

REVIEWS

**CADMIUM PHYTOEXTRACTION: PRESENT STATE,
BIOLOGICAL BACKGROUNDS AND RESEARCH NEEDS**

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Summary. Cadmium is a toxic metal for living organisms and an environmental contaminant. Agricultural soils in many parts of the world are slightly to moderately contaminated by Cd due to long term use of phosphatic fertilisers, sewage sludge application as well as smelter dust spreading. Cd phytoextraction is a promising and environmentally friendly approach for soil decontamination. Plant potential for Cd extraction generally depends on shoot Cd concentration and shoot biomass yield. Some ecotypes of the metal hyperaccumulator species *Thlaspi caerulescens* J. & C. Presl possess an extraordinary Cd accumulation and tolerance, but have low biomass yield. Some fast growing trees (*Salix*, *Populus*) and high biomass producing crops (*Brassica*, *Helianthus*, *Nicotiana*) are also considered as suitable species for phytoextraction as they might compensate lower Cd accumulation with much higher biomass yields. Moreover, they deliver “production” which eventually can be utilised, making the phytoextraction process more feasible from economical point of view. At present, plant potential for Cd phytoextraction is limited by: (1) low biomass yield and some undesirable characteristics of *Thlaspi caerulescens*, (2) low shoot Cd concentration and ability to withstand Cd of biomass plants, (3) economical factor. There are two general approaches for further development of Cd phytoextraction: genetic transformations to improve plant factor and agronomy-related optimisations in many different as-

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pects. To overcome the main biological bottlenecks limiting successful phytoextraction, a good knowledge about Cd uptake, plant defence network against Cd as well as some possibilities to reduce chronic Cd toxicity is fundamental. This paper provides information about the plant potential for Cd phytoextraction, the biological backgrounds of this approach as well as some recent achievements and prospects.

Key words: Cd, phytoextraction, *Thlaspi caerulescens*, biomass plants, *Salix*, Cd phytotoxicity, photosynthetic performance

Abbreviations: APOD – ascorbate peroxidase; AsA – ascorbate; γ -ECS – γ -glutamylcysteine synthetase; CAT – catalase; GS – glutathione synthetase; DHAR – dehydroascorbate reductase; DW – dry weight; EDTA – ethylenediaminetetraacetic acid; GPOD – guaiacol peroxidase; GR – glutathione reductase; GSH – glutathione; K_m – ion affinity; MDHAR – mono-dehydroascorbate reductase; MTs – metallothioneins; NTA – nitrilotriacetate; PCs – phytochelatin; PODs – peroxidases; ROS – reactive oxygen species; SOD – superoxide dismutase; V_{max} – transport capacity

Introduction

The background cadmium (Cd) level in agricultural soils is less than 1 mg.kg^{-1} (Adriano, 2001). However, higher values were observed in many agricultural soils due to long term use of phosphatic fertilisers and sewage sludge application (Chaney, 1980). Increased Cd levels were also found in the surface soils near the metal processing industry all over Europe. High mobility of this metal in soil-plant system allows its easy entering into food network (Ryan et al., 1982), where it may provoke both human diseases (Nogawa et al., 1987) and well known toxicity effects on animals, microorganisms and plants.

Increasing international concern about the risks associated with long-term consumption of crops contaminated with Cd has led the international food standards organisation, Codex Alimentarius Commission, to propose a $0.1 \text{ mg Cd.kg}^{-1}$ dry weight limit for cereals, pulses and legumes (Harris and Taylor, 2001). As this criterion may put under pressure the market of some agricultural products, several strategies have been proposed for the successful management of the Cd-contaminated agricultural soils.

One approach is aiming to screen and use low Cd-accumulating genotypes of crops, known to accumulate unacceptable high Cd levels in grain (Archambault et al., 2001). The second approach recommends profitable use of both non-food crops and cereals for seed production (Zheljazkov and Nielsen, 1996; Vassilev et al., 1996). The third option is metal phytoextraction, based on the natural or “induced” ability of plants

to uptake metals (and organics) from soil and to concentrate them in the harvestable parts (Salt et al., 1998; Robinson et al., 1998).

There is some evidence that metal phytoextraction is a promising approach applicable to slightly or moderately contaminated soils as an alternative to the *ex situ* decontamination techniques, which are very expensive and unacceptable from ecological point of view (McGrath et al., 2001). Two groups of plant species are considered for metal phytoextraction purpose: hyperaccumulators species, able to accumulate and tolerate extraordinary metal levels, and high biomass producing species (referred in the text as biomass plants) compensating lower metal accumulation by high biomass yields. An opinion exists that phytoextraction will be more economically feasible if, in addition to metal removal, plants produce biomass with an added economical value. For example, the biomass of fibres, oil or fragrance producing crops could be used to recover these valuable products (Schwitzguébel et al., 2002).

Agricultural soils may be contaminated with just Cd (by phosphatic fertilisers) or a mixture of heavy metals (by sewage sludge and dust spreading), where in the most cases Cd is the first metal of concern. On the other hand, there is some evidence that Cd phytoextraction from agricultural soils could be easily implemented due to both lower Cd contamination and higher Cd mobility in the soil-plant system as compared, for example, to lead (Pb) or zinc (Zn) (Robinson et al., 1998; 2000).

This paper summarises available information concerning the plant potential for Cd removal, identifies the main biological bottlenecks and proposes the approaches for improvement of Cd phytoextraction. As fundamental knowledge is a basis for technological development, we also provide here some generalised information about the biological backgrounds and open questions regarding Cd uptake and the functioning plant defence network. Some promising results are also presented and the prospects are discussed.

Plant potential for Cd extraction

Metal phytoextraction

Metal phytoextraction is a branch of the concept of phytoremediation (Chaney, 1983) based on plant's ability to work as a solar-driven pump, extracting and concentrating particular elements from the environment. It may be applied to metals such as Zn, Cd, Ni, Cu and Pb. As a technology, metal phytoextraction is still in its infancy stage, being commercially available technology only for Pb removal. Generally, the metal phytoextraction protocol consists of the following elements (1) plant cultivation on contaminated site, (2) removal of harvested metal-rich biomass, (3) post harvest treatments and subsequent disposal of the biomass as a hazardous waste, and (4) eventual recuperation of metals from the metal-enriched biomass (Blaylock and Huang, 2000).

