Phytoremediation of Soils with Mixed Contamination

BY

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THESIS

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This thesis is dedicated to my husband, Abhilash Premnath, without whom it would never have been accomplished.
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RESHMA CHIRAKKARA
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>xiii</td>
</tr>
</tbody>
</table>

## CHAPTER

1. INTRODUCTION
   1.1 Problem Statement | 1 |
   1.2 Research Objectives | 7 |
   1.3 Thesis Organization | 8 |

2. ASSESSING THE APPLICABILITY OF PHYTOREMEDIATION OF SOILS WITH MIXED ORGANIC AND HEAVY METAL CONTAMINANTS: LITERATURE REVIEW | 12 |
   2.1 Introduction | 12 |
   2.2 Complexities with Mixed Contamination | 15 |
   2.3 Phytoremediation and Phytotechnologies for Contaminated Soils | 17 |
   2.3.1 Phytoremediation of Heavy metals | 21 |
   2.3.2 Phytoremediation of Organic Contaminants | 23 |
   2.4 Phytoremediation of Mixed Contaminants | 25 |
   2.5 Methods for Enhancing Phytoremediation | 40 |
   2.5.1 Chelate Assisted Phytoremediation | 43 |
   2.5.2 Surfactant Enhanced Phytoremediation | 46 |
   2.5.3 Bacterial Endophyte Enhanced Phytoremediation | 49 |
   2.5.4 Enhancement of Phytoremediation by the Biomass Enhancement | 52 |
   2.5.5 Combination of Phytoremediation with Other Technologies | 53 |
   2.5.6 Electrokinetic Enhanced Phytoremediation | 58 |
   2.6 Conclusions | |

3. PLANT SPECIES IDENTIFICATION FOR PHYTOREMEDIATION OF MIXED CONTAMINATED SOILS | 83 |
   3.1 Introduction | 83 |
   3.2 Background | 85 |
   3.3 Materials and Methods | 88 |
   3.3.1 Selected Plant Species | 88 |
   3.3.2 Soil Selected | 89 |
   3.3.3 Soil Spiking Procedure | 89 |
   3.3.4 Pots Setup and Monitoring | 91 |
   3.3.5 Analytical Testing | 93 |
   3.4 Results and Discussion | 94 |
   3.5 Conclusions | 113 |
# TABLE OF CONTENTS (continued)

## CHAPTER

4. INTERACTIVE EFFECTS OF CO-CONTAMINANTS ON PHYTOREMEDIATION OF MIXED CONTAMINATED SOILS  
4.1 Introduction.................................................................................................................. 123  
4.2 Background.................................................................................................................... 124  
4.3 Experimental Methods................................................................................................. 130  
  4.3.1 Soil Used.................................................................................................................. 130  
  4.3.2 Soil Spiking Procedure............................................................................................ 130  
  4.3.3 Selected Plant Species............................................................................................. 134  
  4.3.4 Pot Setup and Monitoring....................................................................................... 134  
  4.3.5 Analytical Testing................................................................................................... 135  
4.4 Results and Discussion................................................................................................. 136  
  4.4.1 Interactive Effects of Organic Contaminants and Heavy metals......................... 136  
  4.4.2 Interactive Effects of Pb, Cd, and Cr...................................................................... 157  
4.5 Conclusions................................................................................................................ 159

5. EFFECTS OF VARYING INITIAL CONCENTRATIONS ON PHYTOREMEDIATION OF MIXED CONTAMINATED SOILS  
5.1 Introduction.................................................................................................................. 169  
5.2 Background.................................................................................................................... 170  
5.3 Experimental Methods................................................................................................. 173  
  5.3.1 Selected Plant Species............................................................................................. 173  
  5.3.2 Soil Selected............................................................................................................ 174  
  5.3.3 Soil Spiking Procedure............................................................................................ 174  
  5.3.4 Pots Setup and Monitoring....................................................................................... 177  
  5.3.5 Analytical Testing................................................................................................... 178  
5.4 Results and Discussion................................................................................................. 179  
5.5 Conclusions................................................................................................................ 194

6. BIOMASS AND CHEMICAL AMENDMENTS FOR ENHANCED PHYTOREMEDIATION OF MIXED CONTAMINATED SOILS  
6.1 Introduction.................................................................................................................. 200  
6.2 Background.................................................................................................................... 201  
6.3 Experimental Methods................................................................................................. 207  
  6.3.1 Soil Used ................................................................................................................ 207  
  6.3.2 Soil Spiking Procedure............................................................................................ 207  
  6.3.3 Amendment Application.......................................................................................... 209  
  6.3.4 Selected Plant Species............................................................................................. 211  
  6.3.5 Pot Setup and Monitoring....................................................................................... 211  
  6.3.6 Analytical Testing................................................................................................... 212  
6.4 Results and Discussion................................................................................................. 213
TABLE OF CONTENTS (continued)

CHAPTER

6.4.1 Biomass Amendments ................................................................. 213
6.4.2 Chemical Amendments ............................................................... 229
6.4.3 Combination of Biomass and Chemical Amendments ...................... 232
6.5 Conclusions .................................................................................. 233

7. ELECTROKINETIC AMENDMENT IN PHYTOREMEDIATION OF MIXED CONTAMINATED SOILS ................................................................. 244
7.1 Introduction .................................................................................... 244
7.2 Background .................................................................................... 245
7.3 Materials and Methods ................................................................. 250
  7.3.1 Plant Species ............................................................................. 250
  7.3.2 Soil .......................................................................................... 251
  7.3.3 Soil Spiking Procedure ............................................................. 251
  7.3.4 Cell Setup and Monitoring ...................................................... 254
  7.3.5 Analytical Procedures .............................................................. 256
7.4 Results and Discussion .................................................................. 257
7.5 Conclusions .................................................................................. 281

8. ENHANCED PHYTOREMEDIATION OF FIELD SOIL WITH MIXED CONTAMINATION ................................................................. 288
8.1 Introduction .................................................................................... 288
8.2 Background .................................................................................... 292
8.3 Materials and Methods ................................................................. 295
  8.3.1 Soil Used .................................................................................. 295
  8.3.2 Selected Plant Species ............................................................. 295
  8.3.3 Pot Setup ................................................................................ 298
  8.3.4 Monitoring ............................................................................. 300
  8.3.5 Analytical Testing ................................................................. 300
8.4 Results and Discussion .................................................................. 301
8.5 Conclusions .................................................................................. 317

9. OVERALL CONCLUSIONS AND RECOMMENDATIONS .................... 326
9.1 Overall Conclusions ...................................................................... 326
9.2 Recommendations for Future Research ......................................... 330

APPENDIX A
VITA ................................................................................................. 332
<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>24</td>
</tr>
<tr>
<td>2.2</td>
<td>26</td>
</tr>
<tr>
<td>2.3</td>
<td>29</td>
</tr>
<tr>
<td>2.4</td>
<td>41</td>
</tr>
<tr>
<td>3.1</td>
<td>86</td>
</tr>
<tr>
<td>3.2</td>
<td>90</td>
</tr>
<tr>
<td>3.3</td>
<td>92</td>
</tr>
<tr>
<td>3.4</td>
<td>99</td>
</tr>
<tr>
<td>3.5</td>
<td>104</td>
</tr>
<tr>
<td>4.1</td>
<td>131</td>
</tr>
<tr>
<td>4.2</td>
<td>133</td>
</tr>
<tr>
<td>4.3</td>
<td>142</td>
</tr>
<tr>
<td>4.4</td>
<td>145</td>
</tr>
<tr>
<td>5.1</td>
<td>175</td>
</tr>
<tr>
<td>5.2</td>
<td>176</td>
</tr>
<tr>
<td>5.3</td>
<td>184</td>
</tr>
<tr>
<td>5.4</td>
<td>186</td>
</tr>
<tr>
<td>5.5</td>
<td>190</td>
</tr>
<tr>
<td>TABLE</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>6.1</td>
<td>Important properties of soil used for the experiments</td>
</tr>
<tr>
<td>6.2</td>
<td>Properties of soil at the time of seeding</td>
</tr>
<tr>
<td>6.3</td>
<td>Average biomass of plant grown in different soil treatments</td>
</tr>
<tr>
<td>6.4</td>
<td>Average pH, oxidation reduction potential, and electrical conductivity</td>
</tr>
<tr>
<td>7.1</td>
<td>Important properties of soil used for the experiments</td>
</tr>
<tr>
<td>7.2</td>
<td>Measured properties of soil at the time of seeding</td>
</tr>
<tr>
<td>7.3</td>
<td>Average pH, oxidation reduction potential and electrical conductivity values for soil samples</td>
</tr>
<tr>
<td>8.1</td>
<td>Phytoremediation studies on mixed contaminated soils</td>
</tr>
<tr>
<td>8.2</td>
<td>Important properties of soil used for the experiments</td>
</tr>
<tr>
<td>8.3</td>
<td>Measured properties of soil at the time of seeding</td>
</tr>
<tr>
<td>8.4</td>
<td>Average pH, oxidation reduction potential and electrical conductivity values for clean and contaminated soil samples at harvest time</td>
</tr>
<tr>
<td>FIGURE</td>
<td>DESCRIPTION</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1.1</td>
<td>Mechanisms in phytoremediation</td>
</tr>
<tr>
<td>3.1</td>
<td>Percentage germination of different plants in clean soil vs. contaminated soil</td>
</tr>
<tr>
<td>3.2</td>
<td>Percentage survival of different plants in clean soil vs. contaminated soil</td>
</tr>
<tr>
<td>3.3</td>
<td>Final maximum plant height for clean soil vs. contaminated soil</td>
</tr>
<tr>
<td>3.4</td>
<td>Percentage reduction of total biomass in contaminated soil</td>
</tr>
<tr>
<td>3.5</td>
<td>Exchangeable nutrients in control and contaminated soil</td>
</tr>
<tr>
<td>3.6</td>
<td>Total concentration of metals in soil</td>
</tr>
<tr>
<td>3.7</td>
<td>Percentage reduction of Pb, Cd &amp; Cr by plants</td>
</tr>
<tr>
<td>3.8</td>
<td>Reduction of heavy metal concentration per plant</td>
</tr>
<tr>
<td>3.9</td>
<td>Exchangeable metals in soil</td>
</tr>
<tr>
<td>3.10</td>
<td>PAH Concentrations in Soil</td>
</tr>
<tr>
<td>4.1</td>
<td>Percentage germination of plants in different treatments</td>
</tr>
<tr>
<td>4.2</td>
<td>Percentage survival of plants in different treatments</td>
</tr>
<tr>
<td>4.3</td>
<td>Increase in plant height with time for different treatments</td>
</tr>
<tr>
<td>4.4</td>
<td>Final maximum plant heights in different treatments</td>
</tr>
<tr>
<td>4.5</td>
<td>Percentage reduction of total biomass in contaminated soils</td>
</tr>
<tr>
<td>4.6</td>
<td>Heavy metal concentrations in different soil samples</td>
</tr>
<tr>
<td>4.7</td>
<td>Nutrient concentrations in different soil samples</td>
</tr>
<tr>
<td>4.8</td>
<td>Exchangeable metals in different soil samples</td>
</tr>
<tr>
<td>4.9</td>
<td>Phenanthrene concentration in different soil samples</td>
</tr>
<tr>
<td>FIGURE</td>
<td>PAGE</td>
</tr>
<tr>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td>5.1</td>
<td>180</td>
</tr>
<tr>
<td>5.2</td>
<td>181</td>
</tr>
<tr>
<td>5.3</td>
<td>183</td>
</tr>
<tr>
<td>5.4</td>
<td>185</td>
</tr>
<tr>
<td>5.5</td>
<td>187</td>
</tr>
<tr>
<td>5.6</td>
<td>189</td>
</tr>
<tr>
<td>5.7</td>
<td>192</td>
</tr>
<tr>
<td>5.8</td>
<td>193</td>
</tr>
<tr>
<td>6.1</td>
<td>214</td>
</tr>
<tr>
<td>6.2</td>
<td>215</td>
</tr>
<tr>
<td>6.3</td>
<td>218</td>
</tr>
<tr>
<td>6.4</td>
<td>219</td>
</tr>
<tr>
<td>6.5</td>
<td>220</td>
</tr>
<tr>
<td>6.6</td>
<td>224</td>
</tr>
<tr>
<td>6.7</td>
<td>225</td>
</tr>
<tr>
<td>6.8</td>
<td>228</td>
</tr>
<tr>
<td>7.1</td>
<td>255</td>
</tr>
<tr>
<td>7.2</td>
<td>258</td>
</tr>
<tr>
<td>7.3</td>
<td>259</td>
</tr>
<tr>
<td>7.4</td>
<td>261</td>
</tr>
<tr>
<td>FIGURE</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>7.5</td>
<td>Final maximum plant height of oat plant and sunflower in the four planted cells at the end of the tests</td>
</tr>
<tr>
<td>7.6</td>
<td>Biomass production of oat plant and sunflower in the four planted cells at the end of the tests</td>
</tr>
<tr>
<td>7.7</td>
<td>Exchangeable fraction of nutrients (N, P and K) in soil in all tests</td>
</tr>
<tr>
<td>7.8</td>
<td>pH variation in side chambers in all tests</td>
</tr>
<tr>
<td>7.9</td>
<td>ORP variation in side chambers in all tests</td>
</tr>
<tr>
<td>7.10</td>
<td>Electrical conductivity variation in side chambers in all tests</td>
</tr>
<tr>
<td>7.11</td>
<td>Voltage variation in side chambers for the phytoremediation tests enhanced with AC electric current</td>
</tr>
<tr>
<td>7.12</td>
<td>Electric current variation in side chambers for the phytoremediation tests enhanced with AC voltage application</td>
</tr>
<tr>
<td>7.13</td>
<td>Heavy metal concentration in soil at the end of the tests</td>
</tr>
<tr>
<td>7.14</td>
<td>Exchangeable fraction of heavy metals in soil at the end of the tests</td>
</tr>
<tr>
<td>7.15</td>
<td>Phenanthrene concentrations in soil at the end of the tests</td>
</tr>
<tr>
<td>8.1</td>
<td>Map showing site boundaries</td>
</tr>
<tr>
<td>8.2</td>
<td>Percentage germination and survival of plants in unamended and composted soil</td>
</tr>
<tr>
<td>8.3</td>
<td>Maximum plant height in unamended and composted soil</td>
</tr>
<tr>
<td>8.4</td>
<td>Biomass of plants in unamended and composted soil</td>
</tr>
<tr>
<td>8.5</td>
<td>Exchangeable nutrients in unamended and composted soil</td>
</tr>
<tr>
<td>8.6</td>
<td>Heavy metal concentrations in unamended and composted soil</td>
</tr>
<tr>
<td>8.7</td>
<td>Percentage reduction of Cr in unamended and composted soil</td>
</tr>
<tr>
<td>FIGURE</td>
<td>PAGE</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>8.8</td>
<td>313</td>
</tr>
<tr>
<td>8.9</td>
<td>314</td>
</tr>
</tbody>
</table>

Exchangeable Cr concentrations in unamended and composted soil

PAH Concentrations in unamended and composted soil
SUMMARY

The rise of industrialization has resulted in polluted sites worldwide, and in many of these sites the soil is contaminated by a mixture of organic and heavy metal contaminants, some with potentially far reaching, negative health effects. Very few technologies have proven to be efficient solutions to the problem of mixed contamination. Phytoremediation can be adopted as a green and sustainable approach to decontaminate and restore contaminated sites. It can improve the biological activity and physical structure of the soil. However, its effectiveness to address the problems of mixed contaminants is not well understood since the interaction of co-contaminants can result in different phytoremediation responses based on concentration of the contaminants and the capabilities of the plant species used in phytoremediation. This study presents a series of laboratory experiments that probe the applicability of phytoremediation to soil co-contaminated with naphthalene, phenanthrene, lead, cadmium, and chromium; five contaminants that are commonly found at industrial sites. The specific research objectives are to investigate which plant species can best survive and remediate these typical mixed industrial contaminants in soils; examine the synergistic effects of mixed contaminants; consider the effect of the initial contaminant levels on the phytoremediation of mixed contaminants in soil; weigh strategies to enhance that phytoremediation process, including the use of biomass, chemical and electrokinetic amendments; and, finally, to study the effects of enhanced phytoremediation on historically contaminated soil that is polluted with mixed contamination.

To begin, twelve species of plants were cultivated in pots filled with contaminated soil or in uncontaminated soil as a control. Results showed that five species (sunflower, oat plant, rye grass, tall fescue, and green gram) were able to survive in the mixed contaminated conditions. Of
SUMMARY (continued)

these, the oat plant had the best growth characteristics and only minimal stress signs in mixed contaminated soil. Even though sunflower plants did not show superior growth characteristics, it achieved the maximum contamination removal from the soil. Exchangeable heavy metals were also lowest in the soil in which sunflowers were grown, compared to those in the contaminated, unplanted soil.

To investigate the interactive effects of co-contaminants on phytoremediation, soil was spiked with (1) representative organic contaminants- naphthalene and phenanthrene, (2) representative heavy metals- Pb, Cd and Cr, or (3) a combination of these contaminants. Four plant species (oat plant, perennial rye grass, tall fescue, and sunflower) were grown in contaminated or uncontaminated soil for comparative study. The plants propagated in the soil with organic contamination alone had growth and biomass similar to those grown in uncontaminated soil. Plants grown in the mixed contaminated soil performed better than those in soils with metal contaminants alone. The success of the phytoextraction of heavy metals was higher in the metal contaminated soil than in the mixed contaminated soil. Naphthalene was completely degraded from all the final samples. The phenanthrene concentration in all the final samples was less than in the initial samples.

To understand which heavy metal inhibits plant growth, a similar study was conducted on oat plant and sunflower where the soil was contaminated with Pb, Cd or Cr or a combination of the three heavy metals. Here, Cr as the sole contaminant was more phytotoxic to the plants than all the combinations. Sunflower did not germinate, and germinated oat plants did not survive in the single Cr contaminated soil. The interaction of Cr with other metals in the soil created a better environment for the plants in terms of toxicity levels. The phytoextraction of Pb did not change
SUMMARY (continued)

significantly when Cd and Cr were also present in the soil. However, phytoextraction of Cd was inhibited in the presence of other metals.

The soil was spiked with different concentrations of naphthalene, phenanthrene, Pb, Cd, and Cr to study the effect of varying contaminant concentrations on phytoremediation using the oat plant and sunflower. The results confirmed that an increase in the level of contaminant concentrations negatively affected plant growth and biomass, and of the two species, the sunflower had the lower germination, survival, growth, and biomass rates under increasing contaminant concentrations. Despite these findings, the sunflower was more effective in the phytoextraction of heavy metals at all of the contaminant concentrations studied. Both plants achieved higher Cr reduction than Pb and Cd reduction regardless of the soil treatments. The final PAH concentrations indicate that the presence of plants did not cause any improvement in PAH degradation. Naphthalene was detected only in the initial sample of the maximum contaminated soil treatment, and the phenanthrene concentrations in final planted and unplanted pots were considerably less than in the initial samples. Both plant growth and heavy metal phytoextraction changed gradually with an increase in the contaminant concentration.

The effectiveness of phytoremediation is sometimes limited due to low plant biomass and the low bioavailability of contaminants. Experiments were conducted to investigate the impact of biomass amendments (biochar, compost and a nutrient solution) and chemical amendments (Ethylenediaminetetraacetic acid (EDTA) and Igepal CA-720) on the phytoremediation of soil co-contaminated with naphthalene, phenanthrene, Pb, Cd, and Cr by sunflower and oat plant. To
study the biomass amendments, the soil was amended with biochar or compost. To study chemical amendments, plants raised in contaminated soils received a solution of EDTA, Igepal CA-720 or both. The combined effectiveness of amendments was studied by treating composted soil with either EDTA or Igepal CA-720. Plants cultivated in clean, unamended soil and contaminated unamended soil were used as controls. Both biochar and compost amendments improved the growth characteristics and biomass of the plants. The rate of Cd and Pb removal was best in the presence of biochar and compost amendments, but Cr removal was unaffected by the use of amendments. Nutrient amendments did not improve ability of the plants to remove the heavy metal contamination from the soil. The overall growth and biomass of the plants were less for the plants grown in soil was treated with EDTA and Igepal CA-720 compared to those propagated in untreated soil. A combination of compost and chemical amendments also did not provide positive results in terms of plant growth or contaminant dissipation. PAH degradation improved in the presence of all of the amendments. The results suggest that biochar and compost amendments can improve the plant growth characteristics and enhance phytoremediation of mixed contaminated soils.

Effects of electrokinetic amendments for phytoremediation of mixed contaminated soil were also studied. The contaminated soil was treated with compost and placed in electrokinetic cells seeded with oat plant and sunflower. Thirty days after germination, a 25 V alternating current was applied to selected cells using graphite electrodes for 3 h per day. The plants were harvested after 61 days. One cell remained unplanted to evaluate the effect of the electric current on the soil, alone. There was no noticeable improvement in the phytoextraction of heavy metals
SUMMARY (continued)

or PAH degradation attributed the application of an electric field despite the increase in biomass production by the plants that were treated with the electric current. It is likely that the electric potential application time and frequency should be increased to produce noticeable effects in heavy metal uptake and PAHs degradation.

Most existing studies in phytoremediation are conducted on spiked soils in a laboratory setting. Soils that are newly spiked with chemicals may not represent the actual field conditions and state of the aged contaminants found in the soil. So, experiments were conducted to investigate the effect of enhanced phytoremediation on a mixed contaminated soil extracted from a historically polluted site. The site soil was contaminated with a mixture of Pb, Cr and PAHs. The sample of contaminated soil was homogenized and part of it was amended with yard waste compost. Pots were filled with the soil and phytoremediation experiments were conducted using oat plant and sunflower over two months, with plants grown in unamended pots used as controls. The germination, survival and biomass of the plants were better when the soil was amended with compost, as compared to those in the control soil. The Pb content was not affected by the presence of plants or the amendment. Cr removal was achieved by both plant species in amended and unamended soil with the level of Cr removal by the oat plant considerably improved in the presence of compost. The PAH degradation in the soil did not show any noticeable trend in the presence of plants or compost amendment. The results suggest that phytoremediation of soil in combination with a compost amendment is a promising technology for mixed contaminated sites with aged contaminants. Phytoremediation efficiency varies with the type of plant, type of contaminant or number of contaminants in the mix and the age of those contaminants in the soil. A comparison of the present results with the results of previous experiments on spiked soil...
showed that the behavior of contaminants varies between the historically contaminated field soil and spiked soils from laboratory experiments.

Overall, five plant species (oat plant, sunflower, rye grass, tall fescue, and green gram) were identified as plants that can survive in the typical mixed contaminated soil considered. Although the sunflower produced the least biomass when it was sown in mixed contaminated soil, it achieved the best heavy metal dissipation. Rye grass, tall fescue, and green gram did not effectively reduce the heavy metal concentrations from the soil. However, they enhanced the microbial degradation of organic contaminants. The growth of plants propagated in mixed contaminated soils was better than plant growth in soil contaminated only with heavy metals, showing the antagonistic effects of organic and heavy metal contaminants on plant growth. Cr alone in the soil produced the most phytotoxic soil in which no plants survived to the end of test period. Heavy metal phytoextraction by the plants reduced in presence of organic contaminants. Plant growth characteristics decreased gradually as the contaminant concentrations rose. The chemical amendments used in the study increased the phytotoxicity symptoms and reduced phytoextraction efficiency. Biomass growth and phytoextraction efficiency improved with the application of compost as a soil amendment. Experiments on the aged industrial soil confirmed this finding. The study suggests that for the mixed contaminated soil considered here, phytoremediation using the sunflower plant is a promising approach for heavy metal dissipation. Among all the amendments that were examined, compost improved the biomass of the plants without compromising the success of the heavy metal phytoextraction. However, since no improvement was observed in the PAH degradation in soil samples planted with sunflower, further studies that intercrop sunflower with other plant species capable of degrading PAHs are
SUMMARY (continued)

suggested. Further studies are also recommended to understand the preference of plants for the extraction of different metals, and the optimum conditions for plants to degrade the organic contaminants in the rhizosphere.
1.1 Problem Statement

Soil contamination is an environmental problem, an occurrence that is growing around the world. Industrial use of land typically leads to contamination of soil in many ways. There are different environmental laws and regulations that define the allowable limit of contamination based on risk assessment at a particular site. Contaminated land needs remediation to meet the regulatory requirements before it can be used for another purpose. Specifically, recreational, agricultural, residential, and commercial land use necessitates that human exposure to toxic compounds and elements remain at a minimum in order to provide a safe environment.

Contamination can be found in the form of heavy metals, other inorganic compounds and organic compounds. The most common contaminants are heavy metals, petroleum-based hydrocarbon compounds and solvents and agricultural pesticides. Heavy metals are a major public health concern due to their toxic nature, and they are generated by many sources (USEPA, 1995a; Reddy et al., 2003a). They are very stable and do not break down into other compounds. If ingested or consumed over a long period of time, even in small amounts, heavy metals can accumulate in the liver and other organs and may build up to toxic levels. Organic contaminants are physically and chemically different from heavy metal contaminants, but can also negatively affect human health and the ecosystem as some are known carcinogens. Due to the increased production of synthetic organic chemicals over the last few decades, large numbers of organic compounds have been released into the environment. The most common pollutants among them are associated with fossil fuels derived in the form of petroleum compounds (Schwarzenbach et
al., 1993). Most organic compounds have low reactivity to other chemicals and high stability (Gillette et al., 1999; Sawyer and McCarty, 1978; Connell, 1997). These characteristics are the major reasons for their persistence in nature.

There are a broad range of methods available to treat heavy metals and organic contaminants individually. These include soil washing, soil vapor extraction, thermal desorption, incineration, bioremediation, electrokinetic remediation, stabilization/solidification, and monitored natural attenuation, among others (Sharma and Reddy, 2004). The applicability of each remediation technique depends on a short list of factors such as contaminant type and site-specific conditions (soil type and depth to groundwater table from the surface), cost, and end use. Considering these issues, the remediation of a particular site that is contaminated with a single type of contaminant is difficult and challenging.

Typically, most of the sites contain a variety of mixed contaminants. In the U.S. Environmental Protection Agency (USEPA) National Priority List (NPL), 40% of the hazardous waste sites are co-contaminated with organic and metal pollutants (Sandrin et al., 2000). In such cases, the factors that are considered in a remediation plan should include the factors that affect the remediation of sites contaminated with a single contaminant plus the synergistic effects of multiple, co-existing contaminants on the removal efficiencies. Remediation of co-contaminated sites is a complex technical goal because of the presence of physically and chemically different contaminants and the potential interactions between them. “The fate and transport of mixed contaminants in subsurface environments can be quite complex due to various physical, chemical and biological processes” (Reddy, 2011). The presence of organic contaminants may influence heavy metal mobility in the soils (Galvez-Cloutier and Dube, 2002; Dube et al., 2002), and this can be problematic for the physical/chemical treatment of heavy metals. For instance, volatile
organic compounds affect the solidification/stabilization process, while organic substances with high viscosity affect soil washing techniques. In these cases, the organic pollutants must be removed prior to the treatment of metals (Dermont et al., 2008). In batch sorption experiments, Poly and Sreedeep (2011) found that the sorption of individual contaminants can be affected by the presence of co-contaminants in soil. Additionally, the biodegradation of the organic contaminants can be inhibited by the presence of toxic metals (Said and Lewis, 1991; Sandrin and Maier, 2003), which can affect remediation technologies such as bioremediation, phytoremediation and monitored natural attenuation.

“Although numerous technologies have been developed to remediate contaminated sites, their applicability is often limited to a particular type of contaminant or site condition. Very few technologies are proven to efficiently address the problem of mixed contamination. The methods available to treat mixed contaminants include soil washing, stabilization and solidification, electrokinetic remediation, vitrification, bioremediation, phytoremediation, pump and treat, in-situ flushing, permeable reactive barriers, and monitored natural attenuation” (Cameselle et al. 2013). Some of these methods, like soil washing, in-situ flushing, and stabilization/solidification, require the treatment of soil with chemicals. The major limitation of this technology is the potential for the improper delivery of reagents into low permeability and heterogeneous soils, resulting in further contamination due to the chemicals used in the treatment. Other methods are so intense that they change the texture and property of the soil mass (e.g., stabilization/solidification and vitrification). This type of remedial measure may make the land unsuitable for certain kinds of end use such as agriculture use or creation of a nature preserve. Some methods like stabilization/solidification, bioremediation and vitrification etc. will not destroy or remove all the contaminants, but will leave them in the soil in a stabilized form. In
such cases, the stabilized contaminants can get mobilized later, causing future risk. In addition, most of the methods adopted for remediation of mixed contaminated soil are expensive technologies that require high amounts of energy consumption. Conventional practices for remediating heavy metal-contaminated soils are excavation and disposal or stabilization/solidification. Both these methods do not decontaminate the soil. These methods are only suitable and practical for small and highly contaminated areas. Proper delivery of reagents to the contaminated area is difficult to achieve with these techniques. Some methods, such as soil washing, have an adverse effect on biological activity, soil structure and fertility, and some require significant engineering costs.

There are many mixed contaminated sites with a large area and shallow contamination that await a cost effective and sustainable remediation. A sustainability evaluation of such a site in Chicago was performed in order to identify a green and sustainable remedial strategy (Reddy and Chirakkara 2013). That site investigation revealed that many of the contaminant concentrations exceeded the applicable regulatory limits that are protective of public health and the local ecology. Polynuclear aromatic hydrocarbons, pesticides and heavy metals were found in the soil throughout the site. The potential technologies to remediate the contaminated soil and groundwater were identified and evaluated for sustainability based on qualitative and quantitative analyses. The factors considered for these analyses included greenhouse gases and other air pollutant emissions, water use and personal injury, among others. The results showed that phytoremediation is the most cost effective and sustainable technique that can be adopted for most sites with moderate contamination.

The in-situ approach to phytoremediation can decontaminate and restore a site, maintaining the biological activity and physical structure of the soils. Various processes
contribute to the removal, stabilization and degradation of the contaminants by plants (Figure 1), and use of plants is both cost effective and visually pleasing. Phytoremediation is a green and sustainable natural process, used to remove contaminants from the soil (USEPA 2000). Compared to the typical mechanical approaches to hazardous waste management, phytoremediation is primarily solar powered and, thus, more sustainable. It remediates the site without disturbing the natural micro flora and fauna (Hussain et al., 2009). When it comes to a large contaminated area with shallow contamination, the mechanical approaches may not be always practical. Phytoremediation is a passive technique in which the plants accumulate, degrade or stabilize the contaminants (Sharma and Reddy, 2004). Plants can affect the rhizosphere by changing the local biogeochemistry, availability of water and nutrients, and local microclimate (McCutcheon and Schnoor, 2003). The inherently aesthetic nature of a planted site makes phytoremediation more attractive than other cleanup methods (ITRC, 2009). Vegetation can also offer other benefits at contaminated sites as the plants used for phytoremediation can increase the amount of organic carbon in the soil, which, in turn, can stimulate microbial activity. In addition, the plant roots can hold the soil together, stabilize it and reduce erosion and windblown dust. All these moderate the danger to human exposure pathways such as ingestion and inhalation. Plants can also mitigate groundwater contamination by controlling the downward migration of chemicals by absorption and transpiration of groundwater (Schnoor et al., 1995a).

Research in the field of phytoremediation has accelerated in the last few decades. Even though much progress has been made, there are lots of uncertainties. The health of plants that propagate in an unpolluted atmosphere is dependent on factors such as soil media, temperature, sunlight, rain, wind, and nutrients. In addition to these common plant growth factors, new factors
Figure 1.1 Mechanisms in phytoremediation
are introduced in the case of phytoremediation since this soil is contaminated. The new factors are related to the contaminant types at the site of interest, and the diversity of contamination and its varied effects cannot be classified easily. In addition, these contaminants rarely exist alone.

Most of the existing phytoremediation studies are applicable either for the remediation of heavy metals or of organic contaminants. The effect of co-contamination on phytoremediation is difficult to fully understand. The complex interactions of organic and heavy metal contaminants make it difficult to predict phytoremediation results. More investigations are necessary to optimize phytoremediation on soils polluted with mixed organic and heavy metal contaminants.

1.2 Research Objectives

Although several researchers have explored the effects of different contaminants on phytoremediation, the combined effects of mixed contamination on plant uptake and the subsequent effect on growth have not been studied in detail. Also, the enhancement of phytoremediation in mixed contaminated soil needs to be investigated further in order to develop an optimum strategy for the effective phytoremediation of mixed contaminated sites.

The research hypothesis is that engineered phytoremediation has great potential to be a green, sustainable and effective remediation technology for soil that is contaminated by a mixture of heavy metals and organic compounds such as polycyclic aromatic hydrocarbons (PAHs). The specific objectives of the research are to:

1. Perform a comprehensive literature review on phytoremediation of mixed contaminated soils, including strategies employed to enhance phytoremediation.

2. Investigate plant species that can survive and remediate mixed contaminants in soils (a combination of heavy metals such as lead, chromium and cadmium as well as PAHs such
as naphthalene and phenanthrene, which are commonly found at the Calumet sites in Chicago and similar industrial sites).

3. Investigate the synergistic effects of mixed contaminants (heavy metals and PAHs) in soils on plant growth and contaminant uptake.

4. Investigate the effect of initial contaminant levels on the phytoremediation of mixed contaminants in soils.

5. Investigate strategies to enhance phytoremediation of mixed contaminated soils, including the use of: (a) soil amendments such as fertilizer, compost and biochar, and (b) chelating agents, surfactants and their combination.

6. Investigate the combination of electrokinetics with phytoremediation to enhance remediation of mixed contaminated soils.

7. Enhance phytoremediation on historically contaminated soil with mixed contamination.

1.3 Thesis Organization

This thesis is organized in nine chapters as follows:

- Chapter 2 provides an overview of the existing phytoremediation studies in mixed contaminated soil.

- Chapter 3 explains the investigation of plant species, for phytoremediation of soil co-contaminated with a mixture of organic and heavy metal contaminants. Twelve plant species were studied for this purpose.

- Chapter 4 describes the study done to understand the interactive effects of organic and heavy metal contaminants to identify the critical contaminant condition in soil for phytoremediation.
• Chapter 5 reports the effect of varying initial contaminant concentration in phytoremediation of mixed contaminated soil.

• Chapter 6 explains enhancement of mixed contaminated soil phytoremediation using biomass amendments, chemical amendments, and a combination of the two.

• Chapter 7 explains the feasibility of enhancement of the phytoremediation of mixed contaminated soil by the application of an external electric field.

• Chapter 8 describes enhanced phytoremediation of a historically contaminated field soil with mixed contamination in laboratory conditions.

• Chapter 9 gives the overall conclusions and recommendations.

1.4 Cited References


microbial degradation of organic chemicals.” *Applied and Environmental Microbiology*, 57, 1498-1503.


CHAPTER 2

ASSESSING THE APPLICABILITY OF PHYTOREMEDIATION OF SOILS WITH MIXED ORGANIC AND HEAVY METAL CONTAMINANTS: LITERATURE REVIEW

2.1 Introduction

As the human population continues to grow exponentially, so does the per capita demand for available and habitable land. A primary consequence of increased industrialization and overpopulation is the contamination of soil and groundwater, which presents health risks to humans and the environment. Removal of these toxins is essential to ensure the safety of the public and permit continued use and development of urban and rural lands. Contamination can ensue from organic or inorganic compounds, where the most common contamination is from heavy metals, petroleum-based hydrocarbon compounds and solvents and agricultural pesticides (EGWRTAC, 1997; USEPA, 1997; Khan et al., 2004).

Heavy metals are a major concern for public health and the environment due to their toxicity. Metals can originate from many sources (USEPA, 1995; Reddy et al., 2003). For example, high Pb levels in the soil can be a result of lead paints, pipes and automobile exhaust (USEPA, 1996). The presence of Cd can originate from automobile exhaust, commercial fertilizers and batteries (Lu et al., 2007). And, the presence of As in soil can be attributed to pesticide use, burning coal and smelting processes (Garelick, 2008). Heavy metals are exceptionally stable and do not break down into other compounds. If ingested or consumed over a long period of time, even in small amounts, heavy metals can accumulate to potentially toxic levels in the liver and other organs (Singh, 2011). Different methods commonly adopted for the remediation of heavy metals include stabilization and solidification, vitrification, soil washing,
pump and treat, electrokinetic remediation, phytoremediation, monitored natural attenuation, in situ flushing, and permeable reactive barriers (EGWRTAC, 1997; USEPA, 2006; Wuana and Okieimen, 2011).

In addition, many organic pollutants found at contaminated sites also cause great concern for public safety and health as the increased production of synthetic organic chemicals over the last few decades has led to the release of large quantities of them into the environment. Organic contaminants of special concern in soils and groundwater include hydrocarbons, which are associated with the extraction, distribution and use of fossil fuels (Kamath et al., 2004; Banks and Schultz, 2005), organic solvents, volatile organic compounds (VOCs), halogenated organics (pesticides, PCBs), and polycyclic aromatic hydrocarbons (PAHs) (Schwarzenbach, 1993; Pignatello et al., 2010). Most of these compounds cause acute toxicity to living organisms and the exposure to these compounds, even at low concentrations, results in accumulation in tissues and can lead to toxic concentrations. VOC contamination is especially problematic due to the transference from soil and water to air with inhalation risks for the public (Lee et al., 2002).

Most of the more problematic organic contaminants have a very low solubility in water, forming the group of the so-called hydrophobic organic compounds (HOCs). In addition to their hydrophobic nature (Saichek and Reddy, 2005), HOCs show low reactivity with other chemicals, and have relatively high stability (Sawyer et al., 1978; Gillette et al., 1999). Given these unique characteristics, HOCs remain concentrated in the soil and are neither diluted nor transported readily by flowing water (Saichek and Reddy, 2005). Both, their hydrophobic and persistent nature creates great challenges for their removal from the environment (USEPA, 1997; Luthy et al. 1994; Loehr and Webster, 1996). Nonetheless, while insoluble in water, HOCs still tend to leach into groundwater or surface water slowly, which results in contamination of the subsurface
that may persist for as long as 100 years (National Research Council, 1997).

Sites contaminated with organic pollutants can be remediated with innovative
technologies such as soil vapor extraction, soil washing, stabilization and solidification,
electrokinetic remediation, thermal desorption, bioremediation, in situ chemical oxidation,
phytoremediation, pump and treat, in situ flushing, permeable reactive barriers, in situ air
sparging, and monitored natural attenuation (Sharma and Reddy, 2004). However, some of these
methods are only applicable to specific organic contaminants.

The applicability of remediation techniques depends on factors including contaminant
type and site-specific conditions, such as soil type and depth of groundwater table from the
surface, cost, and end use of the land. Usually, the remediation results depend so much on
contaminants and site characteristics that the technology and operating conditions at one site
cannot be extrapolated to other contaminated sites (Hyman and Dupont, 2001). Given these
complications, remediation of even a single contaminant class remains challenging. Typically,
the most substantially polluted sites contain a variety of mixed contaminants. The U.S.
Environmental Protection Agency (USEPA) National Priority List (NPL) indicates that 40% of
the hazardous waste sites are co-contaminated with organic and metal pollutants (USGAO,
2010). In such cases, all the above factors for the remediation of sites contaminated with a single
type of contaminant plus the possible synergistic effects that occur when more than one type of
contaminant is present must be considered to evaluate the technologies and strategies needed to
achieve a satisfactory removal efficiency in sites that are co-contaminated with organic and metal
pollutants.
2.2 Complexities with Mixed Contamination

Sites with mixed contamination (e.g. hydrophobic organics and heavy metals) pose technical challenges associated with each class of contaminant present in the soil and/or groundwater. Moreover, new problems and challenges arise from the presence of two (or various) classes of contaminants because they are physically and chemically different and, therefore, will respond in different way to the remediation technology. Additionally, the physicochemical interactions among the contaminants might create new and unexpected problems that could limit the efficiency of the remediation technology. As a result, the fate and transport of contaminants in the subsurface in mixed contamination sites can be complex and unpredictable (Reddy, 2011).

Several reports in literature demonstrate interactions between organic and inorganic contaminants that increase the complexity of their use for remediation. Galvez-Cloutier and Dube (2002) found that organic compounds influence the mobility of metal in soil, interfering with the implementation of physical and chemical treatments to remove or permanently immobilize those metals in the soil. As an example, volatile organic compounds (e.g. benzene) can impair the solidification/stabilization of the soil mass, while organic substances with high viscosity reduce the effectiveness of soil washing techniques that rely on the effective desorption of organics from the soil particles. These considerations were highlighted in batch experiments performed by Poly and Sreedeep (2011), who demonstrated that sorption isotherms of individual contaminants differ across multi-contaminated soils.

Other difficulties are related to the inherent toxicity of heavy metals that can inhibit the biodegradation of organic contaminants by the microorganisms in soil (Said and Lewis 1991; Sandrin and Maier 2003) and can hamper efforts at bioremediation, phytoremediation and monitored natural attenuation. A possible solution to heavy metal toxicity is found in a two-stage
approach. In the first stage, heavy metals are removed with a physicochemical technology, followed by a second stage in which the organic contaminants are extracted or degraded by a biological remediation technology that assures its efficacy once the metal toxicity can no longer hinder the biological activity (Dermont et al., 2008).

On the other hand, the presence of organic contaminants may positively or negatively affect the transportation and removal of heavy metals in soils. Organic contaminants such as light and dense organic liquids (LNAPLs, DNAPLs) are frequently found in mixed contaminated soils accompanied by heavy metals. Dube et al. (2002) investigated the interactions between three residual LNAPLs and three heavy metals (Cd, Cu and Pb) in a carbonaceous soil, evaluated the interactive processes that affected the behavior of the contaminants and focused on the influence of residual LNAPL on heavy metal transport. Using soil column experiments, they showed that the LNAPL with the highest residual saturation enhanced heavy metal mobility and decreased metal retention by the soil. They attributed changes in the geochemical distributions of Pb and Cu to soil hydrodynamic changes induced by residual LNAPL rather than those caused by chemical interactions between the metals and LNAPLs. Similar observations were reported by Galvez-Cloutier and Dube (2002), who investigated the influence of residual NAPL on the transport of various heavy metals through mixed contaminated soil under saturated conditions. Parameters such as soil structure, preferential flow, heavy metal transfer, retention, transport, and multiphase hydrodynamics were considered. They determined that the hydrophobicity of residual NAPL in soil causes water to move towards regions in the soil where there is less flow resistance, which creates preferential flow and transport. It is evident that no remediation of heavy metals occurs in the dead-zones. Furthermore, residual NAPL also obstructs small pore entries and masks active surface sites, making the removal of the heavy metals even more
difficult. Overall, the mobility of heavy metals is altered by the presence of NAPL and the result of that alteration can be only determined by considering the contaminants and site characteristics of each specific case.

