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Nickel Pollution, Resistance and its Bioremediation Mechanisms - A Review

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Abstract

Nickel (Ni) is an essential element which is required in low, controlled amounts for various cellular process in both the human body and in microorganisms, respectively, where it also serves its function as a cofactor for the regulation of many different enzymes, including hydrogenases and ureases. In the environment, its presence owing to anthropogenic activities over many decades has resulted in its accumulation at potentially high levels, which has since given rise to different resistance mechanisms in microbial species having adapted themselves for their survival in environmentally high Ni concentrations. In the wake of high metal concentration inside the cell, many Gram-positive and Gram-negative bacterial species have resistance mechanisms that degrade, precipitate, or pump out the toxic heavy metal ions out from the cell. In this review, we summarize the various mechanisms that enable the entry of Ni ions into bacterial cells when it is needed for biological processes, its resistance and efflux systems, as well as the various studies which have reported its bioremediation by both Gram-positive and negative bacteria, respectively, thus presenting these Ni-resistant bacterial species as potential candidates for the efficient removal of Ni from Ni-polluted environments.

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Introduction

The major anthropogenic processes due to the industrial revolution in the last century have given rise to many changes in the world. The environment is comprised of four major spheres and their subsequent interaction results in creating the balance of the ecosystem, where the living and non-living organisms interact with each other (Gadd, 1990). Over many years, these activities have brought forth an unwelcome menace that is known as environmental pollution, where the main culprit seems to be the said industrialization-induced activities. This results in the disturbance of the ecosystem, which is in turn affected by this pollution (Sharma *et al.,* 2018). Soil degradation, air pollution, biodiversity loss, erosion, and waterlogging are an all-natural phenomenon that makes the man-made pollution so drastically severe. Furthermore, the use of harmful pesticides in the environment leads to the build-up of contaminants in the food chain, which further disrupts the harmony of the ecosystem. The toxic chemicals pose a viable threat to all animals and microorganisms interacting with them (Akhtar *et al.,* 2009).

Heavy metals are reported to be those metals that have a density greater than 5 gm/cm^3. Over the years, their excessive use has been listed in various applications and industries, with their long-term usage wreaking havoc on our natural ecosystems and habitats. Heavy metal pollution is, without a doubt, one of the biggest environmental issues of our time and a cause of grave concern. The major source of heavy metals in our soil used to be natural processes, but the passage of time has shifted the blame to major anthropogenic activities taking place all over the world. The industrial wastewater from dyes, pigments, mining, electrical plating, metal cleaning, and leather industry contains large amounts of untreated heavy metals (Naidu and Bolan, 2008). Previously, natural processes like volcanic eruptions, soil erosion and run-off due to changing weather, forest wildfires, the deep-sea sinking of ships and marine vessels, as well as hot geysers had all contributed majorly to the presence of heavy metals in the environment (Shamim, 2014). Furthermore, heavy metals cannot be degraded easily which makes them persist in water, exposing them to marine and aquatic life and disrupting the marine ecosystem (Adriano *et al.,* 2004). Industrial processes like smelting, alloy production, mining, tanning, battery production operations, sewage and the lack of proper disposal of industrial waste (Ahemad and Malik, 2012) contribute to the incessant release of heavy metals into soils and water (Wierzba, 2015). The dumping of industrial wastewater is the biggest source through which living beings can interact with these contaminants. Moreover, this industrial wastewater is also used for the irrigation of plants and crops in many underdeveloped countries of the world (Gupta and Kumar, 2012). These heavy metal ions can render themselves mobile if given any slight optimal conditions, and thus interact with animals to accumulate in their food chain and for the potential interaction with microorganisms, leading to the development of resistance in them (Hookoom and Puchooa, 2013), demonstrating the potentially harmful effects of heavy metal pollution in our environment (Issazadeh *et al.,* 2014). There are many metals found on Earth that are termed toxic metals, which are stated as those metals which cast a harmful effect on living beings and do not have a positive role in their nourishment. Toxic heavy metals like cadmium (Cd), mercury (Hg), and arsenic (As) are characterized as toxic, non-essential metals that are known as hazardous agents for health. Other metals such as chromium (Cr), cobalt (Co), nickel (Ni), copper (Cu) and zinc (Zn) are termed as essential heavy metals as these metals are also required in trace amounts for the growth and rejuvenation of living beings. That being mentioned, it is also important to state that any concentration of these essential metals that crosses the normal limit is also termed to be toxic for living beings. Therefore, these metals should be degraded to permissible limits before their subsequent discharge into the environment to avoid their harmful effects upon living beings and their fragile ecosystems (Alam *et al.,* 2012).

