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Isolation and Characterization of Lead and Cadmium Resistant Bacteria from the Root Zone of Constructed Wetlands (CWs)

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ABSTRACT
Twenty four heavy metal resistant bacteria were isolated from root zone of constructed wetlands (CWs) wastewater treatment system, Charak Udhyan Jiwaji University Gwalior, India, against lead and cadmium. Samples were streaked on selective media; dominant and distinct colonies were identified as Bacillus sp., Pseudomonas sp., Proteus sp. and Klebsellia sp. on the basis of their biochemical and morphological characteristic .The antibiotic resistance pattern and minimum inhibitory concentration (MIC) of the potent isolates were also investigated. Five isolates out of the total showed strong tolerance to heavy metals. Pseudomonas sp. was found to have high resistance pattern against Lead (2400µg/ml) and Cadmium (200µg/ml).It was shown that isolates with high MIC values for a particular group of metals displayed high antibiotic resistance.
Keywords: *Heavy metal, Constructed wetland, Metal resistant, Antibiotic resistant.*

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INTRODUCTION

The vital elements of life, air, water, and land, are constantly poisoned as a result of rising population, rapid urbanisation, and industrialisation [1].Heavy metal bioaccumulation in the environment is currently a severe threat to human life [2, 3]. Water pollution caused by industrial wastage, is frequent [4] by toxic sludge, heavy metals, and solvents as they fall into natural water sources and agricultural environment. Metals are involved in all areas of biota development, metabolism, and differentiation, either directly or indirectly [5]. Some heavy metals are important micronutrients required by organisms (zinc, manganese, iron, nickel and cobalt) and are referred to as 'trace elements' [6]. Some, on the other hand, have no biological function and are harmful to organisms even at very low concentrations (cadmium, copper, lead etc.). Both necessary and non-essential metals, however, become harmful to organisms at excessive amounts.

Bacteria are one of the most common organisms that may be found almost anywhere on the planet. Heavy metals are becoming more common in microbial habitats as a result of a variety of natural and anthropogenic processes; as a result, microbes have evolved mechanisms to tolerate heavy metals by efflux, complexion or reducing metal ions, or by using them as terminal electron acceptors in anaerobic respiration [7]. Transport across the cell membrane, bio-sorption to the cell walls and entrapment in extracellular capsules, precipitation, complexation, and oxidation-reduction reactions are some of the ways microorganisms respond to heavy metals [8, 9, 10,11].

Heavy metal contamination in the environment has become a severe problem due to the increase in the addition of these metals to the surroundings, which cannot be despoiled like organic pollutants and persist in the system having accumulated in different parts of the food chain [12].These heavy metals affect not only the microbial population, but also plants and animals, affecting their growth, morphology, biochemical activities, and ultimately resulting in decreased biomass and diversity [13].The degree of toxicity varies for different organisms. Heavy metals can influence the qualitative and quantitative structure of microbial communities, as well as reduce metabolic activity and diversity[14].Heavy metals have been shown to influence cellular organelles and components such as the cell membrane, endoplasmic reticulum, mitochondria, lysosomes nuclei, and several enzymes involved in metabolism, detoxification and damage repair in biological systems [15].Metal ions have been discovered to interact with cell components such as DNA and nuclear proteins, resulting in DNA damage and conformational changes that may lead to cell cycle regulation, cancer or apoptosis [16,17].The main sources of heavy

metal contamination in soil include wastewater irrigation, solid waste disposal,sludge applications, automobile exhaust, and industrial activities, and enhanced metal uptake by food crops cultivated on such polluted soils is frequently reported.

Heavy metals like cadmium and lead are difficult for microbes to ingest or trap. Heavy metals have the potential to harm cell membranes, alter enzyme specificity, disrupt biological activities, and affect DNA structure. Heavy metal toxicity is caused by the displacement of important metals from their native binding sites or by ligand interactions. Toxicity can also result from changes in nucleic acid and protein conformational shape, as well as interference with oxidative phosphorylation and osmotic equilibrium [18].Metals can interfere with the operation of pigments and enzymes by replacing necessary metals [19]. As a result, metals make the land unfit for plant growth and deplete biodiversity. The use of heavy metal tolerant microorganisms in heavy metal supplemented soils is a promising method for enhancing heavy metal bioavailability. The goal of this research was to isolate and characterize Cadmium and Lead resistant bacteria from heavy metal-contaminated soils, as well as to identify heavy metal resistant bacterial strains that could help improve Pb and Cd-polluted soils under adverse environmental conditions.

