PLANT GROWTH CONTROL: BIOLOGICAL, CHEMICAL, PHYSICAL AND POST-HARVEST PARAMETERS

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Summary

Plants growing on polluted sites must be tolerant to metals or organic contaminants and adapted to local climate conditions. A sufficient growth and biomass production of these plants are the most important requirements for the success of phytoextraction. This decontamination technology uses plants to extract and translocate metals to their harvestable parts. There are number of factors including agronomic practices affect the biomass production and productivity of plants. This chapter is primarily focused on the appropriate use of agronomic practices that can guarantee an effective phytoremediation. The sowing, planting strategy and optimal plant density play very important roles for plant growth during the whole cultivation period. Irrigation and weed control need to be also included in the agronomic approach, especially during the

first vegetation period of plants used for phytoremediation. Irrigation can also help to achieve adequate soil moisture which affects the movement of soluble metals in soil solution to root surface. The application of appropriate fertilizers which are a key source of nutrients provides a sufficient plant growth. Moreover, an appropriate fertilizer treatment can also additionally increase, or decrease metal uptake and accumulation by plants, depending on the chemical properties of the individual fertilizer.

Plants growing on polluted area can become more sensitive to certain pest and disease that leads to a reduced quantity and quality of their product. Therefore a careful plant growth control is needed to minimize environmental abiotic and biotic stress and ensure a sufficient biomass production of plants used for phytoextraction. Regular monitoring of the presence of pests and disease on plants is very important during the growing period. The last important step of the phytoextraction procedure is the removal of harvested metal rich biomass and subsequent disposal of the biomass. Possible methods of the disposal of plants used for soil decontamination are also proposed and discussed in this chapter.

1. Introduction

Soils contaminated with metals and various radionuclides are nowadays a major environmental and human health problem. In contrast to the organic pollutants, which can undergo biodegradation, heavy metals cannot be destroyed and remain in the environment. Therefore there is a need for an effective and affordable technological solution for soil remediation. Phytoremediation is a soil remediation technique that uses green plants and their associated micro-organisms, soil amendments and agronomic techniques to remove, contain and render harmless environmental contaminants.

Plants used for phytoremediation must be tolerant to the mineral element(s) to be removed, adapted to local soil and climate conditions and able to take up large amounts of the target element(s). Their roots must be especially adapted to the spatial distribution of pollution in the substratum. Different root systems and geometry allow plants to adapt to the environment and be more or less efficient in element uptake.

Biomass production and the bioconcentration factor, which is defined as the ratio of metal concentration in plant shoot to metal concentration in soil, are two key factors determining the efficiency of phytoextraction. Therefore, a high biomass (growth) is the first prerequisite for plants to be able to extract metals from contaminated soil. An additional optimization of growth and biomass production can increase phytoextraction potential of plants.

The yield of plants used for phytoextraction can be affected by a number of factors including agronomic practices, such as (1) methods of sowing plants and the length of growing period, (2) irrigation, (3) weed control, (4) application of fertilizers or of chemical agents mobilizing metals from the soil, (5) pest and disease control and (6) plant harvest and post-harvest control. This chapter describes the factors affecting the yield of plants growing on a metal contaminated field and also gives some advices to perform field experiments and use plants for phytoremediation.

Two groups of plants can be used for phytoremediation: (1) high yielding plants producing high biomass, but accumulating only moderate metal amount in shoot; and (2) hyperaccumulating plant species, which tolerate and accumulate a high metal amount in the shoot, but with a limited growth. Standard and practical knowledge of phytoremediation using fast growing crops and metal hyperaccumulators are presented and discussed below. Some results and experience from our own research, focusing mainly on sunflower and tobacco are also shown here.

2. Methods of Sowing Plants

The biomass production as well as the productivity of plants depends on water availability, sowing date, sowing strategies and length of growing period. An important factor that affects the biomass production is plant density (number of plants/m^2). In general, a higher density tends to minimize yield per plant and maximize yield per hectare. It has been shown that plant density affects the pattern of plant growth and development. For example, plants growing at higher density will compete more strongly for light and then more energy and nutrients will be used for plant growth than for developing processes. An optimal distance between plants is also important for sufficient development of root architecture.

Phytoremediation designs commonly involve higher planting densities than standard agronomic rates for various species to overcome decreased germination because of contaminated soils and to maximize overall production of biomass per hectare. Consulting an experienced agronomist is essential to design planting methods for an effective phytoremediation. The method of planting depends on the type of vegetation. For example, grasses are usually dispersed as seeds, and trees such as willow or poplar are transplanted from cuttings.

