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RESEARCH ARTICLE

APPLICATION OF BIOREMEDIATION IN MITIGATION OF HEAVY METAL STRESS ON PLANTS

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Abstract

Heavy metals are toxic metals which are persistent in nature due to their nondegradable nature.Due to increase in industrialization and urbanization heavy metals are being added to theenvironment and consequently causing heavy metal pollution of water and soil. These heavy metals cause significant stress on crop plants and effect various parameters such as growth and yield. Manymethods like physical, chemical and biological methods are available to degrade these heavymetals. Recently, bioremediation is being widely used because of its cost-effective andenvironment friendly approach. Bioremediation is a biological process which makes use ofmicroorganisms to remove hazardous substances or environmental pollutants from air, water,soil, industrial effluents etc. Bacteria and fungi are mostly employed for bioremediation.These organisms are genetically engineered for effective use in bioremediation. This not onlydiscussed about the importance of bacteria for bioremediation of heavy metals but alsodiscussed about the challenges and limitations of bacteria for bioremediation. In this chapter, a variety of mechanisms responsible for adaptation of microorganisms to high heavy metal concentrations, e.g. metal sorption, uptake and accumulation, extracellular precipitation and enzymatic oxidation or reduction, will be reported. Moreover, molecular mechanisms responsible for their metal tolerance will be described.

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Introduction:-

The rapid growth of industrialization is always coupled with the persistent problem of environmental pollution. Accumulation of toxic metals released by the industries entering the ecosystem may lead to biomagnification. Very few heavy metals function as trace elements but, many of them are of great concern in human biochemical processes because of their toxicity. In this respect, the important point that needs to be taken care of for the wellbeing of the ecosystem is the treatment of the processed wastes released into the environment from these industries. "Heavy metals" are a group of metals and metalloids with a specific gravity that is at least four to five times the specific gravity of water at the same temperature and pressure (1). High levels of heavy metals causedegradation of soil quality, leading to reduction of yield and quality of agricultural products, posing significant hazards to human, animaland ecosystem health. The crop plants growing in HM contaminated areas usually show low growth, substantial oxidative stress and genetic changes. These HMs also enter the crop produce and ultimately harm the human health. Heavy metal pollution of agricultural soils is a widespread environmental

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threat. Heavy metals induce deleterious effects on plants. In view of all these ill effects caused by HMs such as inhibition of germination, retardation of growth and metabolism, we should prioritize the need for sustainable techniques to mitigate its hazardous effects. So, it is an urgent necessity to remediate the plants.

The methods of HM remediation are broadly divided into three types physical, chemical and biological. The physical processes of HM remediation are leaching, precipitation, ion exchange, reverse osmosis, etc. Chemical methods are chemical oxidation, reduction chemical precipitation, etc. These technologies typically result in wastes that have high metal concentrations, which are a major cause of pollution in the environment. Moreover, the previously mentioned techniques could not be efficient or cost-effective, particularly in cases when the concentrations of heavy metals are below 100 mg/l (2).

Bio Remediation

Biological methods offer promising solution to HM remediation as they are known to be faster, cheaper and safer methods. In this context phytoremediation and the use of rhizosphere microbes have emerged as an important alternative to ensure high efficiency and better performance. While it has been known that plants provided with some strategies to avoid, tolerate and reduce the accumulation of heavy metals in their tissues (3), these mechanisms, however, have certain limitations and plants have to depend on rhizospheric bacteria for detoxification or heavy metal transformation mechanisms (4). It was experimentally proved by them in rice plants by killing bacteria with chloramphenicol that plants require rhizospheric bacteria for methylation of Arsenic (As) into a less toxic form. Several studies have shown that microorganisms have unique capacities to absorb and transform toxic heavy metals into less toxic forms (5).

Research on PGPB suggests that the usage of plant growth-promoting bacteria has begun to be a promising alternative for the alleviation of plant stress caused by heavy metals Scientists have developed several strategies to minimise the adverse effects of HMs on plant growth, including the application of metal accumulator plants, the use of nanoparticles, the use of biofilms and the recent application of Plant Growth Promoting Bacteria (PGPB) (6). Therefore, it is important to continually assess and monitor the levels of heavy metals in an environment and evaluate the effects of human exposure and should come up with probable solutions for sustainable environment.

