

COULD GREY OYSTER
MUSHROOM (PLEUROTUS
OSTREATUS) BE A VIABLE
OPTION FOR
MYCOREMEDIATION OF SOUTH
WALES COAL SPOIL

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by



Figure 1.1: View of ex-colliery site at Varteg, Torfaen (Macfarlane, 2023a)

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ABSTRACT

This paper assesses the mycoremediation potential of Grey Oyster Mushroom (*Pleurotus ostreatus*) in the South Wales coalfields. Coal tips are currently a global concern in terms of pollution, land stability, and land use change. Currently, very little research has been carried out into implementing mycoremediation in South Wales.

This study demonstrates that *Pleurotus ostreatus* can adsorb various contaminants in differing content ratios, and argues that it produces significant adsorption rates for many elements found in the South Wales coalfields and cannot be ruled out as a supplementary remediation effort.

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1 INTRODUCTION

1.1 BACKGROUND

Healthy soil is a vital global resource, responsible for providing food, nutrient cycling, tackling climate change, carbon storage, and a defence against both flood and drought through filtration and absorption (Kopittke, *et al.*, 2023). It is also one of the most complex and diverse habitats on the planet (Rojas, *et al.*, 2016). However, through wars, agriculture, and industrialisation, soil pollution has been listed as a top 10 threat to soil function in North Africa, the Northwest Pacific, North America, sub-Saharan Africa, Europe and Eurasia, Asia, and Latin America (FAO(a), 2018). Soil pollution poses an immediate threat to ecosystems, biodiversity, agriculture, and human health (FAO(b), 2018), through damage to crop yields, compaction leading to flood and drought events, intensive agriculture stripping the nutrient content, and industrial processes contaminating soils with chemicals and heavy metals (Sokolov, *et al.*, 2010).

Coal mining is the extraction of a non-renewable fossil fuel from deposits on the surface of the planet and from underground (Britannica, 2024). From the 19th century, the South Wales coalfield was extensively mined due to the coal being bituminous, with 20%-40% volatiles, and a carbon content ranging from 84%-91% (BGS, 2007). These characteristics cause the coal to burn faster, at higher temperatures, and produce less ash and smoke, making it ideal for both industrial and residential use (Thomas, 1956).

The most common impact of coal mining on soil is contamination with heavy metals and polycyclic aromatic hydrocarbon (PAH), and potential combustibility from excess mixed coal (Beriro, 2020). Degradation of soil quality reduces viable land for food production, lowers biodiversity, and increases the risk of contaminants spreading further from the site of origin (Bian, *et al.*, 2010). This impact is most noticeable in heavily mined areas, such as India (Mehta, 2002), China (Layke, 2019), and most of Europe (Alves Dias, *et al.*, 2018).

Current management methods include capping, chemical oxidation, soil washing, or bioremediation (ENVA, 2023). Capping is the isolation of the soil so that it neither improves in quality nor spreads (Lee & Jones-Lee, 2006). However, liability and long-term viability are issues that can inhibit capping. For example, Brofiscin quarry in Groesfaen, South Wales, almost remained uncapped as the organisations involved in dumping of pollutants refused to accept liability (Levitt, 2015). To reduce the environmental impact of pollutants leaching into neighbouring areas, the Environment Agency and Rhondda Cynon Taff council grouped forces to cap the area and pursue reimbursement from all organisations later (Rhondda Cynon Taff Council, 2014).

Additionally, capping doesn't function as an overall solution, as the soil remains poor quality, which leads to potential hazards if there is a loss of cap integrity or surface work impacts the lower layer, as seen in the Tir John landfill in Swansea (HazardEx, 2012). However, when successfully capped, whilst the soil quality remains poor, the site can be utilised for green infrastructure, such as the Tir John landfill site which is now earmarked for a 3MW solar farm development (WSP & Swansea Council, 2022).

Soil washing is a low-cost removal of contaminants in soil via water, sometimes with a chemical additive (ENVA, 2023), usually surfactants as seen in Appendices 1-3 (Mao, *et al.*, 2015). This process is effective and can be performed on-site. However, the process can produce a lot of wastewater that requires treatment, and the efficacy depends on the soil type (Liu, *et al.*, 2021); soils containing a high clay or silt

content are difficult to process as the particles are much smaller and have less ability to settle after the extraction has been completed.

Chemical oxidation is the use of oxidants such as hydrogen peroxide (H_2O_2), potassium permanganate ($KMnO_4$), sodium permanganate ($NaMnO_4$), and persulfates (Alazaiza, *et al.*, 2022) to change contaminants into less harmful substances (Lemaire, *et al.*, 2019). Whilst shown to be effective, research has shown that waste products can still be harmful and that it is not entirely effective as a single remediation method (Rada, *et al.*, 2019).

Bioremediation has many forms (Juwarkar, *et al.*, 2010), most commonly phytoremediation through the introduction of plants, usually trees, to remove pollutants from soil (Kafle, *et al.*, 2022). This can be through processes such as extraction, degradation, or stabilisation of the contaminants (Peuke & Rennenberg, 2005). Mycoremediation focuses on degrading or sequestering contaminants depending on both pollutant and fungi species (Boamponsem, *et al.*, 2013). Recent research has shown that mycoremediation is a quicker and potentially more effective method of remediation (Dykes, 2021); the 2007 Cosco Busan oil spill saw Grey Oyster Mushroom (*Pleurotus ostreatus*) utilised to help remove the oil in 12 weeks (Norton, 2012).

1.2 STUDY AREA

The UK has extensive records into the extent of its coal mining (Gombert, *et al.*, 2019). This has made remediation efforts difficult to compare across the Europe where records may not be as robust. However, mycoremediation studies have involved coal-impacted soils from Norway (Eggen & Majcherzyk, 1998), Romania (Radulescu, *et al.*, 2010), Lithuania (Tiso, *et al.*, 2015), and the UK (Canet, *et al.*, 2001). As seen in Appendices 4 and 5, studies have shown potential, but there is limited research into areas with ongoing issues and continued mining, such as Wales (Twigg, 2020). Remediation in Wales saw a large increase in developments between 2009-2010 (Natural Resources Wales, 2016). However, this was followed by a rapid decline in the years following. As such, areas of Wales are still heavily contaminated from the coal industry with the focus primarily on stabilisation rather than remediation due to recent years of extreme wet weather (Welsh Parliament, 2021). Whilst this provides safety for communities in proximity to the coal tips, remediation efforts would also provide the potential for land-use change (Beames, *et al.*, 2015).

Approximately 85% of disused coal tips are in the South Wales coalfield; as such, pollution and potential destruction of the environment is an ever-present threat (Welsh Government, 2022). Similarly, with a 13% increase in annual rainfall in Wales since the 1960s (Forest Research, nd), saturation of colliery waste/coal spoil has led to the leaching of more pollutants into surrounding soil (Li, *et al.*, 2018), particularly metals such as copper (Cu), lead (Pb), zinc (Zn), manganese (Mn), nickel (Ni), mercury (Hg), arsenic (As), cadmium (Cd) (Kou, *et al.*, 2022).

As a result of this, large areas of Wales contain contaminated soils which are unsuitable for farming/agriculture (Welsh Government, 2022). Under current definitions, these sites are classed as brownfield (Ford, 2021) and would have limited redevelopment potential (Sustainable Build, 2006). However, another issue is that the stability of the site means that it is often unsuitable for building development (WHOCC, 2023). With 13% of the 2,456 confirmed disused coal tips in Wales being deemed high risk due to their assessed instability (Welsh Government, 2023), restoration provides an alternative that can be used to increase green infrastructure, or to remediate to the required level for farming

suitability (Hou, *et al.*, 2021). This work investigating *Pleurotus ostreatus* as a mycoremediation option provides a low-cost and fast-acting method for remediating soil in economically deficient (Beatty, *et al.*, 2019) and environmentally poor (Haigh, *et al.*, 2015) areas of South Wales.

1.3 AIMS & OBJECTIVES

The aim of this research is to determine the effectiveness of *Pleurotus ostreatus* in mycoremediation through pollutant sequestration (Akpasi, *et al.*, 2023). This provides a low-cost and low-risk method of restoring those sites (Sadañski, *et al.*, 2019) without compromising any of the newly developed biodiversity that has inhabited the area (Messenger & Fischer, 2023).

- The first objective is to cultivate 5 samples of *Pleurotus ostreatus* with varying ratios (control, 20:80, 40:60, 60:40, and 80:20) of straw pellets and coal spoil that are also hydrated with replicated run-off water. This will determine whether any direct inoculation would need a high volume of straw pellets as an additive, as well as an indication of what ratio would be most effective. Success would be determined by cultivation of *Pleurotus ostreatus* achieving noticeable and sufficient development across all samples to allow for analysis of adsorption rate of contaminants in the fruiting bodies with ICP-MS/MS.
- The second objective is to transfer cultivated mycelium onto agar plates to measure differences in growth rates after exposure (Mycology Start, 2023). Success will be determined by measurements showing steady growth across all samples, enabling an estimated calculation reflecting any potential growth delay across contaminant levels.
- The third objective is to use ICP-MS/MS to measure the heavy metal content in the water, spoil, and all the fungi samples. This provides a baseline of the heavy metals present in the water and spoil samples that will be compared with regional toxicity thresholds. The heavy metals and contaminants to be studied are arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn). Success would be determined by the presence of these contaminants across all samples, increasing in quantity with the coal spoil substrate.
- The final objective asks the question 'does *Pleurotus ostreatus* grown on coal spoil contain more contaminants?' The fungi ICP-MS/MS data will then be analysed with a Welch's t-test (Mishra, *et al.*, 2019) using the below null hypothesis to establish any statistical significance between the samples:

Null Hypothesis H_0 : Sample contaminant means are not different - $H_0: \mu_1 = \mu_2$

Alternative Hypothesis H_A : Sample contaminant means are different - $H_A: \mu_1 \neq \mu_2$

Success would be determined by there being statistical significance, reflecting that the amount of coal spoil used as substrate impacts the amounts of contaminants adsorbed by *Pleurotus ostreatus*.

2 LITERATURE REVIEW

This literature review provides a recapitulation of coal mining and remediation efforts at Varteg Hill, Torfaen, along with an overview of mycoremediation, and a critical examination of the use of *Pleurotus ostreatus* in remediation efforts.

2.1 VARTEG HILL, TORFAEN



Figure 2.1: Aerial view of coal tips with highlighted Cradle for Nature site at Varteg Hill (Jones, 2023)

The site at Varteg Hill has records of mining dating from 1840 until closure in 1957 (Welsh Coal Mines, 2006). From 1963 the area was converted to remediated grassland with little further input until Cradle for Nature started planting tree species such as Sessile Oak (*Quercus petraea*), Black Alder (*Alnus glutinosa*), and Scotch Pine (*Pinus sylvestris*) from 1990 onwards (Cradle for Nature, 2023a). This area, as seen in Fig 2.1, was chosen as its condition as a poorly reclaimed and actively degrading ex-colliery site is significantly problematic even amongst the industrial blackspots of Germany, Poland, and the Czechia (Singh & Haigh, 1995). With three known colliery sites surrounding the sampling site, the land has a thin layer of topsoil (<150mm) over dense clay (40-70cm and 1.6-2.0 g/cm³), and then loose surface sandstone and shale coal spoil (Haigh & Kilmartin, 2015). This site was also chosen as it follows the same layering and coal type as neighbouring Aberfan (Finch & Fookes, 1989), a site world renowned for the disaster of 1966 which highlighted the necessity for remediation of coal tips in Wales (Lee, 2023).

Similarly, the site soil quality and tree growth has been monitored over approximately 20 years, allowing for a valuable and detailed record of environmental changes as a direct result of phytoremediation.

Table 2.1: Soil chemical analysis at UWTSO Dart site, (Cradle for Nature, 2012a)

		Air Dry soil	Air Dry Soil	Air Dry Soil	Air Dry Soil
		01-SEP-12 11:00	01-SEP-12 11:15	01-SEP-12 11:15	01-SEP-12 11:30
		S13	S15	S18	S20
Analyte	Units				
Fluoride, acid extractable : Dry Wt	mg/kg	<20	<20	<20	<20
Bromide : Dry Wt	mg/kg	<0.3	<0.3	<0.3	<0.3
Chloride : Dry Wt	mg/kg	24.3	<10	<10	<10
Sulphate : Dry Wt	mg/kg	28.2	20.4	28	20.8
Nitrogen : Dry Wt as N	mg/kg	3140	2160	3070	3180
Carbon, Organic : Dry Wt as C	%	7.63	4.27	7.12	9.03
Conductivity : Solid sample	m S/cm	0.027	0.0351	0.0639	0.0454
pH : Solid sample	pH Units	4.78	5.13	4.94	5.56
Aluminium : Dry Wt	mg/kg	12100	10100	9600	8990
Antimony : Dry Wt	mg/kg	<10	<10	<10	<10
Arsenic : Dry Wt	mg/kg	7.93	8.76	9.03	8.25
Barium : Dry Wt	mg/kg	305	157	200	170
Beryllium : Dry Wt	mg/kg	0.724	1.21	0.825	1.13
Boron : Dry Wt	mg/kg	8.57	5.53	6.53	6.77
Cadmium : Dry Wt	mg/kg	<0.25	<0.25	<0.3	<0.25
Calcium : Dry Wt	mg/kg	1410	1160	1590	1390
Chromium : Dry Wt	mg/kg	14.6	23.9	16.3	20.1
Cobalt : Dry Wt	mg/kg	7.14	14.1	15.2	20.4
Copper : Dry Wt	mg/kg	31.2	37.7	36.5	33.8
Iron : Dry Wt	mg/kg	13400	26000	19700	26400
Lead : Dry Wt	mg/kg	50.2	36.5	53.1	46.4
Lithium : Dry Wt	mg/kg	20	9.36	9.26	8.41
Magnesium : Dry Wt	mg/kg	645	815	770	679
Manganese : Dry Wt	mg/kg	281	752	566	1150
Mercury : Dry Wt	mg/kg	<2	<2	<2	<2
Molybdenum : Dry Wt	mg/kg	<2	<2	<2	<2
Nickel : Dry Wt	mg/kg	15.1	24.5	23.6	36.6
Phosphorus : Dry Wt	mg/kg	225	310	344	332
Potassium : Dry Wt	mg/kg	2970	2200	2590	2210
Selenium : Dry Wt	mg/kg	<1	<1	<1	<1
Silver : Dry Wt	mg/kg	<10	<10	<10	<10
Sodium : Dry Wt	mg/kg	163	86.8	85.8	74.4
Strontium : Dry Wt	mg/kg	82.2	64.2	80.2	71.8
Thallium : Dry Wt	mg/kg	<3	<3	<3	<3
Tin : Dry Wt	mg/kg	<20	<20	<20	<20
Titanium : Dry Wt	mg/kg	4.44	11.2	5.9	9.03
Vanadium : Dry Wt	mg/kg	25.1	46	29.6	35.1
Zinc : Dry Wt	mg/kg	62.8	103	102	121
Dry Solids @ 30°C	%	98.4	98.7	98.6	96.2
Accreditation Assessment	No.	3	3	3	3

As per Table 2.1, soil chemical analysis was carried out at the site in 2012 (Cradle for Nature, 2012a) which provided insight into the fluctuations across the area, as reported from soil analysis research on other coal tips (Chowdhury, *et al.*, 2021). The pockets of altering contaminants would be problematic for tree species that are vulnerable to certain metals (Filcheva, *et al.*, 2021), and so additional efforts would be required to address higher metal concentrations in certain areas. Fungi species such as *Pleurotus ostreatus* are capable of being trained to cultivate on virtually any substrate such as cigarette butts (Sheldrake, 2020), and so could be a potential candidate for addressing contaminant 'hot spots.'

As seen in Appendix 6, a paper by the Cradle for Nature team showed that phytoremediation over a 20–27-year period had improved the soil, but areas between closed canopy were not showing as positive results (Filcheva, *et al.*, 2021). This would suggest that remediation efforts utilising alternative or smaller species in tandem would be beneficial in targeting those pockets. In addition to tree planting, the addition of fungi species could potentially improve those areas, with a quicker turnaround time and the benefit of introducing a new food source for a range of fauna, thus dispersing metals evenly throughout the environment (Akhtar & Mannan, 2020). This addition of a food source, particularly for invertebrates, would be a valuable addition with the findings showing that phytoremediation has increased bird and small mammal numbers (Haigh, *et al.*, 2020), which in turn increases biodiversity and soil remediation (Garbisu, *et al.*, 2020).

A study into phytoremediation efforts at Varteg (Filcheva, *et al.*, 2021) showed that mortality for a range of tree species was high, partially due to planting method, but overall due to the compacted soil in the area. This evidence showed that trees required more assistance to counteract the auto-compaction occurring at Varteg Hill to survive long enough to provide remediation assistance. It would have been beneficial at this stage to explore both mycorrhizal and saprobic fungi as a solution to this problem; ectomycorrhizal fungi such as *Boletus spp.* (which is now naturally present at the site) would have provided a sheath around the tree root system (Sayner, 2023), allowing for extra growth and sharing nutrients whilst also aerating compacted soils (Incredible Mushrooms, nd). Comparatively, saprobic fungi such as *Pleurotus ostreatus* would have broken down decaying or dead organic matter to create healthier soils (National Park Service, 2021).

Further, research from leaf and soil sampling at Varteg (Desai, *et al.*, 2019) showed that phytoremediation had decreased certain heavy metals by a significant amount over 14 years; 52% for Cd, 48% for Cu, 47% for Zn, 44% for Pb, and 35% for Mn. It was found that leaves had increased concentrations of all sampled metals, with different species absorbing at varying levels and speed.

However, these concentrations were only detected in older and more established trees; similarly, the improvement in soil quality was not even across the area. This would suggest that not only are certain species not as efficient in remediating the soil, but that close planting techniques that restrict the movement of leaf litter would result in the metals returning to the soil through leaf decomposition. Whilst mycoremediation is also vulnerable to contaminants returning to the soil this way, harvesting of fruiting bodies is a simple process with multiple uses for the material elsewhere (Stépien, *et al.*, 2016). Based on this observation, combining both phytoremediation and mycoremediation would provide a more balanced approach to the remediation of this site whilst minimising the potential return of metals to the soil.

2.2 PREVIOUS RESEARCH INTO MYCOREMEDIATION

Whilst remediation of contaminated land has been utilised to improve environmental conditions since the Roman times (Litchfield, 2020), modern remediation has mostly been researched since the 1970s. In contrast, the term ‘mycoremediation’, coined by Paul Stamets, is a term created relatively recently when research focus broadened to include different species of fungi (Anderson & Juday, 2016) as a method to remediate polluted soils. As can be seen in Table 2.2, research mostly investigated remediating soils damaged by industrial processes, and the species of fungi used are varied; the species determines whether the pollutants are absorbed into the mycelium through bioaccumulation, or adsorbed onto the fungi surface through biosorption (Kulshreshtha, *et al.*, 2014).

Table 2.2: A summary of various studies into the applications of mycoremediation, (Macfarlane, 2024a)

REFERENCE	SAMPLE COLLECTION SITE	FUNGI SPECIES	CONTAMINANTS TESTED	ANALYSIS METHOD	STATISTICAL ANALYSIS
Bhatt <i>et al</i> (2002)	Soil from former tar-producing plant, Ostrava, Czechia	<i>Irpex lacteus</i> / <i>Pleurotus ostreatus</i>	PAH	Bioluminescence and seed-germination tests	
Leonardi <i>et al</i> (2007)	Two aged PAH-contaminated soils from sites in the Czech Republic	<i>Irpex lacteus</i> / <i>Pleurotus ostreatus</i>	PAH	Extracts were evaporated under reduced pressure in Rotavapor (Buchi, Switzerland), dissolved in acetonitrile and analyzed using HPLC	Statistica (StatSoft Inc., Tulsa USA) was used for all statistical evaluations
Singh <i>et al</i> (2015)	Arsenic contaminated soils were collected from the paddy fields in the middle Indo-Gangetic plains (IGP) of the Northern India	54 fungal strains	Arsenic	ICP-MS	Tukey's test
Khan <i>et al</i> (2019)	Soil samples from discharge effluent sites of Volta Industry Co. Ltd. and Steel Mills Co. Ltd., Hattar Industrial Estate, District Haripur, KPK, Pakistan	<i>Aspergillus</i> spp.	Hg, Pb, Ni, Cu, Cd, Zn, Cr	AAS	One-way ANOVA at $p < 0.05$ level of significance was applied by using least significant difference (LSD) and Tukey's test by using SPSS 20 (SPSS, Inc.) and GraphPad Prism (GraphPad software)
Elsahie <i>et al</i> (2020)	Silica sand samples artificially contaminated with commercial diesel-fuel obtained from a gasoline station at a concentration of 6000 mg kg ⁻¹	<i>Trichoderma harzianum</i>	Diesel	Gas chromatography	Tukey B Post Hoc multiple comparison test with a probability of $p < 0.05$ using SPSS statistical software package (version 13.0; IBM, Armonk, NY, USA)

An early study into mycoremediation was carried out investigating the possibility of removing PAH from soil samples taken from a tar-producing plant in Czechia (Bhatt, *et al.*, 2002). This research focused on two species of saprobic white-rot fungi, namely *Pleurotus ostreatus* and Milk-White Toothed Polypore (*Irpex lacteus*). To measure the amount of PAH that had been extracted by the fungi, high performance liquid chromatography (HPLC) was used to separate the components (GenTech Scientific, 2023).

