Research Article   **OMICS International**

Rhizoremediation of Contaminated Soils by Comparing Six Roots Species in Al-Wafra, State of Kuwait

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Abstract

Toxic heavy metals concentrations in soils are locally quite high in Kuwait due to the gulf war in 1991. There are many reasons that make heavy metals high in Kuwait soils such as massive oil spills and direct dumping of untreated sewage and hydrocarbons. Phytoremediation may offer a possible solution to this problem because it might treat the soils through the use of plants even without the demand of removing the contaminated material and disposing it elsewhere. This research project will discuss the best contribution among six types of plant (tree) species (*Conocarpus, Tamarix, Phoenix, Rhamnus, Vitex Agnus-Castus and Salix*) collected from Wafra 14 km south Kuwait and another six reference trees from the same plant species collected for correlation from Sabah Al -Salem residential area. The samples are divided into two sample categories (roots and soil). From the chemical analysis it was found that rhizoremediation is a unique process in decreasing the level of soil contamination of hydrocarbons and trace metals. The most effective part of the plant in accumulating hydrocarbons and heavy metals were the roots while the appropriate plant was *Tamarix*. As a recommendation there is a need for studying the fruits of Phoenix to detect accumulates of TPH and Trace Metals and to decrease the harmful effects of the fruits on the human.

Keywords: Bioremediation; Pollutant; Rhizoremediation; Hydrocarbons; Trace metals

Introduction

The study area is located in south of Kuwait along Wafra/Mina Abdullah road at 14 km (Figure 1). The area is about 1 km^2 and it receives over than 4500 m^3 daily of industrial hazardous liquid waste and untreated sewage in one of the largest unlined evaporation ponds in the State of Kuwait. The study area now is completely rehabilitated and planted by several types of plants in four main sectors comprises 45000 trees. This research project will focus on the most efficient and beneficial plant out of six plants that were planted in Wafra/ Mina Abdullah 14 km, to treat the contaminated soils in many areas of Kuwait. Thus, reducing and/or eliminating contaminants and restoring or partly restore the ecological service function of soil. The State of Kuwait is in Western Asia located at the north eastern corner of the Arabian Gulf on the Arabian Plate. It has a strategic location at the head of the Arabian Gulf and shares borders with Iraq and Saudi Arabia. Kuwait's area is about 17,818 km^2 and it lies between latitudes 28°30' and 30°05' N, and longitude 47°30' and 48°36' E.

The main objectives of this study:

- To understand the behavior of the six tree plants roots in absorbing pollutants.
- To study the rate of fate and flux of pollutants.
- To evaluate the roots and soils of the different six plants in cleaning the contaminated soil.
- To make a recommendation for the best out of six tree plants selected in the study area in absorbing hydrocarbons and trace metals from contaminated soil.

This will be achieved through:

- Determining the level of total petroleum hydrocarbon (TPH) in plant's (roots and soil).
- Measuring Trace Metals accumulation (TM) in plant's (roots and soil).
- Chemical comparisons between the plants and soil samples.

This research will focus on studying the plant roots specially and identifying the most efficient tree out of the six trees that were planted in the study area in absorbing contaminants (Figure 2).

Literature Review

Refractory organic harmful and toxic chemicals have accumulated for decades as a result of industrial activities and caused soil contamination. Soil contamination is the essential source that transmits toxins like heavy metals from environment to living beings. Plants adsorb heavy metals from soil. Heavy metals enter the animal's kingdom including people through the food chain, and cause a lot of health risks [1].

Many published research have been proved that plants play an important role in removing heavy metals from contaminated soils. Plants and Microorganisms are possessed by many mechanisms to avoid heavy metal poisoning. Metal efflux particularly which is represented in eubacteria is a famous example in addition to synthesis of metal-binding peptides like metallothionein (MTs). Also, it is representing in plants, fungi, blue-green algae and phytochelatins [2,3]. Additionally, Plant roots cause changes at the soil-root interface as they discharge inorganic and organic mixes (root exudates) in the rhizosphere. The number and activity of the microorganisms, the accumulation and stability of the soil particles around the root, and the availability of the contaminants are affected by the root exudates. The availability of the contaminants in the root zone (rhizosphere) of the plant can be increased or decreased directly or indirectly by root

Figure 1: Location map of Kuwait and its physiography (Source: mapofworld.com).
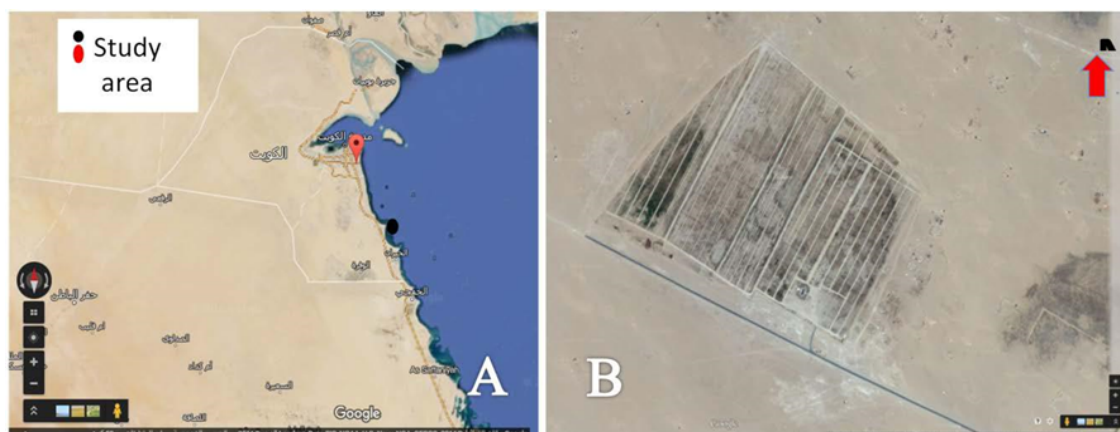

Figure 2: (A) Map showing the study area location across Wafra/Mina Abdullah road area (Source: Google maps, 2016). (B) Map showing the study area location across Wafra/Mina Abdullah road area in a close view (Source: Google maps, 2015).

exudates themselves through changes in soil characteristics, release of organic substances, changes in chemical composition, and/or increase in plant-assisted microbial activity (International Environmental Technology Centre (IETC).

