Phytoremediation: metal decontamination of soils after the sequential forestation of former opencast coal land

1. Mansi Desai, 2. Martin Haigh* and 3. Helen Walkington

* corresponding author: mhaigh@brookes.ac.uk

1,2,3. -Department of Social Sciences (Geography), Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford, UK OX3 0BP, UK.

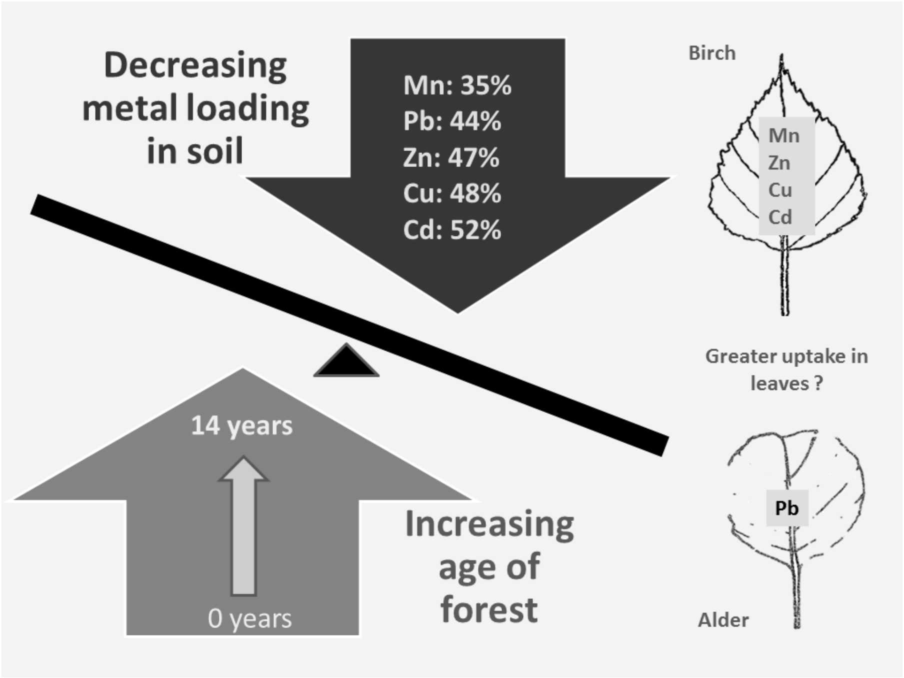
Abstract.

Forest phytoremediation through forestry may be an effective means for reducing the metal loading in lands reclaimed after surface-coal-mining in the UK. Planted with mixed woodland, soil loadings of 5 key metals (Zn, Cd, Mn, Pb and Cu) decreased, significantly and progressively, compared to soils left as grassland, through a 14 year forestation chronosequence on land reclaimed from the former Varteg opencast coalmine, South Wales, UK. Fourteen years after initial tree planting, soil metal loadings decreased by 52% for Cd (4.3 mg.kg -1 per year), 48% for Cu (2.1 mg.kg-1 per year), 47% for Zn (7.3 mg.kg-1 per year), 44% for Pb. (7.1 mg.kg-1 per year) and 35% for Mn (45 mg.kg-1 per year). Analysis of metal loadings in the leaves of Alnus glutinosa (L. Gaertn) (Common Alder) and Betula pendula (Roth) (Silver Birch) found both species involved in metal uptake with birch taking up more Cd, Cu, Zn and Mn and Alder taking up more Pb. Concentrations of Zn, Mn and Cd (Birch only) increased significantly in leaves from, but not soils under, older plantings. Since different tree species take up metals at different rates, mixed plantings may be more effective in forest phytoremediation.

Keywords: forest phytoremediation; chronosequence; technosols; coal-land reclamation; South Wales; metal loadings in soils; metal loadings in leaves.

Highlights

- Isolates the effect of forestation in reducing soil metal loadings of five key metals.
- 14 year forestation chronosequence.
- Foliar analysis provides a direct link between tree growth and reduced metal loadings in soils.
- Mixed species plantings may be more effective in forest phytoremediation.
- Decontamination of former surface-coal-mine land in Wales



Graphical Abstract

1. Introduction

This project aims to determine whether forestation is an effective means of reducing metal contamination in the soils of former surface coal-mine (opencast) sites. It employs a series of forestry test plots planted at different times across a 14-year period (1993 – 2007) on the same terrace-bench of a surface-coal-mine land that was created and reclaimed in 1963. This planting created an experimental framework where the only difference between test plots is the length of time that they have been planted to trees. This study shows how this forestation is associated with changes in metal concentrations in the developing soils on this site.

This study explores the effectiveness of two tree species, Common Alder (*Alnus glutinosa* L. Gaertn.) and Silver Birch (*Betula pendula* Roth) in mitigating the levels of five key metals: Cadmium (Cd), Copper (Cu), Manganese (Mn), Lead (Pb) and Zinc (Zn) in the marginally-contaminated soils of a former opencast coal mine in Torfaen, South Wales, UK. The Varteg Hill Extension Opencast mine was restored as grassland, albeit without great success. In recognition, in 1970, the UK's National Coal Board established a series of grass seeding trials just a kilometre north of Varteg in an attempt to remedy the problem. These National Coal Board test plots remain visible but their outcomes have never been published.

This study forms part of a larger research project that aims to determine best practices for reversing land degradation on formerly reclaimed opencast coal-lands in South Wales. This employs forestation as a means for the self-sustainable reconstruction of soils as well for its wider benefits to environmental regeneration and carbon sequestration (Haigh, 2018). Here, in order to reverse on-going land degradation, experimental tree plantings in trial plots were planted semi-annually between 1991 and 2007 (Haigh et al., 2018; Plamping et al., 2017).

Previous studies of mine-soil chronosequences often involved sites created from the same parent materials but at different times (Schafer et al, 1980; Walker et al., 2010). However, this describes a 'forestry chronosequence', which is a series of forestry plantings where the trees are planted in different years but the soils beneath created simultaneously by a single land reclamation event (Karu et al., 2009). Many previous studies have explored the negative impacts of metal polluted soils on tree growth (Menon et al., 2007, Liu et al, 2013, Pulford and Watson, 2003). However, there are few comparable longitudinal studies of the long-term impacts of forestation on soil metal loadings. Insam and Domsch (1988) compared microbial biomass and soil carbon levels on a 30-year chronosequence of reclaimed opencast brown-coal mine spoils near Cologne, Germany, while Klaminder et al. (2006) used a 20-220 year forestry chronosequence to calculate a 250-year residence time for Pb in the mor organic layer of coniferous forest in northern Sweden. This also found that the response could be much faster (Mean Recovery Time <50 years) in the early stages of forest succession when broadleaved litter fall dominates over conifer. In India's Jharia Coalfield, Bandyopadhyay, Rana and Maiti (2018) compared metal concentrations beneath 3 and 25 year old Eucalyptus trees. They found that Zn, Pb and Cu soil concentrations in the older reclaimed mine soils were, respectively, 3.46, 1.55 and 1.44 times lower.

The UK's surface- coal-mining industry is rapidly disappearing. However, it has created a legacy of disturbed land, some contaminated, but most 'reclaimed', if not always to a high standard (Haigh, 2000). Metal contamination is not, usually, a major problem for former surface-coal-mines in Wales. While deep-mine coal-spoils may contain high levels of toxic metals, the mineral ores associated with such contamination are a relatively small part of surface-coal-mine spoils. These are dominated (90-95%) by 'overburden', the shale and sandstone rocks that overlay the coal-seams. Consequently, generally, these spoils have lower levels of contamination. This said, many surface-coal spoils contain 'hot spots' of severe contamination, which are often associated with coal and metal minespoils that have become mixed into the fill of the opencast pit.

Traditionally, in the UK, most former surface-coal-mine sites have been restored to grassland, especially in the uplands. This may not be the best policy for restoration where there is present or potential contamination by metals. In general, because of their greater productivity and biomass, trees have far greater potential value than grass for the amelioration of metal contaminated soils (Raskin, Smith and Salt, 1997; Pulford and Dickinson, 2006; Pajević et al., 2016). They need only accumulate small amounts of metal to be effective (Pulford and Dickinson, 2006). In addition, their roots penetrate much more deeply into the soil and involve more of the substrate in soil forming processes.

Currently, the main alternatives to the phytoremediation of metal-contaminated soils are physical approaches such as "dig-and-dump" and/or quarantining by containment, physico-chemical immobilisation or extraction (Wuana and Okieimen, 2011). However, while phytoremediation is relatively low-cost, these alternatives are expensive and better suited to the treatment of small problem sites than the large areas of marginal contamination typical of the UK's former surface-coal-mines (Pulford and Watson, 2003, p.529).

