Short Communication

A case study: Role of the sewage dwelling plant growth-inducing rhizospheric microbe in environmental heavy metal bioremediation with special accentuation on chromium

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Abstract:

The chromium tolerant sewage microbes can remove soil chromium by implementing effective chromium absorption and reduction capability. As the soil microbes inhabited at anoxic to hypoxic heavy metal-containing sewage sludge, they have been adapted themselves and utilized the redox potential of chromium reduction to fulfil their own physiological needs. The sewage dwelling microbe secret different chelating compounds like siderophore, EPS (exopolysaccharides) and organic acids that help in microbial soil heavy metal absorption through cation mobility. Soil cation mobility decreases plant chromium absorption and helps in plant well-being in chromium polluted environments because of microbial chromium affinity. The soil isolated microbes eliminate environmental heavy metals and govern plant growth by secreting plant growth-promoting substances like phytohormone, Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), siderophores that make iron available for plants and control soil cation mobility. Consequently, the eco-friendly, sewage dwelling microbes can be applied commercially in heavy metal contaminated crop fields because they can remove soil heavy metals through bio-accumulation, improve plant growth and, decrease the risk of biomagnification.

Keywords:

Chromium, Bioremediation, Reduction, PGPR, Sewage-soil, Siderophore,

Introduction:

As a result of abrupt industrialization and different human activities like unplanned cropping and chemical manure application, the deposition of various heavy metals in the environment has been increased substantially day by day. The untreated wastewater discharged from several commercial outlets for a long time possesses the capability to affect the environment adversely with heavy metals and other unsafe chemicals [Wuana and Okieimen, 2011]. In this manner, the wastewater bodies get contaminated with different heavy metals like chromium, copper, zinc, manganese, iron [Devi, 2011]. The plants that grow in heavy metal contaminated soil can absorb heavy metals from both surface-soil and underground soil micro-climate [Patra et al. 2004]. The absorbed heavy metals accomplished serious plant metabolic disorders by forming substances like reactive oxygen species (ROS) or cytotoxic methylglyoxal (MG) that disturbed the ionic homeostasis within plant tissue and ceased plant growth and metabolism [Hossain et al. 2012; Star et al. 2013]. The heavy metals enter the human system through skin penetration, food chain, or inhalation and cause different fatal consequences [Gadd, 2010; Kapahi and Sachdeva,2019]. Non-biodegradable heavy metals can persist within the environment along with tissues of plants, animals, or human beings and are magnified through each trophic level. The concentration of heavy metals within dry fruit and other edible plant parts have been exceeded the values approved by the World Health Organisation (WHO) and the Food and Agriculture Organization of United States (FAO) [Naeem et al. 2009; Hussain et al. 1995]. That's why the removal of hazardous heavy metals from the environment becomes a great challenge to us. The traditional environmental metal removing methods like ion exchange technique, precipitation, electrodialysis is convenient for metal removal but time and energy-consuming, pH-dependent, non-specific, and costly [Aziz et al. 2008]. Cautious disposal and a considerable amount of metallic contaminants should be needed for conventional contaminated slur development [Gunatilake, 2015; Gray, 1999]. The cost-effective and eco-friendly alternative approach of heavy metal removal by bio-agents can be more useful to eliminate different environmental heavy metal contaminants. Microbes can be used as efficient and eco-friendly metal biodegrading agents because they have the ability to remove metallic contaminants from the environment and control plant metal absorption. Microbial metal conversion is dependent on environmental factors like pH, temperature, presence or absence of other metal ions, acids, moisture content, etc. [Gadd, 2010].

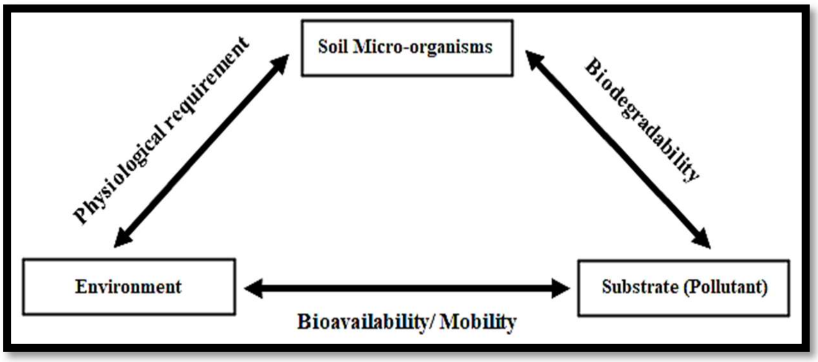

Figure 1: Inter-relationship between Microorganisms, metal pollutants, and environment in their natural habitat [Figure constructed with the basic idea of Tiedje, J. M. (1993)].

