

## **ENDOPHYTES AS AGENTS OF PHYTOREMEDIATION**

### **Abstract**

Phytoremediation is the use of plants for the clearing of toxic contaminants from the environment. The major advantage of phytoremediation compared to other methods is that it is solar-powered and does not leave behind any significant waste. But phytoremediation also has its shortcomings. Contaminants accumulating in plant tissues can at some point of time become toxic to plants, ultimately killing them and making phytoremediation insignificant. Today, along with advancements in technology, pollution is happening at such a fast pace that finding alternative effective method for reversing pollution is an inevitable endeavor. This is where the study of endophytes and endophyte-aided phytoremediation becomes significant. It is estimated that the endophytes living in symbiotic relations with plants that have evolved to tolerate the presence of toxic contaminants might also have evolved to be capable of similar functions as they both invariably live in similar conditions.

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## I. INTRODUCTION

Technology today grows at a breathtaking pace and along with it pollution due to trace metals as well (Khalid et al., 2017; Wuana and Okieimen, 2011). These pollutants are non-biodegradable and hence they are extremely persistent (Rehman et al., 2017). Toxic substances let into the soil as part of anthropogenic activities such as urbanization, variations in agricultural practices and industrialization can cause serious effects on human health and the environment (Kang, 2014). Some of the major organic contaminants reported from aquatic sources include plasticizers used in plastic industry (Peydayesh et al., 2020) antibiotics (Danner et al., 2019), estrogen hormones (Ting and Praveena, 2017), perfumes, sunscreens and other antimicrobial agents (Muir et al., 2017; Wang et al., 2019), petroleum hydrocarbons (Varjani, 2017) and pesticides (Johnson, 2018). The aforementioned compounds also contaminate soil, along with inorganic compounds such as phosphate and ammonia and heavy metals such as copper, selenium, lead, zinc and mercury (Salam and Varma, 2019). Heavy metal pollution is the most threatening of all. A source of toxic metal pollution is the processing of ores, which creates fine grains of minerals. This can leach out toxic metals such as antimony and arsenic (Babel et al., 2016; Li et al., 2022; Sun et al., 2022). Plants require some metals such as copper, iron, manganese and zinc at particular concentrations for the normal functioning of their metabolic activities but these can become toxic when present in excess (Fasani et al., 2018). There are also other metals such as lead, cadmium, arsenic and mercury that have no role in plant metabolism and are highly toxic for plants at any concentration (Clemens, 2006). Heavy metals are non-biodegradable and cause severe pollution in soil and water ecosystems even at a low concentration. This will adversely affect the health of native organisms and can even cause mutagenicity, carcinogenicity and neurological disorders (Saxena et al., 2020). The presence of heavy metals in soil can also be a grave concern in agricultural production (Proshad et al., 2019). As early as 1985, Clijsters and Von Assche reported the potentiality of heavy metals to interfere with and inhibit photosynthesis. Accumulation of cadmium and nickel can disrupt the functions of cells in a plant (Jan et al., 2019) Soil incineration, soil washing, excavation and landfilling and solidification are some of the common methods adopted for the removal of these heavy metals from the soil. But they come with certain disadvantages such as higher costs and adverse changes brought about to the ecosystem (Sheoran et al., 2011). Generally, the three methods adopted for the removal of contaminants from soil, water and air can be categorized as physical, chemical and biological. Of these physical methods are very expensive and chemical methods pose much threat to the environment. Biological methods include phytoremediation and it is considered as a sustainable method (Ma et al., 2016; Tiwari et al., 2016).

Phytoremediation is an economically feasible and eco-friendly method adopted for the removal of toxic compounds from the soil or aquatic environment with the help of tolerant plants (Singh and Singh, 2017). Weyens et al. (2009) put forward one of the most accepted definitions of modern times for phytoremediation as “An *in situ* solar powered remediation technology that requires minimal site disturbance and maintenance resulting in low cost and high public acceptance”. Plants that are tolerant or plants that are hyperaccumulators of heavy metals are used for phytoremediation. That is, plants act by either stabilizing the contaminants in the soil by making them less bioavailable known as phytostabilization or by accumulating the pollutants in plant tissues known as phytoextraction. Rhizospheric microbes are able to degrade the contaminants and this is termed rhizodegradation (Pilon-Smits, 2005).