Building up vegetation spread on generally contaminated perilous waste destinations may be a proficient way and low support strategy to waste remediation. Connection between plant roots and rhizosphere microflora altogether upgrades corruption of unsafe organic compounds in polluted soil [4]. Also, metals are cycling at low rates inside and between bio, geo, atmospheric and hydrosphere frameworks. However, still metals that are collected in soils are dependable to higher dangers since they are drained into ground and surface water. After that, uptake process assimilates and a store metal in plants and after that direct or indirect intake by human population occurs. According to Karenlampi et al. [5] metals are dangerous in the event that they are available at expanded levels of bioavailability for example essential (Mn, Zn, Cu, Mo, Fe, Ni) and non-essential metals (Pb, Cd, Cr, Hg). Metal

hyper aggregating discovery properties in certain plants expressed that it might be conceivable to utilize these plants for the cleanup of heavy metal pollution in soil and water. This way to deal with environmental restoration has been termed phytoremediation [6,7].

The use of plants in bioremediation of soils has been proposed as an attractive technique and because of expanded metal fixations on most living creatures, numerous strategies were produced to remediate or phytoremediate contaminated soils. However, plants do not have the uncommon biodegradative abilities that microorganisms have, so another innovation, called rhizoremediation has emerged. The available methods are costly, environmentally invasive, and labor intensive. However, remediation technique is low. The utilization of green plants to expel, i.e., phytoremediation contains or diminishes innocuous contaminants from environment.

Plants cover can be an effective technique to prevent contaminants spread by reducing erosion process done by wind and surface run off. Furthermore, it is reducing percolation to the ground water. Plants may also be used to remove contaminants from soil by phytoextraction and then harvested for processing [8-11]. There are some promising results suggesting that these techniques might become practical alternatives to mechanical and chemical approaches in remediation of metal contaminated soils [5]. Recently, rhizoremediation objective is to upgrade and develop innovative, economical, and environmentally compatible approaches to remove heavy metals from the environment. Thus, phytoremediation targets currently include contaminating metals, metalloids, petroleum hydrocarbons, pesticides, explosives, chlorinated solvents, and industrial byproducts as stated by Cunningham et al. [8]. The biological technologies that can be employed in the remediation of contaminated soils are Bioremediation, phytoremediation and rhizoremediation. This study will focus on rhizoremediation.

Rhizoremediation

Rhizoremediation is a process where microorganisms degrade soil contaminants in the rhizosphere (Figure 3). It is use plant roots and associated microbial consortium to degrade environmental pollutants from soil with an aim of restoring area sites to a condition useable for intended purpose. Rhizoremediation takes advantage of plant roots
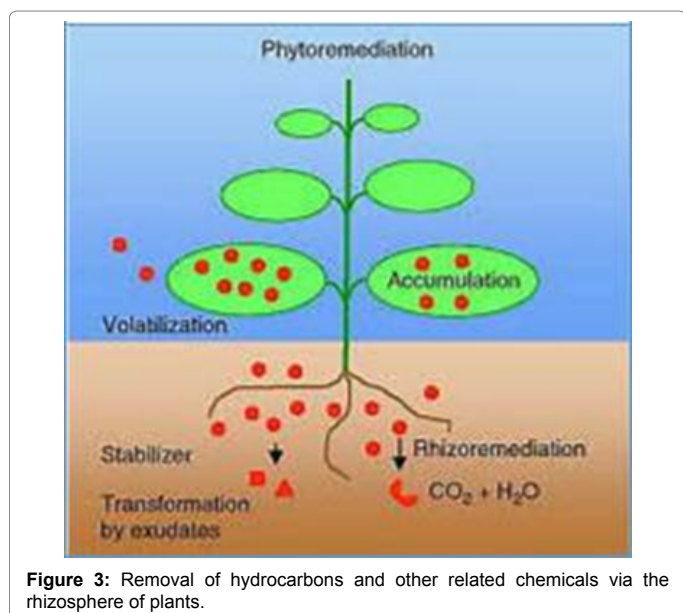

Figure 3: Removal of hydrocarbons and other related chemicals via the rhizosphere of plants.

natural symbiosis with a fungus which grows in association with the roots of a plant called mycorrhiza and root associated natural microbial flora for the enhanced degradation of pollutants in the rhizosphere [12]. This method can remediate the soil pollutants which are generally organic compounds that cannot enter the plant because of their high hydrophobicity. Generally, plants are not considered as the main mode of remediation in this procedure. Rather, the plant creates a niche for rhizosphere microorganisms to do the degradation. Rhizosphere microorganisms are served by the plant acting as a solar-powered pump that draws in water and the pollutant while producing substrates that benefit microbial survival and development. Root exudates and root turn over can serve as substrates for microorganisms that perform toxin degradation.

Determination of organisms has been attempted with great achievement to be useful in rhizoremediation. Utilizing pollutant soil as an initial media to select from, enrichment of bacteria that can survive on the contaminant. This enrichment can then be inoculated onto plants where selection for root colonization can be done. Utilizing this process will result in a host plant that backings a toxin degrader in its rhizosphere. Wild type organisms are selected this procedure and accordingly there is no constraint for usage as with genetically modified microorganisms [13] depicts the successful use of this technique to identify the rhizosphere PAHs degraders (Table 1). Rhizoremediation is usually less costly than competing alternatives such as soil excavation, pump-and-treat, soil washing, or enhanced extraction.

Role of plants in rhizoremediation: Certain plants are better at removing contaminants than others. There are many factors must be able in the plants used for phytoremediation. They must be able to tolerate the concentrations of contaminants present. Also, they must be able to grow and survive in the local climate. Another factor is

Plant	Pollutant	Microbes
Rice (cv.Supriya)	Parathion	Not identified
Mixture of grass, legume, herb and pine	TCE	Not identified
Prairie grasses	PAHs	Not identified
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Grasses and alfalfa	Pyrene, anthracene, phenanthrene	Not identified
Sugar beet (cv.Rex)	PCBs	*Pseudomonas fluorescens*
Unidentified wild plant (*compositae*) and *Senecus glaucus*	oil	Arthobacter/Penicillium
Barley (Hordeum vulgare)	2,4-D	Bukholderia cepacia
Alfalfa and alpine bluegrass	Hexadecane and PAHs	Not identified
Wheat (*Triticum aestivum*)	2,4-D	*Pseudomonas putida* strains
Poplar (Populus deltoides nigra)	1,4-dioxane	Actinomycetes
Wheat	TCE	*P. fluorescens*
Oat, lupin, rape, dill, pepper, radish, pine	Pyrene	Not identified
Reed (*Phragmitis australes*)	Fixed nitrogen	*Nitrospira sp. And Nitrosomas sp.*
Poplar root extract	1,4-dioxane	*Actinomycete Amycolata sp. CB 1190*
Corn (Zea mays)	3-methylbenzoate	*P. putida*
Astragalus sinicus	Cd+	*Mesorhizobium huakuii*
Fern (*Azolla pinnata*)	Diesel fuel	Not identified

Table 1: Rhizoremediation of various environmental pollutants [15].

important such as the depth of contamination. If the contamination is shallow, small plants like ferns and grasses will be used. Trees such as poplars and willows are used to clean up deeper soil contamination and contaminated groundwater because tree roots grow deeper [14].

