

The Remediators Inc

Department of Ecology
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Re: Port Angeles Harbor Cleanup Site Cleanup ID 11907

The preferred cleanup remedies of intertidal excavation, intertidal capping and the final selection of cleanup actions under consideration are intended to provide for the long term safety of the public and restoration of the environment in the most comprehensive and cost effective way. The modern bioremediation technologies we employ have been reviewed and approved by the EPA and the California Department of Toxic Substance Control. We have many successful projects in Superfund Sites, large industrial cleanup sites and private cleanup sites in 8 states within the lower 48 states and Alaska with several more beginning this year. The Integrated Biological Approach to bioremediation used by us and project partners at NASA/Ames Research Center relies on a combination of plants, microbes, and fungi for remediation of mixed contaminants of soil and groundwater. The technology combines patented plant/microbe pairings originating from and licensed through the University of Washington Forest Science Laboratory with fungi from our library of thoroughly tested fungal strains for remediation use. This process can deconstruct organic toxins completely as well as remove inorganic toxins and metals and concentrate them within the plant tissue effectively and at less cost than most other remediation technology. A trial using specially prepared biochar made for use within a thin-layer sand cap trial conducted from the Ashland Chemical Superfund Site on Lake Michigan outperformed 'Sedimite' and Granulated Activated Carbon (GAC), both for prevention of migration of toxins as well as providing a restorative function to the cap. These technologies are flexible, efficient, and restore the sites they are used on to a healthy condition. By combining these technologies as appropriate we are able to treat hard to treat as well as mixed contamination simultaneously with visible improvements to the site, often with minimal disturbance to the ground through our specialized application methods. Information on specific contaminants, site specific treatments, and supporting validation literature will be provided on request as well as presenting a synopsis of our previous and ongoing work. The addition of these technologies within existing treatment options provided for in the Port Angeles Harbor Cleanup will ensure that when completed the contamination is not simply covered up, rather the site is clean and restored to a healthy safe condition.



Ashland Laboratory Study

Estimation of Floatable Sheen from Sediment Disturbance and Sand Cap Efficacy Studies

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9/22/2011

Results of the laboratory trials indicate that a floatable sheen will be produced during any sediment removal project and mitigation controls should be instituted. All cap treatments prevented breakthrough of contaminants to sediment surface but the amended sand treatments of biochar, granular activated carbon and SediMite™ (in that order) prevented vertical migration of contaminants better than the unamended sand cap.

Mycoremediation and the Integrated Biological Approach

Mycoremediation of Environmental Pollutants

Petroleum based contamination of soil and water are a major threat to the health of our ecosystems and human health. Cleanup costs for these often hard to treat contaminants have imposed an enormous financial burden on society with negative effects on land values. As a standalone treatment for petroleum contamination mycoremediation has achieved ‘non-detects’ in as little as a few months’ time. The fungal metabolization of hydrocarbons creates no toxic waste stream with carbon dioxide and water being the final product of decomposition. Mycoremediation in an integrated bioremediation system represents the state of the art in bioremediation technology. We combine the use of specifically selected fungal treatments with phytoremediation plant / microbe combinations that have been proven successful in field applications to treat a variety of pollutants. This newly developed approach allows an effective solution for a broad range of organic and inorganic pollutants as well as being the least costly.

Fungi are nature’s recyclers. They secrete enzymes into their environment that break down organic compounds. These compounds are chemically broken down into simpler ones which then become available to the growing fungi and other organisms. The degradation of lignin and cellulose are primary sources of energy for most fungi and lignin is a natural analogue of petroleum based hydrocarbons. Fungi can degrade a variety of petroleum hydrocarbons including aromatic (PAHs, dioxins) and chlorinated (PCBs, DDT) compounds. Enzymes responsible for this can likewise deconstruct inorganic compounds and metals which then become available to microbes and plants within our combined bioremediation systems.

