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Screening, Isolation and characterization of Soil microbes at the banks of the Song River

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ABSTRACT
The Song River is a river in Uttarakhand, India, that drains the central and eastern sections of the Doon Valley. It is located in the Dehradun district and it flows from Dhanaulti to Narendranagar, beginning as a spring-fed stream on the southern slopes of the Radi Top on the Mussoorie crest of the Himalayan range which is a Ganges tributary. The aim of this research is Isolation and characterization of soil microbes at the banks of the Song River in Dehradun. Different soil samples were taken from various locations along the Song River's bank Microbes are evaluated in the samples that have been gathered. Using serial dilution agar plating procedures, the microorganisms found in the soil samples are screened in Petriplates. The morphological and staining characteristics of the bacteria identified are used to identify them. In this study, we identified various type of soil borne pathogens in the banks of the song river. The relationship between soil microorganisms and human health is being investigated. A more comprehensive knowledge of the soil ecology and its ties to agronomic productivity and human health is required.
Key Words: *Soil borne Pathogens, Song River, Human health, Dehradun*

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INTRODUCTION

The soil is a critical part of the atmosphere because it acts as a home for plants, animals, and microbial life. Microscopic life (bacteria, fungus, algae, protozoa, and viruses) coexists with macroscopic life (earthworms, nematodes, mites, and insects), along with plant root systems, in the soil. Several external conditions influence the quantity and types of microbes found in soil, including the volume and type of nutrition availability, obtainable humidity, oxygenation, acidity, and temperature. Micro colonies form around sand grains, making soil a suitable environment for the multiplication of microbes [1]. An immense collection of organic materials in the ground supports the establishment of a big microbial population in the soil. The majorities of these microbes are bioactive and may be found in the top few inches of agricultural soil. The activities and diversity of soil microbes are regulated by abiotic and biotic stimuli. The abundance of microorganisms in soil is determined by the types of plants present, the structure and chemical makeup of the soil, the nutrient availability, pH, moisture content, climate, and temperatures. All of these factors influence the physiology of the soil, which changes from season to season in the same location. Furthermore, the disposal of organic residues from farming activities assures that increased nutrient contents soil is available for microbial development [2].

Soil has a wide range of microorganisms, especially bacterium, which may thrive in any naturalistic way. Among all of the microorganisms that live in the atmosphere, bacteria are the most prevalent [3].Animal manure is improperly disposed of, and its inappropriate use as fertilizer results in the propagation of resistance in soil microbes, which subsequently operate as a chronic source of antimicrobial resistance. Furthermore, antibiotic-treated poultry, cattle, and pigs have massive numbers of antibiotic-resistant bacteria, which are transferred to people via direct communication with the animals as well as their meat, eggs, including milk [4]. Antibiotic resistance is a growing problem that threatens to people's health and agricultural output. As a result, finding and developing novel antibiotics is critical in the fight against drug-resistant bacteria. The microbiological variability in seafloor environment is hardly understood,

despite the fact that it is an infinite resource that has been underutilized. The true potential of this sector as a biotechnological foundation is virtually untapped, especially in India [5].

Pseudomonads are a kind of free-living bacteria that may be found in soil, marine, and freshwater. P. *aeruginosa*, in instance, is found in a variety of settings, including soil, saltwater, and sewer, and is linked to several flora. Despite being separated from the sea, the species' obvious range has been limited to stream outfalls and coastlines [6]

Pseudomonas has a lengthy history as a potential for microbial control agents, with several evaluations and findings of extensive study [7].

Antibiotic resistance has risen among pathogenic bacteria in today's world as a result of its abuse and exploitation in the medical, agricultural, and pharma sectors. These germs pose a significant risk to humanity. Drug-resistant microorganism diseases claim the lives of around 700,000 individuals yearly [8].As we combine rising food requirements with the need to sustain soil lifespans while also providing key ecosystem services like carbon storage, how we manage our soil health is becoming increasingly important. Livestock production consumes a lot of land and accounts for around 15 percent of all human greenhouse gas emissions, yet it is critical for worldwide nourishment and economic security [9].Because certain soil microbes are closely linked to harmful microbes that infect humans, it's crucial to understand their ecosystem in natural environments, as well as the health risks they bring. Many soils pathogenic bacteria are spread by dust and enter the body through the skin or lungs, causing illnesses including nocardiosis and sporotrichosis. Living species interact in a complex network of biotic and abiotic interactions in the soil, making it a dynamic system. The soil microflora is an important aspect of the environment, and microorganisms constitute the most numerous and diverse group of soil microorganisms. Bacteria can survive in a wide variety of environmental circumstances, such as extreme temps, severe acidity, as well as a lack of water [10].