MATERIAL AND METHODS

Sample Collection

The present study was carried out at School of studies in Microbiology, Jiwaji University Gwalior (M.P.) India. The Rhizospheric soil samples were collected from CWs planted with *Phragmites karka* (CWPK) and constructed wetlands planted with *Chrysopoigon zizanoides* (CWCZ). All the samples were collected using sterilised spatula and stored in sterile plastic zip bag before being processed.

Isolation and Identification of Heavy Metal Resistance Bacteria

The soil sample was mixed with 50 ml distilled water and then filtered. For enrichment, the filtrate was inoculated in Nutrient Broth for 24 hours. For sufficient enrichment, 1 ml of each sample was added to 9 ml of Nutrient Broth and incubated at 37^0C. Individual bacteria colonies of various shapes and colours were picked and streaked on selective media using a calibrated loop, then incubated at 37^0C for 24 hours to obtain potent isolates. The biochemical and morphological characteristics of the most common bacterial genera isolated were investigated, and the bacteria were then described and identified using standard identification procedures [20].

Heavy Metal Resistance

All the five isolates were checked for metal tolerance. MIC was determined by the agar-dilution method against Cd and Pb heavy metals on Luria-Bertani (LB) agar plates until strain fail to give colonies on plates.The initial concentration used was 50µg/ml and thereby gradual increasing the concentration each time on LB plates. Bacterial growth was observed when the plates were incubated overnight at 25^0C.Lowest concentration of the heavy metal that completely inhibited the growth of bacteria was defined as minimal inhibitory concentration (MIC). All the metal salts were added to LB agar after autoclaving and cooling to 50^0C from filter-sterilised stock solutions [21].

Antibiogram of Bacterial Isolates

The Kirby-Bauer disc diffusion method was used to investigate antibiotic sensitivity and resistance in heavy metal resistant isolates [22]. After a 24-hour incubation period, the organisms were classified as sensitive or resistant. It may be antibiotic resistant depending on the diameter of the inhibitory zone. The usual antibiotic disc contains an inhibitory zone.

RESULT AND DISCUSSION

Twenty four heavy metal resistant bacteria were isolated from the root zone of (*Phragmites karka* and *Chrysopogon zizanoides*) CWs wastewater treatment system of Charak Udhyan of Jiwaji university. They were identified as *Bacillus* sp., *Pseudomonas* sp., *Proteus* sp. and *Klebrsellia* sp. Among all, five isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for heavy metals ranging from 100µg/ml to 2400 µg/ml(Table-1).*Pseudomonas* sp. (Ps/PRZ) was found to be have high resistance pattern against Pd (2400µg/ml) and Cd (200µg/ml).*Bacillus* sp. (Ba/PRZ) isolated from root zone of *C. zizanoides* also showed high MIC value against Pb(1800µg/ml) and Cd(200µg/ml). In case of Pb *Bacillus* (Ba/CRZ) from *C. zizanoides* root zone and *Proteus* (Pr/CRZ) showed high metal resistant pattern for Lead following *Pseudomoas*. The MIC value for *Klebsellia* sp. (K/CRZ) against Lead and Cadmium was found to be 1200µg/ml and 150µg/ml respectively.

All the five bacterial isolates were tested for antibiotic sensitivity. The predominant isolates that are tolerant to Lead and Cadmium were found to be multi-antibiotic resistant (Ba/PRZ,

Table 1. Resistance pattern of bacterial isolates to heavy metals (Cd and Pb).