Mycelium, where mushroom meets toxin. Mycelium, the rootlike structure that comprises the bulk of these fungal organisms, exist in an interconnected web of microscopic threads called hyphae that penetrate their environment. A gram of healthy soil can contain hundreds of meters of fungal hyphae. Fungal growth is dependent upon nutrients and minerals that the mycelium encounters that are degraded by enzymes secreted by the mycelium and then reabsorbed as their primary food source. It is in and around the mycelial network that the remediation occurs. Our Mycoremediation treatments consist of live fungal mycelium in cellulosic carriers optimized to meet specific project needs.

- Eliminates the need for offsite disposal of soil.
- There are no downstream negative effects from the process. The conversion of toxins transforms them to mostly CO₂ and Water.
- MycoRemediation A “Green” technology. The materials used can be helpful in restoring soil health.
- The decontaminated soils may be reused, or left in place as an in-situ process.
- Minimal monitoring and no mechanical infrastructure.



Each fungal strain is thoroughly tested for the ability to decontaminate a range of toxins and for growth under different conditions.

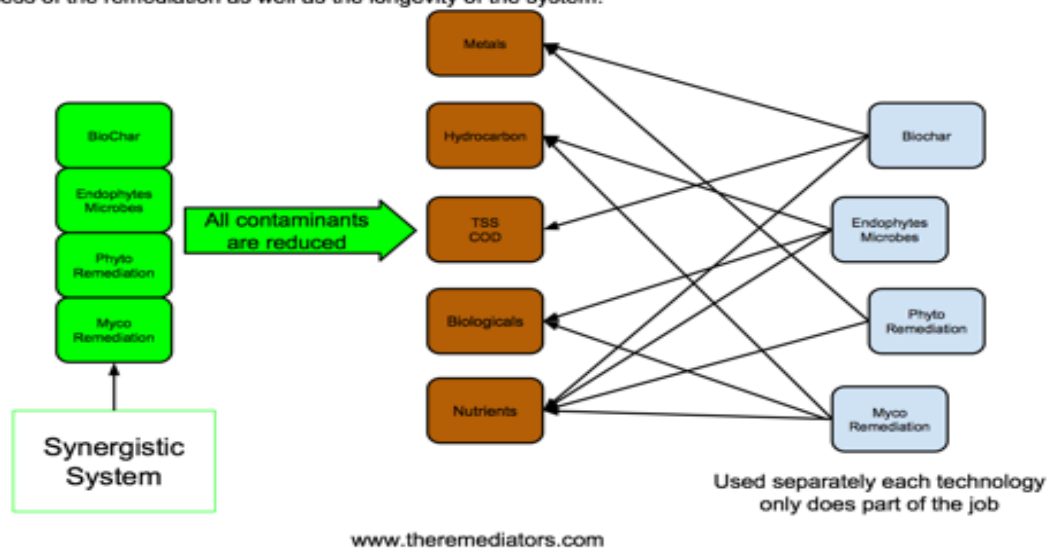
Our process has been used successfully in the United States and Canada in the remediation of petroleum hydrocarbons and was approved by the US Navy’s Facilities Engineering Service Center (NFESC) as an innovative technology suitable for their environmental program.

A Living Partnership: The Integrated Biological Approach.

Soil bacteria grow and travel in the film surrounding the mycelial hyphae more efficiently than in soil or water without hyphae, giving these microbes direct access to their food source. These interactions also support the transfer of genetic material within these populations which supports greater diversity and vitality. These factors translate to more rapid decomposition of toxic compounds that are also made

Integrated Biological Approach

When incorporated into one system each technology supports the success of the remediation as well as the longevity of the system.



accessible for uptake into the roots of plants used in the remediation. The partnership between fungi, soil bacteria, and hyper accumulating plants allows for the successful treatment of many hard to treat toxins as well as increasing the performance of each component of the system. The Integrated Biological Approach is our remediation ‘toolbox’ and constitutes latest state of the art of bioremediation.

For effective and affordable treatment of contaminated soil, sediments and water feel free to contact us for more information.

