to the host roots from a larger soil volume are some of the important aspects of mycorrhizae colonized plants [42]. Cysteine-rich proteins present in the fungal mycelium promote the uptake of nitrate and ammonium from the soil [43]. The glutamine synthetase activates carbon metabolism in *Glomus intraradice* and transports nitrogen to the host plant [44]. AM fungal colonized roots involve two major nutrient uptake pathways, namely the plant uptake pathway and the mycorrhizal uptake pathway. The plant uptake pathway involves the uptake of nutrients via low affinity uptake transporters present in the epidermis or root hairs. The uptake of P with low mobility via the plant pathway is often limited due to the development of depletion zones around the roots. In contrast, the mycorrhizal uptake pathway having evolved high affinity with nutrient transporters in their extraradical mycelium, enhances translocation along the intraradical mycelium hyphae in the root cortex [45]. A plant is simultaneously colonized by several communities of AM fungi with differing efficacy in the extent of nutrient uptake through the mycorrhizal pathway [46].

2.2.1 AM fungi as natural biofertilizers

AM fungi increase the nutrient acquisition to the host plant and are thus known as natural biofertilizers [47]. AM fungi help maintain the soil structure and reduce the nutrient leaching from soil as leaching harms the soil fertility and contaminates surface and groundwater [48]. Thus AM fungi maintain sustainable agriculture by reducing dependence on chemical fertilizers and pesticides. Besides several roles, mycorrhiza is mainly known for enhancing the phosphorus uptake in plants [49]. According to one estimate, approximately 50% of phosphate fertilizer application in croplands may be minimized by treating the plants with specific AM fungal inoculants [50]. The AM fungal symbiosis also increases the uptake of macronutrients like N, P, K, Ca, and Mg as well as micronutrients significantly, leading to enhanced carbon fixation and higher biomass accumulation even under abiotic stress [51]. Adequate nutrient acquisition, proportionately improves growth and yield of the host plants. AM fungi also improve yield and growth of food, fodder and fuel crops [52]. AM fungi also increase the tolerance or resistance in plants to different soil-borne diseases and also restrict the growth of pathogens. The AM fungi colonizes with the host root system and defend against the pathogens present in the soil rhizosphere [53]. But, some environmental conditions adversely affect the establishment of a specific AM fungal strain in forming a mutualistic association with the host [54]. Indigenous AM fungi with evolved physiological adaptations have capability to withstand the adverse environmental conditions of their habitat [55]. AM fungi also activate the soil biota and play a critical role between soil and plant roots in a natural ecosystem. In anthropogenically metal contaminated soil, AM fungi also enhance phytoremediation by mitigating metal toxicity (bioalleviation) and enhancing plant growth [56].

2.2.2 AM fungi and plant stress tolerance

Several abiotic stresses, like drought, salinity, high temperature and pollutants deteriorate soil quality leading to a serious threat to agriculture. All these stresses are among the major causes of crop loss, worldwide [57]. The tolerance mechanisms of mycorrhizal plants to abiotic stress have been reported by Malhi et al. [58]. The occurrence and abundance of AM fungi are influenced by prevalent agricultural practices, environmental conditions, soil type and with species of the host plant [59]. The symbiosis between plant and AM fungi improves photosynthesis and growth of the host plant by enhancing its tolerance to stress [60]. The AM fungi also enhance antioxidant defense mechanisms in host plants [61, 62]. In majority of cases, the arbuscular mycorrhizal fungi are known to increase heavy metals uptake in their host plant [63].

In fact, some AM fungi are also known to have adapted to metal contaminated soil by producing the glycoproteins "Glomalins" to immobilize heavy metals [64]. "Metallothionine", a protein released by AM fungi, reduces the heavy metal toxicity in soil [65]. AM fungi are among the most common biological methods of reclaiming heavy metals contaminated soil. Both AM fungi and hyperaccumulating plants have some unique abilities, making them suitable for treating heavy metals [66, 67]. Plants have in fact developed a symbiotic association with AM fungi as a defense strategy to combat stress. AM fungi can effectively immobilize the toxic heavy metals and minimize their uptake into the host plants by binding metal ions to the hyphal cell wall and excreting several extracellular exudates [68]. These studies suggest that AM fungi have evolved several mechanisms to adapt in contaminated soil and continue to benefit their hosts in turn of a small amount of carbon.