Plants may contribute to remediation in several ways, by reducing the leaching of contaminants, aerating soil, phytodegradation, phytovolatilization, evapotranspiration, and rhizoremediation. Nutrients in the rhizosphere are given by the mucigel emitted by root cells, lost root cap cells, the starvation of root cells, or the decay of complete roots [15,16]. In addition, plants discharge a variety of photosynthesis derived organic compounds [10,17]. Root exudates contain water soluble, insoluble, and volatile compounds including sugar, proteins, alcohols, amino acids, organic acids, nucleotides, flavonones, phenolic compounds and certain enzymes. Prevailing conditions that support the application of both microbes and plants depend on the selection of bioremediation or phytoremediation for cleanup of the contaminated site.

Factors affecting rhizoremediation: The different physical, chemical and biological properties of the root related to soil are soil condition, temperature, pH, organic matter, weather condition. Schroll et al. [18], Alberty [19], Guillot et al. [20], Mastronicolis et al. [21], Singh et al. [22] are responsible for changes in microbial diversity and for expanded numbers and metabolic activities of microorganisms in the rhizosphere microenvironment, this phenomenon called the rhizosphere effect [10,15,17,23]. Many studies have investigated the effects of soil moisture, temperature, aeration, pH, and organic matter content on the biodegradation of pesticides [11,24]. The age of a plant, the availability of mineral nutrients and the presence of contaminants are responsible of the rate of exudation changes [25]. The nature and the quantity of root exudates, and the timing of exudation are crucial for a rhizoremediation process. Plants might respond to chemical stress in the soil by changing the composition of root exudates controlling, in turn, the metabolic activities of rhizosphere microorganisms [25]. The root exudates mediate acquisition of minerals by plants and stimulate microbial growth and activities in the rhizosphere in addition to changing some physicochemical conditions. Some organic compounds in root exudates may serve as carbon and nitrogen sources for the growth and long-term survival of microorganisms that are capable of degrading organic pollutants [10,17,26].

Improvement in rhizoremediation: Rhizoremediation procedure can be intended to enhance in several perspectives like bioavailability of contaminant molecules, expression and support of genetically engineered plant microbial frameworks and root exudates for the effectiveness of the procedure. An interesting alternative to improve the removal efficiency is selection of bacteria. Bacteria are produce biosurfactants in the plants rhizosphere. Kuiper et al. [15] applied bioremediation and distinguished bacteria developing in contaminated area that produces biosurfactants that encourage the solubilisation of PAHs and thus biodegradation by microbes. This property is additionally

of interest because various biodegradative microorganisms show positive chemotaxis towards the contaminations. Thus, the consolidated activity of biosurfactant and chemotaxis can add to bacterial expansion and to microbial spread in contaminated soils, all together that more abundant zones can be cleaned. Microbial degradation of pollutants in the rhizosphere gives a constructive outcome to the plant; the toxin concentration is diminished in the zone close to the roots and the plant can develop better to those in contaminated zones. Because of this shared advantage it has been recommended that plants can choose particular genotypes to be available in their roots. Many experiments performed by Siciliano et al. [27] exhibited that the presence of the alkane monooxygenase quality was more pervasive in endophytic and rhizosphere microbial groups than in those present in bulk soil contaminated with hydrocarbons. This recommends if plants are impacting the rhizosphere, this impact is subject to the contaminant. Additionally, a few researchers concluded that the impact depended on the sort of the plant. This has led to the hypothesis that the viability of rhizoremediation methodologies is identified with the determination of the best plant bacterium pair in each case.

An environmental evaluation for the industrial and sewage wastes in Kuwait [28]

This study examined Al- Wafra 14 km since it is the main area that gets the industrial waste and the fluid from all other areas. The main point of this study was to evaluate and contribute groundwater, soil and air which are the probable contaminants that came from human activities to enhance the environment and people's health and helping the authorized people to plan for the future of country and environment actions by taking the fitting activities for ensuring these resources.

TPH analysis: TPH results showed high percentages in Wafra 14 km (Table 2). The acceptable TPH amount on earth should be 10 mg/l according to the International Bank while in Wafra the mean values of TPH showed 97.46 mg/l as shown in Table 3. Besides, natural Kuwaiti laws express the need to treat the consumed oils that contains high measure of hydrocarbons substances because of its hurtful impact on environment. Subsequently, they accepted a rate of 5 mg/l TPH.

Trace metals analysis (TM): Heavy metals such as Cd, Cr, Cu, Ni, Pb, and Zn in soils cause noteworthy dangers to human, animals as well as to environment. Human activities have expanded as an aftereffect of the Industrial Revolution and were the main causes of heavy metal deposition. Modern exercises, smelting, electroplating and Agriculture have brought about the deposition of undesirable concentrations of metals in soil. Addition of Trace metals might be hurtful to Humans, animals, plants and different life forms such as organisms reaching the soil or groundwater. In Table 3, the results of trace metals concentrations in Wafra 14 km showed that all of the values are above the acceptable local limits.

Phytoremediation for hazardous liquid industrial waste Al-

ID	V	Cr	Fe	Ni	Cu	Zn	Cd	Pb	TPH
Unit	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Mean	1.16	0.31	71.29	2.83	0.39	7.69	0.5	0.4	97.4
Minimum	0.07	0.15	19.9	0.49	0.11	3.98	0.0069	0.18	1.69
Maximum	6.55	0.81	282.9	9.95	1.10	17.72	3.1	1.07	523
Std.Dev.	1.76	0.19	78.2	2.7	0.26	4.41	1.04	0.26	164.5
1-KEPA standards	-	0.15	5	0.2	0.2	2.0	0.01	0.5	5
2-KEPA Standards	0.1	0.15	5	0.2	0.2	2.0	0.01	0.5	10

Table 2: Statistics analysis of trace metals (TM) and total petroleum hydrocarbons (TPH) samples conducted in Wafra 14 km [28].

Wafra [29]: In 2012 unpublished thesis study done by Alia Bado had done at the same study area Al-Wafra. The chemical analyses were done systematically at the laboratory following the Vista-MPX CCD Simultaneous ICP-OES for five plant samples collected (*Salix Fragilis, Albizzia, OleaEuropaea, Ziziphus and Tamarix*). Table 4 showed the trace metals which were analyzed for the five plant species. The study concluded that the high concentration of trace metals absorbed by the Tamarix plant and is the most satisfactory plant that could be utilized in Kuwait for phytoremediation process [29].

