REMEDIATION AND RESTORATION OF AN OIL CONTAMINATED WETLAND AND PINE FOREST SITE

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Abstract

Spills from oil production and exploration are common in Louisiana. Accidental spills, leaks, or discharges can expose sensitive environments, such as wetlands and forests, to petroleum and brine contamination. Development of remediation plans for these types of environments is thus necessary. The goal of this project is to introduce a successful remediation and restoration plan for a contaminated wetland and pine forest site impacted by an oil well blowout.

The research site, located in Kisatchie National Forest, was polluted by an oil and brine spill (13,000 barrels of oil and 600,000 barrels of brine). The release resulted in oil and brine contamination of a 1.7 hectare (4.3 acre) freshwater depressional wetland. Aerial spray of oil, brine, and gas from the blowout also killed or severely stressed numerous loblolly and longleaf pine trees in the vicinity of the well. Remediation and restoration of the wetland and pine forest required both a field study and a greenhouse study. The field study examined the effectiveness of ammoniated bagasse (ABG) at enhancing the bioremediation of the contaminated wetland. A comprehensive soil and vegetation greenhouse study is monitoring both the effects of oil and brine on loblolly pine tree seedlings and the effectiveness of ABG at removing and remediating oil contaminated soils.

Burning was the most effective, ecologically sound, and economical method for removing the oil from the wetland. Once the area was burned, a combination of ABG, lime, and topsoil was applied *in situ* to 20 research plots. The results of the study showed that the bagasse reduced the total petroleum hydrocarbons (TPH) over 300% in the top 10 cm of the soil.

Current greenhouse studies are investigating the effects of foliar and soil applications of oil and brine on one year old loblolly pine tree seedlings. Preliminary results show that foliar application of oil has no effect on the trees (i.e. death, signs of stress). However, when 100% of the surface area of five trees was covered with oil, the new shoot growth on these trees died; other areas of the trees showed no signs of stress. When oil was applied to the soil at a rate of 400 ml/tree, the trees died or showed signs of severe stress within one week. Brine applications are currently being analyzed.

The results of the wetland and greenhouse studies will help to identify beneficial and efficient methods for restoring oil contaminated wetlands and pine forests.

1.0 Introduction

1.1 Background

For most of this century, Louisiana has provided the natural resources to support an extensive and successful petroleum industry. Louisiana is rich in oil reserves from both offshore sites in the Gulf of Mexico and onshore oil wells in northern and central areas of the state. Increased petroleum exploration and production increases the potential hazards to ecologically sensitive areas, such as wetlands and forests. As the petrochemical industry has grown, so has the need for increased protection of natural resources.

Louisiana contains over 30% of the nation's wetlands. The state's only national forest, the Kisatchie National Forest, covers much of the northern and central region of the state. Oil production and exploration take place in both of these environments, and as a result, these regions are at risk from petroleum contamination due to accidental spills, leaks, or discharges.

Offshore oil spills are more common than onshore spills. Consequently, extensive research and development has taken place to prevent and clean up offshore spills (Quina 1973). Inland spills are less common, especially those that involve interactions with wetlands and forests. There is an inadequate amount of literature describing the impacts of and remediation practices for oil contamination in these inland areas. The few writings that do exist can only be found in literature (Davis 1998) that has not been formally published in recognized scientific or academic publications. Remediation practices have been developed for onshore spills, but they are not always applicable to all onshore sites. Remediation methods for inland contaminated sites may include incineration of the oil, landfarming the contaminated soil, burial, containment in surface impounds, or deep well injection (Breitenbeck and DeSilva 1995). These practices are not always viable for contaminated wetlands or forests. These environments are ecologically sensitive, and they offer limited access for equipment. As a result, it is difficult to determine the most beneficial cleanup methods for petroleum contaminated wetlands and forests.

Spills in these environments offer the chance to develop appropriate remediation practices. One such opportunity occurred when an oil well blowout occurred in Cravens, LA in August 1997. Approximately 24 to 30 hectares (60 to 75 acres) of the Kisatchie National Forest were impacted by this oil and brine spill, including a 1.7 hectare (4.3 acre) freshwater depressional wetland adjacent to the oil well. Due to this blowout, numerous loblolly and longleaf pine trees in the vicinity died or were severely stressed. It has been assumed that this was the result of oil and brine drift onto the trees and soil, but the mechanism within the plants that caused death is not well known. All of the wetland vegetation died after the spill. Since a spill of this nature and magnitude has never been recorded in the literature, it offers the opportunity to develop a remediation plan that will restore both the upland pine plantation and the wetlands.