3 Heavy metals in environment

Contamination of soil, water and air with heavy metals has become the world's most consequential environmental problem [69]. Excess amounts of heavy metals in soil and water bodies can negatively impact the terrestrial and aquatic animals. These toxic metals, once established in the surroundings, are non-biodegradable and prevail for a prolonged period [70]. Heavy metals also pollute underground and surface water reservoirs. The contamination of surface water bodies ultimately results in degradation of soil sediments. The release of large amounts of industrial as well as domestic sewage without requisite treatment into the marine community consistently leads to a high level of increase in the quantity of heavy metals in it [71].

A small quantity of heavy metals occurs in the soil naturally; however, the addition of a larger amount from manmade sources leads to a problem of serious global concern. Anthropogenic activities like mining, unsafe disposal of waste, industrial pollutants, excessive use of pesticides and fertilizers in agriculture and irrigation with sewage water containing heavy metals are some of the major reasons for the loss of soil properties and its erosion [72]. The cycling of heavy metals in air, water and soil and their subsequent transfer into the food chain is a prime concern [73–75].

3.1 Sewage sludge

Due to the rapid increase in urbanization and industrialization across the world, large amount of solid wastes are being dumped into the environment particularly on land and in water bodies. Sewage sludge constitutes a major part of the total solid waste generated from such activities. A massive amount of these wastes are produced and discharged in closer proximity of urbanized areas and thus, cause a non-stop menace in the surroundings [76]. Hence, a proper sustainable disposal of the massive amount of sewage sludge in an eco-friendly way is important. Sewage sludge contains nearly all the nutrients needed for plant health. Due to some beneficial properties, sewage sludge is widely applied as fertilizers in agricultural land. In addition to nutrients, solid wastes also have potentially toxic non-essential heavy metals and other organic pollutants [77].

3.1.1 Major constituents

Water constitutes major bulk of the sewage sludge in addition to organic composites, macronutrients, micronutrients, heavy metals, organic micro-toxins as well as microbes [78]. Domestic wastewater includes black water from toilets and greywater from kitchens, bathrooms and cleaning wastes. Sewage sludge is a semi-solid sediment and is generated abundantly [79]. The solid contents in sewage sludge typically constitute 0.25–12% of weight, depending upon effluent type and different processes used for sewerage treatment [80]. Sludge properties usually depend upon the source of sewage discharge and treatment processes. During wastewater treatment, approximately 50–80% of the total heavy metals present in wastewater are immobilized in sludge through certain physicochemical and biological actions [81].

In urban societies, metals primarily come from human waste, cosmetics, detergents and kitchen wastes. The industrial waste sludge varies in its chemical constituents subject to the kind of industrial activities, climate and other factors [82]. Some of the major constituents present in sewage sludge includes nutrients like Nitrogen (N), Phosphorus (P), Potassium (K) along with heavy metals like Cadmium (Cd), Chromium (Cr), Lead (Pb), Nickel (Ni) and Zinc (Zn) [24, 77]. The data on the typical concentration of organic carbon, nutrients and heavy metals present in sewage sludge has been described earlier by Antoniadis et al. [83]. The total amount of sludge produced worldwide has increased significantly over the past several years and is expected to raise many-fold over the next few years [84]. Due to presence of harmful contaminants in

it, the greatest concern is the removal of its toxic substances in an economical and environmentally sound manner [85]. Proper recycling or environmental disposal of urban sewage sludge is lagging behind its required goals and people are facing a greater challenge in adequate treatment of consistently increasing amounts of sewage sludge.