2.3 Phytoremediation and Phytotechnologies for Contaminated Soils

Of the many technologies for the remediation of contaminated soils that have been developed over the last 3 decades, their applicability is often limited to a particular contaminant or specific site conditions. In the case of contaminated sites with mixed contaminations, a few technologies have proven to be efficient, but they too have important limitations. Some of them require the use of chemicals (e.g. soil washing, stabilization and solidification and in-situ flushing); others are so intense that they change the texture and physicochemical properties (i.e., pH or organic content) of the soil mass (e.g. stabilization and solidification, vitrification, and electrokinetic remediation). Certain methods like stabilization and solidification, bioremediation of heavy metals and vitrification etc. do not destroy or remove the contaminants, but stabilize them in soil, causing risk of future contaminant re-mobilization. Additionally, most of the methods mentioned above require high amounts of energy and thus are expensive. In this context, phytoremediation arises as a benign, cost effective alternative for the treatment of contaminated sites with mixed contamination (Cameselle et al., 2013).

Phytoremediation utilizes a passive, low cost, in situ approach that can decontaminate and restore the site while it sustains the existing biological activity and physical structure and fertility of the soil (Marmiroli, 2006; Ouvrard et al., 2011). Because plants utilize solar power for growth and contaminant uptake and/or degradation, phytoremediation is considered more sustainable than the typical mechanical approaches to hazardous waste management, which are
generally impractical for large sites with shallow contamination. The inherently aesthetic nature of a planted site also makes phytoremediation more attractive than the alternative cleanup methods (Cunningham and Ow, 1996; Pradhan et al., 1998).

Several phytotechnologies that have been tested and reported in literature focus on the restoration of contaminated sites as well as surface and subsurface water. The main phytotechnologies include phytoaccumulation, rhizofiltration, phytostabilization, phytodegradation, rhizodegradation, and phytovolatilization, each of which is discussed below.

Phytoaccumulation, also called phytoextraction, is based on the uptake of metals and other inorganic contaminants in the soil by the plant roots, and its subsequent accumulation in plant tissues. It is preferable to use plants that translocate the contaminants from the roots to its shoots (the part of the plant above ground), so the now contaminated plant can be harvested, treated and disposed of properly. Phytoaccumulation uses hyperaccumulators or plant species that can absorb larger amounts of contaminants than other plant species (Bedmar et al., 2009; Mehmood et al., 2013)

Rhizofiltration is the adsorption or precipitation on plant roots or absorption into the roots of contaminants that are in solution surrounding the root zone. Rhizofiltration effectively removes radionuclides, such as uranium (Lee and Yang 2010) and heavy metals (Dushenkov et al., 1995) from soil and groundwater.

Phytostabilization refers to the immobilization of contaminants such as heavy metals in the soil and groundwater through the adsorption by roots or precipitation in the root zone. This process reduces the mobility and bioavailability of the contaminants and is appropriate for the restoration of soil in mines, mine dumps or other areas where the natural vegetation is missing. Usually, metal-tolerant species are used to restore the vegetation as they reduce the mobility of
the contaminants and erosion (Gomes et al., 2014; Wójcik et al., 2014).

Phytodegradation, also called phytotransformation, is the breakdown of organic contaminants absorbed by the plant due to its metabolic activity. External enzymes excreted by the plant can also carry out the degradation of contaminants (Lee, 2013).

Rhizodegradation is plant-assisted biodegradation or bioremediation in the rhizosphere (the soil around the roots of a plant). Root exudates and enzymes released by the roots of the plants can stimulate bacterial and fungal activity in the rhizosphere that aid in the degradation of organic contaminants (Qiu et al., 2004; Weyens et al., 2009). This process is also known as phytostimulation.

Phytovolatilization involves uptake of volatile organics contained in soil and groundwater by plants and the subsequent release of the gaseous form of the contaminant through stomata in the leaves (Batty and Dolan, 2013). In phytovolatilization, plants or trees act as an organic pump that takes up the contaminants from soil and groundwater and disperses them into the atmosphere.

Phytotechnologies bring about additional benefits associated with the increase of vegetation during the remediation. Among these, plant growth will increase the organic carbon content of the soil and stimulate root zone microbial activity. Plant roots also confer structural stability to the soil that helps to reduce erosion and the generation of windblown dust. These benefits of vegetation can minimize human exposure to soil contaminants via ingestion and inhalation. Plants can also mitigate groundwater contamination by reducing the downward migration of chemicals by absorption and transpiration of groundwater (Schnoor et al., 1995).

Effective phytoremediation and the associated benefits for soil and environment can only be achieved if the plant can grow and develop appropriately in the contaminated soil and
groundwater. Normal plant growth is dependent on multiple factors that include soil structure and composition, temperature, sunlight, rain, wind, and nutrient availability. At contaminated sites, new factors that counter plant growth are introduced into the environment and must be assessed for their relative impact on growth in order to construct an effective and often site-specific phytoremedial system. The new factors are specifically related to the types and concentrations of the contaminants on-site. The diversity of contamination and its varied effects is not easily classified. In all, their complex interactions make it difficult to predict phytoremediation results with accuracy (McGrath et al., 2001).

Many studies have explored the phytoremediation potential of various plants on both organic and inorganic contaminants, the plant species that are best able to remediate certain contaminant classes, and effective methods to heighten phytoremediation. Contaminants that can be targeted for phytoremediation include heavy metals (Pulford and Watson, 2003; Robinson et al., 1998), explosives (Medina and McCutcheon, 1996; Bhadra et al., 2001; Rylott and Bruce, 2009; Van Aken, 2009), crude oil and oil products (Nedunuri et al., 2000; Pichtel and Liskanen, 2001; White et al., 2005; Merkl et al., 2005; Memarian and Ramamurthy, 2012), pesticides (Schnoor et al., 1995; Chaudhry et al., 2002), radioactive nuclides (Lee and Yang, 2010; Singh et al. 2008), and PAHs (White et al., 2005; Huesemann et al., 2009). A few select studies on phytoremediation of heavy metals or organic contaminants are reviewed below to create an understanding of the mechanisms of the phytoremediation of these contaminants when each type of contaminants exists individually. This information will be essential in the design and assessment of strategies for the phytoremediation of contaminated soils and ground water with mixed contaminants (co-existence of both heavy metals and organic contaminants).
2.3.1 *Phytoremediation of Heavy Metals*

The phytoremediation of heavy metals in soils is based on the use of plant species that are capable of the uptake and accumulation of contaminants in the plant tissues, not only in the roots, but chiefly in the aerial part or shoots. In order to enhance the remediation process, it is important to use plants species that can accumulate high concentrations of heavy metals with minor effects on their growth and development or hyperaccumulators. In general, hyperaccumulators are plant species that accumulate heavy metal concentrations in their shoots at rates 100 times higher than non-hyperaccumulator plants with no significant negative effect on their growth and development (Barcelo and Poschenrieder, 2003). However, there are three definitions of hyperaccumulator species presently found in the literature on accumulation capability, bioaccumulation and translocation factors. In the case of accumulation capacity, hyperaccumulator plants are those species that can accumulate more than 10,000 mg/kg (dry wt.) for Zn and Mn, 1000 mg/kg for Co, Cu, Ni, As and Se; and 100 mg/kg for Cd in their shoots (Baker et al., 2000). With regard to the bioaccumulation factor, hyperaccumulators are those whose ratio of metal concentration in tissue plant to that in soil is greater than 1.0, and can reach values as high as 50 to 100 (Brooks, 1998). Considering the translocation factor, hyperaccumulators are those species in which the metal concentration in the shoots is greater than that found in its roots (Wei and Zhou, 2006).

During the phytoremediation of contaminated soils, hyperaccumulators are capable of accumulating large amount of heavy metals because they have strongly expressed metal sequestration mechanisms and, sometimes, greater internal requirements for specific metals (Shen et al., 1997). Some species may be able to mobilize and solubilize metals from less-soluble forms than can the non-hyperaccumulating species (Rascio and Navari-Izzo, 2011). However,
their effectiveness also depends on the metal elements. For example, different heavy metals have varied patterns of behavior and mobility within tree tissues: Cd, Ni and Zn are more easily translocated to the aerial tissues while Pb, Cr and Cu tend to be immobilized and held primarily in the roots (Pulford and Watson, 2003). After entering the plant, metals commonly bind to cell wall components (free -COOH or -OH groups), sulfur ligands in cytosol (phytochelatins, thiols) or are stored in vacuoles where they are bound to organic acids (Callahan et al., 2006). It is also possible, although less common, for precipitates with phosphate, sulfate or carbonate to form and occupy intracellular or extracellular spaces (Marques et al., 2009). An ideal plant for the successful phytoaccumulation of heavy metals should possess high metal tolerance, an ability to grow on low quality soils, high bioaccumulation into aerial tissues (root-to-shoot metal translocation), and the capacity for high yield of biomass (Karenlampi et al., 2000; Pilon-Smits, 2005).

Metals accumulated in plant tissues are not degraded or transformed and plant tissues may require harvesting and proper disposal. The harvested biomass can be incinerated and the ashes deposited in a landfill. The volume of ashes with heavy metals is much less than that of the plant biomass or contaminated soil, moreover the cost of the process is much less than the excavation and disposal of the contaminated soil in a landfill (USEPA, 1999). According to Pulford and Watson (2003), willow plants can be used in phytoextraction of heavy metals and the harvested wood can be burned to produce renewable bioenergy. The biorecovery of the metals from the harvested plant is another possible benefit of phytoremediation to remove heavy metals (Baker et al., 1994; Kikuchi and Tanaka, 2012).

When dealing with a site that is contaminated with heavy metals, a phytoremediation study is must determine the ability of the plant to remediate the soil under the specific site
conditions before any large scale implementation occurs. This is because a plant that readily uptakes one or more metals at a specific site may not perform equally well at another. In some cases, even though the plant can accumulate a particular metal, the rate may be so slow that remediation is not possible within an economically feasible time frame (Robinson et al., 2000). A hyperaccumulator plant propagated in different soils may hyperaccumulate different metals (Knight et al., 1997). So, the efficiency of a particular species needs to be tested in the targeted soil type and under similar contaminant concentrations before it can be implemented on a field-scale basis (McGrath and Zhao 2003). The phytoremediation potential of different plant species for heavy metal contaminants are summarized in Table 2.1.

Phytostabilization is an alternative phytotechnology for heavy metal contaminated soil that is based on chemical changes in the rhizosphere that cause the precipitation and immobilization of heavy metals and make them less bioavailable. Chaney et al. (1997) suggested that Cr and Pb may be immobilized by a vegetative cover. Plants achieve Cr immobilization by promoting the reduction of Cr(VI) to Cr(III), which is much less soluble and, therefore, less bioavailable.

2.3.2 Phytoremediation of Organic Contaminants

The degradation organic contaminants can be achieved with phytoremediation due to a combination of mechanisms that include plant-promoted microbial degradation, plant uptake and accumulation, phytovolatilization, and phytodegradation (Kang, 2014). Organic contaminants are either degraded in the rhizosphere (rhizodegradation) by root exudates, i.e. enzymes that catalyzed contaminant degradation to simple organic molecules, or by the action of microbes in the rhizosphere. The microbial activity in the rhizosphere is enhanced by the root exudates, so
Table 2.1: Potential Plant Species for Phytoremediation of Heavy Metals

<table>
<thead>
<tr>
<th>Species</th>
<th>Phytoremediation potential</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Solanum Nigrum</em> (Black Nightshade)</td>
<td>Cd</td>
<td>Wei et al. (2010)</td>
</tr>
<tr>
<td><em>Linum usitatissimum</em> (Flax)</td>
<td>Cd</td>
<td>Bjelkova et al. (2011)</td>
</tr>
<tr>
<td><em>Albizia amara</em></td>
<td>Cr</td>
<td>Shanker et al. (2005).</td>
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<tr>
<td><em>Casuarina equisetifolia</em></td>
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<tr>
<td><em>Tectona grandis</em></td>
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<tr>
<td><em>Leucaena leucocephala</em></td>
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<td></td>
</tr>
<tr>
<td><em>Spirodela polyrhiza</em> (Duckweed)</td>
<td>Ni</td>
<td>Appenroth et al. (2010)</td>
</tr>
<tr>
<td><em>Allium fistulosum</em> (Green Onion)</td>
<td>Pb</td>
<td>Cho et al. (2009)</td>
</tr>
<tr>
<td><em>Pteris cretica</em> (Moonlight Fern)</td>
<td>Pb</td>
<td>Cho et al. (2009)</td>
</tr>
<tr>
<td><em>Pinus sylvestris</em> (Pine)</td>
<td>Cd, Pb,</td>
<td>Ostrowska et al. (2006)</td>
</tr>
<tr>
<td><em>Ricinus communis</em> (Ricinus)</td>
<td>Cd, Pb,</td>
<td>Niu et al. (2007)</td>
</tr>
<tr>
<td>Grasses:</td>
<td></td>
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<tr>
<td><em>Pennisetum americanum</em></td>
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<tr>
<td><em>Paspalum atratum</em></td>
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<tr>
<td><em>Silphium perfoliatum</em></td>
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<tr>
<td><em>Stylosanthes guianensis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Brassica rapa</em> (Field Mustard)</td>
<td>Cd, Cu, Zn</td>
<td>Meers et al. (2005)</td>
</tr>
<tr>
<td><em>Phragmites australis</em> (Common Reed)</td>
<td>Cu, Hg, Pb</td>
<td>Weis and Weis (2004)</td>
</tr>
<tr>
<td><em>Spartina alterniflora</em> (Smooth Cordgrass)</td>
<td></td>
<td></td>
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<tr>
<td><em>Amorpha fruticosa</em></td>
<td></td>
<td>Shi et al. (2011)</td>
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<tr>
<td><em>Vitex trifolia</em></td>
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<tr>
<td><em>Glochidion puberum</em></td>
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<tr>
<td><em>Broussonetia papyrifera</em></td>
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<tr>
<td><em>Styrax tonkinensis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species from <em>Brassica</em> genus</td>
<td>Heavy metals</td>
<td>Palmer et al. (2001)</td>
</tr>
<tr>
<td><em>Vetiveria zizanioides</em> (Vetiver Grass)</td>
<td>Pb, Cu, Zn, Cd, Mn</td>
<td>Andra et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chen et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roongtanakiat and Chairoj (2001)</td>
</tr>
<tr>
<td></td>
<td>Cd, Cr, Cu, Se</td>
<td>Zhu et al. (1999)</td>
</tr>
<tr>
<td><em>Brassica napus</em> (Canola)</td>
<td>Cd, Cr, Cu, Ni, Pb, Zn</td>
<td>Marchiol et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saathoff et al. (2011)</td>
</tr>
<tr>
<td><em>Thlaspi caerulescens</em> (Alpine Pennygrass)</td>
<td>Cr, Cd, Co, Cu, Mo, Ni, Pb,</td>
<td>Robinson et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>Zn, Mn</td>
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</tr>
</tbody>
</table>
the combination of the growing plant and the microflora creates an environment in the rhizosphere that is appropriate for the degradation of contaminants (Dzantor, 2007). Plants may also uptake the organic contaminants where it will be degraded to simpler molecules by enzymatic transformation in the plant tissues (phytodegradation) (Macek et al., 2004).

The efficiency of the remediation of organic contaminated soil is affected by the solubility and bioavailability of the contaminants. In the case of moderately hydrophobic organic chemicals with octanol-water partition coefficients in the range of log $K_{ow} = 0.5$-$3.0$ in shallow subsurface soils, the direct uptake of organics (e.g. pesticides, PCBs, dioxins) by plants is a proven efficient removal mechanism (Gao et al. 2008a; Dettenmaier et al., 2009; Chang et al., 2013). Thus, most BTEX chemicals, chlorinated solvents and short-chain aliphatic chemicals are considered amenable to phytoaccumulation. Hydrophobic chemicals with log $K_{ow} > 3.0$ are bound so strongly to the surface of roots that they are not easily translocated to aerial tissues. Water soluble chemicals with log $K_{ow} < 0.5$ are not sufficiently sorbed to roots or actively transported through plant membranes. The expected end product of the degradation of organic components is generally nontoxic constituents such as carbon dioxide, nitrate, chloride, and ammonia (Dhankher, 2012). Several studies that investigated the phytoremediation of organic contaminants with different plant species are summarized in Table 2.2.

2.4 Phytoremediation of Mixed Contaminants

Phytoremediation of sites with mixed contamination is expected to be more complex than remediation where there is only one kind of contaminant due to the different properties of the kinds of contaminants (heavy metals and organic compounds) and the possible interactions of the contaminants with each other as well as with soil and microbiota in the rhizosphere. As noted
Table 2.2: Potential Plant Species for Phytoremediation of Organic Pollutants

<table>
<thead>
<tr>
<th>Species</th>
<th>Phytoremediation potential</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brachiaria brizantha</em> (Palisade Grass)</td>
<td>Petroleum</td>
<td>Merkl et al. (2005)</td>
</tr>
<tr>
<td><em>Cyperus aggregatus</em> (Inflated-scale Flatsedge)</td>
<td>Petroleum</td>
<td></td>
</tr>
<tr>
<td><em>Gaillardia aristata</em> (Blanket flower)</td>
<td>Petroleum</td>
<td>Liu et al. (2012)</td>
</tr>
<tr>
<td><em>Echinacea purpurea</em> (Eastern Purple Cone Flower)</td>
<td>Petroleum</td>
<td></td>
</tr>
<tr>
<td><em>Festuca arundinacea schreb</em> (Fawn)</td>
<td>Petroleum</td>
<td></td>
</tr>
<tr>
<td>Combined <em>F. arundinacea</em> (Fire Phoenix)</td>
<td>Petroleum</td>
<td></td>
</tr>
<tr>
<td><em>Pinus sylvestris</em> (Pine)</td>
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</tr>
<tr>
<td><em>Trifolium Repens</em> (White Clover)</td>
<td>Fuel Oil</td>
<td>Lin et al. (2002)</td>
</tr>
<tr>
<td><em>S. alterniflora</em> (Smooth Cordgrass)</td>
<td></td>
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<tr>
<td><em>Zea mays</em> (maize)</td>
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<tr>
<td><em>Trifolium pretense</em> (Red Clover)</td>
<td></td>
<td></td>
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<tr>
<td><em>Lolium arundinaceum</em> (Fescue)</td>
<td>Anthracene, Naphthalene, Phenanthrene</td>
<td>White et al. (2005)</td>
</tr>
<tr>
<td><em>Cannabis sativa</em> (Industrial Hemp)</td>
<td>Benzo (a) pyrene, Chrysene</td>
<td>Campbell et al. (2002)</td>
</tr>
<tr>
<td><em>Trifolium Repens</em> (White Clover)</td>
<td>Phenanthrene, Pyrene</td>
<td>Gao et al. (2008b)</td>
</tr>
<tr>
<td><em>Festuca arundinacea</em> (Tall Fescue)</td>
<td>Phenanthrene, Pyrene</td>
<td>Cheema et al. (2009)</td>
</tr>
<tr>
<td><em>Brassica napus</em> (Rapeseed)</td>
<td>Phenanthrene, Pyrene</td>
<td>Sheng-Wang et al. (2008)</td>
</tr>
<tr>
<td><em>Phragmites communis</em> (Common Reed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Typha orientalis</em> (Bullrush)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vetiveria zizanioides</em> (Vetiver grass)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rohdea japonica</em> (Sacred</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant</td>
<td>Chemicals</td>
<td>Reference</td>
</tr>
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</tr>
<tr>
<td><em>Bolboschoenus planiculmis</em></td>
<td></td>
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<tr>
<td><em>Morus</em> (Mulberry)</td>
<td>PAHs</td>
<td>Olson and Fletcher (1999)</td>
</tr>
<tr>
<td><em>Cucurbita pepo</em> (Zucchini)</td>
<td>p,p’-DDE</td>
<td>White (2009)</td>
</tr>
</tbody>
</table>
above, for sites contaminated with only one kind of contaminant, plant selection is critical for an effective remediation, since a plant that is good for the remediation of a particular contaminant may not be effective or even survive in the presence of another contaminant or co-contaminants. Table 2.3 identifies plants that have been used in heavy metal removal and organic contaminant degradation where the soil is contaminated with one kind of contaminant, either heavy metals or organic pollutants. Those plant species are potential candidates for phytoremediation of mixed contaminated soil once their effectiveness with both heavy metals and organic contaminated soils has been proven. The phytoremediation results may be different, however, when the organic and inorganic contaminants examined in the studies identified in Table 2.3 are present together. There have been a few recent studies on phytoremediation of co-contaminated soils with mixed metals and organic contaminants (Chirakkara and Reddy, 2014; Ramamurthy and Memarian, 2014). In these studies, the presence of mixed contamination led to very different results when compared with only one kind of contaminants. The differences are related to plant growth and biomass production, metal uptake, organic contaminant degradation, synergistic or antagonistic effects in phyto-toxicity, and physico-chemical interactions among the contaminants that impact their mobility and/or bioavailability. A review of the literature by Batty and Dolan (2013) suggests that sustaining a diverse microbial community within the rhizosphere to promote endophyte-plant symbioses is critical for the successful phytoremediation of mixed contaminated soils. This is because soil microorganisms often aid organic contaminant degradation, and may serve to bolster plant health against contaminant toxicity, thus improving the survivability of the plants (Mastretta et al., 2006; Germaine et al., 2013).

When dealing with mixed contaminated sites, it seems that the simplest approach could be to stabilize the heavy metals before inducing organic contaminant degradation. This approach
Table 2.3: Potential Plant Species for Phytoremediation of Heavy Metals and Organic Contaminants

<table>
<thead>
<tr>
<th>Species</th>
<th>Phytoremediation potential</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Avena sativa</em> (Oat)</td>
<td>Zn</td>
<td>Ebbs and Kochian (1998)</td>
</tr>
<tr>
<td></td>
<td>Phenanthrene</td>
<td>Miya and Firestone (2001)</td>
</tr>
<tr>
<td><em>Lolium perenne</em> (Rye Grass)</td>
<td>Cu, Cd, As</td>
<td>O’Connor et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Cu, Zn</td>
<td>Zhou et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Petroleum hydrocarbons (Naphthalene, Phenanthrene, Anthracene)</td>
<td>White et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Organic contaminants (creosote)</td>
<td>Huang et al. (2004)</td>
</tr>
<tr>
<td><em>Medicago sativa</em> (Alfalfa)</td>
<td>Pyrene</td>
<td>Fan et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Cd, Cr, Ni, Zn</td>
<td>Peralta-Videa et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>Phenanthrene, Pyrene</td>
<td>Sheng-Wang et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Petroleum contamination</td>
<td>Liu et al. (2012)</td>
</tr>
<tr>
<td><em>Salix spp.</em> (Willow)</td>
<td>Cd, Organics(Oil)</td>
<td>Kuzovkina and Quigley (2005)</td>
</tr>
<tr>
<td></td>
<td>Zn, Cd, Ni, Cr, Pb, Cu</td>
<td>Pulford and Watson (2003)</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>Robinson et al. (2000)</td>
</tr>
<tr>
<td><em>Populus spp.</em> (Poplar Trees)</td>
<td>BTEX</td>
<td>Moore et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>Robinson et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>BTEX, Nutrient contamination</td>
<td>Schnoor et al. (1995)</td>
</tr>
<tr>
<td><em>Helianthus annuus</em> (Sunflower)</td>
<td>Zn, Cu, Cd</td>
<td>Meers et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Zn, Pb</td>
<td>Adesodun et al. (2010)</td>
</tr>
<tr>
<td><em>Brassica juncea</em> (Indian Mustard)</td>
<td>Cd, Cr, Cu, Ni, Pb, U, Zn</td>
<td>Blaylock et al. (1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liu et al. (2000)</td>
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<tr>
<td></td>
<td></td>
<td>Lim et al. (2004)</td>
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<td></td>
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<td>Singh and Singha (2005)</td>
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</table>
was suggested by Palmroth et al. (2006) who tested the feasibility of phytoremediation in a field scale at a site contaminated with organics and metals from bus maintenance activities. The concentrations of hydrocarbons in the soil were 11,400±4,300 mg/kg and the concentrations of Cu, Pb and Zn in the soil were 170 ± 50 mg/kg, 1,100±1,500 mg/kg and 390 ± 340 mg/kg, respectively. Six plant species: *Pinus sylvestris* (pine), *Populus deltoides x Wettsteinii* (poplar), *Festuca rubra* (red fescue), *Poa pratensis* (smooth meadow grass), *Lolium perenne* (rye grass) and *Trifolium Repens* (white clover) where tested in the soil with and without amendment (NPK fertilizer or bio-waste compost). Metal concentrations were analyzed in the plants, soil and leachate to assess the effectiveness of the phytostabilization. Over the 39 month study, a higher hydrocarbon removal rate was observed where the soil had been amended with NPK fertilizer and municipal bio-waste compost than in the amendment-free soil. That data, coupled with observations of greater plant cover in the compost and NPK amended soils relative to the unamended control (which had areas devoid of vegetation), indicate that increased phytodegradation of the hydrocarbons occurred in response to the organic amendments. Though hydrocarbon degradation was apparently enhanced, the plant tissues did not contain the expected high metal contents indicative of hyperaccumulation. In this case, the compost addition may have resulted in reduced metal mobility, implying that a two-phase approach that targets the organic and inorganic contaminants separately may be the most appropriate process. This suggests that the presence of two kinds of contaminants will result in physico-chemical interactions that will affect the remediation itself, and those interactions must be studied and their effect evaluated.

The physico-chemical interactions among contaminants and their effect on mobility and bioavailability usually result in a reduction in the effectiveness of the phytoremediation of heavy
metals compared to similar tests where there is only one kind of contaminant. This effect was highlighted in a study by Chen et al. (2004), who investigated the response of Cu and Zn in phytoremediation of soil contaminated with Cu, Zn and 2,4-dichlorophenol (DCP). The sample of heavy metal contaminated soil was extracted from an industrialized area, and subsamples were spiked with 100 μg/g 2,4-DCP. *Lolium perenne* (rye grass) seeds were sown in pots containing soil contaminated with heavy metals or co-contaminated with 2,4-DCP to evaluate its effect on metal mobility. The results showed that the water soluble fraction of Cu and Zn was greater in planted soil than in unplanted soil, which indicates increased metal mobility due to phytoremediation. While the treatment with 2,4-dichlorophenol did not affect the growth of the plants, there was an apparent impact on metal uptake, with lower metal accumulation noted in the plants grown in pots that were treated with 2,4-dichlorophenol. Similar phenomena were observed by Kobylecka and Skiba (2008), who investigated the effect of herbicides 2,4-dichlorophenoxyacetic acid and 4-chloro-2-methylphenoxyacetic acid on the uptake of Zn, Cu and Mn by *Triticum Aestivum* (wheat). They found that the heavy metal contents of the plants treated with the organic compounds were significantly lower than that of the untreated plants. One explanation for this is that the formation of less water soluble heavy metal-organic complexes that reduced the phytoextractability of the metals and limited the metal accumulation by the plant.

The co-occurrence of the two kinds of contaminants has been found to affect the phytotoxicity during phytoremediation and, therefore, the plant growth and remediation efficiency. An increase in phytotoxicity is expected when the plant is exposed to a soil contaminated with heavy metals and organic contaminants, but surprisingly, several authors have reported a reduction in the phytotoxicity and better remediation results at specific levels of
contaminants. Thus, Lin et al. (2006) investigated the dissipation mechanisms for pentachlorophenol (PCP) in copper co-contaminated soil using *Lolium perenne* L (rye grass) and *Raphanus sativus* (radish) in a greenhouse experiment in which they monitored the growth response of the plants and evaluated its efficiency. Microbial activity and the residual PCP contents in strongly and loosely adhering rhizosphere soil (after gentle shaking) were determined. The soil was contaminated with PCP at two concentrations: 50 mg/kg and 100 mg/kg. Three Cu levels (0, 150, 300 mg/kg) were tested at each of the PCP levels. The removal efficiency of PCP ranged from 62% to 96% in the ryegrass planted soil and 45% to 94% in the soil planted with radish, depending on the concentration of PCP and Cu after 12 weeks incubation. At the initial PCP level of 50 mg/kg, the plant growth improved with the increase in Cu concentration, which suggests that Cu actually improves PCP degradation. Conversely, in soils with an initial PCP level of 100 mg/kg, the soil microbial activity and plant growth decreased with the rise in Cu. These contradictory trends illustrate the sensitivity of plants to low levels of certain micronutrients (i.e. Cu) and the resultant variation in microbial responses to mixed contamination due to the differences in both the type and concentrations of the individual contaminants.

The different response of plants to specific levels of mixed contamination was reported by Batty and Anslow (2008), who studied the effect of pyrene, a polycyclic aromatic hydrocarbon, in soil on the remediation of Zn by *Brassica juncea* (Indian mustard) and *Festuca arundinacea* (tall fescue). Zn was effectively removed from the co-contaminated soils by both plant species, which were able to accumulate higher concentrations in the shoots than in the Zn contaminated soil. However, these plants grew even better in clean soil or pyrene contaminated soil, which confirmed the phytotoxicity of Zn at the concentration tested (8000 mg/kg). The most
interesting result from the study by Batty and Anslow (2008) was the varied response of the two species to the Zn contamination. Zn was predominantly associated with root tissues for F. arundinacea and shoot tissues for B. juncea. The translocation of Zn from roots to shoots in B juncea was the reason for the major phytotoxicity of Zn and lower biomass production in co-contaminated soil. Differences in metal accumulation and distribution throughout the soil-plant systems for each species likely reflect the different metal tolerance mechanisms as F. arundinacea did not translocate Zn from the root to shoot as effectively as B. juncea, and that minimized its detrimental effects on plant growth. However, though F. arundinacea may minimize metal phytotoxicity and increase plant survivability, it is not as effective as a hyperaccumulator in the aerial tissues as the metals remained concentrated within the root zone and could be re-mobilized following the plant’s death or removal.

Zhang et al. (2009) studied the concurrent removal of Cd and pyrene from soil spiked with different concentrations of Cd (0, 2 and 4.5 mg/kg) and pyrene (0, 10, 50, and 100 mg/kg) by Zea mays L (maize). The maize presented minor toxicity when grown in the pyrene-spiked soil, whereas a more significant decrease appeared in maize propagated in Cd-spiked soil. In the co-contaminated soil, a rise in the concentration of pyrene decreased the root dry weight, a confirmation of the synergistic toxic effects on plant health that negatively impact the phytodegradation potential. Phytoremediation of pyrene was possible with maize since the final pyrene concentrations were significantly lower in the planted soil than in unplanted soil. This trend occurred for both pyrene contaminated soil and that co-contaminated with Cd and pyrene. A comparison of the single pyrene contamination and pyrene-Cd co-contamination indicated that, irrespective of the presence of plants, the residual pyrene in soil tended to become higher as the Cd content increased. This result was due to the mounting phytotoxicity of the co-
contaminated soil. In Cd contaminated soils, the Cd concentrations increased in the shoot and root as the Cd level in the soil elevated. However, the accumulation of Cd decreased with as the pyrene contamination in the co-contaminated soil intensified; this is attributed to competitive adsorption between the Cd and pyrene in the root zone. Overall, these results prove that maize can be an effective remediation tool in soil that is co-contaminated with Cd and pyrene, although a decrease in efficiency due to phytotoxic effects can be expected at high contaminant levels.

Similar results were obtained by Chigbo et al. (2013) in a greenhouse experiment that investigated the effect of 50 and 100 mg/kg of Cu or 250 and 500 mg/kg of pyrene and the combined effect of copper and pyrene on the growth of Brassica juncea, as well as the uptake and accumulation of copper and dissipation of pyrene. The negative effect of the copper-pyrene co-contamination on shoot and root dry matter and the inhibition of Cu phytoextraction were observed. Plant tests confirmed the enhanced pyrene dissipation during phytoremediation compared to samples that remained unplanted. The presence of Cu increased the residual pyrene in the planted soil. Yet, when the concentration of Cu was high (100 mg/kg), the residual pyrene concentration in soil were similar to those in unplanted soil. The results suggested that changes in the root physiology or rhizospheric microbial activity that result from Cu stress could negatively affect pyrene dissipation.

Hechmi et al. (2013) investigated the pollutant removal ability of Zea mays L in soil contaminated with Cd and pentachlorophenol (PCP) in pot culture experiments where the plants were grown in soil contaminated with Cd or PCP or a combination of the two. The initial concentrations of PCP were 0, 50 or 100 mg/kg and those of Cd were 0, 2 or 6 mg/kg, taken in different combinations. Both Cd and PCP contaminations significantly reduced the plant growth. The PCP dissipation was significantly affected by the presence of plants and the presence of Cd:
it decreased considerably with the increase in the Cd level. The Cd toxicity was so negative for the plant that the residual PCP was higher in planted soil than in unplanted soil when Cd was also present in the soil. On the other hand, the concentration of Cd in the plant tissues decreased with the PCP application.

These authors also conducted pot culture experiments to evaluate the phytoremediation potential of a wetland plant species *Phragmites australis* in Cd and PCP co-contaminated soil under greenhouse conditions for 70 days (Hechmi et al., 2014). The treatments used were Cd (0, 5 and 50 mg/kg) and PCP (50 and 250 mg/kg) separately or in combination. Apart from the concentrations of Cd and PCP, soil dehydrogenase activity (DHA) was also measured, as it is considered to be a good indicator of soil microbial activity. The growth of *P. australis* decreased significantly with the addition of either Cd or PCP. Compared to the control, the plant biomass was reduced by 89% and 92% in the low and high Cd treatments and by 20% and 40% in the low and high PCP treatments, respectively. In co-contaminated soil with low Cd and PCP, the Cd toxicity was less than for Cd treatment alone, which resulted in a 144% plant growth improvement. This result is a clear example of the antagonist effect of phytotoxicity in co-contaminated soil. However, the improvement seen in plant growth in the co-contaminated soil was not useful for soil remediation because the Cd uptake and translocation by *P. australis* were weak. Since a low proportion of Cd was found in the above ground biomass, the researchers concluded that *P. australis* would not be useful for Cd phytoextraction. The removal rate of PCP was very significant (70%) in planted soil, and they observed significant positive correlations between the DHA and the removal of PCP in planted soils. This confirms that plant root exudates promote the rhizosphere microorganisms and enzyme activity and improve the biodegradation of PCP. Considering the inhibition of plant growth at high contaminant concentrations and the
reduced phytoextraction of Cd under co-contamination, they concluded that *P. australis* may not be effective for phytoremediation of soil co-contaminated with Cd and PCP.

Sun et al. (2011) investigated the effect of different concentrations of benzo[a]pyrene on the growth of *Tagetes patula* (marigold) and its uptake and dissipation of benzo[a]pyrene. They also studied the phytoremediation of soil contaminated with benzo[a]pyrene (0, 2, 5, 10, and 50 mg/kg) and three heavy metals: Cd (20 and 50 mg/kg), Cu (100 and 500 mg/kg) or Pb (1000 and 3000 mg/kg) using marigold. Here, a low concentration of benzo[a]pyrene (≤10 mg/kg) improved the plant growth and increased biomass at the rate of 10.0% - 49.7% compared to the control. Significant positive correlations were observed between the soil benzo[a]pyrene concentration and benzo[a]pyrene accumulation in plant tissues. However, the heavy metals inhibited plant growth and benzo[a]pyrene uptake and accumulation. While only Cd was hyperaccumulated from the co-contaminated soils, the marigold did not absorb Cu and Pb effectively. Most of the benzo[a]pyrene dissipation (79.2% - 92.4% and 78.2% - 92.9%) was due to plant-promoted biodegradation in single benzo[a]pyrene and metal–benzo[a]pyrene contaminated soils. Consequently, they concluded that marigold can be a good remedial option for benzo[a]pyrene and benzo[a]pyrene- sites contaminated with Cd.

The phytotoxicity effects in co-contaminated soil can be reduced by the addition of hormones that enhance the tolerance mechanisms of the plant. This possibility was tested by Ahammed et al. (2013) when they explored the interactions of Cd and phenanthrene in the phytoremediation of co-contaminated soil with tomato plants. According to their findings, the application of Cd alone was more phytotoxic than the application of phenanthrene alone; but, the combined application of Cd and phenanthrene resulted in improved photosynthetic activity when compared to the single Cd contaminated soil. They suggest that the application of
brassinosteroids (a plant hormone related to tolerance mechanisms for a number of abiotic stresses) can reduce phytotoxicity in co-contaminated soil as it stimulates the plant’s natural defense mechanisms against cellular stress.

In addition to variations in contaminant removal due to interactions between mixed contaminants, further complications arise from interspecies variation in metal tolerance, uptake and organic contaminant degradation. This is especially true for poplars in the *Salix* family, which are often favored for groundwater remediation due to high growth and transpiration rates and a demonstrated ability to remove both metal and organic contaminants (Zacchini et al., 2009; Castiglione et al., 2009; Marmiroli et al., 2011). For example, Huang et al. (2011) investigated the phytoremediation potential of *Ricinus communis* (castor oil plant) grown from seed in soil spiked with 2.8 mg/kg Cd and 1.7 mg/kg DDT for two months. Their experiment compared the ability of 23 genotypes of *R. communis* to mobilize and take up Cd and DDTs. The total uptake of DDTs varied from 83.1 to 267.8 μg/pot and the total uptake of Cd varied from 66.0 to 155.1 μg per pot. There were significant variations among the accumulation of Cd and DDT across the genotypes, but they concluded that the bio-energy crop *R. communis* can be considered as a plant species that accumulates DDTs and Cd.

An additional aspect in phytoremediation of co-contaminated soils is the possible benefits of using several species in the same treatment. Many studies suggested certain plants can accumulate heavy metals and other species can enhance the degradation of heavy metals. Based on that, intercropping of plant species is a possible option to remediate mixed contaminated soil. Lee et al. (2007) studied four different plant species: *Echinochloa crusgalli* (barnyard grass), *Helianthus annuus* (sunflower), *Abutilon avicennae* (Indian mallow), and *Aeschynomene indica* (Indian jointvetch), in single plant cultures and mixed plant cultures on soil co-contaminated
with 10 mg/kg Cd, 1100 mg/kg Pb, 90 mg/kg Zn, 30 mg/kg Cu, and 50 mg/kg 2,4,6-trinitrotoluene. In single culture, germination rates ranged from 92% for *H. annuus* to 38% for *A. indica*, whereas in the four plant mixture, seed germination was less than 20% for all the four species. The growth rates of *E. crusgalli* and *H. annuus* were higher in the four-plant mix whereas *A. avicennae* and *A. indica* grew much slower in the 4-plant mix culture. *A. avicennae* and *E. crusgalli* accumulated more Cd than the other two species. Pb concentrations were comparatively higher in *A. avicennae* and *H. annuus*. All plant species removed TNT and its metabolites, both in single and mixed cultures. Results suggested that single plant cultures are better than mixed plant cultures for soil co-contaminated with Cd, Pb and TNT.

Wu et al. (2012) found a positive effect of intercropping for the removal of Cd, Cu and PCB from the soil. The authors tried both pot experiment and field trials that used different cropping patterns to remediate an aged soil that was contaminated with Cd, Cu and PCB. The initial contaminant concentrations were $7.67 \pm 0.51$ mg/kg Cd and $369 \pm 1$ mg/kg Cu in the pot experiment, and $8.46 \pm 0.31$ mg/kg Cd, $468 \pm 7$ mg/kg Cu and $323 \pm 12$ μg/kg PCBs for the field experiment. Different treatments were established with single plant and mixed cropping patterns of *Sedum plumbizincicola*, *Elsholtzia splendens*, *Medicago sativa*, and *Houttuynia cordata*. Some trials were also conducted with lime amended soil. *Sedum plumbizincicola* showed pronounced Cd phytoextraction in the pot experiment and significantly lowered the Zn and Cd concentrations compared to the unplanted control in a monoculture and when intercropped with *E. splendens*. In these two treatments, the Cd removal rates were $52.2 \pm 12.0\%$ and $56.1 \pm 9.1\%$, respectively. Soil PCBs in pots decreased from $323 \pm 11$ μg/kg to $49.3 \pm 6.6$ μg/kg, but with no significant difference between treatments. Intercropping of these three plant species in the field microcosm experiment reduced the yield of *S. plumbizincicola*, with a
consequent decrease in soil Cd removal. The highest shoot Cd uptake (18.5 ± 1.8 mg/pot) after 6 months post-planting was observed for *S. plumbizincicola* intercropped with *E. splendens*. Adding lime to the *S. plumbizincicola* intercropped with *M. sativa* considerably improved the PCB degradation by 25.2%. Overall, amendments with lime to adjust soil pH to 5.56 combined with intercropping with *S. plumbizincicola* and *M. sativa* produced the best removal rates of Cd, Cu and PCBs.

In a recent study, Lu and Zhang (2014) also conducted phytoremediation studies by intercropping to two plant species: the hyperaccumulator (*Sedum alfredii*) and BDE degrader tall fescue (*Festuca arundinaceae*) associated with a BDE degrader (*Bacillus cereus* strain JP12). The experiment soil was co-contaminated with decabromodiphenyl ether (BDE-209) and heavy metals (Cd, Pb and Zn). The plants were grown in a monoculture and in intercropping for 120 days under greenhouse conditions. They measured the plant biomass, concentration of BDE, density of soil bacteria, soil enzyme activity, and physiological profile of the soil microbial community. The results showed that the inoculation with JP12 significantly increased the dissipation of BDE-209 in the soil. Due to the improved plant growth, the phytoextraction of metals was also enhanced by the JP12 inoculation. Compared to unplanted soil, soil planted with tall fescue had enhanced BDE-209 dissipation due to the increased soil microbial activity. The Pb phytoextraction was more successful with the tall fescue when compared to the *S. alfredii*, but the Pb was principally retained in the roots of tall fescue. The highest levels of BDE-209 dissipation and metal phytoextraction were observed when the intercropped *S. alfredii* and tall fescue were inoculated with the strain JP12. The microbial community analysis showed that the inoculated JP12 could functionally adapt to the soil against competition with the indigenous microorganisms in soil. They concluded that intercropping *S. alfredii* with tall fescue combined
with the BDE-degrading bacterial strain JP12 is a promising approach for remediation of soil co-contaminated with BDE-209, Cd, Pb, and Zn.

2.5 Methods for Enhancing Phytoremediation

The phytoremediation of contaminated soil with heavy metals or organics or a combination of the two types of contaminants can be enhanced with strategies that increase contaminant mobility and bioavailability (surfactants or chelating agents), increase overall plant growth (and thus uptake capacity) via nutrient amendments or management strategies (e.g. irrigation), or by genetic modifications to the plant or the associated rhizosphere or endophytic microorganisms that increase contaminant tolerance and accumulation or degradation by the plant (Karenlampi et al., 2000; Kotrba et al., 2009).

