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Phytoremediation of heavy metals principles, mechanisms, enhancements with several efficiency enhancer methods and perspectives: A Review

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ABSTRACT

Heavy metals pollution is one of the hot areas of environmental research. Despite natural existence, various anthropomorphic sources have contributed to an unusually high concentration of heavy metals in the environment. Contaminated soils with heavy metals is a widespread environmental issue resulting from global industrialization. Conventional techniques for reclamation of contaminated soils are expensive and environmental non-friendly. Phytoremediation is a technique utilizing green plants to clean up the environment from contaminants and is offered as a cost-effective and non-invasive alternative to the conventional engineering-based remediation methods. It is a viable, relatively low cost approach to removing heavy metals from contaminated sites, and is aesthetically pleasing approach most suitable for developing countries.. The mechanisms by which plants promote the removal of pollutants are varied, including uptake and concentration, transformation of pollutants, stabilization, and rhizosphere degradation, in which plants promote the growth of bacteria underground in the root zone that in turn break down pollutants. Integrated processes are the combination of two different methods to achieve a synergistic and an effective effort to remove heavy metals. Most of the review articles published so far mainly focus on individual methods on specific heavy metal removal, that too from a particular environmental matrix only. However, more research focus on the process is needed to challenge the in situ operative conditions.

Keywords: Phytoremediation aromatic halophyte plants, heavy metals, environmental contamination

Introduction

Environmental contamination is of serious ecological concern worldwide with a continually rising public outcry to ensure the safest and healthiest environment. Organic and inorganic pollutants have been reported to cause environmental pollution and severe health hazards in living beings Maszenan *et al*. (2011), Saxena and Bharagava (2017). Among them, heavy metals are highly notorious pollutants due to their high abundance and non-biodegradable and persistent nature in the environment. Its cause soil/water pollution and toxic, genotoxic, teratogenic, and mutagenic effects in living beings Dixit et al. (2015); Sarwar *et al*. (2017). They also cause endocrine disruption and neurological disorders even at low concentration Yadav (2010); Maszenan *et al*. (2011); Dixit *et al*. (2015), Sarwar *et al.* (2017). Any naturally occurring metal/metalloid having an atomic number greater than 20 and elemental density greater than 5 g cm^{-3} is termed as heavy metal. They include copper (Cu), cadmium (Cd), chromium (Cr), cobalt (Co), zinc (Zn), iron (Fe), nickel (Ni), mercury (Hg), lead (Pb), arsenic (As), silver (Ag), and platinum group elements Ali *et al*. (2013); Ali and Khan (2018a). Among them, Cd, As, Hg, and Pb do not have any biological function in the body and thus, are nonessential elements. They can cause severe health hazards and are listed as priority pollutants by many environmental protection agencies worldwide Jaishankar *et al*. (2014); Dixit *et al.* (2015); Sarwar *et al.* (2017). Therefore, the removal of heavy metals from the contaminated sites is an urgent need to safeguard the environment and human health.

Phytoremediation has been identified as an emerging, low-cost, and eco-sustainable solution for heavy metal pollution prevention and control. It is the most suitable alternative to conventional physicochemical remediation technologies, which are highly expensive and technically more suited to

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small areas, create secondary pollution and deteriorate soil fertility, and thus adversely affect agro ecosystem Ali *et al*. (2013); Chandra *et al*. (2015); Mahar *et al*. (2016); Muthusaravanan *et al.* (2018). Phytoremediation is the engineered use of green plants with associated soil beneficial microbes to remove toxic pollutants through degradation and detoxification mechanisms from contaminated soil and water/wastewaters Bharagava *et al*. (2017a); Mukhopadhyay and Maiti (2010); Ali *et al*. (2013). It is an eco-friendly, non-intrusive, and aesthetically pleasing remediation technology that removes metal pollutants from the contaminated sites Lee (2013); Chandra *et al*. (2015); Chirakkara *et al*. (2016). It can be commercialized, and income can be generated, if metals removed from contaminated sites could be used to extract usable form of economically viable metals (i.e., phytomining) Chandra *et al*. (2015); Mahar *et al.* (2016). In addition, energy can be generated through the burning of plant biomass, and land restoration could be achieved for sustainable agricultural development or general habitation Stephenson and Black (2014), Mahar *et al*. (2016). The rationale, mechanisms, and economic feasibility of phytoremediation have been discussed elsewhere Ali et al. (2013); Wan et a*l*. (2016), Sarwar *et al*. (2017). However, extensive research is currently underway to testify the phytoremediation potential of hyperaccumulating plants at field scale for the treatment and management of heavy metal contaminated soils. This review highlights better understanding of the problems associated with the toxicity of heavy metals to the contaminated ecosystems and their viable, sustainable and eco-friendly bioremediation technologies, especially the mechanisms of phytoremediation of heavy metals, to briefly review basic processes of phytoremediation with special emphasis on rhizore-mediation and plant-microbe interactions in plant–assisted biotransformation of organic and inorganic pollutants in soil. In addition, the potential and challenges of phytoremediation strategy for enhanced removal of organic and inorganic pollutants from water in treatment wetlands are addressed.

2-Heavy Metals Contamination, Sources and Toxicity

Heavy metals, especially Cu, Ni, Cd, Zn, Cr and Pb considered as one of the main sources of soil pollution Hinojosa *et al.,* (2004) and Karaca *et al.,* (2010). Heavy metals affect microorganism's behavior in soil. They change in their population size, diversity and in their activities in the soil Ashraf and Aly, (2007).Increasing Pb in soil decrease soil productivity and may inhibit some vital plant processes *i.e.* photosynthesis, mitosis and water absorption with toxic symptoms Battachryya e*t al.,*(2008). Heavy metals uptake by plants from heavy metals contaminated soils resulted in a great health risks considering food chains implication Jodao, *et al.,* (2006). The consumption of heavy metals contaminated foods can seriously depleted some essential nutrients in body, which responsible for decreasing immunological defense system, intrauterine growth retardation, and disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates Khan, *et al.,*(2008).

Moreover, heavy metals are not subject to bacterial degradation and hence remain permanently in the marine environment Woo *et al.,* (2009). Contamination of a river with heavy metals may cause devastating effects on the ecological balance of the aquatic environment, and the diversity of aquatic organisms becomes limited based on contamination level Ayandiran, *et al.,* (2009). Heavy metals contaminants in aquatic systems stimulate the production of reactive oxygen species (*ROS*) that can damage fishes and other aquatic organisms Woo *et al.,* (2009).The consumption of fish containing elevated levels of heavy metals is a concern and cause health problems Morin *et al*., (2007).

Heavy metals in soil take place naturally from pedogenetic weathering processes of parent materials at concentrations considered traceable and rarely poisonous Pendias and Pendias, (2001),. Because of human interference, most heavy metals in rural and urban soils can accumulate to pose hazards to human health, crops, livestock and ecosystems D'Amore *et al.,* (2005). Excess heavy metals in the soil originate from many sources, which include atmospheric deposition, animal's wastes, and sewage irrigation, improper stacking of the industrial solid waste, mining activities, the use of long-lived pesticides, herbicides, fungicides, nematocides etc. Fig (1). In addition, fertilizers and some other agriculture practices.

Fig. 1: Illustrates sources of environmental contamination (after Antoniadis *et al*. (2017).

Heavy metals in the atmosphere are mainly from gas and dust produced by energy, transport, metallurgy and production of construction materials. Excepting mercury, heavy metals go into the atmosphere in the form of aerosol and deposit to the soil through natural sedimentation and precipitation.

Natural or anthropogenic processes can introduce heavy metals into the environment either. Natural processes are geological activities, for instance, mineral weathering, erosion, volcanic eruptions, and continental dust. Anthropogenic activities include industrial operations such as mining, smelting, electroplating, and industrial effluent discharge as well as agricultural practices like use of pesticides and phosphate fertilizers and release of agricultural wastes Ali *et al*. (2013); Mahar *et al*. (2016.

Industrial activities are the major source of heavy metals pollution (water and soil) in the environment. If heavy metals enter the food chain, they may bio accumulate and/or bio magnify at higher trophic levels resulting in severe health threats and thus are of serious ecotoxicological concern. The indiscriminate discharge of toxic metal-rich industrial effluents is one of the major sources of environmental pollution. The effluent discharged from metal-based industries, especially leather industries (Cr used in leather tanning), causes serious soil and water pollution, and hence, its treatment and management is a key challenge to pollution control authorities Sahu *et al*. (2007); Saxena *et al*. (2016). Beg and Ali (2008) reported that high concentration of heavy metals in sediments of river Ganga and its tributaries receiving Cr-loaded tannery effluent. In addition, heavy metals beyond the permissible limits also deteriorate water quality and make it unfit for drinking and irrigation purpose Nazeer *et al*. (2014). The effluent released from electroplating and distillery industries also constitutes a highly rich source of heavy metals and, hence, is considered as hazardous to living beings Venkateswaran *et al*. (2007); Chandra *et al*.(2008). Furthermore, effluent released from domestic activities is also responsible for heavy metal pollution and thus is of serious ecotoxicological concerns Bhardwaj et al. (2017).

In an aquatic ecosystem, heavy metals adversely affect gamete production, sperm quality, and embryonic development, delay hatching, and cause physical deformities in fishes that ultimately leads to the death of newly hatched larvae Segura et al. (2006); Jezierska *et al*. (2009); Fatima *et al*. (2014). Heavy metals also cause endocrine disruption, oxidative stress and genotoxicity in fishes Jezierska et al. (2009); Łuszczek- Trojnar *et al*. (2014); Javed *et al*. (2016). Further, heavy metals also cause a reduction in hematological parameters and glycogen reserve and thus make the fishes weak, anemic, and vulnerable to diseases Javed and Usmani (2015).