Bacterial Strain	Isolates Code	Minimum inhibitory concentration (MIC) against Lead (Pb)µg/ml	Minimum inhibitory concentration (MIC) against Cadmium(Cd)µg/ml
Bacillus sp.	Ba/PRZ	1800	200
	Ba/CRZ	1800	100
Pseudomonas sp.	Ps/PRZ	2400	200
Proteus sp.	Pr/CRZ	1400	200
Klebsellia sp.	K/CRZ	1200	150

Ba/CRZ, Ps/PRZ, Pr/CRZ, K/CRZ).It was discovered in this study that isolates with high MIC values for a group of metals have a strong resistance pattern to a group of antibiotics (Table-2). Resistance to high levels of Cu (II) and Pb (II) and antibiotic resistance in bacterial species present in drinking water have long been known [23].Multiple metal resistance bacterial isolates exhibit strong resistance to a range of antibiotics, according to Karbasizaed *et al*., 2003 [24].All the isolates (Ba/PRZ ,Ba/CRZ, Ps/PRZ, Pr/CRZ, K/CRZ) were resistant to Ampicillin ,Co-trimoxazole, Penicillin-G and Oxacillin. Amikacin showed high sensitivity to all five isolates .Gentamycin and Kanamycin were also very sensitive, however Gentamycin was resistant to Ba/PRZ and Pr/CRZ, whereas Kanamycin was resistant to Ps/PRZ. With the exception of Ba/PRZ and Ba/CRZ, practically all isolates tolerated chloramphenicol, tetracycline, vancomycin and erythromycin. Streptomycin also tolerate by most of the isolated strains except Ps/PRZ and Pr/CRZ .Multiple tolerances are only seen in toxic substances with similar mechanisms of harm. Multiple tolerances are frequent among heavy metal resistant bacteria due to the comparable harmful mechanisms of all heavy metals.

Table 2. Antibiotic sensitivity of Cadmium and Lead resistant isolates

Antibiotic	Bacterial isolates				
	Ba/PRZ	Ba/CRZ	Ps/PRZ	Pr/CRZ	K/CRZ
Chloramphenicol	S	S	R	R	R
Ampicillin	R	R	R	R	R
Tetracycline	S	S	R	R	R
Gentamycin	R	S	S	R	S
Streptomycin	R	R	S	S	R
Kanamycin	S	S	R	S	S
Co-trimoxazole	R	R	R	R	R
Amikacin	S	S	S	S	S
Penicillin-G	R	R	R	R	R
Methicillin	R	R	R	R	R
Vancomycin	S	S	R	R	R
Oxacillin	R	R	R	R	R
Erythromycin	S	S	R	R	R

Microorganisms that are resistant to heavy metals are crucial in the bioremediation of heavy metal polluted soils [25, 26]. Toxic metal ions, organic wastes, organic ions and antibiotics accumulate in contaminated areas such as those near industries or industrial dump grounds [27]. Heavy metal ions react in cells at high quantities to generate hazardous chemicals [28]. Because of their high mobility, especially in agricultural settings, both Cd and Zn are considered to be among the most hazardous heavy metals, and they can occur in water or soil of any polluted location, posing a serious threat to human health via food chains [29] .Heavy metal levels that are poisonous metal-resistant bacteria can be used for bioremediation, which has a lot of potential [30,31], reviewed the role of soil microorganisms in phytoremediation affect structural and permeability properties of inner membranes and organelles, cause inhibition of enzymatic activities, nutrient imbalances, decreases in rates of photosynthesis and transpiration[32], stimulate formation of free radicals and reactive oxygen species resulting in oxidative stress [33], suppress seed germination and seedling growth[34], reproductive development [35],seed yield and seed quality[36] and induce deleterious anatomical and ultra-structural changes in crop plants [37,38].

CONCLUSION

Heavy metals exert their toxic effects on microorganisms through various mechanisms, and metal-tolerant bacteria could survive in these habitats and possibly be isolated and selected for their potential application in the bioremediation of contaminated sites [39]. The concentration of a toxic metal that

affects the growth and survival of different microorganisms varied greatly. It is clearly indicated that domestic waste and industrial waste are responsible for the development of bacterial resistance along with the risk of human health and environment. The long term effect of pollutants has led to emergence of multi-metal and multi-antibiotic resistant bacteria in the study areas. The use of microbial populations specifically adapted to high concentrations of heavy metals will increase the ability to remediate heavy metal contaminated soils. Consumption of food crops contaminated with heavy metals is a major food chain route for human exposure. Thus, from the present study it can be concluded that the application of microbial populations specifically adapted to high concentrations of heavy metals will increase the ability to remediate heavy metal contaminated soils.

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