Research Article

A Comparative Review on Potential Role of Environmental Species and Proven Probiotic Species of Genus *Bacillus* in Bioremediation of Heavy Metals with Special Emphasis on Chromium and Lead

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ABSTRACT

Industrialization has considerably increased the levels of soil, water and air pollution leading to heavy metal accumulation. These heavy metals are creating havocon all living organisms including humans. However, microorganisms have shown great tolerance to these heavy metals by evolving different mechanisms for adaptation to heavy metal stress conditions. They use strategies like biosorption, bioaccumulation, biotransformation and biomineralization for bioremediation of these metals. Species of the genus *Bacillus* are the most prevalent ones which exist in metal stress conditions in environment and are therefore used to remove heavy metals like chromium, lead, cadmium, nickel and zinc from industrial effluents. In terms of *in vivo* heavy metal detoxification in animal models various LABs (lactic acid bacteria) have shown great potential. LABs are lactic acid producing probiotic bacteria, which by the virtue of their extracellular polymers can bind to toxic metal cations with great specificity and eliminate them from the organism. Similarly, few probiotic sp. of genus *Bacillus* have exhibited their role in heavy metal bioremediation of industrial effluents including tanneries. Their potential to detoxify heavy metals *in vivo* can be greatly exploited using species of this genus like *B.clausii* and *B. coagulans* which are being used as commercial probiotics for treating some gastrointestinal disorders.

Keywords: *Bacillus*, Bioremediation, Heavy metals, Lactic Acid Bacteria, Probiotics.

INTRODUCTION

In recent years rapid industrialization has led to discharge of heavy metal containing effluents in soil and water especially in urban areas causing their accumulation in the environment. Although their depletion takes place continuously through processes like leaching, erosion and plant uptake, they cannot be degraded into nontoxic forms as they last in the ecosystem for very longtime causing great health concerns (Table 1). All heavy metals are not toxic as some are required, in very small amounts, for growth and optimum performance.[16]

Many conventional methods can be used to remove heavy metals including Cr (VI) and Pb (II) from industrial effluents. These include chemical precipitation, redox reactions, ion exchange, reverse osmosis and electrochemical treatment.[17] However, the efficiency of these processes get reduced as the range of heavy metals present in the effluents is in the range of 1-100mg/L.[18] As a result of this, the focus has shifted to bioremediation by using microorganisms surviving in the presence of Cr (VI) and Pb (II).

Various microorganisms belonging to different genera are also known to survive in and tolerate heavy metal stress conditions. In order to adapt themselves to heavy metal stress conditions, bacteria have evolved various mechanisms such as (i) metal ion efflux outside the cell (ii) its accumulation and complexation inside the cell (iii) its reduction to less toxic state (Fig. 1).[19-30]

Many of the species employed for bioremediation of these heavy metals are LABs.[31-35]LABs are lactic acid producing bacteria that are divided into four genera viz. *Lactococcus, Lactobacilli, Leuconostoc and Pediococcus. Bifidobacterium*, though phylogenetically unrelated to LABs, they produce lactic acid and are thus included under the category of LAB. LABs, many of which are administered as probiotics, include Gram positive cocci and rods which are present in nature as normal inhabitants of human and animal gut, mucous membrane and skin.[36, 37]

Lactobacillus, a common intestinal bacterial species also used as probiotic, has been reported to bind and detoxify heavy metals like Pb, Cr, As and Cd.[38-43]

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit to the host.[44] They are used as live microbial dietary supplements or food ingredients and have a beneficial effect on the host by influencing the composition and/ or metabolic activity of the flora of gastrointestinal tract. There are some genera of microorganisms in nature *e.g. Bacillus* which have the property of bioremediation of heavy metals, while their species have also shown probiotic efficacy.

TOXIC EFFECTS AND BIOREMEDIATION OF Cr (VI) AND Pb (II) BY *BACILLUS* SP.