Whilst the results seen in Appendix 7 showed that both species could remove PAH from soil, it was noted that artificially polluted soils produced better results. This would suggest that soils on industrial sites are inconsistent with the spread of pollution levels, or that certain contaminants had already degraded (depending on the age of the site) and existing vegetation was also altering these levels; alternatively, it is possible that this process had no effect. For site specific data, multiple soil samples taken from various areas across the site would be beneficial to identify the fluctuations and therefore be able to determine what toxicity threshold either fungi species could adequately remediate.

Another study carried out using soils sourced in Czechia (Leonardi, *et al.*, 2007) followed on from the previous research by focusing on aged PAH-contaminated soils. It also used certain mobilizing agents, such as Tween 80, Tween 20, soybean oil, and OMW (olive mill waste) as previous research had shown that the addition of these agents improved the fungal degradation of PAHs (D'Annibale, *et al.*, 2005).

The results seen in Appendix 8 showed that the mobilizing agents either had little effect or inhibited fungal growth; however, the addition of these agents did show that certain PAHs could be degraded more efficiently with *Pleurotus ostreatus* regardless of stunted development. Similarly, the absence of the mobilizing agents resulted in higher degradation of alternate PAHs.

Whilst the results showed that using mobilizing agents have significant potential for certain PAHs, it should be understood that by hindering the adsorption of others overall would only partially remediate the soil. It would therefore be more beneficial to determine what pollutants are in the target site and their quantities before deciding whether the use of a mobilizing agent would be suitable.

Research in India examined the effectiveness of mycoremediation in several pre-existing fungi species (seen in Appendix 9) on arsenic pollution in rice paddy fields (Singh, *et al.*, 2015). Rather than selecting a specific fungi species, this research collected samples of multiple species already present on sites polluted with arsenic. The tolerance and remediation potential of these species was then evaluated using ICP-MS and statistical analysis with a Tukey's Test (Statistics How To, nd).

The results as seen in Appendix 10 showed that several of the 54 strains used were effective in the remediation of arsenic without compromising neighbouring vegetation growth. From this it was determined that further study into remediating arsenic-polluted soils should focus on the highest performers from this group as a refined collective. Whilst this provided valuable insight into effective strains, it should be acknowledged that all 54 strains were tested separately, and so knowledge on how effective they are combined is limited to the soil samples initially collected.

Research into remediating industrial discharge effluent from sites in Pakistan (Khan, *et al.*, 2019) was conducted to determine the remediation potential of various Common Molds (*Aspergillus spp.*), particularly for the presence of heavy metals lead (Pb) and mercury (Hg). Rather than cultivate the strains in the soil itself, the soil was sterilized and 2.5g was added to the mycelium being grown on Sabouraud dextrose broth (SDB), yeast peptone glucose (YPG), and Czapek yeast extract agar (CYE).

The results as seen in Appendix 11 found that certain strains of *Aspergillus spp.* were effective in the uptake of Pb and Hg and could be used in situ or ex situ to remediate soils. However, no indication was given to what would be done with the mold once uptake was completed; as around 40 strains of *Aspergillus spp.* are hazardous to humans and animals (of which several were used in the study), and can cause aspergillosis (CDC, 2021), future use or management of the strains would not involve composting or addition to animal feed as is used for other species (Hernández, *et al.*, 2021).

Combining mycoremediation with ozonation (Elshafie, *et al.*, 2020), research has also investigated the effect of Toxic Mold (*Trichoderma harzianum*) on artificially diesel-contaminated sand. Ozonation was added to the process to oxidise organics within the diesel which are usually difficult to remove.

The results as shown in Appendix 12 displayed that despite most fungal species being vulnerable to crude oil, the strain of *Trichoderma harzianum* used was effective in remediating the diesel, especially after the ozonation treatment; after this process the diesel removal were around 70.16% and 88.35%.

Whilst this research showed great potential for removing diesel contaminants, the process meant that it cannot be conducted in situ and effective treatment would have to be conducted ex situ. For large-scale sites that require remediation, the need for ex situ processing would be cost intensive, evidencing that in situ research such as this project are more commercially viable. Like *Aspergillus spp.* this species of fungi is also hazardous to humans (Sal, *et al.*, 2022), and so there would be further processing required once the remediation has concluded.

2.3 PREVIOUS RESEARCH INTO *PLEUROTUS OSTREATUS*

Table 2.3 presents a summary list of several studies into the mycoremediation potential of *Pleurotus spp.* Significantly, common themes have emerged, particularly regarding remediating former coal mining sites. Being a low-cost and high-yield species, *Pleurotus ostreatus*' biosorption method means that higher quantities of contaminants can be adsorbed, making it a useful mycoremediation species to study (Kapahi & Sachdeva, 2017).

Table 2.3: Summary of various experiments into the use of *Pleurotus spp.* as a species for mycoremediation (Macfarlane, 2023)

REFERENCE	SAMPLE COLLECTION SITE	FUNGI SPECIES	CONTAMINANTS		ANALYSIS	
			TESTED	METHOD	STATISTICAL ANALYSIS	
Vavrková et al (2017)	Leachate from Kuchyňky landfill, Czech Republic	<i>P. ostreatus</i>	Cd, Cr, Ni, Pb, and Zn	AAS	Image Tool 3.0	
Li et al (2017)	Soil artificially polluted with heavy metals	<i>P. ostreatus</i>	Cd and Cr	AAS	SPSS: Unspecified	
Jaikaran et al (2017)	Gold mining soil from Region Seven, Kurupung Creek, Guyana. Bauxite mining soil from Region Ten, East Montgomery, Guyana	<i>P. ostreatus</i>	Co, Pb, Cu, Fe, and Pb	AAS	T-Test	
Vaseem et al (2017)	CWE from Bharat Coking Coal Limited, Dhanbad, India	<i>P. ostreatus</i>	Pb, Mn, As, Al, Cr, Cd, Co, Cu, Zn, Ni, and Fe	FTIR	Duncan's Multiple Range Test (DMRT)	
Vaseem et al (2020)	CWE from Bharat Coking Coal Limited, Dhanbad, India	<i>P. ostreatus</i>	Cr, Ni, Pb, Co, Mn, Zn, and Cu	ICP-MS	Duncan's Multiple Range Test (DMRT)	
Sithole et al (2022)	Various metal mining sites, Pretoria, South Africa	<i>P. ostreatus</i>	Cr, Mn, Co, Ni, Cu, Zn, As, Cd, and Pb	ICP-MS	SPSS v.23: Student T-Test	

Whilst the contaminants were not sourced from a mining site, the study at Kuchyňky landfill (Vavrková, *et al.*, 2017) tested for a similar list of heavy metals found in coal spoil, including: Cd, Cr, Ni, Pb, and Zn. This research also provided a method of inoculating *Pleurotus ostreatus* with the leachate via soaking it into the substrate that attempted to replicate the natural environmental conditions. This *in vivo* process mimicked more natural adsorption by the fungi, as opposed to an *in vitro* method, which would not account for more realistic and natural conditions experienced by the fungi and therefore may produce different results (MPKB, 2022).

One weakness of this study was the lack of information as to whether there was any delay in the lifecycle of *Pleurotus ostreatus* when mixed with the leachate; this information would provide a valuable insight into approximately how much of a delay can be expected, or how much less yield would be produced overall. This information is crucial to determine how much extra development time is required, as well as how much contamination can be adsorbed by a potentially stunted yield. Importantly, in this study the

mushrooms were harvested at maturity thereby demonstrating the viability of *Pleurotus ostreatus*. However, a further weakness is that this does not provide a baseline of time compared with a control.

Furthermore, the use of atomic absorption spectroscopy (AAS) as a means of analysing heavy metal content means that any other non-metal contaminants would not be detected, and the process would destroy any samples used, removing the possibility of recovery post-analysis or of re-testing the same sample (Measurlabs, 2023). Overall, whilst AAS is a more cost-effective process, as per Appendix 13, it is understood that ICP-MS is preferred due to the ability to detect more elements while delivering much faster analysis (Agilent, 2023).

Research focusing on artificially polluted soils (Li, *et al.*, 2017) attempted to calculate both the removal and tolerance of Cd and Cr by *Pleurotus ostreatus*. This research isolated certain contaminants to concentrate on the potential of remediating them individually and was carried out in two parts- mycelium was grown in 100ml of liquid culture containing different levels of Cd and Cr and incubated for 10 days, and soil was divided and contaminated with 10 mg g⁻¹ of either CdCl₂·2·5H₂O or CrCl₃·6H₂O, inoculated with fungus and cultivated for 7 days.

This research, like that carried out at Kuchyňky landfill (Vaverková, *et al.*, 2017), used AAS to analyse the heavy metal content in the fungal samples. This process is better suited to this research given that it is only aiming to measure Cd and Cr rather than a range of contaminants, however the limitations and relative slowness of AAS has been described above.

Whilst Li *et al* (2015) provided a process for artificially replicating contaminated soils, it is evident that isolating certain metals does not produce a true representation of contaminated soil. As seen in Table 1, concentrations are not evenly dispersed throughout the site, and so pinpointing one contaminant will not account for interactions with any other materials or compounds that may be present.

Similarly, harvesting *Pleurotus ostreatus* after only 7 and 10 days of respective growth does not provide sufficient time to allow for fruiting bodies to develop (Bhatti, *et al.*, 2007). Research has shown that most of the heavy metal adsorption potential of this species is in both the stem and the cap rather than the hyphae (Sithole, *et al.*, 2022) and as such, harvesting prior to the development does not give an accurate indication of the adsorption potential.

A study in Guyana focused on growth of *Pleurotus ostreatus* and remediating soils around gold/bauxite mines (Jaikaran, *et al.*, 2017). The process involved a mixture of equal parts contaminated soil and straw substrate being combined prior to inoculation with mushroom spawn. This method aimed to replicate the process that would be used should it become a large-scale method for remediation. After inoculation, *Pleurotus ostreatus* was allowed to reach fruiting stage (approximately 4 weeks for this study) before harvesting for testing with AAS and statistical analysis with ANOVA.

The results seen in Appendix 14 showed that the adsorption capacity of *Pleurotus ostreatus* was not statistically significant across either sample, and so the addition of another remediation method would be required for these soils. It was also noted that fruiting bodies from the bauxite samples were smaller in size than the gold samples, but also that the second harvest was noticeably smaller for both samples. The reason for the difference in size was determined to be due to the bauxite soil containing higher concentrations of cobalt (Co), copper (Cu), manganese (Mn) and iron (Fe).

Whilst this study displayed differences in growth and yield in different contaminants, it would have been beneficial to also vary the concentration of contaminants mixed with the substrate; a 50:50 mix would be a labour-intensive process on a commercial scale. By reducing the straw substrate and/or increasing the soil substrate, mortality and growth could have been effectively measured to determine the minimum required added substrate to produce a similar result.

Studies using *Pleurotus ostreatus* have also attempted to remediate coal wash effluent (CWE). The first of these studies listed on Table 3 was performed *in vitro* with material from Bharat Coking Coal Ltd, Jharkhand (Vaseem, *et al.*, 2017) and cultivated mycelium on agar contaminated with difference concentrations of CWE. After 28 days the samples were tested via ICP-MS and the results were analysed using Duncan's mean range test (DMRT).

The results as seen in Appendices 15-17 showed promising outcomes, with the 50% CWE samples performing the best by remediating the following percentages: 57.2% manganese (Mn), 82.6% zinc (Zn), 98.0% nickel (Ni), 99.9% copper (Cu), 99.3% cobalt (Co), 99.1% chromium (Cr), 89.2% iron (Fe), and 35.6% lead (Pb) (Vaseem, *et al.*, 2017).

A weakness of this study is that the samples were cultivated over 28 days, but mycelium was only taken for metal analysis from the 5th to the 20th day. No rationale was provided for excluding the final 8 days, and hence a fuller representation of what was adsorbed during the cultivation timescale is missing over 25% of collectable data. As a result, this severely limits the inferences that can be drawn from the data. It may also mean that the data is skewed in favour of those metals which are adsorbed early in the lifecycle of *Pleurotus ostreatus* compared with those adsorbed at maturity. Furthermore, it is known that adsorption by *Pleurotus ostreatus* is most effective at maturity and hence sampling prior to reaching full maturity compromises the potential for further adsorption of heavy metals and contaminants.

Another issue was the impracticality of carrying out remediating CWE on a larger scale. Whilst there is potential for a high level of remediation through metal uptake, there's no indication as to how this would be implemented.

The second study remediating CWE listed on Table 3 was also performed with material from Bharat Coking Coal Ltd as it was conducted by the same team (Vaseem, *et al.*, 2020). This research differed from the first by being carried out *in vivo* rather than *in vitro*. Straw substrate was submerged overnight in water containing different concentrations of contaminants with the substrate being inoculated with *Pleurotus ostreatus* within 24 hours. Following harvesting at 35 and 42 days, the samples were again tested using ICP-MS and the data was analysed using DMRT.

In contrast to their previous study (Vaseem, *et al.*, 2017) the results in this study (see Appendices 18-20) differed. The present study showed that the 25% contaminated sample provided the best rates of remediation: 100% nickel (Ni), 99.04% zinc (Zn), 100% copper (Cu), 100% cobalt (Co), 100% chromium (Cr), 84.66% manganese (Mn), and 67.92% lead (Pb) (Vaseem, *et al.*, 2020). It was noted that all samples remediated to some degree, but the higher concentrations had less effective results. These results differ from the previous study which identified the 50% contaminated sample providing the best rates of remediation with greater variance in the metal adsorption. As this study also focused on permissible thresholds for human consumption, the differences between the two pieces of research have identified that an *in vitro* methodology does not produce results that reflect the natural environment and can falsify results that can potentially be dangerous.

Though the present study reflected a more realistic model through an *in vivo* method, scalability remains problematic. However, should a course for making this commercially viable occur, the levels across all samples being within a safe consumption threshold provides an outlet for handling the fungi later (Vaseem, *et al.*, 2020).

Research into metal concentrations of mushrooms grown from coal spoil in Pretoria, South Africa (Sithole, *et al.*, 2022) obtained soil samples from four different mining sites (platinum mining, chromium mining, iron/chromium mining, and a control site). This was combined with a straw substrate to cultivate *Pleurotus ostreatus* over a 5-month period. After harvesting, the samples were tested with ICP-MS and the data was analysed using a T-test.

The results as seen in Appendix 21 showed that the concentrations of cadmium (Cd), cobalt (Co), chromium (Cr), manganese (Mn), nickel (Ni), zinc (Zn), mercury (Hg), and lead (Pb) were statistically significant across all sites, with the fungi adsorbing enough to not pose a risk to human health unless consumed in large quantities. Whilst this was not strictly a remediation study, the intended use for human consumption could be regarded as a method for remediating contaminated areas after further study in the ethics and overall need has been performed.

A major weakness of this research was that the ratio of soil and straw was not disclosed, leaving ambiguity with the results and relative effectiveness. The samples were grown as per a previous study conducted by the same team (Sithole, *et al.*, 2017); however, this project also failed to disclose the quantities used. Without this information, it may be the case that the heavy metal values adsorbed by *Pleurotus ostreatus* were directly affected by how much soil was used.

Furthermore, the use of a student T-test has assumed a normal distribution for the data and the research has not identified in the current study or the previous (Sithole, *et al.*, 2017) why this assumption has been made.

2.4 SUMMARY OF LITERATURE REVIEW

The data and literature available for the site at Varteg consistently and accurately identified the area as heavily impacted from coal mining and an excellent example of industry along the South Wales coalfield. It has also shown that despite remediation efforts spanning several years, the site has remained polluted and alternative remediation methods may be beneficial.

The work reviewed around mycoremediation displayed that whilst several species have been tested for remediation potential, white-rot species appear to produce the best results. This is due to their higher adsorption capacity, the low costs associated with production, and also re-use and management potential having no harmful impact on the environment.

Literature focused on *Pleurotus ostreatus* showed that whilst using an *in vitro* process has shown that this is useful to isolate certain contaminants and accurately measure adsorption potential, those using the *in vivo* process achieved a result that achieved a closer replica of the natural conditions of the contaminant collection sites.

3 MATERIALS AND METHODOLOGY

3.1 RESEARCH DESIGN

Using quantitative data, the methodology followed an empirical experimental approach (Melville & Goddard, 1996); a systematic trial and observation, the research was planned with an intended purpose with carefully recorded results as the answer to the research question was not already known. The independent variable for this methodology was *Pleurotus ostreatus*, whilst the dependent variable was the coal spoil content added to each sample of the independent variable. Identified prior to proceeding, the nuisance variables were light exposure, humidity, and temperature. To convert these into controlled variables, the samples were all cultivated in conditions to keep them constant, such as being given equal exposure to light, kept in a moderately temperature-controlled lab, and grown in monotubs of the same size/design with the same humidity control.

3.2 MUSHROOM CULTURE AND COLLECTION OF SOIL

The *Pleurotus ostreatus* spawn was obtained from commercial business Gourmet Woodland Mushrooms, who source refined strains from Bratislava (Gourmet Woodland Mushrooms, 2023a). This strain was chosen due to being a reliable and healthy sample.

The grow tubs used were a combination of a branded Boomer Shroomer monotub (Mycopunks, 2023), and an unbranded version with the same specifications (NAIXU, 2023). This style of monotub allowed for easy hydration and drainage, as well as limited humidity and ventilation control (Boomer Shroomer, 2023).

Black agar plates were used to achieve a visual representation of mycelial growth. Due to time constraints, rather than self-production, black agar plates were sourced from UK company AgarInAJar (AgarInAJar, 2023). However, after plate contamination occurred both prior to and during sample transfer (see Appendices 22 and 23), these were replaced with light malt extract agar (LMEA) plates from DIY Science (DIY Science, 2024). Mycelium was taken from the monotubs at fruiting after 32 days and was transferred to the plates within a still air box (Mycology Start, 2024). Once transferred, the plates were sealed as shown in Fig 3.1 and measured over a course of 15 days.

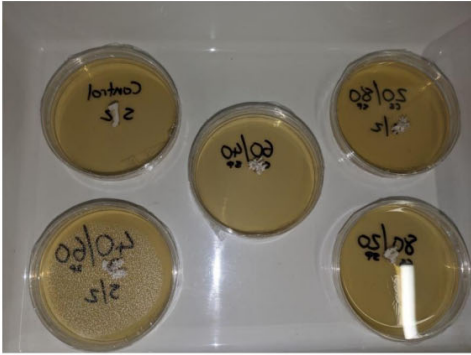


Figure 3.1: Sections of sampled *Pleurotus ostreatus* added to LMEA plates (Macfarlane, 2024b)

Approximately 20kg of contaminated soil was collected from Varteg Hill, Blaenavon (coordinates 51°44'44.9"N 3°04'41.4"W). As shown in the QGIS map in Fig 3.2 (Macfarlane, 2024), the soil was collected from the Cradle for Nature research site (Cradle for Nature, 2023b).

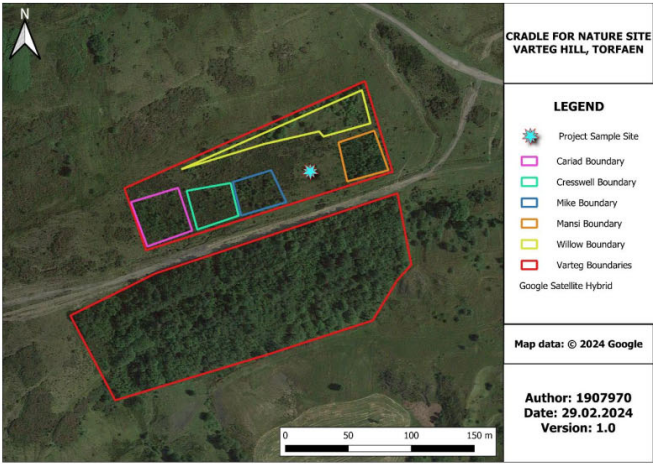


Figure 3.2: Map of Cradle for Nature's site at Varteg Hill, Torfaen, with pinpointed soil collection site (Macfarlane, 2024)



Figure 3.3: Soil collection site at Varteg Hill, Torfaen (Macfarlane, 2023b)

The site was chosen for several reasons- firstly, as shown in Fig 3.3, it is subject to excessive water run-off pooling, especially in bad weather (collection was carried out after 5 days of moderate to heavy rain). Additionally, it is in an area of the Cradle for Nature site that is not currently being disturbed by ongoing projects. Furthermore, the existing datasets from several years of research carried out by Cradle for Nature and UWTSO provided insight into the condition of the site over several years (Cradle for Nature, 2023a). Finally, by taking the averages of the chosen contaminants from Table 2.1, a comparison could be made between their levels and the UK's normal background concentrations set out by the UK and Welsh governments. As shown in Table 3.1, it can be seen that contaminants marked with green are within a relatively safe range, amber is within 25% of the threshold level, and red is at or beyond the threshold toxicity level, meaning it will have a negative impact on wildlife and soils (Welsh Government, 2021). Notably, these thresholds are the point at which each individual contaminant would have a negative impact; it can therefore be reasoned that multiple high concentrations in the amber range would cumulatively have a similar negative effect.