However, forest phytoremediation requires many years to be effective and this is dependent on both the species planted and local site conditions that affect metal bioavailability (Pulford and Watson, 2003). Until recently, it was considered suitable only for very slightly contaminated soils (Ernst, 1996). Different species take up different metals in different proportions at different rates, which may mean that mixed plantings are more effective than monospecific (Samecka-Cymerman and Kempers, 2003). However, there remain questions about the phytoremediation effectiveness of tree species other than *Salix* and *Populus* spp (Raskin and Ensley, 2000). Further, forest phytoremediation has not always proved effective for sites where pollution is on-going. For example, a 23-year study of acid-soluble heavy metals (Cu, Pb, Cd, Zn) in the forest litter and humus horizons of soils affected by atmospheric deposition from the Middle Ural Copper Smelter, Russia, found that metal levels in soils remained largely unaffected by afforestation with spruce (Vorobeichik and Kaigorodova, 2017). This may be important because local research suggests that, even in the absence of the atmospheric deposition of pollutants, natural weathering of the mine-stones may lead to increased soil contamination through residual enrichment. Dang et al. (2002, 1996) used SEM (Scanning Electron Microscope) and EPMA (Electron Probe Micro-Analysis) to compare ashed samples of weathered and unweathered mine-stones from two former South Wales surface-coal-mine sites just 8 km north of Varteg: Pwll Du (restored 1948) and Walters Group (restored 1994). They found that while some metals exist as sulphides, which can be oxidised to sulphates through microbial action (Haigh, 2000), much is complexed with organic carbon, which is not mobilised by weathering and remains to enrich the weathered residual. This process carried Cu (145-675 mg/kg) and Zn (96-485 mg/kg) (cf. Table 1) to join Pb (23.4-74.9 mg/kg) above the thresholds for soil contamination. The present study asks if forest phytoremediation is an effective means for reducing soil decontamination in these circumstances.

2. Site Description

The test site lies 3 km south of the 'Blaenavon Industrial Landscape World Heritage Site' *"one of the best preserved, relict industrial landscapes in Wales, … and [amidst] the only sizeable, abandoned, multiple period, opencast mineral workings in South Wales"* (Torfaen County Borough Council, 2013, p. 183). The forestry plots are located on a terrace bench created in 1963 during the reclamation of the Varteg Hill (Waun Hoscyn Extension) Opencast Coal Mine (51°44'50-53"N 03°07'72-98"W). This bench lies between 360- 370 metres above mean sea level on the western flank of Cwm Afon Llywd in Torfaen and on the western outcrop of the South Wales Coalfield (Figure 1). Pre-mining site geology was Carboniferous Coal Measure (Westphalian and Namurian) strata, mainly shales, often poorly consolidated mudstones, with harder sandstone and some thin coal seams (Neame, 2015). Spoil tips from coal and earlier iron mines were common in the pre-mining land surface and these wastes are mixed into the fill of the former surface-mine creating pockets of highly acid and metal-loaded spoil (Haigh and Kilmartin, 2017).

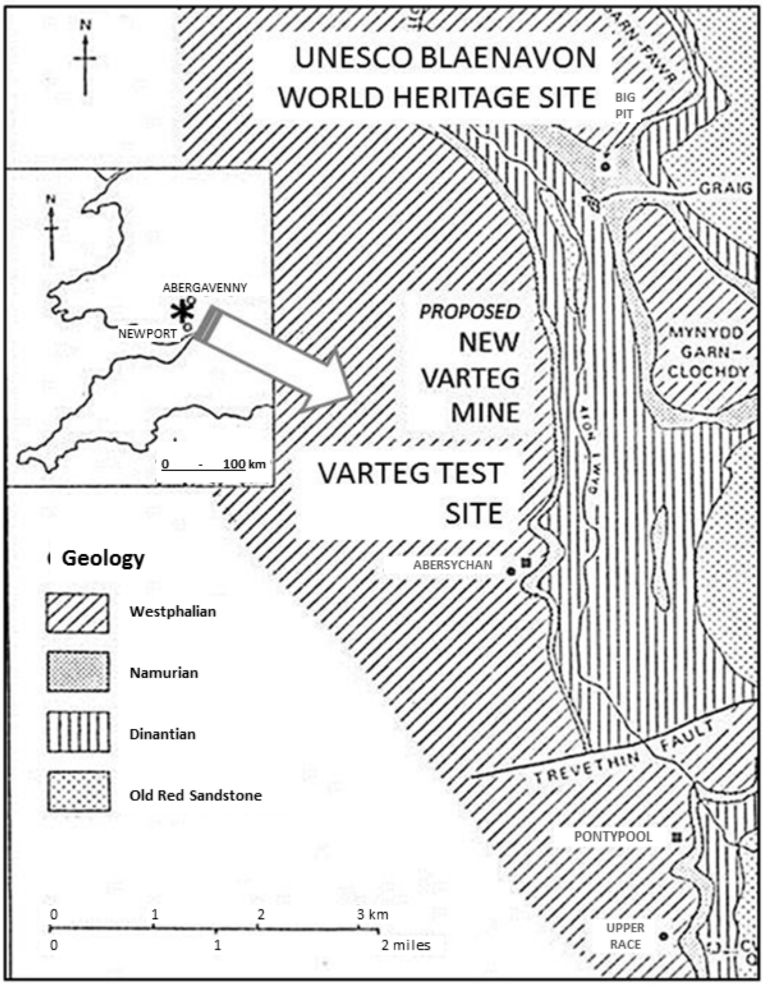


Figure 1. Location of test site, Varteg, S. Wales

Immediately north of the test site is the land of the proposed 'Varteg Hill Reclamation and Coal Recovery Scheme', which includes large parts of the former Varteg Opencast mine area and which has been subject to intensive investigation for an Environmental Impact Assessment (Smart, 2014). This reports high levels of Fe and Mn in many groundwater samples, especially, in waters draining Coal Measures strata and former coal-mine workings (Smart, 2014). Deep-mine coal spoils are judged slightly contaminated, with high levels of As (Ove Arup and Partners Ltd, 2008). Twenty test pit samples found contamination 'hot spots' for Cu, Zn. B and high ambient levels of iron and sulphate (Smart, 2014). The report concluded, controversially, that ground contamination posed no "significant risk either to human health or controlled waters" (Smart, 2014, p68). However, as Table 1 shows, several of the levels reported exceed guideline values recognised by the Environment Agency (UK), CLEA (UK) or MAFF/EU, and the 'Dutch List' and suggest contamination issues for Cu, Zn, Pb, and (possibly), As, Ni and Se.

Table 1: Metals (mg/kg^{-1}) recorded in 20 samples from test pits on adjacent 'Varteg Hill Reclamation and Coal Recovery Scheme' lands (Smart, 2014).

Metal (Emboldened metals are those examined by this study)	Range	Environment Agency 'Prompt Value' for soil of pH 6.5 (Nicholson et al., 2008)	CLEA Guideline Value (UK) (Residential or allotment land use).	'Dutch list' (target and) intervention values for soil remediation (VROM, 2000)	Maximum metals allowable in UK soils (MAFF, 1993) (nb. same as: EC Directive 86/278/EEC for soil of pH5.5).
As	6.7 – 62.5	n/a	500	(29) 55	50
Cadmium (Cd)	**<0.1 – 0.76**	**1.0-1.1**	**1.0-1.8**	**(0.8) 12**	**3**
Cr	3.8 – 28.9	n/a	130	(100) 380	400
Copper (Cu)	**11.7 – 4630**	**58**	**n/a**	**(36) 190**	**100**
Lead (Pb)	**18.1 – 1075**	**260.7**	**450**	**(85) 530**	**300**
Hg	<0.1 – 0.43	n/a	10-26	(0.3) 10	1
Ni	5.1 – 85.6	21	130-230	(35) 210	60
Se	<0.5 – 3.1	n/a	120	(0.7) 100	3
Zinc (Zn)	**18.5 – 742.5**	**n/a**	**n/a**	**(140) 720**	**250-300**

Currently, soil profiles on the reclaimed land consist of a thin organic layer, typically 2-3 cm thickness, above weathered clays with clasts of sandstone, coal shale and some coal. Many soil profiles display a clay-enriched, high density, essentially water-impermeable, layer at 18-30 cm. Deeper, the proportion of large cobbles of sandstone and coal-shale increases. Below 50 - 70cm are unweathered, often clay-veneered, mine-spoil cobbles with bridged voids (Haigh et al., 2015; Haigh and Sansom, 1999).

Autocompaction, caused by the breakdown of water-unstable minestones near the soil surface, is ubiquitous on local former surface coal-mine sites (Haigh and Sansom, 1999). At Varteg, soil bulk density is typically high: 1.63 g/cm^3 at 35-40cm and 1.81 g/cm^3 at 50 cm depth. These high bulk densities negatively affect soil organic carbon levels, which fall below 1% when bulk density >1.5 g/cm^3 (Merrington, 2006). Moffat and Bending (1992) suggest that, ideally, the soil bulk density for forestry on disturbed land should be <1.5 g/cm3 (<50 cm depth) and not >1.7 g/cm^3 at 100 cm depth. Here, the higher bulk densities may reduce root penetration, except through fissures; indeed, 1.8 g/cm^3 is often considered the limiting maximum bulk density for root penetration (Haigh, 2000).

Soil pH is a key influence on metal partitioning between the soil and soil solution (Bolan et. al., 2003). Soil pH recorded in 63 field tests under 3m high trees averaged pH 5.8 (sd. 0.3), while under dense forest in test plot PA93, Humphrey (1997) recorded pH 6.6 (sd. 0.2) in 24 tests. Moffat and Bending (1992) advise that soils for reclamation forestry should have a pH in the range of 3.5 – 8.5. Merrington (2006) proposes trigger values of pH>6 for metal retention and pH>5 for effective microbial functioning.

Organic matter increases a soil's moisture holding capacity and soils used for tree planting should contain >0.8 % organic matter. However, the Varteg soils have only 0.3 % to 0.5 % organic matter, perhaps even less because Loss on Ignition (LOI) tests can be affected by the combustion of mineral coal fragments. Soil fertility levels (N, P, K and Mg) are also very low (Haigh et al., 2018).

Monthly mean air temperatures range from 2.5°C to 15°C. Rainfall (1971-2000) was 1543 mm/year at Cwmavon Reservoir, 0.8 km to the north on the Afon Lwyd valley floor. Locally, natural soils remain at field capacity for 285-325 days/year; evaporation is estimated as 472 mm/year on rough grazing land (Robinson, 1989).