Textile industries generate huge amounts of wastewater owing to the dyes and aquatic plants are used for the phytoremediation of such polluted water (Pandey, 2007). Phytoremediation can be more effectively carried out using pioneer plants that can better tolerate metal toxicity and can help in the re-vegetation of an affected environment (Rodriguez, 2007). The World Health Organization reported 4.3 million deaths across the globe due to indoor air pollution in 2012 (WHO, 2016). Though studies related to the use of plants for removal of indoor air pollutants started in the 1980s (Wolverton and McDonald, 1982) it is less studied compared to soil and water phytoremediation. In order to detoxify contaminants that accumulate in soil, new sustainable and affordable remediation methods should be adopted. Cost-effective and more reliable technology to remove toxic substances from soil would be remediation using plants and microorganisms associated with plants (Fester et al., 2014). Though phytoremediation procedures are famed for being economic and sustainable, these procedures are not devoid of shortcomings. Efficacy and economic factors are still of concern which demands studies in multidirectional ways. It is inferred that the endophytes of plants used in phytoremediation could also show properties of tolerance to contaminants as they invariably survive in the same environment (Idris et al., 2004).

Endophytes are those microbes that colonize plant tissues without any apparent symptoms (Stone et al., 2000). They include bacteria, fungi and actinomycetes (Raghukumar, 2008). Endophytes maintain a symbiotic relationship with the plant where they help plants in growth promotion and tolerance to biotic and abiotic stress in return for the carbohydrates they receive from the plant (Hamilton and Bauerte, 2012). The low cost and environment-friendly nature of endophytes make endophyte-mediated phytoremediation an encouraging technology to improve heavy metal-deposited soil (Feng et al., 2017). The capability of phytoremediation is synchronized by endophytes through carbon dioxide fixation, nutrient absorption and environmental adaptation (Luo et al., 2016). Many endophytic bacteria are reported to have the potential of degrading pollutants in their host's environment (Tripathi, 2020). The mechanisms bacteria use for metal tolerance vary. Metals may be bio precipitated before contact with cells, may be degraded using exopolysaccharides (Bhaskar and Bosle, 2005; Pal and Paul, 2008) or will be dealt with reflex pumps such as PIB-ATPase after entry into the cell (Palmgren and Nissen, 2011). Bacteria inhabiting plants as endophytes are reported to produce siderophore compounds such as catecholate and hydroxamate which are potential heavy metal reducers (El Attar et al., 2019; Ferreira et al., 2019). Endophytes can also convert heavy metals to organic acids, iron carriers and other substances that are bioavailable for the plants (Sheng et al., 2008). Wang et al. (2019) reported the inoculation of soil with endophytes to be an effective method for solubilising contaminants in the soil and making them available for plant absorption. Plants do not metabolize most of the organic contaminants whereas microbes have harnessed the power of converting them to carbon dioxide and water (Afzal et al., 2013). Another advantage of endophytic microbes is that, even when the contaminants reach plant tissues such as xylem evading the microbes in the rhizosphere, endophytes can still play a role in detoxification as they themselves colonize within the plant tissues (Taghavi et al., 2011). Thus, endophytes are generally used for either the direct degradation of pollutants or for their potentiality in plant growth promotion of tolerant plants (Stepniewska and Kuzniar, 2013).

## **II. POLLUTION - A GLOBAL CONCERN**

Today, anthropogenic activities are resulting in the contamination of soil, water and air at an unimaginable degree. When contaminants such as heavy metals are present in soil or water, it is likely to enter the food chain through agricultural crops, fish and meat, which can result in adverse health issues (Abdel-Rahman, 2022). Population rise and technological development are both happening at a lightning pace, which is also adding to the increase in pollution (Zaheer et al., 2020).

The two major environmental pollutants can be classified under the groups, elemental pollutants and organic pollutants. Radioisotopes, heavy metals and metalloids come under the class of elemental pollutants. Radioisotopes of uranium, delirium and tellurium are more toxic and common than others. Mercury, lead and chromium are the common heavy metal pollutants (Baker et al., 2000). Polycyclic aromatic hydrocarbons (PAHs), halogenated hydrocarbons, polychlorinated biphenyls (PCBs) etc. come under the class of organic pollutants (Meagher, 2000).

