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Detection of Heavy Metals in Ganga- Yamuna Water and It's Effect on Bacteria Isolated from River Water

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Abstract
To maintain safety, Ganga- Yamuna river water is traditionally stored in certain metal pots. In order to assess the impact of oligodynamic metals like lead (Pb), cadmium (Cd), and chromium (Cr), measurements were made. Some Gram-negative and Positive isolates of Ganga- Yamuna river water found in Prayagraj. Water samples were collected in from five locations: Jhunsi, Daraganj, Sangam, Naini, and Baluaghat. In compliance with recognized environmental protocols, they were acid-digested using a solution of hydrochloric acid (HCl) and nitric acid (HNO_3). To conduct the test, Four heavy metals viz. cadmium, chromium, lead, and cobalt were detected from the river water sample. Further Gram positive and Gram negative bacteria were isolated from the river water sample. The antimicrobial activity of heavy metals was screened against the isolated bacterial isolates using standard protocols. All the heavy metals were found to be inhibitory against bacterial isolates. In order to manage the gram positive and negative pathogenic bacteria in the water of the Ganga- Yamuna River, this study recommended the usage of water pots constructed of oligodynamic metals including cadmium, chromium, lead, and cobalt.

Keywords: Heavy metals, Oligodynamic action, River bacteria

Introduction
Metals are defined as metallic elements that have a relatively high density relative to water. [1]. Environmental contamination brought on by these metals has become a major ecological and public health concern in recent years. Sediment re-suspension, metal evaporation from water supplies to soil and underground water, air precipitation, erosion of soil of metal ions and heavy metal leaching, and metal corrosion are further types of environmental contamination [2].

Metals refineries, coal-fired power plants, thermal energy crops, overhead lines, petroleum, plastics, textiles, microelectronics, wood preservation, and paper mills are examples of industrial sources [3-4]. The elements Co, Cu, Cr, Fe, Mg, Mn, Mo), Ni, Se, and Zn are necessary for many biological and physiological processes [5]. Because heavy metals are present in a variety of environmental matrices at trace levels (ppb range to less than 10ppm), they are also regarded as trace elements. [6]. Nevertheless, this characteristic additionally, copper makes it potentially dangerous since the changes between Cu (II) and Cu(I) can produce hydroxyl and superoxide radicals [7-8]. There is a very small range of concentrations for certain elements, such as copper and chromium, between having harmful and advantageous effects [8, 9]. Other metals that are regarded as non-essential because they lack known biological functions include Lead (Pb), lithium (Li), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), strontium (Sr), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V), uranium (U), aluminium (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), gallium (Ga), germanium (Ge), gold (Au), indium (In) [9]. The United States Environmental Protection Agency (U.S. EPA) and the International Agency for Research on Cancer (IARC) have also classified certain metals as "known" or "probable" human carcinogens based on epidemiology. There is a link between exposure and the risk of cancer in both humans and animals, according to several experimental studies. The chemical characteristics of heavy metals vary greatly, The issues are made worse by the fact that metals are persistent in the environment, have the ability to bioaccumulate in the food chain, and have a propensity to travel with sediments Even at low concentrations, these metals are harmful to both humans and animals. Lead is exceedingly poisonous and can harm the kidneys, reproductive system, and nervous system. Lead exposure results in encephalopathic symptoms and permanent brain damage. [10]. Cadmium can also be found in rechargeable batteries that uses nickel-cadmium compounds. [11-13] Exposure to cadmium results in liver and blood damage, bone deterioration, and renal dysfunction. There is sufficient evidence that cadmium causes cancer, according to studies. Large-scale production of battery cells made from nickel-cadmium also makes extensive use of nickel. [14]. In contrast to heavy metals, there are several possible "light metal" toxicants that, at high concentrations, represent a major risk to the environment and ecosystems. One such metal is nickel. As the planet's 24th most abundant element, nickel and nickel-containing compounds are found naturally on the Earth's surface and are continuously released into the atmosphere at low concentrations through a variety of natural processes. [15] Effluents containing nickel are released into adjacent bodies of water by the paint and enamelin industries. [16]. Coal burning, mining and smelting activities, pesticide use, and the weathering of rocks naturally are some of the ways it enters the environment. One major worry is the toxicity of arsenic caused by water contamination. In the majority of groundwater, arsenic is found as arsenate (As(V)) and arsenide (As(III) [17-19]. By generating toxic compounds that could harm human tissues, acting as parasite inside cells of humans, or establishing colonies in the body that could interfere with regular human processes, pathogenic bacteria have the capacity to infect or sick people. Numerous harmful bacteria, such as *Salmonella typhi*, *Escherichia coli*, and *Vibrio cholerae*, can be found in water and can cause cholera, typhoid, diarrhoea, and other water-borne illnesses. [20]. Gram-negative and frequently found in nature, Escherichia was formed like a rod with a tiny tail when viewed under a microscope. Antibiotic resistance was innate in gram-negative bacteria both human and cow faces may contain ETEC. ETEC was the most prevalent bacterial pathogen in children under five in many underdeveloped nations, causing hundreds of millions of cases of diarrhoea and thousands of fatalities annually. Additionally, it was the frequent cause of "travellers' diarrhoea," which afflicted visitors from industrialized nations who were visiting developing nations. Another curve-shaped gram-negative bacterium was called Vibrio that primarily caused gastroenteritis was *V. parahaemolyticus*. According to [21]. Moreover, salmonella was a rod-shaped gram-negative bacterium. Typhoid and paratyphoid fever, gastroenteritis, and two forms of salmonellosis (a symptomatic illness caused by Salmonella) could result from it. *Salmonella enterica* (*S. enterica*) and *Salmonella bonsoir* (*S. bonsoir*) were the only two species found in the genus. The earliest human-specific pathogen was Shigella, a rod-shaped, gram-negative bacterium. In light of the aforementioned information, the current investigation was conducted to assess the effectiveness of heavy metals against the bacteria that were isolated from river water.

