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# Making light work of heavy metal contamination: The potential for coupling bioremediation with bioenergy production.

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## Abstract

Intense anthropogenic activity continues to expose the natural environment to heavy metal contamination. Whilst a number of physical and chemical solutions for remediation exist, the use of higher plants and algae for clean-up of contaminated landscapes, termed “phytoremediation” and “phycoremediation”, respectively, offer an attractive and sustainable alternative. However, these remediation processes will always lead to a high-moisture, heavy metal-contaminated biomass, which must be further processed to partition, or render inert, the metal contaminants. Conversion of this metal-rich biomass into second-generation biofuels offers a useful route to subsidise the economics of remediation activities. Here we briefly review the various methods for bioremediation of heavy metals, and discuss the potential to produce bioenergy from these biomass sources. Ultimately, coupling the bioremediation activity to bioenergy production gives far-reaching social and economic benefits; however, established processes such as direct combustion and anaerobic digestion risk releasing heavy metals back into the environment. Alternatively, thermochemical conversions such as pyrolysis or hydrothermal liquefaction (HTL) offer significant advantages in terms of the segregation of metals into a relatively inert and compact solid phase while producing a biocrude oil for bioenergy production. In addition, preliminary work suggests that the HTL process can also be used to partition essential macronutrients, such as N, P and K, into an aqueous medium, allowing additional nutrient recycling.

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# 1 Introduction

Rapid industrialisation and unsustainable mining practices have led to the large-scale deposition of metal-contaminated waste into soil, water and the atmosphere, causing widespread damage to ecosystems and reducing the natural capital of terrestrial and marine environments (Table 1). The sheer scale of contaminated land globally is a growing concern.[1] Land contamination by metals has led to increasing areas becoming unsuitable for agriculture,[2] while water contamination by heavy metals from domestic or industrial sources can have a significant impact on the biodiversity and function of aquatic ecosystems. Agriculture and aquaculture activities in contaminated environments can lead to bioaccumulation of toxins throughout the food chain, posing major environmental and health risks. Developmental delays, kidney damage, damage to the central nervous system and decreased lymphocyte counts in humans have all been associated with exposure to heavy metals such as cadmium, lead and chromium.[3-7,8, 9]

**Table 1.** Most common metal pollutants released in 2014 (reported in tonnes): data represent the total values from all EU member states, Iceland, Liechtenstein, Norway, Serbia and Switzerland. Data adapted from the European Pollutant Release and Transfer Register[10]

| | **As** | **Cd** | **Cr** | **Cu** | **Hg** | **Ni** | **Pb** | **Zn** |
|---|---|---|---|---|---|---|---|---|
| **Airborne** | | | | | | | | |
| Energy sector | 19.7 | 4.02 | 33.4 | 21.2 | 14.1 | 119.0 | 55.1 | 55.1 |
| Production and processing of metals | 5.71 | 4.40 | 34.7 | 56.7 | 4.70 | 34.0 | 210 | 480 |
| Chemical industry | 49.1 | 0.10 | 1.06 | 2.06 | 2.46 | 4.44 | 0.65 | 28.7 |
| Mineral industry | 2.04 | 0.36 | 3.27 | 6.17 | 3.02 | 3.20 | 18.8 | 10.2 |
| Waste and waste water management | 0.32 | 0.09 | 0.69 | 2.54 | 0.97 | 0.76 | 1.32 | 10.8 |
| Paper and wood production processing | 0.28 | 0.49 | 0.53 | 0.49 | 0.22 | 2.92 | 7.66 | 8.38 |
| Animal and vegetable products (food and beverage) | - | - | - | - | - | 1.97 | | 1.29 |
| Other | 0.02 | - | - | 0.95 | - | - | - | 2.64 |
| Total | 28.1 | 9.45 | 73.6 | 90.1 | 25.5 | 167 | 293 | 709 |
| **Watercourse** | | | | | | | | |
| Energy sector | 33.2 | 2.54 | 20.7 | 37.8 | 1.00 | 16.3 | 9.10 | 93.3 |
| Production and processing of metals | 2.98 | 2.42 | 278 | 19.0 | 0.08 | 96.5 | 33.5 | 191 |
| Chemical industry | 8.60 | 0.63 | 29.9 | 13.4 | 0.73 | 20.0 | 9.15 | 117 |
| Mineral industry | 5.07 | 3.2 | 36.2 | 115 | 0.25 | 18.2 | 42.7 | 326 |
| Waste and waste water management | 28.5 | 9.28 | 85.2 | 220 | 2.92 | 175 | 74.8 | 1064 |
| Paper and wood | 1.72 | 0.86 | 4.16 | 14.4 | 0.15 | 6.51 | 2.92 | 117 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| production processing | | | | | | | | |
| Intensive livestock production and aquaculture | - | - | - | 69.1 | - | - | - | 227 |
| Animal and vegetable products (food and beverage) | 0.12 | 0.01 | - | 2.47 | 0.01 | 1.80 | 0.03 | 8.77 |
| Other | 0.01 | 0.06 | 32.10 | 156 | 0.02 | 0.50 | 0.28 | 2.31 |
| Total | 80.2 | 19.0 | 487 | 492 | 5.13 | 335 | 172 | 2146 |

