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Detection of Heavy Metals in Soil Samples and their antimicrobial activity against bacteria isolated from soil

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Abstract
The present study aimed to assess the agricultural and environmental quality of soils from different regions of Prayagraj by examining microbial diversity and heavy metal contamination. Soil samples were collected from five selected sites—Arail, Jhunsi, Naini, Phaphamau, and Kaushambi using sterilized tools, properly labeled, and sealed to prevent cross-contamination. Heavy metals, including cadmium (Cd), chromium (Cr), lead (Pb), and cobalt (Co), were quantified using an Atomic Absorption Spectrometer (AAS). Microbial analysis was performed using serial dilution and culture-based techniques on selective media, and bacterial isolates were identified based on cultural and morphological characteristics. The oligodynamic effect of selected heavy metals was also evaluated, and significant antimicrobial activity was observed.

Keywords: Atomic absorption spectrometry [AAS], heavy metals, soil bacteria, oligodynamic action

Introduction
Metallic elements having relatively large densities, atomic weights, or atomic numbers are referred to as heavy metals, a contentious and confusing phrase. According to some, the "Heavy metals" should be avoided and the criteria employed and whether metalloids are included differ which is depending on the source and context [1]. Density, atomic number or chemical behavior can all be used to identify a heavy metal. Certain heavy metals including iron, cobalt, copper and zinc either necessary nutrients or generally innocuous (like ruthenium, silver, and indium) but in higher concentrations or at specific forms, they can be harmful. Lead, mercury, cadmium, arsenic and other heavy metals are extremely toxic [2].
Sources of heavy metal in soil can be both natural and man-made. Soil erosion, volcanic eruptions and parents' material weathering are of natural sources. However the most important sources anthropogenic and they include poor waste disposal, industrial pollutants and agricultural activities [3].
Many soils naturally contain heavy metals in varying amounts. The lithosphere or the mineral portion of the soil that creates the rocks and minerals that comprise the earth's crust is the exclusive sources of the metals founds in naturals and unpolluted soils. Because of Pedogenetic weathering process of parents materials, naturally occurring elements enter the soil from the parent substrate at amounts considered trace and harmful. Over (99%) of the earth's crust is composed of the Metal traces are present in soils by nature. Therefore the presence of metals in soils does not always mean that it is contaminated [4].
Anthropogenic sources of anything which usually pollution or environmental impact that comes from the sources of human activities. Human activities can cause the soil's heavy metal concentrations to be higher than usual [5]. Heavy metal and metalloid accumulation in soils can be caused by a number of factors, including sewage sludge, pesticides, irrigation of waste water, coal combustion residues, runoff from terrestrial systems, petrochemical spills, accidental leaks, atmospheric deposition, releases from mine tailings that are leaded into gasoline and paints, and quickly expanding industrial areas. Since anthropogenic heavy metals are often mobile than (pathogenic or lithogenic) ones they are more bioavailability in soils. Because they don't break down like organic contaminants do the heavy metals pose a persistent risk to human health and the environment?
Mercury, cadmium, chromium, nickel, Heavy metals, such as Pb and As, are characterized

by their high density and relatively wide atomic environment. Because of human activity, they are released into the air, water, and land after existing naturally in the environment. Such as mining, forming industrial separations and the usage of certain goods [6]. Due to their poisonous nature they damage soil microbes which are essential for our ecosystem functions and soil fertility. The microbial population in the soil Because heavy metals damage microbial cells, they effectively stop bacteria from growing and developing, which is why soil is diminished when they are present. The breakdown of organic materials in the soil, which modifies the nitrogen cycle, nitrification and Denitrification, which affect the soil's overall nutrient availability, and the breakdown of contaminants are just a few of the critical processes that may be disrupted by this decreased microbial biomass [7]. Lead, mercury, and cadmium are among the metals that seriously affect microorganisms. These dangerous heavy metals have an impact on soil enzyme activity, which is essential for carrying out essential metabolic processes. in addition to inhibiting development. Among these metabolic activities are the conversion of nutrients and the breakdown of organic substances into forms that plant can use for their development. The availability of nutrients and this cycle may become unbalanced if there are too many heavy metals present because of this the soil fertility state declines which lower its productivity as well as the plant's general growth and development. Some microbial species vulnerable to increase heavy metal concentration [8]. Degradation of soil and water and ecological dysfunction are frequent outcomes consequences of the buildup of heavy metals. Additionally, because heavy metals enter food chains from polluted land and air, contaminated food poses a danger to both human and animal health [9, 10, 11].

