RESEARCH

Hyperaccumulation of lead using *Agrostis tenuis*

Lorna Anguilano[1*], Uchechukwu Onwukwe[1], Aghis Dekhli[2], Susanna Venditti[1], Danny Aryani[1] and Alan Reynolds[1]

Abstract

In recent years the quest for a circular economy approach and the upcycling of secondary raw materials have been pushed in the global political agenda. Increased interest has been taken by the recovery of materials from sludges, brines, contaminated waters and other media, all included in the larger umbrella identified as "low-grade" mineralisation. Contaminated soils have an interesting role in this process, and various methodologies have been developed using chemical, bacteriological and pyrometallurgical cleaning procedures. However, these procedures all involve the movement of high volume of materials and the disruption of the industrial landscape; furthermore, they often require the use of hazardous solvents and high energy processes. This work proposes to identify less impactful methods aimed at the recovery of metals from mining areas while preserving the landscape and avoiding environmental impacts such as the increase of CO_2 for transport and increase hazard through use of solvents, this takes particular importance in areas of industrial heritage status. In particular, this work focuses on the use of *Agrostis tenuis*, an autochthonous species in mining areas of the UK, as a "mining tool" for the removal of lead. The selection of this plant is due to its visually non-invasive nature, as the plant is already present in these areas, it doesn't grow above 10/15 cm and it also grows very fast and can be easily harvested with existing agricultural equipment. The experiments and results presented in this paper indicate: (1) Agrostis Tenuis shows the ability to adjust to contamination and increase its accumulation capacity; (2) the metal collected by the plant is fully transposed in the aerial part of the plant in a stable compound form and can consequently be harvested and further processed.

Keywords: Phytomining, Phytoremediation, Hyperaccumulators, *Agrostis*, Lead contamination

Introduction

Hyperaccumulator plants can accumulate high concentration of contaminants (especially metals) in their shoots without showing significant signs of toxicity (Ebbs et al. 1997; Thijs, et al 2017); the term was coined by Brooks and co (1977) to indicate plants that uptake large amounts of heavy metals from the soil, a behaviour contrary to that of excluder plants. In quantitative terms, the level of metal concentration achievable by hyperaccumulators is metal-dependent. A plant is classified as

hyperaccumulator if it achieves: 10,000 mg/kg dry weight for Mn and Zn concentrations, 100 mg/kg dry weight for Cd, and 1000 mg/kg dry weight for As, Cu, Co and Ni (Asad et al. 2019). Aside from the "high" metal concentration that these plants can achieve, another intrinsic feature is the ability to translocate the metals from their roots system to their shoots (Asad et al. 2019). The main characteristics of plant hyperaccumulators are detailed by Ernst (2005) as:

- A well branched root system;
- High transfer/efficient translocation from root to shoot;
- High capacity of metal accumulation in the aerial part of the plant without hindering growth;

*Correspondence: lorna.anguilano@brunel.ac.uk

[1] Wolfson Centre for Sustainable Materials Development and Processing, Brunel Univeristy London, Kingston Lane, Uxbridge UB8 3PH, UK
Full list of author information is available at the end of the article

- Low transfer of the metals to the seeds of the plant.

These characteristics make hyperaccumulators the ideal candidates for phyto-remediation and even phyto-mining (Thijs, et al 2017). Hyperaccumulating mechanism has been studied thoroughly for plants accumulating nickel, where approximately 350 taxa are known to accumulate between 1000 and 38,000 mg/kg of dry leaf biomass (Reeves 1992). However, not all metals have the same level of scientific interest and significantly fewer taxa have been identified for the hyperaccumulation of lead. Studies of herbaceous species growing in mining areas have indicated that lead accumulates 600 times more in some of the grass species (Yanquan et al. 2005). In comparison, studies showing the accumulation in bush-like plants indicated a much lower accumulation ability (Yanqun et al. 2004). In Wales, UK, *Agrostis tenuis* has been recorded in mining areas as resistant to lead and zinc poisoning (Bradshaw 1952; Gregory and Bradshaw 1965), particularly at the Welsh sites of Goginan mine and Parys Mountain—where the relative concentration of lead was estimated at 3250 and 1600 ppm respectively, this is in comparison for instance, with the 6000 ppm of the mines in Yunnan (Robinson et al. 2006). *Agrostis* fits well with the characteristics of an hyperaccumulator, however its accumulation ability is limited to the first few centimetres of soil.

In the UK, around 200,000 sites have been identified as potentially at risk of soil contamination (Crane et al. 2017), but the problem is also recognised worldwide with an extent of 3.5 million sites identified as being potentially at risk of contamination in the EU and half a million recognised as highly contaminated and requiring remediation (Mahar et al. 2015), hence the challenge is wide spread with the levels and combination of contaminants being highly variable.

Examples of the level of contamination present at ancient mining sites are given by the assessments of the ancient mining activities in Wales where a lead concentration of 4 wt % in the Frongoch and 0.9 wt % in the Parys Mountain mine (Crane et al. 2017) was discovered.

Environmental Agency data (McGrath and Zhao 2006) indicates a maximum concentration of lead in fine loamy sediments in close proximity to ancient metal mines reaching above 16,000 ppm and averaging 3500 ppm in coarse and fine silty sediments, fundamentally evidencing extensive diffusion of lead contamination due to water percolation through open adits and leachate from tailings: enriching the loamy and silty sediments of waterbeds in proximity of the mines.

The literature indicates that 14 species of hyperaccumulators have been identified for lead (Mahar et al. 2015), with the four main species being: *Betula occidentalis* (1000 mg/kg d wt) (Koptsik 2014), *Brassica juncea* (10,300 mg/kg d wt) (Ernst 2005), *Medicago sativa* (43,300 mg/kg d wt) (Ernst 2005) and *Thlaspi rotundifolium* (8200 mg/kg d wt)(Wenzel and Jockwer 1999); however, it is always best practice to evaluate the presence of autochthonous species in abandoned mining areas to ensure the avoidance of environmental issues arising due to the use of exotic species becoming invasive in the long term (Li 2006). For this study, the correspondence of lead contamination and the presence of grass hyperaccumulators and in particular *Agrostis* in areas such as the discharges at Cwmystwyth mine (Ceredigion, Wales) shows a potential route for the use of this common taxon for the phytoextraction of metals. *Agrostis* is known for the multi-selective accumulation of Cd, Cu, Mn, Ni, As, Pb and Zn (Gregory and Bradshaw 1965; Li 2006), a summary of the types of *Agrostis* species and the metals accumulated is presented in Table 1. In particular, *Agrostis* grown on Pb-rich soil shows the preferential formation of chloropyromorphite ($Pb_5(PO_4)_3Cl$) in the roots of the plant (Cotter-Howells et al. 1999). The formation of this compound within the roots indicates a way forward for the stabilization of the metal contaminant in the soil and for this specific task *Agrostis tenuis* Sibth is already commercially available (Prasad and De Oliveira Freitas 2003). Although other plants species like *Thlaspi alpestre* and *Minuartia verna* have been recorded as establishing successful populations on metal rich soils such as "the acidic [soils] in Central Wales and Snowdonia", the almost consistent proliferation of *Agrostis tenuis* increases its attractiveness for use in lead bioremediation (Jowett 1964) and potential phytomining practices. The prevalence of *Agrostis tenuis* and *stolonifera* was also observed near the metal refineries at Prescot, where heavy metal aerial pollution and a Cu concentration in the soil of 4000 ppm was detected. The *Agrostis* species were shown to evolve increasing metal tolerance by selecting for metal tolerant genotypes that could thrive. It was observed that although a large number of plant species were present around Prescot before pollution, the *Agrostis* species were the only genus able to develop the genetic variability required to survive in such heavily polluted conditions within a relatively short period of time (sometimes within a single generation)(Wu et al. 1975).

Overall, *Agrostis* shows great capabilities to adapt and increase its accumulation capacity under stress from soil pollution, exhibiting a major advantage as a versatile hyperaccumulator in soils displaying different metal mixing (association of metals) and metal concentrations (ratio and absolute concentration of the different metals) (Austruy, et al. 2013). The innovation of this study is to indicate the mechanism and the speed of adaptation of Agrostis in lead contaminated soils. In

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Table 1 Reference to Agrostis spp

Agrostis type	Metal extracted	References	Type of trial
Capillaris	Cd, Cu, Mn, Ni, Pb, Zn	(Ernst 2005)	In situ
Castellana; Tenerrima	As	(Asad et al. 2019)	Literature
Tenuis; Stolonifera	Pb, Zn	(Li 2006)	Literature
Stolinifera; Capillaris	As, Cd, Cu, Pb, Zn	(Pérez-de-Mora et al. 2006)	In situ; Containers
Spp	As; Cd; Cu; Pb; Zn	(Boshoff et al. 2014)	In situ
Capillaris	Pb	(Rodríguez-Seijo et al. 2016)	In situ
Capillaris	As, Pb, Cu, Cd, Sb	(Austruy et al. 2013)	Containers
Spp	Cu; Zn; Pb	(Mahar et al. 2015)	Literature
Tenuis	Zn; Pb	(Wenzel and Jockwer 1999)	Literature
Capillaris; Canina	As; Cu; Pb; Sb	(Bech et al. 2016)	Literature
Durieui	Pb	(Fernández et al. 2017)	In-situ
Capillaris	Pb	(Miransari 2011)	Literature
Capillaris	Pb	(Chaney et al. 1997)	In-situ
Capillaris	Pb	(Tangahu et al. 2011)	Containers
Capillaris; Gigantea	Cu	(Lange et al. 2017)	Literature

As hyperaccumulator in the literature, indicating the type of Agrostis, the metal/s targeted and the type of experimental setting. When "Literature" is indicated it means that the authors did not perform an experiment but they are reporting previous data from the literature themselves

particular, differing from pervious literature indicating the selection of metal tolerant genotypes as a route of adaptation, this study indicates that commercially available seeds already show this capability.