Impact of sewage dwelling microbes on environmental heavy metal removal:

Bioremediation is the in-situ eco-friendly [Verma and Kuila, 2019] approach through which microbes degrade the hazardous environmental metal contaminants or converted them to less-hazardous, bio-available organic substances. Bioremediation help in the growth and bio-mass formation of the other residual flora and fauna.

Though bacterial exposure with the metallic contaminants is required before contamination to induce the bacterial enzymatic system, a minimum amount of metallic contaminant can initiate enzymatic reaction [Adenipekun, 2012]. Heavy metals chromium, cadmium, lead, zinc, and copper can rapidly remove from the environment by bio-accumulation [Ozer and Ozer, 2003].

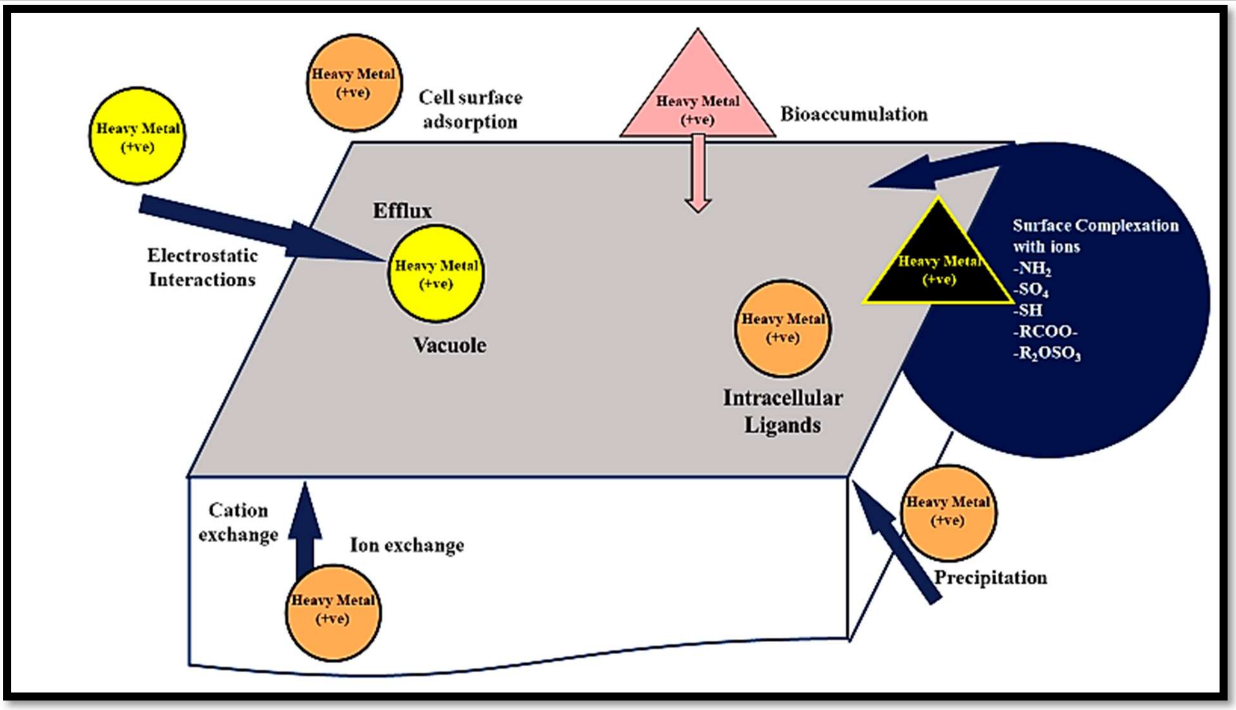

Figure 2: The mechanisms through implying which soil microbe can uptake heavy metals [Based on the findings of Yang et al. (2015) and Ayangbenro and Babalola (2017)]

Different chemical constituents [teichoic acid (in Gram-positive bacteria), N-acetylmuramic acid (NAM), poly-N-acetylglucosamine (NAG), anionic group-containing peptidoglycan (in both Gram-positive bacteria and Gram-negative bacteria), phospholipids (in Gram-negative bacteria), lipopolysaccharide (in Gram-negative bacteria)], [Sherbet, 1978] and functional groups [carboxyl group, hydroxyl group, amine group, phosphate group] which are present on the bacterial cell wall and increased the metal-binding capability of bacteria [Doyle et al. 1980]. The amino group of microbial cell walls plays a dynamic role in chromium removal by creating Van Der Waals interaction and forming a chelate with chromium ion [Kang et al. 2007].

