Chapter nine:

Aquatic Phytoremediation by Accumulator Plants

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Introduction

Phytoremediation of metal-contaminated waters is readily achieved by use of aquatic and terrestrial plants because there is no problem of bioavailability of the target element. In terrestrial systems involving hyperaccumulator plants growing over polluted soils, the plant must first solubilise the target element in the rhizosphere and then have the ability to transport it to the aerial tissues. No such problem exists when the plant grows naturally, or is made to grow in, or on, an aqueous medium. It is not surprising therefore that some quite spectacular hyperaccumulations of heavy metals from aqueous systems can be achieved, and indeed this has been known for at least 50 years.

In the treatment of the subject of aquatic phytoremediation systems, there are two natural divisions that involve: 1 - purely aquatic plants such as the floating water hyacinth (*Eichhornia crassipes*); 2 - submersion of the rhizosphere of terrestrial plants in order to remove metal pollutants (*rhizofiltration*). Each of these two subjects will be discussed independently below.

There is a vast literature on the subject of trace elements in marine plants (mainly seaweeds) but these are described separately in Chapter 5.

Hyperaccumulation by Aquatic Plants

Introduction

A useful review of hyperaccumulation of elements by aquatic plants (Outridge and Noller, 1991) is the basis of much of the material in this first section of this chapter. Freshwater vascular plants (FVPs) when combined with macroscopic algae are known collectively as *macrophytes*. Their ability to concentrate elements from the aquatic environment was first reviewed by Hutchinson (1975) who reported that levels of potentially toxic elements such as cadmium, lead and mercury were at least an order of magnitude higher than in the supporting aqueous medium.

Building on the review of Hutchinson (1975), Outridge and Noller (1991) sought to establish the following facts:

1 - the "normal" concentrations of selected elements in natural fresh water systems;

2 - "normal" levels of elements in macrophytes growing in uncontaminated ecosystems;

3 - the median and maximum concentrations of elements in macrophytes growing in contaminated waters;

4 - pathways and rates of elemental uptake and excretion;

5 - environmental factors that control uptake of elements by these plants;

6 - the importance of these plants in terms of biogeochemistry or entry of trace elements into food chains;

7 - elements that are the most toxic to FVPs and how this compares with their toxicity to algae;

8 - the significance of trace element uptake for the field of wastewater treatment and biomonitoring of pollution.

In practical terms, the last topic is perhaps of greatest interest to scientists engaged in the theory and practice of bioremediation of aqueous systems and will be given some prominence in this chapter. It may be appropriate at this stage to define two of the terms used.

Freshwater vascular plants comprise mainly angiosperms (flowering plants) with a few fern species. They have a vascular system (xylem and phloem) as well as well defined roots. They exclude macroscopic algae.

Heavy metals is a term often used to describe some trace elements. It is an imprecise term that can include quite "light" elements such as copper. It has been suggested by Nieboer and Richardson (1980) that heavy metals are often associated with toxicity to biota, although some "heavy metals" such as manganese and uranium are not known to be particularly toxic to life forms.

Life forms include:

1 - rooted emergents that are rooted in bottom sediments;

2 - free-floating emergents such as water hyacinth and duckweed that are not rooted in bottom sediments;

3 - rooted submergents with leaves and flowers under water;

4 - free-floating submergents;

5 - rooted floating-leaved plants such as water lilies;

6 - free-floating floating-leaved plants.

Heavy metals in natural uncontaminated fresh water systems

The elemental composition of uncontaminated fresh waters has been calculated by Turekian (1969) and is shown in the second column of Table 9.1. The values are of course rather imprecise, partly because of analytical error involved in estimations in the ng/g (ppb) range and partly because of the problem of extrapolating to the entire fresh hydrosphere from a relatively small number of rivers.

Table 9.1. Elemental concentrations (μg/mL [waters] and μg/g dry weight for freshwater vascular plants - FVPs).

Element	A	B	C	D	E	C/A
Ag	0.003	0.06	0.15	0.12	67	500
As	0.002	0.20	2.7	1.4	1200	1350
Cd	0.0002	0.64	1.0	1.4	90	5000
Co	0.0002	0.48	0.32	0.37	350	1600
Cr	0.001	0.23	4.0	2.8	65	4000
Cu	0.007	14	7.9	42	190	1128
Hg	0.0001	0.015	0.50	0.58	1000	5000
Mn	0.007	630	370	430	8370	52,857
Mo	0.001	0.90	12	-	-	12,000
Ni	0.0003	2.7	4.2	6.1	290	14,000
Pb	0.003	2.7	6.1	27	1200	2033
Se	0.0002	0.2	1.0	0.30	21	5000
U	0.00004	0.04	0.50	0.05	1.1	12,500
V	0.0009	1,6	3.6	-	-	4000
Zn	0.02	100	52	47	7030	2600

A - uncontaminated river waters (Turekian, 1969), B - terrestrial plants (Bowen, 1966), C - median values for uncontaminated FVPs, D - median values for contaminated FVPs, E - maximum value for contaminated FVPs, C/A - accumulation factor for FVPs growing in uncontaminated waters. After: Outridge and Noller (1991).

It will be seen from Table 9.1 that in natural uncontaminated fresh waters, elemental abundances are extremely low and in most cases are in the ng/mL (μg/L) range. Under these conditions, the FVPs appear to be invariably hyperaccumulators of the heavy metals even in the uncontaminated environment.

Heavy metals in freshwater vascular plants

Several patterns were established by Outridge and Noller (1991) using median values for the data shown in Table 9.1 and elsewhere in their paper.

1 - manganese was the most strongly absorbed element followed in descending order by zinc, molybdenum, copper and lead;

2 - FVPs contained higher concentrations of Ag, As, Cd, Cr, Hg, Ni, Pb, Se and U than did terrestrial plants, whereas Co, Cu, Mn and V were similar in both groups.

3 - concentrations of As, Cd, Co, Cr and U were generally higher in submerged plants than in other FVP life forms;

4 - roots usually contained higher concentrations of heavy metals than above-

ground plant parts;

5 - maximum elemental abundances in FVPs were usually one or two orders of magnitude higher than naturally occurring levels, though the median concentrations were not usually very different;

A further observation needs to be made on the findings of Outridge and Noller (1991). The generally higher levels of elemental concentrations in roots would seem to indicate that these heavy metals are absorbed from the sediments rather than the waters. However, the sediments are in most cases derived from the settling of particulates from the waters and there must be some degree of constant proportionality between the two phases.