Phytoremediation for hazardous liquid industrial waste using six famous trees species in Al Wafra [30]: Another unpublished thesis study done by Mohammed Al-Ibrahim in 2015 conducted in the same contaminated study area Wafra 14 km. He collected six types of plant tree species (*Conocarpus, Tamarix, Phoenix, Rhamnus, Vitex Agnus-Castus and Salix*) and studied different plant part (leaves and stem) from the contaminated area. The study showed that the best part absorbed and stored the contaminants and the Trace Metals is the leaves. *Vitex Agnus-Castus* species leaves got the highest storage of TPH amount. Stems samples TPH analysis revealed that the highest TPH storage is of *Rhamnus* and the least one is *Tamarix*. However, TPH analysis of soil samples collected from the study area showed that the least plant could absorb the contaminants from soil got the highest value of TPH, which is *Conocarpus* species.

The study concluded that Vitex Agnus-Castus species is the most adequate plant that could be used for phytoremediation process in Kuwait according to the high concentration of the TPH and trace metals absorbed by the plant leaves [30].

Regional case study of phytoremediation

Does phytoremediation work at every site? [31]: A study has been carried out examining the growth characteristics and performance of mangroves, halophytes and other plants in soil irrigated with saline water in the United Arab Emirates. Salinity is a serious threat for crop production in arid regions, where the high rate of evaporation, combined with the demand for scarce water reserves, causes salt build-up during the cultivation process. The study showed that some plants have the necessary physiological mechanisms and capacities to accumulate significant concentrations of iron, manganese and magnesium, calcium, sodium and chloride ions, thereby reducing the overall salinity of the soil system and potential related effects on crop physiological and growth performance. Based on this work, it has been suggested that by introducing highly salt-tolerant species (i.e., *Conocarpus erectus*, *Atriplex lentiformis*, etc.) that can be irrigated with saline water, higher plant and agricultural production levels in arid regions can be achieved.

International case study of phytoremediation

Petroleum hydrocarbons pollution in soil and its bioaccumulation in mangrove species, *Avicennia marina* from Alibaug mangrove ecosystem, Maharashtra, India [32]: A study done by Lotfinasabasl [32] had investigated the capability of mangrove species, *Avicennia marina* for bioaccumulation of petroleum hydrocarbons from contaminated soil. The results showed that the TPH concentration was vary in leaves, roots and seedlings at the same plant and it was showing higher uptake of petroleum hydrocarbons by roots took after by seedlings and leaves.

The study concluded that *Avicennia marina* is observed to be a potential species for protection of coastal ecosystem. Phytoremediation has been recognized as a practical, naturally benevolent, stylishly satisfying procedure for evacuation of ecological toxins [32].

Materials and Methods

From the study area Wafra 14 km as shown below (Figure 4), six types of plant tree species were collected (*Conocarpus, Tamarix, Phoenix, Salix, Rhamnus, and Vitex Agnus-Castus*). Another six reference trees were collected for correlation from Sabah Al -Salem residential area from the same plant species. The samples were divided

ID	V	Cr	Fe	Ni	Cu	Zn	Cd	Pb	TPH
Unit	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
IA	0.5707	0.2476	37.9017	0.8922	0.4025	5.5232	0.0186	0.6762	54.3
1B	0.9354	0.4209	282.9234	6.2901	0.3624	15.7374	2.2618	0.6585	17.9
1C	6.5557	0.8118	176.6287	2.5396	1.1045	17.7218	0.0231	1.0749	9.73
2A	0.9241	0.5019	27.0217	0.4911	0.6518	4.1066	0.0156	0.2224	10.1
2B	0.0748	0.2803	45.081	2.7268	0.3044	7.7437	0.0747	0.3535	18.5
2C	0.5753	0.3197	34.2768	9.9575	0.1704	4.9234	0.0069	0.2792	14
3A	0.1764	0.1555	58.0832	1.8628	0.3318	6.374	3.1094	0.2613	206
3B	0.1015	0.1598	40.3729	1.8628	0.3318	7.9874	0.0242	0.3132	523
3C	0.7205	0.2089	58.9177	3.2001	0.1138	5.3476	0.4703	0.208	303
4A	1.0831	0.1916	29.3609	1.9591	0.338	6.4256	0.0114	0.2648	1.69
4B	0.4262	0.1587	19.9392	0.9415	0.3265	3.9842	0.0207	0.182	2.4
4C	1.8826	0.2657	45.05	1.8394	0.4364	6.5064	0.0079	0.3202	9

Table 3: Trace metals analysis results of Wafra 14 km [28].

Sample ID	Cr	Mn	Cd	Cu	Fe	Ni	V	Zn	Pb
Tamarix	6.607	52.77	0.204	41.201	181.146	4.432	BDL	94.05	BDL
Albizzia	3.945	49.486	BDL	24.234	201.538	0.957	BDL	28.027	BDL
Salix Fragilis	2.396	40.095	BDL	15.187	78.617	2.152	BDL	26.623	BDL
Ziziphus	1.682	67.103	BDL	9.192	69.121	0.213	BDL	33.776	BDL

All values are given in ppm (mg/g)

BDL: Below detection limit. In the method used for the analysis the minimum detection level of Cd is 0.205 ppm. V is 0.800 and Pb is 1.400 ppm.

Table 4: Trace metals analysis of plant samples from Al-Wafra 14 km [29].

into two sample categories (plant roots and soil) of each plant in the study. Therefore, the total numbers of samples collected are 24 samples including mixed treated soil (Figures 5 and 6). Total Petroleum Hydrocarbons (TPH) and Trace Metals analysis will be performed for duration of four months, starting from (November 2015 – March 2016). The samples collected and analyzed for determining the concentration of total petroleum hydrocarbon (TPH) and trace metals (TM).

Getting the plant roots samples from the study area was difficult so a bulldozer was used to extract the roots (Figures 5A and 5B) then the

Figure 4: Landscape view of plants in the contaminated study area Wafra 14 km.

Figure 5: Study area Wafra 14 km A) Getting a root sample using the bulldozer. B) The bulldozer while taking roots samples of *Conocarpus* species. C) Labours digging using the shovel to get the roots. D) Collecting the plant roots.
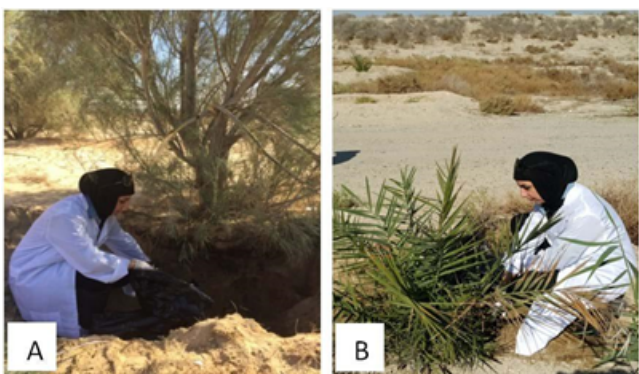

Figure 6: Getting different plants roots from the study area A. *Tamarix* B. *Phoenix.*

plant was replanted again at the same time. Several labors were helping to continue the root extraction by using shovels (Figure 5C). Finally, the root samples were collected very carefully in a plastic bag (Figure 5D).