Many studies suggest the minimization of heavy metal stress of plants by the application of PGPB. The main objective of this study is to assess the effect and mechanisms of PGPB inoculants of on improving growth, managing oxidative stress and improving the yield.

Bioaugmentation, bioventing and bioreactor are the possible biological methods of HM remediation. In this article bioremediation process is explained.

Microbial remediation:

Microbial organisms are very important and natural recyclers. Many bacteria growing in heavy metal contaminated areas possess mechanisms operates by them to survive in hostile environments and resistant genes acquired through horizontal gene transfer methods.

Various microorganisms are used for heavy metals biosorption.Saccharomyces cerevisiae (yeast), Streptococcus (bacteria) and Aspergillus niger (fungi) is widespread. Genetically engineered organisms can be generated which can likely to reduce different types of polycyclichydrocarbons (PAHs). **Flavobacterium,** Pseudomonas**, Bacillus, Arthrobacter, Corynebacterium, Mycobacterium, Stereumhirsutum, Pleurotus ostreatus, Azotobacter, Phormidiumvalderium, Ganoderma applantus** are some microbial species that help to remediate heavy metals more efficiently.

Different microbes have been proposed to be efficient and economical alternatives for the removal and transformation of heavy metals from soil and water. There were quite a few studies carried on with regard to bacterial application in remediation of phytotoxicity induced by heavy metals. Further exploration of bacteria is needed for more efficient methods under field conditions, as well as the understanding of resistance mechanisms are still required.

Mechanisms operated by bacteria

Microbial bioremediation is mainly about converting harmful substances into substances that are less toxic to human health and the environment. Bacteria found to have several distinct mechanisms including physiological, biochemical and genetic to deal with extreme environmental conditions.

It has been reported that some of heavy metal resistant-PGPRs can also reduce both uptake and transportation of H Ms to aerial parts of plant by decreasing metal bioavailability in soil. (7). Rhizospheric bacteria initiate mutualistic relationships with plants and minimize the stress. By improving nitrogen fixation, phytohormone synthesis, and mineral uptake, PGPRs also support plant growth. Since one of the most well-known effects of auxins is stimulation of rhizogenesis, the most common way that rhizobacteria are thought to stimulate root growth is through their ability to synthesize indole acetic acid (IAA), the most common, naturally occurring plant hormone of the auxin class (8). Additionally, studies demonstrating that Azospirillum mutants weak in auxin synthesis did not improve wheat root development further supported the significance of bacterial auxins in boosting plant root proliferation (9). Thus, mutational analyses of the significance of auxin in plant/microbe interactions gave consistent results across two bacterial genera and different plant species. PGPR can increase the tolerance of plants to salinity, acting primarily through the accumulation of osmolytes, increasing the absorption of nutrients, the nitrogen fixation, the solubilization of P and other essential elements, but similarly with the activity of ACC deaminase, the production of auxins, siderophores, and exopolysaccharides (10).

The mechanisms operated by bacteria to alleviate toxic effects on plants of important HMs are discussed below.

Arsenic (As)

Arsenic is known as one of the most important toxic metalloids. Anthropogenic sources of arsenic are mainly mining industries. Arsenic is available in both inorganic and organic forms. Inorganic forms primarily elemental form (As 0), arsenite (As III) and arsenate (As V). As (III) is much more toxic and mobile than As (V), and hence, microbial arsenic redox transformation has a major impact on arsenic toxicity and mobility which can greatly influence the human health. The organic form of arsenic is methyl arsenic acid (CH_3ASH_2). Usually As enters food chain of humans through water and crop plants cultivated soils.

Alleviation of As stress of plants by inoculation with As resistant bacteria

Bioremediation with special reference to bacteria hold promise to alleviate HM induced phytotoxicity. Among microbial approaches, oxidation, reduction and biomethylation are the major detoxification processes which have direct implications on amelioration of arsenic contaminated soils.

Bioremediation by microbes (bacteria, fungi& yeast) are quite effective and relies on deliberate action of natural or engineered microbial activity to reduce, mobilize, or immobilize, volatilize As through sorption, bio-methylation and redox reactions.