The amount of metals that a plant is able to accumulate can be improved with procedures that increase their metal-tolerance. In the case of organic contaminants, a reduction in phytovolatilization can be accomplished by genetic modification that will enhance the degradation of organics at the same time. Inoculation with engineered endophytic bacteria is another alternative to enhance the degradation in the rhizosphere (Weyens et al., 2010, 2011). Further, when coupled with the manipulation of soil conditions via chemical treatments, plant uptake can even be increased in non-hyperaccumulator plant species, which enables the use of high-biomass crops for metal uptake (Sheoran et al., 2012). Some of the significant studies that involve soil amendments are summarized in Table 2.4.
Table 2.4: Soil Amendments Used for Enhanced Phytoremediation

<table>
<thead>
<tr>
<th>Amendment</th>
<th>Contaminants / Plant</th>
<th>Reference(s)</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Amendments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEDTA, DTPA, EDTA, EDDHA, EGTA</td>
<td>Pb, Pea, Corn, Sunflower</td>
<td>Huang et al. (1997)</td>
<td>After 0.5g/kg of HEDTA, not much increase in accumulation.</td>
</tr>
<tr>
<td>EDTA, NTA</td>
<td>Cd, Poplar and Willow</td>
<td>Robinson et al. (2000)</td>
<td>Agents caused temporary increase in uptake. EDTA and NTA reduced growth with amendments</td>
</tr>
<tr>
<td>EDTA</td>
<td>Cd, Cu, Fe, Mn, Ni, Pb, Zn, Barley</td>
<td>Madrid et al. (2003)</td>
<td>EDTA mobilized Cd, Fe, Mn, Ni, Pb, and Zn</td>
</tr>
<tr>
<td>EDTA, oxalic, citric and malic acid</td>
<td>Cu, Zn, Pb, Cd Indian Mustard</td>
<td>Wu et al. (2004)</td>
<td>EDTA significantly enhanced the mobility of soil Cu and Pb, but not of Zn and Cd.</td>
</tr>
<tr>
<td>EDTA and EDDS</td>
<td>Zn, Cu, Cd, Pb, Ni Field mustard, <em>Cannabis sativa</em>, Sunflower and <em>Zea mays</em></td>
<td>Meers et al. (2005)</td>
<td>The mobilizing effects induced by EDTA in the soil were found to be too long-lived for application as a soil amendment</td>
</tr>
<tr>
<td>Tween 80, Brij35, sodium dodecyl sulfate, cetyltrimethylammonium bromide</td>
<td>Phenanthrene, Pyrene, Ryegrass</td>
<td>Gao et al. (2006; 2007)</td>
<td>Tween 80, Brij35 (&lt; 74.0 mg /l) effective at low concentrations. high concentration less effective. SDS, CTMAB are ineffective and phytotoxic.</td>
</tr>
<tr>
<td>Sorbitan trioleate, salicylic acid and histidine</td>
<td>Ni, phenanthrene, chrysene, and Candargy</td>
<td>Singer et al. (2007)</td>
<td>Histidine extractable Ni showed high correlation with phytoextractable Ni.</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------</td>
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<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Tween 80</td>
<td>Phenanthrene, Pyrene Tall Wheat Grass</td>
<td>Cheng et al. (2008)</td>
<td>Tween 80 increased removal of pyrene but not phenanthrene. Maximum Pyrene removal at 100 mg/kg tween 80.</td>
</tr>
<tr>
<td>GA₃ and Tween 80</td>
<td>Cd, benzo[a]pyrene</td>
<td>Sun et al. (2013)</td>
<td>Maximum contamination removal when Soil amended with GA₃ and Tween 80</td>
</tr>
<tr>
<td>Tween 80, Triton X-100</td>
<td>Cd, Pb, Engine Oil Indian Mustard</td>
<td>Ramamurthy and Memarian (2012)</td>
<td>Tween 80 effective for improved remediation of mixed metal (Pb, Cr) and petroleum contaminants (engine oil).</td>
</tr>
<tr>
<td><strong>Organic Amendments</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken Manure, Urea</td>
<td>Cd <em>Solanum nigrum</em></td>
<td>Wei et al. (2010)</td>
<td>Extraction increased due to increased biomass.</td>
</tr>
</tbody>
</table>
2.5.1 Chelate Assisted Phytoremediation

Chelating agents are soluble chemicals that are able to bind and mobilize other molecules (including both metals and several organic contaminants) into the soil solution, increasing their availability for plant uptake and root-to-shoot translocation (Huang et al., 1997; Evangelou et al., 2007). Both natural and synthetic chelators are available, though their effectiveness varies between plant and soil types. Ethylene diamine tetraacetic acid (EDTA) is a commonly used synthetic chelating agent used to increase plant metal uptake since it forms stable chelates with most of heavy metals (Evangelou et al., 2007; Wu et al., 2007; Huang et al., 1997). Others include hydroxylethylene diamine tetraacetic acid (HEDTA), diethylene triamino pentaacetic acid (DTPA), trans-1,2-cyclohexylene dinitrilolo tetraacetic acid (CDTA), ethylene bis[oxyethylenetrinitrilo] tetraacetic acid (EGTA), ethylenediamine- N, N’bis (o-hydroxyphenyl) acetic acid (EDDHA), N-(2-hydroxyethyl)iminodiacetic acid (HEIDA), and N,N’- di(2-hydroxybenzyl) ethylene diamine N,N’-diacetic acid (HBED).

Natural chelating agents such as ethylene diamine disuccinate (EDDS), nitrilotriacetic acid (NTA), citric acid, oxalic acid, and malic acid are often preferable to synthetic ones due to their lower toxicity and lifetime in the soil. The fate of the chelating agent and toxicity to plant and soil microorganisms after its application are highly significant factors in selecting the appropriate chelating agent. It should not persist in the soil system for a long time without degradation because if it does remain, the risk of heavy metal migration in the subsurface with the possible dispersion into and contamination of groundwater is high (Evangelou et al., 2007).

Huang et al. (1997) investigated the effectiveness of chelates in Pb contaminated soils to increase Pb accumulation in plants. Chelates were very effective since the concentrations of Pb in the shoots of Zea mays (corn) and Pisum sativum (pea) increased from less than 500 mg/kg to
more than 10,000 mg/kg after the addition of chelates. The rise in phytoaccumulation was directly caused by the increase in Pb bioavailability in the soil solution due to the addition of chelates. The effectiveness of different chelating agents was EDTA > HEDTA > DTPA > EGTA > EDDHA. Furthermore, the EDTA significantly increased the Pb translocation from roots to shoots. The Pb concentration in the corn xylem sap increased 140-fold and the net Pb translocation from roots to shoots increased 120-fold as compared to the control without EDTA, all within 24 hours after the application of the EDTA solution (1.0 g of EDTA/kg of soil) to the contaminated soil. Their results indicate that the use of chelates augmented the Pb desorption from soil to soil solution, facilitated Pb transport into the xylem and increased Pb translocation from roots to shoots, all of which are important factors in phytoaccumulation.

A greenhouse pot-based experiment and a laboratory leaching column experiment were conducted by Wu et al. (2004), who studied the EDTA enhanced phytoextraction of heavy metals by Brassica juncea (Indian mustard) in pots and leaching columns. They added 3 mmol/kg of EDTA to pots of a paddy soil polluted with Cu (169 mg/kg total Cu) and spiked with Zn (500 mg/kg), Pb (500 mg/kg) and Cd (50 mg/kg). The application of EDTA significantly enhanced the mobility of the Cu and Pb, but not Zn and Cd. The concentrations of Cu and Pb in the shoots of the plants were also boosted by the EDTA, but the uptake rates were too low for an effective remediation of the soil. The authors estimated at least 200 crops would need to be planted over time to remediate the soil. They also investigated the addition of oxalic, citric and malic acid to the soil at the same rate (3 mmol/kg), but with virtually no effect on uptake of the metals by B. juncea. Rainfall was simulated in the column leaching experiments, which showed that the concentrations of Cu, Zn, Pb, and Cd in the leachate increased linearly as the dosage of EDTA increased. Soil macronutrients, including Fe, were lost due to the application of EDTA. Since the
shoot uptake of Pb and Cu were low and the chelating agent posed the threat of groundwater pollution, the study concluded that chelate-assisted phytoremediation was unsuitable for the combination of pollutants in the soil used in the study and the Indian mustard, especially during periods of high rainfall.

Chigbo and Batty (2013) evaluated the role of single and combined applications of chelates to single or mixed contaminated soils where the contaminants were Cr and benzo[a]pyrene (B[a]P). After growing *Medicago sativa* in this contaminated soil, the soil was amended with 0.3 g citric acid, 0.146 g EDTA or their combination for 60 days. In the Cr contaminated soil, the application of EDTA + citric acid significantly decreased the shoot dry matter of *M. sativa* by 55%, so this also decreased the Cr removal potential from the soil. Even though the soluble Cr concentration in single Cr or Cr + B[a]P contaminated soil was enhanced with the amendment with all chelates, only the application of citric acid to the Cr contaminated soil (44%) or EDTA and EDTA + citric acid in co-contaminated soil improved the removal of Cr (34% and 54%, respectively). The dissipation of B[a]P was effective even without plants or amendments in the single B[a]P-contaminated soil. In the co-contaminated soil, the B[a]P dissipation improved with the application of either EDTA or EDTA + citric acid.

Overall, several studies have demonstrated that the addition of chelating agents increased the potential for metal mobility as well as leaching into the subsurface. The high risk for contaminant remobilization and the persistence of certain synthetic chelating agents in the environment necessitate the careful selection of the proper chelating agent for the particular soil and plant type employed. Natural chelating agents such as NTA, EDDS and citric acid are much less harmful to the environment than synthetic agents (Alkorta et al., 2004). NTA has high biodegradability and good chelating strength (Bolton et al., 1996). Kos and Lestan (2003)
indicated that the use of a biodegradable chelating agent like EDDS might allow environmentally safe chelate-induced Pb phytoextraction. However, given the risks of metal remobilization via chelating agents, the addition of these chemicals should be carefully evaluated on a case by case basis before field implementation is pursued.

2.5.2 **Surfactant Enhanced Phytoremediation**

Surfactants are a group of natural and synthetic chemicals that can dissolve, desorb, solubilize, and/or emulsify poorly soluble substrates (Mulder et al., 1998; Noordman et al., 2002), and can be used to remediate both organic and metal contamination (Miller, 1995), but their main application is to raise the solubility and bioavailability of hydrophobic organics. Mass transfer and the rate of plant uptake are the major factors that limit the phytoremediation of HOCs (Gao et al., 2007). In phytoremediation, surfactants can assist the mobilization of contaminants into the soil solution where they are more accessible and easily accumulated by plants. However, there are some possible negative effects of surfactant use, such as surfactant phytotoxicity and preferential biodegradation of the surfactant itself by soil microflora (Volkering et al., 1998). The toxicity of surfactants can be minimized by the use of biosurfactants: surfactants produced by plants, animals and many microorganisms. Their merits over synthetic surfactants include biodegradability, cost effectiveness and the potential for in situ production (Miller, 1995).

Differences in improvements to plant uptake and the biodegradation of HOCs among types of surfactants were illustrated by Gao et al. (2007), who showed that the phytoremediation of pyrene contaminated soil was significantly enhanced by amending the soil with low concentrations of nonionic surfactants, like polyoxyethylene sorbitan monooleate (Tween 80) and polyoxyethylene(23)dodecanol (Brij35). However, the anionic surfactants (sodium dodecyl
sulfate, SDS) and the cationic surfactants (cetyltrimethylammonium bromide, CTMAB) were phytotoxic, which led to much lower removal rates. They concluded that surfactants can stimulate the microbial biodegradation of the HOCs and promote the plant uptake of HOCs.

Ramamurthy and Memarian (2012) studied the influence of non-ionic surfactants Triton X-100 and Tween 80 on the removal of mixed contaminants (Cd, Pb and used engine oil) from sandy soil by *B. juncea* (Indian mustard) in a greenhouse study. After 30 days of growth, surfactants were applied to the test pots where the soil had been spiked with 50 mg/kg of CdCl₂, 500 mg/kg of PbCl₂ and 500 mg/kg of used engine oil. Control tests were also conducted; one set consisted of unplanted pots of the contaminated soil without surfactants to demonstrate natural attenuation, and the other consisted of planted pots with contaminated soil, but without surfactants. Tests used different concentrations of the surfactants, and the results showed that Triton X-100 and Tween 80 enhanced the Cd and Pb accumulation in the plant roots; better results were obtained when the surfactant concentrations were above the critical micelle concentration. The Cd contaminants, but not the Pb, were translocated to plant shoots. When applied at equal concentrations, Tween 80 was more effective than Triton X-100 in facilitating the rhizodegradation of used engine oil. This demonstrated that the phytoremediation of mixed contaminated soil can be enhanced with the non-ionic surfactant Tween 80. Leaching test results indicated that the enhanced phytoremediation could remove the mixed contaminants safely without causing groundwater contamination.

Another greenhouse study examined the uptake of phenanthrene and pyrene from an aqueous solution containing the nonionic surfactant Tween 80 by the *Trifolium pretense* L (red clover) plant (Gao et al., 2008b). The mixture of 1.0 mg/l of phenanthrene and 0.1 mg/l of pyrene were amended with a wide range of Tween 80 concentrations (0–105.6 mg/l). At the test
concentration, Tween 80 did not cause any apparent phytotoxicity to the plant. Below a concentration of 13.2 mg/l, Tween 80 improved the plant uptake based on the concentrations of the two PAHs. Still, at higher concentrations, Tween 80 inhibited the uptake of both PAHs. The maximum plant uptake was observed at a Tween 80 concentration of 6.6 mg/l. With the increase in the concentration of the Tween 80, the total mass removal of phenanthrene and pyrene by T. pretense root or shoot increased initially and then decreased. This study concluded that surfactants can effectively enhance phytoremediation of PAH contaminated soils.

The combination of surfactants with amendments to boost plant growth was investigated by Sun et al. (2013). They conducted pot experiments to evaluate the effectiveness of GA\(_3\) (a vegetable hormone that can promote plant growth) and Tween 80 as soil amendments for enhanced phytoremediation of soil co-contaminated with Cd and benzo[a]pyrene using Tagetes patula (marigold). The co-contaminated soil used contained 200 mg/kg of Cd and 5 mg/kg of benzo[a]pyrene. One experimental set consisted of three levels of single GA\(_3\) treatment (1, 3 and 5 mmol/kg), while another consisted of three combined treatments of GA\(_3\) and Tween 80 (1 mmol GA\(_3\)/kg + 5 mmol Tween-80/kg, 3 mmol GA\(_3\)/kg + 5 mmol Tween-80/kg and 5 mmol GA\(_3\)/kg + 5 mmol Tween-80/kg). Treatments included one control set without GA\(_3\) and Tween 80. The plants were harvested 92 days after planting and analyzed for the Cd and benzo[a]pyrene contents in their roots and shoots. Treatment with GA\(_3\) and GA\(_3\)+Tween 80 enhanced the plant growth by 14% to 32% and 23% to 55%, respectively, relative to the control plants. However, Cd and benzo(a)pyrene (B[a]P) concentrations in the shoots decreased by 15% to 33% and 15% to 53%, respectively, compared with control, under independent GA\(_3\) treated soils. The accumulation of Cd and B[a]P in the shoots under the GA\(_3\)+Tween 80 treatment increased by 1.33 to 1.55 times, compared with those of the control treatment. Phytoextraction efficiency here
is quantified in terms of the remediation factor (RF), which is the ratio of element accumulation in roots to that found in the soil. The maximum removal of Cd from the soil was achieved under the combined 5 mmol/kg Tween 80 and 1 mmol/kg GA₃, in which case the RF was highest (5.21%). The best removal rate of B[a]P was obtained with the application of 5 mmol/kg Tween 80 and 5 mmol/kg GA₃, in which case the residual B[a]P concentration in soil was 0.37 mg/kg. These results support the use of combined treatments in co-contaminated soils to bolster plant health and improve both phytoextraction and phytodegradation.

2.5.3 Bacterial Endophyte Enhanced Phytoremediation

The enhancement of the rhizodegradation of organics by endophytic bacteria and fungi that exist within the plant root zone is gaining interest as a potential phytoremediation enhancement strategy. If the contaminants are degraded in the rhizosphere, the amount of pollutants taken up by the plant is reduced, avoiding possible phytotoxicity and limited plant growth, as well as reducing the possible volatilization of the chemicals through the plant leaves. Thus, the risks associated to inhalation and remobilization of contaminants upon plant death is minimized.

The enhanced rhizodegradation can be achieved via the stimulation or inoculation of plants with natural or engineered endophytic bacteria that favor the degradation of organic contaminants, as well as aid in the bioavailability of some contaminants by extending the reach of the root zone and secreting organic acids and enzymes that can enhance local mobility. One successful demonstration of endophyte-enhanced rhizodegradation of toluene was conducted by Barac et al. (2004). They introduced engineered endophytic bacteria, which degraded toluene and resulted in a marked decrease in its phytotoxicity as well as a 50-70% reduction of its evapotranspiration through the leaves. This strategy improves the efficiency of phytoremediation.
when treating soil for volatile organic contaminants.

Similar improvements were noted with other organic contaminants by Germaine et al. (2006), who studied bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid, which is widely used throughout the world as a herbicide for the control of broad-leaf weeds. This compound is particularly toxic to some other broad-leaved plants, such as poplar and willow, which are often used in phytoremediation projects. In their test for a solution to this problem, P. sativum (pea) was inoculated with a genetically tagged bacterial endophyte that naturally possessed the ability to degrade 2,4-dichlorophenoxyacetic acid. The bacterial strain colonized both the plant and rhizosphere, leading to a significant increase in the plant biomass relative to the control. They observed a higher rate of removal of 2,4-dichlorophenoxyacetic acid in the inoculated plants without any contaminant accumulation in the aerial tissues of inoculated plants. This study demonstrates the effectiveness of bacterial endophytes in enhancing phytoremediation of herbicide contaminated soil and groundwater.

Research efforts were made by Weyens et al. (2010) to reduce the effects of phytotoxicity and evapotranspiration by inoculating Lupinus luteus (yellow lupine) plants with engineered endophytic bacteria where the soil was contaminated with Ni and trichloroethylene (TCE) for trichloroethylene degradation and Ni resistance/sequestration system. After 14 days of growth in pots filled with sand, the plants were supplied with 40 mg/L NiSO₄ and 10 mg/L TCE. The plants were harvested after 2 additional weeks. The root mass of the inoculated plants increased by 30% indicating decreased phytotoxicity. The inoculated plants showed a decreasing trend for TCE evapotranspiration when compared to un-inoculated controls. The inoculated plants also had 5 times higher Ni uptake than the control plants: the Ni concentrations in the roots were
approximately 7-fold higher and the concentrations in the shoots increased 5-fold in the inoculated plants than in the control plants. This study confirmed that engineered bacteria can be equipped with mechanisms that will successfully degrade metal tolerance and organic contaminants and yield promising results for application at mixed contaminated sites.

A related study found similar improvements in Ni uptake in co-contaminated soil that contained Ni and toluene after the root zone was inoculated with engineered endophytic bacteria, and using Lupinus luteus (Weyens et al., 2011). The plants were exposed to 0, 0.05 or 0.25 mM NiSO₄, and 0, 250 and 500 mg/L toluene solutions. Ni concentrations in the roots rapidly increased with the increase of Ni in soil, while the concentrations found in the shoots were similar in all plants exposed to Ni via the soil. The Ni concentration varied from approximately 1000 to 3000 mg/kg biomass in roots and 200 to 500 mg/kg biomass in shoots. The amount of toluene that evapotranspired from the shoot varied from approximately 50 to 200 μg. The interesting result of this study is that the inoculation with some strains of bacteria considerably reduced the evapotranspiration and increased the Ni uptake. Although further research to identify the most effective inoculum for different plant and contaminants is suggested by the authors, these results suggest that engineered endophytes can help the host plant to deal with co-contamination of toxic metals and organic contaminants.

Pot-based experiments by Zhu et al. (2012) examined the combined effect of phytoremediation and bacterial inoculum in soil co-contaminated with Cd and DDT. Sedum alfredii, a known hyperaccumulator plant, was selected for the experiments. The pots, some with plants and others without, were prepared with and without microbial inoculums. Two soils were used: low Cd level (0.895 mg/kg) and high Cd level (3.225 mg/kg) soil. The initial concentration of DDT in the soil was 0.715 mg/kg. After six months, the plants were harvested and the soil,
roots and shoots were analyzed. The presence of microbes did not seem to affect Cd removal, which was 32\% and 39\% for the low and high Cd soils, respectively. The inoculation decreased the concentration of DDT in the soil by 27.5\% to 51.9\% compared to the un-inoculated control. The study demonstrated that even though the bacterial inoculum did not have any noticeable effect on the Cd extraction, it helped to increase the rhizodegradation of DDT, possibly by conferring protection against Cd toxicity to the plant. Overall, these studies illustrate that the combination of phytoremediation and bacterial inoculation is a promising approach for remediation of co-contaminated soils.

2.5.4 Enhancement of Phytoremediation by the Biomass Enhancement

Increasing the biomass of the plant through the use of fertilizers, compost or other amendments is a way of augmenting the efficiency and uptake capacity of phytoremedial systems. Increased plant growth leads to increased rates of uptake and organic contaminant degradation in the soil. Amendments used to increase the biomass include NPK fertilizer (Pichtel and Liskanen, 2001), chicken manure, urea (Wei et al., 2010), farmyard manure, and biochar (Hamzah et al., 2012). The application of vegetable (Sun et al., 2013) and plant hormones (Ahammed et al., 2013) to stimulate plant growth has shown promising results. The effects of biomass enhancement in phytoremediation of post-oil spill habitat restoration site soil were evaluated in an early study by Lin et al. (1998). Two years after application of the oil, plants were transplanted into oil contaminated and oil-free soil samples, with fertilizer applied 1 and 7 months after transplantation. They observed increased microbial numbers and activities in response to an increase in vegetation spurred by the fertilizer application, resulting in increased oil degradation. Several recent studies have confirmed the effectiveness of organic amendments for improved
growth, which is often incorporated with other technologies to improve remediation (e.g. Rentz et al., 2003; Willscher et al., 2013).

2.5.5 Combination of Phytoremediation with Other Technologies

Phytoremediation can be combined with certain other remediation technologies to optimize removal efficiency. Most of these strategies employ a coupled technology (e.g. chemical amendments or electrokinetic remediation) to increase the availability of contaminants to the plants used for phytoremediation. Electrokinetic remediation in conjunction with phytoremediation has shown significant promise for promoting heavy metal mobility and thus plant uptake (Cameselle et al., 2013), although its application for mixed contamination has been limited. Phytoremediation can also be used to clean up residual contaminants after the primary remedial treatment has been applied as a final ‘polishing’ step. Along with residual contaminant clean up, phytoremediation can help recover the soil structure and texture after physical or chemical treatment by providing organic nutrients and encouraging the growth of endophytic microorganisms in the rhizosphere.

2.5.6 Electrokinetic-enhanced Phytoremediation

Electrokinetic (EK) remediation has received recent attention as a means of increasing plant uptake of both inorganic and organic compounds, though the majority of studies coupling electrokinetics with phytoremediation have focused on enhancing plant uptake of heavy metals (Gomes et al., 2012). Evidence for increased plant growth and contaminant mobility in the presence of an electric field (e.g. Lemström, 1904) indicate a possible applicability for use with mixed contaminants (Cameselle et al., 2013). When an electric field is applied to the soil, the
movement of the pore fluid (electroosmosis), ions (electromigration) and colloids (electrophoresis) within the pore fluid can be induced, which allows greater metal accumulation in the rhizosphere and uptake by the plant as noted by Hodko et al. (2000). They suggested that the effectiveness of phytoremediation can also be improved if the soil is prevented from becoming strongly acidic or basic through the manipulation of the electric field. However, increased exposure to heavy metals due to the electric field may also increase the stress on the plants. As such, researchers have found that only plants that are able to tolerate high metal concentrations, (i.e. hyperaccumulator plants with rapid growth periods) are suitable for use in electrokinetic-enhanced phytoremediation (Bedmar et al., 2009).

Laboratory experiments of electrokinetic remediation and phytoremediation were combined to remediate two metal-polluted soils by O’Connor et al. (2003). One soil was contaminated with Cu (2500 μg/g), while the other sample was co-contaminated with Cd (300–400 μg/g) and As (230 μg/g). The test reactor had two chambers with a capacity of 5.25 kg soil each that were filled with either a mixture of the polluted soil and control topsoil (3:1) or topsoil alone. *Lolium perenne* (perennial ryegrass) was sown in the chambers and 30 V was continuously applied across the soil. Initial and terminal soil samples were taken for each test run (98 days for Cu soil, 80 days for Cd soil), and the foliage was sampled after about 3 weeks of growth and at the end of the full test period. Tests of the metal content in the soil and leaves revealed a significant redistribution of the metals from the anode to cathode after the electrokinetic treatment. Though no clear patterns in Cd uptake were noted, an upsurge in Cu uptake was observed in the cathode region. Significant soil acidification near the anode due to the EK treatment negatively affected plant growth, although the growth and soil pH were apparently unaffected elsewhere within the reactor. Further, no visual signs of metal toxicity
were noted in either polluted soil in response to the combined treatment, which indicates the feasibility of this approach for enhanced metal uptake with minimal oxidative stress on the plants.

The addition of an electric field around *B. juncea* (Indian mustard) plants in conjunction with a chelating agent (EDTA) to increase the uptake of lead was studied by Lim et al. (2004). The studies were conducted at different ranges of the parameters such as operating current/voltage, concentration, application time of EDTA, and electric potential. When 0.5 mmol/kg EDTA was used with electric potential, the accumulation of Pb in the shoots was 2-4 times higher than in the experiments where EDTA was used without electric potential. The optimal results were obtained with 5 mmol EDTA/kg, 30–40 V and an electric field application time of 1 h per day where the plants were harvested 9 days after the EDTA application. This shows that the combined procedure resulted in better remediation.

Zhou et al. (2007) studied the effect of direct current (DC) on metal uptake by *Lolium perenne* (rye grass) in greenhouse experiments. They combined phytoremediation with electrokinetic remediation and the application of EDTA or EDDS to strengthen the uptake of Cu and Zn from the contaminated soil. There were five treatment sets: 1) a control set without any chelator or applied voltage; 2) application of 5 mmol EDTA; 3) application of 5 mmol EDDS per kg of 0-20 cm surface soil; 4-5) one treatment with either EDTA or EDDS at 5 mmol/kg with 1 V/cm DC voltage. Soil solutions were sampled at three depths of the soil column (0-20, 20-40 and 40-60 cm bgs). The results showed that the combination of a chelating agent and the electric field had better phytoremediation results than the control or individual treatments. EDTA/EDDS application significantly increased the uptake of Cu and Zn as it increased the aqueous concentrations of each metal even though the increase in uptake was greater for Cu than for Zn.
The redistribution of Cu and Zn concentrations were due to the vertical electric field. The study also showed that better control over the leaching of Cu and Zn was achieved with the application of an electric field.

A set of laboratory-scale greenhouse experiments by Aboughalma et al. (2008) combined electrokinetic remediation and phytoremediation to decontaminate soil polluted with Zn, Pb, Cu, and Cd. *Solanum tuberosum* (potato tubers) were planted in plastic containers filled with contaminated soil. The study compared the application of alternating current (AC) versus DC: triplicate samples were treated with each electric field, in additional to an un-treated control set. The results showed that the soil pH varied from 3 near the anode to 8 near the cathode in the DC treated samples. They observed an accumulation of Zn, Cu and Cd at about 12 cm from anode when the soil pH was 5 in the DC treated soil. The biomass production of the plants was 27% lower with the DC treatment that the control. The AC treated samples showed no significant metal redistribution or pH variation between the anode and cathode regions, and the biomass production of the plants was 72% higher than the control. In both the AC and DC treatments, there was higher metal accumulation in the plant roots than was found in the control. Plants with AC treatment had a higher accumulation of metals in their shoots than those with DC treatment. Specifically, the Zn uptake in plant shoots that received the AC treatment was higher than in the control and DC treated plants. With both the AC and DC treatment, Zn and Cu accumulation in the plant roots had similar high values (>800 mg/kg of Zn and ~60 mg/kg Cu) relative to the controls (<600 mg/kg Zn and ~55 mg/kg Cu), though the increase in Zn uptake was more significant. The Cd content in plant roots, no matter what the treatment, was higher than that measured in the soil, while the Pb accumulation in the roots and the uptake into the shoots was lower than its content in the soil. Overall, even though differences existed among the metals, the
use of electrokinetics improved both the plant growth and metal removal.

The combination of phytoremediation and electrokinetic remediation to remediate soil contaminated with heavy metals was demonstrated in a laboratory experiment by Bi et al. (2011) using *Brassica napus* (rapeseed) and *Nicotiana tabacum* (tobacco). The three soils used for the experiments included contaminant-free soil from a forest area (S1), soil artificially contaminated with 15 mg/kg Cd (S2) and co-contaminated soil (Cd, Zn and Pb) from an industrial area (S3). The plants grown in containers with contaminated soil were subjected to three treatment conditions: AC electric field (1 V/cm), DC electric field (1 V/cm), or no electric field (control). The polarity of the DC electric field was switched every 3 hours to eliminate the known pH variation from the anode to cathode region. The electrical fields were applied for 30 days for rapeseed and 90 days for tobacco. The plants were harvested after a total growth of 90 days for rapeseed and 180 days for tobacco. The plants had different responses to the electric field. The rapeseed biomass was not affected significantly by the DC electric field, but under the AC treatment, its biomass increased. In the case of tobacco plant, its biomass was decreased under DC electric field, but did not evidence enhancement in the plants subjected to the AC electric field. In general, the metal uptake of rapeseed was higher in the AC treated samples, a point attributed to the increased biomass. The application of the DC electrical field was found to be unfavorable for plant growth. Better performance of electrokinetic assisted phytoremediation was found in the Cd contaminated soil where there were no other contaminants present than when multiple metals were present in the soil.

Cang et al. (2012) conducted laboratory experiments to study the combined effect of phytoremediation and electric potential application on a heavy metal contaminated soil. They monitored the changes in physicochemical properties and microbial activities of the soil. *B.*
*juncea* (Indian mustard) was grown in pots for 35 days and treated with a 0, 1, 2, or 4 V/cm DC electric field for 8 hours per day for 16 days. Another treatment consisted of application of an electric field of 2 V/cm to unplanted soil. Upon measurement after the treatment, the extractable concentrations of Cd and Zn had increased from cathode to anode, whereas the inverse was observed for Cu. Soil respiration and key enzyme activities (i.e. urease, invertase and neutral phosphatase) were negatively affected by the electric field, while the plant growth did help to minimize any drop in the enzymatic activity relative to the activity in the control plants. Thus, in some cases, electrokinetic remediation may negatively impact contaminant bioavailability (in this case, Cu) and soil microbial health, and so it is not appropriate for all metal or contaminant mixtures. Further work should be conducted with different contaminant mixtures using site-specific soils to better understand field-relevant geochemical interactions that may be stimulated using EK-enhanced phytoremediation.

### 2.6 Conclusions

Researchers have undertaken many studies that use the mixed contaminants typically found at hazardous waste sites. Overall, the observed interactive effects of these different contaminant mixtures and resultant phytoremedial efficiencies vary significantly due to the differences among site-specific soil types, geochemistry, plant species employed, and extent of the co-contamination. Consequently, it is difficult to identify any general trends in plant uptake and/or degradation in mixed contaminated soils. For instance, according Lin et al. (2006), when the PCP levels tested were 50 mg/kg, there was an increase in the Cu level enhanced PCP degradation; but conversely, under the initial or lowest PCP levels of 100 mg/kg, both the soil microbial activity and plant growth decreased with an increase in Cu, thus limiting the PCP degradation.
The effective enrichment of phytoremediation with amendments or coupled technologies is also dependent on the contaminant and soil chemistries (Ramamurthy and Memarian, 2012). In some studies, a combination of enhancement chemicals proved successful in the phytoremediation of co-contaminated soils (e.g. Sun et al., 2013). The use of engineered endophytic bacteria is another option for the reduction of phytotoxicity and volatilization (Weyens et al., 2010; 2011). Cherian and Oliveira (2005) suggest that transgenic plants may be a possible option for the remediation of sites co-contaminated with organic and inorganic contaminants. And, Zhang and Liu (2011) successfully engineered transgenic alfalfa plants that showed enhanced resistance to mixed contamination of heavy metal and organic pollutants. Given the sensitivity of plant growth and contaminant uptake or removal to soil geochemistry and contaminant interactions, contaminant-specific and site-specific investigations may be necessary to successfully implement phytoremediation at a particular mixed contaminated site. Coupling phytoremediation with soil amendments and other technologies (i.e. electrokinetic remediation) can improve plant survival and growth rates, and may be used to optimize the remediation process for recalcitrant contaminants in complex subsurface conditions.

2.7 Cited References


annuus) for metals in soils contaminated with zinc and lead nitrates.” Water Air and Soil Pollution, 207 (1-4), 195-201.


hyperaccumulator Thlaspi caerulescens in contaminated soils and its effects on the concentration and chemical speciation of metals in soil solution.” *Plant and Soil*, 197, 71-78.


3.1. Introduction

Soil contamination is a global problem. Most of the sites in question are adulterated by a mixture of organic and heavy metal contaminants. Even though many remediation methods are available, most of them decontaminate only a particular type of pollutant. So it becomes difficult to select a remedial strategy when the soil is co-contaminated with a mixture of organic and heavy metal contaminants. Commonly adopted remediation technologies for co-contaminated soils are expensive and energy intensive. So, it may be impractical for large sites with low to moderate contamination to use the common remedial strategies. In such cases, phytoremediation can be adopted as a viable option to remediate mixed contaminated sites. Phytoremediation is a passive and less expensive method that can address both organic and heavy metal contaminants. Phytoremediation is a technology that uses plants to degrade, extract, contain, or immobilize contaminants from soil and/or water. This technology is now considered as an alternative to more established treatment methods used at hazardous waste sites (EPA/600/R-99/107). Apart from being a green and sustainable method, visually pleasing appearance of planted sites makes phytoremediation a better option compared to other remedial strategies (ITRC 2009).

Plant species for phytoremediation are normally selected based on regional climate, root depth, and the nature of the contaminants. The approximate cleaning depths are 3 feet for grasses, 10 feet for shrubs and 20 feet for deep rooting trees (Sharma and Reddy 2004). For shallow contamination, grasses or shrubs may be preferable as trees take more time to grow and
establish. Nature of contaminants is another important factor to be considered while selecting the plant species since the remediation mechanisms are different for different types of contaminants. Organic contaminants are either accumulated in the plant tissue or degraded in the root zone (rhizodegradation) or in the plant tissue (phytodegradation). It can also be stored in the plant tissue as is or after being transformed to other forms (Pilon-Smits 2005). Metal contaminants are either transformed into harmless forms by phytostabilization or they are accumulated in the plant tissue by phytoaccumulation. The most successful plant species for the phytoremediation of heavy metals are high biomass plants, which can accumulate more contaminants by increased uptake of water, nutrients and contaminants. Hyperaccumulator plants can accumulate potentially phytotoxic elements to concentrations more than 100 times than those found in non-accumulators (Barman et al. 2000). These plants usually have strong metal sequestration mechanisms. The reason that hyperaccumulation occurs include the possible greater internal requirements for specific metals (Shen et al. 1997). Some plant species can mobilize metals from less-soluble soil fractions by producing chelating agents (McGrath et al. 1997). The phytoaccumulation of heavy metals may be hindered in the presence of organic contamination (Chen et al. 2004). Similarly, plant species that can degrade the soil organic contamination by stimulating soil microbes may not be able to perform if heavy metal contaminants are also present in the soil. Heavy metals can hinder organic contamination degradation in the soil by changing the microbial composition and action directly. Indirect effects can include the reduction of rhizosphere soil volume due to the reduced root biomass in presence of heavy metals (Lin et al. 2008). So for phytoremediation in a mixed contaminated site, it is important to carefully select the plant species that can tolerate and remediate contaminants that are physically and chemically different.
3.2. Background

“Hundreds of acres of some historically industrialized former wetland and grassland sites in Chicago have been found to be contaminated with a mixture of organic and heavy metals contaminants” (Chirakkara and Reddy 2014). The most common contaminants observed at many of these sites are naphthalene, phenanthrene, lead (Pb), cadmium (Cd), and chromium (Cr). Studies have been done of soils that are co-contaminated with heavy metals and organic contaminants (Table 3.1). Some of them compared the phytoremediation of heavy metals in presence of PAHs (Chen et al. 2004; Batty and Anslow 2008; Kobylecka and Skiba 2008) or the degradation of organic contaminants in presence of heavy metals (Lin et al. 2006). Some of these studies considered the dissipation of both the heavy metals and the organic contaminants in presence of plants (Palmroth et al. 2006; Lee et al. 2007; Singer et al. 2007; Zhang et al. 2009; Huang et al. 2011; Sun et al. 2011; Ramamurthy and Memarian 2012; Wu et al. 2012; Chigbo et al. 2013; Chigbo and Batty 2013; Hechmi et al. 2013; Sun et al. 2012; Hechmi et al. 2014; Lu and Zhang 2014).

Some studies have also compared different plants species for their efficiency in the remediation of mixed contaminated soils (Lin et al. 2006; Palmroth et al. 2006; Lee et al. 2007; Batty and Anslow 2008; Wu et al. 2012; Lu and Zhang 2014). However, those studies do not address the typical contaminants of current interest. Objective of this study is to compare the germination rate, growth rate and phytoremediation efficiencies of twelve selected plant species in soils that are polluted with a mixture of organic contaminants (naphthalene and phenanthrene) and heavy metals (Pb, Cd and Cr).
<table>
<thead>
<tr>
<th>Plant</th>
<th>Contaminants</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lolium (rye grass)</td>
<td>Cu, Zn and 2,4-dichlorophenol</td>
<td>Chen et al. (2004)</td>
</tr>
<tr>
<td>Lolium perenne L (rye grass) and Raphanus sativus (radish)</td>
<td>Cu and pentachlorophenol</td>
<td>Lin et al. (2006)</td>
</tr>
<tr>
<td>Pinus sylvestris (pine), Populus deltoides x Wettsteinii (poplar), Festuca rubra (red fescue), Poa pratensis (smooth meadow grass), Lolium perenne (rye grass) and Trifolium Repens (white clover)</td>
<td>organic and metal contamination from bus maintenance activities</td>
<td>Palmroth et al. (2006)</td>
</tr>
<tr>
<td>Alyssum lesbiacum (Candargy)</td>
<td>Ni, phenanthrene, and chrysene</td>
<td>Singer et al. (2007)</td>
</tr>
<tr>
<td>Echinochloa crusgalli (barnyard grass), Helianthus annuus (sunflower), Abutilon avicennae (Indian mallow), and Aeschynomena indica (Indian jointvetch)</td>
<td>Cd, Pb, and 2,4,6-trinitrotoluene</td>
<td>Lee et al. (2007)</td>
</tr>
<tr>
<td>Brassica juncea (Indian mustard) and Festuca arundinacea (tall fescue)</td>
<td>Zn and pyrene</td>
<td>Batty and Anslow (2008)</td>
</tr>
<tr>
<td>Triticum Aestivum L (wheat straw)</td>
<td>Zn, Cu, Mn, 2,4-dichlorophenoxyacetic acid, and 4-chloro-2-methylphenoxyacetic acid</td>
<td>Kobylecka and Skiba (2008)</td>
</tr>
<tr>
<td>Zea mays L. (maize)</td>
<td>Cd and pyrene</td>
<td>Zhang et al. (2009)</td>
</tr>
<tr>
<td>Lupinus luteus (yellow lupine)</td>
<td>Ni and trichloroethylene</td>
<td>Weyens et al. (2010)</td>
</tr>
<tr>
<td>Lupinus luteus (yellow lupine)</td>
<td>Ni and toluene</td>
<td>Weyens et al. (2011)</td>
</tr>
<tr>
<td>Ricinus communis (castor oil plant)</td>
<td>Cd and DDT</td>
<td>Huang et al. (2011)</td>
</tr>
<tr>
<td>Tagetes patula (marigold)</td>
<td>Cd, Cu, Pb, and benzo[a]pyrene</td>
<td>Sun et al. (2011)</td>
</tr>
<tr>
<td>Medicago sativa (Alfalfa)</td>
<td>Cd and trichloroethylene</td>
<td>Zhang and Liu (2011)</td>
</tr>
<tr>
<td>Brassica juncea (Indian mustard)</td>
<td>Cd and Pb and used engine oil</td>
<td>Ramamurthy and Memarian (2012)</td>
</tr>
<tr>
<td>Elsholtzia splendens, Houttuynia cordata, Medicago sativa (Alfalfa) and Sedum plumbizincicola</td>
<td>Cd, Cu, and polychlorinated biphenyls</td>
<td>Wu et al. (2012)</td>
</tr>
<tr>
<td>Plant Species</td>
<td>Chemicals</td>
<td>References</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>--------------------------------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td><em>Sedum alfredi</em></td>
<td>Cd and DDT</td>
<td>Zhu et al. (2012)</td>
</tr>
<tr>
<td><em>Solanum lycopersicum</em> (tomato)</td>
<td>Cd and phenanthrene</td>
<td>Ahammed et al. (2013)</td>
</tr>
<tr>
<td><em>Tagetes patula</em> (marigold)</td>
<td>Cd and benzo[a]pyrene</td>
<td>Sun et al. (2013)</td>
</tr>
<tr>
<td><em>Brassica juncea</em> (Indian mustard)</td>
<td>Cu and Pyrene</td>
<td>Chigbo et al. (2013)</td>
</tr>
<tr>
<td><em>Medicago sativa</em> (Alfalfa)</td>
<td>Cr and benzo[a]pyrene</td>
<td>Chigbo and Batty (2013)</td>
</tr>
<tr>
<td><em>Zea mays L.</em> (maize)</td>
<td>Cd and Pentachlorophenol</td>
<td>Hechmi et al. (2013)</td>
</tr>
<tr>
<td><em>Medicago sativa</em> (Alfalfa)</td>
<td>Cd and trichloroethylene</td>
<td>Zhang et al. (2013)</td>
</tr>
<tr>
<td><em>Phragmites australis</em></td>
<td>Cd and Pentachlorophenol</td>
<td>Hechmi et al. (2014)</td>
</tr>
<tr>
<td><em>Sedum alfredi</em> and <em>Festuca arundinacea</em> (tall fescue)</td>
<td>Cd, Pb, Zn, and decabromodiphenyl ether</td>
<td>Lu and Zhang (2014)</td>
</tr>
</tbody>
</table>
3.3. Materials and Methods

3.3.1 Selected Plant Species

The plant species for the study were selected as per the literature review, based on biomass and phytoremediation efficiency for different contaminants. Sunflower (Lee et al. 2007), Indian mustard (Batty and Anslow 2008; Ramamurthy and Memarian 2012), marigold (Sun et al. 2011), white clover (Palmroth et al. 2006), rye grass (Chen et al. 2004; Lin et al. 2006), and tall fescue (Batty and Anslow 2008) were chosen since they had previously undergone studies on the remediation of soils co-contaminated by metals and organic contaminants. Oat plant (Ebbs and Kochian 1998; Miya and Firestone 2001) and alfalfa (Fan et al. 2008; Videa et al. 2002; Sheng-Wang et al. 2008) were successfully investigated for phytoremediation potential with heavy metals in some studies and organic contaminants in others. Field mustard (Meers et al. 2005), black nightshade (Wei et al. 2006) and green onions (Cho et al. 2009) were studied for phytoremediation of heavy metal contamination, only. Like other plants in the legume family (Dzantor et al. 2000; Palmroth et al. 2002), green gram is expected to rhizodegrade the organic contaminants in the soil.