Materials and Methods

Collection of Sample Method

Total 5 randomly selected water samples were collected in sterile, wide-mouthed glass stopper sample vials from the Jhunsi, Daraganj, Sangam, Naini, and Baluaghat areas. As soon as the water was collected, it was taken out of the lab for additional chemical and microbiological processing.

Sample processing for heavy metal detection

Digestion of water

A clean digesting flask was filled with 100 millilitres of each sample for the digestion. The material in the digestion flask was mixed with 0.4 ml of a concentrated HNO3 and 1 ml of concentrated HCL. The entire sample was roasted on a hot plate until all of the nitrogenous compound (brownish fumes) were released, indicating that the sample had been digested. After that, it was left to cool at room temperature. The liquid was filtered into a 100 ml standard flask and then put into a polypropylene reagent bottle for Atomic Absorption Spectrometry (AAS) after a few ml of distilled water were added.Certain hollow cathode lamps (HCL) are used in Atomic Absorption Spectroscopy (AAS) to identify heavy metals according to their distinct absorption wavelengths. Deuterium or hydrogen discharge lamps are used for background correction; they work especially well in the UV spectrum (190-320 nm), where the majority of heavy metals absorb light. Heavy metals like lead (Pb), cobalt (Co), chromium (Cr), and cadmium (Cd) can be precisely identified using AAS in a digested water sample [22].

Every element functions at its unique absorption wavelength and needs a different HCL

ALead HCL is used to detect lead (Pb) at 283.3 nm. A Cobalt HCL is used to detect cobalt (Co) at 240.7 nm. A chromium HCL is used to test chromium (Cr) at 357.87 nm. A Cadmium HCL is used to analyse cadmium (Cd) at 228.8 nm. The effective range of deuterium background adjustment includes these wavelengths. All heavy metals are guaranteed to be in a detectable ionic form upon proper sample digestion, allowing for accurate quantification. In heavy metal analysis employing AAS, precise lamp wavelength combinations are critical for sensitivity and specificity, and background correction is necessary to remove matrix interferences.

Isolation of the bacteria from water sample

One milliliter of the dried water sample was combined with nine milliliters of distilled water, and the combination was well mixed. A ten-fold serial dilution was carried out using the pour plate technique. One milliliter of each dilution was added to a petri plate after water was serially diluted up to a 10-7. The uninoculated plate served as a medium control while the plates were incubated for 24 hours at 37 °C. Following this, a cultural enumeration of the various isolated colonies was conducted, and they were streaked over nutrient-agar medium. As a control, an uninoculated medium plate was incubated for 24 hours at 37 °C, and the growth was assessed [23].

Identification of Bacteria from Water Samples

Based on their physical and cultural traits, bacterial isolates from water samples were identified. For initial identification, colony characteristics including color, texture, and size were noted on culture plates. The isolates underwent further Gram staining, and cellular morphology and Gram reactivity were assessed under a microscope with a 100× oil immersion objective.

