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REVIEW ARTICLE
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Agromining: Agroremediation for Heavy Metal Contaminated Ecosystems: A Review

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ABSTRACT

Heavy metals can be extracted from the earth using hyperaccumulator plants. These plants can grow in soil with high concentrations of metals and concentrate trace elements in their biomass. Agromining is a branch of phytoremediation that aims to remove toxic metals from polluted ecosystems. The plants can be used to produce valuable metals such as Ni and Zn. The potential of agromining can be enhanced by improving biomass yield through agronomic practices. The phenological stage of the crop at harvest influences metal concentration in the tissues. Inoculation with microorganisms especially *Arthrobacter* sp. strain LA44 improved the metal yield in *Alyssum murale*. Observed high Ni yield in *Phyllanthus rufuschaneyi* and *Rinorea bengalensis* by the application of major nutrients. Agromining is still at an early stage of development. Even then, it created a new era in the recovery of metals from natural resources. Hyperaccumulator plants changed their recognition from a botanical curiosity to tangible socio-economic and environmental applications. However, rigorous and dedicated research is required for its wide utilization and to improve quantitative and qualitative performance.

Key words: Agromining, Agronomic practices, Hyperaccumulators, Hypernickelophores.

Ecosystem contamination with heavy metals has seriously threatened the environment and food security. These metals have been observed to have deleterious impacts on human and environmental health, but when extracted from the earth can produce significant economic and environmental benefits. Arsenic in the soils of West Bengal in India and Bangladesh affects the leguminous crops like lentil and chickpea which are grown in these regions and cause cancer to people (Ghritlahre and Singh, 2022). As a result of studies conducted in the western part of the Ryazan region of Russia, which is under the RGRES (Ryazan State District Power Plant) influence, it was found that the level of contamination of agricultural land of this area of the farm with heavy metals is moderately dangerous (Vinogradov and Zubkova, 2022). Traditional physical and chemical remediation technologies involve relatively high capital expenditure and manpower. Hence efforts are underway to develop cost-effective approaches to recover metal-contaminated ecosystems. The use of plants for decontamination and extraction of metals is identified as a promising alternative and widely practiced both in developed and developing countries.

Agromining first came into notice in 1948, when botanist Ornella Vergnano came across a plant called *Alyssum bertolonii* in Tuscany, Italy. This was a relative of kale and cabbages and it had 10 mg of nickel in every gram of its dried tissue. That is surprisingly 2000 times more than a typical plant. Hundreds more of these plants have been discovered since then. For decades, these plants were considered as mere curiosities. Then in 1997, Rufus Chaney, an agronomist at the US Department of Agriculture and his colleagues found that it is possible to extract a nickel-rich “bio-ore”.

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Agromining is a branch of phytoremediation that specifically aims to remove toxic metals and metalloids from polluted media (*e.g.* soils, sediments, wastes) using hyperaccumulator plants. The biomass derived from phytoremediation operations is considered contaminated waste, but in phytomining, it is a source of valuable metals-a true bio-ore. Agromining can be used to produce As, Se, Cd, Cu, Co, La, Mn, Ni, Pb, Tl and Zn, as hyperaccumulator plants are known for all of these elements (Table 1). Profitability depends on the element market price, the annual yield per unit area (biomass produced and contained amount of target element) and the availability of surface areas enriched in this element.

Hyperaccumulators

Hyperaccumulator plants are capable of growing in soil or water with very high concentrations of metals, absorbing these metals through their roots and concentrating extremely high levels of metals in their tissues. Hyperaccumulator plants have a special ability to 'biopurify' and concentrate certain trace elements (*e.g.* nickel) from the soil into their biomass (van der Ent *et al*., 2015). Cultivating these plants as 'metal crops' by periodically harvesting and incinerating their biomass generates a high-grade bio-ore (Li *et al*., 2003). Hyperaccumulator species are known with high concentrations of metals such as Ni, Mn, or Zn in their aboveground biomass. Such plants may be used in locating orebodies, phytoextraction and agromining.

Approaches in agromining

Two main approaches may be considered in agromining:

1. Agromining on degraded or mined land

This can be part of rehabilitation strategies for degraded mined lands. Agromining could also form a first stage in the development of lateritic mining projects and then continue as part of the rehabilitation strategy during the mining operation. This gives an opportunity for the generation of income from agromining projects during the project development phase. It will not hinder the mining project, as agromining would first use the overburden that would be cleared before extracting the underlying minerals.

