Development of Field Platforms for Bioremediation of Heavy Metal-Contaminated Site

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Shazia Iram

Abstract

An irrigation system in areas near urban periphery is partial or totally relies on untreated sewage effluents. There is very less data available about heavy metal status in raw sewage used for soil irrigation in Pakistan. On the other hand, soil of arid areas and semiarid areas is rich in metals like nickel, zinc, copper, and lead. The bioavailability of these heavy metals is affected largely by physical and chemical characteristics of soil and partially affected by characteristics of plants. This issue is a major concern for the health of humans and animals. Therefore, in order to prevent the possible health hazards of metals in agrarian land monitoring of soil, water and plant quality is essential. Heavy metal-contaminated soils need to be remediated. In Pakistan as a developing country, soil reclamation methods include physical and chemical management that cannot be brought into action because of expensive technologies involved. Phytoremediation, in general, phytoextraction, and microbial remediation in particular offer a promising alternative to conventional engineering-based technologies. Phytoremediation is an emerging technology that may be used to clean up contaminated soil in which plants are used for removing pollutants from the contaminated soils. Phytoextraction remediation technique has two strategies such as natural phytoextraction and chemically enhanced phytoextraction. In one study (Rawalpindi, Pakistan), tolerance potential of plants (*Zea mays*, sorghum, *Helianthus*, *Brassica*) was assessed against deleterious effects of heavy metals (Pb, Cd, Cr, Cu) on plant growth, and role of chelator (EDTA, DTPA, and NTA) and tolerant fungal strains was also checked to increase the tolerance index. By 3 years of research, it was assessed that heavy metal uptake and their translocation in biomass of plant enhanced the phytoremediation process from contaminated soil. Phytoremediation research in field can provide capacity building to youth and farmer community. By the bioremediation of soil and water, it is possible to

S. Iram (✉)
Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi, Pakistan

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produce biofuel, biomass, and gasification for energy production. Bioremediation techniques will provide training and capacity building to youth and serve an important role at field level for technology transfer and as a broker of emerging technologies.

8.1 Introduction

The agriculture sustainability largely depends on two natural resources: water and land; agricultural production is affected badly if one of them is limited. On Earth for existence and survival of life, water is a very important factor. It is being used for agricultural, domestic, industrial, and recreational purposes, though agricultural sector is using 90% of water (Dara 1993).

There is shortage of irrigation water in Pakistan because surface water does not fulfill the water requirements for crops. This water shortage is being fulfilled by combined use of groundwater and wastewater (domestic and industrial) of urban areas. This mixed water is being used for vegetable and crop growth in peri-urban agricultural areas (Lone 1995). In Pakistan it is estimated that in big cities like Lahore, Karachi, Multan, Peshawar, Faisalabad, Hyderabad, Kasur, Quetta, Sukkur, and Islamabad/Rawalpindi, sewage is being produced 116,590 million gallons per day, and 32,000 hectares of land is being irrigated with this water. Big cities have no proper disposal and management systems but produce constantly huge volumes of wastewater. In Pakistan only 2% of cities are using wastewater treatment plants (Clemett and Ensink 2006). So, 90% of untreated wastewater is being used in agricultural activities in more than 80% of cities of Pakistan (Ensink et al. 2004).

Currently 0.3 million hectares of agricultural land is irrigated with wastewater. The use of wastewater and its disposal ultimately boost agricultural production and minimize the threats of environmental contamination. This wastewater is used for irrigation as it is a rich source of nutrients which are beneficial for plant growth. There are various types of industries situated in and around industrial cities. These industries discharge their untreated effluent which will ultimately mix with urban wastewater and contain exceeding quantity of heavy metals such as chromium, cadmium, copper, lead, etc. This industrial wastewater may be poured directly into water courses without pretreatment, while on the other hand, farmers use this contaminated water in their fields (Malik et al. 2009). Soil acts as filter of toxic chemicals it may adsorb and retains heavy metals from wastewater (Rattan et al. 2005), but when the capacity of soil to retain toxic metals is reduced, then these toxic metals are released into groundwater. These toxic metals may enter the plants and whole food chain making it poisonous for human beings. Thus, environmental and human life quality simultaneously is under threat by increasing soil pollution (Zia et al. 2008).