Oligodyanmic action of heavy metals

Nutrient agar plates were sterilized for a whole day. A sterile swab was used to swab an old liquid culture onto a hardened nutritional agar plate. After placing a small quantity of heavy metals in the center of the swabbed plate, the plate was incubated for 24 to 48 hours at 37 °C. The outcome was then evaluated [24].

Table 1: Culture characteristics

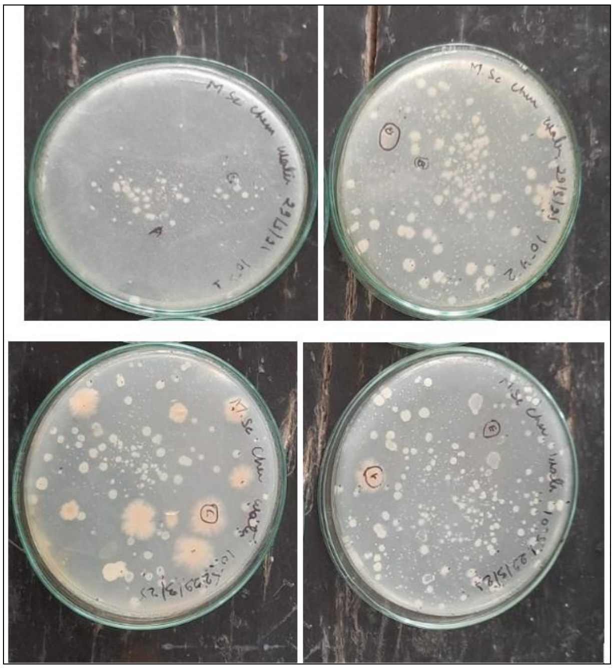
S. No.	Isolates	Shape	Size	Color
1	Isolates 1	Spherical	Small	White
2	Isolates2	Swarming, spherical	Medium and large	White, slight oranges
3	Isolates 3	Spherical,	Pinpoint	White

A water sample was serially diluted, and the resulting spherical colonies of different sizes—small, medium, and large—as well as white and white, slight organges were seen using the spread plate technique on nutrient agar media and nutritional broth.

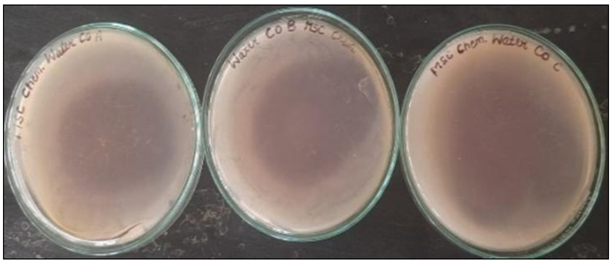
Table 2: Morphological characteristics

S. No.	Isolates	Shape	Arrangement	Gram Reaction
1	Isolates1	Cocci	Cluster	Gram+ve
2	Isolates2	Bacilli	Single, dipolobacilli	Gram+ve
3	Isolates3	Bacilli	Single cell	Gram-ve

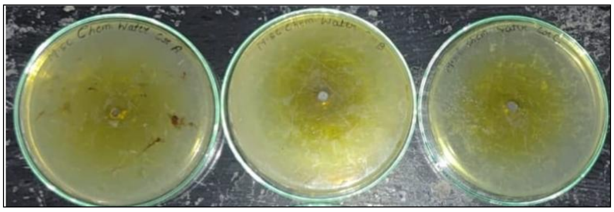
A gram-positive and gram-negative result was demonstrated by the rod-shaped bacilli grouped in single dipole bacilli or cluster form in a water sample that had been serially diluted and then subjected to the spread plate technique.



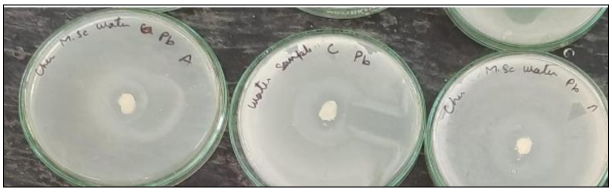
Isolations growing on medium with heavy metals Cr, Co, Cd, Pb