Nickel as a heavy metal

The element Nickel (Ni) is the 24th most abundantly found element on Earth. It was discovered for the first time by Axel Frederik Cronstedt, a chemist from Sweden in 1751. The metal was mistaken for copper before its classification as a separate metal, by copper miners who had termed it as “kupfernickel” (Barceloux, 1999). The pure form of Ni is silver to white metal, hard on the surface which makes it an ideal metal for the formation of alloys with other metals such as Fe, Cu, Cr and Zn. The alloys of Ni are used in the manufacture of coins, pennies, metal ornaments as well as its use in various applications on the industrial side. Naturally, Ni is found in an amalgamated state with other metals in the Earth's crust, eventually being found in soils and also being emitted from within the Earth in the form of volcanic emissions (Agency for Toxic Substances and Disease Registry, 2005). It is naturally found in the form of compounds, like halides, sulphates and nitrates, interacting with other elements such as Cl, S, and N, respectively.

It is also present on the ocean bed as build-ups of minerals known as “sea floor nodules”. The emanation of Ni results from its various uses at an industrial level, and also during the mining of Ni from soils and deep Earth. The manufacture of Ni alloys and their related compounds (which have a characteristic green colour) leads to their discharge in industrial wastewater. These compounds are routinely employed for Ni plating, battery manufacturing and as catalysts in industrial processes (Reis *et al.,* 2017). The pollution of Ni has been accredited to the various industrial processes, as well as many other anthropogenic activities such as the burning of coal as well as heavy traffic of automobile vehicles on roads, which leads to the accumulation of Ni in soils nearby (Antić-Mladenović *et al.,* 2017). The railways are also considered to be a source of Ni pollution (Stojic *et al.,* 2017).

Ni pollution in pakistan

The metal Ni is widely distributed in the environment due to the natural and anthropogenic activities including mining, smelting, electroplating as well the discharge of Ni and Ni-rich compounds. Human interaction and exposure to the metal is the result of accidental and harmful ingestion of Ni contaminated food, water and air. The infants can directly consume Ni-rich soils which can result in their potentially harmful exposure to Ni. The National Standards for Drinking Water Quality (NSDWQ), Pakistan suggests an average value of Ni of 0.02 ppm in drinking water, all across Pakistan (Pakistan Environmental Protection Agency, 2008). The different sources of water have variable ranges of Ni. In the groundwater, it is said to be between < 0.001-4 ppm and in surface water, Ni concentration ranges from <0.001 – 1.5 ppm, respectively (Haq *et al.,* 2005). In the study conducted by Waseem *et al.* (2014), it was reported that groundwater was observed to be contaminated with values of Ni above the average values recommended by either the World Health Organization or NSDWQ.

In a similar pattern, the surface waters of a metropolitan city of Pakistan were reported to be contaminated by elevated values of Ni (Haq *et al.,* 2005). In the study conducted in Punjab to assess the value of Ni in soil, the mean value was recorded to be more than 80 mg/kg, which presented no potentially harmful health risk for people living in that area (Khan *et al.,* 2010). In another study, the highest concentration of Ni in soil was observed to be more than 300 mg/kg at a contaminated site in Lahore, where the mean values were much higher than the normal permissible limits of Ni set by governmental bodies (Mahmood and Malik, 2014). This may be due to the emanation of metals in the soil, present before mining, posing potential threats to the environments of Pakistan. High Ni values were also reported for the Karachi coastal and port areas (Siddique *et al.,* 2009). The air was also found to be contaminated with Ni in a study, with a range of 0.01-0.15 ng/m^3 of particles found in the urban area of a metropolitan city of Pakistan (Shah and Shaheen, 2007).

The mean levels of Ni were also found to be increased in foods like fruits and vegetables that were irrigated by sewage water, which eventually lead to the accumulation of heavy metals like Ni in foods (Toor and Tahir, 2009). Another previously conducted study had also reported elevated Ni concentration in vegetables that were irrigated with untreated industrial wastewaters (Lone *et al.*, 2003).