Certain heavy metals have a crucial role in soil systems and plant growth when present in trace or optimal concentrations [12]. Elements such as zinc (Zn), copper (Cu), and nickel (Ni) act as essential micronutrients for plants, where they are involved in enzyme activation, protein synthesis, and photosynthetic processes, thereby supporting normal plant growth and development [13, 14]. Heavy metals such as manganese (Mn) are involved in soil enzyme activation, while molybdenum (Mo) serves as an essential cofactor for several soil microbial enzymes that regulate key biochemical reactions, including organic matter decomposition and biological nitrogen fixation [15]. At regulated or optimal levels, certain heavy metals contribute to the maintenance of soil nutrient balance and stimulate microbial activity, which indirectly enhances soil fertility, productivity, and overall plant health [16, 17]. Furthermore, soils enriched with specific metals support the cultivation of medicinal plants and metal-accumulating species, which are of importance for industrial, pharmacological, and phytoremediation applications [18].

Soil pollution is the term used to describe the contamination of soils with substances, primarily chemicals that are either inappropriate or present in higher quantities than usual and may have harmful consequences on people or other living things [19]. Other than the direct addition of Heavy metal concentrations in household garbage are typically lower than those in industrial waste. Those plants are able to absorb soil solutions. Long-term use of sludge can lead to excessive metal accumulation in soils. As micronutrients, heavy metals are necessary components of biological systems and are derived from soil through agricultural products, which are the main sources of various heavy metal bioaccumulation and transfer to the human body [20, 21 22]. We inhale them in the air and always consume them to some degree through food and water. Certain micronutrient components may be poisonous to both people and animals in addition to being necessary for plant development and/or human nutrition. at higher concentrations. Other trace elements e.g. As, Cd, Hg and Pb may also inadvertently enter the food chain and pose health risks to humans and animals [23]. Applications of heavy phosphate decrease the availability of cationic metals, phosphorus levels can contaminate water. Greater amounts of metals are transferred by plants to their leaves as opposed to their fruits or seeds [24].

Materials and methods

Collection of Soil Sample

Five specific locations in the Prayagraj district—Arail, Jhunsi, Naini, Phaphamau, and Kaushambi—were used to gather soil samples. To guarantee representativeness and reduce geographic variability, duplicate soil samples were taken from three distinct locations within a 100-meter radius at each site. To prevent cross-contamination, the samples were taken with sterile instruments, promptly marked, and packed in sterile polythene bags. After that, every sample was brought to the lab for additional examination [24, 25, 26, 27].

Soil sample preparation

In the preparation of the soil samples Hand picking was used to remove unwanted elements including stones, Leaves and debris from the soil samples which were then dried in an air dried oven. After being mixed and slightly homogenized, the samples were sieved by using a (2mm) mesh sieve. The samples were initially allowed to dry before spending around [30 minutes] in an electric oven set at [40 °C]. For the digestion process the fine powder of soil sample is made by the [mortal –pastel] which will be started at room temperature [28].

Processing of Sample

In the laboratory, soil samples were air-dried, ground using a clean mortar and pestle, and sieved through a 2 mm mesh. The triplicate samples from each site were either analyzed separately or composited depending on the analysis requirements. Heavy metal concentration in the soils was determined following the U.S. Environmental Protection Agency (EPA) Method 3050B. Heavy metals including lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) were measured using an Atomic Absorption Spectrophotometer (AAS) after the soil was broken down using strong nitric acid and hydrogen peroxide [29].