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TABLE 9 SUMMARY OF TOC/TC AND TOTAL SOLIDS DATA

Core Depth	Sand			SediMite™			Biochar			GAC		
	TOC	TC	TS	TOC	TC	TS	TOC	TC	TS	TOC	TC	TS
0-1	0.02 U	0.02 U	73..3	0.02 U	0.02 U	76.0	0.02 U	0.02 U	78.7	0.02 U	0.02 U	77.8
1-2				0.02 U	0.02 U	76.2	0.02 U	0.02 U	77.3	0.02 U	0.02 U	78.1
2-3				7.28	7.30	72.5	5.47	6.21	77.6	12.0	12.9	75.4
3-4				0.02 U	0.02 U	76.6	0.028	0.022	77.2	3.44	4.11	77.0
4-5				0.02 U	0.02 U	75.5	0.02 U	0.023	77.0	0.02 U	0.024	77.2

In addition to physical analysis, the deepest section of each core was analyzed for SVOCs to establish if the contaminant layer was migrating through the cap. The results of this analysis are provided in Table 10. The SVOCs were detected at a much higher concentration in the sand cap (10,590 ug/kg dry wt.) than the amendments. All of the amended sediment had reduced concentrations of SVOCs compared to the sand cap by at least 25 and as much as 50-fold. Biochar appeared to be the most efficient at reducing the migration of the contaminated layer followed by GAC and then SediMite™ (approximately half the concentrations observed in the other amended sand cap treatments).

The dominant compounds within the different treatments changed depending on treatment type; phenanthrene was found at the highest concentration in the sand, GAC, and biochar treatments whereas naphthalene compounds were the most dominant in the SediMite™ treatment. This observation may be related to SediMite™ pellets moving somewhat vertically through the cap and absorbing or entraining the contaminants. In any case, the sand cap had higher concentrations of contaminants than any of the amended treatments; therefore if the restorative layer is selected as the preferred option for the Ashland waterfront site, the restorative layer should include the addition of an amendment.

CONCLUSIONS

The summary findings as a result of laboratory studies conducted on selected cores from Chequamegon Bay, Lake Superior are:

1. There is a distinct silty sand contamination layer of free floating product mixed with pebbles varying in depth from approximately 1 ft to 3 ft below the sediment surface depending on the station location. This layer is comprised of PAH compounds similar to sediment samples collected from URS in 2001; most likely derived from petrogenic sources related to on-shore activities. *Based on boring logs advanced both on shore at Kreher Park and off shore in the bay sediments, this sandy material corresponds to granular materials underlying the wood chip/reworked sediments at varying depths from elevation 591 to 595 msl. This granular unit is in turn underlain by soft to firm silts and/or stiff to hard clay. At some sediment sample points contaminants have penetrated the silt. Monitoring wells screened in the sand at the shoreline have historically encountered both dissolved and more recently free-phase hydrocarbon. Prior to initiating any sediment dredging or removal operation, the granular materials at Kreher Park should be contained*
2. The measured PAH compounds within the contamination layer are mobile and move within the sediment column both in an upward as well as downward direction.
3. The PAH compounds will migrate from the sediment to the water surface during dredging operations (4% of measured compounds were found in the elutriate preparations). *Mitigation measures such as containment booms should be employed to address floatable products that will surface during dredging.* Under this method at least 4% of the contamination in the sediment will be mobilized to the surface of the water.