Environmental and physiological factors affecting elemental toxicity and uptake by FVPs

Outridge and Noller (1991) have shown that heavy metal uptake and retention by FVPs and macrophytes in general, are controlled by four main factors:

1 - sediment geochemistry;
2 - water physicochemistry;
3 - plant physiology;
4 - genotypic differences.

The first two control metal speciation in sediments and waters, whereas the last two control the ability of plants to accumulate plant-available forms of the metals.

The question of speciation is very important in deciding to what extent a given elemental species will be taken up by macrophytes or be toxic to them. It is less understood that any given elemental species will have a whole range of different bioavailabilities because of physiological differences with respect to uptake sites and uptake mechanisms (Leppard, 1983).

The bioavailability of elemental species to FVPs are less well understood than in the case of other aquatic biota such as fish. However, it is known that FVPs usually accumulate heavy metals by absorption followed by passive or active transport across membranes (Forstner and Wittman, 1981; Smies, 1983).

In their summary of their benchmark paper, Outridge and Noller (1991) showed that physicochemical factors that increase metal solubility (such as lake acidification) also increase elemental concentrations in FVPs presumably because of greater solubility of metals in the sediments.

Aquo metal and soluble metal chelate complexes were the most plant-available forms, whereas metals in reducing sediments were almost completely unavailable to plants, perhaps because of the great insolubility of most metal sulphides.

Elemental levels in most macrophytes exhibited a typical seasonal pattern of a spring maximum followed by a steady decline during the summer. This was ascribed to environmentally driven physiological changes including translocation between above-ground and below-ground tissues. Iron root plaque displayed an important role in availability and uptake of elements from reduced sediments.

Macrophytes play an important role in sediment-water trace element cycling through root uptake, excretion into water and detrital absorption of metals that may be conveyed to the sediments. Elemental toxicity experiments have shown that Ag, As, Cr, Cu, Hg and Ni are about 10 times more toxic to macrophytes than are lead or zinc. Copper is one of the most toxic of these elements and its effects are visible at concentrations of 0.05-0.15 μg/mL. Compared with phytoplankton, macrophytes are 10-100 times less sensitive to most elements except copper to which they are equally sensitive.

Practical applications of FVPs for water purity monitoring and decontamination

Water purity monitoring

Freshwater vascular plants can be used for biomonitoring of contaminated waters and possess several innate advantages over algae:

1 - they have longer life cycles;

2 - they have a higher degree of tolerance to most elements including those heavy metals largely responsible for pollution;;

3 - their biomass is much larger than in the case of algae, so that a larger sample is available for chemical analysis.

Phillips (1977) has proposed the following essential requirements for a suitable FVP biomonitor:

1 - the species must be endemic to the area;

2 - it should be easily cultivated or abundant in the field;

3 - it should concentrate the elements to above the threshold of the limits of detection of the analytical method that is to be employed;

4 - there must be a statistically significant relationship between the abundance

Table 9.2. Freshwater vascular plants proposed for biomonitoring of trace elements in waters.

Species	As	Cd	Co	Cu	Hg	Mn	Ni	Pb	Zn
Callitriche platycarpa				+					
Ceratophyllum demersum		+						+	
Eichhornia crassipes	+	+			+			+	
Elodea canadensis	+		+	+			+		
E.nutallii		+	+					+	+
Equisetum arvense				+					+
E.fluviatale								+	+
Myriophyllum exalbescens									+
M.verticillatum	+		+	+		+			
Nuphar lutea				+					
Potamogeton perfoliatus						+			+
P.richardsonii			+	+				+	+

Sources: various, summarised by Outridge and Noller (1991).

of a given target element in the plant and its concentration level in the surrounding water.

Several FVPs have been suggested as suitable for biomonitoring of trace elements in waters. These are summarised in Table 9.2 above. Some will also be discussed later in this chapter under the individual species.

As pointed out by Outridge and Noller (1991), the species in Table 9.2 were sometimes selected on the basis of their very high accumulation of trace elements from the surrounding waters. This criterion is not necessarily valid as it is far more important that there be a statistically significant relationship between the plant-water system of elemental abundances.

There is not usually a good correlation between elemental abundances in sediments and those in rooted aquatic plants. Indeed Campbell *et al.* (1985) found fewer than 30 significant correlations in the 100 cases that they investigated.

For monitoring purposes, it seems logical that free-floating plants should be used in place of rooted species since such plants derive their nutrients solely from the water column. Sprenger and McIntosh (1989) found a significant relationship for the free-floating *Utricularia purpurea*.

Decontamination of polluted waters

The use of FVPs for removal of pollutants has been established for over 20 years following the pioneering work of Wolverton (1975), Wolverton and McDonald (1975a, 1975b) and Wolverton *et al.* (1975), several species, notably the water hyacinth (*Eichhornia crassipes*), have been proposed for this purpose and will be discussed further below.

There are two main ways in which FVPs may be used for remediation of polluted waters. The first of these involves monospecific pond cultures of free-floating plants such as water hyacinth. The plants accumulate the pollutants until a steady state of equilibrium is achieved. They are then harvested by removal from the pond.

There are various problems inherent in the above method. The first is associated with how to dispose of the waste matter. One solution was achieved at a sewage treatment plant where the toxic waste was used to generate methane gas. Further problems associated with the "free floating" procedures include the presence of unwanted pathogens that may destroy the whole of a monospecific culture and there is also the problem of continual harvesting that requires specialised equipment.

The second methodology employing FVPs for pollutant removal involves growing rooted emergents in trickling bed filters. Uptake of trace elements in these systems is usually caused by rhizosphere microbes with relatively little uptake caused by the plants themselves. The rhizosphere methods do not necessarily have to employ FVPs and are discussed below in a separate section.

There will now be a discussion of selected FVP species that have significant promise for phytoremediation of polluted waters.

The water hyacinth (*Eichhornia crassipes*)

The water hyacinth is perhaps one of the most commonly cited species for phytoremediation of polluted waters (Gupta, 1980; McDonald and Wolverton, 1980). The plant has a rapid growth rate and can hyperaccumulate nutrients (Cornwell *et al.*,1977) as well as heavy metals (Wolverton, 1975; Wolverton *et al.*,1975).

The water hyacinth has a number of problems that tend to hinder its commercial use. The first of these is that in many countries it is a noxious weed that has choked large areas of waterways. For example in Sudan it has completely covered a large area of the Nile River with a dense mat known as the *sudd*. A further disadvantage of this plant is that it will only grow in tropical or warm-temperate parts of the world that are frost-free in winter.