Field work

The fieldwork conducted on the contaminated soil in the Wafra/ Mina Abdulla road 14 km. The study area includes 12 samples which were divided as 6 samples of plant roots and 6 soil samples. In addition, 12 reference samples were collected from Sabah Al-Salem residential area, another six plants roots samples from the same species and 6 soil samples.

Results and Discussion

The utilization of plants for remediation might be particularly appropriate for soils polluted by organic chemicals to profundities of less than 2 m. Accordingly, the collaboration between plant roots and rhizosphere microflora fundamentally improves degradation of dangerous natural mixes in polluted soil. The root arrangement of plants can spread microbes through soil and infiltrate generally impermeable soil layers. The immunization of contamination degrading microscopic organisms on plant seed can be an essential added substance to enhance the proficiency of phytoremediation [15]. Through degradation or gathering plants can cooperate with dangerous natural mixes. Uptake of poisons in plants roots is an immediate capacity of the contaminant amount and concentration in the solution of soil and large contains compound isolating connected to the root surfaces took after by development over the cortex to vascular arrangement of plants [33]. The contamination may be bound or processed at whatever time among transference. Toxins in plants might be found as uninhibitedly extractable stores, extractable conjugate bound to plant material, and unextractable developments combined in plant tissue.

Plants may by implication add to the dispersal of contaminants in vegetated soil. The usage of plants to concentrate toxic mixes from soils (phytoextraction) is being made as a framework for remediation of metal sullied soils [11,34,35]. Phytoremediation is a suitable remediation technique for petroleum contaminated soil based on evidence from greenhouse and field studies. The utilization of vegetation for remediation of polluted sites is attractive because it is cheap and passive. Plants can gather trace metals, in or on their issues as a result of their capacity to adjust to variable chemical properties of the environment: in this way, plants supplies through which trace elements from soils, waters and air, move to man and animals [36]. According to Srinivas et al. [37] air and soil are the main sources of trace metals to plants from which the root foliage take up the trace metals. Generally, metals are concentrated in the root and absorbed by plant through root uptake.

Total petroleum hydrocarbons analysis

TPH is well -defined as the quantifiable amount of petroleum -based hydrocarbon in an ecological media. It is, thus, reliant on analysis of the medium in which it is found. This section explains the TPH analysis results that were conducted in the study area on different six plants species compared to similar ones from different areas as references samples. In this section we would explain the total petroleum hydrocarbons analysis of the six plants samples collected from the study area in Wafra 14 km (*Conocarpus, Vitex Agnus-Castus, Salix, Rhamnus, Tamarix and Phoenix*).

Conocarpus: *Conocarpus* or Button mangrove is a widespread

species of terrestrial mangrove. On the coastal mainland, it grows as a small tree and has a variety of sizes on islands. *Conocarpus* has downy smooth leaves. It tolerates many things such as lowly drainage, compressed soil, air contamination and drought so that it is exceptionally adjustable as a decorative [38,39]. From total petroleum hydrocarbons analysis (TPH) of *Conocarpus* samples collected from the contaminated study area the soil had high rate of TPH value than the roots. On the other hand, the reference from same species collected from Sabah Al-Salem residential area depicted that the roots possessed the highest TPH value compared to soil which is about 9036 mg/kg as shown in Figure 7. The reference sample showed higher amount of TPH and that could be due to the age of plant and/or its nearness of a sewage area.

Vitex Agnus-Castus: *Vitex Agnus-Castus* is one of the common flowering plants and has many uses such as medical and herbal uses. It varies in size from small to tall tree. *Vitex Agnus-Castus* leaves are shaped liked a hand or palmate, and were once accepted to have sedative impacts. If *Vitex Agnus-Castus* soil kept too moist it would decline due to the root rot [38]. The results of Total Petroleum Hydrocarbons (TPH) of study area and reference samples collected from Sabah Al-Salem residential area showed that *Vitex Agnus-Castus* roots stored the highest amount of TPH 4565-2091 mg/kg compared to soil 600-570 mg/kg (Figure 8).

Salix: Around 400 species of deciduous trees and shrubs form the genus *Salix* were found in many regions [40]. *Salix* leaves are typically elongated and sometime may be round to oval. The roots grow from aerial parts of the plant and they are remarkable for their toughness and size. *Salix* as a plant have many environmental uses. It is used for biofiltration, wastewater treatment systems, phytoremediation, windbreak and soil building. From the roots TPH results, the value ranges from 1820-1477 mg/kg whereas soil TPH ranges from 960-810 mg/kg (Figure 9). As mentioned before, the reference sample showed higher amount of TPH and that might be due to the age of plant and/or its nearness of a sewage area.

Rhamnus: *Rhamnus* species are varying in size from shrubs to medium sized trees. The *Rhamnus* leaves are undivided and innately veined. The collected sample of *Rhamnus* roots from study area and the reference area are highly stored the contaminants 2770-18822 mg/kg and the low amount of contaminants stored in soil 250-270 mg/kg (Figure 10).

Tamarix: *Tamarix* is one of flowering plants that composed of many species. It grows as tree in the arid area. *Tamarix* can tolerate a lot of environmental conditions. It is capable to exploit
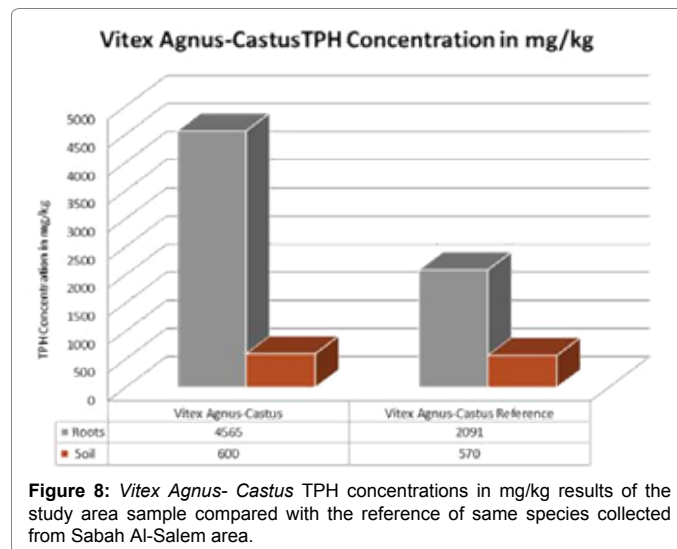

Figure 8: *Vitex Agnus- Castus* TPH concentrations in mg/kg results of the study area sample compared with the reference of same species collected from Sabah Al-Salem area.