The sowing, planting strategies and optimal plant density are described here in more detail manner for the following plants: fast growing crops, such as sunflower and tobacco, and the hyperaccumulating plant *Thlaspi caerulescens*.

Sunflowers

Growing sunflowers from seeds is quite easy. Seeds are big and relatively quick to germinate. Sunflower sowing starts when soil temperature at sowing depth is higher than 7°C; sunflower seeds for phytoremediation purpose can thus be sown already in April in many countries. They should be sown 60 cm apart in lines with a plant to plant spacing of 13-20 cm. Seeds should be sown at 3-4 cm depth for a better stand. Sowing can be done manually or with a corn planter in the furrows. After 10-12 days of germination, extra seedlings should be uprooted to provide a space of 20 cm between plants in rows. The sowing density is 10-15% higher than the expected canopy density at harvesting (45,000-50,000 plants/ha in rained fields, 50,000-60,000 plants/ha in irrigated fields).

Sunflower sowing also works in contaminated fields very well; therefore additional pre-growing of sunflower seedlings in pots in greenhouse is not necessary. Directly after seed sowing, snail control is important to save the sown seeds for germination.

Tobacco

Tobacco seeds are produced in abundance and are very small. The sowing of tobacco is much more difficult than for sunflowers, and needs at least a cultivation period of 12 weeks in a protected greenhouse with a night temperature above 18^{0}C. Therefore, the appropriate pre-cultivation method of tobacco for phytoremediation purpose is pre-growing of the seeds and seedlings in cultivation plates, followed by transplantation to mini-pots, both on seedling soil. Before explanting in the contaminated field a free-land conditioning on a protected site without night frost is needed for another fortnight. An alternative and promising technique for fast production of tobacco seedlings for phytoextraction is micropropagation. This *in vitro* technique uses the axillary buds for propagating plants. Sterile tobacco seedlings are cultivated on a basic Murashige-Skoog medium at 14-h photoperiod under *in vitro* conditions for a further micropropagation. The shoots need to be cut into single node pieces containing lateral buds. With this method mother plants can be vegetatively propagated at regular intervals (every 4-5 weeks) to obtain sufficient amounts of plant material for field assessment. The same pre-cultivation procedure is needed for *in vitro* produced plants as for tobaccos grown from the seeds. Tobacco clones, developed by *in vitro* micropropagation, have shown sufficient growth on two contaminated fields in Rafz (Switzerland) and Balen (Belgium) within field experiments performed during 3 years (2002-2005) in the context of the EC project PHYTAC by research groups of Herzig (CH) and Vangronsveld (BE).

Pre-grown seedlings of tobacco (9-week old) can be planted in the field in mid of May (after the end of soil frost) with an optimized plant density up to 40,000 plants per hectare (row space = 0.5 m; between plant space = 0.5 m). Prior to planting into free-land, the *in vitro* propagated tobacco seedlings (4-5 weeks old) need to be transplanted for 2 weeks to the soil in covered plates for rooting on the soil under a 14 h photoperiod (14 h light / 10h dark) at 25/22 ºC in a growth chamber, followed by a 2-3 weeks free-land conditioning, shadowed from direct sun at the beginning.

Thlaspi caerulescens

This metal hyperaccumulator plant can be directly sown out into the soil or seedlings can be pre-grown in pots and transplanted in the field. Prior to sowing a cooling period of 2-3 weeks (vernalization) is needed. Pre-grown seedlings of *T. caerulescens* can be planted in the field at 1,000,000 seedlings per ha (row space = 0.1 m). The effect of two different sowing strategies on Cd and Zn uptake by *T. caerulescens* has been studied. Published data show that metal tissue concentrations in plants grown from the seeds are higher than in transplanted plants. However, differences of metal uptake by plants sown with different strategies can be due to environmental conditions, such as rainfall and temperature. Compared to high yielding crop plants, *T. caerulescens* shows a very low biomass that restricts the metal extraction from soil.

3. Irrigation and Weed Control

Irrigation and weed control are probably the two most important tools to guarantee a sufficient growth of plants for phytoremediation. Irrigation may be required to achieve adequate soil moisture, which affects the movement of soil solution containing soluble heavy metals from the bulk soil to root surface. Water should compensate for normal losses by evaporation and transpiration. The method of irrigation must also be carefully considered. Excessive water delivery may restrict root growth and development and thereby decrease metal extraction. In addition, intensive irrigation practice increases the cost of phytoremediation.