Heavy metals can be released into the environment by a number of industrial and societal practices, through mechanisms such as run-off from agricultural and urban areas,[11] acid mine drainage,[12] and industrial waste disposal.[13] Heavy metals including cadmium, mercury, lead and arsenic are now commonly present at toxic concentrations in a wide range of terrestrial and aqueous environments as a result of anthropogenic activities.[14] The presence of these metal contaminants, even at lower levels, has a negative effect on local ecosystems and causes environmental degradation.[15]

As intense anthropogenic activity continues to expose the natural environment to heavy metal contamination, the driving force behind it continues to be fuelled by excessive consumption of fossil resources, which, in turn, are non-renewable and finite. If the current rate of fossil fuel consumption is not tempered, supply is likely to become uneven and problematic in the near future, and the global effects of fossil emissions (climate change and air, land and water pollution) will continue to worsen; therefore sourcing alternatives is of utmost importance.

Biomass is an extremely promising route to liquid fuel replacements, and biofuels have gained huge attention in recent decades. Currently, approximately 100M tonnes of biofuel is produced globally each year – predominantly biodiesel and bioethanol – typically sourced from crop plants (vegetable oils and sugar cane, respectively). This is also problematic: the use of arable land to grow crops for biofuels leads to competition between “food vs. fuel” production. Together with the use of clean water, fertilizers, pesticides, and potential deforestation for fuel crop cultivation, biofuels derived from edible crops are an unattractive alternative.[16] Biofuels sourced from wastes and agricultural residues (mainly composed of lignocellulosic biomass), as well as marine biomass (micro- and macroalgae) have the potential to supply a proportion of the global energy needs without impacting food production.

Although a number of mechanical and chemical solutions to metal remediation exist, the use of plants and algae for clean-up of contaminated landscapes (termed “phytoremediation” and “phycoremediation”, respectively) are an extremely promising technology, with various advantages over traditional methods. Remediation by biomass is simple, inexpensive and solar-driven, and presents a viable strategy for long-term improvements to soil and water quality. Biomass cultivated on contaminated land or in water can subsequently be used for direct energy generation or biofuel production, and this strategy has attracted an increasing amount of attention in recent years.[17] The potential for the recovery of high-value metals could also present a lucrative opportunity.

In this review, the current state of the art in remediation of metal-degraded landscapes and waterways will be reviewed, and the possibility of converting the biological fraction into a biofuel while recovering the metals critically evaluated.

# 2 Remediation methods

A large number of technologies are currently used for the non-biological remediation of contaminated land.[18] The most basic excavation-based methods involve physically removing contaminated soil to landfill, then surface-capping the site to prevent the release of contaminants, or using a binding agent to render the metals inert in the soil. Another method in use is thermal treatment of the soil to volatilise metal contaminants, or chemical remediation *via* the addition of compounds which react with the metals to produce more chemically stable products.[19]

Metal-contaminated waters may be treated using "pump and treat" methods, in which water is pumped through vessels containing activated carbon, alternative sorbents such as clays, or nanoparticles specifically designed to interact with the metal contaminants.[20-22] The most common approaches to metal-contaminated water remediation are still physicochemical. By far the most widely used is chemical precipitation,[23, 24] brought about by changing the effluent pH (e.g. through the addition of lime or NaOH to form metal hydroxides). This is used particularly for remediation of acid mine drainage (AMD), which is a significant source of aqueous metal contamination in countries with active or former mining activities.[25] More advanced physicochemical methods of metal-contaminated water treatment include solvent extraction, ion exchange (by synthetic resins or abundant, naturally occurring zeolites),[26] or adsorption by organic compounds,[27] as well as flocculation, membrane filtration,[28] and electrochemical methods.[26] However, all of these methods tend to be expensive, difficult to scale, and are inefficient in cases where metal concentration is low.

## 2.1 Bioremediation of contaminated soil and watercourses

Relieving the requirement to add chemical remediation agents into soil and water could greatly reduce the cost of treatment. It is possible, for example, to stimulate the growth of microbes already present in the contaminated site, which can facilitate the breakdown of toxic compounds (bioremediation). Microbial growth may be accelerated by supplying additional oxygen-releasing compounds into the soil (this can be carried out either *in situ* or *ex situ*).[29] If the necessary microorganisms do not exist naturally in the target area, they can be introduced (bioaugmentation),[30] although the ecological implications of introducing a new microbe species into an ecosystem must be well understood.

Regular monitoring is necessary for this type of bioremediation, as it is possible for contaminants to break down only partially, generating compounds that are more mobile than the initial contaminants, but no less harmful.[31] Furthermore, the effectiveness of bioremediation depends on the degree to which the target contaminants are biodegradable – certain contaminants may not be metabolised, and microbe activity can be inhibited by some metals. This issue can potentially be overcome through genetic modification; however, such an approach may limit bioremediation to *ex situ* applications, as *in situ* bioremediation would involve the release of genetically modified microbes into an already compromised ecosystem.