8.2 Effects of Heavy Metals on Environment

Soil contamination with heavy metals is a common practice and difficult to treat because soil is a source and also sink for the heavy metals. With concern to human health, heavy metal assessment in the soil is a very important issue, because they are toxic in nature and their degradation is difficult. Therefore, these heavy metals remain persistent in the soil and in the ecosystem. Bioconcentration in different levels of food chain threatens all the living beings (Aragay et al. 2011). Soil contaminated with metals is a primary route of toxic metal exposure to humans causing cytotoxic, mutagenic, and carcinogenic effects in animals. Heavy metals could enter into the body of human beings when they consumed food contaminated with heavy metals. Dietary crops and vegetables grown in heavy metal-contaminated soils pose serious health problems (Zia et al. 2008). Heavy metal pollution increases in biological and ecological systems and exerts harmful effects. Even a very low level of these heavy metals causes serious health disorders. Heavy metals can persist in the soil for many thousands of years and dangerous for the higher organisms. These heavy metals also affect the plant growth and soil microbiota. It is a well-known fact that heavy metals cannot be degraded chemically, so they are physically removed and can be transformed into less toxic and nontoxic forms (Ghani 2010). Contamination of soil with heavy metals poses serious effects on living organisms and the ecosystem. Prolonged human exposure to heavy metals causes renal dysfunction disease – tubular proteinuria. Similarly inhaling dust and fumes having high metal concentrations can cause destructive lung disease, that is, pneumonitis. Cadmium pneumonitis can be identified as pain in the chest, with reddish sputum and an ultimately destroyed inner layer of tissues of the lungs due to excessive watery fluid accumulation. Excessive metal exposure may cause pulmonary edema which can lead to death. Contamination of soil with heavy metals needs the implementation of suitable remedial techniques (Vaxevanidou et al. 2008). Hence, there is a need to maintain the soil quality that is not a one-time course rather a continuous process.

8.3 Field Platform for Bioremediation

A variety of treatment technologies has been developed for the remediation of metal-contaminated soils, and bioremediation by plants (phytoremediation) is an economically feasible technique. It is more acceptable publically because overall it increases the aesthetic beauty of the contaminated area and also has potential to clean the environment (Chen and Cutright 2002). Phytoremediation is a plant-based remediation strategy which uses the plants for environmental remediation (Rauf et al. 2009).

Phytoextraction of heavy metals is a technique under phytoremediation plants act as a solar energy driven pumps and extract and accumulate different elements from the soil and environment (Luo et al. 2005). Heavy metals, for example, cadmium, lead, copper, nickel, and zinc, can be extracted through this technique. Among the phytoremediation categories, phytoextraction is used to extract heavy metals from the soil system by plants because some metals like Mn, Fe, Mg, Mo, Ni, Zn, etc. are essential plant nutrients. But for Pb removal, it is a commercially available technique. Phytoremediation efficiency depends on different factors like climate, time period, soil type, and root depth. Vassilev et al. (2002) reported the phytoextraction metal protocol that follows four main strategies:

1. Plant cultivation on heavy metal-contaminated soil
2. Harvest of metal-rich plant biomass
3. Postharvest treatments and successive plant biomass disposal as hazardous or toxic waste
4. Recovery of metals from metal-loaded plant biomass

Phytoremediation is an affordable and effective technical solution for heavy metal extraction from soil. Phytoremediation is an economical and environmental-friendly technique. Plant root's ability of uptake of heavy metals is being used in this process of phytoremediation along with the transformation, accumulation, and biodegradation capability of the whole plant body (Tangahu et al. 2011). Efficiency of remediation depends on different factors such as soil type, nutrient status of soil, and plant tolerance against heavy metals. For the management of contaminated agricultural land, the use of heavy metal-tolerant crops for remediation of heavy metals is a new emerging technique. It is indicated from the recent studies that different high-biomass-producing crop varieties have potential for heavy metal accumulation such as oat (*Avena sativa*), Indian mustard (*Brassica juncea*), sunflower (*Helianthus annuus*), maize (*Zea mays*), ryegrass (*Lolium perenne*), and barley (*Hordeum vulgare*) (Meers et al. 2005). In this technique by using high-biomass-producing crops, with better management of soil and improvement of plant husbandry, an alternative strategy could be developed for remediation of heavy metal-polluted soils (Evangelou et al. 2007). Effectiveness of phytoremediation depends on root zone of the plants. This may be from few centimeters to many meters. This phytoremediation technique is a long-term strategy, and it is more beneficial than other physical and chemical technologies. Hyper-accumulators are those plants which have 50–500 times greater capability to absorb metals than average plants (Lasat 2000). Hyper-accumulators have greater than one bioconcentration factor; sometimes it reaches 50–100 (McGrath and Zhao 2003). Hyper-accumulator plants are ideal model organisms for scientists and have acquired attention all over the world for their use in phytoremediation technology.