1.2 Objectives

The general objective of this study is to develop and implement a remediation and restoration plan for the impacted upland forest and wetland. The study has the following specific objectives:

1. to determine the exact cause of death of the loblolly and longleaf pines impacted by the blowout using nutrient concentration analysis;
2. to develop an approved remediation and restoration plan (for the impacted wetland) in conjunction with the National Forest Service;
3. to develop recommendations for the restoration and management of the affected upland forest areas.

2.0 Previous Studies

2.1 Remediation and Restoration of Oil Contaminated Sites

Most inland oil spills use traditional cleanup and remediation methods: recovery of oil by skimming or absorbence; and disposal of oil by incineration, land farming, burial in land fills, containment in surface impoundments, deep well injection, and solidification (Bartha and Bossart 1984). However, employing such practices in Louisiana's wetlands is complicated because most wetlands provide limited access for cleanup equipment. In the case of oil spills in wetlands or forests, these techniques are not always applicable due to limited access and the ecological sensitivity of these areas. Therefore, alternative methods must be developed.

The natural pathway for petroleum decontamination is through microbial action. Many fungi and bacteria can either completely or partially metabolize the hydrocarbons from petroleum oils and fractions. Many reports describe the activity and distribution of oil degrading microorganisms in water (Atlas 1975) and in uncontaminated and contaminated soils (Davis 1967; Odu 1978a). However, natural biodegradation and volatilization of petroleum hydrocarbons may not occur soon enough to prevent extensive environmental damage to contaminated wetlands. Generally, the rehabilitation of a contaminated surface area relies on promoting the *in situ* biodegradation of contaminant oil (Atlas 1977; Atlas *et al*. 1978; Westlake *et al*. 1978; Atlas 1981). The ability of microorganisms to change the chemical composition of oil and promote its degradation is gaining recognition as an effective remediation strategy (Marconi *et al*. 1977; Bartha *et al*. 1976; Westlake *et al*. 1978; Odu 1978a). The principal reason that oily materials do not decompose in aerated environments is related to the hydrophobic nature of oil and grease. These materials flocculate together excluding water, soluble nutrients, and oxygen. Only a fraction of the flocculated material offers an environment suitable for colonization and degradation by microorganisms. Using sediment slurries with controlled redox potentials and pH, Delaune *et al*. (1980), Hambrick *et al*. (1980), and Ward *et al*. (1980) found that up to 15% of added hydrocarbons degraded at Eh 220 and pH 8.0. The rate of hydrocarbon degradation was significantly higher at more positive redox potentials. Studies using oil contaminated soils (Jobson *et al*. 1972) consistently showed that the highest rate of oil degradation occurred when soil aeration was improved. Similarly, Atlas *et al*. (1978) showed that hydrocarbon utilization was sharply reduced in soils when O_2 was depleted.

Besides O_2 and water, microorganisms require N, P, and other inorganic nutrients for growth when using petroleum as a substrate. Hydrocarbons typically contain a low amount of these essential elements. Laboratory experiments have shown that the addition of nutrient salts (KNO_3 and Na_2HPO_4) to broth media increases the biodegradation of the petroleum by 70% (Bartha and Atlas 1976). Similarly, Odu (1978) reported the result of a laboratory study showing that addition of nutrient solutions to soils amended with light crude oil enhanced O_2 uptake and CO_2 evolution. Treatment of an oil contaminated field site with urea and phosphate fertilizers led to a rapid increase in bacterial density followed by a rapid disappearance of n-alkanes, isoprenoids, and a continued loss in weight of saturated hydrocarbons (Westlake *et al*. 1978).

The stimulatory effect of mineral nutrients on biodegradation varies depending on the methods and quantities applied. According to Dibble and Bartha (1979), the highest biodegradation rate in culture appeared to be at a C:N ratio of 15:1 and a C:P ratio of 200:1. To achieve those ratios, application of 1400 kg N and 100 kg P per hectare would be required on a field contaminated with 100 metric tons of hydrocarbons. However, Hunt *et al*. (1973) found that although addition of low amounts of NH_4NO_3 stimulated biodegradation, an increase of N levels greater than 100 mg N/kg actually depressed soil respiration, possibly because of NH_3 or NO_3 toxicity. The American Petroleum Institute recommends a C:N ratio of 160:1, indicating that approximately 500 kg N or 5000 kg of 10:5:5 fertilizer is needed for remediation of a one hectare site contaminated with 100 metric tons of hydrocarbons. The potential danger of groundwater contamination may be an important consideration when using readily available inorganic forms of nutrients to enhance bioremediation of contaminated soils.

