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# Effect of Heavy Metal Tolerance of Bacterial Pathogens Isolated from Agricultural Soil

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## Abstract

This study was conducted to investigate the antibiotic and heavy metal tolerance profile of bacterial pathogens isolated from farm land in Gwalior Soil samples were collected from different farm lands within Presco campus with sterile spatula container and were transported to Microbiology Laboratory Unit of Jiwaji University, Gwalior for bacteriological analyses Bacteria species isolated were characterized and identified using standard microbiological techniques. Bacteria isolated were E. coli, Klebsiella sp, Staphylococcus spp and Shigella spp. Antibiotic susceptibility studies was conducted using Kirby and Bauer method according to Clinical Laboratory Standards Institute (CLSI). The result of antibiotic studies showed that Shigella sp was resistant to ceftazidime, ampicillin, cefuroxime and amoxicillin/clavulanic acid. Klebsiell asp was most susceptible to the antibiotic used. Metal tolerant studies were conducted using silver nitrate, copper II sulphate, zinc sulphate and lead acetate at varying concentrations of 0.5, 1.0, 3.0 and 5.0 mM, respectively. The result of the metal tolerance showed that all the bacterial isolates had growth at the metals concentration of 0.5 mM, but at higher concentration, there was no growth. Our results revealed that the isolates could be potential agents for the development of soil inoculants applicable in bioaugmentation of heavy metals polluted agricultural and industrial sites.

## Introduction

An increasing body of evidence suggests that microorganisms are far more sensitive to Heavy metal stress than soil animals or plants growing on the same soils. Not surprisingly, most studies of heavy metal toxicity to soil microorganisms have concentrated on effects where loss of microbial function can be observed and yet such studies may mask underlying effects on biodiversity within microbial populations and communities. The types of evidence which are available for determining critical metal concentrations or loadings for microbial processes and populations in agricultural soil are assessed, particularly in relation to the agricultural use of Sewage. Much of the confusion in deriving critical toxic concentrations of heavy metals in soils arises from comparison of experimental results based on short-term laboratory ecotoxicological studies with results from monitoring of long-term exposures of microbial populations to heavy metals in field experiments. The laboratory studies in effect measure responses to immediate, acute toxicity (disturbance) whereas the monitoring of field experiments measures responses to long-term chronic toxicity (stress) which accumulates gradually. Laboratory ecotoxicological studies are the most easily conducted and by far the most numerous, but are difficult to extrapolate meaningfully to toxic effects likely to occur in the field. Using evidence primarily derived from long-term field experiments, a hypothesis is formulated to explain how microorganisms may become affected by gradually increasing soil metal concentrations and this is discussed in relation to defining “safe” or “critical” soil metal loadings for soil protection.

## Review of literature

Heavy metals are well known to be toxic to most organisms when present in excessive concentrations. It is thus hardly surprising that heavy metals toxicity to microorganisms in soil has often been reported. First observations of the effects of heavy metals on soil microbial processes date back to the beginning of this century (Lipman and Burgess, 1914; Brown and Minges, 1916). But only when the large adverse effects of emissions of heavy metals from smelters on surrounding ecosystems was observed in the 1960–70's was it realized how severely soil microorganisms and soil microbial processes can become disrupted by elevated metal concentrations, sometimes resulting in severe ecosystem disturbance. Extreme metal contamination in the vicinity of smelters caused clearly visible effects such as accumulation of deep layers of organic matter on the soil surface through inhibition of the activity of soil microorganisms and soil fauna (Tyler, 1975; Strojan, 1978; Freedman and Hutchinson, 1980).

When measures to limit the metal loading rates of soils due to the use of sewage sludge in agriculture were first introduced in many European countries during the 1970's, these limits were focused on protecting against negative effects on crop plants, on animals grazing on land to which sewage sludge had been applied and to protect man from metal exposure through the food chain. It was not until 20 yr later that the effects of elevated heavy metal concentrations on soil microorganisms were taken into consideration in the drafting of legislation to regulate the agricultural use of sewage sludge (Witter, 1992; MAFF/DoE, 1993). A considerable body of information has now been accumulated on the effects of heavy metals on soil microorganisms and microbially-mediated soil processes from both laboratory studies and field experiments. A summary of the results from such studies (Bååth, 1989) shows the enormous disparity between studies as to which metal concentrations are toxic. Given this disparity how can we establish critical metal concentrations for soil microorganisms? Do the toxicity effects seen in short-term laboratory tests also apply to the field situation? And how can we ensure that metal limits protect not only specific populations and processes, but protect all those which are important for the fertility of soils, now as well as in the future? These are some of the questions which we attempt to answer here.

This is not intended to be an exhaustive review of the subject but rather, by looking in detail at some of the more commonly used bioassays in studies of metal toxicity, we attempt to explain the disparities between the results and conclusions from different types of scientific studies and to set priorities for further research. We first discuss the problems and pitfalls of research on this subject and then assess the evidence which indicates the great sensitivity of soil microorganisms to heavy metals in soil, in the light of our comments on methodology. Finally we suggest ways in which "ideal" evidence for the setting of metal limits for environmental protection can be achieved.