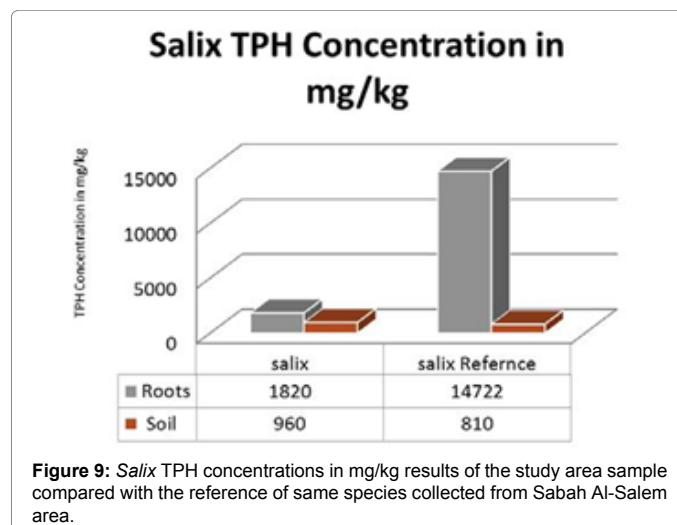|	Vitex Agnus-Castus	Vitex Agnus-Castus Reference
Roots	4565	2091
Soil	600	570

Figure 9: *Salix* TPH concentrations in mg/kg results of the study area sample compared with the reference of same species collected from Sabah Al-Salem area.

	salix	salix Refernce
Roots	1820	14722
Soil	960	810

Figure 7: *Conocarpus* TPH concentrations in mg/kg results of the study area sample compared with the reference of same species collected from Sabah Al-Salem area.

	Conocarpus	Conocarpus Refernce
Roots	1065	9036
Soil	1900	200

Figure 10: *Rhamnus* TPH concentrations in mg/kg results of the study area sample compared with the reference of same species collected from Sabah Al-Salem area.

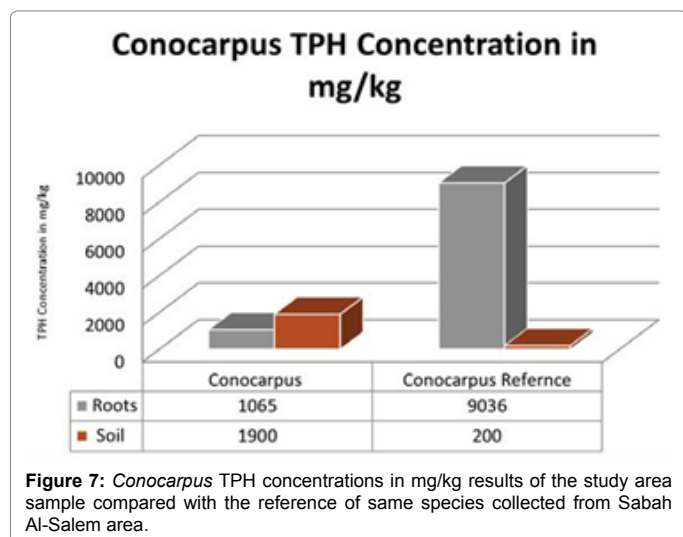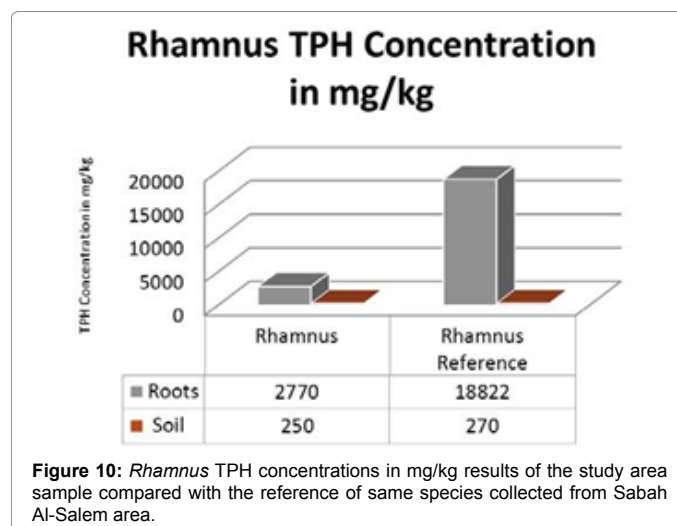|	Rhamnus	Rhamnus Reference
Roots	2770	18822
Soil	250	270

a wide spectrum of habitats because of the genotype in saltcedar [41]. Moreover, *Tamarix* TPH analysis reveals that the roots stored efficiently the soil contaminants in the study area and reference area at Sabah Al-Salem. Total Petroleum Hydrocarbons (TPH) values of *Tamarix* roots were the highest 7600-11263 mg/kg whereas the soil comprises 340-370 mg/kg. Soil sample collected from the contaminated study area is little lower than the TPH amount the one collected as a reference from the similar species in Sabah Al-Salem area (Figure 11).

Phoenix: *Phoenix* is a genus of palms family. *Phoenix* is widespread and commonly grown palm in the world. They are growing in many varied climates such as tropics, arid and even in cold climate. The TPH results of *Phoenix* samples collected from the study area showed that the plant's root and soil were storing the same amount of Total Petroleum Hydrocarbons (TPH) 350 mg/kg (Figure 12). However, the *Phoenix* reference sample collected from Sabah Al-Salem area were having higher amount of TPH 4573-210 mg/kg compared to the sample collected from Al-Wafra 14Km and that might be due to age of plant and/or its nearness of a sewage area.
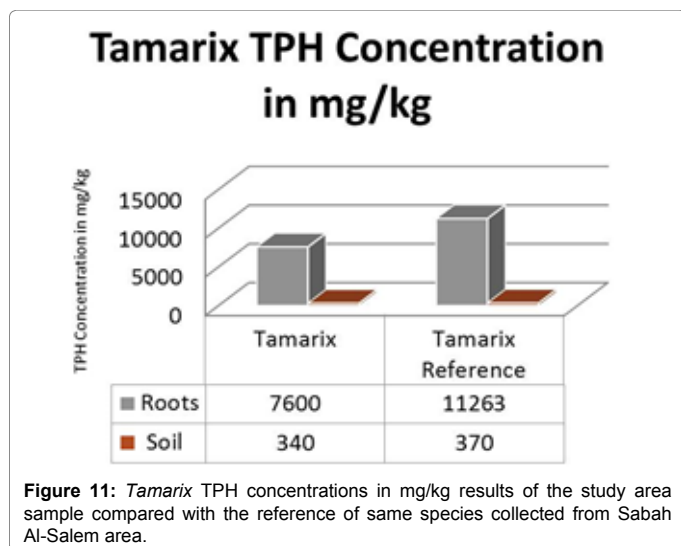