8.4 Native Plant Species of Pakistan for Bioremediation

Escalating heavy metal contamination in the environment is stated in most of the developing countries including Pakistan (Jamali et al. 2007; Kausar et al. 2012). In Pakistan tremendously higher concentration of mercury (Hg) has been noticed in marine and riverine ecosystems (Mubeen et al. 2010). Soil and water of Pakistan are most probably in direct use of fertilizers, herbicides, and pesticides in agrarian sector.

Soil heavy metal contamination became a very grave environmental concern in Pakistan. The instant increment in population together with untreated effluent disposal from textile and tannery industrial sector enhances the threat of soil pollution (Khan 2001). The decontamination and removal of heavy metal-contaminated soil are very rare in Pakistan.

Various hyper-accumulative species of plants have been broadly explored that led to the considerable advancement in this field. Plants with BCF (bioconcentration factor) greater than 1000 are hyper-accumulators, whereas those with less than 1000 and more than 1 BCF are accumulators. BCF targets the plants efficiency to uptake metals from soil. There are total of 17 biomes in the world, while Pakistan has 9 out of them, so it shows the unique geographical landscape of Pakistan, and it has over 6000 higher plant species. Currently over 400 plant species of angiosperms had been examined and characterized as hyper-accumulators all over the world (Freeman et al. 2004). In Pakistan however almost 50 plant species were identified and characterized as metal accumulators of soil and water.

Mubeen et al. (2010) stated that utilizing locally accessible wild plants for uptake of Cu from industrial wastewater city Lahore has proved a succesful technique. *Calotropis procera* roots were used as biosorbent for removal of heavy metals from wastewater. The synthetic chelators prepared increased the heavy metal uptake and translocation in plant biomass which speed up the phytoremediation process of nickel and lead.

Substantial progress has been made in the field of metal remediation by plants, and various number of plant species have been widely investigated all over the world to date. However, for indigenous flora of Pakistan, inadequate information is available. About 400 species of plants from angiosperms have been inspected and identified as hyper-accumulators from all over the world. However, limited data is available about the use of flora of Pakistan, and about 30 reported studies have been carried out for investigation of potential flora for phytoremediation from Pakistan. Still there is a large figure of plant species present in diverse localities of the country that needed to be tested for phytoremediation process.

Among those unfamiliar species of plants, most of them are used for the purpose of phytoremediation all over the world, e.g., *Brassica juncea* used for remediation of Pb, Zn, and Cu from soils by many researchers (Zaidi et al. 2006). This species is found in different regions of Pakistan (Rawalpindi, Islamabad, Quetta, Karachi, and Lahore). Different studies stated the heavy metal assessment in contaminated soils and water from Pakistan. Younas et al. (1998) and Malik et al. (2010) investigated that soil samples from industrial areas of Rawalpindi, Lahore, and Islamabad have been heavily contaminated with Ni, Cd, Cu, Pb, and Zn. It could be useful for remediation of the mentioned *Eschhornia crassipes* which has been sighted in industrial and urban regions of the province of Punjab. Although this is not very common to use for remediation, it has been cited as heavy metal accumulator (Zn, Cr, Cd, Ni, Hg, Pb, P, pesticides) from various parts of the world (Xia and Ma 2006; Odjegba and Fasidi 2007; Mishra and Tripathi 2009).