Molecular mechanisms of As bacterial remediation mainly catalize various metabolic reaction oxidation, reduction, methylation and volatization(11). The bioremediation processes carried by bacteria are the oxidation of more toxic As (III) to less toxic form As (V) with the help of an enzyme arsenic oxidase (AioBA). Thermus thermophiles, Th. Aquaticus and A. faecalis and Bacillus arsenic oselenatis are the effective bacteria employed in arsenic remediation. In furthering detoxification processes there are bacteria which convert inorganic arsenate to organic form of As. Bacteria convert more toxic form of methylated arsenate to less toxic methylated forms. This process is catalysed by an enzyme S. adenosyl methionine transferase. The final form of sequential methylation of arsenic is the trimethyl arsenic which was least toxic. The bacterial genes arsM genes present in bacteria are responsible for synthesizing the S. adenosyl methionine transferase enzyme.

The genome of several microorganisms metabolising As has been characterised from various ecosystems(12). Previous studies documented the arsenic resistant bacterium, Herminiimonasarsenicoxydans which is isolated from an industrial water treatment plant. It is resistant to high As concentrations and able to oxidise As(III) to As(V) (13). In another research, a Rhizobium strain isolated from a gold mine in Australia carries the genes involved in the resistance and detoxification of As on a plasmid. Such a genetic tool could be interesting from a phytoremediation perspective by transferring the As detoxification capacity to related plant-associated bacteria (14). In series of studies, arsenic tolerant strains are isolated which can bring about the oxidation ofarsenite to arsenate named as Halomonas A3H3 and Pseudomonas xanthomarina S11, respectively isolated from the Mediterranean Sea-contaminated sediments (15) and a French old gold mine (16).

Arsenic resistant bacteria could be utilized for promoting plant growth. By increasing the IAA production, siderophore production, potassium solubilization and P solubilization, the chromium resistant bacterial species Bacillus thuringiensis, B. ceres and B. Subtilis and Aspergillus niger PMI -118 in Cicer arietinunm plant. They improved growth of root and shoot and dry weight. Several experiments proved that As resistant bacteria regulate the arsenic induced oxidation stress and significant reduction of activity of antioxidant enzymes of SOD, CAT, APX and GPX. Inoculation of bacterial species such as Pseudomonas, Bacillus and Bruvindimonas(17)

Chromium (Cr)

Among the heavy metals, chromium is considered as one of the most toxic heavy metals, exists in Cr (III) and Cr (VI). being the most stable states. Cr (III)is less toxic and poorly mobile and is less toxic. however, Cr (VI) is highly soluble and mobile, is more toxic than Cr (III) and reported to be mutagenic, carcinogenic, and teratogenic (18).

Alleviation of Cr stress of plants by inoculation with Cr resistant bacteria

Recent studies evaluated the effect of application of bacteria in alleviating crop plants under chromium stress. Stenotrophomonas maltophilia, Bacillus thuringiensis B. cereus, and B. subtilis are the chromium resistant strains applied for this purpose. The treatment of Cicer seeds with these chromium resistant bacteria showed significant improvement in all the growth parameters by increasing the IAA production, Siderophore production, increasing the P and K solubility.

Chromium reductase enzyme in chromium resistant bacteria, such as Pseudomonas putida and Bacillus subtilis, can reduce the toxic Cr(VI) to the less toxic and immobilized trivalent form of chromium Cr(III) through reductive immobilization. (19).Plant growth is adversely affected by Cr due to its impairment of critical metabolic processes. Reactive oxygen species (ROS) production is linked to the harmful effects of Cr and results in oxidative stress in plants. Changes in many physiological and biochemical processes have been noted in Ocimumtenuiflorum (20), Vallisneria spiralis (21) and Triticum aestivum (22), and where the production of MDA by Cr metal induced the degradation of membrane permeability.

Inoculation of wheat seeds with a bacterium strain resistant to Cr, Staphylococcus aureus strain K1 has shown the detoxification potential of chromium-induced stress by increasing growth parameters and the production of carotenoid and chlorophyll. The mechanisms behind the reduction of bacterial Cr6+ are particularly important because they convert harmful and mobile chromium derivatives into reduced species that are harmless and less mobile (23). Microbial cell walls are mainly composed of polysaccharides, lipids and proteins, providing many functional groups that can bind heavy metal ions, including carboxylate, hydroxyl, amino and phosphate (24).

