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Phytoremediation

Molecular biology, requirements for application, environmental protection, public attention and feasibility

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Over centuries, human industrial, mining and military activities as well as farming and waste practices have contaminated large areas of developed countries with high concentrations of heavy metals and organic pollutants. In addition to their negative effects on ecosystems and other natural resources, these sites pose a great danger to public health, because pollutants can enter food through agricultural products or leach into drinking water (EC, 2002; EEA, 2003). In the EU alone, an estimated 52 million hectares—more than 16% of the total land area—are affected by some level of soil degradation. The largest and probably most heavily contaminated areas are found near industrialized regions in northwestern Europe, but many contaminated areas exist around most major European cities (EEA, 2003). There could be between 300,000 and 1.5 million of these sites in the EU (EC, 2002)—the uncertainty in this estimate is due to the lack of common definitions and a scarcity of accurate data on the size and the level of contamination of affected sites.

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Cleaning up contaminated soil is a costly enterprise—the overall cost to remediate affected sites in the EU is estimated to be between €59 and €109 billion (EC, 2002). Furthermore, current methods of soil remediation do not really solve the problem. In Germany, for instance, only 30% of soils from contaminated sites are cleaned up in soil remediation facilities (SRU, 2004); the remaining soil must be stored in waste disposal facilities. This does not solve the problem, it merely transfers it to future generations. Obviously, there is an urgent need for alternative, cheap and efficient methods to clean up heavily contaminated industrial areas.

This could be achieved by a relatively new technology known as phytoremediation, which uses plants to remove pollutants from the environment. Due to its elegance and the extent of contaminated areas, it has already received significant scientific and commercial attention (Salt *et al*, 1998; Gleba *et al*, 1999; Meagher, 2000; Dietz & Schnoor, 2001; Guerinot & Salt, 2001; Krämer & Chardonnens, 2001; McGrath & Zhao, 2003; Peuke & Rennenberg, 2005). Phytoremediation uses wild or genetically modified plants (GMPs) to extract a wide range of heavy metals and organic pollutants from the soil. Initial experiments with transgenic plants have shown that they are indeed efficient in drawing metals from heavily contaminated soils. However, despite this and other advantages, the progress and application of this technology to tackle widespread environmental problems is being hampered by ideology-driven, restrictive legislation over the use and release of GMPs in Europe, and particularly in Germany.

Phytoremediation comes in several forms. Phytoextraction removes metals or organics from soils by accumulating them in the biomass of plants. Phytodegradation, or phytotransformation, is the use of plants to uptake, store and degrade organic pollutants; rhizofiltration involves the removal of pollutants from aqueous sources by plant roots. Phytostabilization reduces the bioavailability of pollutants by immobilizing or binding them to the soil matrix, and phytovolatilization uses plants to take pollutants from the growth matrix, transform them and release them into the atmosphere.

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Most scientific and commercial interest in phytoremediation now focuses on phytoextraction and phytodegradation, which use selected plant species grown on contaminated soils. These are then harvested to remove the plants together with the pollutants that have accumulated in their tissues. Depending on the type of contamination, the plants can either be disposed of or used in alternative processes, such as burning for energy production. In essence, phytoextraction removes pollutants from contaminated soils, concentrates them in biomass and further concentrates the pollutants by combustion.

It is also possible to recover some metals from plant tissue (phytomining), which humans have done for centuries in the case of potassium (potash), and which may even become economically valuable (Meagher, 2000). In addition to accumulating toxic minerals in their tissues, plants are also able to take up a range of harmful organic compounds, including some of the most abundant environmental pollutants such as polychlorinated biphenyl (PCB), halogenated hydrocarbons (trichloroethylene, TCE) and ammunition wastes (nitroaromatics such as trinitrotoluene (TNT) and glycerol trinitrate (GTN)). Subsequent metabolism in plant tissues then mineralizes or degrades such pollutants to non- or less-toxic compounds (Salt *et al*, 1998; Meagher, 2000; Dietz & Schnoor, 2001).