Table 3.1: Mean contaminant level compared with generic UK SSVs- majority of SSV data from Wales (Welsh Government, 2021), *data from National Soil Resources Institute (NSRI, 2017), **data from Environment Agency (Environment Agency, 2007)

Analyte	Units	Varteg Mean	Generic SSV
Arsenic : Dry Wt	As mg/kg	8.4925	79
Cadmium : Dry Wt	Cd mg/kg	0.2625	0.6
Chromium : Dry Wt	Cr mg/kg	18.725	21
Cobalt : Dry Wt	Co mg/kg	14.21	4.2
Copper : Dry Wt	Cu mg/kg	34.8	35.1
Iron : Dry Wt	Fe mg/kg	21375	28147.8*
Lead : Dry Wt	Pb mg/kg	46.55	630
Manganese : Dry Wt	Mn mg/kg	687.25	598**
Nickel : Dry Wt	Ni mg/kg	24.95	28.2
Zinc : Dry Wt	Zn mg/kg	97.2	59.7

The sample location was in a clear section of the site, surrounded by less established heather (*Calluna vulgaris*) and bilberry (*Vaccinium myrtillus*). The sample was taken from one of many waterlogged troughs to ensure a lower chance of removing any established species, as patches on the edge of this area were shown to have yellow club fungus (*Clavulinopsis helvola*) present. Furthermore, the site was chosen due to the lack of biodiversity or environmental development, evidencing that the soil quality was of a low quality. Additionally, using this patch helped to determine viability in soil with a high clay content.

As per Appendix 24, topsoil of approximately 3" was removed prior to collection so that the layer could be returned to the site; this was to reduce the amount of relatively healthy topsoil being removed from the site, which is an ongoing issue with other remediation techniques (Alexander, 2019).

3.3 PREPARATION OF STRAW SUBSTRATE AND SOIL MIX

As per Table 3.2, adequate hydration of the straw substrate required 1.733l of water for every 1kg with the exception of the samples with a higher concentration of coal spoil; similarly, to ensure optimum conditions, maximise full inoculation and improve yield capacity, 250g of *Pleurotus ostreatus* spawn was added per 1kg of substrate (Gourmet Woodland Mushrooms, 2023). The quantity of water was decreased by 50% for both samples 4 and 5 to compensate for the increase in clay/coal spoil and decrease in straw pellets, which had a direct impact on the hydration threshold (RHS, 2023). As the substrate was unable to hold as much water, reducing the total water added minimised the risk of creating an unsuitable environment for *Pleurotus ostreatus* (Saputera, et al., 2020).

Table 3.2: Quantities and concentrations of materials and contaminants across all samples, (Macfarlane, 2024g)

Samples	Materials			
	Straw Pellets	Coal Spoil	<i>P.ostreatus</i> Spawn	Distilled Water
1	1500g	N/A	375g	2600ml
2	1200g	300g	375g	2600ml
3	900g	600g	375g	2600ml
4	600g	900g	375g	1300ml
5	300g	1200g	375g	1300ml

After combining the substrate and spawn, half of the water mixture was added and allowed to soak for 30 minutes, upon which the remaining water was added. A total of 1500g of substrate, 375g of *Pleurotus ostreatus* spawn, and 1300/2600ml of distilled water was used per grow tub for the inoculation process.

The water used to hydrate the substrate was produced from a mixture of distilled water and water collected after being mixed with a sample of the soil for a period of 28 days. Whilst understood that full leaching of contaminants into water takes several years or chemical additives (as seen with soil washing) (Shipitalo & Bonta, 2008), this process was designed to replicate standing water from both seasonal weather and run-off. The water purity was to be matched to the level of soil mixed into the substrate; as shown in Table 5, where the sample had a soil/straw ratio of 80:20, the water would also hold a contamination solution of 80% (it is noteworthy that this percentage is regarding the overall toxicity level of the water and not a direct match to the soil). As previously stated, the process to fully contaminate the water would take several years or the use of chemical additives. Therefore to produce a hydration solution that replicated runoff the process was adapted from the studies both in South Africa (Sithole, *et al.*, 2022), and India (Vaseem, *et al.*, 2020) to ensure that dilution of contaminants didn't occur through the hydration process.

To create the water solution, a combination of techniques was carried out. Firstly, 8kg of soil was mixed with 8kg water, stirred weekly, and left in a combined state for 28 days to allow for natural leaching that would simulate coal wash effluent. Additionally, by following a basic soil washing technique of filtration, the spoil mix was processed through a series of filters to separate the smaller particles. This process is not the most efficient at separating heavy metals from soils and more intensive techniques with chemical additives are recommended (Dermont, *et al.*, 2008); however, due to the limited facilities such as no access to a centrifuge, the filtering process was carried out manually. Manually filtering the water from the spoil was a highly labour-intensive and time-consuming process; in 24hrs, only 2.5l of water was filtered, approximately 2l of which was water that was above the settled clay layer, as shown in Fig 3.4.



Figure 3.4: Water and spoil mixture after clay settling (L) and after manual mixing (R), (Macfarlane, 2024f)

As shown in Fig 3.5, only around 500ml of the clay layer was filtered in a 24hr period from the mixture, continuing with this method would take too long and push back the inoculation date to a point that would compromise the entire project. As a result, inoculation of the substrate was performed with distilled water, and the 2.5l of filtered water that was produced was used as described above- diluted into matching concentrations and used to indirectly mist spray the tubs.



Figure 3.5: Manual filtration of water from spoil sample (Macfarlane, 2024e)

3.4 PLEUROTUS OSTREATUS GROWTH ANALYSIS ON SUBSTRATE

In vivo cultivation of *Pleurotus ostreatus* provided a more realistic result of contaminants not being evenly spread across the substrate and gave insight into how the mushrooms would grow with any other undetected materials. This process was supported by previous research in Pretoria, South Africa (Sithole, *et al.*, 2022) which accounted for natural fluctuations in the soil content. However, a key difference from both this and previous research (Sithole, *et al.*, 2017) was that the ratios of coal spoil and substrate was set in rising increments of 20% from the control up to the highest value of 80% coal spoil.

Harvesting of *Pleurotus ostreatus* can occur after as little as 21 days (Urban Farm It, 2023). Allowing a delayed growing cycle due to exposure to contaminants, harvesting was carried out after 42 days for all samples except the 80% coal spoil sample which required a further 8 days to achieve full fruit. Recent studies have harvested in a similar timeframe (Vaseem, *et al.*, 2020), as well as waiting far longer for repeated fruiting and/or full adsorption potential to be achieved (Sithole, *et al.*, 2022). However, due to time constraints the latter process was not replicated.

Following both general cultivation advice (Urban Farm It, 2023) and specific research methodology after the work of Sithole *et al* (2022), indirect spray watering of the individual monotubs was performed. To ensure even and regular spraying, inoculation wasn't started until January 2024; this both avoided campus closure days over the Christmas period which would impact initial growth. To maintain the high humidity required, daily indirect spraying was performed 7 days after inoculation until the spawn had reached the pinhead stage (Ahlawat & Tewari, 2007), after which spraying was reduced to every 3 days. Waiting 7 days after inoculation minimised the risk of flooding the samples and allowed the distilled water added for initial hydration to be absorbed (Hamel, 2023).

Upon reaching pinhead stage, it was noted that growth deformities were present across all samples except the 80% contaminated samples. Based on the growth pattern becoming more coral shaped than the initial and more typical 'star' stemming, it was decided that relocating the monotubs to an area with more natural light and a slighter higher temperature would minimise these irregularities (Spore Shift, 2023).

3.5 PLEUROTUS OSTREATUS MYCELIAL GROWTH ANALYSIS ON AGAR PLATES

Samples of *Pleurotus ostreatus* were cut from each monotub when they had reached the end of the colonisation stage and had started to develop hyphal knots (Mycologic, 2019); a small section from each monotub was then added to an individual agar plate (see Appendix 12).

Growth on the agar plates was monitored via both microscope and diameter measurement over 15 days. The black agar plates were all contaminated, as seen in Appendices 22 and 23, and thus growth comparison could not be measured against these. Cultivation on agar is easily contaminated (Shi, *et al.*, 2019); to rectify this, transfer of samples was repeated in a makeshift still air box (Kosel & Ropret, 2021) on light malt extract agar (LMEA) plates. The LMEA plates replaced the black agar to minimise the yeast content and the potential for reactivation in humid conditions. The control was added to a compromised LMEA plate as seen in Appendix 25, and thus a replacement control was reproduced with no contamination, as shown in Appendix 26.

Any delays in growth in the contaminated samples were compared with the control to estimate an average delay per contaminant strength.

3.6 CONTAMINANT ANALYSIS IN FRUITING BODIES, WATER, AND SUBSTRATE

Initial basic testing was to be conducted using the Palintest Complete Soil Management Kit (Palintest, 2019). However, upon starting this research the Palintest Kit was unfortunately discontinued, and further reagents were unavailable (Palintest, 2023). As a result, all testing was carried out via ICP-MS/MS (inductively coupled plasma mass spectrometry) as the literature showed that this method is a more effective and efficient process and able to detect more contaminants and heavy metals than AAS; this would be beneficial should further research be carried out requiring more in-depth analysis.

A minimum of 5g of *Pleurotus ostreatus* was collected from each of the samples upon harvesting and digested in nitric acid (HNO₃) to be processed for analysis via ICP-MS/MS. Additionally, the water sample was also digested via this process (Thermo Fisher, nd). Due to the soil sample containing coal and other silicates, ideally it should have been digested in perchloric acid (HClO₄) (Anton Paar, 2023), however this process was not available. An alternative method was therefore implemented, utilising aqua regia (Cl₃H₄NO₃) to carry out digestion via EPA's Method 3051A standards (EPA, 2007).

ICP-MS/MS and the required digestion process was unable to be performed at UWTSO; therefore, to perform analysis the samples were sent to Cambridge University's Trace Element Analysis Facility (Cambridge University, 2023) for commercial testing using their Agilent 8900 Triple Quadrupole ICP-MS (Agilent, 2024). Based on studies described in the literature and supported by soil chemical analysis carried out on the site in 2012 (see Table 1), the contaminants tested for were: As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn.

Data provided through ICP-MS/MS was statistically analysed and visualised via Welch's T-test using both Excel 365 and PAST software, version 4.14 (Oslo Natural History Museum, 2023). A parametric method was chosen over non-parametric as the sample size was too small to confirm or reject normal distribution (Krithikadatta, 2014).

4 RESULTS

4.1 CULTIVATION OF *PLEUROTUS OSTREATUS* ON COAL SPOIL

As shown in Figures 4.1-4.4, four of the five samples achieved full fruiting stage within 40 days and were able to be harvested on the 42nd day. As shown in Figure 4.5, the sample cultivated on 80% coal spoil was delayed and achieved a lower yield full fruiting stage by the 50th day; harvesting was also carried out on the 50th day. All samples produced sufficient fruiting bodies to harvest for ICP-MS/MS analysis.

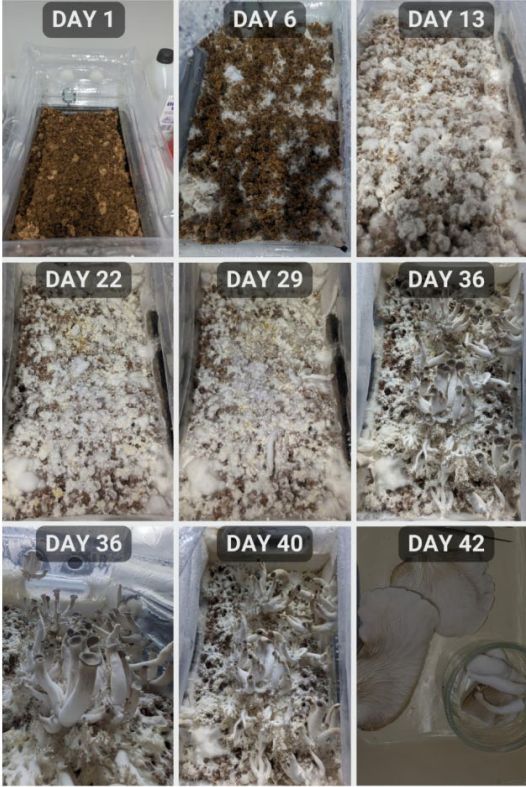


Figure 4.1: Growth progression and harvesting of *Pleurotus* on control substrate (Macfarlane, 2024n)



Figure 4.2: Growth progression and harvesting of *Postreatus* on 20% coal spoil substrate (Macfarlane, 2024n)



Figure 4.3: Growth progression and harvesting of *Postreatus* on 40% coal spoil substrate (Macfarlane, 2024n)



Figure 4.4: Growth progression and harvesting of *Postreatus* on 60% coal spoil substrate (Macfarlane, 2024n)



Figure 4.5: Growth progression and harvesting of *Postreatus* on 80% coal spoil substrate (Macfarlane, 2024n)

4.2 AGAR PLATE MYCELIAL GROWTH

The cut samples from each of the fruiting monotubs, as shown in Figs 4.6-4.10, all achieved mycelial growth to the full extent of the plates within 15 days. The control, shown in Fig 4.6, was able to achieve pinhead fruiting by day 10.

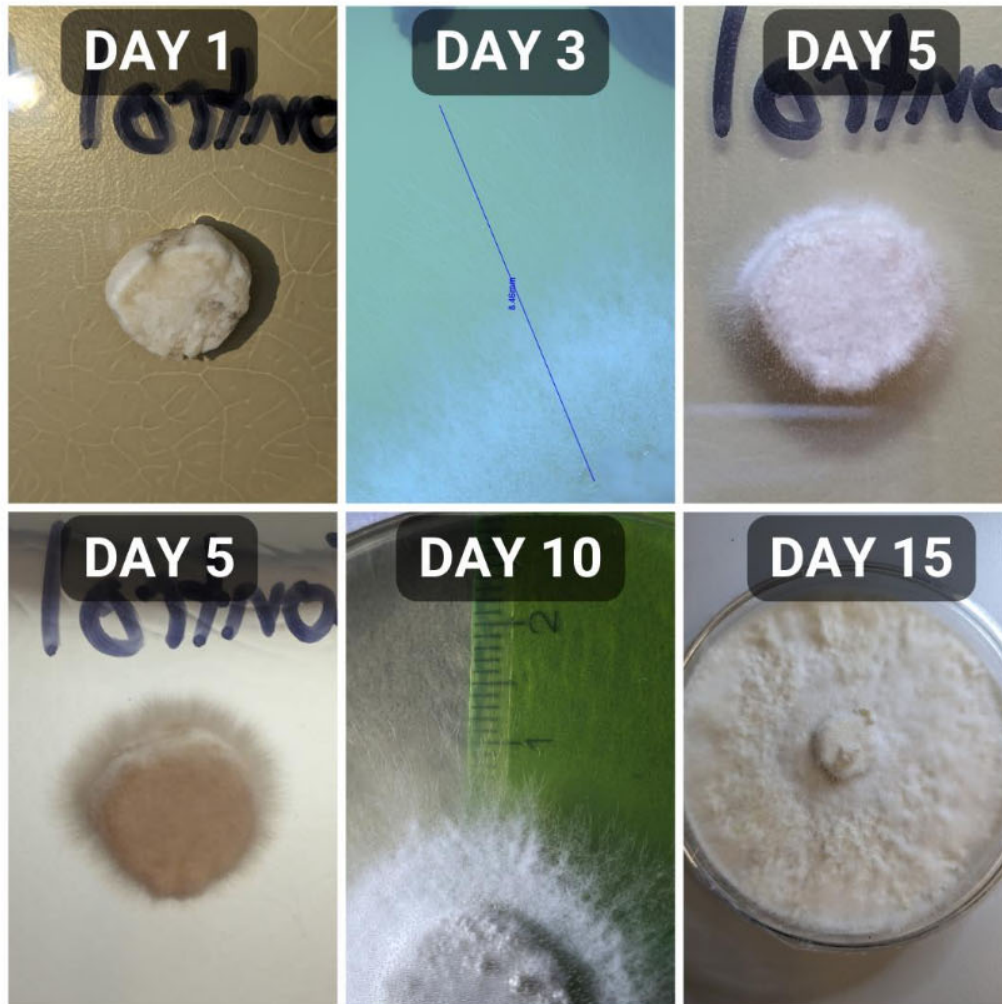


Figure 4.6: Hyphae and mycelial development of control on LMEA (Macfarlane, 2024p)

The 20% contaminated sample, as shown in Fig 4.7, was also able to achieve pinhead stage within 15 days despite some contamination to the plate. The hyphae also spread 5.97mm from the source by day 5 and was unable to be microscopically measured by day 10, having reached 32.9mm.

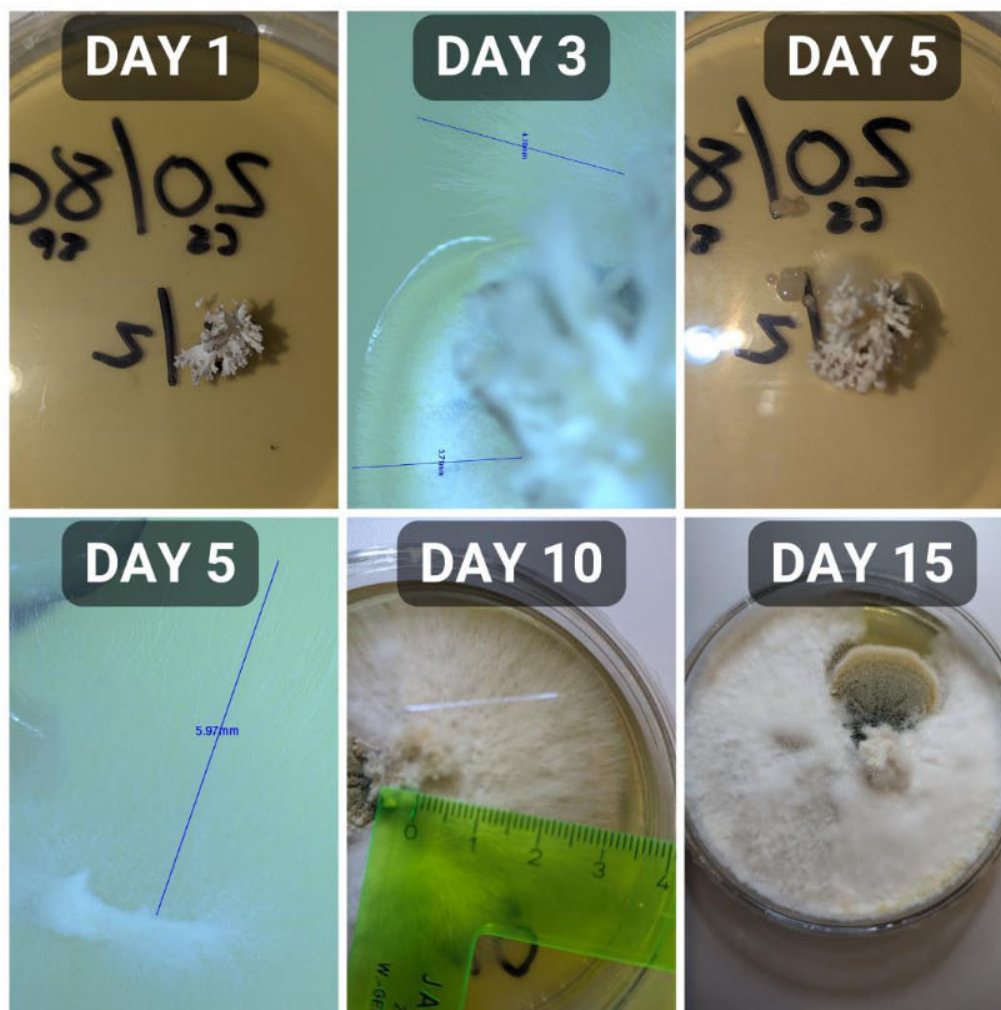


Figure 4.7: Hyphae and mycelial development of 20% contaminated *Postreatus* on LMEA (Macfarlane, 2024p)

In addition, the 40% contaminated sample, as shown in Fig 4.8, was also able to achieve pinhead stage within 15 days. Without any contamination to the plate, the hyphae spread significantly between days 5-10 and was unable to be microscopically measured by day 10, having reached 33.8mm.



Figure 4.8: Hyphae and mycelial development of 40% contaminated *Postreatus* on LMEA (Macfarlane, 2024p)

Furthermore, the 60% contaminated sample, as shown in Fig 4.9, achieved pinhead stage within 15 days. The hyphae spread 7.7mm from the source by day 5 and was unable to be microscopically measured by day 10, having reached 34.8mm.