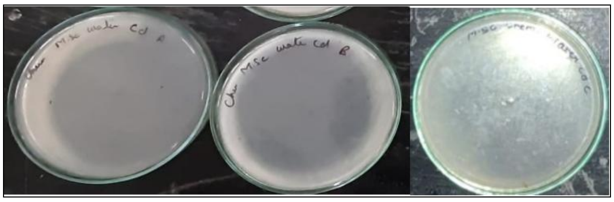
Cobalt



Chromium

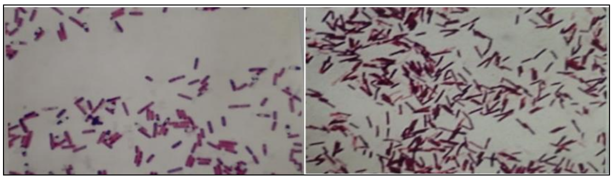


Lead

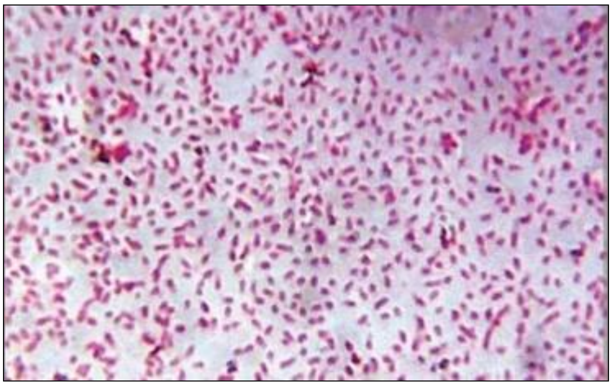


Cadmium

Bacterial colonies isolated from water sample kept in different pots



Gram positive



Gram negative

Result and Discussion

The following table displays the amounts of a few chosen heavy metals—lead (Pb), chromium (Cr), cobalt (Co), and cadmium (Cd)—in a variety of water samples taken from various locations based on the results of Atomic Absorption Spectroscopy (AAS) examination. The presence and fluctuation of metal pollution in the river's water at particular geographic regions are shown by these figures, which are given in mg/L.

Table 1: of metal concentration of heavy metal (Pb, Cr, Co, and Cd) different site of Prayagraj.

S. No.	Name of Place	Heavy Metals Concentration(mg/l)			
		Pb	Cr	Co	Cd
1.	Jhunsi	0.14	0.053	0.03	0.020
2.	Sangam	0.05	0.080	0.016	0.007
3.	Naini	0.00	0.076	0.027	0.002
4.	Baluaghat	0.01	0.007	0.040	0.006
5.	Daraganj	0.03	0.027	0.027	0.005

As a result of different regulation methodologies, the USEPA and Indian standards have different allowable levels for heavy metals in water. The Indian standard allows up to 0.1 mg/L of lead (Pb), while the USEPA only allowed 0.05 mg/L. Even low levels of exposure to lead, a strong neurotoxic, can be hazardous, particularly for young people. According to USEPA rules, the limit for cadmium (Cd), which is known to have nephrotoxic and carcinogenic effects, is 0.005 mg/L, but in India it is 0.01 mg/L. Both agencies uniformly restrict chromium in its hexavalent form (Cr) at 0.05 mg/L, demonstrating worldwide agreement regarding its toxicity and environmental concern. In contrast, neither Indian nor USEPA guidelines specify any acceptable levels for cobalt (Co). Despite being a necessary trace metal, high levels of cobalt can cause health issues like cardiomyopathy. To guarantee safe amounts of cobalt in drinking water, further research and defined norms are required, as evidenced by the lack of regulation values. All things considered, the disparities in allowable limitations point to the necessity of unified international water quality standards in order to effectively safeguard human health. Heavy metals Pb, Cr, Co, and Cd were examined in water samples taken from five locations along the river: Jhusi, Daraganj, Sangam, Naini, and Baluaghat. There was notable geographical variation in the concentrations (in mg/L). The amounts of lead (Pb) varied from 0.00 to 0.14 mg/L. Jhusi 1 had the highest Pb levels (0.14 mg/L), which was more than the 0.05 mg/L allowable limit according to both Indian and international norms. This high concentration suggests possible urban or industrial contamination. Pb levels at other locations were within or near permissible bounds. Jhusi 2 had the highest value of chromium (Cr), which ranged from -0.007 mg/L (probably below the detection limit) to 0.203 mg/L. exceeding the 0.05 mg/L regulatory limit. Such high chromium levels point to potential pollution from nearby metal processing operations or tannery effluents. The amounts of cobalt (Co) varied between 0.012 and 0.04 mg/L. The discovered levels are regarded as normal and not immediately alarming because cobalt has no precise regulatory limitations and the readings stay below 0.05 mg/L. The amounts of cadmium (Cd) varied from 0.002 to 0.02 mg/L. The maximum cadmium content of 0.02 mg/L was reported by Jhusi 1, surpassing the allowable limit of 0.01 mg/L. The elevated presence of cadmium necessitates attention and additional monitoring because it is harmful even at low quantities.