Compared with conventional methods of soil remediation, the use of plants provides several striking advantages. It is cheap: after planting, only marginal costs apply for harvesting and field management, such as weed control. It is a carbon-dioxide neutral technology: if the harvested biomass is burned, no additional carbon dioxide is released into the atmosphere beyond what was originally assimilated by the plants during growth. Phytoremediation is also a potentially profitable technology as the resulting biomass can be used for heat and energy production in specialized facilities. A major disadvantage of phytoremediation is its relatively slow pace, because it requires several years or even decades to halve metal contamination in soil (McGrath & Zhao, 2003). Furthermore, during the process of phytoremediation, a contaminated site is not available for sale or rent, which can cause problems for economic development (SRU, 2004). The challenge for plant scientists is therefore to improve the plants' performance in removing toxicants from the soil, which will require more basic research and knowledge on the natural detoxification mechanisms of plants.

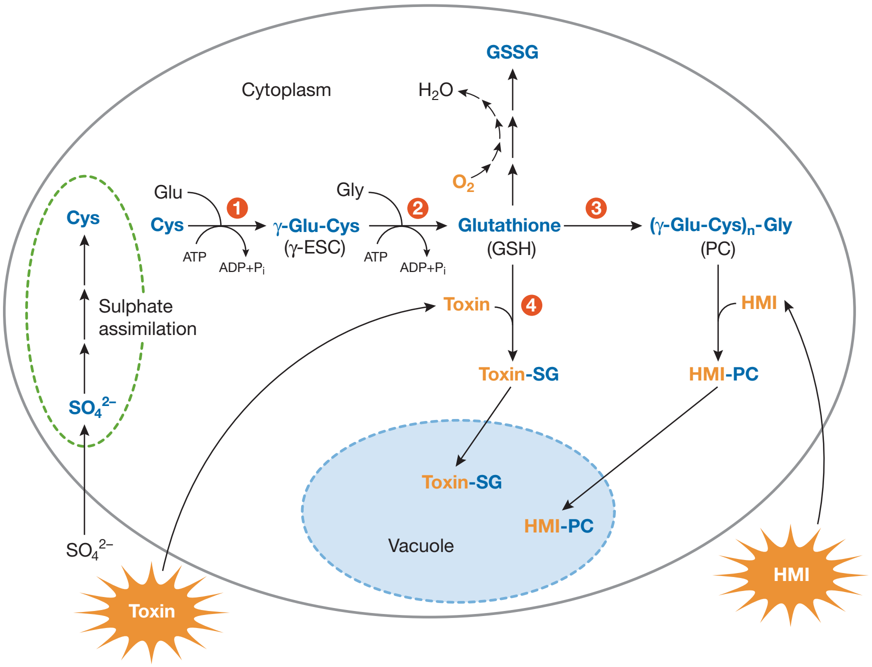

Fig 1 | Mechanism of detoxification of heavy metals, organic pollutants and oxidative stress in plant cells by glutathione. Cys, cysteine; γ-Glu-Cys, γ-L-glutamyl-L-cysteine; γ-ECS, γ-glutamylcysteine synthetase; GSH, glutathione; GSSG, oxidized glutathione; PC, phytochelatine; HMI, heavy metal ion; HMI-PC, heavy metal–phytochelatine complex; Toxin, xenobiotics; Toxin–SG, toxin–GSH conjugate. (1) γ-Glutamylcysteine synthetase; (2) glutathione synthetase; (3) phytochelatine synthase; (4) glutathione *S*-transferase (GST).

These mechanisms evolved to tolerate naturally occurring heavy metals in the soil, which either disrupt or inhibit enzymatic activity by displacing other metal cofactors, or generate reactive oxygen species and free radicals that bind to the sulphur and/or nitrogen atoms of proteins (Clemens, 2001; Hall, 2002; Rea *et al*, 2004). Plants have several cellular structures and physiological processes to maintain homeostasis and detoxify supra-optimal metal concentrations. These include metal binding to mycorrhizal fungi, metal binding to cell walls, exudation of metal chelating compounds and a network of processes that take up metals, chelate them and transport these complexes to above-ground tissues where they are sequestered into vacuoles (Clemens, 2001; Guerinot & Salt, 2001; Hall, 2002; Clemens *et al*, 2002).

