**Plants to mine metals and remediate land**

Engineered plants can clean up pollution and recover technology-critical metals

By Elizabeth L. Rylott and Neil C. Bruce

Centre for Novel Agricultural Products, Department of Biology, University of York, York, UK.

Email liz.rylott@york.ac.uk, neil.bruce@york.ac.uk

Anthropogenic activities such as mining have released vast amounts of metals and metalloids, into the ecosystem. The cost to human health is acute (1), and reserves of valuable technology-critical metals are now running out (2). Phytoremediation using synthetically engineered plants to clean up polluted environments and phytomining, a method to recover valuable metals, offer a solution to help alleviate these problems.

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and dioxins are toxic carcinogens produced predominantly from industrial processes and chemical manufacturing, that resist biodegradation in the environment for decades. These xenobiotic compounds are also often diluted into ecosystems, making their remediation logistically challenging. The use of soil-penetrating root networks of plants can address these issues. Furthermore, planting polluted land can restore biodiversity and soil health, reduce pollution expo-sure, and is aesthetically pleasing with high public acceptance. Plants lack the ability to degrade most POPs and instead follow a classic three-step detoxification pathway in planta that results in sequestration or in-corporation into macromolecular structures such as lignin. In this state, the original pollutant is considered biologically unavailable, with any further catabolism or mineralization occurring at the end of the plant’s life through fungal and bacterial de-composition.

There is a wealth of biochemical diversity within the plant kingdom to detoxify xenobiotics, but where plants lack sufficient enzymatic activities, there is a seemingly bottomless pool of microbial enzymes that can tackle even the most ubiquitous and challenging pollutants. Thus, genetically modified (GM) plants can be engineered to express microbial xenobiotic-degrading enzymes to extend their physiology to de-grade or detoxify organic pollutants. Rice (*Oryza sativa*) engineered with an apoplastic-targeted thiocyanate hydrolase from *Thiobacillus thioparus* can effectively degrade thiocyanate from industrial mine wastes (3). For the military explosive and toxic pollutant, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), expression of a bacterial cytochrome P450 was shown to mineralize the pollutant in planta; a technology that has now been successfully tested on military ranges using native switchgrass (Panicum virgatum) (4).

Industry-critical metals and finite re-sources, include platinum group metals (PGMs), noble metals such as gold and silver; and rare earth elements (REEs; lanthanide metals, as well as yttrium and scandium) (2), are often geopolitically controlled. These elements are increasingly used in developing technologies with no current substitutes and they are vulnerable to supply chain disruptions. Moreover, the mining process has a high carbon footprint and causes considerable environmental damage (2). Indeed, metal pollution has arisen from wastes from commercial mining; metal smelting and processing; dispersion along roadside verges from catalytic exhaust emissions; and wastes from artisanal gold mining activities. These wastes contain high-value metals but at levels that are too low for current mining practices to be economically viable. Moreover, the number of catastrophic mine tailings dam failures has increased globally. Phytoremediation could be combined with phytomining to revegetate and restore often denuded environments, reduce dam breeching, and recover valuable metals. The process can be carried out by small holders in high-metal sites, sometimes in difficult-to-access locations, and increasingly, the “eco-metal” recovered can attract a premium price.

Plant metal uptake starts in the rhizosphere where plant roots and associated microbes exude compounds that solubilize metals in the soil. These metal cations enter the plant via an assortment of transporters. At uncontrolled concentrations metals be-come toxic, interacting with electron transport activities and generating reactive oxygen species that indiscriminately dam-age cellular components. Metals can also disrupt sulfhydryl, carboxyl, amino and phosphoryl groups in proteins, inhibiting critical cellular enzyme activities. Thus, cellular levels of all metals need to be tightly regulated. The first point of controlling metal homeostasis is regulating influx transporters to reduce uptake, and efflux transporters to pump metals out of the plant roots. This enables some “excluder” species to successfully inhabit metalliferous environments. Once taken up, metals are de-toxified by complexing with chelators such as phytochelatins, metallothioneins and amino acids, then further transported via the xylem, and compartmentalized into the vacuole or cell wall (see the figure).