As pointed out by Kay *et al.* (1984), much of the research on the water hyacinth has been faulty insofar as estimates of biomass production have been carried out in the field in unpolluted waters, whereas uptake experiments are usually carried out in the laboratory where there is concomitant, but unmeasured, reduction in biomass.

There is little doubt that there is a rapid rate of removal of heavy metals following absorption by the water hyacinth. This is demonstrated in Table 9.3 that shows the rate of removal of six heavy metals from a system in which the biomass production was 600 kg/ha/day.

Table 9.3. Rate of removal of heavy metals from the aqueous phase by use of the water hyacinth (*Eichhornia crassipes*).

Element	mg/g of dry biomass/day	g/ha/day
Cadmium	0.67	400
Cobalt	0.57	340
Lead	0.18	90
Mercury	0.15	110
Nickel	0.50	300
Silver	0.44	260

After: Outridge and Noller (1991).

The effect of lead, copper and cadmium on metal uptake and growth of the water hyacinth was studied by Kay *et al.* (1984). Although lead had no effect on plant growth, they found that cadmium and copper were toxic to this plant and that the thresholds were respectively 0.5 and 1.0-2.0 μg/mL in the ambient water. Beyond the threshold concentrations the effects were chlorosis, suppressed development of new roots, and greatly reduced growth rates.

Muramoto and Oki (1983) studied the uptake of cadmium, lead and mercury by the water hyacinth. Their data are summarised in Table 9.4. The absorption factors appear to be quite low compared with the average of several thousand shown in Table 9.1. However, it must be remembered that the data were on a fresh-weight basis and that dry weights of aquatic plants are usually about 5% of the fresh weight. This would give concentration factors of the order of 1000 for the tops of plants growing in cadmium and lead, and about 5000 for the roots.

It can also be seen that increasing the heavy metal content of the aqueous medium actually has the effect of significant reduction of concentration factors owing to the toxicity of the metals concerned.

When the laboratory data were extrapolated to field conditions in Japan, Muramoto and Oki (1983) calculated (Table 9.4) the maximum removal of heavy metals from heavily contaminated waters. The findings are perhaps somewhat overoptimistic because we are again confronted here with the problems of extrapolating from the laboratory to the field where the biomass reported, was based on cultivation under metal-free conditions.

Table 9.4. Fresh-weight concentration factors (plant/water) for water hyacinth grown in different concentrations of cadmium, lead and mercury.

Treatment		Tops	Roots	Max. removal*
Cadmium	1.0 μg/mL	44	101	6.6
	4.0 μg/mL	47	47	17.7
	8.0 μg/mL	35	29	5.6
Lead	1.0 μg/mL	42	296	47.5
	4.0 μg/mL	19	220	336
	8.0 μg/mL	20	127	627
Mercury	0.5 μg/mL	0.044	438	3.5
	1.0 μg/mL	0.032	488	105
	2.0 μg/mL	0.048	340	7.5

*These values are in g/m^2 of whole plants under field conditions assuming a biomass yield of 45 kg (fresh weight)/m^2. After: Muramoto and Oki (1983).

Before leaving the discussion of water hyacinth, some mention should be made of a proposal to use this plant to recover gold from tailings (Anon, 1976). The Gold Hill Mesa Corp. of Colorado Springs undertook a survey in which gold tailings were to be washed free of cyanide and stored in settling ponds to which the *Eichhornia* was to be added. The initial vat leaching procedure removes only 60% of the gold and it was hoped that some of the remainder could be removed by the water hyacinth and later recovered by burning the plant material. No information is currently available about the success or otherwise of the operation.

Ceratophyllum demersum, Egeria densa *and* Lagarosiphon major

The above plants are extremely common in temperate waterways and are mentioned here because of their extraordinary accumulation of arsenic derived from geothermal activity in New Zealand. The unusual accumulation of arsenic by FVPs in this area was first reported by Reay (1972) who found 650 μg/g (dry weight) in *C.demersum*. Later, Liddle (1982) reported arsenic concentrations of 265-1121 μg/g (dry weight) for the same species collected from this area.

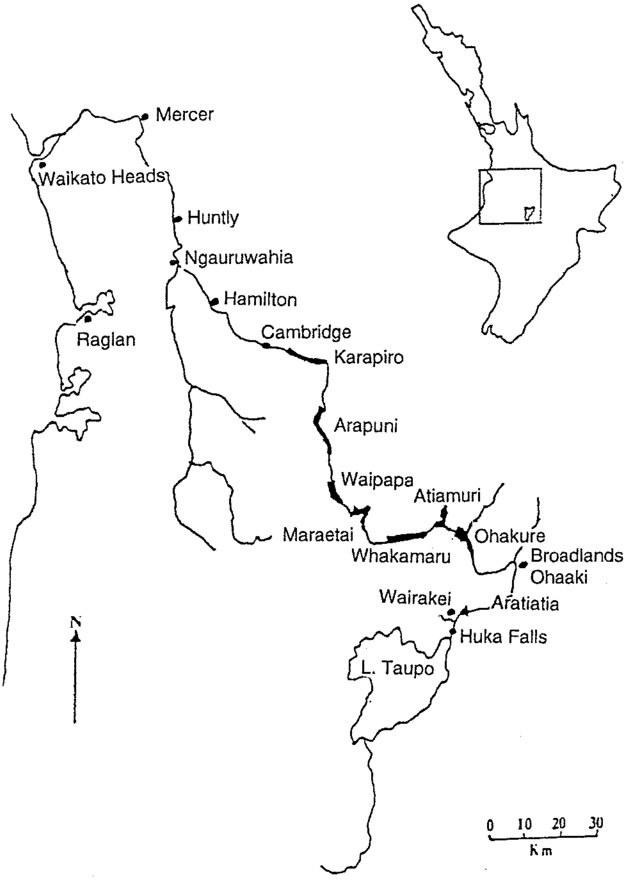

Fig.9.1. Map of the Waikato River system, North Island, New Zealand.

A more recent survey by Robinson *et al.* (1995) involved a study of *Egeria densa*, *Ceratophyllum demersum* and *Lagarosiphon major* taken from the Waikato River (Fig.9.1). Means and ranges (μg/g dry weight) were as follows: *Ceratophyllum demersum* 378 and 44-1160; and *Egeria densa* 488 and 94-1120. *Lagarosiphon major* from the Huka Falls upstream from the geothermal area contained 11 μg/g arsenic, whereas the same species from Lake Aratiatia below the geothermal activity, had 300 μg/g of this element. *Ceratophyllum demersum* occurred from Lake Aratiatia northwards. *Lagarosiphon major* occurred between Lake Taupo and Broadlands where it was replaced by *E. densa*. All species had arsenic concentrations of up to 1200 μg/g (0.12%) dry weight.