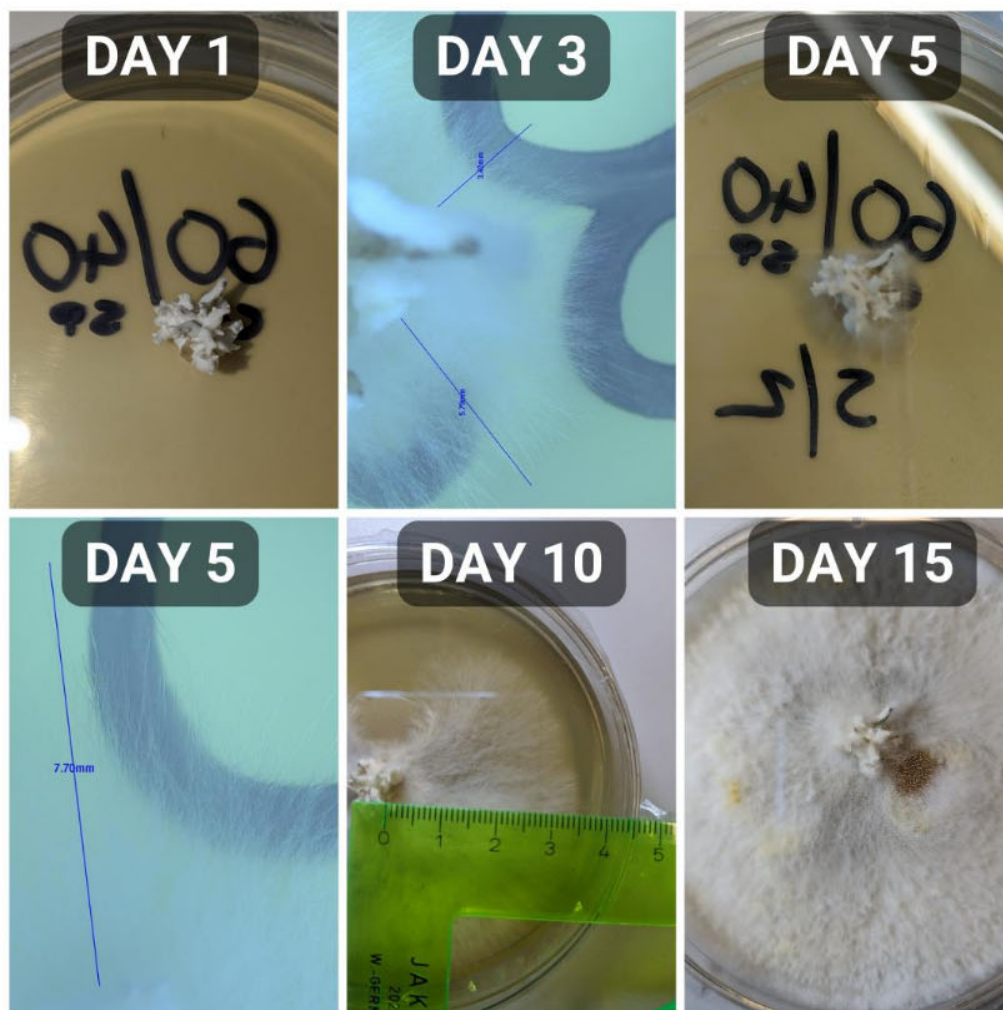


Figure 4.9: Hyphae and mycelial development of 60% contaminated *Postreatus* on LMEA (Macfarlane, 2024p)

Additionally, the 80% contaminated sample, as shown in Fig 4.10, did not achieve pinhead stage within 15 days. However, even with plate contamination the hyphae spread 7.18mm from the source by day 5 and was unable to be microscopically measured by day 10, having reached 30.9mm.

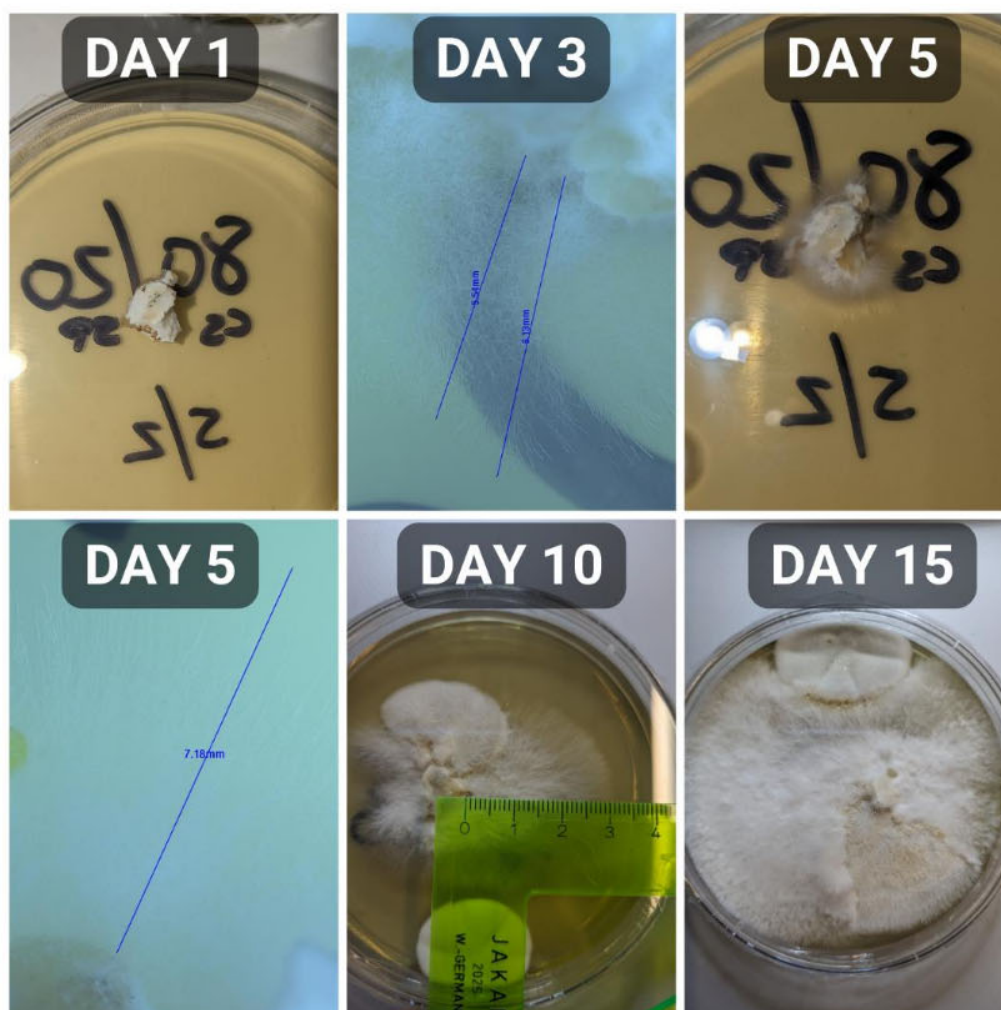


Figure 4.10: Hyphae and mycelial development of 80% contaminated *P.ostreatus* on LMEA (Macfarlane, 2024p)

4.3 ICP-MS/MS ELEMENTAL ANALYSIS

The ICP-MS/MS data provided by Cambridge University, as seen in Appendix 27, was separated into water, spoil, and fungi samples. As shown in Fig 4.11, concentrations of each pollutant vary greatly, with Fe, Zn, and Mn being the most potent, requiring conversion to mg/kg whilst the remaining elements are displayed in $\mu\text{g}/\text{kg}$.

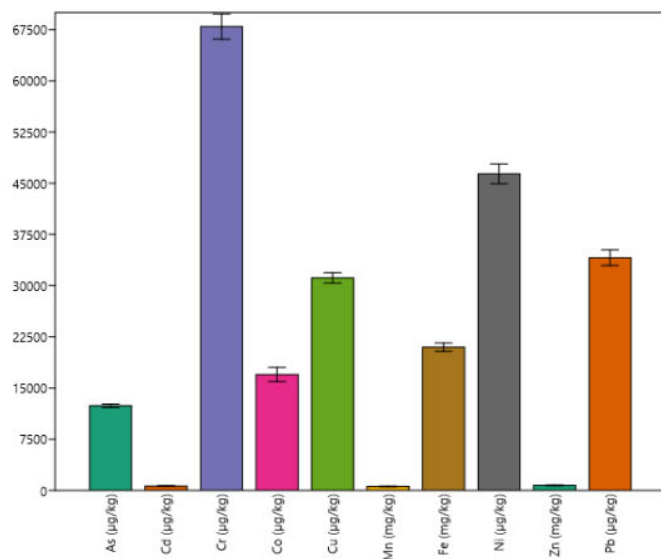


Figure 4.11: Concentrations of pollutants in mine spoil samples (displayed in µg/kg and mg/kg) (Macfarlane, 2024d)

Fig 4.12 shows that the replicated water run-off contains smaller quantities of most contaminants, whereas the uptake of manganese was substantially higher.

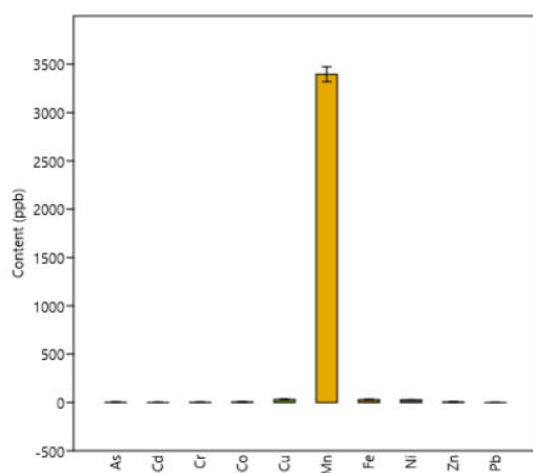


Figure 4.12: Concentrations of pollutants in replicated water run-off samples (Macfarlane, 2024h)

The triplicate tested *Pleurotus ostreatus* samples were separated by each contaminant. As shown in Fig 4.13, the mean for each sample ratio increases substantially for As, whilst Cd uptake peaks at 60%.

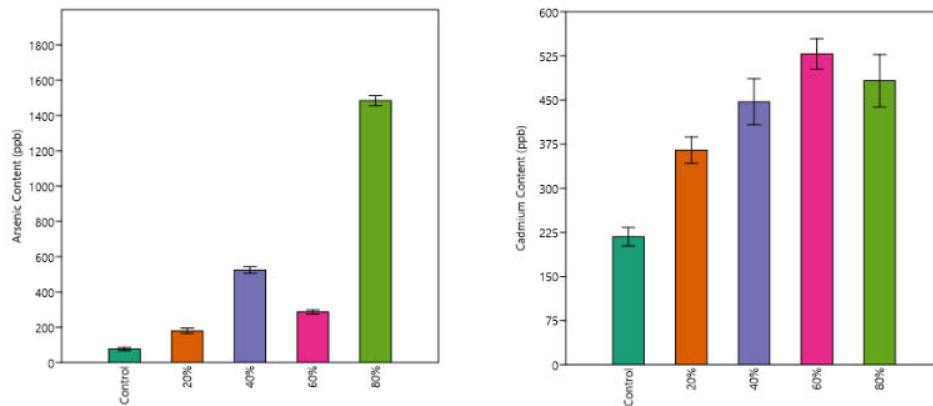


Figure 4.13: Concentrations of As and Cd in harvested *P.ostreatus* samples (displayed in $\mu\text{g/kg}$) (Macfarlane, 2024i)

Similar to As, the Co concentrations shown in Fig 4.14 peak with the highest contaminated fungi. However, Cr concentrations peak at 40% and reflect a normalised distribution despite the small sample size.

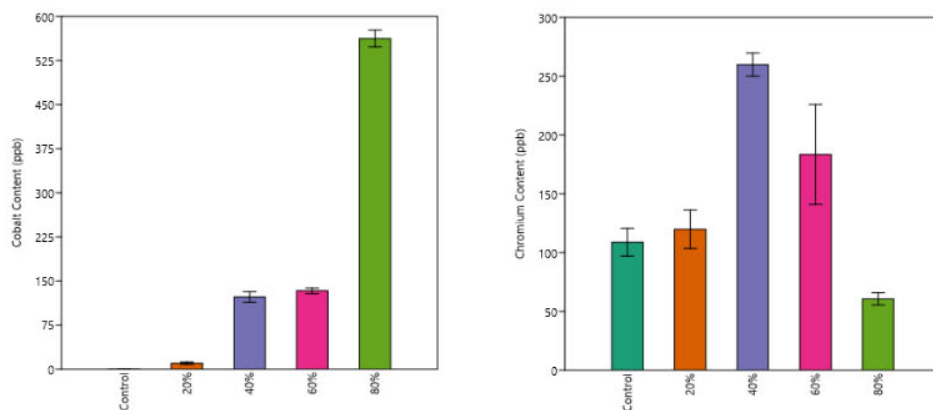


Figure 4.14: Concentrations of Co and Cr in harvested *P.ostreatus* samples (displayed in $\mu\text{g/kg}$) (Macfarlane, 2024j)

The Cu concentrations shown in Fig 4.15 also reflect a normalised distribution, whereas the Mn concentrations are higher at both 40% and 80%, with a drop in adsorption at 60%.

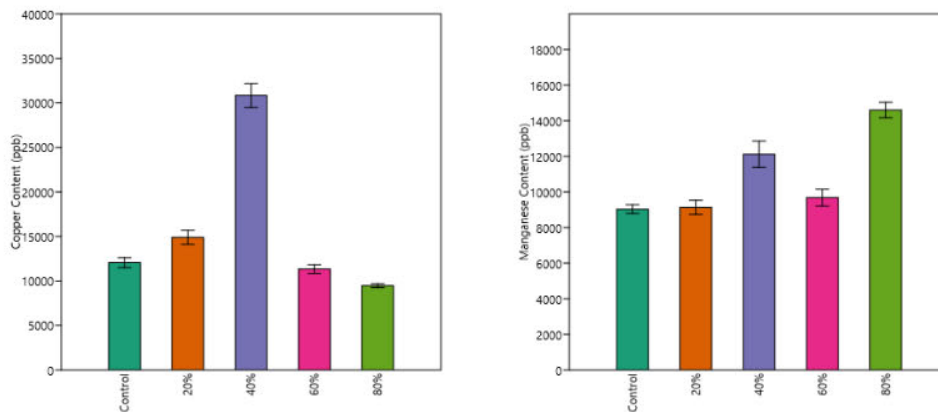


Figure 4.15: Concentrations of Cu and Mn in harvested *Postreatus* samples (displayed in $\mu\text{g/kg}$) (Macfarlane, 2024k)

The concentrations of Ni and Pb, shown in Fig 4.16, are similar to As and Cd in that nickel peaks at 80% whilst lead peaks more around the centre at 40%.

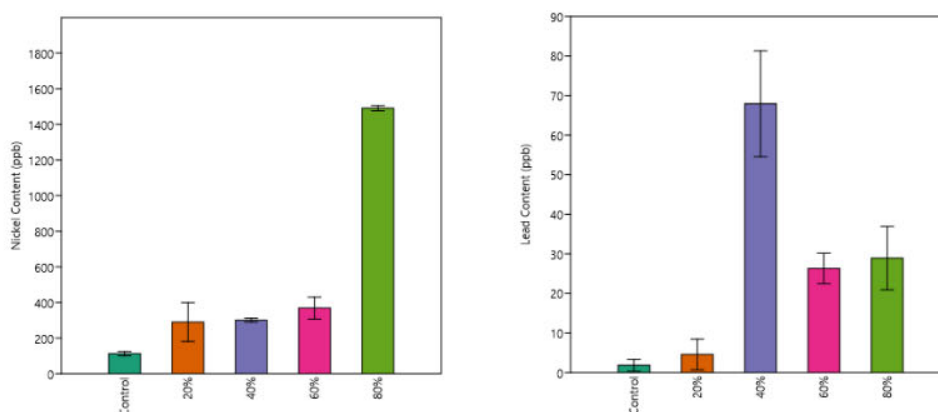


Figure 4.16: Concentrations of Ni and Pb in harvested *Postreatus* samples (displayed in $\mu\text{g/kg}$) (Macfarlane, 2024l)

In addition, the Zn and Fe concentrations shown in Fig 4.17 show that zinc adsorption started to decline after increasing the contamination in the substrate beyond 20%, whereas iron follows more of a bell curve and peaks at 40%.

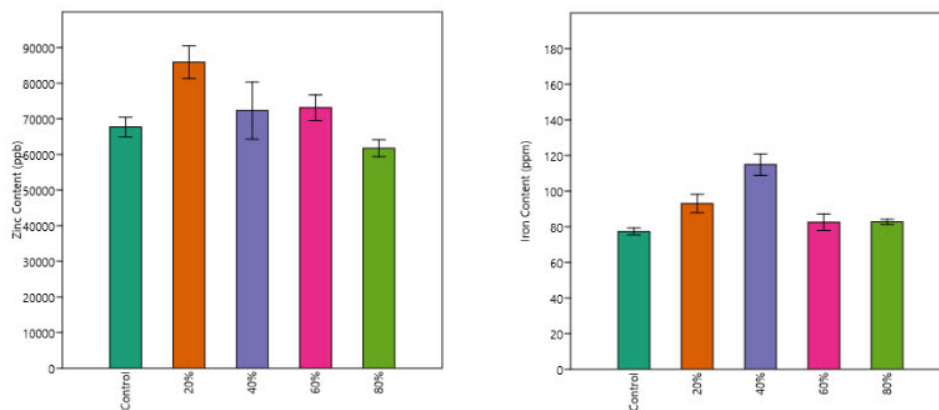


Figure 4.17: Concentrations of Zn and Fe in harvested *Postreatus* samples (displayed in $\mu\text{g/kg}$ and mg/kg) (Macfarlane, 2024m)

4.4 STATISTICAL ANALYSIS

A Welch's T-test was performed for each element against the control, as shown grouped in Fig 4.18 and separately in Figs 4.19-4.23, with the independent variable being the ratio of contamination in the substrate, and the dependent variable being the element content. Grouped together, Fig 4.18 shows that the 40% and 80% contaminated samples achieved the most significance.

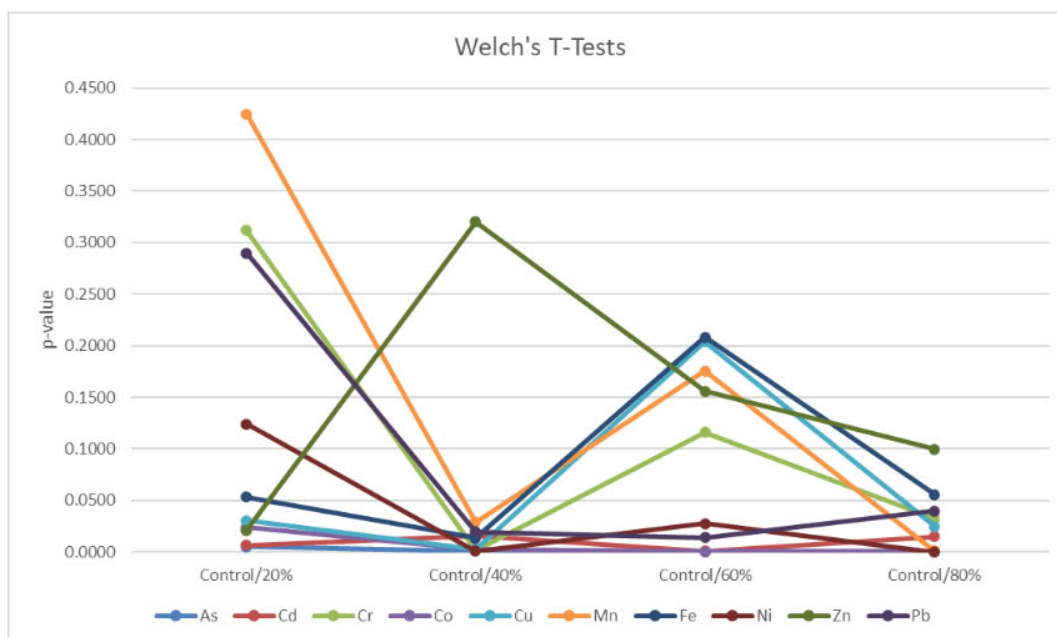


Figure 4.18: Line chart of contaminant t-test p-values (Macfarlane, 2024n)

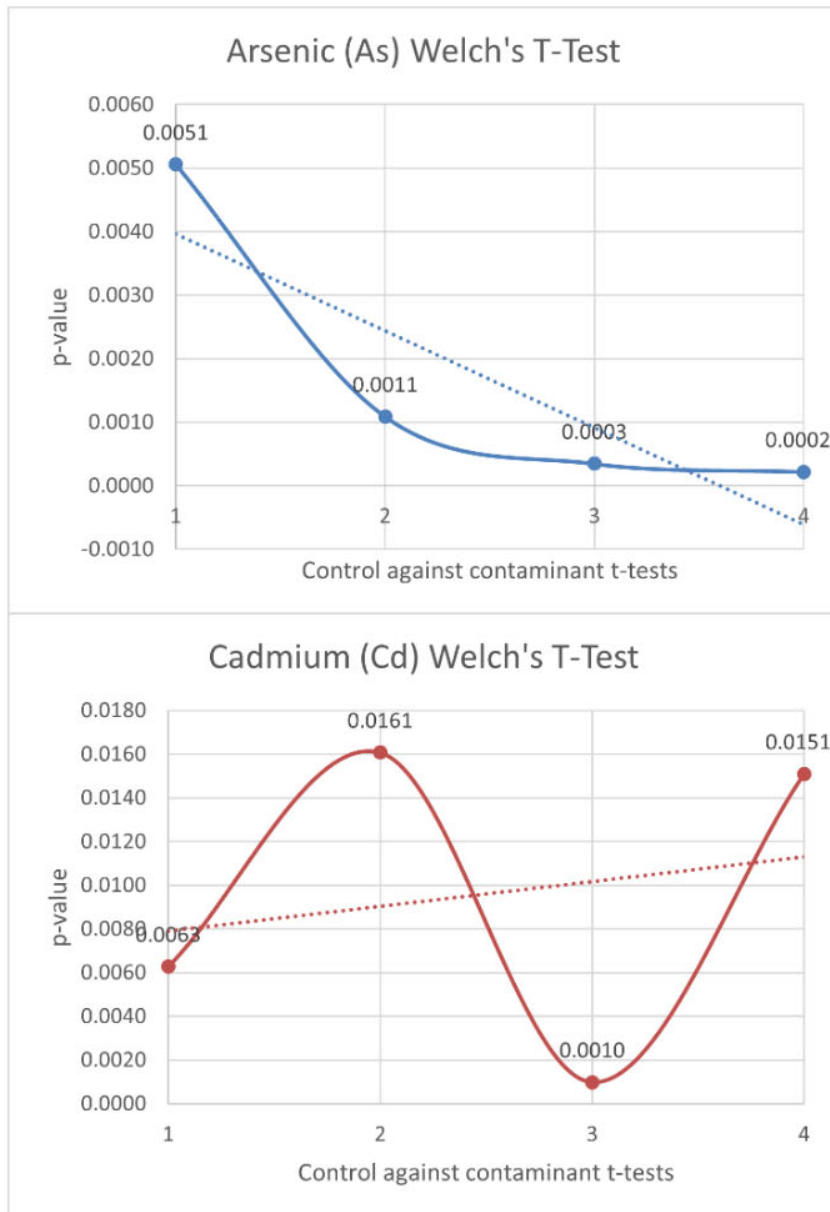


Figure 4.19: As and Cd T-test results across all samples (Macfarlane, 2024a)

The t-test results displayed in Fig 4.19 showed that p-values achieved more significance across higher concentrations for As, whilst the most significant result for Cd was 60%.

