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Effect of heavy metals on the growth of Bacillus spp. isolates

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ABSTRACT

The objective of this study was to investigate the effects of different concentrations of heavy metals,: namely Cobalt (Co), Cadmium (Cd), manganese (Mn), Copper (Cu), Mercury (Hg), Iron (F)e, Zinc (Zn), and Lead (Pb), on four different species of Bacillus (*Bacillus subitils* sub sp. *subitilus, B. atrophaeus, B. mycoides, B. weihenstephananensis*). Various metal concentrations (75, 125, 250, 500, and 750 µg ml^{-1}) of each heavy metal salt were prepared and tested against the target organisms. This study showed that *Bacillus* spp. exhibited different responses to the different concentrations of heavy metals. Among the tested strains, *B. atrophaeus* and *B. subtilils* sub sp. *subitils* were the most sensitive to varying concentrations of the studied heavy metals. Cd was the most effective heavy metal on all tested strains, whereas all isolates were resistant to all concentrations of Mn and Pb. Most of the strains were resistant to different concentrations of Cu and Fe.

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1. INTRODUCTION

Heavy metals are a well-defined group of metals with atomic densities exceeding 5 g cm^{-3}. Generally, they encompass numerous metals and metalloids in groups IIIB, IVB, VB, and VIB of periodic table elements. Many of them represent micronutrients, but they are of interest because of their toxicity to living organisms. Approximately 50 heavy metals are important for their toxicological effects on all living organisms [1, 2]. They play an integral role in different life processes of living organisms. Their importance comes from their serving as micro-nutrients, used in redox processes, stabilizing molecules through electrostatic interactions; as components of various enzymes; and regulating osmotic pressure. These are potassium K, sodium Na, magnesium Mg, nickel Ni, calcium Ca, manganese Mn, cobalt Co, chromium Cr, cupper Cu, iron Fe, and zinc Zn and are essential. However, other metals such as silver Ag, aluminum Al, cadmium Cd, gold Au, lead Pb, and mercury Hg have no biological role and are nonessential [3]. At certain concentrations, these elements participate in some enzyme activity [4]. They may cause enzyme disruption in structure and function, where they bind to thiol and other groups on protein molecules, leading to the replacement of naturally occurring metals in enzyme prosthetic groups. [5], they also bind with and disrupt deoxyribonucleic acid (DNA) [6]. When there is excess concentration, the toxic effects of these ions are revealed. They cannot be destroyed or degraded because they are stable and resistant to environmental contaminants [4]. They cannot be biologically degraded and enter the environment; however, their potential toxicity is controlled by biological and geochemical factors [4, 7]. Their toxicity in plants [8, 9] and animals [6] has been well studied. They are known to cause various diseases in humans, where cadmium causes kidney and bone disease, headache, hypertension, and lung and prostate cancer [10–12]. Chromium causes liver diseases, renal failure, chronic bronchitis, skin irritation, and lung cancer [13, 14], while lead causes learning disorders, renal damage, chronic nephropathy, insomnia, reduced fertility, and is a risk factor for Alzheimer's disease [10, 12, 15]. The toxicity of some metals is attributed to their bioaccumulation [16, 17]. Prolonged exposure leads to the appearance of symptoms and may mobilize through the food chain [18], which could have possible effects on higher organisms. Mining, power station smelters, and the application of pesticides containing metals, fertilizers, and sewage sludge are the main anthropogenic sources of soil and water pollution with heavy metals [3, 19]. Industrialization and mining have continued to increase the charge on environmental pollution by metals [20]. The production of pesticides, fertilizers, and mining industries liberates Cd into the environment [10]. Industries of batteries, tanning, and textile as well as mining, electroplating, paints, and pigments liberate Cr and Pb into the environment [10, 11, 13]. The microbial population is affected by heavy metals, leading to an effect on growth, morphology, and biochemical activities, which results in a decrease in biomass and diversity. This nonessential heavy metal toxicity occurs by the displacement of essential metals from their native binding sites or by ligand interactions, alterations in morphological structure, and changes in the conformation of nucleic acids and proteins. In addition, they inhibit enzyme activity, disrupt membrane function, cause oxidative phosphorylation, and alter the osmotic balance of bacterial cells [3, 21, 22]. Microbes have developed different mechanisms that help them tolerate heavy metals either by reduction of metal ions, by using them as terminal electron acceptors in anaerobic respiration, or by the presence of heavy metals through efflux and complexation [23]. They also accumulat them inside the cell, effluxing metal ions outside the cell and reducing them to a less toxic state [24, 25]. Previously, different genera of heavy metal-tolerant bacteria have been reported, such as *Bacillus*, *Pseudomonas*, *Micrococcus*, *Athrobacter*, and *Enterobacter*, [26–28]. Bacteria that are resistant to and able to grow on metals play an important role in the biogeochemical cycling of these metal ions [19]. *Bacillus spp*. are Gram-positive, rod-shaped, spore-forming, aerobic or facultative anaerobic bacteria. They inhabit soil and can be isolated from air, water, vegetables, food, human and animal intestines [29–34]. The aim of this study was to examine the effect of different concentrations of different heavy metals on the growth of *Bacillus spp*. isolates.