Sewage water, industrial effluents, oil spills, fertilizers and pesticides are major sources of various inorganic and organic toxic compounds including heavy metals in the soil (Khan et al., 2021). Soil contaminated with lead is directly related to the presence of lead in the blood composition of children which can result in cognitive dysfunction, Alzheimer's and Parkinson's disease in them (Laidlaw and Taylor, 2011; Bakulski et al., 2012; Chen et al., 2017). Gasoline, smelting and mining are some of the major sources of lead contamination in soil. Cadmium released from chemical wastewater discharge, mines and batteries are carcinogenic and can cause bone injury, kidney stone, emphysema and the like (Sarwar et al., 2017). Cosmetics, lighting lamps, dental materials, instruments and meter plants can leach out mercury to the environment. This can cause severe toxicity, depression, hair loss, ulcers and kidney damage in humans. It can also adversely affect other organisms and plants as well (Kim et al., 2016). Another important toxic heavy metal is arsenic which is released from mines, chemical pharmacies, chemical fertilizers, insecticides and arsenic drugs. Anorexia, gastrointestinal disorders, corneal sclerosis, and cardiovascular and respiratory disorders can be caused due to arsenic poisoning (Balali-Mood et al., 2021). Mining, gas emission, pesticide use, municipal waste discharge are ongoing processes that have contributed much to pollutants in soil, water and air (Shah et al., 2020).

The contaminants released into the soil can flow with the rainwater and pollute groundwater or even lakes (Khan et al., 2021). When harmful chemicals are present on water surfaces, they affect both the plants and other organisms in the water bodies by both the obstruction of light and due to the presence of toxic substances (Malik et al., 2017).

Not just the soil and water bodies but the very air we breathe is also being polluted at an alarming rate. The World Health Organization identifies that 92% of the entire population across the globe are breathing air of very poor quality, that has pollutants in excess of WHO limit (WHO, 2016). Aerosol pollutants in the atmosphere, not just affect the health of organisms directly, but can also be a major cause of climatic changes (Mhawish et al., 2018). As heavy metals or other pollutants enter the food chain they will be subjected to biomagnification causing huge threat to the very survival of organisms (Singh and Kalamdhad, 2011).

### **III. PHYTOREMEDIATION - STRATEGIES AND DRAWBACKS**

Removal of toxic contaminants from the environment is a significant requirement in the present world and those three general methods are adopted. They come under the classes of physical, chemical and biological. Physical remediation includes soil washing, soil covering, soil replacement and encapsulation. Soil washing demands a high cost and it is difficult in the case of Wells or underground drains (Rajendran et al., 2021). Surface covering can be done only in very small areas (Liu et al., 2018), soil replacement and encapsulation are also economically demanding and limited to small areas (Rajendran et al., 2021; Li et al., 2019). Chemical remediation includes thermal remediation, which requires large amounts of energy and causes secondary pollution (Gong et al., 2018), and chemical fixation and vitrification techniques, which are of higher cost (Nejad et al., 2018). Compared to both these methods bioremediation is more economically feasible and eco-friendly and it includes phytoremediation and microbial remediation. Phytoremediation is the use of plants for the removal of contaminants from air, water or soil. It will develop the least amount of waste while detoxification of the environment is carried out and is economically more feasible (Shah and Daverey, 2020). Plants use various strategies to counter contaminants in the soil and aquatic environments. The major strategies include phytostabilization, phytoextraction, phytovolatilization, phytofiltration and rhizodegradation (Poria et al., 2022).