Metal phytoextraction has received increasing attention in recent years, being in development to meet the growing market for phytoremediation products (Glass, 2000).

The permissible Cd value in agricultural soils in Bulgaria is 2 mg.kg^{-1} soil (Instruction RD-00-11; see references). If the soil is assumed to have bulk density of 1.3 and to be penetrated by roots to a depth of 15 cm, total Cd content will be about 4 kg.ha^{-1} at contamination level of 2 mg.kg^{-1} soil and about 10 and 20 kg.ha^{-1} at 5 and 10 mg.kg^{-1} soil, respectively. Thus, to decrease soil Cd level from 5 or 10 mg.kg^{-1} (slight and moderate contamination) to 2 mg.kg^{-1} (permissible limit), it will be necessary to extract from 6 kg up to 16 kg Cd.ha^{-1} .

Use of natural hyperaccumulators in Cd phytoextraction

Hyperaccumulation of Cd is a rare phenomenon in higher plants. So far, only *Thlaspi caerulescens* J. and C. Presl (*Brassicaceae*), known also as pannycress, has been identified as Cd hyperaccumulator able to meet Cd hyperaccumulation criterion of 100 mg.kg^{-1} shoot dry weight (Baker et al., 2000). For comparison, more than 300 taxa are hyperaccumulating Ni and about 20 – Zn, Cu and Co. Another possible Cd hyperaccumulator could be *Arabidopsis halleri* (L) O’Kane and Al-Shenbaz, which was found to hyperaccumulate Cd in hydroponics (Küpper et al., 2000), but according to the strong hyperaccumulation definition, this ability has to be proved in its natural habitat, not at artificial conditions (Reeves and Baker, 2000). Hyperaccumulator plants are usually found on metalliferous soils, where the natural exposure to a surplus of various metals has driven the evolution of metal hyperaccumulation as well as plant resistance to heavy metals (Ernst, 1998).

The ability of *T. caerulescens* to hyperaccumulate Cd (and Zn) is known for a long time (Ernst, 1968, and references therein). Robinson et al. (1998) have found Cd accumulation in the leaves of *T. caerulescens* up to $1600 \text{ mg Cd.kg}^{-1}$ DW without detectable decrease of its dry biomass up to $50 \text{ mg extractable Cd kg}^{-1}$ soil. Recently, Lombi et al. (2000) found that in the hydroponics experiment one French population of *T. caerulescens* (Ganges ecotype) was able to accumulate Cd in the shoots over 10000 mg.kg^{-1} without biomass reduction. Moreover, in the field trial, this population was able to accumulate up to $500 \text{ mg Cd.kg}^{-1}$ in the shoot at 12 mg Cd.kg^{-1} soil, which is encouraging for the Cd phytoextraction from agricultural soils.

Very different values have been reported about dry mass potential of *T. caerulescens*. Robinson et al. (1998) estimated that dry biomass production of this species averaging 2.6 t.ha^{-1} , whereas in field trials Kayser et al. (2000) found less than 1 t.ha^{-1} . On the other hand, Bennett et al. (1998) showed that the yield of fertilised crop of *T. caerulescens* could be easily increased by a factor of three without significant reduction in Cd tissue concentration. Robinson et al. (1998) accepting dry mass potential of *T. caerulescens* of about 5 t.ha^{-1} calculated that soil contaminated by 10 mg Cd.kg^{-1} would be cleaned in only 2 years. Saxena et al. (1999) have recently reported that the

potential of *T. caerulescens* for Cd extraction, observed in field trial was $2 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ under optimum growth conditions. Controversially, Ernst (1998) pointed that this species is not suitable for phytoextraction due to its rosette characteristics, making difficult the mechanical harvesting. Furthermore, it was mentioned that *T. caerulescens* has low resistance to hot and dry environments (Kayser et al., 2000).

Use of biomass plants in Cd phytoextraction

A/ Woody biomass plants

The ideal plant for metal phytoextraction has to be highly productive in biomass and to assimilate and translocate to shoots a significant part of metals of concern. Additional favourable traits are fast growth, easy propagation and a deep root system. Some tree species, mainly willows (*Salix*) and poplars (*Populus*) exhibit these traits and are in use in phytoremediation programs. Short-rotation coppice of willow has shown particular promise as a renewable energy crop having ability to accumulate higher levels of some metals, for example Zn and Cd (Riddell-Black et al., 1997; Greger and Landberg, 1999; Rulford et al., 2002).

In fact, *Salix* species are not metal hyperaccumulators, but it was shown that some clones are able to accumulate Cd up to $70 \text{ mg} \cdot \text{kg}^{-1}$ DW in leaves (Landberg and Greger, 1994; Landberg and Greger, 1996). Due to big variation in shoot Cd concentrations ($5\text{--}70 \text{ mg} \cdot \text{kg}^{-1}$) found in different *Salix* species and clones, very different values concerning Cd removal have been calculated in the literature: $222 \text{ g Cd} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ (Felix, 1997), $61.7 \text{ g Cd} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ (Rulford et al., 2002) and about $1060 \text{ g Cd} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ (Robinson et al., 2000).

The resistance of willow plants to excess Cd is very important characteristic, as they have to be able to grow continuously on Cd-contaminated land and to deliver an additional economical value (dry matter for energy, chip wood and paper production), but this aspect is not very well addressed. We have recently initiated study on the ability of *Salix viminalis* clones to resist increasing Cd concentrations. Data presented in Fig. 1 showed dry mass accumulation in the hydroponically grown cuttings of the two selected clones was retarded by excess Cd. After 14 days exposure at external concentration of $15 \mu\text{M}$ Cd the observed inhibition was 30 and 50% in the first and the second clone, respectively. Furthermore, the photosynthetic performance of these plants was seriously disturbed. For example, both net photosynthetic rate and chlorophyll content at $15 \mu\text{M}$ Cd treatment were diminished by 28–36% and 29–49%, respectively (data not shown). Further studies are in progress to characterise profoundly the ability of these clones to withstand excess of Cd.

