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Effect of Heavy Metal on Bacterial Characterization in Soil

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Abstract: Soil sample of Gopalpur soil series was taken to understand heavy metal effect on bacterial characterization. The soil was collected in the laboratory using a thermos flask. The sample was sieved and sorted properly and mixed throughly. Analytical-grade sulfate (SO_4^{2-}) salts of zinc, copper, and cadmium were applied to designated pots, both individually and in combinations. The soil sample that received no metal treatment served as the control. These heavy metals (Cu, Cd & Zn) and their combinations were applied to the soil. 0 day, 15 days, 30 days, 45 days and 60 days were selected as incubation periods. The experiment was conducted using a Completely Randomized Design (CRD) with three replications. Combined applications of heavy metals are found more toxic to bacterial population than single application. The treatments, both Cd (3000ppm) and Cu+Cd+Zn (1000ppm+1000ppm+1000ppm), were found more toxic to both Gram- positive bacteria and Gram-negative bacteria. If we compare with Gram- positive bacteria, Gram-negative bacteria were found more resistant. Treatments, both Cd (3000ppm) and Cu+Cd+Zn (1000ppm+1000ppm+1000ppm), were more toxic to both non spore forming bacteria and spore forming bacteria. Here, spore forming bacteria were found more resistant. Treatment,Cu+Cd+Zn(1000ppm+1000ppm+1000ppm), was more toxic to both non capsulated bacteria and capsulated bacteria. We found capsulated bacteria bacteria as more resistant. Gram-positive bacteria, non spore forming bacteria, non capsulated bacteria were very sensitive whereas Gram-negative bacteria, spore forming bacteria and capsulated bacteria were available as resistant to heavy metals.

Keywords: *Heavy Metal, Bacteria, Characterization, Gram Stain, Capsule Stain, Spore Stain*.

Introduction

Pollution by heavy metal is introduced in the environment through human partcipations, including industrial works, household waste with other refuse. Elevated amount of heavy metals of soil is responsible for the disruption of terrestrial ecosystems of nature (Wei *et al.*, 2007; Yadav *et al.*, 2009). At elevated quantities , heavy metals exhibit strong toxicity and cause environmental pollutants (Nedelkoska and Doran, 2000; Chehregani *et al.*, 2005). Heavy metals represent a hazardous group of soil pollutants. They cannot be naturally degraded and tend to accumulate within various levels of the food chain. Under stress conditions resulting from harmful human activities—such as the release of chemical pollutants—the growth and biochemical functions of soil microorganisms can be significantly altered. To mitigate adverse ecological impacts, microbiological indicators should be included in assessments of soil quality (Filip 2002). Heavy metals disrupt the biochemical processes of various groups of microbes isolated from nature (Alloway, 1995; Sani *et al.*, 2003; Utgikar *et al.*, 2004; Pennanem *et al.*, 1996). Elevated concentrations of heavy metals are to affect microbial populations of soil and their activities, that can has an impact on soil health (Smith, 1996). Soil microorganisms play a crucial role to fix nitrogen, assimilate nutrient, and decompose organic matter with a view to releasing nutrients. As heavy metals accumulate into the soil due to repeated with unregulated inputs, they disrupt vital biochemical processes, leading to ecological imbalances. These metals also pose a risk to human health by entering and moving by the food chain. The toxicity of heavy metals on microbes are evident in many ways, including impaired nitrogen fixation, and less nutrient cycling (Baath, 1989; Brookes, 1995). Among all microorganisms, bacteria are distributed widely, simple in morphology, small in size, challenging to classify, and hard to identify (Joklik *et al.*, 1992). The life and ecosystems maintenances in land and water depends on bacteria, as they have a vital role in decomposition. Additionally, the cycling of essential elements like carbon, nitrogen, and sulfur in nature is sustained by their continuous activity (Stevenson, 1986). Bacteria are successful and ancient forms. They being small, single-celled, prokaryotic bodies have no distinct nucleus. The bacterial morphology is quite easy, with measuring only 0.5 to 2.0 micrometers in their diameter. Bacteria typically exhibit shapes: bacillus (rod-shaped), spherical, and spiral. In environment, bacteria can exist either individual or aggregated forms. Bacteria actively participate in nearly all organic processes that contribute to a healthy soil system. Due to their enzymatic capabilities, experts are exploring paths to harness and even enhance bacterial metabolic activities to aid in the remediation of soils contaminated by organic toxins. They are usually the most important group involved in breaking down hydrocarbon compounds (Brady and Weil, 2002). Bacteria possess various cultural, morphological and physiological characteristics which provide useful information about the different species of bacteria. Before proceeding with the characterization of bacterial species, it is essential to isolate pure cultures using various techniques. Without pure cultures, studying bacteria becomes ineffective, as distinct and separate isolates are required for the multiple tests employed in bacterial identification in all microbiology laboratories. Unfortunately, soil microbiology has