Phytostabilization is also known as phytoimmobilization. This is a process in which, instead of degrading or accumulating contaminants within plants, the contaminants are merely immobilized in the ecosystem, restricting their spread in the food chain. The pollutants like toxic heavy metals are stabilized in the underground regions of the plant by deactivating them. Thus, they are not permanently removed from the ecosystem (Shackira and Puthur, 2019; Kafle et al., 2022). Whereas phytodegradation is a process by which the contaminants are literally degraded using enzymes such as halogenases, nitroreductases and peroxidases (Jhila et al., 2021; Nedjimi, 2021). This is mostly carried out by associated microbes (Poria et al., 2021). Another method adopted is phytoextraction which is also known as phytoaccumulation. As the name suggests in this process the contaminants are extracted from the soil and accumulated in tissues of tolerant plants (Ali et al., 2020). In a different method termed phytovolatilization, the plants absorb heavy metals from contaminated soil and convert them to volatile forms. This is then released as less toxic volatile gasses through transpiration (Bortoloti and Baron, 2022). Rhizodegradation is a method wherein organic contaminants in soil or water are degraded especially with the aid of root-associated microorganisms that are capable of secreting required enzymes (Ashraf et al., 2019).

One of the major shortcomings of phytoremediation is that when the toxic compounds in the environment are present at higher concentrations it will disrupt the normal plant metabolism and reduce plant growth. Thus, the ultimate aim of achieving phytoremediation will either be slowed down or will become insignificant (Deng and Cao, 2017; Dubchak and Bondar, 2019). Microorganisms can play a role where plants by themselves fail, as they have evolved to better tolerate soil metal toxicity. This is achieved by reducing cell permeability, reducing metal toxicity, through biosorption and complex formation. Moreover, phytoremediation as such can be limited to a single contaminant alone, but it can be made applicable for multiple contaminants as well with the introduction of endophytes (Ijaz et al.,

2016). We can thus infer that microbe inhabiting plants as endophytes can help plants in phytoremediation by reducing toxicity and by improving plant growth (Roskova et al., 2022).

#### **IV. PLANT-ENDOPHYTE INTERACTION**

Endophytes are microbes that are capable of inhabiting plants in a symbiotic relationship without the formation of any apparent symptoms on the host (Hardoim et al., 2015). Microbes enter plants through roots or through shoots. They may make entry, especially through natural openings such as stomata and hydathodes or through natural discontinuities in roots (Compant et al., 2010; Mercado-Blanco and Prieto, 2012). Endophytes may spend their entire life cycle inside plants. They are known as systemic endophytes. Some spend only part of their life cycle inside plants and are known as non-systemic endophytes (Wani et al., 2015). These microbial wonders inside plants are famed for their role in plant growth promotion. Endophytes are reported to produce plant growth-promoting hormones such as auxin, cytokinin and gibberellins. They also play a role in phosphate solubilization and the production of secondary metabolites that are antimicrobial in nature (Latha et al., 2019). The defence mechanisms of plants against pathogens are also influenced or upregulated in the presence of a healthy endophytic community (Jacob et al., 2020). Endophytes also have the potential to help host plants better survive stressed conditions and toxic contaminants through the production of secondary metabolites such as siderophores and carboxylic acids (Rajkumar et al., 2012). Vandenkoomhuysen et al. (2015) report endophytes as a significant factor in the stress tolerance ability of a plant. ACC deaminase produced by endophytic bacteria can bring down the concentration of ACC which is a precursor for ethylene. Ethylene is produced in excess during stress and this can harm plant health. ACC deaminase will ultimately bring down ethylene concentration in stressed conditions thus aiding plants to better resist abiotic stress (Hardoim et al., 2008).

Endophytes can also bring down the toxicity level in plants. Plants used for phytoremediation often accumulate contaminants such as heavy metals inside their tissues. This can at some point of time become toxic to the plant itself. Microbiota in plants can bring down the toxicity through various methods such as the production of iron chelators, siderophores, or other enzymes that can degrade toxic metals (Yousaf et al., 2010). The three major ways in which endophytes help plants better perform in phytoremediation are through the promotion of plant growth, by bringing down phytotoxicity and by increasing the tolerance of plants. Thus, it can be clearly concluded that phytoremediation can be better achieved when microbes are used along with plants. Most microbes associated with plants, that are capable of tolerating contaminated conditions, would have the same ability as well. This is because they invariably survive in the same stressed condition. Endophytes that show resistance to heavy metals have been identified even from plants that are not exposed to heavy metal contamination (Moore et al., 2006). Besides the potentiality of endophytes to aid plants in phytoremediation, these microbes can also be subjected to genetic engineering much easier than plants. The ability of plants to tolerate toxic contaminants and the ability of microbes to complement this attribute needs to be studied and applied together (Fatima et al., 2016).