3.1.2 Major effect on plants

Spreading of sewage sludge as fertilizer or manure in farmlands is considered as an ecofriendly way of its disposal [85, 86] due to presence of large amounts of organic matter, macronutrients such as nitrogen and phosphorus in addition to other essential nutrients [87]. Many findings have suggested that supplication of sewage sludge showed considerable improvements in the growth of several crops including faba bean [88], cucumber [89], corn [90], poplars [59], wheat [91] and mung bean [92]. Sewage sludge as an organic fertilizer promotes plant growth and productivity [24, 77, 93, 94]. The use of sewage sludge has resulted in a significant boost in the development and yield output of various plants in recent years. With soil amendment containing sewage sludge up to 12 kg m^{-2} , the shoot length, leaf area, and total biomass of mung beans (*Vigna radiata*) and rice (*Oryza sativa*) improved [92, 95]. The soil treated with sewage sludge has been linked to higher growth of oats (*Avena sativa*) and barley (*Hordeum vulgare*) [96]. According to Gu et al. [97], adding 300 tons ha^{-1} of sewage sludge to the soil boosted the fresh weight of the ryegrass's roots and above-ground sections.

The addition of sewage sludge in the soil increased chlorophyll contents in the host plant more efficiently than on application of other fertilizers. Photosynthesis and other biochemical parameters improved in rice (*Oryza sativa*) grown in soil with varied sludge concentration [98]. Similarly, enhancement in the pigment biosynthesis, total sugar and protein contents in wheat and Jews mallow plants grown in sludge amended soil has been reported by Mazen et al. [99]. The amount of sugars and ascorbic acid was higher in the roots and leaves of sweet potatoes planted in soil enriched with sewage sludge [100]. Increasing sludge concentrations in the soil led to corresponding increase in lipid peroxidation, ascorbic acid, peroxidase activity and amount of proline indicating oxidative stress proportional to the quantity of applied sludge, besides the reduction of thiol and phenolic content in rice plants [98]. Mazen et al. [99] found that the amount of proline reduced in wheat and mallow plants treated with 25% of sewage sludge but increased on application of 75% sewage sludge, indicating that plants at this level were affected by coercion.

Sewage sludge application increases crop yields of *Phaseolus vulgaris* [101]. Yamur et al. [102] reported a significant increase in seed yield of lentils (*Lens culinaris*) grown in soil treated with 6 kg m^{-2} sewage sludge. When soil enriched with 2.51 kg m^{-2} sewage sludge, Lady's finger (*Abelmoschus esculentus*) production also rose to 135% [103]. Growing rice (*Oryza sativa*) in soils containing different quantities of sewage sludge resulted in an increase in yield; the maximum yield was obtained when 12 kg m^{-2} of sewage sludge was applied [95]. Mung bean (*Vigna radiata*) growth and yield also increased with a 12 kg m^{-2} application of sewage sludge [92]. Hence, increased productivity on sludge treatment has been attributed to increased plant nutrient availability on sludge application. Higher plant growth performance is thought to result from the sludge's early commencement of the reproductive cycle [86]. Certain plants had a shorter life span and an early commencement of the reproductive cycle due to the presence of heavy metals [82] and other physico-chemical or ecological pressures [104].

3.1.3 Major effect on soil

3.1.3.1 Physical properties The physicochemical properties of soil get altered on sewage sludge application in agricultural land as a fertilizer. Because of the persistence of organic compounds in biosolids, the incorporation of sewage sludge boosts the physical properties of soil, such as its overall density, permeability, capacity for water retention, and formation of soil aggregates and stability [105]. These properties can also outperform farm manure applications to soil. Higher amounts of sewage sludge in wheat croplands reduced bulk density and improved soil porosity [106]. The increase in aggregate stability and total porosity is a contributing factor in the decrease in bulk density. Cameron et al. [107] reported that the addition of sludge increased the stability of soil aggregates by 41% in sandy loam soil but not on addition in clay soil and inferred that the beneficial impact of sewage sludge depends upon the soil type. Sewage sludge amendment improved soil physical characteristics, which led to higher soil filtration rates, less surface runoffs and overall lesser water erosion.