Nickel toxicity

The presence of Ni as an essential element is required for the regulation of selected processes in the human body. Nevertheless, it is required as a trace element in the human body which means that its high concentration can be toxic for health. It is also considered a high-level pollutant by the EPA. Ni is termed to be highly toxic and can poison the central nervous system through the inhalation of ash particles and the interaction of Ni compounds (Zhong and Jiang, 2017). It also tends to accumulate in the food chain through the action of consumption of various fruits and vegetables that have accumulated Ni, causing toxicity of the metal in living beings (Kelepertzis, 2014). Furthermore, high Ni concentration is also reported to have unpleasant effects on the brain functioning of toddlers and infants (Hong *et al.,* 2016; Chen *et al.,* 2019). The average level of Ni in drinking water usually comprises Ni at a concentration of > 10 ppm, with an average daily intake for adults to be in the range of 7 to 14 ppm (Cempel and Nikel, 2006). Accidental exposure is the usual premise for Ni induced toxicity, but Ni concentrations can also rise in the body after food and water intake (Agency for Toxic Substances and Disease Registry, 2005).

The elevated Ni levels in the body can give rise to several diseases and ailments like pulmonary fibrosis, dermatitis, nausea, fever, and neuronal disorders in children. It is also a well-reported genotoxic, neurotoxic, carcinogenic, and hepatotoxic agent. The acute toxicity of Ni results in the formation of ROS in humans, animals and microorganisms, as well as nausea, headache, respiratory distress in humans (Das *et al.,* 2008). In the air, Ni is found to have adhered to small particles which leads to its inhalation when particles in the air are elevated. In the United States, the mean values of Ni concentrations in both urban and rural areas ranged from 6 to 12 ng/m^3. In water bodies like the rivers and lakes, the mean value of Ni concentration to be reported is approximate> 10 ppb (0.01 ppm), which is a minute value to be detected. However, this value is reported to drastically increase if a water body is situated near industry or an area where Ni is routinely mined from Ni-rich soils. The mean Ni value in soils range from 1-80 ppm, and the highest Ni concentrations are reported from soils which are rich in Ni (Agency for Toxic Substances and Disease Registry, 2005).

Nickel import, homeostasis and resistance in bacteria

Similar to the way Ni is used and considered to be toxic for the growth of living beings, microorganisms have had a certain way of living in the presence of Ni over certain periods. They have evolved by adaptation and resistance, a mechanism deemed necessary for their survival, requiring Ni for several metabolic processes but its high concentrations tend to act as oxidative stress, facilitating the production of ROS in microorganisms like bacteria. For combating the stress that the metal generates in its presence, bacteria have several resistance mechanisms that degrade, break down or pump out the toxic heavy metal ions out from the cell. Many well-reported resistance mechanisms mediate the toxicity of Ni ions out of the cell, such as CBA efflux system of *C. metallidurans* CH34, nccCBA of *A. xylosoxidans* 31A, and cznABC from *H. pylori* (Salvador *et al.,* 2007; Alboghobeish *et al.,* 2014). In *E. coli*, the identification of the RcnA efflux pathway which was mediated by Ni was suggestive of its homeostasis mechanisms in Gram-negative bacteria.

The RcnR protein, a crucial part of the efflux pathway, is activated from the *rcnA* promotor, which allows for the transcription to occur in the presence of Ni (Iwig *et al.*, 2006). The effective uptake of Ni in certain bacteria is dependent upon the Ni-specific, high-affinity ABC transporter system which is encoded by the *nikABCDE* operon (Fig. 1) (Wu *et al.*, 1994). In DNA binding protein, the binding of Ni induces many conformational changes, where Ni proteins induce metal homeostasis and resistance mechanisms, with the best Ni metalloregulatory protein being NikR (De Pina *et al.*, 1999). NikABCDE was originally characterized in *E. coli*, where it was found to be an amalgamation of various proteins working together as part of the ABC transporter family (sub-type 2). NikB and NikC are transmembrane proteins that help to form a Ni pore (Navarro *et al.*, 1993). NikD and NikE tend to bind and hydrolyze ATP molecules, whereas NikA is a periplasmic protein that binds with one Ni per protein molecule (Cherrier *et al.*, 2008). Various bacteria have incorporated Nik systems that aid in the regulation and homeostasis of Ni, such as *Helicobacter*, *Brucella*, *Vibrio*, *Yersinia* as well as *Staphylococcus* species (Hiron *et al.*, 2010). NikR is crucial for the regulation of NikABCDE production in a Ni-dependent manner so that the expression of the transporter is reduced when the metal is present in abundance at extracellular levels (Rowe *et al.*, 2005). In *E. coli,* it tends to bind with four Ni molecules on its high-affinity sites, with the other sites being reserved for potassium and low-affinity Ni, respectively. Therefore, the Ni-bound protein plays a key role as a repressor protein when it binds to operator regions. This feature of NikR is found to be different in various bacteria, with variations in the properties and metal-binding features, respectively (West *et al.*, 2010). Moreover, the assemblage of the Ni-Fe metallocluster hydrogenase isoenzymes' active site competes with NikR for the surety of optimum expression of *nikABCDE* genes for suitable growth conditions of bacterial fermentation.