Toxic Effects of Cr (VI)

The two stable valences of chromium in nature are Cr (VI) and Cr (III). Of these, Cr (VI) has high permeability in cells as compared to Cr (III). As a consequence, Cr (VI) is approximately 1000 times more toxic than Cr (III).[45,46] Inside the cells Cr (VI) reacts spontaneously with ascorbate and glutathione and generates free radicals, reactive oxygen species (ROS) and Cr (III).[47-49] These ROS combine with DNA-protein complexes and create mutagenic, carcinogenic and teratogenic effects.[50] Thus it can be concluded that Cr (VI) is highly toxic to humans while the effect of Cr (III) is relatively less.[51,52]

Microbial Bioremediation of Cr (VI)

Many studies have identified various bacterial species showing capacity of bioremediation of Cr (VI) from either contaminated soil or water. Amongst the bacteria identified, *Bacillus* sp. has shown a very high potential for reduction of toxic Cr (VI) to nontoxic Cr (III). It is because of this potential it has been used as a component of commercial biosorbent.[53]

Various researchers have successfully isolated such microorganisms from contaminated environments and have identified them as *Bacillus* sp. either by biochemical characterization[54] or by 16sr DNA sequencing.[55,56] In a study, a Gram-positive Cr- resistant isolate showed potential capacity for reduction of Cr (VI) concentration in aqueous medium. This isolate reduced 87% of Cr (VI) after 72 hrs, as estimated by Diphenyl Carbazide method.[57] However, as the concentration of Cr (VI) was increased, its capacity for reduction of Cr (VI) was reduced.[54]Researchers concluded from these findings that in order to grow in Cr (VI) rich environment microorganisms either reduce or accumulate Cr ion in its cell.[58,59] Other researchers performed similar studies on various contaminated environment and have identified various *Bacillus* sp. that are able to grow in and reduce Cr (VI). They have also reported similar findings and have confirmed this results.[55, 60, 61]

Mechanism of Bioremediation of Cr (VI)

In the presence of oxygen, microbial reduction of Cr (VI) is commonly catalyzed by soluble enzymes. Microbes are known to catalyze reduction of Cr (VI) to Cr (III) and its subsequent precipitation[62,63] which was first reported in a *Pseudomonas* sp.[64] Since then various species of genus *Bacillus* have been reported to detoxify chromium using this mechanism. This catalytic protein is induced in the presence of chromium and has a molecular weight of about 25kDa.[61] This protein has been identified as enzyme chromate reductase.[64, 65] A study showed that even the cell free extract exhibited chromate reductase activity which increased by 2.2 times after $(NH_4)_2SO_4$ fractionation.[55] The optimum pH and temperature of the enzyme were observed as 6 and 30°C, respectively.

Heavy metals impose a selective pressure leading to the formation of a metal resistant population. This population could result from (i) vertical gene transfer (reproduction) (ii) horizontal gene transfer by transposons and plasmids and (iii) selection pressure on spontaneous mutants.[66] The bacterial strains capable of expressing both the resistance and the reduction of chromate are very useful for bioremediation. Under heavy metal stress the population adapts faster by spread of R factors than by mutation or natural selection.[67-70] A group of researchers observed that *B. cereus*, which was isolated from diesel polluted soil, showed resistance to both, heavy metals i.e. Cr (VI) and antibiotics especially penicillin and cephalosporins, indicating that heavy metal resistance is present together with antibiotic resistance.[71]

However, in a work using laboratory procured *L. acidophilus, L. rhamnosus and L. casei*, found that these species did not acquire resistance against various antibiotics upon chronic Cr (VI) exposure.[72]

Toxic Effects of Pb (II)

Natural as well as anthropogenic activities are responsible for increase in Pb (II) concentration in the environment. Natural processes like soil erosion and volcanic emissions contribute a small percentage to environmental Pb (II) concentrations[73-75] while in reality the main sources are manmade i.e. activities of industries like metal smelting, battery and paint industries.

