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Heavy Metal Uptake Efficiency of Alfalfa, Barley, Indian Mustard and Atriplex from Contaminated Desert Soil

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Abstract: *Phytoremediation is a cost effective and environmental friendly tool for the remediation of contaminated environment. Certain plant species have the ability to uptake toxic pollutants into their biomass and the rhizosphere microbes can degrade complex pollutants into simpler and less toxic forms. Contamination of soil with petroleum hydrocarbons and associated heavy metals is a major cause of concern in oil producing countries. In our ongoing research, Alfalfa, Indian mustard, Barley and Atriplex plants were screened for remediation of heavy metal contaminated soil and data on heavy metal uptake efficacy is presented. The presentation is focused on the experiments designed to examine the degree of toxicity of oil contaminated soil on alfalfa, Indian mustard, barley and Atriplex species and their ability to uptake heavy metals when administered in a mixture form. Plants were subjected to heavy metal treatment in a growth chamber under controlled conditions. Treatments included: control, heavy metal mix in 1x and 10x concentration with and without EDTA. Each treatment was done on five replicate pots. Treatments were given once in 10 days for one month. At the end of the experiment, roots and shoots were collected, dried, powdered and used for the measurement of heavy metals. In conclusion, amendment of soil with EDTA enhanced uptake of heavy metals when mixture of heavy metals were introduced to the growth medium. The results indicate that Barley, Alfalfa, Mustard and Atriplex plants can be efficiently used for remediation of soil contaminated with heavy metals mainly originating from crude oil spills.*

Keywords: *Phytoremediation; Environmental cleanup; Kuwait; oilcontaminated soil*

INTRODUCTION

Cleanup of oil contaminated soil is a challenging task. Conventional physical and chemical means of soil remediation can irreversibly damage the environment. The mechanical methods involve excavation and displacement of contaminated soil which is more destructive, causes additional environmental damage and requires a huge amount of energy and labor. Bioremediation is an alternative method used for cleaning contaminated soil. Plants and its associated rhizosphere microbes have the ability to accumulate/ degrade various organic and inorganic chemical pollutants present in contaminated soil.

Phytoremediation is a plant-based technology that offers an environment friendly and cost-effective solution to clean the environment contaminated with moderate amounts of chemical pollutants [1-5]. Several plant species have the capability to accumulate various types of heavy metals and other harmful chemicals within their tissues [6-21]. According to Phytochem database developed by Environment Canada, there are more than 750 terrestrial and aquatic plants with potential value for phytoremediation.

Phytoremediation can be combined with microbial assisted bioremediation approach in a cost-effective and environment-friendly manner for remediating a polluted environment [3-5]. The microbial population associated with the roots can actively degrade and eliminate pollutants from the contaminated soil.

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Kuwait has experienced a catastrophic environmental disaster during the Iraqi invasion [22]. Millions of gallons of crude oil were released into Kuwait's fragile ecosystem. In spite of large scale cleanup efforts, the large areas contamination Crude oil contains a significant amount of heavy metals such as nickel, vanadium, copper, cadmium, and lead. Hence, the oil spills lead to the contamination of soil and ground water with heavy metals, and they can enter into the food chain and pose a health hazard [23-24].

Plant species demonstrate a great degree of variability in terms of their remediation potential. For the cleanup of heavy metal contaminated soil, one of the most important criteria is the metal tolerance and uptake ability of the plant. Similarly, the root abundance and microbial population present in the rhizosphere is important for degradation of petrochemicals. Hence, the selected plants for remediation process need to be thoroughly screened for metal tolerance and uptake ability under controlled experimental conditions. It is important to know that in an arid region such as Kuwait, the weather conditions do not support the growth of many plant species. Therefore, the plants that are suitable for this region should be chosen carefully for phytoremediation. Considering these factors, alfalfa, Indian mustard, barley and Atriplex plants have been chosen for the current study.