*Helianthus annuus* (sunflower), *Brassica Juncea* (Indian mustard), *Brassica Rapa* (field mustard), *Tagetes patula* (marigold), *Avena sativa* (oat plant), *Lolium perenne* (perennial rye grass), *Festuca arundinacea* (tall fescue), *Medicago sativa* (alfalfa), *Trifolium repens* (white clover), *Vigna radiata* (green gram), *Allium fistulosum* (green onions) and *Solanum nigrum* (black nightshade) were the twelve plants selected for the study. Sunflower seeds were ordered from Carolina Biological Supply Company. Alfalfa and oat plant seeds were supplied by Seedville USA. Mustard seeds were obtained from Living Whole Foods. Green onion and
marigold seeds were obtained from Seed Needs. Green gram seeds were purchased from a local grocery store. Rye grass and white clover seeds were supplied by Sheffield’s Seed Company. Tall fescue seeds used were Amturf 77022 Ultra. Black nightshade seeds were obtained from Tropical Island Exotics.

3.3.2 Soil Selected
Grey silty clay, typical of the Chicago glacial till was selected for the pot experiments. Important physical properties of the soil are presented in Table 3.2.

3.3.3 Soil Spiking Procedure
Mixed contaminated soil was prepared by spiking the soil with naphthalene, phenanthrene, Pb, Cd, and Cr. The required amount of naphthalene and phenanthrene was measured and dissolved in hexane using magnetic stirrer. The hexane containing naphthalene and phenanthrene was mixed into the soil to get a final concentration in the soil of 50 mg/kg naphthalene and 100 mg/kg phenanthrene. The mixed soil was dried for 3 to 4 days in the fume hood. To ensure uniformity, the soil was mixed once every day during drying.

Quantities of PbCl₂, K₂Cr₂O₇ and CdCl₂. ½ H₂O were measured to create a final concentration of 500 mg/kg Pb, 200 mg/kg Cr and 50 mg/kg Cd in the soil. K₂Cr₂O₇ has oxidation state of six for Cr. These chemicals were mixed in water (to get approximate water content of 15% in soil) for one hour using magnetic stirrer. This solution was added to the soil previously spiked with naphthalene and phenanthrene and mixed well to ensure uniform contaminant distribution. All the chemicals used for spiking and analytical testing were purchased from Fischer Scientific.
Table 3.2: Important Properties of Soil Used for the Experiments

<table>
<thead>
<tr>
<th>Property</th>
<th>ASTM Standards</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil organic content</td>
<td>ASTM D 2974</td>
<td>2.4%</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>ASTM D 854</td>
<td>2.7</td>
</tr>
<tr>
<td>Water holding capacity</td>
<td>ASTM D 2980</td>
<td>46.4%</td>
</tr>
<tr>
<td>Porosity</td>
<td></td>
<td>0.55</td>
</tr>
<tr>
<td>pH</td>
<td>ASTM D4972</td>
<td>7.7</td>
</tr>
<tr>
<td>Liquid limit</td>
<td>ASTM D 4318</td>
<td>32.7%</td>
</tr>
<tr>
<td>Plastic limit</td>
<td></td>
<td>19.1%</td>
</tr>
<tr>
<td>Plasticity index</td>
<td></td>
<td>13.6%</td>
</tr>
<tr>
<td>Clay (&lt; 0.002mm)</td>
<td>ASTM D 422</td>
<td>41%</td>
</tr>
<tr>
<td>Silt (0.002 - 0.05mm)</td>
<td></td>
<td>43%</td>
</tr>
<tr>
<td>Sand (0.05 – 2 mm)</td>
<td></td>
<td>14.2%</td>
</tr>
<tr>
<td>USCS Classification</td>
<td></td>
<td>CL</td>
</tr>
<tr>
<td>USDA Classification</td>
<td></td>
<td>Silty clay</td>
</tr>
</tbody>
</table>
Uncontaminated soil was mixed with water (approximate water content of 15%) for the control experiments. Properties of control and contaminated soil at the time of seeding are given in Table 3.3.

Pots of 8 cm diameter and 9 cm height were filled with the prepared soil for seeding plants. Two control pots were prepared for each plant species. Ten contaminated pots were prepared for the sunflower and oat plants, five for rye grass and tall fescue, and two each for the Indian mustard, field mustard, marigold, alfalfa, white clover, green gram, green onion, and black nightshade plants. The seeds were placed approximately a half inch below the soil surface. To ensure that the leachate did not get mixed up, each pot was kept on separate trays. Out of the twelve species studied here, only white clover needed darkness for germination. To achieve this, the pots containing the white clover seeds were covered with a tray to provide darkness. The pots of white clover were kept uncovered once leaves started showing out.

The pots were placed under metal halide grow lights with an average light intensity of 400 μmols/m²/s. The lamps were hung ~ 12 inches above the plants to obtain the desired light intensity of 16 hours of light per day, which was provided using an automatic timer. The hanging height of the lamps was adjusted as the plants started growing taller to reduce heat stress. The temperature below the grow lights was measured as 25°C at the height of the plants. Fans were used to blow the hot air away from the plants.

3.3.4 Pots Setup and Monitoring

The plants were grown for 61 days in the laboratory and the growth was monitored. The positions of the pots were changed periodically to ensure uniform light intensity to all the pots.
Table 3.3: Measured Properties of Soil at the Time of Seeding:

<table>
<thead>
<tr>
<th>Property</th>
<th>Clean Soil</th>
<th>Contaminated Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.7</td>
<td>7.5</td>
</tr>
<tr>
<td>Oxidation reduction potential (mV)</td>
<td>-52.5</td>
<td>-40</td>
</tr>
<tr>
<td>Electrical conductivity (milli Siemens/cm)</td>
<td>0.127</td>
<td>0.218</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>16</td>
<td>15.5</td>
</tr>
</tbody>
</table>
The number of plants in each pot was counted and the plant height was recorded every week. Photographs recorded the plant growth and biomass production.

At the end of the growth period, shoots of the plants were cut at the soil surface. The roots were carefully separated from the soil. The roots, shoots and soil were dried in oven at 60°C for 6 days (until it attained constant weight). The dry weights of the roots and shoots were measured and noted as root biomass and shoot biomass. The soil samples were tested for metals and organic contaminants.

3.3.5 Analytical Testing

Tests of physical properties of the soil viz. water content (ASTM D2216), organic content (ASTM D2974), pH (ASTM D4972), and grain size (ASTM D422) were analyzed as per standards. The soil water holding capacity was determined by a method similar to the one used for saturated peat materials (ASTM D2980). For the heavy metal analysis, acid digestion of the soil samples was conducted per EPA method 3050B. The digested and filtered liquid was analyzed for Pb, Cd and Cr with Flame Atomic Absorption (FLAA) spectroscopy. To estimate the exchangeable metals in the soil, 8 ml of 1M sodium acetate solution was added to 1g of soil and mixed continuously for one hour (Reddy et al. 2001). The filtrate was analyzed for Pb, Cd and Cr with FLAA spectroscopy. The organic contaminants were analyzed by solvent extraction and analysis using Gas Chromatography, following EPA method SW8270C. To analyze exchangeable nitrogen, 1g of soil was mixed with 10 ml of 2M KCl solution, and then shaken for one hour (Xu et al. 2013). The filtered extractant was analyzed using Spectronic Genesys spectrophotometers, following the procedure given by Sattayatewa et al. (2011). To determine the exchangeable fractions of potassium and phosphorus, 1g soil was shaken with 1M
ammonium acetate for a period of one hour. That solution was filtered and the extractant was analyzed for phosphorus with Spectronic Genesys spectrophotometers, per Sattayatewa et al. (2011). Exchangeable potassium in the extractant was analyzed using FLAA.

Microsoft Office Excel 2007 was used for the means and standard deviations test results and t-tests. To check whether a significant difference exists between the result sets, t-test was with the alpha value taken as 0.05.

3.4. Results and Discussion
All the plant species showed delayed germination and reduced rate of germination, survival and growth in contaminated soil compared to the control plants. Figure 3.1 shows the percentage germination of the studied plants in contaminated and control soils. In this plot, germination is explained as the rise of a green shoot or leaf above the soil. The germination rate of all the plants was reduced by the soil contamination, but the extent of reduction varied by species. Different germination rates in different species can be explained based on the seed coat permeability of the plant species. Literature shows that seeds of some plants have highly permeable seed coats (Wierzbicka and Obidzinska 1998). For such seeds, the contamination can enter the embryos and affect seed germination. The negative effect of heavy metal on seed germination can be caused by either its general toxicity or by inhibition of water uptake (Kranner and Colville 2011). These facts imply that germination in contaminated soil depends both on the type of plant species and the contaminants. In the present experiments, when compared to the other plants, oat plant had a better germination rate in contaminated soil, followed by rye grass, tall fescue, sunflower and green gram. Green onion, alfalfa and Indian mustard had comparatively low germination rates in contaminated soil. No seeds were germinated in the
Figure 3.1. Percentage Germination of Different Plants in Clean Soil vs. Contaminated soil (SF-sunflower, IM-Indian mustard, FM-field mustard, M-marigold, OP-oat plant, RG-rye grass, TF-tall fescue, A-alfalfa, WC-white clover, GG-green gram, GO-green onion, SN-solanum nigrum)
contaminated soil for field mustard, marigold, white clover and black nightshade. Some germinated plants showed signs of stress, e.g. diminished growth and changes in color, and some of them eventually died.

All of the germinated plants failed to survive to the end of the experimental period. Some plants showed yellowish color and reduced growth, which are symptoms of phytotoxicity. Percentage survival, shown in Figure 3.2, is expressed as the number of surviving plants (green/live plants) from the number of seeds germinated. Out of the twelve plant species, only five species survived in the contaminated soil. Oat plant had a better survival rate, followed by tall fescue, green gram, sunflower and rye grass. Even though some seeds of Indian mustard, alfalfa and green onion germinated in contaminated soil, none survived. Since Indian mustard, field mustard, marigold, alfalfa, white clover, green onion and blank nightshade did not germinate and/or survive in contaminated soils; they were not considered for further growth and contamination removal studies.

The final (after growth period) plant height for the sunflower, oat, rye grass, tall fescue and green gram are presented in Figure 3.3. For oats, the average value of maximum plant height was reduced only by 16% in contaminated soil compared to the clean soil. The percentage reduction in maximum plant height for sunflower, rye grass, tall fescue and green gram were 69, 50, 55 and 38% respectively. Observations show that apart from the oat plant, all other plants had a considerable reduction in final maximum plant height when growing in contaminated soil compared to the clean soil.

The average root and shoot biomass of plants in clean soil and contaminated soil are given in Table 3.4. The sunflower plant had the highest value of root and shoot biomass in clean soil, but its biomass was reduced considerably in contaminated soil. The percent reduction of
Figure 3.2. Percentage Survival of Different Plants in Clean Soil vs. Contaminated soil (SF-sunflower, IM-Indian mustard, FM-field mustard, M-marigold, OP-oat plant, RG-rye grass, TF-tall fescue, A-alfalfa, WC-white clover, GG-green gram, GO-green onion, SN-solanum nigrum)
Figure 3.3. Final Maximum Plant Height for Clean Soil vs. Contaminated Soil (SF- sunflower, OP- oat plant, RG- rye grass, TF- tall fescue, GG- green gram)
Table 3.4: Average Root Biomass and Shoot Biomass of Plants in Clean and Contaminated Soil:

<table>
<thead>
<tr>
<th>Plant</th>
<th>Root Biomass (g)</th>
<th>Shoot Biomass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clean Soil</td>
<td>Contaminated Soil</td>
</tr>
<tr>
<td>Sunflower</td>
<td>1.29</td>
<td>0.46</td>
</tr>
<tr>
<td>Oat Plant</td>
<td>0.65</td>
<td>0.50</td>
</tr>
<tr>
<td>Rye Grass</td>
<td>0.49</td>
<td>0.15</td>
</tr>
<tr>
<td>Tall Fescue</td>
<td>0.44</td>
<td>0.10</td>
</tr>
<tr>
<td>Green Gram</td>
<td>0.80</td>
<td>0.20</td>
</tr>
</tbody>
</table>
total biomass of the plants in contaminated soil, compared to the same plants in clean soil, is presented in Figure 3.4. For the oat plant, the biomass was reduced by 32% in contaminated soil, while the percentage reductions of biomass were 78, 60, 79, and 68% for sunflower, rye grass, tall fescue and green gram, respectively. Except for oat plant, all plants had considerable reduction of biomass in contaminated soil. Based on the tolerance (% germination, % survival, growth and biomass) of the twelve plant species studied here for mixed contaminated conditions, oat plant performed better than all other species.

Summarizing the growth characteristics of the plants, it can be seen that the germination, survival, plant height, and final biomass were noticeably influenced by contamination in the soil. Reduced growth in contaminated soil can be due to the general toxicity of the contaminants or the lack of nutrients in the contaminated soil. To check the availability of nutrients, exchangeable nitrogen, phosphorus and potassium were tested (Figure 3.5). The exchangeable nutrient concentrations were not significantly different (p>0.5) for the control and contaminated soils. There was a slight, non-significant increase in exchangeable nutrient concentrations in the case of contaminated soils. This may be due to the preferential adsorption of heavy metals over nutrient ions (McLean and Bledsoe 1992). This implies that the poor performance of plants in contaminated soils is due to the phytotoxicity caused by the contaminants in the soil.

The phytotoxicity to the plants by the combined contamination condition can be due to the metals or organic contamination or the combination of both. Previous studies by researchers show the evidence for phytotoxicity by different heavy metals. Some heavy metals are essential and others are non-essential for plant growth. Small amounts of essential elements are required for the normal functioning of plants. But, essential micronutrients also affect germination and survival at relatively high concentrations (Kranner and Colville 2011). Pb, Cd and Cr are non-
Figure 3.4. Percentage Reduction of Total Biomass in Contaminated Soil (SF- sunflower, OP-oat plant, RG-rye grass, TF-tall fescue, GG-green gram)
Figure 3.5. Exchangeable Nutrients in Control and Contaminated Soil
essential metals that do not have any metabolic function in plants (Pahlsson 1989), but are toxic to plants. However, there are cases of growth stimulation reported in the literature that are due to slight increases in Cr levels. There studies also report that higher concentrations of chromium inhibit plant growth (Chandra and Kulshreshtha 2004). Organic contaminants can also be toxic to the plants, depending on the type and molecular weight of the contaminant. PAHs are capable of inhibiting plant germination and growth (Henner et al. 1999).

Table 3.5 shows the average values of pH, electrical conductivity and oxidation-reduction potential for the soil samples in this study. It can be observed that the pH value of the contaminated soil is less than the pH value of the control soil by approximately 0.3 pH units. A small change in soil solution pH can affect plant life (Islam et al. 1980) and the heavy metal mobility in the soil (McLean and Bledsoe 1992). For the soil system, pH is a very important parameter that can directly influence sorption/desorption, precipitation/dissolution, complex formation, and oxidation-reduction reactions, which are important in determining the contaminant transport through soils. Microbial activities responsible for the rhizodegradation and rhizostabilization of the contaminants are also dependent on the soil pH to a great extent (Atagana et al. 2003). It can also be seen from Table 3.5 that the planted contaminated soil showed pH values that are similar to that of the control soil. This means that the soil may be able to sustain better varieties of vegetation after a period of phytoremediation or phytorestoration using plants that are capable of surviving in contaminated soils. General trends were not observed for oxidation reduction potential and electrical conductivity values. It is expected that
Table 3.5: Average pH, Oxidation Reduction Potential and Electrical Conductivity Values for Clean and Contaminated Soil samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>ORP (mV)</th>
<th>EC (mS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clean Soil</td>
<td>Contaminated Soil</td>
<td>Clean Soil</td>
</tr>
<tr>
<td>Initial</td>
<td>7.8</td>
<td>7.5</td>
<td>-57.7</td>
</tr>
<tr>
<td>Sunflower</td>
<td>7.7</td>
<td>7.8</td>
<td>-48.2</td>
</tr>
<tr>
<td>Oat Plant</td>
<td>7.8</td>
<td>7.8</td>
<td>-48.2</td>
</tr>
<tr>
<td>Rye Grass</td>
<td>7.8</td>
<td>7.8</td>
<td>-52.3</td>
</tr>
<tr>
<td>Tall Fescue</td>
<td>7.8</td>
<td>7.9</td>
<td>-53.6</td>
</tr>
<tr>
<td>Green Gram</td>
<td>7.6</td>
<td>7.8</td>
<td>-45.7</td>
</tr>
</tbody>
</table>
reducing conditions in the soil can favor the accelerated migration of contaminants (McLean and Bledsoe 1992).

Figure 3.6 shows the heavy metal concentrations in the soil samples from unplanted and planted pots after the phytoremediation period. Only the samples from the pots planted with sunflower showed a significant reduction in Pb concentration (p<0.5) when compared to the unplanted pots. The reduction in Pb concentration by sunflower was approximately 29%. In the case of Cd concentrations, significant reductions were achieved by sunflower (18%) and green gram (7%). Significant Cr reduction was achieved by all the plant species studied. The percentage reduction of metals by different plants is shown in Figure 3.7. This shows that highest reduction of contaminant concentrations were achieved by sunflower compared to the other plants studied. Out of the three metals studied, the best removal by plants was achieved for Cr. This may be due to the higher bio availability of Cr, compared to other two metals at the present pH levels of the samples. Figure 3.8 reports the amount of contaminant removed per plant for all the species studied. This was also best achieved by sunflower. The magnitude of heavy metal reduction achieved per plant here are comparable with the same achieved in studies conducted by Li et al. (2014). From their experiments using two energy grasses, *Arundo donax* and *Miscanthus sacchariflorus*, they achieved Zn reduction of 17.5 and 12.1 mg per plant and Cr reduction of 3.9 and 2.9 mg per plant.

Figure 3.9 shows the exchangeable Pb, Cd and Cr in different soil samples. For all the metals, exchangeable metals were very low compared to the total metals in the samples (Figure 3.6). Exchangeable metal concentrations were highest in pots with oat plants, with values close to that of the samples in unplanted pots. Compared to the unplanted pots, exchangeable Pb reductions were 45% by sunflower, 92% by rye grass, 84% by tall fescue, and 100% by green
Figure 3.6. Total Concentration of Metals in Soil (None-no plants, SF-sunflower, OP- oat plant, RG- rye grass, TF- tall fescue, GG-green gram)
Figure 3.7. Percentage Reduction of Pb, Cd & Cr by Plants
Figure 3.8: Reduction of Heavy Metal Concentration Per Plant
Figure 3.9: Exchangeable Metals in Soil (NP-no plants, SF-sunflower, OP- oat plant, RG- rye grass, TF- tall fescue, GG-green gram)
gram. Exchangeable Cd reductions were 39% by sunflower, 18% by rye grass, 35% by tall fescue, and 29% by green gram. Exchangeable Cr was reduced only by sunflower (21%) and green gram (31%). It can be observed from Figure 3.9 that some planted samples have slightly higher exchangeable metal concentrations than the unplanted samples. This may be due to the secretion of organic ligands in the rhizosphere of the plants, which would have increased the metal solubility. Previous research findings suggest that plants can not only affect the solubility of heavy metals in the rhizosphere, but also change their species distribution in the soil solution (Chen et al. 2004).

In general, the exchangeable Cr concentrations were found to be higher in the solution compared to Pb and Cd. This indicates the preference in adsorption of different heavy metals in exchange sites of the soil particles. According to McLean and Bledsoe (1992), maximum retention of anionic metals (Cr) occurs at pH<7 and maximum retention of cationic metals (Pb & Cd) occurs at pH>7. The pH values of the soil samples can explain the higher availability of Cr in the soil solution compared to Pb and Cd. At a given pH level, there can be competitive sorption and desorption among the cationic metals present in the soil solution, and Pb tends to adsorb to the soil particle compared to Cd ions (Covelo et al. 2007). That may be the reason for the lower exchangeable Pb concentrations in spite of the high total Pb concentration in the soil.

In most of the cases, exchangeable metal concentrations are reduced as a result of plant growth. The possible reasons are reduction of total metal concentrations due to phytoextraction, and/or the immobilization of the contaminants by change in speciation of the metals. Since sunflower is observed to reduce the total metal concentrations considerably, the reductions in exchangeable metals in soil with sunflower plants are expected to be primarily due to the reduction of total metal concentration in the soil. But, rye grass, tall fescue and green gram did
not show any noticeable reduction in total concentrations of Pb and Cd. However, for these plants, there is a significant reduction of exchangeable concentrations of Pb and Cd. This shows that these plants have the capability to alter the species distribution of metals in the soil, indicating phytostabilization of the contaminants. After a period of growing these plants in the soil, a polluted site should be able to sustain more varieties of vegetation, and the contaminant migration from the soil to the ground water will be lesser due to plant-induced immobilization of the contaminants.

Figure 3.10 shows the PAH concentrations in the initial and final samples. The naphthalene concentration was found to be zero in almost all the samples. Even in the initial samples, naphthalene concentration was very low compared to the spiked concentrations, indicating volatilization and microbial degradation of naphthalene (Mozo et al., 2012). The phenanthrene concentrations in final unplanted pots were considerably lesser than the concentration of phenanthrene in initial samples, which again shows the biodegradation/volatilization of phenanthrene in the soil.

Rye grass, tall fescue and green gram each showed a noticeable reduction in soil phenanthrene concentration, which is an expected result of phytodegradation and/or plant promoted rhizodegradation. But, the pots planted with sunflower and oat had higher concentrations of phenanthrene than the unplanted pots. PAH degradation is also expected to be related with the root biomass of different plants since large and dense root systems and high level of degrading enzymes are favorable properties for phytodegradation (Pilon-Smits. 2005). However, the present results did not show any decreasing trend of phenanthrene concentration with increase in root biomass of the plants. This can be a concentration variation in different samples and the variation may not be significant if more samples are analyzed. Another
Figure 3.10: PAH Concentrations in Soil (NP-no plants, SF-sunflower, OP- oat plant, RG- rye grass, TF- tall fescue, GG-green gram)
explanation is the production of phytoalexins by the plants in stressed conditions (Bais et al. 2006). It may be the antimicrobial property of the phytoalexins, which act against the microbial biodegradation of PAHs. Similar results were obtained by Hechmi et al. (2013) where researchers observed higher pentachlorophenol concentration in planted soil compared to non-planted soil after the growth period. Experiments done by Corgie et al. (2004) also suggest that plants can either improve or inhibit biodegradation by affecting the spatial distribution of bacterial communities.

Even though sunflower plants could not enhance the degradation of the organic contaminants in the present study, sunflower is suggested as a promising plant species for phytoremediation of mixed contaminated soil considering its capability of removing heavy metals from the soil. Rye grass, tall fescue, and green gram can be considered for phytorestoration because these plants could lower the exchangeable metals in the soil and also enhance the microbial degradation of phenanthrene in soil.

3.5. Conclusions

The mixed contamination soil had significant effect on the growth characteristics of almost all the plants studied. All of the seeds showed delayed or reduced germination and survival rates in contaminated soil compared to the control. Oat had a better germination rate and growth characteristics in contaminated soil. Out of the 12 plant species studied, only five could survive until the end of the test period. Apart from the oat plant, all others that survived had a considerable reduction in the final maximum plant height and biomass when grown in contaminated soil. Even though the percentage germination was low for sunflower in the contaminated soil, sunflower plants that survived also seemed to have good growth. Based on
reduction of total heavy metals, sunflower performed better than all the other species. Exchangeable heavy metals were also considerably lower in pots with sunflower, compared to the unplanted pots. Sunflower and oat could not enhance the degradation of phenanthrene. Even though rye grass, tall fescue, and green gram were not effective in removing heavy metals from soil, they were successful in reducing the exchangeable metals in soil. Also rye grass, tall fescue, and green gram were found to enhance the microbial degradation of phenanthrene. Sunflower is suggested as a promising plant for phytoremediation of mixed contaminated soil considering its ability of remove heavy metals from mixed contaminated soils. However, soil amendments may be necessary in order to improve the germination and biomass of sunflower.

3.6 References


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*Water Air Soil Pollut.*, 223, 511-518.


CHAPTER 4

INTERACTIVE EFFECTS OF CO-CONTAMINANTS ON PHYTOREMEDIATION OF MIXED CONTAMINATED SOIL


4.1. Introduction

Organic and heavy metal contaminants co-exist in many polluted sites worldwide. The processes that lead to the release of organic and heavy metal contaminants into the soil include industrial operations and agricultural activities (Masciandaro et al. 2013). Various physicochemical processes are used to remediate co-contaminated soils (soil washing, solidification/stabilization, electrokinetic remediation, chemical oxidation, etc.). However, for large sites with shallow and moderate contamination, phytoremediation can be adopted as a green, sustainable option to remediate mixed contaminants (Reddy and Chirakkara 2013; USEPA 2000). Phytoremediation is a low cost solution when compared to the other expensive, energy intense methods.

For phytoremediation of mixed contaminants, the properties and interactions of the different contaminants as well as their interactions with the plant and rhizosphere are important. The plants that are selected for the remediation of mixed contaminated sites should be able to perform in the presence of mixed contamination and when the contaminants are present individually, as well. Naphthalene, phenanthrene, lead (Pb), cadmium (Cd), and chromium (Cr) are the common contaminants found at many of the sites. Individual and combined effects of
these organic and heavy metal contaminants have not been studied in the past. In the earlier studies in which the interaction of organic and heavy metal contaminants or interaction of different heavy metal were tested separately and in combination for phytoremediation, the results varied with the contaminants involved and the plant species. Synergistic effects were observed in some studies and antagonistic effects were observed in other studies for organic and heavy metal contaminants (Batty and Dolan 2013). The combination of different heavy metals in the soil can produce additive, protective or synergistic effects on phytoremediation (Wallace 1982). So contaminant specific studies are required to fully examine the phytoremediation of mixed contaminated soils.

4.2. Background

Some studies done in the past describe phytoextraction of heavy metals in co-contaminated soil and in soil contaminated by a single metal (Chen et al. 2004; Kobylecka and Skiba 2008). Some studies discuss the degradation or uptake of organic contaminants in presence of heavy metals (Lin et al. 2006; Sun et al. 2011). Still others discuss the individual and combined effects of organic and heavy metal contaminants on phytoremediation (Ahammed et al. 2013; Batty and Anslow 2008).

In the study by Chen et al. (2004), soil contaminated by Cu and Zn was taken from an industrial area. Part of the soil was spiked with 2,4-dichlorophenol (2,4-DCP), and rye grass was planted in both spiked and unspiked soil samples. They found that the water soluble fraction of the heavy metals was higher in the presence of 2,4-DCP compared to the unspiked soil. Also, the rye grass activation of Zn was more than that of Cu, which points to the variation of phytoextraction behavior with individual contaminants. They attributed the increase in water
soluble heavy metals in spiked soil to a soluble complex formation with the organic contaminants. Kobylecka and Skiba (2008) investigated the phytoextraction of Mn, Zn and Cu by wheat straw in the presence of herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) or 4-chloro-2-methylphenoxyacetic acid (MCPA). Their results suggested that the formation of metal-organic complexes in mixed contaminated soil can reduce the uptake of metals by plants.

Lin et al. (2006) evaluated the dissipation of pentachlorophenol (PCP) in soil in the presence of Cu. Here, the soil was contaminated with different levels of Cu and PCP. When the initial PCP concentration was 50 mg/kg both the plant growth and PCP degradation improved as the Cu level increased. But, when the initial concentration was 100 mg/kg, plant growth and the degradation of PCP were inhibited by increasing PCP levels. Similar to this, phytoremediation results that varied with different concentrations of the same contaminants were observed by Hechmi et al. (2014). They treated the soil with Cd (0, 5 and 50 mg/kg) with or without PCP (0, 50 and 250 mg/kg). Phragmites australis or common reed plants were grown in the soil. They observed that plant growth decreased with an increase in the concentration of Cd and PCP. In the mixture that contain low amounts of Cd and PCP, the plant stress was less compared to the single treatment of Cd. PCP degradation was higher in planted soil compared to unplanted soil, and the plant’s accumulation of Cd decreased with a rise in the amount of PCP.

In the study by Ahammed et al. (2013), tomato plants were grown in soil contaminated with either phenanthrene or Cd or a combination of the two. Phytotoxicity was highest when the soil was contaminated with Cd alone, and least phytotoxicity was observed when phenanthrene was applied alone. A combination of Cd and phenanthrene in the soil could alleviate the stress in the plants encountered, compared to Cd alone. In another effort to understand the synergistic effects of organic and heavy metal contaminants, Batty and Anslow (2008) studied the
phytoremediation efficiencies of Indian mustard and tall fescue in soil contaminated with Zn or pyrene alone or in combination. According to their findings, higher plant growth was observed when Zn and pyrene appeared together in the soil compared to instances where Zn was the sole contaminant. The plant’s accumulation of Zn was higher in mixed contaminated soil. A similar study was conducted by Zhang et al. (2009) in soil contaminated with Cd and pyrene individually or in combination. Here, the Cd accumulation in maize decreased with an increase in the pyrene concentration. However, the presence of Cd increased the accumulation of pyrene. The main dissipation mechanism of pyrene in the soil was the microbial degradation, and microbial degradation was lesser in the soil as the concentration of Cd increased. Sun et al. (2011) showed that the presence of heavy metals (Cd, Cu, Pb) in the soil inhibited benzo(a)pyrene uptake and accumulation by plants. They found that marigold could accumulate Cd in the presence of benzo(a)pyrene. But, they did not include single heavy metal treatments to compare the heavy metal accumulation without benzo(a)pyrene in their study.

Some studies show lower heavy metal phytoextraction in the presence of PAHs and some studies show improved phytoextraction of metals in the presence of PAHs. Lower heavy metal phytoextraction in the presence of PAHs (Lin et al. 2008; Kobylecka and Skiba 2008) can be explained by the formation of an insoluble metal complex with PAHs leading to the lower bioavailability of heavy metals. But, PAHs also improved phytoextraction (Batty and Anslow 2008; Zhang et al. 2009) if that particular PAH does not form an insoluble complex with metals, and if the PAH can passively penetrate the root cell membrane. In that case, heavy metals can penetrate the root cell membrane without any carriers (Chigbo et al. 2013). Also, when compared to organic contaminants, heavy metals can be more reactive in the plant tissue and, so, more toxic (Pilon-Smits 2005).
There are very few studies that demonstrate the individual and combined effects of heavy metal contaminants on phytoremediation. Some early studies were done to understand the uptake of heavy metals by and their interactions with edible crops. One such study was conducted by Hassett et al. (1976) to learn the interaction of Pb and Cd on maize root growth. Root elongation was recorded when the soil was supplied with Pb or Cd or a combination of both. They found that the effect of these metals when added in combination was greater than the sum of the effects of these metals when they were added separately.

Beckett and Davis (1978) showed that Cu has little effect on the phytoextraction of Ni or Zn by barley. However, increased Ni uptake was observed in the presence of Zn and increased Zn uptake was observed in the presence of Ni. Symeonidis and Karataglis (1992) estimated the toxicity effects of Cd, Pb or Zn or a combination of these metals on two different genotypes of Holcus lanams L, which are grasses. In all of the treatments, the root length of the plants decreased with the increased concentration of metal in the soil. For the first genotype, the effects on root growth of Cd + Pb and Pb + Zn combinations were additive. Synergistic interactions were observed for Cd + Zn and Cd + Pb + Zn combinations. But, for genotype 2, when Cd, Pb and Zn were supplied together, antagonistic effects were observed on root growth. This study shows the variation of results with different genotypes of the same plant.

Experimental results by Luo and Rimmer (1995) also show the interactive effects of different heavy metals on phytoremediation of soil. They conducted a greenhouse study in which spring barley was grown in soil where Cd, Cu, Pb, and Zn were mixed singly and in combination. Significant changes in phytoextraction and toxicity was observed for the Cu-Zn interaction. Zn alone was not toxic to the plants, but when Zn was added to the Cu contaminated
soils, it increased the toxicity. Zn uptake by the plants was increased by Cu additions, but Cu uptake was not affected by the inclusion of Zn in the soil.

Ebbs and Kochian (1997) estimated the toxicity effects of Zn or Cu or a combination of Zn and Cu on the *Brassica* species, which includes mustard. The experiments were run hydroponically. It was observed that Cu inhibited the lateral root elongation of the plants while Zn tended to decrease the lateral root diameter. These plants were found to remove more Zn from the nutrient solution than Cu. The extent of the removal of Zn and Cu from the solution was reduced when both the metals were supplied together. The biomass of the plants decreased significantly in the presence of heavy metals. Combined treatment by Zn and Cu further reduced the biomass compared to exposure to a single metal, which underscored the synergistic effects of metals on plant growth.

The transfer of Cd and Zn from soil to plants was studied by Nan et al. (2002) under actual field conditions. They concluded that the presence of Cd can increase the accumulation of Zn and Zn can increase the accumulation of Cd. An et al. (2004) investigated the individual and combined effects of Cu, Cd and Pb on toxicity to and metal accumulation by cucumber. They considered each metal separately, combinations of two metals at a time, and then a combination of all three metals in soil. They found simple additive, synergistic or antagonistic effects under different combinations of metals.

The reduced accumulation of Pb and Cd in the presence of Se and Zn was observed by He et al. (2004). From their pot experiments using Chinese cabbage and lettuce, they found that a supply of Se and Zn can enhance the accumulation of beneficial trace elements like Mn and Mg by the plants. Experiments done by Sun et al. (2008) also show that the interaction of different heavy metals can considerably affect the phytoremediation of soil. They tested soil contaminated
with Cd and As at different concentrations and proved that a certain level of Cd can facilitate As uptake by *Solanum nigrum* (black nightshade). Experiments by Israr et al. (2011) on *Sesbania drummondii* (poison bean) seedlings to study the interactive effects of Pb, Cu, Ni, and Zn showed the antagonistic effects of multiple metals on plant uptake.

The heavy metals interaction can directly affect the plant cells by its toxicity or indirectly affect phytoremediation by changing the microbial communities in the rhizosphere. *Arbuscular mycorrhizal* fungi can provide a convenient system for the plants to clean up heavy metals from the soil through its symbiotic interaction with the plants (Göhre and Paszkowski 2006). Shalaby (2003) studied the responses of *arbuscular mycorrhizal* fungal spores in soils contaminated with Cd, Pb and Zn. The soil samples were prepared with single metal, combinations of two metals and a mixture of all three metals. They observed that in single Pb or Cd contaminated soil, the toxicity was reduced when Zn was also added to the soil, showing antagonistic effects of metals on toxicity. However, Cd and Pb acted synergistically, showing a further reduction on hyphal growth than did the individually contaminated soils. When the three metals appeared together in the soil, the toxicity was less than the individual contamination cases. They concluded that a toxicity assessment of individual metals may not be sufficient to know the toxicity when in combination with another metal or multiple metals. Toxicity to the *arbuscular mycorrhizal* fungal spores can indirectly affect the plants and phytoremediation due to their symbiotic relationship with the plants. Also, similar interactions of heavy metals can be expected to produce direct plant toxicity.

In general, synergistic effects were observed in some studies and antagonistic effects were observed in others for different organic and heavy metal contaminants. The same is observed for combined effect of different heavy metals. So, it is an obvious conclusion that
contaminant specific studies are required on phytoremediation of mixed contaminated soils. This paper presents a laboratory investigation on phytoremediation using four plant species to identify the effects of organic (naphthalene, phenanthrene) and heavy metal contaminants (Pb, Cd, Cr) when they are present in the soil separately and in combination. Since the heavy metals were found to inhibit plant growth in mixed contaminated soil, an additional study was conducted to investigate the individual and combined effects of heavy metals considered, using oat plant and sunflower. The aim is to understand the best and worst conditions under which plants grow and the uptake, immobilize or degrade the contaminants.

4.3. Experimental Methods

4.3.1 Soil Used

Gray silty clay, which represents typical Chicago glacial till, was selected for the pot experiments. The important physical properties of the soil used in the study are presented in Table 4.1.

4.3.2 Soil Spiking Procedure

Clean control soil was prepared by mixing the soil with 15% tap water. To prepare the organic contaminated soil, naphthalene (50 mg/kg soil) and phenanthrene (100 mg/kg soil) were mixed with hexane. The hexane was mixed into the soil and dried in the fume hood until all the hexane evaporated. To prepare the heavy metal contaminated soil, PbCl$_2$, K$_2$Cr$_2$O$_7$ and CdCl$_2$ $\frac{1}{2}$H$_2$O were measured to produce a final concentration of 500 mg/kg Pb, 200 mg/kg Cr and 50 mg/kg Cd in the soil. The measured chemicals were mixed in water to reach an approximate water
Table 4.1. Important Properties of Soil Used for the Experiments

<table>
<thead>
<tr>
<th>Property</th>
<th>ASTM Standards</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil organic content</td>
<td>ASTM D 2974</td>
<td>2.4%</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>ASTM D 854</td>
<td>2.7</td>
</tr>
<tr>
<td>Water holding capacity</td>
<td>ASTM D 2980</td>
<td>46.4%</td>
</tr>
<tr>
<td>Liquid limit</td>
<td>ASTM D 4318</td>
<td>32.7%</td>
</tr>
<tr>
<td>Plastic limit</td>
<td></td>
<td>19.1%</td>
</tr>
<tr>
<td>Plasticity index</td>
<td></td>
<td>13.6%</td>
</tr>
<tr>
<td>Clay (&lt; 0.002mm)</td>
<td>ASTM D 422</td>
<td>41%</td>
</tr>
<tr>
<td>Silt (0.002 - 0.05mm)</td>
<td></td>
<td>43%</td>
</tr>
<tr>
<td>Sand (0.05 – 2 mm)</td>
<td></td>
<td>14.2%</td>
</tr>
<tr>
<td>USCS Classification</td>
<td></td>
<td>CL</td>
</tr>
<tr>
<td>USDA Classification</td>
<td></td>
<td>Silty clay</td>
</tr>
</tbody>
</table>
content of 15% in the soil for one hour with a magnetic stirrer. This solution containing Pb, Cd and Cr was added to the clean dry soil. For the contaminated soil, the clean soil was mixed with the organic contaminants in hexane and then dried in the fume hood until the hexane evaporated. This soil was then mixed with heavy metal contaminants dissolved in deionized water. The properties of the seven soil treatments were measured at the time of seeding and are presented in Table 4.2. They samples are: clean soil (T1), soil with organic contamination by naphthalene and phenanthrene (T2), soil with multiple metals that is contaminated by Pb+Cd+Cr (T3), soil with mixed contamination of organics and heavy metals that is co-contaminated by naphthalene+phenanthrene+Pb+Cd+Cr (T4), soil that contains Pb (T5), soil spiked with Cd (T6), and soil contaminated with Cr (T7).

Seeds were planted in pots of 8 cm diameter and 9 cm height that were filled with the prepared soil. Four plant species were selected (see section 4.3.3). The numbers of replicates for the mixed contaminated soil (T4) were 10 each for sunflower and oat plants, and 5 each for rye grass and tall fescue. Six replicates for the oat plant and sunflower and three replicates each for rye grass and tall fescue were prepared for the mixed metal contaminated soils (T3). For the PAH contamination (T2) tests, three sets of pots were prepared for each plant species. Three replicates were prepared for single metal contaminated soils (T5, T6 and T7) for oat plant and sunflower. Plants were also seeded in uncontaminated soil (T1) as the control with two sets of pots prepared for each plant species. The seeds were planted approximately a half inch below the soil surface. Ten seeds were sown in each oat plant and sunflower pot and twenty were sown for rye grass and tall fescue. Each pot was kept on a separate tray to ensure that the leachate did not get mixed up. The pots were placed under metal halide grow lamps with an average light intensity of 400 µmols/m²/s that were hung ~12 inches above the plants to obtain the desired
<table>
<thead>
<tr>
<th>Soil Sample</th>
<th>Property</th>
<th>pH</th>
<th>Oxidation Reduction Potential (mV)</th>
<th>Electrical Conductivity (mS/cm)</th>
<th>Water Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Clean</td>
<td></td>
<td>7.9</td>
<td>-66.2</td>
<td>0.08</td>
<td>14.6</td>
</tr>
<tr>
<td>T2 Organic Contamination</td>
<td></td>
<td>7.7</td>
<td>-55.3</td>
<td>0.12</td>
<td>14.8</td>
</tr>
<tr>
<td>(naphthalene, phenanthrene)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3 Heavy Metal Contamination</td>
<td></td>
<td>7.7</td>
<td>-54.5</td>
<td>0.12</td>
<td>15.6</td>
</tr>
<tr>
<td>(Pb, Cd, Cr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4 Mixed Contamination</td>
<td></td>
<td>7.6</td>
<td>-45.3</td>
<td>0.17</td>
<td>15.9</td>
</tr>
<tr>
<td>(naphthalene, phenanthrene, Pb,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd, Cr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T5 Single Metal Contamination</td>
<td></td>
<td>7.4</td>
<td>-46.7</td>
<td>0.16</td>
<td>18.5</td>
</tr>
<tr>
<td>(Pb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T6 Single Metal Contamination</td>
<td></td>
<td>7.5</td>
<td>-53.4</td>
<td>0.12</td>
<td>18.8</td>
</tr>
<tr>
<td>(Cd)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T7 Single Metal Contamination</td>
<td></td>
<td>7.2</td>
<td>-32.1</td>
<td>0.29</td>
<td>18.7</td>
</tr>
</tbody>
</table>
light intensity. A timer was set to provide 16 hours of light per day. The hanging height of the light was adjusted as the plants grew to reduce heat stress. The temperature was measured at the height of the plants and maintained at 25°C. Fans were used to control the temperatures of the grow lights.

4.3.3 Selected Plant Species

The plant species were selected based on their biomass and capability of survival based on previous laboratory results under similar conditions (Chirakkara and Reddy, 2013). The plants selected to study the interactive effects of organic and heavy metal contaminants were *Avena sativa* (oat plant), *Lolium perenne* (rye grass), *Festuca arundinacea* (tall fescue), and *Helianthus annuus* (sunflower). Only the oat plant and sunflower were studied for the effect of single metals on phytoremediation. Number of seeds sown in each pot was ten for oat plant and sunflower and twenty for rye grass and tall fescue. Oat plant seeds were supplied by Seedville USA and rye grass seeds were supplied by Sheffield’s Seed Company. Tall fescue seeds used were Amturf 77022 Ultra. Sunflower seeds were received from Carolina Biological Supply Company.

4.3.4 Pot Setup and Monitoring

The plants were grown for 61 days, during which the growth was monitored and the pots were watered daily. The locations of the pots were rotated periodically to ensure uniform light intensity. Weekly monitoring included counting the number of plants in each pot and measuring the plant height. Weekly photographs recorded plant growth and biomass production. Soil samples were taken at the beginning and end of the plant growth period to test for pH, electrical
conductivity, oxidation reduction potential, and the concentrations of metals and organic contaminants.