The import of Ni in bacterial cells tends to be an intricate yet simple process, with both specific as well as non-specific proteins coming into play for its transport into the cell. HoxN is a high-affinity Ni-permease enzyme which is a crucial member of the NiCoT family, usually reported in *Cupriavidus necator*. This family can be categorized depending on its functional requirements, with the three major transporting affinities being restricted specifically to both cobalt (Co) and Ni, only Ni, and only Co, respectively (Eitenger *et al.*, 2005). This family has been reported to be present in both Gram-positive and negative bacteria, where Ni specific proteins import it into the cell by NiCoT proteins (Fu *et al.*, 1994; Mobley *et al.*, 1995; Cole *et al.*, 1998; Degen *et al.*, 1999; Niegowski and Eshagi, 2007). The non-specific import of Ni into cytoplasmic space can also take place. In *E. coli*, the Mg-importer CorA can facilitate the entry of Ni, indicating that this route likely serves as the only route of Ni entry under extreme or elevated environmental Ni concentrations. Besides, Ni, once imported into the cell, must pass an extra barrier in some bacteria, which also contain an outer membrane. Originally, it was thought that the non-specific entry of Ni is facilitated by the hep of porins. However, it was reported that the activity of urease in *H. pylori* was found to be inhibited by the action of knocking out FecA3 and FrpB4, types of TonB-dependent transporters (TBDT), hence indicating their importance in facilitating Ni entry in microbial cells (Davis *et al.*, 2006; Rodionov *et al.*, 2006; Schauer *et al.*, 2007; Schauer *et al.*, 2008; Macomber and Hausinger, 2011).

However, the regulation of Ni is inhibited at high concentrations in medium, due to Ni being toxic to bacterial growth, leading to its inhibition. In response to Ni toxicity, *E. coli* employs the use of two methods, with the first being the activation of NikA and Tar-dependent negative chemotaxis and the second being the inhibition of Ni entry via the Ni transporter system, which is induced through the expression of the *nikABCDE* operon (De Pina *et al.*, 1999).

Techniques for Ni removal

Over many years, various methods have been employed for the removal of heavy metals from various contaminated environments. The physical-chemical methods such as immobilization, soil substitution, encapsulation are all methods that are used for Ni removal, by detoxifying the metal ions. The removal of heavy metals from the environment, traditional chemical methods such as metal ion precipitation, electro-remediation, whereas physical methods such as ion exchange, adsorption, reverse osmosis technology, and filtration have been used over the years for the effective removal of metal ions from contaminated wastewaters (Shamim, 2018). However, these methods come with their set of pros and cons, with their disadvantages weighing more when applied at a large scale, rendering them not as effective as other processes. There are many other removal methods, among which phytoremediation is another method that is deemed environmentally friendly, as this technique uses plants that accumulate and uptake pollutants from the soil. There have been many plants that have been reported to be in use for the removal of Ni over the years, where younger plants are reported to be more effective in the uptake of metals as compared to older plants (Ziarati and Alaedini, 2014). This aspect of phytoremediation is further strengthened by plant growth-promoting bacteria, also known as PGPR. These bacteria are reported to be ubiquitously present in soil sources, providing a synergistic relationship between beneficial bacteria and plants, increasing the plant growth characteristics, as well as improving metal-resistance and accumulation in plants (Ma *et al.,* 2011).