Tillage is generally not a practical technique for remediation of sites that are inaccessible or that will not support the operation of heavy equipment. Oily contaminants cannot be tilled to mix with soil or other adsorbents, and application of fertilizers would be of little benefit because the water soluble forms of nutrients are segregated from the oily wastes by hydrophobic exclusion.

In the case of an oil and brine spill in Sam Houston National Forest, San Jacinto County, TX, two atypical methods were selected to mitigate the spill. These were: (1) freshwater flushing in areas principally affected by brine, and (2) controlled burning in oil saturated areas (Zehner and Mullings 1987). Approximately 12.5 acres of the forest and an unnamed tributary near the oil well were affected by the spill. The site was divided into three zones: Zone 1 was predominately oil with limited brine, Zone 2 was predominately brine with limited oil, and Zone 3 was brine only. In Zone 2, freshwater was discharged in an attempt to dilute the standing or trapped brine, the relay prevented the brine from saturating the forest soil. By increasing the fluid flow into the drainage pattern, it was expected that the oil in the zone would also be mobilized. During this time, approximately 300,000 gallons of freshwater were flushed into the zone for 2.5 days. This reduced the chloride concentration to 1800 ppm, which is well below the 3000 ppm limit set by the Texas Railroad Commission. Based on these results, this practice was deemed successful.

In Zone 1, it was decided that a controlled burn would be the best oil removal option. The burn appeared to drastically reduce the amount of oil on the surface and subsurface. The recommended restoration of the site after burning the oil and removing the brine included four parts: (1) clear cut the areas to remove all salvageable timber and expose the soil surface, (2) add four to six tons of lime per acre in order to increase the pH and enhance the natural populations of saprophytic fungi and bacteria, (3) reseed the area after four months with a mixture of endemic and weed species of grasses, and (4) reestablish pine trees after six to 12 months (Zehner and Mullins 1987).

2.2 Ammoniation of Cellulolytic Materials

Use of cellulolytic materials as absorbents is a common cleanup technology in aquatic systems (Ericsson *et al*. 1985). Enriching readily available cellulolytic waste materials with N or other nutrients to enhance *in situ* or offsite degradation of contaminant petroleum has received surprisingly little attention. An extensive search of the scientific and U.S. Patent literature found only a few reports of the effects of modifying cellulolytic waste materials for use with oil spills. A patented process was found that treated cellulolytic materials and inorganic fertilizers with paraffin to maintain buoyancy and reduce solubility of nutrients for remediation of oil contaminated seawater (Marconi *et al*. 1977). Similarly, Ericsson *et al*. (1971) patented a method of oil absorption at sea by using cellulolytic materials rendered hydrophobic by treatment with heat decomposed ammonium or amide salts of aliphatic or cycloaliphatic carboxylic acids and oil. No conclusive laboratory or field data demonstrating the efficacy of these materials for enhancing microbial degradation was found in the scientific or engineering literature.

An alternative method for using ammoniated organic wastes was recently developed by Gary Breitenbeck of the LSU Agricultural Center. This process involves ammoniating organic wastes with anhydrous ammonia at high temperatures and pressures. Examples of organic wastes used include bagasse, filterpress cake, rice hulls, and kenaf. Cellulose fibers are a principle component of all these wastes. These fibers have highly porous structures capable of absorbing large amounts of water and oil (Breitenbeck and DeSilva 1995; Breitenbeck and Grace 1997).

When these wastes are ammoniated under high temperatures and pressures, their nitrogen (N) content is increased. This N is bound in slowly available organic forms that ensure a dependable N supply for oil degrading microorganisms (Breitenbeck and DeSilva 1995). "Accelerated degradation results because crude oil or sludge is absorbed into an environment where surface area is greatly increased and microorganisms are provided with water, oxygen and nutrients needed for degradation." (Breitenbeck and DeSilva 1995)

ABG was found to be the most effective of the organic wastes. Its effectiveness was increased when it was incorporated into the soil instead of applied to the surface. ABG was found to consistently reduce hydrocarbon concentrations in coastal sands and saline marsh soils when added at the rate of 2 g ABG per 25 g soil. In these soils, the principle fate of spilled oil was humification (Breitenbeck and DeSilva 1995).

This process was considered to be a viable option for remediating the wetland impacted at the Cravens blowout site. We also considered incorporating gypsum with the ABG in order to reduce the increased salt concentrations caused by the blowout's release of brine. Ammoniated organic wastes offer an inexpensive and effective means of remediating oil contaminated shorelines and wetlands. The ammoniated material is lightweight, can be stored for long periods, has an attractive appearance and pleasant odor, and can be applied safely with negligible risk to personnel. A complement to adding ammoniated organic wastes would be to incinerate the oil contained within the wetland, thus reducing the amount that would require bioremediation. Restoration efforts could then focus on removal of brine from the area.