3.1.3.2 Chemical properties Application of organic matter rich sewage sludge in soil increased the synthesis of humic compounds and other organic acids, essential for modifying the properties of soil. In two contrary findings, application

of municipal sewage sludge in one case increased the soil pH [108] and in another reduced the soil pH while increasing organic carbon, accessible phosphorus and total nitrogen levels [92, 95, 98, 109]. Acid generation during sludge decomposition and the calcium carbonate concentration of the sludge has been linked to changes in soil pH. High amounts (100 t ha^{-1}) of sewage sludge application in a calcareous soil reduced the soil pH from 8.2 to 8.0 [110]. Application of sewage sludge to soil improved cation exchange capacity through additional cation binding sites to vital plant nutrients effectively available within the root zone of higher plants [111]. One of the primary criteria for sewage sludge's suitability for agricultural application is the ability of humic compounds to bind high quantities of nitrogen in it [112].

There are some harmful adverse effects of the addition of sewage sludge in soil. Sewage sludge addition to soil generally increases the amount of heavy metals both in soils and plants [24]. Some soil characteristics like pH, redox potential, sesquioxide content, organic matter and sludge application rate, also affect the bioavailability of the metals. Morera et al. [113] studied the bioavailability of four heavy metals (Cu, Ni, Pb and Zn) to sunflower from sludge applied in soils with four different physico-chemical properties and found that Zn bioavailability was favored by acidic soil and Cu bioavailability by alkaline soils. Hazra et al. [114] also noted that phytoavailable heavy metals (including Cu, Ni, Pb and Zn) increased with the rate of sludge application.

3.1.3.3 Biological properties Implementation of sewage sludge promotes the re utilization of nutrients from sludge into agriculture by boosting microbial biomass carbon (C), basal respiration, N-mineralization, and several soil enzyme activities (urease, BAA-protease, dehydrogenase, alkaline phosphatase, and β -glucosidase) [115]. In contrast, Banerjee et al. [116] reported that sludge application decreased the diversity and microbial population, but did not affect the size of the soil's microbial biomass, its capacity to mineralize nutrients and activity of soil enzymes like arylsulphatase, acid phosphatase and alkaline phosphatase. Kızılkaya and Bayraklı [117] investigated the effects of various doses of sewage sludge applied in soil (0, 100, 200 and 300 t ha^{-1} dry wt.) and C/N ratios (3:1, 6:1 and 9:1) and found significant increase in the activities of β -glucosidase, alkaline phosphatase, arylsulphatase and urease. The highest β -glucosidase activity resulted in soils with highest C/N ratio and sludge dose. The soil modified with the lowest C/N ratio and the largest dosage of sludge showed the greatest action of urease, alkaline phosphatase, and arylsulphatase [117]. Low concentrations of heavy metals in sewage sludge had numerous positive effects on soil microbial biomass, organic carbon and soil microbial activity, whereas higher concentrations significantly reduced organic carbon, soil microbial biomass level, activities of soil enzymes and soil microorganisms [118].

3.1.4 Occurrence of heavy metals in sewage sludge and its impact on health and productivity

Heavy metals interfere with the agronomical values like depreciation of soil parent materials and atmospheric accumulations [119]. Sewage sludge is one of the major and consistent source of heavy metal addition in agricultural soils [120]. Cadmium, chromium, lead, nickel and zinc are constituents of major heavy metals in sewage sludge. The relative proportion of heavy metals varies with the source of sewage (domestic or industrial) and the degree of treatment of sludge in sewage treatment plants [83]. Despite, soil improvement and plant growth promoting abilities, the adverse effects of heavy metals contained in the sewage sludge on food chain organisms cannot be set aside. Organic and inorganic pollutants found in the sludge may enter consumer food chains via consumable sections of agricultural plants growing in the sludge amended lands and such food chains are of serious concern [74, 75, 121, 122]. Heavy metal concentrations may be leached into groundwater or absorbed by plants and transported to other food chains [123]. Concentrations of Cd, Cr, Pb, Ni and Zn in sewage sludge amended soil were significantly higher [124]. The application of sewage sludge increased the amounts of Cd, Cu, Ni, Pb and Zn in soil far within the permissible limits for agricultural soils [125]. Fluctuations in the types and concentrations showed variations in various studies. These fluctuations are attributed to the source and extent of sewage sludge treatment [122].