Although bioremediation can convert metals to a more benign form, and may be helpful in remediating certain contaminants in soil, it does not offer the opportunity to recover metals easily, as the resulting metabolites typically remain in the soil. In order to extract metal contaminants from the soil – and cheaply remediate many of the other contaminants – phytoremediation may be employed.

## 2.2 Phytoremediation of metals in soils by higher plants

A range of higher plants can grow on non-arable land and are often able to remove or degrade contaminants found in their habitat, including metals. Phytoremediation using plants has been demonstrated to be effective at removing or stabilising a large range of contaminants such as metals,[8, 32] metalloids[33] and organics[34, 35] from the surrounding soil.[36] Phytoremediation mechanisms include: storage of contaminants in the body of the plant, specifically in the above-ground biomass (phyto-extraction); metabolism of contaminants into products of lower toxicity, often assisted by symbiotic microbes (phyto-degradation); vapourising contaminants, releasing them into the air (phyto-volatilisation); preventing transport of dissolved contaminants in the soil (phyto-immobilisation); and by mechanically stabilising polluted soils (phyto-stabilisation).[36] Phytoremediation is generally inexpensive, does not require excessive amounts of specialist equipment, and can help reintroduce an effective ecosystem into an otherwise desolated landscape.

An essential property of any phytoremediator is that it must be able to grow on contaminated land containing harmful levels of metal contamination, and usually must also be hardy enough to cope with poor nutrient-lacking soil, harsh weather conditions and insufficient fresh water. Plants that are able to grow under such conditions include *Ricinus communis, Jatropha curcas, Populus linnaeus*,[8] and various species of *Miscanthus*.[37] *Miscanthus × giganteus*, also known as elephant grass, is a phytoremediator crop that is primarily grown as a renewable energy source.[38]

Superville et al. investigated metal accumulation in trees growing in metal- (cadmium, lead, zinc, mercury and copper) contaminated soils surrounding a closed-down smelter.[39] Out of five tree species investigated, *Robinia pseudoacacia* (black locust tree)*, Alnus glutinosa* (black alder), *Acer pseudoplatanus* (sycamore) and *Salix alba* (white willow) were able to survive on the heavily contaminated soil. *Salix alba* accumulated the highest levels of zinc and cadmium. Lead was also accumulated; however, unlike zinc and cadmium, lead accumulation occurred seasonally.

Although trees such as white willow may be well suited for long-term stabilisation of metal contaminants, due to their slow growth rates, trees are not always suitable if remediation needs to be rapid. Faster-growing perennial crops may need to be used in these cases. Jeke et al. evaluated the use of *Panicum virgatum* (switchgrass) and *Typha latifolia* (cattail) for the phytoremediation of biosolids from an end-of-life municipal lagoon containing phosphoprous, nitrogen, cadmium, zinc, copper and chromium.[40] Switchgrass and cattail were both capable of phyto-extracting nitrogen and phosphorus from the biosolids; however, phytoextraction of all metals occurred only to a minimal extent, as these trace elements tend to accumulate in the belowground biomass – phyto-immobilisation/phyto-stabilisation.[41] In addition, Zeke et al. observed that the biomass yields of both species were up to to 75 % lower when grown on contaminated land compared to uncontaminated land. Reductions in biomass yields relatively to uncontaminated conditions are a common phenomenon for crops cultivated on contaminated land.[42, 43]

The majority of phytoremediators are reported to accumulate metals in belowground biomass, reducing the potential for contaminant recovery; however, Karimi et al. reported an arsenic-accumulating species of brassica (*Isatis capadocica*), which favours accumulation in the aboveground biomass.[44] *I. capadocica* populations grown on mining-contaminated land (sourced from the gold-arsenic Zarshuran deposit) and non-contaminated land were studied. The metal tolerance of the two plant populations was compared through exposure to either a hydroponic metal solution, or metal-contaminated soils. The mine population retained >50 % root growth at arsenate concentrations of up to 1.3 mM, and was able to accumulate more than 600 mg $kg^{-1}$ arsenic in its aboveground biomass (dry mass), making it an excellent phytoextractor. The tolerance of both plant populations was probed using arsenate-spiked pottings, and soil from an abandoned lead mine. Only mine populations of *I. capadocica* were able to grow in soil arsenic concentrations of 200 mg $kg^{-1}$, and no plants grew at concentrations of 500 mg As $kg^{-1}$ soil. However, both populations were able to tolerate much higher concentrations of arsenic in soils taken from the mine, compared to the arsenate-spiked pottings. This difference in tolerance was attributed to the majority of arsenic in the contaminated mine soil existing in a non-bioavailable form. Although *I. capadocica* is a good candidate for phytoremediation of arsenic, as accumulation in the shoots allows for phytoextraction to take place with effective removal of the contaminants from the soil, additional remediation measures may be necessary in cases where arsenic is present in soils in a less bioavailable form.