Soil is a nonrenewable resource for sustainable agriculture and acts as a major sink for heavy metals. Contamination of agricultural soil with toxic metals affects its physicochemical and biological properties, reduces land usability for agricultural farming leading to food insecurity, and thus creates land tenure problems Wuana and Okieimen (2011). Moreover, the coexistence and persistence of heavy

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metals in soil are also responsible for the entry of toxic metals into the food chain and thus lead to severe health hazards in living beings Khan *et al*. (2008). Heavy metals inhibit several microbial metabolic processes such as respiration, denitrification, and enzymatic activity and hence retard the bioremediation processes Zhuang *et al*. (2007); Sobolev and Begonia ((2008). Heavy metals also cause a reduction in the number of specific microbial populations and a shift in the microbial community structure. Ding *et al*. (2016) evaluated the effect of Cd and Cr on the microbial community structure in the rhizospheric soil of rice, they observed that the relative abundance of a bacterial genus *Longilinea* was significantly higher in the control soil than in Cd- and Cr- treated soils, whereas the relative abundance of the genus *Pseudomonas* was significantly higher in the Cd-treated soils than in the Cr-treated and control soils.

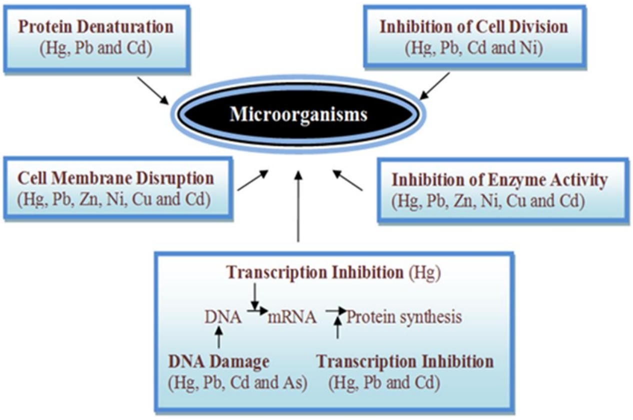

Fig.2: Anthropogenic activities leading to the contamination of soils with heavy metals

Heavy metals also inhibit the cell division, transcription process, and denaturation of protein and adversely affect the cell membrane distribution in microbes Jacob *et al*. (2018) as presented in Fig. (2).Hexavalent chromium (Cr^{+6}) is also reported to cause DNA damage by exerting oxidative stress Abou seeda et al. (2020) in soil bacteria and thus leads to genotoxic effects Quievryn *et al*. (2003).The irrigation of food crops in the agriculture field with water contaminated with toxic metal-rich industrial effluents is a common practice in many developing countries. It may provide a chance for the movement of potentially toxic metals from contaminated soil to edible crops, which ultimately reach into the human/animal body via consumption and thus render severe toxic effects.

Heavy metals affect various metal-sensitive enzymes in plants such as alcohol dehydrogenase, nitrogenase, nitrate reductase, amylase, and hydrolytic (phosphatase and ribonuclease) and carboxylating (phosphoenolpyruvate carboxylase and ribulose-1, 5-bisphosphatecarboxylase) enzymes Nagajyoti et al. (2010), Yadav (2010). Several biochemical/physiological processes in plants, were disrupt and inhibited by heavy metals such as seed germination, enzymatic activities, nitrogen metabolism, electron transport system, transpiration,CO_2 assimilation, antioxidant defense system, photosynthesis, photophosphorylation, cellular metabolism, nitrogen fixation, water balance, mineral nutrition, and cellular ionic homeostasis, which ultimately leads to plant's death Abou seeda (2020), Yadav (2010) ; Lajayar *et al*. (2017). Irrigation of agricultural with sewage effluents and industrial effluents (heavy metal-loaded) disrupts and inhibits also several cytological processes in plants such as root growth and elongation, cell membrane permeability, mitotic activity, and the stability of genetic material and also creates chromosomal abnormalities Nagajyoti *et al*. (2010); Yadav (2010). For example, the irrigation of agricultural crops with the heavy metal -rich distillery and tannery effluent has been reported to cause a reduction in root/shoot growth and biomass, seed germination, and seedling growth and also induced chlorosis and photosynthetic impairment Chandra *et al*. (2009); Bharagava *et al*. (2017b) Abou seeda, (2019) (2020).Heavy metals may cause oxidative stress by forming reactive oxygen species (ROS),which disrupt the antioxidant's defense system, lead to cell damage in humans / animals, and in extreme cases can be fatal Jaishankar *et al*. (2014). For instance, Mishra and, Bharagava (2016) reported that hexavalent chromium (Cr^{6+}) cause cancer in humans and damage cellular components during its reduction into trivalent chromium (Cr^{3+}), leading to generate radicals that cause DNA damage as presented in Fig.(3).

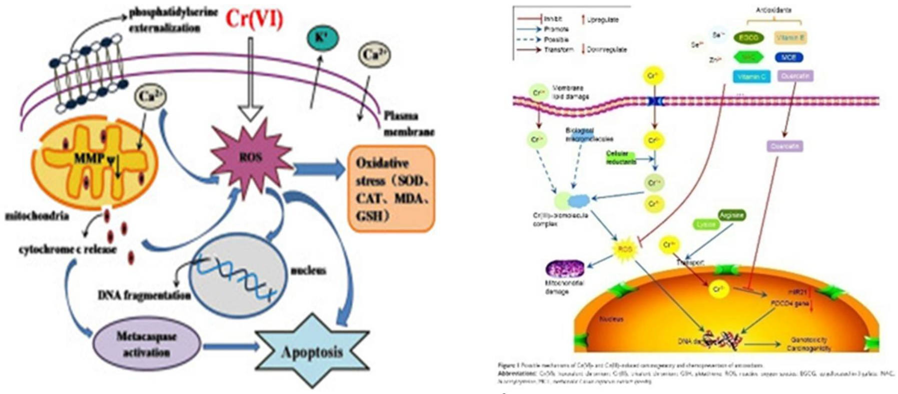

Fig. 3: Schematic diagram of hexavalent chromium (Cr^{6+}) during its reduction into trivalent chromium (Cr^{3+}), leading to the generation of free radicals that cause DNA damage

3- Toxicity of heavy metals transfer and their consequences

Bio transference (trophic transfer) is an ecological phenomenon through the contaminant enters the food chain by uptake either from ambient abiotic environment(bio concentration) or both ambient abiotic environment and organism's food/diet (bioaccumulation) and passage from one trophic level to the next higher trophic level (bio magnification) and consequently, poses risks to human/animal health Aliand Khan (2018a,& b).The trophic transfer of toxic heavy metals from soil to plants to humans and organism's food to humans.

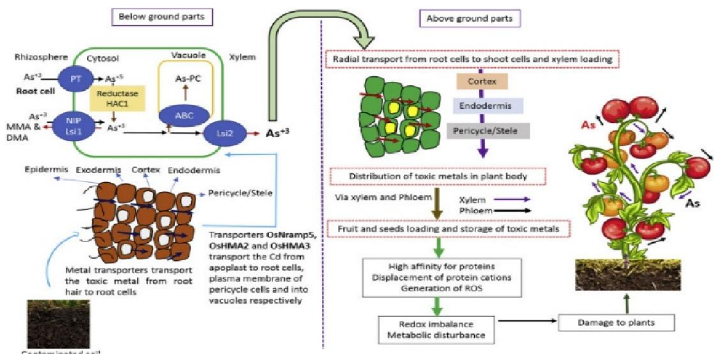

Fig. 4: Schematic diagram representation of hazardous heavy metals contamination of vegetables and food

The primary route of heavy metal entry into the food chain is, through the soil /plant transfer mechanism as presented in Fig (4). In the soil /plant transfer mechanism, heavy metals are transferred from soil to agricultural crops/vegetables that constitute a large source of human diet and thus, may result in catastrophic health hazards. According to a study, the daily intake of metal (DIM) was higher for vegetables grown on soils irrigated with heavy metal-rich, wastewater as compared to control soils Jan *et al*. (2010). Thus, the trophic transfer, bioaccumulation, and bio- magnification of toxic heavy metals in food chains have important implications for wildlife and human health.

4- Phytoremediation approaches for environmental cleanup

Environmental contamination increased due to, industrial revolution and excessive population growth, posing major environmental and human health problems Abdelhafez and Li, (2014).Several contamination sources such as emissions from waste incinerators, car exhaust, residues from mining and military activities, the smelting industry and the use of agricultural amendments (sludge or urban composts, pesticides, and mineral fertilizers Abou-Shanab (2011); Abdelhafez *et al.,*(2012). Organic contaminants, heavy metals are not biodegradable, and pose a critical concern to living organisms and

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the environment. In addition to their action as carcinogenic and mutagenic compounds Diels *et al.,*(2002).Heavy metals in soils at high concentrations affected negatively the growth and agricultural productivity Roy *et al.,*(2005). Due to heavy metals impact on plant cellular activities, plants differ in their tolerance Peixoto *et al.,* (2001); Hall, (2002). Yan-de *et al.,* (2007) reported that Cd, Cr, Cu, Hg, Pb and Zn are the most common heavy metal contaminants. These metals cannot be easily degraded to harmless products, such as carbon dioxide, and the cleanup usually requires their removal from the contaminated areas Lasat, (2002).

Several remediation technologies are available for reclaiming contaminated soils. Generally, the remediation technologies can be classified into three major groups, physical, chemical and biological technologies. However, most of the remediation technologies are expensive, need intensive works, may generate secondary contaminants to the surrounding environment Marques *et al.,* (2009); Haque *et al.,* (2008), or may lead to adverse effect to biological activities, soil structure and infertility problems Pulford and Watson, (2003). Therefore, there is a need for a less expensive and environmental friendly clean-up technique. Phytoremediation is a promising, economically and effective technology through using plant species to decontaminate aquatic or terrestrial sites Salt *et al.,*(1998), Abou-Shanab *et al.,* (2007), (2008)and (2011) Chirakkara, and Reddy, (2015). This technology is cost effective, simple and environmentally friendly with minimal environmental disruption. Abou seeda *et al*. (2019) Fig. (5).

