Evolution of Phytological growth activities of Meerut agricultural soil microbes under heavy metal stress

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Abstract

Heavy metal pollution of soils is of great concern. The presence of the toxic metal species above critical concentration not only harmfully affects human health but also the environment. Phytoremediation approach using metal accumulating plants is much convincing in terms of metal removal efficiency, but it has many limitations because of slow plant growth and decreased biomass owing to metal-induced stress. Soil microorganisms play a central role in maintaining soil structure, fertility, and in remediating contaminated soils. Rhizosphere, an important interface of soil and plant, plays a significant role in Phytoremediation of contaminated soil by heavy metals, in which, microbial populations are known to affect heavy metal mobility. Phytoremediation strategies with appropriate heavy metal-adapted rhizobacteria have received more and more attention. The supplementation of silicon as nutrient to the plants may play a significant role which includes increase in crop growth and yield, improvement of leaf exposure to light, decreased susceptibility to pathogens and pests and amelioration to abiotic and biotic stresses. The understanding of the beneficial effects of silicon is important to improve crop productivity as it may enhance plant nutritional value for a growing world population.

Keywords: Heavy metal pollution, toxic metal species, Phytoremediation, bacterial assisted Phytoremediation, Rhizosphere, rhizobacteria, mobilization of heavy metals.

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Introduction

Agriculture of India is still facing a multitude of problems to maximize productivity to feed the continuously increasing population (Brahman *et al.*, 2013). Farmers are intensifying land use practices without proper nutrient management in their fields which results in nutrient depletion from the soil and it is linked to the decline in crop yield (Meena *et al.*, 2014). Agricultural soil is slightly too moderately contaminated by heavy metal toxicity such as by Cd, Cu, Zn, Ni, Co, Cr, Pb and As. This could be due to long term use of phosphate fertilizers, sewage sludge application, smelters dust, industrial waste and bad watering practices in agricultural lands (Bell *et al.*, 2001; Schwartz *et al.*, 2001; Passariello *et al.*, 2002). Excessive

accumulation of heavy metals is toxic to most of the plants. Heavy metals ions are excessively absorbed by roots and translocated to shoot, leading to impaired metabolism and reduce growth (Bingham *et al.*, 1986; Foy *et al.*, 1978). In addition excessive metal concentrations in soil contamination result in microbial activity and fertility of soil and yield losses (McGrath *et al.*, 1995). Waste water in Meerut city runs through a peripheral water stream (Kali nadi) around the city. This water is lifted directly for agriculture purpose. The wastewater stream and agricultural fields near Abdullapur region, irrigated with this waste water is the study area. In the present study we have investigated the heavy metal content of urban waste water, soils and vegetables under cultivation.

Soil microbes

a) Types of soil microbes

Soil Flora (Microflora): Bacteria, Fungi, Molds, Yeast, Mushroom, Actinomycetes, Streptomyces, (Algae i.e. BGA, Yellow Green Algae, Golden Brown Algae). Bacteria is again classified in (I) Heterotrophic e.g. symbiotic & non - symbiotic N₂ fixers, Ammonifier, Cellulose Decomposers, Denitrifiers; (II) Autotrophic e.g. Nitrosomonas, Nitrobacter, Sulphur oxidizers, etc. Macroflora: Higher plant roots

Soil Fauna (Microfauna): Protozoa and Nematodes, Macrofauna: Earthworms, Moles, Ants and others.

b) Plant growth promoting bacteria:

The beneficial free living soil bacteria that exist in roots association of many different plants which referred to as plant growth promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978). Depending on their host plants relationship, PGPR divided into two major groups:

(i) Symbiotic rhizobacteria, which may invade the cell interior and inside cell survival (also called intracellular PGPR, example, bacterial nodule), and (ii). Free-living rhizobacteria exist outside plant cells (called extracellular PGPR, e.g., Bacillus, Pseudomonas, Burkholderia, and Azotobacter) (Khan 2005; Babalola and Akindolire, 2011). PGPR can positively influence plant growth and development in three different ways, in that they

- a. Synthesize and provide growth – promoting compounds to the plants (Glick, 1995)
- b. Facilitate the uptake of certain environmental nutrients such as nitrogen, phosphorus, sulphur, magnesium and calcium (Bashan and levanony, 1990; Belimov and

Dietz, 2000; Cakmakei *et al.*, 2006), and

- c. Decrease or prevent some deleterious effects caused by phytopathogenic organisms or other diseases (Khan *et al.*, 2002; Lugtenberg and Kamilova, 2009).

