Chapter

Trends in Heavy Metals Tolerance and Uptake by *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is considered as the most potent bacterial strain for solving heavy metals pollution problems. Pollution is the most of problems in our world which causing a lot of risks to human, animal, plant and ecosystem. Heavy metals pollution is an ever-increasing problem in developing nations. Release of heavy metals into the environment has increased in the recent years at an alarming rate. To remove heavy metals from environment, there are different methods such as physical, chemical and biological. The biological method includes microorganisms and plant which recorded high heavy metals removal, safe and low-cost method. Microorganisms remove heavy metals from environment by different mechanisms according to their types. Thus, microbes are used as potential candidates of bioremediation that can adapt quickly to the changing noxious environment and be utilized for toxic metal remediation. In bacterial uptake and tolerance to heavy metals, *Pseudomonas aeruginosa* recorded potential role in bioremediation of different heavy metals with high removal percentage comparison with other bacterial strains. Chapter discusses the roles and trends of *Pseudomonas aeruginosa* in heavy metals tolerance and uptake as potential bacterial strain.

Keywords: pollution, *Pseudomonas aeruginosa*, heavy metals, bioremediation, tolerance, plasmid and resistant genes

1. Introduction

Heavy metals are wide distribution elements in environment have a high density, atomic weight and multiple applications in different fields [1]. Heavy metal pollution causes environmental problems to human, animal, plant and ecosystem [2]. This pollution resulted in accumulation of different heavy metals in to soil and water. Different heavy metals such as copper, cadmium, nickel, lead, chrome and mercury, etc. released into natural sources and recorded high accumulation then have toxic effects on human health and biological system [3].

To remove of this heavy metals, there are different biotechnological methods were done such as bio-mineralization, bio-sorption, phyto-stabilization, hyper-accumulation, bio-stimulation, rhizo-remediation, myco-remediation, cyano-remediation and geno-remediation [4].

In nature, microorganisms play a potential role for a recycling and degradation of accumulated heavy metals which decrease their toxicity. This microorganisms as fungi, bacteria and algae were recognized [5]. Also, bioremediation of heavy metals by microorganisms is an economic and effective strategy because of its high efficiency, low cost and eco-friendly nature. Additionally, microbial bioremediation is done by interaction of microbe-metal for accumulation and detoxification of heavy metals [6].

Subsequently, some of bacterial strains can remove heavy metals from polluted soils as *Pseudomonas* sp., *Arthrobacter* sp., *Alcaligenes* sp., *Corynebacterium* sp., *Bacillus* sp., *Flavobacterium* sp., *Mycobacterium* sp., *Azotobacter* sp., *Rhodococcus* sp. and *Methanogens* [7]. Bacterial strains have different mechanisms to adapt and remove of heavy metals from polluted environments [8]. Different mechanisms are responsible for adapted of bacterial strains to grow at high concentrations from heavy metal. Also, mechanisms include accumulation and uptake, sorption of metal, enzymatic reduction or oxidation and extracellular precipitation. Additionally, metal tolerance was done by molecular mechanisms by having resistant genes [9]. Heavy metals resistant bacterial strains able to grow under exposing to high concentrations and have potentiality in bioremediation of high content of heavy metals in soils [10]. Finally, *Pseudomonas aeruginosa* tolerated and removal the highest dose of heavy this metals as compared with different bacterial strains [11].

2. Problems and risks of heavy metals on environment

2.1 Heavy metals pollution sources

Firstly, pollution of environment is a huge problem in the world because of increasing of industrial activities resulting the toxic compounds which lead to contamination of soils and clean water [12]. Toxic heavy metals pollution is a wide distribution throughout world countries along with progress of different industries which chromium, copper, nickel cadmium and mercury were observed as the most common heavy metals that widespread and used causing environmental pollution [13]. Increased industrial activity and demand for heavy metals like arsenic, nickel, chrome, copper, lead, mercury, manganese, zinc cadmium and many more has recorded increasing in amounts of heavy metals at polluted wastewater [14]. Additionally, metallurgical and mining industries are generated high amounts of heavy metals in wastewaters and accumulate in soil and water. [15]. Also, agricultural activities using pesticides, fertilizers and crop preservatives which participate in releasing of heavy metals in to the environment [16].

