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Review

Approaches for enhanced phytoextraction of heavy metals

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ABSTRACT

The contamination of the environment with toxic metals has become a worldwide problem. Metal toxicity affects crop yields, soil biomass and fertility. Soils polluted with heavy metals pose a serious health hazard to humans as well as plants and animals, and often requires soil remediation practices. Phytoextraction refers to the uptake of contaminants from soil or water by plant roots and their translocation to any harvestable plant part. Phytoextraction has the potential to remove contaminants and promote long-term cleanup of soil or wastewater. The success of phytoextraction as a potential environmental cleanup technology depends on factors like metal availability for uptake, as well as plants ability to absorb and accumulate metals in aerial parts. Efforts are ongoing to understand the genetics and biochemistry of metal uptake, transport and storage in hyperaccumulator plants so as to be able to develop transgenic plants with improved phytoremediation capability. Many plant species are being investigated to determine their usefulness for phytoextraction, especially high biomass crops. The present review aims to give an updated version of information available with respect to metal tolerance and accumulation mechanisms in plants, as well as on the environmental and genetic factors affecting heavy metal uptake. The genetic tools of classical breeding and genetic engineering have opened the door to creation of 'remediation' cultivars. An overview is presented on the possible strategies for developing novel genotypes with increased metal accumulation and tolerance to toxicity.

1. Introduction

The rising rate of human activities in the biosphere is posing unprecedented threats that would ultimately lead to a disturbing imbalance in the biosphere. Industrial activities such as chemical works, service stations, metal fabrication shops, paper mills, tanneries, textile plants, waste disposal sites and intensive agriculture are particularly guilty of polluting the environment (Wong, 2003; Freitas et al., 2004). Heavy metal contamination of soils has become a serious problem in areas of intense industry and agriculture. A heavy metal is a member of an ill-defined subset of elements that exhibit metallic properties, which mainly includes the transition metals, some metalloids, lanthanides and actinides (Babula et al., 2008). These are metallic chemical elements that have a relatively high density and are toxic even at low concentrations. The contamination of the environment with heavy metals has become a worldwide problem that affects crop yields, soil biomass and fertility, and leads to bioaccumulation of metals in the food chain (Gratao et al., 2005; Rajkumar et al., 2009). This is majorly due to pollution of agricultural soils by increasing reliance on chemical fertilizers, which has imposed a long-term risk on environmental health (McLaughlin et al., 1999; Wong et al., 2002). Industrialized countries have regulated the emission of toxic substances, but in developing countries, rapid industrial development and population explosion, coupled with lack of pollution control has caused an enormous increase in heavy metal contamination of agricultural soils (Ji et al., 2000). Soils polluted with heavy metals pose a health hazard to humans as well as plants and animals. Thus, heavy metals need to be removed from the soil for agro-ecological sustainability and human benefit.

Various efficient soil cleanup techniques are available, but most of them are costly, labour intensive and cause soil disturbances, and have thus found limited acceptability among the communities. Conventional remediation methods usually involve pneumatic fracturing, solidification/stabilization, vitrification, excavation and removal of contaminated soil layer, physical stabilization or washing of contaminated soils with strong acids or heavy metal chelators (Steele and Pichtel, 1998; Khan et al., 2004;

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Bhargava et al., 2012). Phytoremediation, using plants to cleanup contaminated environment, is an idea that is attracting increasing attention among scientists, remediation engineers, and environmental professionals in government and industries. In situ remediation using plants has the potential to be less expensive than current technologies and simultaneously initiates both detoxification of hazardous waste and site restoration. Phytoremediation technologies include phytoextraction, phytostabilization, phytovolatilization, phytofiltration and phytodegradation (Fulekar et al., 2009; Marques et al., 2009). Phytoextraction refers to the uptake of contaminants from soil or water by plant roots and their translocation into the shoot, or any other harvestable plant part, to remove contaminants and promote long-term cleanup of soil or wastewater (Sas-Nowosielska et al., 2008). In this approach, plants capable of accumulating heavy metals are grown on contaminated sites and the metal-rich aboveground biomass is harvested on maturity. As a result, a fraction of the soil contaminant is removed. The success of phytoextraction as a potential environmental cleanup technology depends on factors like metal availability for uptake as well as plant ability to absorb and accumulate metals in its aerial parts. As per the economic feasibility, the harvested biomass is usually incinerated or composted and rarely recycled for reuse (Prasad and Freitas, 2003).

Plants ideal for phytoextraction should possess multiple traits like ability to grow outside their area of collection, fast growth, high biomass, easy harvesting and accumulation of a range of heavy metals in their harvestable parts (Jabeen et al., 2009; Seth, 2011). No plant is presently known that fulfils all these criteria. However, a rapidly growing non-accumulator plant could be modified and/or engineered so that it achieves most of the above-mentioned attributes. There has been significant progress in determining the biochemical and molecular basis for metal accumulation, which provides us with a strong scientific basis to outline some strategies for achieving this goal. This article first aims to review studies that are beginning to uncover the detailed mechanism behind phytoextraction of heavy metals in plants. The progress of research focused on the unravelling of genetic and biochemical mechanisms that confer the ability to accumulate heavy metals into plants is described. Plant breeders and environmental researchers have long strived to develop improved plant varieties which can be used for effective phytoextraction. This aspect is been discussed in this review, and the use of classical and biotechnological approaches used for enhancing natural hyperaccumulation of heavy metals is presented.