Furthermore, the direct formation of stable lead salts as a way to store and effectively avoid the toxicity of the metal by *Agrostis* implies that a simple removal method of the lead compound from the harvested biomass is feasible, indicating a route for effective removal of the contaminant from the soils. Of course, this route would be viable only if the salts are present in highest concentration in the harvestable shoots of the plants.

The aim of this paper is then to evaluate the potential of the use of commercially available Agrostis as hyperaccumulator for phytomining purposes due to its visually non-invasive nature, ease of adaptation and fast growth. To achieve this aim the authors have set the following objectives:

- Understand the capability of Agrostis to cope with exceedingly highly contaminated soils (assessed using experiment 1);
- Understand the capability of Agrostis to improve its hyperaccumulator effects through adaptative behaviour during one growth cycle (assessed using experiment 2);
- Investigate the ability of Agrostis to transpose the lead compounds to the aerial part as this is a fundamental property for phytomining processes.

This is indeed the significance of this paper as the results below can be used to indicate the level of concentration of lead in the shoots of commercially available *Agrostis tenuis* (as used in this study) and their composition with the aim of extracting metal salts (from the soil) and stabilising them within the plant. This could be a method not only for remediating soils contaminated by industrial and mining activities, but also tap into a secondary raw material source that can lessen the demand for virgin metals and the environmentally damaging activities linked to their sourcing.

Materials and methods
Experimental Settings
Agrostis tenuis commercially available seeds (REF 6–1836) were planted in off the shelve multi-purpose compost (Verve). The seedlings were kept within a controlled environment with a temperature of 22 ± 0.5 °C using cycles of 12 h of light (T5 light wave, 240 V AC ~ 50 Hz, 216 W 1A) and 12 h of darkness.

Two experiments were performed synchronously
In experiment 1, 500 g of soil was prepared and 3 g of seeds were evenly distributed in the soil. 10 ml of 0.1 M of $Pb(NO_3)_2$ were administered every other day until the 50th day to monitor the plant growth in the presence of cumulative lead dosage (which would reach a maximum of 5 g and mimic the highest concentration of lead measured by the authors in the Cwmystwith mine -1700 ppm)

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and the changes in metal accumulation of the plant during the adjustment to the enrichment in metal concentration in the soil throughout the plant growth. These plants were designated as Pb-CU-AT (Lead- Cumulative dose-*Agrostis tenuis*), the cumulative plant biomass. 4 replicates of this experiment were running concurrently.

In experiment 2, 500 g of soil was prepared and enriched with 30 g of $Pb(NO_3)_2$, delivered via an acidified solution of the salt. 3 g of seeds were evenly distributed in the soil and the plant growth was monitored up to 50 days. The choice of 30 g is to exacerbate the lead concentration found in the mine of Cwmystwyth as analysed by the authors (maximum 1700ppms) and mirror situation such as the concentrations indicated by McGrath and Zhao, (2006), Shi et al. (2021), in the UK and in China respectively (to show the diffusion of such concentrations globally). These plants were designated as Pb-EN-AT (Lead- Enriched dose- *Agrostis tenuis),* the enriched plant biomass*.* This experiment was done in 3 replicates and was aimed at understanding the reaction of plants in high metal concentration in the soil.

A *blank experiment* was also run to ascertain the baseline behaviour in the absence of any external contamination. Here, 500 g of soil was prepared and seeded with 3 g of seeds. They were designated as AT.

In the course of the experiments, a portion of the plants were harvested after 20 days to ascertain the Pb concentration in the aerial portion of the biomass.

Analytical methods

Analyses of the harvested plants were carried out using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), X- Ray Fluorescence (XRF), Scanning Electron Microscopy coupled with Energy Dispersive X-Ray Spectroscopy (SEM–EDX), Transmission Electron Microscopy (TEM) and Fourier Transform Infra-Red spectroscopy (FTIR). The ICP-OES utilised is located within the facilities of the Institute of Health, Medicine and Environments (IHME) at Brunel University, while all the other analytical techniques utilised for this work are housed within the Experimental Techniques Centre in Brunel University.

Soil pH calculation was carried out using a Hanna Instrument™ Hi 2550–02 pH benchtop meter. The methodology was a standard soil sampling procedure where 10 g of oven dried soil (333 k) was mixed with 100 ml of deionised water and then allowed to sit for 30 min (Carter, Gregorich and Gregorich, 2007). pH measurements were then carried out after previously calibrating the pH meter with standard buffer solutions of pH 4, pH 7 and pH 10.

SEM analyses were performed using a Zeiss™ Supra VP35 Field Emission Gun SEM equipped with an EDAX Ametek™ Octane Super EDS detector. Imaging was done on the Smart-SEM software.

To carry out SEM analysis, 1 cm sections were cut with a scalpel from the leaf blade of the harvested biomass and were then placed on an aluminium stub covered with conductive carbon tape Fig. 3h. The samples were then sputter coated for 120 s with a thin layer of Au under vacuum using a Quorum™ sputter coater.

TEM-EDS analyses were performed to analyse the potential intracellular accumulation of Pb within *Agrostis tenuis*. The sample was prepared for TEM analysis using the following procedure: the harvested plant was first flash frozen in liquid nitrogen (≈77 K) and then freeze dried for 12 h at ≈218 K using a ScanVac Coolsafe, afterwards the freeze-dried samples were cut into smaller sections and fixed with 5 mL of 2% glutaraldehyde solution for 12 h at 277 K. The fixed biomass was then washed in deionised water 3 times before being immersed in a 5 mL 30% ethanol solution for 15 min. The biomass was then transferred to a 5 mL 50% ethanol solution, and then a 5 mL 70% ethanol solution for a further 15 min, and finally a 100% ethanol solution for 30 min. The ethanol dehydrated biomass was then imbedded in a propylene oxide: agar Low viscosity resin (1:1) for 12 h. The mix was then exchanged for pure resin and polymerised in an oven at 333 K for 24 h. Ultrathin sections of the embedded samples were obtained using an RMC Power Tome PC ultramicrotome and then floated onto TEM copper grids. The procedure followed for dehydration and embedding were modified from the methods used by Agar et.al (1965).

The sections were examined using a Jeol 2100 TEM at 80 kV, with energy dispersive X-Ray spectroscopy (EDS) carried out using a Thermo Scientific™ Ultra dry I detector. The software used for imaging was the Gatan microscopy suite® GM3 software, and for EDS analysis, the Thermo Scientific™ NSS EDX analysis software was used.

Elemental analysis using XRF was done. Before testing, samples were dried at 333 K for 48 h and then ground using a pestle and mortar. For soil matter, 10 g of the dried sample was collected and mixed thoroughly with 0.5 ml of 10% PVA solution. The mixed sample was then loaded into an XRF pellet forming die. The die was then pressed with a pressure of 10 ton using a Specac hydraulic press. A solid pellet with a diameter of 30 mm is formed which is then dried at 60 °C overnight. This sample is ready to be analysed by XRF.

For plant material, 0.5–2 g of dried sample was used as it was.

XRF analysis was carried out using a Rigaku NEX DE benchtop XRF (60 kV) in Helium. The prepared soil and plant samples were placed in XRF cups, backed by a mylar film, before undergoing examination using a

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Fundamental analysis profile. Pre-calibration of the XRF was carried out using the reference calibration standard MCA R-2128. Results obtained were checked using ICP-OES analysis.

Similar to the XRF analysis, for ICP-OES, the samples were dried and then homogenised using a pestle and mortar. For soil matter, 10 mL of nitric acid (>68%) was added to 0.5 g of soil in a MARS microwave Xpress plus vessel (TFM with an inner Teflon lining). These samples then underwent digestion in a MARS microwave using a power setting of 1200–1800w, with a ramp time of 5 min and 30 s and a hold time of 4 min and 30 s, reaching a temperature of 175 °C. On completion of digestion, the samples were centrifuged for 10 min at 3000 rpm.

For plant material (shoot), 10 mL of nitric acid (>68%) was added to 0.5 g of sample. These samples then underwent digestion in a MARS Xpress plus vessel using a power setting of 290–1800w, with a ramp time of 20 min and a hold time of 10 min, reaching a temperature of 200 °C. On completion of digestion, the samples were centrifuged for 10 min at 3000 rpm.

The centrifuged samples were placed in a tube and diluted to a volume of 15 mL. Calibration using Pb metal standard solution diluted to 0.05, 0.1, 0.5, 1, 2, 5, 10 mg/L was carried to ensure $r^2 > 0.995$. Finally, sample analysis was done using the Perkin Elmer® Optima 5300DV ICP-OES.