B/ Non-woody biomass plants

The use of non-woody biomass plants has been later introduced in the phytoextraction concept in order to overcome the some limitations, such as low dry biomass of hyper-

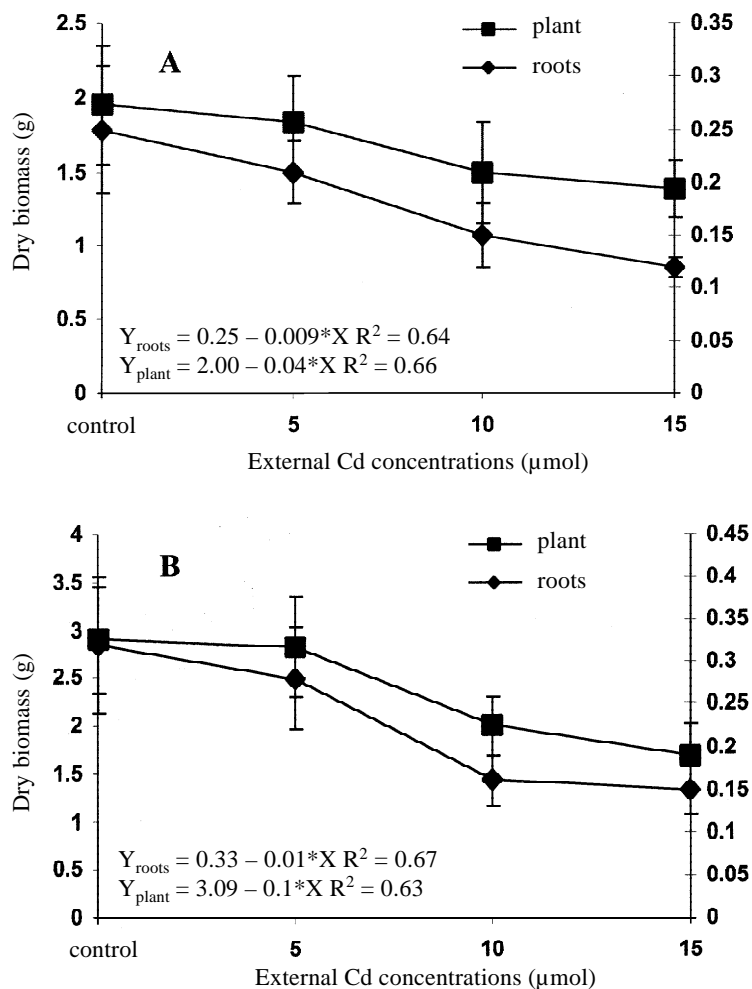


Fig. 1. Dry biomass of plants from two *Salix viminalis* clones at excess Cd. (A – Ist clone, B – IInd clone). On the right axis are presented the roots values. (Vassilev and Vangronsveld, *unpublished data*)

accumulators as well as low phytoavailability of some metals. For the later limitation the chemically assisted approach has been developed (Salt et al., 1998). It has been mostly adjusted to Pb phytoextraction (Blaylock et al., 1997), but later extended to the other target metals, too.

Firstly, the chemically-assisted metal phytoextraction was based only on EDTA (ethylenediamine tetraacetic acid) application, but it has been criticised for some toxic properties of the chemical as well as for the possible leaching of metals to groundwater (Crèman et al., 2001; Gebelen et al., 2002). Now, a significant research effort is forwarded to find efficient and environmentally acceptable amendments (Schmidt, 2002). In fact, the EDTA application dramatically enhanced Pb phytoaccumulation to more

than 1% Pb per dry mass of shoots of Indian mustard, but different opinions exist regarding its effects on Cd solubility in the soil and plant uptake (Blaylock et al., 1997; McGrath et al., 1999). The EDTA applied at rate of 2 g.kg⁻¹ soil caused a temporary increase in uptake in Cd by poplar, but also resulted in significant reduction in growth, as well as abscission of leaves (Robinson et al., 2000). As for *Salix* sp. the crops envisaged for Cd removal should give an added value, thus their ability to withstand excess Cd is very important trait. This is a remarkable difference between Cd and Pb phytoextraction. The latter is based on short time consecutive croppings of Indian mustard (*Brassica juncea*), where EDTA is applied shortly before harvest, so, the resistance of the crop to Pb is of a low importance.

The primarily interest concerning biomass crops now is focused on oilseed rape (*Brassica napus*), tobacco (*Nicotiana tabacum*), flax (*Linum usitatissimum*), peppermint (*Mentha piperita*), cotton (*Gossypium hirsutum*), but also triticale and maize (*Zea mays*), sunflower (*Helianthus annuus*), cereals, Indian mustard (*Brassica juncea*), etc. (Zheljazkov and Nielsen, 1996; Vassilev and Zaprianova, 1999; Yankov et al., 2000; Schmidt, 2002; Stoinova et al., 1998, 1999; Griga et al., 2002). Some information about the Cd phytoextraction potential of several plant species is presented in Table 1, but in general the availability of this kind of data is still limited. Furthermore, most data are from pot-soil experiments, which are known to show increased metal transfer from soil to plants.

The main factors limiting Cd phytoextraction

The acceptable duration of phytoextraction process is considered to be not more than 5–10 years (Robinson et al., 1998; Blaylock and Huang, 2000). The results presented in Table 1 showed that Cd removal by biomass crops is not high enough to meet this requirement. On the other hand, the use of *T. caerulescens* is problematic in term of its slow growth and low yield, the rosette characteristics as well as its sensitivity to heat and drought. That is why Barcelo et al. (2001) has recommended the search for new metal hyperaccumulators with better survival strategies at different climatic conditions. Additionally, the lack of any cultivation protocol limit the use of *T. caerulescens* for practical purposes. At present, it is a preferable model plant for studying the molecular biology of Zn and Cd hyperaccumulation and tolerance (Lasat et al., 1998; Saier, 2000; Zhao et al., 2002).