2.3 Effects of Oil on Plants

The effects of oil on plants has been studied since the early 1900s. But the majority of this work focused on the effects of oil on agricultural crops and coastal salt marshes. As a result, there is little published literature on the effects of oil on forest communities and, in particular, pine trees. Some general observations of the effects of oil on agricultural and salt marsh plants are, however, applicable to pine trees. "Oil pollution effects may vary according to the type and amount of oil involved, the degree of weathering, the time of year, and the species and age of the plants concerned (Baker 1970)." Some of the observed effects include yellowing and death of leaves, reduction of seedlings, and death of the plant. Needle chlorosis and wilting were readily observed among the pine trees impacted by the Cravens blowout.

deOng (1927) was able to distinguish between rapid or acute injury caused by light oils, and slow or chronic injury caused by heavier oils. In general, the smaller the hydrocarbon molecule, the more toxic the oil is to plants. The smaller the oil molecule, the more easily it can enter into the plant; thus making the oil more toxic (van Overbeek and Blondeau 1954). The oil molecule enters the plant more easily through stomata or at the point of contact (Baker 1970). Cuille and Blanchet (1958) identified three factors related to phytotoxicity of oils: (1) the properties of the oil, (2) the quantity of oil applied, and (3) the environmental conditions.

Once a spill has occurred, the oil must penetrate and move within the plant before injury occurs. Once inside the plant, the oil travels through intercellular spaces into the plant cells (Knight 1929). Within the cell, the oil damages the plasma membrane, and cell sap then leaks into intercellular spaces (Baker 1970). The leakage of cell sap causes the leaf to darken and lose turgor (Currier 1951). This process may have contributed to the wilting of the pine needles on trees affected by the Cravens blowout.

Oils have also been found to affect transpiration, respiration, and photosynthetic rates of plants. Transpiration rates were consistently reduced due to physical interference on or in the leaf tissue (Knight 1929; Bartholomew 1936). The effects of oil on respiration rates vary with each plant species. Respiration rates are reduced when oil interferes with gaseous exchange by blocking stomata and intercellular spaces (Baker 1970). Oil has been found to consistently reduce the rate of photosynthesis, primarily by physical interference with gaseous exchange, thus creating an effect similar to respiration reduction. In the case of the loblolly and longleaf pines affected by the Cravens blowout, the deaths and injuries may have been due to one or a combination of these processes.

Nutrient deficiencies or excesses may also have contributed to the trees' injuries. In a similar oil well blowout site in Union Hill, LA, pine needles of impacted trees were analyzed for nutrient concentrations of ammonium (NH_4^+), nitrate (NO_3^-), boron (B), barium (Ba), nickel (Ni), strontium (Sr), sodium (Na), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), sulfur (S), and silicon (Si) (Hudnall, 1997). These trees showed effects similar to those suffered by the pine trees at the Cravens blowout, site. In the trees near the Cravens blowout, the chlorotic appearance of the needles suggested a reduced uptake of N. After analysis, it was concluded that the trees' capacity to utilize N was reduced, which caused the chlorotic appearance. Increased conditions of Boron, Ba, Ni, and Sr were found in the pine needles as a result of the elements' reduced solubility.

The increased levels of Na were obviously the result of brine. The pine needles accumulated Na, which may have contributed to the loss of turgor and caused the needles to assume a distorted shape. Increased concentrations of K may have resulted from physiological accumulations or from surface residuals from the brine. Potassium regulates the opening and closing of stomata in pine needles. The lack of turgor in the pine needles may have been due to the stomata being forced open, which then allowed excessive transpiration of water from the plant. This would also have contributed to the wilting of the needles. Magnesium and P are important to photosynthesis, and their elevated concentrations may have inhibited this metabolic function. Increased S concentrations may have been due to the result of S deposition. The pine trees at the Union Hill site were found to have more than a three-fold decrease of Si. Pine trees use Si to keep needles rigid and brittle. The solubility of Si increases in the presence of Na, and the increased levels of Na may have caused loss of Si from the needles. This process would cause the needles to take on a wilted appearance.