*Miscanthus × giganteus* – a sterile hybrid of *Miscanthus sinensis* and *Miscanthus sacchariflorus* – is a high-yielding C4 perennial grass primarily studied as a second generation fuel crop, which shows promise as a phytoremediator. As it is able to tolerate low temperatures, maintaining photosynthetic activity over long periods at temperatures as low as 4 °C,[45] it has the potential to expand the range of climates in which phytoremediation may be implemented. *M. × giganteus* is tolerant to a wide range of pH levels and is able to grow in shallow, dry, cold and waterlogged soils,[46] has low fertiliser and pesticide requirements, and makes efficient use of available nutrients.[47] As such *Miscanthus* is an ideal candidate plant species to couple bioaccumulation and bioenergy production.

Antonkiewicz et al. compared the use of *Phalaris arundinacea* (reed canary grass) and *M. × giganteus* in the phytoremediation of metal-contaminated soil.[48] The crops were treated with varying amounts of municipal sewage sludge to assess effects on metal uptake and plant growth. Increasing the dose of sewage sludge was found to increase biomass yields from both species, and improved the ability of both crops to accumulate metal contaminants (chromium, nickel, copper, zinc and lead). Overall, *M. × giganteus* displayed superior metal accumulation and higher biomass yields in a greater range of conditions than did *P. arundinacea*; therefore, of the two crops studied, *M. × giganteus* was recommended for the remediation of sewage sludge.

Koco´n et al. also found *M. × giganteus* a viable remediator for soils contaminated with metals.[49] *M. × giganteus* was compared with *Sida hermaphrodita* as a phytoremediator for cadmium, copper, nickel, lead and zinc on clay and sandy soils. Both plants were able to purify soils of zinc, but took up lower levels of the remaining metals. *M. × giganteus* was found to accumulate all metals with greater success than *S. harmephrodita*, with the exception of cadmium. *M. × giganteus* was able to phytoremediate to a similar extent on both soil types, although accumulation of copper and zinc were significantly higher on sandy soil. Compared to *M. × giganteus*, *S. harmephrodita* had increased levels of metal translocation to the belowground biomass; however, while both species successfully accumulated all metals, neither crop could be classed as an accumulator with respect to aboveground biomass, indicating that they are not effective phyto-extractors.

Nsanganwimana et al. investigated the distribution of Cd, Pb, and Zn within *M. × giganteus* plants, finding that all three metals were stored preferentially in the roots.[50] Over 40 mg $kg^{-1}$ (dry matter) of both Cd and Pb were accumulated in the roots, as well as up to 150 mg $kg^{-1}$ of Zn. In contrast, the combined accumulation in leaves and stems of Cd, Pb, and Zn, were 4, 35, and 180 mg $kg^{-1}$, respectively, while rhizome storage levels were 2, 12, and 60 mg $kg^{-1}$. It appears evident that the ability of *M. × giganteus* to translocate Zn from the roots to other parts of the plant is substantially higher than for Pb and Cd, likely due to the biological role of Zn in plants.

Phytoremediation is most viable when using hardy, low-maintenance crops, such as *M. × giganteus*, that grow well under poor conditions. This is because greater biomass yields have a higher capacity to store sequestered material, and lower-maintenance crops reduce the costs of the process. At present, *M. × giganteus* is predominantly used as an energy crop; however, there is possibility promising opportunity to use *M. × giganteus* to combine the services of bioenergy production and phytoremediation, making both more economically viable.

### 2.3 Phycoremediation of heavy metals in water by micro- and macroalgae

One key source of metal contamination in waters is industrial effluent. Their remediation is conventionally carried out through physicochemical processes, including chemical precipitation, ion exchange and adsorption, which are expensive and often not completely effective.[51] Bioremediation methods using constructed wetlands and reed beds are also in common usage, often in conjunction with physical methods such as precipitation to maximise metal removal efficiency. Alternatively, biological remediation by microorganisms – phycoremediation by microalgae and biosorption by bacteria, yeast and fungi – has been found to be effective in the remediation of metal-contaminated waters. Algae in particular have developed a wide spectrum of absorption and adsorption mechanisms to mediate heavy metal toxicity.[52] It is now generally accepted that biological processes have a number of advantages over traditional physicochemical methods, although the uptake of metals is strongly dependent on the provision of adequate light, temperature and nutrients for algal growth.[52] The use of both living and dead biomass has been explored, with living algal cells found to be particularly efficient at remediating water with low (ppb) metal concentrations. *In situ* phycoremediation not only allows for water clean-up, but helps to prevent the leaching of metals from contaminated waters into soils.

The remediation of heavy metal ions using algal biomass processes has been recently reviewed.[51, 53, 54] Both macroalgae (seaweed) and microalgae have been extensively studied for the biosorption of heavy metals, using both living cells as well as algae in its lysed form (Table 2).