Egeria densa and *C. demersum* had arsenic concentrations that had a highly significant inverse correlation (r=-0.86[S**] and r=-0.76[S**] respectively) with the distance of the plant downstream (Fig.9.2). These results show that the above aquatic macrophytes actively extract arsenic from the water in which they grow.

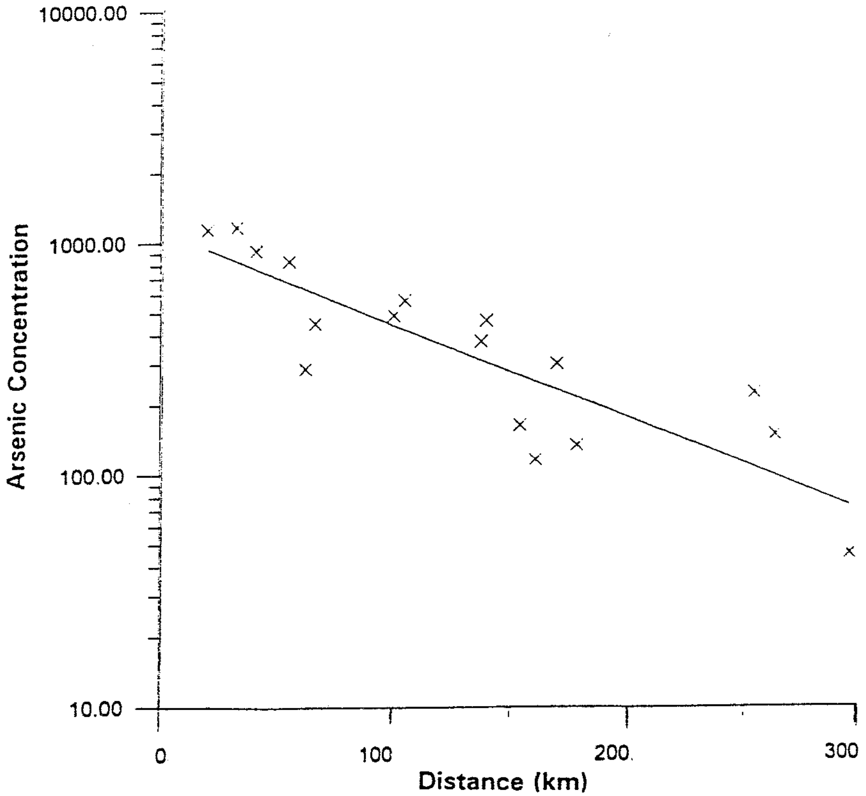

Fig.9.2. The arsenic content (μg/g dry weight) in *Ceratophyllum demersum* collected from the Waikato River. Data are expressed as a function of distance (km) of the collection site downstream from the source in Lake Taupo. Source: Robinson (1994).

The arsenic concentration of *C.demersum* showed a highly significant (r=0.65[S*]) positive correlation with the arsenic content of the water from which the plant was taken. The arsenic concentration in this plant was about 10,000 times the arsenic concentration in the surrounding water. The arsenic concentration in *E. densa* showed no significant correlation (r=0.41[NS]) with that of the water from which it was taken. These results indicate that *C.demersum* can also be used as a biomonitor of arsenic levels in waters.

The high concentrations of arsenic in the weeds may be the result of the element being taken up by the same process as the uptake of an essential element. Arsenic has some chemical similarities with phosphorus, which is an essential plant macro nutrient. Accumulation of arsenic may be incidental to phosphorus uptake. This possibility is supported by the observation that nearly all the plants tested that grow in waters with elevated arsenic levels, accumulate arsenic to some degree.

The concentration of phosphorus in the water may affect the amount of arsenic accumulated by the plant. This may in turn affect the amount of arsenic in the river water. Benson (1953) showed that increasing levels of phosphorus decreased the toxicity of arsenic to barley plants by possibly competing for the binding sites that would otherwise have been occupied by this phytotoxic element.

It is normal farming practice to apply 400 kg/ha phosphate fertiliser to pumice soils (Hill, 1975) and some of the phosphorus leaches into the waterways of the area. The amount of phosphorus applied to farms around the Waikato River system may directly affect the amount of arsenic accumulated by plants, which will affect the arsenic concentration in the waters of this waterway.

Weed eradication programmes that involve the use of herbicides need to take into account the amount of arsenic released into the water as the weeds decay. The performance of carp and other herbivorous fish introduced in an attempt to control the weeds will be affected by the arsenic concentration of weeds in the Taupo Volcanic Zone. Arsenic in weeds may be toxic and/or unpalatable to the fish.

The weeds may also have a use as detoxification agents in waterways with high levels of arsenic. Arsenic may be removed from a body of water by growing, and periodically removing, the macrophytes in a particular area. Lakes such as Lake Rotoroa, which still contains large amounts of arsenic from a weed eradication programme 25 years ago, may be detoxified in this manner.

Water cress

Robinson *et al.* (1995) have found that New Zealand water cress *Rorippa naturtium* subsp. *aquaticum* collected from the Waikato River near the Ohaaki (Broadlands) power station (Fig.9.1) contained >400 μg/g (dry weight) of arsenic. Laboratory trials with water cress grown in tanks containing added arsenic confirmed the ability of this species to accumulate arsenic to a degree at least an order of magnitude higher than the ambient water.

Fig.9.3 shows the relationship between arsenic in the plants and in the surrounding waters. The correlation is extremely good and shows that water cress might also be used for biomonitoring of arsenic pollution in waters.

Arsenic levels in plants grown under laboratory conditions were neverthless about five-fold lower than in plants growing in the Waikato River. This was attributed to a number of factors including the likelihood that the laboratory plants were surrounded by reduced arsenic (As^{3+}) instead of the more strongly absorbed As^{5+} encountered under natural conditions. Another factor is that in the field, the water cress is rooted in sediment containing on average about 95 μg/g arsenic. It was recommended that water cress not be consumed in waters containing >0.05 μg/mL arsenic (essentially the Waikato River between Wairakei and Atiamuri). These studies carry an important message for health authorities.