3.0 Materials and Methods

3.1 Description of Study Area

The study area is located in Cravens, LA and is located within the Kisatchie National Forest (Figure 3.1). The impacted freshwater depressional wetland lies approximately 0.2 kilometers (1/8 mile) south of the oil well. After the oil well blowout, an earthen berm was constructed in order to protect the wetland from any overland oil runoff. After a heavy rain washed the berm away, the oil flowed unimpeded into the wetland (Figure 3.2). Overland flow of oil also occurred west of the well into Little Sixmile Creek, which is located less than 1/8 mile from the well (Figure 3.3).

The soils of the upland area have been mapped as a Ruston fine sandy loam. The soil of the wetland is a Guyton silt loam, a typical wetland soil. Loblolly pine (*Pinus taeda*) and longleaf pine (*Pinus palustris*) dominate the vegetation of the upland area. Dominant vegetation within the wetland includes sweetgum (*Liquidambar styracifula*), black gum (*Nyssa sylvatica*), and tupelo gum (*Nyssa aquatica*).

3.2 Wetland Remediation

The oil well blowout occurred on August 1997. Soil samples from the wetland were taken at depths of 0 to 10, 10 to 20, and 20 to 30 centimeters (cm) in July 1998. The electrical conductivity (EC), pH, and sodium (Na) concentrations of the soil samples were determined. The samples were analyzed for EC in order to determine the extent of brine contamination within the wetland. The 0 to 10 cm soil layer was the most highly contaminated with oil and contained the highest electrical conductivity and soluble Na. After much discussion, the United States Forest Service decided to burn the wetland to remove the oil (Figure 3.4).

Figure 3.1 *Location of research site.*

Figure 3.2 *Oil contaminated wetland.*

Figure 3.3 *Oil contamination of Little Sixmile Creek before the burn.*

Figure 3.4 *Wetland being burned.*

The wetland was burned in December 1998. The burn removed nearly all of the surface oil. Soil samples were taken three days after the burn and analyzed for pH, EC, and Na concentration (Figures 3.5 and 3.6). As a result of natural water flow through the wetland, most of the brine was flushed out of the area, therefore significantly reducing the EC measurements from the first sampling period. It was concluded that the brine was no longer a serious problem within the wetland, and efforts were then focused on the removal of residual oil left after the burn (Figure 3.7).

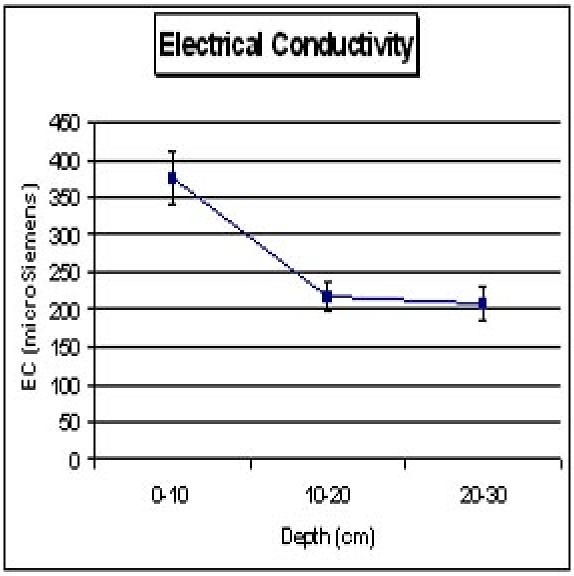

Figure 3.5 *Post-burn electrical conductivity.*

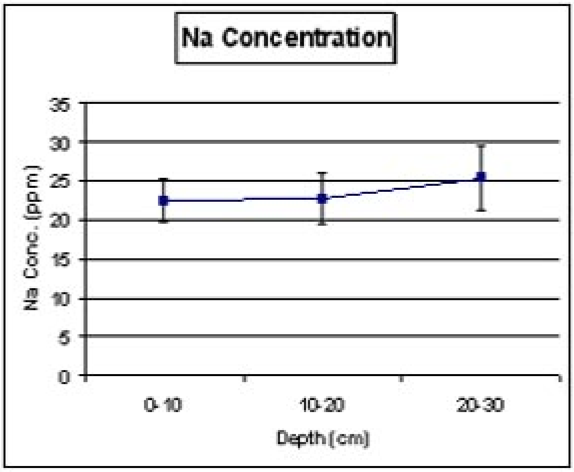

Figure 3.6 *Post-burn sodium concentration.*

ABG was used in combination with lime ($CaCO_3$) and topsoil to test how well bagasse enhanced the bioremediation of the wetland. Test plots were constructed using open-ended cylinders (30 cm diameter / 105 cm height) driven 15 to 20 cm into the soil (Figure 3.8). The bagasse, lime, and topsoil were then added to each cylinder in May 1999. The rates of bagasse added were 0, 50, 100, and 200, kilograms per hectare (kg/ha), with five replications of each treatment randomly placed within the wetland. These rates were based on the post-burn total petroleum hydrocarbon (TPH) analyses performed by a private environmental consulting firm contracted by the owners of the oil well. The $CaCO_3$ was added at a rate of 1500 kg/ha in order to raise the pH of the soil to approximately 6.5. Fifteen grams of topsoil were mixed with the bagasse in order to ensure an adequate microbial population.