Generally, rhizobacteria improve growth of plant by synthesizing phytohormone precursors (Ahmad *et al.*, 2008), vitamins, enzymes, siderophores and antibiotics (Burd *et al.*, 2000; Noordman *et al.*, 2006). PGPR also increase plant growth by synthesizing specific enzymes which generates biochemical changes in plants. For example ethylene plays a critical role in various plant developmental processes such as leaf senescence and abscission, epinasty, and fruit ripening (Vogel *et al.*, 1998). Ethylene also regulates node factor signaling, nodule formation, and has primary functions in defense systems of plants. Moreover, as a result of the plant infection by rhizobacteria, ethylene production is increased (Boller, 1991) which at high concentrations will inhibit plant growth and development (Morgan and Drew, 1997; Grichko and Glick, 2001).

Production of Plant Hormones

Plant hormones are biochemical molecules which are regulating different plant activities. PGPR are also able to produce some of the plant hormones like Auxin and cytokinins. Auxin is among the most important plant hormones affecting growth and development of plant singly or in interaction with the other plant hormones (Miransari *et al.*, 2014; Remans *et al.*, 2007) indicated that the responsiveness of roots of common bean (*Phaseolus vulgaris* L.) to the presence of *Azospirillum brasilense* is determined by the Auxin production by the PGPR bacteria. The loci location which was responsible for root responsiveness to production of Auxin by the bacteria was exactly similar to the location of trait loci, which produces root tips under low phosphorus concentration. That is indicating

there is some kind of interaction between Phosphorus, Auxin and in root formation.

The Advantages of PGPR in Comparison to Chemical Fertilization

The use of PGPR is superior to chemical fertilization due to the following reasons: (i) they can be used more safely, (ii) more recommendable for economic and environmental conditions, (iii) have used wider range, (iv) much affective in smaller amounts, (v) can be multiplied, however their number is controlled by the activity of plant and other soil microbes, (vi) can contribute to soil organic matter, (vii) can be used under different agricultural practices types including organic farming, (viii) are less subjected to chemical reactions in the soil (Berg, 2009).

Nature and classification of Heavy metals

The public concern growing over the degenerating quality of the environment has led to a widespread usage when referring to trace elements. Hence, for all practical purposes, other terms such as “trace inorganics,” “heavy metals,” “microelements,” and “micronutrients” have been treated as with the term trace elements (Nagajyoti *et al.*, 2010). The elementary constituents of plant, animal, and human life may be classified as major and trace elements, the latter group including both essential and nonessential elements (including toxic elements). Some of the heavy metals such Fe, Cu, and Zn are essential for plants and animals (Wintz *et al.*, 2002). The availability of heavy metals in different medium, and metals such as Cu, Zn, Fe, Mn, Mo, Ni, and Co are essential micronutrients (Reeves and Baker, 2000), whose uptake in excess to the plant requirements result in toxic effects (Monni *et al.*, 2000; Blaylock and Huang, 2000). An alternative classification of metals based on their coordination chemistry categorizes heavy metals as class B metals that come beneath nonessential trace elements, which are highly toxic elements such as Hg, Ag, Pb, and Ni

(Nieboer and Richardson, 1980). Some of these heavy metals are bio-accumulative, and they not break down in the environment nor are easily metabolized. Such metals accumulate in the ecological food chain through uptake at primary producer level and then through consumption at consumer levels. Plants are stationary, and plant roots are the primary contact site for heavy metal ions in soil.

a) Types of heavy metals

Lead: Lead is naturally occurring, but it is usually released into the environment from artificial sources. It has been mined, smelted, refined and used as an addition in paints and gasoline, leaded pipes, solders, crystals and ceramics (Alberto *et al.*, 2007). Lead concentrations in uncontaminated soils are generally $Ca\ 20 \pm 50\ mg/kg$ (Norrish, 1968; Nriagu, 1978), Lead addition to agricultural soils in herbicides/ pesticides has been significant in the past with some orchard soils receiving up to $kg\ Pb/year$ resulting in lead concentration in some of these soils exceeding $500\ mg\ kg^{-1}$ (Merry *et al.*, 1983).