4. The concentrations in the sand cap were more than fifty times as high as the biochar treatment and more than 20 times higher than the GAC or SediMite amendment. *A restorative layer should include a sand cap amended with a carbon source. Additionally, the biochar amendment appears to enhance the carbon nutrient quality of the sand which could promote more rapid recolonization of the returning benthic community.*

TABLE 10 SUMMARY RESULTS FOR SEDIMENT CORES

Analyte (conc. = ug/kg, dry)	Clean sand	SediMite™	Biochar	GAC
Phenol	< 60	< 63	< 64	< 64
2-Methylphenol	< 60	< 63	< 64	< 64
4-Methylphenol	< 60	< 63	< 64	< 64
2,4-Dimethylphenol	< 60	< 63	< 64	< 64
Naphthalene	84	270	< 64	< 64
2-Methylnaphthalene	780	130	< 64	80
1-Methylnaphthalene	580	96	< 64	< 64
Dimethylphthalate	< 60	< 63	< 64	< 64
Biphenyl	120 J	12 J	< 64	< 64
Acenaphthylene	< 60	< 63	< 64	< 64
Acenaphthene	1200	< 63	< 64	< 64
Dibenzofuran	77	< 63	< 64	< 64
Diethylphthalate	< 60	< 63	< 64	< 64
Fluorene	520	< 63	< 64	< 64
Pentachlorophenol	< 300	< 320	< 320	< 320
Dibenzothiophene	120 J	< 63	< 64	< 64
Phenanthrene	2100	< 63	140	160
Carbazole	< 60	< 63	< 64	< 64
Anthracene	550	< 63	< 64	< 64
C(1)-Ph/An's	920 J	< 63	< 64	< 64
Cyclopentaphenanthrene	180 J	< 63	< 64	< 64
Di-n-butylphthalate	< 60	< 63	< 64	< 64
Fluoranthene	620	< 63	< 64	< 64
CyclopentaPh/An	240 J	< 63	< 64	< 64
Pyrene	990	< 63	72	86
Butylbenzylphthalate	< 60	< 63	< 64	< 64
Benzo(a)anthracene	270	< 63	< 64	< 64
bis(2-Ethylhexyl)phthalate	< 60	< 63	< 64	< 64
Chrysene	250	< 63	< 64	< 64
Di-n-octylphthalate	< 60	< 63	< 64	< 64
total Benzofluoranthenes	240	< 63	< 64	< 64
Benzo(a)fluoranthene	70 J	< 63	< 64	< 64
Benzo(e)pyrene	140 J	< 63	< 64	< 64
Benzo(a)pyrene	240	< 63	< 64	< 64
Perylene	40 J	< 63	< 64	< 64
Indeno(1,2,3-cd)pyrene	89	< 63	< 64	< 64
Dibenzo(a,h)anthracene	< 60	< 63	< 64	< 64
Benzo(g,h,i)perylene	120	< 63	< 64	< 64
Anthanthrene	50 J	< 63	< 64	< 64
Sum of detected SVOC	10590	508	212	326

RESTORATIVE LAYER RESULTS

The restorative layer experiment was initiated on 5/15/11 and the experiment was terminated on 7/14/11. One hundred grams of contaminated sediment created from contaminated sections typically 13 inches or more below the sediment surface from several stations was used as a worse case residual material. The test aquaria were monitored daily for signs of any contaminant breakthrough of the sand cap including visual observance of product either floating on water surface or present in the sediment, any noticeable odors representative of petroleum, and the volume of water passing through the system was measured daily.

GENERAL OBSERVATIONS

A lighter colored sand layer approximately 0.25 inches in thickness was observed in all of the treatment tanks at the surface of sediment layer. This lighter colored layer is thought to be associated with the finer sand particles settling out of the sand surface. Over time green and red algae were observed in most treatment tanks; the biochar treatments had the most predominant growth.

SediMite™

A SediMite™ pellet was noted on the surface of one tank approximately one week after the start of the experiment. This pellet migrated up through the sand cap from the three inch depth. Additionally, there was a noticeable upward migration pattern from the SediMite™ layer towards the surface of the sand cap. This pattern was noted for all three treatment replicates and is illustrated in Figure 13.