FTIR analysis was used to analyse the chemical changes in *Agrostis tenuis* before and after treatment with Pb were carried out on the samples. The samples examined were rinsed with deionised water and dried in air overnight. They were analysed using a Perkin Elmer® spectrum one FTIR with an ATR accessory. A spectrum was collected in the range 3000 cm^{-1} to 500 cm^{-1} at 30 accumulations.

Results

Soil pH analysis

The pH values of the soil samples were found to be fairly acidic with figures of 4.8, 5.4 and 4.5 recorded for AT, Pb-CU-AT and Pb-EN-AT respectively. It appears that the addition of $Pb(NO_3)_2$ leads to some alteration in the pH properties of the soil which could directly be due to the expected acidic profile of lead nitrate salts that could in turn affect the soil microbiome, eliciting a biotic response.

Plant growth analysis

The growth of the plant was monitored by measuring the overall plant height. This involved measuring the visible portion of the plant shoot above the soil to the tallest part of the blade.

The growth progression of the plant sown on soil intentionally imbued with lead nitrate (Pb-EN-AT &

Pb-CU-AT) and without any metal addition (AT) shows that there is a difference in the growth profile (Fig. 1). It appears that without the inclusion of any contaminant, AT on average was able to reach a maximum height of 18 ± 2.0 cm after 50 days of growth. This was in contrast to Pb-CU-AT (14.6 ± 3.2 cm) and Pb-EN-AT (8 ± 3.0 cm), both of which showed a decrease in the maximum achievable height after a 50 day period—the latter experiencing a drop of 57%, against a 22% of the former.

Aside from the height attainable being affected, the apparent toxicity of Pb is evident in the different germination and subsequent growth behaviour of the experiments as seen in Fig. 2. The inhibition in the germination of seeds can be witnessed when comparing the amount of plant cover between Pb-CU-AT and Pb-EN-AT. Although there is still growth in both instances, the plant cover of Pb-CU-AT is more robust than in Pb-EN-AT which is likely due to the different starting levels of Pb in the potting medium.

The tolerance indices (TI)–a measure of a plant's ability to tolerate a given stressor – was calculated using the average length of the shoot, as the growth parameter, and were shown to both be < 1.

$$TI = \frac{[Growth\,parameter]Pb\,contaminated\,soil}{[Growth\,parameter]Control\,soil}$$

Pb-CU-AT and Pb-EN-AT had values of 0.78 and 0.43 respectively. TI values < 1 are thought to indicate that the plant is stressed, thus experiencing a reduction in biomass.

Further observation following harvesting of only the aerial part and allowing the regrowth has yielded interesting results with regards to improved growth (Fig. 1b) and TI. The new mean shoot lengths for Pb-EN-AT and Pb-CU-AT are 20.0 ± 3.6 cm (previously 8 ± 3.0 cm) and 22.4 ± 2.0 cm (previously 14.6 ± 3.2 cm) respectively. Though the TI had previously returned values < 1, suggesting that both Pb-EN-AT and Pb-CU-AT had experienced a decrease in biomass on exposure to Pb, in this regrowth, there appears to be an improvement in the TI of the experiments: Pb-EN-AT (1.0) and Pb-CU-AT (1.1). This improvement in the tolerance seemingly within a 50 day period is an excellent result and demonstrates the adaptability of *Agrostis tenuis* and consequently its suitability as a soil remedial tool.

SEM–EDS analysis

The morphology of the roots and shoot of the plants were imaged using SEM. The shoot of *Agrostis tenuis* shows a typical cuticular surface found in grass species, with regular folding having cuticular and epicuticular wax particulates scattered across its shoot façade (Fig. 3g). EDS analysis reveals a generally high carbon content,
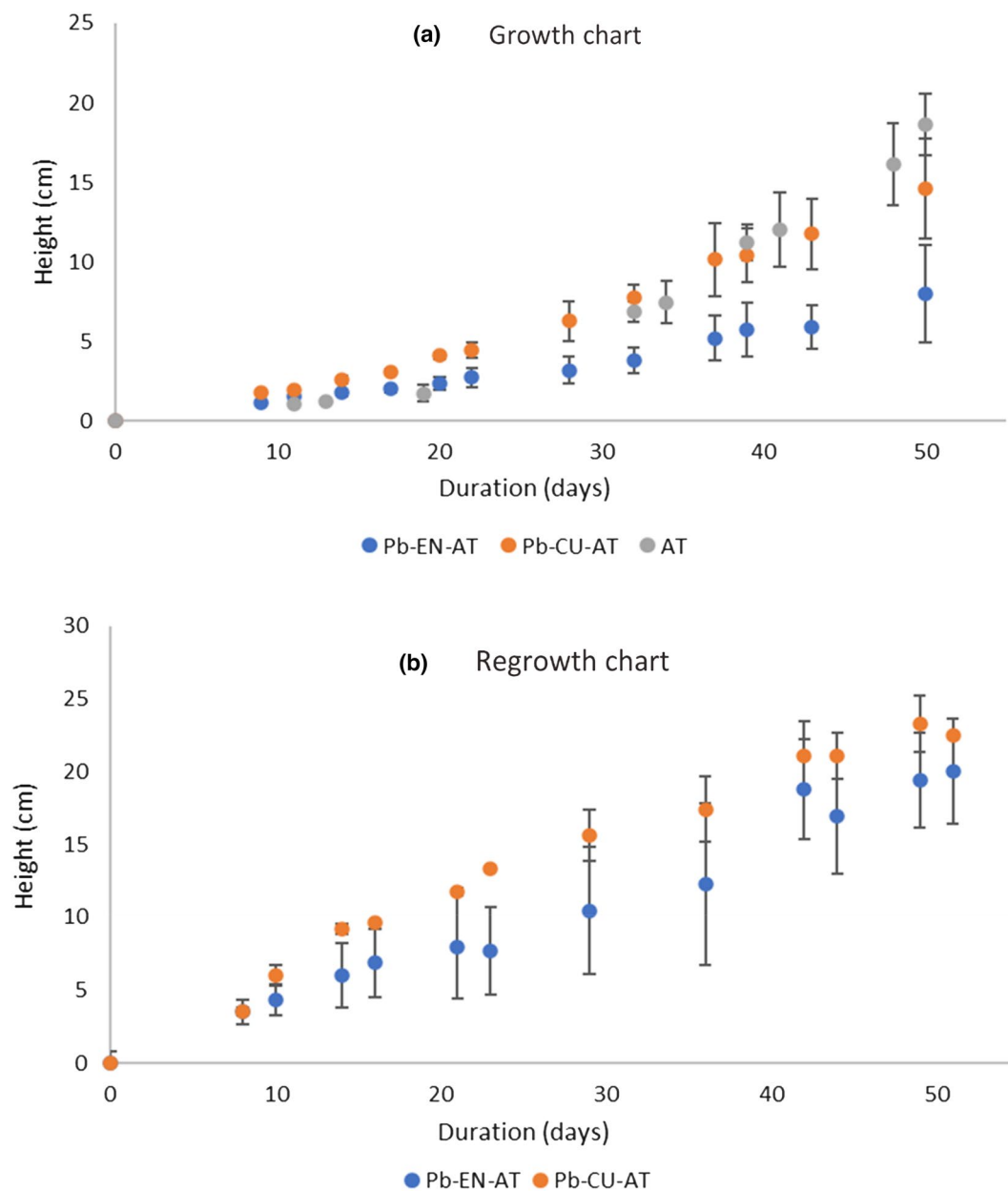
Fig. 1 Growth chart of Pb-EN-AT, Pb-CU-AT and AT over a 50 day (**a**) and a regrowth chart of Pb-EN-AT and Pb-CU-AT, after 1st harvest, covering a 50 day period (**b**)

confirming their organic identity as possible waxes and roots exudates. Additionally present are Mg, Si, P, S, Cl and K, all of which are not out of place in a plant undergoing normal metabolic functions.

Variations in the external characteristics of both the roots and shoots of Pb-EN-AT and Pb-CU-AT appear to be almost visually absent. The roots system, however, does seem to exhibit some disparity with the density of root hairs. We see foreign particulates scattered across

its surface–despite the initial rinsing of the roots. Some of these sits on the surface while others appear to be more intimately bonded to its surface. Elemental analysis shows that these particulate matters contain a significant amount of lead as part of their composition (Fig. 3f).