At the end of the plant growth period, the roots of the plants were separated from the shoots and washed in deionized water. The roots, shoots and soil were dried in oven at 60°C for 6 days (until it attained a constant weight). The dry weights of the roots and shoots are recorded as root or shoot biomass.

### 4.3.5 Analytical Testing

Testing of the physical properties of the soil viz. water content (ASTM D2216), organic content (ASTM D2974), pH (ASTM D4972), and grain size analysis (ASTM D422) was done as per these standards. The water holding capacity of the soil was determined by method similar to the one used for saturated peat materials (ASTM D2980). For conducting the heavy metal analysis, acid digestion of the soil samples was done as per EPA method 3050B. The digested and filtered liquid was analyzed with Flame Atomic Absorption (FLAA) spectroscopy for Pb, Cd and Cr. To estimate the exchangeable metals in the soil, 8 ml of 1M sodium acetate solution was added to 1g of soil and was mixed continuously for one hour per Reddy et al. (2001). The filtrate was analyzed with Flame Atomic Absorption (FLAA) spectroscopy for Pb, Cd and Cr. The organic contaminants were analyzed by solvent extraction and analysis using Gas Chromatography, following EPA method SW8270C. For analyzing exchangeable nitrogen, 1g soil was shaken with 10 ml of 2M KCl solution and was shaken for one hour (Xu et al. 2013). The filtered extract was analyzed using Spectronic Genesys spectrophotometers, following the procedure given by Sattayatewa et al. (2011). To determine the exchangeable fractions of potassium and phosphorus, 1g soil was shaken with 1M ammonium acetate for a period of one hour. The solution was
filtered, and the extract was analyzed for phosphorus with Spectronic Genesys spectrophotometers, as per the procedure given by Sattayatew a et al. (2011). Exchangeable potassium in the extract was analyzed using Flame Atomic Absorption (FLAA) spectroscopy. All the chemicals used to spike the soil and for analytical testing were purchased from Fischer Scientific.

For the test results, means and standard deviations were calculated using Microsoft Office Excel 2007. To check whether a significant difference exists between the result sets, the t-test was performed with Microsoft Office Excel 2007. The alpha value was taken as 0.05 for the t-test.

4.4. Results and Discussion

4.4.1 Interactive Effects of Organic Contaminants and Heavy Metals
Phytoremediation using oat plant, rye grass, tall fescue, and sunflower in organic contaminated soil (T2), multiple metal contaminated soil (T3) and mixed contaminated soil (T4) are compared with each other and with the control sample (T1) in this section. Germination percentages of the plants in contaminated and uncontaminated (control) soils are plotted in Figure 4.1. Here, germination is interpreted as the appearance of a green shoot/leaf above the soil. For most of the species, the plants in the organic contaminated soil had similar germination and growth features as those in the control pots. The germination of all the plants was affected by the heavy metal and the mixed contamination, but the extent of the reduction varied from species to species. Among the four plant species studied, the oat plant had the highest germination rate in heavy metal contaminated (T3) and mixed contaminated (T4) soil. The sunflower germination rate was
Figure 4.1. Percentage Germination of Plants in Different Treatments
considerably less in heavy metal contaminated and mixed contaminated soil than in the control sample and the organic contaminated soil. In general, for all species, the highest germination rate was in clean soil, followed by organic contaminated soil, mixed contaminated soil, and heavy metal contaminated soil.

Figure 4.2 shows the percentage of survival by plants growing in the clean soil and contaminated soil. Here, survival is expressed as the presence of green/live plant in the pot at the end of the test period. Percentage of survival is the number of surviving plants as percentage of the number of the seeds that germinated. All four plant species had survival rates in organic contaminated soil, which was similar to the control soil results. For oat plant, rye grass and tall fescue, the plants had better survival rates in mixed contaminated soil than in heavy metal contaminated soil. However, the sunflower plants grown in the mixed contaminated soil had a lower survival rate than those in the heavy metal and organic contaminated soils. Rye grass did not survive in the heavy metal contaminated soil.

The increase in maximum plant height with time is plotted in Figure 4.3. The final (after 61 days) plant height of oat plant, rye grass, tall fescue, and sunflower are presented in Figure 4.4. All the plants had lesser maximum heights in the contaminated soils than in the control (clean) soil. In general, higher plant heights are observed in organic contaminated soil, followed by mixed contaminated soil and heavy metal contaminated soil. The final plant heights of heavy metal and mixed contaminated soils were considerably less compared to the control. The average root and shoot biomass measurements of the plants grown in clean soil and contaminated soils are summarized in Table 4.3. The percent reduction of total biomass of the plants grown in contaminated soils compared to the same plants in clean soil is presented in Figure 4.5. These
Figure 4.2. Percentage Survival of Plants in Different Treatments
Figure 4.3. Increase in Plant Height with Time for Different Treatments
Figure 4.4. Final Maximum Plant Heights in Different Treatments
Table 4.3: Average Root Biomass, Shoot Biomass, and Total Biomass of Plants in Clean and Contaminated Soils

<table>
<thead>
<tr>
<th>Soil Sample</th>
<th>Treatment</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root Biomass (g)</td>
<td>Oat Plant</td>
<td>0.65</td>
<td>0.63</td>
<td>0.30</td>
<td>0.50</td>
<td>0.50</td>
<td>0.55</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Rye Grass</td>
<td>0.49</td>
<td>0.32</td>
<td>0.00</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tall Fescue</td>
<td>0.44</td>
<td>0.15</td>
<td>0.10</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sunflower</td>
<td>1.30</td>
<td>1.24</td>
<td>0.38</td>
<td>0.46</td>
<td>0.81</td>
<td>0.47</td>
<td>0.00</td>
</tr>
<tr>
<td>Shoot Biomass (g)</td>
<td>Oat Plant</td>
<td>1.15</td>
<td>0.78</td>
<td>0.28</td>
<td>0.72</td>
<td>0.60</td>
<td>0.66</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Rye Grass</td>
<td>0.51</td>
<td>0.30</td>
<td>0.00</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tall Fescue</td>
<td>0.41</td>
<td>0.22</td>
<td>0.09</td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sunflower</td>
<td>3.06</td>
<td>4.25</td>
<td>0.59</td>
<td>0.76</td>
<td>2.04</td>
<td>2.08</td>
<td>0.00</td>
</tr>
<tr>
<td>Total Biomass (g)</td>
<td>Oat Plant</td>
<td>1.81</td>
<td>1.41</td>
<td>0.42</td>
<td>1.22</td>
<td>1.10</td>
<td>1.21</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Rye Grass</td>
<td>1.00</td>
<td>0.62</td>
<td>0.00</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tall Fescue</td>
<td>0.85</td>
<td>0.37</td>
<td>0.19</td>
<td>0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sunflower</td>
<td>5.69</td>
<td>5.50</td>
<td>0.98</td>
<td>1.23</td>
<td>2.85</td>
<td>2.56</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Figure 4.5. Percentage Reduction of Total Biomass in Contaminated Soils
preliminary results of plant growth were presented and reported earlier (Chirakkara and Reddy 2014).

The initial and final (after plant growth) soil samples from all the pots were analyzed for pH, electrical conductivity and oxidation-reduction potential. Table 4.4 shows the average values of pH, electrical conductivity and oxidation-reduction potential (ORP) for the soil samples after the plant growth period. According to this, pH, electrical conductivity and ORP are not considerably affected by contamination or by the presence of plants. This can be favorable for remediation, as microbial activities responsible for the rhizodegradation and rhizostabilization are dependent on the soil pH to a great extent (Atagana et al. 2003). Large electrical conductivity can also inhibit microbial activity (Masciandaro et al. 2013).

Figure 4.6 shows the heavy metal concentrations in planted and unplanted soil samples where there is mixed or metal contamination. It can be noted from the metal concentrations in pots that never contained plants that the same initial concentration for metal contaminated soil and for mixed contaminated soil was not achieved during the mixing process. Examined, this difference was insignificant (p>0.05). The final concentration of Pb in all of the pots that contained either oat plant or sunflower were slightly, but insignificantly higher (p>0.05) for the metal contaminated soil, compared to the mixed contaminated soil. However, this trend was observed even in the unplanted pots. In pots that contained tall fescue, the Pb concentration was significantly higher (p<0.05) for mixed contaminated soil than in the metal contaminated soil. Oat plant, rye grass and tall fescue in mixed contaminated soil did not show any significant difference in Pb concentration from the unplanted soil. But, in metal contaminated soil, there was a significant reduction in Pb concentration in all planted soils compared to the unplanted pots of soil. The average reductions of Pb concentration in metal contaminated soils were 8% by oat
Table 4.4. Average pH, Oxidation Reduction Potential, and Electrical Conductivity Values for Soil Samples

<table>
<thead>
<tr>
<th>Soil Sample</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Plant</td>
<td>7.9</td>
<td>7.7</td>
<td>7.7</td>
<td>7.6</td>
<td>7.4</td>
<td>7.5</td>
<td>7.2</td>
</tr>
<tr>
<td>Oat Plant</td>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
<td>7.6</td>
<td>7.8</td>
<td>8.0</td>
</tr>
<tr>
<td>Rye Grass</td>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tall Fescue</td>
<td></td>
<td></td>
<td>7.9</td>
<td>7.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunflower</td>
<td>7.7</td>
<td>7.7</td>
<td>8.0</td>
<td>7.8</td>
<td>7.6</td>
<td>7.6</td>
<td>8.0</td>
</tr>
<tr>
<td>ORP (mV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Plant</td>
<td>-66.2</td>
<td>-55.30</td>
<td>-54.50</td>
<td>-45.30</td>
<td>-46.7</td>
<td>-53.4</td>
<td>-32.1</td>
</tr>
<tr>
<td>Oat Plant</td>
<td>-48.2</td>
<td>-46.10</td>
<td>-46.03</td>
<td>-50.40</td>
<td>-55.3</td>
<td>-68.6</td>
<td>-76.8</td>
</tr>
<tr>
<td>Rye Grass</td>
<td>-52.3</td>
<td>-48.73</td>
<td>-45.30</td>
<td>-52.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tall Fescue</td>
<td>-53.6</td>
<td>-49.13</td>
<td>-56.23</td>
<td>-54.30</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sunflower</td>
<td>-48.2</td>
<td>-42.97</td>
<td>-56.77</td>
<td>-47.30</td>
<td>-58.4</td>
<td>-65.7</td>
<td>-76.8</td>
</tr>
<tr>
<td>EC (mS/cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Plant</td>
<td>0.08</td>
<td>0.12</td>
<td>0.12</td>
<td>0.17</td>
<td>0.16</td>
<td>0.13</td>
<td>0.29</td>
</tr>
<tr>
<td>Oat Plant</td>
<td>0.20</td>
<td>0.17</td>
<td>0.17</td>
<td>0.10</td>
<td>0.18</td>
<td>0.15</td>
<td>0.11</td>
</tr>
<tr>
<td>Rye Grass</td>
<td>0.10</td>
<td>0.15</td>
<td>0.17</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tall Fescue</td>
<td>0.10</td>
<td>0.15</td>
<td>0.11</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunflower</td>
<td>0.20</td>
<td>0.19</td>
<td>0.11</td>
<td>0.20</td>
<td>0.18</td>
<td>0.14</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Figure 4.6. Heavy Metal Concentrations in Different Soil Samples
plant and 19% by tall fescue. Sunflower significantly reduced Pb concentrations in both the mixed contaminated soils (29%) and metal contaminated soils (33%). The final Cd concentrations in all planted pots were observed to be higher for mixed contaminated soil than in the metal contaminated soil. This difference was significant for oat plant and tall fescue, but not for sunflower. In the case of mixed contaminated soil, only the sunflower achieved a significant reduction in Cd concentration (18%). But, in metal contaminated soil, all the plants reduced Cd concentration significantly. The reduction of Cd in metal contaminated soil was 17% for oat plant, 23% for tall fescue and 24% for sunflower. All the plants reduced Cr in the mixed contaminated soil and metal contaminated soil. In mixed contaminated soil, the reduction of Cr was 27% by oat plant, 29% by rye grass, 22% by tall fescue, and 37% by sunflower. Reduction of Cr concentration in metal contaminated soils were 29% by oat plant, 29% by tall fescue and 32% by sunflower.

The growth characteristics show that the germination, survival, plant height, and final biomass greatly varied with different treatments. Nutrient analysis was performed on soil samples to check if the difference in growth characteristics were due to the non-availability of nutrients in contaminated soils. The exchangeable nutrient concentrations in different soil samples are shown in Figure 4.7. It can be inferred that the nutrient availability in the contaminated soils are not less than that of the clean soil. This implies that the diminished growth characteristics were due to the toxicity caused by the contaminants.

More phytotoxicity to the plants was observed under heavy metal contamination alone than under organic or combined contamination conditions. Phytotoxicity by different heavy metals has been studied by various researchers. Some heavy metal contaminants are essential for plant growth. These essential micronutrients, like Mn, Zn, Fe, are elements that are required in
Figure 4.7. Nutrient Concentrations in Different Soil Samples
small amounts for the normal functioning of plants and animals. However, when present at high concentrations, they can also negatively affect germination and survival (Kranner and Colville 2011). Cr is one of the non-essential micronutrient for plants. However, while low concentrations of Cr are observed to improve plant growth, it can inhibit plant growth if supplied in higher concentrations (Chandra and Kulshreshtha 2004). Pb and Cd are non-essential heavy metals that are not known to have any metabolic function in plants and are toxic to plants at high concentrations (Pahlsson 1989). In the case of organic contaminants, previous research studies show that high molecular weight PAHs (3-5 rings) do not cause phytotoxicity (Henner et al. 1999). This shows that naphthalene (2 rings) is more likely to affect plants negatively than phenanthrene (3 rings).

Pilon-Smits (2005) explains that when a soil is contaminated with a mixture of organics and metals, the metals are most likely to limit plant growth. Organic contaminants tend to be less toxic to the plants because they are less reactive. Also, since organic contaminants are xenobiotic to the plants, there are no transporters for these compounds in plant membranes. So, their movements into and within the plant tissues are driven by simple diffusion, based on their chemical properties. As a result, they do not readily accumulate in the plant tissues. However, inorganic compounds like heavy metals are taken up in the plant by biological processes through membrane transfer proteins. Since the inorganic pollutants are either nutrients or are chemically similar to nutrients, they are taken up via membrane transporter proteins. Non-essential heavy metals can enter the plant through transporters of essential heavy metals due to their chemical similarity (Ali et al. 2013). Thus, there are more chances for these inorganic pollutants to accumulate in tissues where they can cause toxicity by oxidative stress or replacement of other essential nutrients.
Better performance of plants in combined contaminated soil, compared to the plants in metal contaminated soil, is likely due to the lower bioavailability of heavy metals in the presence of organic contaminants. Galvez-Cloutier and Dube (2002) also indicated that the presence of organic contaminants can influence metal mobility in soils. Differences in the sorption of heavy metal contaminants in the presence of organic contaminants shown by Poly and Sreedeep (2011) may be the reason for lower bioavailability of heavy metals in combined contaminated soils. Heavy metals can form complexes with the organic contaminants (McLean and Bledsoe 1992), which can better adsorb to the clay particles, reducing its availability in the soil. The same explanation applies to the higher metal uptake observed in single metal contaminated soil compared to the mixed contaminated soil.

Lin et al (2008) also observed lower metal uptake by plants in presence of organic contamination. They found that in soil co-contaminated with Cu and pyrene, the uptake of Cu by Zea mays L. (corn) was considerably less than the uptake from soil contaminated by Cu alone. Chigbo et al. (2013) also observed an inhibition of Cu phytoextraction by Brassica juncea (mustard greens) when pyrene was also present in the soil with the Cu. Metal-organic complexation also reduced the heavy metal uptake by wheat straw (Kobylecka and Skiba 2008). However, some studies show the trend of higher heavy metal extraction in the presence of organic contaminants (Batty and Anslow 2008; Zhang et al. 2009). Chigbo and Batty (2013) reported that co-contamination with pyrene significantly decreased Cu accumulation in shoots grown in aged soil, whereas the Cu accumulation increased in presence of pyrene for freshly spiked soil. These studies show that there can be synergistic or antagonistic effects on heavy metal accumulation by plants based on the complex interactions that depend on the type of co-contaminants, plants, soil, and age of the contamination in soil.
Figure 4.8. Exchangeable Metals in Different Soil Samples
To check the availability of heavy metals in the pore fluid, exchangeable heavy metals were extracted and analyzed; the results are shown in Figure 4.8. It can be observed that compared to total Pb content of the soil, exchangeable Pb concentrations were very low (approximately 1% of the total concentration) in all the samples, which indicates strong adsorption of Pb to the soil particles. The exchangeable Pb concentration in heavy metal contaminated soils were close to zero in all planted soils, probably because most of the exchangeable Pb was taken up by the plants. This is supported by the fact that the total Pb concentrations were also less for metal contaminated soils in planted pots. In the case of mixed contaminated soils, all of the plants except oat plant reduced the exchangeable Pb concentration in soil. For sunflower plants, the reduction of exchangeable Pb may be due to the Pb extraction by the plants, as indicated by the reduction of total Pb in mixed contaminated pots with sunflower plants. However, rye grass and tall fescue showed reduced exchangeable metal concentrations in mixed contaminated soils even though the total metal concentrations were not reduced by the plants. This indicates that rye grass and tall fescue phytostabilize contaminants. With phytostabilization, leaching of the heavy metals to groundwater will be minimized, thereby reducing the exposure pathways (Ali et al. 2013).

The exchangeable Cd concentrations were also less for all the samples compared to the total Cd concentration (approximately 10% of total concentration). Apart from oat plant, the other plants grown in the metal contaminated and mixed contaminated soils achieved a significant reduction in the exchangeable Cd concentrations when compared to the unplanted pots. Similar to the Pb results, the reduction of exchangeable Cd concentrations in metal contaminated soils by tall fescue and sunflower may be due to phytoextraction or a combination
of phytoextraction and phytostabilization. In mixed contaminated soils only sunflower lowered the total metal concentration of the soil significantly. This indicates that the reduced exchangeable metal concentrations in mixed contaminated soil by rye grass and tall fescue is by phytostabilization.

Compared to the total concentration of Cr, the exchangeable Cr concentration was less (approximately 25%). However, when compared with exchangeable concentrations of Pb and Cd, the highest exchangeable metal concentrations were observed for Cr. A possible reason for this is the preferential adsorption of the three metals to the soil particles. The selectivity sequence for the considered metals for adsorption is Pb>Cd>Cr. This selectivity sequence can be considered for hexavalent Cr. Trivalent Cr tends to adsorb more to the soil particles than Pb ions (McLean and Bledsoe 1992; Gomes et al. 2001). Sunflower plants could significantly reduce the exchangeable Cr concentrations compared to the unplanted pots, both in metal contaminated soil and in mixed contaminated soil. The exchangeable Cr concentrations in all the other planted pots were not significantly different from that of the unplanted pots or those planted in metal contaminated soil or mixed contaminated soils. This shows that there was no plant-promoted immobilization of Cr in the rhizosphere by oat plant, tall fescue or rye grass. The reduction in exchangeable Cr achieved by sunflower may be due to phytoextraction or a combination of phytoextraction and phytostabilization.

The PAH analysis results showed that naphthalene was not found in any of the final soil samples, including the samples from unplanted pots. Concentrations of naphthalene found in initial samples were 2.7 mg/kg in samples with mixed contamination and 2.1 mg/kg in samples with organic contamination, alone. These concentrations were considerably less than the spiked concentration, which was 50 mg/kg. The initial concentration of phenanthrene was close to 45
mg/kg for the samples, which is, again, less than the spiked concentration (100 mg/kg). These reductions in concentration of initial samples are attributed to microbial degradation and volatilization of the organic contaminants (Masciandaro et al. 2013). Also, in the planted pots, microbial degradation is the expected dissipation pathway for naphthalene and phenanthrene since they are not expected to be taken up by the plants due to their hydrophobic nature. Since the plant’s uptake of organic contaminants occurs by simple diffusion, the process depends mainly on the chemical properties of the contaminants, most importantly hydrophobicity. The ideal value of the octanol water partition coefficient (log $K_{ow}$) for diffusion through root membranes and plant tissues ranges from 0.5 and 3 (Pilon-Smits 2005). The log $K_{ow}$ values for naphthalene and phenanthrene are above these limits, which indicates that plant uptake of these contaminants is not probable (De Maagd et al. 1998). PAHs, which can passively penetrate the root cell membranes, can also facilitate the penetration of metals or metal complexes (Chigbo et al. 2013). This may be the reason for the higher metal accumulation in the presence of PAHs that is reported in some of the previous studies.

The phenanthrene concentrations from the different samples are depicted in Figure 4.9. In the pots planted with rye grass and tall fescue, the mixed contaminated soils had a higher concentration of phenanthrene than was found in the organic contaminated samples, which indicated a lower level of degradation of phenanthrene in the mixed contaminated samples. This is an expected result since the heavy metal contaminants in mixed contaminated soil can be toxic to the micro-organisms responsible for the degradation of PAHs (Oyetibo et al. 2013; Almeida et al. 2013). The presence of heavy metals can negatively affect soil microbial activity and microbial diversity (Giller et al. 1998). In the unplanted pots and pots planted with sunflower, the organic contaminated soil had a higher concentration of phenanthrene than did the mixed...
Figure 4.9. Phenanthrene Concentration in Different Soil Samples.
contaminated soil. No phenanthrene was detected in the mixed contaminated soil samples planted with oat plant. Giller et al. (1998) explains that metal exposure can lead to the development of metal tolerant microbial populations, which may have enhanced the phenanthrene dissipation in this case.

In general, the planted pots seemed to have a higher concentration of phenanthrene compared to the unplanted pots, which was unexpected. The plants were expected to stimulate the growth of the micro-organisms responsible for the biodegradation of the organic contaminants (Cheema et al. 2010; Hechmi et al. 2013; Khan et al. 2013). However, polymerization reactions like humification that occur in the rhizosphere can make the organic contaminants less bioavailable compared to non-rhizosphere soil (Walton et al. 1994). Some other studies reported higher PAH contents in planted soils (Lin et al. 2008; Chigbo et al. 2013; Hechmi et al. 2013). Lin et al. (2008) and Hechmi et al. (2013) observed that the inhibition of PAH degradation in planted soils occurred when higher levels of heavy metals were present in soil. However, results by Chigbo et al. (2013) did not support this trend. Instead, their results showed inhibition of PAH degradation in planted soil in presence of lower levels of heavy metal compared to the soil with higher heavy metal levels. They attributed this to micro-organisms that were highly adapted to heavy metals in soil with higher heavy metal content.

According to Corgie et al. (2004) plants can either promote or inhibit the growth of PAH degrading micro-organisms by affecting the spatial distribution of bacterial communities in and around the rhizosphere. Also, the type and concentration of both heavy metals and PAHs can decide the positive or negative effect on PAH degradation (Khan et al. 2009). The quantity and quality of metabolites released by dead and live plant roots depends on the complex interactions of co-contaminants in the mixed contaminated soil (Olsen et al. 2003). This can change the
microbial number and activity in a positive or negative manner, which can affect the degradation process. Since the conditions that cause plants to promote or inhibit micro-organism growth are not clearly understood, further studies in this area are suggested.

4.4.2 Interactive Effects of Pb, Cd, and Cr

In this section, the responses of oat plant and sunflower grown in multiple metal contaminated soil (T3), Pb contaminated soil (T5), Cd contaminated soil (T6), and Cr contaminated soil (T7) are compared. Figure 4.1 shows that oat plant germination was not much affected by Pb and Cd contamination, but occurred least in Cr contaminated soil. In the mixed metal contaminated soil, the germination rate was considerably less than the control, but it was better than in the soil contaminated by Cr alone. Sunflower plants had considerably less successful germination rates in all of the contaminated soil samples. No sunflowers germinated in the single Cr contaminated soil. Some plants germinated in the mixed metal contaminated pots, but the germination rate was considerably less than in the single Pb and Cd contaminated soils.

Examination of the survival rate and maximum plant height also showed trends similar to the germination rate for both the plants (Figures 2 and 3). No plants of either species survived the full growth period in the single Cr contaminated soil. More phytotoxicity was observed under Cr contamination alone than in combined contamination conditions. Similar results of antagonistic plant response under combined contamination of different metals were observed by An et al. (2004) when cucumber plants were exposed to mixed Cu and Cd contamination or mixed Cu and Pb contamination. Plants grown in Pb contaminated soil had the least reduction in biomass compared to other single metal contaminated soils (Table 4.3). The addition of contamination decreased the pH of the soils (Table 4.4). However, planting corrected this as it helped to raise
the pH to close that of uncontaminated soil, which underscores the beneficial effect of plants on soil.

A significant reduction of Pb and Cd was achieved by both the plants grown in single and mixed metal soils (p<0.05). The greater reduction of Pb concentration was achieved by sunflower than the oat plant (Figure 4.6). The reduction in the concentration of Pb was not significantly different for the single Pb contaminated samples and mixed metal contaminated samples. Plants grown in the Cd contaminated soil produced a higher reduction of Cd compared to the plants in mixed metal contaminated soil. This shows that the presence of other metals inhibited the Cd extraction by the plants. Cr reduction by both the plants grown in mixed metal contaminated soils was significant. The higher phytotoxicity measured in the Cr contaminated soil shows that the phytoextractability of Cr was more when Cr was alone in the soil. Similar to the present results, An et al. (2004) reported antagonistic effects on extraction of Cu in the presence of Cd by cucumber plants. He et al. (2004) also observed antagonism in the uptake of Pb and Cd in an experiment with Chinese cabbage and lettuce where Zn and Se were supplied in the soil. Cr uptake by *Sesbania virgata* (wand riverhemp) can be inhibited in presence of Cu or Zn (Branzini et al. 2012).

The higher value of exchangeable Pb in single Pb contaminated soil did not seem to affect the Pb extraction from the sample (Figure 4.8). A considerable difference in exchangeable Cd in the unplanted pots was found only in the mixed metal contaminated pots grown with sunflower. But, this reduction in the exchangeable Cd level could not be related to the total reduction in Cd from the soil. The highest exchangeable metal concentrations were observed for Cr. The higher bioavailability of Cr at the soil pH and the preference for Cr over Pb and Cd by plant transporter proteins can be the reasons for the higher absorption and higher phytotoxicity in
the Cr contaminated soils (Ali et al. 2013). The toxicity and accumulation of metals in the metal contaminated soil mixtures depends both on the interaction of the metals in the soil and on the bioaccumulation pattern within the plants (An et al. 2004). The sorption capacity of metal ions decreases in a competitive process with other metal ions (Flogeac et al. 2007). The main reason for the antagonistic effects of different metals on contaminant uptake by plants is competition between metals at the plant uptake site (Israr et al. 2011).

4.5. Conclusions

In general, better germination and growth characteristics were observed in soils with organic contamination rather than those contaminated with heavy metal or mixed contaminated soils. All of the plants in this study showed delayed and reduced germination, as well as lower survival rates when planted in heavy metal contaminated soil or mixed contaminated soil compared to the control soil. The performance of the plants grown in mixed contaminated soils was better than that of the plants grown in soil contaminated with heavy metals. Cr alone in the soil created the most phytotoxic contamination condition. Phytotoxicity seems to be less when organic contaminants are present along with the heavy metals compared to the situations where heavy metals are the only form of contamination to the soil. This indicates that a plant that is able to survive in the heavy metal contaminated soil may perform better in the heavy metal-PAH co-contaminated soils. Compared to the other plants studied, the oat plant germinated more successfully than the other plants under all contamination conditions. However, when grown in soil that was contaminated with a heavy metal alone, the survival rate of the oat plant was very low. Even though rye grass had good germination and survival rates in organic and mixed contaminated soils, it had a very low germination rate and no plants survived to the end of the study in the heavy metal contaminated soil. Likewise, tall fescue also had low germination rates
and survival rates in heavy metal contamination and mixed contamination. Even though sunflower had reduced germination rates in heavy metal contaminated soil, its survival rates were better in all contamination situations. The biomass of the sunflower plants was considerably higher than that of all the other plants in all contamination conditions. In general, the presence of organic contamination inhibited the extraction of heavy metals from the soil by the plants.

Oat plant and sunflower could reduce Pb and Cd concentrations both in soils solely contaminated with one of these metals or in mixed metal contaminated soils. The rates of Pb reduction were similar in single Pb contaminated soil and mixed metal contaminated soils. Plants in single Cd contaminated soil achieved a higher reduction of Cd compared to the plants in mixed metal contaminated soil. Sunflower plants considerably reduced the total metal concentration and exchangeable metal concentrations in heavy metal contaminated soils and mixed contaminated soils. Interestingly, the plants could not considerably affect the degradation of organic contaminants. However, degradation of the organic contaminants is expected to take place with time. Since the contaminants may be present at a site individually or in combination, sunflower seems to be a good phytoremediation plant considering its growth characteristics and the reduction of heavy metals in individual and combined contamination conditions. These research findings suggest the need for soil amendments for the initial immobilization of the contaminants, and to improve the biomass of the plants to better withstand the toxicity caused by the metal contaminants.

4.6 Cited References
photosynthetic inhibition and oxidative stress in tomato.” *Journal of Experimental Botany*, 64(1), 199-213.


CHAPTER 5
EFFECTS OF VARYING INITIAL CONCENTRATIONS ON PHYTOREMEDIATION OF MIXED CONTAMINATED SOILS

5.1 Introduction

Many sites are contaminated with a mixture of organic and heavy metal contaminants as consequence of urbanization, industrial activities and agricultural activities. For large sites with shallow contamination, the typical mechanical approaches of remediating mixed contaminated sites can be expensive and energy intensive. In such cases, phytoremediation can be adopted as a green and sustainable remediation approach (Reddy and Chirakkara 2013). Phytoremediation is the technology in which suitable plants are grown in a contaminated area to extract, immobilize or degrade the contaminants (Sharma and Reddy 2004). During the remediation period, the plant cover can minimize the exposure pathways of the contaminants in the soil by controlling the downward migration of contaminated water and reducing soil erosion and windblown dust. Also, phytoremediation is more aesthetically pleasing than mechanical approaches to remediation (ITRC 2009).

The nature of on-site contaminants and their concentration levels are governing factors in phytoremediation. Some chemicals are harmless to the plants at lower concentrations and toxic at higher amounts (Kranner and Colville 2011). The higher phytotoxicity at greater concentrations of the chemicals can negatively affect the germination and survival of the plants, which in turn can affect the efficiency of phytoremediation. In the case of mixed contaminated soils, the same contaminants can cause synergistic or antagonistic effects on phytoremediation based on the concentrations of the individual contaminants in the soil (Lin et al. 2006; Hechmi et al. 2014).
Also, plants can show reduced metal absorption rates when the contaminant concentration increases beyond a certain value due to the restriction of metal transport to the shoot (Chigbo et al. 2013). Understanding the contaminant concentrations above which plants can survive and remediate well is important in implementation of the phytoremediation process.

5.2 Background

Only a limited number of studies have examined the phytoremediation efficiencies of plants at varying concentrations of contaminants. Some of these studies are discussed below.

Lin et al. (2006) conducted greenhouse-based phytoremediation experiments on soil contaminated with Cu and pentachlorophenol (PCP) using Lolium perenne L (rye grass) and Raphanus sativus (radish). They tested two concentrations of pentachlorophenol (50 and 100 mg/kg) and three levels of Cu (0, 150, 300 mg/kg), as well as combinations. In co-contaminated experiments with an initial PCP concentration of 50 mg/kg, the plant growth improved as the Cu levels increased, but the addition of Cu caused phytotoxicity when the soil did not contain PCP. Higher microbial activity, better plant growth and PCP dissipation was observed when the soil was spiked with 50 mg/kg PCP, in the presence of Cu. Yet, when the PCP concentration was 100 mg/kg, the plant growth and microbial activities were inhibited when the Cu level increased. This was explained by the PCP toxicity present at the higher PCP levels, combined with the Cu toxicity, which acted against microbial action and plant growth in the soil.

Zhang et al. (2009) studied the concurrent removal of Cd and pyrene from soil through pot experiments where the soil was spiked with different Cd concentrations (0, 2 and 4.5 mg/kg) and pyrene (0, 10, 50, and 100 mg/kg). Zea mays L. (maize) seeds were sown in pots containing combinations of contaminated soil. The results of tests on the plants harvested after 60 days
showed that both pyrene and Cd contamination affected the root and shoot biomass, and the biomass decreased as contaminant concentrations increased. While slight decreases in the dry weights of the roots and shoots were observed in the plants grown in the pyrene spiked soil, a more significant decrease appeared in those raised in the Cd spiked soil. Increases in the concentration of pyrene diminished the root dry weights when the soil was co-contaminated, indicating that pyrene contamination could exert synergistic toxic effects on plant health. The final pyrene concentration was significantly lower in soil that had been planted than in unplanted soil. This trend was observed in both pyrene contaminated soil and that co-contaminated with both Cd and pyrene. In the Cd contaminated soils, the concentrations of Cd found in the shoots and roots increased as the Cd level in the soil was elevated. However, the accumulation of Cd decreased as the pyrene contamination increased in the co-contaminated soil.

Sun et al. (2011) investigated the effect of benzo[a]pyrene in different concentrations on the growth of Tagetes patula (marigold) and its uptake and dissipation of benzo[a]pyrene as well as its phytoremediation of soil contaminated with 5 mg/kg benzo[a]pyrene and Cd, Cu and Pb at two concentrations. They prepared control pots without contamination, contaminated pots with benzo[a]pyrene alone in different concentrations (0, 2, 5, 10 and 50 mg/kg) and co-contaminated soils. In the co-contamination experiments, 5 mg/kg of benzo[a]pyrene treated soil was mixed with Cd (20 and 50 mg/kg), Cu (100 and 500 mg/kg) or Pb (1000 and 3000 mg/kg). The marigolds were grown for 92 days. Results showed that a low concentration of benzo[a]pyrene (≤10 mg/kg) facilitated plant growth and increased the plant biomass relative to the control plants. However, when those concentrations were increased beyond that level, inhibitive effects were observed on plant growth. There were significant positive correlations between the concentrations of benzo[a]pyrene accumulated in tissues of the plants and the amount in the soil.
The occurrence of heavy metals had inhibitive effects on the plant growth as well as benzo[a]pyrene uptake and accumulation. There was a reduction in biomass associated with the increase in heavy metal content. The concentration of heavy metals in the plants increased with the increase in metal concentration in the soils.

In a recent pot culture experiment, Hechmi et al. (2014) evaluated the phytoremediation potential of *Phragmites australis* (common reed) in soil contaminated with Cd and pentachlorophenol (PCP) in different concentrations. The treatments were 0, 5 and 50 mg/kg of Cd with or without PCP (50 and 250 mg/kg). They found that plant growth was inhibited by either Cd or PCP additions. The reduction of plant biomass was 89 and 92% in the low and high Cd treatments and 20 and 40% in the PCP treatments, respectively. The addition of PCP reduced the known Cd toxicity to plants, while the phytoextraction of Cd increased with Cd additions and decreased with PCP additions.

These studies show that an increase in the contamination level can influence phytoremediation positively or negatively, depending on the presence of co-contaminants and soil conditions. There was an instance of improved plant growth with the increase in contaminant concentration initially, and a decrease in growth after a particular level of contaminant concentration was reached (Sun et al. 2011). Also, the variation in responses demonstrated in the scientific studies suggests that the combination of plant species, soil type and pollutants can cause a spectrum of effects.

The concentration of contaminants can vary considerably at even among locations within a polluted site. The germination and survival of plants and the phytoremediation efficiency will depend to a great extent on these initial contaminant concentrations. So, it is important to understand how phytoremediation is affected by the increase in the initial contaminant
concentration. The toxicity can be caused by the contaminant if the concentration exceeds a threshold or it can be a gradual increase of effect with a rise in the concentration. The expected and most observed trend is a gradual upturn in phytotoxicity following a buildup in the concentration of the contaminant in the soil (Pedro et al. 2013; Zhang et al. 2009; Huang et al. 2004). But, in the case of essential heavy metals, there usually is some threshold value up to which the metals can be beneficial to the plants. When the essential metal concentration exceeds the tolerance value, it becomes toxic to the plants (Jadia et al. 2009). The threshold toxicity levels are also dependent on the plant species (Islam et al. 2007). Plant responses to an increase in contaminant concentrations are dissimilar and more complicated when both organic and heavy metal contaminants are present in the soil.

Previous studies conducted with varying concentrations of mixed contaminants all tested a single heavy metal mixed with a single organic contaminant. No studies have experimented with a mixture of multiple metals and organic contaminants with varying concentrations. This study focuses on phytoremediation of soil contaminated with a mixture of the commonly occurring pollutants naphthalene, phenanthrene, Pb, Cd, and Cr in different concentrations.

5.3 Experimental Methods

5.3.1 Selected Plant Species

The plant species for the study, Avena sativa (oat plant), and Helianthus annuus (sunflower), were selected based on their biomass and capability for survival in mixed contaminated soil based on previous results (Chirakkara and Reddy 2013). The oat plant was studied for its phytoremediation efficiency for heavy metal (Ebbs and Kochian 1998) and organic contaminants
Sunflower was also the basis of phytoremediation studies of both organic (Rosado and Pichtel 2004) and heavy metal contaminants (Meers et al. 2005; Adesodun et al. 2010; January et al. 2008). The oat plant seeds were supplied by Seedville USA and sunflower seeds were supplied by Carolina Biological Supply Company.

5.3.2 Soil Selected

Clean gray silty clay, representative of typical Chicago glacial till, was obtained from a field site in Chicago, IL for use in the pot experiments. The important physical properties of the soil used in the study are presented in Table 5.1.

5.3.3 Soil Spiking Procedure

The control sample of uncontaminated soil (T1) was prepared by mixing the soil with 15% tap water. For contaminated treatments, naphthalene and phenanthrene were dissolved in hexane by mixing using a magnetic stirrer prior to being mixed with the dry soil. This was dried for 3 to 4 days in the fume hood to ensure that all hexane has evaporated during which time it was mixed once a day to ensure uniformity. \( \text{PbCl}_2 \), \( \text{K}_2\text{Cr}_2\text{O}_7 \), and \( \text{CdCl}_2 \cdot \frac{1}{2} \text{H}_2\text{O} \) were measured and mixed in water (to yield approximate water content of 15% in soil) for one hour using magnetic stirrer. The solution was added to the soil that was already spiked with the solution of naphthalene and phenanthrene, and again mixed well to ensure the homogenous distribution of contaminants. Contaminant concentrations aimed for different soil treatments and measured properties of soil at the time of seeding are presented in Table 5.2.
Table 5.1: Important Properties of Soil Used for the Experiments

<table>
<thead>
<tr>
<th>Property</th>
<th>ASTM Standards</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil organic content</td>
<td>ASTM D 2974</td>
<td>2.3%</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>ASTM D 854</td>
<td>2.7</td>
</tr>
<tr>
<td>Water holding capacity</td>
<td>ASTM D 2980</td>
<td>45.8%</td>
</tr>
<tr>
<td>Liquid limit</td>
<td>ASTM D 4318</td>
<td>33.1%</td>
</tr>
<tr>
<td>Plastic limit</td>
<td></td>
<td>18.9%</td>
</tr>
<tr>
<td>Plasticity index</td>
<td></td>
<td>14.2%</td>
</tr>
<tr>
<td>Clay (&lt; 0.002mm)</td>
<td>ASTM D 422</td>
<td>42%</td>
</tr>
<tr>
<td>Silt (0.002 - 0.05mm)</td>
<td></td>
<td>42%</td>
</tr>
<tr>
<td>Sand (0.05 – 2 mm)</td>
<td></td>
<td>14.3%</td>
</tr>
<tr>
<td>USCS Classification</td>
<td></td>
<td>CL</td>
</tr>
<tr>
<td>USDA Classification</td>
<td></td>
<td>Silty clay</td>
</tr>
</tbody>
</table>
Table 5.2: Important Properties of Soil at the Time of Seeding

<table>
<thead>
<tr>
<th>Property</th>
<th>Soil Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>Contaminant Concentration (mg/kg)</td>
<td>Naphthalene</td>
</tr>
<tr>
<td></td>
<td>Phenanthrene</td>
</tr>
<tr>
<td></td>
<td>Pb</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
</tr>
<tr>
<td></td>
<td>Cr</td>
</tr>
<tr>
<td>pH</td>
<td>7.622</td>
</tr>
<tr>
<td>Oxidation reduction potential (mV)</td>
<td>-36.8</td>
</tr>
<tr>
<td>Electrical conductivity (mS/cm)</td>
<td>0.239</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>15.2</td>
</tr>
</tbody>
</table>
Pots of 8 cm diameter and 9 cm height were filled with the prepared soil. Three contaminated pots were prepared for each concentration selected for each plant species. The seeds were planted approximately a half inch below the surface. Each pot was kept on a separate tray to ensure that the leachate did not intermingle, and were placed under grow lights (metal halide lamps; average light intensity of 400 µmols/m²/s), which were hung ~ 12 inches above the plants to obtain that light intensity. A timer was set to provide 16 hours of light per day. The hanging height was adjusted as the plants grew to reduce the heat stress, and fans were used to control the temperatures, which was measured at the height of the plants and maintained at 25°C. The pots were rotated periodically to ensure uniform light intensity.

5.3.4 Pots Setup and Monitoring

The plants were watered and were grown for 65 days. The locations of the pots were rotated periodically to ensure uniform light intensity. 100 ml of nutrient solution was applied to each pot, once in a week. All purpose 20-20-20 fertilizer supplied by Carolina Biological Supply Company was used. Weekly monitoring included counting and measuring the plants in each pot and photographing them to record the plant growth and biomass production. Soil samples were taken at the beginning and end of the growth period to test for metals and organic contaminants. At the end of the plant growth period, the roots of the plants were separated from the shoots and washed in deionized water. Then, the roots, shoots and soil were dried in an oven at 60°C for 6 days (until it attained constant weight), and the dry weight of the roots and shoots were noted as root biomass and shoot biomass.
5.3.5 Analytical Testing

Testing of the physical properties of the soil viz. water content (ASTM D2216), organic content (ASTM D2974), pH (ASTM D4972), and grain size (ASTM D422) were done as per these standards. The water holding capacity of the soil was determined by method similar to the one used for saturated peat materials (ASTM D2980). To conduct the heavy metal analysis, acid digestion of the soil samples was done as per EPA method 3050B. The digested and filtered liquid was analyzed with Flame Atomic Absorption (FLAA) spectroscopy for Pb, Cd and Cr. To estimate the exchangeable metals in the soil, 8 ml of 1M sodium acetate solution was added to 1g of soil and mixed continuously for one hour (Reddy et al. 2001). The filtrate was analyzed with Flame Atomic Absorption (FLAA) spectroscopy for Pb, Cd and Cr. The organic contaminants were analyzed by solvent extraction and with Gas Chromatography, following EPA method SW8270C. To analyze the exchangeable nitrogen, 1g soil was shaken with 10 ml of 2M KCl solution for one hour (Xu et al. 2013). The filtered extractant was analyzed using Spectronic Genesys spectrophotometers, following the procedure given by Sattayatewa et al. (2011). To determine the exchangeable fractions of potassium and phosphorus, 1g soil was shaken with 1M ammonium acetate for a period of one hour. The solution was filtered, and the extractant was analyzed for phosphorus with Spectronic Genesys spectrophotometers, as per the procedure given by Sattayatewa et al. (2011). Exchangeable potassium in the extractant was analyzed using Flame Atomic Absorption (FLAA) spectroscopy. All the chemicals used to spike the soil and for analytical testing were purchased from Fischer Scientific.