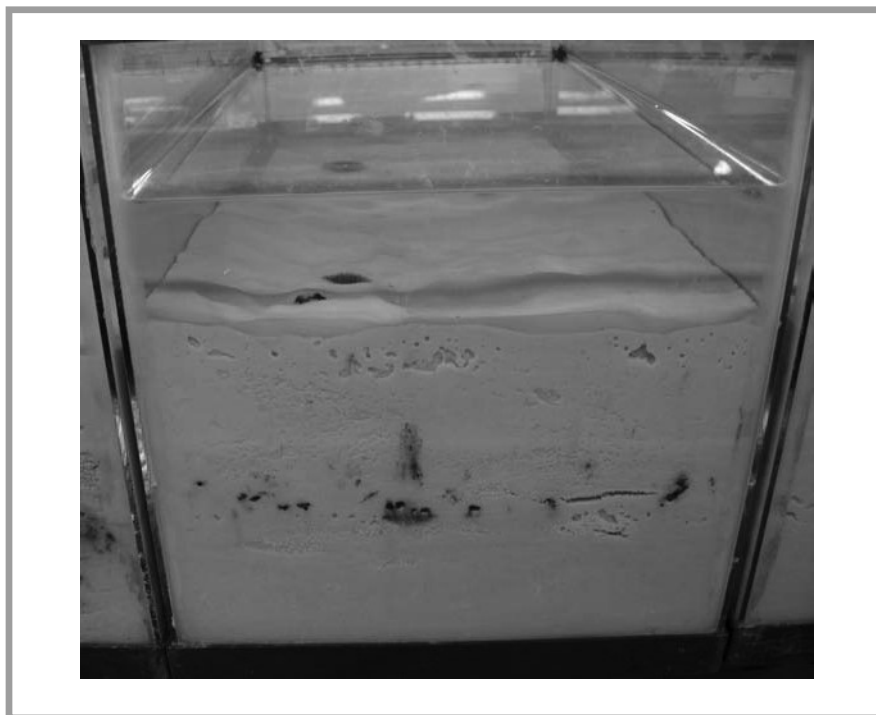


FIGURE 13 SEDI MITE™ VERTICAL MIGRATION PATTERN, PELLETS ON SURFACE

BIOCHAR

The biochar treatments all showed a black cloud pattern diffusing in all directions from the three inch layer (Figure 14). Algal growth was most prevalent in biochar treatments in association with the black cloud pattern which may indicate that this amendment has the most available carbon to promote more rapid benthic recolonization of the cap.

