Fungi as potential bioremediation agents in soil contaminated with heavy or radioactive metals

S. N. Gray
Faculty of Science, Technology and Design, University of Luton, Park Square, Luton LU1 3UJ, U.K.

Origin and impact of heavy metal and radionuclide pollution

Pollution of soil by heavy metals arises from a number of sources, including emissions from motor vehicle exhausts and from manufacturing industry, sewage sludge application, and acid mine drainage. Heavy metals such as zinc, cadmium and lead are toxic and may affect man directly through ingestion after passing through the food chain [1], or indirectly through their effect on other organisms [2,3]. Contamination of soil with radionuclides has occurred as a result of fall-out from nuclear weapons testing, and controlled and accidental discharges from nuclear power stations and nuclear fuel reprocessing plants. The risk to health presented by a radionuclide depends upon the quantity present, its decay products, the potential for entry of the radionuclide into biological systems, its half-life, and the type and energy of the radiation it emits. For example, the release of $^{137}$Cs following the accident at the Chernobyl reactor was of concern because this isotope has a half-life of 30 years, emits high-energy $\gamma$ and $\beta$ radiation and is readily taken up into biological systems [4,5]. Remediation of land polluted with heavy or radioactive metals presents particular difficulties compared with remediation of contaminated water, or land contaminated with organic pollutants. Metals can be removed from aqueous solution either by conventional physicochemical techniques or by biological treatments, for example through bacterial reduction of metal species to form insoluble precipitates [6]. Microbial remediation of soil contaminated by organic pollutants can be achieved through the catabolism of the pollutants to relatively harmless products such as carbon dioxide and water [7]. Heavy metals, unlike hydrocarbons, cannot be removed by degradation. Because soil is a solid matrix, it is not amenable to remediation by filtration or adsorption techniques. Physicochemical methods for the removal of metal pollution from soil include leaching and electoremediation [8,9]. In many cases these approaches are infeasible or uneconomic, especially where remediation in situ is required. The potential for the exploitation of fungi in remediation by leaching of metals from spoil heaps has already been recognized [10]. A cheap and simple method for the remediation in situ of metal-contaminated soil, using fungi, would be of great benefit.

Accumulation of heavy and radioactive metals by fungi

Filamentous fungi constitute 25–82% of the biomass in soil [11]. Fungal mycelia can be very large; an individual mycelium of Armillaria bulbosa has been reported to occupy at least 15 hectares in a mixed hardwood forest [12]. The ability of filamentous fungi to take up and to adsorb heavy metals and radionuclides from solution is well documented [13], and fungi have been shown to solubilize metals in soil which would otherwise be unavailable for uptake by plants [14].

Given the capacity for translocation within the fungal mycelium [15], the activities of filamentous fungi could potentially lead to substantial spatial redistribution of heavy metals or radionuclides in polluted soils. Evidence that this takes place is provided by the high levels of metal pollutants recorded in basidiocarps in the field. Heavy metals detected at elevated concentrations in basidiocarps include lead, cadmium, zinc, mercury, arsenic, copper and manganese [16–18]. Post-Chernobyl monitoring of radiocaesium concentrations in basidiocarps in upland Europe has revealed a number of characteristics of the accumulation of this particular radionuclide by fungi. Concentrations of radiocaesium in basidiocarps were elevated to levels exceeding 50 kBq/kg dry weight in some cases, but were unpredictable, varying both between species and within species at different locations [19–21]. Reports of differences in the ability of different species to accumulate radiocaesium have been contradictory, with some suggesting that basidiocarps of mycorrhizal species contain more radiocaesium than those of saprotrophic species [22], and some reporting the reverse [23]. The concentration of radiocaesium in basidiocarps could not be predicted on the basis of species, soil type or the deposition pattern of radiocaesium. Fungi consistently contained more $^{137}$Cs than plants growing nearby, with ratios of up to 270:1.

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was by mass flow. The maximum velocity of radiocaesium within soil ecosystems is due to the fact that radiocaesium remains bound to the top cm of the soil profile [31].