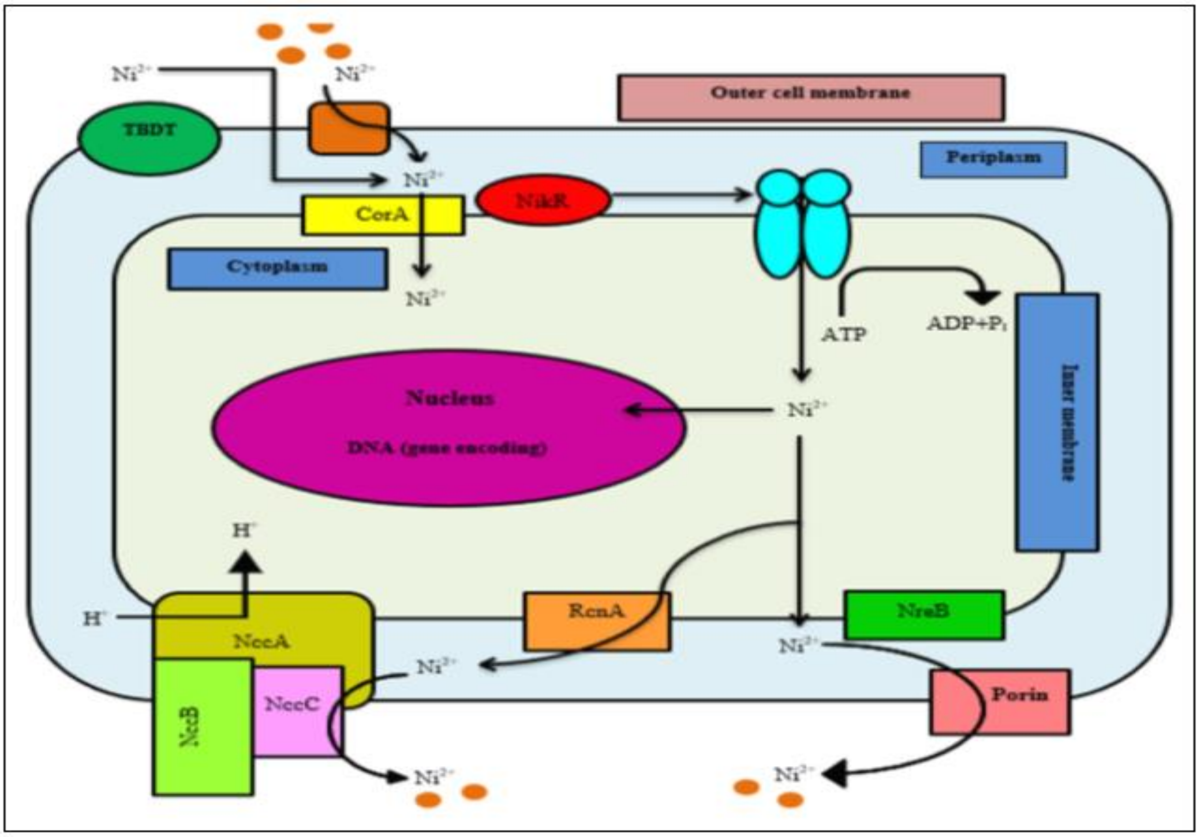

Fig. 1. A summary of selected pathways involved in Ni entry and exit in bacterial cells.

The advantages and disadvantages of the previously mentioned methods should be kept in mind before applying the methods on a large-scale. The development of a cost-effective, environmentally friendly method for the removal of heavy metals is crucially important. The introduction of innovative methods over the years has given rise to new aspects that use natural sources such as microorganisms, plants, and microbial biomass to reduce the problem of pollution (Ahemad and Malik, 2012). The sewage and industrial wastewaters contain many microorganisms that comprise bacteria, fungi, algae, and rotifers which have evolved for maintaining and regulating resistance against high concentrations of heavy metals. Microbial remediation is a process that observes various microorganisms like bacteria, fungi, and algal biomass employing different methods like biosorption, bioaugmentation, and bioleaching for the effective removal of metals from contaminated environments.

Ni bioremediation

Bioremediation is a cost-effective and environmentally friendly method for the removal of heavy metals from contaminated environments (Congeevaram *et al.,* 2007). The availability, cheap sources, and the natural ubiquity of microorganisms are the main advantages that are considered in the remediation of heavy metals using microorganisms to treat large bodies of wastewaters. Some microorganisms are naturally good remediating agents because of their natural characteristics, whereas other microorganisms are genetically engineered to become good remediating agents, where they are modified to remove more than one metal in the consortium, respectively (Afzal *et al.,* 2017). Some of the microorganisms possess efflux systems that pump out metal ions when they reach toxic concentrations, whereas the resistance genes are also found on both chromosomes and plasmids that confer metal resistance to microorganisms (Hookoom and Puchooa, 2013). The other mechanisms include the detoxification of heavy metals by the action of chelating agents (Kavamura and Esposito, 2010), whereas some other microorganisms reportedly reduce the toxic form of metals into their lesser toxic forms (Solecka *et al.,* 2012). Biosorption is termed as the capacity of microorganisms to accumulate and remove heavy metals from the environment from contaminated wastewaters and other environments.