2.2 Heavy metals effects on living organisms

Heavy metals are any inorganic metallic compound that can exert their toxicity via binding to the thiol group of the enzyme and the disulfide bond that contribute the stability of the enzyme. The metals have high affinity to the disulfide bridge between two cysteine residues in any protein compound. Heavy metals are very dangerous to living organisms especially human as certain of them cause DNA damage and their carcinogenic effects in animals and man are probably causally related to their abilities to cause mutation [17]. Also, heavy metals divided into nonessential metals (lead, cadmium, mercury and nickel) and essential metals (zinc, copper, iron and manganese). Because of their high toxicity, lead and cadmium represented the major heavy metals pollutants. Cadmium is released to ecosystem during electroplating, effluents from textile, mine tailing, tannery, leather, and galvanizing industries (cadmium batteries) [18]. Due to their high prevalence as contaminants, heavy metals have an excellent concern to environmental problems in soluble type that area unit terribly venomous to biological systems causing cancer [19]. Also, heavy metal compounds in soluble forms might be harmful for living organisms of ecosystem by entering of the food chain [16].

In 2015, ATSDR prepared the priority list of hazardous substances and the results recorded that cadmium and lead were in the seventh position and second position, respectively. Also, The International Agency for Research on Cancer classified cadmium and cadmium compounds as a group (1) carcinogen and lead compounds as group (2) carcinogens [20]. Additionally, cadmium cause renal dysfunction especially in the proximal tubular cells which considered as the main site accumulation of cadmium. Also, cadmium cause demineralization of bone either indirectly, as a result of renal dysfunction or directly by the bones damaging. Furthermore, nickel has carcinogenic, neurotoxic, hemato-toxic, reproductive toxic, immune-toxic, genotoxic, nephrotoxic, pulmonary toxic and hepatotoxic effects [21]. On the other hand, mercury cause risks effects as neurotoxic substance because it accumulates and increase its content in food chains [22].

Subsequently, if plants exposure to excess copper concentrations, it generates oxidative stress causing damage to macromolecules and metabolic pathways disturbance. Also, excessive manganese accumulates in leaves and causing photosynthetic rate reduction [23]. Plants can uptake high concentration of heavy metals that present in causing adversely effect of symbiosis, the growth and consequently effects on the crops yields [24].

In addition, chromium (hexavalent form) is the most toxic chromium species which used in some industries as leather processing. Chromium is a carcinogenic substance especially in case of the lung and enter through inhalation. Chromium toxicity comes from its potentiality to cause allergic reactions and be corrosive [25].

Furthermore, heavy metals effect on microorganisms and induced physiological and morphological changes in microbial populations [26]. When microorganisms exposed to heavy metal stress, they produce antioxidant enzymes under toxic conditions and tolerance of this stress such as some resistant bacterial strains [27]. In naturally, microbes response to toxicity of heavy metals depends on high concentration and its resistance and tolerance mechanisms [28].

3. Bioremediation of heavy metals by microorganisms

Bioremediation meaning use of microbial metabolism to remove of pollutants. It can occur on its own and this called intrinsic bioremediation or can be done by addition of fertilizers to stimulate of microbial bioavailability inside medium and this called bio stimulation. Also, in some cases the addition of other microbial strains into medium to enhance the resident microbial population's and increase their ability to remove of heavy metals. Microorganisms that used to perform this function of bioremediation known as bio-remediators [29]. These microorganisms have developed unique resistance mechanisms which allowing to survive and remove high concentrations of heavy metals from environments [30]. Subsequently, bioremediation considered as alternative to chemical techniques by using microorganisms for biodegrading and detoxify of heavy metals from polluted soils and wasted groundwater [31].

In addition, biosorption defined as the use of biomass to remove heavy metals from environment by using microorganisms as (bacterial strains, fungal strains and algae) or plant extracts. It represents as a low-cost method and environmentally friendly for bioremediation of heavy metals and management of resource [32]. The need for an efficient and inexpensive method has interested in case of bio-sorption and bio-accumulation processes using microorganisms as profit systems for removing of heavy metal [14].