Mercury: Mercury occurs in a wide range of minerals in the earth's crust, with average crustal concentrations of mercury $\sim 0.08\ mg/kg$ (Mason and Moore, 1982). It is also associated with Zn, Fe and other complex sulphides. Mercury concentrations in limestone are generally $< 20\ mg\ kg^{-1}$, animal manures may have concentrations of mercury in sludge may range from 5 to $10\ mg/kg$, and occasionally values of up to $100\ mg\ kg^{-1}$ are reported (Steinnes, 1990).

Cadmium: Cadmium concentrations in crystal rocks vary from one to $90,000\ mg/kg$ (Page *et al.*, 1981; Cook and Frenay, 1998) with igneous and metamorphic rocks generally having lower Cadmium addition to soils varies widely among the countries and the regions within countries (Jensen and

Bro-Rasmussen, 1992; McLaughlin *et al.*, 1996). Cadmium is an important toxic heavy metals and the warning of cadmium pollution health risks was issued initially in the 1970s.

Arsenic: Arsenic is widely distributed into the nature in form of either metalloids or chemical compounds, which causes a variety of pathogenic conditions including cutaneous and visceral malignancies (Matsui *et al.*, 1999).

Selenium: The primary cause of elevated soil selenium is its natural occurrence most often in sedimentary rocks (especially some cretaceous shale) from which seleniferous soils wither (Mayland *et al.*, 1989).

b) Heavy metal-bacteria interactions

Rhizobacteria have been shown to possess several traits that can alter heavy metals bioavailability (McGrath *et al.*, 2001; Whiting *et al.*, 2001) through the exemption of chelating substances, acidification of the microenvironment, and by influencing changes in redox potential (Smith and Read, 1997). For example, Abou-Shanab *et al.* (2003a) reported that the inclusion of *Sphingomonas macrogoltabidus*, *Microbacterium liquefaciens*, and *Microbacterium arabinogalactanolyticum* to *Alyssum murale* grown in serpentine soil which is significantly increased the plant uptake of Ni when compared with the un-inoculated controls as a result of soil pH reduction.

c) Toxic effects

Contamination of agricultural soil by heavy metals has become a critical environmental concern being their potential adverse ecological effects. Such toxic elements are considered as soil pollutants due to their widespread occurrence and their acute and chronic toxic effect on plants grown of such soils. The regulatory limits of

Cadmium (Cd) in agricultural soil are 100 mg/kg soil (Salt *et al.*, 1995). Plants grown in soil containing high levels of Cd display visible symptoms of injury reflected in terms of Chlorosis, growth inhibition, browning of root tip, and finally death (Wojcik and Tukiendorf, 2004; Mohanpuria *et al.*, 2007). High levels of Zn in soil inhibit many plant metabolic functions; result in moronic growth and cause senescence. Zinc toxicity in plants limited the growth of both root and shoot (Choi *et al.*, 1996; Ebbs and Kochian, 1997; Fontes and Cox, 1998). Copper is considered as a micronutrient for plants (Thomas *et al.*, 1998) and plays important role in CO₂ assimilations and ATP synthesis. Excess of Cu in soil plays a cytotoxic role induces stress and causes plants injury. This leads to plant growth retardation and leaf chlorosis (Lewis *et al.*, 2001). Exposure of plants to excess Cu generates oxidative stress and ROS (Stadtman and Oliver, 1991), oxidative stress causes disturbance of metabolic pathways and damage to macromolecules (Hegedus *et al.*, 2001). Chromium (Cr) is a heavy metal that causes genuine environmental contamination in soil, sediments, and groundwater (Shanker *et al.*, 2005). Toxicity of Cr has been studied in many plants. Toxic effect of Cr on plant growth and development include alterations in the germination process as well as in the growth of roots, stems and leaves. Hence, exposure to high level of Cr affected total dry matter production and yield of plants (Shanker *et al.*, 2005). Lead (Pb) is one of the ubiquitously distributed most generous toxic elements in the soil. The toxic level of Pb in soil results from disposal of municipal sewage sludge, mining and smelting activities, Pb containing paints, pulp and paper,

gasoline and explosive. High level of Pb also causes inhibition of enzyme activities, water imbalance, and alterations in membrane permeability and distributes mineral nutrition (Sharma and Dubey, 2005). Toxic effects of Cr on plant growth and development include alterations in the generation process as well as in the growth of roots, stems and leaves. Hence exposure to high level of Cr affected total dry matter production and yield of plants (Shanker *et al.*, 2005). Arsenate (As) is an analog of Phosphate (P) and competes for the same uptake carriers in the root plasma membrane of plants (Meharg and Macnair, 1992). Nickel (Ni) is a transition metal and being in natural soils at trace concentrations excepting in ultramafic or serpentinitic soils. However, Ni^{2+} concentration is increasing in certain areas by human activities such as mining works, smelters emission, coal burning and oil, sewage, Phosphate fertilizers and pesticides (Gimeno-Garcia *et al.*, 1996). Exposure of Wheat to high level of Ni^{2+} enhanced MDA concentration (Pandolfi *et al.*, 1992).