2. MATERIALS AND METHODS

2.1. Test organisms

Four bacterial isolates (*Bacillus subitils* sub sp. *subitilus, B. atrophaeus, B. mycoides, B. weihenstephananensis*) were obtained from the Microbiology Section, Biological Sciences Department, Faculty of Science, Sana'a University. Bacterial isolates were subcultured on Nutrient agar (NA), which contained (g l^{-1}): Peptic digest of animal tissue, 5.00; Beef extract, 1.50; Yeast extract, 1.50; NaCl, 5.00; and agar, 15.00, pH 7. The medium was sterilized by autoclaving at 121º C and 1.5 bar for 30 min. and maintained on an NA slant at 4º C until further use.

2.2. Preparation of stock solution of heavy metal salts

The heavy metal salts employed in this study were: Cu (vi) copper tetraoxosulphate (aquatic) salt ($CuSO_4.5H_2O$), Cd (vi) cadmium tetraoxosulphate (aquatic) salt ($CdSO_4.8H_2O$), Co (vi) cobalt tetraoxosulphate (aquatic) salt ($CoSO_4.7H_2O$), Fe (vi) iron tetraoxosulfate (aquatic) salt ($FeSO_4.7H_2O$), Hg (vi) mercuric chloride salt ($HgCl_2$), Mn (vi) manganese chloride (aquatic) salt, ($MnCl_2.4H_2O$), Zn (vi) zinc tetraoxosulfate (aquatic) salt ($ZnSO_4.7H_2O$), Pb (VI) lead to two moles of nitrate salt $Pb(NO_3)_2$. The heavy metal salts that gave 1 g of each of the respective heavy metals (metal without the salt) were weighed and dissolved in 1000 ml as described by Odokuma, and Akponah [35].

2.3. Preparation of different concentrations of heavy metals

Various heavy metal concentrations (75, 125, 250, 500, and 750 µg ml^{-1} of each heavy metal salt were prepared from the stock solution (1 g equivalent of heavy metal in 1000 ml of deionized water), kept in dark containers, sterilized in an autoclave (121º C for 15 min), and refrigerated at 4º C for further study.

2.4. Effect of heavy metal ions on the tested microorganisms

The effect of heavy metals on the four isolates of *Bacillus* spp. was tested by streaking on NA plates and then incubating at 28ºC for 24-72 hrs. using the E-test method with modification,s and the inhibition zones of different concentrations of heavy metals were measured in mm [36].

3. RESULTS

3.1. Effect of Cobalt on the growth of *Bacillus spp.*

The results in Figure 1 showed that B. *atrophaeus* was the most affected by different concentrations of Cobalt, with a 180 mm inhibition zone at 750 µg ml^{-1} followed by 160 mm at 500 µg ml^{-1}, whereas B. *mycoids* was the most resistant to Cobalt, with a 120 mm inhibition zone at concentrations of 500 and 750 µg ml^{-1}.