These contaminants may be used for the accumulation of metals even from minute concentrations (Shamim, 2014). This process is observed to be dependent upon many other factors that determine the capability of biosorption such as pH, temperature, metal concentration and the type of microorganism (Gabr *et al.,* 2008). Studies of biosorption by various microorganisms have been reported by many researchers over the years (Shamim, 2018). Microorganisms like bacteria are considered efficacious agents for metal removal which is mainly due to their adaptability and effectivity to perform in highly toxic concentrations (Ayangbenro and Babalola, 2017). Their biosorption ability is largely due to the favorable area ratio and their interactive active sites on the bacterial cell wall. The bacterial cell wall is of two types, which is essential for their characterization. The Gram-positive bacteria comprise of a thicker cell wall, due to the high layer of peptidoglycan and teichoic acids, which ultimately enables them to be comparatively more effective in the interaction of metal ions with the cell wall than Gram-negative bacteria, which do not contain a thick layer, instead, a thin layer of peptidoglycan is present that is not that effective in binding the metal ions to the bacterial cell wall. Many reported genera that are known to remove heavy metals are *Bacillus*, *Pseudomonas*, *Enterobacter*, *Micrococcus* and *Streptomyces*, etc. (Uslu and Tanyol, 2006).

In a recent study, the biosorption capability of *Cystoseria indica* against heavy metals such as Cd and Ni was evaluated. The species was observed to effectively remove Cd from aqueous solutions more than Ni, where the optimum conditions like temperature, contact time and pH were also determined. The results highlighted the effective biosorption ability of *C. indica* against various heavy metals in solution, where it can be used to remove a consortium of metals in solution (Khajavian *et al.,* 2019). The last decade has seen the rise of industries and its major source of pollution that destroyed the environment and natural resources.

The biosorption studies conducted by Ramya and Thatheyus, (2018) effectively highlighted the biosorption potential of different bacterial species and their optimization to describe maximum metal removal. Another study involved the isolation of metal resistant bacteria and their characterization for the removal of heavy metals from a renowned river bank in Nigeria. These bacterial isolates were also characterized at a molecular level, which demonstrated resistance against heavy metals like Ni, Zn, Pb, and Cd. Biosorption study revealed the potent effectivity of metal removal when the bacterial species were used in a consortium (Akudo *et al.,* 2018). The textile effluents were also examined for the potential removal of heavy metals before they are discharged into fertile lands. Heavy metal resistant bacteria were isolated from textile effluents and their biosorption abilities were determined in a study. The results demonstrated a novel bacterial isolate that was effectively used in the bioremediation of many heavy metals (Afzal *et al.,* 2017). The bioremediation ability of two *Bacillus* species was determined in a study conducted in Mexico. The species were examined to be heavy metal resistant and their metal removal ability was examined against various metals like Ni and Vanadium (V). The bacterial species could remove more than 30 and 15% of Ni and V, respectively. Moreover, PCR was also employed to determine the genetic determinants of resistance against Ni in the bacterial species, which demonstrated that the genetic resistance varied and mattered greatly for the metal resistance mechanisms in those bacterial isolates (Fierros-Romero *et al.,* 2016). *Bacillus* species were also studied for their bioremediation potential after they were isolated from solar lanterns for the screening of various metals like Pb, Cr, and Cu. Techniques like AAS, ICP-OES and EDS helped to determine the presence of metals in the bacterial cells, where it was observed that *Bacillus* species were good biosorbents for all of these metals. The maximum results were observed in the case of Pb, followed by Cu and Zn.

The bacterial isolates were found to be potential agents to treat metal contamination in a more efficient and eco-friendly manner (Syed and Chinthala, 2015). From industrial wastewater, more than eight bacterial strains were isolated and were termed as Ni resistant bacteria which were then examined for their resistance and bioremediation ability. These eight strains were found to be resistant to Ni, where one isolate was able to effectively remove more than 80 mg/L from an aqueous medium (Alboghobeish *et al.,* 2014). The Klip River pollution had generated a protective survival mechanism in microorganisms, where metal resistance mechanisms were observed in a study conducted by Chihomvu *et al.* (2014). AAS analysis revealed the presence of many metals in samples, against which several investigated bacterial species demonstrated resistance. The antibiogram also revealed the bacteria to be resistant to several antibiotics.