Soil samples were collected at depths of 0 to 5, 5 to 10, and 10 to 20 cm at 21 day intervals and analyzed for TPH, EC, and pH. After a 90 day study period, the test cylinders were excavated and soil samples were collected at depths of 0 to 10 and 10 to 20 cm. Residual oil for all soil samples collected was determined in aliquots of mixed soil extractions using carbon disulfide (CS_2) as the extractant according to the methods of Breitenbeck and Grace (1997). Total petroleum hydrocarbons for all samples were determined using a Buck Scientific Total Hydrocarbon Analyzer. The Analyzer used a known concentration of weathered crude oil obtained from the research site. Samples taken after the 90 day study period area are also being analyzed for the following water-soluble cations (Na, Ca, Mg, K, Fe, Al, Mn, Zn, Pb, Cu, Ni, Cd, and Se). Based on these results, a remediation plan can be developed for the contaminated wetland.

Figure 3.7 *Wetland post-burn.*

Figure 3.8 *Test cylinder.*

3.2.1 Results

The 90 day wetland study ended in September 1999. Soil samples taken in June, July, August, and September were analyzed for TPH reduction. Results of the analyses show that the ABG decreased the overall hydrocarbon concentration of the contaminated wetland. The mean hydrocarbon concentration for the wetland is 385 parts per million (ppm), with a maximum concentration of 3203 ppm and a minimum concentration of 5 ppm. The standard deviation of 549 ppm illustrates the high variability of oil concentration within the wetland. This high variability was also seen in previous studies using ammoniated organic wastes (Breitenbeck and DeSilva 1995; Breitenbeck and Grace 1997).

The results of the soil analyses are presented in Figures 3.9 and 3.10, with replications of each treatment averaged. The results of the 0 to 5 cm and 5 to 10 cm layer analyses from the first three sampling periods were averaged to form a composite 0 to 10 cm layer. This was done in order to compare results with the last sampling period. As expected, the highest concentration of oil was found in the 0 to 10 cm soil layer, with a mean concentration of 596 ppm. The lowest concentration was found in the 10 to 20 cm layer, with a mean concentration of 175 ppm. Figures 3.9 and 3.10 illustrate the effectiveness of bagasse in decreasing hydrocarbon concentrations within the 0 to 10 and 10 to 20 cm soil layers.

In the 0 to 10 cm layer (Figure 3.9), the wicking action of the ABG is illustrated in the high treatment. In June, the high treatment had the highest concentration of oil after 21 days when compared to the other treatments. From June to July, the high treatment continued to wick oil to the surface, which is represented by the increase in TPH concentration (904 ppm to 942 ppm). From July to August there was a marked decrease (942 ppm to 199 ppm) in TPH in the high treatment a result of accelerated microbial degradation. The increase in TPH from August to September in the high treatment (199 ppm to 1336 ppm) occurred when the microbes exhausted their energy source (the ABG). This effect was also seen in the medium treatment (246 to 1530 ppm). As depicted by the graph, the low treatment had little or no effect on the degradation of oil in the wetland. The TPH values for the low treatment ranged from a high concentration of 515 ppm in June to a low concentration of 290 ppm in July. For the control, the low concentration was 215 ppm in June, and the high concentration was 833 ppm in September.