For example, 2-3 irrigation applications are recommended for sunflower (400-800 m^3/ha for sprinkler irrigation). The interval between irrigations is 7-14 days depending on soil texture. The most sensitive period to water stress for sunflower is the growth period of 40 days after germination, corresponding to the intensive vegetative development and flower initiation.

Weed control is very important in the first vegetation period of plants used for soil remediation. It can be done by mechanical hoeing or by using chemical substances called herbicides which can destroy weeds. For tobacco and sunflower growing on a sewage sludge contaminated field in Rafz (Switzerland) during 5 years (2002-2007), mechanical weed control was successfully applied. No herbicides were applied during these field experiments for weed control. For example, the weed control for sunflower required the following field work: first mechanical hoeing when the plants have two leaves; second hoeing 2 weeks after the first one; and third hoeing when the sunflower crop is 60-70 cm high. Weed control in the field with *T. caerulescens* can also be done manually without any herbicide. It is important to apply irrigation and weed control when it is necessary, to avoid increased costs for this phytoextraction method, which should be achieved at a lower cost than any alternative technology or the cost of inaction (natural attenuation). Some chemical methods for weed control have been proposed beside mechanical methods. Herbicides can be applied before or after the emergence of plants used for phytoremediation. Application of pre-emergent herbicides can guarantee good weed control, whereas quick emergence and post-emergent herbicides control weeds that occur later in the growing season.

4. Application of Fertilizers or Chelating Agents

Eighteen nutrient elements are essential for the normal growth and reproduction of plants. Plants obtain the three most abundant nutrients, carbon (C), hydrogen (H) and oxygen (O), from water and the air. The other elements, including nitrogen, need to be supplied into the soil by soil amendments, such as fertilizers and lime. Exceptions are leguminous plants (e.g., pulses, peas, soybeans, clovers, alfalfa) that obtain the nitrogen from the air with the help of *Rhizobium* bacteria, which are living in symbiosis in the nodules of plant roots and can fix N_2 from the air. Nitrogen (N), phosphorus (P) and potassium (K) are primary nutrients, which are needed in large quantities compared to the other nutrients. These nutrients can most affect plant growth and development in soil systems.

- *Nitrogen* is involved in all the major processes of plant growth and development. It reacts with some products of carbohydrate metabolism and forms amino acids and proteins. A good supply of nitrogen for the plant is also important for the uptake of other nutrients.

- *Phosphorus* is essential for photosynthesis and other biochemical-physiological processes. It also plays an important role for cell differentiation and the development of plant tissues. Phosphorus is deficient in most natural or agricultural soils, where fixation limits its availability.
- *Potassium* activates more than 60 enzymes. Therefore it plays a vital role in carbohydrate and protein synthesis. K improves the water regime of plants and increases the tolerance to drought, frost and salinity. Plants well supplied with K are also less affected by disease.

Calcium (Ca), Magnesium (Mg) and Sulfur (S) are secondary nutrients which are required by the plant in lower quantities but they are also essential for good plant growth. Calcium and magnesium are added in liming materials when soil pH is adjusted and sulfur is usually present in sufficient quantities from the slow decomposition of soil organic matter.

Zinc (Zn), Manganese (Mn), Iron (Fe), Boron (B), Copper (Cu), Molybdenum (Mo), Chlorine (Cl), Cobalt (Co) and Nickel (Ni) are micro-nutrients which are found in plants in very small amounts, but their deficiency also limits plant growth and development in many soil systems. Therefore, an insufficient fertilization can slow down plant growth and cause deficiency symptoms such as leaf chlorosis (yellowing), leaf death and stunting (Table 1). However, the excess of fertilizer often causes a salinity problem because the excess of salts makes water less available to the plant due to an osmotic effect. This is usually the case for mineral nutrients such as potassium, calcium, magnesium, and nitrate-nitrogen, which do not produce a specific toxicity. Excess of these nutrients may induce a deficiency of other minerals. For example, magnesium excess can cause a calcium deficiency and excess of manganese, copper or zinc cause specific toxicity symptoms.