Shoots and roots of *Beta vulgaris* [109], *Oryza sativa* [95] and *Vigna radiata* [92] cultivated on sewage sludge remedied soil had greater concentrations of Cd, Cr, Cu, Ni, Pb, and Zn. Accumulations of Cd, Cr, Cu, Ni, Pb and Zn were higher in roots than in the shoots of chickpea plants grown in heavy metals contaminated soil [126]. On the contrary, it was also discovered that the shoots of numerous crops accumulated more amounts of metal than the permissible limits, rendering them unsafe for human or animal consumption [74, 92, 109]. The convergence of several metals in ryegrass was enhanced by the addition of sewage sludge to the soil [97]. Cd, Cr, Cu, Mn, Ni, and Zn rose by 1130, 12.9, 355, 108, 2230, and 497% in the roots and by 431, 4.3, 92.6, 58.3, 890, and 211% in the aboveground sections of ryegrass with the use of 300 tons ha^{-1} sewage sludge in the soil [97]. The content of heavy metals in the tissues of rice plants was also raised by the usage of sludge. According to Latare et al. [85], rice grain's Cd content exceeded the Indian threshold when 20 tons of sludge was applied to the soil annually. The

periodic application of biosolid compost with a rate of 20 tons/fed in chickpea and maize plants grown at different rates of mineral NPK fertilizers with surface drip or subsurface drip irrigation systems saved about 25% of the mineral NPK fertilizers proved to be the best means of recycling farm and human wastes for a clean environment [127].

4 Microbes and plants-heavy metal interaction

Rhizobium inoculation promotes crop productivity and greater plant biomass accompanied by an increase in nitrogen content in plants. Apart from their innate capacity of fixing nitrogen, *Rhizobium* can also help plant growth through processes such as adsorption and metal tolerance. *Rhizobium* forming root nodules with their hosts increase metal accumulation in roots. In contrast, bacteria in the rhizosphere reduce metal toxicity by processes such as precipitation, chelation, inhibiting metal transport and biosorption. Once rhizobia allied with its host, the nodules can operate as a storage location for metals, reducing their ability to translocate into aboveground plant sections [7]. The uptake of Pb, Cu and Cd in *Mimosa pudica* symbiosis with *Cupriavidus taiwanensis* was 86, 12, and 70%, respectively, higher than in nodule-free host without symbiosis with *C. taiwanensis* [128].

Rhizobial symbionts of the genera *Mesorhizobium*, *Rhizobium* and *Aminobacter* have been discovered to be linked with *Anthyllis vulneraria*. New rhizobial species like *Mesorhizobium metallidurans*, *Rhizobium metallidurans* and *Aminobacter anthyllidis* are among them [5, 129–131]. These new rhizobial species have only ever been discovered in Pb contaminated areas and not in unpolluted soils [6]. When grown in soil contaminated with metals, inoculating *Vicia faba*, *Lens culinaris* and *Sulla coronaria* with consortia of rhizobia and non-rhizobia improved plant growth and pod yield. Additionally, compared to uninoculated plants, the infected *S. coronaria* acquired substantially more cadmium [132]. These findings show that the success of phytoremediation by *Rhizobium* inoculated legumes may be influenced by root associated microbial populations. Researchers working on the recovery of places contaminated with heavy metals found interesting relationship between leguminous plants and symbiotic rhizobia [133]. Low dosages of Ni^{2+} were used in the treatment to increase hydrogenase activity in *Rhizobium leguminosarum* bv. *viciae* and stimulate nitrogen fixation and plant development in soybean [134]. Besides many beneficial roles of *Rhizobium* in mitigating adverse effects of heavy metals on the host plant, the symbiosis is negatively impacted by an excess of heavy metals, which results in fewer symbiotic nodules, decreased percentage of nodulation and nitrogen fixation [7].