Kang et al. (2007) reported that the outer surface of *Pseudomonas aeruginosa* bio-absorbed hexavalent chromium and reduced it to trivalent chromium afterwards. Different metal removing microbial strains like copper and arsenic removing *Bacillus*, *Micrococcus*, *Geobacter*, cadmium, nickel, chromium, copper, lead, and zinc removing *Pseudomonas* was identified previously [Dey et al. 2016; Zouboulis et al. 2004; Lee et al. 2012; He et al. 2019; Chellaiah et al. 2018; Lopez et al. 2000; Pardo et al. 2003]. These heavy metals removing bacteria uptake environmental heavy metal through different strategies like physical adsorption by cell surface, membrane transport, ion exchange or redox reaction, bio-absorption [Ianieva, 2009; Banerjee et al. 2018; Bernard et al. 2018] and utilize the heavy metals to fulfill their physiological needs. Heavy metals or other environmental chemical contaminants stimulate the growth of the bioremediating microbes because they provide microbial food and energy through different reciprocal metabolic pathways [Speight, 2018]. Being the transition element, chromium (atomic number 24) possesses different oxidation states such as trivalent-Cr(III), pentavalent-Cr(V), hexavalent-Cr(VI). Among which, less mobile trivalent form is abundant in the earth. The trivalent chromium generally forms a different crust with other soil minerals like calcium or silicon and remains present as ore within soil or rock. But, the second stable form, the hexavalent chromium, is not the usual component of the environment and does not take part in any biological well-being, and leaves many harmful effects on different life forms. The unusual oxidation state of chromium, hexavalent chromium [Cr(VI)], comes to nature through human activities like random industrialization. The quick and easy spreading of hexavalent chromium happened because of its high water solubility and soil mobility. The heavy metal contaminated, anaerobic waste water-dwelling microbes can fulfil their metabolic requirements by reducing the hexavalent chromium to harmless, less mobile, and non-toxic trivalent chromium [Kanmani et al. 2012]. Recently Novotnik et al. (2019) was reported the microbial reduction of the transition element manganese. They [Novotnik et al. 2019] were described that manganese tolerant microbe utilized the metal reduction potential in their cellular metabolic reaction at anoxic situations even more efficiently than fermentation. In anaerobic conditions, the microbes interact with the environmental hexavalent chromium through bio-absorption, bio-accumulation, and enzymatic redox reaction [Mishra et al. 2012]. When the hexavalent chromium remains absent, the bacteria can take part in fermentation. The chromate reductase enzyme present within the microbial cell membrane can take an important part in chromium reduction under anaerobic conditions and, the activity of this enzyme induced by co-factor NADH or glutathione [Elangovan et al. 2006] and hexavalent chromium behave as terminal electron acceptor of this pathway [Camargo et al. 2004].

While oxygen competes with chromium reduction as an electron acceptor, the presence of oxygen affects the chromium reduction pathway [Han et al. 2016]. One of the key blockades of microbial chromium reduction in neutral soil is oxygen. The oxides of both hexavalent and trivalent chromium are poorly soluble at neutral pH. Due to the insolubility, microbial cell membranes become impermeable to chromium. The hexavalent and trivalent chromium oxides are soluble in an acidic and, alkaline environment respectively. The microbes make the surrounding environment acidic by secreting extracellular siderophores and make chromium available to the microbes. Soil microbes can bind heavy metals by producing siderophores [Glick and Bashan, 1997; Braud et al. 2009a; Rajkumar et al. 2010] and low molecular weight organic acids [Rózycki and Strzelczyk, 1986; Renella et al. 2004] and make chromium available to the microbes by enhancing cation mobility. The soil microbe secreted siderophore also play a significant role in the iron extraction from organic substances and makes it available for the microbes [Dale et al. 2004]. The solubilized iron that has entered the microbial cell enhanced the chromium reduction by performing a redox reaction. Brumovský et al. (2020) reported that sulfur-containing zero-valent iron can reduce hexavalent chromium (Cr^{6+}) and form the immobile complex oxide or hydroxide salts of trivalent chromium (Cr^{3+}) and iron. Thus, the microbe's available iron can help in chromium removal from the environment for the long term by taking part in a simultaneous redox reaction with chromium. Hydroximate siderophore is the foremost constituent that helps in the microbial chelate compound formation and ferric iron uptake through enhancing iron mobility [Powell et al. 1980; Mawji et al. 2008]. Many sulfur-reducing and fermentative bacteria have also taken part in this kind of metabolic reaction. Brumovský et al. (2020) stated the role of reduced sulfur in trivalent chromium immobilization within the microbial cells.