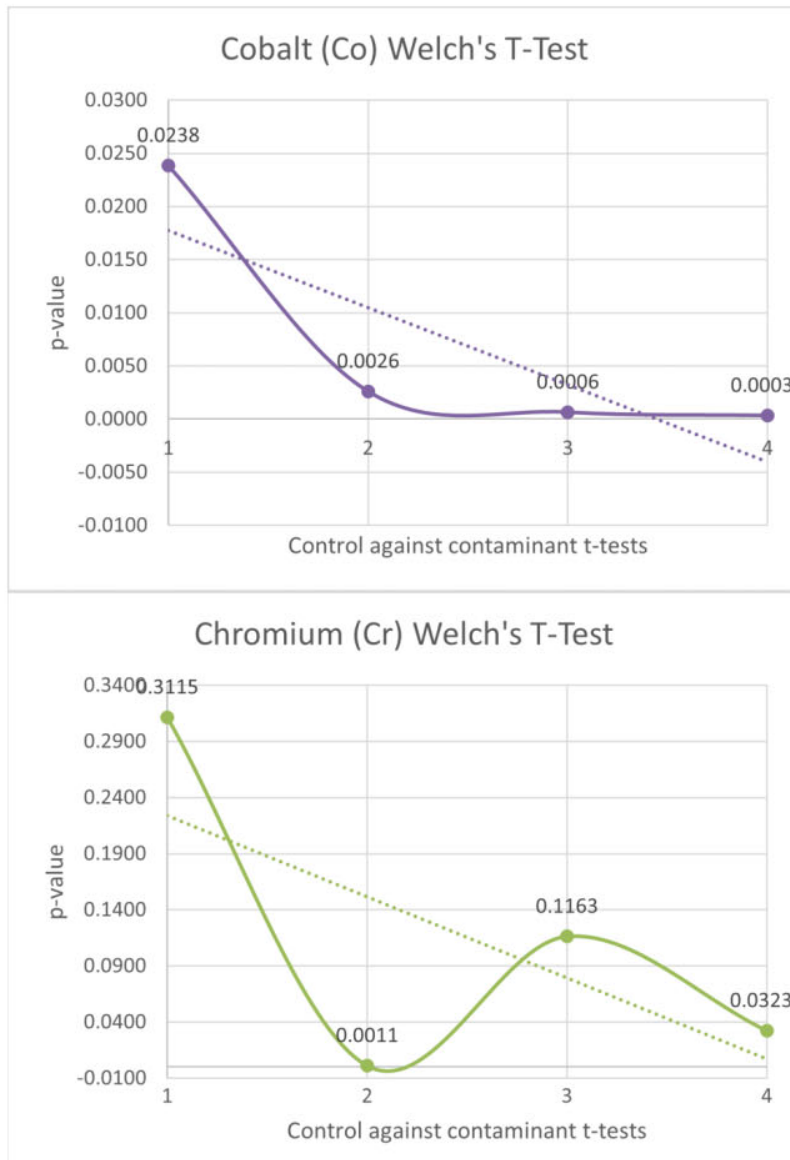


Figure 4.20: Co and Cr T-test results across all samples (Macfarlane, 2024a)

The results shown in Fig 4.20 reflect that Co has greater significance across p-value for higher concentrations. In addition, p-values achieved varying significance across higher concentrations for Cr, with the greatest significance in the 40% sample. However, the 60% and 80% had slightly lower significance, with the 20% having the least significance overall.

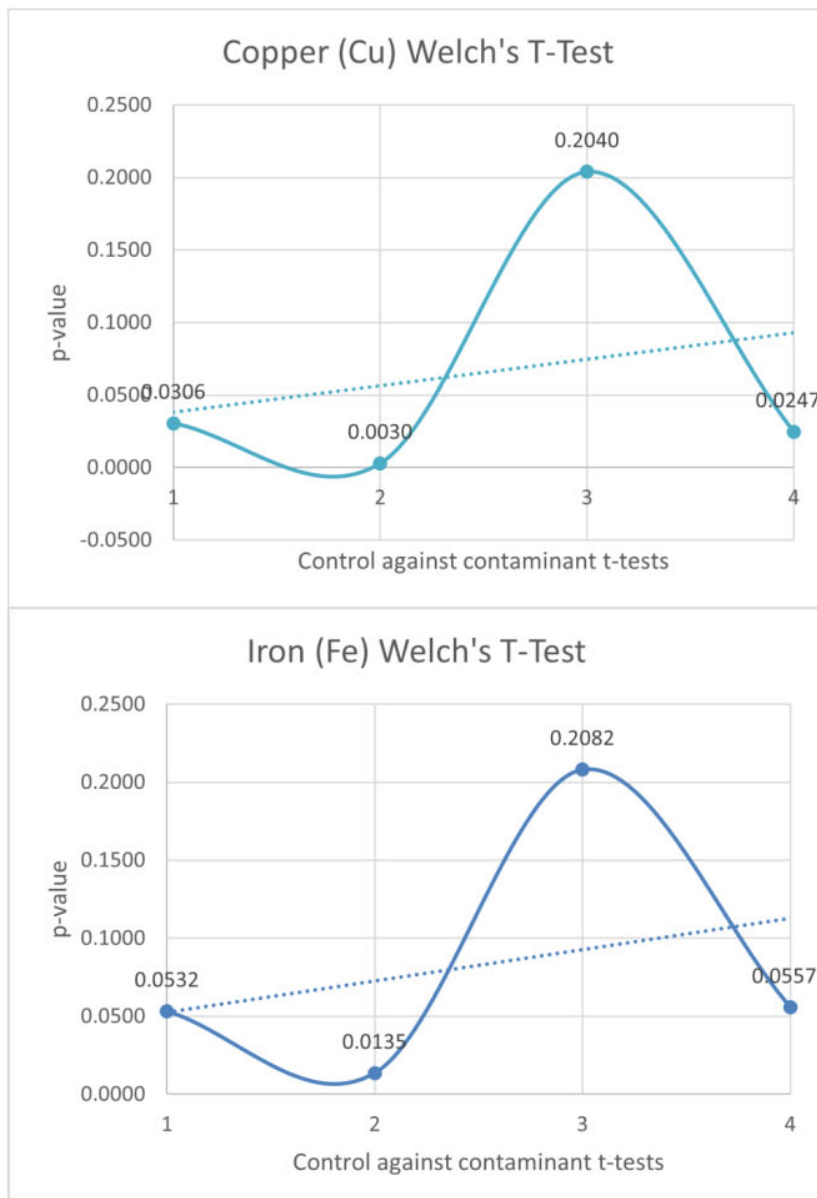


Figure 4.21: Cu and Fe T-test results across all samples (Macfarlane, 2024o)

Additionally, Fig 4.21 shows that the 40% p-value had the greatest significance across the samples of Cu. The 60% notably had the least significance overall. Fe also displays a similar p-value pattern across the samples, with 40% again being the most significant.

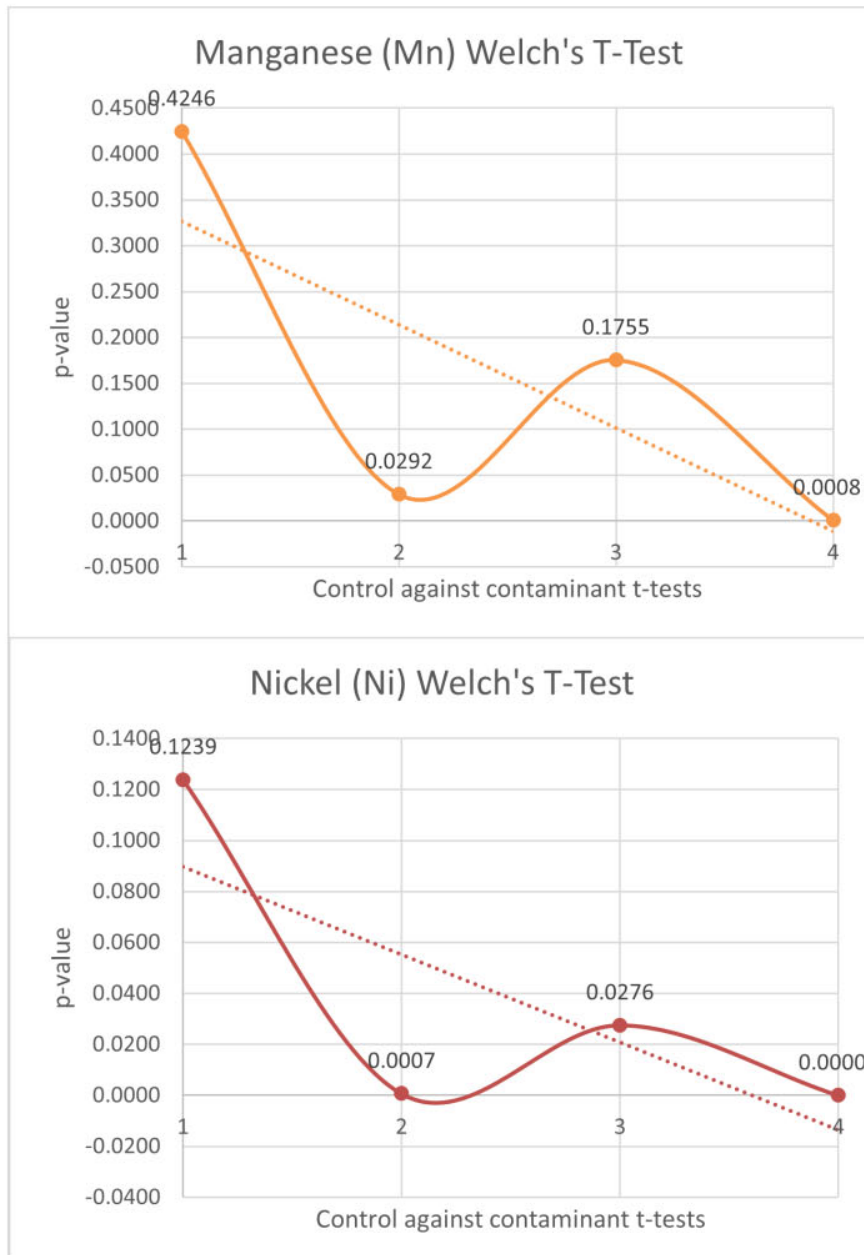


Figure 4.22: Mn and Ni T-test results across all samples (Macfarlane, 2024a)

Furthermore, Fig 4.22 shows that the 80% p-value had the greatest significance across the samples for Mn, with a drop at 60% after a noticeable rise in significance at 40%. Ni also displays a similar p-value pattern across the samples, with 80% again being the most significant.



Figure 4.23: Pb and Zn T-test results across all samples (Macfarlane, 2024o)

Additionally, Fig 4.23 shows greater significance from 40%, whilst the 60% p-value had the greatest significance across the samples for Pb. Zn displays greatest significance at 20% with a marked decrease in significance at 40%. After 40% the p-values start to increase in significance.

5 DISCUSSION

The results indicate that:

- *Pleurotus ostreatus* can be fully cultivated on a mixture of straw pellet substrate and coal spoil and produce sufficient fruiting bodies required for ICP-MS/MS analysis;
- The study demonstrates no correlation between contamination and delayed mycelial development as all samples achieved full LMEA plate colonisation within 15 days;
- The use of a Welch's t-test showed that higher concentrations of substrate contamination produced statistically significant adsorption potential of *Pleurotus ostreatus*. Whilst the 60% sample had statistically weaker results than 40% or 80%, these results are equally stronger than the 20% sample and many of the contaminants still displayed significant adsorption;

The cultivation was successful as sufficient fruiting material was produced from all samples to enable ICP-MS/MS analysis. The results might suggest that the reduced water content at the initial substrate hydration stage caused a development delay in the 60% sample, and that the earliest fruiting displayed in Fig 4.4 was the result of stress, much in the way that certain plants flower when exposed to stress factors (Wada & Takeno, 2010). However, the 80% sample was exposed to stronger stress factors and an equal amount of water yet provided more significant results. As the Cd content was highest in the sample it is possible that, as per findings by Li et al (2017), the effects of Cd stress limited the uptake potential of other elements whilst also causing a fruiting development delay.

The full fruiting stage took longer to complete and produced a lower yield overall compared to all samples with lower contamination levels. Additionally, the 80% sample didn't achieve the fruiting stage until all other samples had fully fruited and were ready for harvesting; the sample was delayed by 20% compared to the control. This confirms some findings by Vaverková *et al* (2017) that higher concentrations result in a noticeable delay in growth.

As shown in Figure 4.5, the structure of the fruiting bodies in the 80% contaminated sample did not form the clustered caps typical of *Pleurotus ostreatus*. Rather, the sample produced individual stalks and caps with browning at the cap edges, all of which were smaller than the other samples. This would also confirm findings by Jaikaran *et al* (2017) that *Pleurotus ostreatus* exposed to higher levels of contamination can produce smaller fruiting bodies. This would suggest that areas with very high toxicity levels would take longer to process in addition to a reduced uptake potential.

The experiment provided an insight into the benefits of *in vivo* studies into the relationship between *Pleurotus ostreatus* and raw soil matter intended for remediation as it supported findings by Vaseem *et al* (2020) that results differ significantly compared to *in vitro* studies. Notably, the agar growth measurement process did not account for the same potential delay of samples cultivated *in vivo* as the conditions were significantly different; however, it provided a visual representation of the process and how the hyphae developed after exposure to varying contaminant concentrations.

There was little difference in growth rate between the plate samples; as shown in Figs 4.7-4.10, by day 10 all contaminated samples ranged between 30.9mm – 34.8mm in mycelial development. By day 15, all samples had fully colonised the LMEA plates. Furthermore, apart from the 80%, all other contaminated samples achieved pinhead stage within 15 days; this would confirm that only the fruiting stages experience a delayed impact by the contaminants, rather than initial mycelial development. These

findings support those of Li *et al* (2017), that contaminated samples cultivated on agar experienced no growth delay over 28 days. This is supported by Vaseem *et al* (2017), who found that growing contaminated samples on agar required no more than 28 days to reach the desired growth stage.

Compared to the soil sample data in Table 2.1, the ICP-MS/MS data in Appendix 27 showed that the means of Cu, Fe, Pb, and Mn were all lower from the sample site, As, Cd, Cr, Co, Ni, and Zn were all higher. As the areas sampled in Table 2.1 had all been subjected to various remediation efforts, it would suggest that these practices have only been successful in remediating a selection of the contaminants. As a result, the species used in these areas would benefit from the addition of a species like *Pleurotus ostreatus* to adsorb the contaminants that would otherwise remain unchanged or be slow to remove.

Statistical analysis of the individual contaminants showed that a higher contamination level did not result in higher levels of adsorption for all elements. For example, whilst As, Co, Mn and Ni achieved more significance with higher concentrations, Cu, Cr, Fe, Pb, and Zn had less significance. This would confirm findings by Jaikaran *et al* (2017) that certain contaminants have a lower adsorption rate depending on the relative concentrations in the substrate, and additionally Vaseem *et al* (2020) that higher concentrations are less effective in remediation potential.

The 40% sample produced the overall most significant results, with the 80% performing similarly well. However, as the fruiting bodies of the 40% sample were more developed and experienced no delay compared to the 80% (which required 50 days before harvesting rather than 42 days) it would suggest that this concentration provides a stronger mycoremediation potential. This would confirm similar findings by Vaseem *et al* (2020) that samples in higher concentrations can adsorb well, but their delayed growth and subsequent size limits the remediation potential.

The 60% sample produced results that were significantly weaker to both the 40% and 80%, and stronger than the 20% samples. As this sample achieved initial fruiting first but took the longest time from initial fruiting to full fruit, this would suggest that this concentration produces the least suitable conditions for *Pleurotus ostreatus*. It should be noted that not all of the measured contaminants achieved weaker significance in this sample; As, Cd, Co and Pb all produced significance to reflect that there was a difference between the control and sample means. The Cr, Cu, Fe, Mn, Ni, and Zn levels showed weaker statistical significance. These results would indicate that further investigation into isolating contaminants would be beneficial to determine what the peak exposure threshold would be for each. By then adding several contaminants together, it would be possible to determine the relationship between these elements and their interaction with the adsorption capacity of *Pleurotus ostreatus*.

The data contribute a clearer understanding of how despite *Pleurotus ostreatus* producing significant adsorption results of the selected contaminants, overall, the quantities that were adsorbed were not significant compared to the levels of contamination in the soil itself. This supports the findings by Jaikaran *et al* (2017) and suggest that any remediation work involving *Pleurotus ostreatus* should consider the fungi as a supportive measure rather than the sole method.

The reliability of the data is impacted by the small sample size, which reduced the robustness of the statistical analysis and limited what statistical analysis could be performed. Additionally, due to the small sample size, normalised distribution could neither be confirmed nor rejected, and so a larger sample size would possibly suggest an alternative statistical analysis to the method that was selected.

The methodological choices were constrained by a lack of facilities and equipment, resulting in adaptations with regards to the replicated water run-off due to no access to a centrifuge. In addition, without a lateral flow hood or commercial still air box, instances of contamination occurred during the sample transfer to LMEA plates.

Moreover, without direct access to ICP-MS facilities, reliance on an external institution to perform the testing within the required timeline was an added stressor, in addition to having no management of the process for the testing for adequate methodological logging. This was further impacted by Cambridge University being unable to perform perchloric acid (HClO_4) digestion, instead adapting to partial digestion with aqua regia ($\text{Cl}_3\text{H}_4\text{NO}_3$).

6 CONCLUSION AND RECOMMENDATIONS

The study revealed that *Pleurotus ostreatus* can adsorb certain elements from soils. Based on the results, it can be concluded that *Pleurotus ostreatus* is effective in remediation of heavy metals and is a viable option for mycoremediation of South Wales coal spoil. However, whilst the results were significant, it is understood that the quantities adsorbed were not a significant amount. As a result, it would be recommended that mycoremediation with *Pleurotus ostreatus* be implemented in addition to alternative bioremediation efforts such as phytoremediation (Desai, *et al.*, 2019) rather than acting as the sole remediation process.

Future studies should consider that it would be recommended to use perchloric acid (HClO_4) digestion. For a more in-depth analysis of future samples, this would allow for full digestion and potentially produce differing data.

Additionally, it is recommended that any further investigation into the mycoremediation potential of *Pleurotus ostreatus* would require a greater number of samples to provide a larger dataset. By confirming normal distribution with the Shapiro-Wilk test (Ahad, *et al.*, 2011), a parametric T-test or non-parametric Mann-Whitney test can then be used based on the results. In addition, with a much larger sample size, further testing can use ANOVA and a post-hoc method such as a Dunn's or Tukey's test will refine the results (Statistics How To, 2024) and provide more robust observations.

Moreover, calculating the loss on ignition at the end of this cycle would provide further data on how much has been adsorbed in the mycelium (Heiri, *et al.*, 2001). As the mycelial development was not impacted by the contaminants and *Pleurotus ostreatus* can adsorb heavy metals via hyphae, this process would give further calculation of uptake potential and can consequently determine best practices for processing the fungi after harvesting.

These results should be considered when targeting smaller areas of high contamination. For example, as per the literature review, areas of Varteg Hill being treated with phytoremediation contain hot spots of differing pollution concentrations, thus the introduction of *Pleurotus ostreatus* would target these areas. Considering the lower levels of adsorption and quicker cultivation cycle of *Pleurotus ostreatus* compared with trees, the remediation process could be performed over a similar timeframe to phytoremediation efforts. It is possible that repeated fruiting and harvesting would produce altering results (Sheldrake, 2020). For overall adsorption efficacy and mycoremediation, it would be recommended to perform a full annual growth and harvest analysis to determine what quantities of contaminants *Pleurotus ostreatus* can remove from the soil in this time.

Avenues for future research include investigation into how to process harvested *Pleurotus ostreatus* and whether it could contribute towards a circular economy model. As healthy soil is a key resource in circular economy, the potential for utilising natural processes with redistributable outputs to improve soil health can be examined in more detail with further investigation into this research. Initial comparisons displayed that whilst samples were not suited for human consumption, lower concentrations were potentially suited for animal feed. If this were to be explored, it would be recommended to explore the ethical impact of this use. Similarly, if added to composting material, further investigation would be recommended to determine what quantities would ensure the least impactful distribution of the contaminants back into the environment.