Fungal and bacterial strains have been reported to remove high concentrations of heavy metals from polluted environment using biosorption and bioaccumulation techniques [33]. Bioaccumulation is a process which involves two aspects; active metal uptake and passive metal uptake and may be carried out by any living organism with the ability to withstand the toxic effects of a particular metal ion [34]. Additionally, utilization of potential microbial populations in biosorption process to transform or adsorb heavy metals either by live and dead biomass or by their products have produced to for detoxify of heavy metals forms whether in particulates or as soluble form. Negative charged of microbial cell surface as a result of the presence of different functional groups such as hydroxyl, amines, carboxylic and phenolics give microorganisms an ability for binding with different cationic heavy metals [35].

As above, microbial strains have different mechanisms for reducing the toxicity of heavy metals through its intracellular and extracellular precipitation, binding of elements to cell wall, adsorption on polysaccharides or by export via various transporters [36]. Also, in wide variety of bacterial strains especially in genus "*Pseudomonas*" resistance to heavy metals, disinfectants, antibiotics, detergents and different toxic substances were observed. *Pseudomonas* considered as one of the most indicators bacterial strains for measuring contamination in environment [37, 38].

4. *Pseudomonas aeruginosa* as the most potent bacterial strain for tolerating and uptake heavy metals

4.1 Bacterial community and *Pseudomonas* classification

Bacteria are microorganism play important role in living world. It represents approximately 10^8 g of the total living world biomass. They used as bio sorbent because their ubiquity, small size, and ability to grow under different conditions such as *Pseudomonas*, *Bacillus*, *Escherichia*, *Micrococcus*, and *Streptomyces* species and used for bioremediation of heavy metals by using functional groups and metal chelating agents present on cell wall to make metal binding [35].

In addition, *Pseudomonas aeruginosa* classified as Gram negative bacteria, Gamma Proteobacteria, aerobic, rod and belonging to family *Pseudomonadaceae* which tolerate some heavy metals such as copper, chromium, cadmium and nickel [39]. Also, it is tolerant to different physical conditions and resistant to high concentrations from most of heavy metals, dyes, salts, weak antiseptics and antibiotics [40]. Several studies reported that *Pseudomonas aeruginosa* has efficiency for metal uptake which biosorption of cadmium (II) and lead (II) ions from solution using lyophilized *P. aeruginosa* (PAO1) cells were observed under different conditions [41].

4.2 Tolerance and resistance mechanisms by *Pseudomonas aeruginosa*

Pseudomonas aeruginosa has three different mechanisms for resistance of heavy metals: Firstly, accumulation of specific ion can be diminishing not by interference with uptake but by using of the heavy metal ion active extrusion from cells and this mechanism is only specific for *Pseudomonas aeruginosa*. Secondly, cations especially the "Sulfur lovers" can be segregated in to complex compounds by thiol-containing molecules and then ejected from the cell. Thirdly, some metal ions could also be reduced to a less deadly aerophilic state by the complicated enzymes and special oxidization mechanisms within the cells. Finally, for many metals, resistance and homoeostasis is a combination of two or three of the mentioned basic mechanisms that is the case which *Pseudomonas aeruginosa* success. *Pseudomonas aeruginosa* produce an extracellular compound with yellowish green fluorescence, called Pyoverdin, which functions as a byproduct. The production of Pyoverdin, formerly called fluorescein, is concomitant with the production of another byproduct, Pyochelin and produce other types of soluble pigments, the blue pigment pyocyanin [40].

Additionally, *Pseudomonas aeruginosa,* yet as different metal-tolerant bacterium, develop varied detoxification and/or tolerance mechanisms, such metal reduction, precipitation as metal salts, animate thing sequestration, binding to metallothioneins and therefore the removal of excessive metal ions out of the cell by transport (efflux pump). Removal of excessive metal ions out of the cell by flow pump is achieved by varied proteins driven by ATP chemical reaction (ATPases) and ion diffusion transporter that acts as chemiosmotic ion-proton money dealer and therefore the Resistance Nodulation Division (RND) transporters that mediate nucleon driven flow [42]. Many mechanisms are evolved to resist metal uptake. These embrace the discharge of metal outside the microbial cell, metal storage within the microbial cell and reduction of virulent metal to fewer virulent forms [43].