## **V. PLANT SELECTION AND ISOLATION OF ENDOPHYTES FOR PHYTOREMEDIATION**

Plants that are able to extract toxic contaminants from the soil or are able to immobilize them and those that can better tolerate heavy metal toxicity are selected for phytoremediation. Some plants are better suited for endophyte isolation as well because the chances of finding tolerant endophytes are higher in such plants. It was proposed that plants that grow at least 5 to 10 m apart and those that are ecotypes must be selected. Ecotypes are groups of plants that are adapted to the particular environment it grows and thus show some variations from other members of the same species. Elimination of collecting clones is the idea behind the selection of plants that grow at a distance from each other. For the phytoremediation of water sources, wetland plants are considered to be the best candidates due to their attributes such as better stress tolerance, exuberant root system, sturdier development characteristics and the like (Ali et al., 2020). Besides these features, any plant selected for endophyte isolation must be healthy and symptomless (Shadmani et al., 2021).

After collection, plants need to be subjected to surface sterilization before endophyte isolation. Successful surface sterilization will ensure that only the endophytes grow in the culture medium and not any of the undesirable microbes on the surface of the plant. One of the most accepted surface sterilants today is sodium hypochlorite (NaOCl). The plant is first washed with water to remove dirt particles under running tap water, treated for 5 minutes with 70% ethanol, 20 minutes with NaOCl and then washed with distilled water. The plant is then ground to a slurry using sterilized mortar and pestle in a laminar airflow chamber and this is plated on Luria-Bertania's (LB) agar (Tryptone 10g/L, Yeast extract 5g/L, NaCl 10g/L, pH - 7.0) for bacterial endophytes. The slurry can be directly plated or can be subjected to serial dilutions. The water from the last wash is also plated to ensure the completion of surface sterilization. LB agar is modified with the addition of components for studying tolerance to heavy metals. For example, Cd (CdCl<sub>2</sub>) can be used for studying tolerance to cadmium toxicity. (Liu et al., 2019).

Potato dextrose agar (PDA) is used for the culture of endophytic fungus. It is supplemented with chloramphenicol (50mg/L) to ensure that undesirable bacterial growth is entirely inhibited. Incubation at 25<sup>0</sup>C for about 9 days will give fungal colonies (Shadmani et al., 2021). Similarly, antifungal compounds can be added to LB agar to remove the prospect of fungal growth and obtain cultures of bacteria alone

For the culture of actinobacteria, starch casein medium is used (SCM; soluble starch 10g/L, K<sub>2</sub>HPO<sub>4</sub> 2g/L, KNO<sub>3</sub> 2g/L, casein 0.3 g/L, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.05g/L, CaCO<sub>3</sub> 0.02g/L, FeSO<sub>4</sub>.7H<sub>2</sub>O 0.01g/L, Agar 20g/L and pH 7.0). Endophytic actinomycetes are reported to better grow in slightly modified starch casein in the medium. 1% powdered host plant can be added to this medium which will allow the growth of endophytes. Microbial growth can be observed in 2 weeks when incubated at 30<sup>0</sup>C (Al-Huqail and El-Bondkly, 2021).

## **VI. IDENTIFICATION AND CHARACTERIZATION OF ENDOPHYTES**

For the identification of bacterial endophytes, the conserved region in their DNA known as 16s region is used. DNA can be extracted using Zymo research fungal/bacterial DNA miniprep kit. Then the 16sDNA region from the extracted DNA is amplified using

PCR. Primers such as 27f, 1498r and the like can be used. For the molecular identification of fungus, the ITS (Internal Transcribed Spacer) region of the DNA is used. PCR amplification of this ITS region is carried out using primers such as ITS1 and ITS4. The concentration of the PCR products is checked using a nanodrop spectrophotometer after purification. The products are then sequenced (Xia et al., 2015). Sequences are run in BLAST of NCBI for identification of fungal or bacterial species by sequence alignment (Shadmani et al., 2021). Cultured endophytic fungus is classified as either DSE (dark septate endophytes) or non-DSE (Sieber and Grunig, 2013).