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7 APPENDICES

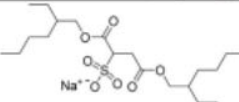
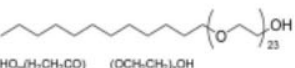
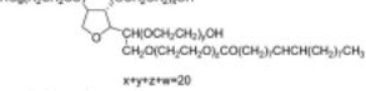
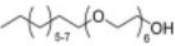
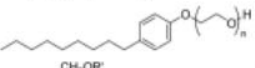
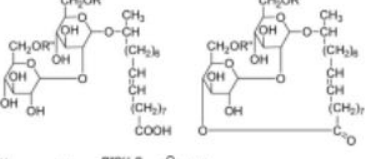
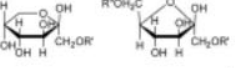
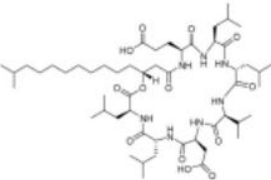
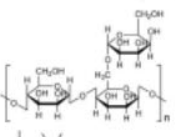
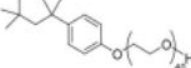
Appendix 1: Table of surfactants used in soil washing, (Mao, et al., 2015)

Table 1
Basic information of the surfactants mentioned in the article.

Surfactant	Name/components	Type	General structural formula/examples of typical structure	Molecular formula/mol. wt.
DPC	1-dodecylpyridinium chloride	Cationic surfactant		$C_{17}H_{30}ClN/283.88$
TX-100	P-tertiary-octylphenoxy polyethyl alcohol	Nonionic surfactant		$C_{26}H_{44}O_4/414.628$
PFOA	Perfluorooctanoic acid	Anionic surfactant		$C_8F_{15}O_2/414.07$
NINOL 40-CO	Cocamide DEA	Nonionic surfactant		$C_{18}H_{32}NO_2/287.44$
CAPB	Cocaoat amide propyl betaine	Zwitterionic surfactant		$C_{18}H_{34}N_2O_2/342.52$
DDAC	Didecyl dimethyl ammonium chloride	Cationic surfactant		$C_{22}H_{48}ClN/362.08$
SLES	Sodium laureth sulfate	Anionic surfactant		$C_{12}H_{25}(OCH_2CH_2)_nOSO_3Na$
SOS	Sodium dodecyl sulphate	Anionic surfactant		$CH_3(CH_2)_{11}OSO_3Na/288.38$
SDHS	Sodium dihexyl sulfosuccinate	Anionic surfactant		$C_{12}H_{24}NaO_5/388.45$
JBR-425	Rhamnolipid	Nonionic biosurfactant		$C_{32}H_{58}O_{11}$ and $C_{26}H_{48}O_8/504.6$ and 650.8
Ammonyx KP	Oleyl dimethyl benzyl ammonium chloride	Cationic surfactant		$C_{27}H_{48}ClNO/436.11$
CTAB	Cetyltriethyl Ammonium Bromide	Cationic surfactant		$C_{18}H_{37}(CH_3)_3NBr$ and $C_{16}H_{33}(CH_3)_3NBr/364.45$ and 406.53
SDBS	Sodium dodecyl benzene sulfonate	Anionic surfactant		$C_{18}H_{29}NaO_2S/348.48$
Texapon-40	Sodium lauryl ether sulfate	Anionic surfactant		$C_{12}H_{23}NaO_5/376.48$

Appendix 2: Table of surfactants used in soil washing (cont.), (Mao, et al., 2015)

Table 1 (Continued)

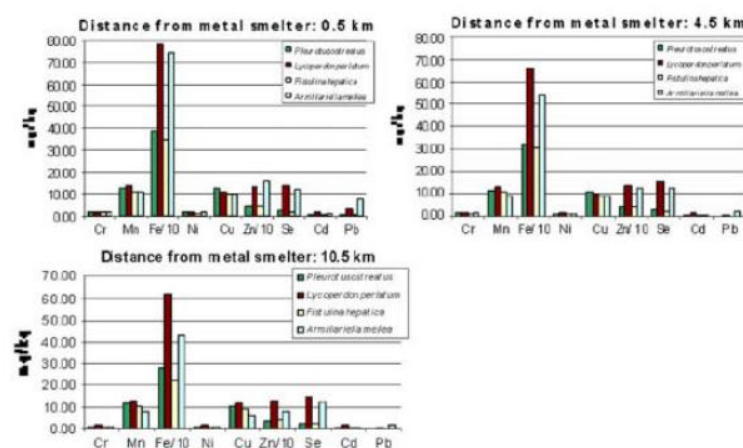
Surfactant	Name/components	Type	General structural formula/examples of typical structure	Molecular formula/mol. wt.
AOT	Bis(2-ethylhexyl) sulfo succinate sodium	Anionic surfactant		$C_{28}H_{52}NaO_7/444.56$
Calfax 16L-35;Dowfax 8390	Sodium hexadecyl diphenyl ether disulfonate	Anionic gemini surfactant		$C_{38}H_{48}Na_2O_7/598.72$
Spolapon AOS 146	Linear sodium alkene sulfonates and hydroxyalkanesulfonates (C12-C16)	Anionic surfactant	$R-CH=CH-SO_3Na$ $R-\underset{H}{\underset{OH}{C}}-CH_2-SO_3Na$ $R = C_{10}, C_{14}$	$CH_3(CH_2)_{8-12}CH=CHSO_3Na$ & $CH_3(CH_2)_{8-12}CH(OH)CH_2SO_3Na$
Brij-35	Poly(oxyethylene) ₂₃ dodecyl ether	Nonionic surfactant		$C_{12}H_{25}(OC_2H_4)_{23}OH/1198$
Tween 80	Polyoxyethylene sorbitan monooleate	Nonionic surfactant		$C_{54}H_{124}O_{26}/1310$
Empilan KR6	Alcohols, C9-C11, ethoxylated	Nonionic surfactant		$CH_3(CH_2)_{8-10}(OCH_2CH_2)_6OH$
Tergitol NP-10	Polyoxyethylene nonyl phenyl ether	Nonionic surfactant		No record (N. R.)
Sophorolipid	Sophorolipid	Nonionic biosurfactant		N. R.
Fructoselipid	Mixture of fructoselipid with different structure	Nonionic biosurfactant		N. R.
Surfactin	Cyclic lipopeptide	Zwitterionic biosurfactant		N. R.
Guar gam	Galactomannan	Nonionic biosurfactant		N. R.
TX-405	Polyoxyethylene (40) isooctylphenyl ether	Nonionic surfactant		$C_{54}H_{121}(OCH_2CH_2)_{40}OH$

Appendix 3: Table of surfactants used in soil washing (cont.), (Mao, et al., 2015)

Surfactant	Name/components	Type	General structural formula/examples of typical structure	Molecular formula/mol. wt.
Brij-58	Polyoxyethylene (20) cetyl ether	Nonionic surfactant		$C_{36}H_{72}(OCH_2CH_2)_{20}OH/1123.5$
Brij-98	Polyoxyethylene (20) oleyl ether	Nonionic surfactant		$C_{38}H_{72}(OCH_2CH_2)_{20}OH/1149.5$
Saponin	Pentacyclic triterpene saponin	Nonionic biosurfactant		N. R.
CAS	Cocamidopropyl hydroxysultaine	Zwitterionic surfactant		$CH_3(CH_2)_{11}-N^+(OH)-CH_2-CH(OH)-CH_2SO_3^-/452.69$

Appendix 4: Concentrations of heavy metals found in mushrooms grown in varying distances from metal smelters, (Radulescu, et al., 2010)

Fig. 1 The concentration of heavy metals in mushrooms species at 0.5, 4.5 and 10.5 km distance from metal smelter



Appendix 5: Growth rate of various species of fungi after exposure to varying concentrations of coal tar, (Tiso, et al., 2015)

Table 4. Daily radial extension rate (cm per day) of fungal isolates on MEA medium, on PDA medium, and in presence of different concentration of coal tar (mg ml⁻¹), naphthalene, anthracene, and phenanthrene in Petri dishes on MEA medium. RSD does not exceed 16.51%.

Code	Strain	Growth rate on MEA medium	Growth rate on PDA medium	Growth rate on control	TR coal tar 0.25 mg ml ⁻¹	TR coal tar 0.5 mg ml ⁻¹	TR coal tar 1 mg ml ⁻¹	TR naphthalene	TR anthracene	TR phenanthrene
SMR 684	<i>Pleurotus ostreatus</i>	0.59	0.59	0.53	68.76	61.52	57.71	50.56	70.77	50.56
VMU 001	<i>Pleurotus</i> sp.	0.66	0.6	0.46	87.53	71.55	65.65	83.78	87.84	48.65
VMU 002	<i>Schizophyllum</i> sp.	0.50	0.51	0.50	70.00	47.40	36.80	95.64	98.47	82.50
VMU 003	<i>Irpex lacteus</i>	0.71	0.83	0.59	67.44	58.14	47.53	70.59	80.00	54.41
VMU 004	<i>Bjerkandera adusta</i>	0.95	0.97	0.9	39.56	43.78	34.33	96.61	102.64	76.62
VMU 005	<i>Fomitopsis pinicola</i>	0.46	0.39	0.33	48.01	28.75	10.09	–	–	–
VMU 006	<i>Hypocrea</i> sp. 1	1.78	1.18	1.48	23.73	13.02	10.31	–	–	–
VMU 007	<i>Trichoderma harzianum</i>	1.63	1.08	1.63	11.82	7.38	4.55	–	–	–
VMU 008	<i>Hypocrea</i> sp. 2	1.33	0.88	1.25	13.68	8.88	3.84	–	–	–

Appendix 6: Sampled soil microflora taken at Varteg Hill, Torfaen, in 1998 and 2018 (Filcheva, et al., 2021)

Table 5. Soil microflora in 1998 and 2018; (CFU per 1000g⁻¹ of absolutely dry soil) and its components (%).

Test Plot	Total microflor [CFU per 1000g ⁻¹ of absolutely dry soil]		Ammonifying Bacteria %		Bacilli %		Actinomycetes %		Fungi %	
	1998	2018	1998	2018	1998	2018	1998	2018	1998	2018
Sv91-1A	94	1480	40	14	7	76	0	7	53	3
Sv91-1B	270	3400	90	9	0	46	3	26	7	19
Sv91-2A	97	2180	70	40	10	53	10	4	10	3
Sv91-2B	241	2820	89	8	2	44	4	30	5	18
Sv91-3A	114	900	71	22	0	53	9	18	20	60
Sv91-3B	100	1160	81	40	0	52	14	5	5	3
Sv92-4	63	1400	36	29	8	39	8	20	48	12
Sh97-2	138	2220	84	32	2	14	10	38	4	16
Sh97-3	93	1620	62	12	0	31	9	32	26	25
Tf94-1	47	2140	62	12	0	25	4	37	34	26
Tf94-2	141	1480	80	14	4	35	6	30	10	22
Tf94-3	251	1040	48	27	48	38	0	25	4	10
Mean	137	1820	68	22	6.8	42	6.4	23	19	18

Appendix 7: Results of PAH removal by *Irpex lacteus* and *Pleurotus ostreatus* (Bhatt, et al., 2002)

Table II. Removal of PAH (mg PAH per kg dry soil $\pm$ SD) in industrial soil A and B by *Irpex lacteus* and *Pleurotus ostreatus* after 14 weeks of treatment

Sample	ANT	BAN	CHR	FLO	FLT	PHE	PYR
A (control)	223 $\pm$ 51	139 $\pm$ 5	160 $\pm$ 15.9	122 $\pm$ 25.9	820 $\pm$ 27.8	125 $\pm$ 11.4	642 $\pm$ 26.6
A + <i>I. lacteus</i>	161 $\pm$ 34.3	93.9 $\pm$ 6.8	136 $\pm$ 14.5	40.2 $\pm$ 12.8	347 $\pm$ 34.6	54.1 $\pm$ 7.5	370 $\pm$ 22.8
A + <i>P. ostreatus</i>	155 $\pm$ 13	136 $\pm$ 2	164 $\pm$ 6.6	91.3 $\pm$ 7.4	574 $\pm$ 50.7	123 $\pm$ 5.8	507 $\pm$ 61.6
B (control)	102 $\pm$ 15.1	171 $\pm$ 5.2	175 $\pm$ 8.7	62.4 $\pm$ 2.6	711 $\pm$ 2.4	252 $\pm$ 7.2	584 $\pm$ 10
B + <i>I. lacteus</i>	50.4 $\pm$ 7.2	124 $\pm$ 17.3	117 $\pm$ 12.5	37.6 $\pm$ 9.8	507 $\pm$ 80.7	205 $\pm$ 41.8	447 $\pm$ 52.9
B + <i>P. ostreatus</i>	35.3 $\pm$ 7.2	117 $\pm$ 14.8	106 $\pm$ 12.6	19.9 $\pm$ 7.2	488 $\pm$ 63.3	199 $\pm$ 34.4	393 $\pm$ 54.7

Appendix 8: Results of extracted PAH by *Pleurotus ostreatus* and *Irpex lacteus* with the addition of mobilizing agents (Leonardi, et al., 2007)

Table2. Initial concentrations of PAHs in S1 soil with relative non-bioavailable fractions and residual concentrations observed after incubation with *P. ostreatus* and *I. lacteus* in the absence and presence of different mobilizing agents (MAs)

S1	Correlation coefficient ^a	PAH sum ^b	PHE ^c	ANT	FLT	PYR	BaA	CHR	BbF	BkF	BaP	DBA
Initial PAH conc.		2320.0	958.0	34.0	789.0	404.0	20.0	24.0	18.0	16.0	26.0	2.0
Non-bioavailable ^d		94.3	8.5	1.0	21.6	15.3	3.7	4.1	8.4	5.4	10.9	1.2
<i>P.ostreatus</i>												
Soil without MAs	0.60	596.5	84.6	0.0	299.0	137.9	6.7	14.3	14.5	8.1	8.1	1.8
Soil + SO ^e	0.54	518.5	185.0	0.0	234.2	52.3	1.3	15.5	10.8	4.6	2.8	0.8
Soil + Tw 80	0.67	599.0	49.5	0.3	313.4	162.5	5.7	17.9	13.6	7.8	8.3	1.9
Soil + Tw 20	0.66	591.4	31.8	0.8	314.1	162.4	8.7	16.2	15.9	9.8	9.7	2.2
Soil + OMW	0.19	650.1	200.2	1.6	273.4	118.8	9.4	16.6	11.1	6.8	1.4	0.4
<i>I. lacteus</i>												
Soil without MAs	0.83	279.2	20.6	0.0	71.9	90.5	8.6	10.8	18.2	9.1	19.9	2.0
Soil + SO	0.55	1037.5	394.6	0.0	400.3	168.7	4.0	18.9	13.6	8.1	7.4	1.3
Soil + Tw 80	0.82	566.2	33.8	0.6	168.9	269.7	7.4	14.4	17.6	10.2	16.7	2.0
Soil + Tw 20	0.81	335.6	30.6	0.7	64.9	145.4	6.6	9.8	19.4	10.6	17.7	2.0
Soil + OMW	0.17	1462.0	582.9	9.7	529.3	271.7	6.4	11.3	11.5	9.3	11.2	1.4

Appendix 9: List of fungi isolates enumerated from arsenic-contaminated soils (Singh, et al., 2015)

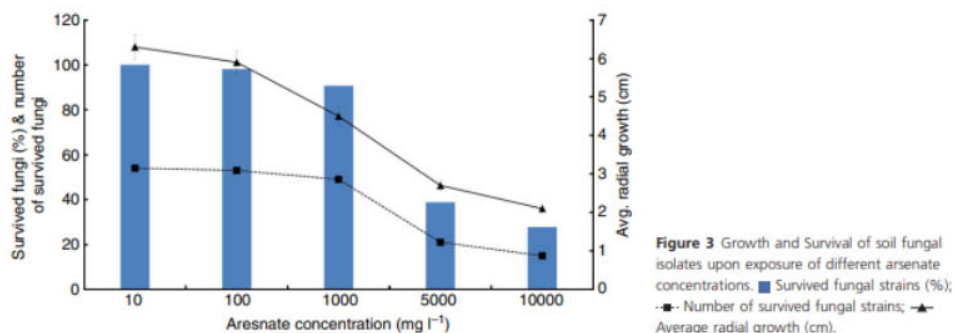
Table 1 Molecular characterization of fungal isolates enumerated from arsenic – contaminated soils

S.No	NBRI Code	Organism with most similar sequences in databases	ITS size (bp)	ITS similarity (%)	Group	E value
1	B-1	<i>Penicillium</i> spp. 4 TMS-2011 voucher BGD1p19-3	598	99	Ascomycetes	0.0
2	BA-1	<i>Penicillium</i> spp. 4 TMS-2011 voucher BGD1p19-3	602	99	Ascomycetes	0.0
3	BA-12	<i>Penicillium</i> spp.	602	98	Ascomycetes	0.0
4	LK-2	<i>Penicillium</i> spp.	594	99	Ascomycetes	0.0
5	BA-10	<i>Penicillium</i> spp.	736	99	Ascomycetes	0.0
6	B-2	<i>Penicillium</i> spp.	550	99	Ascomycetes	0.0
7	B-8	<i>Penicillium minioluteum</i> strain LCP 210	581	93	Ascomycetes	0.0
8	BA-13	<i>Penicillium</i> spp F103	294	100	Ascomycetes	7e ⁻¹²⁶
9	BA-3	Uncultured fungus clone LX042400-122-057-H03	602	99	Ascomycetes	0.0
10	B-3	<i>Trichophyton verrucosum</i> _KJ150712	567	100	Ascomycetes	0.0
11	BA-4	Uncultured fungus clone L042884-122-064-G02	573	99	Fungi	0.0
12	B-5	Uncultured Fungus	574	100	Fungi	0.0
13	LK-5	<i>Arthroderma benhamiae</i> _KJ150718	572	100	Ascomycetes	0.0
14	L-36	Uncultured Fungus Clone seq FUN 1-4	669	98	Fungi	0.0
15	GZ-4	<i>Microdochium</i> spp.	572	100	Ascomycetes	0.0
16	L-82	Fungal strain_KJ679497	574	97	Ascomycetes	0.0
17	L-48	<i>Aspergillus</i> spp.	546	98	Ascomycetes	0.0
18	L-62	<i>Hypocrea lixii</i>	569	95	Ascomycetes	0.0
19	L-11	<i>Aspergillus</i> spp.	623	99	Ascomycetes	0.0
20	L-61	<i>Trichoderma harzianum</i> _KR584665	589	99	Ascomycetes	0.0
21	L-46	<i>Trichoderma harzianum</i> _KM819011	696	97	Ascomycetes	0.0
22	L-81	<i>Trichoderma harzianum</i> _KM819012	600	98	Ascomycetes	0.0
23	B-6	<i>Aspergillus terreus</i> SM-EF 3	672	99	Ascomycetes	0.0
24	BA-5	<i>Emericella</i> spp._KJ679498	532	100	Ascomycetes	0.0
25	BA-6	<i>Emericella</i> spp	568	99	Ascomycetes	0.0
26	L-10	<i>E. corrugata</i>	560	99	Ascomycetes	0.0
27	B-4	<i>Aspergillus quadrilineatus</i>	569	99	Ascomycetes	0.0
28	LK-1	<i>Emericella nidulans</i> _KJ150717	376	100	Ascomycetes	0.0
29	L-31	<i>Emericella</i> spp	465	99	Ascomycetes	0.0
30	BA-7	<i>Aspergillus flavus</i>	566	99	Ascomycetes	0.0
31	L-19	<i>Aspergillus flavus</i> NRRL 21882	563	100	Ascomycetes	0.0
32	L-17	<i>Aspergillus ustus</i> _UWFP 1132	542	99	Ascomycetes	0.0
33	L-41	<i>Aspergillus niger</i>	589	100	Ascomycetes	0.0
34	L-53	<i>Aspergillus</i> spp	616	98	Ascomycetes	0.0
35	L-15	<i>Aspergillus terreus</i> strain	522	100	Ascomycetes	0.0
36	L-35	<i>Aspergillus oryzae</i> _KJ150716	683	99	Ascomycetes	0.0
37	L-83	<i>Aspergillus</i> spp	579	99	Ascomycetes	0.0
38	L-51	<i>Talaromyces trachyspermus</i> , C234	570	99	Ascomycetes	0.0
39	L-34	<i>Talaromyces trachyspermus</i> , C234	945	100	Ascomycetes	0.0
40	BA-11	<i>Taifanglania major</i> strain GZDXIFR-H-57-2	191	89	Ascomycetes	1e ⁻⁵¹
41	LK-3	<i>Myrothecium</i> spp	632	99	Ascomycetes	0.0
42	L-18	<i>Corynascus verrucosus</i>	286	98	Ascomycetes	3e ⁻¹³⁹
43	L-37	<i>Corynascus verrucosus</i> Isolate South-West 0024	535	100	Ascomycetes	0.0
44	BA-2	<i>Simplicillium</i> ID05-F0117	630	99	Ascomycetes	0.0
45	LK-4	<i>Stachybotrys</i> Spp sta_38	587	99	Ascomycetes	0.0
46	GZ-3	<i>Edenia gomezpompae</i> DH08042-3	593	99	Ascomycetes	0.0
47	L-33	<i>Chaetomium</i> spp.	612	99	Ascomycetes	0.0
48	B-7	<i>Fusarium</i> spp._KT778272	403	96	Ascomycetes	0.0
49	BA-8	<i>Bionectria epicholae</i>	477	99	Ascomycetes	0.0
50	B-9	<i>Rhizomucor variabilis</i> _KJ150714	711	99	Fungi	0.0
51	GZ-1	<i>Curvularia</i> spp.	582	99	Ascomycetes	0.0
52	L-12	<i>Rhizopus oryzae</i>	774	99	Ascomycetes	0.0
53	L-44	<i>Gibberella moniliformis</i> strain LYF065	682	100	Ascomycetes	0.0
54	GZ-5	FNBR_GZ5_KJ150715	652	99	Ascomycetes	0.0

The ITS sequence includes 5-8S rDNA gene, the ITS1 and ITS2 regions.