Efficiency of Cd phytoextraction by the biomass crops is limited due to the low Cd concentration in the harvestable parts. If higher shoot Cd concentrations would be achieved by means of induced phytoextraction, Cd phytoextraction potential might be limited by phytotoxicity problems. At present, for many crops the value for Cd toxicity is not well established. For example, for maize the values varied from 25 to 150 mg Cd.kg⁻¹ DW shoots (Macnicol and Beckett, 1985; Lagriffoul et al., 1998). Furthermore, there is a lack of data from experiments where more reliable Cd treat-

ments, able to produce chronic, but not acute Cd phytotoxicity, have been used. Thus, the data shown in Table 2 should be considered as a first approximation only. More detailed information about the biomass crops ability to withstand Cd as well as to accumulate it in the shoots is needed.

The model of phytoextraction crop is also not well consolidated. Several authors have considered hyperaccumulation as more important trait than biomass yield (Chaney et al., 1997), while others do not accept this opinion (Ebbs et al., 1997; Kayser et al., 2000; Ernst, 1998). For example, Ebbs et al. (1997) found in a pot-soil study that *T. caerulescens* has 10-fold higher Cd concentrations, but also 10-fold less biomass production as compared to the crops used, thus both options showed the same result.

It is generally believed that the metal phytoextraction potential might be improved by genetic transformations (if they could achieve public acceptance) as well as appropriate agronomy-related optimisation (Salt et al., 1998; Lasat, 2000; Kärenlampi et al., 2000). For the development of these directions a good knowledge about plant Cd uptake, plant defence network functioning as well as some possibilities for chronic Cd toxicity amelioration is fundamental.

Cd uptake and plant defence network: backgrounds and opened questions

Cd uptake in plants

Cd uptake in plants has been studied for a long time (Cutler and Rains, 1974; Zhao et al., 2002). Many aspects are well known and a number of similarities with other metals have been identified (Siedleska, 1995). Here, we discuss only some aspects having importance for Cd phytoextraction.

In soils, Cd is present mainly as a free ion and soluble complexes or adsorbed at ion exchange sites of inorganic soil constituents (Tessier et al., 1979). It was found

Table 1. Established Cd phytoextraction potential of several plant species

Plant species	Leaf Cd concentration (mg kg ⁻¹)	Reference	Possible DM yield (t.ha ⁻¹)	Possible Cd removal (g ha ⁻¹ .yr ⁻¹)
<i>T. caerulescens</i>	1600	Robinson et al., 1998	2.6–5.2	4160–8320
<i>Salix viminalis</i>	3–40	Riddell-Black et al., 1997	10–16	80–220
<i>Sails viminalis</i>	10–20	Rulford et al., 2002	8	80–160
<i>S. viminalis</i> + EDTA	53	Robinson et al., 2000	20	1060
<i>Nicotiana tabacum</i>	9–40(120)	Kayser et al., 1999a	9–13	90–115
<i>Nicotiana tabacum</i>	50	Gupta et al., 2001	10	500
<i>Brassica juncea</i>	73	Blaylock et al., 1997	4–24	60–80
<i>Zea mays</i>	1–9	Kayser et al., 2000	10	11–74
<i>Hordeum vulgare</i>	3–15	Vassilev, Zapryanova, 1999	5	15–75

that about 50% of Cd accumulation in *T. caerulescens* was taken up from the soil solution (Knight et al., 1997). Cd uptake through roots system depends on many soil factors e.g. pH, redox potential, cation exchange capacity, organic matter content, presence of other metals, etc. (Greger, 1999). Some of these factors, for example pH, can be manipulated to increase Cd phytoavailability in the soil (Schmidt, 2002).

The movement of Cd (as other metals) from external solution to the cell walls is a non-metabolic, passive process, driven by diffusion or mass flow (Marschner, 1995). Using data of Knight et al. (1997) and assuming transpiration efficiency of *T. caerulescens* of 4001 H₂O.kg⁻¹ shoot dry weight McGrath et al. (2001) calculated that mass flow contributed only 0.3–6.5% of the total Zn uptake and suggested that Zn diffusion in the rhizosphere as a limiting process. Soil Cd content generally is much lower than Zn (Zn/Cd ratio in the industrially contaminated soils is about 100/1 – Chaney et al., 1997), so, Cd diffusion in the soil may be limiting, too. On the other hand, it has been shown that the roots of *T. caerulescens* are able to sense and actively forage the Cd-rich patches in the soil (Whiting et al., 2000), but no information is available for biomass crops.

At the cell wall, a part of Cd is bound to the carboxy-groups of mucilage uronic acids. This fraction cannot be transported to the shoots, therefore, cannot be removed by shoot biomass harvesting. Thus, it is possible for a plant exhibiting significant metal accumulation into roots to express a limited capacity for phytoextraction (Lasat, 2000). Because of their charge, metal ions cannot move freely across the cellular membranes, their transport is mediated by membrane proteins known as transporters. Two kinetic parameters are used to characterise their ability to transport ion(s): transport capacity (V_{\max}) and ion affinity (K_m). The former measures the maximum rate of ion transport across the membrane and the latter determines the ion affinity, estimating the external ion concentration at which the transport rate is equal to $V_{\max}/2$.

Cd influx across plasma membrane in roots occurs via concentration-dependant process exhibiting saturable kinetics (Cataldo et al., 1983; Costa and Morel, 1993). According to Greger (1999) Cd is taken up metabolically in low, and non-metabolically in high supply. Some external factors such as light intensity, temperature, etc. may influence mainly the metabolic way.

Cd moves across the membrane through divalent cation channels as an opportunistic transport by a transporter for another divalent cations, such as Zn²⁺, Cu²⁺, Fe²⁺, Ni²⁺ (Welch and Norvell, 1999). There is some evidence that Cd can enter the cytosol also by voltage-gated Ca channels (Blaylock and Huang, 2000). Because of their similar properties, it was suggested that Cd and Zn may be taken up and translocated within the plant via similar pathways (Grant et al., 1998). The ability of *T. caerulescens* to accumulate Cd has so far often been considered as a “side activity” of a hyperaccumulation mechanism for the chemically related metal Zn (Krämer, 2000). However, Lombi et al. (2000, 2001) have identified Ganges and Prayon ecotypes of *T. caerulescens* differed greatly in Cd accumulation and kinetics of Cd uptake, but not in both

Zn accumulation and uptake. Recently, Zhao et al. (2002) have found that both V_{\max} and K_m characterising Cd transport in Ganges ecotype of *T. caerulescens* were 5 to 10 fold higher than the reported values for the non-accumulating plants. As Cd uptake in Ganges ecotype of *T. caerulescens* was not suppressed by the presence of other divalent cations, La^{3+} and verapamil – Ca channel inhibitors, the authors suggested that there may exist a highly selective Cd transport system in the root cell membranes of this ecotype.