3. Methodology

3.1 Tree Species Selection.

The tree species recommended for the reclamation of former coal-lands in the UK include: *"generally undemanding or 'pioneer' species…rowan, birch, alder, poplar, larch and pine"* (Forestry

Commission (Scotland), 2001, p. 7). Willow is also planted, typically at riparian or boggy sites or as part of a mixed planting. However, *Alnus* (Alder) and *Betula* (Birch) are widely used and two key species are selected for this study (Grobelak, 2016; Pulford and Watson, 2003; Haigh et al., 2018).

Alder species are often used in the phytoremediation of metals, where bacterial endophytes are central to the process (Ma et al., 2016). *Alnus glutinosa* (L.) Gaertn., the UK's only native alder, is known to thrive in metal contaminated and waterlogged soils and on coal-land reclamation sites for, at least the first 5 years, >10 years at Varteg (Moffat, 2014; Haigh et al. 2018). Alder also accumulates metals such as Pb, Zn and, especially, Cu in its roots and root nodules, of which a small proportion is transferred to leaves and shoots (Lorenc-Plucińska et al., 2013). Nevertheless, the leaves and leaf litter from *Alnus glutinosa* grown on polluted soil contain greater amounts of Cu, Zn and Cd (but similar amounts of Pb) than trees grown on non-polluted soils. Since *Alnus* is a nitrogen fixer, it tends to increase soil acidity, which may also increase metal mobility, although rates of litter decomposition do not seem to be affected (Scheid et al., 2009).

Silver birch, *Betula pendula* (Roth), like other *Betula* species, is a pioneer coloniser of lighter infertile soils. It has a strong rooting system that enables it to penetrate into the deeper layers of soil, which also helps it access and accumulate larger amounts of metal. Silver birch has been suggested for use in the biomonitoring of soil contamination because of the close correlation between the levels of Cu and Pb in its leaves and in the soil (Klink et al., 2006). Mycorrhizal associates (VAM) facilitate metal uptake by the plant and ameliorate Zn toxicity (Baker and Walker, 1990). Birch, a hyperaccumulator of Zn, has a high tolerance of polymetallic soil contamination, although shoot and root growth in young seedlings can be inhibited, especially by Cd (Dmuchowski et al., 2014, Hermle et al., 2006; Osteras et al. 2000). Hence, within limits, both *A. glutinosa* and *B. pendula* are tolerant of soil metal contamination and, while total metabolic activity can be impaired, the density of associated microorganisms (bacteria and fungi) inhabiting root and rhizosphere, is not (ZŁoch et al., 2014; Scheid et al., 2009).

3.2 Metal Selection

Coal-mine spoils are a common source of heavy metal contamination (Siegel, 2002). The metals found in surface-coal-mine spoils originate, variously, from the geochemistry of the native rocks, past industrial activities including mining, air pollution, and from the addition of soil amending substances during reclamation (Alloway, 2013; Haigh, 1995; Siegel, 2002). An inventory of normal agricultural soils in the UK found that atmospheric deposition was the major source (25-85%) followed by manure and sewage sludge (Nicholson et al., 2003). However, in coal-mine spoils, many metals are mobilised by weathering and microbiological processes in the relatively oxidizing and sometimes acidic environments of the newly displaced and fractured minespoils (Dang, et al., 2002; Haigh, 2000). Acids caused by pyrite dissolution are aggressive; they can degrade aluminosilicates and other minerals releasing previously stabilised metal components (Johnson, 2003). For example, Cadmium (Cd) is mobilised at pH 6.0 - 5.5, and its mobility increases by 60% -90% at pH 4.0, while Zn, Ni and Cu become mobile at 5.0-5.5 (Blake and Goulding, 2002).

Different researchers have selected different clusters of metals for analysis in similar contexts. For example: Alvarez et al (2003) selected: Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn; Moreno-Jiménez et al. (2009): Cd, Cu, Fe, Mn, and Zn; Maiti et al. (2016): Cd, Cu, Fe, Mn, Ni, and Zn; Niu et al (2015): Cd, Cr, Ni, Pb and Zn, and Pietrzykowski et al. (2014): Cd, Cu, Pb and Zn. This study selects Cd, Cu, Mn, Pb, and Zn, all five are among the 11 key metals listed by Alloway (2013, p.3). Four, Cd, Cu, Pb, and Zn are counted strongly hazardous, while Mn is only problematic at very high loadings (Vodyanitskii, 2016). As for bioavailability, Cottenie et al. (1982) regard two, Cadmium (Cd) and Zinc (Zn), as readily mobilised by plants, two, Lead (Pb) and Copper (Cu), much less so while Manganese (Mn) is 'average'.

3.3 Experimental Design and Methods

This project evaluates the forest phytoremediation potential of five key metals (Cd, Cu, Mn, Pb and Zn) by two tree species commonly used in land reclamation: Common Alder (*Alnus glutinosa* L. Gaertn.) and Silver Birch (*Betula pendula* Roth). It explores soil and foliar samples from 5 forestation plots planted between 1993 and 2007 (Table 2) into the (marginally) contaminated soils of a former surface-coal-mine in South Wales, UK. (Haigh et al, 2018). These data are compared with data from the soil prior to planting plot MD07 (BL07: 'Background Level').

Table 2. Varteg Test Plots and their date of plantation

Site Code	Age of Plantation	Year of Planting
PA93	*16-17*	1993
TA94	*15-16*	1994
SH97	*12-13*	1997
CA03	*6-7*	2003
MD07	*2-3*	2007
BL07	*0*	*Not planted (Control)*

Successive plantings of similar, adjacent, mixed species, forestation plots in 1993, 1994, 1997, 2003 and 2007 has created a set of trees and soil that differ only in the length of time that they have been forested. This study aims to establish whether soil metal loadings of Zn, Cd, Mn, Cu and Pb are different beneath Alder and Birch trees of different age and through foliar analysis to determine whether these trees are directly involved in these changes. The null hypothesis is that there is no significant difference in metal loadings between test plots.

Soil samples were collected at a depth of 20-30cm, using random number tables to create co-ordinates for sampling, and replicated in 2009 and 2010**.** Soil compaction made it difficult to collect samples from deeper layers. The 210 soil samples were tested for standard physical and chemical parameters including: soil pH, soil texture, soil moisture content, soil organic content - loss on ignition and carbonate content (following: Heiri, et al., 2001). Metals were assayed by an Atomic Absorption Spectrophotometer (AAS) using an Agilent Technologies SpectrAA 220FS and EDTA-Na_2 extraction (Quevauviller, 1996). Soil test results were calibrated against tests undertaken, independently, at the University of Wales Trinity St Davids and data published for the adjacent Varteg Hill Reclamation and Coal Recovery Scheme (Faber-Maunsell, 2006). Results were compared with the contamination action/treatment thresholds suggested by the widely used "Dutch List" (VROM, 2000).

Foliar samples (>10g per tree – dry weight, n=234) were collected in 2009 and 2010 from mature larger leaves in the upper three quarters of the tree, where metal content might be maximal. These samples were collected from the 'control' sections of each plot, where the only additives supplied to the trees were 750g of spent mushroom compost on planting (cf. Haigh et al., 2018). Foliar samples were digested using the MLS-1200 MEGA Microwave Digestion System. Soil and foliar sample collection was replicated in 2009 and 2010. During analysis, soil and foliar reagent blanks were analysed after every 15 tests and standard solutions after each 10 samples, to check against instrument drift.

4. Results.

The results are reported in three stages. First, soil results prior to the 2007 planting (BL07) are contrasted with those measured on the same site after planting (MD07) in2009 and 2010. Second, these results are compared with those from soil testing on four older plantings (CA03—2003; SH97 - 1997; TA94 - 1994 and PA93 – 1993). Finally, results from the foliar tests are considered.

Table 3. Soil metal loadings before planting (BL07) and 2 and 3 years after planting (mg kg^{-1})

Mean (sd.)	**Cd**	**Cu**	**Mn**	**Pb**	**Zn**	**Sample n=**
BL07 (2007)	1.16 (2.63)	63.92 (1.52)	1879 (385)	224 (34)	192.8 (29.2)	213
MD07 (2009)	1.12 (2.33)	58.00 (2.00).	1598 (275)	224 (32)	177 (24.0)	210
MD07 (2010)	1.07 (1.98)	51.21 (1.98)	1532 (242)	200 (28)	169.7 (30.51)	234
Significance of difference: 2007 to 2010 (p=)	0.004	0.003	0.002	0.025	0.005	-

Table 3 shows that, in all cases, recorded metal levels are highest in the pre-plantation tests and lowest in the third, 2010, data collection: 2007>2009>2010. A Wilcoxon Signed Ranks test suggests that the sequence 2007>2009>2010 is significant ($p<0.01$). Overall, levels of Cu, Mn, Pb and Zn in the soil decreased by 14-18% but Cd just 6%. In the case of Cu, and possibly Mn and Zn, the total reduction is greater than (or close to) one standard deviation (sd.) of the mean. T-testing confirms that the decrease in all metal levels, from before planting (2007) until 2-years after planting (2010), is significant.