Isolation of the Bacteria

A total of five water samples were gathered from various locations across Prayagraj, and the bacteria were isolated from the samples. Using pour plate techniques on nutrient agar plates, isolation was carried out. Using the Streak Plates method on Nutrient Agar plates, bacterial isolates were purified. Bacterial isolates' incidence was computed. G -ve rods had the highest occurrence (25%), followed by G +ve rods (45%), while G+ve cocci in the cluster only had (30%) incidences.

Incidence

According to reports, the Gaughat region had the highest incidence of *Salmonella*, followed by Arail Ghat and Mahewa ghat. At Arail and Gaughat, the *Vibrio* was also observed.

Table 2: Table of isolation of the Bacteria

Total No. of sample	No, of isolates	Distribution of Bacteria		
		Gram + Ve cocci	Gram + Ve rod	Gram - Ve rod
5	20	30%	45%	25%

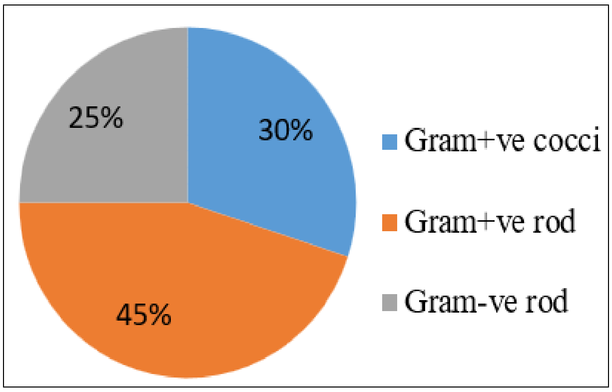


Chart 1: of isolation of bacteria

Effect of heavy metals on isolates

Table 3: Table of Effect heavy metals on isolation

Heavy Metals	No. Of isolates (zone of inhibition in mm)		
	Isolates 1	Isolates 2	Isolates 3
Cd	58	67	47
Cr	45	46	15
Pb	-	-	-
Co	45	55	68

All three isolates exhibited resistance against the greatest toxicity of lead, followed by the maximal zone of inhibition against Co, Cd and Cr. Due to the massive amount of industrial waste the Ganga Yamuna River is now so polluted that it can mass levels of heavy metals that can be lethal to any animal that depends on the river for water in one form or another.The degree of heavy metal contamination in the Ganga Yamuna River has already been demonstrated by earlier research. Previous reports suggests that the most notable isolates were those of *Bacillus* sp. Sphenodon's sps and Stenotrophomonas sps, which were discovered to be found in the Ganga Yamuna River's barrages and have demonstrated resistance to heavy metals. Certain G+ve and G-ve rods demonstrated chromium resistance. Chromium-resistant bacteria utilize diverse mechanisms to cope with chromium-related stress. [25] Biosorption, bioaccumulation, biotransformation, efflux, enzymatic and non-enzymatic reduction, precipitation, cytosolic binding, biofilm formation, and so These include the following mechanisms. To cope with chromium toxicity, Bacteria resistant to it may utilize one or a combination of multiple strategies [26, 27]. The current study sought to find a variety of bacteria that would be helpful for bioremediation as well as to ascertain whether bacteria isolated from water that had been extensively contaminated with lead had adapted to the high lead content.

Conclusion

The present study reveals site-specific heavy metal contamination in river water from Prayagraj. Elevated concentrations of Pb (0.14 mg/L) and Cd (0.020 mg/L) at Jhunsi exceeded permissible limits, indicating significant anthropogenic influence. Chromium levels at Sangam (0.080 mg/L) and Naini (0.076 mg/L) also surpassed recommended standards. Cobalt was consistently detected (0.016-0.040 mg/L) across all sites despite the absence of regulatory limits. The findings highlight deteriorating water quality at certain locations and emphasize the need for continuous monitoring, stricter pollution control, and improved river management strategies to protect environmental and public health.

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