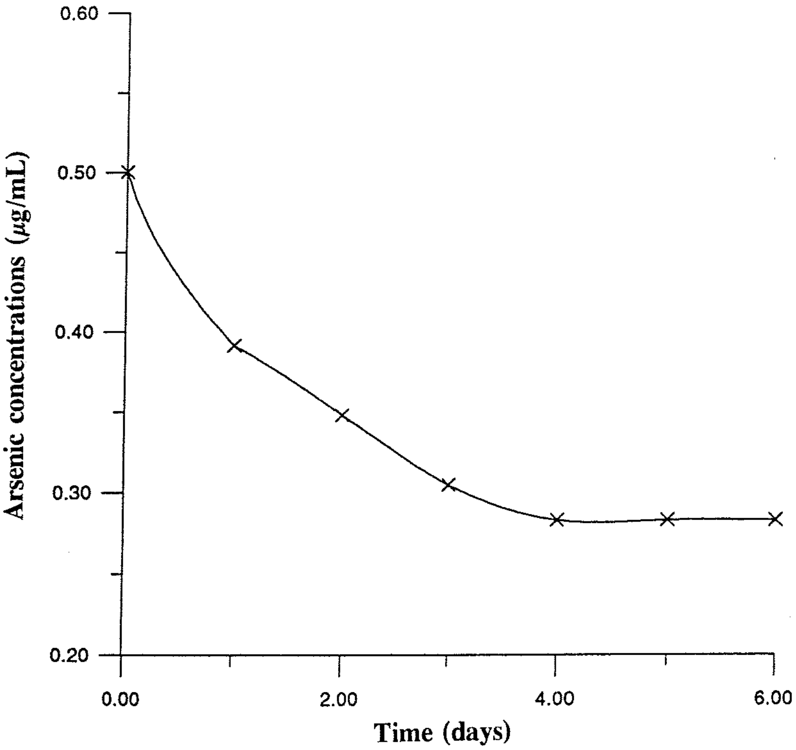

Fig.9.3. Laboratory study of arsenic concentrations in water cress compared with the surrounding water. Source: Robinson *et al.* (1996).

Other floating FVPs

Before leaving the subject of metal uptake by specific plants, brief mention should be made of use of duckweed (*Lemna minor*) and water velvet (*Azolla pinnata*).

Wahaab *et al.* (1995) studied copper and chromium (III) uptake by duckweed. They found that the light incidence was a major factor in growth, with the plants doubling in size every 3 days under the most favourable conditions. When grown in water containing 0.25 and 1.0 μg/mL of copper and chromium (III) respectively, uptake rates were 80-333 and 250-667 mg/day/m^2 of water surface. Maximum levels of uptake were 1-2 g of metal/kg of dry matter.

A somewhat analogous series of experiments were carried out by Jain *et al.* (1989) using duckweed and water velvet in which laboratory trials studied uptake of iron and copper from solutions containing 1.0, 2.0, 4.0 and 8.0 μg/mL of these two elements. Growth rates decreased slightly with increasing concentrations of either element for both species. After 14 days, bioaccumulation coefficients (plant/water) approached about 1000 for both elements in both species for waters containing 8 μg/mL of the element concerned.

The authors suggested that both species would be useful for bioremediation of polluted waters and might be employed by passing effluent over a bed of these plants contained in ponds.

Rhizofiltration

Introduction

The discussion so far has been centred around freshwater vascular plants (FVPs) that are able to take up significant amounts of heavy metal pollutants from the aqueous medium. Although quite high concentration factors are achieved so that these species can be considered as hyperaccumulators, their biomass is usually very small so that the actual mass of pollutant removed is not large. In addition, these plants are often slow-growing.

Terrestrial plants usually have much larger root systems than FVPs. These roots are often fibrous and are covered with a multitude of root hairs that present a large surface area to the medium in which they grow. A new and burgeoning field has been described as *rhizofiltration* and is currently being studied at various institutions such as at Rutgers University, New Jersey, where I.Raskin is very active in this field. Much of this work at the university, and in their associated commercial enterprise (Phytotech Inc.), has been concerned with the possibility of extracting lead commercially from polluted waters using the root systems of terrestrial plants.

Rhizofiltration usually involves the hydroponic culture of plants in a stationary or moving aqueous environment wherein the plant roots absorb pollutants from the water. The principles of the technique have been described by Dushenkov *et al.* (1995) and by Salt *et al.* (1995).

Ideal plants for rhizofiltration should have extensive root systems and be able to remove metal pollutants over an extended period. Such plants should be capable of producing up to 1.5 kg (dry weight) of roots per month per m^2 of water surface.

Suitable candidates for rhizofiltration have included the Indian mustard (*Brassica juncea*) and sunflower (*Helianthus annuus*). Both of these species tend to concentrate heavy metals in the root systems and translocate only a small part of the metal burden to the shoots. If extensive translocation does occur, this has the effect of making the system less efficient.

Apart from physical absorption of heavy metals at root systems, plants are also able to precipitate metals such as lead at the root systems by exuding phosphate that can form the highly insoluble lead phosphate. Concentration factors (*bioaccumulation coefficients*) defined as elemental content of roots divided by that of the water, are as high as 60,000 for some elements (Salt *et* *al.*,1995).

Although the emphasis in this chapter is on bioremediation by metal uptake, a great deal of work has been carried out on removal of organic compounds and nutrient elements by rhizofiltration (Schnoor *et al.*,1995), often by the use of large trees such as willow and poplar. The same large trees can however also remove metals and will be discussed further below.

The field of rhizofiltration can be divided into three main divisions:

1 - the use of large annual or perennial herbs or grasses;

2 - the use of trees;

3 - the use of trees, large herbs, reeds or grasses to remove contaminated water whereby the water is recycled into the atmosphere and the metals retained within the plant.

Rhizofiltration with large annual or perennial herbs or grasses

Brassica juncea (Indian mustard)

Lead

Some of the earliest experiments on the use of *Brassica juncea* for rhizofiltration of lead were carried out by Dushenkov *et al.* (1995). They report the study of 24 different herbs, grasses and crop plants in order to discover which absorbed the greatest amount of lead in their root systems. Values ranged from 60 mg Pb/g of dry biomass to 136, 140 and 169 mg/g for *Brassica juncea, Helianthus annuus* and *Agrostis tenuis* respectively. The much smaller total biomass of the grass *Agrostis* precluded its potential use for rhizofiltration, though other grasses of greater biomass might be useful for this purpose.

Table 9.5. Shoot and root bioaccumulation coefficients for hydroponically grown specimens of *Thlaspi caerulesecens* and *Brassica juncea* exposed for 8 days to various metal solutions.