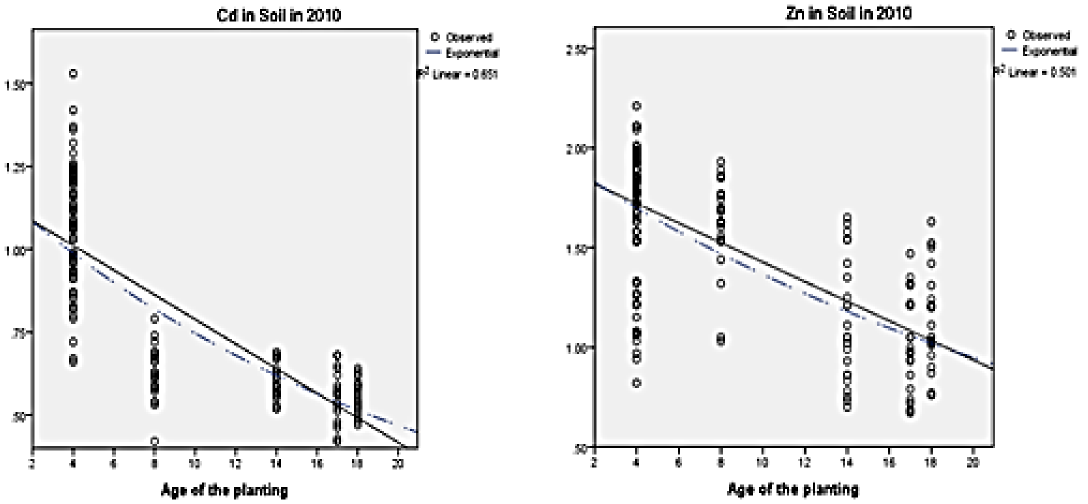
Table 4 displays data from tests conducted in 2009 and 2010 on all five plots. Again, the metal loadings recorded in 2009 are, as a whole, significantly greater than those of 2010 ($p<0.0005$), even if relative differences are small. The decrease in metal concentrations across progressively older plantations continues; in 46 of 50 cases the next older plot displays a lower level of each metal. This is significant (Wilcoxon Signed Ranks: $p<0.05$). The four exceptions cases all come from the relatively unsuccessfully forested CA03 plot, where concentrations of Cu (slightly) and Mn (considerably) are higher on CA03 (planted 2003) than the younger MD07 (planted 2007).

Table 4. Metal loadings (mg kg^{-1}) in soils across 14-year forestation chronosequence (n=423)

MD07	**Cd - 2009**	**Cd - 2010**	**Cu -2009**	**Cu – 2010**	**Mn - 2009**	**Mn - 2010**	**Pb - 2009**	**Pb - 2010**	**Zn - 2009**	**Zn - 2010**
MEAN	1.12	1.07	58.23	51.21	1598.3	1533.8	223.62	199.49	176.75	169.68
Min	0.43	0.28	32.39	30.42	1233.5	1003.0	134.11	131.16	83.64	75.43
Max	2.85	2.45	78.61	69.66	2353	2171.5	285.38	265.32	232.03	221.82

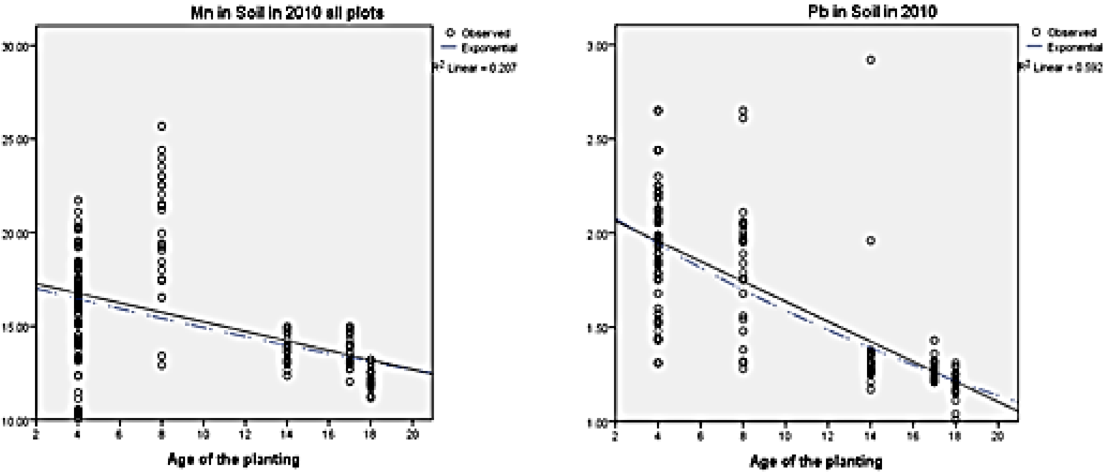
sd	2.33	1.98	5.66	7.13	275.3	241.7	32.36	27.60	24.43	30.46
CA03										
MEAN	0.70	0.63	60.46	52.31	2058.46	2012.69	197.86	189.25	155.45	146.94
Min	0.30	0.42	32.53	31.58	1332.13	1292.06	122.74	128.74	73.53	75.52
Max	1.27	1.25	68.68	63.85	2686.42	2568.73	278.62	268.17	203.33	193.17
sd	0.87	0.77	7.22	7.70	299.33	277.23	34.56	37.46	43.35	35.30
SH97										
MEAN	0.64	0.60	49.16	43.00	1366.02	1340.04	142.8	131.95	124.56	111.74
Min	0.58	0.52	32.32	31.16	1034.75	1048.92	104.96	117.03	66.08	70.8
Max	0.73	0.69	67.28	59.83	1557.69	1498.63	176.44	168.89	176.05	165
sd	0.34	0.49	4.68	5.84	687.54	928.43	8.68	9.78	28.43	27.79
TA94										
MEAN	0.61	0.59	41.00	37.58	1374.56	1337.48	130.83	123.52	100.03	97.3
Min	0.32	0.37	22.05	25.93	1033.96	1048.82	100.25	104.96	63.86	67.08
Max	0.82	0.78	65.73	59.32	1532.03	1498.04	157.09	143.04	155.75	147.55
Sd	0.87	0.99	6.99	7.43	790.44	845.72	10.26	8.05	24.55	22.45
PA93										
MEAN	0.57	0.56	36.53	33.27	1276.02	1227.10	127.23	120.4	94.32	89.43
Min	0.11	0.14	22.56	27.37	1132.32	1116.48	99.4	101.24	78.2	67.32
Max	0.68	0.64	43.52	40.71	1353.84	1322.83	137	131.57	174.11	163.79
sd	0.48	0.47	3.50	3.56	432.92	493.92	5.50	6.11	24.53	25.32

Regression analysis demonstrates the negative trend linking the amount of time trees have been planted and the amount of metals present in soil. Figure 2, which combines the data collected in 2009 and 2010, shows the significant (p=0.002), negative, best-fit trend line linking increasing age of trees with decreasing soil metal concentration. It depicts both linear and exponential best-fit lines, and, while the differences are small, they suggest that the rate of decrease in soil metal concentrations could be declining with time after forestation. In sum, 14 years after forestation with Alder and Birch, soil metal concentrations have decreased relative to background levels (BL07) by 52% for Cd (0.043 mg.kg $^{-1}$ per year), 48% for Cu (2.07 mg.kg^{-1} per year), 47% for Zn (7.21 mg.kg^{-1} per year), 44% for Pb. (7.16 mg.kg^{-1} per year) and 35% for Mn (44.82 mg.kg^{-1} per year).



a) Cd in soil

b) Zn in soil



c) Mn in soil

d) Pb in soil

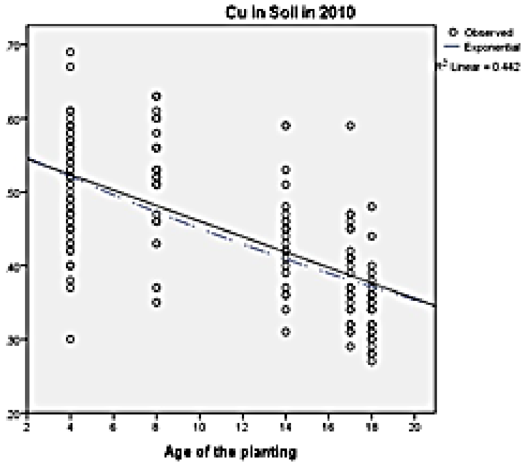


Figure 2: Regression between metal loadings in soil (mg/kg) and age of tree plantation (years) (with linear R^2 values: all cases: $p<0.005$).

e) Cu in soil

The pre-planting data (BL07) indicate baseline metal loading in grassed soils. Of course, these are not the initial soil concentrations on site; they are what remain after 44 years. Similarly, the data from the forestation plots show the soil metal loadings that remain after 46-47 years, including between 2-3 years under forest (MD07) and up to 16-17 years under forest (PA93). These results, however, do show the impact, through time, of changing the land use from grass to forest.

The significant and progressive decrease in metal loadings following forestation suggests that trees play a role but it does not prove the connection. Hence, foliar analysis was undertaken to prove that uptake by trees is directly involved.

Table 5 describes the average metal loading (mg kg^{-1}) of Alder and Birch leaves across the whole series of forest plantings from 1993 to 2007 as recorded in 2009 and 2010. The Alder leaf data shows that metal uptake remains fairly consistent across the whole chronosequence; there is a moderate uptake of Mn, Zn and, to some extent, Cd, although Pb may show a slight negative trend. Findings for Birch are similar, although only four of the six test plots include this species. For both species, the sequence of foliar volumetric uptake is Mn>Zn>Cu>Pb>Cd. However, Birch leaves contain higher levels of Cd, Cu, Mn and Zn, while Alder leaves contain more Pb. Unlike Alder, Birch shows a stronger uptake in the leaves of trees planted 2-3 years previously (MD07) than those planted 15-16 years ago (TA94). The exception is Mn, where the trend is reversed. In theory, of course, the total uptake of trees increases with age because of their greater biomass (Haigh et al., 2018; Plamping et al., 2017).