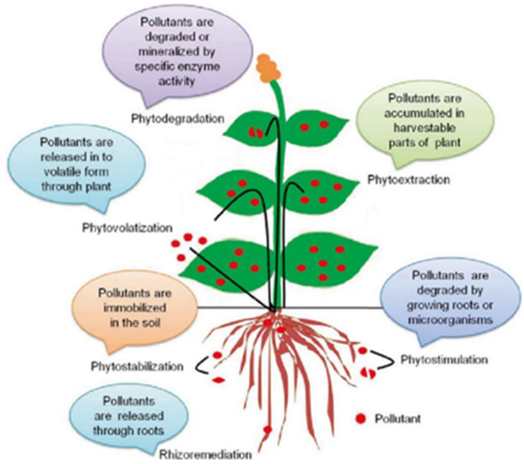

Fig. 5: Schematic diagram of different approaches of phytoremediation

Green remediation, botanoremediation, agro remediation, or vegetative remediation can be describe as in situ remediation strategy that uses vegetation and associated micro biota, soil amendments, and agronomic techniques to remove, contain, or render environmental contaminants harmless Cunningham and Ow, *et al*., (1996) and Chaney, *et al*., (1997). Phytoremediation is energy efficient, aesthetically pleasing method of remediating sites with low to- moderate levels of contamination, and it can integrated with other technology as a finishing step to the remedial process.
Hyperaccumulator plant species are used on metalliferous sites due to their tolerance of relatively high levels of pollution. Approximately 400 plant species from 45 plant families have been, reported to hyperaccumulate heavy metals Lasat , (2000) and Ghosh and Singh, (2005), some of the families are *Brassicaceae, Fabaceae, Euphorbiaceae, Asterraceae, Lamiaceae, and Scrophulariaceae* Salt, *et al*., (1998); Dushenkov, (2003). Crops like alpine pennycress (*Thlaspi caerulescens),* Ipomea alpine*, Haumaniastrum robertii, Astragalus racemosus, Sebertia.* Willow *(Salix viminalis L.),* Indian mustard *(Brassica juncea L.),* corn *(Zea mays L.), and* sunflower *(Helianthus annuus L.)* have reportedly shown high uptake and tolerance to heavy metals Schmidt, (2003).

Phytoremediation can be classified into different applications, such as (*a*) rhizofiltration, (*b*) phytoextraction, (*c*) phytovolatilization, (*d*) phytodegradation, and (*e*) phytostabilization. Aided phytostabilization is a biological method included in the Gentle Remediation Options *(GRO),* which, among others, are safer and least interfere with the natural environment Gołda and Korzeniowska, (2016), Abou seeda (2019). Phytoextraction plants can absorb the concentrated metals in their aboveground parts that can then be harvested. Phytodegradation is also known as phytotransformation, plants degrade organic pollutants directly via their enzymatic activities. Some enzymes break down and convert ammunition wastes, others degrade chlorinated solvents such as trichloroethylene, and others degrade herbicides.

Phytovolatilization refers to the uptake and transpiration of contaminants, primarily organic compounds, by plants, the contaminant, present in the soil solution, is taken up and modified by the plant and is released to the atmosphere through the plant leaves by evaporation or vaporization processes. Phytostimulation refers to stimulation of rhizospheric microorganisms capable of degrading the contaminants by the growing roots releasing exudates/nutrients such as carbon sources; this method is useful for removing organic contaminants, such as pesticides, aromatics, and poly-nuclear aromatic hydrocarbons from soil and sediments. Phytostabilization by using plant roots for reducing the mobility or leaching the contaminants in the soil, plants can decrease the amount of percolating water through the soil profile, which may act as a barrier and reduce the leaching of the contaminant. Phytostabilization can occur through sorption, precipitation, complexation, or metal valence reduction. It is helpful in the treatment of contaminated land areas affected by mining activities. Rhizofiltration used for metals such as Pb, Cd, Cu, Ni, Zn, and Cr, which retained within the roots. It is useful for both terrestrial and aquatic plants for in situ or ex situ purposes and not translocate to the shoots. Rhizoremediation is the integration between plants and microorganisms since organic pollutants characterized by high hydrophobicity (hence, unable to be absorbed by the plant) are remediated by this method. Microbes can play major role for remediation process.

This technique is based on the chemical stabilization of heavy metals using various non-organic and/or organic soil additives in connection with using the proper plant species Radziemska *et al*., (2017). , which will be adapted to specific conditions prevailing in the soil, such as low pH and high concentrations of heavy metals. Moreover, such plant species should not accumulate heavy metals in their aboveground parts, thus preventing their further passage to subsequent elements of the food chain, and should be characterized by a fast increase in biomass, ensuring good coverage of the area in a short period of time Gil Loaza *et al*., (2016). An example of such plants are grasses from the fescue family of grasses, which are commonly used to create a vegetation cover in post-mining areas and slag heaps. Various species of grass, such as red fescue (*Festuca rubra* L.) are the most useful in the process of the aided phytostabilization of heavy metals in soils Gil Loaza *et al*., (2016). Some literature reports Radziemska *et al*., (2017); Golda and Kozeniowska (2016) show that *F. rubra* is a suitable species for the vegetation of metal-contaminated soils contaminated by industrial activities such as mining, energy, and fuel production. Furthermore, *F. rubra* has the ability to accumulate Cu, Pb, Mn, and Zn from contaminated soils Wong et al., (1994); Padmavathiamma and Li (2009); Yin *et al*., (2014).

The phytoextraction efficiency of green plants primarily depends on the bio concentration factor *(BCF)* and translocation factor *(TF).* Bio concentration factor represents metal concentration in root/soil and denotes metal accumulation, whereas TF represents metal concentration in shoot/root and denotes metal translocation Goel *et a*l. (2009); Ali *et al*. (2013), Antoniadis *et al*. (2017). Plants with high biomass, fast growth rate,, high metal tolerance and accumulation are chiefly preferred for metal's phytoextraction Mukhopadhyay and Maiti (2010); Lee (2013), Chandra *et al*. (2015).Phytoextraction is performed in two different ways natural through metals accumulate under natural conditions and induced or assisted by using enhancers to increase metal accumulation. Enhancers are used to increase the phytoextraction efficiency and include chelators or soil amendments Sarwar *et al*. (2017). Chelators are the organic and mineral acids that increase the bioavailability of insoluble or unavailable form of metals in soil making them available for plant uptake Ali *et al*. (2013); Mahar *et al*. (2016), thus, enhancing the phytoremediation efficiency by solving the low metal phytoavailability issue.

The ability of chelators to enhance the metal accumulation in plants has reported by many researchers among them Xie *et al*. (2012); Ramamurthy and Memarian (2014); Sun *et al*. (2015); Chirakkara *et al*. (2016). Organic soil amendments are cheaper, eco-friendly, and non- or less toxic and degradable in nature. These help to minimize environmental pollution and reduce toxicity to remediating plants and ultimately enhance phytoremediation efficiency Wiszniewska *et al*. (2016). Some specific kinds of organic soil amendments include agro- and industrial wastes such as compost, humic substances, plant extracts, and exudates and are of great significance in heavy metals phytoremediation Wiszniewska *et al.* (2016). The use of organic soil amendments to enhance the phytoremediation efficiency has been evaluated Park *et al*. (2011); Paz-Ferreiro *et al*. (2014); Wiszniewska *et al*. (2016); Chirakkara *et al*. (2016); Reddy *et al*. (2017).The biotic and abiotic factors also affect the efficiency of phytoremediation (Fig. 6). Biotic factors include plant and root zone characteristics, whereas abiotic factors comprise pollutant and chelators characteristics, properties of the medium (e.g., soil), and climate conditions. The environmental risks associated with synthetic chelators such as low biodegradability of chelators, will cause the groundwater contamination due to leaching. However, to minimize the associated environmental risks and toxic effects selection of chelators with optimum dose and application time could help.

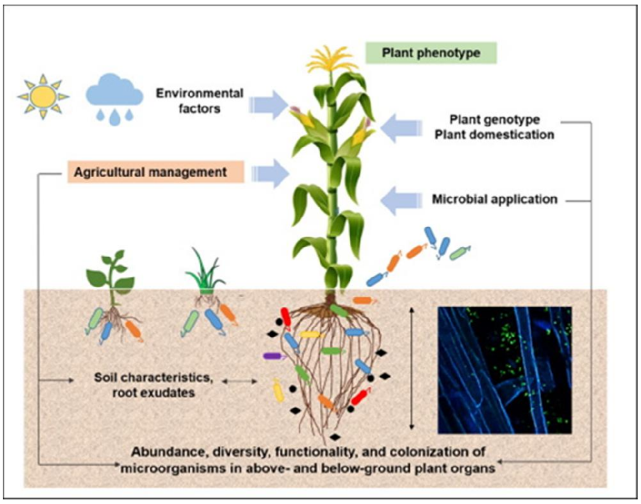

Fig. 6: Illustrates factors affecting phytoremediation efficiency

5-Phytoremediation of heavy metals using hyperaccumulating plants

5.1 Classification of metallophytes

According to growth potential in heavy metals-contaminated sites, metallophytes can be grouped into deferent categories: (*a*) metal excluders (heavy metals accumulating in roots but restrict transport and entry into their aerial parts possibly by altering cell membrane permeability and changing cell wall metal-binding capacity via modulating ionic channels, ion pump activity, and activation of new ionic conductance or exudating more chelating substances in soil); (*b*) metal indicators (accumulating metals in their aerial parts by releasing intracellular metal-binding chemicals, i.e., chelators, or altering the pattern of metal compartmentalization by storing them in non-sensitive plant parts such as vacuoles and cell wall and generally reflect metal concentration in soil); and (*c*) metal accumulators (accumulating exceedingly large concentration of metals from the soil in the aboveground plant parts, especially leaves with no symptoms of phytotoxicity).Fig. (7)