Several references indicate the toxic effects of lead leading to neurodegenerative diseases, reproductive disorders as well as renal failures in humans.[76, 77] Prolonged exposure of lead leads to anaemia, cancer and impairment of vitamin D metabolism. If blood level of lead goes beyond 70 µg/dl, it causes coma and even death.[78-80]

Pb (II) has also been shown to affect cellular functioning at molecular level causing conformational changes of protein and nucleic acids, inhibition of enzyme activity and disruption of membrane function.[81, 82]

Microbial Bioremediation of Pb (II)

Inspite of the high toxicity of Pb (II), microorganisms especially those belonging to the genera *Bacillus*, are able to survive in high concentration of Pb (II).[83] Researchers have isolated Pb (II) resistant *Bacillus* sp. from effluents of various industries, identified them by either 16s rDNA sequencing method[84] or by morphological and biochemical characterization.[85,86]

A pure culture of *B. subtilis* was tested for its biosorption capacity using 600, 700 and 800 ppm Pb (II) every 12hrs for 72 hrs.[87] It was observed that as the concentration of Pb (II) increased, the rate of % absorption decreased. This was attributed to the toxicity of Pb (II) which caused a decrease in number of cells during exponential phase of bacterial growth. Various factors also affected the rate of biosorption of Pb (II). Maximum biosorption was observed at pH 4.5, at optimum temperature of 40°C and at 60rpm.

Isolates have been isolated from heavy metal rich sediments of Guilan province in Iran and were identified by 16s rDNA sequencing.[66] The organisms were identified as those belonging to *Bacillus* genus i.e. *B. licheniformis*, B. cereus, *B. amyloliquefacians* and *B. subtilis* and showed good potential for bioremediation of heavy metals such as copper, cadmium, zinc, lead and chromium.

A similar result was obtained for B. cereus isolated from various industrial effluents. The organism showed maximum biosorption of Pb (II) at a concentration of 100mg/L at 30°C and at pH 5.0.[88] These factors are important as they affect the physiochemistry of the cell wall, which is the main site of for biosorption of Pb (II).

Different species of genus *Bacillus* e.g. *B. subtilis, B. carotarum, B. lentis* and *B.licheniformis* have shown similar results, indicating that this genus possesses potential bioremediation capacity for Pb (II).[89-96]

Mechanism of Bioremediation of Pb (II)

Different mechanisms are involved in the bioremediation of Pb (II). The mechanism which is used by *Bacillus* sp. is biosorption. Biosorption is a process by which biological materials accumulate heavy metals on their surface and is based on their metal binding capacities of various biological materials.[97] It is a passive and non-metabolic process by which microorganisms passively concentrate and bind constituents onto its cellular structure.[98] This is a complexing process in which both living and dead cells are involved.[97,99] Biosorption of heavy metals is a phenomenon which involves mechanisms like ion exchange, adsorption, chelation and microprecipitation.[37]

In a study, biomass of *B. subtilis* was prepared by lyophilization and portions of this were used for determination of biosorption capacity of the organism for Pb (II). The results suggest that *B. subtilis* showed high biosorption potential. This was attributed to the thick peptidoglycan cell wall of *Bacillus* genera which is characteristic of Gram positive bacteria.[100] Apart from peptidoglycan the cell wall contains other components like teichoic acid and S layer. These components create a net negative surface charge which leads to electrostatic attraction between positive metal ions and their negatively charged binding sites. Thus, Pb (II) was removed from waste water by binding to the external surfaces of the cell.[101, 102]

The role of these sites in biosorption of heavy metals was further corroborated by extracting the phosphodiester groups of teichoic acid and reducing the number of carboxyl groups, which lead to a decrease in metal ion uptake by isolated *B. subtilis* cell walls.[19] Another study reported that, as compared to other heavy metals under study, Pb (II) was maximally adsorbed by *B. licheniformis* NSPA5, *B. cereus* NSPA8 and *B.subtilis* NSPA13. SEM-EDS (Scanning Electron Microscopy-Energy Dispersive Spectroscopy) analysis also confirmed that the mechanism of bioremediation of Pb (II) by *Bacillus* sp. is through biosorption.[96] These findings were in corroboration to the results obtained by earlier conducted studies.[103, 104.]