## Material and Methods

### Soil Sampling

To isolate potent bacteria, surface soil samples (0–15 cm) were collected from wastewater irrigated agricultural soils of major industrial area. Five random sub-samples collection with the help of a wooden core borer from each sampling site within a radius of 500 The soil samples keep on, ice and store at Gravel and stones remove from the soils and samples. The physicochemical analysis of soil samples was carried.

### Isolation of Bacteria

Bacteria isolation from soil using a serial dilution method. Briefly, 10 g of soil was added to 90 mL of 1% normal saline solution in a 250 mL flask. The flask was then shaken for 20 min on a rotary shaker. One mL of the suspension was taken out of the flask and added to 9 mL of normal saline solution, and serial dilutions were made up to 10−9 dilution. One mL of each dilution was spread on the nutrient agar and MacConkey agar media plates and were incubated for up to 3 days at 28 ± 2 °C to observe bacterial growth [20]. Bacterial colonies were studied for their colony morphology such as size, shape, margins, elevations, texture and opacity. Single colonies were re-streaked on to fresh LB agar plates and incubated under similar conditions. The process was repeated three times to purify the colonies. Bacterial colonies were preserved at −20 °C in 50% sterile glycerol solution for future use.

Minimum inhibitory concentration (MIC) of above-mentioned heavy metals for bacterial isolates with at least one tested plant growth promoting trait was determined with the plate dilution method [28]. Various concentrations of heavy metals ranging from 0.1 in LB agar inoculated with 18 h old bacterial culture. Inoc incubated at 28 ± 2 °C for 48 h.

**Isolation of Microorganisms**

Microorganisms occur in natural environment like soil. They are mixed with several other forms of life. Many microbes are pathogenic. They cause a number of diseases with a variety of symptoms, depending on how they interact with the patient. The isolation and growth of suspected microbe in pure culture is essential for the identification and control the infectious agent. The primary culture from natural source will normally be a mixed culture containing microbes of different kinds. But in laboratory, the various species may be isolated from one another. A culture which contains just one species of microorganism is called a pure culture. The process of obtaining a pure culture by separating one species of microbe from a mixture of other species, is known as isolation of the organisms.

## Results

Industrialization and modern agriculture have increased environmental contamination with heavy metals, which can accumulate in living organisms, causing the emergence of toxicity symptoms [[1], [2], [3], [4], [5], [6]]. Although several heavy metals (Co, Cu, Cr, Ni, Fe, Mn, Zn etc.) play a vital role in the metabolic processes in enzymatic reactions and in providing osmotic balance [[7], [8], [9]], some others (Cr, Cd, Hg, Ni, Pb) are toxic, inhibiting growth to different extents, even at very low concentrations [1,3,[8], [9], [10], [11], [12]]. An excessive accumulation of heavy metals in aquatic and soil environments can induce adverse phytotoxic effects, such as growth inhibition, photosynthesis disturbance, biomass decrease, and nutrient uptake deficiency [[13], [14], [15]].

Plants growing on soils contaminated with heavy metals are able to absorb significant amounts of metal ions, which thus enter the food chain affecting human health [[16], [17], [18], [19], [20]]. Likewise, some microorganisms living in the soil can accumulate heavy metals, since they are able to initiate and develop various mechanisms for metal mobilization or immobilization (e.g. biosorption, bioprecipitation), depending on soil properties (pH, type, salinity etc.) [21]. Hence, the cleanup of contaminated soils using plants, microorganisms or other biological systems, within the limits of their tolerance for heavy metals and under controlled conditions, remains a constant challenge for researchers and for regulatory authorities [1,3,13,[21], Cr and Cd were selected for this study since they are recognized as frequently-encountered toxic heavy metals and were categorized as human carcinogens by the International Agency for Research on Cancer in 1993 [[20], They are very toxic to both plants and microorganisms, whose response to any stress generated by heavy metals depends on the heavy metal concentration, type and speciation, but also on environmental factors and organism species [[21], Cd can play the role of cofactor for oxidative reactions that disrupt and damage living tissues, and can increase the oxidative capacity in the generation of reactive-oxygen species (ROS), lipid peroxidation and depleting glutathione, enhancing and linking protein sulfhydryl groups [[3], The reduction/oxidation of Cr from Cr(VI) to Cr(III) is possible from a thermodynamic point of view in certain physiological conditions. Cr(VI) is the most toxic form of Cr, often found as oxyanions associated with oxygen, as chromate ($CrO_4^{2}$) or dichromate ($Cr_2O_7^{2-}$) [17]. It is acknowledged that Cr(III) is indispensable for sustaining the glucose metabolism of lipids and proteins. In addition, Cr(III) can stabilize the tertiary structure of proteins, RNA and DNA conformation. On the other hand, the compounds of Cr(VI) are toxic. Interactions between bacteria, algae, fungi and plants, with Cr and its compounds have been thoroughly reviewed in the literature [12,]

## Conclusions

The need for information about heavy metal levels in the environment (water, soil), their mobility, availability and toxicity in plants and microorganisms was addressed in this study as a preliminary approach in supporting decision making which demands the most appropriate bioremediation strategies. The studies revealed the adverse effects of heavy metal ions, Cr(VI) and Cd(II) on an edible plant (*L. sativum)* and growth of two microorganisms (*Azotobacter* sp., *Pichia* sp.), depending on metal

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