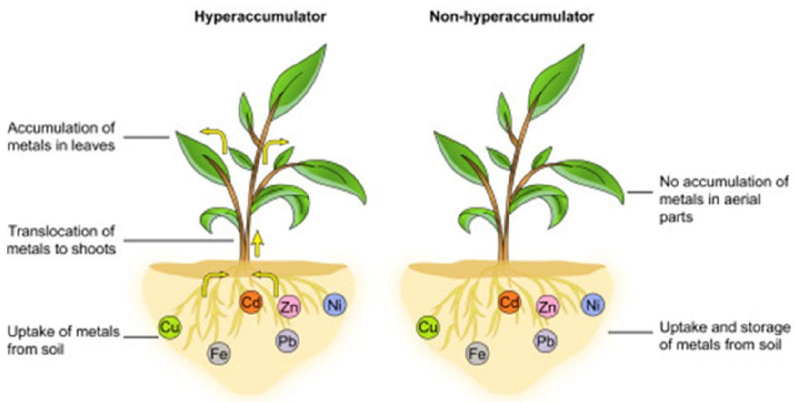

Fig.7: Schematic diagram between hyperaccumulators and non-hyperaccumulators plants for phytoremediation

Forest ecosystem usually possessed unique vegetation and/or species composition coupled with high rates of species endemicity. However, due to high mineral content, ultramafic soils are exploited to mining hence, among the extremely threatened ecosystems. Ultramafic soils contain high concentrations of heavy metal elements and are classified as metalliferous soils, which is extremely poor conditions. Plants can adapted to extreme poor conditions and are called metallophytes. Metallophytes growing naturally on metal-rich soils accumulating heavy metals in their biomass without effects on physiological processes, or exhibiting phytotoxicity Baker and Brooks, (1989); Reeves and Baker, (2000). Some metallophytes can accumulate very high concentrations of metallic or metalloid elements in their aerial tissues to levels exceeding their normal physiological requirement Reeves, (2003). Hyperaccumulators represent plant tolerance to extremely hostile edaphic environments and would kill many other species Bondada and Ma, (2003). Lambinon and Auquier (1964) differentiated the plant species colonizing metalliferous soils into metallophytes and pseudo-metallophytes. Metallophytes are species found only on metal-rich soils while the pseudo-metallophytes found on both contaminated and uncontaminated soils. According to Baker and Brooks (1989), metal hyperaccumulation is generally restricted to species growing at a given locality due to a great variation in physical, chemical and biological factors that exist among contaminated areas Fig (8 a & b). Although they are widely distributed in different genera and families, hyperaccumulators represent low percentage on all angiosperm Baker *et al*.,(1999).

About 450 angiosperm species have been identified so far as heavy metal (As, Cd, Co, Cu, Mn, Ni, Pb, Sb, Se, Tl, Zn,) hyper accumulators, accounting for less than 0.2% of all known species. Mukhopadhyay and Maiti (2010); Chandra *et al*. (2015); Antoniadis *et al*. (2017), they reported that using metallophytes alone or in combination with microorganisms is an excellent strategy for the phytoremediation and heavy metals pollution prevention and control.

Fig. (8b): The Ni hyperaccumulator Pycnandra acuminata (Sapotaceae) from New Caledonia has a peculiar blue-green latex with up to 25% Ni (after Reeves e-tal (2018)

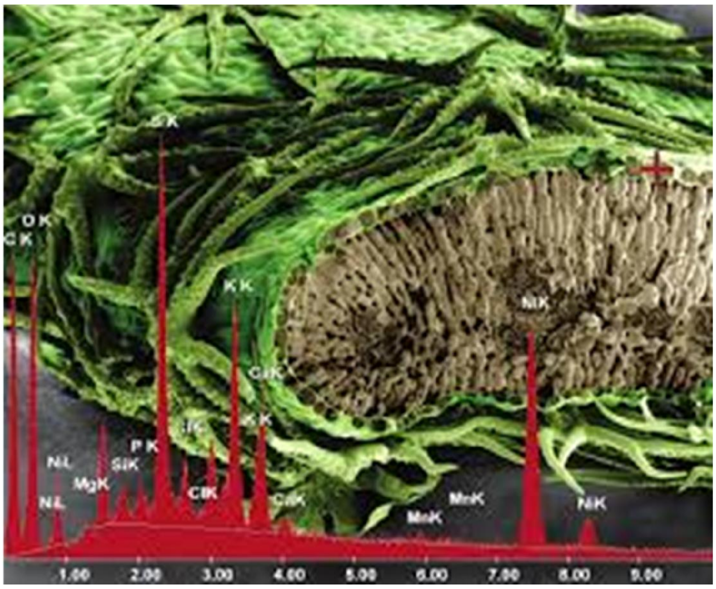

Fig. (8 a): SEM image (2.0 kV) of a frozen-hydrated leaf cross section from the Ni hyperaccumulator (Alyssum murale hyperaccumulator) showed vacuoles containing high concentrations of Ni

Several metallophytes have been identified and used in the phytoremediation of heavy metals contaminated sites. Some specific include *Pteris vittata*, which can accumulate Cr and As up to 35,303 and 20,707 mg kg^{-1} dry weight (DW), respectively Kalve *et al*. (2011); *Alyssum murale*, accumulate Ni in range of 4,730–20,100 mg kg^{-1} DW Bani *et al*. (2010) Fig.(8a and b); *Tagetes minuta*, also accumulate as up to 380.5 mg kg^{-1} DW Salazar and Pignata (2014); *Eleocharis acicularis*, accumulate Zn up to 11,200 mg kg^{-1} DW Sakakibara *et al*. (2011);*Corrigiola telephiifolia,* accumulate as up to2,110 mg kg^{-1} DW Garcia-Salgado *et al*. (2012),and *Noccaea caerulescens*, which can accumulate Pb in range of 1,700–2,300 mg kg^{-1} DW Dinh *et al*. (2018).

5.2 Selection criteria for hyperaccumulating plants for phytoremediation

Woody and herbaceous can accumulate high concentration of heavy metal in their shoot higher than non-hyperaccumulating species (100–1,000-fold) without any visible symptoms, hyper accumulators (represented by <0.2% of angiosperms), and the overall process is termed as hyper accumulation Ghosh and Singh (2005); Mukhopadhyay and Maiti (2010); Lee (2013).

Metal hyperaccumulating plants for heavy metals are specially preferred for phytoremediation of contaminated soil. However, some of hyper accumulating plants are not successfully applied due to some reasons:(*a*) low biomass, (*b*) slow growth rate, (*c*) metals (such as Ni, Zn, and Cu) under scope of phytoextraction that are not the priority pollutants, and (*d*) agronomic practices and crop protection measures for their cultivation and protection that have not been developed Goel et al. (2009); Marques *et al*. (2009); Mahar *et al*.(2016); Yadava *et al.* (2018).The ideal plants for phytoremediation should possess a series of characteristics: (*a*) ability to hyperaccumulate of heavy metals preferably in the aboveground parts; (*b*) tolerance to high pH, salt, and accumulated toxic metal concentration; (*c*) fast growth and high biomass; (*d*) widespread highly branched roots; (e) easy to cultivate and harvest; and (*f*) resistant to diseases and pests Vangronsveld *et al*. (2009); Chandra *et al*. (2015); Mahar *et al*. (2016). Metal hyperaccumulating plants are often preferred over non-accumulators because they produce a high volume of metal rich biomass and are economic to process for metal recovery and safe disposal, which have an additional eco-environmental benefit. More than 500 plant species have been identified as metal hyper accumulators, such as *Brassicaceae, Asteraceae, Caryophyllaceae, Lamiaceae, Euphorbiaceae, Poaceae, etc.*, Ali *et al*. (2013);Chandra *et al*. (2015); Mahar *et al*. (2016).

6- Plant–microbe interactions in phytoremediation

Bioremediation is an attractive tool to overcome the challenges posed by the traditional methods such as incineration and excavation. It has been widely used to remediate the organic and inorganic pollutants from the environment, but certain compounds and heavy metals tend to inhibit the growth of the plants. Rhizoremediation, which involves the mutualism between microorganisms and plants that degrades the polluted materials in the soil and makes eco-friendly environment. The important factors such as temperature, pH, and organic matter present in the soil, which affects the growth and metabolism not only the organism but also the plants, interaction between plant and microorganisms, and role of endophytic and rhizobacteria in bioremediation of heavy metals and organic pollutants. Rhizoremediation is mainly affected by various physical, chemical, and biological properties/compositions of the root-associated soil.

Many studies were carried out to interpret the effects of climatic factors on the breakdown of pesticides; many factors such as mineral nutrients, the age of plants, and polluted material affect the quantity and quality of root exudates. Rhizoremediation is majorly dependent on the nature and quality of the root exudates. The root exudates mediate the acquirement of minerals by plants, thus, stimulating the microbial growth and activities in the rhizosphere, besides changing of some physicochemical conditions. Under stress condition, plants respond by varying the composition of root exudates, in turn controlling the metabolic profile and activities of rhizosphere microorganisms.

Interactions between plants and microbes are an integral part of terrestrial ecosystem. Several types of plant–microbe interactions: competition, commensalism, mutualism and parasitism. More common interactions are commensalism or mutualism, at least one or both species benefit from the relationship respectively Campbell, (1995). Sorensen and Sessitsch, (2007), reporting excellent reviews on lifestyles and molecular interactions of plant-associated bacteria rhizosphere interactions Singh *et al.*, (2004a), plant responses to bacterial quorum-sensing (QS) signals Bauer and Mathesius,(2004), endophyte applications Ryan et al., (2008), and rhizosphere bacteria responses to transgenic plants Fillion, (2008).

The physicochemical characteristic of soil may play a crucial role in the success of bioremediation. Since microbial metabolic activity and physicochemical characteristic depends on factors, such as, moisture, redox conditions, temperature, pH, organic matter, nutrients and clay content. Fillion, (2008) evaluated the aerobic microbial mineralization / degradation of selected pesticides (benzolin-ethyl, isoproturon, and glyphosphate) in different types of soil at different moisture content. He found that mineralization/degradation of pesticide, increasing with soil moisture content ($p < 0.0001$) (within a soil water potential range of −20 and −0.015 MPa).Temperature also plays a vital role in biodegradation of recalcitrant chemical compounds by microbial consortia since biochemical reactions and metabolic activity of microbes depends on thermal thermodynamics. The cell membrane permeability and cell physiology-altering proteins are manly impacted by temperature (Ryan *et al*., (2008),).

