

# EFFECT OF HEAVY METAL ON BACTERIAL POPULATION AND DIVERSITY OF GOPALPUR SOIL SERIES

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## ABSTRACT

Soil contaminated with heavy metals is emerging hazardous tendency across the world due to various activities. Soil are providing habitat to many organisms and its contamination leads to effect the growth, yield and performance. The heavy metal deposition in soil effects microbial diversity and production. In this study the heavy metal, zinc (Zn), copper (Cu) and cadmium (Cd) were selected to observe their effect on bacterial population and diversity from Gopalpur soil series. The incubation period of heavy metals was for every 15 days. Bacterial population and diversity were determined carefully by viable count and colony characteristics method. The combined applications of heavy metal were found more toxic to bacterial population than single application. The bacterial abundance including diversity decreases with time.

**Keywords:** Gopalpur soil, heavy metal, bacterial population and bacterial diversity

## 1. INTRODUCTION

Heavy metals are non-biodegradable and abounding in nature due to severe different anthropogenic activities, such as industrial manufacturing process, domestic refuse, waste materials, etc released into the environmental sources [1]. Their presence effect many living being like human, plant, birds, amphibian and microbes at various level. The deposition of heavy metals in soil creates an unexpected disruption of terrestrial ecosystems [2]. However at higher concentrations, all types of heavy metals are

responsible for serious dangerous effects and are considered as environmental pollutants [3]. Bangladesh is one of the most densely populated countries with various case reports on deposition of heavy metal into ecosystem and its exposure increased in recent decades [4]. The effluents from various industrial are channelized to river and soil leading misbalance in ecosphere. The metadata collected from government reports and publications reported high presence of As, Pb, Cd, Zn and Cr in soil. Meanwhile, agricultural fields of various regions are contaminated by wide dispersion of cadmium (Cd), nickel (Ni), iron (Fe), lead (Pb), zinc (Zn) and chromium (Cr)

high concentrations, which may affect human health directly or indirectly [5-8].

Soil microorganisms are closely related to nitrogen fixation, assimilation, and degradation of organic residues with a view to releasing nutrients. In the repeated and uncontrolled additional process of heavy metals into the soil, it must interfere with the key biochemical processes altering the balance of our ecosystem. The involvement of heavy metals into the food chain of human health is also severally fatal and can alter many genetic matters [9]. Decrease in litter decomposition and nitrogen fixation, less efficient nutrient cycling are the results of the effects of heavy metals on microorganisms [10]. Higher concentration of heavy metals not only affects microbial population but also their related activities, directly influencing the soil fertility [11]. From many earlier investigations, it is observed that the abundance and the diversity of soil bacteria decreased by heavy metals [12] as they interfere with the biochemistry of different group of microorganisms [13]. The microbial community of soil should be considered as a sensitive indicator of metal contamination on both bioavailability and biogeochemical processes [14]. Therefore our study focuses on understanding the effect of heavy metal (Cu, Cd and Zn) on soil bacterial population and diversity.

## 2. MATERIALS AND METHODS

### 2.1. Sample collection and preparation:

Fresh top soil samples (0-15 cm) were collected from the Gopalpur (24.5629° N, 89.9277° E) of Tangail district in Bangladesh and taken aseptically into laboratory using thermo flask and kept for further study. The soil was sieved, sorted and mixed thoroughly with hand trowel. Eight different treatments were prepared as control (0 ppm), Cu (3000 ppm), Cd (3000 ppm), Zn (3000 ppm), Cu+Cd (1500+1500), Cd+Zn (1500+1500), Zn+Cu (1500+1500) and Cu+Cd+Zn (1000+1000+1000).

One kg of the sieved soil was taken in different plastic pots. The solutions of analytical grade Sulphate (SO<sub>4</sub><sup>2-</sup>) salts of zinc (Zn), copper (Cu) and cadmium (Cd) were applied to the respective pots both individually and in combination in 0 day, 15 days, 30 days, 45 days and 60 days were selected as incubation periods. The experiment was laid out in a Complete Randomize Design (CRD) with three replications.

### 2.2. Bacterial population:

Bacterial population was determined by Viable Count method [15]. The plates with 25 to 250 colonies were selected for counting and total bacterial count was calculated by equation (1). The bacterial diversity was determined as described by [16].

$$\text{Total bacteria/g soil} = \frac{\text{no. of colonies} \times \text{dilution factor}}{\text{volume of sample(ml)}}$$

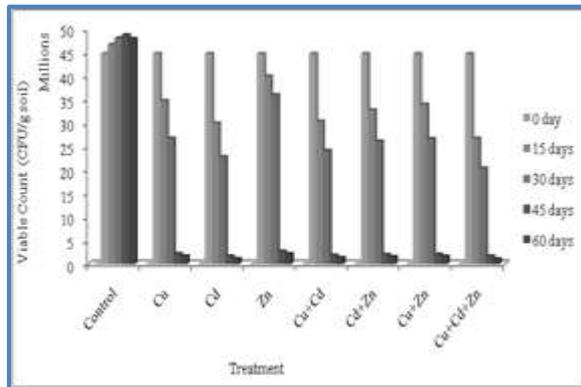
### 2.3. Statistical analysis:

The collected data on different parameters were analyzed following analysis of variance (ANOVA) technique by using MSTAT-C program.