This leads one to suspect that the particulates present on the root structure of Pb-EN-AT are formed as a direct result of the interaction with lead ions. Similarly, in Pb-CU-AT, more particulates are found on the roots
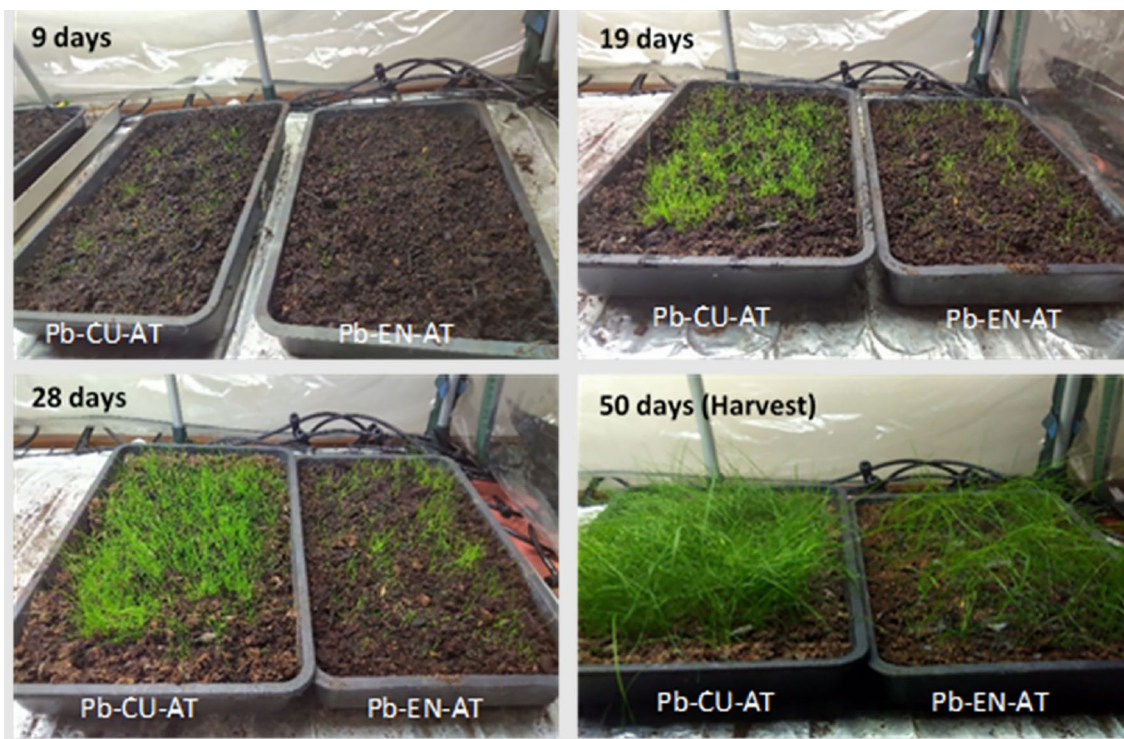
Fig. 2 Pictures showing the shoot growth progress of experiment 1 (Pb-CU-AT) and experiment 2 (Pb-EN-AT) over 50 days

meaning that the precipitation of lead containing compounds are uniform between Pb-CU-AT and Pb-EN-AT, thus reinforcing the extensive role the rhizosphere plays in triggering this soil remediation process. On the shoots, we also see Pb bearing particulates. (Fig. 3e).

Closer analysis of the EDS appears to show that Pb is incorporated into a matrix different from the form it was delivered as—Pb(NO$_3$)$_2$. In particular, lead-bearing particulates detected also embedded in the shoots show a composition corresponding to chloropyromorphite.

TEM-EDS analysis

Small sections of *Agrostis tenuis* were examined after extensive preparatory measures detailed in the methodology section. The TEM micrographs (Fig. 4a, b) confirms the accretive ability of *Agrostis tenuis* when exposed to heavy metal contaminants. In this case, we can see the sequestering of lead as dark contrast regions within the cellular structure of *Agrostis tenuis.* One can see parallelepipedal lead nanoparticles (highlighted in the red rectangle in Fig. 4b) lying next to the cell wall and what appears to be the actual cystol of the cell.

Closer inspection of these particulates show that they are elongated, a phenomenon also witnessed in *Salvinia minima,* primarily in the lead particles found in the plant cystol. Elemental analysis, using EDS, confirms the presence of Pb and other potential elements that co-precipitate with it.

The signals from Si, C, O (naturally present) and Cu (from the TEM grid) can be discounted. This would leave P, Pb, Cl and Ca as the relevant elements to consider as making up the composition of the particles accumulated within the plant cell. These particles show a size of < 30 nm and their elemental composition established by EDS analysis can be seen as a positive identification of the precipitation of the mineral chloropyromorphite as the ideal lead stabilising form. This also supports the EDS analysis of the particulate seen in The presence of the calcium recorded could be due to the formation of calcian pyromorphite.
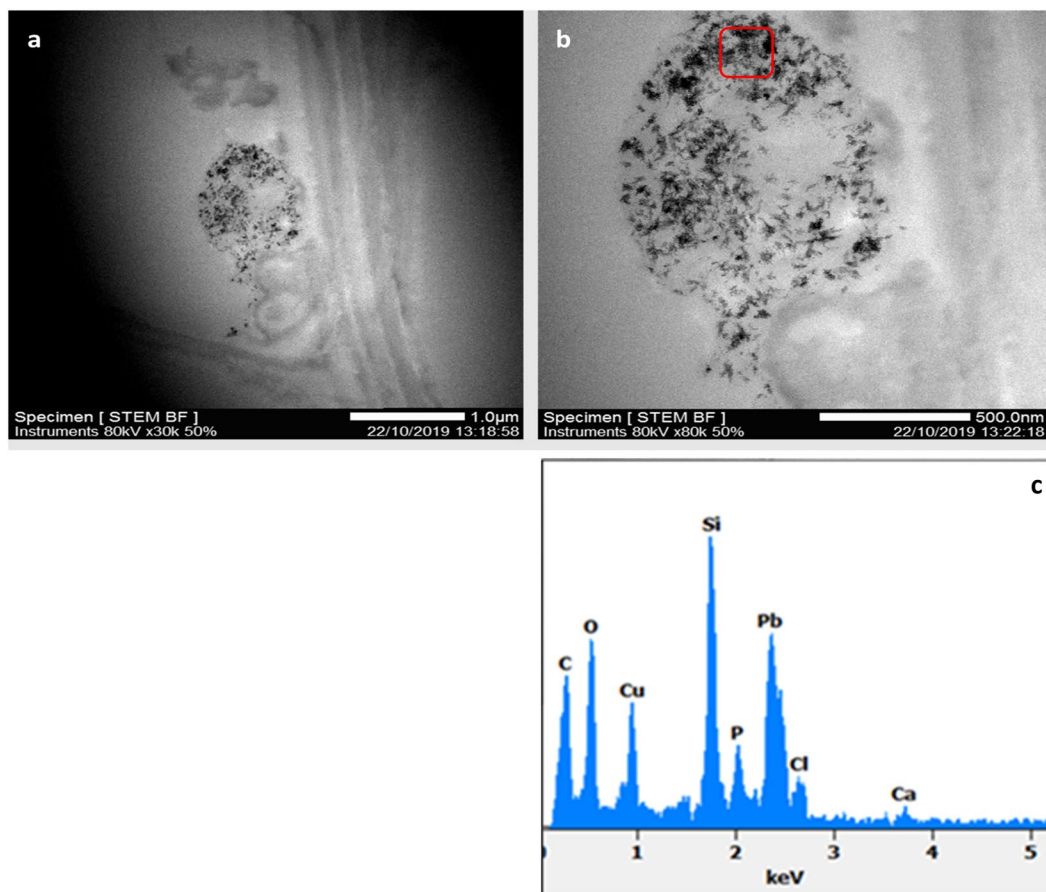
(See figure on next page.)
Fig. 3 SEM micrographs showing the surface of the shoots (left) and roots (right) of Pb-CU-AT (**a**, **b**) and Pb-EN-AT (**c**, **d**), with a typical EDS showing the presence of Pb from the highlighted particulates in the micrographs (**f**); a close up of the Pb bearing particulate is seen in (**e**); a micrograph of AT (**g**); biomass sample on SEM stub (**h**)