4.3 Calculation of removal percentage by *Pseudomonas aeruginosa*

Firstly, *Pseudomonas aeruginosa* has a good ability to resist and accumulate metal ions such as HgCl, $MgSO_4$, Zn_2O_3, $MgCO_3$, $CuCl_2$ and $CdCl_2$ [35]. In addition, study suggest that *Pseudomonas aeruginosa* can be an effective measure for heavy metals compensation and recorded best achieved with 15% metal concentration of copper and zinc, which showed a reduction in free ion concentration about 79.1% at 48 hours, respectively, 52.4% at 72 hours of an incubation. There was a biodegradable chromium of 41.6% at 72 hours of incubation with a 5% concentration of ion and with reduced concentrations of metal reduction of reduced Cr-ion. The reduction of the concentration of free metal ions was observed at 61.0% of the 10% solution after 24 hours of incubation [39]. In another study, *Pseudomonas aeruginosa* presents a potential sorbent for the removal of heavy metals contained in groundwater. The results of the experiments showed that these bacteria can break an average of 81% of heavy metals as low-cost, highly-efficient [44].

In addition, the results of the study show the potential for the isolated *Pseudomonas aeruginosa* (S7) which resistance to heavy metals in the treatment of heavy metals contaminated solutions. Further study investigates their ability to remove heavy metals in pollution area and genetic traits for tolerance to heavy metals were recorded [45].

Also, bacterial strains isolated from the drainage of Kakuri characterized and subjected to the salt concentration of various heavy metals and limited its ability to carry heavy metal and recorded minimally inhibitory concentrations (MIC). This demonstrates their ability to tolerate and live in an atmosphere with high metal salts. Eight (8) heavy metals were examined and included; $ZnSO_4$, $CdCl_2$, $CoCl_2$, $K_2Cr_2O_7$, $CuSO_4$, $HgCl_2$, $NiCl_2$ and $PbCl_2$ [46].

Furthermore, other study showed that 90.4% of mercury biosorption was observed on combinations of cultures *Bacillus subtilis* and *Pseudomonas aeruginosa* 78.5 and 99.3% respectively. Also, the time required for maximum sorption of maximum mercury amount is 40 and 60 minutes for a mixture of cultures and *Bacillus subtilis* and *Pseudomonas aeruginosa*. In addition, biosorption of chromium showed that 77.6% of cultures, 60.5 and 81.3 for *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively. Also, Arsenic biosorption is carried out using the same biomass as described above by achieving sorption of 30, 32 and 28% of mixed cultures (*Pseudomonas aeruginosa* and *Bacillus subtilis*) [47].

In a test in which the removal of heavy metals from waste water is an important target, heavy metals biosorption on biomass of *Pseudomonas aeruginosa*, immobilized carbon activated on granular, it has been studied in batch and column systems. In this batch system, the adsorption of heavy metals is reached between 20 and 50 minutes, and the best dose of bio solids is 0.3 g/l. so, the efficiency of the biosorption is 84, 80, 79, 59 and 42% For Cr, Ni, Cu, Zn and Cd respectively [48].

In another study, *Pseudomonas aeruginosa* isolated from the waste water of electroplating industry, is able to absorb chromium, nickel and zinc, by 20% concentration. The highest percentage of the reduction was observed in nickel after 10 days and lowest for 10 days chromium, so the bacteria can be used as bio sorbents [49].

In addition, another study was investigated for biosorption of ionic cadmium by *P. aeruginosa* under varying conditions which the values have the first pH of the cadmium solution ranges from 1 to 7, the maximum removal of cadmium is obtained at pH 6. From the perspective of the application of the procedure, the time for bio sorbents was 70 minutes and biosorption concentrated (1 g/l) is a suitable bio sorbent for treatment of cadmium ion (up to 100 ppm) [50].

Additionally, it has been found that the adsorption of heavy metals by *Pseudomonas aeruginosa* bio flocculant is influenced by the first metal focus, the concentration of bio flocculant and the pH of the solution. The study showed that microbial potential bio flocculant has been used as a bio remedial tool in the treatment of contaminated wastewater with heavy metals [51].

4.4 Plasmid mediated heavy metals in *Pseudomonas aeruginosa*

Pseudomonas aeruginosa launches resistance to heavy metals such as cadmium, chromium, nickel and lead. DNA plasmid was isolated from *P. aeruginosa* and has been defined as pBC15 and the plasmid size is about 23 kb [52]. Also, results of heavy metal tolerance and accumulation experiments concluded that *Pseudomonas aeruginosa* bacterial strain has the tendency for tolerate heavy metals due to it has plasmid that carry genes and play important role in tolerance of heavy metals, so it will be promising for new trends in heavy metals bioremediation and bioaccumulation in the future [2].