In contrast to excluder species, “hyper-accumulator” plants have the ability to grow on naturally-occurring metalliferous environments and take up metals with exquisite specificity to many thousand-fold above background environmental levels. Their remarkable biochemistry is hypothesized to confer antiherbivory properties, but could also be the key to successful phytomining (5). Over 700 hyperaccumulator species, including many in the Brassicaceae and tropical tree species of Phyllanthaceae, have been identified. They store a range of metals and metalloids including arsenic (As), cadmium (Cd), copper, colbalt (Co), manganese, nickel (Ni), selenium (Se), thalli-um, zinc and the REEs lanthanum (La), cerium (Ce) and neodymium (Nd) (6). The efficacy of Ni-phytomining was demonstrated with a seven-year field-study in Pojska, Albania where the hyperaccumulator *Odontarrhena chalcidica* growing on ultramafic (geologically Ni-rich) soils extracted ~100 kg Ni ha-1 annually (5). Following harvest, the plant biomass can be ashed, producing heat as a by-product, then high-purity nick-el salts recovered using acid-based chemical treatments. In South Africa, Berkheya cod-dii has been successfully used to phytomine and remediate Ni and Co from contaminated soils around a Ni-smelting plant, with the metals recovered from the biomass via the smelting plant (7).

Geobotanical prospecting--identifying metalliferous soils by observing the presence of indicator species known to grow in those conditions--has revealed species containing >1000 μg g−1 of REEs (8). The fern *Dicranopteris linearis*, found growing on tailings at a former REE mine in southeast-ern China (9) accumulates predominantly La, Nd and Ce, producing biomass at commercially harvestable levels. Although relatively little is known of the mechanisms be-hind REE hyperaccumulation, potassium, sodium, calcium and aluminium channels are involved in their uptake (9).

Gene expression studies on the most-studied Ni-hyperaccumulator species in the *Noccaea* and *Odontarrhena* genera indicate that Ni accumulation is achieved predominantly by increased expression of the genes that exist in non-hyperaccumulators, rather than increased metal-specificity per se (10). This upregulation includes low affinity transporters such as the zinc regulated (ZRT) and iron regulated (IRT)-like (ZIP) family, and the metal chelators histidine and nicotianamine. How Ni is loaded into the xylem, and unloaded into the aerial tissue has not yet been conclusively determined, but tonoplast iron-regulated (IREG) transporters are implicated in transport into the vacuoles of aerial tissues in three families of hyperaccumulator species (10). Within the vacuole, Ni-citrate and Ni-malate are the predominant forms. In addition to enhanced detoxification pathways, hyperaccumulators often have upregulated antioxidant systems, presumably to ameliorate metal-produced reactive oxygen species.

There has been little evidence of successful commercialization of hyperaccumulator plants to phytomine metals. Key factors hindering the development of successful phytomining include the availability of agronomically-suitable varieties that will grow in broad geographical locations and soil types; the absence of hyperaccumulator species for PGMs and noble metals; the volatile prices of technology-critical metals; and the presence of phytotoxic levels of co-contaminating metals and, on industrial sites, organic pollutants. Increasing demand is likely to further increase metal prices, and thus the viability of phytomining, but gaining additional value would improve the financial stability. Towards this goal, the catalytic activity of in planta metals such as Pd or Ni can be used to enhance production of value-added platform chemicals and bio-fuels from biomass (11).

Although the agronomic traits of some species, such as *O. chalcidica*, have been developed into commercial crops, there are arguably no hyperaccumulator species that can produce the same amount of biomass as non-hyperaccumulator, fast-growing species such as willow (Salix spp), poplar (Pop-ulus spp) and miscanthus (Miscanthus × giganteus), which are currently widely cultivated across temperate regions. There are also genetic transformation protocols available for these biomass species, and thus enormous potential exists to use synthetic biology tools to create artificial hyperaccumulators, combined if necessary, with degrading abilities for organic pollutants. AlfaFold, an artificial intelligence pro-gram that can predict protein structures (12), could be used to design proteins with metal-binding abilities or activities to specific xenobiotics. Together with gene editing techniques such as CRISPR-CAS (13), these tools could be used to confer traits into biomass species. For example, expression of metal-binding proteins such as lanmodulin, a La-binding protein from *Methylobacterium extorquens* (14), or upregulating tar-get metal transporters and chelators, could boost metal specificity and accumulation. Alternatively, plant artificial chromosomes containing synthetic promoters, transcriptional activators and repressors could be assembled into a gene stack encoding complete metabolic pathways (13) for metal accumulation and xenobiotic degradation.