Studies have been conducted to determine the effects of different environmental conditions such as pH, incubation temperature and biomass for growth and metal uptake ability of microorganisms under heavy metal stress. As most of the surface moieties of *Bacillus* sp. are ionic in nature, the charge on the cell wall is affected by pH. At lower pH the binding capacity of cell wall for Pb (II) is more. Maximum absorption of Pb (II) by *Bacillus* sp. was reported at pH 5.0[105] while similar results were obtained by various researchers.[100, 106-108]

Temperature is another important factor affecting heavy metal biosorption.[109] Increase in percentage biosorption of heavy metals was reported from 25-40°C.[110] Similar study conducted on the effect of various incubation temperature showed that for *B. cereus* optimum temperature for maximum Pb (II) uptake is 30°C.

Increase in biomass is an important factor which affects rate of biosorption of heavy metals. This can be attributed to the number of functional groups involved in biosorption, present on active sites on microbial cell wall. A study was performed in which it was observed that with increase in bacterial concentration of *L. rhamnosus* GG there was increase in Cd and Pb binding by the microorganism.[41] Work done by other researchers have validated these findings.[111,112]

ROLE OF LACTIC ACID BACTERIA (LABS) IN BIOREMEDIATION OF Cr (VI) AND Pb (II) *IN VIVO*

LABs are natural inhabitants of different parts of humans and survive in the host by showing symbiotic association with the mucosal membranes of gastrointestinal tract and skin[37]. LABs are a large group of beneficial bacteria which are non-toxic and fermentative in nature.[113] They are called so as they produce lactic acid as end product of fermentation of sugars.[114,115] The beneficial properties of LABs are largely due to their ability to bind and adhere to mucous membranes.[116] It is due to the above properties that LABs including *Lactobacillus* and *Bifidobacterium* are used as probiotics.[117,118] LABs are beneficial to humans as they stimulate the immune system, help in preventing or reducing diarrhea and lactose tolerance[119] *Lactobacilli* also exhibit probiotic properties, i.e. they can survive at pH 2-3 and are bile tolerant. They can inhibit pathogens by producing inhibitory compounds by competing with them for energy and adhesion sites response.[120-122]

Mechanism of Bioremediation by LABs

Although LABs is a group of heterogenous bacteria belonging to different genera e.g. *Lactobacillus*, *Leuconostoc, Pediococcus, Lacctococcus* etc., their cell wall is typically of Gram positive bacteria. Its main components are peptidoglycan, teichoic acid and some exopolysaccharides.[123] Many micro-organisms synthesize extracellular polymers (EPs) that bind cations of toxic metals, thus protecting metal-sensitive and essential cellular components.[82] The composition of EPs is very complex, including proteins, humic acids, polysaccharides and nucleic acids, which chelate metals with different specificity and affinity.[124-126] Pb(II) binding by EPs has been reported for *Bacillus firmus*.[126] This EP exhibits a characteristic feature, namely a high content of uronic acids (28.29%), which are considered to play an important role in its Pb(II) binding specificity. These exopolysaccharides contain glucose, galactose, rhamnose, N-acetylglucosamine and N-acetylgalactosamine.[128] Thus the charge on cell wall is net negative charge due to functional groups of these components. This has been supported by electrophoretic studies at neutral pH.[39]

A study suggested the role of metallothionein like protein in Pb (II) binding in *Bacillus megaterium*.[124] Metallothioneins were first discovered in *Synechococcus* PCC 7942. They are primarily proteins with a role in Zn homeostasis and protecting the cell against Zn toxicity.[129]