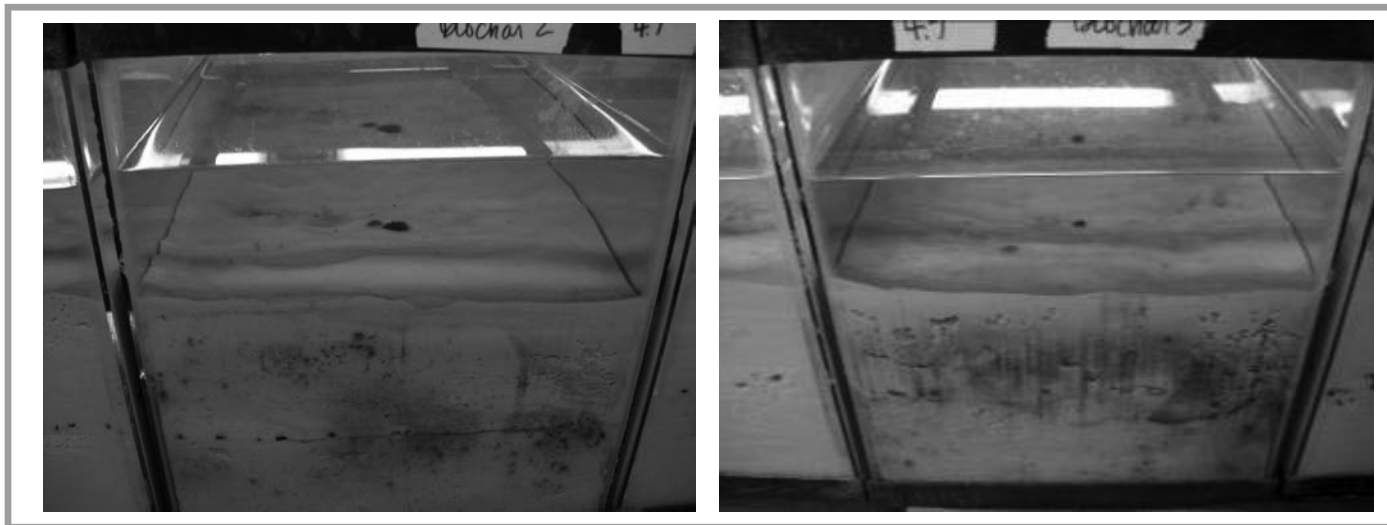


FIGURE 14 BIOCHAR DIFFUSION PATTERNS AND ALGAL GROWTH

Granular Activated Carbon (GAC) – Pellet Form

The GAC treatments had no vertical movement from the three inch depth layer as shown in Figure 15. There was also no observed algal growth associated with any of the GAC replicates.



FIGURE 15 GAC TREATMENT REMAINS AT THREE INCH DEPTH INTERVAL

Oil and Gas Endophyte-Enhanced Tree Phytoremediation plus Mycoremediation

Crude oil and gas pollution originate from many sources and can include activities such as unintentional spills of organic pollutants, leaking storage tanks, and oil and gas exploration, extraction, and transportation that can contaminate soils and sediments, groundwater, and surface water. Traditional cleanup of petroleum hydrocarbon pollutants is costly, not only financially but also environmentally. In some cases, traditional remediation treatments are unsuccessful at removing a sizable portion of pollutants that were accidentally released into the environment. In other cases, low, yet reportable, levels of recalcitrant petroleum hydrocarbon pollutants persist after initial cleanup, making it difficult to close sites. Our team provides remediation assessment, direction and new solutions to assist in the stabilization and remediation of these contaminants from polluted environments, and the mitigation of risks associated with these sites. Intrinsyx and PPCU utilize poplar tree-endophytic bacteria in many different groundwater loving trees combined with the Remediators fungal soil mycelium to effectively remove and degrade petroleum hydrocarbon pollutants (BTEX, TPH, PAH's, etc.) in groundwater and soil using a combined poplar tree phytoremediation and mycoremediation system.

Advantages of using plants inoculated with endophytic bacteria that degrade petroleum hydrocarbons.

Trees inoculated with our highly-specialized bacteria significantly increase the degradation of petroleum pollutants in soil and water by as much as 40% versus controls containing un-inoculated plants, and considerably more than no treatment at all. In addition, plants containing these specialized endophytic bacteria have demonstrated higher root and shoot growth as well as no signs of phytotoxic effects from petroleum pollutants, even at traditionally phytotoxic concentrations. In fact, these endophytic bacteria even facilitate increased uptake of pollutants into the plant tissues for degradation, which is especially important for recalcitrant hydrocarbons. In the image shown above, willow trees were inoculated (left 3 trees), or un-inoculated (right 3 trees), and grown in soil containing phytotoxic concentrations of phenanthrene (Khan et al. 2014). Thankfully, these specialized endophytic bacteria can be used with any plant species, and the inoculation of plants can occur at the time of planting or on established trees, shrubs, herbs and grasses!



Plant endophytic bacteria that degrade chlorinated solvents and pesticides. Some petroleum hydrocarbon impacted sites also contain chlorinated solvents or persistent organic pesticides, or even explosives! In addition to our petroleum hydrocarbon degrading poplar endophytes we have tree endophytes that degrade chlorinated molecules and explosives like TNT and RDX.



Soil mycoremediation. Fungi naturally breakdown organic compounds from the soil in which they reside. They inherently degrade a variety of petroleum hydrocarbons including aromatic (PAHs, dioxins) and chlorinated (PCBs, DDT) compounds. Degradation of these organic pollutants results in the creation of water and carbon dioxide, leaving no contaminants behind. In addition to the degradation activities of these beneficial fungi for remediation of organic pollutants, they also provide benefits to the plants used for phytoremediation by helping to make mineral nutrients more bioavailable as well as confer greater environmental stress tolerance to biotic and abiotic factors.