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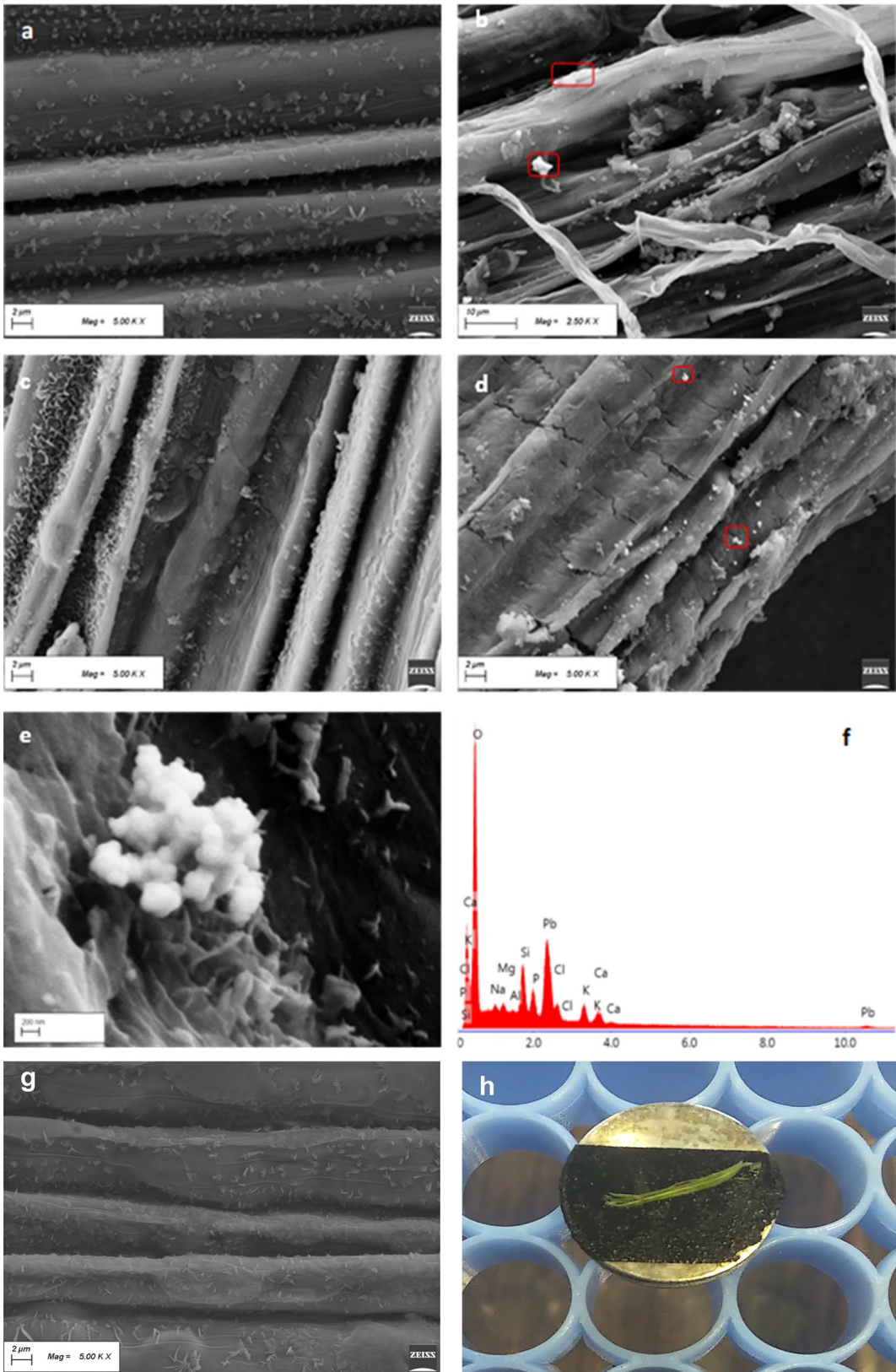
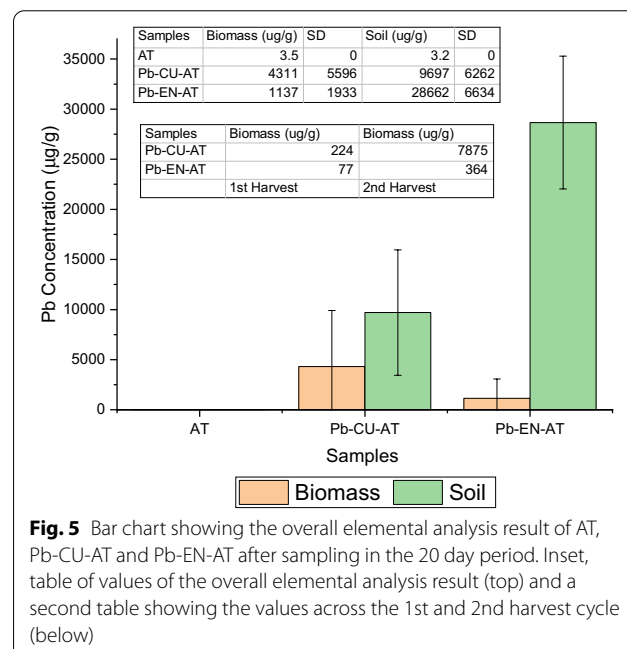**Fig. 3** (See legend on previous page.)

Fig. 4 TEM micrographs of the shoot section from the Agrostis tenuis plant exposed to lead nitrate during growth. (**a**–**c**). Sections of Pb-AT showing internal localisation of lead nanoparticles close to the plant cell wall; EDS spectrum of the selected area from (**b**) is shown in (**c**)

Elemental analysis using XRF

From the results (Fig. 5), it appears that the storing potential of the plants is quite obvious with Pb-CU-AT accumulating 4311 μg/g and Pb-EN-AT seen to take up 1137 μg/g after 20 days of growth in their respective soil medium. Interestingly, the uptake abilities showed variation between the samples, Pb-CU-AT and Pb-EN-AT. Whilst Pb-EN-AT started with the highest amount of Pb enrichment, the amount concentrated in the shoot was several orders of magnitude lower than the sample that started with a low but increasing amount of Pb added to it (Pb-CU-AT) indicating a faster ability of adjusting to a changing environment compared to the ability to adapt to a highly stressing environment within one cycle of growth.

To evaluate the phytoremediation potential and suitability of *Agrostis tenuis* for use as a hyperaccumulator, the bioaccumulation coefficient (BAC) was calculated using the values presented for the biomass in Fig. 5 and the formula below

Samples	Biomass (ug/g)	SD	Soil (ug/g)	SD
AT	3.5	0	3.2	0
Pb-CU-AT	4311	5596	9697	6262
Pb-EN-AT	1137	1933	28662	6634

Samples	Biomass (ug/g)	Biomass (ug/g)
Pb-CU-AT	224	7875
Pb-EN-AT	77	364
	1st Harvest	2nd Harvest

Fig. 5 Bar chart showing the overall elemental analysis result of AT, Pb-CU-AT and Pb-EN-AT after sampling in the 20 day period. Inset, table of values of the overall elemental analysis result (top) and a second table showing the values across the 1st and 2nd harvest cycle (below)

$$BAC = \frac{Pb\,concentration\,in\,plant\,(shoot)}{Pb\,concentration\,in\,soil}$$

Plants having a BAC of > 1 are thought to be better adapted for phytoextraction of metal contaminants to within their plant tissue.

After the first harvest, the experiments indicate a BAC value > 1 in the AT (1.09), while the BAC values for Pb-CU-AT and Pb-EN-AT are calculated at 0.3 and 0.002, respectively. However, at the second harvest Pb-CU-AT's BAC double to 0.59, while Pb-EN-AT increases one order of magnitude to 0.01. Despite both values still being below 1, this increase indicates a quick ability of Agrostis to increase its accumulation capacity.

FTIR analysis
The most prominent bands across all of the FTIR spectra seen in Fig. 6 are those that could be ascribed to the presence of the cellular wall structure primarily in cellulose (1033 cm^{-1}, 1162 cm^{-1}, 1369 cm^{-1}, 3300 cm^{-1}), and in lignin (1425 cm^{-1}, 1500 cm^{-1} and hemicellulose (1630 cm^{-1} and 1734 cm^{-1}). A robust cuticular layer is confirmed by waxes and cutine assignments at 2850 cm^{-1} and 2917 cm^{-1}. With regards to the plants that had been fed on a diet consisting of lead (Pb-CU-AT and Pb-EN-AT) and on a lead-free diet (AT), some changes appear to be affecting the band at 1370 cm^{-1}, with it broadening in Pb-EN-AT but shrinking in Pb-CU-AT. This is a

band that belongs to cellulose. Additionally, other bands undergoing further transformations, in the lead treated samples are seen at 1542 cm^{-1} and 1637 cm^{-1}, both of which being indicative of vital structural components in a cell wall, lignin and hemicellulose. A peak appears at 1542 cm^{-1} in Pb-EN- AT, while a broadening of the peak at 1637 cm^{-1} is exhibited by Pb-CU-AT and Pb-EN-AT. This disruption of bands linked to the biomass cell wall components are telling of the role of the cell wall in stabilising the accreted lead.

