Phase 1: Fungal Growth Evaluation in Vactor Waste

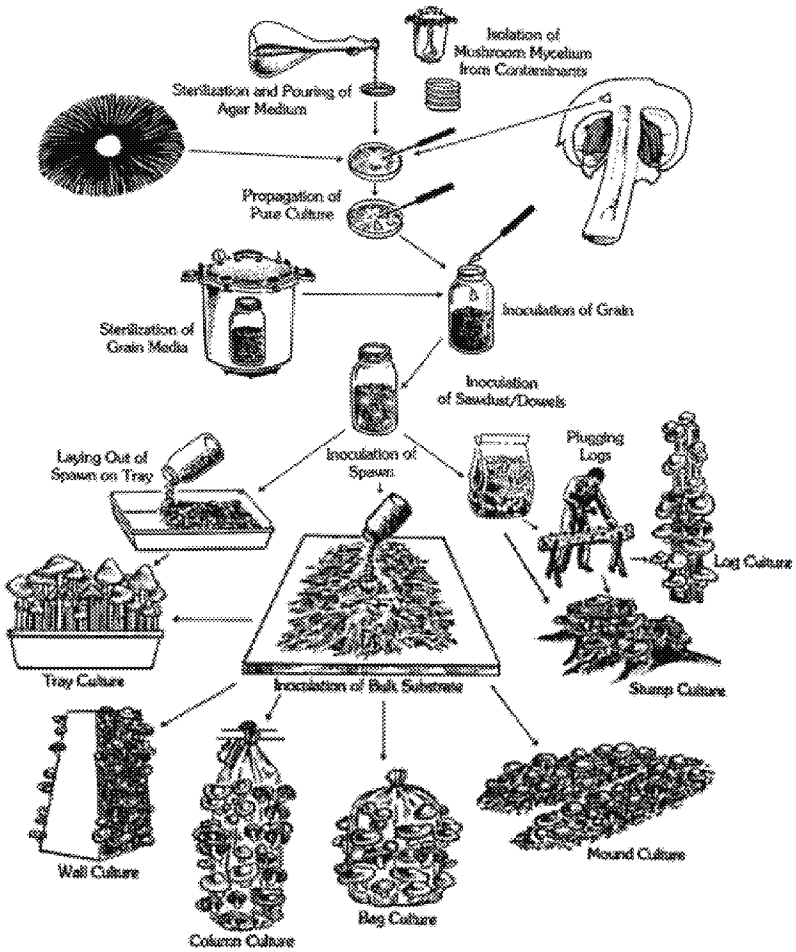
During Phase 1 we will demonstrate our ability to cultivate fungi, and assess several species of fungi for their ability to grow in PCB laden vactor waste from the City of Spokane.

A: Cultivation of Fungi

Purpose: Demonstrate our ability to successfully cultivate mycelium (vegetative body of fungus) on several types of substrate, ensuring our ability to conduct experiments and pilot projects with fungi.

Overview: Transfer mycelium of each species from agar petri dish to sterilized grain. Allow to fully "colonize" grain, and then transfer to straw or wood (sawdust/ chips) depending on species.

Approach/ reasoning: In order to test our newly built lab space, equipment, and cultivation skills, we will show that we can successfully grow several species of white rot fungi. This will demonstrate our competence, and ability to carry out projects that require the cultivation of fungi.



B: Vactor Waste Observation Experiment

Purpose: To observe the growth of 12 species of fungi in the presence of "vactor waste" (storm drain sludge) from the City of Spokane's decant facility. *(Analytical testing for PCB degradation will take place at a later stage.)*

Overview: We will 'train' or 'acclimate' each strain to digest the components of vactor waste by introducing them to a small amount of sterilized vactor waste in petri dishes. We will then transfer these 'acclimated' fungal cultures to jars of grain, then pasteurized sawdust/ woodchips to expand the mycelium. After sawdust jars are colonized (2-4 weeks) we will add this mycelium to various ratios of raw vactor waste and woodchips. We will visually observe fungal growth over several weeks (4-8).