In addition, genes are set for the degradation of environmental pollution, such as heavy metals, toluene, acids, and pesticides, Halogen and this toxic waste. So, plasmids are required for each compound. It is not that one plasmid reduces all toxic compounds from other groups [53].

In bioremediation of chromium, bacterial strains show chromosome plasmid resistance and reduced enzyme coordination. In molecular engineering, it can now extract stress by improving even under stress conditions [4]. Also, it has been reported that the plasmid resistance gene is determined in pathogenic bacteria of the genus *Escherichia*, *Salmonella*, *Shigella*, *Klebsiella*, *Aeromonas* and *Pseudomonas* which determining factors for resistance to heavy metals such as cadmium, cobalt, nickel, zinc, and mercury, also different groups of drugs, such as tetracycline, quinolones, aminoglycosides and β-lactam [54].

Additionally, *Pseudomonas aeruginosa* use different types of mechanisms in response to heavy metals stress. These mechanisms can be encoded with chromosomal genes, but more often resistance is located on plasmid [55]. Plasmid curing in *Pseudomonas aeruginosa* is a testament to the relationship between genetic presentation and the transmission of a specific feature in heavy metals tolerance and removal. Various approaches have been developed to cure of plasmid, including chemical and physical agents for the elimination of plasmid [56].

4.5 Evaluation of resistant genes in *Pseudomonas aeruginosa*

Firstly, metals-microbial interactions might have several environmental implications. Main resistance mechanisms for some heavy metals as (Cu^{+}, Zn^{+} and Ni^{2+})

were active efflux transporters. Also, in bacterial strains, molecular basis of zinc resistance determined by presence of znt-related genes. In addition, it investigates adaptation of *Bacillus cereus* and has znt genes. Heavy metal resistant genes identified in *Pseudomonas aeruginosa* as CZC genes [57].

Subsequently, the ncc, czc, mer and chr genes responsible for heavy metals resistance to different heavy metals as Cr, Zn, Hg and Ni and which the genes have high homology to the chrB, czcD, mer and nccA genes [58].

In *Pseudomonas*, there are 6 genes in resistance of cadmium were identified formed from 3 gene clusters as cadA2R, czcCBA1 and colRS. The homologs of the first two gene clusters were predicted as metal efflux systems [59].

Finally, conjugative plasmid (pUM505) isolated from *Pseudomonas aeruginosa* possesses a putative (31.292 kb) mobile element in addition to possessing chr genes that confer chromate resistance to *Pseudomonas* contains two putative mer operons which could confer resistance of mercury. Furthermore, the Mpe contains genes related with the virulence of *Pseudomonas aeruginosa* [60].

5. Conclusion

Heavy metals pollution cause problems and effect on soil, water, plant, animal, human and ecosystem. Heavy metals cause health risks to human lead to cancer. Also, the highest removal percentage of heavy metals from environment is recorded by microorganisms and plants. Microbial community recorded high tolerance and uptake to different heavy metals such as bacteria, fungi and algae. *Pseudomonas aeruginosa* is the most potent bacterial strains which tolerating and removal heavy metals. Tolerance and removal of heavy metals occurred by different mechanisms. Additionally, *Pseudomonas aeruginosa* recorded high removal percentage from different heavy metals such as cadmium, nickel, lead, chromium, mercury, copper and zinc. Also, different studies insured that high removal recorded at optimum conditions for growth and biomass produced. Conditions included pH, temperatures, biomass dose and incubation periods. Finally, *Pseudomonas aeruginosa* can tolerate heavy metals using resistant genes and genes that carry on plasmid which play important role in increase efficacy of strain in bioaccumulation and tolerance. In future prospections, *Pseudomonas aeruginosa* will be promising in heavy metals bioremediation and bioaccumulation from environment and achieve high removal percentage after using genetic engineering and gene transfer.

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Conflict of interest

The author declares no conflict of interest.

Abbreviations

Cd	cadmium
Cu	copper
Ni	nickel
Zn	zinc
Hg	mercury
Pb	lead
Mn	manganese
Cr	chromium
Kb	kilobase pairs
MIC	minimum inhibitory concentration

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