very limited research in Bangladesh, and the develop of this field is negligible compared to other scopes of soil science. Experiments on the effect of heavy metal on bacterial populations are closely related to soil management. Copper (Cu), cadmium (Cd), and zinc (Zn) are common heavy metals that contaminate agricultural soils. Understanding how these heavy metals affect soil fertility requires knowledge of their impact on bacterial populations, diversity, and characterization. Therefore, the goals of this study are to examine the effects of interactions among Cu, Cd, and Zn on bacterial characterization through Gram staining, capsule staining, spore staining, and acid-fast staining.

Materials and Methods

General description of the location, the methods and material concerning the experiments are discussed in this section.

Location and extent

Soil sample was collected from 5-15 cm depth in composite manner. A generalization of the location is in Table 1.

Table 1. Description of the location.

Sample No.	GPS Reading	Address	Physiography	Soil Series
1	N: 22º 55.040′ E: 89º 31.704′	Village: Barakpur Union: Barakpur Thana: Dighalia District: Khulna	Ganges meander floodplain	Gopalpur

Soil preparation

Surface soil was collected from field. The soil was taken in laboratory using thermo flask. The sample was sieved using a mesh size of less than 2 mm, sorted for removing stones, debris of plant, and visible fauna, and then mixed using a trowel. Some physical with chemical properties of initial sample were in determination. Those properties are presented in table 2. One kg of the sieved soil sample was taken in each of the plastic pots. Analytical grade Sulphate (SO_4^{2-}) salts solutions of Zink, Copper and Cadmium were used to the pots singly and combinations. Proper labeling was done by using treatment code.

Table 2. Some physical and chemical properties of soil sample.

Soil properties	Value
Textural class	Silty Loam
Soil pH	6.5
Soil moisture	28.78%
Field capacity	52.75%
EC	2.52 dS/m
Ca	368 ppm
Mg	40.57 ppm
Zn	4.409 ppm
Cd	0.036 ppm
Cu	1.22 ppm
Pb	0 ppm

Experimental design

The experiment was laid out with three replications in Complete Randomize Design (CRD). The codes of the treatment are shown in table 3. For this experiment the incubation periods were 0 day, 15 days, 30 days, 45 days and 60 days.

Table 3. Treatments code.

Code	Treatment	Concentration (ppm)
0	Control	0
A	Cu	3000
B	Cd	3000
C	Zn	3000
D	Cu+Cd	(1500+1500)
E	Cd+Zn	(1500+1500)
F	Zn+Cu	(1500+1500)
G	Cu+Cd+Zn	(1000+1000+1000)

Isolation of bacteria

First, the sample preparation was done using soil with physiological water (distilled water + 0.9% NaCl) as described by Dubey and Maheshwari (1999). Next, nutrient agar media was prepared following the method outlined by Prescott and Harley (2002). Sterilization of agar media and all equipment for bacterial culture were by steam at 121°C and 15 psi (pounds per square inch) of pressure, according to Prescott and Harley (2002). Staining characteristics of the bacteria were determined using Gram stain, capsule stain, spore stain following the procedures outlined by Cappuccino and Sherman (1999).

Gram stain procedure

Using sterile technique, a heat-fixed smear was made on a glass slide. With crystal violet smear was flooded and left for one minute, rinsed with tap water. Next, Gram's iodine was applied and allowed to sit for one minute, followed by another rinse with tap water. Ninety-five percent ethyl alcohol was then added drop by drop until the crystal violet no longer washed off the smear. The slide was rinsed with tap water and counterstained using safranin for 45 seconds. After final rinse by tap water, slide was air-dried. This procedure was repeated on two additional slides. Finally, the slides were examined under oil immersion.