2. Agromining on low-productivity agricultural soils

This would target large areas, which have low productivity for food production. Agromining would be preferable than crop production, generating higher economic income for farmers. Intercropping can also be adopted: for example, in Greece, olive plantations could be intercropped with *Alyssum*; and in Malaysia, palm oil estates could be intercropped with *Phyllanthus*.

Tools for determining hyperaccumulator plants

The high percentage of identified Ni hyperaccumulator species as compared to hyperaccumulators that accumulate other elements is primarily thanks to the very fact that the field testing method for Ni using dimethylglyoxime (DMG)-treated paper.

After initial field screening employing a spot test (*e.g.* DMG-paper), verification of hyperaccumulator status has usually been attained using Atomic Absorption Spectrophotometry (AAS) (Kelly *et al*., 1975) and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (van der Ent *et al*., 2015) after acid digestion of dried leaf samples. Although the analysis with AAS or ICP-AES itself takes only minutes, the sample preparation will take more time and resources (100 samples take 2-3 days to digest).

Table-top XRF (X-ray fluo-rescence) was also used for this intention in the past. XRF analysis can be done on undamaged specimens, but for more reliable results homogenisation and preparation of pellets is proposed. Recent innovations in more sensitive and compact X-ray fluorescence (XRF) instruments enable non-destructive elemental screening of an extensive numbers of samples, including herbarium specimens, in a relatively short time. The use of handheld XRF instruments can save time and resources. It can measure a range of different elements concurrently within 30-60 s in dry samples. Recently, handheld XRF systems are validated for the measurement of elements in plant samples, although this has involved powdering and pelletization of sample material before measurement (Guerra *et al*., 2014).

The XRF instrument works by exposing the sample to a beam of focused, high-energy X-rays produced from an X-ray tube in the device. The spectrum of excited fluorescent X-rays is then examined to determine the presence of different elements and to compute their relative concentrations in the sample.

The elemental distribution in selected hyperaccumulator plant tissues can further be studied using techniques like desktop or synchrotron micro-XRF, nuclear microprobe (PIXE), scanning/transmission electron microscopy with energy-dispersive spectroscopy (SEM/TEM-EDS), secondary ion mass spectrometry (SIMS) or laser ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS).

Mechanisms of hyperaccumulation

Plants growing on high metal substrate can adopt three strategies to deal with high metal concentrations:

i) Exclusion.
ii) Indication.
iii) Accumulation (Baker, 1981).

Excluders inhibit metal accumulation in photosynthetically active shoot tissues by:

i) Limitation of metal absorption by roots.
ii) Increased metal efflux from root tissues.

Table 1: Metals produced by agromining.

Metals	Source plant	Common name	Family	Reference
Se	*Astragalus* sp.	Milkvetch	Fabaceae	Byers *et al*., 1938
As	*Ceratophyllum demersum*	Hornwort	Ceratophyllaceae	Lancaster *et al*., 1971
Co	*Nyssa sylvatica* var. biflora	Swamp blackgum	Cornaceae	Brooks *et al*., 1977
Ni	*Streptanthus polygaloides*	Milkwort jewelflower	Brassicaceae	Reeves *et al*., 1981
Zn	*Thlapsi goesingense*	Tiny wild mustard	Brassicaceae	Reeves and Baker, 1984
Cd	*Noccaea praecox*		Brassicaceae	Schwartz *et al*., 2006
Mn	*Beaupreopsis* sp.	Spider flower	Proteaceae	Losfeld *et al*., 2015
Li	*Brassica napus*	Rapeseed	Brassicaceae	Kavanagh *et al*., 2018

iii) Higher storage of metals in non-active root cell walls and vacuoles (Merlot *et al*., 2018).

Second, indicators increase uptake and accumulation of elements to aerial plant parts as a response to elevated soil contents. Third, (hyper)- accumulators concentrate enormous amounts of metals in their aboveground biomass, independent of soil metal levels (Baker, 1981). In contrast to excluders, the metal homeostasis network in (hyper)-accumulators is altered to enable the translocation of metals to shoot tissues for sequestration (Merlot *et al*., 2018).

Furthermore, Merlot *et al*. (2018) suggested that hyperaccumulation requires:

i) Increased mobilization, uptake and radial transport of metals in roots, towards root vascular tissues (with reduced sequestration in root vacuoles).

ii) Enhanced metal translocation from roots to shoots through xylem, to enable metal storage in shoot tissues.

iii) Unloading of xylem to store metals in shoot vacuoles with high storage capacity.