3.2. Effect of Cadmium (Cd) on the growth of *Bacillus spp.*

B. atrophaeus was the most sensitive to different concentrations of Cd, with even the lowest concentration at 75 µg ml^{-1} has an inhibition zone of 80 mm. This

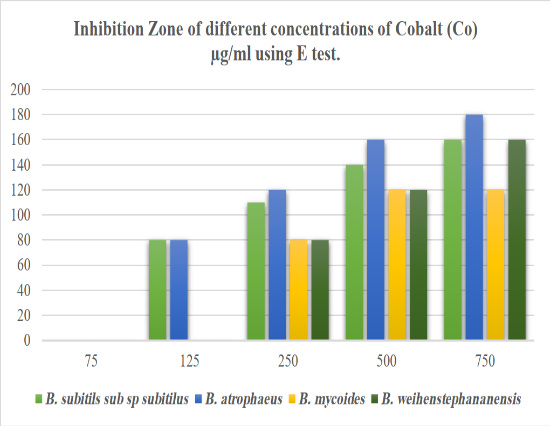

Figure 1. Effect of Cobalt (Co) on the growth of *Bacillus spp.*

result was observed even with B. *subitils* sub sp. *subtilis* which was sensitive to different concentrations of Cd, with the highest inhibition zone of 270 mm at 750 µg ml^{-1}. B. *mycoids* was the most resistant, followed by B. *weihenstephananesis* Figure 2.

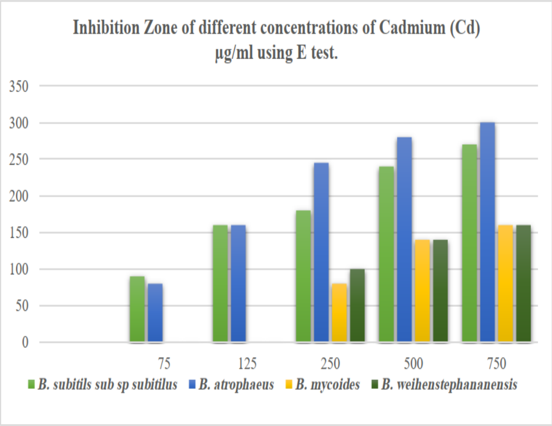

Figure 2. Effect of Cadmium (Cd) on the growth of *Bacillus spp.*

3.3. Effect of Manganese and Lead on the Growth of *Bacillus spp.*

Both Mn and nor Pb do not affected all *Bacillus spp.*, and all isolates grew even at the highest concentrations of both heavy metals.

3.4. Effect of Copper (Cu) on the growth of *Bacillus spp.*

The results in Figure 3 showed that B. *atrophaeus* was the most sensitive to Cu with an 80 mm inhibition zone at 250 µg ml^{-1} followed by B. *subitils* sub sp. *subtilis* with an 80 mm inhibition zone at 500 µg ml^{-1}. B. *mycoids* and B. *weihenstephananesis* were the most resistant to different concentrations of Cu with an 80 mm inhibition zone at the highest concentrations.

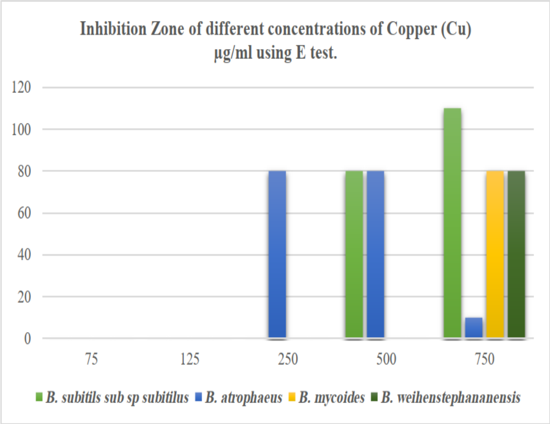

Figure 3. Effect of Copper (Cu) on the growth of *Bacillus spp.*

3.5. Effect of Mercury (Hg) on the growth of *Bacillus spp.*

Hg was the most effective heavy metal that inhibited the growth of all *Bacillus* spp. with the highest inhibition zone on B. *atrophaeus*, whereas B. *weihenstephananesis* was the most tolerant to the highest concentration of Hg (Figure 4).