The molecular biology of Cd transport remains to be investigated, but some research efforts have recently addressed this aspect. The members of the so-called ZIP family of membrane transporters have been shown to be capable of transporting several metals including Cd in *Arabidopsis thaliana* (Korshunova et al., 1999). It has been also found that plants from Prayon ecotype of *T. caerulescens* and related non-accumulator *T. arvensis* differed in transcriptional regulation of *ZNT1*, a member of the ZIP family (Saier, 2000). The *CAX2* transporter, related to Ca^{2+}/H^+ antiporter *CAX1* in *Arabidopsis thaliana*, has been shown to possess transport activity for Cd, probably due to chemical similarities between Ca and Cd (Hirschi et al., 2000).

Movement of Cd from roots to shoots is a critical point for Cd phytoextraction. Instead of the apoplast-bound fraction of the metal, another part, which is stored by compartmentation in the vacuole of the root cells (see below) is not available for translocation and thereafter for phytoextraction. To be able to reach xylem vessels, metal uptake is taking place at the younger parts of the root, where Casparian strips are not well developed (Hardiman et al., 1984). The absorbed metals are unloaded from the xylem parenchyma into mature xylem vessels, but this process is not well understood (Salt and Krämer, 2000). There is some evidence for elevated loading rates for some metals in hyperaccumulator plants: Zn in *T. caerulescens* (Lasat et al., 1998) and Ni in *Alyssum lesbiacum* (Salt and Krämer, 2000), but no information is available for Cd.

After metal's loading to the xylem, its translocation to the shoots is driven by transpiration and root pressure. Using data of Lombi et al. (2000) and assuming transpiration efficiency of *T. caerulescens* of $4001 H_2O.kg^{-1}$ shoot dry weight (McGrath et al., 2001) we calculated that each 150 parts water transported 1 part Cd in the shoot of the high Cd-accumulating Ganges ecotype of this species. Salt et al. (1995) showed that ABA-induced stomatal closure dramatically reduced Cd accumulation in shoots of Indian mustard. Within the xylem sap Cd is transported as the chelate form with organic acids and amino acids (Cataldo et al., 1988; Senden et al., 1995) or as free ion (Leita et al., 1996).

Cd movement through the phloem has been intensively studied in order to explain its accumulation in the seeds (Hart et al., 1998; Cakmak et al., 2000; Harris and Taylor, 2001). For the phytoextraction purpose this aspect has no significant importance, as the share of seeds in Cd removal would be negligible.

Generally, the gradient of Cd concentrations in the plant declines in the order root > leaves > seeds (Hart et al., 1998), but there are some differences among the

species and genotypes regarding Cd allocation in the shoot. For example, Cd transport to the shoots represents 2% in soybean (Cataldo et al., 1981), 10–20% in sugarbeet (Greger and Lindberg, 1986), 17–20% in oilseed rape (Larsson et al., 1998), 50% in rice (Chien et al., 2001), and 1 to 72% in different *Salix viminalis* clones (Greger and Landberg, 1999). Within the canopy the maximum Cd content was found in senescing leaves (Vassilev et al., 1998), but in *Brassica juncea* Cd was preferably accumulated in the youngest leaves (Salt et al., 1995). Hyperaccumulators tend to accumulate highest metal concentrations in epidermal tissue; high Cd content was detected in leaf trichomes of Indian mustard (Salt et al., 1995; Heath et al., 1997). One probable explanation of this fact is a role in defence against herbivorous insects, bacterial or fungal diseases (Boyd and Martens, 1994).

Plant defence network against Cd

Generally, plant defence against Cd includes two strategies: (1) Cd detoxification and (2) coping with Cd-induced oxidative stress. Several mechanisms for Cd detoxification in plants exist, the best known are complexation with phytochelatins (PCs) and metallothioneins (MTs). PCs constitute a family of peptides with the general structure $(\gamma\text{-Clu-Cys})_n\text{-Gly}$, where $n=2$ to 11 (Rauser, 1990). PCs were induced by Cd in all the plants tested; glutathione (GSH) is their precursor (Zenk, 1996). General mode of action of PCs is the following: PCs bind Cd and after that PCs-Cd complex enters the vacuole; Cd is released from the PCs, which is returned to the cytoplasm and the metal may become bound to an organic acid in the vacuole (Rauser, 1995). In addition to PCs, MTs – cysteine-rich peptides with low molecular weight identified in numerous animals and more recently also in bacteria and plants – take part in metal detoxification (Hamer, 1986). Plant MTs-like genes have been recently isolated from several crop species including maize, soybean, rice, wheat, tobacco and oilseed rape (Nedkovska and Atanassov, 1998). Briefly, Cd detoxification is achieved by Cd binding to the cell wall, complexation with PCs followed by compartmentalisation in the vacuole and complexation with MTs and GSH in the cytosol (Vögeli-Lange and Wagner, 1996, and references therein). Despite of the achievements made concerning the elucidation of Cd tolerance, some aspects are still under discussion. For example, Ebbs et al. (2002) have recently reported that PCs synthesis is not responsible for Cd tolerance in the Prayon ecotype of *T. caerulescens* and suggested a role for organic acids in Cd detoxification.

Cd has low redox potential and therefore it can not participate in biological redox reactions as, for example Cu does (Clijsters et al., 1999). Interestingly, evidence has been reported suggesting that Cd stress is performed as oxidative stress (Hendry et al., 1992; Gallego et al., 1996; Lagriffoul et al., 1998; Di Cagno et al., 2001; Pereira et al., 2002; Fediuc and Erdei, 2002). It was proved by an over-production of reactive oxygen species (ROS), such as $^1\text{O}_2$, $^{\bullet}\text{OH}$, H_2O_2 and $\text{O}_2^{\bullet-}$. Since Cd (and other metals

as Zn, Ni) do not interfere directly with cellular oxygen metabolism, the question arises to the reasons of the observed oxidative stress.