Microbial soil activities are intimately linked to soil fertility and environmental quality. Fungi and bacteria make up the majority of the soil microbial biomass, and they serve as excellent models for researching metal toxicity at the cellular level [11].Previous research of microbes that relied on microbiological cultures on specified medium excluded a large section of the microflora that was deemed uncultured. And over 90 percent of bacteria are unable to be cultivated under standard laboratory settings, and many are unidentified or untested by any current means. The recent advancement of metagenomics allowed for the investigation of the genomes of a large number of bacteria found in the atmosphere. The genetic diversity of bacteria may be measured by sequencing variable regions in their genomes without having to cultivate them. This data may also be used to evaluate the microbe's population makeup and functional biodiversity [12].

MATERIAL AND METHODS

Collection of Sample

Take upper layer of the soil from the n=10 different banks of the song rivers sites in the UV sterile plastic bags. The sampling sites were banks of song river Dehradun. The Song River is a river in Uttarakhand, India, that drains the central and eastern sections of the Doon Valley. It is located in the Dehradun district. It flows from Dhanaulti to Narendranagar, beginning as a spring-fed stream on the southern slopes of the Radi Top on the Mussoorie crest of the Himalayan range which is a Ganges tributary.

Most Probable Number of soil Isolates

In the most probable number (MPN)15 tubes of MacConkey broth are required, 10 tubes of single strength and 5 tubes of double strength. 0.1 gram and 1 gram are contained in a set of 5 tubes for single strength, and 10 tubes for double strength used 10 gram of soil sample. We cultured positive fermentation tube cultures for 48 hours at 44.5°C for confirmation of faecal coliforms. Total and faecal coliform gas positive tubes were used to determine MPN per 100 ml. E. coli contamination was confirmed by growing inoculums on EMB Agar at 35°C for 24 hours.

Total Soil Bacterial Count

Cells that were viable were counted with a tenfold dilution. Various enrichment medium plates were spread with bacterial cultures after overnight growth. After incubating the plates for 24 hours inverted at 37°C, we stored them in an incubator.

Isolation of Soil bacteria

On an enrichment medium, water samples were spread out and incubated under ideal development conditions. After subcultures were performed, each isolate was further investigated.

Characterization of Soil isolates

Gram's staining studied the colony morphology (shape, color, and texture) of the acquired bacterial colonies were studied macroscopically and microscopically. In order to examine the morphological

characteristics of the isolates, a single colony was chosen for smear preparation and staining. Further biochemical characterization of bacterial isolates was accomplished through the use of Indole, Methyl Red, VogesProskauer, Citrate Utilization test (IMViC), and Triple Sugar Iron Agar (TSI) tests. Positive and negative controls were utilized to distinguish positive from false-positive reactions.

Carbohydrates of a Soil isolate

The oxidation of glycol creates various secondary metabolites due to the metabolism of carbohydrates. We used 5 different types of carbohydrates for soil isolates such as galactose, adonitol, fructose, dextrose, and mannose.

Antibiotic sensitivity of Soil isolates

The bacterial isolates from soil samples were tested against 5 antibiotics: Azithromycin, Gentamycin, Levofloxacin, Vancomycinand Tetracycline.

RESULT

Most Probable Number of soil Isolates

The highest range of faecal coliform in Song River water was found in Site 7 which was 170, and lowest range is 10 from Site 1. The highest range of Coliform is 46 in Site 7, and lowest was 1.8 in Site 1. **(Fig.1).**