Similarly, AM fungi aid in the phytoremediation of heavy metal contaminated soil by attaching the heavy metals to their chitin based cell walls. The mechanism known as passive adsorption binds around 0.5 mg of Cd per mg dry mass in fungal mycelial branches [135]. The AM fungus has a vast hyphal network that binds heavy metal contaminants from soil and works as a heavy metal sink. Heavy metal resistant AM fungus may withstand 2–4 times more of the host roots [136]. Pollutants infiltrating the ecosystems through the ingestion of heavy metals affect food crops and endanger the entire food chain, making heavy metal removal from the terrestrial land critical [137]. Metal transformation in the rhizosphere is influenced by AM fungi in a variety of ways, including immobilization, acidification, hyphal sequestration, change of root exudates and chemical coagulation inside the ground [136, 138]. The symbiotic relationship between the AM fungus (*Funneliformis mosseae* and *Rhizophagus intraradices*) with lettuce resulted in varied Zn uptake, while application of *R. intraradices* increased Zn levels, *F. mosseae* did not [139]. The AM fungus aids in mineral nutrient uptake and lowers systemic toxicity in plants. Metals such as Cu, Fe, Mn and Zn are necessary for a variety of biological activities in plants, but they can be poisonous in large amounts. AM fungi regulate their transport by acting as a homeostat [63]. Table 1 shows certain studies on interaction in leguminous plants (chickpea and lentil) by *Rhizobium* and AM inoculants and their effect on plant growth and productivity.

5 Implication for phytoremediation

Heavy metal-contaminated soils can be remedied using a variety of phytoremediation techniques, such as (i) phytostabilization, which uses plants to lower the bioavailability of heavy metals in the soil, (ii) phytoextraction, which uses plants to draw out and eliminate heavy metals from the soil, (iii) phytovolatilization, which uses plants to capture heavy metal from the soil and emit it into the atmosphere as volatile substances, and (iv) phytofiltration, which uses hydroponically cultivars to absorb or adsorb ions of heavy metals from underground water and liquid wastes [140]. Various inorganic and organic pollutants are responsible for soil pollution, and their phytoremediation is regarded as an efficient and safer way for the elimination of these pollutants [141]. Repeated cycles of plantation and harvesting of heavy metals accumulating plants reduce the heavy metals concentration and toxicity in soils [142]. The effective phytoremediation depends upon

Table 1 Representative studies on the interaction of *Rhizobium* and arbuscular mycorrhizal (AM) fungi inoculants and its mechanism of action on the growth and yield of selected leguminous plants

Host Plant	Interaction of symbiotic microbes	Mechanism of action of symbiotic microbes	Effect on plant	References
<i>Lens culinaris</i>	<i>Rhizobium</i> and AM fungi	Tolerance strategy by accumulating heavy metals (Cd, Pb and Zn) from fly ash amended soil in plant root system	Improved plant growth, biochemistry and symbiotic microbial population	[13]
<i>Lens culinaris</i>	N ₂ -fixing bacteria and AM fungi (<i>Glomus intraradices</i>)	Enhanced water and nutrient uptake under water scarcity	Improved growth and productivity	[14]
<i>Lens culinaris</i>	<i>Rhizobium</i> and AM fungi	Enhanced nutrient availability (N, P) to plants	Increased in growth, chlorophyll content, and quality of lentil plant	[17]
<i>Cicer arietinum</i>	<i>Rhizobium</i> and AM fungi	Increased P uptake	Increased plant growth and dry matter yield of chickpea plant	[19]
<i>Cicer arietinum</i>	<i>Mesorhizobium ciceri</i> and AM fungi (<i>Glomus fasciculatum</i>)	Stimulatory effect of dual inoculation due to increased population of these microbes	Increased growth, yield and nutrient uptake	[20]
<i>Lens culinaris</i> ; <i>Cicer arietinum</i>	<i>Rhizobium</i> and AM fungi (<i>Glomus bagyarajii</i> / <i>Funneliformis caledonius</i>)	Tolerance strategy by chelating heavy metals (Cd, Cr, Pb, Ni and Zn) from sewage sludge in roots and preventing translocation to shoots	Increased growth, biochemical content, nutrient uptake and yield	[24, 77]
<i>Cicer arietinum</i>	<i>Rhizobium</i> and AM fungi	Increased NPK uptake	Increased plant growth	[93]

the bioavailability of the contaminating heavy metal, besides other factors. As, Cd, Cu, Ni and Zn are highly bioavailable, Mn moderately and Cr as well as Pb are least bioavailable for plants [143, 144].