8.5 Enhanced Bioremediation for Field

Many chemical and biological treatments, such as inoculums of fungi and bacteria, EDTA, DTPA, NTA, and other organic compounds, have been used in pot and field experiments to facilitate the heavy metal extraction and to acquire the higher phytoextraction efficiency (Ke et al. 2006; Wu et al. 2006). It is known that microbial populations affect the solubilization of heavy metals and their availability to plants, through acidification, releasing chelaters, and reduction-oxidation changes (Peer et al. 2006). It is reported that presence of microbes in the rhizosphere increases the levels of Zn, Cu, Pb, Ni, and Cd in plants. Heavy metal tolerance and production of biomass could be enhanced by improved interaction among the plants and rhizosphere microbes. It is also considered as an important phytoremediation technology factor (Whiting et al. 2003).

8.6 Chemically Enhanced Bioremediation

The bioremediation effectiveness becomes limited often because of low solubility of metals and their sorption on surfaces of soil particles; however metal solubilization could be increased by adding complexing/chelating agents with time (Pivetz 2001). Several chelating agents have been reported which enhance the rate of phytoextraction. However EDTA and DTPA have been investigated widely, and they have high chelating ability toward most of the metals, like Cd, Cu, Cr, and Pb, which ultimately leads to increased translocation of metals from soil to plant (Wong et al. 2004).

From the literature it is seen that chelating agents may pose potential risk when they are applied in situ, because during extending period of time, chelating agents leech down to the groundwater. However, some studies showed that ammonium application to soil might promote the heavy metal phyto-availability from polluted soils (Wenzel 2009). A lot of researches on phytoextraction are based on greenhouse experiments; few tested the hyper-accumulator plants in the field and actually determined their heavy metal accumulation potential (Zhuang et al. 2007).

Different chelates such as DTPA, EDTA, and EDDS have shown enhancement in the uptake of heavy metals by solubilizing them from the soil solid phase because of the formation of water-soluble complexes (Lestan et al. 2008). Primarily, heavy metals are distributed mainly in two phases which are reversible and irreversible. The chelates try to extract metal ion from reversible phase first and then from irreversible phase. Based on these criteria, ability of chelates is analyzed. If a chelate dissolves metals more from irreversible phase, it is more efficient. Recently NTA and EDDS are used because of their ability to be biodegraded as compared to the EDTA (Evangelou et al. 2007).

Synthetic chelants, such as EDDS, EDTA, and NTA, have been used in facilitating the heavy metal solubility from the soil system and their uptake and translocation in the shoot parts of plants (Shen et al. 2002). For example, Cu metal could be toxic for many plant species. The recommended threshold limit of Cu metal for the plants is 30 mg per kg of plant dry matter. In multimetal-polluted soil system, Cu toxicity for the plants might be a constraint in the phytoremediation process (Lombiet al. 2001).

Blaylock et al. (1997) reported the results of his study that concentration of Cu in *Brassica juncea* shoots in Cu-contaminated soil containing 200 mg kg^{-1} of Cu reached 1000 mg kg^{-1} dry matter 1 week after 2.5 mm application of EDTA. Chelant concentration is also an important factor to develop an effective model for remediation of metal-polluted soils. As reported (Greman et al. 2003), EDDS enhanced the phytoremediation and increased Cd, Pb, and Cu solubilization. Tandy et al. (2004) reported that chelate application rate affects the metal extraction efficiency. However, a single conclusion cannot be drawn from their concentrations; it varies in different conditions (Nowack 2002). Chelants have high affinity for different metals; chelate metal ratio is important. Consequently, concentration of chelating agent should be higher than metals for optimum extraction (Kim et al. 2003). For soil conservation, lower chelate concentration was more favorable (Lim et al. 2005).

Another important factor governing the solubilization of metal ions in the soil is shaking time. Stability of the complex in soil determines the ability of chelate to sustain the metals in soluble form. As reported by the Kim et al. (2003), a continuous steady-state condition between Pb and EDTA was not achieved within 1 day in Pb-contaminated soil. So by monitoring the time factor, we can predict the persistent and availability of the chelating agent in the soil matrix.

Incubation time is another important factor, which should be considered while evaluating the solubilization efficiency of chelating agent. Chaney et al. (1997) demonstrated that oxidation of metal ion and formation of metal-chelate complex may take different time period for different heavy metals. Therefore, incubation period should not be ignored while evaluating other environmental factors. In addition to this, incubation period helps in determining the biodegradation period of chelants.