The summary of these processes in roots and shoots enables detoxification *via* storage of metals in non-photosynthetic active parts (*e.g*. vacuoles) (Merlot *et al*., 2018).

Hypernickelophores

Globally, >700 hyperaccumulator plant species are now known, about 70% of which are Ni hyperaccumulators (Reeves *et al*., 2018). Notably, only 10% of the ~500 Ni hyperaccumulators reported to date are able to accumulate Ni to concentrations exceeding 1 wt% in the shoot (Nkrumah *et al*., 2016) and are termed 'hypernickelophores (Jaffre and Schmid, 1974). For Ni, a plant is considered as hyperaccumulator if the concentration of nickel is higher than 0.1% in the dried leaves. For phyto or agromining, hypernickelophores (concentration higher than 1%) are more suitable candidates.

Ni-Hyperaccumulation

Plants growing on Ni-rich substrate evolved strategies to regulate homeostasis and hyperaccumulation of Ni. To transport Ni from roots to shoots, metal transporters and chelators are needed to bind Ni at different stages of transport and pH environments. Regarding the latter, Ni can be complexed to organic acids, such as the carboxylic acids citrate and malate, to ensure binding in acidic environments (*e.g*. vacuoles and xylem). While, NA (nicotianamine) complexes Ni (NA - Ni) in compartments with neutral pH (*e.g* cytoplasm and phloem) (Merlot *et al*., 2018). Uptill now, it is not clear, which transporters are specifically involved in Ni hyperaccumulation. Merlot *et al*. (2018) claimed that generally two types of transporter can be distinguished: divalent metal importers (*e.g*. ZIP/IRT, NRAMP) or transporters for complexed Ni phases (*e.g*. Yellow Stripe-Like (YSL) family). Hence, Merlot *et al*. (2018) proposed the following strategy for Ni transport in hyperaccumulators.

i) First, Ni is taken up from soil solution by metal transporters (ZIP/IRT, NRAMP), which are located at the plasma membrane of root epidermal cells.

ii) Then Ni is chelated (*e.g*. by nicotianamine to form [NA-Ni]-complex) to reduce its reactivity for the transport through cortex and endodermis and to inhibit vacuolar sequestration.

iii) Next, Ni and chelator molecules (*e.g*. NA, Citrate) are loaded in xylem, where the complexes (*e.g*. NA-Ni, Ni-Citrate) are translocated to the shoot by YSL transporters.

iv) Finally, Ni is unloaded in xylem and transported to the leaf epidermal cell, where Ni is eventually stored in the vacuole by iron transporters (IREG).

Processing of Ni biomass and bio-ores

Ashing

In the majority of processes, biomass is ashed yielding Ni content up to 20% as NiO. In order to get the desired Ni phase and mineralize organic matter without volatilizing, reactor conditions have to be controlled. Heat energy acquired from this reaction could potentially be retrieved as the combustion is exothermic. Hyperaccumulator plants can be incinerated along with the municipal waste. With high-temperature ashing (>500°C), the residue consists mainly of carbonates and oxides of the major metallic elements present in the plant: K, Ca, Mg and Ni. There may be an advantage in lower temperature ashing (<400°C), where C is retained in a reduced form, which can be used as fuel or a reducing agent in successive pyrometallurgical processing stages. Prewashing can help increase the Ni content of the ash, as K_2CO_3 is present in it and is highly water-soluble.

Smelting

The thermochemical treatment of bio-ore to produce Ni metal needs heat supply as well as an apt reductant and flux to control the slag properties. The production of ferro-nickel or nickel pig-iron from laterite ore is economically viable for grades above 1.5% Ni. Once dried, biomass or bio-ore could be used in the feed of existing Ni smelters, reducing the expenditure at the initial stages.

Leaching

Another extraction method is to leach the metal into a solution. Hydrometallurgical processing of Ni uses H_2SO_4, NH_4OH-$(NH_4)_2CO_3$, or NH_4OH- $(NH_4)_2SO_4$. Existing hydrometallurgical operations could put bio-ore into their feed; for example, pressure acid leaching is done where concentrated H_2SO_4 is applied into an autoclave at 250°C would probably solubilize the Ni. The drawback of this is the Ni recombination with impurity elements like Fe, which must be separated later. Common hydrometallurgical Fe removal processes result in 2-10% of the leached Ni being lost to the tailings stream.