For the test results, means and standard deviations were calculated using Microsoft Office Excel 2007. To check whether a significant difference exists between the result sets, the t-
test was performed with Microsoft Office Excel 2007. The alpha value was taken as 0.05 for the t-test.

5.4 Results and Discussion

Both species suffered delayed germination and reduced rates of germination, survival and growth when propagated in contaminated soil. Germination percentages of the plants seeded in contaminated and uncontaminated soils are plotted in Figure 5.1. Here, germination is explained as the appearance of a green shoot/leaf above the soil. Germination of the oat plant did not seem to be much affected when there was a rise in the contamination in the soil, but the sunflower showed a distinct decrease in germination with an increase in the contamination. Germination of plants in contaminated soils can show species variation because seed coat permeability varies with species (Wierzbicka and Obidzinska 1998). Plants with permeable seed coats will be affected to a greater extent by the concentration of contaminants present in the soil.

Not all of the plants that germinated survived to the end of the experimental period. Some in contaminated soils showed phytotoxicity symptoms like yellowish color and reduced growth and eventually dried up. Here, survival is expressed as the presence of green/live plant in the pot at the end of 65 days. The percentage of survival is the number of surviving plants as the percentage of the seeds germinated, and Figure 5.2 shows that percentage in soils with varying contamination. The percentage survival of the oat plants is better than that of sunflower plants in contaminated soils as the sunflower showed diminishing rate of survival with the intensification of contamination. The survival rate of plants in the T4 and T5 experiments were considerably less that those sown in the control (T1) soil.
Figure 5.1 Percentage of Germination of Plants by Soil Treatment
Figure 5.2 Percentage Survival of Plants by Soil Treatment
The increase in plant heights over time are plotted in Figure 5.3. It is evident that growth rates decreased as the contamination mounted for both plant species. The final (after 65 days) maximum plant height of the oat plant and sunflower are presented in Figure 5.4. The reduction in the maximum plant height of those propagated in contaminated soils, compared to those grown in the control soil, appears in Table 5.3. Sunflower showed greater reduction in plant height, compared to the control plants, in all contaminated soils with the greatest reduction in the case of soil mixes T4 and T5.

The average root and shoot biomass of plants grown in clean and contaminated soil are given in Table 5.4. Figure 5.5 shows the percentage reduction of total biomass for both plants in contaminated soils as compared to those grown in clean soil. The sunflower had a lower percentage reduction of biomass in T2 than the oat plant, however, for all other contaminated soils, the sunflower plants showed the greater biomass reduction.

Summarizing the growth characteristics, the tests demonstrated that the germination, survival, plant height, and final biomass were influenced by the combined contamination conditions and there was a gradual increase in toxicity with the increase in contaminant concentrations. Nutrient concentrations of the soils were tested to determine if the lower plant growth noted for plants grown in contaminated soils is due to the non-availability of nutrients when contamination is present in the soil. For the clean soil (T1), the average value of N, P and K were 22.84, 0.85 and 161 mg/kg, respectively. For treatment T5, the average value of N, P and K were 25.9, 0.86 and 209 mg/kg, respectively. The exchangeable nutrient concentrations were not less in contaminated soil than those in the clean soil. This shows that the lower plant growth in contaminated soil is due to the phytotoxicity caused by the metals or organic contamination or a combination of both. Phytotoxicity by different heavy metals has been studied. Non-essential
Figure 5.3 Increase in Plant Height with Time by Soil Treatment
Table 5.3: Average Values of Percentage Reduction of Maximum Plant Height for Contaminated soils Compared to Control

<table>
<thead>
<tr>
<th>Plant</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat plant</td>
<td>2.6</td>
<td>14.7</td>
<td>17.7</td>
<td>36.8</td>
</tr>
<tr>
<td>Sunflower</td>
<td>15.7</td>
<td>32.4</td>
<td>60.3</td>
<td>63.2</td>
</tr>
</tbody>
</table>
Figure 5.4 Final Maximum Plant Heights by Soil Treatment
Table 5.4: Average Values of Root Biomass, Shoot Biomass and Total Biomass for Oat Plant and Sunflower for Different Soil Treatments

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Plant</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>Root Biomass (g)</td>
<td>Oat plant</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Sunflower</td>
<td>3.7</td>
</tr>
<tr>
<td>Shoot Biomass (g)</td>
<td>Oat plant</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Sunflower</td>
<td>7.9</td>
</tr>
<tr>
<td>Total Biomass (g)</td>
<td>Oat plant</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>Sunflower</td>
<td>11.6</td>
</tr>
</tbody>
</table>
Figure 5.5 Percentage Reduction of Total Biomass Compared to Control
heavy metals like Pb, Cd and Cr are not known to have any metabolic function in plants and are toxic to plants at high concentrations (Pahlsson, 1989). In the case of organic contaminants, phytotoxicity is dependent on the type and molecular weight of the contaminant. The presence of low molecular-weight, volatile, water-soluble hydrocarbons (<3 rings) such as benzene, toluene, naphthalene, etc., can strongly inhibit seed germination and plant growth. But, high molecular weight PAHs (3-5 rings) does not show phytotoxicity (Henner et al. 1999). Phytotoxicity also varies by species. If the growth characteristics of both species are compared, the oat plant seems to have better growth and survival rates in higher contamination soils than the sunflower. These preliminary results of plant growth were presented and reported earlier (Chirakkara and Reddy 2014).

The final soil samples taken from all the pots after the plant growth period were analyzed for pH, electrical conductivity and oxidation-reduction potential (ORP) as shown in Table 5.5, which reports the averages. The pH variation was not considerable, but there was a tendency toward a lower pH value in the soil as the contamination increased, especially in pots used to raise sunflower plants. This may be due to the difference in rhizosphere acidification by different species (Nye 1981). Pb and Cd are expected to be less soluble at these soil pH conditions. Even though reduction in redox potential is expected to increase metal solubility, the variation in redox potential obtained here across different treatments was not sufficient to change the solubility of heavy metals (Chuan et al. 1996).

Figure 5.6 shows the final concentrations of heavy metals in the planted and unplanted pots with varying initial contaminant concentrations. At all of the initial concentration levels except for the T4, sunflower was best at reducing the Pb from the soil than the oat plant, while the the difference in final Pb concentration produced by the sunflower and oat plant for the T4
Figure 5.6 Soil Metal Concentrations After Plant Growth Period
Table 5.5: Average Values of pH, Oxidation Reduction Potential and Electrical Conductivity for Oat Plant and Sunflower for Different Treatments

<table>
<thead>
<tr>
<th>Value</th>
<th>Plant</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>pH</td>
<td>Oat plant</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>Sunflower</td>
<td>8.0</td>
</tr>
<tr>
<td>ORP (mV)</td>
<td>Oat plant</td>
<td>-57.6</td>
</tr>
<tr>
<td></td>
<td>Sunflower</td>
<td>-58.3</td>
</tr>
<tr>
<td>EC (mS/cm)</td>
<td>Oat plant</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Sunflower</td>
<td>0.10</td>
</tr>
</tbody>
</table>
pots was not significant (p>0.05). Overall, the sunflower produced the greater reductions of Cd and Cr from the soil at all initial contaminant concentration levels. Both plant species proved more adept at removing Cr than Pb and Cd.

The exchangeable heavy metal concentrations found in the different soil samples post-plant growth period appear in Figure 5.7. Exchangeable Pb concentrations were zero in all of the planted pots, suggesting that the plants, regardless of species, assisted with the immobilization, and/or extraction of Pb. At higher Pb concentrations, exchangeable Pb was found in unplanted pots, but the concentrations were much less than the total Pb concentration in the soil. Exchangeable Cd and Cr were found in all the samples, but their concentrations were considerably less than the total metal concentrations. In general, the exchangeable Cr concentrations were higher in the solution compared to the exchangeable concentrations of Pb and Cd found at all initial concentration levels. This may be due to the preference in adsorption of different heavy metals in exchange sites of the soil particles. The solubility of anionic metals, like Cr, will increase and that of cationic metals, like Pb and Cd, will decrease at pH>7 (McLean and Bledsoe 1992; Chuan et al. 1996). So, at the present pH of the soil, Cr tends to be found in a more exchangeable form than Pb and Cr. The exchangeable metal concentrations increase as the total metal concentrations rise. This is because the affinity of a metal to the soil surface is concentration dependent and that affinity decreases with increasing concentration (McLean and Bledsoe 1992). This should be the reason for the gradual increase in toxicity that was found with the increase in the concentration of contaminants.

The PAH analysis showed that the naphthalene concentration was zero in all the initial samples and final samples, except for initial sample T5 where the concentration detected was very low (5.2 mg/kg) compared to the spiked the concentration of naphthalene (50 mg/kg). This
Figure 5.7 Exchangeable Metal Concentrations After Plant Growth Period
Figure 5.8 Soil Phenanthrene Concentrations by Treatment
is indicative of volatilization and the microbial degradation of naphthalene (Mozo et al. 2012). Figure 5.8 shows the phenanthrene concentrations in initial and final samples. The final phenanthrene concentrations in both the planted and unplanted pots were considerably less than the concentration seen in the initial samples, which again shows the biodegradation or volatilization of phenanthrene in the soil. But, there was no indication of plant-promoted microbial degradation, as there was no considerable reduction of phenanthrene in planted pots when compared to the measurements from the unplanted pots.

5.5 Conclusions

The mixed contaminants had a significant effect on the growth characteristics of the oat plant and sunflower. All of the plants showed delayed germination and reduced germination and survival rates when seeded in all of the contaminated soils, as compared to the plants sown in the control pots. The oat plant showed the better germination rate in all of the contaminated soils and its survival rate did not seem to depend upon the magnitude of contaminant concentrations in the soil. The survival rate for the sunflower was comparable to that of oat plant at lower concentrations of contaminants, yet its survival was greatly affected at higher concentrations. Both species showed reduced growth rates and reduced biomass as the contaminant level rose. The results suggest that oat plant has better survivability in soil with a higher level of contamination than the sunflower. It may not be appropriate to use sunflower plants in contaminations above the T4 concentrations considered here since the biomass dropped by more than 80% in that case. Soil amendments that increase the biomass and survivability of sunflower plants may be considered to improve both the biomass and phytoremediation efficiency at higher concentrations of contaminants. This is important as the phytoextraction of heavy metals was
better by sunflower at all of the contaminant concentrations studied. The maximum reduction was achieved for Cr concentrations, compared to those for Pb and Cd. No clear trend was observed that would indicate plant-assisted rhizodegradation of the PAHs. In general, the plant growth and phytoremediation efficiency followed a gradual trend as the contaminant concentrations became elevated.

5.6 Cited References


cadmium co-contaminated soils.” Environmental Science and Pollution Research, 21(2), 1304-1313.


6.1 Introduction

Phytoremediation is a green, sustainable remedial option that can be adopted to remediate soil that is contaminated with a mixture of organic and heavy metal contaminants (Reddy and Chirakkara, 2013). Phytoremediation is the use of plants to degrade, extract, contain, or immobilize contaminants from soil and water (Sharma and Reddy, 2004). The capability of the plants to uptake contaminants, survive in contaminated soil and the low bioavailability of the contaminants in the soil are some of the limiting factors that influence phytoremediation efficiency. Phytoremediation can be enhanced either by increasing the capability of contaminant uptake by the plant or amending the soil to increase the bioavailability of the contaminants. Plant uptake of contaminants can be increased by the use of transgenic plants (Bhargava and Srivastava 2014) or the inoculation of engineered entophytic bacteria (Bell et al. 2014).

By improving the growth of the plants, their water and nutrient uptake will be increased, which leads to increased contaminant uptake. But, it can also affect plant uptake of contaminants via ion competition at the soil and plant level (Pilon-Smits 2005). The addition of organic matter to the soil can improve the soil texture and increase the plant biomass in phytoremediation (Masciandaro et al. 2013). Biochar can immobilize heavy metals in the soil through the surface sorption of the metals, and improve the biological activity in the soil (Paz-Ferreiro et al. 2014). Compost can improve plant growth and microbial activity (Ghanem et al. 2013). Compost is also expected to immobilize heavy metal contaminants in the soil (Alvarenga et al. 2014).
The bioavailability of heavy metals can decrease in the presence of organic contaminants and become a limiting factor in the phytoremediation of mixed contaminated soils (Batty and Dolan, 2013). Due to the low bioavailability of heavy metals, the accumulation of heavy metals by plants is less in the presence of organic contaminants (Chen et al. 2004; Kobyłecka and Skiba 2008). Further, the addition of chelating agents or surfactants to the soil can increase the heavy metal bioavailability by desorbing it from the particle surfaces or by complex formation. Research shows that the mobility of heavy metals in the soil can be considerably increased by the addition of chelating agents (Lombi et al. 2001). Surfactants can also dissolve, desorb, solubilize, and/or emulsify poorly soluble substrates in soil (Noordman et al. 2002; Mudler et al.1998), and surfactants remediate both organic and metal contamination (Miller 1995).

Compost is expected to immobilize the heavy metal contaminants in the soil, thus plant germination and growth are expected to improve in composted soil. But, immobilization of the contaminants by compost can also reduce phytoextraction (Karami et al. 2011). This can be overcome by supplementing the soil with chelating agents or surfactants after the plants have germinated and are established.

6.2 Background

Biochar has received considerable attention in recent years as a material for soil remediation due to its surface sorption and immobilization properties. In particular, Biochar can be used to reduce the mobility of both organic and heavy metal contaminants (Beesley et al. 2011). It can also release essential nutrients that are beneficial for plant growth (Uchimiya et al. 2010). The biochar amendment of the soil can improve its water holding capacity and porosity and improve the soil structure to promote better plant growth. These properties make biochar a suitable option to
amend soil in order to enhance phytoremediation. Only a limited number of researchers have addressed the combination of biochar application and phytoremediation for the remediation of heavy metal contaminated soils.

To this end, Karami et al. (2011) studied the effects of biochar amendments on the uptake of Pb and Cu using rye grass. Plant uptake of Pb and Cu was reduced in biochar treated soil, compared to untreated soil. The pore water concentrations of the metals were also reduced, showing the immobilization of the metals by biochar. Houben et al. (2013) studied the effects of biochar application on the bioavailability of Cd, Pb and Zn in a metal polluted soil as well as its impact on the biomass production of Brassica napus L. (rapeseed) planted in that contaminated soil. They observed that the phytoextraction of Pb and Zn was inhibited in biochar amended soil and the bioavailable metal concentrations in the soil decreased in the biochar treated soils.

Fellet et al. (2014) studied three types of biochars on the uptake of elements by known metal accumulator plant species (Anthyllis vulneraria subsp. polyphylla (Dc.) Nyman, Noccaea rotundifolium (L.) Moench subsp. cepaeifolium and Poa alpina L. subsp. alpina) that were grown on mine tailings. Pot experiments were conducted using soil amended with three types of biochars: ROM, ABE and MAN. The feedstocks were pruning residues from orchards for ROM, fir tree pellets for ABE and manure pellets (70%) mixed with fir tree pellets (30%) for MAN. Plants raised in unamended soils were used as controls. Although to different extents, the biochars induced significant changes in the bioavailability of the metals as ROM and MAN biochars reduced metal accumulation in the shoots and MAN biochar also led to a higher biomass production by all the plants. ROM and ABE had statistically equal biomass productions, and their mean biomasses were less than that of control. Plants grown in the soil amended with ABE biochar had increased shoot metal accumulations compared to the control. Metal
accumulations were also greatly affected by plant species. MAN biochar immobilized heavy metals in the soil while ABE biochar improved phytoextraction from the soil. This study shows the importance of the careful selection of biochars, based on the project requirements. The literature review about combined biochar application and phytoremediation for heavy metal contaminated soils that was compiled by Paz-Ferreiro et al. (2014) concludes that more understanding on the heterogeneous properties and changes of properties associated with the aging of biochars is required.

A number of researchers in the past decade have examined the combination of phytoremediation with a compost application to the soil. Most of these studies concentrate on the enhanced biodegradation of organic compounds using the combined method. Vouillamoz and Milke (2001) showed that composting can improve plant growth and organic contaminant degradation by rye grass. Parrish et al. (2004) investigated the effect of phytoremediation on composted soil contaminated by PAHs where tall fescue (Festuca arundinacea), annual ryegrass (Lolium multiflorum), and yellow sweet clover (Melilotus officinalis) were planted in the composted soil. Unplanted composted soil was taken as the control. The highest reduction of PAH concentrations were observed in planted soils, and there was a positive relationship implication between the production of plant biomass and PAH degradation.

Parrish et al. (2005) compared the concentrations of labile PAH in composted unplanted soil and in composted samples, planted with tall fescue. Their results indicated that the combination of compost and phytoremediation can reduce the labile PAH concentrations in the soil. In another study, Wang et al. (2012) compared the dissipation of pyrene by rye grass and alfalfa in composted and unamended soil using three types of soils: quartz sand, alluvial soil and red soil. The higher dissipation of pyrene in the composted soil showed that the combined effect
of plant root exudates, micro-organisms and compost amendments can result in the enhanced degradation of pyrene.

Phytorestoration of a metal polluted site by a combination of composting followed by planting with Indian mustard was studied by Clemente et al. (2006). The compost raised the pH of the soil, making it able to sustain vegetation. Diethylene triamine pentaacetic acid (DTPA) extractable metal contents were less in the composted soil than in the non-composted soil. Karami et al. (2011) investigated the effect of compost amendments on the uptake of Pb and Cu by rye grass. Their results showed that the compost amendment significantly reduced the phytoextraction of Pb, but the compost had less effect on Cu phytoextraction.

Nutrient solutions were applied to the soil to improve the growth of plants for phytoremediation (Ahammed et al. 2013; Batty and Anslow 2008). Even though NPK fertilizer application is expected to improve remediation due to improved plant biomass, there are instances of reduced concentration of metals in plants produced in fertilized soils due to increased yield (Chigbo et al. 2013). Even in that case, the total removal of metals from the soil was higher in fertilized than unfertilized soil. Most of the existing studies that involve nutrient applications do not concentrate on the effect of nutrient application on phytoremediation. In the present study, different soil treatments are compared, with and without nutrient application, to investigate the specific effect of nutrient solution in the growth and phytoremediation efficiency of plants.

Chelating agents are soluble chemicals that can bind and mobilize other molecules (including both metals and organic contaminants) into the soil solution, increasing their availability for plant uptake and root-to-shoot translocation (Huang et al. 1997; Evangelou et al. 2007). A number of natural and synthetic chelating agents are available, although their
effectiveness varies with plant and soil types. EDTA is a chelating agent commonly used for increasing plant metal uptake of heavy metals (Zaier et al. 2014; Huang et al. 1997).

According to the research by Huang et al. (1997), EDTA was most effective in increasing Pb accumulation by plants than many other chelating agents. It has been used in studies to enhance the heavy metal accumulation efficiencies of high biomass crops like *Avena sativa* (Ebbs and Kochian 1998). Still, there are very few published studies where EDTA enhanced phytoremediation was tried on mixed contaminated soils. Chigbo and Batty (2013) investigated the effect of EDTA in the phytoremediation of a soil that was contaminated with Cr and Benzo(a)pyrene using plant *Medicago sativa* (alfalfa). In that test, the EDTA solution was applied to each pot once in a week for three weeks, but did not affect the biomass of the plants in the mixed contaminated soil. The soluble concentration of Cr was significantly affected by the EDTA, and the concentration of Cr in the shoots of the plants was markedly increased with that application. Specifically, the EDTA application significantly reduced the root concentration of Cr, indicating high root to shoot translocation of the metal, due to the application. The addition of EDTA to the soil also reduced the Benzo(a)pyrene concentration, when compared to the control.

Surfactants are the other group of chemicals that can assist in increasing the water solubility of contaminants, so they can be more easily accumulated by plants. These are biodegradable soil amendments for enhancing the bioavailability of the contaminants (Agnello et al. 2013). Most of the phytoremediation studies involving surfactants are conducted on soil contaminated solely with organic contaminants (Gao et al. 2007; Gao et al. 2008; Cheng et al. 2008). Ramamurthy and Memarian (2012) studied the influence of two non-ionic surfactants, Triton X-100 and Tween 80, on the removal of mixed contaminants (Cd, Pb and used engine oil)
by *Brassica juncea* (Indian mustard). These surfactants were applied to the test pots in which the soil had been spiked with those contaminants. The results showed that Triton X-100 and Tween 80 enhanced Cd and Pb accumulation in the plant roots and that Cd was translocated to the plant shoots, but not Pb.

Another study of surfactants used in the phytoremediation of mixed contaminated soil was performed by Sun et al. (2013). They conducted pot experiments with *Tagetes patula* (marigold) to evaluate the effectiveness of GA₃ (a vegetable hormone that can promote plant growth) and Tween-80 as soil amendments for the enhanced phytoremediation of soil co-contaminated with Cd and benzo[a]pyrene. GA₃+Tween-80 treatment enhanced the growth of *Tagetes patula* by 23 to 55% relative to the control plants. Compared to the control treatment, the accumulation of Cd and B[a]P in the shoots under GA₃ + Tween-80 treatment increased by 1.33 to 1.55 times. These results support the use of surfactants in co-contaminated soils to improve both phytoextraction and phytodegradation. Igepal CA-720, used in the present study, is a non-ionic surfactant used in previous studies where it was effective in removing phenanthrene from soils co-contaminated with nickel and phenanthrene (Khodadoust et al. 2004; Maturi et al. 2009).

Naphthalene, phenanthrene, Pb, Cd, and Cr are the most common contaminants discovered at many mixed contaminated sites, such as abandoned industrial sites. The existing studies on enhanced phytoremediation do not address the typical contaminants of current interest. Of the existing studies that involve biochar and compost amendments, all were completed on soils contaminated with either heavy metals or organic contaminants, not both although sites are likely to be co-contaminated. Some studies discuss the usage of compost, chelating agents or surfactants for enhancement of phytoremediation (Walker et al. 2003; Yang et al. 2005; Cheng et al. 2008). But, they do not consider the usage of a chelating agent or
surfactant in the composted soil. The objective of this study is to fill some of the missed areas of research by comparing the germination rate, growth rate and phytoremediation efficiencies of *Avena sativa* (Oat plant) and *Helianthus annuus* (sunflower) in soils contaminated with a mixture of organic contaminants (naphthalene and phenanthrene) and heavy metals (Pb, Cd, Cr), and to investigate the possibilities of enhanced phytoremediation through the application of biomass amendments, chemical amendments, or a combination of both.

6.3 Experimental Methods

6.3.1 Soil Used

Gray silty clay, which represents typical Chicago glacial till, was selected for the pot experiments. The important physical properties of the soil are presented in Table 6.1.

6.3.2 Soil Spiking Procedure

The clean control soil required for the pot experiment was prepared by mixing the soil with 15% tap water. Mixed contaminated soil was prepared by spiking the soil with naphthalene, phenanthrene, Pb, Cd, and Cr. For that, a measured amount of naphthalene and phenanthrene were dissolved in hexane using a magnetic stirrer, and once dissolved it was then mixed into a measured quantity of soil to produce a final concentration of 50 mg/kg naphthalene and 100 mg/kg phenanthrene in the soil. The mixed soil dried in a fume hood for 3 to 4 days. To ensure uniformity, the drying soil was mixed daily. After that, PbCl₂, K₂Cr₂O₇ and CdCl₂. ½ H₂O were measured to produce a final concentration of 500 mg/kg Pb, 200 mg/kg Cr and 50 mg/kg Cd.
Table 6.1. Important Properties of Soil Used for the Experiments

<table>
<thead>
<tr>
<th>Property</th>
<th>ASTM Standards</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil organic content</td>
<td>ASTM D 2974</td>
<td>2.4%</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>ASTM D 854</td>
<td>2.7</td>
</tr>
<tr>
<td>Water holding capacity</td>
<td>ASTM D 2980</td>
<td>46.4%</td>
</tr>
<tr>
<td>Liquid limit</td>
<td>ASTM D 4318</td>
<td>32.7%</td>
</tr>
<tr>
<td>Plastic limit</td>
<td></td>
<td>19.1%</td>
</tr>
<tr>
<td>Plasticity index</td>
<td></td>
<td>13.6%</td>
</tr>
<tr>
<td>Clay (&lt; 0.002mm)</td>
<td>ASTM D 422</td>
<td>41%</td>
</tr>
<tr>
<td>Silt (0.002 - 0.05mm)</td>
<td></td>
<td>43%</td>
</tr>
<tr>
<td>Sand (0.05 – 2 mm)</td>
<td></td>
<td>14.2%</td>
</tr>
<tr>
<td>USCS Classification</td>
<td></td>
<td>CL</td>
</tr>
<tr>
<td>USDA Classification</td>
<td></td>
<td>Silty clay</td>
</tr>
</tbody>
</table>
These chemicals were mixed in water (to get approximate water content of 15% in soil) for one hour using the magnetic stirrer before being added to the soil that was previously spiked with naphthalene and phenanthrene. The soil was again mixed well to ensure that the contaminant distribution was uniform.

### 6.3.3 Amendment Application

To prepare the biochar amended soil, a part of the mixed contaminated soil was amended with pine wood biochar (50 g/kg soil). Another part was mixed with yard waste compost (200 g/kg soil) to produce the compost amended soil. For the pots to be amended with nutrients alone, 100 ml of nutrient solution (20-20-20 fertilizer supplied by Carolina Biological Supply Company) was applied once a week to the otherwise unamended pots.

For tests involving application of the chemical amendments, plants were raised in contaminated but unamended soil for four weeks before beginning periodic treatment with the chemical solution composed of 0.4 g/l of EDTA solution or 1 CMC solution of Igepal CA-720. Equal amounts of EDTA and Igepal CA-720 solutions were mixed to study the combined application of both amendments. The planted pots were supplied with 100 ml of one of the prepared chemical solution, once every week.

To study the combined effect of biomass and chemical amendments, plants were grown in composted soil for four weeks. After that, 100 ml of 0.4 g/l EDTA solution or 100 ml of 1 CMC solution of Igepal CA-720 was supplied to the pots once every week.

All the chemicals used for spiking and analytical testing were purchased from Fischer Scientific. Soil treatments, the number of replicate pots and measured properties for each treatment are given in Table 6.2.
Table 6.2. Properties of Soil at the Time of Seeding

<table>
<thead>
<tr>
<th>Soil Treatment</th>
<th>Replicates</th>
<th>pH</th>
<th>Oxidation Reduction Potential (mV)</th>
<th>Electrical Conductivity (mS/cm)</th>
<th>Water Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Clean unamended</td>
<td>2</td>
<td>7.7</td>
<td>-52.5</td>
<td>0.127</td>
<td>16.0</td>
</tr>
<tr>
<td>T2 Contaminated unamended</td>
<td>10</td>
<td>7.5</td>
<td>-40.0</td>
<td>0.218</td>
<td>15.5</td>
</tr>
<tr>
<td>T3 Contaminated biochar amended</td>
<td>3</td>
<td>7.9</td>
<td>-64.1</td>
<td>0.082</td>
<td>16.5</td>
</tr>
<tr>
<td>T4 Contaminated compost amended</td>
<td>3</td>
<td>8.1</td>
<td>-72.4</td>
<td>0.060</td>
<td>17.0</td>
</tr>
<tr>
<td>T5 Contaminated nutrient solution amended</td>
<td>3</td>
<td>7.5</td>
<td>-40.0</td>
<td>0.218</td>
<td>15.5</td>
</tr>
<tr>
<td>T6 EDTA amended</td>
<td>2</td>
<td>7.5</td>
<td>-30.5</td>
<td>0.302</td>
<td>15.9</td>
</tr>
<tr>
<td>T7 Igepal CA-720 amended</td>
<td>2</td>
<td>7.5</td>
<td>-30.5</td>
<td>0.302</td>
<td>15.9</td>
</tr>
<tr>
<td>T8 EDTA+Igepal CA-720 amended</td>
<td>2</td>
<td>7.5</td>
<td>-30.5</td>
<td>0.302</td>
<td>15.9</td>
</tr>
<tr>
<td>T9 Compost+EDTA amended</td>
<td>3</td>
<td>8.1</td>
<td>-72.4</td>
<td>0.06</td>
<td>14.2</td>
</tr>
<tr>
<td>T10 Compost +Igepal CA 720 amended</td>
<td>3</td>
<td>8.1</td>
<td>-72.4</td>
<td>0.06</td>
<td>14.2</td>
</tr>
</tbody>
</table>
6.3.4 Selected Plant Species

The plant species (*Avena sativa* (oat plant) and *Helianthus annuus* (sunflower)) were selected based on their biomass and capability of survival based on previous laboratory results under similar conditions (Chirakkara and Reddy 2013). Each pot was planted with 10 seed. The oat plant seeds were supplied by Seedville USA and sunflower seeds were supplied by Carolina Biological Supply Company.

6.3.5 Pot Setup and Monitoring

Pots of 8cm diameter and 9 cm height were filled with the prepared soil and then the seeds were placed approximately a half inch below the soil surface. Each pot was kept on a separate tray to ensure that the leachate did not get mixed up. The pots were placed under grow lights (metal halide lamps; average light intensity of 400 µmols/m²/s) hung ~12 inches above the plants to obtain the desired light intensity. A timer was set to provide 16 hours of light per day. The hanging height was adjusted as the plants started growing taller to reduce the heat stress caused by the hanging lamps. The temperature below the grow lights, at the height of the plants, was measured as 25°C. Fans circulated air to control the heat. The location of the pots was rotated periodically to ensure uniform light intensity. The plants were propagated for 61 days during which time the growth was monitored and the pots were watered daily. Weekly monitoring included counting the number of plants in each pot and measuring their height. Weekly photographs recorded the plant growth and biomass production. Soil samples were taken at the beginning and end of the growth period to test for pH, electrical conductivity, oxidation reduction potential (ORP), and the concentrations of metals and organic contaminants.
At the end of the plant growth period, the roots of the plants were separated out from the shoots and washed in deionized water. The roots, shoots, and soil were dried in oven at 60°C for 6 days (until it attained a constant weight). The dry weights of the roots and shoots are recorded as root or shoot biomass.

6.3.6 Analytical Testing

Tests of the physical properties of the soil viz. water content (ASTM D 2216), organic content (ASTM D 2974), pH (ASTM D 4972), and grain size (ASTM D 422) were done as per these standards. The water holding capacity of the soil was determined by a method similar to the one used for saturated peat materials (ASTM D 2980). To conduct the heavy metal analysis, acid digestion of the soil samples was done as per EPA method 3050B. The digested and filtered liquid was analyzed with Flame Atomic Absorption (FLAA) spectroscopy for Pb, Cd and Cr. To estimate the exchangeable metals in the soil, 8 ml of 1M sodium acetate solution was added to 1g of soil and was mixed continuously for one hour (Reddy et al. 2001). The filtrate was analyzed with Flame Atomic Absorption (FLAA) spectroscopy for Pb, Cd and Cr. The organic contaminants were analyzed by solvent extraction and analysis using Gas Chromatography, following EPA method SW8270C. To analyze exchangeable nitrogen, 1g soil was shaken with 10 ml of 2M KCl solution for one hour (Xu et al. 2013). The filtered extractant was analyzed using Spectronic Genesys spectrophotometers, following the procedure given by Sattayatewa et al. (2011). To determine the exchangeable fractions of potassium and phosphorus, 1g soil was shaken with 1M ammonium acetate for one hour. The solution was filtered, and the extractant was analyzed for phosphorus with Spectronic Genesys spectrophotometers, as per the procedure given by Sattayatewa et al. (2011). Exchangeable potassium in the extractant was analyzed using
Flame Atomic Absorption (FLAA) spectroscopy. For the test results, means and standard deviations were calculated using Microsoft Office Excel 2007. To check whether a significant difference exists between the result sets, the t-test was performed with Microsoft Office Excel 2007. The alpha value was taken as 0.05 for the t-test.

6.4. Results and Discussions

6.4.1 Biomass Amendments

Enhancement of phytoremediation using biochar amendment (T3), compost amendment (T4) and nutrient solution amendment (T5) are discussed in this section. Germination percentages for the plants are plotted in Figure 6.1. Here, germination is interpreted as the appearance of a green shoot/leaf above the soil. It reveals that the plants seeded in contaminated unamended soil have very low germination rates compared to the plants in clean soil. The worst effect was observed for sunflower, where the contamination reduced the germination rate by 77%. Plant germination in contaminated soil improved with the addition of amendments.

Figure 6.2 shows the percentage of survival of the plants raised in clean soil (T1) and contaminated soils (T2 through T5). Here, the survival is expressed as the presence of green or living plant in each pot at the end of the test period. The percentage of survival is the number of surviving plants as percentage of the number of seeds germinated. The survival rates of all the plants in the contaminated soil improved with the addition of biochar and compost, most considerably for the sunflower. Nutrient amended soil did not produce any significant increase in the survival rate compared to the unamended samples.
Figure 6.1. Percentage Germination of Plants in Different Soil Treatments
Figure 6.2. Percentage Survival of Plants in Different Soil Treatments
Figure 6.3 shows the typical increase in plant height over time for plants receiving different treatments. The final (after 61 days) maximum heights of the plants are presented in Figure 6.4. The maximum plant heights for those raised in control (clean) soil were all higher than for the plants grown in contaminated soils. The maximum plant heights improved for sunflower in biochar and compost amended soils, but this trend was not observed for oat plant. The height of the sunflowers cultivated in the unamended soil and nutrient amended soil were not significantly different, but nutrient amendment improved the final maximum plant height of the oat plant.

The average root and shoot biomass of plants in amended and unamended soil are summarized in Table 6.3. The biomasses of the plants in all of the contaminated soils proved to be less than that found in those produced in clean soil. The addition of compost improved the biomass of sunflower plants, but its total biomass was not significantly different in contaminated unamended pots and biochar amended pots. The oat plant biomass, where the plant was raised in contaminated soil, did not improve through the amendment with compost or biochar. Instead, biochar and compost amendments significantly decreased the total biomass of oat plant, while the nutrient amendment significantly increased the total biomass of oat plant. The total biomass of the sunflower did not change significantly with the nutrient amendment.

The nutrient concentrations shown in Figure 6.5 demonstrate that there was a slight increase in exchangeable nutrient concentrations resulting from the addition of biomass and compost amendments. The addition of a nutrient solution produced a considerable increase in the soil exchangeable nutrient concentration. This may be the reason for the better growth of the oat plant in the nutrient amended soils. But, in the case of sunflower, plant growth was not improved.
<table>
<thead>
<tr>
<th>Soil</th>
<th>Oat Plant</th>
<th></th>
<th>Sunflower</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root Biomass</td>
<td>Shoot Biomass</td>
<td>Total Biomass</td>
<td>Root Biomass</td>
<td>Shoot Biomass</td>
</tr>
<tr>
<td>T1</td>
<td>0.653</td>
<td>1.153</td>
<td>1.806</td>
<td>1.295</td>
<td>3.063</td>
</tr>
<tr>
<td>T2</td>
<td>0.499</td>
<td>0.724</td>
<td>1.223</td>
<td>0.464</td>
<td>0.763</td>
</tr>
<tr>
<td>T3</td>
<td>0.476</td>
<td>0.467</td>
<td>0.943</td>
<td>0.262</td>
<td>0.536</td>
</tr>
<tr>
<td>T4</td>
<td>0.353</td>
<td>0.464</td>
<td>0.816</td>
<td>0.604</td>
<td>2.082</td>
</tr>
<tr>
<td>T5</td>
<td>0.961</td>
<td>1.159</td>
<td>2.119</td>
<td>0.532</td>
<td>0.834</td>
</tr>
<tr>
<td>T6</td>
<td>0.199</td>
<td>0.396</td>
<td>0.594</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T7</td>
<td>0.191</td>
<td>0.339</td>
<td>0.530</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T8</td>
<td>0.283</td>
<td>0.378</td>
<td>0.661</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T9</td>
<td>0.486</td>
<td>0.562</td>
<td>1.047</td>
<td>0.546</td>
<td>2.273</td>
</tr>
<tr>
<td>T10</td>
<td>0.520</td>
<td>0.640</td>
<td>1.159</td>
<td>0.497</td>
<td>2.250</td>
</tr>
</tbody>
</table>
Figure 6.3. Variation of Maximum Plant Height with Time in Different Soil Treatments

(a) Oat plant

(b) Sunflower
Figure 6.4. Final Maximum Height of Plants in Different Soil Treatments
Figure 6.5. Exchangeable Nutrients in Unamended and Amended Soil Samples
by the nutrient addition. The sunflower showed improved growth characteristics in the biochar and compost amended soils. The contaminant immobilization caused by biochar and compost would have caused the improved growth characteristics seen with the plants. In nutrient amended soils and in unamended soils, mobile contaminants in the soil solution would have caused phytotoxicity to sunflower plants.

The phytotoxicity of plants bred in the contaminated soil is expected to be mainly due to the heavy metals present in the soil (Chirakkara and Reddy 2014). Non-essential heavy metals like Pb, Cd and Cr are toxic to plants at high concentrations (Pahlsson 1989). Biochar can reduce phytotoxicity by adsorbing toxic contaminants to its surface (Beesley et al. 2011). Mobilized metals due to soluble complex formation with organic ligands may be the reason for the reduced biomass of the oat plant grown in the amended soil (McLean and Bledsoe 1992). In the case of biochar amended soil, the biochar adsorption of signaling compounds secreted by the plant host to the symbioants is another possible cause of reduced plant biomass (Beesley et al. 2011). While the biochar amendment initially improved the germination and survival of the sunflower plants, the final biomass of sunflower was less for biochar amended soil than in unamended soil. The decreased ability of biochars to sequester the metals with time due to aging process can be the possible reason for this finding (Kookana et al. 2011).

Table 6.4 shows the average values of pH, electrical conductivity and ORP for the soil samples after the plant propagation period. According to this, the pH value of the soil with the biochar and compost amendments increased slightly. The magnitude of the reduction potential was also higher for the biochar and compost amended soil than the unamended soil. Soil samples from pots with the sunflower had slightly lower pH values compared to the soil planted with oats. This may be due to the production of chelating agents by sunflower plants. All the pH
### Table 6.4. Average Values of pH, Oxidation Reduction Potential, and Electrical Conductivity

<table>
<thead>
<tr>
<th>Soil</th>
<th>No Plant</th>
<th>Oat Plant</th>
<th>Sunflower</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>ORP (mV)</td>
<td>EC (mS/cm)</td>
</tr>
<tr>
<td>T1</td>
<td>7.8</td>
<td>-48.2</td>
<td>0.20</td>
</tr>
<tr>
<td>T2</td>
<td>7.8</td>
<td>-48.1</td>
<td>0.15</td>
</tr>
<tr>
<td>T3</td>
<td>8.1</td>
<td>-50.2</td>
<td>0.12</td>
</tr>
<tr>
<td>T4</td>
<td>8.0</td>
<td>-55.3</td>
<td>0.10</td>
</tr>
<tr>
<td>T5</td>
<td>7.9</td>
<td>-52.1</td>
<td>0.13</td>
</tr>
<tr>
<td>T6</td>
<td>7.6</td>
<td>-48.2</td>
<td>0.15</td>
</tr>
<tr>
<td>T7</td>
<td>7.6</td>
<td>-50.7</td>
<td>0.14</td>
</tr>
<tr>
<td>T8</td>
<td>7.6</td>
<td>-51.0</td>
<td>0.14</td>
</tr>
<tr>
<td>T9</td>
<td>8.1</td>
<td>-65.7</td>
<td>0.09</td>
</tr>
<tr>
<td>T10</td>
<td>7.6</td>
<td>-56.3</td>
<td>0.11</td>
</tr>
</tbody>
</table>
values reported here are greater than 7, which means that the anionic metals like hexavalent Cr will be more mobile in the soil solution compared to the cationic metals like Pb and Cd (McLean and Bledsoe 1992). At lower pH values, the mobility of cations in the soil pore water increases due to the replacement of cations at the soil exchange sites by the H+ ions. The negative value of the ORP indicates that reducing conditions exist in the soil, which favors more reduced elemental forms of the metals.

Figure 6.6 shows the heavy metal concentrations of the soil at the conclusion of the plant growth period for both the planted and unplanted soils. In the unamended soil, only the sunflower achieved a significant (p<0.05) reduction in Pb concentration compared to unseeded soil. But, in the biochar and compost amended soil, both the plants were able to reduce the Pb concentration significantly (p<0.05). The nutrient amendment did not change the soil Pb concentrations significantly (p>0.05) compared to the unamended samples. Similar results were observed for the reduction of Cd concentration by the plants. However, the Cr concentration of the soil was not affected considerably by the addition of the amendments for the sunflower. The oat plant achieved a greater Cr reduction when raised in the biochar and compost amended soils compared to nutrient amended soils or unamended soils. This variation may be due to the difference in the bioavailability of different metals in the presence of amendments. The bioavailability and mobility of the metal contaminants in soil are dependent on the speciation of metals based on their complex formation and pH values. At the present pH conditions of the soil, Cr is more bioavailable for extraction by plants. With the addition of organic amendments like biochar and compost, Pb and Cd are expected to form soluble complexes with organic ligands (McLean and Bledsoe 1992). The concentrations of exchangeable heavy metals in the soil samples appear in Figure 6.7, which shows the highest concentrations for Cr, followed by Cd and
Figure 6.6. Soil Heavy Metal Concentrations in Different Soil Treatments
Figure 6.7. Exchangeable Heavy Metal Concentrations in Different Soil Treatments
Pb. The selectivity sequence for the considered metals for adsorption is Pb>Cd>Cr, considering hexavalent chromium (McLean and Bledsoe, 1992; Gomes et al. 2001). A possible reason for this is the preferential adsorption of the three metals to the soil particles at this pH level.

A comparison of the nutrient amended pots of sunflower and oat plant shows that in pots with sunflower, the exchangeable nutrient concentrations are higher and exchangeable and total heavy metal concentrations are lower than in the pots with the oat plant. This means that the sunflower has taken up fewer nutrients than the oat plant, while it has taken up more of the heavy metals. A possible explanation is the membrane transporter proteins, which are responsible for the absorption of heavy metals and nutrients from soil and their transport within the plant (Pilon-Smits 2005). These proteins are supposed to take up and transport required nutrients; they transport the heavy metals due the chemical similarity of heavy metal ions to nutrient ions. This similarity can cause competition that, in turn, can affect the plant uptake and transport of nutrients and heavy metals in contaminated soils. The membrane transporter proteins are also species dependent, which might also explain the reason for the lower nutrient uptake and higher heavy metal uptake found in the sunflower plants.