Discussion
From the results obtained thus far, it is apparent that *Agrostis tenuis* is not only able to survive in the presence of heavy metal contamination but is in fact able to successfully thrive. Starting with the height profile of *Agrostis tenuis* grown in the absence of Pb (AT) against those grown in a Pb medium (Pb-CU-AT and Pb-EN-AT), one of the limitations known to hyperaccumulators, i.e., a lag in growth rate becomes quite obvious (Tangahu et al. 2011). This is a common feature noted amongst hyperaccumulators and indeed in plants in general that have been exposed to heavy metals above a certain threshold. Toxic effects such as: a reduction in the population of useful soil microorganisms that would normally assist in the breaking down of organic matter to release essential nutrient for plant growth, a substitution of essential cations with heavy metals, inhibition of enzymic activities
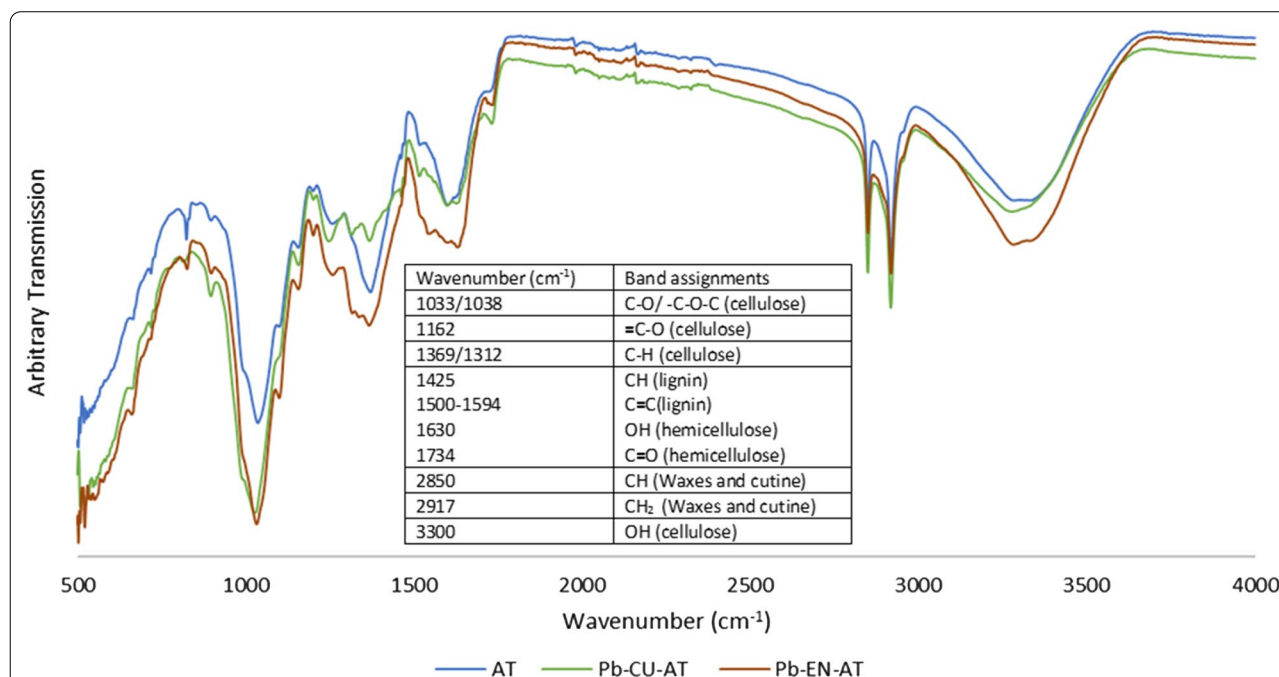
Fig. 6 FTIR spectra of the shoot section of AT, Pb-CU-AT and Pb-EN-AT with inset of infrared band assignments *(Alonso-Simón et al. 2011; Traoré et al. 2018; Chakradhari et al. 2019)*

and proliferation of damages rendered by the increase in oxidative stress due to the presence of the heavy metals could all account for inhibition in the growth rate witnessed in Pb-CU-AT and Pb-EN-AT and are all perfectly within previously observed behavioural pattern (Van Ginneken et al. 2007; Chibuike and Obiora 2014). It seems as though the issue of the lagging growth rate might have less to do with the soil chemistry, at least in terms of the pH which appeared to show that both the plant grown in a soil free of lead and that grown with lead had similar values. It appears that the initial concentration of lead in the soil is the most important factor impacting growth; thus, while the Pb-CU-AT experiment shows plants ability to adapt in spite of the continuously increasing pollution, growing at almost the same rate as within the clean compost, Pb-EN-AT shows a lower germination rate, and also a limited final height. Indeed, this inhibition of germination in plants exposed to lead and other traits like roots elongation, chlorophyll production, seedling development, cell division etc. are just some of the characteristic traits that show the toxic effects of heavy metal contaminants (Amin et al. 2018). However, this effect appears to be mitigated within the second cycle of growth (see Fig. 1b) which confirms the high adaptability of this plant to stressful conditions.

Additionally, the appearance of lead-based deposits within the plant structure satisfies the phytoextraction function of using *Agrostis tenuis* and confirms its suitability for the decontamination of Pb polluted sites. From the particles within the cellular structure of *Agrostis tenuis* (Fig. 4), one can see that there appears to be a preference for lead storage closer to the plant cell wall which is likely why we see perturbation of bands linked to the plant cell wall, see the FTIR analysis section. This is in contrast to what was seen in the case of the plant species *Salvinia minima* (Castro-Longoria et al. 2014), where *Salvinia minima* was shown to accumulate more lead within the cell wall structure (middle lamella, primary and secondary wall) and less in the actual cystol of the cell. In the case of *Aspergillus* species, it was thought that this preference for accumulation in the cell wall was due to an ionic interaction; the negative factions present in the cell wall (carboxylate groups) acted to attract the positively charged lead ions to the cell wall structure (Pavani et al. 2012). Although accumulation in the cell wall is one of the many mechanism plants have to cope with heavy metal stress, usually its first defence, aggregation in the cystol or rather within a vacuole does appear to occur; Fig. 4a, b) and is thought to be linked to the presence of phytochelatins (Estrella-Gómez et al. 2009; Fahr et al. 2013; Castro-Longoria et al. 2014). The existence of these lead-based deposits and Fig. 4) in the shoot means that *Agrostis tenuis* was able to take up Pb via its root

system and translocate it to its aerial portion, thus satisfying the description of phytoextraction, which is "the uptake/absorption of contaminants by plants into the above ground portion of the plants (shoots) that can be harvested " (Tangahu et al. 2011). The movement to the aerial portion, a trait beneficial to the soil clean-up process, is likely assisted by an evapotranspiration process which again is thought to be more enhanced in hyperaccumulator due to their ability to maintain a metal "shoot-to-root concentration" of above one (Tangahu et al. 2011), though as we have seen this condition is not fully achieved within one life cycle of the plant. SEM showed that there were significant Pb containing particulate primarily on the roots this appears to be quite similar to what occurred in *Phyllostachys pubescens* (Liu et al. 2015) and *Eichhornia crassipes* (Baruah, Hazarika and Sarma, no date), both of which showed morphological magnification of the uptake and localization of Pb in the root and shoot system. The appearance of these inclusion both in the roots and shoots in the aforementioned instances and within *Agrostis tenuis* shows that the plants are able to store this metal extracellularly and intracellularly (Fig. 4).

Elemental analysis further substantiates the accumulating abilities of the *Agrostis tenuis* used in this study. While there does appear to be some latent Pb content in the starting soil AT (< 3.2 μg/g), the amount remains lower than the mean concentration of Pb found in urban soils in the UK (110 mg/kg [μg/g]), from soils in London gardens (654 mg/kg[μg/g]), and also lower than the proposed safe levels (600 mg/kg) found in areas frequented by children (Madhavan et al. 1989; Ross et al. 2007). The same goes for the plant tissue, which here has been evaluated as having a value (3.5 μg/g) that is lower than mean urban herbage values for the UK (8.6 mg/kg[μg/g]).

Furthermore, the ability of the plants to store in multiple locations might have caused some underestimation in the results (Fig. 5), as only the shoot was used to ascertain the amount of Pb in the biomass. From electron microscopy we are able to see lead bearing particulates in/on the roots indicating that not all lead was readily bio-available and one part of the accumulated lead was still stored in the roots and not yet translocated in the shoots; more than one growth cycle of the plant might be required to obtain a full translocation. Indeed, the case of Phyllostachys pubescens (Liu et al. 2015) showed that the calculated bioaccumulating factor was significantly augmented by the higher amount of lead accumulated in the roots. The same could be said for water hyacinth where the lead content in the roots were higher than in the petioles or leaves (Malar et al. 2016). The indications gathered by these experiments mean that in one life cycle *Agrostis tenuis* accumulates Pb when subjected to low concentration in the soil (AT) and adapts to higher concentrations

when these are slowly increased (Pb-CU-AT) although it has a slower start when the initial concentration is high (Pb-EN-AT). Regardless, the added benefit of leaving the root without harvesting is to ease the regeneration of the plants after the aerial portion has been harvested, thus continuing the remediation process and potentially increasing it through time since the adaptability of the plant is already noticeable within one cycle, as seen after comparing Pb-EN-AT and Pb-CU-AT. The improvement in the TI for Pb-EN-AT and Pb-CU-AT from < 1 to > 1 in the second growth cycle, shows the development of tolerance in the presence of Pb contamination (Audet and Charest 2007; Amin et al. 2018). This improvement is also highlighted in the BAC values for both Pb-EN-AT and Pb-CU-AT which both underwent very significant improvements after the first harvest. It is highly probable that the high concentration in the soil of Pb-EN-AT is not readily bioavailable within the first cycle of growth hence limiting the accumulation ability of the plant. This could be confirmed by the high volume of salt particles visible surrounding the roots of the plants for both Pb-CU-AT and Pb-EN-AT in While the Pb-bearing particles visible in the figure are changing their nature from nitrate to a more bio-available form, they do not have enough time to be accumulated by the plants. Further cycles of accumulation need to be trialled and examined in order to correctly establish the BAC of the plants under evaluation, and this can be established by investigating plants growing naturally in polluted sites such as Agrostis capillaris L exposed to Pb pollution due to shooting activities (Rodríguez-Seijo et al. 2016), where after examination of 13 different sites it was found that 5 of the sites exhibited BAC values > 1, with 9 sites showing phytostabilising abilities as indicated by a bioconcentration factor (BCF) also > 1. Even though the phytoextraction abilities of *Agrostis capillaris L* exceeds that of the samples studied here, the initial amount of Pb present in the soil needs to be considered. Rodríguez-Seijo et al (2016) found Pb soil concentration of 402–724 mg/kg (µg/g), due to shooting activities, these are significantly smaller than those utilised in this paper (> 9697 µg/g). Thus, while the samples used here display BAC values that would lump them in a category as moderate accumulator plants (Gawryluk et al. 2020), the reality is that the excessive amount of soil Pb (in our paper) when compared to Pb content in literature implies that the actual performance of *Agrostis tenuis,* here, moves it quite comfortably from a moderate tier to an above average tier.