To understand the role of endophytes in phytoremediation, their plant growth-promoting characteristics need to be analyzed. They are important in providing the plant with the ability to grow better in the presence of toxic contaminants. Endophytes are first characterized for the production of plant growth-promoting phytohormones such as IAA (Brick et al., 1991) and gibberellic acid (Frankland and Wareing, 1960). Lettuce hypocotyl bioassay is the proposed method for the estimation of gibberellic acid production. Production of ACC deaminase is estimated using a colourimetric ninhydrin assay (Anwar et al., 2016). Another important characteristic to be estimated is the phosphate solubilization ability of the endophytes. Phosphate in the soil Pikovskaya's plate method is one of the most sought-after methods for the estimation of phosphate solubilization (Al-Huqail and El-Bondkly, 2021). Production of enzymes such as chitinase, mannosidase and amylase also contribute to plant growth promoting characters. Quantitative estimation is done for these enzymes. Synthetic agar medium is used for chitinase, casein for mannosidase and starch agar medium is used for amylase (Al-Huqail and El-Bondkly, 2021).

The ability of endophytes to tolerate toxic metal ions can be studied using the method described by El-Gendy and El-Bondkly (2016). Metal ions of nickel, iron, copper, cadmium, lead, cobalt, mercury, manganese, arsenic, chromium, aluminium, zinc and silver can be supplemented in cultural media such as starch casein broth or Kuster's media for actinomycetes culture. The minimum inhibitory concentrations and IC<sub>50</sub> values need to be calculated which would give a picture of how well the endophytes are able to tolerate toxic metals (Al-Huqail and El-Bondkly, 2021). Siderophore production is one of the most important endophyte characteristics to be looked out for, in regard to phytoremediation. It can be determined using methods of Schwyn and Neilands (1987). 1 ml King B medium is used for culture of endophytes to which 1 ml of Chrome azurol-S solution is added and is allowed to react. The optical density of the cultures is measured and the siderophore production index is calculated.

One of the major limiting factors in studying the microbiome of a plant is that most of the endophytes are not culturable (Thomas et al., 2008). It is estimated that culturable bacteria sum up to a mere 0.0001% to 1% of the total endophyte community of a plant (Alain and Querellou, 2009; Torsvik and Ovresas, 2002; Afzal et al., 2019). Another major concern is the growth of undesirable microbes in the culture plates. Extreme sterile conditions are necessary throughout the culture period. (Afzal et al., 2019). Metagenomic approaches can be used for the study of such unculturable endophytes. Whole DNA is extracted from the plant directly and then this is used for the construction of 16srDNA libraries for bacterial endophytes and ITS for fungal endophytes. Next-generation sequencing, transcriptomics and RNA sequence analysis can be used to assign gene functions and study potential genes and gene products that could play a role in phytoremediation (Ijaz et al., 2016).



## VII. ENDOPHYTES IN PHYTOREMEDIATION

Plants that are capable of absorbing and storing heavy metals in their tissues are known as hyperaccumulators and they are capable of phytoremediation. Two such hyperaccumulators are *Astragalus bisulcatus* and *Stanleya pinnata*. These plants are selenium hyperaccumulators and many of their endophytic species such as *Bacillus*, *Pseudomonas*, *Pantoea*, *Staphylococcus*, *Paenibacillus*, *Advenella*, *Arthrobacter*, and *Variovorax* are also reported to have properties of selenium accumulation by production of elemental selenium that plants can use for growth promotion (Sura-de Jong et al. 2015). Textile effluents are a major pollutant in the aquatic ecosystem. Wetland plants are better suited for the phytoremediation of contaminated water bodies. *Typha domingensis* is one such wetland plant that could be used for phytoremediation, but most of the time plant health was adversely affected and mutagenesis was reported. The addition of endophytic bacteria, *Microbacterium arborescens* TYSI04 and *Bacillus pumilus* PIRI30 significantly improved both these factors. It also enhanced the degradation of pollutants by the plant (Shehzadi et al. 2014). *Pantoea stewartii* ASI11, *Microbacterium arborescens* HU33, and *Enterobacter* HU38 are endophytes isolated from the plant *Prosopis juliflora* that survived in soil contaminated with toxic metals such as Cr, Cd, Cu, Pb, and Zn. When these endophytes were inoculated in the ryegrass, it was reported to have significantly improved Cr toxicity resistance and uptake by the grass (Khan et al. 2015). Fungal endophytes such as *Plectosphaerella* sp., *Cladosporium* sp., and *Verticillium* sp isolated from *Dysphania ambrosioides* L. plants collected from Huize County, Yunnan province, Southwest China, exhibited heavy metal tolerance (Li et al., 2016).