**Table 2** Overview of remediation capabilities of various algal genera, data adapted from[54-56]

| | | **0–20 mg g$^{-1}$** | **20–50 mg g$^{-1}$** | **50–100 mg g$^{-1}$** | **>100 mg g$^{-1}$** |
|---|---|---|---|---|---|
| **Microalgae** | *Chlorella* sp. | | Cr(VI), Cu(II), Zn(II) | Cd(II), Cr(III), Ni(II) | Pb(II) |
| | *Spirulina* sp. | Cd(II) | Zn(II) | Cu(II) | Cr(III), Ni(II), Pb(II) |
| | *Claidomonas* sp. | Cr(VI), Cu(II) | Cd(II) | Hg(II), Pb(II) | |
| | *Scenedesmus* sp. | Cr(VI), Cu(II), Hg(II) | Zn(II), Ni(II) | Cd(II), Pb(II) | |
| | *Spirogyra* sp. | | Zn(II), Cd(II), Hg(II), Cu(II) | Ni(II), Pb(II) | |
| **Macroalgae** | *Sargassum* sp. | | Zn(II), Ni(II) | Cu(II), Cd(II) | Pb(II) |
| | *Fucus* sp. | | Cd(II), Ni(II) | Cu(II) | Zn(II), Pb(II) |
| | *Ulva* sp. | Ni(II) | Cd(II), Pb(II) | Cu(II) | |
| | *Chondrus* sp. | | Zn(II), Cu(II), Ni(II) | Cd(II) | |

Live algae, while suitable for remediation of low concentrations of metals, are susceptible to heavy metal poisoning; growth rates tend to be somewhat slower than in unpolluted waters. The biosorption capacity of living algal cells is strongly dependent on a number of environmental factors, and the "active" biosorption mechanism involves a large number of metabolic pathways, which are associated with specific timeframes. In contrast, dead algae are not susceptible to poisoning, and the "passive" biosorption mechanism involves the interaction of the heavy metal ions with functional groups located on the cell walls. Proteins, carbohydrates and lipids present on the walls contain hydroxyl, carboxyl, sulfhydryl, amino and phosphate groups that interact with the metal cations *via* ion exchange and complexation.[57] It follows that for this mechanism, uptake depends on type and number of active sites, and therefore on the specific algae, but also on the metal ion (size, charge), together with other environmental factors. To gain an understanding of these highly variable interactions, a large number of both algae and metals have been studied.

Absorption experiments are generally conducted on single metal solutions in order to understand the influence of characteristic parameters. It has been demonstrated that on increasing the initial metal concentration, the metal uptake increases, but overall efficiency of metal absorption decreases.[58, 59] One of the most important parameters is pH, as it influences functional group ionization and the complex formation constants, and thus may need to be carefully optimized. Metal biosorption is highly time-dependent; whilst with live algae, the process is usually slow, with dead biomass it becomes much faster, typically occurring within tens of minutes.[60, 61] Under optimised conditions, many algae strains have been shown to sequester up to tens (sometimes hundreds) of milligrams of metal per gram biomass.[51, 53] This elevated remediation potential has recently been attracting industrial interest, with some processes beginning to be commercialized.[53, 62] However, many challenges have yet to be addressed, especially regarding biomass reuse and metal recovery.

For example, Henriques et al. studied the bioaccumulation and biosorption of mercury by three species of macroalgae – *Ulva lactuca*, *Gracilaria gracilis* and *Fucus vesiculosus* – in waters with high salinity. The mercury concentrations of the contaminated water ranged between 10–100 µg $L^{-1}$.[63] Biosorption was a faster process than bioaccumulaton; however, bioaccumulation was deemed a more advantageous method overall. After 12 h, mercury removal from the solution resulted 45–80 % by biosorption, and 37–45 % by bioaccumulation; however, the total mercury removal by bioaccumulation was 97–99 %, whereas maximum total removal by biosorption was around 90%. The fastest species to bioaccumulate mercury was *Ulva lactuca*, removing 99 % of mercury, corresponding to 209 µg of mercury per gram of macroalgae (dry matter).

Nevertheless, irrespectively of the efficiency of the bioremediation, any process generates contaminated biomass which needs to be dealt with. Bio-mining, or the recovery of metals through biomass, is one potential route, and phytoremediation-derived biomass has been receiving increasing attention in recent years. Brooks *et al.* discussed biomass incineration to recover valuable metals.[64] More recently, Keller *et al.* found that pyrolysis was a preferable method to incineration to recover Cd and Zn from terrestrial bioremediation biomass, mainly due to the lack of metals in the exhaust stream.[65] Hydrothermal processing of biomass containing high metal concentrations has also been explored.[17]

# 3 Combining phyto-/phycoremediation and bioenergy production

The implementation of bioremediation could be encouraged further if economic viability could be improved. One solution is to couple phytoremediation with bioenergy production. Income generated from biofuel production could then help lower the costs of the environmental remediation.[66] Recent advances in cellulosic ethanol, anaerobic digestion, syngas fermentation and various thermochemical routes have demonstrated the suitability of the industrial conversion of lignocellulose and whole algal biomass on a large scale.[67] It is therefore plausible that lignocellulose and algae used as remediators could be a further source of energy.