Mercury (Hg)

Mercury (Hg) is the most poisonous because of its non-degradable nature. Mercury is a highly toxic trace metal that is ubiquitous in nature (25) and is the main cause of severe pollution all around the world (26).

Accumulation of heavy metals more particularly mercury in agriculture soils has become a major concern for food crop production. Once they enter the plant body they start to show visible toxic symptoms.

Since autotrophic plants function as the primary and principal entry point of heavy metals, they get accumulated once they enter the food chain. Hence, a significant amount of these heavy metals is found in the animal body leading to biomagnification. Though reports related to the mechanism of accumulation of mercury in plants and their tissue distribution are scanty, literature pertaining to the effect of mercury on the plant systems are plentily available in comparison to animal systems. Some of the possible mechanisms through which mercury can impair important biochemical and physiological processes in living organisms include inhibition of germination (27), a decrease of biomass production (28), inhibition of photosynthesis (27), hindrance of protein function and induction of oxidative stress (29) anddamaging effect on DNA (30).

Alleviation of Hg stress of plants by inoculation with Hg resistant bacteria

There are many instances to isolate mercury resistant bacteria from contaminated areas. Mercury tolerant bacterial strains were isolatedfrom mercury contaminated areas and identifiedthe bacterial strain showing high mercury resistance which showed growth up to 600 ppm mercury chloride concentration in growth medium. Based on the high tolerance, they identified the bacterial isolate named as HG 2 that showed tolerance to 600 ppm mercuric chloride concentration. This bacterial isolate identified as Brevundimonas by phylogenetic analysis using the MEGA 6.0 software using 16S r RNA analysis (31).

Results of mercury stress mitigation experiments (32), showed improved growth of Cajanus plants under mercury stress caused by mitigating effect of Brevundimonas bacterium coupled with increased production of IAA. The seeds of the pigeon pea plant under Hg stress were inoculated with mercury resistant Bacillus sp. bacterium observed to show enhanced rates of germination and growth in terms of length and biomass compared to Hg exposed pigeon pea seeds (untreated).

Rhizospheric bacteria play a great role in alleviation of stress by means of accumulation and transform them into less toxic forms. There are many instances of minimization of stress by using bacterium to improve plant growth parameters (32). Enhanced production of oxidative enzymes is an important parameter in evaluation of heavy metal stress. Oxidative stress is a direct mechanism of plants facing heavy metal stress. Oxidative stress is the increased production of oxygen free radicles. Reactive oxygen species damage enzymes and cell membranes by binding with them. An experiment conducted on pigeon pea(33) has proved that when seeds exposed to stress are treated with Hg resistant Brevundimonas bacterium showed lowered production of antioxidant enzymes such as CAT, POX and SOD and also reduced lipid peroxidation. In Cajanus seeds exposed to mercury stress. The plant antioxidant response has been analyzed by quantifying the catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) enzyme activity in pigeon pea seedlings exposed to mercury and different PGPB treatments. These studies indicated that the bacteria acted as a shield and protect the Cajanus plants from mercury toxicity and hence extent of lipid peroxidation and antioxidant enzyme activities, MDA content and proline content were increased in the mercury exposed pigeon pea and a parallel reduction was observed in Brevundimonasbacterium treated pigeon pea seedlings under mercury stress indicate that these plants faced less stress in presence of Brevundimonas bacterium.