Most of the putrefaction of compounds are due to the enzymes secreted by the plant-microbe interactions. The catalytic activities of these enzymes are pH dependent; the optimal bacterial growth is at pH 6.5 and 7.5 for most of the organisms. Sorensen and Sessitsch, (2007), noticed that the *Pandoraea* sp. Isolated from an enrichment culture degrade the HCH isomer in the pH range of 4–9. They also observed that growth and biodegradation of α- and γ-isomers of HCH seem to be optimal at pH 9. Singh *et al*. (2004 a) while, studying the putrefaction of organophosphate pesticides in the soil showed similar observation. They understood that the degradation was slow at acidic pH compared to that of neutral or alkaline pH. The organic matter in soil affects the adsorption/desorption process of pesticides in the soil including the nutrients for cell growth. Singh et al. (2004 a) monitored putrefaction of isoproturon (herbicide) by introducing phosphorus (P), nitrogen (N), and sewage sludge separately, thus, observed that P and N had the greatest effect on the process of isoproturon degradation. Fig. (9). All HCH isomers are acutely toxic to mammals, due to their mutagenic, teratogenic and carcinogenic properties. Although nowadays its use is restricted or completely banned in most countries, it continues posing serious environmental and health concerns. This is because γ-HCH was widely used as insecticide, which added to its high environmental persistence.

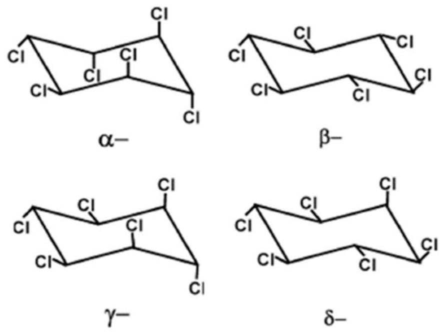

Fig. 9: Structures of hexachlorocyclohexane (HCH) isomers (after Manickam *et al*. 2006).

6-1 Role of endophytes in rhizoremediation

Utilization of endophytic microbes/bacteria in phytoremediation to degrade xenobiotic compounds from the environment has much more attention. Fig, (10) illustrate schematic diagram showing the integration between phytoremediation and strains treatment. These bacteria are nonpathogenic and find its existence in all higher plant species. Some of these species such as *Pseudomonas*, *Burkholderia*, *Bacillus*, and *Azospirillum*are found most abundantly in soil (Chandra *et al*. (2015).The endophytes possess plant growth-promoting ability and pathogen controlling capability (Ryan *et al*. 2008). The major advantage of employing endophytes over other rhizospheric bacteria in phytoremediation is that, in rhizospheric bacteria, there will be competition among the strains and furthermore reduces the number of desired strains. Conversely, endophytic bacteria are acquainted in the internal membranes/tissues of plants thus reducing the problem of competition between bacterial strains (Chandra *et al*., 2015).

Genetic modification strategies of these endophytes have gained more attention in phytoremediation process. Ryan et al. (2008) reported that introduction of toluene degradation plasmid (pTOM) from B. cepacia G4 into a natural endophyte such as yellow lupine is capable of degrading toluene up to 50–70%. While Germaine *et al.* (2006) reported that interaction of natural endophytes with a genetically modified endophyte possessed the capability of degrading 2,4-dichlorophenoxyaceticacid. The same group has also reported 40% higher degradation of2, 4-dichlorophenoxyacetic acid by using *Pseudomonas putida* VM1441 (pNAH7).

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The genetic engineered endophytes were used to improvise the phytoremediation of organic/inorganic pollutants and toxic metals. Incorporation of modified yellow lupine was inoculated with pTOM-Bu61 plasmid (encoding for trichloroethylene degradation constitutively) and ncc-nre (Ni resistance/sequestration in B. cepacia VM1468), along with the natural yellow lupine showed significant reduction in TCE and Ni phytotoxicity. This also promoted 30% enhancement in root biomass and 50% decrease in the enzyme activities involved in antioxidative defense in the roots. In addition, to the decreasing trend in TCE evapotranspiration, it showed about a fivefold higher Ni uptake after inoculation of two types of yellow lupine plants together (Soleimani *et al*. 2015). The bioaugmentation of two grass species (*FestucaarundinaceaSchreb. and FestucapratensisHuds*) along with the endophytic fungi (*Neotyphodiumcoenophialum and Neotyphodiumuncinatum*) showed 80–84% and 64–72% of PAH and TPH reduction as compared to control plants, which showed only 30% removal (Soleimani *et al*. 2015). A part from the rhizosphere endophytes, the culturable endophytes in aquatic plants show enhancement in phytoremediation (Chen *et al*. 2012). It was shown that genetically engineered endophytic bacteria possess much easier in application than genetic plants because it has the ability to colonize multiple plants, and it also benefits plants by reducing stress hormones, nitrogen fixation, and phosphate solubilization (Dimkpa *et al*. 2009; Chen *et al*. 2012).

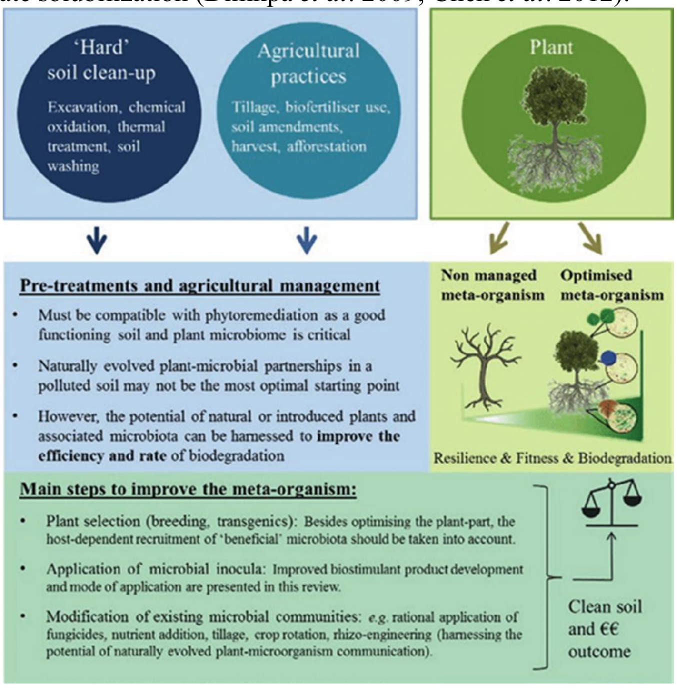

Fig. 10: Schematic diagram showing the integration between phytoremediation and Strains treatment and optimization of the plant microbiome.in soil cleanup (Thijs *et al*. 2017)

Examination of these interactions helps to understand natural phenomena that affect our daily lives and could lead to applications resulting in sustainable resources, less impact on the environment, cleanup of pollution and influence on atmospheric gases on a global scale. Advantages of using these interactions for biotechnological applications are many-fold. The use of naturally existing plant-microbe symbiosis for plant growth and biocontrol reduces synthetic fertilizer and pesticide treatments leading to cost-effectiveness and less impact by nutrients Boddey *et al.*, (2003) and pesticides Whipps and Gerhardson, (2007); Elmer and Reglinski, (2006) on surrounding fauna and flora. The production of useful compounds with pharmaceutical and industrial relevance using plant–bacteria symbiosis is energy efficient Wu *et al.*, (2007); Del Giudice *et al.*, (2008) and diminishes the need to add expensive precursors and catalysts. Remediation through conventional method, such as excavate and treat, is expensive and labour intensive. Conversely, plant– microbial remediation strategies can be less intrusive and much more economical Anderson *et al.*, (1993).Carbon sequestration through plant–rhizosphere processes is a potentially sustainable method to lowering atmospheric carbon Kumar *et al.*, (2006).

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Phytoremediation, use of plants to immobilize, extract metals or degrade organic pollutants, provides a cost-effective eco-benign alternative to traditional methods. In most cases, plants act indirectly by stimulating beneficial rhizosphere and endophytic microbes, which could facilitate/accelerate phytoremediation process by improving plant growth, altering soil metal bioavailability or facilitating the degradation of organic pollutants (known as bioaugmentation). Plant growth-promoting microorganisms (PGPM) [e.g. plant growth-promoting bacteria (*PGPB*), rhizobia and arbuscular mycorrhizal fungi (*AMF*)] exhibiting plant growth-promoting (*PGP*) traits [e.g. synthesis of indole-3-acetic acid (*IAA*), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, siderophores, surfactants, nitrogen (*N*) fixation, solubilization of phosphate (*P*) and potassium (*K*)] can enhance plant biomass production. Furthermore, AMF can contribute considerably to the short-term underground carbon (*C*) sequestration by retaining photosynthate C transferred by their host plant and/or stabilizing soil aggregate in the phytoremediation systems.

In the case of organic pollutants, the application of pollutant-degrading bacteria and fungi can improve phytoremediation due to their ability to partially degrade organic pollutants or metabolize pollutant degradation products to CO_2 and water. Regarding heavy metal decontamination, the release of organic acids and acidification of rhizosphere soils by metal-mobilizing microbes may facilitate phytoextraction, whereas the release of root exudates (such as sugars, amino acids, and enzymes) and precipitation of metal-immobilizing bacteria are beneficial to phytostabilization.

Plant-microbe interactions play a critical role in plant adaption to metalliferous environments, stimulation of plant growth, and thus can be explored to accelerate microbe-aided phytoremediation. Ma *et al*., (2011).