Experimental procedure digestion process

Preparation of Aquaregia

The corrosive acid combination known as Aquaregia which is created by mixing hydrochloric acid and nitric acids. The typical ratio of acid is one part of nitric acid to the three parts hydrochloric acid. Nitric acid should be added to hydrochloric acid. Do not add [Hydrochloric acid and nitric acid [30]. The final mixture will be a bubbling liquid that is either red or yellow. Aquaregia mainly used to dissolve [Palladium, Platinum and gold] after being weighed the samples were put in a [250ml] beaker and digested by using

an Aquaregia acid solution. In a [3:1] ratio [10ml] of Aquaregia [35% HCL and 65%] of high quality of [HNO_3] were used to heat the soil samples. What kind of filter paper was used to filter the final solution once the sample had cooled? After cooling, the samples were transferred to a [50ml] volumetric flask, and their volume was measured by diluting them with deionized water. The concentration of [Cd, Cr, Pb, and Co] in the sample solution was then determined using an atomic absorption spectrometer.

Preparation of standard solutions

The standard solution for Cadmium, Chromium, Lead and cobalt was prepared by using intermediate stock solution. A concentrated solution used to make working solution with lower concentration is called an intermediate stock solution. It serves as a translational solution between the highly concentration. Stock solution and final working solution required for a process or experiment. To get the appropriate concentration we will essentially dilute the intermediate stock solution.

Analysis of Heavy Metals

One quantitative method for analyzing heavy metals is atomic absorption spectrometry (AAS). Element-specific radiation is absorbed by neutral atoms produced by atomization, and the absorbance is directly correlated with the concentration of the metal. For determining soil metals, this technique offers good sensitivity, selectivity, and accuracy [31].

Oligodynamic Action

The biocidal activity of heavy metals was evaluated on the basis of principle of the oligodynamic effect, which describes the antimicrobial action of metals at very low concentrations. This effect results from the interaction of metal ions with thiol and amine groups of cellular proteins, leading to protein denaturation, enzyme inactivation, and disruption of membrane integrity. The strong affinity of metal ions for biological macromolecules causes an increase in intracellular metal concentration, ultimately resulting in cellular damage and death. Plasmid-mediated mechanisms may contribute to microbial resistance against metal toxicity. Silver ions interact with sulfhydryl groups to form silver sulfides, adversely affecting proteins, enzymes, and cell membranes. Copper ions bind to negatively charged moieties on the bacterial cell surface, causing cell wall disruption and increased membrane permeability, which facilitates the entry of silver ions into the cell. Once inside the cell, silver ions interact with proteins, enzymes, DNA, and RNA, leading to irreversible cellular damage. In the current investigation, silver was deemed an effective antiseptic since it has antibacterial action at doses much lower than those that are harmful to humans [34].

Preparation of Medium

Nutrient agar medium was used for bacterial growth and isolation. Five grams of peptone, three grams of beef extract, five grams of sodium chloride, and eight to eighteen grams of agar-agar were dissolved in one thousand milliliters of distilled water to create the medium. Before sterilization, the medium's pH was raised to 7.0–7.5. The prepared medium was autoclaved for 25 to 30 minutes at 120 °C and 15 to 30 psi of pressure to sanitize it. The medium was used for bacterial culture and isolation after it had been sterilized and allowed to cool to room temperature. Isolation of microorganisms was carried out using serial dilution followed by culture-based techniques to obtain both pathogenic and agriculturally beneficial microbial isolates. Appropriate selective and general-purpose media, including nutrient agar, were employed for microbial cultivation. The isolates were identified on the basis of their cultural characteristics, including colony morphology, pigmentation, and growth pattern, as well as microscopic and morphological features.