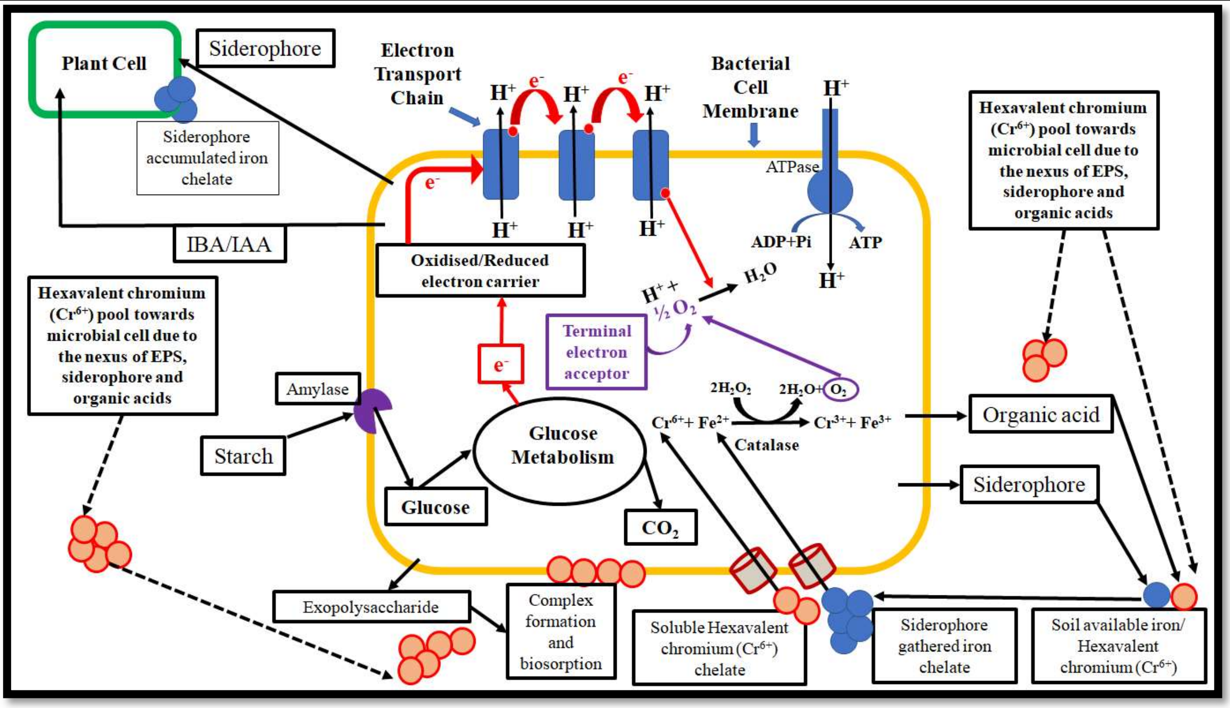

Figure 3: Schematic representation of the inter-relationship among the anaerobic soil microbe, chromium pollutant, and plants in hexavalent chromium contaminated sewage regions.