Mleczek et al. used two species of willow – *Salix alba* and *Salix viminalis* – as potential phytoremediators; one cultivar of the former, and eight of the latter were investigated.[68] The cultivar *S. viminalis* “Turbo” was identified as the best overall metal-accumulator, based on stored metal concentrations (Cd, Cu, Hg, Pb and Zn). However, total metal accumulation in *S. alba* was higher due to a greater dry matter yield. The authors went on to suggest that the wood produced during phytoremediation could be used as an energy source, or by the pulp and paper industry. However, the environmental and human health implications of using contaminated wood for such purposes were not discussed.[69]

Gizinska-Górna et al. reported that biomass grown in a constructed contaminated wetland system in Poland could be used also to produce bioenergy as either a solid biofuel, or in the form of biomethane generated through anaerobic fermentation.[70] Of the plants tested, willow had the greatest higher heating value (19.2 MJ kg$^{-1}$), and common reed produced the most biomethane (108 m$^3$ Mg$^{-1}$). However, the ultimate fate of the remediated contaminants was not discussed, and so it is not clear whether or not they were present in the bioenergy products. The most likely fraction containing the metals is the leftover sludge, which is commonly repurposed as a fertiliser for plant growth, thereby exacerbating the initial metal contamination problem.

Ginneken et al. identified that large areas of contaminated land could be treated cheaply and effectively using phytoremediation, with potential for the resulting biomass products (rapeseed, maize and wheat) to be used to produce biodiesel.[70] However, metal retention in the biodiesel was highlighted as a major concern of this process, due to a previous study by Angelova et al. which revealed that Cd, Cu and Pb contents in the seeds – the plant organ containing the lowest levels of metal contamination – of phytoremediating rapeseed plants exceeded maximum permissible concentrations for human consumption[88-89]. Previously, *M. × giganteus* grown as a phytoremediator has been used as an energy source, in the form of dried biomass pellets. However, there have again been concerns over the metal content of the resulting pellets.[36] While the metal content of *M. × giganteus* is lower than that of coal, due to the lower energy density of *M. × giganteus*, the metal content per unit of energy is still high.

Recently, a range of other bioenergy production techniques have been explored, including gasification,[71] bioelectricity generation[72] and direct combustion,[73] although it is unclear how the heavy metals in these systems can be retained without release back into the environment. One potentially more promising route appears to be pyrolysis of the dry plant biomass. Pyrolysis reactions occur at between 400–750 °C under an inert atmosphere and result in the formation of a bio-oil, a solid char phase and gaseous products. A handful of investigations have demonstrated the processing of post-phytoremediation lignocellulose: for example, pyrolysis of contaminated *Broussonetia papyrifera* (6.0 ppm Cu and 1.6 ppm Cd) resulted in up to 68 % of Cd and 78 % of Cu being retained in the solid char. The majority of the biomass was converted to a bio-oil, and the yield was only slightly reduced compared with uncontaminated biomass.[74]

The implementation of phytoremediation as a method of processing heavy metals present in other waste streams has also been investigated; Tian et al., co-processed a heavy metal-rich hydrocarbon waste stream (containing 748 ppm Cu, , 826 ppm Cr, , 828 ppm Ni, 789 ppm Pb, and 865 ppm Zn,)with virgin wood. This afforded extensive metal immobilization into the solid char (approximately 40–80 % depending on the metal and operating conditions). A higher pyrolysis temperature led to a decreased oil recovery, but more non-bioavailable metal species in the char residue. The authors found that a low temperature co-pyrolysis (400 °C) was the most effective oily waste disposal strategy with satisfactory oil recovery.[75]

While fast pyrolysis is favourable for high oil yields, slow pyrolysis has also been examined for enhanced metal retention. Grottola et al. investigated the production of a clean combustible gas phase and the stabilisation of heavy metals (Pb, Cu, Cd, Zn) in the solid char. In this study, both branches and leaves of *Populus Nigra* L., and rhizomes and culms of *Arundo donax* L., were pyrolysed between 380-600 °C under steam-assisted slow pyrolysis conditions. While partial recovery of the metals was obtained, there was a reduced energy recovery in the oil phase from these types of systems due to the large deposition of carbon in the solid residue.[76] Further analysis suggests that there are significant advantages to a combined system, and integrated phytomanagement could generate significant income streams, provide a sustainable solution to waste management and give further environmental and social benefits.[77]

A number of studies reported bioenergy production from wet algal biomass used for remediation of watercourses. For example, a combination of biodiesel production and metal removal was reported by Kim et al.,[78] where residual biomass of *Nannochloropsis oculata* post-lipid extraction was used to remove chromium from aqueous solutions. Richard and Mullins were the first to propose a theoretical combination of metal biosorption by living algae followed by lipid extraction and transesterification.[79] Similarly, Yang et al. cultured the oleaginous species *Chlorella minutissima* and obtained high metal removal efficiencies for Zn, Mn, Cd and Cu (62–84 %).[80] They observed that lipid accumulation was not inhibited by heavy metals, but enhanced with the addition of cadmium and copper (21 % and 94 %, respectively). Lipids were extracted, and their metal content was found low enough to satisfy the commercial standards for fuels. A similar approach was used by Upadhyay et al.[81] who studied the effect of arsenic on growth of *Nannochloropsis sp*., finding that increasing As concentrations result in lower biomass productivities but higher lipid contents. At 100 µM As, lipids showed a 3-fold increase in content and a 2-fold increase in productivity. A similar activity was observed for *C. minutissima* and *Scenedesmus sp*., which were able to accumulate high concentrations of As and almost double their lipid productivity.[82] Additionally, the extracted lipids were transesterified, obtaining biodiesel with high oxidative stability, in compliance with biodiesel standards.