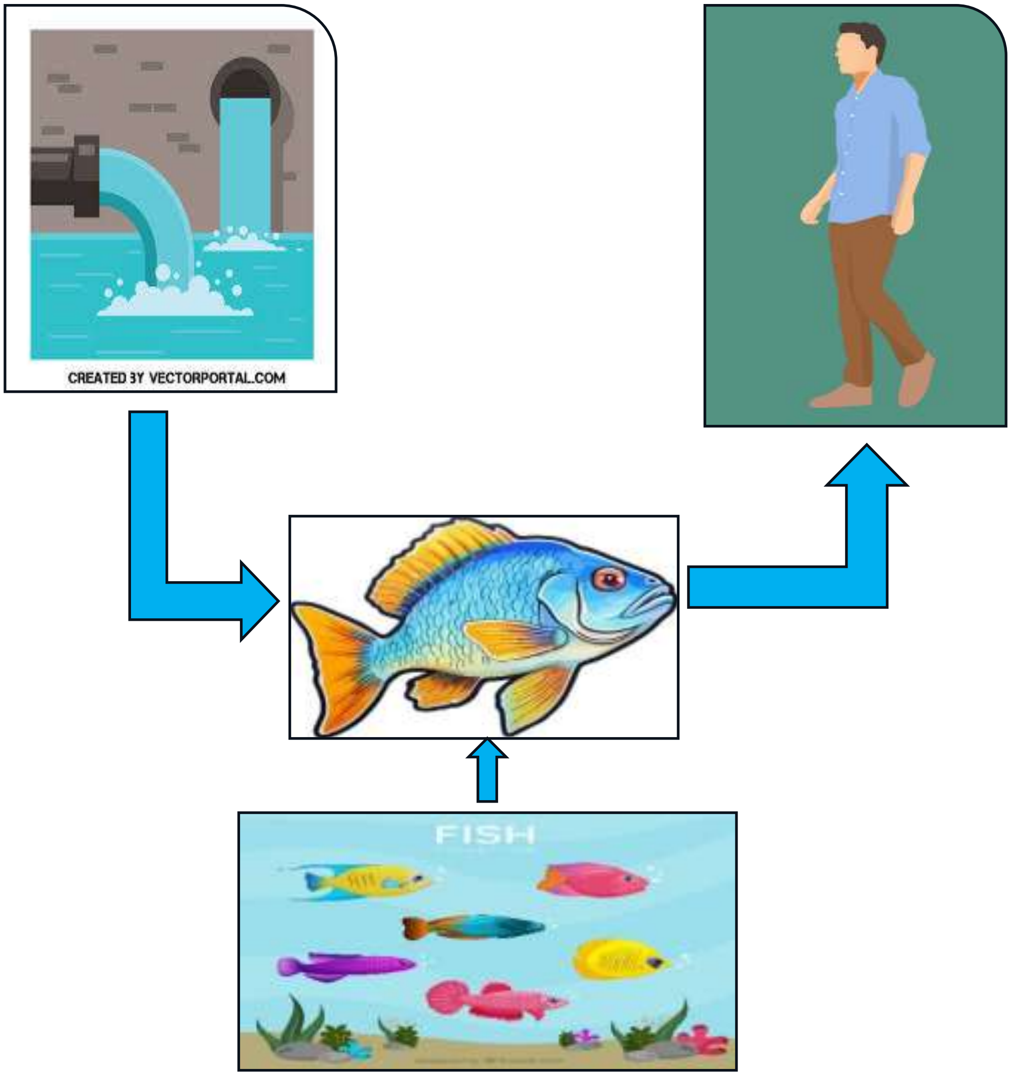
Studies conducted to know the mechanisms of mercury resistance showed that resistance is not due to genes responsible for detoxification. In this research it was identified that two membrane proteins account for conferring mercury resistance. These proteins make the membrane impermeable to mercury compound may be responsible for the mercury resistance of the strain (34).

Enzymatic reduction of Hg^{2+} to Hg^0.