Not just heavy metals, but endophytes are also capable of degrading organic contaminants. *Burkholderia cepacia* is an endophyte isolated from *Zea mays*. Wang et al. (2010) reported the ability of *Burkholderia cepacia* to degrade organic contaminants such as phenol and toluene. Another example is the *Sphingopyxis* sp. and *Pseudomonas* sp. Isolated from the root and shoot of *Lolium multiflorum* and *Lotus corniculatus* capable of degrading alkanes (Yousaf et al. 2010). Instead of using one or two species of endophytic bacteria, often a bacterial consortium of certain endophytic bacteria and rhizospheric bacteria is often used for phytoremediation. Bacteria from the endosphere and rhizosphere of *Acer pseudoplatanus*, proved to potentially alleviate toxicity due to the presence of TNT (2,4,6-trinitrotoluene) was reported by Thijs et al. (2014). Due to years of military training activities millions of hectares of land that are remote and hard to access are said to be polluted with TNT in the US. There is a high requirement of alternate sources for the remediation of such polluted lands.

Besides the accumulation and degradation of contaminants, an important feature of endophytes is that they are capable of improving plant growth through the production of phytohormones. *Acinetobacter*, *Agrobacterium tumefaciens*, *Bacillus* sp., *B. subtilis*, and *B. megaterium* all reside as endophytes in the plant *Commelina communis*. These endophytes are reported to aid plant growth promotion through the production of the hormone IAA and thus phytoremediation can be better achieved (Zhang et al. 2011). Not just the production of hormones but endophytes can downregulate certain hormones such as ethylene which also aids in plant growth promotion. *Methylobacterium oryzae* CBMB20, *Burkholderia* sp. obtained from tissues of *Oryza sativa* when inoculated in *Lycopersicon esculentum* is reported to produce ACC deaminase that brings down ethylene emission under stressed conditions (Madhaiyan et al. (2007). *Pseudomonas tolaasii* ACC23, *P. fluorescens* ACC9,

*Mycobacterium* sp. ACC14 isolated from the roots of some Gramineae grasses that grow in meadows polluted with cadmium, nickel and copper has been studied by Dell'Amico et al. (2008) and identified that they produce IAA, siderophores and ACC deaminase. All these ultimately reduce the production of ethylene, promote root elongation in plants, increase shoot and root dry biomass and can also play a role in phytoextraction of cadmium. Contaminants pose a major threat to the growth of plants. Plant growth-promoting activities of endophytes become significant in this context. Phosphate is required by plants in soluble form for proper growth and endophytes are capable of phosphate solubilization. Thus, phosphate-solubilizing endophytes also aid in phytoremediation. *Fusarium* sp. CBRF44, *Penicillium* sp. CBRF65 and *Alternaria* sp. CBSF6 isolated from *Brassica napus* L. in China was reported to have the ability to solubilise phosphate, and produce IAA and siderophores and are thus potential candidates in improving phytoremediation methods. (Shi et al., 2017). *Piriformospora indica* inhabiting *Zea mays* L. provide that plant with tolerance against petroleum hydrocarbons, which are organic contaminants, through its plant growth-promoting properties (Said et al., 2017).

Plants and their endophytes are known for the production of small molecular weight compounds capable of metal chelation, known as siderophores. One of the major metal ions scavenged by siderophores are of iron the others being that of copper, nickel, zinc, cadmium and cobalt. The presence of siderophores in plants can reduce metal ion composition in soil. This is because, when plants absorb metal ions into their cells, siderophores chelate them and restrict them within the cell membranes itself (Hofmann et al., 2020). It was reported in 2018, by Gu et al. that siderophores from *Pseudoalteromonas* sp. found in the marine environment were involved in phytoremediation of the contaminant Tetrabromobisphenol A (TBBPA).