8.7 Biologically Enhanced Bioremediation

Soil upper layer is an interface of plant and soil, and the process of bioremediation takes place in this layer. Microorganisms in soil horizons release different organic compounds and make the contaminated metals present in the rhizosphere available for the plants through phytoremediation. Soil microorganisms play very effective roles in different processes and have effects on human beings. Soil microbes involve in different nutrient availability for the plants and develop symbiotic relationships with plant roots; however these processes are needed to fully explore.

Fungi play a very important role in solubilization and fixation of heavy metal ions and change the availability of these ions for the plants. Different soil and plant factors affect the phytoremediation process, and these also include the soil fungi. There is a need of information about symbiotic relationships between soil microbes (bacteria and fungi) and roots of plants. Heavy metals are also in compound forms in soil which also affects the metal behavior in their solubilization and uptake processes

(Boruvka and Drabek 2004). Different types of components present in the fungal cell walls like carboxyl, hydroxyl, amino, and other functional groups. Through these functional groups, fungi can bind with toxic heavy metals such as Pb, Cu, Cd, Ni etc.

A large number of filamentous fungi may absorb heavy metal ions and used commercially. Protein present in the fungal cell wall has potential to sorb the heavy metal ions; this is in accordance with those fungi that can tolerate with the toxic heavy metals (Prasad 2017, 2018). Gonzalez-Chavez et al. (2004) reported that hyphae of the arbuscular mycorrhizal fungi consist of glomalin which can sequester the heavy metal ions. Fungi play a very important role in the phytostabilization of toxic heavy metals in the contaminated soils by sequestration and ultimately help mycorrhizal plants survive in contaminated soils.

The phytoremediation efficiency depends mainly on plant characteristics like growth rate, biomass production, harvesting ease, metal resistance, and shoots' ability of heavy metal accumulation (Heet al. 2005). Due to some constraints like large-scale cropping technique deficiency, less biomass and slow rate of plant growth researches are directed to using amendments like addition of chelates which enhanced biomass production of agronomic crops, and their yield also increased (Neugschwandtner et al. 2008).

Many research studies have been conducted, and very few species of plants have been used for bioremediation or phytodegradation. These few plant species depend on their rhizosphere microorganisms (fungi) for heavy metal remediation of soils. Crops used in the laboratory experiments and greenhouse experiments (maize, sorghum, wheat, mustard, canola, sunflower, etc.) could be used for multitasking like for the management of heavy metal-polluted soils (phytoremediation) as well as for biomass production which would be ultimately used for biogas and biofuel production. Based on preliminary data, inoculums of best fungal species would be used to enhance the process of metal uptake by the pretested plants in the field, and in addition to these, pretested fungal inoculums would be prepared, and their effect on biomass production of tested crops may be evaluated.

8.8 Bioremediation of Wastewater

In Pakistan first time bio-treatment ponds were constructed to understand the reuse of treated water for agriculture and aquaculture purpose. Environmental assessment studies on the reuse of wastewater for agriculture and aquaculture were conducted by the National Agricultural Research Center (NARC), Islamabad, Pakistan (Fig. 8.1). The bio-treatment pond performance was assessed after 1 year at NARC (National Agriculture Research Centre, Islamabad). The physical and chemical parameters including color, pH, EC, TDS, turbidity, Zn, Cu, Ni, Cd, Mn, Pb, and Fe were all within defined limits that were not sublethal for rearing fish. *Lemna* accumulated large amount of heavy metals and suggested as best for phytoremediation of wastewater. The treated water is now used for rearing fish and for plant and vegetable cultivation (Iram et al. 2012).