The hexavalent chromium can take part in cellular energy production by breaking down organic macro-molecules. The organic macro-molecules were come from surrounding soil within the microbial cell by different extra-cellular starch or protein degrading enzymes like amylase, gelatinase, and urease. In the anoxic to hypoxic sewage environment, the microbes can produce the required metabolic energy by reducing hexavalent chromium to trivalent chromium instead of oxygen. Alam and Ahmad (2012) was reported that the chromate reducing *Staphylococcus gallinarum* W-61, *Stenotrophomonas maltophilia* ZA-6, *Pantoea* KS-2, and *Aeromonas* KS-14 had decelerated the reduction rate in the presence of sodium azide, and sodium cyanide also depleted the chromium reduction rate whereas, uncoupling agent dinitrophenol (DNP) triggered it. The chromate reduction stimulating capability of uncoupler dinitrophenol (DNP) suggested a strong association-ship among electron transport chain (ETC) and chromium bio-reduction. As Han et al (2016) reported that in anaerobic microbes, oxygen competes with hexavalent chromium for the electron, it could be inferred that, like oxygen, the hexavalent chromium can be attached at the terminal complex (generally complex IV) of the electron transport chain and react with the proton to form water. The significance of chromate reduction in the anaerobic conditions is that the microbial electron transport chain (ETC) can collect electrons from hexavalent chromium, which is behaved as the terminal electron acceptor in the absence of oxygen with the help of an uncoupler like DNP, the anaerobic microbes can continue the respiratory pathway. The rate of chromium reduction acceleration in the presence of DNP revealed that chromium reduction might take a significant part in the respiratory electron transport chain (ETC). The anaerobic bacteria produced energy by reducing transgenic metals like chromium [Naz et al. 2021] but, not preserve the energy gained by metallic reduction. Rather, utilize the metal reduction procedure as an electron-donating step that enhances fermentation [Novotnik et al. 2019]. Some chromium reducing *Shewanella* (Hunt et al. 2010) and *Geobacter* (Esther et al. 2015) species were also reported that conserved the energy gained through chromium reduction for further cellular metabolic purposes.

Microbial impact on plant metal accumulation and plant well-being:

The entrance of heavy metal within the food chain depends on the composition of flora and fauna of regional micro-climate because the plant-alliance soil microbes play an important role in soil metal mobility, plant metal uptake, and biogeochemical cycling of soil heavy metal. Chromium also competed with other elements like phosphorus, sulfur, or iron [Shanker, 2005] for the plant or microbial cells and finally entered by forming chelate compounds or through active transport. The presence of hexavalent chromium may also increase the plant's soil mineral consumption [Wyszkowski and Radziemska, 2009] which is not good for plant health. Many soil microbes, including *Bacillus*, compete for nutrient minerals like sulfur, phosphorus, chromium with plants. Different *Bacillus* species played a significant role in soil mineral mobility, mineral absorption and accumulation in their vicinity [Wani and Khan 2010]. The soil microbe-mediated plant metal absorption reduction has a good impact on human and plant health because it lowers the chances of heavy metal bio-magnification. The Bio-concentration Factor (BCF) value, which is the ratio of the metal concentration in the vegetation to the concentration in the soil supporting that vegetation, indicates whether the heavy metal bio-magnification occurred through the vegetation or not. A lesser than one BCF value in plants indicates that they probably only absorb but do not accumulate heavy metals within the cell [Glick and Bashan, 1997]. Gupta et al. (2012) was evaluated the role of sewage microbe in decreasing bio-magnification by calculating BCF.