Figure 11: *Tamarix* TPH concentrations in mg/kg results of the study area sample compared with the reference of same species collected from Sabah Al-Salem area.
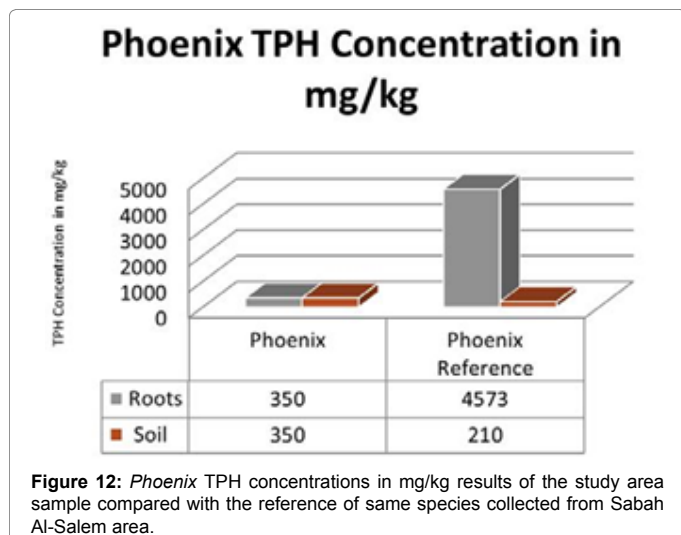

Figure 12: *Phoenix* TPH concentrations in mg/kg results of the study area sample compared with the reference of same species collected from Sabah Al-Salem area.

Total petroleum hydrocarbons (TPH) results comparison between plant's root and soil

Plant's root: TPH concentration results of the six plants species collected from the study area and their reference samples of the same species collected from Sabah Al-Salem area will be compared in this section. This comparison will show TPH concentration results for plant's root of all the plants species and the higher TPH value will be determined. TPH results of plant roots showed that the highest value goes for *Tamarix* 7600-11236 mg/kg in both reference and study area samples (Figures 13).

Soil: Soil TPH concentration results depicted that the introduced planted *Conocarpus* soil was having the highest amount of contaminants in the study area 1900 mg/kg (Figure 14).

Comparisons between TPH results in plant roots and soil from this study and TPH results in plant roots and soil from another study [32]

A study by Lotfinasabasl [32] for different plant species roots (mangrove) find out that the highest amount of TPH was 16300 and the TPH amount in the soil was 3760, while from this study the highest amount of TPH in plant root collected from the study area Al-Wafra 14 km was 7600 mg/kg and TPH amount in the soil was 1900 (Figure 15).

Trace metals analysis in plants and soil

In geochemistry, the term "trace metals" has been used for the chemical elements that occur in the Earth's crust. The amount of the trace metals in the Earth's crust is less than 0.1% which is about 1000 mg/kg [36]. Nowadays, the term "heavy metals" are used widely and that because of chemical hazards [42]. Plants are capable of absorbing a quantity of elements from soil, some are known to be lethal at low concentrations and some have no recognized biological purpose. Plants can accumulate high concentrations of heavy metals in their tissue. Serious danger to individual human health can result from the
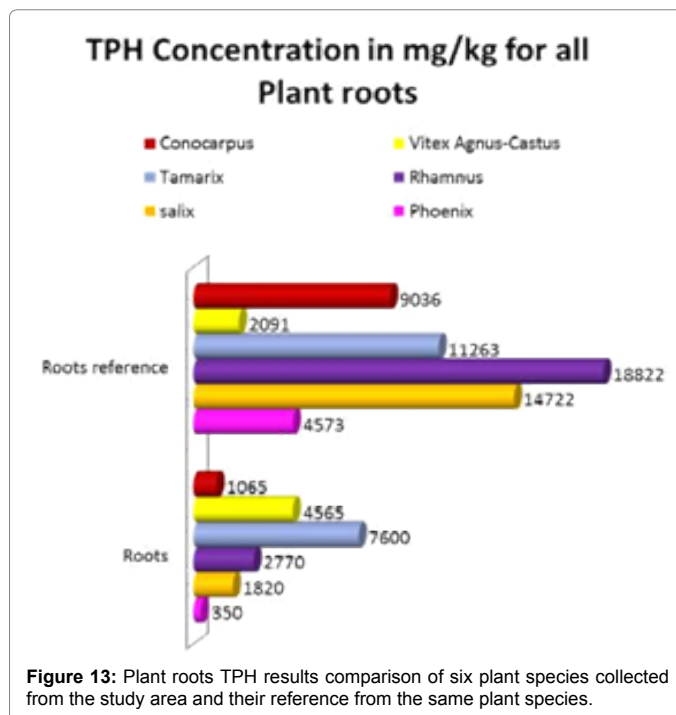

Figure 13: Plant roots TPH results comparison of six plant species collected from the study area and their reference from the same plant species.
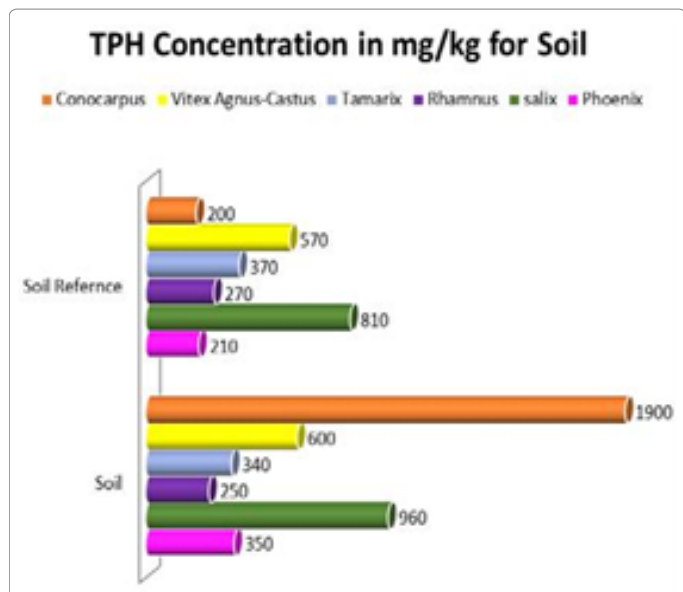
Figure 14: Soil TPH results comparison of six plant species collected from the study area and their reference from the same plant species.
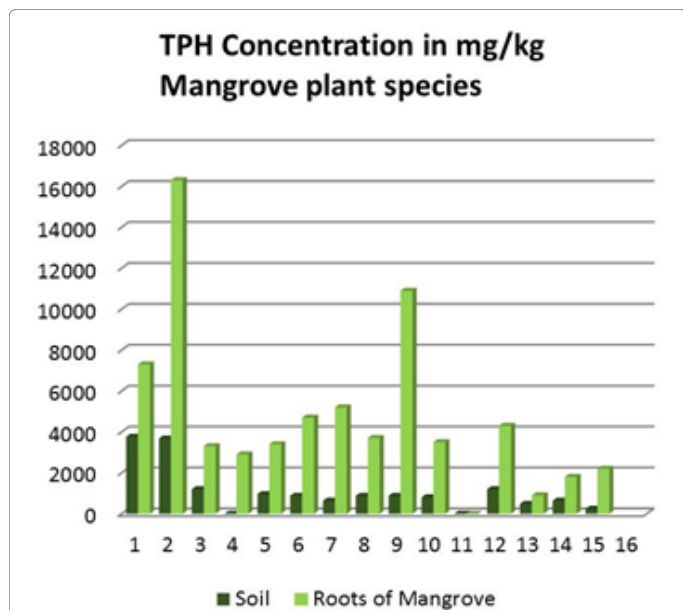

