From green to clean: a promising and sustainable approach to remove toxic metals from contaminated soils

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Abstract: Phytoextraction is an *in situ* decontamination technique using metal accumulating plants and appropriate soil amendments to transport and concentrate metals from the soil into the aboveground parts of plants, which are harvested with conventional agriculture methods. Since most of the metal hyperaccumulating plants only produce very low biomass, and most of plants producing high biomass accumulate only moderate amounts of metals, the current research is mainly focused on the overcoming of this deficiency to optimise metal phytoextraction. The main goal of our study aimed at improvement of phytoextraction through improved metal accumulation in sunflowers producing a high biomass. The potential use of their oil and biomass for technical purpose (biodiesel, biogas and energy) allows to produce an added value and to improve the economical balance of phytoextraction. Chemical mutagenesis (non-GMO approach) and appropriate fertilization treatments stimulating metal bioavailability in the soil were used as an alternative to genetic engineering to enhance both metal accumulation and extraction efficiency of oil crops. The effect of chemical mutagenesis on metal accumulation and extraction potential of two sunflower cultivars was directly assessed on a metal contaminated field. Theoretical calculations for phytoextraction potential of new variants show that the best sunflower mutants of the 2nd generation can produce up to 26 t dry matter yield per ha and remove 13.3 kg Zn per ha and year at the sewage sludge contaminated site; that is a gain factor of 9 compared to Zn removal of sunflower controls. Results of field experiments on the same metal contaminated site confirmed the improved yield, metal accumulation and metal extraction efficiency by new sunflower mutant lines in the 3rd and 4th generation. Sunflowers still showed a yield improvement by a factor of 4-6 and metal extraction by a factor of 3-4 for Cd, 5 for Zn and 5-6 for Pb.
Keywords: Phytoextraction, toxic metals, sunflower, contaminated soils

1. Introduction

Soils contaminated with metals (such as cadmium, chromium, nickel, zinc, lead, etc.), arsenic, and various radionuclides are nowadays a major environmental and human health problem. Therefore there is a need for an effective and affordable technological solution for soil remediation. Main sources of the soil contamination are the metal smelting industry, residues from metalliferous mining, combustion of fossil fuel, sewage sludge, waste incineration, car exhausts as well as some pesticides and fertilisers used in agriculture. In contrast to the organic contaminants, which can undergo biodegradation, heavy metals cannot be destroyed and remain in the environment. Long-term deposition of metals in soil can lead to the accumulation, transport and biotoxicity/zootoxicity caused by mobility and bioavailability of significant fraction of the metals (1). Toxic metals have to be either significantly removed from the contaminated soils or to be reliably immobilised to ensure minimal ecological risks presented by polluted soil (2). There are different conventional remediation techniques available for contaminated soil, but relatively few are applicable to soils contaminated with heavy metals. Moreover, classical *ex situ* decontamination techniques are expensive and involve digging up the entire contaminated area and taking it away to another location for chemical treatment.

2. Plant potential for remediation of metal contaminated soil

Phytoremediation is an environment-friendly technology using green plants and their associated microorganisms, soil amendments and agronomic techniques to remove pollutants, such as heavy metals, organic xenobiotics or radionuclides, from the environment (3-5). The main advantage of this technology is *in situ* application without further disturbance of the natural surroundings of the contaminated site, i.e. saving the soil structure, fertility and soil organisms. Another advantage is a lower cost than conventional methods to decontaminate land. The U.S. EPA (Environmental Protection Agency) estimates phytoremediation costs to be 50-80% lower than the traditional treatment techniques. From the environmental, economical and social point of view, phytoremediation is a sustainable approach, which is economically viable and environmentally compatible. Moreover, this promising technology provides a private benefit for the owner or user of the land and less-polluted site means a less-risky surrounding for humans. There are several specific approaches of metal phytoremediation being developed.

Phytoextraction is one of them, which uses metal accumulating plants and appropriate soil amendments to transport and concentrate metals from the soil into the above-ground parts of plants, which are harvested with conventional agriculture methods (6-7). Two basic strategies of metal phytoextraction have been developed: (1) natural or continuous phytoextraction and (2) induced or chemically assisted phytoextraction (6). Two groups of plant species are considered to be useful for metal phytoextraction purpose: (a) hyperaccumulator species, which are able to accumulate and tolerate extraordinary levels of metals (4, 8), but produce low biomass and (b) high biomass producing species, like *Helianthus annuus* L. (9-11), *Nicotiana tabacum* L. (12-13*)*, *Brassica juncea* L. (14) and some fast growing trees (Salix), compensating lower metal accumulation by high biomass (15).