Approach/ reasoning: Each selected species shows promising traits for PCB breakdown. We aim to learn if they will survive and grow in the presence of vactor waste, which contains a multitude of substances including PCBs. Research suggests that fungi can potentially be 'trained' to digest certain substrates if introduced to them in small amounts at an early growth stage. This can trigger the production of enzymes that are effective at digesting the specific food source. Introducing a small amount of sterilized vactor waste to the cultured fungi in petri dishes could give them a better chance of survival when mixed with large amounts of vactor waste later in the experiment. Observing mycelial growth in jars of various ratios of vactor waste and woodchips will be very informative to our long term goal of digesting PCBs in vactor waste.

Task 1: train fungi to sterilized vactor waste (1-2 weeks)

- -Homogenize (20mL?) of vactor waste, add to agar mix and sterilize together
- - make normal malt agar
- -Pour petri dishes (36 normal agar , 36 spiked agar)
- -Grow each species on 3 dishes of normal agar and 3 dishes of vactor waste spiked agar
- -observe growth of each species, and use spiked cultures for the following steps

'Training' to vactor waste treatment groups:

Species:	**Group A:** Agar+Fungus	**Group B:** Agar+ Vactor waste+ Fungus	**Species totals:**
Pleurotus ostreatus			**6**
Pleurotus pulmonarius			**6**
Pleurotus djamor			**6**
Wild local oyster(sp?)			**6**
Hypsizygus ulmarius			**6**
Grifola frondosa			**6**
Stropharia r-annulata			**6**

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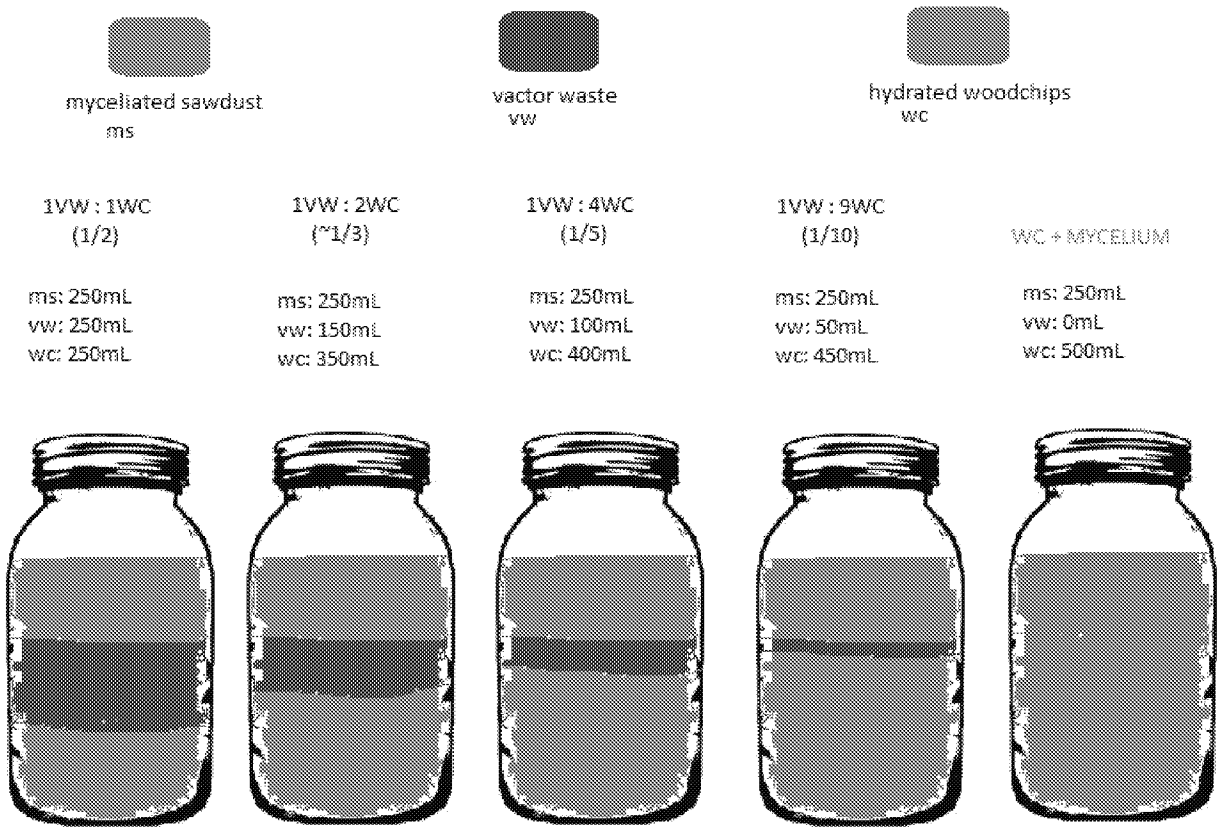
Trametes versicolor			**6**
Lentinula edodes			**6**
Lentinus lepidus			**6**
Flammulina velutipes			**6**
Coprinus comatus			**6**
Group Totals:	**36**	**36**	**Grand total: 72**