The ability to tolerate large concentrations of heavy metals is a rare phenomenon in the plant kingdom as a whole, but is widespread in particular plant groups: some hyperaccumulating or metal-tolerant species have been investigated for several years: *Silene vulgaris*, *Thlaspi caerulescens*, *Alyssum lesbiacum*, *Arabidopsis halleri* and *Brassica* spp. (Clemens *et al*, 2002; Krämer, 2003). Their ability to accumulate high concentrations of metals was observed for both essential nutrients, such as copper (Cu), iron (Fe), zinc (Zn) and selenium (Se), as well as non-essential metals, such as cadmium (Cd), mercury (Hg), lead (Pb), aluminium (Al) and arsenic (As; Salt *et al*, 1998; Meagher, 2000; Clemens, 2001; Guerinot & Salt, 2001; Hall, 2002; Clemens *et al*, 2002; McGrath & Zhao, 2003). Metal concentrations in the shoots of accumulating plants can be 100–1,000-fold higher than in non-accumulating plants: 1% for Zn (up to 4%) and manganese (Mn); 0.1% for cobalt (Co; up to 1.2%), Cu, nickel (Ni; up to 3.8%), As (up to 0.75%) and Se (up to 0.4%); and 100 ppm for Cd (up to 0.2%).

Chelating compounds, most notably metallothioneins and phytochelatins, have a significant role in the detoxification of metals, and their synthesis in the plant is induced by exposure of root cells to heavy metals (Rauser, 1999; Cobbett, 2000; Clemens, 2001; Hall, 2002; Cobbett & Goldsbrough, 2002; Rea *et al*, 2004). These cysteine-rich polypeptides exploit the property of heavy metals to bind to the thiol-groups of proteins—one of the toxic effects of heavy metals—for detoxification. Metallothioneins are sulphur-rich proteins of 60–80 amino acids that contain 9–16 cysteine residues and are found in plants, animals and some prokaryotes (Rauser, 1999; Cobbett, 2000; Cobbett & Goldsbrough, 2002). Phytochelatins (PCs) are a family of γ-glutamylcysteine oligopeptides with glycine or other amino acids at the carboxy-terminal end, in which γ-Glu-Cys units are repeated 2–11 times. They are synthesized from glutathione (GSH) and its derivates by phytochelatine synthase in the presence of heavy-metal ions (Cobbett, 2000; Rea *et al*, 2004). After exposure to Cd or Cu, PCs were found in yeast, algae and lower and higher plants (Cobbett, 2000). PCs form ligand complexes with these metals, which are then sequestered into the vacuole. GSH also occupies a central role in defence against oxidative stress, heavy metals and xenobiotics. It is synthesized in two ATP-dependent steps that are catalysed by γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase (Fig 1; May *et al*, 1998; Noctor *et al*, 1998; Foyer *et al*, 2001). Other low-molecular-weight chelators, including organic acids (malate, citrate), amino acids (O-acetylserine, histidine) and nicotinamine, are used in detoxification, sequestration or transport (Cobbett, 2000; Clemens, 2001; Hall, 2002; Krämer, 2003). The transformation to less harmful forms is another approach to detoxifying heavy metals, particularly As, Hg, Fe, Se and chromium (Cr), which exist in a variety of cationic and oxyanionic species and thio- and organometallic forms (Meagher, 2000; Guerinot & Salt, 2001).

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The detoxification of organic pollutants by plants is achieved—similar to the detoxification of heavy metals—by uptake and translocation, sequestration into the vacuole and metabolization, including oxidation, reduction or hydrolysis and conjugation with glucose, GSH or amino acids (Salt *et al*, 1998; Meagher, 2000; Dietz & Schnoor, 2001). GSH and the glutathione *S*-transferase (GST) isoenzymes have a crucial role in the degradation of many pesticides, as they are able to form conjugates between various xenobiotics and GSH by nucleophilic addition reactions (Edwards *et al*, 2000; Dietz & Schnoor, 2001; Dixon *et al*, 2002). These pesticide–GSH conjugates are generally much less toxic and more water-soluble than the original molecules and are sequestered into the vacuole where they can be further degraded (Edwards *et al*, 2000; Foyer *et al*, 2001). The expression of GST-encoding genes is induced by a wide range of endogenous and xenobiotic chemicals, including phytohormones, heavy metals and herbicides (Noctor *et al*, 1998; Foyer *et al*, 2001; Kopriva & Rennenberg, 2004).