Plants using for phytoextraction have to be finally harvested and plant biomass has to be disposed and/or covert. On one hand, volume reduction of the contaminated plant biomass can be achieved by composting, compaction or pyrolysis (16). On the other hand, a slightly contaminated plant biomass can be used as a source of energy that is relevant for the phytoremediation cost recovery (17). We can solve a waste disposal problem and in the same time create energy for use in homes, farms and factories. We can get energy directly from plants, for example by burning wood (willow, poplar) for cooking and heating or indirectly from plants by production of a liquid (alcohol) or a gaseous (biogas) fuel. Biogas (methane) is a well-established fuel for cooking and lighting in a number of countries. It is produced by anaerobic digestion, when organic matter is broken down by microbiological activity in the absence of air. Whereas the energy potential of biodiesel is 10 000 kWh per hectare, bio-methane can reach a 10-fold higher value.

The main advantage of metal phytoextraction is *in situ* application of phytoextraction without further disturbance of the site. Another advantage is a lower cost than conventional methods to decontaminate land. The possible recycling of metals, recovery of bioenergy and of any additionally valuable products could provide further economic advantages of phytoextraction. One of the possible limitations of this method is its applicability restricted to the upper soil layers and to low or moderately contaminated soils (18). The greatest disadvantage of metal phytoextraction is the need of a relatively long cleaning up time. The phytoextraction process should preferably not exceed 10 years (13, 19-20). However, metal removal by many plants is not efficient enough to fulfil this requirement. Therefore an enhancement of phytoextraction efficiency is the turning point for a practical use of this green technology.

3. The optimisation and perspectives of metal phytoextraction

In the case of optimisation of natural phytoextraction, agronomic practises of plant cultivation like plant density per hectare, possible multi-cropping, harvest time and harvesting practises need to be optimised. The choice of the suitable cultivation approach as well as the appropriated plant species including the selection of the cultivar with the naturally highest metal uptake potential are the first steps of a successful phytoextraction.

An increasing bioavailable fraction of metals may provide a way to enhance the phytoextraction efficiency. Metal availability to the plant can be increased by means of lowering the soil pH by the application of specific fertilisers (11, 13, 21), synthetic chelators (22-23) or by the use of soil microorganisms and root-associated bacteria, which are essentially stimulated by root exudates including a wide range of organic molecules (24-26)

Other possibility to optimise metal phytoextraction is the improvement of insufficient metal uptake characteristics of high yielding crops and the enhancement of low biomass of hyperaccumulating wild plants using plant biotechnology. For the last ten years improvement of phytoextraction focused on the development of efficient transgenic plants for phytoremediation (27-29). Several possibilities of genetic engineering of plants have been proposed. Transgenic plants with increased phytochelatin levels showing enhanced Cd tolerance were also obtained (30). Another possibility of genetic modification of metal tolerance and accumulation in plants is the genetic manipulation of metal transporters (31). Alterations of metabolic pathways, oxidative stress mechanisms and of biomass production can also lead to an improvement of transgenic plants for phytoremediation (32).

Although the successful development of transgenic plants with enhanced metal tolerance was reported, but in most cases metal uptake and metal extraction of transgenic plants was not markedly altered. Additionally a main limitation of the study and use of transgenic plants is the restriction of GM plant for real field experiments in most of European countries and they can be tested on the laboratory scale only. The use of transgenic plants for phytoextraction still remains an open question as its answer strongly depends on public perception (18). Therefore a non GMO-approach is needed as a good alternative to a genetic engineering to develop new plant variants with a high biomass production and an enhanced metal accumulation efficiency. Such non-GM plants can be directly tested on the field and be used for metal removal from contaminated sites.

Conventional breeding techniques (classical breeding, *in vitro* breeding and mutagenesis), which are presently not classified as genetic modification within Europe, are appropriate technologies to develop new plant variants with enhanced metal accumulation and extraction properties (33-38).

4. Sunflower mutants promising for phytoextraction use

New sunflower variants promising for phytoextraction use were developed by means of chemical mutagenesis by Nehnevajova et al. (36, 38) and tested in years 2003-2006 on the metal contaminated Rafz field in Switzerland. This study was part of the EC Project “PHYTAC – Development of systems to improve phytoremediation of metal contaminated soils through improved phytoaccumulation" (Project No QLRT-2001-00429) and EC Project COST Action 859 "Phytotechnologies to promote sustainable land use and improve food safety".

Prior to mutation breeding, 15 commercial sunflower cultivars, commonly cultivated in Switzerland, were tested on a sewage-sludge contaminated field in Rafz (CH). Highly significant differences of heavy metal accumulation and extraction were found between sunflower cultivars. Cadmium extraction (cadmium accumulation* yield) varied by a factor of 4, Zn extraction by a factor of 3 and Pb extraction by a factor of 14 between the cultivars with the highest and lowest metal extraction treated with the same fertiliser (11). To meet the claim of enhanced metal bioavailability, two different fertilisers (ammonium sulphate and ammonium nitrate) were additionally tested in the field experiment with the sunflower cultivars. Sulphate fertilisation significantly enhanced Zn and Pb extraction, whereas ammonium nitrate enhanced Cd extraction by most of the sunflower cultivars (11).