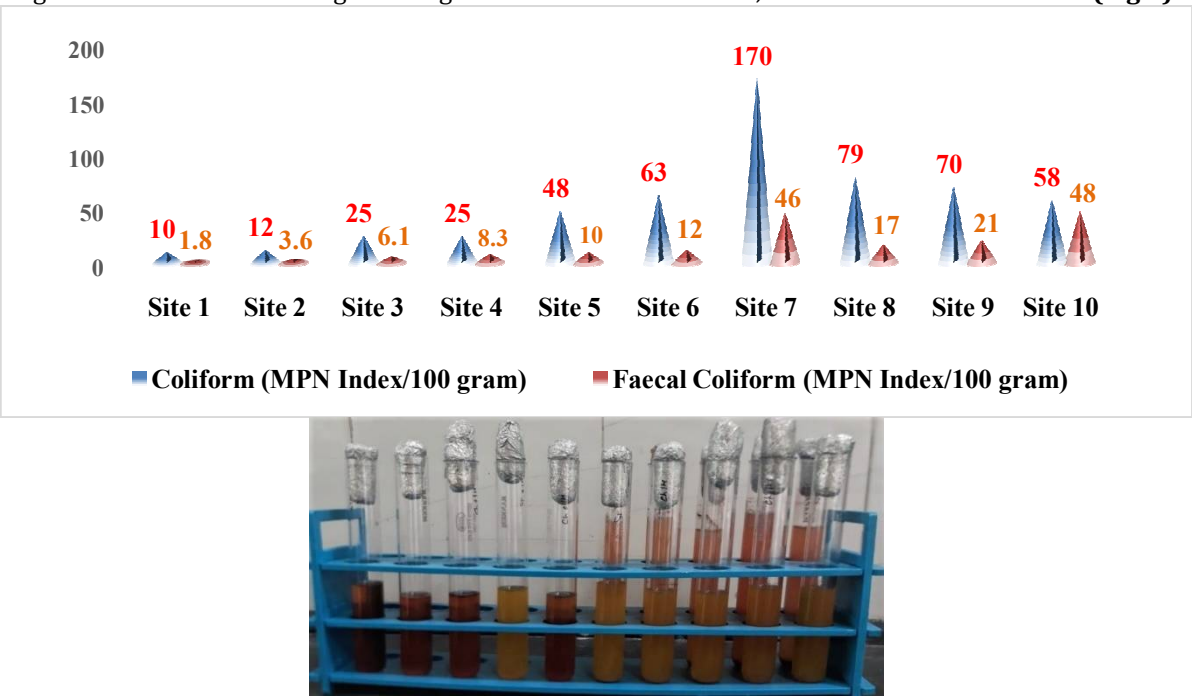
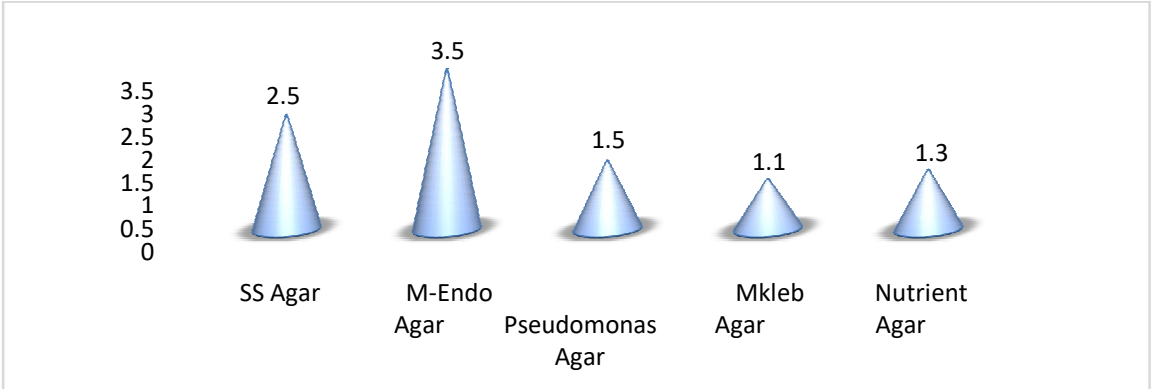


Fig.1.Coliform and Faecal ColiformandMost Probable Number in Song bank Soil sample

Total Bacterial Count / Standard Plate Count

On the different enrichment medium, we have found different bacterial colonies in the soil samples of Song River banks. There were Salmonella Shigella agar, M-Endo agar, Pseudomonas agar, M-kleb Agar Base and Nutrient agar2.5, 3.5, 1.5, 1.1, 1.3 colonies per gram on 10^{-7}dilution plate were found.**(Fig.2)**