METHODS

The degree of toxicity of oil-contaminated soil was measured using Phytotox Kit (MicroBioTests, Belgium). The percentage inhibition of seed germination and root growth on control and oil-contaminated soil was measured. The test soil had 1.3% total petroleum hydrocarbons (TPH). The data was collected from 30 replicates for each plant species. The percentage inhibition of seed germination and root growth in the test soil was calculated using the formula (A - B)/A x 100. Where A = mean seed germination or root length in the control soil; B = mean seed germination or root length in the test soil.

To test the metal uptake and oil degradation ability of selected plants from oil contaminayed soil, the seedlings were grown on control (C) clean and oil-contaminated soil [0.8% and 3% TPH] for 50 d. At the end of the experiment, heavy metals in the soil were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Total petroleum hydrocarbons were measured using gas chromatography with Flame Ionization Detection (GC-FID) using a gas chromatograph. Fourier transform infrared spectroscopy (FT-IR) was performed for routine soil analysis for TPH. Ten replicates were used for each treatment.

To study the heavy metal uptake efficiency of alfalfa, Indian mustard, and barley seeds planted in commercial soil mix and subjected to heavy metal treatment in a growth chamber under 16:10 h light and 23^{o}C temperature. Two week old seedlings were used in this experiment. Treatments included: control (mock treatment with water) and heavy metal mix at the concentration reported in published literature (Wangeline et al., 2004). Another treatment consisted of a tenfold increased content of all five heavy metals and designated as 10H. The experimental setup also consisted of ethylenediaminetetraacetic Acid (EDTA) 50 mM (50 ml/pot). Each treatment was given on six replicate pots. Treatments were given once in 10 d for one month. At the end of the experiment, roots and shoots were collected, dried, powdered in duplicate sets, and used for the measurement of heavy metals. The average accumulation of each of the heavy metals under study was done separately for the root and shoot tissues of the plants under the aforementioned four treatments, in comparison with the control mock treated plants. The data was subjected to Analysis of Variance (ANOVA).

RESULTS

Germination potential and root growth were severely affected in Indian mustard in oil-contaminated soil. Barley and alfalfa seeds showed efficient germination and successful initial establishment in oil-contaminated soil. The test indicated that barley and alfalfa can be directly germinated on oil-contaminated soil, and Indian mustard can be only used after pre-germinating the seeds in clean soil followed by replanting of seedlings in the contaminated site.

The oil-contaminated soil did not affect the growth of barley plants severely. However, the growth suppression in alfalfa and Indian mustard was evident at the time of termination of the experiment. These results are in line with the phytotox assay results where barley showed maximum tolerance to oil-contaminated soil. Levels of heavy metal in the control and test soil used for growing plants were measured after 50 d. There was a decrease in heavy metal content in the soil under phytoremediation. Significant reduction was noticed in nickel, lead, and vanadium in the soil used for growing alfalfa and Indian mustard. Although barley can tolerate the toxicity of contaminated soil comparatively better than the other two species, the heavy metal removal process was not very efficient. Despite severe growth reduction, maximum reduction in heavy metals, C8-C40 petroleum range organics and TPH in soil was noticed with Indian mustard and alfalfa. A 50 d period may be very short for a remediation experiment involving persistent residual contaminants like weathered hydrocarbons and associated heavy metals.

All the four species demonstrated accumulation of heavy metals in the vegetative parts when grown on soil containing mixture of five heavy metals. However, the degree of uptake and translocation of heavy metals to shoots varied between the tested plant species. EDTA treatment enhanced the accumulation of heavy metals in vegetative parts of all the four tested plants. Interestingly, *Atriplex* roots are highly efficient in accumulating the tested heavy metals with enhanced accumulation in presence of EDTA. The presentation would include data pertaining to all these aspects.

In conclusion, plant species like barley and Atriplex are ideal for the remediation of oil contaminated soil in the arid environment. These plants can be used along with microbial assisted bioremediation process for environmental cleanup in an economical and ecofriendly manner.

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