The sunflower plants showed improved biomass and also better contamination removal when raised in the compost amended soil. This indicates that the complex formed by the heavy metals with organic ligands were nontoxic, yet available for extraction by the plants. The complexation of metals with ligands can decrease free ion activity, thus lessening the toxicity to the plants. The metal complex can be stored in root cells or xylem saps without causing toxicity to the plants (McGrath et al. 2002).

The naphthalene concentration in the initial unamended sample (T2) was 8.9 mg/kg, which is considerably less than the spiked concentration of naphthalene. No naphthalene was
detected in initial amended samples or any of the final samples except for the unamended samples planted with sunflower where that concentration was 3 mg/kg.

Figure 6.8 shows the initial and final phenanthrene concentrations for the samples. The expected dissipation mechanisms of the PAHs in soil are either one or a combination of microbial degradation, volatilization and phytodegradation/accumulation. With the unamended soils, all the final samples have less phenanthrene concentration than the initial samples, indicating the degradation of phenanthrene. In the unamended soils, the planted pots have higher PAH contents, possibly due to production of phytoalexins by the plants in stressed conditions (Bais et al. 2006). Phytoalexins have antimicrobial properties that can inhibit the degradation of organic matter in soil. In the case of planted soils, phenanthrene degradation improved when the soil was supplemented with the amendments. This demonstrates that the amendments reduced the stress conditions to the plants, inhibiting the production of phytoalexins. The unplanted pots show that phenanthrene degradation is higher in both unamended samples and nutrient amended samples. Thus, the microbial degradation of phenanthrene is lesser in the unplanted biochar and compost amended samples. Biochar tends to adsorb the organic contaminants to its surface, reducing the bioavailability of the organic contaminants for degradation (Beesley et al. 2011). The presence of humic acid in the soil due to composting might have reduced the biodegradation of phenanthrene in the compost amended soils (Shimp and Pfaender 1985). Also, organic materials like biochar and compost can contain lignin, which can bind to the hydrophobic organic compounds and reduce its bioavailability (Pilon-Smits 2005). In planted samples amended with biochar and compost, the plant-promoted microbial degradation is expected to be the dissipation pathway for phenanthrene.
Figure 6.8. Phenanthrene Concentrations in Different Soil Treatments
6.4.2 Chemical Amendments

Phytoremediation efficiencies in chemical amended soil samples (T6, T7 and T8) are discussed in this section. There were an insufficient number of sunflower replicates to study the EDTA/Igepal amendments in non-composted soil. So, as a consequence, the contaminated EDTA/Igepal amended tests could not be conducted for sunflower. All the plants treated with chemical amendments showed phytotoxicity symptoms such as a yellowish color and diminished growth, and those cultivated in contaminated soils all had lower survival rates compared to the plants grown in clean soil (Figure 6.2). The survival rate improved for Igepal CA-720 amended soil compared to unamended soil and EDTA amended soil. Of these, the greatest height was achieved by plants grown in clean soil, followed by those in contaminated unamended soil (Figure 6.4). EDTA and Igepal amendments adversely affected the plant growth. Plant height in contaminated soil was reduced by 16% compared to the plants in clean soil. The addition of EDTA reduced the plant height by 18%, compared to the plants in contaminated unamended soil. The reduction of plant height was 26% for Igepal CA-720 treated soil compared to the plants in the contaminated but unamended soil. A plant height reduction of 28% occurred when the soil was treated with a mixture of both EDTA and Igepal CA-720.

In general, the plants raised in the contaminated soils had a lower biomass than the plants in clean soil (Table 6.3). And, the biomass was further reduced when the chemical amendments were added to the soil. The plants in contaminated unamended soil had 20% less root biomass compared to the plants grown in clean soil. The root biomass was reduced by approximately 60% when either EDTA or Igepal CA-720 solution was added to already contaminated soil and the reduction was 43% when a combination of EDTA and Igepal solution was applied. The shoot biomass for plants bred in contaminated unamended soil was 40% less than those in the clean
soil. The shoot biomass reduction was 53% when EDTA solution was added to the contaminated soil and 45% when Igepal solution was added. The combination of EDTA and Igepal reduced the shoot biomass by 48%.

The plant growth characteristics clearly indicate increased phytotoxicity symptoms in plants grown in chemically amended soil, compared to those in unamended soil. This indicates that the chemical amendments have caused an increase in the bioavailability of the contaminants. Previous research studies support this hypothesis that chelating agents like EDTA have the potential to desorb the heavy metals in soil and enhance their uptake by plants (Thayalakumaran et al. 2003). EDTA was proven to increase the plant accumulation of Pb and increase the Pb translocation from roots to shoots (Huang et al. 1997). Higher heavy metal accumulation can cause phytotoxicity by affecting chlorophyll content (Oncel et al. 2000), photosynthesis, stomata closure (Bazzaz et al. 1970; Bazzaz et al. 1974), water potential, and transpiration rate (Chatterjee and Chatterjee 2000). Similar results of reduced biomass were observed by Gunawardana et al. (2010) in the chemically enhanced phytoextraction of Cu, Cd and Pb by *Lolium perenne* (rye grass), due to the increased plant accumulation of metals in amended soil.

Table 6.4 shows that EDTA and Igepal amendments reduced the soil pH by 0.2 units. This change in pH can affect the heavy metal mobility in the soil solution and, thus, the plant growth (McLean and Bledsoe 1992). The reduction potential and electrical conductivity values were not much different for the amended and unamended samples, and the contaminant migration in soil could not be commented on given these values.

While comparing the metal concentrations, unplanted unamended pots used as the control. Pb concentrations were not significantly different (p> 0.05) in planted unamended pots, and Igepal CA-720 treated plots compared to the control. EDTA treatment has shown a
significant reduction of Pb (p<0.05) concentration compared to the control. EDTA treated pots had a 30% reduction in Pb concentration compared to the control. The reduction in Pb concentration was 27% in the case of pots treated with a combination of EDTA and Igepal. Zaier et al. (2014) observed similar results of increased Pb phytoextraction in EDTA amended soil. The Cd concentrations were not significantly affected by the presence of plants or by the application of amendments. There was a significant reduction of Cr (27%) in the planted unamended pots compared to the control group. But, all of the measurements from the chemically amended pots showed that there was no significant difference in the contaminant concentration when compared to the control. This confirms that chemical amendments reduce the efficiency of the plants to uptake Cr from the soil. The reduced mobility of Cr in the solution, due to the reduced pH caused by the chemical amendment application, can be a reason for this occurrence. Another potential reason is the reduced biomass of the plants bred in amended soils that is caused by the increased availability of Pb and Cd during the initial stage of plant growth.

The chemically amended samples had significantly higher concentrations of exchangeable Pb compared to the unamended samples (Figure 6.7). Here, the unamended planted pots did not show any significant difference in exchangeable Pb concentration when compared to the control pots. The exchangeable Pb concentration was highest in the EDTA amended soil compared to all other samples. For Cd, the planted unamended samples had concentrations of exchangeable Cd that were similar to the measurements of the control samples. The chemical amendments significantly increased the concentrations of exchangeable Cd in the soil. The exchangeable Cd concentrations were higher for EDTA amended soils than the samples with Igepal amendment alone. None of the samples showed a significant difference from each other in the case of exchangeable Cr concentrations. This means that EDTA or Igepal did not
mobilize the adsorbed Cr from the soil particles.

Phenanthrene degradation was higher when the pots were treated with chemical amendments. This supports the hypothesis that chemical amendments can improve the biodegradation of organic contaminants by increasing their bioavailability for microbial degradation (Chigbo and Batty 2013).

### 6.4.3 Combination of Biomass and Chemical Amendments

This section discusses the application of EDTA or Igepal CA-720 to composted soil (T9 and T10) and the effect of these solutions on the performance of the oat plant and sunflower. The addition of EDTA or Igepal CA-720 to the soil did not seem to affect the survival rate of either species (Figure 6.2). The outcome for the oat plant shows that when the soil was not initially amended with compost, both EDTA and Igepal adversely affected its growth. But, when the soil was amended with compost first, there was no reduction in the growth characteristics when either EDTA or Igepal was added.

For sunflower plants in compost amended soil, the total biomass did not change significantly given the EDTA or Igepal amendments. The biomass of oat plant in composted soil increased slightly when fed with the EDTA or Igepal. The analysis of the nutrient concentrations (Figure 6.5) illustrates an increase in exchangeable concentrations of P and K driven by compost amendment. The improved growth of plants in compost amended soils can be due to the improved nutrients or the immobilization of contaminants caused due to the organic ligands in the compost.

The overall efficiency of both plants to take up metals from the soil was reduced significantly (p<0.05) when the composted soil was enhanced with chemical amendments.
Compared to the performance of plants in uncomposted soil, plants in composted soil performed better in terms of Pb reduction when the chemical amendments were added to the soil.

The concentrations of exchangeable heavy metals in different soil samples appear in Figure 6.6. The exchangeable Cd concentrations increased significantly by the addition of EDTA, however the exchangeable Cr concentrations increased only slightly and insignificantly when EDTA was added to composted soil. There was a significant increase in exchangeable Cr when Igepal CA-720 was added to the composted pots growing the sunflower plants. The addition of chemical amendments to the composted soil did not help to improve the phytoextraction of heavy metal by the soil, probably due to the reduced plant biomass in found in the chemically amended soil.

The degradation of phenanthrene was best observed when chemical amendments were added in combination with the compost. This signifies that the addition of compost amendments reduced the stress conditions to the plants, and that the chemical amendments improved the biodegradation of organic contaminants by increasing their bioavailability for microbial degradation (Chigbo and Batty, 2013). In the amended planted samples, the plant-promoted microbial degradation is expected to be the dissipation pathway for phenanthrene.

6.5. Conclusions

All of the plants cultivated for this study showed delayed germination and reduced germination and survival rates in the mixed contaminated soil compared to the control pots. The germination, growth and biomass of sunflower plants were greatly improved by the addition of biomass amendments. There was improved germination for the oat plant grown in biochar and compost amended soils, but the final biomass was less than that of the plants in unamended soil. The
addition of chemical amendments increased the phytotoxicity symptoms of the plants. Sunflower reduced the concentrations of Pb, Cd and Cr in unamended soil. With the addition of biochar and compost amendments, more reduction of Pb and Cd was observed in pots planted with sunflower. The Cr reduction by sunflower plants did not change considerably with the addition of the biomass amendments. The oat plant failed to remove Pb and Cd from the unamended soil, but there was a measurable reduction in the concentration of Pb and Cd when the soils were amended with biochar and compost. Cr reduction was achieved by all the plants in unamended soil and this did not change considerably with the addition of biomass amendments. The nutrient amendments did not cause a considerable change in the reduction of heavy metals by either plant. The addition of chemical amendments to the soil reduced the phytoextraction efficiency of the plants, possibly due to the reduction in the plant biomass. The exchangeable metal concentrations were considerably increased by the chemical amendment application for Pb and Cd. The addition of chemical amendments to composted soil did not improve phytoextraction. PAH degradation in planted soils was improved by the addition of biomass amendments. The chemical amendments also enhanced the degradation of phenanthrene from the soil. PAH degradation was best achieved when composting was combined with the application of a chemical amendment. Overall, the results suggest that due to the phytotoxicity caused by the mobilized metals, EDTA or Igepal CA-720 are not recommended as a soil amendment for the enhanced phytoremediation of mixed contaminated soil when the plant is the oat plant. Biochar and compost amendments provide a promising approach for enhancing phytoremediation of mixed contaminated soils using oat plant and sunflower.
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CHAPTER 7
ELECTROKINETIC AMENDMENT IN PHYTOREMEDIATION OF MIXED CONTAMINATED SOILS


7.1. Introduction

Rapid urbanization and industrialization have resulted in sites that have contaminated soil and ground water. Heavy metals and polycyclic aromatic hydrocarbons (PAH) are major concerns at these sites and their remediation is a widely researched topic. Since these two groups of contaminants are physically and chemically different, it becomes difficult to select suitable remedial strategies when these are present together in the soil. Most of the available methods like soil washing, solidification/stabilization, vitrification, in-situ flushing, etc., are energy intensive and expensive. Compared to those methods, phytoremediation is a green and sustainable option to decontaminate mixed contaminated soils (Reddy and Chirakkara 2013). Electrokinetic remediation is another method that is proven beneficial to treat mixed contaminated soils (Maturi and Reddy 2006; Wang et al. 2007).

In phytoremediation, suitable plants are grown in the contaminated area to uptake, degrade, immobilize, or volatilize the contaminants (Sharma and Reddy 2004). The term phytoremediation includes different technologies, namely: rhizofiltration, phytodegradation, phytoaccumulation, phytovolatilization, phytostabilization and rhizodegradation. The
unavailability of heavy metal contaminants for uptake and low bioavailability of organic contaminants for microbial degradation are often limiting factors for phytoremediation of co-contaminated soil (Baker et al. 1994). Some limitations of phytoremediation, like the unavailability of contaminants for phytoextraction or degradation or the limitation in depth of treatment to the root zone, can be overcome by combining electrokinetic remediation with phytoremediation (Denvir et al. 2000). In-field phytoremediation can be used after electrokinetic remediation to remove residual contamination and achieve cleaner soil. Phytoremediation can contribute to the recovery of soil properties and improve the soil structure through the influence of the root system. This study concentrates on combining a electrokinetic remedial strategy with phytoremediation for a soil co-contaminated with heavy metals and PAHs.

7.2. Background

Phytoremediation of co-contaminated sites is an area of active research (Batty and Dolan 2013). Considering the influence of an electric field on plant characteristics, some research efforts have focused on a combination of electrokinetic remediation with phytoremediation. Early studies of electric field application to planted soil were conducted for agricultural purposes. Lemstrom (1904) found that plants treated with an electric field were greener, with an increase in yield. In electrokinetic-assisted phytoremediation, the electric field mobilizes the contaminants into more bioavailable fractions. The electric field can efficiently drive more and more soluble heavy metals toward the plant roots to facilitate the accumulation of heavy metals by plants (Bedmar et al. 2009). Studies also investigate the application of electrokinetic remediation for the removal of organic contamination from the soil (Kim et al. 2000; Ribeiro et al. 2005). Electric field application can stimulate and improve microbial metabolism by improving the degradation of
organic contaminants (Cang et al. 2012). Experimental studies were also conducted on the effectiveness of electrokinetic methods to remediate soils contaminated with a mixture of organic and heavy metal contaminants (Maini et al. 2000; Wang et al. 2007; Maturi and Reddy, 2008).

Denvir et al. (2000) proposed a method of remediating soil, water and other porous media that contain organic and/or inorganic contaminants using plants in conjunction with an electric field applied through the medium. That electric field was used to control the movement of and enhance the removal of the contaminants. The hypothesis was that the electric field can be beneficially utilized to control the transport of charged and/or non-charged contaminants in soil within the rhizosphere and bring the contaminants into the root zone from a contaminated zone located deeper in the soil below the root zone. They suggested that the effectiveness of phytoremediation can also be enhanced to prevent the soil from becoming strongly acidic or basic by manipulating the electric field.

O'Connor et al. (2003) combined electrokinetic remediation and phytoremediation to decontaminate two metal-polluted soils in laboratory-scale reactors. One soil sample was contaminated with copper and the other with cadmium and arsenic. The contaminated soils were filled in test reactors with two separate chambers. Rye grass seeds were sown in the reactors and a constant DC voltage of 30 V was applied continually across the soils. The tests ran for a period of 98 days for the Cu spiked soil and 80 days for the Cd-As soil. The plant Cu uptake was enhanced near the cathode region for the Cu soil. However, the result of Cd uptake was not clear. The plant growth was affected at the anode region due to soil acidification. Also, a fungal infection seen on the rye grass in the cathode region was attributed to the alkaline pH conditions. Lim et al. (2004) examined the addition of a DC electric field around the plants as an approach to increase the uptake of lead by mustard plants. The application of electrolytoremediation with
EDTA was tested in the Pb contaminated soil. The effects of parameters such as operating current/voltage, application time of EDTA, electric potential, and daily application time of the electric potential were studied. They found that the maximum lead accumulation in the plant shoots was obtained with the application of an electric field for 1 h per day for 9 days supplemented by EDTA.

Zhou et al. (2007) studied the effect of vertical direct current on metal uptake by rye grass. The anode was placed at the top, close to the surface, and the cathode was placed at bottom. EDTA or [S,S]-ethylenediaminedisuccinic acid (EDDS) was used to enhance rye grass uptake of Cu/Zn from contaminated soil. The results showed that application of EDTA/EDDS significantly increased the rye grass uptake of Cu/Zn when compared with samples without the EDTA/EDDS application. A vertical DC electric field (1.0 V cm$^{-1}$) passed through the sample caused the redistribution of Cu/Zn concentrations in the soil column. In the bottom sections of the column, Cu/Zn concentrations were significantly decreased in the soil pore fluid. This study suggests that the application of a vertical electric field can control the leaching risk of heavy metal complexes. Moreover, the Cu concentration in the rye grass shoots increased with the application of the electric fields.

Aboughalma et al. (2008) studied the use of AC and DC electric fields for the phytoremediation of soil contaminated with Zn, Pb, Cu, and Cd using potato tubers. In DC treated soils, the pH varied from 3 near the anode to 8 near the cathode. Biomass production of the plants was 27% lower in DC treatments compared to the control. On the other hand, plants treated with AC had 72% higher biomass than the control. In general, the soil treated with either electric fields showed higher metal accumulation by plants roots than the control. However, the shoot accumulation of metals was lower under DC treatment compared to AC treatment and
control. The Zn uptake was higher in plants raised in AC treated soil. Compared to soil Cd content, the plant roots had higher Cd content in all the treatments. The Pb accumulation in either the roots or shoots was less than its content in the soil.

Cang et al. (2011) investigated the effect of DC electric current on growth of Indian mustard and the speciation of soil heavy metals in multiple metal contaminated soil (Cd, Cu, Pb, and Zn). After growing the plant in contaminated soil for 45 days, four DC voltage gradients of were applied to the soils. The extractable soil metals showed a significant redistribution from the anode to the cathode after the electric field treatment. This demonstrated that electric fields can enhance the plant uptake of metals. In a comparison, a voltage gradient of 2 V/cm produced the highest phytoaccumulation. They commented that voltage gradient was the most important factor affecting plant growth, soil properties and metal concentrations in the soil and plant.

Bi et al. (2011) studied the combined application of phytoremediation and electrokinetic (AC and DC) remediation in heavy metal contaminated soil using rapeseed and tobacco. They chose three kinds of soil: un-contaminated soil from a forest area (S1), artificially contaminated soil spiked with 15 mg/ kg Cd (S2) and multi-contaminated soil with Cd, Zn and Pb from an industrial area (S3). The plants, grown in soil filled experimental vessels, received one of three treatment conditions: control without electrical field, AC electric field (1 V/ cm) or DC electrical field (1 V/cm). The polarity of the DC electric field was switched every three hours to minimize the pH variation to the soil caused by the DC field. The electrical field application continued for 30 days for the rapeseed and 90 days for tobacco. The plants were harvested after a total growth of 90 days for the rapeseed and 180 days for tobacco. The plant reactions varied with the applied electric field. The AC electric field had a positive effect on biomass of the rapeseed, and no negative effects were seen for rapeseed biomass under the DC electric field. However, the
tobacco plants did not show biomass enhancement under the AC electric field and the biomass was reduced under DC electric field. In the artificially contaminated soil (S2), Cd uptake was higher for both plant species that were treated with the AC electric field compared to control. The application of the AC electric field enhanced the metal uptake by rapeseed in the soil from industrial area (S3).

The impact of the electrokinetic-assisted phytoremediation of a heavy metal contaminated soil (Cd, Cu, Pb, and Zn) on its physicochemical properties, enzymatic and microbial activities was examined by Cang et al. (2012). Indian mustard plants were grown in contaminated soil for 35 days. Then, three DC voltage gradients (1, 2, and 4 V) were applied across the soil for 16 days for 8 h per day. Samples without the electric field application and samples without plants were treated as the controls. The concentrations of Cd and Zn increased from cathode to anode, while the extractable concentrations of Cu decreased from cathode to anode. Soil enzyme activities were inhibited under the electric field application. They noted that the plant growth partially counteracted the impact of the electric field on the soil properties.

In a recent effort to improve the uptake of Pb from a contaminated sandy soil with Kentucky bluegrass, Putra et al. (2013) combined phytoremediation with electrokinetic remediation. They first evaluated the two dimensional electrode configuration with DC electric field using agar media for 48 hours. After that, the electrokinetic-assisted phytoremediation experiment was run for 15 days. The results where compared with plantings in contaminated soil in which the bluegrass was grown for 30 days without electric potential application. They also discussed the effectiveness of common agrochemical urea that was used to facilitate healthy growth of plants. The root and shoot accumulation of Pb was higher in the electrokinetic-assisted phytoremediation system compared to an unassisted phytoremediation system. The addition of
urea helped the plants to overcome the stress caused by the contamination.

The literature demonstrates that the combination of electrokinetic remediation and phytoremediation is a very promising approach to the decontamination of metal polluted soils. Since the separate use of phytoremediation (Batty and Dolan, 2013) and electrokinetic remediation (Maini et al. 2000; Wang et al. 2007; Maturi and Reddy, 2008) were successfully studied for remediating co-contaminated soils, that combination seems applicable for mixed contaminated soils, too (Cameselle et al. 2013). However, there are no studies available that combine the use of electrokinetic remediation with phytoremediation for soils contaminated with a combination of heavy metals and organic contaminants. An effort has been made in this study to enhance the phytoremediation of soil contaminated with naphthalene, phenanthrene, Pb, Cd, and Cr (common contaminants found at many sites) with use of an electric field as well as the effects of the oat plant and sunflower.

7.3. Materials and Methods

7.3.1. Plant Species

The plant species in this study, *Avena sativa* (the oat plant) and *Helianthus annuus* (sunflower), were selected based on previous studies about biomass production and survival capability in mixed contaminated soil with heavy metals and PAHs (Chirakkara and Reddy, 2013). The oat plant was studied for its phytoremediation efficiency for heavy metals (Ebbs and Kochian, 1998) and organic contaminants (Miya and Firestone, 2001). The sunflower species was reported to be effective in phytoremediation of both organic (Rosado and Pichtel 2004) and heavy metal contaminated soils (Meers et al. 2005; Adesodun et al. 2010; January et al. 2008). The oat plant
seeds were supplied by Seedville (Green, OH, USA) and sunflower seeds were supplied by Carolina Biological Supply Company (Burlington, NC, USA).

7.3.2. Soil
Grey silty clay soil, typical of the Chicago glacial till, was selected for the phytoremediation and electro-phytoremediation tests. Its soil characteristics are listed in Table 7.1.

7.3.3. Soil Spiking Procedure
Mixed contaminated soil was prepared by spiking the silty clay soil with naphthalene, phenanthrene, Pb, Cd, and Cr. Measured amounts of naphthalene and phenanthrene were dissolved in hexane and thoroughly mixed with a measured amount of soil to get a final concentration of 50 mg/kg naphthalene and 100 mg/kg phenanthrene (dry soil basis). The spiked soil specimen was dried for 3 to 4 days in a fume hood until complete drying. To ensure uniform distribution of the naphthalene and phenanthrene, the soil was mixed once a day during the drying process. Measured amounts of PbCl₂, K₂Cr₂O₇ and CdCl₂·½ H₂O were dissolved in DI water and mixed with the previously spiked soil. The heavy metal solution concentration was selected to get the final concentration in the soil of 500 mg/kg Pb, 200 mg/kg Cr and 50 mg/kg Cd (dry soil basis) and moisture content of approximately 15%; all thoroughly mixed to ensure uniform contaminant distribution. This contaminated soil was then mixed with yard waste compost (200 g/kg soil) before it was used for the tests. The properties of contaminated soil at the time of seeding are given in Table 7.2. All of the chemicals were purchased from Fischer Scientific (USA).
Table 7.1: Important properties of soil used for the experiments

<table>
<thead>
<tr>
<th>Property</th>
<th>ASTM Standards</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil organic content</td>
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</tr>
<tr>
<td>Specific gravity</td>
<td>ASTM D 854</td>
<td>2.7</td>
</tr>
<tr>
<td>Water holding capacity</td>
<td>ASTM D 2980</td>
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</tr>
<tr>
<td>Liquid limit</td>
<td>ASTM D 4318</td>
<td>33.1%</td>
</tr>
<tr>
<td>Plastic limit</td>
<td></td>
<td>18.9%</td>
</tr>
<tr>
<td>Plasticity index</td>
<td></td>
<td>14.2%</td>
</tr>
<tr>
<td>Clay (&lt; 0.002mm)</td>
<td>ASTM D 422</td>
<td>42%</td>
</tr>
<tr>
<td>Silt (0.002 - 0.05mm)</td>
<td></td>
<td>42%</td>
</tr>
<tr>
<td>Sand (0.05 – 2 mm)</td>
<td></td>
<td>14.3%</td>
</tr>
<tr>
<td>USCS Classification</td>
<td></td>
<td>CL</td>
</tr>
<tr>
<td>USDA Classification</td>
<td></td>
<td>Silty clay</td>
</tr>
</tbody>
</table>
Table 7.2: Measured properties of soil at the time of seeding

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.4</td>
</tr>
<tr>
<td>Oxidation reduction potential (mV)</td>
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</tr>
<tr>
<td>Electrical conductivity (milli Siemens/cm)</td>
<td>0.226</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>17.3</td>
</tr>
</tbody>
</table>
7.3.4 Cell Setup and Monitoring

The remediation tests were carried out in a rectangular cell divided into three compartments. The cell dimensions are 30 cm (length) x 5 cm (width) and 18.5 cm (height). The central compartment (20 cm long) was filled with the spiked soil. The other two compartments (each 5 cm long) were filled with water up to the 10 cm mark and used to facilitate the voltage application. Graphite electrodes were immersed in the water and connected to the AC electric power source. Water was added to these cells daily to maintain the water level of 10 cm high in the left and right chambers. Five cells were used to carry out 5 phyto- and electro-phytoremediation experiments. Two cells (cell 1 and cell 3) were seeded with twenty seeds of the oat plant and two cells (cell 2 and cell 4) were seeded with twenty sunflower seeds. Cell 5 was left unseeded to study the effect of AC electric potential application alone, without plant growth. The cells were placed under metal halide lamps with average light intensity of 400 µmol/s m² hung about 12 in above the growing plants to obtain the appropriate light intensity for 16 hours per day, with the light provided using an automatic timer. The height of the lamps was adjusted as the plants grew in order to reduce the heat stress to the plants. The temperature below the grow lights was measured as 25°C at the height of the plants, and fans were used to blow out the hot air away from the plants.

After 30 days of growth, 25 V AC current was applied for 3 h per day to one cell with the oat plant (cell 3), one cell with sunflower (cell 4) and the unplanted cell (cell 5). Figure 7.1 shows the cell set up with the growing plants after 30 days of seeding. Once a week, the number of plants in each cell was counted and the height measured. Photographs recorded both growth and biomass production. The pH, oxidation reduction potential (ORP) and electrical conductivity (EC) were measured in the water-filled chambers before and after refilling. The plants were
Figure 7.1. Photographs of the Four Planted Cells
harvested after 61 days. Their shoots were cut at the soil surface, while the roots were carefully separated and washed with water to completely remove soil particles. The roots, shoots and soil were dried in an oven at 60°C for 6 days, until reaching a constant weight. Soil samples were analyzed for metal and organic contaminant concentration.

7.3.5 Analytical procedures

Physical properties of the soil, namely water content (ASTM D2216), organic content (ASTM D2974), pH (ASTM D4972), and grain size distribution (ASTM D422) were analyzed using the appropriate ASTM method. The water holding capacity of the soil was determined using the method for saturated peat materials (ASTM D2980). The heavy metal soil concentration was determined by extracting the metals from the soil by acid digestion, following EPA method 3050B. After the filtration of the extracting solution, the heavy metal concentration was analyzed by Flame Atomic Absorption (FLAA) spectroscopy for Pb, Cd and Cr. The exchangeable metal fraction in the soil was determined by the extraction with 8 mL of 1 M sodium acetate solution per 1 g of soil. The soil sample and extraction solution were continuously mixed for one hour and, then, the supernatant solution was separated by filtration and analyzed for Pb, Cd and Cr by FLAA spectroscopy (Reddy et al. 2001). The organic contaminants in soil were determined through solvent extraction and analysis using Gas Chromatography, as per EPA method SW8270C. The exchangeable nitrogen in soil was extracted by mixing 1 g of soil and 10 ml of 2 M KCl solution for 1 hour (Xu et al. 2013). The nitrogen concentration in the filtered extractant was analyzed by UV spectroscopy, following the procedure reported by Sattayatewa et al. (2011). The exchangeable fractions of potassium and phosphorus were determined by extraction with 1 M ammonium acetate solution per 1 g of soil for one hour. The extractant solution was
filtered and analyzed for phosphorus by UV spectroscopy, following the procedure of Sattayatewa et al. (2011). The potassium in the extractant was analyzed using FLAA. The heavy metal and organic contaminant concentrations are reported here as the mean and standard deviation for the soil samples from each test. In order to evaluate whether there is a statistically significant difference among the treatments in the 5 remediation tests, a t-test with a confidence level of 95% (p value: 0.05) was performed with Microsoft Office Excel 2007 software.

7.4. Results and Discussion

The oat plant and sunflower were seeded in the contaminated soil with heavy metals and PAHs, and the germination was checked regularly given the known difficulty of growing plants in highly polluted soil. In this study, the germination of a seed was considered as the appearance of a green shoot or leaf above the soil. Figure 7.2 shows the germination rate of the plants in different cells. The oat plants showed much better germination, about 100%, compared to sunflower, which was about 60-65%. The literature reports large variations in the germination rate of seeds from species to species in contaminated soils. Some plants have highly permeable seed coats (Wierzbicka and Obidzinska, 1998) and the contamination may easily enter the embryos to inhibit the germination. Heavy metals in soils can affect seed germination either by the general toxicity of the heavy metal in the metabolic processes or by the inhibition of water uptake (Kranner and Colville, 2011). Thus, germination in contaminated soil depends both on the type of plant species and the contaminants and their concentration. Figure 7.3 shows the percentage of survival of the plant species in the four planted cells. The percentage of survival is expressed as the number of surviving plants (green/live plants) from the number of germinated seeds. Both the oat plant and sunflower had high survival rates, close to 100% in all the cells,
Figure 7.2. Germination of Oat Plant and Sunflower in the Four Planted Cells
Figure 7.3. Survival of Oat Plant and Sunflower in the Four Planted Cells
confirming that these species are well-adapted to the mixed contamination in the composted soil, and that the application of the electric field during their growth period did not have a negative effect on survival. Figure 7.4 maps the growth of the plants expressed as plant height over the four planted cells. Even though sunflower plants showed a lower growth rate in the initial stages of the experiment, the growth rate improved after a few days, bringing the two species to a similar height at the end of the tests. The final maximum height of plants after the 61 day growth period is shown in Figure 7.5. Again, no negative effect was seen in the growing plants as a result of the electric field. The biomass production of the oat plant and sunflower in the four cells appears in Figure 7.6. The biomass of sunflower plants was greater than that of the oat plant, whereas the ratio of root biomass to shoot biomass was much higher for the oat plant. In all, the root biomass for sunflower was about 40%, while it was 65% of the total biomass. These differences in biomass production are only due to the biological characteristics of each species, but these results are important in the selection of the plant species for a large scale phytoremedial application. Phytoremediation in the field requires fast growing plants in order to achieve the quick removal of contaminants from the soil. Figure 7.6 also shows that the AC application enhanced the production of biomass for both species. The total biomass increased 28% for the oat plant and 13% for the sunflower in the electric current tests. This result can be interpreted as the improved metabolism in plants due to changes in ion activity at extracellular and intracellular level (Bi et al. 2011). AC electric field can enhance water movement and ionic movements in the soil mass, which can benefit the plant. The root cell membrane, which is negatively charged, is also expected to be affected by the electric potential application (Kinraide et al. 1992) with the subsequent effects in the mass transportation between soil and plant.
Figure 7.4. Height of the Growing Oat Plant and Sunflower in the Four Planted Cells
Figure 7.5. Final Maximum Plant Height of Oat Plant and Sunflower in the Four Planted Cells at the End of the Tests.
Figure 7.6. Biomass Production of Oat Plant and Sunflower in the Four Planted Cells at the End of the Tests.
Overall, the application of electric potential did not negatively affect the germination, survival and plant heights of either plant species. However, a significant increase in biomass production was found in the cells that received the electric potential application. These results confirm that the coupled technology electro-phytoremediation may be an interesting alternative for the remediation of mixed contaminated sites.

The enhancement of biomass production may be a result of the improved availability of nutrients in the soil. Thus, exchangeable nitrogen, phosphorus and potassium in the soil were measured at the end of the experiments (Figure 7.7) and the results were compared with those from the unplanted soil that did not receive the electrical potential application. The blank column in Figure 7.7 corresponds to the plant free soil that did not receive the electric application. In general, the exchangeable nutrient concentration was lower in planted than unplanted cells due to the plants’ consumption for biomass production. Cell 5 (EK column in Figure 7.7), which operated with voltage application alone, had a nutrient status similar to the blank column as there were no plants to consume the nutrients. The small decrease in nutrients from the soil may be interpreted as a leaching to the adjacent chambers filled with water. The cells that contained the oat plant showed a significant decrease in the exchangeable N and P compared to the unplanted cell. However, the sunflower cells showed similar concentrations of N and P to the unplanted cell. Conversely, the exchangeable K was considerably less for sunflower than was found in the unplanted samples, while the oat plant showed an intermediate value. In all, the data did not portray any general trend of higher nutrient availability with electric potential application. This may be due to the continuous consumption of nutrients while they are mobilized by the effect of the electric field.
Figure 7.7. Exchangeable Fraction of Nutrients (N, P and K) in Soil in All Tests.
Figures 8 through 12 show the daily variation of pH, ORP, EC, voltage, and current intensity, measured in the water-filled chambers of the 5 cells. In order to maintain the water level of the chambers, the amount of water was measured and refilled to the appropriate level daily. Then, the AC voltage was applied for 3 h per day. The pH, ORP, EC, voltage, and current intensity were measured before and after the voltage application, except in cells 1 and 2 as these cells corresponds to the phytoremediation tests with no electrical application. In these two cells, the measurement was done before and after the water cells were refilled.

The daily pH variation was in the range of pH = 8 - 10, while the unplanted cell had slightly lower pH values between pH = 8 - 9. The planted cells had slightly higher pH values, between pH = 8.5 - 9.5 for both plant species (Figure 7.8). There was not a significant variation between the planted cells with and without electricity. The daily variation in ORP (Figure 7.9) was slightly higher (less negative) in the unplanted cell (cell 5: EK) than the four planted cells. However, in this case, slightly higher ORP values were observed the planted cells with electricity, especially for the oat plant. The electric conductivity in the water-filled chambers (Figure 7.10) was clearly higher in the unplanted cell, which confirmed the effect of the electricity on the mobilization of ions. The presence of plants in the other four cells decreased the electric conductivity of the solutions, probably due to the retention of ionic species by the roots of the plants. The voltage variation in the 3 cells with electricity was very close to the actual value of the applied voltage (Figure 7.11). These measurements were taken at the start and completion of the 3 h voltage application period, and a minor influence of the soil in the voltage readings was expected during the application of a constant AC voltage. The current intensity profile was significantly different in the 3 cells with electricity (Figure 7.12). The unplanted cell
Figure 7.8. pH Variation in Side Chambers in All Tests
Figure 7.9. ORP Variation in Side Chambers in All Tests
Figure 7.10. Electrical Conductivity Variation in Side Chambers in All Tests
Figure 7.11. Voltage Variation in Side Chambers for the Phytoremediation Tests Enhanced with AC Electric Current
Figure 7.12. Electric Current Variation in Side Chambers for the Phytoremediation Tests
Enhanced with AC Voltage Application
showed a steady decrease in the current intensity that suggests a depletion of the mobile ions in the soil, where the two cells with the oat plant and sunflower showed higher electric current values. The latter confirms the higher concentration of mobile of ions in the soil. The oat plant showed significant higher current values than the sunflower, probably due to the higher consumption of nutrients, especially potassium, by the sunflower for production of its higher biomass.

At the end of the experiments, soil samples were taken from different spots within the central compartment of all 5 cells. Of these, three soil samples were taken from the bottom of the cell, coming from its right, middle and left sides and another three were extracted from the upper layer of soil, again on the right, middle and left sides. Table 7.3 shows the values of pH, electrical conductivity and ORP for the 6 soil samples from each of the 5 cells. In general, the pH values remained basically the same as the original pH value of the soil (pH = 7.4), although the pH value from the top sample was about pH = 8.5. The EC values were higher at the bottom of the cells compared to top, likely due to the leaching of ions from top and the anaerobic conditions created at the bottom. There was no considerable variation of pH, ORP or EC from the left or right samples from any of the cells. The average values of pH, ORP and EC were uniform in all the cells; therefore, the average values could not be related to the electric potential application. Aboughalma et al. (2008) and Bi et al. (2011) also saw no considerable variation in the pH in the soil between the electrodes when the AC electric field was applied. However, other authors who used DC electric current in their electro-phytoremediation tests, found lower rates of plant growth near the anode due to acidification and the increasing concentrations of heavy metals, which became toxic to the plants (O'Connor et al. 2003). Cang et al. (2012) also reported
Table 7.3: Average pH, Oxidation Reduction Potential and Electrical Conductivity Values for Soil Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Average ORP (mV)</th>
<th>Average EC (mS/cm)</th>
<th>Average</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell 1: OP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.028</td>
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</tr>
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<td>0.023</td>
<td></td>
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</tr>
<tr>
<td>Top right</td>
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<td>0.048</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom left</td>
<td>8</td>
<td>-60.2</td>
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<td></td>
</tr>
<tr>
<td>Bottom middle</td>
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<td>0.013</td>
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</tr>
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<td>0.126</td>
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<td>-42.4</td>
<td>-67.5</td>
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<tr>
<td><strong>Cell 3: OP+EK</strong></td>
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</tr>
<tr>
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</tr>
<tr>
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<tr>
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<tr>
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<td>0.038</td>
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<td><strong>Cell 5: EK</strong></td>
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<tr>
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<td>8.4</td>
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<td>0.038</td>
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<tr>
<td>Bottom left</td>
<td>8.3</td>
<td>-79.5</td>
<td>0.046</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom middle</td>
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<td>-49.8</td>
<td>0.144</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom right</td>
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<td>-57.6</td>
<td>-73.5</td>
<td></td>
<td>0.108</td>
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</tbody>
</table>
that the treatment with DC electric current depressed the enzymatic and microbial activities in
the soil, mainly due to pH variation to very acidic or alkaline values. The application of AC
voltage did not result in a significant variation of the pH in the soil, and this is probably the
reason for the unhindered plant growth observed in this study.

Figure 7.13 shows the heavy metal concentrations in all of the cells based on
measurements registered at the conclusion of the tests. The data reported in Figure 7.13
corresponds to the average concentration measured in all the samples taken from the soil during
each experiment. The error bars corresponds to the standard deviation. The blank bar in Figure
7.13 represents the metal concentration in the unplanted soil without electric potential
application. In general, the unplanted cell shows similar values to the blank soil or just a small
decrease that can be related to some heavy metal leaching into the water chambers. The four
planted cells revealed some reduction in the final metal concentration compared to the initial
soil. However, the statistical analysis of the data confirmed that the oat plant did not produce a
significant reduction ($p>0.05$) in Pb or Cd in the case of cells without the electric potential
application. Conversely, the oat plant achieved a significant reduction ($p<0.05$) in Pb and Cd
compared to the control for the test with the electric potential application. Sunflower achieved
better results as it produced a significant reduction ($p<0.05$) in Pb and Cd both with and without
electric potential application. There was an approximate reduction of 24% Pb and 17% Cd for
sunflower without electricity. With the electric potential application, the sunflower removed 25%
Pb and 17% Cd. The Cr concentration in the soil was reduced significantly in all of the planted
cells: approximate 23% by the oat plant and 18% by sunflower without electricity, and 19% by
the oat plant and 16% by sunflower, respectively, with the electric potential application.
Figure 7.13. Heavy Metal Concentration in Soil at the End of the Tests.
Figure 7.14 shows the exchangeable fraction of heavy metals at the end of the test. The exchangeable Pb was zero in all of the cells. This is an important result considering the toxicity of lead for living organisms. It demonstrates that the exchangeable Pb can be removed by plant uptake or be immobilized in the soil by biological or electrochemical processes. In either case, the lower bioavailability of Pb is an important result in the remediation of soils. The exchangeable Cd content in the planted cells and the control were similar with no significant difference. The unplanted cell that received the electric potential application had a higher measure of exchangeable Cd compared to the control as the electric field can increase the mobility of metals. Anyway, this result has to be analyzed in the time frame of a relatively short tests reported in this study compared to longer treatment times of phytoremediation tests. It would be necessary to evaluate the same in extended tests to determine whether the availability of Cr decreased by plant uptake. All of the cells showed less exchangeable Cr concentrations than the control. Specifically, all of the planted cells had significantly less exchangeable Cr concentrations compared to the cells that only had electric potential application. This may be due to the extraction of available metal fractions by the plants or a change in speciation of the metals due to the presence of plants (Chen et al. 2004).

In general, the exchangeable Cr concentrations were higher than those for Pb and Cd. This indicates the preference in adsorption of different heavy metals in the exchange sites of the soil particles. According to McLean and Bledsoe (1992), the maximum retention of anionic metals (i.e., Cr) occurs at pH < 7 and maximum retention of cationic metals (Pb and Cd) occurs at pH > 7. The pH values of the soil samples can explain the higher availability of Cr in the soil solution compared to Pb and Cd. At a given pH level, there can be competitive sorption and desorption among the cationic metals present in the soil solution, and Pb tends to adsorb to the
Figure 7.14. Exchangeable Fraction of Heavy Metals in Soil at the End of the Test
soil particle compared to Cd ions (Covelo et al. 2007). That may be the reason for the lower exchangeable Pb concentrations in spite of the high total Pb concentration in the soil. The reduction of the total metal concentrations by the plants suggests that the electric potential application did not affect the phytoextraction of the metals, even though there was a slight and insignificant improvement of metal extraction by the oat plant.