The rhizosphere is increasingly recognized as an essential part of the metal up-take process, and one that could be manipulated, for example by using cyanogenic bacteria to solubilize metals in soils. A step further is the design of artificial organelles that bind and concentrate specific metals. Additionally, organ-level compartmentalization could be engineered to promote metal accumulation in harvestable tissues, such as above ground, woody tissues.

Although there is still controversy over the use of GM crops, their use is established in the US, Brazil, Argentina, and India, and there is evidence of increasing public sup-port (15). Furthermore, there is higher public acceptance for non-food GM-crops; and environmental pollution is understandably an issue many would like addressed. But what of the relatively low-value, toxic metals such as cadmium, zinc, lead, and chromium; and the metalloid arsenic? Often found together, these elements comprise the majority of inorganic pollutants; they cause adverse health effects in millions globally, and remediating them is a substantial challenge. There are many plant species that can be used to take up these elements, and genetic modifications to further increase plant tolerance and uptake. However, they have little current commercial value. Focusing on high-value metals could provide the knowledge and financial incentive to develop cost-effective technologies to phytoremediate lower-value metals and metalloids. However, the answer will be a multi-pronged approach, with phytotechnologies filling a valuable niche.

REFERENCES AND NOTES

1. Global Assessment of Soil Pollution. Food and Agri-culture Organization (FAO) and United Nations Environment (UNEP), Rome, Italy, (2021).

2. T. E. Graedel, Nat. Resour. Res. 27, 181 (2018).

3. J.-J. Gao et al., Sci. Total Environ. 820, 153283 (2022).

4. T. J. Cary et al., Nat. Biotechnol. 39, 1216 (2021).

5. A. Bani et al., in Agromining: Farming for Metals: Extracting Unconventional Resources Using Plants, Springer International Publishing, Cham. 221 (2018).

6. R. D. Reeves et al., New Phytol. 218, 407 (2018).

7. M. Rue et al., Metallomics 12, 1278 (2020).

8. P. N. Nkrumah et al., Plant Soil 464, 375 (2021).

9. Z. Chour et al., J. Environ. Chem. 8, 103961 (2020).

10. L. van der Pas and R. A. Ingle, Plants (Basel) 8: 11 (2019).

11. V.-N. Edgar et al., Appl. Sci. 11, 2982 (2021).

12. J. Jumper et al., Nature 596, 583 (2021).

13. W. Liu et al., Nat. Rev. Genet. 14, 781 (2013).

14. C. Deane Nat. Chem. Biol. 15, 2 (2019).

15. S. Evanega et al., GM Crops Food 13, 38 (2022).

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Fig. 1. Generalized overview of nickel (Ni) and organic pollutant (X) detoxification in plants. Back-ground image shows a crop of the nickel hyperaccumulator *Berkheya codii* growing around, and later processed at, the nearby Rustenburg Base Metals Refiners refinery in South Africa. Image courtesy of C.W.N. Anderson. Xenobiotics are detoxified firstly by an activation step which converts these generally lipophilic polluting molecules into more polar forms. Next, glutathione or chemical groups such as glucosyls are added, which increases solubility and often decreases toxicity. Then the trans-formed conjugated compounds are stored, often transiently in the vacuole, via carriers such as ATP-binding cassette (ABC) transporters, before sequestration or incorporation into macromolecular structures such as lignin. The foreground schematic shows the potential targets where synthetic biology (SynBio) could be used to increase uptake, de-toxification and metal accumulation. ZIP: ZRT-IRT-like (ZIP) and IREG1; iron-regulated transporters.