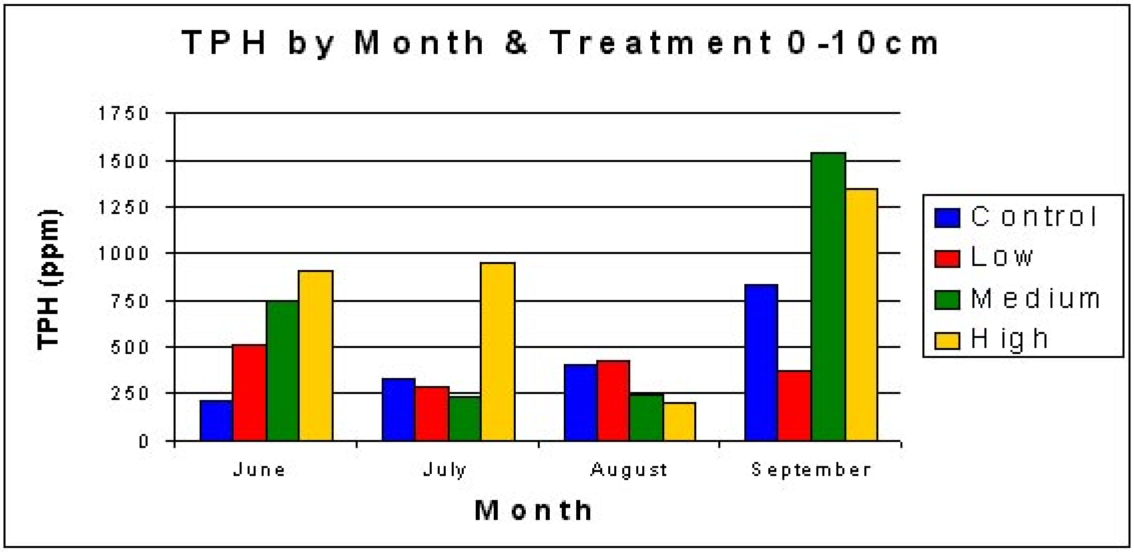

Figure 3.9 *TPH results for the 0 to 10 cm soil layer.*

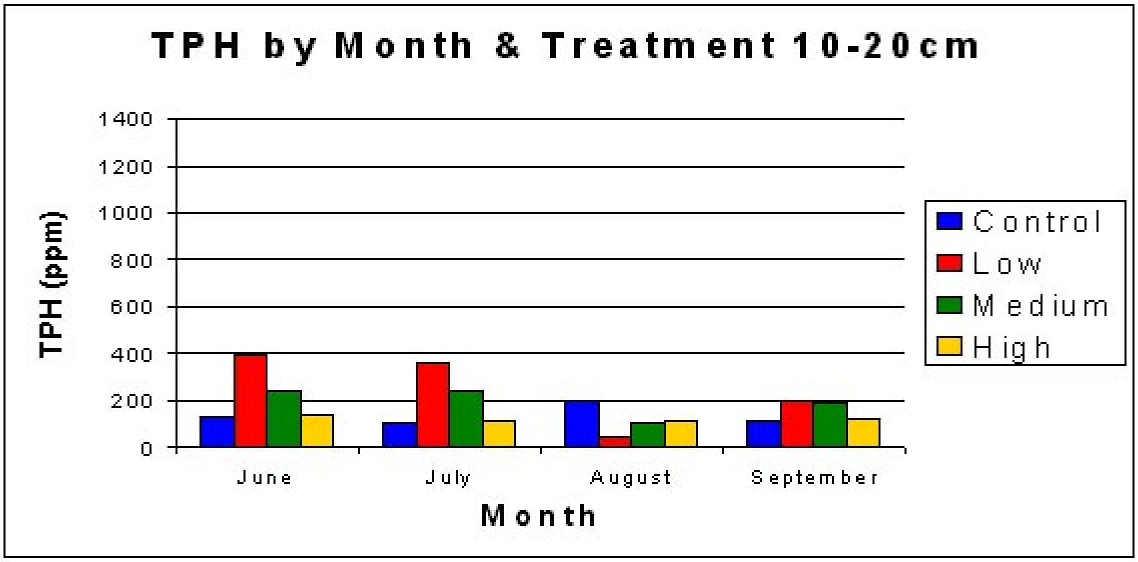

Figure 3.10 *TPH results for the 10 to 20 cm layer.*

For the 10 to 20 cm layer (Figure 3.10), all of the treatments remained below a TPH concentration of 400 ppm. The lowest concentration was 40 ppm, and the highest concentration was 394 ppm. The June and July data support the conclusion that the oil was being removed by the ABG, and that the efficiency or amount wicked by the ABG was proportional to the amount of ABG added. The results for August and September suggest that equilibrium had been reached with the amount of oil in the subsoil. This equilibrium depended on two factors: (1) the amount of ABG added, and (2) the topographic position of the treatment.

3.3 Greenhouse Studies

3.3.1 Foliar Oil Pre-Study

Because the effect of oil on pine trees has never been investigated, there is no data describing how much oil is lethal to pine trees. As a result, preliminary data had to be obtained. This involved determining the LD_{50} of oil for one year old loblolly pine trees. Ten fascicles from each of ten randomly selected trees were removed, and the surface area of the needles was determined according to Johnson (1984). The total number of fascicles for each of the trees was then counted. Using a regression equation, the total needle surface area of each tree was calculated. The fascicles from each tree were then immersed in oil to determine the amount of oil necessary to cover 100% of the needle surface area. Using this data in a regression equation, the amount of oil necessary to cover 100% of the total needle surface area for each tree was determined. Based on this data, we were able to determine how much oil to apply to cover various percentages of needle surface areas with oil.