Metal	Initial conc.	BS	TS	BR	TR
Cd	5 μg/mL	175	59	20574	4258]
Cu	1 μg/mL	159	623	55809	60716
Cr	0.4 μg/mL	80	89	5486	8545
Ni	1 μg/mL	587	2739	11475	8425
Pb	5 μg/mL	3	29	1432	7011
Zn	3 μg/mL	49	770	1816	2990

BS-*Brassica* shoots, TS-*Thlaspi* shoots, BR-*Brassica* roots, TR-*Thlaspi* roots. After: Salt *et al.* (1995).

The uptake of lead and five other elements in roots and shoots of *Brassica juncea* was compared with corresponding values for *Thlaspi caerulescens*, a well known hyperaccumulator of cadmium and zinc. The results are shown above in Table 9.5 where it can be seen that differences in metal uptake are much greater for the shoots than the roots and that the latter have comparable uptakes for both species. The root biomass of *Brassica* is however, far greater than for *Thlaspi* and this is the greatest factor in its favour.

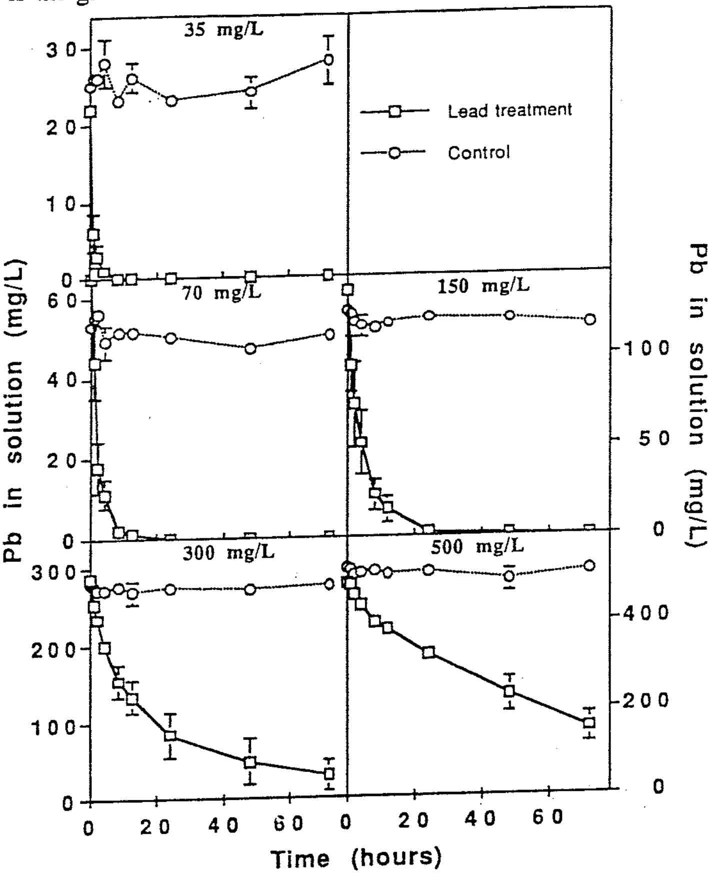

Fig.9.4. Removal of lead by roots of *Brassica juncea* grown hydroponically for up to 72 hours in test solutions containing 0-500 mg/L (μg/mL) lead. Source: Dushenkov *et al.* (1995).

Experiments described by Dushenkov *et al.* (1995) have demonstrated the rapid removal of lead from solutions containing 0-500 μg/mL (ppm) lead in which *Brassica juncea* was grown hydroponically (Fig.9.4). The roots were able to remove most of the lead in 24 hours for concentrations of lead up to 150 μg/mL. At higher concentrations, extraction was slower and less complete. There was a linear relationship between the initial lead concentration and the time needed for the *Brassica* roots to remove half of the initial lead content of the water. This is shown in Fig.9.5.

Other elements

Although most of the work described by Dushenkov *et al.* (1995) was devoted to lead uptake, they also described analogous experiments in which rhizofiltration of cadmium, nickel, copper, zinc and chromium, as well as lead was described. These experiments are shown in Fig.9.6. The initial elemental concentrations (mg/L [μg/mL]) in the aqueous media were: Cd^{2+} (2), Ni^{2+} (10), Cu^{2+} (6), Zn^{2+} (100), Cr^{6+} (4) and Pb^{2+} (2).

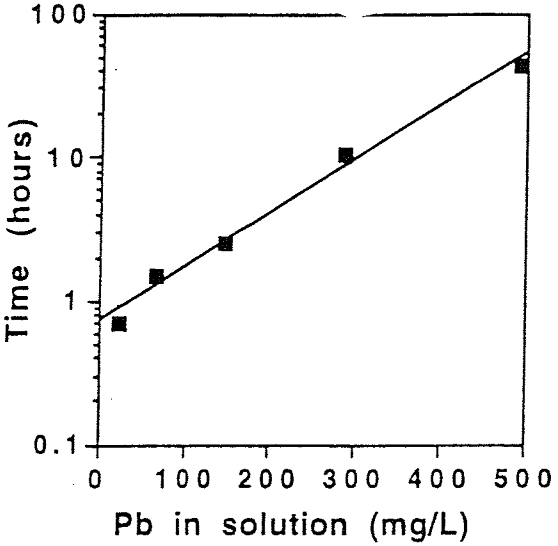

Fig.9.5. Relationship between the initial lead concentration and the time required for roots of *Brassica juncea* to reduce the lead level by 50%. Source: Dushenkov *et al.* (1995).

There were no visible signs of toxicity in the plants. After 8 hours there was a drastic reduction in concentration levels of all of the test metals. The bioaccumulation coefficients were as follows: Pb (563), Cu (490), Ni (208), Cr (179), Cd (134), Zn (131). When a mass balance was carried out, it was found that significantly less metal was found in the plant material than had disappeared from the solutions. This could be explained by precipitation of some of the ions by plant exudates such as phosphate (see also above).

It should be further mentioned that chromium extracted into plant roots was found to be present as the Cr^{3+} ion, thus indicating that the plant reduces chromium during the process of absorption.

Washed dried roots of *Brassica juncea* were much less effective at removing heavy metals from solution. For example, a solution initially containing 300 μg/mL lead decreased immediately to 250 μg/mL (due to precipitation) in all experiments and then decreased to about 20 μg/mL after 10 hours when live roots were used, and still had 200 μg/mL after dried roots had been employed.

Helianthus annuus *(sunflower)*

Extensive experiments have been carried out with the sunflower (*Helianthus annuus*). This large annual herb has about the same biomass as *Brassica juncea* and will readily absorb trace metals in its rhizosphere. Salt *et al.* (1995) have described experiments in which this plant was able to remove five elements from the solutions in which they were grown hydroponically (Fig.9.7).