E-value- is the number of hits one can expect to see just by chance when searching a database of a particular size (Altschul et al. 1997).

Appendix 10: Growth and survival of fungi isolates when exposed to various arsenic concentrations (Singh, et al., 2015)



Appendix 11: Removal efficiency of *Aspergillus* spp for Hg and Mg (Khan, et al., 2019)

Table 2 Removal efficiency of heavy metals by fungal isolates

From: **Mycoremediation: a treatment for heavy metal-polluted soil using indigenous metalotolerant fungi**

Sample code	Heavy metal	Initial metal con.	Fungal isolate	Media	Final metal con.	Removal efficiency
S1	Pb	170.90 mg L ⁻¹	<i>A. fumigatus</i>	CYE	2.40 ± 0.012 mg L ⁻¹	98.5%
				SDB	1.22 ± 0.021 mg L ⁻¹	99.2%
				YPG	1.86 ± 0.013 mg L ⁻¹	98.9%
			<i>A. flavus</i>	CYE	1.67 ± 0.021 mg L ⁻¹	99%
				SDB	1.10 ± 0.010 mg L ⁻¹	99.3%
				YPG	1.24 ± 0.031 mg L ⁻¹	99.2%
S2	Hg	26.66 mg L ⁻¹	<i>A. niger</i>	CYE	1.78 ± 0.042 mg L ⁻¹	93.3%
				SDB	2.10 ± 0.037 mg L ⁻¹	92%
				YPG	1.00 ± 0.013 mg L ⁻¹	96%
			<i>A. terreus</i>	CYE	2.23 ± 0.017 mg L ⁻¹	91.6%
				SDB	1.25 ± 0.035 mg L ⁻¹	95.3%
				YPG	1.44 ± 0.053 mg L ⁻¹	95.5%

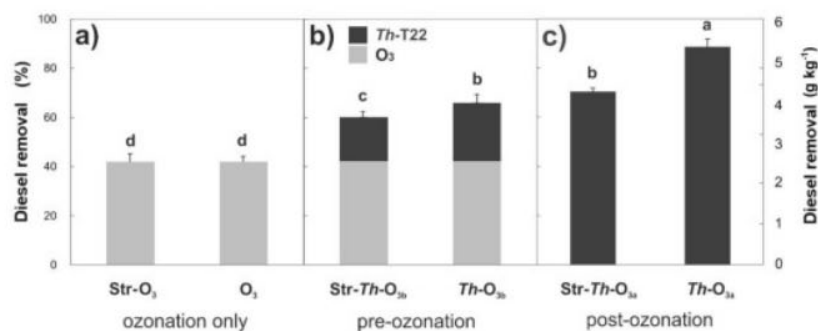


Fig. 1. Diesel removal, expressed as percentage or absolute value, by *Trichoderma harzianum* strain T22 (black columns) coupled with pre- or post-ozonation treatments (grey columns). The values represent means ($n=5$) $\pm$ SD of five independent replicates. Bars with different letters are significantly different at $P<0.05$. Treatments: (a) Str-O₃=200mL PDB+250ppm streptomycin (control 1); O₃=200mL PDB (control 2); (b) Str-Th-O_{3b}=200mL PDB+250ppm streptomycin+*Th*-T22; *Th*-O_{3b}=200mL PDB+*Th*-T22; (c) Str-Th-O_{3a}=200mL PDB+250ppm streptomycin+*Th*-T22; *Th*-O_{3a}=200mL PDB+*Th*-T22.

Appendix 13: Side by side comparison: Graphite Furnace AAS vs ICP MS (Agilent, 2023)

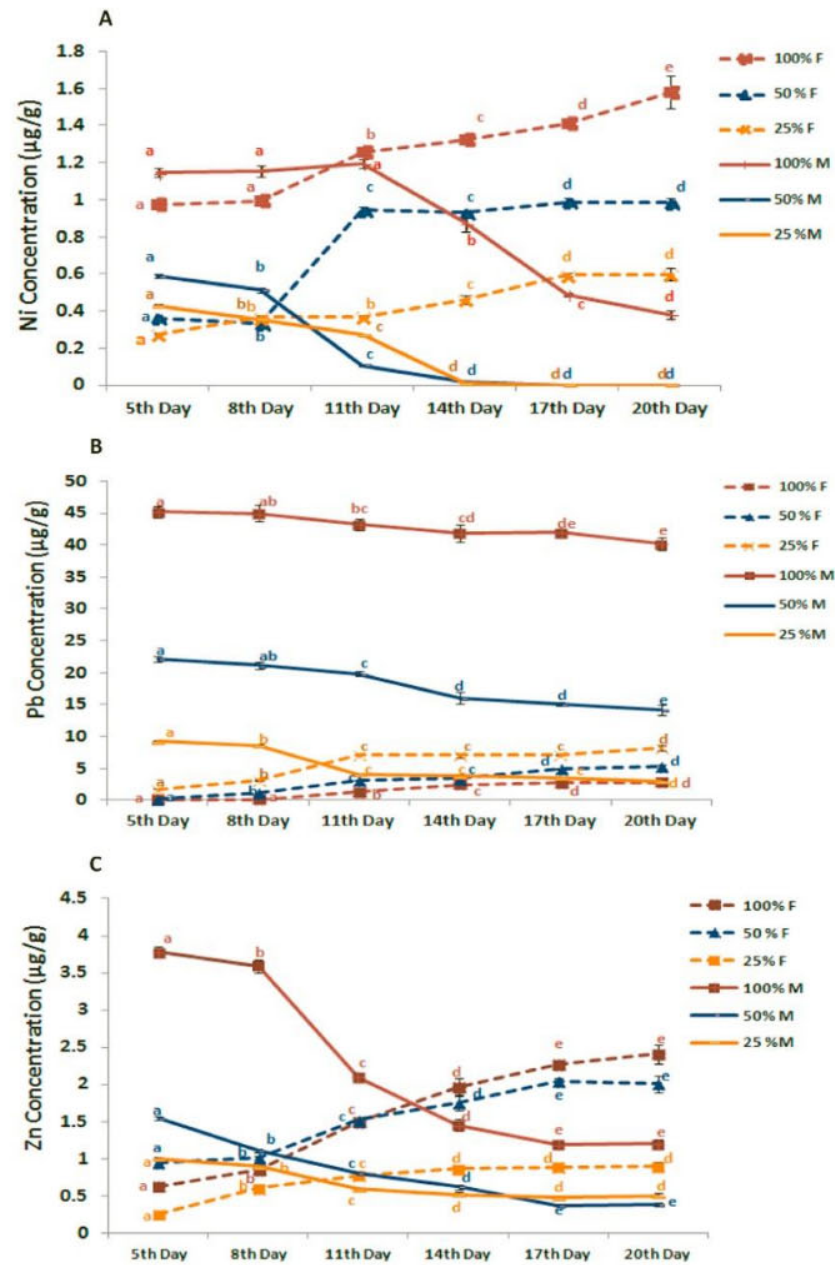
	GFAAS View product	ICP-MS View product
Relative Price	\$\$	\$\$\$\$
Relative Cost per sample	\$\$	\$\$\$
Relative Sensitivity	QQQQ	QQQQQ
Reviews	SelectScience	SelectScience
Maximum samples per day ¹	60 (4 elements)	1200 (50+ elements)
Dynamic range of measurement	10 ppt - 1000 ppb	<1 ppt – 1000ppm
Relative sample volume required	💧	💧💧
Relative tolerance of solids in sample		
Element measurement	Sequential	Simultaneous
How many elements can be measured?	48	86
Relative routine maintenance requirements	🔧🔧	🔧🔧🔧🔧
Relative operator skill required	👤👤👤	👤👤👤 ²
Can be left unattended	Yes	Yes
Part 11/Annex 11 GMP compliance	Yes (with optional software)	Yes (with optional software)
Specifications		
Relative operational power use	⚡⚡	⚡⚡⚡
Dimensions (mm – width x depth x height)	1030 x 600 x 590 ³	730 x 600 x 595
Weight	119 kg	100 kg
Gas requirements	99.99% Argon or 99.99% Nitrogen	99.99% Argon ⁵
Warranty ⁴	12 months	12 months
Accessories		
Autosampler	Included	Optional
Water cooling system	Cool water supply required	Required, not included

Appendix 14: ANOVA results of *Pleurotus ostreatus* grown on substrate from gold/bauxite mining sites (Jaikaran, et al., 2017)

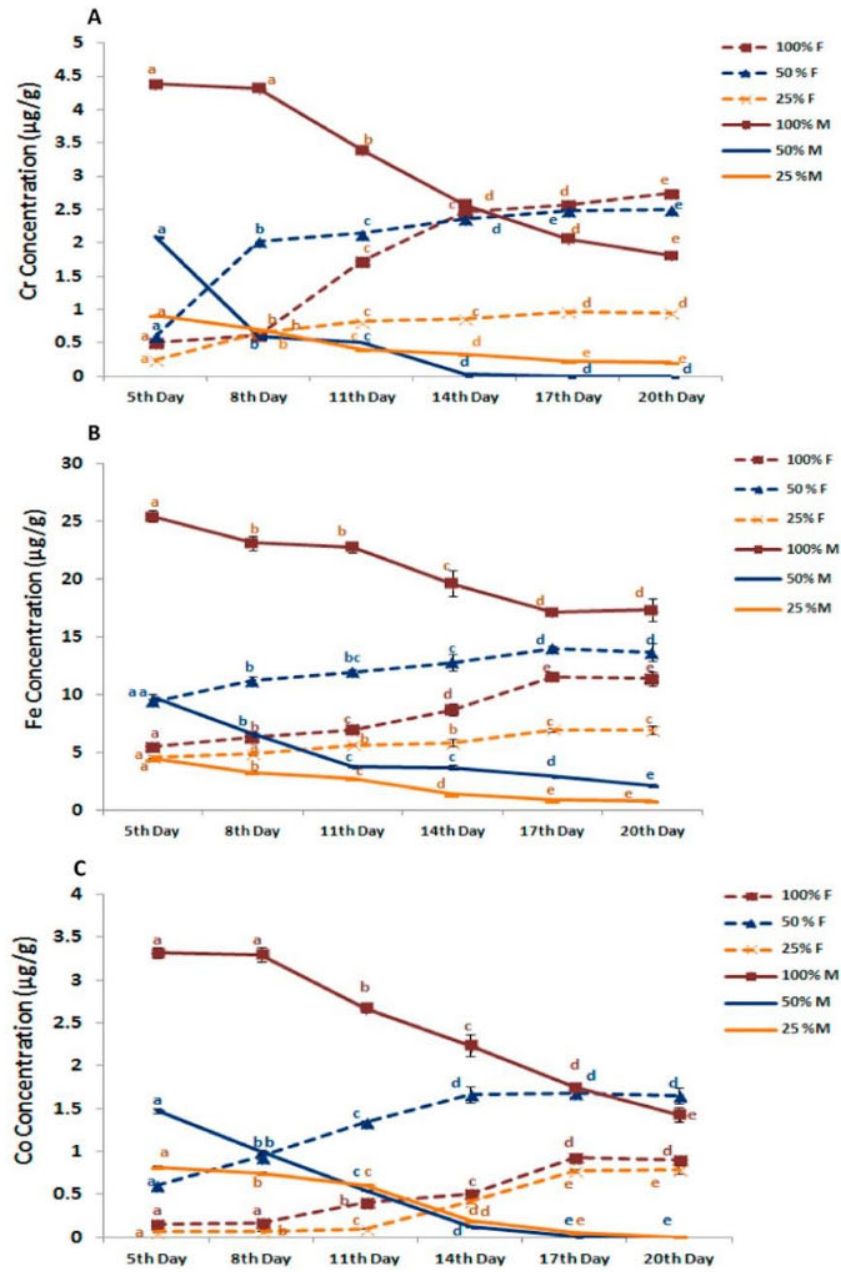
Table 12. Table showing ANOVA Two-way for coefficients of accumulation for Co, Pb, Cu, Fe and Mn in Treatment AGO and ABO

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	7.80366	4	1.950915	0.545919	0.713961	6.388233
Columns	0.40401	1	0.40401	0.113053	0.753593	7.708647
Error	14.29454	4	3.573635			
Total	22.50221	9				

Appendix 15: Zinc, Lead and Nickel concentrations in harvested *Pleurotus ostreatus* samples (Vaseem, et al., 2017)



Appendix 16: Cobalt, Iron and Chromium concentrations in harvested *Pleurotus ostreatus* samples (Vaseem, et al., 2017)



Appendix 17: Copper and Manganese concentrations in harvested *Pleurotus ostreatus* samples (Vaseem, et al., 2017)

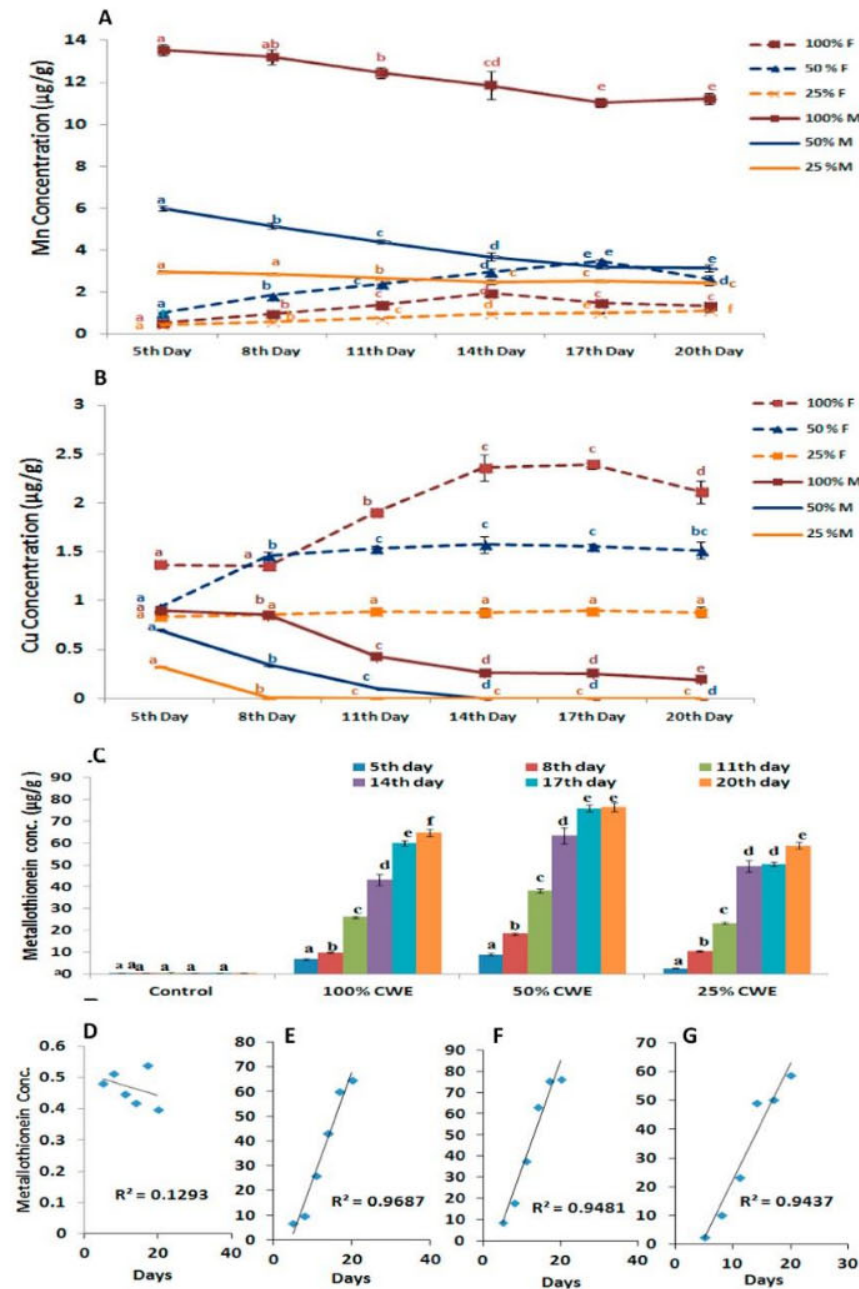
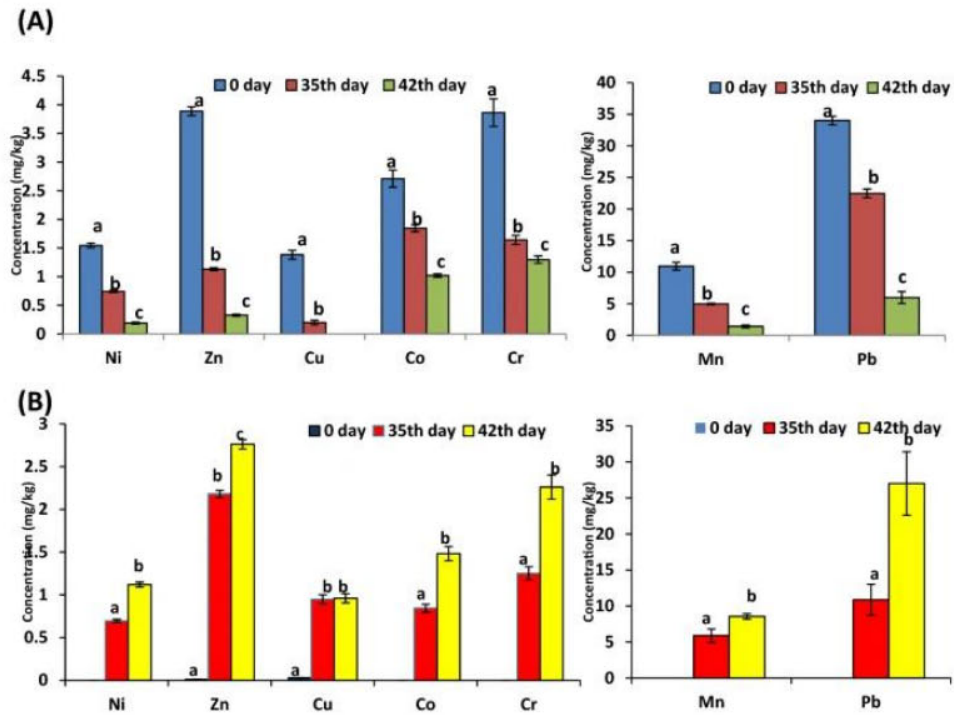
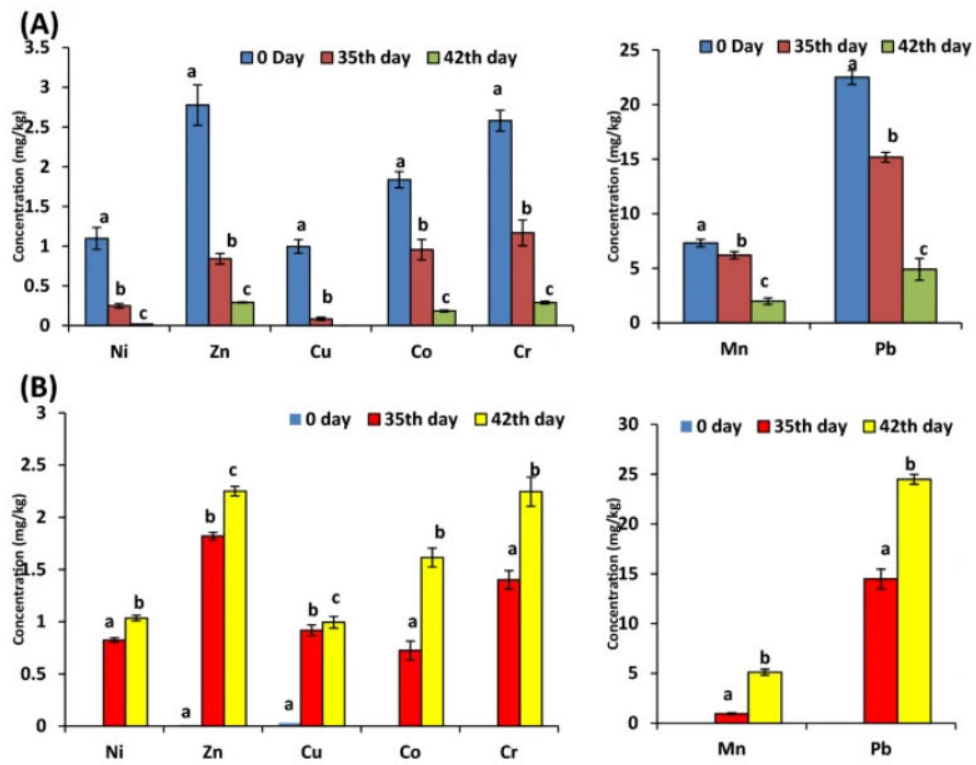


Fig. 2



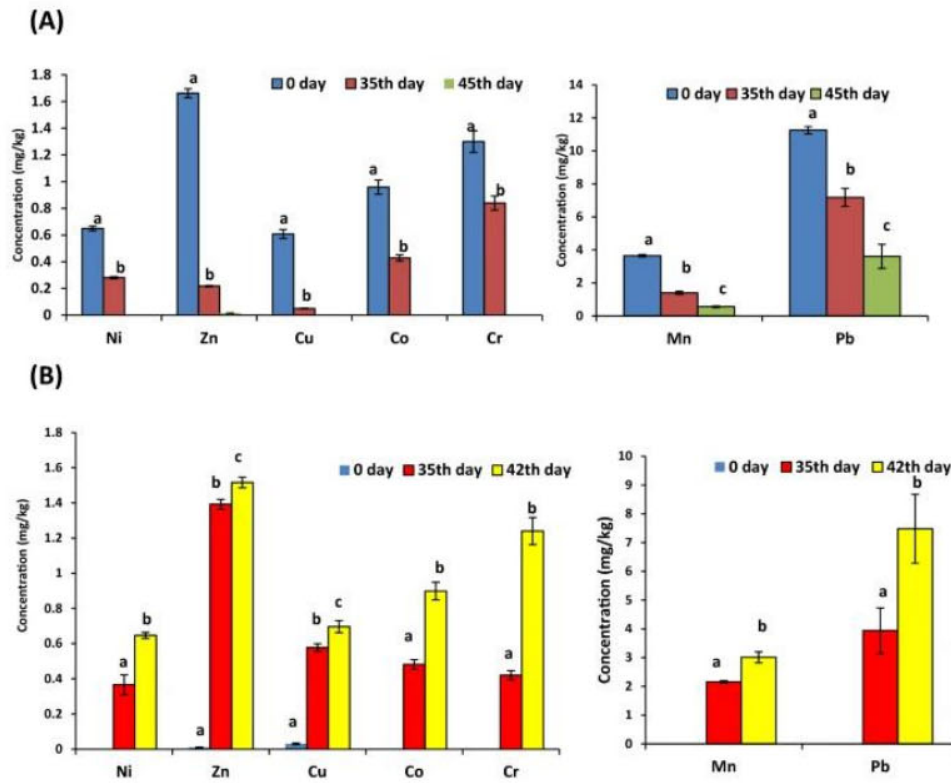
a Metals concentration in substrate soaked with 75% CWE. b Metals concentration in fruiting bodies grown on substrate soaked with 75% CWE

Fig. 3



a Metals concentration in substrate soaked with 50% CWE. b Metals concentration in fruiting bodies grown on substrate soaked with 50% CWE

Fig. 4



a Metals concentration in substrate soaked with 25% CWE. b Metals concentration in fruiting bodies grown on substrate soaked with 25% CWE

Table 2. Mean trace metal concentrations ($\mu\text{g/g}$ dry weight) in the caps of *Pleurotus ostreatus* (oyster) mushroom grown on mine soils.