Capsule stain procedure

Using sterile technique, an air-dried bacterial smear was made on a glass slide. The smear was flooded by crystal violet and left for five to seven minutes. It was then rinsed with twenty percent copper sulfate solution. Ensuring air-dried slide, the procedure was repeated on two additional slides. Once dried, all slides were examined under oil immersion.

Spore stain procedure

Using sterile technique, a heat-fixed smear was available on a glass slide. The smear was flooded using malachite green and then placed on a hot plate, allowing it to steam for two to three minutes without any boiling. The slide was removed from hot plate, cooled,

rinsed with water. Next, smear was counterstained using safranin for 30 seconds, rinsed again with tap water. After ensuring the slide was air-dried, the procedure was repeated on two additional slides. Once dried, the slides were examined under oil immersion.

Statistical analysis

The collected data for various parameters were in an analysis using analysis of variance (ANOVA) with the MSTAT-C program.

Results and Discussion

Effect of heavy metal interactions on Gram- positive bacteria and Gram-negative bacteria

Populations of both Gram-positive bacteria (Fig. 1) and Gram-negative bacteria (Fig. 2) decreased significantly ($p \leq 0.01$) over the incubation period due to having antagonistic effects of heavy metals in the soil. Treatments with cadmium (Cd) at 3000 ppm and the combined treatment of copper, cadmium, and zinc (Cu+Cd+Zn at 1000 ppm each) were found to be more toxic to Gram-positive and Gram-negative bacteria. Gram-negative bacteria exhibited greater resistance. According to Samad (2000), the cells of Gram-negative bacteria possess an impermeable membrane, which makes them more resistant to heavy metals, antibiotics, and adverse environmental conditions than Gram-positive bacteria. Yamina et al. (2012) reported that seventy seven percent of bacteria in a metal-polluted sample were Gram-negative, while 23% were Gram-positive.

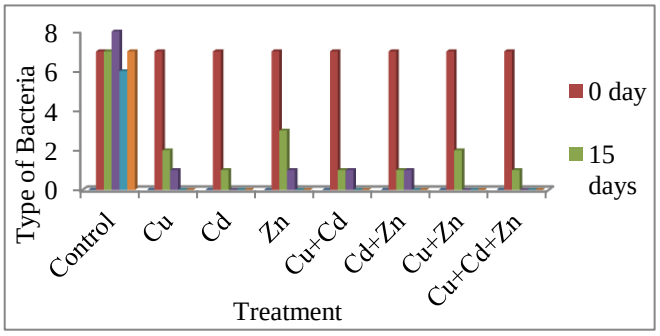

Fig. 1. Effect on Gram- positive bacteria.

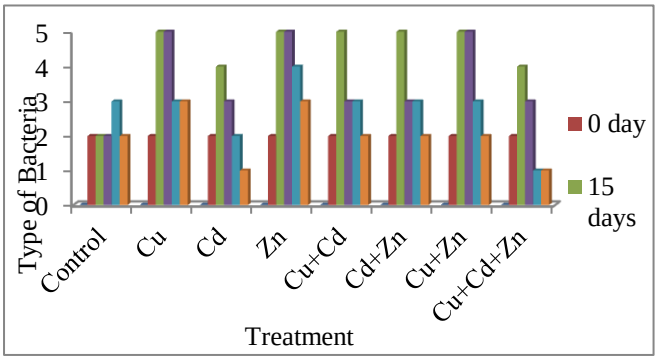

Fig. 2. Effect on Gram-negative bacteria.

Effect of heavy metal interactions on spore forming bacteria and non spore forming bacteria

Populations of both spore-forming bacteria (Fig. 3) and non-spore-forming bacteria (Fig. 4) decreased significantly ($p \leq 0.01$) with increasing days of incubation. Treatments with cadmium (Cd) at 3000 ppm and the combined treatment of copper, cadmium, and zinc (Cu+Cd+Zn at 1000 ppm each) were more toxic to spore-forming and non-spore-forming bacteria. Spore-forming bacteria showed greater resistance. According to Shaha (2010), spore-forming bacteria are more resilient to adverse natures, such as heavy metals, antibiotics, and pollution than non-spore forming bacteria.