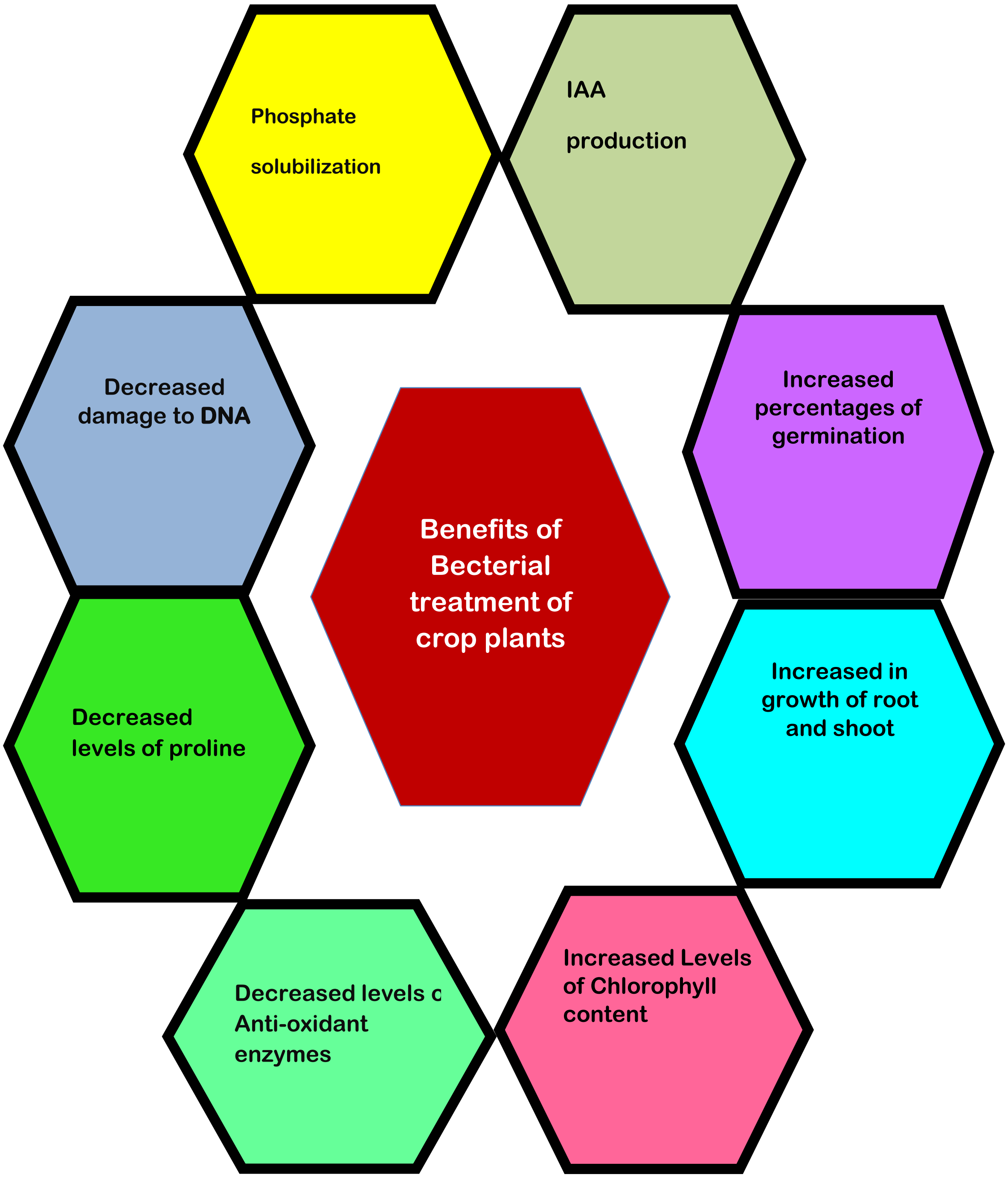
The central enzyme in the microbial mercury detoxification system is the mercuric reductase protein encoded by mer A genes, which catalyzes the reduction of Hg(II) to volatile Hg(0). In addition to merA, mer operons encode for proteins involved in regulation, Hg binding and degradation of organomercury. The detoxification processes operated by mercury resistant bacteria are mainly due to mer operon which consists mer A and the two regulatorgenes (merR and merD) in Pseudomonas putida. In this no mer B gene is detected (35).

The possible mechanisms operated in bacteria for the mitigation of heavy metal stress in crop plants treated by heavy metal resistant bacteria were represented diagrammatically for the quick understanding.

Mercury pollution cycle



Biomagnification


Phosphate
solubilization
IAA
production
Decreased damage to DNA
Benefits of Becterial treatment of crop plants
Increased percentages of germination
Decreased levels of proline
Increased in growth of root and shoot
Decreased levels o
Anti-oxidant enzymes
Increased Levels of Chlorophyll content

Conclusions:-

The growth and output of crop plants are significantly altered by heavy metal pollution in agricultural soils, which eventually affects human health. Thus, it is necessary to look for mitigation techniques for the harmful effects that heavy metals have on plants. As a potential treatment option, rhizospheric bacterial remediation shields plants from heavy metal stress. In addition, intriguing insights can be gained from techniques for separating metal-resistant microorganisms for bioremediation from contaminated areas. These bacteria use a variety of strategies and genetic mechanisms to mitigate the harmful effects caused by heavy metals to plants.

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