ROS are produced naturally during cell metabolism in processes such as photosynthesis, photorespiration, fatty acid oxidation and senescence. Plants, like all aerobic organisms, possess the means to protect themselves from the toxic effect of ROS like antioxidant molecules and enzymes. The antioxidative system in plants comprises several enzymes. Superoxide dismutases (SOD), catalases (CAT) and peroxidases (PODs) are involved in detoxification of O_2^- , and H_2O_2 , respectively, thereby preventing the formation of $\cdot OH$ radicals. Accumulation of H_2O_2 is also prevented by the functioning of the ascorbate-glutathione pathway (Noctor and Foyer, 1998). It includes the metabolites GSH and ascorbate (AsA) and the enzymes ascorbate peroxidase (APOD), dehydroascorbate reductase (DHAP), glutathione reductase (GR) and monodehydroascorbate reductase (MDHAR).

The activities of the antioxidative enzymes have been found to increase or decrease in response to excess Cd (Lagriffoul et al., 1998; Dixit et al., 2001; Schützendübel et al., 2001; Sandalio et al., 2001; Pereira *et al.*, 2002; Fediuc and Erdei, 2002). The induction of an “unspecific” guaiacol peroxidase (GPOD) in root tips of Cd-treated pine plants was explained by their higher phenolics content due to enhanced lignification processes (Schützendübel et al., 2001). The physiological meaning of the induction of the other enzymes in response to Cd (and other heavy metals) is not well understood (Clijsters et al., 1999). On the other hand, the inhibition of the antioxidative enzymes activities could be easily explained by the known effects of Cd (and other metals) on the enzymes e.g. binding to the functional groups, induction of metal deficiency in metalloenzymes and/or substitution of the toxic metal for an essential metal in the enzyme complex (Vangronsveld and Clijsters, 1994).

The current knowledge reveals the important role of AsA and GSH in the defence of plants against Cd as well as other metals and xenobiotics. Instead of its role in scavenging H_2O_2 through the activity of APOD, AsA has the capacity to eliminate several ROS and to maintain the membrane-bound antioxidant α -tocopherol in the reduced state (Finckh and Kunert, 1985). GSH has been shown to have several roles in plant defence against Cd: being precursor of PCs, taking part in the glutathione-ascorbate pathway as well as being a component of the redox balance of the cell. The important roles of GSH has been illustrated through the observation that Cd-sensitive mutant of *Arabidopsis thaliana* was defective in GSH biosynthesis (Howden et al., 1995).

Although the indicators of oxidative damages in Cd-exposed plants have been already well detected, the mode of its action is still under discussion. Chien et al. (2001) have recently suggested that Cd toxicity in detached rice leaves may require the participation of transition metal, probably iron. An analogy between Cd-induced over-oxidative production and the “oxidative burst” in pathogenesis has been also suggested (Piqueras et al., 1999). Using metabolic modelling as a means to predict changes in oxidant levels from measured Cd-induced changes in “antioxidative capacities” the following scenario has been recently proposed by Schützendübel and Polle (2002):

Cd exposure resulted in a severe transition depletion of GSH due to its increased consumption for PCs production; this in turn leads to insufficient redox balance of the cell and thereafter rise of oxy radicals production. On the other hand, due to Cd-induced loss in the activities of defence enzymes, they are not able to control the “over-oxidative production” and thereafter it produces numerous toxic effects on cell and whole plant physiology. Several examples showing Cd-induced overproduction of ROS, lipid peroxidation and modulation of antioxidative enzymes in plants are presented in Table 2.

Cd phytotoxicity and some possibilities to reduce it

When the plant defence network is not able to maintain the concentration of free Cd ions in the cytosol below the toxic threshold level, Cd-induced over-production of ROS results in numerous toxic effects. Among them the most known are lipid peroxidation (Sandalió et al., 2001), altered enzymes structure and activities (Van Assche and Clijsters, 1990), disruption in membrane integrity (Vangronsveld and Clijsters, 1994), etc. Further these primary cell toxic effects are multiplied on the cardinal physiological processes in plants, such as photosynthesis, water relations, mineral nutrition, etc. and in turn lead to integral metal phytotoxicity (for review see: Clijsters and Van Assche, 1985; Baszynski, 1986; Barcelo and Poschenrieder, 1990; Breckle, 1991; Siedleska, 1995). At present, the photosynthetic performance is recognised as one of the most important factor limiting growth of Cd-exposed plants (Stoynova and Merakchiiska-Nikolova, 1992; Vassilev and Yordanov, 1997). Thus, the partial amelioration of toxic Cd effects on photosynthesis may improve whole plant performance at excess Cd as well. Some data on that aspect have been recently obtained.

Chen and Huerta (1997) showed that sulphur (S) is a critical nutritional factor for reduction of Cd toxicity. These authors observed that the negative effects of Cd on growth and photosynthesis are stronger at barley plants supplied with 0.1 mM S than in plants receiving 1 mM S. The positive effect of higher S nutrition on barley resistance to Cd was expressed by weaker depressions on plant morphology, leaf gas exchange and chlorophyll fluorescence parameters. A positive role of S nutrition on Cd detoxification in sugar beet plants have been also established (Popovic et al., 1996). It has been found that at sub-optimal S nutrition Cd-exposed plants preferably allocate S to PCs synthesis, which may provoke S deficiency (McMahon and Anderson, 1998). Probably, the improved S nutrition allows an adequate plant defence response to Cd, but also prevention of S deficiency onset. Recently El-Shintinawy (1999) has given an information showing that exogenous GSH counteracted all retardation effects in soybean seedling induced by Cd. He provided data allowing to conclude the dual function of GSH: first, as being an antioxidant and oxy radicals scavenger, it protects chloroplasts from oxidative damage by trapping the hydroxyl radicals, and secondly, as a substrate for PCs synthesis that mainly sequesters and detoxifies excess Cd ions. When