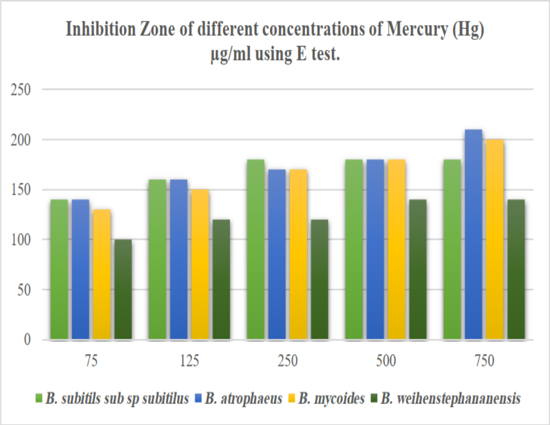

Figure 4. Effect of Mercury (Hg) on the growth of *Bacillus spp.*

3.6. Effect of Iron (Fe) on the growth of *Bacillus spp.*

The results in Figure 5 showed that B. subitils sub sp. *subtilis* and B. *mycoids* were sensitive to Fe at high concentrations with an 80 mm inhibition zone at 500 µg ml^{-1} followed by B. *atrophaeus* with 100 mm inhibition zone at 750 µg ml^{-1}, whereas *B. weihenstephananesis* was the most resistant one in all tested concentrations.

3.7. Effect of Zinc (Zn) on the growth of *Bacillus spp.*

B. atrophaeus was the most affected by different concentrations of Zn, with a 220 mm inhibition zone at 750

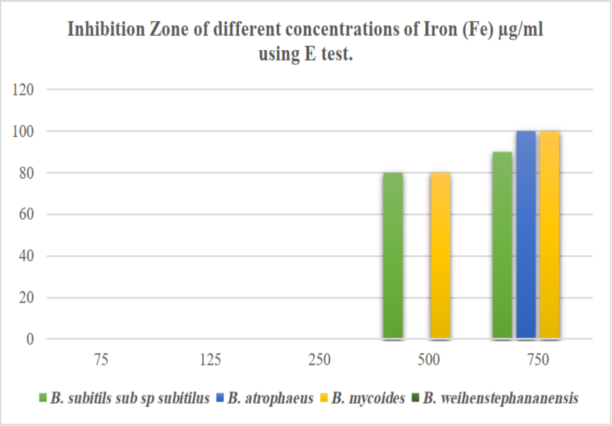

Figure 5. Effect of Iron (Fe) on the growth of *Bacillus spp.*

µg ml^{-1} followed by 160 mm at 500 µg ml^{-1}, whereas B. *mycoids* was the most resistant to Zn with an 80 mm inhibition zone at a concentration of 120 at 750 µg ml^{-1}. B. *weihenstephananesis* was the most tolerant to the highest concentration of Zn (Figure 6).