Table 5. Comparison of foliar metal uptake (mg kg^{-1}) by Alder and Birch (2009-2010) (Alder n= 111; Birch n=123).

Plot	**Alder**	**Birch**	**Alder**	**Birch**	**Alder**	**Birch**	**Alder**	**Birch**	**Alder**	**Birch**
MD07	**Cd**	**Cd**	**Cu**	**Cu**	**Mn**	**Mn**	**Pb**	**Pb**	**Zn**	**Zn**
MEAN	0.20	**0.62**	4.51	**14.00**	239.65	**448.78**	**4.25**	2.38	67.72	**88.86**
MIN	0.00	0	2.01	9.22	167.01	305.13	1.99	1.02	38.54	39.02

MAX	0.75	1.13	6.88	20.01	405.00	572.30	6.47	4.75	107.53	145.23
Std Dev	0.41	0.58	0.91	2.01	50.31	70.66	1.31	0.60	13.49	23.67
CA03	**Cd**		**Cu**		**Mn**		**Pb**		**Zn**	
MEAN	0.32		4.91		307.74		4.65		77.13	
MIN	0.06		3.02		189.00		2.15		52.12	
MAX	0.52		7.64		433.21		6.92		98.56	
Std Dev	0.36		1.08		58.51		1.33		11.92	
SH97	**Cd**	**Cd**	**Cu**	**Cu**	**Mn**	**Mn**	**Pb**	**Pb**	**Zn**	**Zn**
MEAN	0.22	**0.58**	4.72	**14.70**	243.68	**465.20**	**3.82**	2.78	61.76	**81.60**
MIN	0.04	0.01	2.01	10.21	201.19	322.01	2.53	1.95	31.24	31.21
MAX	0.42	0.84	7.94	18.45	354.00	599.00	6.49	4.52	89.43	125.63
Std Dev	0.48	0.54	1.32	2.91	39.72	63.31	1.39	0.70	17.86	23.01
TA94	**Cd**	**Cd**	**Cu**	**Cu**	**Mn**	**Mn**	**Pb**	**Pb**	**Zn**	**Zn**
MEAN	0.23	**0.52**	4.80	**10.95**	266.74	**498.12**	**3.59**	2.41	60.05	**71.86**
MIN	0.05	0	2.04	6.8	195.02	211.03	1.53	1.01	32.19	22.06
MAX	0.31	0.6	8.43	13.56	371.42	699.53	6.78	3.21	78.42	120.40
Std Dev	0.48	0.54	1.42	1.39	30.06	98.71	1.26	0.65	9.54	25.01
PA93	**Cd**		**Cu**		**Mn**		**Pb**		**Zn**	
MEAN	0.25		5.33		291.02		3.75		58.49	
MIN	0 .00		0.00		136.22		1.94		32.45	
MAX	0.30		10.30		398.05		5.77		70.41	
Std Dev	0.41		2.02		64.36		1.22		15.57	

Table 6 displays correlations between the metal loadings in soils and leaves across the full range of plantings. The first column displays, after applying a Bonferroni correction, the significant negative correlations between metal loading in the soil and age of the plantation (cf. Figure 2). The top row explores correlations between plantation age and foliar metal levels by tree species. It shows positive correlations between the age of plantation and the loading of Mn and Zn in both Alder and Birch leaves and Cd in Alder alone. Table 7 also displays several positive significant correlations between metal levels in soils and leaves for all metals except Cd. This means that as the plantation ages, there are higher levels of metals engaged with the biological system.

The loading of Mn and Zn in soils also correlates with that in leaves for both species, which suggests a relationship with the level of contamination, while the positive correlation (p=0.002) between Zn in soil and Mn in leaves implies a mutuality of some kind. These data, however, confirm that the trees are engaged actively with the mobilization of, at least some, of the metals in the soils.

Table 6: Correlations between soil metal loadings and foliar metal concentrations for Alder and Birch trees across all Varteg plots. (Values show Pearson's 'r' and significance where p<0.005 is rated 'significant'**).**

	Age of Trees	Mn Alder	Mn Birch	Cd Alder	Cd Birch	Zn Alder	Zn Birch
Age of Trees		0.573 p=0.002	**0.385 p=0.001**	**0.468 p=0.001**		**0.302 p=0.001**	0.205 p=0.004
Cd Soil	**-0.830 p=0.001**						
Cu Soil	**-0.651 p<0.0005**						
Mn Soil	**-0.388 p<0.0005**	0.453 p=0.003	0.348 p=0.005				
Pb Soil							
Zn Soil	**-0.672 p=0.001**	0.366 p=0.004	0.393 p=0.002			0.489 p=0.005	0.589 p=0.002

5. Discussion

This investigation explored a 14 year forest chronosequence on land of the same age and character where parts were forested with similar species at different dates and other parts left as grassland. This allowed the assessment of the long term impact of forest upon metal loadings in the soil. Common Alder and Silver Birch were studied, both are native species widely used in UK land reclamation. Metal selection was guided by their plant availability; Cd and Zn are readily mobilised, Pb and Cu much less easily, while Mn is 'average' (Cottenie et al., 1982). Four of the five metals are rated strongly hazardous, while Mn is hazardous only at very high loadings (Vodyanitskii, 2016).

The plantation of mixed woodland on marginally metal contaminated lands, which were reclaimed in1963 from the Varteg Hill Extension Opencast Coal-mine, was associated with a significant long term reduction in the loadings of all five metals compared to land left under grass. The reduction ranged between 35% (Mn) and 52% (Cd). By contrast, Baltrėnaitė et al., (2016), whose focus was uptake by plant tissues only, found that the effectiveness of Birch for the purification of a sewage-sludge polluted soil was 0.2-0.3% per year (<4.2% across 14 years) for Mn and Zn but only 0.04-0.07% per year for Cu and Pb (<0.98% across 14 years) while that for Alder reached only 0.01-0.04% per year for Cu, Mn and Pb (<0.6% across 14 years). Clearly, there is more involved in metal decontamination than simply the planted trees. Elsewhere in the UK, *Populus* sp. has been estimated to reduce soil Cd and Zn contamination by 5.6 mg Cd kg^{-1} and 96 mg Zn kg^{-1} over 20-years (French, 2004; French et al. 2006). The non-linear regression graphs of Figure 2 and negative correlations in Table 6 confirm that the rate of metal depletion is decreasing as soil loadings decrease. However, if present depletion rates continued: Cd and Zn would be depleted in about 15 years, Cu and Pb within 20 and Mn within 30 years. If Placek et al. (2016) are correct in suggesting that the 'self-cleaning of soils' can take several hundred years, then it is clear that forestation with a mixed woodland is an effective catalyst of these processes.

Table 7 compares the uptake of metals by tree leaves on the Varteg with data collected for the same species in other contexts. The table shows that, typically, Birch leaves contain higher loadings of all the metals tested except Pb. The Varteg data, in general, fits within this spectrum of other results and with other data from its local context (Smart, 2014). Several studies report low uptake of Pb and Cu in Alder leaves (Huang et. al., 1997; Schmidt, 2003; French, 2004). Butkus and Baltrėnaitė (2007) also found Alder leaves to contain more Pb but also more Cu and Mn. Samecka-Cymerman et al (2003) also found low uptake Pb and Cu into leaves of Birch (*Betula pendula*) grown on contaminated black coal spoils in Poland. However, on the Varteg, the Cu loading of Birch leaves and amount of Mn in the soil is high, while the amount of Zn is relatively low (Table7).

Table 7. Comparisons of metal loadings (mg.kg^{-1}) in contaminated soils (italics) and in the leaves of Alder and Birch (means and +/- standard deviations)

	Varteg Soil Range of Means (Data from Smart (2014)	Varteg Birch (and Alder) leaves: Range of Means	*Silesia, Black Coal Dumps (Samecka-Cymerman et al., 2003)*	Silesia, Birch leaves (Samecka-Cymerman et al., 2003)	*Lithuania, sewage sludge amended soils (Butkus and Baltrėnaitė, 2007).*	Lithuania Birch (and Alder) leaves (Butkus and Baltrėnaitė, 2007).	*Belgium Dredged river sediment (Depth: 30-45cm) (Mertens et al., 2006a)*	Leaves (Alder) (Mertens et al., 2006b)
Cd	*1.16 +/- 0.32 (<0.1 - 0.8)*	0.5-0.6 (0.2. - 0.3)	*0.6-1.2*	0.3-0.6	*n/a*	n/a	*5.9 +/-0.7*	(<0.23)
Cu	*64 +/- 1.5 (11.7 - 4630)*	11-14 (5.3 - 4.5)	*41-68*	4.6-5.3	*25.3 +/- 5.9*	2.3 (2.8)	*53.9 +/- 6.1*	(5.8 +/- 0.9)
Mn	*1879 +/-385 (n/a)*	465-498 (308-244)	*265-715*	170-650;	*108 +/- 28*	108 (113)	*n/a*	n/a
Pb	*216.0 +/-34.2 (18.1 - 1075.0)*	2.4-2.8 (4.7-3.6)	*35-189*	10-15	*4.4 +/- 1.3*	0.7 (1.0)	*74.3 +/- 8.1*	(5.0 +/- 0.5)
Zn	*200 +/- 29 (19-743)*	72-78 (77-59)	*85-181*	160-409	*114 +/- 47*	n/a (214)	*359 +/- 46*	(65 +/- 12)

Leaves are important but not the only parts of plants where metals accumulate and different metals accumulate in different proportions in different plant tissues (Rana and Maiti, 2018). For Birch, the Zn accumulation sequence is branches<fine roots<large roots <stem< leaves and, for Alder, fine roots<large roots<stem <leaves<branches. Mn accumulates more in the fine roots than leaves of Birch while for Alder the Mn sequence is large roots<fine roots<stem<leaves<branches (Butkus and Baltrėnaitė, 2007). Pb accumulates most in the branches of Alder but in the leaves of Birch while, in both species, Cu accumulates more in leaves. Rosselli, Keller and Boschi (2003) report that while Betula and Salix transfer Zn and Cd into leaves and twigs, Alnus tends to exclude them from above-

ground tissues. They conclude that it is not possible to convert metal uptake in leaves to uptake of the tree from the soil. So, the foliar data from the Varteg does no more than demonstrate that trees are directly involved in the uptake of metals, and that there are differences in the uptake of different metals by different tree species. They do, however, support the argument for using mixed species plantations in forest phytoremediation.