Table 2. Examples of Cd-induced changes in plant oxidative metabolism

Cd concentration	Exposure time (days-d; hours-h)	Species/organ	Parameters	Changes (% from control)	Reference
50 μ M	28 d (plants)	<i>Pisum sativum</i> leaves	TBARS	150	Sandalo et al., 2001
			Carbonyl-groups content	260	
			CAT	51	
			GPOD	64	
			GR	NSD	
3 mM	48 h (leaf segments)	<i>Oryza sativa</i> (leaf segments)	TBARS	230	Chien et al., 2001
			GPOD	218	
			SOD	33	
			APOD	57	
			GR	44	
			CAT	40	
40 μ M	1-7 d (plants)	<i>Pisum sativum</i> leaves	TBARS	NSD	Dixit et al., 2001*
			GPOD	102-126	
			SOD	113-172	
			APOD	128-172	
			GR	110-150	
			CAT	105-208	

NSD – no significant difference; TBARS – thiobarbituric acid reacting substances

* Data showed the minimal and maximal increase of the parameters over the period of 7 days

both functions are realised, repairing membrane damage, and sequestration of Cd are achieved by GSH, the electron transport rate as well photosynthetic characteristics are not perturbed and this results in a photosynthetic performance similar to that in the control plants.

Panković et al. (2000) have shown that optimal nitrogen (N) supply decreased the inhibitory effects of Cd on photosynthesis of sunflower plants. The authors studied the effects of Cd on sunflower plants grown at optimal, sub-optimal and supra-optimal N supply. They found the smallest inhibition of photosynthetic activity by Cd at optimum N supply, when N investment in soluble proteins and Rubisco were in their maximum. Higher N supplies did not alleviate the toxic Cd effect, therefore the authors concluded that N nutrition can be manipulated as a means of decreasing Cd phytotoxicity. Thus, the optimal ratio of N over Cd must be determined for actual plant species and growth conditions.

Instead of mineral nutrition, there is some evidence that plant ability to withstand Cd may be improved by plant growth regulating substances. Recently, Ghorbanli et al. (1999) have found that exogenous gibberelin positively affected growth rate and reduced the toxic effects of Cd on soybean plants. They suggested that this effect might result from cooperative effects on leaf area development, photosynthetic rate, modification in partitioning of photosynthates, etc. It was also shown that the pre-treatment of Cd-exposed leaf discs with exogenously added polyamine spermin reverted the lipid peroxidation almost to the control values (Groppa et al., 2001). Other promising results are achieved by treating Cd-suffering plants with a natural product, named biomin (Kamenova-Jouhimenko et al., 1997/1998). The addition of biomin improved photosynthetic performance of Cd-exposed pea plants through a stabilisation of chloroplast ultrastructure as well as the increased share of the C₄ pathway for CO₂ fixation at the expense of C₃ pathway.

Approaches for improvement of Cd phytoextraction potential

Biotechnological approach

It has been proposed that genetic engineering could greatly contribute to the development of metal phytoextraction (Salt et al., 1998; Kärenlampi et al., 2000). Different approaches have been followed in order to improve plant Cd tolerance and uptake. Some promising results tested at a laboratory scale have been already obtained.

Firstly, the attention was focussed on the overexpression of MTs as a means to increase Cd tolerance. The first report about stable integration of human MTs into tobacco and oilseed rape showed that the growth of transformed seedling was unaffected up to 100 µM Cd (Misra and Gedamu, 1989). The expression of mammalian MTs in transformed *Nicotiana tabacum* plants also resulted in improved Cd resistance (Pan et al., 1994). *Brassica oleraceae* expressing yeast MTs gene has better tolerance to Cd than that of the wild-type (Hasegawa et al., 1997).

Secondly, modification or over-expression of the enzymes involved in the synthesis of GSH and PCs has been considered as a good approach to enhance Cd tolerance and accumulation. GSH is synthesised from its constituent aminoacids in two steps by ATP-dependant enzymatic reactions catalysed by γ -glutamylcysteine synthetase (γ -ECS) and glutathione synthetase (GS). Transgenic *Brassica juncea* plants overexpressing bacterial (*Escherichia coli*) GS gene (*gsh2*) were found to have both higher shoot Cd concentration (25% increase) and enhanced Cd tolerance (Zhu et al., 1999a). Following an expression of another gene encoding γ -ECS (*gsh1*) the authors found that *Brassica juncea* transformants have higher Cd tolerance and up to 50–70% higher Cd concentration (Zhu et al., 1999b). Recently Arisi et al. (2000) reported that poplars overexpressing bacterial γ -ECS showed better Cd accumulation, but not improved Cd tolerance. Pilon-Smith et al. (2000) overexpressed *E. coli* gene encoding the enzyme GR in the cytosol (cytGR) and the plastids (cpGR) of *Brassica juncea*, hypothesising that the enhanced GR activity will alleviate Cd-induced oxidative stress. They found Cd tolerance at whole plant level was not affected, but was enhanced at the chloroplast level as judged by chlorophyll *a* fluorescence parameters. This effect was explained by the observations for lower Cd content in the chloroplast and higher GSH in cpGR plants as compared to the untransformed plants.

Another biotechnological approach for improving performance of the metal-extracting plants overexpressing of metal transporters. The first results showed some evidence for increased metal accumulation and plant tolerance: Cd in the roots of tobacco overexpressing *CAX2* under the control of a 35S promoter (Hirschi et al., 2000), Ni in tobacco overexpressing *NtCBP4* (Arazi et al., 1999) and Zn in *A. thaliana* overexpressing *ZAT1* (Van der Zaal et al., 1999).

However, these promising results from the biotechnological research efforts should also be studied and proved on Cd-contaminated soils. On the other hand, the use of genetically modified plants for metal phytoextraction strongly depends on their public acceptance.

Agronomy-based approach

In short term perspective Lasat (2000) suggested agronomy-based optimisations as a tool for further improvement of metal phytoextraction. There are several aspects where such improvements should be made.