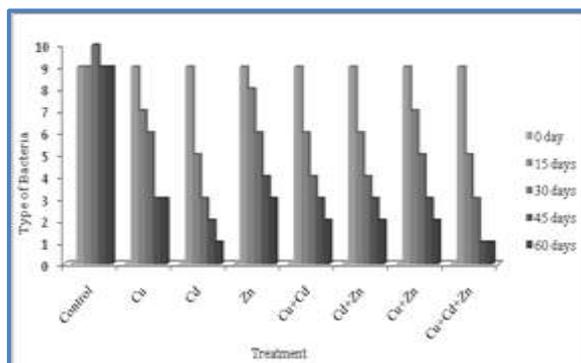
## 3. RESULTS AND DISCUSSION

There is significant changes in bacterial population due to the application of heavy metal as well as with the days of incubation were observed (Fig. 1). The total number of bacteria decreased gradually with days of incubation due to the antagonistic effect of heavy metal in soil. The lowest value ( $1.01 \times 10^6 \pm 0.58$  CFU/g soil) of the viable count of bacteria (Fig. 3) was found at 60 days of incubation under the treatment of Cu+Cd+Zn (1000ppm+1000ppm+1000ppm) and the highest value ( $4.5 \times 10^7 \pm 10.82$  CFU/g soil) was found at 0 day of incubation under all of the treatments. Here, the toxicity series of heavy metal interactions to the viable count of bacteria was found to be in order of Cu+Cd+Zn > Cd > Cu+Cd > Cd+Zn > Cu+ Zn > Cu > Zn. According to Walpola, B.C *et al.*, (2011) [17], metal toxicity is responsible for the decrease in microorganisms and higher

concentration of metal exposure will cause immediate death of cells by disrupting necessary functions. It provides gradual changes in population sizes by changing the viability or their competitive ability. It is observed in this study that the effect of Cd and its interactions were more toxic to bacteria as compared to others.



The Cd toxicity to soil biota was found varying with time, soil type, speciation, ageing, Cd-source, organisms and the environmental factors [18, 19]. The yield and N<sub>2</sub>-fixation capacity of clover *Rhizobium liguminosarum*-*Biover* trefoil association was hampered by applying heavy metals [20]. There is decreased level of *Bacillus subtilis*, *E. coli*, gram negative bacteria and other *actenomyciate* are decreased due to long term exposure of heavy metals [21].



The significant ( $p \leq 0.01$ ) changes in diversity of bacteria due to the application of heavy metal as well as with the days of incubation (Fig.2). The diversity of bacteria decreased gradually with days of incubation due to the antagonistic effect of heavy metal in soil.

The lowest value (1 type of bacteria) of the type of bacteria was found at 60 days of incubation under the treatments of both Cd (3000ppm) and Cu+Cd+Zn (1000ppm+1000ppm+1000ppm). The highest value (10 types of bacteria) of the type of bacteria was found at 30 days of incubation under control. Here, the toxicity series of heavy metal interactions to the diversity of bacteria was  $Cu+Cd+Zn > Cd > Cu+Cd = Cd+Zn > Cu+ Zn > Cu > Zn$ . The investigation demonstrated that heavy metal had decreased bacterial diversity and Cd was more toxic to diversity of bacteria. Similar result was observed by [22]. The soil contaminated with Pb, Ni, Zn and other heavy metals have lower bacterial diversity [23]. The high concentration of Pb in combination of As and Cu was observed for consistently reduced number of bacteria and fungi [24]. In comparison of our study, Lampkin and Sommerfeld (1982) [25] also recorded reduced species diversity in the soil containing high levels of Cu, Mn, Zn and Fe. In some reports there is decreased level of bacterial diversity from soil in long-term exposure of Cr [26] and high content of Zn decreased the diversity of bacterial communities in arable soils [27]

#### 4. CONCLUSION

Combined applications of heavy metals were more toxic to bacterial population than single application. Generally, heavy metal decreased the bacterial abundance and diversity in soil with time. This study will be helpful for increasing knowledge about the harmful effect of Cu, Cd and Zn on soil fertility and thus will ensure awareness to minimize the toxicity of heavy metal.

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#### 6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of

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