Differentiation of the fungi led to increased heterogeneity in the distribution of 137Cs through the mycelia. Formation of rhizomorphs by Armillaria resulted in the appearance of radial ridges in the distribution of label, attributed to translocation of radiocaesium through the rhizomorphs. The flux of 137Cs through rhizomorphs of Armillaria was estimated to be five times greater than that through undifferentiated hyphae [29]. Of 14 Armillaria rhizomorph systems studied in the field, two translocated 134Cs both acropetally and basipetally; seven translocated 134Cs only acropetally; one translocated 134Cs only basipetally; and in four cases no translocation of 134Cs was detected. The direction and magnitude of the translocation of radiocaesium were determined largely by the ongoing translocation of other nutrients within the mycelium, which in turn was determined by the location of nutrient sources and sinks such as decaying wood and exploratory mycelium[29]. This explains why the levels of radiocaesium in basidiocarps in the field cannot be predicted solely on the basis of soil type and the pattern of deposition of fall-out. When mycelia of Schizophyllum commune were exposed to blue light to induce fruiting, 137Cs accumulated at the sites of initiation of basidiocarp primordia [28]. The high levels of radiocaesium recorded in basidiocarps in the field are due to translocation and accumulation of radiocaesium within fungal mycelia.

Translocation of radiocaesium by filamentous fungi

The translocation of radiocaesium through intact mycelia of Schizophyllum commune and Armillaria spp has been studied in the laboratory [28,29]. Translocation of 137Cs added to mature mycelia of Schizophyllum commune was by diffusion; when mycelia were labelled at inoculation, translocation was by mass flow. The maximum velocity of translocation of 137Cs through mycelia of Schizophyllum commune was an order of magnitude slower than that reported for 86Rb added as a tracer for potassium to mycelia of Neurospora crassa [23]. The distribution of 137Cs within undifferentiated mycelia of Schizophyllum commune eventually became even across the colony [28]. In contrast, an elevated concentration of radiocaesium remained at the centre of mycelia of Armillaria ostoyae and Armillaria gallica for at least 1950 h after the addition of label. This was attributed to immobilization of the label in melanized regions of the mycelium [29]. Unmelanized regions of fungal mycelia were not highly conservative for radiocaesium, which leaked out of the hyphae slowly; the persistence of radiocaesium within soil ecosystems is due to the fact that radiocaesium remains bound to the top 15 cm of the soil profile [31].

Translocation and accumulation of zinc and cadmium by filamentous fungi

Translocation of zinc (65Zn) and cadmium (109Cd) through mycelia grown on agar plates was studied using image analysis and autoradiography, as described previously [28]. For measurement of translocation, label was added to a well located either centrally or 15 mm from the edge of the plate. All plates were inoculated centrally; labelling was carried out either at inoculation or when the mycelium had grown to 10 mm from the edge of the plate. To investigate accumulation of label by fungal mycelia, label was added to the medium before inoculation.

Translocation of 65Zn through centrally labelled, undifferentiated mycelia of the litter-decomposing basidiomycete Schizophyllum commune, the wood-rotting basidiomycete Pleurotus ostreatus and the mycorrhizal basidiomycete Suillus luteus was similar to that of 137Cs through Schizophyllum commune [28] in that the distribution of label through the mycelium showed rotational symmetry around an axis drawn through the centre of the Petri dish. In contrast with the behaviour of 137Cs, transloca-
tion of $^{65}$Zn through mature mycelia could not be accounted for by diffusion. A high density of $^{65}$Zn remained at the centre of the colony, with a lower but relatively constant density of label across the remainder of the plate (Figure 1). The data obtained were consistent with immobilization of $^{65}$Zn at or near the site of addition, followed by rapid translocation of a mobilized fraction of the label. Comparison of the shape of the tracer front with those predicted by a number of models proposed for phloem translocation [32] suggested that $^{65}$Zn translocation was analogous to flow through a pipe with reversible loss of radiotracer through the walls. In this context a hypha, capable of both taking up and releasing zinc, would correspond to the pipe in the model.