Cd solubility and plant uptake

In spite that Cd generally has a higher mobility in the soil-plant system as compared with Pb or Cu, for the phytoextraction purpose it is important to increase its plant-availability. Blaylock et al. (1997) found increased shoot Cd levels up to 480 mg Cd.kg DM⁻¹ in Indian mustard by adding 0.73 g.kg⁻¹ soil EDTA, but McGrath et al. (1999) did not observe any effect on Cd solubility in the soil. Recently, some more environmentally acceptable amendments as NTA (nitrilotriacetate), organic acids, inorganic

agents as elemental sulphur and ammonium sulphate, etc. have been introduced in the chemically-assisted phytoextraction (Schmidt, 2002). Kayser *et al.* (2000) have shown that NTA is able to increase the solubility of Cd by factor of 58, but plant accumulation was increased only by a factor of 2. Nigam *et al.* (2001; citation of Schmidt, 2002) found that citric and malic acids enhanced Cd accumulation in maize shoots up to 19 mg.kg⁻¹. On carbonate rich soils, transition soil acidification by elemental sulphur was found to be useful for metal (including Cd) mobilization, and on the other hand without any negative effects on soil environment as it is gradually oxidised by sulphur-oxidising bacteria. In a pot experiment addition of 12.8 g S₈.kg⁻¹ temporary lowered the pH from 7.2 to 3.6 and increased Cd mobility and concentration in Indian mustard 10- and 27-fold (Kayser *et al.*, 1999), but in a field trial the observed effect was weaker (Kayser *et al.*, 2000). It is necessary the efforts for selection of appropriate rhizosphere manipulation to be continued. There is a need to find cheaper, environmentally benign chemical compounds with chelating properties (Lasat, 2000).

Screening for high Cd uptake and tolerance

As plant species and even different genotypes within the same species differ in Cd accumulation, screening tests might be used to identify genotypes exhibiting high Cd accumulation and /or tolerance.

Jarvis *et al.* (1976) established remarkable differences in shoot Cd content between 23 plant species varying almost a factor of 10. A great difference in Cd accumulation and tolerance in *T. caerulea* ecotypes with different origin has been already mentioned (Lombi *et al.*, 2000). For example, Ganges ecotype is able to accumulate approximately 3 times more Cd in the shoots than the Prayon ecotype without suffering phytotoxicity. Screening up to 150 *Salix* genotypes Landberg and Greger (1994, 1996) found that there is no correlation between Cd accumulation and tolerance. Moreover, no correlation was found between root and shoot Cd accumulation. However, genotypes having both traits high Cd uptake and tolerance have been identified. Significant differences in Cd uptake and transfer among maize genotypes have been also established (Hinesly *et al.*, 1978; Florin and Van Beusichem, 1993; Guo and Marschner, 1996). Recently, we have reported well expressed cultivar-dependant Cd accumulation and resistance in barley, but concluded that Cd phytoextraction capacity of this crop is not sufficient for short-term phytoextraction purpose (Vassilev *et al.*, submitted). There is not enough information about cultivar-dependant Cd uptake and tolerance in many crops envisaged for Cd phytoextraction, that is why the screening studies should be developed.

Mineral nutrient optimisation

In general, crop cultivation technologies are well established, but in connection with the phytoextraction purpose, some innovations aiming to improve plant performance might be introduced.

It is well known that Cd (and other metals) can induce essential nutrient deficiency and even to decrease concentrations of many macronutrients in plants (Siedleska, 1995). It was shown that Cd disturbs N assimilation (Boussama et al., 1999), induced micronutrients decrease (Greger and Lindberg, 1987; Vassilev et al., in press) and at sub-optimal S status provoked S deficiency (McMahon and Anderson, 1998). Thus, it seems that through optimising mineral nutrition of Cd-exposed plants it could be possible to reverse or ameliorate (at least partly) some of the negative Cd effects on the extracting plants. There is some evidence for this suggestion regarding S and N nutrient optimisation (Chen and Huerta, 1997; Pankovic et al., 2000), but the large mineral nutrition database offers many other examples, too. Burzynski and Buszek (1989) found that Mo appeared to be a good antidotium for Cd-treated plants because it stimulated translocation of some essential nutrients like Fe, Cu, Mn, Zn from roots to leaves, resulting in a strong stimulatory effect on plant metabolism without decreasing Cd concentration in leaves. Summarising, an optimisation of the mineral nutrition of the actual Cd-extracting plants, should be proposed.

Use of plant growth regulators

It has been mentioned above that some phytohormones and physiologically active substances such as gibberelins, polyamines, biomin have protecting effects against Cd phytotoxicity (Kamenova-Jouhimenko et al., 1997/1998; Ghorbanli et al., 1999; Groppa et al., 2001). It seems logical to further develop this approach using phytohormone synthetic analogies in order to prevent or to ameliorate the toxic Cd effects.

Conclusions

In conclusion, the information presented in this review paper showed plants in a new vision – as environmental counterbalances to industrial pollution. In particular, as a promising approach for soil decontamination the use of plants for Cd phytoextraction from agricultural soils was shown. However, its implementation requires further optimization. As phytoextraction needs a quite interdisciplinary approach such improvements might be addressed to many plant and soil sciences. In the plant physiology field there are many open questions related to the mechanisms of plant Cd hyperaccumulation and tolerance, phytotoxicity and its amelioration.

Metal phytoextraction might be applied not only to metals like Pb and Cd, but also to other target metals, including radionuclides ^{137}Cs , ^{90}Sr , as local soil contaminations with particular metal(s) have been observed in many occasions. Depending on the case, the other branches of phytoremediation, such as phytostabilisation, phytovolatilization, phytofiltration, etc. also might be used (Vangronsveld and Cunningham, 1998; de Souza et al., 2000; Duschekov et al., 1999).

To support the phytoremediation development significant number of scientific teams in Europe is involved in fruitful coordination of national efforts in the frame of COST Action 837 (<http://ibewww.epfl.ch/COST837>) (Schwitzguébel, J-P., 2001). In Bulgaria, this research until now is not well enough targeted in spite that several plant physiological aspects have been investigated. To authors' point of view shows there is a need to enhance research efforts to further contribute to this emerging and environmentally friendly "green" technology.

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