More importantly, trees are not the only active biological agent involved. Forestation provides the soil with a sheltering canopy, a biologically active forest floor and litter layer, a rhizosphere of roots, fungal mycorrhiza and associated organisms, collectively a biochemical reactor that controls the mobilisation of metals and, separately, uptake by trees (Richter, 1987). Successful reclamation depends on the formation of a living soil system. At Varteg, results show that, while humus formation remains at an initial stage, there is a large and active microflora, especially in those plots where the trees have received fertiliser. Here, Noustorova et al (1999) recorded a total microflora of 196-286*10^3g, of which >80% were ammonifying bacteria. Earthworm biomass is also greater under Varteg's trees than outside the plantations (Plamping et al., 2017). The reduction of soil metal loadings is a system process; many organisms may be involved.

Birch, generally, has a higher loading of metals in its leaves than Alder, which has, occasionally, been called a metal excluder, despite many studies, including this one, showing it capable of significant metal uptake (cf. Gamalero et al., 2009; Lorenc-Plucińska et al., 2013). The key observation is that these species take up different amounts of different metals, which means that mixed plantings may be most effective for phytoremediation.

Metal loadings in the leaves collected at Varteg are not unusually high apart from Mn (Millaleo, et. al. 2010; Migeon, et. al. 2010) and Cd and Zn in Birch (Boregard and Rydin, 1989; Butkus and Baltrėnaitė, 2007). In northern France, Mignon et al. (2009), investigated 25 woody species grown on Cd, Zn and Pb contaminated land and found the highest accumulations of Zn and Cd in Poplar leaves (*Populus tremula × Populus tremuloides)* ,Pb concentration low across all species, and that Birch (and Oak – also planted at Varteg) accumulated more Mn than other woody species. It is possible that the characteristics of mine spoils encourage relatively high metal uptake. Borgegard and Rydin (1989) report on heavy metal uptake in Birch in a soil cover over Cu tailings and found that the leaves contained more Zn, Pb and Cd than comparable uncontaminated soils; the BL07 test show that the same is true for Mn, Cd and Zn on the Varteg plots. However, in this study, foliar metal loadings increase over time whereas soil metal loadings decrease.

There is a well-known, albeit complex, positive association between the uptake of Cd and Zn which may be species dependent (Liu et al., 2013). Mn has been commended used as a soil amendment for the remediation of metal contaminated soils and, here, there is a significant positive correlation between Zn in soil and Mn uptake, while (McCann et al., 2015). Rana and Maiti (2018) also find a correlation between Cu and Pb in Eucalyptus. High levels of Mn are found in soils with low organic matter and soils that are easily waterlogged, which is certainly the case at Varteg, and also in soils with pH <5.5 (Schulte and Kelling, 1999). Soil pH is thought to affect the availability of Zn more than any other factor, with maximum Zn availability at pH 5.4 (Schulte, 2004). Similarly, low pH facilitates the availability of Cd in soil (Kirkham, 2006). On the Varteg pH is generally in the range of 5.2– 5.7*,* which may help explain the uptake of these metals.

In sum, these decreases in soil metal loadings may be attributed to two processes. First, the mobilization effected by organic activity may transform the metals into forms that are easily carried away by leaching, subsurface and surface runoff. Dispersed into the wider environment, these contaminants are, here, rendered undetectable by dilution (Haigh and Kilmartin, 2017). Second is the uptake or absorption of metals by leaves, other plant tissues, and/or by the trees fungal and rhizospheric associates (Liu et al., 2013). Some of this mobilised metal may be recycled into the soil by the decay of organic tissues, while some may be bio-transformed into inert forms and/or held outside the soil within the standing biomass (Sinha et. al., 2007). Indeed, the input of organic matter by trees could counter the mobilisation of some metals including Pb, which can form insoluble complexes with organic matter; this is why the residence time of Pb in rich organic soils can be very long (Manceau et al., 1996). However, if this were the case here, of course, the result would be enrichment of the top-soils by inputs of metals taken up from the buried minespoils. In fact, metal loadings decline and the likely reason is leaching into the soil solution and surface or near –surface runoff (Ross, 1994). However, given high rainfall, runoff and ambient metal pollution levels offsite, it is probable that any contribution to off-site pollution loadings is too small to be detected (Haigh and Kilmartin, 2017).

6. Conclusion.

In coal mine-spoil derived soils of the same character and age, the plantation of mixed woodland results in large, significant and progressive decreases in the soil loadings of five key metal contaminants. As the age of the tree plantation increases, the level of soil metal contaminant loading decreases progressively, so confirming that forestation ameliorates soil metal loadings. Here, soil loadings of five key metals (Zn, Cd, Mn, Pb and Cu) decrease, significantly, under *Alnus glutinosa* (L. Gaertn) (Common Alder) and *Betula pendula* (Roth) (Silver Birch). Fourteen years after tree planting, soil metal loadings, relative to land left under grass, decreased by 52% for Cd (0.043 mg.kg $^{-1}$ per year), 48% for Cu (2.1 mg.kg^{-1} per year), 47% for Zn (7.3 mg.kg^{-1} per year), 44% for Pb. (7.1 mg.kg^{-1} per year) and 35% for Mn (45 mg.kg^{-1} per year). Foliar analysis indicated that trees were directly involved in for drawing down soil metal loadings for all five of the metals. There were significant positive correlations between the age of the trees and the amount of Mn, Zn and Cd

(Alder only) in leaves. However, there was little other evidence for metal accumulation on site and it is probable that most of the mobilised metals were dispersed into the larger environment by runoff and leaching (cf. Haigh and Kilmartin, 2017). More generally, differences in the metal uptake of Birch and Alder suggest that mixed plantings may be more effective than monospecific for forest phytoremediation.

Here, in South Wales, the forestation of marginally contaminated soils on reclaimed former surface-coal-mine soils has resulted in a significant decline in soil metal loadings on even on the most recently planted test sites. Measurement from soils across the full 14 year forestation chronosequence found that metal levels declined significantly (p= 0.003 to p=0.0002) through time. This paper has demonstrated that forestation with Alnus glutinosa (L. Gaertn) (Common Alder) and Betula pendula (Roth) (Silver Birch) is an effective means of (Cd, Cu, Zn, Pb and Mn) metal remediation on the moderately contaminated lands produced by opencast coal-mining in South Wales.

References

Alloway, B. J. (ed) (2013). *Heavy metals in soils: trace metals and metalloids in soils and their bioavailability (3e)*. Dordrecht, Springer.

Alvarez, E., Marcos, M. F., Vaamonde, C., Fernández-Sanjurjo, M. J. (2003). Heavy metals in the dump of an abandoned mine in Galicia (NW Spain) and in the spontaneously occurring vegetation. *Science of the total environment*, 313(1), 185-197.)

Baker, A. J., Walker, P. L. (1990). Chapter 11. Ecophysiology of metal uptake by tolerant plants. In Shaw, A.J. (ed). *Heavy metal tolerance in plants: evolutionary aspects.* Boca Raton, FLA. CRC Press, pp 155-175.

Baltrėnaitė, E., Baltrenas, P., Lietuvninkas, A. (2016). *The sustainable role of the tree in environmental protection technologies*. Cham, Switzerland: Springer International.

Bandyopadhyay, S., Rana, V., Maiti, S.K. (2018). Chronological variation of metals in reclaimed coal mine soil and tissues of eucalyptus hybrid tree after 25 years of reclamation, Jharia Coal Field (India). *Bulletin of environmental contamination and toxicology*, 101(5), 604-610.

Blake, L., Goulding, K. W. T. (2002). Effects of atmospheric deposition, soil pH and acidification on heavy metal contents in soils and vegetation of semi-natural ecosystems at Rothamsted Experimental Station, UK. *Plant and Soil*, 240(2), 235-251.

Bolan, N. S., Adriano, D. C., Curtin, D. (2003). Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. *Advances in Agronomy*, 78(21), 5-272.

Borgegard, S. O. and Rydin, H. (1989). Biomass, root penetration and heavy metal uptake in birch, in a soil cover over copper tailings. *Journal of Applied Ecology*. 26(2), 585 -595.

Butkus, D., Baltrėnaitė, E. (2007). Accumulation of heavy metals in tree seedlings from soil amended with sewage sludge. *Ekologija*, 53(4), 68-76.

Cottenie, A., Velghe, G., Verloo, M., Kiekens, L. (1982). *Biological and analytical aspects of soil pollution*. Ghent, Belgium, Universitieit Gent, Laboratory of Analytical and Agrochemistry.

Dang, Z., Liu, C., Haigh, M. J. (2002). Mobility of heavy metals associated with the natural weathering of coal mine spoils. *Environmental Pollution*, 118(3), 419-426.