Oil was foliar applied, using a spray bottle, in order to achieve needle surface area coverages of 20 to 100%, with five replications of each treatment. To our surprise, none of the trees sprayed with oil showed any signs of stress after six weeks (Figure 3.11). The only effect observed was on the trees whose needle surface area was 100% covered. On these trees, the new shoot growth died, but no other parts of the trees were stressed (Figure 3.12). Therefore, we could not determine an LD_{50} for oil on one year old loblolly pine trees.

Figure 3.11 *Foliar oiled trees after six weeks.*

Figure 3.12 *Oil added at 100% the needle surface area.*

3.3.2 Foliar Oil Study

As result of the pre-study, we decided to replicate the experiment using needle surface area coverages of 0, 25, 50, 75, and 100%, with 15 replications of each treatment. The oil was applied May 15, 2000. The height and diameter of each tree was measured before treatment. The trees were sprayed with oil a second time on May 22, 2000. The trees will be harvested 60 days after the initial treatment, and nutrient concentrations will be analyzed for the following elements: N, P, K, Ca, Na, Mg, Fe, Zn, Si, Al, Mn, Cu, Pb, Cd, Ni, and Mo.

3.3.3 Oil Soil Application Study

A second oil experiment is underway. This experiment is evaluating the effect and interaction of oil within the soil and ABG. A 4x4 factorial design with five replications is being employed. The rates of oil added to the soil were 0, 100, 200, and 400 ml/tree. The oil was applied May 8, 2000. Within two weeks, the trees treated with 400 ml of oil died or showed severe signs of stress (Figure 3.13). The ABG was added to the soil 14 days after the oil treatment on May 22, 2000 at rates of 0, 100, 200, and 400 g/tree. The bagasse was mixed into the upper 0 to 5 cm of the soil. The trees will be harvested 60 days after the oil treatment, and nutrient concentrations will be analyzed for the following elements: N, P, K, Ca, Na, Mg, Fe, Zn, Si, Al, Mn, Cu, Pb, Cd, Ni, and Mo. TPH for each treatment will be determined by the same method used to analyze the wetland soils.

Figure 3.13 *Trees with oil applied to soil at a rate of 400 ml/tree after two weeks.*

3.3.4 Foliar Brine Study

A foliar brine study similar to the foliar oil study was initiated May 15, 2000. Brine from the oil well was obtained, and the electrical conductivity was found to be 60 ms. Brine was foliar applied using needle surface area coverages of 0, 25, 50, 75, and 100%, with 15 replications of each treatment. The height and diameter of each tree were measured before treatment. The trees were sprayed again on May 22, 2000. The trees will be harvested 60 days after the initial treatment, and nutrient concentrations will be analyzed for the following elements: N, P, K, Ca, Na, Mg, Fe, Zn, Si, Al, Mn, Cu, Pb, Cd, Ni, and Mo.

3.3.5 Brine Application to the Soil

A study investigating the effects of soil applied brine on pine trees will be initiated in June 2000. Six treatments of brine will be applied to the soil, with five replications of each treatment. The six concentrations of brine will be 0, 2, 4, 8, 16, and 32 ms as determined from a saturated paste. The trees will be harvested 60 days after the initial treatment, and nutrient concentrations will be analyzed for the following elements: N, P, K, Ca, Na, Mg, Fe, Zn, Si, Al, Mn, Cu, Pb, Cd, Ni, and Mo.

All data from all experiments will be statistically analyzed using SAS.

4.0 Conclusions

The use of ammoniated organic wastes, specifically bagasse, has been found to enhance the remediation of ecologically sensitive environments. Results from this study demonstrate that bagasse promoted a decrease in the overall total petroleum hydrocarbon concentration of the contaminated wetland. A remediation plan using 500 to 1000 kg/ha of ABG in combination with lime, topsoil, and a seed bank has been proposed to the U.S. Forest Service to remediate the contaminated wetland at the Cravens site. This treatment method could be adopted as the standard Best Management Practice (BMP) for oil spills occurring in sensitive wetland environments.

Preliminary results of the greenhouse studies show that foliar oil contamination was probably not the cause of death of the upland trees surrounding the oil well. Oil applied to the soil at high rates caused severe stress within one week and death within two weeks. It is the hope of the authors that as a result of the greenhouse studies, the process(es) by which the pine trees died can be determined.

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