Microbial Bioremediation of Cr (VI) by LABs

It was observed that pure cultures *of L. acidophilus, L. rhamnosus* and *L. casei* were able to reduce the concentration of Cr (VI) under in vitro conditions. Enzyme chromate reductase was purified from these strains indicating that the mechanism of chromium bioremediation is likely through the reduction of Cr (VI) to Cr (III).[72] This was further supported by the findings that total chromium concentration remained constant in the media and indicates possible existence of efflux pump.[55] Gut LABs were also found to reduce Cr (VI) concentration *in vivo* too, acting as the first line of defense by rapidly converting Cr (VI) to Cr (III).[130] When a group of mice was stressed with chromium, *Pseudomonas* sp. isolated from caecum of mice showed better tolerance to chromium as compared to *Lactobacillus* and *E. coli,* which too were isolated from rats.[131] However, if the rats were continually stressed with Cr (VI), the tolerance of these microorganisms increased overtime, providing better resistance to chromium toxicity.[43] Researchers have reported that human fecal and gut *Lactobacilli* perform similar functions; thus highlighting the importance of dead LABs in sequestering chromium from gut, just like *Bacillus* sp. in environment.[132-134]

Microbial Bioremediation of Pb (II) by LABs

Reports suggest that probiotic LABs have the capacity to bind heavy metals from water.[135] This feature of LAB was further studied by using three species of genus Lacto *Bacillus* I.e. *L. rhamnosus* GC, *L. casei* Shirota *and L. fermentum* ME3 and three species of genus *Bifidobacterium* i.e. B*. longum* 2C*, B. longum* 46 *and B. lactis* Bb12.[41] These LABs were found most efficient in binding Pb (II) at pH 5.0 where up to 97% of initial Pb (II) was removed within 1 hr. This fast uptake suggests that the primary method for removal of Pb (II) by LABs is biosorption rather than bioaccumulation.

Ion exchange is one of the mechanisms employed by LABs in metal biosorption. This mechanism is affected by pH; lower the pH more will be biosorption due to increase in negatively charged binding sites on cell surface.[136] As biosorption is a surface phenomenon, it is also affected by biomass since increase in biomass means more binding sites available for removal for heavy metals.

Another interesting observation was made regarding sequestration of Pb (II) by the LABs in the study. Even after 48 hrs the metal ions did not dissociate, indicating strong binding of the metal ions with the cells. A study was performed on another LAB, *Lactobacillus* kefir strains CIDCA 8348 and JCM 5818 and it was found that the strains bind Pb (II) in the S-layer, which undergoes changes in conformation and secondary structure after metal precipitation. This was confirmed by Fourier Transform Infrared Spectroscopy (FTIR).[137] Rapid binding of Pb (II) was also observed in *L. rhamnosus* LC-705 and *Propionibacterium freundenreichii* which was pH dependent.[138] Similar observation was made in *B. subtilis*.[139] LABs like *L. rhamnosus* GG and *B. longum* produce exopolysaccharide and have higher percentage of negatively charged groups, thus leading to increased capacity of Pb (II) binding.[140,141] These complexes do not remain for long in the gut and get eliminated upon defaecation,[35] further reducing the toxicity of lead ions.

POTENTIAL OF PROBIOTIC SPECIES OF GENUS *BACILLUS* IN BIOREMEDIATION OF Cr (VI) AND Pb (II)

Among bacteria, those belonging to genus *Bacillus* are most widespread in nature. They are omnipresent they are present predominantly in soil[142] followed by water[143] and air[144], through which they find their way to food products. *Bacillus* sp. are spore formers; hence they can survive in extreme procedures such as baking[145] and canning. Therefore, they are also found in gastrointestinal tract and respiratory tract of healthy adults[146] and children.[147] These strains have also shown some properties like resistance to acid and bile allowing them to remain viable in gut.[148, 149] Thus researchers focused their studies on establishing strains of *Bacillus* sp. as probiotic organisms. For an organism to be considered as probiotic it must exhibit certain characteristics such as acid and bile tolerance, adherence, production of enzymes which support digestive function of gut and production of antibiotics. Many *Bacillus* sp. have been reported to possess these properties. As many as 795 antibiotics have been identified by some researchers from *Bacillus* sp,[150] while others have found them to produce amylolytic[151], pectinolytic,[152] lipolytic, cellulytic[153,154] and proteolytic enzymes.[155] At the same time there has been no report of infectivity of *Bacillus* sp. from environment except *B. anthracis* and *B. cereus*.[145] However, every *Bacillus* strain must be fully tested before declaring it as probiotic.