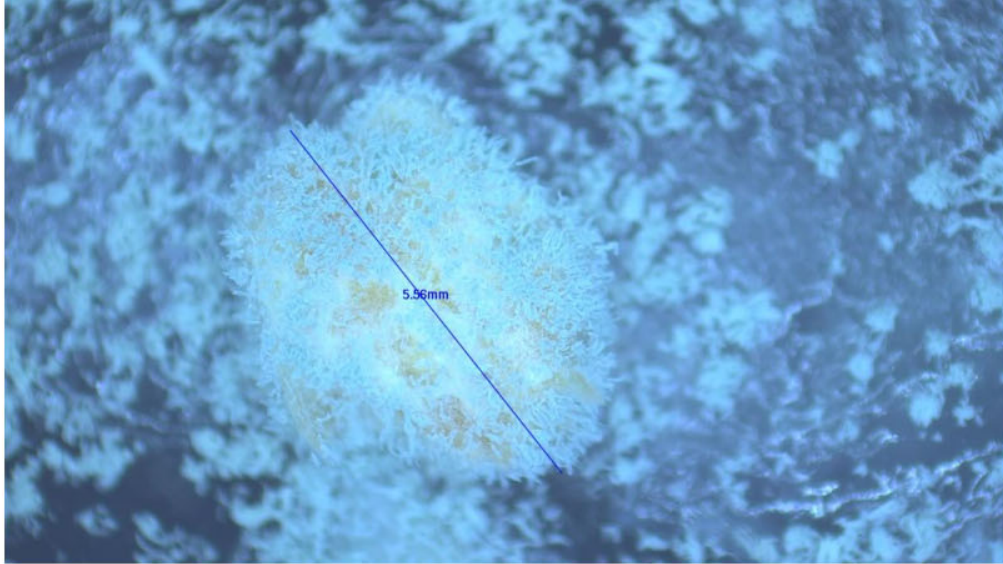
Trace metals							
Sites	Plant Part	Cr	Mn	Co	Ni	Cu	Zn
A	Cap	1.22 $\pm$ 0.02 ^c	8.32 $\pm$ 0.06 ^c	3.13 $\pm$ 0.06 ^b	4.30 $\pm$ 0.49 ^b	17.33 $\pm$ 0.08 ^a	65.8
	Stalk	2.36 $\pm$ 0.13 ^c	15.59$\pm$0.21^c	2.92 $\pm$ 0.09 ^b	9.86 $\pm$ 0.24 ^b	29.52 $\pm$ 0.51 ^b	29.2
B	Cap	2.87 $\pm$ 0.03 ^a	9.10 $\pm$ 0.05 ^b	1.51 $\pm$ 0.03 ^c	2.65 $\pm$ 0.24 ^d	12.9 $\pm$ 0.13 ^d	68.1
	Stalk	7.85 $\pm$ 0.37 ^b	9.50$\pm$0.11^d	0.75 $\pm$ 0.03 ^c	3.92 $\pm$ 0.35 ^d	15.06 $\pm$ 0.67 ^d	22.0
C	Cap	2.46 $\pm$ 0.14 ^b	9.58 $\pm$ 0.21 ^b	0.57 $\pm$ 0.02 ^c	2.16 $\pm$ 0.17 ^c	16.87 $\pm$ 0.53 ^b	77.6
	Stalk	9.99 $\pm$ 0.87 ^a	17.63$\pm$1.34^b	0.77 $\pm$ 0.05 ^c	5.15 $\pm$ 0.29 ^c	36.64 $\pm$ 2.09 ^a	36.1
D	Cap	1.42 $\pm$ 0.02 ^c	10.01 $\pm$ 0.12 ^a	3.87 $\pm$ 0.08 ^a	4.63 $\pm$ 0.07 ^a	14.66 $\pm$ 0.36 ^c	66.1
	Stalk	1.84 $\pm$ 0.14 ^c	40.12$\pm$0.53^a	4.16 $\pm$ 0.19 ^a	14.93 $\pm$ 0.34 ^a	25.62 $\pm$ 0.98 ^c	24.0

Values in the same column with different letter (s) are significantly different ($p < 0.05$) and values in the same column with the same letter (s) are not significantly different ($p < 0.05$).

Appendix 22: *Pleurotus ostreatus* on black agar plate (Macfarlane, 2024)



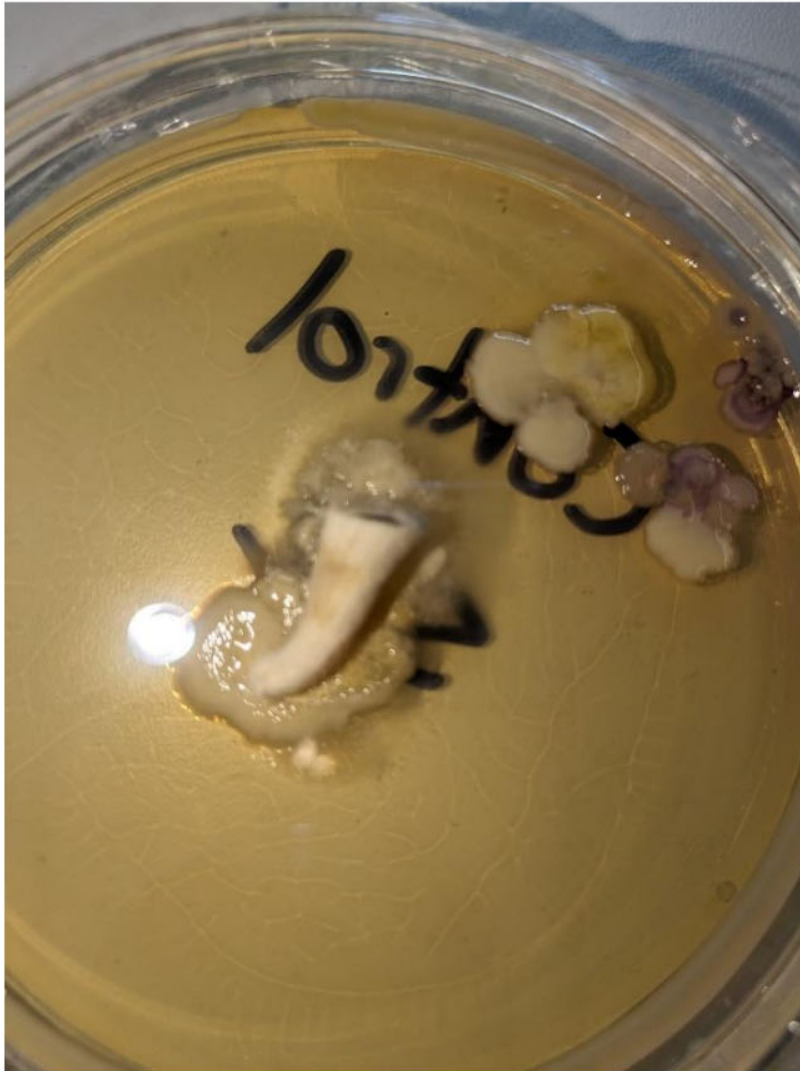
Appendix 23: *Pleurotus ostreatus* on agar plate contaminated with active yeast, (Macfarlane, 2024)



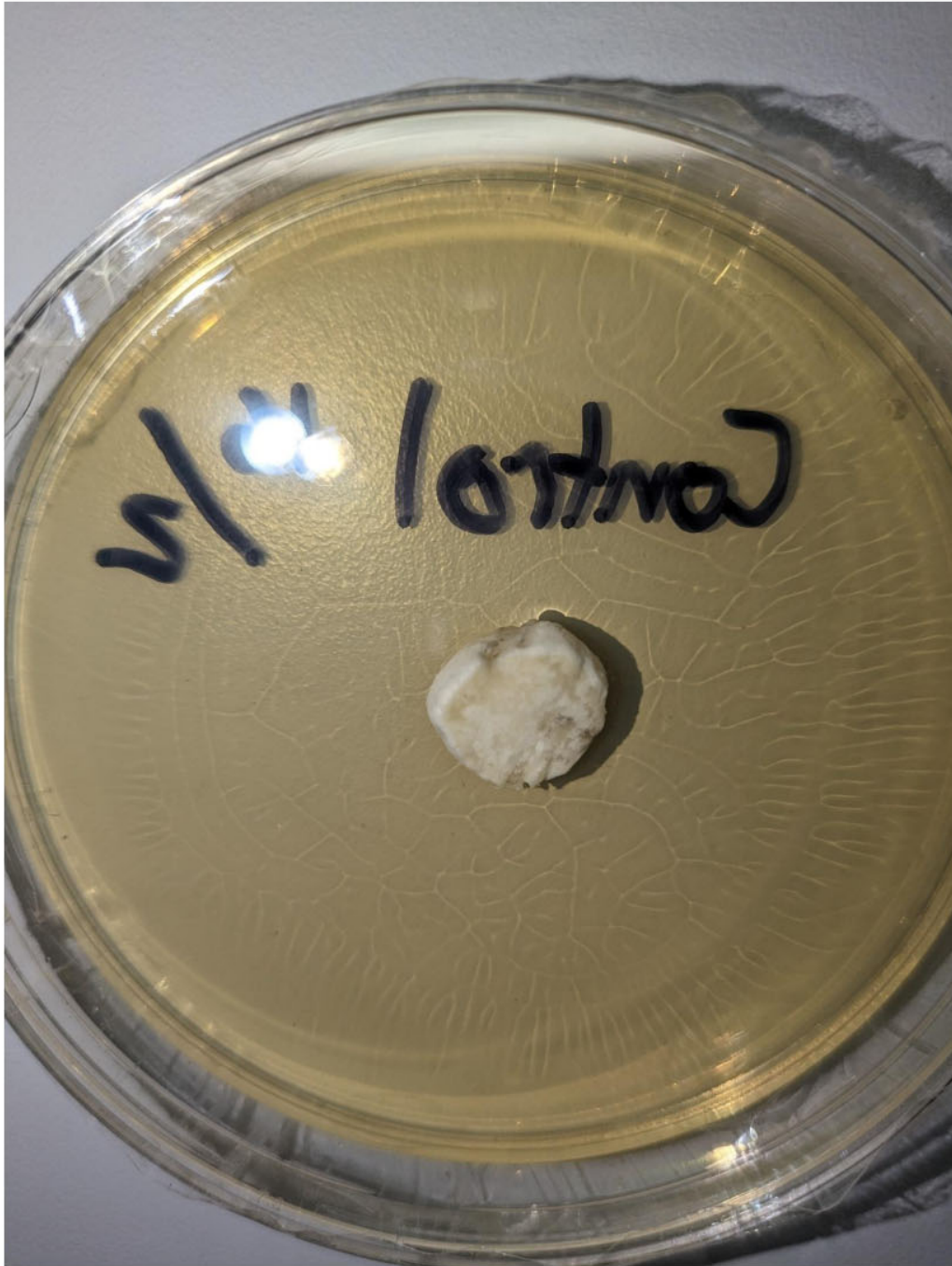
Appendix 24: Photo of topsoil removed from sample site at Varteg, (Macfarlane, 2023c)



Appendix 25: Contaminated *Pleurotus ostreatus* sample on LMEA plate (Macfarlane, 2024)



Appendix 26: Reproduced control sample of *Pleurotus ostreatus* added to LMEA plate (Macfarlane, 2024c)



Appendix 27: ICP-MS/MS test results (Jugdaohsingh, 2024)

ppb	Run off water sample			S1 Mine Spoil			mushroom/Control			mushroom, 20/80			mushroom, 40/60			mushroom, 60/40			mushroom, 80/20		
	mean	sd		mean	sd		mean	sd		mean	sd		mean	sd		mean	sd		mean	sd	
As	1.96	0.09		12409.10	522.57		76.20	23.97		179.81	34.42		524.35	43.85		286.33	29.42		1484.09	49.93	
Cd	0.15	0.02		640.30	110.82		217.49	27.65		364.77	38.87		446.89	68.01		528.42	45.04		482.72	77.27	
Cr	0.75	0.02		67943.57	3720.87		108.85	20.41		119.85	28.29		259.88	17.18		183.51	73.60		60.61	9.02	
Co	4.60	0.33		16968.90	2069.19		0.32	0.19		9.92	3.85		122.68	15.36		133.26	8.29		562.34	25.05	
Cu	30.77	1.03		31142.11	1532.98		12059.93	968.00		14887.72	1365.30		30839.55	2327.89		11343.84	856.59		9474.80	366.08	
Mn	3430.61	127.88		585266.12	102260.82		9033.82	423.25		9130.32	687.66		12118.66	1283.03		9678.11	822.39		14598.23	744.05	
Fe	28.95	1.70		20984801.59	1225836.96		77379.20	3445.78		93016.01	9063.60		114889.64	10397.38		82486.14	8126.47		82747.01	2630.50	
Ni	26.47	1.10		46371.17	2903.10		112.61	21.23		289.94	189.04		300.58	18.53		368.64	106.56		1490.69	25.62	
Zn	5.59	1.08		75272.76	103015.10		67682.23	4709.63		85857.06	7932.29		72301.91	13915.20		73128.66	6184.39		61732.10	4149.25	
Pb	0.01	0.01		34088.47	2278.78		1.87	2.62		4.60	6.74		67.93	23.18		26.30	6.68		28.94	13.86	
Li	5.21	0.05		96640.69	3875.93		0.00	0.00		0.00	0.00		48.77			8.73			26.14	45.28	
Be	0.01	0.00		1964.59	118.41		0.00	0.00		0.00	0.00		2.14	1.64		0.46	0.79		2.27	1.11	
Be	36.53	0.65		63241.18	2983.88		1853.53	274.03		1310.38	476.24		1181.93			770.34	822.27		588.19	780.98	
Mg	6576.33	400.89		2208550.83	210349.87		1194087.85	76886.93		1180008.07	78004.83		1608213.57	119419.39		1278857.23	101649.14		1438811.78	85364.91	
Al	0.00			51712576.70	4203110.83		1787.16			6635.33			49906.00	8304.32		6846.53	6150.56		12736.69	4708.84	
Si	1.59	0.07		3006.44	181.72		0.82			11.03	8.89		136.36	25.33		16.53	12.71		19.83	13.53	
P	0.02	0.00		315.96	22.23		3830771.45	332367.48		7256.14	429.51		6621.42	952.25		6752.34	532.17		6086.66	439.25	
K	2196.27	31.43		13434801.14	1521834.06		19394094.27	1373903.85		20466352.66	909407.52		21503516.05	1066007.20		21144618.78	869160.42		22559615.95	8889958.01	
Ca	82.89	3.99		10612.86	1689.11		8.56	6.59		17.22	7.70		488.96	43.64		66.59	11.98		39.51	7.51	
Sc	0.13	0.01		11077.90	326.02		1.79	2.33		4.37	3.51		17.50	2.47		8.04	2.47		9.17	0.64	
Ti	0.020	0.029		132705.45	51647.94		90.07	31.84		204.62	44.17		1497.07	170.95		301.80	42.06		578.94	106.83	
V	0.48	0.01		97866.80	5926.18		1667.47	250.20		1803.87	1590.00		1979.82	1536.33		1703.25	1405.87		1828.21	1325.70	
Se	1.70	0.33		1433.75	530.36		257.48	198.54		84.29	86.58		239.87	147.69		582.37	99.20		494.86	238.67	
Rb	6.28	0.04		81732.13	9713.42		4600.37	462.99		3511.50	292.84		2960.87	347.38		2762.42	205.87		1156.29	53.35	
Sr	215.13	3.35		134985.86	9735.13					492.24	107.21			66.97					56.59	45.46	
Mo	1.01	0.03		1231.86	190.91		60.22	4.96		56.23	11.78		87.89	18.49		67.37	13.00		112.26	15.51	
Ru	0.00	0.00		0.53	0.80		0.21	0.27		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00	
Rh	0.00	0.00		3.47	0.32		0.16	0.01		0.20	0.06		0.35	0.16		0.09	0.07		0.05	0.07	
Pd	1.42	0.07		3741.68	262.49		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00	
Ag	0.02	0.00		300.27	26.47		44.40	5.34		136.17	19.50		293.07	37.14		548.46	30.87		814.91	70.91	
In	0.00	0.00		57.95	3.37		0.29	0.21		0.59	0.47		4.74	0.51		1.05	0.35		0.94	0.40	
Sn	0.52	0.01					3.55	4.37		16.48	8.85		1413.16	163.80		120.23	17.78		19.36	8.01	
Sb	0.29	0.01		747.80	36.64		0.57	0.79		0.76	1.32		2.31	0.71		0.58	0.75		1.57	2.03	
Te	0.00	0.00		63.02	12.53		0.05	0.09		0.11	0.19		0.32	0.42		0.00	0.00		0.79	1.09	
Cs	0.17	0.00		11565.74	767.12		6.49	1.77		29.38	3.33		42.46	3.96		47.78	3.52		31.29	1.50	
Ba	369.28	4.55		309321.20	23110.82		51.17			441.10	258.25		243.80	214.22		133.52			136.28		
La	0.00	0.00		31.19	4.68		0.00	0.00		0.00	0.00		0.02	0.01		0.00	0.00		0.01		
Ce	0.04	0.01		56717.23	8385.90		0.00	0.00		1.30	2.25		37.47	21.84		13.88			20.80		
Er	0.00	0.00		1491.88	93.40		0.00	0.00		0.00	0.00		0.79	0.75		0.04	0.07		0.41		
Yb	0.00	0.00		1211.47	81.13		0.00	0.01		0.00			0.99	0.71		0.04	0.07		0.17	0.16	
Lu	0.00	0.00		164.87	10.97		0.00	0.00		0.00	0.00		0.19	0.06		0.03	0.04		0.03	0.04	
Re	0.02	0.00		2.95	0.58		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.02	0.04	
Os	0.11	0.06		32.46			0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		9.45		
Ir	0.01	0.00		0.94	0.61		0.00	0.00		0.00	0.00		0.00	0.00		0.04	0.07		0.08	0.08	
Pt	0.01	0.00		0.56	0.56		0.11	0.20		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00	
Au	0.03	0.01		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00	
Hg	0.12	0.03		0.00	0.00		1.83	3.17		0.00	0.00		23.06	39.94		94.73			60.83	60.62	
Tl	0.10	0.00		767.08	54.40											0.06	0.10		3.00	2.58	
Bi	0.00	0.00		352.38	16.28		0.09	0.09		0.08	0.13		0.79	0.05		0.23	0.20		0.42	0.01	
Th				7974.22	426.32		0.13	0.22		0.47	0.81		6.87	1.89		1.28	1.22		2.52	1.06	
U	2.45	0.18		1134.84	56.51		0.04	0.07		0.58	0.04		5.31	0.25		2.20	0.28		10.15	0.43	

COULD GREY OYSTER MUSHROOM (PLEUROTUS OSTREATUS) BE A VIABLE OPTION FOR MYCOREMEDIATION OF SOUTH WALES COAL SPOIL

GRADEMARK REPORT

FINAL GRADE

GENERAL COMMENTS

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