Dang Zhi, Watts, S., Haigh, M.J., Wan Guo-Jiang, Xioa, B.H. (1996). The interaction of coalmine spoil and different acidity solutions II: mineralogy of natural weathered and unweathered coal-mine spoil. *Science in China* Series D: 39, 6, pp. 610-617.

Dmuchowski, W., Gozdowski, D., Brągoszewska, P., Baczewska, A. H., & Suwara, I. (2014). Phytoremediation of zinc contaminated soils using silver birch (Betula pendula (Roth)). *Ecological Engineering,* 71, 32-35.

Ernst, W. H. O. (1996). Bioavailability of heavy metals and decontamination of soils by plants. *Applied Geochemistry*, 11(1-2), 163-167.

Faber Maunsell (2006) *Varteg Hill Reclamation and Coal Recovery Scheme, Blaenavon, South Wales: Hydrogeological and Contaminated Land Assessment – Final Interpretative Report*. Birmingham, Faber Maunsell AECOM

Forestry Commission (Scotland) (2001). *Derelict and Reclaimed Land* . Edinburgh, Forestry Comission Guidance Note 19. Retrieved 12 October 2016: http://scotland.forestry.gov.uk/images/corporate/pdf/guidancenote19.pdf.

French, C. J. (2004). *Tree planting for phytoremediation: The fate of soil contaminants on brownfield sites*. Liverpool, University of Liverpool, School of Biological and Earth Sciences,Ph. D. Thesis..

French, C. J., Dickinson, N. M., Putwain, P. D. (2006). Woody biomass phytoremediation of contaminated brownfield land. *Environmental Pollution*, 141(3), 387-395.

Gamalero, E., Lingua, G., Berta, G., Glick, B. R. (2009). Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. *Canadian Journal of Microbiolog*y, 55(5), 501-514.

Grobelak, A. (2016). Organic soil amendments in the phytoremediation process. In Ansari, A. A. Gill, S. S., Gill, R., Lanza, G. R. & Newman L. (eds) *Phytoremediation: Management of Environmental Contaminants*. Cham, Switzerland, Springer International, Vol. 4, pp. 21-39.

Haigh, M.J. (2000). Soil stewardship on reclaimed coal-lands. In: Haigh. M.J. (ed). *Reclaimed Land: Erosion Control, Soils & Ecology.* Rotterdam: A. A. Balkema, pp 165-274

Haigh, M.J. (1995). Soil quality standards for reclaimed coalmine disturbed lands: a discussion paper. *International Journal of Surface Mining, Reclamation & Environment* 9,4, 187-202.

Haigh, M.J. (2000). Soil stewardship on reclaimed coal-lands. In: Haigh. M.J. (ed). *Reclaimed Land: Erosion Control, Soils & Ecology*. Rotterdam: A. A. Balkema (Land Reconstruction & Management 1). , pp 165-274,

Haigh, M., Kilmartin, M.P. (2017). Illustrating the water quality impacts of reclaimed opencast coal lands, SE Wales. In: Palán L., Křeček, J. (eds) *EFC/FAO Working Party on the Management of Mountain Watersheds: Management of municipal watersheds in mountain regions, 31st Session, Prague (Czech Republic), September 4–6, 2017, Proceedings*. Food and Agriculture Organisation of the United Nations (FAO) Rome, pp. 87-115.

Haigh, M. J., Sansom, B. (1999). Soil compaction, runoff and erosion on reclaimed coal-lands (UK). *International Journal of Surface Mining, Reclamation and Environment*, 13(4), pp. 135-146.

Haigh, M., Reed, H., Flege, A., D'Aucourt, M., Plamping, K., Cullis, M., Woodruffe, P., Sawyer, S., Panhuis, W., Wilding, G. and Farrugia, F. (2015). Effect of Planting Method on the Growth of Alnus glutinosa and Quercus petraea in Compacted Opencast Coal-Mine Spoils, South Wales. *Land Degradation & Development*, 26(3), pp.227-236.

Haigh, M., Reed,H., D’Aucourt, M., Flege, A., Cullis, M., Davis, S., Farrugia, F., Gentcheva-Kostadinova, S., Zheleva, E., Hatton, L., Plamping, K.,. Powell, S., Panhuis, W., Sansom, B., Sawyer, S., Wilding, G., & Woodruffe, P. (2018). Effects of initial fertiliser treatment on the 10-year growth of mixed woodland on compacted opencast coal-mine spoils, S. Wales. *Land Degradation and Development* 29, pp 3456-3468.

Heiri, O, Lotter, A.F,. Lemcke G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of Paleolimnology* 25: 101–110.

Hermle, S., Günthardt-Goerg, M. S., Schulin, R. (2006). Effects of metal-contaminated soil on the performance of young trees growing in model ecosystems under field conditions. *Environmental Pollution,* 144(2), 703-714.

Huang, J. W, Chen, J, Berti, W. R., Cunningham, S. D. (1997). Phytoremediation of lead contaminated soils: role of synthetic chelates in lead phytoextraction. *Environment Science and Technology*, 31, 800– 805.

Humphrey, M. (1997). *An investigation into the impact of soil quality on tree growth on land reclaimed from opencast coal-mining at Varteg, South Wales*. Birmingham, University of Central England, MSc Thesis.

Insam, H., Domsch, K. H. (1988). Relationship between soil organic carbon and microbial biomass on chronosequences of reclamation sites. *Microbial ecology*, 15(2), 177-188.

Johnson, D. B. (2003). Chemical and microbiological characteristics of mineral spoils and drainage waters at abandoned coal and metal mines. *Water, Air and Soil Pollution: Focus*, 3(1), 47-66.

Karu, H., Szava-Kovats, R., Pensa, M., Kull, O. (2009). Carbon sequestration in a chronosequence of Scots pine stands in a reclaimed opencast oil shale mine. *Canadian Journal of Forest Research*, 39(8), 1507-1517. https://doi.org/10.1139/X09-069.

Kirkham, M. B. (2006). Cadmium in plants on polluted soils: effects of soil factors, hyperaccumulation, and amendments. *Geoderma*, 137(1), 19-32.

Klaminder, J., Bindler, R., Emteryd, O., Appleby, P., Grip, H. (2006). Estimating the mean residence time of lead in the organic horizon of boreal forest soils using 210-lead, stable lead and a soil chronosequence. *Biogeochemistry*, 78(1), 31-49.

Klink, A., Letachowicz, B., Krawczyk, J., Wisłocka, M. (2006). The content of heavy metals in soil and silver birch leaves (*Betula pendula* Roth) from Wałbrzych and Głogów. *Polish Journal of Environmental Study*, 15, 347-350.

Liu, W. T., Ni, J. C., Zhou, Q. X. (2013). Uptake of heavy metals by trees: prospects for phytoremediation. *Materials Science Forum* 743, 768-781.

Lorenc-Plucińska, G., Walentynowicz, M., Niewiadomska, A. (2013). Capabilities of alders (*Alnus incana* and *A. glutinosa*) to grow in metal-contaminated soil. *Ecological engineering*, 58, 214-227.

Ma, Y., Rajkumar, M., Zhang, C., & Freitas, H. (2016). Beneficial role of bacterial endophytes in heavy metal phytoremediation. Journal of environmental management, 174, 14-25.

Maiti, S. K., Kumar, A., Ahirwal, J. (2016). Bioaccumulation of metals in timber and edible fruit trees growing on reclaimed coal mine overburden dumps. *International Journal of Mining, Reclamation and Environment*, 30(3), 231-244.

Manceau, A., Boisset, M.C., Sarret, G., Hazemann, J.L., Mench, M., Cambier, P. Prost, R. (1996).Direct determination of lead speciation in contaminated soil by EXAFS spectroscopy. *Environmental Science and Technology* 30, 1540–1552.

McCann, C. M., Gray, N. D., Tourney, J., Davenport, R. J., Wade, M., Finlay, N., Hudson-Edwards, K.A., Johnson, K. L. (2015). Remediation of a historically Pb contaminated soil using a model natural Mn oxide waste. *Chemosphere*, 138, 211-217.

Menon, M., Hermle, S., Günthardt-Goerg, M. S., Schulin, R. (2007). Effects of heavy metal soil pollution and acid rain on growth and water use efficiency of a young model forest ecosystem. *Plant and Soil*, 297(1-2), 171-183.

Merrington, G. (2006). *The development and use of soil quality indicators for assessing the role of soil in environmental interactions: Science report SC030265*. Bristol, Environment Agency.

Mertens, J., Vervaeke, P., Meers, E., Tack, F. M. (2006a). Seasonal changes of metals in willow (Salix sp.) stands for phytoremediation on dredged sediment. *Environmental science & technology*, 40(6), 1962-1968.

Mertens, J., Springael, D., De Troyer, I., Cheyns, K., Wattiau, P., Smolders, E. (2006b). Long-term exposure to elevated zinc concentrations induced structural changes and zinc tolerance of the nitrifying community in soil*.* *Environmental Microbiology*, 8(12), 2170-2178.

Migeon, A., Richaud, P., Guinet, F., Chalot, M., Blaudez, D. (2009). Metal accumulation by woody species on contaminated sites in the north of France. *Water, air, and soil pollution*, 204(1-4), 89-101.

Millaleo, R., Reyes-Diaz, M., Ivanov, A.G., Mora. M.L., Alberdi, M. (2010). Manganese as essential and toxic element for plants: Transport, accumulation and resistance mechanisms. *Journal of soil science and plant nutrition* 10 (4): 470 - 481

Moffat, A. (2014) *Native and non-native trees: why and how to choose*. Farnham, Surrey, Forest Research, Best Practice Guidance for Land Management, BPG 8. Retrieved 2. October, 2016: http://www.forestry.gov.uk/pdf/LRU_BPG08.pdf/$FILE/LRU_BPG08.pdf.