Liu et al. studied the growth of *Chlorella sp*. on wastewaters containing different concentrations of heavy metals.[83] They found that low to medium concentrations of Mn(II), Cu(II) and Zn(II) can enhance algae growth, while either the complete absence or high concentrations of these ions inhibit growth. Under optimal conditions, *Chlorella* was able to sequester up to 97 %, 100 %, 95 % and 46 % for Mn(II), Zn(II), Cu(II) and Cr(VI), respectively. Liu et al. propose the adoption of a two-stage process, starting from low metal concentration to obtain a significant amount of biomass, then increasing the concentration to maximise biosorption. They also conducted a study on the effect of metal ions on lipid production, finding that high concentrations of Mn and Zn increase lipid production, probably due to inducted stress.

The crucial issue remains in that, in addition to removing metals from soils and watercourses and depositing in the biomass, on conversion of the biomass to an energy product, metals must not be re-released into the environment.

One potential solution for the processing of wet biomass is through hydrothermal liquefaction (HTL). HTL is a thermochemical process in which wet biomass is converted under high temperatures (up to 350 °C) and high pressures (approximately 180 bar) to produce a bio-oil, an aqueous phase, a gas and a solid residue. Due to the inherently high water content within the system, HTL is particularly suitable for algae processing, although HTL of lignocellulosic materials has also been reported. Similar to pyrolysis, HTL generates a bio-crude oil suitable for upgrading to biofuels, whilst partitioning and concentrating metals and other inorganics in the solid phase products.

### 3.1 Hydrothermal liquefaction of metal contaminated biomass

One of the most promising routes to bioenergy and metal recovery is through hydrothermal liquefaction of algae, where the contaminated algal biomass is processed at a solids loading of up to 25 %. The positive economics of this process for bioenergy production are well-established; due to the closed nature of the system, the process also offers the potential to retain the metals remediated and prevent their release into the environment.

Initially, algal metal remediation was examined solely for metal recovery. Le Clercq et al. examined the recovery of nickel from the microalgae *Berkheya coddii*. Pre-treated algal biomass containing 630 ppm Ni was liquefied in a batch reactor at 25 MPa, heated to 375 °C at approximately 10 % solid loading. Using a model solution of Ni in histidine, the group suggested that Ni(II) was reduced to Ni(0) by biomass decomposition products, which partitioned into the char phase, rather than forming a separate metal layer. Around 80 % recovery of Ni from the biomass was obtained. However, no information was provided concerning the isolation of Ni metal from the char.[84]

Another study focused on the recovery of metals from *Sedum plumbizincicola*, a flowering plant and a heavy metal hyperaccumulator. Under hydrothermal processing conditions, over 99 % removal efficiency of Zn, Pb and Cu from the biomass was obtained, with efficiency increasing with processing temperature and duration. The optimum conditions for maximising both oil yield and metal removal from the biomass were found to be 370 °C, 22.1 MPa. In this case, the metals were found dissolved in the aqueous phase post-processing.[85] A 62 % oil yield was obtained.[86] In a similar study on hydrothermal upgrading of an arsenic hyperacculumator, *Pteris vittata*, maximum metal recoveries in the aqueous phase for As, Zn, Pb and Cu were found to be 99.9 %, 94.9 %, 95.4 % and 95.5 %, respectively, with a 84 % yield of bio-crude.[87] As the main goal of the study was detoxification of secondary wastes, rather than metal recovery, removal efficiencies are cited; however, no research on metal recovery from the aqueous raffinate was carried out. The HTL process has also been demonstrated to be an excellent method of recovery from Cd- and Cu-contaminated rice straw, recovering over 95 % of the metals into the solid residue phase, though this phase also contained a large amount of carbon.[88] Similarly, the hydrothermal gasification (termed supercritical water gasification) of *Pteris vittata* L., an As-hyperaccumulator was examined. The biomass, which contained elevated levels of As, Pb, Cd and Zn, was processed at between 395–445°C and up to 270 bar. Like HTL, the metals could be collected from the solid phase, with up to 80 % of the As partitioning into this phase. However, while only 50 % of the Pb partitioned into the solid phase, the authors noted that this was in a far more stable oxidised form.[89]

We recently assessed the integration of heavy metal remediation from acid mine drainage (AMD) with bio-crude production *via* HTL (Fig 1a).[17] An initial set of experiments assessed HTL of *Spirulina* sp. with a synthetic wastewater containing Fe, Zn, Pb and Sn, demonstrating an increase in bio-crude oil yield in the presence of metals, potentially due to catalytic effects of the metal ions. A mixed microalgal community (mainly *Chlamydomonas* sp.) isolated from AMD was then cultured on synthetic AMD containing Fe, Zn, Pb and Cu, and successfully used as a feedstock for HTL. Notably, heavy metals partitioned exclusively into the solid residue. This typically occurred *via* the formation of metal phosphates. The metal toxins were effectively concentrated from a dilute aqueous solution into a more concentrated and easily processable form in the solid phase, suitable for recovery and/or disposal. Further to this study, we recently developed a more efficient synergistic approach for the bioremediation and products (Fig 1b).[90]