Combined Tree Bio-Phytoremediation and Mycoremediation Applications. Combining endophyte-enhanced phytoremediation with mycoremediation has the potential to dramatically increase the remediation efficiency and effectiveness of organic polluted sites over any other green technology on the market; and, this system is vastly less expensive than traditional remediation approaches. Our system is designed to work together to increase remediation efficiency from the time of implementation to closure and reduces the total time to remediate using biological organisms. To top it all off, the technologies discussed in this paper are isolated from nature and are completely safe to humans and the environment, and do not require specialized permitting for use. Many sites we encounter are contaminated with multiple pollutants and we have found that this multifaceted approach is ideal because we can address multiple contaminants of concern concomitantly.

Our endophytic plant bacteria and soil fungi are compatible with most plant species. That means we can customize our remediation approach specific to the site's geographic region, site conditions, chemical characteristics, and depth of pollutant(s). Plant selection can take into consideration any desire for native plants as well as future plant biomass use for timber or bio-fuel related applications. This combined system allows us to address multiple pollutants at many depths. We can address:

- Soil contamination at shallow depths and deeper due to the trees
- Groundwater contamination at 30 feet below ground using high-transpiration water loving trees. Trees like Poplar, Willow, Ash and Alder are quite useful in this regard. These trees generally grow in freshwater aquifers where the water table depth is not more than ten meters.
- Aquatic systems requiring water and/or sediment remediation

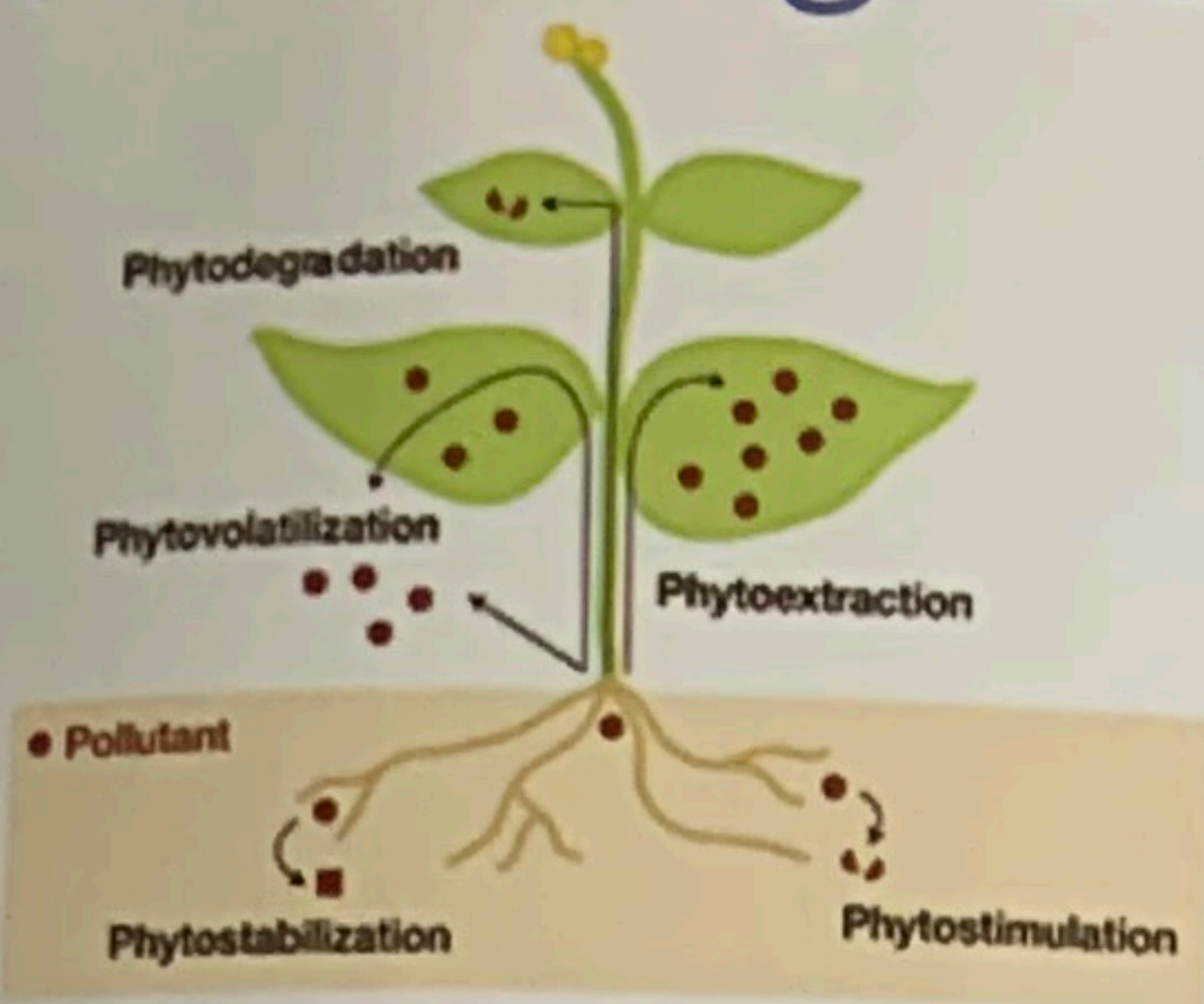
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CLEANING UP THE CLEANUP

