Bioremediation of Contaminated Soil with Fungi

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Many urban and industrial activities lead to soil contamination. Problems are caused by filling stations, repair shops, waste disposal and landfill areas, sawmill areas, market gardens and many more. Soils contaminated with organic pollutants, such as oil hydrocarbons, polyaromatic hydrocarbons, various solvents, wood preservatives and pesticides, can be theoretically remediated with biological methods. The ultimate aim in bioremediation is the mineralisation of organic compound, i.e. the degradation to carbon dioxide and water. The technology is both sustainable (pollutants are degraded) and eco-efficient (bioremediation consumes less energy and creates less pollution than alternative technologies, e.g. combustion in high temperature). However, the use of bioremediation plays only a minor role in handling contaminated soils. First of all, landfilling is still the most common way disposing contaminated soils. Secondly, the most persistent organic contaminants cannot be degraded by composting, which is the currently used bioremediation technology.

Figure 1. Fungi growing on pine bark, ready for inoculation to the soil.

Figure 2. Fungal inoculum is set inside the soil pile in layers (fungal treatment pile behind).

Composting is used commercially to treat particularly oil contaminated soils. However, oil contaminated soils might contain also polyaromatic hydrocarbons (PAH). If the PAH concentration is high, these soils can no longer be treated by composting. In addition to oil hydrocarbons, also chlorophenols, which have been used as active ingredients in wood preservatives, can be degraded in a well optimised composting process. Nevertheless, many sawmill areas are also contaminated with polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F), which are extremely persistent. Other examples of persistent organic pollutants are polychlorinated biphenyls (PCB), which were used as coolants and insulating fluids in transformers and capacitors, tributyltin (TBT) used in antifouling paints for ships, and trinitrotoluene (TNT) used in explosives.

The aim of the mycoremediation research, in co-operation between the University of Helsinki, Aalto University and Finnish Environment Institute, was to widen the applicability of bioremediation to the most persistent organic contaminants. Mycoremediation takes advantage of the lignin degradation capabilities of white-rot and litter-decomposing fungi, which live on wood and forest floor litter respectively. Both groups of fungi are able to degrade lignin with extracellular oxidising enzymes. In addition to lignin, these enzymes are able to degrade other compounds with structural similarities to lignin such as many organic pollutants. Although these fungi do not naturally grow deep into soil, they are able to survive in soil if suitable substrates are available. In fact, one of the key issues in mycoremediation research was to find a suitable carrier material to enable extensive fungal growth in the soil.

The starting point for this research was a screening of potential fungal strains. The University of Helsinki has a unique culture collection (FBCC = Fungal Biotechnology Culture Collection)

of approximately 2000 fungal strains isolated from mainly Finnish forests. Approximately 150 strains were tested for their capability to grow in soil and to produce lignin modifying enzymes (Valentín et al., 2008). Several promising fungal strains were found and *Phanerochaete velutina, Stropharia rugosoannulata* and *Gymnopilus luteofolius* were selected for further bioremediation experiments in laboratory scale. *P. velutina* is classified as a white-rot fungus, *S. rugosoannulata* as a litter-decomposing fungus, and *G. luteofolius* grows on stumps and falls between both ecophysiological groups of white-rot and litter-decomposing fungi.

Sawmill soils contaminated either with PAHs or PCDD/Fs were chosen as target soils for the experiments. Both soils were obtained from former sawmill areas with well documented sawmilling activity for decades. PAHs originated from the use of coal-tar creosote. The main compounds in the PAH contaminated experimental soil were fluoranthene and pyrene with a total concentration of 5000 mg/kg (sum of 16 PAH). Although fungi in average are able to tolerate high concentrations of PAHs, the toxicity for a fungus depends also on the bioavailability of PAHs in the soil. Since both bioavailability and the total concentration of PAHs were high, the original soil was too toxic for the fungi. However, after dilution with composted green waste to a concentration of 3500 mg/kg (sum of 16 PAH), fungi grew well in the soil and degraded 95 % of PAHs in 3 months (Winquist et al., 2014). After the dilution, also indigenous micro-organisms were able to degrade 70 % of PAHs in a control treatment without fungal inoculum.

The other experimental soil was contaminated with PCDD/Fs. The chlorophenol containing wood preservative Ky-5 used in this area had contained PCDD/Fs as impurities. While the chlorophenols had degraded during the years, the PCDD/F contamination was still present. The main congener was 1,2,3,4,6,7,8-heptachlorodibenzofuran and the total concentration accounted for 75 000 ng/kg WHO-TEQ. This soil was not too toxic for the fungus and was therefore not diluted. Tested fungi (*S. rugosoannulata and P. velutina*) were able to degrade up to 64 % of PCDD/Fs in 3 months (Anasonye et al., 2014). No degradation was observed in a control treatment without fungal inoculum.

After promising laboratory scale results, field scale trials followed. The two major challenges in the field scale are 1) production of fungal inoculum in larger quantity and 2) correct application of inoculum for obtaining adequate growth in the soil. The selection of a suitable carrier material is crucial for both of these steps. We used pine bark as a growth substrate (Valentín et al., 2010). Pine bark is a by-product from pulp, paper and timber manufacturing. It is composed mainly of lignocellulose but contains also phenolic extractives with antimicrobial properties. The composition of pine bark makes it a selective growth substrate. Therefore it is possible to use semi-aseptic growth conditions during the production of fungal inoculum and even added to soil pine bark is only slowly colonised by other soil micro-organisms.

Field experiments, described more in detail by Winquist et al. (2014), were carried out with PAH contaminated soil. The excavated soil was placed in two piles (each 2000 kg in size) of which one contained fungal inoculum (80 kg) and the other was left without serving as a control pile. The original soil was diluted with composted green waste according to the laboratory experiments, but the starting concentration was lower (1400 mg/kg). Due to the lower starting concentration, fungal treatment did not enhance the biodegradation, but both treatments, with or without the fungal inoculum, resulted in an efficient remediation and 94 % of PAHs were degraded. However, the inoculation of the soil in the field scale worked out well and we were able to achieve an extensive growth in the soil.

Figure 3. Three months later the fungal mycelia are growing on the surface of and inside the fungal treatment pile (fungal treatment pile in front).

As a conclusion, promising results were obtained in the laboratory scale, particularly with PCDD/Fs, which are extremely persistent in the environment. The next step would be to continue with the field experiments before the technology is ready for commercial use. The research on this topic still continues at the University of Helsinki and in addition to PAHs and PCDD/Fs also TNT contaminated soil has been remediated successfully by fungi (Tuomela, 2013).

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