Nutrient broth was prepared for microbial cultivation by dissolving 13 g of dehydrated nutrient broth powder, equivalent to a composition of 5.0 g peptone, 3.0 g beef extract, and 5.0 g sodium chloride, in 1000 mL of distilled or deionized water. The medium was stirred thoroughly and gently heated to ensure complete dissolution of all components. After complete dissolution, the nutrient broth was dispensed into suitable containers such as test tubes or conical flasks as required. The distributed medium was autoclaved for 15 minutes at 121 °C to sanitize it. Following sterilization, the medium was allowed to cool to room temperature and was stored at 10–25 °C in a dark environment until use.

For microbial growth, the cooled nutrient broth was inoculated with the bacterial culture or environmental sample using aseptic techniques. The inoculated broth was incubated at 35 ± 2 °C for 18–24 hours, or longer when necessary, depending on the growth characteristics of the microorganism.

Table 1: Of Effect of Heavy Metals on isolation of bacteria (Zone of Inhibition in mm)

Heavy metals	Isolates 1	Isolates 2	Isolates 3
Cd	60	64	58
Cr	52	46	40
Pb	45	48	52
Co	48	52	56

Preparation of Slides for the Gram Staining

Cleaning and labeling the slide is the first step in getting it ready for Gram staining. Next, apply a thin layer of the bacterial culture on the surface. To bind the bacteria to the slide, the smear needs to be air-dried before being heat-fixed. After the heat-fix process, the slide is prepared for the Gram staining process.

Steps involved in preparation of slides

1. **Preparation the Bacterial Smear:** Clean the Slide: Ensure the slide is free from the entire grease free glass slide. Sterilize Loop: Loop is used for the sterilize which is sterilizing by the flame near about [1 to 2 minutes loop near the flame].
2. **Transfer of Bacteria:** Liquid Culture: Transfer a loop full of the culture to the slide and spread it over the slide thinly. Solid Culture: If using a Petri dish or slant place a drop of distilled water on the slide and transfer a small amount of colony into the water droplets and spread it gently.
3. **Heat fixing the smear:** Purpose of Heat: Heat Fixation helps the bacteria adhere to the slide and prevents them from being washed away during the staining process. Method: Pass the slide gently over a flame and moving it back and prevent it from the overheating.
4. **Staining procedure:** The Next steps involve flooding

the Slide with Crystal Violet, Grams iodine, Decolorize (alcohol or acetone) and counter strain (Safranin). Each step is timed and the slide is rinsed with water in between the steps.

- **Crystal violet**: Slide is dipped in Crystal white near about [1 minute] and washed with water and it is the primary stain added to specimen smear.
- **Grams iodine**: Slide is dipped in Grams iodine for near about [1 minute] and washed it with water and it is the mordant dye which is less soluble so it adhere the cell walls.
- **Alcohol**: Slide is dipped in alcohol for near about [30 sec] and washed it with water and alcohol works as decolorize and washes away from the cell walls.
- **Safranin**: It is the last steps in the Gram staining process in which slide is dipped in safranin in near about 15 sec and counterstained allows dye adherence to pink in colour.

Results

The table below displays the amounts of certain heavy metals—lead (Pb), chromium (Cr), cobalt (Co), and cadmium (Cd)—in different water samples based on the findings of Atomic Absorption Spectroscopy (AAS) examination. Collected from different sites. These values, expressed in mg/L, reflect the presence and variation of metal contamination in the river water at specific geographic locations.

Table 2: Concentration Value of Heavy metals in soils samples

Sample No	Lead concentration range ppm.	Cadmium concentration range ppm.	Chromium concentration range ppm.	Cobalt concentration range ppm.
Sample 1 Naini	0.53	0.693	0.252	0.121
Sample 2 Arail	0.72	0.757	0.214	0.115
Sample 3 Jhunsi	0.10	0.011	0.110	0.091
Sample 4 Phaphamau	0.18	0.010	0.760	0.105
Sample 5 Kaushambi	0.13	0.004	0.098	0.109