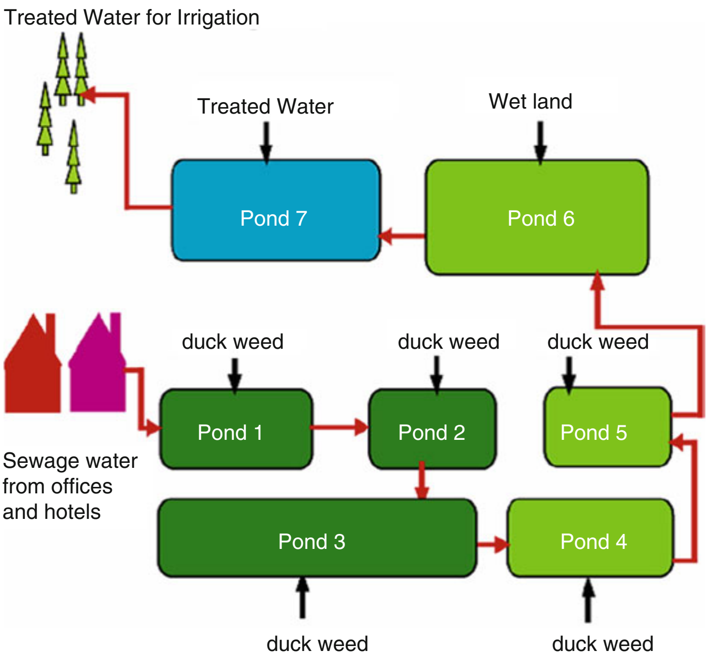

Fig. 8.1 Treatment of wastewater by plants (bioremediation ponds)

8.9 Bioremediation at Contaminated Field

In present time for rapid progress, industries are keys for achieving development. In major cities of Pakistan, industrial estates are established. Besides contributing a major share of the economy to the country's GDP, these industries are creating pollution problem. The increase level of contamination is making land useless for better yield production. Heavy metal contamination on lands is a threatening issue. Many local and conventional technologies seem to be expensive and environmentally unfriendly. Thus, bioremediation is an uprising technology to clean the environment and is a cost-effective and noninvasive alternative technique. The use of plants and tolerant microbes (bacterial and fungal strains) to remove, contain, inactivate, or degrade harmful environmental contaminants and to revitalize contaminated sites is gaining more and more attention in the world. The main purpose of proposed research is to provide the experience of the use of plants, chelates, and fungi for the remediation of contaminated soils.

From a 2012 to 2015 (Akhtar 2015) study, soils contaminated with heavy metals were considered, and remediation with fungi and plants was carried out because these technologies were untested in Pakistan and were beneficial in the economic aspects, uses, and processing of the biomass. Furthermore, development of a model of plant-contaminant-soil interaction was used in remediation technology for rapid and successful remediation of polluted peri-urban arable soils. The study helped in developing new cost-effective strategy for heavy metal-contaminated agrarian land by selecting an appropriate indigenous plant to extract metals and harvest the biomass as valuable wealth. The generated biomass could be either subjected to biomethanation or composting to reduce the volume and then processed for recycling of heavy metals. Ethanol would be then extracted and used as a biofuel in the future. The use of plants on different sites would serve to restore wetlands and other habitats, create natural parks and other green areas, and resolve the pollution problems. This research would help the farmers in selection of best germinated seeds on contaminated agricultural lands. This further promoted the research and development in the future about implementing phytoremediation which makes use of local plants to extract, transfer, and stabilize potentially toxic metals from contaminated soil. This study also advocated the development of a model of plant-contaminant-soil interaction and future remediation programs for rapid and successful remediation of polluted peri-urban soil.

8.10 Conclusions

In the field it is possible to test and accelerate the implementation of bioremediation technologies and enhance biofuel production. Much of the investigation was conducted, and very few species of plants have been discovered as phytodegrading plants. These few plants species depend on their rhizosphere microorganisms (fungi) for heavy metal remediation of soils. Crops used in laboratory experiments and greenhouse experiments (maize, sorghum, wheat, mustard, canola, sunflower, etc.) could be used for multitasking like for the management of heavy metal-polluted soils (phytoremediation) as well as for biomass production which could be ultimately used for biogas and biofuel production. Plant species, maize, mustard, sunflower, sorghum etc., have the ability to remove metals from soils; they also have potential as a bioenergy crop in Cu-, Cd-, Cr-, and Pb-contaminated land. Also sorghum proved to be good for bioethanol production. Thus, resource conservation and their sustainability are very crucial goals; hence the prospect of higher plants for pollution cleanup also served as source of biofuel in a useful prospect. The innovation is to develop a complex technique which will cover the whole value chain from setting the heavy metal-degraded soil management target through successful crop production and biofuel feedstock preparation up to conversion to energy in a local small-scale gasification installation.

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