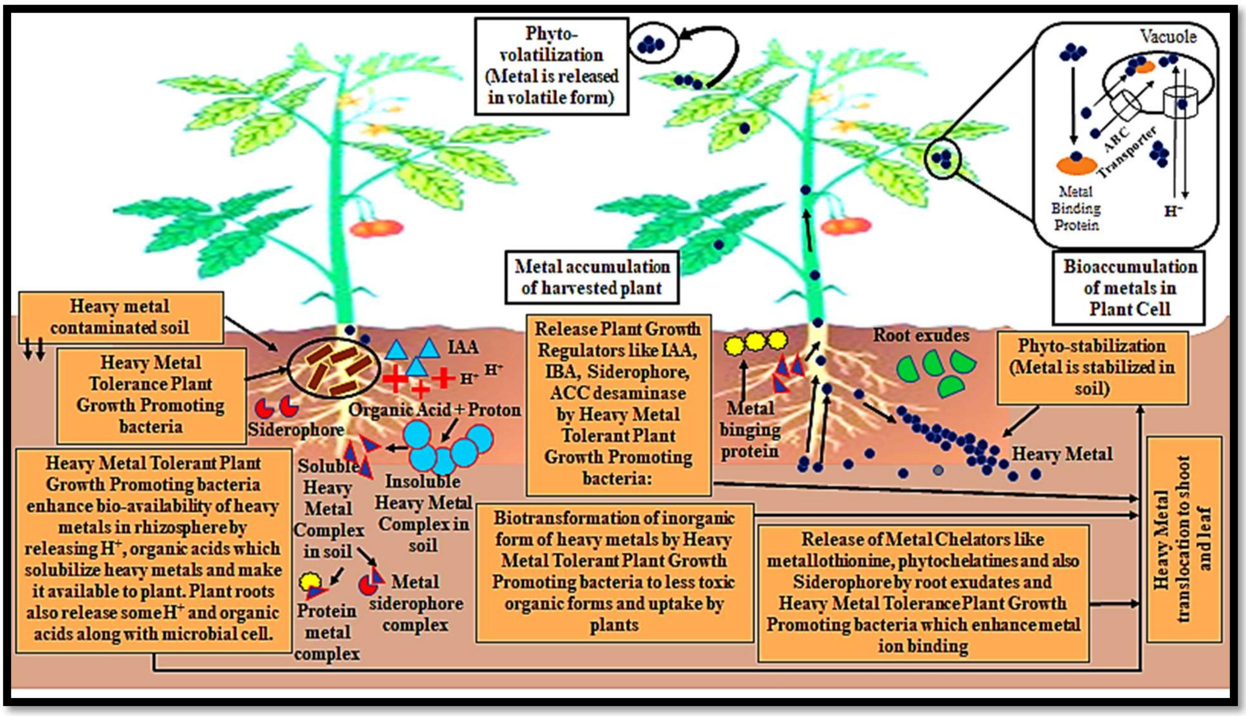

Figure 4: The multiple roles of heavy metal tolerant plant growth-promoting rhizobacteria in soil heavy metal detoxification and plant growth-promotion represented schematically [Figure is constructed based on the findings of Mishra et al. (2017)].

Some heavy metal resisting soil rhizospheric microbes play a potent role in the survival and growth of the metal-sensitive plant by alleviating the effect of heavy metal and providing nutrients to the plants [Benizri and Kidd 2018]. The plant growth-promoting rhizospheric bacteria (PGPR) promote plant growth by fabricating extracellular plant growth stimulating substances like Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), siderophore, phosphate degrading enzymes in the root-adjacent soil. Different Heavy metal stresses tolerating, plant growth-promoting rhizobacteria like (PGPR) *Bacillus*, *Streptomyces*, *Pseudomonas*, *Methylobacterium* strain have been reported for their potent role in the plant growth improvement in the presence of heavy metal [Sessitsch et al. 2013]. The rhizospheric soil-borne microbes play a significant role in plant cell division and elongation by secreting Indole compounds in low concentrations [Susilowati et al. 2002; Spaepen et al. 2007]. The metal-resistant sewage bacteria produce siderophore and phytohormones to help the survival of the plants in heavy metal contaminated soil. Siderophore protects the plant from the deleterious effects of soil heavy metals and also provides growth stimulators like iron to plant [Rajkumar et al. 2010]. Some stress-tolerant Gram-positive and Gram-negative microbe also form a biofilm with different exopolysaccharides (EPS) that convert the toxic heavy metal to the non-toxic form and protect the microbe and surrounding plants [Whitfield et al. 2015; Gupta and Diwan, 2017].

In this way, soil microbes protect plants in stressful environments by lowering the absorption of high-risk heavy metals and producing different growth inducers. This type of complex ecological relationship and its interaction with environmental abiotic factors should play a key role when implemented in environmental management.

Concluding remarks:

In several ways, the plant and its rhizospheric bacteria help each other and maintained a symbiotic association. Many of the bacterial genes have been co-evolved with plant in a conserved and stable manner and has considered as selection unit [Rosenberg and Zilber-Rosenberg, 2016]. Because of the dependence of the plant with their rhizospheric microbe, recently different long-term and short-term plant-microbial volt have been created which have tried to help out the plant to grow in consortia with their rhizospheric microbes and form microbiome just as in their natural habitat [Gopal and Gupta, 2019].

In the future, more investigation has to be required to check the expression of genes responsible for conducting the respiratory electron transport chain (ETC), chromium reduction pathway, and plant chromium accumulation. The cross-talk mechanisms of these genes might possess a considerable impact on human well-being and crop development.

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