Some plants, along with their rhizospheric symbiosis with microorganisms are more helpful in the remediation of contaminated soil and water [145]. Some symbiotic microbes have ability to change absorptive properties of the host roots by increasing their length, root surface area and number of root hairs and thus, increase metal uptake by the plants [146]. Therefore some symbiotic microorganisms and their host plants have greater potential to carry out the bioremediation of heavy metals in any contaminated soil [147].

Rhizobium species resistant to heavy metals act directly on these metals by chelation, precipitation, transformation, biosorption and accumulation and hence can be used for phytoremediation. In addition, *Rhizobium* acts as a plant growth promoter as it helps in nitrogen fixation, phosphorus solubilization, synthesis of phytohormones and some enzymes enhances legume growth by reducing heavy metal toxicity [7]. This mutualistic association between *Rhizobium* and roots of leguminous crops favors the plant–microbe relations and has widely been used in agricultural lands as a source of nitrogen for crops which in turn increases plant growth. Besides this, symbiosis is also important for areas polluted with heavy metals for increasing soil fertility as well as alleviating metals concurrently. Thus, *Rhizobium* can augment phytoremediation by fixing nitrogen and producing substances that encourage plant growth. Because of the shift in accessibility, would cause an increase in the absorption of metals and their transfer from soil to plant [148]. A few strains of *Rhizobium* have the ability to absorb and store metals, which aids in phytostabilization in an indirect way [149].

Similarly, several AM fungi are known to promote nutrient uptake to increase growth of their host plant and thus, decrease heavy metal toxicity by dispersing in larger plant body and let the host plants survive in metal contaminated soils [150]. AM fungi intensify heavy metals intake in the host plants and encourage processes like phytostabilization and phytoextraction in several plant species [151]. Total Pb and Cd accumulated by the roots were about 2 and 3 times higher in mycorrhizal plants than in non-mycorrhizal plants. A small amount was transferred to the shoots of *Limonium sinuatum* [152]. Greater concentrations of Cd, Co, Cu, Pb, U and Zn in soil and roots were directly related to lower mycorrhiza infection [153]. Cd negatively affects the root colonization and development of AM extraradical hyphae, impacting mycorrhizal symbiosis with the host plant [154]. *Gigaspora margarita* inoculated plants had high shoot dry weight with high P, Cu, Zn and Pb concentrations except Cd concentration [155]. Inoculation with AM fungi significantly increased activities of antioxidant enzymes than the non-mycorrhizal ones, which indicates that AM fungi phytostabilized Cd stress in *Trigonella* plants [156]. Sharma et al. [157] inoculated one Cd tolerant and other non-tolerant wheat genotypes with AM fungi and revealed that higher accumulation of stress metabolites like sugar, proteins, proline and glycine betaine elevated plant growth in tolerant genotype and managed the Cd stress than non-tolerant wheat species.

6 Conclusions and future prospects

Recycling of sewage sludge is a key component of the circular economy. Stabilization of sewage sludge can restore its agronomic value which can be utilized in achieving sustainable agricultural activities for its use as a fertilizer to improve soil properties and grow crops. Sewage sludge is rich in nutrients like potassium, calcium, phosphate, and nitrogen. However, sewage sludge consists of both heavy metals and nutrients in it, heavy metals can affect growth of host plant directly or indirectly but the nutrients present in it promote the growth of host plant, although excessive nutrients in it can often hamper the establishment of symbiotic association between the host plant and soil microbes. The soil symbionts like *Rhizobium* and AM fungi help in plant growth and protect the plants against the stress caused by heavy metals. But, the beneficial roles of symbiotic microbes in mitigating heavy metals stress in the host plants and the adverse effects of heavy metals on population of microbes may vary from species to species of the host, *Rhizobium* and AM fungi as well as sewage sludge source and rate of its application in addition to environmental variables. This review concludes with the benefits of using legumes inoculated with symbiotic microbes. However, to obtain the greater benefits from legumes accompanied with the symbiotic microbes for phytoremediation of heavy metals, it is crucial to have a thorough understanding of interactions between the host plant–*Rhizobium*–AM fungi relationship with the heavy metals. In future, a long term research vision should include the use of sewage waste that build on a thorough understanding of sewage sludge properties and its appropriate doses along with their interactions with soil micro-organisms when utilized in agriculture.

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