7- Halophytic for Phytoremediation of Contaminated Sites

Halophytes are the plants naturally grow under salt stress, by developing different strategies to complete their life cycles, under very high salinity level reached over seawater salinity level. *Sporobolus virginicus*, *Spartina patens* and *Atrriplex nummularia* are halophyte species which can remove excess salt from the soil by various strategies. Plants have salt glands through which salt is excreted from their leaves. This capability to uptake salt from soil and translocated from root to the aboveground shoot without, any differences of shoot biomass production is an advantage not available in conventional crops. Moreover, halophytes are found usually in arid and semi-arid regions.

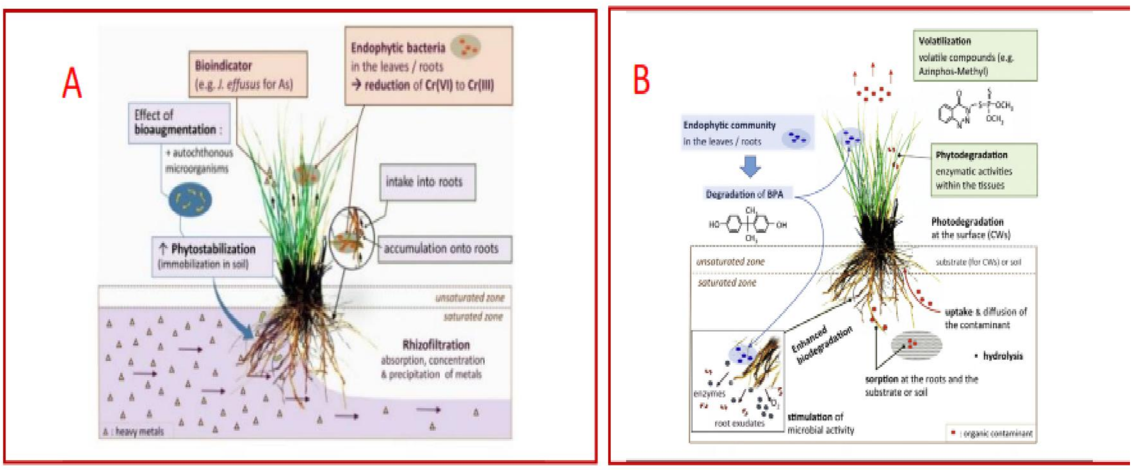

Fig.11 a & b: Schematic diagram represent a mechanisms remediation of helophyte -Juncus spp in **(A)**;Contaminated soils, groundwater or wastewater,**(B);**and organic contaminants removal from soil, groundwater or wastewater of plant associated microorganisms and root secretions. (After Evdokia *et al*. 2019).

Phytoremediation can be viewed as a solar-powered technology that exploits plants and their associated microorganisms to degrade/detoxify/remove organic and inorganic contaminants from soil and/or water bodies, including wastewater Fig. (11a & b). Cost effectiveness, soil stabilization, demand for simple monitoring methods, production of biomass with an economic value, maintenance and increase of the local biodiversity are all among the benefits that this alternative provides over the conventional mechanical or bioremediation approaches Liang et al. (2017). Contaminated soils present a strong environmental stress to plants and hence helophytes, halotolerant helophytes and halophytes that have a higher tolerance to environmental stressors are particularly suitable for phytoremediation purposes Wang *et al.* (2014); Salt marshes are dynamic systems, developed in sheltered areas from the Arctic to sub-tropics Chai *et al*. (2013), and are characterized by a small number of highly productive species Liang *et al*. (2017). Furthermore, salt marsh vegetation is responsible for ecosystem auto-remediation.

Successful phytoremediation in the field relies on several factors including plant species with specific characteristics (high biomass and fast growth rate), with the ability to tolerate a wide range of pollutants at elevated concentrations Fang *et al.* (2016). Many species have been found to be able to grow on polluted sites and accumulate high concentrations of metals, yet they are inappropriate for field applications due to their small biomass and slow growth rate., Autochthonous species, or species that are naturally abundant in the respective site area, should preferably be chosen in order to protect the local biodiversity and avoid the expansion of exotic species and the spread of their genes.

Halophytes are cultivable in soils irrigated with highly saline water as compared with soil irrigated with good water quality (due to high urban requirements and climate change) Manousaki and Kalogerakis (2011). It represents an additional eco-environmental benefit for phytoremediation. High salinity increases the mobility of heavy metals in soil and therefore, facilitates their greater uptake and translocation from root to shoot to achieve phytoremediation Wang *et al*. (2014); Liang *et al*. (2017). Several researchers reported that, halophyte *Tamarix smyrnensis* removes 9.4, 19.7, and 38.3 µg of Cd in a solution containing 0, 0.5, and 3% NaCl, respectively Manousaki *et al*. (2008).High salt content in forms of (NaCl) gradually increased heavy metal accumulation in halophytes either by enhancing metal mobility or by modifying root functions and alleviates metal-induced phytotoxicity through improved management of osmotic solutes and oxidative status Chai *et al*. (2013).

7-1 Salt secretion through specialized salt glands

A small group of halophytes have evolved specific salt excretory structures, termed salt glands, which can excrete excess salt from plant tissues to enhance salinity tolerance Yuan *et al*. (2016) .Halophytes with salt glands are collectively termed Recreto halophytes. Salt glands have originated from the epidermis of these plant species; however, the structure and mechanism of salt exclusion differs between Recreto halophytes species Fig. (12a & b).

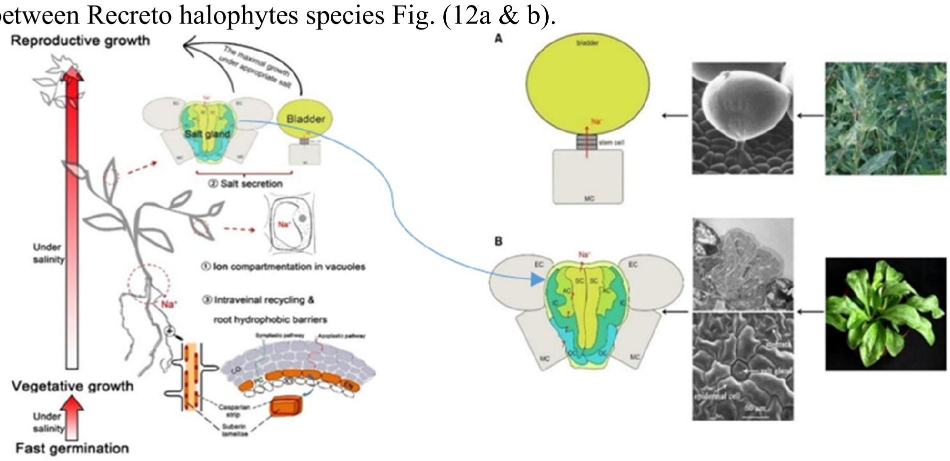

Fig. (12 a & b): Structure and Na^+ secretion pathway of a salt bladder (A) and a salt gland (B).

(A) The large balloon represents the typical structure of the salt bladder. Na^+can be transported into the balloon and released after bladder rupture. The representative Plant is Atriplexcentralasiatica.

(B) The typical multi-cellular salt gland and the Na+ pathway. (After Fang *et al*. (2016) and Yuan *et a*l. (2016)

Salt glands can be categorized into 4 groups: salt bladders, multicellular salt glands, bicellular salt glands and unicellular vacuolated secretory hairs Dassanayake etal (2017) .Salt bladders consisting of a large vacuolated cell with or without 1 or 2 stalk cells are only found in *Aizoaceae* and

Amaranthaceae, in which salt is sequestered in the bladder cell vacuole upon salt stress Park *et al*. (2009).A mutant *M. crystallinum* plant deficient in bladder cells was highly sensitive to salt under salt stress compared to the wild type *M. crystallinum*, which indicates the critical importance of salt bladders for salt compartmentalization and ion homeostasis Agarie *et al*. (2007) . Most salt glands consist of multiple cells (varying from 4-40 cells) which have cell types differentiated into basal collecting cells and distal secretory cells. The secretory cells have numerous plasmodesmata connections with surrounding mesophyll cells. Thus, it appears that salt is actively transported through the collecting cells into the secretory cells Dassanayake *et al.* (2017) .The outer surface of the secretory cells is covered with cuticle. Research by Feng *et al*. (2014) in *Limonium bicolar* showed that each of the secretory cells has a pore in the center of the cuticle and observed salt crystals located above the pores. In addition to secretion from the pore, extra salt also could be stored in the cuticular chamber on top of the secretory cells as observed in *Aeluropus littoralis* Barhoumi *et al*. (2008). The bicellular salt gland with a basal cell and a cap cell is found in *Chloridoid grasses*. The continuous cuticle on the epidermis in some species thickens on top of the cap cell and forms a cuticular chamber that stores secreted salts Amarasinghe *et al*. (1988).The unicellular hairs are found in the wild rice species *Porteresia coarctata*, and appear to lack specific organelles and be completely filled with vacuoles Dassanayake *et al*. (2017). Molecular genetic studies of salt glands have been limited in the past. However, new methods are increasing our ability to study the detailed function of salt glands at the cellular and molecular level. For instance, scanning electron microscopy has identified a potentially important feature of L. bicolor salt glands showing that salt glands in these plants emit fluorescence under UV excitation (330– 380 nm) Yuan *et al*. (2016). This auto fluorescence arises from ferulic acid localized in the cuticle, which plays an crucial role in salt secretion Deng *et al*. (2015) .Salt secretion is an energy-intensive process that is associated with high levels of water efflux. To recover from water loss, aquaporin's play an critical role in re-uptake of water into cells Yuan *et al*. (2016), Tan *et al*. (2013).