MPC Brings Sustainability to Environmental Remediation



Environmental remediation involves removing contaminants from water and soil to protect human health and restore the environment; however, certain remediation activities can generate emissions or other waste products that impact the environment. MPC is moving to the forefront of the oil and gas industry with plans for a new company-wide approach to remediation projects that incorporates a sustainability evaluation.

Framing the Bigger Picture

The program's foundation is an assessment that identifies opportunities for reducing a project's anticipated amount of greenhouse gas, air emissions, energy use, waste, water use and raw materials. This can lead to switching remediation technology, exploring greener options in the supply chain or introducing new habitat features. The assessments aim to go beyond environmental concerns to address the broader potential of this work to positively affect surrounding communities.

"We are looking to factor in stakeholder inclusion and evaluating software tools that could gauge economic and social factors for more complex sites," said Kyle Waldron, HES professional in Environment, Safety, Security and Product Quality (ESS&PQ). "Considering economic and social factors may allow remediation projects to provide value to the local community through the use of low-impact solutions and by identifying reuse opportunities for the remediation resources."

Advantages Made in the Shade

In developing its sustainable remediation program, MPC is conducting pilot testing at two company remediation sites. It is also drawing upon successful initial results from a remediation effort at a former Andeavor fuel terminal in Alaska. This undertaking has shown sustainability can create substantial cost advantages, especially when it includes planting trees (phytoremediation) to mitigate the effects of petroleum contaminants. Remediation has added 550 balsam poplar trees to the three-acre site.

"Prior to planting the trees, they were inoculated with specific bacteria that have the ability to degrade the contaminants of concern at the site," Waldron said. "When the trees are fully mature after approximately four years, they will take up groundwater at a rate that significantly reduces groundwater flow across the site, and treat the water through their natural processes, eliminating the expense of operating a mechanical pump and treatment system."



The trees planted at the Alaska site support phytoremediation, which relies on the natural processes of plants and trees to mitigate the effects of contaminants.



This tactic is expected to help shorten the use of mechanical systems at the site by at least seven years,

adding to the benefits of using more energy-efficient equipment for other tasks. Together, these measures are calculated to reduce carbon dioxide (CO₂) emissions during the project's lifecycle by roughly 90 tons, which is equivalent to the energy use of 10.8 average homes in one year.