d) Beneficial effects

The elements which promote growth and act as essential are Al, Co, Na, Se, and Si, these elements promote growth of various plant species under certain environmental conditions, however their function and concentration varies in various plant species (Pilon-Smits Elizabeth *et al.*, 2009). According to Epstein and Bloom (2005) silicon acts as a quasi-essential element for plants because its deficiency can cause different abnormalities with respect to plant growth, development and reproduction. Silicon is an agronomically essential fertilizer because it enhances plant tolerance capacity to abiotic and biotic stresses

(Liang *et al.*, 2005). *Bacillus clausii*, *Bacillus mucilaginosus*, *Protens mirabilis*, *Pseudomonas* and *Penicillium* were found to release silicon, silicate minerals contain K, Ca, Mg, Fe and Zn and therefore inoculation of silica solubilizing bacteria into the soil may benefit the crop by releasing several nutrients (Muralikannan and Anthomiray, 1998). Silicon and Aluminium interact in the soil and form sub-colloidal and inert aluminosilicates and reduce the concentration of phytotoxic aluminium in soil solution (Liang *et al.*, 2007).

e) Mechanism of heavy metal toxicity in plants

The toxicity of heavy metals is manifested in many ways when plant cells accumulate them at high levels heavy metals can be divided into two groups: redox active (Fe, Cu, Cr, Co) and redox inactive (Cd, Zn, Ni, Al, etc). The redox active heavy metals are directly elaborate the redox reaction in cells and result in the formation of $\text{O}_2^{\cdot-}$ and subsequently in H_2O_2 and OH production via the Haber-Weiss and Fenton reactions (Schutzendubel and Polle, 2002) exposure of plants to redox inactive heavy metals also results in oxidative stress through indirect mechanisms. Such as interaction with antioxidant defense system, disruption of the electron transport chain or induction of lipid peroxidation. The latter can be due to a heavy metal induced increase in lipoxygenase (LOX) activity. Another important mechanism of heavy metal toxicity is the ability of heavy metals to bind strongly to oxygen, nitrogen, and sulphur atoms. This binding affinity is related to free enthalpy of the formation of the product of heavy metal and ligand with low solubility of these products.

Because of these features heavy metals can inactive enzymes by binding to cysteine residues. For example, Cd binding to sulfhydryl groups of structural proteins and enzymes leads to misfoldings and inhibition of activity and / or interference with redox-enzymatic regulation (Dalcorsio *et al.*, 2008; Hall, 2002). Many enzymes use cofactors to work properly for both heavy metals ions (such as Fe^{2+} , Mg^{2+} , Cu^{2+} , Ca^{2+}) and organic molecules (such as haem, biotin, FAD, NAD, or Co enzyme A). The displacement of one heavy metal ion by another leads to the inhibition or loss of enzymes activities. Divalent cations such as Co^{2+} , Ni^{2+} , and Zn^{2+} displace Mg^{2+} in ribulose-1-5-bisphosphate-carboxylase/ oxygenase (RuBisco) and result in a fall of activity. Displacement of Ca^{2+} by Cd^{2+} in Calmodulin, an important protein in cellular signaling, led to the inhibition of Calmodulin-dependent phosphodiesterase activity in radish (Rivetta *et al.*, 1997).

f) Techniques to cure plant species

Heavy metals cannot be destroyed biologically (no “degradation” change in the nuclear structure of the element shows) but are only transformed from one to another oxidation state or organic complex (Garbisu and Alkorta, 2001) remediation of heavy metal contamination in soil is more difficult.