Moffat, A. J., Bending, N. A. D. (1992). *Physical site evaluation for community woodland establishment*. Farnham, Forestry Commission Research Division.

Moreno-Jiménez, E., Peñalosa, J. M., Manzano, R., Carpena-Ruiz, R. O., Gamarra, R., & Esteban, E. (2009). Heavy metals distribution in soils surrounding an abandoned mine in NW Madrid (Spain) and their transference to wild flora. *Journal of hazardous materials*, 162(2), 854-859.

Neame, T. (2015) *Varteg Hill Reclamation and Coal Recovery Scheme, Blaenavon, South Wales: Phase 1 – Desk Study for Glamorgan Power Co Ltd.* Birmingham, Faber-Maunsell / AECOM

Nicholson, F. A., Smith, S. R., Alloway, B. J., Carlton-Smith, C., Chambers, B. J. (2003). An inventory of heavy metals inputs to agricultural soils in England and Wales. *Science of the total environment*, 311(1), 205-219.

Nicholson, F. A., Boucard, T., Chambers, B. J., Merrington, G. (2008). *Road testing of 'trigger values' for assessing site specific soil quality: Phase 1 – metals*. Bristol Environment Agency, Science Report – SC050054SR1. Retrieved 27 October 2016 from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/291141/scho1008bost-e-e.pdf.

Niu, S., Gao, L., Zhao, J. (2015). Risk analysis of metals in soil from a restored coal mining area. *Bulletin of environmental contamination and toxicology*, 95(2), 183-187.

Noustorova, M., Filcheva, E., Gencheva, S., Zheleva, E. (1999). Microbiological characteristics and composition of organic matter on materials from coal-mining in Wales, United Kingdom. *Journal of Balkan Ecology* 2(1): 57-61.

Osteras, A.H., Ekvall, L., Greger, M. (2000). Sensitivity to, and accumulation of, cadmium in *Betula pendula*, *Picea abies*, and *Pinus sylvestris* seedlings from different regions in Sweden. *Canadian Journal of Botany* 78, 1440-1449.

Ove Arup and Partners Ltd (2008). *The British Reclamation Scheme: Environmental Statement*. Abersychan, Spring (Talywain) Ltd. Retrieved "6 October 2016 from: http://www.planapps.torfaen.gov.uk/thebritish/applicationforms/0003%20Environmental%20Statement/0001%20Reclamation%20Scheme%20Environmental%20Statement.pdf.

Pajević, S., Borišev, M., Nikolić, N., Arsenov, D. D., Orlović, S., & Župunski, M. (2016). Phytoextraction of heavy metals by fast-growing trees: A Rreview. In: Ansari, A. A. Gill, S. S., Gill, R., Lanza, G. R. & Newman L. (eds). *Phytoremediation: Management of Environmental Contaminants* Volume 3. Cham, Switzerland, Springer International, pp. 29-64.

Placek, A., Grobelak, A., Kacprzak, M. (2016). Improving the phytoremediation of heavy metals contaminated soil by use of sewage sludge. *International Journal of Phytoremediation*, 18(6), 605–618. http://doi.org/10.1080/15226514.2015.1086308

Pietrzykowski, M., Socha, J., van Doorn, N. S. (2014). Linking heavy metal bioavailability (Cd, Cu, Zn and Pb) in Scots pine needles to soil properties in reclaimed mine areas. *Science of the Total Environment*, 470, 501-510.

Plamping, K., Haigh, M., Reed, H., Woodruffe, P., Fitzpatrick, S., Farrugia, F., D'Aucourt, M., Flege, A., Sawyer, S., Panhuis, W., Wilding, G., Cullis, M., Powell, S. (2017). Effects of initial planting method on the performance of mixed plantings of alder and oak on compacted opencast coal-spoils, Wales: 10-year results*. International Journal of Mining, Reclamation and Environment*, 31(4): 286-300, https://doi.org/10.1080/17480930.2016.1139528.

Pulford, I. D., Dickinson, N. M. (2006). Phytoremediation Technologies Using Trees, Chapter 20 in: Prasad, M.N.V. Sajwan, K, S., Naidu R. (eds). *Trace elements in the environment: biogeochemistry, biotechnology, and bioremediation*, Boca Raton, FL, CRC (Taylor and Francis), pp. 383 – 404.

Pulford, I. D., Watson, C. (2003). Phytoremediation of heavy metal-contaminated land by trees—a review. *Environment International*, 29(4), 529-540.

Quevauviller, P. (1996). Atomic spectrometry hyphenated to chromatography for elemental speciation: performance assessment within the Standards, Measurements and Testing Programme (Community Bureau of Reference) of the European Union. *Journal of Analytical Atomic Spectrometry*, 11(12), 1225-1231.

Rana, V., Maiti, S.K. (2018). Differential distribution of metals in tree tissues growing on reclaimed coal mine overburden dumps, Jharia coal field (India). *Environmental Science and Pollution Research*, 25(19): 9745-9758.

Raskin, I., Ensley, B. D. (eds) (2000). *Phytoremediation of toxic metals*. New York, John Wiley &Sons.

Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. *Current Opinion in Biotechnology* 8:221–226.

Richter, J. (1987). *The soil as a reactor: modelling processes in the soi*l. Weisbaden, Catena Verlag.

Robinson, J. (1989). *Agricultural land classification and physical characteristics and soil forming materials survey: Pwll Du Opencast Coal Site, Blaenavon, Gwent*. Cardiff, ADAS Land Management Services.

Ross, S.M. (1994). 3. Retention, transformation and mobility of toxic metals in soils. In: Ross, S. M. (ed.). (1994). *Toxic metals in soil-plant systems.* Chichester: Wiley, pp 63-152.

Rosselli, W., Keller, C., Boschi, K. (2003). Phytoextraction capacity of trees growing on a metal contaminated soil. *Plant and Soil*, 256(2), 265-272.

Samecka-Cymerman A., Kempers A.J.(2003). Enrichment ratios of elements in selected plant species from black coal mine dumps in Lower Silesia (Poland). *Polish Journal of Ecology* 51, 377-383.

Schafer, W. M., Nielsen, G. A., Nettleton, W. D. (1980). Minesoil genesis and morphology in a spoil chronosequence in Montana. *Soil Science Society of America Journal*, 44(4), 802-807. doi:10.2136/sssaj1980.03615995004400040029x .

Scheid, S., Günthardt-Goerg, M. S., Schulin, R., Nowack, B. (2009). Accumulation and solubility of metals during leaf litter decomposition in non-polluted and polluted soil. *European Journal of Soil Science*, 60(4), 613-621.

Schmidt, U. (2003). The effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. *Journal of Environmental Quality* 32, 1939-1954.

Schulte, E.E. (2004). Soil and applied Zinc. *Understanding Plant Nutrients (A2528)*. Madison, WI, University of Wisconsin – Extension. Retrieved 20 October 2016: http://corn.agronomy.wisc.edu/Management/pdfs/a2528.pdf.

Schulte, E. E., Kelling, K. A. (1999). Soil and applied Manganese. *Understanding plant nutrients (A2526)*. Madison, WI, University of Wisconsin – Extension. Retrieved 20 October 2016: http://corn.agronomy.wisc.edu/Management/pdfs/a2526.pdf

Siegel, F.R. (2002) *Environmental geochemistry of potentially toxic metals*. Berlin, Springer-Verlag.

Sinha, R. K., Herat, S., Tandon, P. K. (2007). Phytoremediation: Role of plants in contaminated site management. In Singh, S.N. and Tripathi, R.D. (eds) *Environmental Bioremediation Technologies* Springer Berlin Heidelberg, pp. 315-330. DOI: 10.1007/978-3-540-34793-4.

Smart, P. (2014) *Varteg Hill reclamation and coal recovery scheme, Blaenavon, South Wales: Hydrogeological and contaminated land assessment: Interpretative report (2e).* Birmingham, AECOM.

Torfaen County Borough Council (2013). *Local Development Plan (to 2021) adopted plan: Written statement, December, 2013*. Cwmbran, Torfaen County Borough Council.

Vorobeichik, E.L., Kaigorodova, S.Y., 2017. Long-term dynamics of heavy metals in the upper horizons of soils in the region of a copper smelter impacts during the period of reduced emission. *Eurasian Soil Sci*. 50, 977–990.

Vodyanitskii, Yu.N. (2016) Standards for the contents of heavy metals in soils of some states. *Annals of Agrarian Science* 14(3), 257-263.

VROM (2000). *Soil remediation circular.* Den Haag, Netherlands Ministry of Infrastructure and the Environment Retrieved 12 November 2018: http://esdat.net/Environmental_Standards.aspx.

Walker, L. R., Wardle, D. A., Bardgett, R. D., Clarkson, B. D. (2010). The use of chronosequences in studies of ecological succession and soil development. *Journal of Ecology*, 98(4), 725-736.

Wuana, R. A., Okieimen, F. E. 2011. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *Isrn Ecology*, 2011. ISRN Ecology, Article 402647, doi:10.5402/2011/402647.

ZŁoch, M., Kowalkowski, T., Szymańska, S., Hrynkiewicz, K. (2014). Response of Birch and Alder root endophytes as well as rhizosphere and bulk soil microorganisms to heavy metal pollution. *Polish Journal of Ecology* 62(1):37-53. 2014. doi: http://dx.doi.org/10.3161/104.062.0105.