**Table 1:** Some Other Recently Reported Endophytes in Phytoremediation

Endophyte	Host Plant	Phytoremediation Activity	Reference
<i>P. plecoglossicida</i>	<i>Z. mays</i>	Tolerance against Al heavy metals through the production of IAA and ACC-deaminase	Zerrouk et al., 2019
<i>M. anisopliae</i> SLR04, R. vinctus SLS02, <i>Phomopsis</i> sp. SLS05, <i>D. nobilis</i> SLS18, and <i>C. nigricolor</i> SLS22	<i>Polygonum hydropiper</i>	Production of plant growth promoting hormones in phosphorus accumulating species.	Ye et al., 2019
<i>Rhodotorula mucilaginosa</i> CAM4	<i>L. sativa</i>	Tolerance against Al heavy metals through the production of IAA and siderophores	Silambarasan et al., 2019
<i>Microbacterium oxydans</i> JYC17, <i>P. thivervalensis</i> Y1-3-9, and <i>B. cepacia</i> J62	<i>Brassica napus</i>	Tolerance against heavy metals of Cu	Ren et al., 2019
<i>P. fluorescens</i>	<i>Sedum</i>	Tolerance against Cd ions	Wu et al.,

	<i>alfredii</i>	through production of IAA	2020
<i>Aspergillus sp.</i> A31, <i>C. geniculata</i> P1, <i>Lindgomycetaceae</i> P87 and <i>Westerdykella sp.</i>	<i>Aeschynomene fluminensis</i>	Bioremediation of mercury	Pietro-Souza et al., 2020
<i>Pseudomonas fluorescens</i> , <i>Kosakonia radicinans</i> , <i>Paraburkholderia tropica</i> , and <i>Herbaspirillum frisingense</i>	<i>Saccharum officinarum</i>	Tolerance against Al toxicity	Labanca et al., 2020
<i>Serratia sp.</i> IU01	<i>Brassica napus</i> L.	Tolerance against Cd toxicity, plant growth promotion, protection against oxidative damage	Shah et al., 2020
<i>B. contaminans</i> ZCC	Soy beans	Tolerance against Cd toxicity	You et al., 2021
<i>Pseudomonas sp.</i> K32	<i>Oryza sativa</i>	Tolerance against Cd toxicity	Pramanik et al., 2021
<i>B. atrophaeus</i> GQJK17 S8, <i>E. asburiae</i> QB1	<i>Chenopodium quinoa</i> willd.	Tolerance against Cu and Cd toxicity	Mahdi et al., 2021
<i>Providencia sp.</i>	<i>Z. mays</i>	Tolerance against Cr toxicity	Vishnuprad eep et al., 2022

### VIII. CONCLUSION

Pollution is a major concern for people around the globe. Both elemental contaminants like heavy metals, radioisotopes metalloids and other organic contaminants are being released to the environment at an alarming rate. The air we breathe, the water we drink and the soil we walk on are all not safe enough anymore solely due to anthropogenic activities. Various equipment we use, industries we run and other factors such as the use of pesticides are all so intimately connected to our day-to-day life and the kind of pollution these activities cause is painstakingly huge. People have devised various approaches for the slowing down of such pollution and also for the removal of contaminants from the environment. Physical, chemical and biological practices are being used today. But both the physical and chemical processes are costly and sometimes cause the generation of more waste. Biological remediation that includes phytoremediation is a major advantage in both these aspects. It is both eco-friendly and economically feasible. Phytoremediation as such uses plants alone for extraction or stabilization of contaminants from air, soil or water. However, phytoremediation can be aided by endophytes. Endophytes are capable of

improving plant growth, thus helping tolerant plants grow better in contaminated conditions. Production of IAA, production of ACC deaminase and phosphate solubilization activity contributes to this attribute. They are also capable of degrading or stabilizing contaminants by themselves through the production of secondary metabolites such as siderophores. Thus, endophyte-aided phytoremediation works much better than phytoremediation by itself. It is an area with immense potential of further breakthrough studies.

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