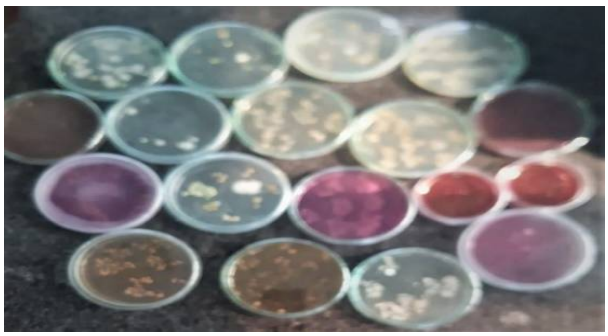


Fig.2. Cfu/gram (10^{-7}) in different Agar Medium and Serial Dilution Plate

Isolation of soil bacteria

After the colonies were found in the dilution plates and picked, the bacterial colonies were stick on the different enrichment medium, then incubate the plates in 37°C - 24 hrs. Soil isolates are *E. coli, Salmonella, Pseudomonas, and Klebsiella sp.* were found.**(Fig.3).**

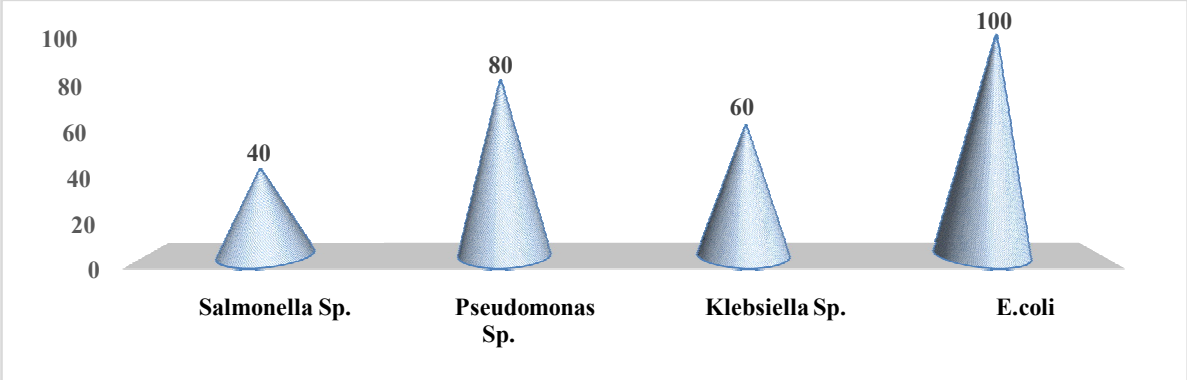
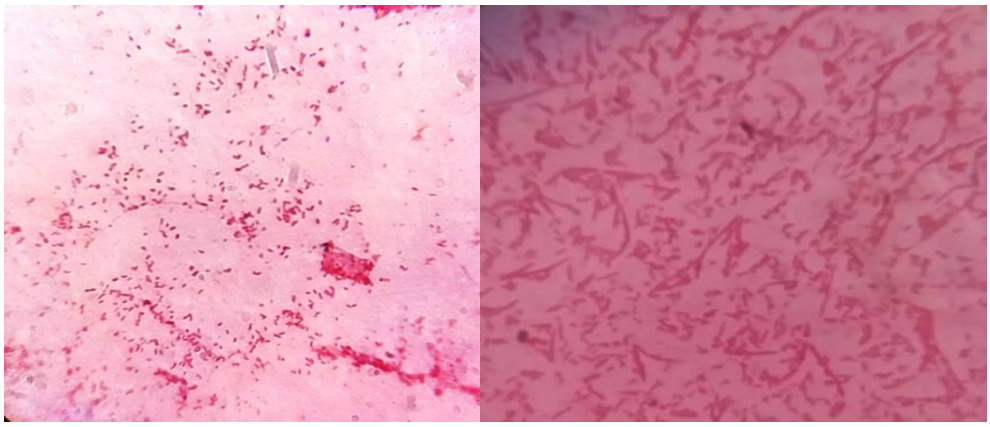


Fig.3. % of Song bank Soil samples isolate

Characterization of soil isolates

The bacteria characterized by colonies morphology, and gram straining of the following isolated *sp.* as *Salmonella, Pseudomonas, E. coli, Klebsiella*were giving different types of color developed in selective mediums. Black centre with opaque colonies by *Salmonella sp.*, Green metallic by *E. coli sp*., Mucoid and pink color by *Mkleb sp.*, and Irregular, slightly green by *Pseudomonas sp*. As per the microscopic observation all isolates are showing rod shaped and pink color**(Fig.4).**



a. ***Pseudomonas sp.*****b.** ***Klebsiella sp.*****c.*****Salmonella sp.*** **d.** ***Escherichia coli Sp.***
Fig.4.Microscopic Observation &Soil samples isolate