- (i) Phytoremediation:** Phytoremediation is an in situ bio mediation process that uses green plants and the microorganisms that are associated with them to extract, sequester, or detoxify pollutants. Plants have the capacity to take up, accumulate, reduce, or eliminate metals, pesticides, solvents, crude oil, and many industrial contaminants. There are many successful examples where

phytoremediation has been occupied and where it has been documented to work well for remediating contaminated industrial environments (Macek *et al.* 2000; Suresh and Ravishankar, 2004). Depending on the method used and contaminated nature involved, phytoremediating areas where metals and other inorganic compounds exist, may utilize one of several techniques (Glick,2003; Newmann and Reynolds, 2004) as follows:

- Phytoextraction,
Phytostabilization,
Phytostimulation,
Phytovolatilization/
Rhizovolatilized,
Phytodegradation,
Rhizofiltration.
- When research is dedicated to finding optimal hyper-accumulator plants, key study goals should include both (1) evaluating the impact of metal stress on beneficial rhizospheric microbes and crop and (2) concluding the application of bioremediation technologies that could be used to clean up metals from the polluted soils.

- (ii) Mobilization of heavy metals:** Plants have developed mechanisms by which they can effectively absorb metals from the soil solution and transport them to other parts within the plant. Most metal accumulating species were discovered in which areas having immense metal concentration, and majority of such areas exist in tropical regions for examples, Indian mustard (*Brassica juncea*) (Salt *et al.*, 1995; Salt and Kramer,1999). Uptake of metals into root cells which is the point of entry into living tissue is a major step in the Phytoextraction process. The mechanisms by which

metals are absorbed into complex plant root. This process involves transfer of metals from the soil solution to the root surface interface, and then penetration through the membranes of root to root cells. Metal ions cannot move openly across the cellular membrane because of their charge. Therefore, ion transport into cells must be mediated by membrane proteins that have a transport function,

and these referred to as transporters. These transporters possess an extracellular domain to which the ions attach just before the transport, and a trans membrane binding structure that comes connects extracellular and intracellular media. This is an oversimplification, and the uptake process complex by nature of the rhizosphere (Laurie and Manthey, 1994).

Table 1: Physicochemical analysis of Kali Nadi water, Abdullapur region, running peripheral to Meerut city

Parameter	September	October
pH	7.38 ±(0.81)	7.81± (0.84)
Cu (mg L ⁻¹)	7.54± (1.82)	5.23 ± (1.05)
Cr (mg L ⁻¹)	24.23± (5.98)	13.02± (6.32)
Cd (mgL ⁻¹)	18.22 ± (5.19)	4.54 ± (4.24)
Pb (mg L ⁻¹)	11.13 ± (6.88)	5.32± (6.24)
Zn (mgL ⁻¹)	21.01± (6.44)	8.54 ± (7.32)
Ni (mg L ⁻¹)	18.32 ± (8.22)	4.98± (4.72)

Table 2: Accumulation of heavy metals in vegetables grown in wastewater irrigated soils

Metal concentration (mg/ Kg dry weight)				
Vegetable	Cr	Cd	Pb	Zn
Allium cepa L.	11.82± (5.44)	4.10 ± (1.97)	11.30± (2.98)	28.32 ± (7.34)
Coriander sativum L.	17.23 ± (6.21)	14.99 ± (3.98)	20.46 ± (6.24)	23.22± (8.01)
Spinacia oleracea L.	18.31 ± (5.45)	24.46 ± (6.41)	49.92 ± (8.41)	18.71 ± (4.04)

Conclusion

Plants grow on heavy metal polluted soils resultant in reduction in growth due to changes in their physiological and biochemical activities especially true when the heavy metal involved does not play any beneficial role against the growth and plant development. Thus, it is evident from the several research findings that judicious use and presence of heavy metals having toxic effects on plants after certain limits. There are two aspects on the interaction of plants and heavy metals, one hand; heavy metals show negative effects on

plants and other hand, plants have their resistance mechanisms towards toxic effects and for detoxifying heavy metal pollution. Our review showed that both growth and photosynthetic pigments are affected by the presence of heavy metals. The toxicity of heavy metals which is caused by their accumulation in soil can be removed by using hyper accumulator plant through bioremediation/phytoremediation process effectively used for the treatment of heavy metal polluted soil. Plants employ different mechanisms in there mediation of heavy metal polluted soils and Phytoextraction is the most

common method of phytoremediation used for treatment of heavy metal polluted soils which ensures the complete removal of the pollutant. Microorganisms establish associations with plants and promote plant growth by means of several beneficial characteristics. Finally the search for beneficial bacteria is important for the development of new and efficient inoculants for agriculture. Thus, the introduction of beneficial bacteria in the soil tends to be less impact to the environment than chemical fertilizers, which makes it a sustainable agronomic practice and a way of reducing the production costs.

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