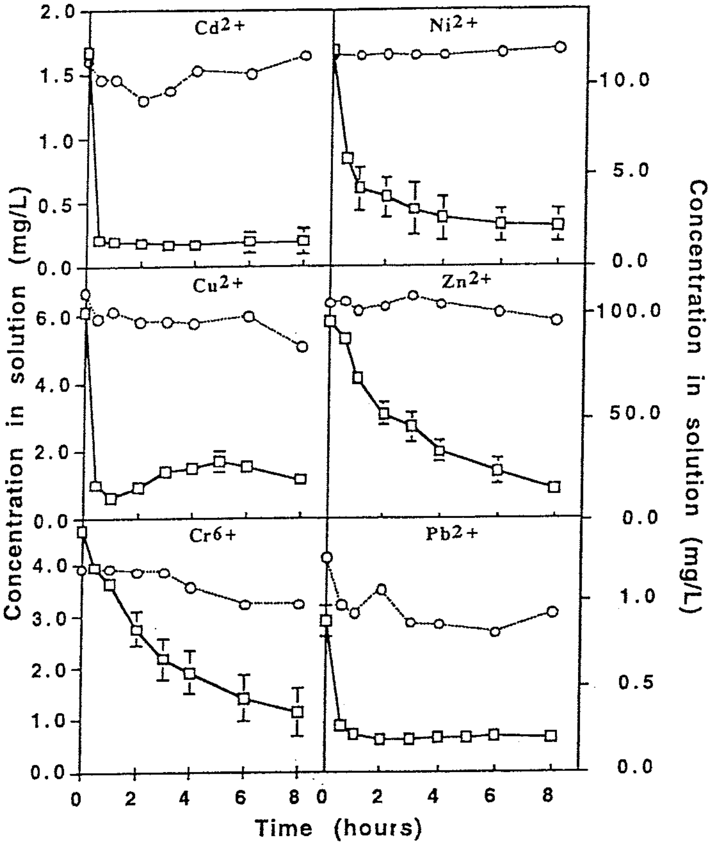

Fig.9.6. Removal of metal ions by roots of *Brassica juncea* (open squares) grown hydroponically in solutions of six elements for up to 8 hours. Controls (open circles) do not contain roots. Source: Dushenkov *et al.* (1995).

As can be seen from Fig.9.7, the sunflower was able to drastically reduce elemental concentrations of manganese, chromium, cadmium, copper and nickel to extremely low levels after only 24 hours. Similar results were obtained for uranium (VI), lead, zinc and lead.

Because rhizofiltration can be employed most economically in situations involving large volumes of water and relatively low levels of contaminants, it would seem to be a possible method of removing radioactive nuclides from polluted waters such as at Chernobyl. Cooney (1996) has reported on field tests carried out near Chernobyl by B.D.Ensley (Phytotech Inc.) and I.Raskin and others from Rutgers University. In these tests, a pond contaminated with radioactive strontium and caesium was covered with 1 m^2 styrofoam rafts in which seedlings of *Helianthus annuus* were inserted. After 4-8 weeks the plants were harvested, dried and analysed for both radioisotopes. The highest bioconcentration coefficient for caesium was found in sunflower roots and the highest for strontium was in the shoots.

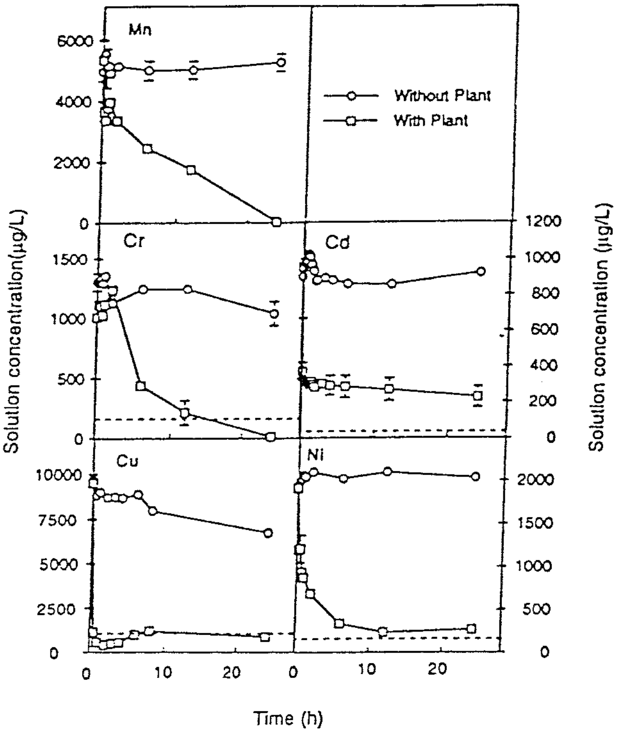

Fig.9.7. Removal of metal ions by roots of *Helianthus annuus* (open squares) grown hydroponically in solutions of five elements for up to 24 hours. Controls (open circles) do not contain roots. Source: Salt *et al.* (1995).

In tests carried out in Ashtabula, Ohio, sunflower plants had their roots submerged in waters contaminated with 100-400 ng/mL (ppb) uranium (Cooney, 1996). Within the first 24 hours, the uranium content decreased by 95 % to below the EPA standard of 20 ng/mL.

Practical proposals for rhizofiltration using large herbs

An advertising leaflet issued by Phytotech Inc. proposes a commercial rhizofiltration system in which polluted water runs through a series of cells in which plants are grown hydroponically and spray fertilised from above. The cleaned water then passes out of the system or can be recycled if not sufficiently pure. The benefits of the system according to the company's claims are that it allows *in situ* treatment minimising environmental disturbance. Further claimed benefits are that the procedure is inexpensive yet more effective than comparable technology, and that the system is suitable for removal of low-level radioactive contamination.

It is not clear from the advertising brochure to what degree this system has been proven by commercial applications, but the concept is impressive in its design and obvious future potential.

Rhizofiltration by use of large trees

Rhizofiltration by use of large trees is also a viable, and better proven, emerging technology. The technique is already well established for remediation of organic contaminants such as TNT (Schnoor *et al.*,1995). There are certain essential differences between the use of large trees and large herbs for rhizofiltration. The trees are usually *in situ* so that the "mountain comes to Mohammed rather than Mohammed to the mountain" insofar as circulating ground waters seek out the rhizosphere of the plant rather than vice versa. Furthermore, the tree, unlike the annual herbs described above, is not usually removed after rhizofiltration.