Inorganic elements extruded through the salt glands include a variety of cations and anions, but high selectivity for Na^+ and Cl^- compared to other ions has been observed by Feng *et al*. (2015).Additionally, recent transcriptomic Yuan *et al*. (2015), Yuan *et al*. (2016), Yamamoto *et al*. (2015) proteomic Barkla *et al*. (2012),(2016), and metabolomic Barkla *et al*. (2015), analyses have reported many candidate genes, proteins and metabolites expressed specifically in salt glands; these candidate genes, proteins and metabolites may play key roles in salt gland development and salt secretion.. In salt bladders cells of *M. crystallinum*, active metabolic changes related to energy generation, UV protection, organic osmolyte accumulation and stress signaling have been identified to be regulated by a number of genes of unknown function in response to salt stress Barkla et al (2012), (2015).In addition, recretohalophyte *L. bicolor* mutants exhibiting altered salt secretion can be obtained by physical and chemical methods and used to identify potentially critical genes that contribute to salt secretion pathways Yuan *et al*. (2013),(2015). The functions of these genes can then be validated by combining established transformation protocols with the leaf disk secretion model Yuan *et al.* (2015),

7-2 Alterations in ion homeostasis and osmotic pressure contribute to salt tolerance

Intracellular compartmentalization of toxic ions using specific transporters represents another key pattern used by halophytes to maintain a moderate cytosolic K^+/Na^+ ratio in the cytosol. Thus, membrane ATPase's and ion transporters play essential roles in salinity tolerance in some halophytes. Expression and activity of plasma membrane and vacuolar membrane H^+-ATPase has significantly increased in *Suaeda salsa* in response to NaCl treatment Chen *et al*. (2010), Yang *et al*. (2010). ATPase activity is required to establish the proton gradient that maintains electrochemical and pH differences across the membrane. Membrane transporters can couple this electrochemical gradient to movement of substrates against their concentration gradients Palmgren (2001). Thus, the activities of ion transporters or antiporters localized in the plasma membrane and vacuolar membrane are tightly regulated and essential for plant growth and development Ren *et al*. (2013), Lu *et al.* (2016). Many such ion transporters, including the vacuolar Ca^{2+}/H^+ antiporters Han *et al*. (2011) the vacuolar H^+/Ca^{2+} transporter Han *et al*. (2012) the K^+ transporter Shao *et al*. (2014) and others Mishra *et al*. (2017), Kong *et al*. (2011) have been cloned and shown to reduce concentrations of Na^+ and Cl^- in the cytosol. Over expression of these transporters can improve salt tolerance by maintaining cytosolic ion homeostasis during salt stress Patel *et al*. (2015), Pandey *et al*. (2016) .Under salt stress conditions the osmotic pressure is also severely compromised due to the influx of high concentrations of salt ions.

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Halophytes have evolved a defense mechanism involving accumulation of osmoprotectants, such as proline, glycine betaine, polyphenols, and soluble sugars, in the cytosol to reduce and balance the osmotic pressure. Over expression of halophyte genes for enzymes involved in the synthesis of glycine betaine or raffinose, such as choline monooxygenase (CMO) Wu *et al*. (2010) obetaine aldehyde dehydrogenase (BADH) Li *et al.* (2003) and galactinol synthase (GOLS) Sun *et al.* (2013) have been shown to enhance salt stress tolerance in glycophytic plants. Furthermore, expression of these genes is also induced in response to cold, drought, and heat, in addition to salinity, resulting in a concomitant increase in galactinol, raffinose, and α-ketoglutaric acid in transgenic plants Sun *et al*. (2013)

7-3 Detoxification of ROS and alterations in membrane

Halophytes have demonstrated their capability to survive under extremely saline conditions and considered as one of the best germplasm for saline agriculture. Salinity is a worldwide problem, and the salt-affected areas are increasing day-by-day due to low and scarcely rainfall, salt ingression, poor irrigation system, water contamination, and other environmental factors. The mechanism of salinity stress is a very complex phenomenon, and some pathways are coordinately linked for imparting salinity tolerance. Though a number of salt responsive genes have been reported from the halophytes, there is always a quest for promising stress-responsive genes that can adapt plant physiology according to the salt stress. Halophytes such as *Aeluropus, Mesembryanthemum, Suaeda, Atriplex, Thellungiella, Cakile, and Salicornia* serve as a potential candidate for the salt-responsive genes and promoters. Several known genes like antiporters (NHX, SOS, HKT, VTPase), ion channels (Cl^-, Ca^{2+}, aquaporin's), antioxidant encoding genes (APX, CAT, GST, BADH, SOD) and some novel genes such as USP, SDR1, SRP etc. were isolated from halophytes and explored for developing stress tolerance in the crop plants (glycophytes). It is evidenced that stress triggers salt sensors that lead to the activation of stress tolerance mechanisms, which involve multiple signaling proteins, up- or down-regulation of several genes, and finally the distinctive or collective effects of stress-responsive genes. Halophytes are an excellent platform for salt responsive genes, which can be utilized for developing salinity tolerance in crop plants through genetic engineering Fig.(13 a & b), represent schematic diagram of salinity stress tolerance mechanism in a plant and its adaptation to salts stress, this mechanisms include cell integrity, phytohormones, antioxidants, synthesis of osmolytes and ion homeostasis. The coordinated action leads to re-establish the cellular homeostasis, protection of functional and structural proteins and membranes, and ultimately the tolerance to salinity stressed for developing salinity tolerance in crop plants through genetic engineering.

Composition ROS detoxification pathways play a protective role in the response to salt stress by scavenging toxic radicals generated from the electron transport chains of mitochondria and chloroplasts. Antioxidative defense systems include both non-enzymatic and enzymatic components. One such system is termed the ascorbate-glutathione pathway and acts in chloroplasts.

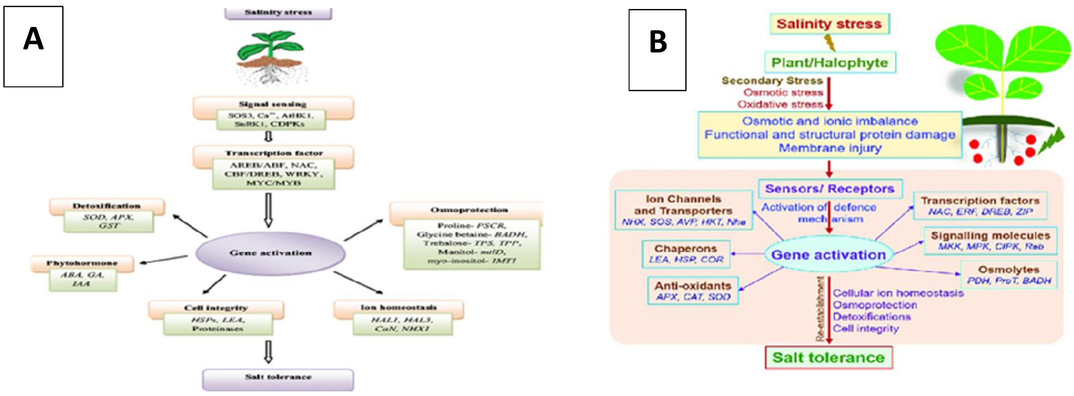

Fig. 13a &b: Schematic diagram represent Salinity Stress tolerance mechanism in a plant (Modified after - Chen *et al*. 2014).

A series of enzymes belonging to this system, including monodehydroascorbate reductase (Am-MDAR) Kavitha *et al*. (2010)glutathione transferases (SbGST, SsGST) Jha *et al*. (2011) Qi *et al*. (2010)ascorbate peroxidases (SssAPX and PtcAPX) Li *et al*. (2012), Cao *et al*. (2017) and superoxide dismutases (TaSOD) Wang *et al*. (2010) have been identified in several kinds of halophytes and have been shown to play important roles for protecting against salt inducing oxidative stress in higher plants.

Overexpression of these genes leads to enhanced NaCl tolerance under salt stress. Over expression of the SssAPX gene, that normally encodes the stromal APX in *S. salsa*, can increase the germination rate, cotyledon growth, survival rate, and salt tolerance of transgenic Arabidopsis Li *et al*. (2012).In addition to enzymes that scavenge ROS directly certain other types of proteins/enzymes have also been shown to improve a plants antioxidative capacity.

Metallothioneins (MTs) can bind to heavy metals and are involved in the homeostasis of essential metals (Cu and Zn), as well as cellular detoxification of nonessential metals (Cd and Hg). For example, cloning of the *Salicornia brachiata* metallothionein gene sbMT-2 and expression in tobacco resulted in significantly enhanced salt tolerance, a higher membrane stability index, and decreased levels of H_2O_2 and lipid peroxidation (MDA), implicating sbMT-2 in H_2O_2 detoxification. Furthermore, mechanistic analysis revealed elevated expression of key antioxidant enzymes, specifically SOD, POD, and APX, in sbMT-2-expressing transgenic plants, further confirming the role of the SbMT-2 gene and its protein product in ROS scavenging/detoxification Chaturvedi et al. (2014). In addition to metallothioneins, S-adenosylmethionine synthetase Qi *et al*. (2010) glycosyltransferase Zheng *et al*. (2017).At Fes1A Fu *et al*. (2015) and CCCH-type zinc finger protein have also been shown to participate in salt tolerance by limiting oxidative stress and, additionally, helping to maintain the ionic and osmotic balance Han *et al*. (2014).Membrane structure and fluidity regulated by varying the composition and degree of fatty acid saturation of membrane lipids affects membrane permeability and contributes to plant resistance to environmental stressors Mikami and Murata (2003). Tang *et al.* (2012).Comparative analysis of the membrane lipid and fatty acid composition in the halophyte *Thellungiella halophila* and the glycophytes *Arabidopsis thaliana* under high salinity conditions revealed higher levels of phosphatidyln glycerol (PG) and unsaturated fatty acids, as well as a higher double-bond index for monogalacto syldiacyl glycerols and PGs in *T. halophila* Sui and Han (2014). Consistent with these observations, transgenic Arabidopsis plants expressing the *S. salsa* gene that encodes glycerol-3-phosphate acyltransferase (GPAT), anacyl-esterifying enzyme required for PG synthesis expressed under high-salt conditions, exhibit tolerance to NaCl Sui *et al*. (2017).