Recently many *Bacillus* sp. have been declared as probiotic and approved for human use. *B.clausii*, *B. cereus* and *B. coagulans* have been medically accepted in various countries. After clinical trials, *B. coagulans* was found to be effective in the treatment of diarrhea and dysbiosis, [156] while *B. clausii* has shown to have anti diarrhoeal activity.[157]

Another characteristic feature of a probiotic organism is the residence time in the gut after ingestion. Most probiotic organisms survive for small time period in gut of the organism after which they are eliminated, if not regularly ingested. A group of researchers observed that the spores of *B. clausii* were reduced to negligible numbers in ileum, colon and faeces after 1 week of ingestion while vegetative cells were not isolated after 72 hrs.[158] These results were also reported in other studies.[159] This allows control over amount of bacteria present in gut and reduces incidence of ill effects due to horizontal and vertical gene transfer. These findings, along with earlier reports that *Bacillus* sp. are efficient in removing heavy metals including lead and chromium from the environment, suggesting that the probiotic strains of this genus have the potential to reduce the toxicity of Pb (II) and Cr (VI) from humans.

Role of a probiotic species, *B. coagulans*, in biosorption of Cr (VI) has been well documented. This species was originally isolated and described as Lacto*Bacillus* sporogenes, [160] which was later reclassified as *Bacillus* sporogenes, that is evidenced to share some characteristics of *B. coagulans* and therefore, the species has been moved into *B. coagulans* group. In a study, three strains of *Bacillus* genus were isolated; one of which was *B. coagulans*. Its live and dead cells biosorbed 23.8 and 39.9 mg Cr/ g dry weight respectively from tannery effluent, indicating that the dead cells showed better biosorption than the living cells. The reason for this was that dead cells were conditioned to acidic pH by treating them with deionized water acidified with H_2SO_4 at pH 2.5.133 In continuation to this work in 2003, immobilized *B. coagulans* biomass was used for biosorption in treating tannery effluents ascertaining that immobilized B. coagulans retains the ability to biosorb Cr (VI) even on different matrices. This biosorption was found best on agarose matrix where it remains stable and retains its integrity in acidic pH.[161] This finding confirmed the results of earlier reports.[162]

Additional studies have provided compelling evidence showing the role of other probiotic sp. of genus *Bacillus* i.e. *B. clausii* in heavy metal adsorption. This study investigated the role of *B. clausii* S-4 as a biosorbent for Zn ions in aqueous solution. The study was aimed at finding the optimum pH value for biosorption at different temperatures. The results showed maximum biosorption of 57.5 mg/g by *B. clausii* S-4 at temperature of 40oC and pH 4.5. These results were also confirmed by FTIR and Energy Dispersive X-ray (EDX) analysis. Thus, the study concluded that *B. clausii* S-4 biomass had a great potential in the removal of Zn ions and hence can be used for wastewater treatment.[163] In 2011, a multiple heavy metal resistant *B. clausii* was isolated establishing high resistance against various heavy metals including Pb (II).[164] These studies emphasized the role of *B. clausii* in heavy metal bioremediation and since it is a proven probiotic it is believed to remove heavy metal from gut of an organism.

Researchers conducted a study emphasizing the importance of probiotic species of genus *Bacillus* in excreting toxic heavy metals. They reported self-eliminating antagonistic behavior of these species as well as their antibiotic influence that actively excreted heavy metals from the organism. Their study was done using three commercial probiotic preparations namely Sporobacterin containing *B. subtilis* 534, Biosporin which has*B. subtilis* and *B. licheniformis* and *Bactisubtil* containing B. cereus IP 5832. The experiments were conducted under Zn and Pb stress on rat models. *B. subtilis* i.e. Sporobacterin showed highest accumulation for both the heavy metals i.e. 58.9% reduction of Pb (II) and 60.5% reduction of Zn (II) as compared to other commercial probiotic supplemented groups. Their results indicate that probiotic *B. subtilis* 534 has a better potential to reduce heavy metal concentration in vivo as compared to other probiotic strains studied.[165]