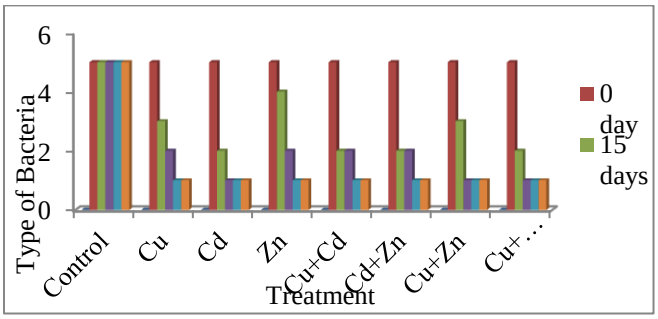

Fig. 3. Effect on spore-forming bacteria.

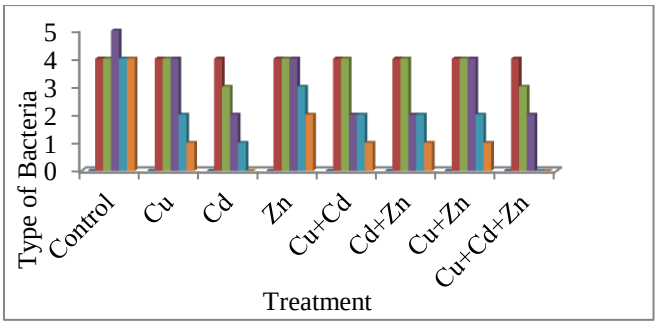

Fig. 4. Effect on non spore-forming bacteria.

Effect of heavy metal interactions on capsulated bacteria and non capsulated bacteria

Populations of both capsulated bacteria (Fig. 5) and non-capsulated bacteria (Fig. 6) decreased significantly ($p \leq 0.01$) with increasing days of incubation. The combined treatment of copper, cadmium, and zinc (Cu+Cd+Zn at 1000 ppm each) was more toxic to capsulated and non-capsulated bacteria. Capsulated bacteria showed greater resistance. According to Shaha (2010), capsulated bacteria are more resistant to environmental stress due to the protective capsule or slime layer surrounding them.

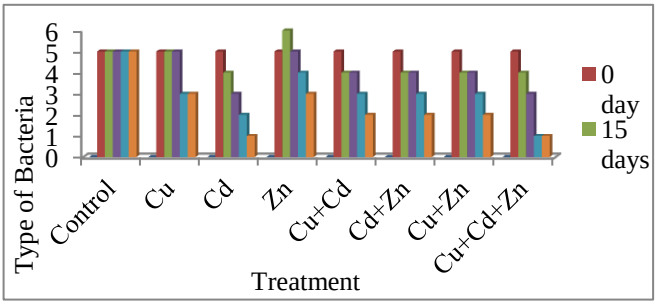

Fig. 5. Effect on capsulated bacteria.

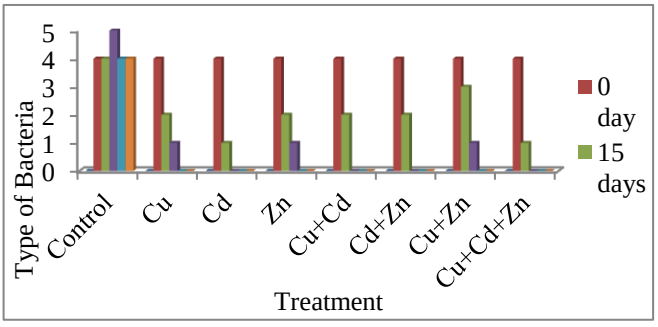

Fig. 6. Effect on non capsulated bacteria.

Summary and Conclusion

Three heavy metals (Cu, Cd & Zn) and their combinations were applied as treatment to Gopalpur soil to understand the effects on bacterial population, diversity and characterization with time. The findings are noted that combined applications of heavy metals are more toxic to bacterial population than single application. Some bacterial populations become much active with the progress of heavy metal treatment duration. Gram-positive bacteria, non spore forming bacteria and non capsulated bacteria are very sensitive whereas Gram-negative, spore forming bacteria and capsulated bacteria are found more resistant group of bacteria to heavy metals.

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