2. Plants as accumulators of heavy metals

The uptake of metals by plants is selective, with some being preferentially acquired over others. Many heavy metals like nickel (Ni), copper (Cu), manganese (Mn) and zinc (Zn) are essential micronutrients and required by plants to grow and complete the life cycle. According to Baker (1981), plants growing on metalliferous soils can be grouped into three categories: (i) Excluders – in which the metal concentrations in the shoot are maintained up to a critical value, at a low level across a wide range of soil concentration. Excluders prevent uptake of toxic metals into root cells (de Vos et al., 1991). Excluders can be used for stabilization of soil and to avoid further spread of contamination due to erosion (Lasat, 2002). (ii) Accumulators – in which the metals are concentrated in aboveground plant parts from low to high soil concentrations. Accumulators do not prevent metals from entering the roots and thus allow bioaccumulation of high concentration of metals. (iii) Indicators – where internal concentration reflects the external levels (McGrath et al., 2002).

2.1. Hyperaccumulators

The discovery of hyperaccumulator plant species has revolutionized phytomediation technology since these plants have an innate capacity to absorb metal at levels 50–500 times greater than average plants (Lasat, 2000). Hyperaccumulators are a subgroup of accumulator species often endemic to naturally mineralized soils, which accumulate high concentrations of metals in their foliage (Baker and Brooks, 1989; Raskin et al., 1997). Metal hyperaccumulators are naturally capable of accumulating heavy metals in their aboveground tissues, without developing any toxicity symptoms. A metal hyperaccumulator is a plant that can concentrate the metals to a level of 0.1% (of the leaf dry weight) for Ni, Co, Cr, Cu, Al and Pb; 1% for Zn and Mn; and 0.01% for Cd and Se (Baker and Brooks, 1989; Baker et al., 2000). The time taken by plants to reduce the amount of heavy metals in contaminated soils depends on biomass production as well as on their bioconcentration factor (BCF), which is the ratio of metal concentration in the shoot tissue to the soil (McGrath and Zhao, 2003). It is determined by the capacity of the roots to take up metals and their ability to accumulate, store and detoxify metals while maintaining metabolism, growth and biomass production (Gleba et al., 1999; Guerinot and Salt, 2001; Clemens et al., 2002). With the exception of hyperaccumulators, most plants have metal bioconcentration factors of less than 1, which means that it takes longer than a human lifespan to reduce soil contamination by 50% (Peuke and Rennenberg, 2005). Hyperaccumulators have a bioconcentration factor greater than 1, sometimes reaching as high as 50–100. The relationship between metal hyperaccumulation and tolerance is still a subject of debate. Views range from no correlation between hyperaccumulators and the degree of tolerance to metals (Baker et al., 1994) to strong association between these traits (Chaney et al., 1997). It is increasingly being realized that to cope with high concentrations of metals in their tissue, plants must also tolerate the metals that they accumulate.

There has long been a general agreement that metal hyperaccumulation is an evolutionary adaptation by specialized plants to live in habitats that are naturally rich in specific minerals that confers on them the qualities of increased metal tolerance, protection against herbivores or pathogens, drought tolerance, and allelopathy (Boyd and Martens, 1992; Macnair, 1993). The hypothesis of protection against pathogens and herbivores is considered the most accepted one (Boyd and Martens, 1994; Huitson and Macnair, 2003; Boyd, 2007; Noret et al., 2007; Galeas et al., 2008). However, the mechanisms of metal uptake, tolerance to high metal concentrations, and the exact roles that high level of elements play in the survival of hyperaccumulators have continued to be debated.

Hyperaccumulation of heavy metal ions is a striking phenomenon exhibited by approximately $<0.2\%$ of angiosperms (Baker and Whiting, 2002; Rascio and Navari-Izzo, 2011). Metal hyperaccumulators have been reported to occur in over 450 species of vascular plants from 45 angiosperm families (Table 1) including members of the Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunoniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae (Padmavathiamma and Li, 2007), but are well represented in Brassicaceae especially in the genera *Alyssum* and *Thlaspi*, wherein accumulation of more than one metal has been reported (Reeves and Baker, 2000; Prasad and Freitas, 2003; Verbruggen et al., 2009; Vamerali et al., 2010) (Table 1). *Pteris vittata* (Chinese brake fern) is known to accumulate up to 95% of the arsenic taken up from soil in its fronds (Ma et al., 2001; Zhang et al., 2002). The best known angiosperm hyperaccumulator of metals is *Thlaspi* (now: *Noccaea*) *caerulescens* (pennycress), which can accumulate large amounts of Zn (39,600 mg/kg) and Cd