Figure 7.15 shows the PAH concentrations in the initial soil and in the cells at the end of the tests. The blank bar corresponds with the actual PAH concentrations in the untreated soil at the conclusion. The dissipation mechanisms of organic contaminants in phytoremediation are phytodegradation, phytovolatilization, accumulation, or rhizodegradation. For the first three mechanisms, the organic contaminant has to enter the plant. The absorption of organic contaminants into the plant tissues is not driven by transporter proteins. It occurs by simple diffusion, mostly based on the hydrophobicity of the contaminant. The ideal value of log $K_{ow}$ for the organic contaminant to enter the plant tissue is between 0.5 and 3 (Pilon-Smits 2005). However, naphthalene and phenanthrene have log $K_{ow}$ values that are higher than 3 (De Maagd et al. 1998). This indicates that the possible dissipation mechanisms of these contaminants in soil are direct volatilization or microbial degradation, but not plant uptake. The naphthalene concentration was zero in all of the samples, indicating volatilization and microbial degradation of naphthalene in soil (Mozo et al. 2012). The phenanthrene concentration in the untreated soil (blank bar) was considerably less than the concentration of phenanthrene in the initial soil, which again establishes the biodegradation/volatilization of phenanthrene in the soil. The four planted cells and the cell with only the electric potential application (cell 5: EK) produced similar results for the residual phenanthrene in soil, which was significantly higher than the phenanthrene concentration in the untreated soil. This may be a concentration variation across the samples and
Figure 7.15: Phenanthrene Concentrations in Soil at the End of the Tests
might not be significant if more samples were analyzed. Another explanation is the production of phytoalexins by the plants, in stressed conditions (Bais et al. 2006). It may be a result of the antimicrobial property of the phytoalexins, which acts against the microbial biodegradation of PAHs. Experiments done by Corgie et al. (2004) also suggest that plants can either improve or inhibit biodegradation by affecting the spatial distribution of bacterial communities. The plants can behave contrarily in mixed contaminated soils due to the stress caused by metals. Lin et al. (2008) and Chigbo et al. (2013) have also reported lower PAH degradation in planted samples compared to unplanted samples, depending on the co-contaminants present in the soil. The lower phenanthrene degradation in cell 5 with the electrical potential alone is also expected to be due to the variation in soil sampling. The degradation of organic contaminants was expected to increase with the electric potential application due to the increased mobility and bioavailability in the presence of the electric field (Maini et al. 2000).

The results suggest that the applied voltage and duration of the electric potential may not be sufficient to increase the availability of contaminants for plant uptake or plant-promoted degradation. The strength and duration of the applied electric field are very important parameters for determining the fate and transport of contaminants in electrokinetic-assisted phytoremediation (Cang et al. 2011). Other properties that affect the success of electrokinetic-assisted phytoremediation are plant species, soil properties and presence of co-contaminants (Bi et al. 2011). According to Cameselle et al. (2013), the voltage level should be selected to optimize the plant growth and contaminant removal efficiency. Low voltage may not mobilize much contaminant, which can lead to less bioavailable fractions of contaminants in the soil pore water and less uptake by plants. Higher fractions of contaminants are bioavailable under higher voltage, which can lead to higher uptake, but can also increase plant stress. In the present
experiments, no plant stress occurred, which suggests that the increase in the application time of the electric potential and the frequency of its application will have an enhanced effect on the bioavailability of contaminants.

7.5 Conclusions

The germination and survival of the plants studied were not considerably affected by the presence of the electric field. The results suggest that there was a slight increase in the biomass of the plants due to the electric potential application, but this increase was not enough to improve the heavy metal phytoextraction. The electric conductivities of the samples from cells that received the electric potential application were comparatively higher, which indicates the higher mobility of metals in the soil pore solution. However, the electric potential application did not improve the heavy metal phytoextraction. The naphthalene concentration was zero in all the samples, which indicates microbial degradation and volatilization. The final concentrations of phenanthrene in the soil samples did not suggest any improvement in degradation with the introduction of either plants or electric potential to the soil. Instead, the treatments caused a slight increase in the phenanthrene concentrations compared to the untreated soil. To obtain noticeable effects, an increase in the electrical potential application and frequency is suggested.

7.6 Cited References


EPA/600/R-99/107 (2000), Introduction to Phytoremediation


CHAPTER 8
ENHANCED PHYTOREMEDIATION OF FIELD SOIL WITH MIXED CONTAMINATION

8.1. Introduction
Soil contamination is a common problem at former industrial sites, especially in urban areas. The risks to human beings and the ecosystem posed by the contaminants make it necessary to carry out remedial actions to clean the soil. There are many remedial alternatives available to treat organic or heavy metal contamination. However, in reality, soil contaminated with organic contaminants like PAHs usually contain heavy metals because they are discharged from the same sources such as vehicle emissions, industrial processes, power generation, etc. (Sun et al. 2011). When organic and heavy metal contaminants are present together, it becomes difficult to select a suitable remedial strategy. Compared to the other expensive and/or energy intense methods for remediation of mixed contaminated soil, phytoremediation is a cost effective, sustainable option (Reddy and Chirakkara 2013). For large areas of land awaiting remediation of mixed contaminated soil, phytoremediation may be the only practical, sustainable option because the funds available for environmental cleanup projects are usually limited. Phytoremediation is a technology in which plants are grown in the contaminated area specifically for their characteristics that are known to degrade, extract, contain, or immobilize contaminants from soil and/or water (Sharma and Reddy 2004). Phytoremediation may be slow compared to mechanical remedial approaches, but during the remediation period the plants can reduce the contaminant exposure routes by minimizing windblown dust and controlling the downward migration of contaminated water by natural pumping through evapotranspiration. Reduced soil erosion due to
plant growth can also minimize the migration and spreading of contaminated soil. In addition, planted sites are aesthetic and this makes phytoremediation a better option compared to other remedial strategies (ITRC 2009). In phytoremediation, organic contaminants are either accumulated in the plant tissue or degraded in the root mass (rhizodegradation) or plant tissue (phytodegradation). Metal contaminants are either transformed into harmless forms by plant induced stabilization (phytostabilization) or accumulated in plant tissue by phytoaccumulation.

There have been many research studies conducted on the phytoremediation of either heavy metals or organic contaminants from the soil. But, due to the complex interactions of the contaminants in the soil, the behavior of phytoremediation plants in mixed contaminated soil becomes unpredictable. Past studies on mixed contaminated soils prove that heavy metal and organic contaminants can pose synergistic or antagonistic effects, depending on the nature of contaminants and the plants. So, it is important to conduct contaminant specific, plant specific, and site specific studies before phytoremediation implementation. For this purpose, a series of laboratory phytoremediation studies were conducted earlier to understand phytoremediation mechanisms of different plants in soil spiked with a mixture of organic and heavy metal contaminants (Chirakkara and Reddy 2013; Chirakkara and Reddy 2014). Also, there are some studies that involve phytoremediation in soil contaminated with a mixture of organic and heavy metal contaminants (Table 8.1). However, most of the existing studies on mixed contaminated soil were done on once healthy soils that were spiked with mixed contaminants in the laboratory. Phytoremediation experiments done using newly spiked, contaminated soil may behave differently from the experiments conducted on a soil with aged contamination as would be found in older industrial sites, for example. In the case of sites with aged contamination, it is important to understand the phytoremediation mechanisms of mixed contamination as aged contaminants.
Table 8.1: Phytoremediation Studies on Mixed Contaminated Soils

<table>
<thead>
<tr>
<th>Plant</th>
<th>Contaminants</th>
<th>Contamination</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lolium (rye grass)</td>
<td>Cu, Zn and 2,4-dichlorophenol</td>
<td>Spiked with 2,4-dichlorophenol</td>
<td>Chen et al. (2004)</td>
</tr>
<tr>
<td>Lolium perenne L (rye grass) and Raphanus sativus (radish)</td>
<td>Cu and pentachlorophenol</td>
<td>Spiked</td>
<td>Lin et al. (2006)</td>
</tr>
<tr>
<td>Pinus sylvestris (pine), Populus deltoides x Wettsteinii (poplar), Festuca rubra (red fescue), Poa pratensis (smooth meadow grass), Lolium perenne (rye grass) and Trifolium Repens (white clover)</td>
<td>organic and metal contamination from bus maintenance activities</td>
<td>Field</td>
<td>Palmroth et al. (2006)</td>
</tr>
<tr>
<td>Alyssum lesbiacum (Candargy)</td>
<td>Ni, phenanthrene, and chrysene</td>
<td>Spiked</td>
<td>Singer et al. (2007)</td>
</tr>
<tr>
<td>Echinochloa crusgalli (barnyard grass), Helianthus annuus (sunflower), Abutilon avicennae (Indian mallow), and Aeschynomene indica (Indian jointvetch)</td>
<td>Cd, Pb, and 2,4,6-trinitrotoluene</td>
<td>Spiked with 2,4,6-trinitrotoluene</td>
<td>Lee et al. (2007)</td>
</tr>
<tr>
<td>Brassica juncea (Indian mustard) and Festuca arundinacea (tall fescue)</td>
<td>Zn and pyrene</td>
<td>Spiked</td>
<td>Batty and Anslow (2008)</td>
</tr>
<tr>
<td>Triticum Aestivum L (wheat straw)</td>
<td>Zn, Cu, Mn, 2,4-dichlorophenoxyacetic acid, and 4-chloro-2-methylphenoxyacetic acid</td>
<td>Spiked with 2,4-dichlorophenoxyacetic acid and 4-chloro-2-methylphenoxyacetic acid</td>
<td>Kobylecka and Skiba (2008)</td>
</tr>
<tr>
<td>Zea mays L. (maize)</td>
<td>Cd and pyrene</td>
<td>Spiked</td>
<td>Zhang et al. (2009)</td>
</tr>
<tr>
<td>Ricinus communis (castor oil plant)</td>
<td>Cd and DDT</td>
<td>Spiked</td>
<td>Huang et al. (2011)</td>
</tr>
<tr>
<td>Tagetes patula (marigold)</td>
<td>Cd, Cu, Pb, and benzo[a]pyrene</td>
<td>Spiked</td>
<td>Sun et al. (2011)</td>
</tr>
<tr>
<td>Brassica juncea (Indian mustard)</td>
<td>Cd and Pb and used engine oil</td>
<td>Spiked</td>
<td>Ramamurthy and Memarian (2012)</td>
</tr>
<tr>
<td>Plant Species</td>
<td>Contaminants</td>
<td>Application Method</td>
<td>Reference</td>
</tr>
<tr>
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</tr>
<tr>
<td><em>Sedum alfredi</em></td>
<td>Cd and DDT</td>
<td>Field and spiked</td>
<td>Zhu et al. (2012)</td>
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<tr>
<td><em>Solanum lycopersicum</em> (tomato)</td>
<td>Cd and phenanthrene</td>
<td>Spiked</td>
<td>Ahammed et al. (2013)</td>
</tr>
<tr>
<td><em>Brassica juncea</em> (Indian mustard)</td>
<td>Cu and Pyrene</td>
<td>Spiked</td>
<td>Chigbo et al. (2013)</td>
</tr>
<tr>
<td><em>Zea mays L.</em> (maize)</td>
<td>Cd and Pentachlorophenol</td>
<td>Spiked</td>
<td>Hechmi et al. (2013)</td>
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<tr>
<td><em>Tagetes patula</em> (marigold)</td>
<td>Cd and benzo[a]pyrene</td>
<td>Spiked</td>
<td>Sun et al. (2013)</td>
</tr>
<tr>
<td><em>Medicago sativa</em> (Alfalfa)</td>
<td>Cd and trichloroethylene</td>
<td>Spiked</td>
<td>Zhang et al. (2013)</td>
</tr>
<tr>
<td><em>Phragmites australis</em></td>
<td>Cd and Pentachlorophenol</td>
<td>Spiked</td>
<td>Hechmi et al. (2014)</td>
</tr>
<tr>
<td><em>Sedum alfredi</em> and <em>Festuca arundinacea</em> (tall fescue)</td>
<td>Cd, Pb, Zn, and decabromodiphenyl ether</td>
<td>Spiked</td>
<td>Lu and Zhang (2014)</td>
</tr>
</tbody>
</table>
are more recalcitrant than contaminants in a newly polluted area. Over time, bioavailable pollutants tend to decrease in concentration due to physical, chemical and biological processes. These aged industrial contaminants are usually difficult to mobilize (Batty and Dolan 2013). Most of the existing phytoremediation studies involving a mixture of organic and heavy metal contaminants have been carried out on laboratory spiked soils. An effort has been done here to study the phytoremediation potential of a soil with aged contamination using *Avena sativa* (oat plant) and *Helianthus annuus* (sunflower) to remove Pb, Cd, and multiple PAHs. The possibility of enhancing the phytoremediation using compost as a soil amendment is also explored.

### 8.2. Background

Heavy metal bioavailability can be greatly influenced by soil characteristics and the aging period. Bioavailable fractions of contaminants in the soil pore water can lessen over a period of time and that can affect the phytoremediation efficiency when compared to newly spiked soils. Lock et al. (2001) conducted a study in which soil characteristics and the aging period were modeled by comparing heavy metal bioavailability in metal spiked soil and historically contaminated field soils. Their results indicated that extractable Zn fractions of the contaminated field soils were lower when compared to those of artificially spiked soils. They attributed this to Zn fixation in aged soils depending on the metal oxyhydroxide formation.

Palmroth et al. (2006) conducted phytoremediation experiments on aged soil contaminated with hydrocarbons and heavy metals in a field study using pine, poplar, white clover and a grass mixture. They also conducted experiments with soils amended with NPK fertilizer and municipal biowaste compost. During the 36 month study, they noticed that there was a significant 30% reduction of hydrocarbon in the unamended soil that occurred only in the
last 4 months of the study. There was noticeable reduction of hydrocarbons from the amended soils as well; those removal rates were 65 and 60%, respectively, for soil amended with NPK fertilizer and compost. The metal analysis showed that heavy metals did not accumulate in grasses and clover. Metal accumulation in the trees was not tested. They recommended that phytoremediation of weathered contamination requires more treatment time compared to the newly spiked contamination. They suggested the use of compost addition combined with grass and legume crops for stabilization of combined hydrocarbon and metal contaminated soil.

Zhu et al. (2012) studied the effect of the hyperaccumulator plant *Sedum alfredii* on soil co-contaminated with Cd and dichlorodiphenyltrichloroethane (DDT) and its metabolites with the latter two together called DDs. They considered two soils with two Cd levels. For the low Cd soil, they sampled contaminated soil from a field that had a Cd concentration of 0.895 mg/kg and DDs concentration of 0.715 mg/kg. For the high Cd soil, they spiked the field soil with Cd to raise the concentration of Cd to 3.225 mg/kg. The phytoremediation experiment ran for six months. At the end, a Cd removal rate of 32 and 39%, respectively, was achieved for the low Cd and high Cd treatments. The higher percentage removal in high Cd soil may be due to the higher bioavailability of the Cd in the spiked soil (Lock et al. 2001). Another explanation is the increase in exchangeable metal concentrations with increasing total metal concentrations (McLean and Bledsoe 1992). In this case, since one soil sample was from a historically contaminated field and the other soil sample contained both historic and spiked contamination, the reason for the higher Cd removal from the high Cd level soil could not be clearly interpreted. The removal of DDs was found to be similar for both the low Cd soil and high Cd soil.

In a recent effort to compare freshly spiked soil with aged soil, Chigbo and Batty (2013) conducted phytoremediation experiments using Indian mustard in soil contaminated with Cu and
Pyrene. They stored spiked soil in sealed bags in the dark for 8 months prior to planting for the ageing studies. The response of plants in this soil was compared with plants in freshly spiked soil. Different concentrations of Cu (0, 50, 100 mg/kg) and pyrene (0, 250, 500 mg/kg) were used in combination. They observed a significant decrease of plant biomass (> 50% reduction) in the freshly spiked soil compared to that in the aged soil. Also, the shoot accumulation of Cu was significantly reduced (60-88%) in the aged soil after 60 days of growth. The removal results of Cu from the soil did not give similar trends for different combinations of contaminant concentrations. When 50 mg/kg Cu was co-contaminated with 250 or 500 mg/kg pyrene, the removal rate of Cu from the soil was higher in the aged soil than in the freshly spiked soil. In all other co-contamination treatments, the removal of Cu from the aged soil was significantly lower than from the freshly spiked soils. In aged soil, the presence of co-contamination decreased the Cu accumulation in plant shoots, whereas co-contamination increased the shoot Cu accumulation in the freshly spiked soil. Also, the shoot biomass decreased with an increase in pyrene concentration in freshly spiked soil, while it increased with a rise in the pyrene concentration in the aged soil. The presence of plants significantly decreased the pyrene concentrations in freshly contaminated soils. However, in aged soil, there was no significant effect of the plants observed on the pyrene dissipation. These results suggest that the behavior of contaminants in soil with freshly spiked contaminants may not reflect the true situation in the field.

In historically contaminated field soil, plant growth and microbial activities are usually scarce due to the poor physical and chemical conditions of the polluted soil. The bad physical condition of the soil can also restrict the root proliferation depth in contaminated field soil. The stress caused by the contamination can alter the nutrient cycles, resulting in structural and functional diversity of microorganisms. The addition of organic amendments, like compost, to
the contaminated soil can improve the physical, chemical and biological properties of the soil, resulting in better plant growth that leads to better phytoremediation efficiency (Masciandaro et al. 2013). Apart from providing nutrients and organic matter to the soil, these amendments can improve the soil structure and can modify the solubility of contaminants by direct adsorption or by changes in pH, redox conditions, salinity, etc. (Pardo et al. 2014).

8.3. Materials and Methods

8.3.1 Soil Used

The soil used for the study was collected from a historically contaminated wetland site called Big Marsh located on South Stony Island Avenue, Chicago, Illinois. Site boundaries are marked in Figure 8.1. The site contains approximately 289 acres of vacant wooded and marsh land. The surrounding properties have been heavily industrialized since the late 1800s. Current and historic activities in the surrounding area include heavy manufacturing, illegal dumping and usage of underground storage tanks. As a result, the site is contaminated with heavy metals and PAHs. The site is intended to be used as an ecological open space reserve after the required remedial action (City of Chicago 2005). The soil required for the experiments was collected from the northwest corner of the site. The collected soil was air dried, passed through a sieve no. 4 and then mixed well to ensure uniform contaminant distribution. The important physical properties of the soil are presented in Table 8.2.

8.3.2 Selected Plant Species

The plant species for the study were selected for their biomass and capability of survival in mixed contaminated soil based on previous results (Chirakkara and Reddy 2013). They are
Figure 8.1. Map Showing Site Boundaries
Table 8.2: Important Properties of Soil Used for the Experiments

<table>
<thead>
<tr>
<th>Property</th>
<th>ASTM Standards</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Content</td>
<td>ASTM D 2216</td>
<td>22 %</td>
</tr>
<tr>
<td>Soil organic content</td>
<td>ASTM D 2974</td>
<td>6.1 %</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>ASTM D 854</td>
<td>2.6</td>
</tr>
<tr>
<td>Water holding capacity</td>
<td>ASTM D 2980</td>
<td>43.2 %</td>
</tr>
<tr>
<td>Liquid limit</td>
<td>ASTM D 4318</td>
<td>33.2 %</td>
</tr>
<tr>
<td>Plastic limit</td>
<td></td>
<td>22.8 %</td>
</tr>
<tr>
<td>Plasticity index</td>
<td></td>
<td>10.4 %</td>
</tr>
<tr>
<td>Clay (&lt; 0.002mm)</td>
<td>ASTM D 422</td>
<td>22 %</td>
</tr>
<tr>
<td>Silt (0.002 - 0.05mm)</td>
<td></td>
<td>28 %</td>
</tr>
<tr>
<td>Sand (0.05 – 2 mm)</td>
<td></td>
<td>48.1 %</td>
</tr>
<tr>
<td>USCS Classification</td>
<td></td>
<td>CL</td>
</tr>
<tr>
<td>USDA Classification</td>
<td></td>
<td>Sandy Clay Loam</td>
</tr>
</tbody>
</table>
Avena sativa (oat plant), and Helianthus annuus (sunflower). Oat plant was studied for its phytoremediation efficiency for heavy metal (Ebbs and Kochian 1998) and organic contaminants (Miya and Firestone 2001), in the past. Sunflower species was also involved in phytoremediation studies of both organic (Rosado and Pichtel, 2004) and heavy metal (Meers et al. 2005; Adesodun et al. 2010; January et al. 2008) contaminants. Oat plant seeds were supplied by Seedville USA and sunflower seeds were supplied by Carolina Biological Supply Company.

8.3.3 Pot Setup

For the pot experiments, control soil was prepared by mixing the field soil with approximately 22% water. One part of this soil was mixed with yard waste compost (200 g/kg soil) to conduct the enhanced phytoremediation experiments. Table 8.3 shows the measured properties of the prepared soil samples. The pots filled with the prepared soil and used for seeding were 8cm diameter and 9 cm height. Ten replicates were prepared for each plant species, both for amended and unamended soil. Three additional control pots were prepared with amended and unamended soil, but without any plants. Ten seeds were placed approximately a half inch below the soil surface in each pot. To ensure that the leachate does not get mixed up, each pot was kept on a separate tray.

The pots were placed under metal halide grow lights with an average light intensity of 400 μmols/m²/s, which were hung ~12 inches above the plants to obtain the desired light intensity. Sixteen hours of light per day was provided using an automatic timer. The lamp height was adjusted to reduce the heat stress as the plants grew. The temperature below the grow lights was measured as 25°C at the top of the plants. Fans blew the hot air away from the plants.
Table 8.3: Measured Properties of Soil at the Time of Seeding:

<table>
<thead>
<tr>
<th>Property</th>
<th>ASTM Standards</th>
<th>Unamended Soil</th>
<th>Composted Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>ASTM D 4972</td>
<td>7.6</td>
<td>7.7</td>
</tr>
<tr>
<td>Oxidation reduction potential (mV)</td>
<td></td>
<td>-60.3</td>
<td>-62.9</td>
</tr>
<tr>
<td>Electrical conductivity (milliSiemens/cm)</td>
<td></td>
<td>0.173</td>
<td>0.160</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>ASTM D 2216</td>
<td>17.7</td>
<td>22.3</td>
</tr>
</tbody>
</table>
8.3.4 Monitoring

The plants were grown for 61 days in the laboratory with the growth monitored. The positions of the pots were changed periodically to ensure uniform light intensity to all the pots. The number of plants that germinated in each pot was counted and the plant heights were recorded weekly. Photographs documented the plant growth and biomass production.

At the end of the growth period, shoots of the plants were cut at the soil surface. The roots were carefully separated from the soil. The harvested shoots, roots and soil were dried in oven at 60°C for 6 days, until it attained constant weight. The dry weights of roots and shoots were measured and noted as root biomass and shoot biomass. The soil samples were tested for metals and organic contaminants.

8.3.5 Analytical Testing

Physical properties of the soil like water content (ASTM D 2216), organic content (ASTM D 2974), pH (ASTM D 4972), and grain size (ASTM D 422) were tested as per standards. The water holding capacity of the soil was determined by a method similar to the one used for saturated peat materials (ASTM D 2980). Here, the water holding capacity is expressed gravimetrically in order to compare it with other water content values. For the heavy metal analysis, the acid digestion of the soil samples was examined per EPA method 3050 B. The digested and filtered liquid was analyzed with Flame Atomic Absorption (FLAA) spectroscopy for Pb, Cd and Cr to estimate the exchangeable metals in the soil. 8 ml of 1M sodium acetate solution was added to 1g of soil and mixed continuously for one hour (Reddy et al. 2001). The filtrate was analyzed with Flame Atomic Absorption (FLAA) spectroscopy for Pb, Cd and Cr. The organic contaminants were analyzed by solvent extraction and analysis using Gas
Chromatography, per EPA method SW8270C. To analyze the exchangeable nitrogen, 1g soil was shaken with 10 ml of 2M KCl solution for one hour (Xu et al. 2013). The filtered extractant was analyzed using Spectronic Genesys spectrophotometers, following the procedure given by Sattayatewa et al. (2011). To determine the exchangeable fractions of potassium and phosphorus, 1g soil was shaken with 1M ammonium acetate for one hour. The solution was filtered and the extractant was analyzed for phosphorus with Spectronic Genesys spectrophotometers, as per Sattayatewa et al. (2011). Exchangeable potassium in the extractant was analyzed using Flame Atomic Absorption (FLAA) spectroscopy. All the chemicals used for analytical testing were purchased from Fischer Scientific.

For the test results, means and standard deviations were calculated using Microsoft Office Excel 2007. To check whether a significant difference exists between the result sets, t-test was performed with Microsoft Office Excel 2007. The alpha value was taken as 0.05 for the t-test.

### 8.4. Results and Discussion

The germination percentages of both the plants in unamended and composted soil are shown in Figure 8.2a. Oat plant had very good germination rates in both the amended and unamended soils. The average germination rate of sunflower, which was 50% in unamended soil, improved to 62% in the composted soil. Different germination rates in different species can be explained based on the seed coat permeability of the plant species. Literature shows that seeds of some plants have highly permeable seed coats (Wierzbicka and Obidzinska 1998). For such seeds, the contamination can enter the embryos and affect seed germination. The effect of heavy metal on seed germination can be attributed to either its general toxicity or the inhibition of water uptake.
Figure 8.2. Percentage Germination and Survival of Plants in Unamended and Composted Soil
(Kranner and Colville 2011). These facts imply that germination in contaminated soil depends on both the type of plant species and the contaminants.

Not all of the germinated plants survived. The survival rate for those green living plants that survived to the end of the 61 day experiment period is expressed as a percentage of the number of seeds germinated. Figure 8.2b shows the percentage of survival of each plant species in unamended and composted soil. A slight improvement in survival rates for both the plants can be observed in the case of composted soil. Figure 8.3a shows the increase in plant height with time. Here, the growth rate was better for plants in the composted polluted soil compared to the plants in the unamended contaminated soil. The final (after 61 days) plant heights of sunflower and oat plant are presented in Figure 8.3b. This shows a considerable increase in maximum height of both the plants with the compost application. Figure 8.4 shows that the biomasses of both the plants also improved considerably when compost was applied as an amendment to the soil. However, the total biomass enhancement in the composted soil was not significant (p>0.05) for oat plants, while the sunflower plants showed significant (p<0.05) improvement. The root biomass to shoot biomass ratio only had significant improvement (p<0.05) in the case of sunflower.

Similar results of better biomass of plants in a heavy metal contaminated soil with compost amendment was observed by Pardo et al. (2014) in a recent field scale study conducted over two years. They tested the applicability of compost as soil amendment to promote the growth of native species for the phytorestitution of a mine where the affected soil was contaminated with As, Cd, Cu, Mn, Pb, and Zn. Their results proved that the use of compost as a soil amendment is useful for the promotion of vegetation cover for moderately contaminated soils. Better germination, survival and biomass observed in composted soil can be due to the
Figure 8.3. Maximum Plant Height in Unamended and Composted Soil
Figure 8.4. Biomass of Plants in Unamended and Composted Soil
direct and indirect effect of compost on the contaminated soil. Compost amendment can reduce the amount of toxic contaminants in the pore water of the soil due to the affinity of most of the contaminants towards the organic matter in the compost (Karami et al. 2011). This would have reduced the direct phytotoxicity effects caused by the soil contaminants.

The improved nutrient status of the soil after the compost addition can be observed in Figure 8.5, which shows the exchangeable nutrients in different soil samples. There was an improvement in exchangeable P and K in composted soil samples. The enhanced organic matter and nutrients in the rhizosphere due to the compost addition would also have enhanced the plant biomass and plant growth as well as promoted rhizobacterial activities in the soil. Plant growth that promotes rhizobacteria then benefits those plants through different mechanisms, including production of secondary metabolites, nitrogen fixation, phosphate solubilization, antagonism to soil borne root pathogens, and production of siderophores (De Brito et al. 1995).

The average values of pH, electrical conductivity (EC) and oxidation-reduction potential (ORP) for the soil samples after the plant growth period are shown in Table 8.4. The pH and EC values ranges were similar to those obtained by Pardo et al. (2014) for the unamended and composted soil samples. The pH, ORP and EC remained in a narrow range with no considerable difference between each other. There was a significant increase in pH value (p<0.05) only in the case of 0.1 unit of composted soil with an oat plant, compared to unamended soil with oat plants. The magnitude of ORP values were also significantly higher (p<0.05) for composted soil in which oat plants grew than in the unamended soil with oat plants. In the case of ORP, the unplanted soil also had significantly higher (p<0.05) values when it was composted soil rather than when it was not amended. However, ORP values were not significantly different for
Figure 8.5. Exchangeable Nutrients in Unamended and Composted Soil
Table 8.4: Average pH, Oxidation Reduction Potential and Electrical Conductivity Values for Clean and Contaminated Soil samples at Harvest Time

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>ORP (mV)</th>
<th>EC (mS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unamended</td>
<td>Composted</td>
<td>Unamended</td>
</tr>
<tr>
<td>No Plant</td>
<td>7.6</td>
<td>7.8</td>
<td>-55.5</td>
</tr>
<tr>
<td>Oat Plant</td>
<td>7.6</td>
<td>7.7</td>
<td>-52.3</td>
</tr>
<tr>
<td>Sunflower</td>
<td>7.6</td>
<td>7.7</td>
<td>-53.8</td>
</tr>
</tbody>
</table>
unamended and composted samples in soil planted with the sunflower plants. A significant difference of EC was observed only for the oat plant in both the unamended and composted soil. Though not significant, there was a considerable reduction in EC value with the addition of compost in unplanted soils. PH, ORP and EC are important factors for deciding the fate and transport of contaminants in the soil as speciation of metals can change based on these factors. Changes in speciation can affect the mobility, bioavailability and toxicity of metals (McLean and Bledsoe 1992). Also, high electrical conductivity and high or low pH values can inhibit microbial activity (Masciandaro et al. 2013; Luna-Guido and Dendooven 2001). However, the values of EC cited in the literature (Luna-Guido and Dendooven 2001) that can inhibit the microbial activities were two orders of magnitude higher than the values that were obtained in the present experiments.

The total concentrations Pb and Cr in different soil samples are presented in Figure 8.6. There was no detectable Cd present in any sample, including the initial samples. The Pb concentrations in the soil were not affected significantly by the presence of plants or soil amendment (p>0.05). The Cr in the soil was significantly reduced by both plant species in the amended and unamended soils (p<0.05). The percentage removal of Cr achieved by the oat plant and sunflower in unamended and composted soil are compared in Figure 8.7. Cr removal by the oat plants improved considerably when the soil was amended with compost. Cr removal was better achieved by the sunflower, when compared to the oat plant, in both amended and unamended soils. The results show that the bioavailability of Cr is higher than that of Pb for phytoextraction at the present pH levels.

The exchangeable concentrations of Pb were zero in all the samples, supporting the hypothesis that the bioavailability of Pb is less than that of Cr. This indicated the preference in
Figure 8.6. Heavy Metal Concentrations in Unamended and Composted Soil
Figure 8.7. Percentage Reduction of Cr in Unamended and Composted Soil
adsorption of different heavy metals in exchange sites of the soil particles. According to McLean and Bledsoe (1992), the maximum retention of anionic metals (Cr) occurs at pH<7 and maximum retention of cationic metals (Pb) occurs at pH>7. The pH values of the soil samples can explain the higher availability of Cr in the soil solution compared to Pb. This is supported by the fact that no detectable Pb was found in the extract for testing exchangeable Pb. Figure 8.8 shows the exchangeable Cr concentrations in different soil samples. For both unplanted and planted soil samples, the exchangeable Cr increased significantly (p<0.05) with the application of compost. This finding agrees with the results obtained by Pardo et al. (2014) where DTPA extractable concentrations of heavy metals were higher in the composted samples. The slightly higher value of pH in the composted soil can be the reason for the increased Cr mobility in the composted soil. In general, the planted samples had significantly higher exchangeable Cr concentrations than the unplanted samples. This may be due to the complexation with organic ligands in the rhizosphere of the plants, which would have increased the metal solubility. Similar results showing higher extractable metal concentration in rhizosphere soil were observed by Cattani et al. (2006) while studying Cu bioavailability in the rhizosphere of maize. Other research findings also suggest that plants can not only affect the solubility of heavy metals in the rhizosphere, but also change their species distribution in the soil solution (Chen et al. 2004; Lin et al. 2008).

Figure 8.9 shows the total PAH concentrations in the initial and final samples. In the final samples, most of the planted soil samples had lesser PAH concentrations than the unplanted samples, indicating plant-induced microbial degradation and/or volatilization (Mozo et al. 2012). A general trend of improved PAH degradation with compost addition was observed, showing that compost improved the microbial activity in the soil (Masciandaro et al. 2013). However, in
Figure 8.8. Exchangeable Cr Concentrations in Unamended and Composted Soil
Figure 8.9: PAH Concentrations in Unamended and Composted Soil

(UI-unamended initial, CI-composted initial, UB-unamended unplanted, CB-composted unplanted, UO-unamended with oat plant, CO-composted with oat plant, US-unamended with sunflower, CS-composted with sunflower)
the final composted sample from the sunflower pot, the PAH concentration was considerably higher than expected. This may be due to a non-representative sample or an analytical error. Another explanation is the production of phytoalexins by the plants under stressed conditions (Bais et al. 2006). It may be the antimicrobial property of the phytoalexins, which acts against the microbial biodegradation of PAHs. Experiments done by Corgie et al. (2004) also suggest that plants can either improve or inhibit biodegradation by affecting the spatial distribution of bacterial communities.

Similar results that showed higher PAH content in planted soil compared to unplanted soils were observed by Chigbo et al. (2013). They conducted co-contamination experiments with different concentrations of Cu and pyrene. When the soil was spiked with 50 mg/kg Cu and 250 mg/kg pyrene, the residual pyrene was higher in the planted soil than in the unplanted soil. However, when the Cu concentration was 100 mg/kg, there was no considerable difference in the residual pyrene in soils between the planted and unplanted soil. There is also possibility of reduced biodegradation in composted soil due to the presence of lignin on the compost, which can bind hydrophobic organic compounds and reduce its bioavailability (Pilon-Smits, 2005). Water content is another very important factor that decides the biodegradation of organic contaminants (Masciandaro et al. 2013). The ideal moisture level for the biodegradation of hydrocarbons is 45 to 85% of the soil water holding capacity (www.epa.gov/swerust1/cat/landfarm.htm). However, in the present experiment, the soil water contents were close to the soil water holding capacity, as reported in Table 8.1. The soil water content at the top, middle and bottom of the pot were determined to be 48, 54.7 and 55.8%, respectively, for the unamended soil. The results for the pot with composted soil were 51.4, 71.1
and 74.1%, respectively. This higher water content is another probable cause of the low biodegradation of organic contaminants in the soil.

The results of the present experiments for unamended soil were compared with previous experimental results of soil spiked with Pb, Cd, Cr, naphthalene and phenanthrene at different concentrations. At similar initial concentrations of Pb as those in the field soil sample taken in this study, considerable Pb removal (22%) was achieved by the sunflower planted in the spiked soil. Aging of the contaminated soil is expected to be the main reason for the ineffectiveness of the plants to extract Pb from that soil. However, Cr removal was found to be higher in aged soil compared to that found in the spiked soil. At similar initial concentrations of Cr, sunflower and oat plant achieved 22 and 19% reduction of Cr from the spiked soil, respectively. On the other hand, the Cr removals achieved by sunflower and oat plant in the present experiments on field soil were 52 and 30%, respectively.

Soil characteristics and the presence of different co-contaminants and ageing should be the important factors that decide the extractability of the heavy metals in soil. Experimental results by Chigbo and Batty (2013) also suggest that phytoextraction behavior of plants can differ considerably in freshly spiked soil and in aged soil. A comparative study of the phenanthrene concentrations of the present field soil experiments and the earlier spiked soil experiments revealed that for the spiked soil experiments (initial concentration of 7.3 mg/kg), the final concentration of phenanthrene was zero in both planted and unplanted soil after the conclusion of the 61 day plant growth period. However, in the field soil experiments, there was no phenanthrene dissipation observed in either the planted or unplanted soil with initial phenanthrene concentration of 2.9 mg/kg. This finding reinforces that fact that the bioavailability
of organic contaminants for degradation decreases with their residence time in the soil (Smith et al. 2011).

8.5. Conclusions

The study of soil with aged industrial contamination revealed that the germination, survival and growth of the oat and sunflower plants improved with the application of compost. Pb was not extracted by either plant in the unamended or composted soil, indicating that Pb immobilization occurred in aged soils. The non-detection of exchangeable Pb in unamended and composted soils also supports this hypothesis. Cr removal by the oat plant considerably improved when the soil was amended with compost, but compost did not significantly improve the Cr reduction by sunflower plants. The exchangeable Cr in the soil increased with the compost application. In most of the cases, the PAH results indicated that both the presence of plants and the compost amendment improved biodegradation. A comparison of the present experimental results on field soil with an aged soil of similar contamination level showed that the effect of soil aging varied for different contaminants. Pb extraction by the plants was better in the newly spiked soils used in the earlier research, whereas the rate of Cr extraction was better in the aged soils. The results suggest that phytoremediation efficiency depends on the complex interactions of soil, co-contaminants, microbes, plants and soil amendments. These interactions can also be affected considerably by the ageing of the contaminated soils. Phytoremediation in combination with compost application can be adopted as a promising technology for remediating sites with aged contamination.
8.6. Cited References


9.1 Overall Conclusions

The applicability of phytoremediation to soil co-contaminated with naphthalene, phenanthrene, lead, cadmium, and chromium, contaminants that are commonly found at many industrial sites, was investigated. Different techniques to enhance phytoremediation through the use of soil amendments were also studied successfully. The research was carried out systematically; the first step was the selection of plant species that can survive in the mixed contaminated soil. Since contaminants can be present at the site individually or with co-contaminants, the individual and combined effects of these contaminants on the success of phytoremediation were evaluated. Co-contamination experiments were carried out using different initial concentrations of the contaminants to learn if there is a threshold concentration that causes phytotoxicity.

The studies showed that the oat plant displayed the least phytotoxicity and best growth characteristics, but despite this, its ability to phytoextract metal was less than that of other plant species. The sunflower has the capability to phytoextract metals, but it proved to have both diminished growth characteristics and biomass in presence of metals. Amendments were introduced to the soil to improve the bioavailability of the contaminants and biomass of plants. Three biomass amendments, biochar, compost or a nutrient solution, were tested for their ability to enhance biomass, and EDTA and Igepal CA 720 were studied as potential chemical amendments to improve the bioavailability of metals for extraction and the degradation of organic contaminants. An additional effort was made to improve the bioavailability of contaminants by applying a low voltage AC potential in the rhizosphere. Among all of the likely
amendments studied, compost produced the best results as it improved the plant growth characteristics and contaminant dissipation. Compost also enhanced the phytoremediation of typical contaminated field soil sampled from a historically polluted site.

The general conclusions from this research are summarized below:

• Mixed contaminated soil has a significant negative effect on the germination, survival and the growth characteristics of all the studied plant species. Seeds had delayed or reduced germination and survival rates in contaminated soil as compared to those sown in the control pots.

• Of the 12 plant species studied, only five (oat plant, sunflower, rye grass, tall fescue, and green gram) survived for the full test period. When grown in contaminated soil, sunflower, rye grass, tall fescue, and green gram showed a considerable reduction in their final maximum height and biomass, and the oat plant had best germination rate and growth characteristics.

• Although the percentage of sunflower seeds that germinated was low when seeded in contaminated soil, the surviving sunflower plants showed good growth. The sunflower was more successful at heavy metal extraction than all the other plant species.

• Neither the sunflower nor the oat plant enhanced the degradation of organic contaminants.

• Rye grass, tall fescue and green gram were not effective species for the removal of heavy metals from soil. However, they successfully reduced the exchangeable metals in that soil. Also, they were all able to enhance the microbial degradation of phenanthrene. Therefore, rye grass, tall fescue and green gram can be considered for immobilization or restoration projects.
• Better germination and growth characteristics were observed among plants grown in soil tainted with individual organic contaminants than when the plants were grown in soil contaminated with heavy metal or mixed contaminants.

• The growth characteristics of the plants propagated in mixed contaminated soils (mix of organic and heavy metals) were better than those of the plants grown in soil solely contaminated with heavy metal.

• The presence of Cr created the most phytotoxic contamination condition when it was the sole contaminant in the soil. No plants survived.

• The presence of organic contamination inhibited the extraction of heavy metals by the plants.

• The oat and sunflower plants reduced the Pb and Cd concentrations in soil that was solely contaminated with one of these metals and in mixed metal contaminated soil.

• The rates of Pb reduction were similar in soil contaminated with Pb alone and in mixed metal contaminated soils.

• Plants grown in soil contaminated with Cd alone achieved a higher reduction of Cd than plants in mixed metal contaminated soil.

• The plants grown in unamended soil did not considerably affect the degradation of organic contaminants.

• The plant growth and phytoremediation efficiency followed a gradual reduction as the contaminant concentrations rose. There was no threshold concentration of the contaminants for phytotoxicity.

• The sunflower’s rates of germination, growth and biomass were greatly improved by the addition of biochar and compost.
• Biochar and compost amended soils improved the germination of the oat plants, but its final biomass remained less than that of the oat plants sown in unamended soil.

• The EDTA and Igepal CA-720 amendments increased the phytotoxicity symptoms in plants.

• The addition of biochar and compost amendments improved the reduction of Pb and Cd. The addition of the biomass amendments did not alter the Cr reduction considerably.

• Soil amendment with a fertilizer solution did not cause a considerable change in the reduction of heavy metals by either the oat plant or sunflower.

• The addition of EDTA and Igepal CA-720 to the soil reduced the phytoextraction efficiency of both plant species, possibly due to a reduction in the plant biomass.

• The exchangeable Pb and Cd concentrations were considerably increased by the application of EDTA and Igepal CA-720.

• The degradation of PAHs in planted soils was improved with the biomass amendments. Applications of EDTA and Igepal CA-720 enhanced the degradation of phenanthrene from the soil.

• PAH degradation was best achieved when compost was combined with the application of a chemical amendment.

• Due to the phytotoxicity of the mobilized metals, EDTA and Igepal CA-720 are not recommended as a soil amendment for the enhanced phytoremediation of mixed contaminants with oat plant.

• Biochar and compost amendments provide a promising approach for enhancing the phytoremediation of mixed contaminated soils using oat plant and sunflower.
• Though there was a slight increase in the biomass of the plants due to the electric potential application, the increase was not enough to improve the heavy metal phytoextraction.

• The application of electric potential to soil did not lead to any improvement in degradation of organic contaminants.

• Experiments with field soil confirmed that phytoremediation in combination with compost amendment is a promising approach to remediate mixed contaminated soil.

9.2 Recommendations for Future Research

Based on the above facts, the following recommendations are made for future research:

• The success of heavy metal phytoextraction can vary based on the co-contaminants and types of soil and plants. Consequently, the present results are applicable for the typical soil with the considered plants and the selected co-contaminants. However, when more co-contaminants are present, the results can vary. More research is required to improve understanding of the phytoextraction mechanism by plant transporter proteins and their preference for some metals over others.

• The PAH data suggests that plants can affect the biodegradation of organic contaminants positively or negatively. The conditions under which plants behave differently are not clearly understood. More research is needed to clarify the mechanisms that can create optimum conditions for the phytodegradation of organic contaminants.

• The electric potential application in this research showed that it could enhance the biomass of the plants, but was insufficient to cause a change in the dissipation of contaminants by plants. The application of a more frequent and higher voltage AC is suggested in order to produce a noticeable effect on contaminant removal or degradation.
• Phytostabilization using rye grass, tall fescue and green gram is a promising approach that can be further researched to determine the range of its potential use in restoration projects.

• Intercropping of plant species that are capable of metal extraction and organic contaminant degradation is also recommended for future research.
APPENDIX A

VITA

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