The biochemical tests produced varying results for different species of bacteria when it came to producing acid, alkali, and gas. The citrate test produced a negative result, but the Indole production test produced a positive result, the TSI agar test produced a negative result, and the MR-VP test produced a negative result. The alkaline gas produced by *Salmonella* in the TSI agar was positive in the citrate utilization, Indole synthesis, and MR-VP tests. *Pseudomonas* produced no gas, but it was capable of utilizing citrate, producing Indole, and making MR-VP. MR-VP production by *Klebsiella* was positive, but Indole production failed. In addition to acid, it produced gas on TSI agar **(Table.1, Fig.5).**

Fig.5. Biochemical Testof Soil isolates

Carbohydrates of soil Isolates

Salmonella and *E. coli sp.* gave -ve to adonitol and +ve to all other 4 carbohydrates, *Klebsiella sp.* gave -ve to mannose and +ve to all other 4 carbohydrates, and *pseudomonassp.* Gave +ve to dextrose and -ve to other 4 carbohydrates **(Table.1, Fig.6).**

Table.1. Biochemical and Carbohydrate Test of Soil isolates

#	*Isolates* / *Tests*	*Salmonella sp.*	*Escherichia coli*	*Klebsiella sp.*	*Pseudomonas sp.*
1	Indole test	+ve	+ve	*-ve*	*-ve*
2	Methyl Red test	*-ve*	+ve	*-ve*	*-ve*
3	Voges Proskaur test	+ve	*-ve*	+ve	*-ve*
4	Citrate test	+ve	*-ve*	+ve	+ve
5	Catalase test	+ve	+ve	+ve	+ve
6	Urease test	*-ve*	*-ve*	+ve	*-ve*
7	TSI test	Alk/H2S	A/G	A/G	No gas/H2s
8	Adonitol	*-ve*	*-ve*	+ve	*-ve*
9	Galactose	+ve	+ve	+ve	*-ve*
10	Fructose	+ve	+ve	+ve	*-ve*
11	Dextrose	+ve	+ve	+ve	*+ve*
12	Mannose	+ve	+ve	*-ve*	*-ve*

Fig.6. Carbohydrate Test

Antibiotic sensitivity of Soil isolates

The bacterial isolates from soil samples were tested against 5 antibiotics: Azithromycin, Gentamycin, Levofloxacin, Vancomycin, and Tetracycline. *E. coli sp.* was found resistance to Levofloxacin, Vancomycin and sensitive to other three antibiotics. *Salmonella, klebsiella sp*. was found sensitive to all five antibiotics. *Pseudomonas sp.* was found intermediate toGentamycin and sensitive to other four antibiotics. **(Table 2, Fig.7).**

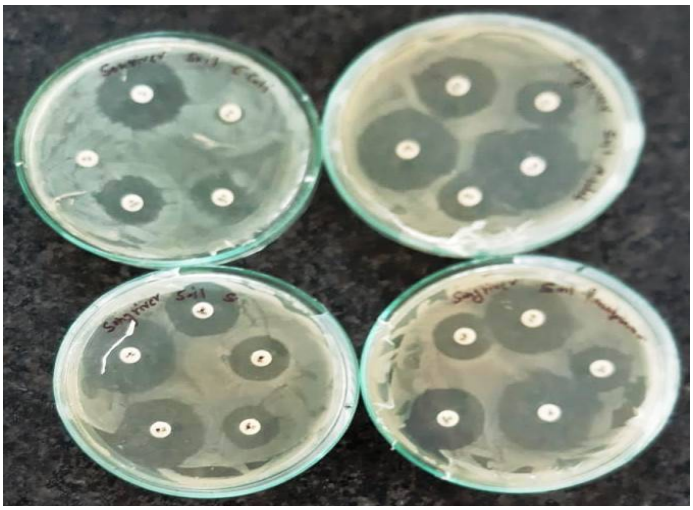


Fig.7. Antibiotic sensitivity Test

Table.2. Antibiotic sensitivity of Soil isolates

#	*Isolates* / *Antibiotic*	*Salmonella sp.*	*Escherichia coli*	*Klebsiella sp.*	*Pseudomonas sp.*
1	Azithromycin	S	S	S	S
2	Gentamycin	S	S	S	I
3	Levofloxacin	S	R	S	S
4	Tetracycline	S	R	S	S
5	Vancomycin	S	S	S	S