Finally, sunflower cultivar Salut with the naturally highest potential to accumulate and extract metals from contaminated soil and three sunflower inbred lines were selected for chemical mutagenesis. Sunflower seeds were treated with chemical mutagen ethyl methanesulphonate (EMS), which can induce point mutation in DNA leading to changed genetic information in new mutants.

The field experiments (2003-2006) were focused on the screening of sunflower mutants of M_1-M_4 generations on the metal contaminated field in Rafz (CH) to assess the effect of mutagenesis on yield, metal accumulation and extraction characteristics by sunflowers.

We found that chemical mutagen EMS affected biomass production and metal accumulation efficiency of sunflower mutants. The sunflower mutants of the first generation showed a 2-3 times higher Cd, Zn and Pb concentration in shoots, but, as expected, a considerably reduced growth, as compared to control cultivars due to the phytotoxic effect of the mutagen (36). The main mutant screening was done in the second generation of sunflower mutants. Three phenotypic groups of new sunflower variants with alterations in growth and metal uptake were obtained: (1) sunflower mutants with a significantly enhanced biomass and no changed metal tissue concentration; (2) mutants producing a higher biomass with an enhanced metal concentration (for phytoremediation use); and (3) mutants with a reduced metal concentration (exclusion) in the shoots interesting for improved food quality (36). The same phenotypic groups of new sunflower variants were also found in the 3rd mutant generation.

Biomass production and the ability of metal shoot accumulation in plants are two key factors for an efficient phytoextraction. Metal extraction (yield*metal shoot concentration) was the main criterion for our sunflower mutants screening in the field experiments. In the 2nd generation the best sunflower mutant showed a strongly enhanced metal extraction, as compared to the control sunflower inbred line IBL 04 (Cd 7.5 x, Zn 9.2 x and Pb 8.2 x) (36). In the next 3rd and 4th generations sunflower mutants also produced a high biomass leading to an improved metal extraction, as compared to control. The best M_3 sunflower mutants showed the following improvement of metal extraction: 3-5 x for Cd, 4-5 x for Zn, 3-5 x for Pb (38). The best M_4 sunflowers also provided enhanced metal extraction: Cd 3-4 x, Zn 5-7 x, Pb 6-8 x and Cr 5-7 x higher, as compared to control sunflowers (Fig. 1).

5. Conclusions

Theoretical calculations of phytoextraction potential of sunflowers point out that the best M_2 sunflower mutant could produce up to 26 t dry matter yield per ha and year and remove 13.3 kg Zn and 31.2 g Cd per ha and year from the metal contaminated site in Rafz (CH), containing the following concentrations: Cd 0.9 mg/kg, Zn 813 mg/kg, Pb 492 mg/kg, for a soil pH of 5.8. The biomass production and metal removal of the best M_2 sunflower mutant was 9 times higher than by control sunflowers. The sunflower mutants of 3rd generation could still produced up to 16.8 t dry matter yield per ha and year and could remove 10 kg Zn, 176.9 g Pb and 16.1 g Cd per ha and year on the same metal contaminated site, as compared to the control sunflower IBL 04 producing 4.1 t dry matter yield per ha and year and with the following metal removal: 4.8 g Cd, 2.2 kg Zn and 16.9 kg Pb per ha and year. M_4 sunflower mutants could also produced up to 20.9 t dry matter yield per ha and year and could remove 11 kg Zn, 147 g Pb and 25.1 g Cd per ha and year.

In contrast sunflowers tested by Marchiol et al. (39) can remove 1 kg Zn per ha and year on a metal contaminated soil with the total Zn concentration of 625 mg /kg. Kayser et al. (12) have shown a possible metal removal of 15 g Cd and 3 kg Zn per ha and year by sunflowers, 35 g Cd and 1.2 kg Zn per ha and year by tobacco and 9 g Cd and 2 kg Zn per ha and year by maize on a contaminated calcareous soil at Swiss Dornach site. They have also shown that *T. caerulescens* accumulates 2 g Zn per kg, but shows very limited growth. Therefore the Zn removal by *Thlaspi* shoots is finally 2.2 kg per ha and year.

New sunflower mutants obviously show a high potential for Zn removal and partly for Cd removal, as compared to the metal removal efficiency by other sunflower cultivars, tobacco, maize and hyperaccumulator *T.caerulescens*.

The combined use of sunflowers for soil decontamination and sunflower biomass and oil as renewable fuels looks very promising a sustainable land use and management in the near future.

Figure 1. Sunflower mutants growing on a soil contaminated with heavy metals. Phytoextraction is the absorption of trace elements into plant roots, then translocation into shoots, followed by harvest and destruction of the plant or its use for biofuel production (e.g. biodiesel from sunflower oil). The recovery of valuable metals from the contaminated biomass or ash appears as a promising recycling strategy.

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