CONCLUSION

As a side effect of industrialization heavy metals have become an integral part of our ecosystem. They are continuously entering the food chain via air, food and water, resulting in their accumulation leading to various disorders. *Bacillus* species play a prominent role in bioremediation of heavy metals in the environment. Many LABs have been proven to reduce the concentration of heavy metals *in vivo*. In view of this the current review focuses on potential use of probiotic bacteria of genus *Bacillus* for detoxification of heavy metals, emphasizing on bioremediation of chromium and lead, *in vivo*.

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Table 1: Source and effect of heavy metals on humans

Heavy metal	Source	Effect
Lead (Pb)	Auto exhaust, hair dyes, paints, vermilion	Loss of coordination and concentration, infertility and memory loss[1]
Chromium (Cr)	Electroplating, resistant alloys e.g. Stainless steel, tanneries and dye production	High toxicity, mutagenicity, carcinogenicity, nasal irritation and ulcers, neural, respiratory and dermal toxicity, malformation and fetal death[2-9]
Mercury (Hg)	Dental amalgams, laxatives, tattooing	Headache, irritability, fatigue, confusion, insomnia, behavioral disorders, tremor, polyneuropathy, reduction or loss of hearing and vision[10]
Nickel (Ni)	Stainless steel cookware, tea, tobacco	Disruption of hormone and lipid metabolism, lipid dysfunction and intestinal cancer[11]
Cadmium (Cd)	Ni-Cd batteries, plating, pigments and plastics production, phosphate fertilizers	Carcinogenic, mutagenic, endocrine disruptor, lung damage and fragile bones, affects calcium regulation in biological systems[12-13]
Zinc (Zn)	Marine antifouling coatings, anticorrosion paints, glow in the dark products	Dizziness, fatigue[14]
Arsenic (As)	Mining, coal burning, arsenic pesticides	Affects essential cellular processes such as oxidative phosphorylation and ATP synthesis[15]

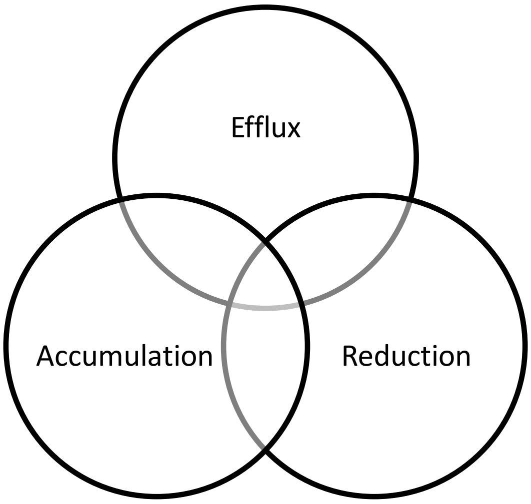

Figure 1: Mechanisms Adapted by Microorganisms to Survive in and Tolerate Heavy Metal Stress

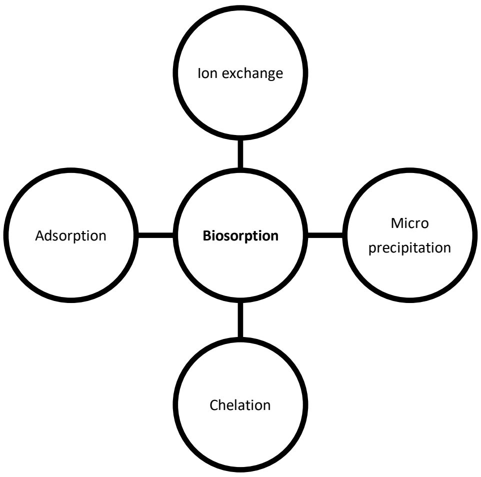

Figure 2: Mechanism of Biosorption by *Bacillus* sp.

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