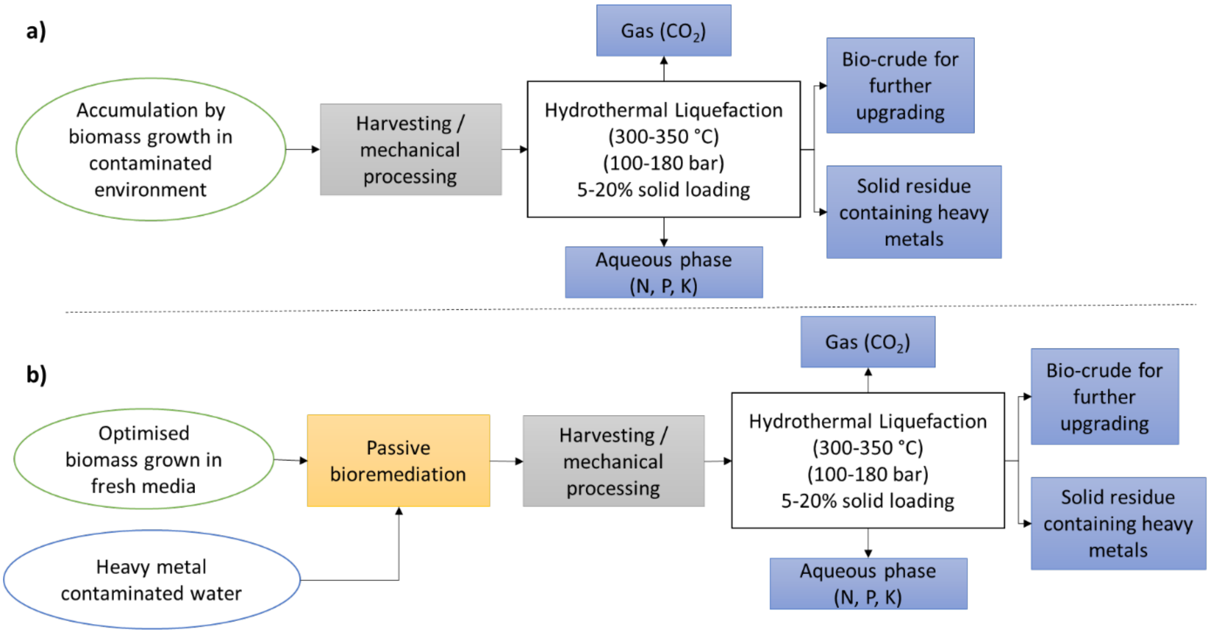

*Figure 1. Simplified flow chart demonstrating the two approaches for bio-remediation of contaminated environments using hydrothermal liquefaction*

In this study, two microalgae, *Chlorella vulgaris* and *Arthrospira platensis* (Spirulina), and two macroalgae, *Ulva lactuca* and *Sargassum muticum*, were used as passive bioremediation agents for the metals Ni(II), Zn(II), Cd(II) and Cu(II). The metal-contaminated biomass was then processed using hydrothermal liquefaction. Whilst the remediation efficiency depended heavily on species, over 99%

of the target metals partitioned to the solid phase products, predominantly as phosphates, while bio-crude production was maintained. This metal recovery was far in excess of any other recovery system to date. Combining a high P-containing biomass, such as microalgae or macroalgae, with hydrothermal liquefaction is a potentially highly effective method of remediating contaminated wastewaters. The recovery and partitioning of the target metals coupled with bio-crude oil formation (as well as the production of a nutrient-rich aqueous phase for use as a potential agricultural fertiliser) improves the economic viability of the process, thereby subsidising the environmental clean-up and contributing to a sustainable circular bioeconomy.

# 4 Conclusions

Phytoremediation and phycoremediation are effective methods of removing metal contaminants from soil and water using biomass. To aid the economics of remediation, biomass can subsequently be converted into second generation biofuels. A range of fuel conversion methods have been demonstrated, however, most technologies to date, such as direct combustion or anaerobic digestion, ultimately release metals back into the environment at the point of energy generation or in one of the marketable products. Pyrolysis is a promising alternative, where bio-oils can be produced and up to 80 % of the target metals deposit in the char phase. However, an alternative thermochemical route, hydrothermal liquefaction, has been demonstrated to be highly effective for converting microalgae, macroalgae and higher plants used in the remediation of heavy metal-contaminated water, generating bio-crude for upgrading to advanced biofuels, and depositing the majority of the metal contaminants in the solid residue for ease of recovery or further disposal. In summary, heavy metal pollution is a global environmental burden which needs innovative, scalable and local solutions; solar-driven, natural biological systems coupled with thermochemical processing offer a promising solution and show great potential to lighten this load.

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