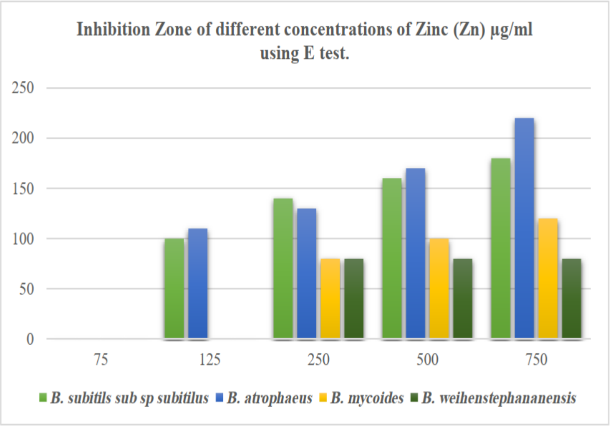

Figure 6. Effect of Zinc (Zn) on the growth of *Bacillus spp.*

4. DISCUSSION

Microbes have been used to remove heavy metals from the environment through various approaches, such as biosorption, oxidation and reduction, bioaccumulation, methylation, and demethylation [26–28]. Some bacteria have evolved mechanisms to control and respond to heavy metal uptake and accumulation. These mechanisms include the production and secretion of organic acids, polysaccharides, melanins, or proteins, and the subsequent binding/complexation and precipitation of metal ions. They also involve the chemical transformation of metals, metal binding to cell walls, transport of metal cations, organellar compartmentation, and synthesis of thiol-containing compounds such as glutathione, phytochelatins, and metallothionein proteins of families 8–13, which can sequester metal ions [37].

Bacteria play a significant role in modifying, activating, and detoxifying heavy metals in the environment; however, they may also be subjected to the toxic effects of metals. This is important for key processes such as biological waste treatment [38–40] and medicine [41].

This study showed that different strains of *Bacillus spp.* varying responses to different heavy metals concentrations. Among the tested strains, B. *atrophaeus* and B. *subtilils* sup sp. *subitils* were the most sensitive to the different concentrations of the heavy metals studied. The results in Figure 4 showed that B. *subtilils* sup sp. *subitils* exhibited tolerance to different concentrations of Hg, even at higher concentrations. This tolerance may be due to the presence of HgR genes, which are often associated with genes that confer resistance to antimicrobial drugs [42].

Another finding in this investigation was that Cd was the most effective heavy metal for all *Bacillus spp*. This result is in agreement with those of previous studies by Fashola et. al., [43] Imam et. al., [44] whose examined the tolerance of different *Bacillus spp.* to Cd, and Sizentcov et. al. [45] tested the biotoxicity of heavy metals on different strains of B. *subitilus.* In this study, B. *mycoids* and B. *weihenstephananesis* were more sensitive to heavy metals, including Co, Cd, Hg, and Zn, whereas they were more resistant to Pb, Mn, Fe, and Cu. This result is consistent with those of other studies [35] that recorded the tolerance of tested *Bacillus sp.* to the presence of certain metals. The mechanisms of resistance to metals in bacteria are sometimes encoded by plasmid genes, where the transfer of toxic metal resistance factors from one cell to another is facilitated [46, 47]. However, after plasmid curing, the *Bacillus sp.* became more sensitive to all the heavy metals. Other bacteria showed multiple metal resistances, and the mechanisms of resistance and genes involved are typically not common [48]. This heavy metal-resistant organism could be a potential agent in the bioremediation of heavy metal-polluted environments [49]. The results in Figure 3 and Figure 5 showed that most strains were resistant to different concentrations of Fe and Cu. This finding is in agreement with a previous report that B. *subtilis* can be grown in the presence of a wide range of metals, namely, zinc, copper, and iron, making it suitable for the remediation of heavy metals [50]. In addition, Gram-positive bacteria, especially *Bacillus spp.*, have a high adsorptive capacity to sorb heavy metals because of the high peptidoglycan and teichoic acid content in their cell walls. In the present study, B. *subtilis* showed greater tolerance to heavy metals, which may be due to the involvement of anionic surface groups in heavy metal binding [51].

5. CONCLUSION

Different tested isolates of *Bacillus* showed different degrees of response to the heavy metals. B. *mycoids* was the most tolerant isolate for most heavy metals tested in this study, followed by B. *weihenstephananesis* whereas B. *atrophaeus* and B. *subtilils* sup sp. *subitils* were the most sensitive ones. B. *subtilils* sup sp. *subitils* were tolerant to the high concentrations of Hg when they showed the same growth at different concentrations. All isolates showed tolerance to the different concentrations of Pb and Mn; these isolates need to be further investigated as they could be a good tool in bioremediation studies in case of environmental pollution with heavy metals.

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