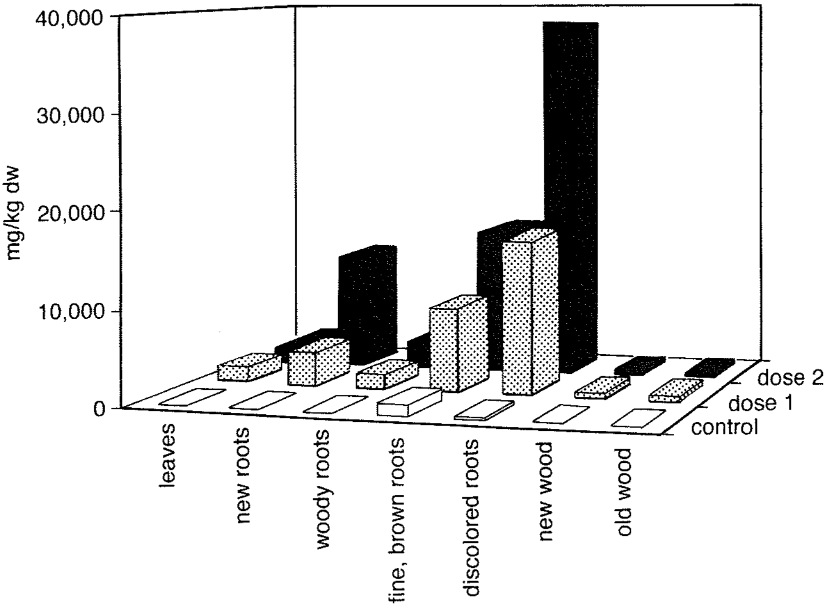

Fig.9.8. Zinc concentrations in poplars exposed to contaminated waters. Source: Negri *et al.* (1996).

Nyer and Gatliff (1996) have reported work carried out by Negri *et al.* (1996) in which zinc uptake by a hybrid poplar (*Populus* sp.) was investigated. Laboratory experiments involved growing the plants in quartz sand to which nutrients and varying amounts of zinc solutions circulated through the rhizospheres. A single pass of a solution containing 800 μg/mL zinc showed almost complete removal of the zinc in a period of 4 hours. For solutions containing >1000 μg/mL of the metal, the leachates always had <100 μg/mL zinc.

At the end of the experiment, the poplars were harvested and various plant organs analysed for zinc. The results are shown in Fig.9.8 from which it will be seen that by far the greatest concentration of zinc (38,055 μg/g) was to be found in the roots. In the aerial parts of the plants, the metal concentrations did not exceed 2250 μg/g in dry leaf tissue and 900 μg/g in the woody branches.

Rhizofiltration using plants to remove and purify contaminated waste waters

There are situations in which it is desired not only to purify waste water but also to reduce its volume. Phreatophytic trees such as poplar, willow and cottonwood are very efficient natural pumps that remove subterranean water and return it to the atmosphere by transpiration. Nyer and Gatliff (1996) have reported that a single willow tree uses and transpires as much as 22,500 litres (5000 gallons) of water in a single day.

Negri and Hinchman (1996a) have introduced the interesting concept of a plant-based system of water treatment that includes a bioreactor in which selected plants are grown hydroponically. The process was designed originally to deal with the large volume of salt-rich water emerging from drilling natural gas wells.

The water evaporates through plant transpiration and rapidly reduces the volume of water. At the same time the heavy metals and other salts are absorbed in the fine root system. The generated plant biomass is harvested periodically, dried, and composted or burnt to produce a small amount of ash that can be buried or even sold in some cases.

It is well known that fresh and saltwater marshes can act as efficient bioremediators of contaminated waters (Kirschner, 1995). One of the commonest plants in these marshes is the bulrush (*Scirpus validus*). This was one of the species selected by Negri and Hinchman (1996a) for their experiments.

Water passes through two cells in parallel containing hydroponically grown bulrush. The water enters with about 1.5% solutes (calculated as the sodium chloride equivalent) and on leaving has a salt content doubled to 3%. At this stage the water enters the final treatment cell containing the highly tolerant saltwater cordgrass (*Spartina alterniflora*). It leaves the unit with 6% salt content and a total volume reduction of 75% carried out in only 7.6 days.

A final assessment of rhizofiltration

Rhizofiltration, like the more general field of phytoremediation, is a new and burgeoning concept that is exciting worldwide interest because it is not only "green", but it appears to be far cheaper than many other alternatives, and in some cases there can even be a financial return if the final product is saleable. The initial euphoria of describing a new technique must be tempered by the realisation of the disadvantages as well as advantages of the new technology. These have been outlined by Black (1995) for phytoremediation in general rather than specifically for rhizofiltration. Nevertheless, the general picture is the same for both.

Advantages

1 - B.Ensley of Phytotech Inc. has estimated that the cost of remediating a cubic metre of contaminated soil would be about $US80 compared with $250 for conventional methods. No figure is given for contaminated waters but we could assume that rhizofiltration would cost about 25% of that of other methods as was the case for soils;

2 - the basic simplicity and cheapness of phytoremediation might well speed up the current rate of hazardous waste remediation;

3 - the volume of biomass that has to be removed and stored or sold is several orders of magnitude less than the original volume of water;

4 - phytoremediation by rhizofiltration, particularly of water contaminated by toxic metals such as lead will do much to improve public health;

5 - rhizofiltration is a much more rapid method of bioremediation than techniques that involve growing and harvesting crops grown over contaminated soils where several years might be required.

Disadvantages

1 - Rhizofiltration is temperature-dependent. The plants will probably grow the whole year in tropical and warm-temperate regions, but in cool-temperate climates may well grow only half the year unless heating is installed;

2 - The right plants have to be discovered that will be suitable for the climate and tolerant to the heavy metals that are to removed;

3 - The method is still in its infancy and will need to be proven by extensive testing under field conditions rather than in the laboratory.

The future

It would seem that the future is bright for rhizofiltration though care must be taken not to overstate its potential at this early stage in case it suffers the fate of other new techniques that have initially been greeted with euphoria and then discarded after disillusionment. A new idea that has recently surfaced (Negri and Hinchman, 1996b) is the use of chelating agents to increase uptake of heavy metals by plants grown as crops over polluted soils. There is no reason why the same procedure might not be applied to rhizofiltration system with perhaps a drip-feed of chelators to the filtration unit.

We should look forward to other new ideas in the next few years that may serve to give rhizofiltration a due place in the armoury of those engaged in phytoremediation of the environment.

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