The pattern of translocation of $^{65}$Zn through *S. commune* and *P. ostreatus* during the first 900 h after labelling was similar irrespective of whether label was added at inoculation or when the mycelium was mature. In contrast with translocation of $^{137}$Cs through undifferentiated, unmelanized mycelia, an elevated density of $^{65}$Zn remained at the centre of the plate for more than 1000 h after labelling. Elevated concentrations of $^{65}$Zn began to appear near the edge of mycelia of *S. commune* 950 h after labelling centrally. When mature mycelia were labelled, $^{65}$Zn was accumulated in localized regions, each approximately 3 mm in diameter. Where labelling had been carried out at inoculation, an entire ring of elevated $^{65}$Zn density, again approximately 3 mm wide, appeared close to the colony margin. When $^{109}$Cd was added to mature mycelia of *S. commune*, the concentration of label in the region of the colony surrounding the central well remained low, but two to five peaks of cadmium concentration, each approximately 4 mm in diameter, accumulated at the edge of the colony. This implied directional translocation of cadmium through defined pathways within the mycelium, despite the lack of macroscopic differentiation (for example cord formation) which might provide such pathways. Similar behaviour has been reported previously for $^{32}$P and $^{14}$C-amino isobutyric acid translocation in *S. commune* and *P. ostreatus* [30]. When $^{65}$Zn was added to the periphery of mycelia of *S. commune*, translocation initially occurred mainly around the circumference of the mycelium. This strongly suggested the presence of frequent anastamoses between hyphae as routes for translocation, as predicted by Rayner [33].

The greatest velocity and flux of translocation of $^{65}$Zn were observed in mature mycelia of *Suillus luteus* (Table 1). Efficient translocation of micronutrients would be a selective advantage for fungi involved in mycorrhizal symbioses. The

![Figure 1](image_url)

**Figure 1**
Distribution of $^{65}$Zn through a microcosm containing *P. ostreatus* grown from a central well and labelled when the mycelium was mature, 1094 h after labelling.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Position of well</th>
<th>Colony diameter at labelling (mm)</th>
<th>Time after addition of label (h)</th>
<th>Velocity of front (mm/h)</th>
<th>Time after addition of label (h)</th>
<th>Flux (pmol/cm² per s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. ostreatus</em></td>
<td>Central</td>
<td>130</td>
<td>86.4</td>
<td>0.511</td>
<td>86.4</td>
<td>$1.4 \times 10^{-3}$</td>
</tr>
<tr>
<td><em>S. commune</em></td>
<td>Central</td>
<td>130</td>
<td>271.3</td>
<td>0.342</td>
<td>271.3</td>
<td>$2.5 \times 10^{-3}$</td>
</tr>
<tr>
<td><em>S. commune</em></td>
<td>Peripheral</td>
<td>130</td>
<td>90.9</td>
<td>0.101</td>
<td>90.9</td>
<td>$3.6 \times 10^{-4}$</td>
</tr>
<tr>
<td><em>S. commune</em></td>
<td>Central</td>
<td>5</td>
<td>712.9</td>
<td>0.126</td>
<td>271.3</td>
<td>$2.7 \times 10^{-3}$</td>
</tr>
<tr>
<td><em>Suillus luteus</em></td>
<td>Central</td>
<td>130</td>
<td>34.7</td>
<td>1.579</td>
<td>34.7</td>
<td>$3.2 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

**Table 1**
Velocity and flux of $^{65}$Zn translocation through mycelia of *P. ostreatus*, *S. commune* and *Suillus luteus*
velocity of translocation of $^{65}$Zn was up to two orders of magnitude greater than that of $^{137}$Cs through mycelia of *S. commune* [28] or *Armillaria* spp. [29]. The velocity of translocation of $^{65}$Zn through mycelia of *Suillus luteus* and *P. ostreatus* was similar to that reported for translocation of $^{86}$Rb through *N. crassa* [25]. The complex pattern of translocation of $^{109}$Cd precluded analysis of the translocation velocity and flux using the same approach as that taken for the other isotopes.