Nutrient	Deficiency Symptoms
Primary macronutrients	
Nitrogen (N)	Deficiency symptoms are developed predominantly in the older and mature leaves; older leaves become uniformly yellow (chlorotic); young leaves at the top maintain a green color, but become smaller in size; branching is reduced, stunted growth and poor fruit development occur
Phosphorus (P)	Leaf tips look burned; leaves may develop reddish-purple coloration; a stunted plant growth and delay in plant development appear
Potassium (K)	Deficiency symptoms are mainly in older leaves: they turn yellow initially around margins, may wilt and look scorched; irregular fruit development
Secondary macronutrients	
Calcium (Ca)	Deficiency symptoms first occur in the growing regions and in new leaves; calcium-deficient leaves show necrosis around the base of the leaves; Ca-deficient plants show reduced growth; terminal bud death

Magnesium (Mg)	Initial yellowing of older leaves between leaf veins spreading to younger leaves can occur; mottling or chlorosis with or without spots of dead tissue on lower leaves; poor fruit development and production.
Sulfur (S)	Young leaves do not wilt; necrosis not commonly present; leaves are pale green; initial yellowing of young leaves spreading to whole plant; difficult to distinguish from nitrogen deficiency
Micronutrients	
Zinc (Zn)	The younger leaves become yellow; necrosis may be present, generally within interveinal tissue of the mature leaves; necroses on older leaves; leaves become very small and the internodes shorten, producing rosette-like appearance
Manganese (Mn)	Yellowing occurs between the veins of young leaves; the early stage of chlorosis by manganese deficiency is similar to iron deficiency; young leaves do not wilt; necrosis commonly present, in spots scattered over the leaf; growth reduction of plant organs (leaves, shoots, fruits)
Iron (Fe)	Deficiency symptoms are first occurring in new leaves; terminal bud commonly remains alive; necrosis may be present, often confined to leaf tip or margins; interveinal tissue yellowish
Boron (B)	Deficiency symptoms first occur in new leaves; young leaves of terminal bud becoming light green in later growth; leaves become twisted; stalk finally dies at terminal bud
Copper (Cu)	Young leaves permanently wilt and can be curled, without spotting or chlorosis; plant is stunted
Molybdenum (Mo)	General yellowing (chlorosis) of the older leaves, similar to the symptom for nitrogen deficiency but generally without the reddish coloration on the undersides of the leaves
Chlorine (Cl)	Chlorosis and wilting of young leaves; in advanced case, a characteristic bronzing often appears on the upper side of mature leaves
Cobalt (Co)	Co deficiency can result in nitrogen deficiency symptoms, because Co is required for nitrogen fixation in root nodules of legumes
Nickel (Ni)	Plants show growth depression; chlorosis of the youngest leaves and premature senescence; grains and seeds development is inhibited

Table 1. Nutrient deficiency symptoms in plants

Prior to the selection of appropriate fertilizers, a soil analysis is thus required. It will help determining the need for amendments, such as primary nutrients, manure, sewage sludge, compost, straw, which are then added as required to improve the performance of the plant. For example, mineral soil has a strong influence on plant nutrition. It can store, transform and supply nutrients to plants, as well as affect the availability of applied fertilizers. A measurement of soil pH and nutrient status is important: if pH is too high or low, added nutrients such as iron may be ineffective. If a soil already contains a high level of a specific nutrient (e.g., phosphorus), there is little need or advantage to plants in adding more in the form of fertilizer.

Commercial fertilizers, providing nitrogen (N), phosphate (P_2O_5) and potash (K_2O) for sufficient plant growth may also increase the metal uptake and accumulation by plants. The appropriate dose of fertilizers can improve plant growth and root development, thus can lead to an enhanced metal accumulation and extraction. Plant growth regulators can also improve shoot biomass and may additionally counteract the negative effect of heavy metals stress in plants growing on a metal contaminated area. It has been proposed that the commercially available plant growth regulators can increase biomass production and metal extraction.

The application of fertilizers can enhance the metal accumulation in plants not only by enhanced plant growth, but also by lowering soil pH. A low pH decreases adsorption of heavy metals on soil particles and increases their concentration in the soil solution. Fertilizers with a high NH_4 content can decrease soil pH, leading to an increased Cd uptake. It has been observed that an increase in fertilizers dose (NPK) generally causes an increased Cd soluble concentration in the soil and in the plants. An excess addition of fertilizers cations (K and NH_4) also causes a substantial increase of heavy metals in soil solution and concentration in plant tissue. The NH_4 ion has an acidifying effect in soil, due to either the nitrification process or the release of H^+ ions to the soil solution. The use of $(NH_4)_2SO_4$ as a soil additive has been proposed to provide nutrients needed for high yield and to increase metal bioavailability. Two different fertilizers (ammonium sulfate and ammonium nitrate) have also been used to enhance the metal bioavailability for sunflowers grown on a sewage sludge contaminated soil. The pH of soil samples taken from subplots treated with both fertilizers decreases by half a unit after fertilization treatment. Ammonium sulfate can improve Zn and Pb accumulation and extraction (metal accumulation*biomass) by sunflowers, whereas ammonium nitrate leads to an enhanced Cd accumulation and extraction.