The process of metal accumulation within *Agrostis tenuis* appears to involve the transformation of the intentionally inoculated lead salt, $Pb(NO_3)_2$, into the less bioavailable form pyromorphite, which is incidentally one of the more stable forms of lead salts with a solubility

(K_{sp}) of − 84.4 and a slightly higher solubility of > − 76.8 when Cl is exchanged for − OH and − F (Traina and Laperche 1999). Amongst some of the other common means of lead remediation of contaminated sites, one method of remediation is the intentional addition of phosphate to amend the soil. The premise behind this process involves the use of a source of phosphate (e.g. rock phosphate, phosphoric acid, apatite etc.) to form stable lead phosphate phases, reducing free Pb. Usually, the rapid and preferential formation of pyromorphite above all other types of phosphates occurs due to thermodynamics. The higher stability of pyromorphite in comparison to other lead phosphate phases (e.g. plumbogummite and tsumebite) results in the eventual transformation into pyromorphite (Ryan et al. 2001; Rhee et al. 2012) or calcian pyromorphite seen in Charterhouse mine (Kampf et al. 2006). Thus, the formation of pyromorphite in mine-waste, though preferred, does not readily occur unless with the presence of an ample source of phosphorus–specifically in the form of phosphates. As we have demonstrated, *Agrostis tenuis* grown without the additional seeding of the soils with phosphate has been shown to capture quite a significant amount of Pb in the form of chloropyromorphite. It helps that *Agrostis* plant roots are capable of exuding phosphatase enzymes that are capable of converting organic phosphorus to phosphates (within their rhizosphere) which can then be taken up by the plant; also, the presence of soil organism around the rhizosphere is capable of converting organic phosphorus to phosphates (Cotter-Howells and Caporn 1996). All of this makes the use of *Agrostis tenuis* quite self-sufficient, or at least without the problem of intentionally adding phosphates which normally involves prior acidification of the soil to promote the dissolution of Pb and P sources; this could inadvertently cause the leaching of other metal contaminants in the soil (Rhee et al. 2012).

As long as lead is not just sequestered but housed in a relatively stable form (pyromorphite), half of the problem of having Pb in a bioavailable format within the soil is eliminated; the transformation through the plant exuding phosphatase enzymes requires time, which is confirmed by the fact that the *Agrostis* can cope with an incremental addition of aliquots of lead salts (Pb-CU-AT), while it has a slower reaction to a larger amount of lead salt being present directly in the soil (Pb-EN-AT).

Conclusion

The literature confirms *Agrostis* spp. as a highly adaptable hyperaccumulator in a variety of metal combinations but particularly associated with the accumulation of lead. This study focuses on the evaluation of the behaviour of *Agrostis tenuis* as lead hyperaccumulator, specifically on the speed of adaptability to high lead concentration and

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how and where the lead-bearing particles are stored in the plant. In order to achieve these objects, three experiments were performed on *Agrostis tenuis* in the following conditions:

- A blank sample [AT] the plant was sown and grown on a commercially available multi-purpose compost;
- A cumulative sample [Pb-CU-AT] 10 ml of 0.1 M of $Pb(NO_3)_2$ was added to the soil on alternating days for a 50 day growth period;
- An enriched sample [Pb-EN-AT] 30 g of $Pb(NO_3)_2$ was added to the soil on the sowing day for 50 day growth period.

The growth of the plants shows (Fig. 1a) a lagging effect in the high lead concentration soil of Pb-EN-AT, while Pb-CU-AT and AT show a similar growing curve. The elevated adaptability of *Agrostis tenuis* is visible in the second growth cycle (Fig. 1b) here the curve becomes significantly similar, especially for Pb-EN-AT.

Lead bearing nanoparticles (∼ 30 nm) are visible through TEM analyses (Fig. 4) in the shoots of Pb-EN-AT and Pb-CU-AT, indicating the ability to translocate lead-bearing particles from the roots to the shoots, storing them close to the cellular walls in a chloropyromorphite form (Cotter-Howells et al. 1999)–this mineral form is less bio-available and can be present as a non-hazardous compound within the plant itself (Ryan et al. 2001; Rhee et al. 2012).

The evaluation of the quantity of lead sequestrated in the shoots of the plants within the first and second harvest cycle, and the consequent BAC calculations reveal a very interesting phenomenon. In the blank (control) experiment, the plants were able to successfully accumulate all the available lead, the same is not the case for Pb-CU-AT and Pb-EN-AT; in these samples, the significantly higher amount of lead in the soil is not completely removed within the 1st harvest but instead we witness a progressive uptick in accumulation from the 1st (Pb-CU-AT: 224 µg/g, Pb-EN-AT: 77 µg/g) to the 2nd (Pb-CU-AT: 7875 µg/g , Pb-EN-AT: 364 µg/g) harvest cycle Three phenomena can explain these data that seem to indicate a very low capacity of phytoextraction by *Agrostis*:

- The plant needs time to adapt to the stressful environment,
- The plant needs time to transform the lead in the soil to a bioavailable form in order to sequestrate it,
- The plant needs time to translocate the metal to the shoots.

These phenomena seem to take place all at the same time and the experiments conducted thus far are unable to indicate the most predominant amongst the aforementioned occurrences. Where aliquots of lead nitrate solution are added to the soil (Pb-CU-AT), the translocated lead cation can react with the bioavailable phosphate to form the more stable lead pyromorphite, this is visible in the change of nature of the lead-bearing particles present in close proximity to the root system These same particles are present in larger volume in the Pb-EN-AT, indicating that the plant is attempting to gather the lead, however, the transformation rate is slow in comparison with the overall lead concentration. This appeared to have been resolved within the second regrowth cycle (Fig. 1b), where a growth similar to Pb-CU-AT was witnessed, indicating the improved capability to cope in the high metal stress environment.

Declarations

Author details
[1]Wolfson Centre for Sustainable Materials Development and Processing, Brunel Univeristy London, Kingston Lane, Uxbridge UB8 3PH, UK. [2]Polytech Marseille-Luminy Science and Technology Park, 13288 Marseille Cedex 09, France.

References

Agar AW, Alderson RH, Basset GA, Bradley DE, Brammar IS, Glauert AM, Halliday JS, Home RW, Howie A, Philips R (1965) Techniques for electron microscopy. In: Kay D (eds) Oxford. Blackwell Scientific Publication Ltd. Oxford

Alonso-Simón A, García-Angulo P, Mélida H, Encina A, Álvarez JM, Acebes JL (2011) The use of FTIR spectroscopy to monitor modifications in plant cell wall architecture caused by cellulose biosynthesis inhibitors. Plant Signal Behav 6:1104–1110

Amin H, Arain BA, Jahangir TM, Abbasi MS, Amin F (2018) Accumulation and distribution of lead (Pb) in plant tissues of guar (*Cyamopsis tetragonoloba* L.) and sesame (*Sesamum indicum* L.): profitable phytoremediation with biofuel crops. Geol Landscapes 2:51–60

Asad SA, Farooq M, Afzal A, West H (2019) Integrated phytobial heavy metal remediation strategies for a sustainable clean environment—a review. Chemosphere 217:925–941

Audet P, Charest C (2007) Heavy metal phytoremediation from a meta-analytical perspective. Environ Poll 147(1):231–237

Austruy A, Wanat N, Moussard C, Vernay P, Joussein E, Ledoigt G, Hitmi A (2013) 'Physiological impacts of soil pollution and arsenic uptake in three plant species: Agrostis capillaris, Solanum nigrum and Vicia faba. Ecotoxicol Environ 90:28–34

Baruah S, Hazarika KK, Sarma KP (2012) Uptake and localization of Lead in Eichhornia crassipes grown within a hydroponic system. Adv Appl Sci Res 3(1):51–59

Bech J, Roca N, Tume P, Ramos-Miras J, Gil C, Boluda R (2016) 'Screening for new accumulator plants in potential hazards elements polluted soil surrounding Peruvian mine tailings', Catena (Amst). Elsevier 136:66–73

Boshoff M, De Jonge M, Scheifler R, Bervoets L (2014) Predicting As, Cd, Cu, Pb and Zn levels in grasses (Agrostis sp. and Poa sp.) and stinging nettle (Urtica dioica) applying soil-plant transfer models. Sci Total Environ 493:862–871

Bradshaw AD (1952) Populations of Agrostis tenuis resistant to lead and Zinc poisoning. Nature 169(4313):1098–1098

Brooks RR, Lee J, Reeves RD, Jaffre T (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J Geochem Explor 7:49–57

Carter MR, Gregorich EG, Gregorich EG (2007) 'Soil reaction and exchangeable acidity in soil sampling and methods of analysis. CRC Press, Boca Raton