8- Aromatic plants for phytoremediation of metal contaminated Sites

Various plant species are cultivated especially for their secondary metabolites. They are called “aromatic plants” due to their specific use for production of essential oils, cosmetics, personal care products, etc. Lubbe and Verpoorte (2011). As these crops are not directly linked to food chain, they hold an add-on position in comparison to food crops for phytoremediation purpose. In the recent past, a large number of aromatic plants have been tested for their phytoremediation potential. Most promising aromatic plants for phytoremediation of heavy metal contaminated sites have been identified from families – *Poaceae, Lamiaceae*, *Asteraceae, and Geraniaceae*. Perennial aromatic grasses produce huge biomass and are widely grown for high value essential oil production like Vetiver *(Chrysopogon zizanioides (L.) Nash),* Lemon g*rass (Cymbopogon flexuosus (Nees ex Steud.) Watson),* Palmaros*a (Cymbopogon martinii (Roxb.) Watson),* and Citronella *(Cymbopogon winterianus Jowitt ex Bor).* These aromatic grasses hold a great potential for phytoremediation of heavy metal contaminated sites. Apart from grasses, plants like Ocimum, Mentha, Lavender, Salvia, Rosemary (Family – *Lamiaceae*), Chamomile (Family – *Asteraceae*), and Geranium (Family – *Geraniaceae*) have been identified for the same Fig. (14). The cultivation of non-edible economic aromatic crops at heavy metal contaminated sites has often been suggested as a profitable and feasible option. The benefits of using aromatic plants for phytoremediation purpose can be categorized under two main headings as environmental aspect and economic aspect.

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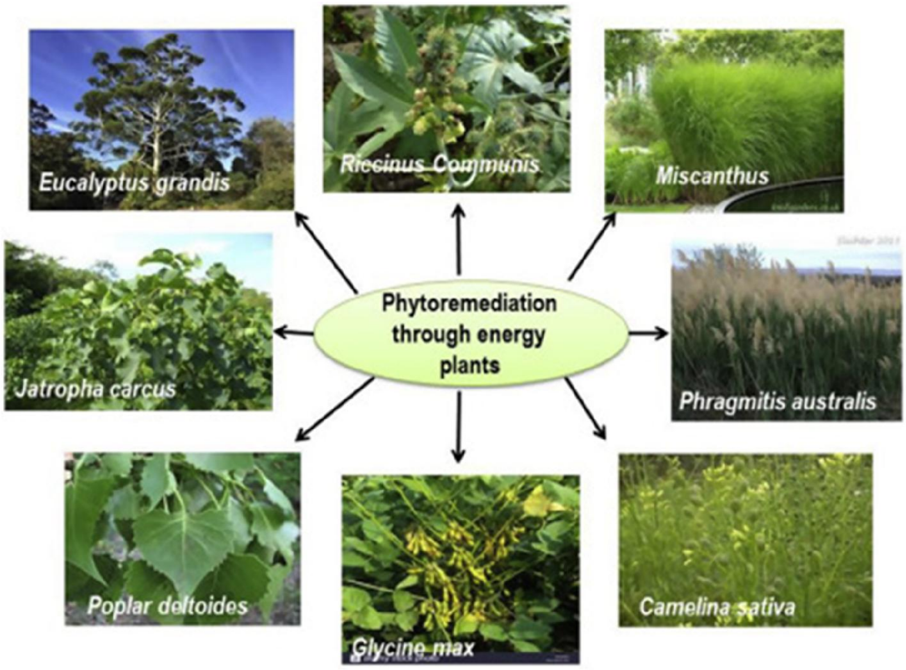

Fig. 14: Illustrate some of aromatic plants for phytoremediation of heavy metal contaminated sites

8-1 Environmental aspect

The major environmental aspect regarding use of aromatic plants for phytoremediation is that by using them, contaminant's entry into the food chain can be reduced (Lal et al. 2008; Verma *et al.* 2014).Many researchers have suggested use of aromatic plants for cleanup of heavy metal polluted sites in the recent past. Many studies have indicated that Lemongrass and Vetiver can tolerate toxic environments as well as reduce soil erosion and simultaneously enhance the quality of soil (Chiang et al. 2006). Zheljazkov *et al*. (2006) reported that there are no significant risks of metal contamination in essential oil and change in oil constituents by growing aromatic plants in contaminated sites. Some aromatic plants significantly accumulate heavy metals from heavy metal polluted sites, which are then removed after harvesting (Zheljazkov and Nielsen 1996a; Zheljazkov and Warman 2004). Various methods have been suggested recently through which accumulated metals from the harvested biomass can be extracted efficiently. Hence, aromatic plants could be suggested as better methods for phytoremediation of heavy metal contaminated soil as they lower the risk of food chain contamination as well as check soil erosion simultaneously enhancing the soil quality Fig. (15).

Economic aspect (Demand and availability).

A growing gap has been observed in the global production and demand of the essential oils (3900 metric tons; Barbosa *et al.* 2009). Essential oils are high value products, which are widely used as aromatic agents in various industries, like perfumery, cosmetics, and aromatherapy. There is a continuous increase in global demand of essential oils and it is expected to reach the mark of US$5 trillion by the end of year 2050 (Verma *et al.* 2014). Therefore, to meet the ever-increasing demand for essential oil, aromatic grasses can be grown on contaminated sites that can help in their restoration. It has been confirmed that there is least risk of heavy metal contamination in essential oil obtained through steam distillation process as heavy metals remain in the extracted plant. Hence, it can be acceptable in the market (Scora and Chang 1997; Zheljazkov and Nielsen 1996b).

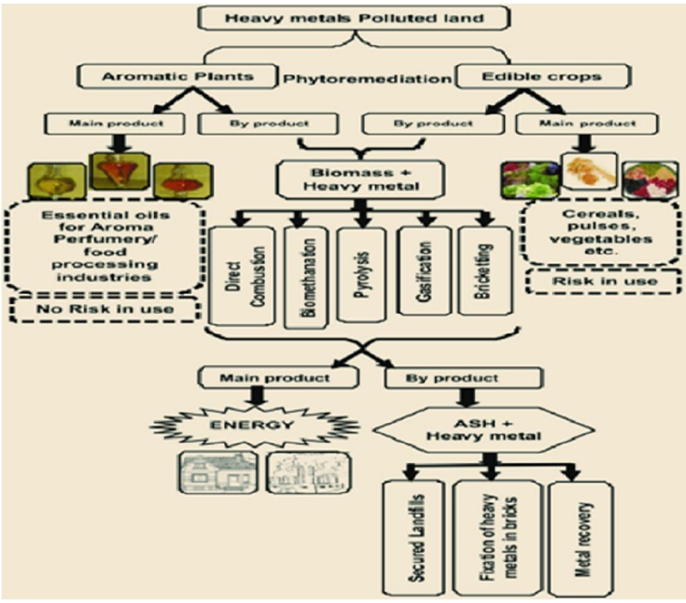

Fig. 15: Conceptual diagram showing benefits of aromatic plants for phytoremediation

8-2 Risk assessment and sustainable utilization of contaminated biomass

The major concern regarding feasibility of phytoremediation has been the safe disposal of contaminated plant biomass as with each consecutive harvest, large amount of contaminated biomass accumulates. Various studies have been done suggesting many methods which can be implemented for safe disposal of large amount of polluted biomass which includes burning (Yan *et al*. 2008), high-temperature decomposition (Yang *et al*. 2003; Thewys and Kuppens 2008), chemical extraction, and phytomining (Jaffre *et al*. 1976). Many researchers have done risk assessment of using aromatic crops for phytoremediation purposes. The major by-product of aromatic crops is essential oil, which is mainly used for non-edible purposes like soaps, and detergents manufacturing, preparation of insect repellents, cosmetics and perfumes hence, they can be considered as a putative selection for minimizing food chain contamination (Lal *et al*. 2013). Zheljazkov *et al.* (2005) reported that aromatic crops could be grown on heavy metal contaminated sites without causing any significant risks of metal transfer to by-product and alterations in essential oil composition. The critical limit of heavy metals for food is Cr: 1.5 ppm; Cd: 1.5 ppm, Ni: 2.5 ppm, and Pb: 1.3 ppm (Lone *et al*. 2003; FSSAI 2011). Lal *et al*. (2013) observed that heavy metals in essential oil extracted from aromatic crops grown on heavy metal contaminated soil were well within these critical limits. Rattan *et al.* (2005) also suggested this point by doing experiments on crops irrigated with municipal sewage. In this regard, hydro-distillation process for essential oil extraction seemed to be the main reason for less contamination of essential oil by heavy metals (Scora and Chang 1997; Bernstein *et al.* 2009). Hence, aromatic crops hold a superior position over food crops for phytoremediation purposes as there is minimum risk of food chain contamination by using them.

Conclusions

Contaminated environments have become common, are of global concern due to their ecosystem impact, and associated socio-economic loss. Phytoremediation is an attractive and potent tool for remediating toxic materials present in the environment. The adoption of biological approaches aids in environmental restoration, and is environmentally resilient. Most remarkable biotransformation technologies suitable for plant remediation of metal-contaminated soils include phytotransformation, phytostimulation, phytoextraction, phytostabilization, and phytovolatilization. Whereas bioaugmentation and bio stimulation are the most suitable methods for microbial restoration of metal-contaminated environments and the least suitable technique because of time constraints. Established case studies revealed that biotransformation and removal of heavy metals would aid in the restoration of polluted wetlands, ex-mining lands, agricultural soils, and residential areas. These approaches are recommended for field use, where industrialization and rapid economic development cause environmental deterioration.

Rhizoremediation, a special phytoremediation technique that involves both plants and microbes, elucidates their usage in removing hazardous materials. Heavy metal - contaminated sites are often inhibited and microbial biomass is reduced. Inhibited cytoplasmic enzymes and damaged cell structures are caused by oxidative stress occurring in plants due to heavy metal contamination. However, with the exponential increase of population and ever-increasing pollution, the progress made in remediating is gloomy. On the other hand, it is promising to note that the allocation of assets and awareness in the society toward such eminent concerns is augmenting day by day.

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