The Role of Mycoremediation in Alaska

The applications, strengths and limitations of mushroom-based bioremediation in cool climates and high latitudes

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Fungi Alliance
OUTLINE

1. Fungi Alliance Business
2. Principles of Mycoremediation
3. Potential of fungi in remediation
4. Mycoremediation development in Alaska
5. Discussion
A social enterprise is an organization that addresses a basic unmet need or solves a social or environmental problem through a market-driven approach.

*Social Enterprise Alliance*

The Fungi Alliance is a social enterprise for-profit collaboration providing services, consulting, and products using fungi for environmental soil remediation.

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PRINCIPLES OF MYCOREMEDIATION

Fruitbody

Young mushroom forms

Spores

Spores (enlarged)

Hyphae

Spores germinate if moisture and food present

Mating between hyphae from different spores

Hyphae feed and grow in soil
PRINCIPLES OF MYCOREMEDIATION
MYCORRHIZER

ECTOMYCORRHIZA

ACCUMULATION

Zn
Cd
Ag
Cl

RHIZOSPHERE

5% (w/w) FUNGUS
95% (w/w) PLANT
PRINCIPLES OF MYCOREMEDIATION

Largest Organism in the World at over 2000 acres
PRINCIPLES OF MYCOREMEDIATION

Fruiting Body

Mycelium

- Cells secrete digestive enzymes
- Hyphae
- Cells absorb nutrients
- Enzymes cause hydrolysis reaction, break down nutrients in soil
Principles of Mycoremediation

Enzymes
LIGNINOLYTIC ENZYMES

• Laccase,
• Manganese Peroxidase (MnP),
• Lignin Peroxidase (LiP)
• Versatile Peroxidase (VP)

➢ Extracellular, low substrate specific, oxidize many recalcitrant xenobiotic organic compounds
PERSISTENT ORGANIC CONTAMINANTS

Laboratory and benchtop studies

- **Petroleum Hydrocarbons**
  - Gasoline, Diesel, Crude Oil, Polynuclear Hydrocarbons

- **Chlorinated Compounds**
  - Polychlorinated Biphenyl's (PCBs), chlorinated solvents, Dioxane, chlorinated pesticides, insecticides.

- **Pharmaceutical & Personal Care Products**
  - Antibacterial, Antidepressants, Anticonvulsant, and more

- **Bacterial contamination**
  - E.coli and other pathogens
PETROLEUM HYDROCARBONS

Number of Carbons

Crude Oil

Gasoline

Diesel

JP - 4

Stoddard Solvent

Fresh Creosote

Kerosene

JP - 8 or Jet A

Heating Oils

Heavy Fuel Oils

Bunker C

Lubricating Oils

Waxes and related products

Asphalts and Pitch
PETROLEUM HYDROCARBONS

• Gasoline, Diesel  
  Seidu et al 2015

• Polycyclic Aromatic Hydrocarbons (PAHs) >100 different
  • Gramass et al. 2009
  ➢ Compared 58 fungi for PAHs degradation, different fungi prefer different PAH compounds, 19-95% degradation.

• Crude Oil
  • Isikhumhen et al 2003, Ezekoye et al 2018,
  ➢ Decline of contamination, making soil more fertile, increases growth of other vegetation.
Chlorinated Compounds

Polychlorinated Biphenyls (PCBs)
209 congeners

With Ligninolytic Enzymes
(Sredlova et al 2020, Cvancarove et al 2012)
- Basidiomycetes e.g. fresh and spent
  *Pleurotus ostreatus* (oyster mushroom) in water and soil
  ➢ Splitting of aromatic rings & De-chlorination

Without Ligninolytic Enzymes
(Tigini et al 2009, Sredlova et al 2020,
Marco-Urea et al. 2015 and more)
- Ascomycota (sac) fungi
  *Penicillium* (chrysogenum, digitatum)
  *Scedosporium apiospermum, Fusarium solani*
  ➢ De-chlorination
Chlorinated compounds

Trichloroethylene (TCE) highly toxic compound derived from industrial cleaner (dry cleaner, auto shops) very soluble and found as groundwater contaminant
White Rot Fungi *Tramets versicolor* produced CO$_2$ and 2,2,2 Trichloroethanol
Successful remediation combining *Poplar sp.* and TCE degrading endophyte. (Doty et al 2017)

[Chemical Structures: Trichloroethylene, Vinyl Chloride, Ethylene]
Metals
Cadmium, Copper, Chromium, Lead, Mercury, Nickel, Tin, Zinc

Biosorption and Sequestration:
- Modified mushroom material (mycelium and fruiting body): Dried mycelia, live mycelia, spent mushroom substrate, biomass immobilized on calcium alginate.
- Mushrooms take up and accumulate metal concentrations above permissible concentrations.

Process: Ion-exchange and chemisorption

- Adsorption due to functional groups, and ionizable groups (carboxyl, amino groups) pH dependent, pH 5-6 favorable for most metals

- Heavy metals are mostly enriched in fruiting body or stipe

- Metal concentrations may reduce growth of mycelia can stimulate or reduce enzyme production
Pilot Study 2018-2019
Anchorage International Airport Landspread Site

Plastic and passive aeration

Weed guard and passive aeration

Weed guard

Control

Tilling
47 DAYS

SITE VISIT IN AUGUST 8\textsuperscript{TH}, 2018
100 DAYS
SITE VISIT OCTOBER 1ST 2018
SAMPLING OCTOBER 10TH 2018

Control

Plastic

Weed-Guard

Weed Guard

no aeration
Degradation Pattern

DRO Concentration PPM

130 ppm/month
RESULTS: THE PROCESS

**selection**
- Soil characterization and fungi strain selection
- → soil sampling, analysis, strain cultivation

**cultivation**
- Cultivation and extension
- → preparing grain bags
  
  **monitoring**
- Site preparation and inoculation
- → 1 part inoculum and 4 parts soil

**inoculation**
- Soil and contaminant monitoring
- → moisture

2 month
RESULTS: FUNGI-BIOPILE

Complies with ADEC requirements for Biopiles/bioremediation, 18 AAC 75.370/ 18 AAC 75.360.11(E)
Crude Oil Experiment

Agar-Agar with 1000 ppm Crude Oil
SUSTAINABILITY

- Waste materials
- Invasive species
- Faster (2 weeks)
- Minimal maintenance
- Retaining the soil on site
WHAT NEXTS?

➢ There are many many positive studies

➢ Nobody developing/applying this in Alaska

➢ Establishing a business that brings Mycoremediation to Alaska

➢ Fulfilling agency regulations

➢ Real Applications – Pilot Study
QUESTIONS?

Phanerochaete chrysosporium

Trametes trogii

Lentinus tigrinus

Penicillium

Trametes Versicolor

Pleurotus ostreatus

Aspergillus awamori

Fly Agaric
REFERENCES


Tigini, V., et al. (2009). "Isolation and characterisation of polychlorinated biphenyl (PCB) degrading fungi from a