ATP-binding cassette (ABC) transporters are the best-characterized system to transfer toxic organics out of root cells and into vacuoles after conjugation by GSTs (Meagher, 2000; Foyer *et al*, 2001; Dixon *et al*, 2002). This combination of glutathione, GSTs and ABC transporters has a prominent role in detoxifying most heavy metals and organic pollutants in plant cells (Fig 1; Noctor *et al*, 1998; Edwards *et al*, 2000; Foyer *et al*, 2001; Dixon *et al*, 2002).

The time it takes for plants to reduce the amount of heavy metals in contaminated soils depends on two factors: how much biomass these plants produce and their metal bioconcentration factor, which is the ratio of metal concentration in the shoot tissue to the soil (McGrath & Zhao, 2003). The latter factor is determined by the ability and capacity of the roots to take up metals and load them into the xylem, by the mass flow in the xylem to the shoot in the transpiration stream, and by the ability to accumulate, store and detoxify metals while maintaining metabolism, growth and biomass production (Gleba *et al*, 1999; Guerinot & Salt, 2001; Clemens *et al*, 2002). With the exception of hyperaccumulators, most plants have metal bioconcentration factors of less than 1, which means that it takes longer than a human lifespan to reduce soil contamination by 50%. To achieve a significant reduction of contaminants within one or two decades, it is therefore necessary to use plants that excel in either of these two factors—for example, grow crops with a metal bioconcentration factor of 20 and a biomass production of 10 tonnes per hectare (t/ha), or with a metal bioconcentration factor of 10 and a biomass production of 20 t/ha.

Two possible strategies have emerged to improve the phytoextraction of heavy metals: growing plant phenotypes that are able to accumulate large concentrations of heavy metals in their above-ground parts, or using phenotypes that are able to produce high biomass with average heavy-metal concentration in their harvestable tissue. Of course, it would be desirable to combine both features and design plants that are specialized for fast growth and hyperaccumulation. This is the fundamental aim that underlies efforts to generate transgenic plants for phytoremediation. Other than plant growth, which depends on numerous genetic and non-genetic factors, the accumulation of heavy metals is controlled by only a few gene loci and is therefore more easily accessible for genetic manipulation (Clemens, 2001). Phytoremediation strategies that have recently been put into practice are the genetic manipulation of GSH and phytochelatine production in plant tissues (Noctor *et al*, 1998; Cobbett, 2000; Cobbett & Goldsbrough, 2002).

Trees are probably the best-suited plants for transgenic approaches to improve the heavy-metal accumulation and metabolism of organic compounds. Forest trees already have several mechanisms for stress defence, ranging from morphological changes to the synthesis of defence compounds. Tree biotechnology is thus becoming an increasingly important tool for the remediation of contaminated environments (Peuke & Rennenberg, 2005). Fast-growing trees, such as various *Populus* species, are also good candidates for phytoremediation applications due to their extensive root systems, high rates of water uptake and transpiration—which result in efficient transport of compounds from roots to shoot—rapid growth and large biomass production. Poplars can be grown in a wide range of climatic conditions and are used with increasing frequency in 'short-rotation forestry' systems for pulp and paper production. This raises the possibility of using plantations of transgenic poplars across several multi-year cycles to remove heavy metals from contaminated soils. In addition, a dense tree cover would also prevent erosion and the spread of contaminated soil by wind. After the first planting, the costs for field management are relatively low and the products (biomass/wood) can be used for the production of electricity and heat by burning in wood power stations. Another important point is that it is very unlikely that poplars will enter the human food chain or end up as feedstock for animals.

Plant scientists who work on phytoremediation have therefore spent considerable efforts to enhance GSH levels in trees to increase their stress tolerance. The transformation of grey poplar trees (*Populus tremula* x *P. alba*) to overexpress γ-ECS from *Escherichia coli* resulted in higher levels of GSH and its precursor γ-L-glutamyl-L-cysteine compared with wild type (Noctor *et al*, 1998), and an elevated capacity for phytochelatine production and detoxification of organic pollutants. These new transgenic trees are indeed

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Fig 2 | Plantation with wild-type as well as transgenic poplars on a field site with high amounts of heavy metals in the soil, in the former copper mining area Mansfelder Land, Saxonia Anhalt, Germany.