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Biographical Sketches

Erika Nehnevajova was born in Slovakia (Nova Bana) on 28 January 1978. Her educational background is following:

Master degree in Teaching Biology and Chemistry, at the Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia (1996 – 2001)

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PhD degree in Environmental Biotechnology at Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland (2002-2005). Her PhD thesis: “Non-GMO approach for the improvement of heavy metal accumulation and extraction of high yielding crop species for efficient phytoextraction of contaminated soil” was awarded by the Dimitris N. Chorafas Foundation.

She started her scientific career as scientific assistant at the Institute of Chemistry at the Slovak Academy of Sciences in Bratislava (Slovakia). In the years 2002 – 2005, she was doing her postgraduate research in the Laboratory of Phytotech-Foundation (PTF) in Berne and in the Laboratory for Environmental Biotechnology, EPFL in Lausanne (Switzerland) within EC-Framework program PHYTAC. After her PhD research, she worked as PostDoc in the Laboratory in Phytotech-Foundation & AGB in Berne (2005-2007) within EC project COST 859: *Phytotechnologies to promote sustainable land use and improve food safety*. Since September 2007, she is working as PostDoc in the Institute of Biology, Applied Genetics at the Free University of Berlin in Germany on the research project supported as fellowship by Swiss National Science Foundation. Her scientific interests are mainly focusing on plant biology, metal tolerance, transport and accumulation in plants; field assessment of metal uptake efficiency by plants, mainly sunflowers and tobacco; plant breeding (*in vitro*, mutagenesis, genetic engineering).

Dr. Nehnevajova is member of American Society of Plant Biologist (ASPB) since January 2008.

Dr Rolf Herzig, Swiss citizen, is an environmental botanist, trace element specialist, high school teacher and electronician. During his studies at Berne University he became founder of the AGB Institute of Biomonitoring in 1985. Nowadeys head of the PT-F laboratory and owner of the AGB Institute. He has published numerous peer reviewed papers, reviews, books, book chapters and reports in the field of air pollution biomonitoring, certified reference materials, and phytoremediation. Dr Rolf Herzig has been co-ordinator or partner of several national and international R&D projects of the EC, such as BCR482, BCR679, PHYTAC, and was co-cordinator and national delegate of the COST Actions 837, and supervisor of 2 Phd thesis in phytoremediation. Presently he is a member of the management committee of COST action 859 “Phytotechnologies to promote sustainable land use management and improve food chain safety”, and Swiss Delegate of working group „Measurement of Immission Effects“ of the VDI-Commission Rheinhaltung der Luft (D). He has long-term experience in environmental biomonitoring and statistics, trace element analyses, QC-measures, CRM production and phytoremediation. Fianally he is acting as a reviewer of several international scientific Journals, such as Int. Journal of Phytoremediation, Plant and Soil, Environmental Science and Technology, and others.

Dr Jean-Paul Schwitzguébel, French and Swiss citizen, is a plant and microbial biochemist. After his studies at the University of Geneva (CH), he worked as a postdoctoral researcher at the Swiss Federal Institute of Technology (ETH) in Zurich (CH), at the Imperial College of Science and Technology in London (UK), and at the University of Neuchatel (CH), focusing on the metabolism and bioenergetics of higher plants and micro-organisms.
He moved then to the Swiss Federal Institute of Technology at Lausanne (EPFL) as a permanent senior scientist in the Laboratory for Environmental Biotechnology. Most of his work is now devoted to research and development in the fields of phytoremediation and bioenergy.

He is the initiator and coordinator of the European COST Actions 837, from 1998 to 2003, (http://lbewww.epfl.ch/COST837) and 859, from 2004 to 2009, (http://w3.gre.ac.uk/cost859/) on phytotechnologies to promote sustainable land use and improve food safety. Dr Jean-Paul Schwitzguébel is also the Swiss delegate on the COST Domain Committee on Food and Agriculture.

Finally, he is subject editor for "Environmental Science and Pollution Research" and "Journal of Soils and Sediments", and member of the editorial board of the "International Journal of Phytoremediation" and of "Agrochimica".