The permissible limits of heavy metals in soil samples differ between the USEPA and Indian standards, reflecting varying regulatory approaches. For Lead (Pb), the USEPA allows a maximum of 0.05 mg/l, while the Indian standard permits up to 0.1 mg/l. Lead is a potent neurotoxin, and even low-level exposure can be harmful, especially to children. Cadmium (Cd), known for its carcinogenic and nephrotoxic effects, has a limit of 0.005 mg/l under USEPA guidelines, compared to 0.01 mg/g in India. Chromium in its hexavalent form (Cr) is uniformly regulated by both agencies at 0.05 mg/l, indicating global consensus on its toxicity and environmental risk. In contrast, Cobalt (Co) lacks defined permissible limits in both USEPA and Indian standards. Although cobalt is an essential trace element, elevated concentrations may lead to health complications such as cardiomyopathy. The absence of regulatory values highlights the need for further research and standardized guidelines to ensure safe levels of cobalt in soils. Overall, the differences in permissible limits suggest a need for harmonized global soil quality standards to better protect public health.

Soil samples collected from five sites along the river—Jhunsi, Arail, Phaphamau, Naini, and Kaushambi—were analyzed for heavy metals Pb, Cr, Co, and Cd. The concentrations (in mg/g) showed significant spatial variation.

Lead (Pb) concentrations ranged from. The highest Pb concentration was recorded at Naini 2 (0.72), exceeding the permissible limit of 0.01 mg/l as per both international and Indian standards. This elevated level indicates potential contamination from industrial or urban sources. Other sites showed Pb levels within or close to acceptable limits

Chromium (Cr) levels varied between 0.098 mg/l (likely below detection limit) and 0.760 mg/l, with Arail 2 showing the highest value, surpassing the standard limit of 0.01 mg/l. Such elevated chromium levels suggest pollution possibly linked to tannery effluents or metal processing activities in the area.

Cobalt (Co) concentrations ranged from 0.091 to 0.166 mg/L. Since there are no specific regulatory limits for cobalt, and the values remain below 0.05 mg/L, the detected levels are considered typical and not immediately concerning.

Cadmium (Cd) concentrations ranged from 0.757 to 0.006 mg/L. Naini 2 recorded the highest cadmium concentration of 0.02 mg/L, exceeding the permissible limit of 0.01 mg/L. Cadmium is toxic even at low concentrations, so its elevated presence requires attention and further monitoring.

Oligodynamic Action against isolated microorganisms

Microbial Analysis of soil stored in different storage Petri plate like Cadmium Chromium, Lead and cobalt was assessed for their Oligodynamic potential after the regular interval of time (24 to 36 hours) of incubation. There was a very significant decrease in the bacteria population in the soil samples following a 24-hour incubation period. Nonetheless, the following soils were kept for almost the same amount of time in (cadmium, chromium, lead, and cobalt):

- **Cadmium (Cd):** Cadmium exhibited significant Oligodynamic action even at low doses (5 mg/kg), which resulted in a noticeable decrease (around 45%). in microbial biomass carbon (MBC) compared to the control. Dehydrogenates activity dropped by 52%, indicating pronounced microbial inhibition.
- **Chromium (Cr):** Chromium showed moderate Oligodynamic effects. At 10 mg/kg, microbial biomass was reduced by 28%, and soil respiration activity was suppressed by 31%. The toxic impact was more pronounced in soils with lower organic matter content.
- **Lead (Pb):** Lead demonstrated a weaker Oligodynamic effect compared to Cd and Cr. At 50 mg/kg, a marginal decrease (15%) in microbial activity was observed. However, Pb accumulation still led to a measurable reduction in soil urea's activity.

Cobalt (Co) Cobalt showed significant inhibitory effects at higher concentrations. At 25 mg/kg, microbial biomass declined by 33%, with a 40% drop in phosphates activity, indicating interference in nutrient cycling processes.

Overall, the order of Oligodynamic activity was: Cd > CO> Cr > Pb, with cadmium being the most toxic to soil microbes even at trace levels. From the current study it can be concluded that cadmium was the metal is the most promising metal in inhibiting the bacterial population within (48 to 60 hours) of incubation.