'all-purpose performers' for phytoremediation in controlled greenhouse conditions: they showed a high potential for the uptake and detoxification of both heavy metals and pesticides (Peuke & Rennenberg, 2005). We are now conducting field trials with these poplars in former copper-mining regions with different levels of contamination and under different climatic conditions to measure their capacity to remove heavy metals from the soil (Fig 2). Further aims of the project are to assess the biosafety risk of transgenic poplars for the phytoremediation of soils by elucidating the stability of the transgene under field conditions and the possibility of horizontal gene transfer to microorganisms in the rhizosphere. Three field trials, each with low (control), middle and high amounts of heavy metals in the soil were set up in former mining areas in Germany (Saxonia Anhalt, district Mansfelder Land) and Russia (Middle Urals, Swerdlovsk oblast).

Preliminary results from these trials show that the transgenic poplars are genetically stable and there are no indications so far of any impact on the environment. The transgenic trees have a higher capacity than wild-type trees for accumulating heavy metals, but only on the most contaminated soils. On control sites or sites with low contamination, there were no differences in heavy-metal concentration in the shoot between wild-type and transgenic trees.

Phytoremediation, in combination with burning the resulting biomass to produce electricity and heat, could become a new environmentally friendly form of biotechnology. If genetic engineering is eventually successful in producing plants that are able to reclaim contaminated lands in tractable time frames, then we may also see a better public acceptance of GMPs with respect to environmental protection. In countries with low fossil energy resources and agricultural overproduction, such as most of the EU states, the production of biomass for wood power stations on soils that are no longer suitable for human food production may also provide financial benefits for farmers. At the moment, many farmers receive money from the EU for not using up to 15% of their land, so as to avert overproduction.

At present, the main concerns about the release of GMPs are their potential impact on the environment and their risks to human health, which are reflected both in past German legislation and in European directives (EC, 2001). The present discussion in Germany on the use of GMPs shows less concern for human health and focuses more on the potential impact on the environment in general and in particular on agriculture forms that are free of GMPs and subject to ecological management (SRU, 2004). Unfortunately, these debates have largely slowed down or even halted the use of this environmentally friendly technology. As public discussions are often dominated by categorical arguments based on ideology rather than scientific facts, it makes no difference that the genetically modified poplars in our field trials are not a food plant, will not come to reproductive age within the experimental period, are female and therefore cannot produce pollen, and do not generate toxic substances.

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The situation deteriorated in 2004, when the German government passed new legislation concerning the release of genetically modified organisms (GMOs), which could create legal problems for those developing and testing GMPs. Under the new law, all users of GMPs can be made legally liable if pollen or other biological material contaminates products from GMO-free agriculture. Moreover, all German sites in which GMPs are planted—scientific field trials as well as commercial cultivation—must now be reported to a governmental agency, and information on these sites and the GMPs grown there will be made publicly available. The authorities may even pass on personal data about GMP users to interested parties, which is almost an invitation for militant opponents of the technology to protest against or uproot these fields.

These new policies pose an incalculable financial and social risk for research on the molecular engineering and application of GMOs in Germany, a country that relies heavily on developing new technologies and scientific innovation for economic progress due to its lack of natural resources. To undermine or block the potential of new biotechnological products based on categorical prejudices and ideological extremism, rather than leading an objective debate that is driven by scientific facts, is a significant political error. GMPs that are modified for improved performance in phytoremediation are clearly and demonstrably beneficial to the environment. Even in conventional agriculture, a strong case can be made that the use of GMPs will reduce our reliance on pesticides and herbicides and therefore improve the environmental compatibility of agricultural practices. A final irony of the current situation is that any collateral damage will hamper further research on biological safety. Germany has had extensive programmes in this field for several years (www.biosicherheit.de) and the mere presence of active biotechnology research has the potential to stimulate novel safety and containment approaches that can be applied to a broad range of biomedical and industrial hazards.

It is therefore greatly disappointing that, given the rich heritage of German biotechnological research over the past decades, virtually no scientific insight was incorporated into the law-making process that led to the current legislation. In a society whose advancement is critically dependent on the informed exchange between scientists and politicians, it is imperative that people remain open to new perspectives and that knowledge is respected. The lack of these imperatives in the proceedings that hatched this legislation has probably rendered environmental health the unfortunate victim of an ill-informed political process.

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