Castro-Longoria E, Trejo-Guillén K, Vilchis-Nestor AR, Avalos-Borja M, Andrade-Canto SB, Leal-Alvarado DA, Santamaría JM (2014) 'Biosynthesis of lead nanoparticles by the aquatic water fern, Salvinia minima Baker, when exposed to high lead concentration. Colloids Surf B Biointerfaces 114:277–283

Chakradhari S, Deb MK, Patel KS, Martín-Gil J, Towett EK, Martín-Ramos P (2019) Evaluation of the phytochemical and mineral characteristics of some selected sapotaceae plants. Eur J Med Plants. https://doi.org/10.9734/ejmp/2019/v27i430122

Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS, Baker AJM (1997) Phytoremediation of soil metals. Curr Opin Biotechnol 8(3):279–284

Chibuike GU, Obiora SC (2014) Heavy metal polluted soils: effect on plants and bioremediation methods. Hindawi Publishing Corporation, Appl Environ Soil Sci

Cotter-Howells J, Caporn S (1996) Remediation of contaminated land by formation of heavy metal phosphates. Appl Geochem 11:335–342

Cotter-Howells JD, Champness PE, Charnock JM (1999) Mineralogy of Pb-P grains in the roots of Agrostis capillaris L. by ATEM and EXAFS. Mineral Mag Mineral Soc 63(6):777–789

Crane RA, Sinnett DE, Cleall PJ, Sapsford DJ (2017) Physicochemical composition of wastes and co-located environmental designations at legacy mine sites in the south west of England and Wales implications for their resource potential. Resour Conserv Recycl 123:117–134

Ebbs SD, Lasat MM, Brady DJ, Cornish J, Gordon R, Kochian LV (1997) Phytoextraction of Cadmium and Zinc from a contaminated soil. J Environ Qual 26(5):1424–1430

Ernst WHO (2005) Phytoextraction of mine wastes—options and impossibilities. Geochemistry 65:29–42

Estrella-Gómez N, Mendoza-Cózatl D, Moreno-Sánchez R, González-Mendoza D, Zapata-Pérez O, Martínez-Hernández A, Santamaría JM (2009) The Pb-hyperaccumulator aquatic fern *Salvinia minima* Baker, responds to Pb2+ by increasing phytochelatins via changes in SmPCS

expression and in phytochelatin synthase activity. Aquatic Toxicol Aquat Toxicol 91(4):320–328

Fahr M, Laplaze L, Bendaou N, Hocher V, Mzibri ME, Bogusz D, Smouni A (2013) Effect of lead on root growth. Front Plant Sci 4:175

Fernández S, Poschenrieder C, Marcenò C, Gallego JR, Jiménez-Gámez D, Bueno A, Afif E (2017) Phytoremediation capability of native plant species living on Pb-Zn and Hg-As mining wastes in the Cantabrian range, north of Spain. J Geochem Explor 174:10–20

Gawryluk A, Wyłupek T, Wolański P (2020) 'Assessment of Cu, Pb and Zn content in selected species of grasses and in the soil of the roadside embankment. Ecol Evol 10(18):9841–9852

Gregory RPG, Bradshaw AD (1965) Heavy metal tolerance in population of Agrostis Tenuis sibth and other grasses. New Phytol 64(1):131–143

Jowett D (1964) 'Population studies on lead-tolerant agrostis tenuis evolution (N Y). JSTOR 18(1):70

Kampf AR, Steele IM, Jenkins RA (2006) Phosphohedyphane, Ca2Pb3(PO4)3Cl, the phosphate analog of hedyphane: description and crystal structure. Am Mineral Geoscienceworld 91(11–12):1909–1917

Koptsik GN (2014) Problems and prospects concerning the phytoremediation of heavy metal polluted soils: a review. Eurasian Soil Sci 47:923–939

Lange B, van der Ent A, Baker AJM, Echevarria G, Mahy G, Malaisse F, Meerts P, Pourret O, Verbruggen N, Faucon MP (2017) 'Copper and cobalt accumulation in plants: a critical assessment of the current state of knowledge. New Phytol 213:537–551

Li MS (2006) Ecological restoration of mineland with particular reference to the metalliferous mine wasteland in China: a review of research and practice. Sci Total Environ 357(1–3):38–53

Liu D, Li S, Islam E, Chen J, Jia-sen W, Ye Z, Peng D, Yan W, Kou-ping Lu (2015) Lead accumulation and tolerance of Moso bamboo (Phyllostachys pubescens) seedlings: applications of phytoremediation. J Zhejiang Univ Sci B 16(2):123–130

Madhavan S, Rosenman KD, Shehata T (1989) Lead in soil: recommended maximum permissible levels. Environ Res 49(1):136–142

Mahar A, Wang P, Ali A, Awasthi K, Hussain Lahori A, Wang Q, Li R, Zhang Z (2015) 'Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. Ecotoxicol Environ Safety. https://doi.org/10.1016/j.ecoenv.2015.12.02

Malar S, ShivendraVikram S, FavasPerumal JCV (2016) Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [Eichhornia crassipes (Mart.)]. Bot Stud Springer Nature 55(1):54

McGrath, S. P. and Zhao, F. J. (2006) Science Report SC050054: Ambient background metal concentrations for soils in England and Wales. Environmental Agency.

Miransari M (2011) 'Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnol Advpp. https://doi.org/10.1016/j.biotechadv.2011.04.006

Pavani KV, Sunil Kumar N, Sangameswaran BB (2012) Synthesis of lead nanoparticles by *Aspergillus* species'. Polish Soc Microbiol 61(1):61–63

Pérez-de-Mora A, Madejón E, Burgos P, Cabrera F (2006) Trace element availability and plant growth in a mine-spill-contaminated soil under assisted natural remediation. II. Plants. Sci Total Environ 363(1–3):38–45

Prasad MNV, De Oliveira Freitas HM (2003) Metal hyperaccumulation in plants—biodiversity prospecting for phytoremediation technology. Electron J Biotechnol. https://doi.org/10.2225/vol6-issue3-fulltext-6

Reeves R (1992) The hyperaccumulation of nickle by serpentine plants. In: Baker A, Proctor J, Reeves R (eds) The vegetation of ultramafic (serpentine) soils. Intercept Ltd., Morristown

Rhee YJ, Hillier S, Gadd GM (2012) Lead transformation to pyromorphite by fungi. Current Biol Cell Press 22(3):237–241

Robinson B, Kim N, Marchetti M, Moni C, Schroeter L, van den Dijssel C, Milne G, Clothier B (2006) Arsenic hyperaccumulation by aquatic macrophytes in the Taupo Volcanic Zone, New Zealand. Environ Exp Bot 58(1–3):206–215

Rodríguez-Seijo A, Lago-Vila M, Andrade ML, Vega FA (2016) Pb pollution in soils from a trap shooting range and the phytoremediation ability of Agrostis capillaris L. Environ Sci Poll Res 23(2):1312–1323

Ross SM, Wood MD, Copplestone D, Warriner M, Crook P (2007) UK Soil and Herbage—pollutant survey environmental concentrations of heavy metals in UK Soil and Herbage. J Bryol 33(2):170

Ryan JA, Zhang P, Hesterberg D, Chou J, Sayers DE (2001) Formation of chloropyromorphite in a lead-contaminated soil amended with hydroxyapatite. Environ Sci Technol Am Chem Soc 35(18):3798–3803

Shi J, Du P, Luo H, Chen J, Zhang Y, Wu M, Xu G (2021) Characteristics and risk assessment of soil polluted by lead around various metal mines in China. Int J Environ Res Public Health 18(9):4598

Tangahu, B. V, Sheikh Abdullah, S. R, Basri, H, Idris, M, Anuar N. and Mukhlisin M. (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. International Journal of Chemical Engineering

Thijs S, Langill T, Vangronsveld J (2017) The bacterial and fungal microbiota of hyperaccumulator plants. Adv Bot Res 11(22):3024

Traina SJ, Laperche V (1999) 'Contaminant bioavailability in soils, sediments, and aquatic environments. Proc Natl Acad Sci USA 96(7):3365–3371

Traoré M, Kaal J, Martínez Cortizas A (2018) Differentiation between pine woods according to species and growing location using FTIR-ATR. Wood Sci Technol 52(2):487–504

Van Ginneken L, Meers E, Guisson R, Ruttens A, Elst K, Tack FMG, Vangronsveld J, Diels L, Dejonghe W (2007) Phytoremediation for heavy metal-contaminated soils combined with bioenergy production. J Environ Eng Landsc Manag 15(4):227–236

Wenzel WW, Jockwer F (1999) Accumulation of heavy metals in plants grown on mineralised soils of the Austrian Alps. Envirn Poll 104(1):145–155

Wu L, Bradshaw AD, Thurman DA (1975) 'The potential for evolution of heavy metal tolerance in plants heredity (Edinb). Nat Publ Group 34(2):165–187

Yanquan Z, Yuan L, Jianjun C, Haiyan C, Li Q, Schvartz C (2005) Hyperaccumulation of Pb, Zn and Cd in herbaceous grown on lead–zinc mining area in Yunnan, China. Environ Int 31(5):755–762

Yanqun Z, Yuan L, Schvartz C, Langlade L, Fan L (2004) Accumulation of Pb, Cd, Cu and Zn in plants and hyperaccumulator choice in Lanping lead–zinc mine area, China. Environ Int 30(4):567–576

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