Discussions

The current work was carried out to identify antimicrobial bacteria from the same soil settings and to determine the presence and heavy metal's level in soil samples, with a focus on investigating the interactions between these bacterial isolates and heavy metals. The results of this research contribute to the understanding of soil contamination, microbial ecology, and the potential of native soil bacteria in bioremediation processes.

Analysis of the collected soil samples revealed the presence of various heavy metals including lead (Pb), cadmium (Cd), chromium (Cr), Cobalt (C0), with concentrations varying across different sampling sites. The detection of these metals suggests contamination likely due to anthropogenic activities such as industrial discharge, improper waste disposal, agricultural runoff, and vehicular emissions [40]. Some sampling locations showed metal concentrations exceeding the permissible limits set by environmental regulatory bodies, indicating potential ecological and public health risks (WHO, 2017). The variation in heavy metal content across the samples highlights the influence of land use patterns, proximity to pollution sources, and soil physicochemical properties on metal accumulation. Similar trends have been reported in other studies, reinforcing the global concern over soil contamination by heavy metals [41]

Several bacterial isolates were successfully obtained from the soil samples, including species belonging to the genera Pseudomonas, Bacillus, Escherichia, and Staphylococcus. Some of these isolates, particularly Escherichia coli and Staphylococcus aurous, are recognized as opportunistic pathogens. The presence of these pathogenic bacteria in soil environments poses a potential risk for transmission to humans, animals, and plants, especially in agricultural or recreational areas and the isolation of these bacteria from heavy metal-contaminated soils aligns with findings from previous studies, which have reported the persistence of pathogenic bacteria in polluted environments [42]. Heavy metals can exert selective pressure on microbial communities, often leading to the survival of metal-tolerant and potentially antimicrobial species.

Interaction between Antimicrobial Bacteria and Heavy Metals

When assessing the impacts of heavy metals on the isolated bacterial strains, it was observed that some bacteria exhibited tolerance to elevated metal concentrations. The mechanisms behind this tolerance may include metal efflux systems, enzymatic detoxification, and sequestration of metals within bacterial cells [43]. Interestingly, certain isolates demonstrated the ability to bioaccumulation or even biotransform heavy metals, suggesting a potential application in bioremediation [44] conversely Elevated levels of heavy metals inhibited the development of of several pathogenic isolates, indicating the toxic effects of metals on microbial viability. The degree of inhibition varied among different bacteria and metals, with cadmium and lead showing higher toxicity levels compared to zinc and copper. These findings are consistent with despite the fact that their bioaccumulation and environmental, Permanence raises concerns about possible impacts on environment and the human health. Additionally, the study suggests that heavy metal contamination may have an effect on how the microbial community is organized in soils. There by favoring pathogenic strains that are resistant to metals. Public health is significantly impacted by this, since the spread of metal-tolerant existing literature that documents the antimicrobial properties of heavy metals [45], pathogenic bacteria can contribute to the spread of antimicrobial resistance, a growing global health issue.

Conclusion

The present study clearly demonstrates significant heavy metal contamination in soil samples collected from different sites of Prayagraj, with marked spatial variation. Elevated concentrations of Pb (up to 0.72 ppm at Arail), Cr (0.760 ppm at Phaphamau), and Cd (0.757 ppm at Arail) indicate strong anthropogenic influence, likely from industrial discharge, urban runoff, and agricultural activities. Although cobalt lacks regulatory limits, its consistent presence (0.091–0.121 ppm) is noteworthy. Oligodynamic studies confirmed that heavy metals, particularly cadmium, exert strong inhibitory effects on soil microbial activity, with the toxicity order Cd > Co > Cr > Pb. The isolation of metal-tolerant and pathogenic bacteria suggests selective pressure imposed by heavy metals, highlighting potential ecological imbalance and public health risks. Overall, the findings emphasize the need for continuous soil monitoring, stricter regulatory control, and the exploration of native metal-tolerant microbes for bioremediation strategies.

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