Synthesis of ANSH (Ammonium Nickel Sulphate Hexahydrate) from *Odontarrhena muralis*

The patented procedure to produce ANSH from *Odontarrhena muralis* ashes will be implemented in the design (Barbaroux *et al*., 2012). Modifications suggested by Zhang and the team will be used to reduce cost and to increase nickel extraction. This patented process consists of four major processes: ashing, leaching, purification and crystallization. The final product will contain 13.2% nickel and have a purity of 99%. The process has been developed to conserve water, energy and chemicals, while producing a high purity salt. The biomass is dried and ashed, potassium will be separated by washing ash with pure water following a cross current pathway and nickel will be extracted by acid leaching (2M H_2SO_4, 95°C, 2 h). The leachate is then neutralized by Ca $(OH)_2$ to come to a pH of 4-5 and magnesium is removed by precipitating MgF_2 after addition of NaF. Then volume is reduced by evaporation and ANSH crystallization will be done at 2°C for 4 h. The crystals will be dissolved and a second crystallization will be done. The final ANSH was characterized by combined techniques (ICP-AES, XRD and gravimetric analysis) and the purity was 99.1±0.2%.

Agronomic practices for improving biomass yield of hyperaccumulator plants

Appropriate agronomic practices, based on findings from laboratory and field tests, have been recommended to maximize the yields of the selected 'metal crop' (Nkrumah *et al*., 2016).

- Application of fertilizers (mineral or organic).
- Irrigation management.
- Weed control (*e.g*. herbicide application).
- Harvesting at the right stage.

The hyperaccumulator crop will deplete soil Ca, P, N and K and these elements will need to be applied to retain good growth, as soils are innately deficient in these elements. Organic amendments are suggested to increase biomass production by various processes. Organic fertilizers enhance soil quality and structure, decrease compaction and erosion and enhance biological activity (Nkrumah *et al*., 2016). Furthermore, weed control is needed to reduce competition for nutrients and water between weeds and hyperaccumulators (Bani *et al*., 2015). Additionally, irrigation management can support plant growth, which is usually restricted by drought (Kidd *et al*., 2018).

Nkrumah *et al*., (2018) observed high Ni yield in *Phyllanthus rufuschaneyi* and *Rinorea bengalensis* by the application of major nutrients and organic matter compared to control.

Nkrumah *et al*. (2016) have observed that with increase in phosphorus, calcium and magnesium treatments, there will be increase in biomass yield hence an increase in metal yield.

The study conducted by Pardo *et al*. (2017) aimed to assess the potential of PGPB for increasing the establishment and yield of the Ni-hyperaccumulating Mediterranean species *Alyssum murale* for agromining intentions at field scale. The inoculation treatments were: (i) non-inoculated plants (NI); (ii) *Arthrobacter* sp. strain LA44; (iii) *Arthrobacter* sp. strain SBA82; and (iv) *Variovorax paradoxus* strain AB30 (all originally isolated from rhizosphere of Ni hyperaccumating plant species).

All plants had similar Ni concentrations in their shoots, however, the phytoextracted Ni per plant and Ni yield was significantly increased by LA44 strains. Therefore, the inoculation of *A. murale* with LA44 strains improved the agromining success exhibits the potential of PGPBs in agromining systems.

The experimental field was installed in Pojskë (Pogradec, East of Albania) by Bani *et al*. (2014). Three plots were fertilized and treated with anti-monocot herbicide (FocusTM ultra 33 ml applied in 3 L water sprayed onto 108 m^2) (FH), three were treated with herbicide alone with no fertilization (NFH) and a further three were not treated at all (NFNH). They have found that phyto extracted Ni was higher in FH than NFNH plots throughout the years.

According to Bani *et al*. (2014), phenological stage of the crop influence metal concentration in the tissues. In *A. murale*, Ni found to be at highest concentration in mid-flowering stage, Ca at vegetative stage and Mg at germination stage.

Major disadvantage of agromining is that process of reclamation by biological technologies requires time (Paul *et al*., 2021).

CONCLUSION

Agromining is still at an early stage of development. Even then, it created a new era in the recovery of metals from natural resources. Hyperaccumulator plants changed their recognition from a botanical curiosity to tangible socio economic and environmental applications. However, rigorous and dedicated research is required for its wide utilization and to improve quantitative and qualitative performance.

Conflict of interest

All authors declared that there is no conflict of interest.

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