When *S. commune* and *P. chrysogenum*, an ascomycete, were grown on medium containing $^{65}$Zn or $^{109}$Cd, the heavy metals were accumulated within the mycelia to concentrations higher than those found in the surrounding medium; this was not the case with *P. ostreatus*. The concentration of $^{65}$Zn per unit volume of hypha exceeded that in the medium by a factor of up to 4.5 in *S. commune* and up to 7.5 in *P. chrysogenum*, consistent with reports of heavy metal accumulation by fungi in the literature [16]. The site of greatest accumulation was at the centre of the colony for zinc, in contrast with cadmium, which was accumulated in an annular region 10 mm from the colony edge (Figure 2). This suggests that there are profound differences in the way fungi accumulate and translocate the biologically inessential, soft cation cadmium and the biologically essential, intermediate cation zinc.

The concentration of $^{65}$Zn per unit volume in basidiocarps of *S. commune* formed *in vitro* was at least seven times greater than that in the medium. Furthermore, the concentration of $^{65}$Zn in the hyphae at the centre of the mycelium was 30 times greater than that in the medium. This is again consistent with reports of heavy metal accumulation by macrofungi [16]. These results suggest that basidiocarp formation enhances zinc accumulation by the mycelium as a whole. Mechanisms which could contribute to the accumulation of zinc and cadmium include adsorption to the fungal cell wall, extracellular precipitation and complexation, and intracellular accumulation, possibly within the vacuole.

**Application to bioremediation**

In order to be successful, an agent for remediation of soil polluted with heavy metals or radionuclides would have to survive in the presence of the pollutant and to accumulate it from the soil. Fungi possess a number of mechanisms for resistance and tolerance to heavy metal toxicity [16], and are efficient at solubilizing metal ions in soil [14]. Mycelia of all but one of the fungal species studied in our laboratory accumulated zinc and cadmium. Fungi translocate radiocaesium, zinc and cadmium; translocation can lead to concentration of heavy metals and radionuclides in particular regions of the mycelium. Where translocation is very rapid as with zinc, or occurs only through particular pathways within the mycelium as with cadmium, the capacity for concentration of pollutants is increased. Finally, heavy metals and radionuclides are concentrated in basidiocarps both in the field and in the laboratory.

It has been suggested previously that plants able to hyperaccumulate nickel could be used for phytoremediation *in situ* of soil polluted by heavy metals [34]. The evidence presented above suggests that it is likely that filamentous fungi, introduced into polluted soil in an appropriate manner, would take up and translocate heavy metals or radionuclides, which would then be concentrated in the basidiocarps. Basidiocarps could then be harvested and the metals extracted for recycling, or disposed of appropriately. It remains to be seen whether the concentration factors we have observed *in vitro* can be reproduced in the field, and whether this will lead to an acceptable degree of remediation of contaminated soil.

In conclusion, these findings indicate a key role for fungi in determining the fate of radionuclides and heavy metals in soil ecosystems, and significant potential for exploiting fungi as bioremediation agents in metal-contaminated soil.
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Biomolecular site-recognition in the prediction of environmental oestrogen mimicry

A. Wiseman*†, P. Goldfarb*, T. Ridgway† and H. Wiseman††
*Molecular Toxicology Group, School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, U.K., †BioWise Associates at SBS, University of Surrey, Guildford, Surrey GU2 5XH, U.K. and ††Nutrition, Food and Health Research Centre, King's College London, Campden Hill Road, Kensington, London W8 7AH, U.K.

Introduction

Novel, rapid and inexpensive bioassessment techniques need to be developed to facilitate the identification of oestrogen mimics (full or partial agonists or antagonists) by biomonitoring, and where necessary, subsequent bioremediation of aquatic environments [1]. For total human exposure to oestrogenic material and for water-environment ecology and ecotoxicology, environmental oestrogen effluent derivatives (xenoestrogens) may be of less significance than

Abbreviation used: ER, oestrogen receptor.

*To whom correspondence should be addressed.