The study conducted by Margaryan *et al.* (2013) observed the bioaccumulation ability, expression of heavy metal resistance genes, and growth response in the presence of different concentrations of heavy metals in *Bacillus* sp. The metal ions concentration in the medium was in µM, which did not affect the bacterial growth. In *Bacillus subtilis,* Cu and Zn biomass was observed to be 6.8 and 3.0 mg/g, respectively. The PCR amplification used a specific primer set to identify *nikA* and *copA* genes but not *czcD*. The main expression of *nikA* and *copA* genes observes in a different quantity of µM with the help of RT-qPCR.

The expression of both genes potentially enhances the accumulation potential of maximum high metal ion concentrations. *Bacillus* sp. showed the high microbial resistance against various heavy metals, which was isolated from industrial wastewater in a study conducted by Alzahrani and Ahamad, (2015). The potential bioaccumulation of Zn and Al by freshwater plants was carried out in a study. Both metals were accumulated to lead the dry weight under higher metal treatment and showed the antioxidant response of the plant species especially superoxide dismutase and peroxidases, which demonstrated better results than catalase. The oxidative stress results showed induction in the presence of both metals. The antioxidant capacity increased due to high bioaccumulation potential and tolerance. Conclusively, phytoremediation too resulted in the low levels of Zn and Al in the water bodies, respectively (Radic *et al.,* 2009).

The use of industrial wastewater for irrigation was examined in a study conducted by Ansari and Malik, (2007). The presence of heavy metals and the presence of heavy metal resistant bacteria were also investigated. More than thirty-five bacterial species were isolated from soil samples irrigated with contaminated wastewater. The maximum MIC was observed of 200, 400, and 800 mg/L for Cd, Zn, and Ni, respectively. The Ni concentration increased from 7 to 56 mg/g of cells during biosorption, in a single solution where the concentration range was 50-400 mg/L for passing 2 h of incubation. No significant effect was observed on the biosorption of metals after further increasing the incubation time.

The study conducted by Patel *et al.* (2006), concluded the absorption and bioaccumulation of heavy metals in wastewater are due to microbes. The different industrial waste was used to isolate Ni resistant bacteria. They were further grown in nutrient broth with different concentrations of Ni salts. The growth of bacteria (Ni resistant bacteria, also known as NiRB) was observed in the medium that had 2.5 mmol/L of Ni. The NiRB was also investigated using SDS-PAGE. The two stress-induced proteins demonstrated a molecular weight of 48 KDa and 18 KDa, respectively. The metalloregulatory proteins are controlled by metal ion homeostasis and are controlled by the ion uptake in the cell. *B. subtilis* was used to check the rapidly induced effect of heavy metals for transcriptional analysis (Moore and Helmann, 2005). The study of Yilmaz *et al.* (2005) studied more than 20 species of *Bacillus* which were isolated from soil and were analyzed using the agar diffusion method with varying concentration of Ni. The extensive inhibition effects against Gram-negative bacteria were also observed. When compared with known antibiotics, some other isolates were found to be more effective against test organisms.

The study of Yilmaz, (2003) studied *Bacillus* isolated from heavy metal contaminated soil that was from the southeast region of Turkey. The *Bacillus* strain EB1 showed the maximum MIC value or antibiotic resistance spectrum value too. The order of heavy metals toxicity in bacterium was Cd>Co> Cu> Ni> Zn>Mn in solid media. The resistance of the bacterial growth rate was observed by increasing the concentration of the metal in liquid cultures. The course of growth was used for metals biosorption. More than 80% Mn, 70% Zn, 70% Cu, more than 40% Ni was removed after the action of the bacterial isolate, respectively.

Conclusion

Nickel is a metal that is vital in limited amounts to all biological organisms. Several different distinctive mechanisms are present in bacteria that help to import and regulate Ni when it is needed for these cellular processes, but its high concentrations in the cells can create an environment of metal toxicity. Bacterial species isolated from Ni-polluted environments tend to have resistance mechanisms that enable them to survive and remove Ni from those environments. In this light, future studies on these resistant bacterial species can lead to the efficient, cost-effective and swift removal of Ni with minimal harm to the environment.

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