Task 2: transfer 'acclimated' (spiked) cultures of each species from petri dishes to sterilized grain and allow colonization (2-4 weeks), then transfer to pasteurized hardwood sawdust/ chips to 'bulk up' mycelium in preparation for vactor waste ratios (2-4 weeks).

Task 3: grow each species of fungi in quart jars with the following Volume: Volume ratios and observe growth (2-4 weeks)

-use 'vactor-waste-acclimated' mycelium from jars

Task 4: Send samples for analytical PCB testing



(visual representation of ratios)

Species Descriptions:

Pleurotus ostreatus Wood decomposer. Common across North America. Can thrive on wide variety of wood types mainly hardwoods, and other substrates (paper products, straw, ag wastes, coffee grounds, etc) Aggressive, fast growing, many studies done on PCB and other degradation. Fruits in summer and fall. Incubation: 75 degrees

Pleurotus pulmonarius Wood decomposer north america. Exceedingly easy to cultivate, most hardwoods (aspen) at lower elevations, and sometimes firs and spruces (1,200-3,000 meters), other substrates (paper products, straw, ag wastes, coffee grounds, etc) Aggressive, fast growing, favors slightly warmer temperatures and higher altitudes than *ostreatus*. Fruits in Spring. 75-85 degrees

Pleurotus djamor Pan tropical hardwood decomposer, Can thrive on hardwoods, and other substrates (paper products, straw, ag wastes, coffee grounds, etc) Aggressive, fast growing, will outcompete contaminants on unpasteurized cereal substrates, tolerance for high temperatures. 75-85 degrees

Pleurotus (sp? Wild local) Found locally (by friend) in Spokane County at California Creek! *Was not able to identify to species, but plant to after fruiting....*

Hypsizygus ulmarius wood decomposer hardwoods, very easy to grow, aggressive and similar to ostreatus in habits, etc. 70-80 degrees

Grifola frondosa dead or dying hardwoods and sometimes larch, grain to hardwood sawdust, highly medicinal, cited in literature to have pcb degrading qualities. 70-75 degrees

Stropharia ruggosa-annulata Extremely adaptive to outdoor cultivation, benefits from presence of other microorganisms in soil, and from being disturbed. Liquid culture to grain recommended, growth in lab is slow, benefits from frequent shaking until fully colonized. Grows well in woodchips (mixed hardwood and conifer) or straw mulch, mycelium activated by microflora in soils, particularly bacteria. 70-80 degrees

Trametes versicolor highly adaptive wood decomposer hardwoods and conifers, found on much of the globe. Pcb degrading qualities in scientific lit, its enzymes can break down wood very effectively to a pulp. Effective anti cancer drug, competes well with other organisms. 75-85 degrees

Lentinula edodes saprophytic (never parasitic) to hardwoods, particularly oak, and not fruit woods. Shown to have PCB degrading qualities in scientific studies. Anti cancer properties perhaps useful for degradation...70-80 degrees

Lentinus Lepidus aka trainwrecker, known to grow on railroad ties treated with creosote, aggressive.

Flammunlia velutipes widespread in temperate regions sea level to tree line, on softer hardwoods (aspen, alder, cottonwood, birch, willow, poplar etc), occasionally conifers doug fir, **growing in late fall to early winter**. 70-75 degrees

Coprinus comatus Compost/ manure/ soil mushroom, grows well in manure enriched soils or 4-6in deep beds of hardwood sawdust, fertilized and frequently watered lawns, cow or horse manure mixed with

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straw or sawdust is ideal, benefits from peat moss based casing soil. 70-80 degrees (grain or manure straw/sawdust)

Desired species : phanerochaete chrysosporium,

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