DISCUSSION

When compared to certain other habitats, the soil is proven to be a rich source of diverse sorts of microbial flora, and they are highly suited to continually changing soil conditions. Antimicrobial substances must be produced in order to destroy the rival in order for them to survive. One of several explanations, why soil microbes are favoured for antimicrobial activities assessment, is because of this. Annually, almost 500 antibiotics are made from microorganisms found in the soil [1, 5]. The need for microbial antibiotics is continuing to rise throughout the world as pathogenic bacteria develop resistance to medicines and many drugs have shown to be ineffective against illnesses. Most labs have used microscopic identification and biochemical characterization to identification of bacteria for years.

Because microorganisms lack adequate anatomical traits to validate their identification, numerous approaches based on their nutrients, metabolic activity and products, or enzymatic reactions have been developed to aid in the categorization and identification of microbes at the species levels [7, 9].
Pseudomonas infections have been linked to pollution of recreational and potable waterways. With this in consideration, it's worth noting that Pseudomonads are quite adaptable and may develop in a variety of environments, even in purified water. Because of their versatility, they are widespread in nature and have a significant influence on ecology, farming, and economics. *P. aeruginosa* is an opportunistic pathogen that has a role in the pathogenesis of a variety of human contagious diseases. Blue pus is prevalent in illnesses caused by these bacteria, and the organism is frequently recovered from clinical samples [12, 15].
Pseudomonas aeruginosa is one of the most common bacteria responsible for drug-resistant hospital-acquired infections causing bacteremia and pneumonia in hospitalized patients. Despite having a lower pathogenic potential than *Pseudomonas aeruginosa*, Pseudomonas putida is resistant to many antimicrobial drugs. Siderophores and Pseudomonas have attracted much interest and relevance in current decades in the field of study all over the globe. Pseudomonas is a bacterial community that has revealed its advantage for glycerol usage and also physiological, metabolic, andgenetic properties, providing us with a bigger platform for future study on numerous different components generated by them for industry and therapeutic application throughout the globe [18, 20].
The microorganisms E. coli are often found in the human gut. E. coli might become harmful, meaning it can cause illness, under specific circumstances. E. coli is an essential model organism that has been around for over 120 years. E. coli has been a widespread environmental inhabitant for a long time. Furthermore, because the environmental factors are drastically different from those found in the human gut, E. coli is unable to thrive outside of the host body for lengthy periods of time. E. coli has been utilized as an indication of recent fecal pollution for these factors, and it poses a risk to human and environmental health [17].
But, the MPN approach, which is not widely used, could be explored for quick identification of indicator organisms in potable water and soil. It's more like a qualitative than a quantitative test, showing merely the existence of coliforms instead of their numerical representation. Any source of water that is utilized for cleanliness or consumption should be free of fecal organisms. According to WHO recommendations for evaluating the microbiological water quality parameters, the existence of enteric coliforms, particularly E. coli, renders the samples taken unfit for human consumption. E. coli may be found in tap and surface water, which is used for consumption, washing, swimming, and a variety of other household activities [16].
Salmonella enterica, a human pathogenic microbe, has the ability to invade agricultural plants. Between, 2008 – 2011, the frequency of illness occurrences in the EU linked to non-animal fresh food polluted with human pathogens rose. Raw consumed leafy greens infected with Salmonella ssp. rated first among pathogen-food correlations.

CONCLUSION

As we combine rising food requirements with the need to sustain soil life expectancies while also providing key ecosystem functions like carbon storage, how we handle our soil health is becoming increasingly important. Soil biodiversity is important for soil health because it supports a wide range of ecological processes. It is important for health care workers to be aware of the growing resistance of nosocomial microorganisms and the proper use of antibiotics in treating such infections, regardless of what factors contribute to infection pathogenesis. Human pathogen contamination of soil may be caused by compost used as organic fertilizer and polluted irrigation water.

Conflict of interest

Authors declared no conflict of interest.

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