Figure 15: TPH concentration in plant roots and soil of mangrove plant species.

plants which are growing in a contaminate environment and collect trace elements at high concentrations. Roots depend on speciation of metal and soil types and kind of plant species to uptake of metal concentration root [37].

This section will explain the chemical analysis for the six plants root samples and soils were collected from the study area and the references area (*Conocarpus, Vitex Agnus-Castus, Salix, Rhamnus, Tamarix and Phoenix*).

Chromium (Cr): Chromium (Cr) is the 22nd most abundant element in Earth's crust. It has an average concentration of 100 ppm [6,7]. Chromium is a remarkable metal for being profoundly impervious to oxidation even at high temperatures. It has a high melting point. Chromium is discharged into the environment by industries. It

affects the soil fertility and the microbial activity of the soil. The high concentration of Chromium in the soil may cause the losses of plants. The permissible limit concentration for Cr in plants is 1.5 ppm (mg/g) [43]. Chromium (Cr) concentrations in study area plant's root samples are ranging from -0.7- 358.2 mg/kg and soil samples 13.6-31.8 mg/kg. The highest level of Cr recorded storage in roots goes for *Phoenix* 358.2 mg/kg whereas the lowest is *Rhamnus* at -0.7 mg/kg. The highest Cr recorded in the soil of the study area samples is *Conocarpus* 31.8 mg/kg and the lowest is *Tamarix* 13.6 mg/kg (Figure 16).

Manganese (Mn): Manganese is a naturally occurring metal that has silver color with no taste or smell. Manganese is usually found in the nature as a compound with elements such as iron, sulfur, oxygen, or chlorine [44]. It is hard and very brittle but easy to oxidize. Manganese has two forms in the environment. Inorganic manganese compounds are used in the manufacture of steel, batteries, ceramics, and nutritional supplements [44]. According to WHO [45] the Mn concentration in tend to range from 20-500 mg/kg in terrestrial plants (Figure 17).

As shown in Figure 18, Manganese (Mn) concentration in study area root samples ranges between 13-139.4 mg/kg while the reference root samples collected from Sabah Al-Salem ranges from 28.5-481.3 mg/kg. Manganese concentration of soil sample collected from study area ranges from 83.3-138.6 mg/kg whereas the reference soil sample is

	Root	Soil	Root Refernce	Soil Refernce
Conocarpus	13.3	31.8	166.2	13.6
Vitex Agnus-Castus	31.1	22.7	96.4	21
Salix	68.2	18.3	607.1	21
Rhamnus	-0.7	21.1	48.4	26.5
Tamarix	17.3	13.6	384.3	12.2
Phoenix	358.2	20.3	1591	22.6
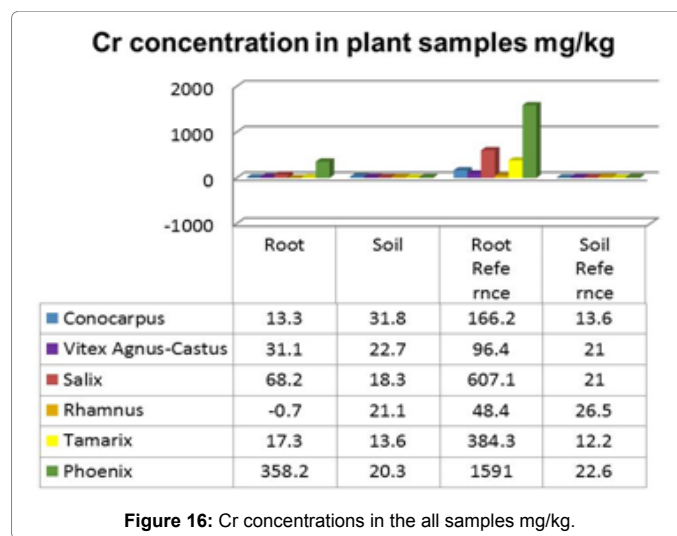
Figure 16: Cr concentrations in the all samples mg/kg.

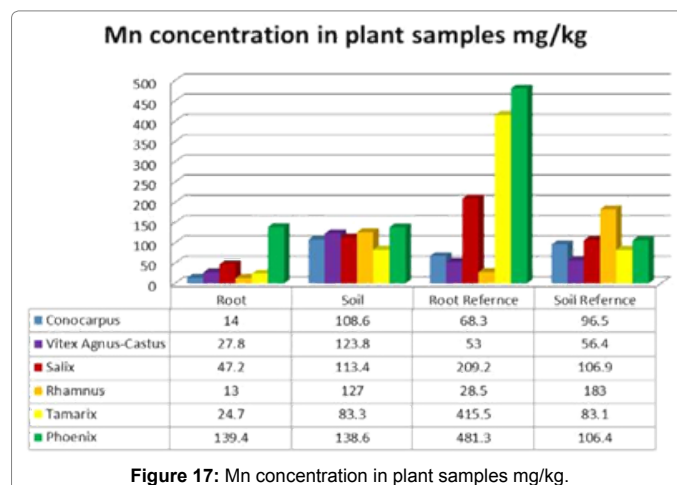|	Root	Soil	Root Refernce	Soil Refernce
Conocarpus	14	108.6	68.3	96.5
Vitex Agnus-Castus	27.8	123.8	53	56.4
Salix	47.2	113.4	209.2	106.9
Rhamnus	13	127	28.5	183
Tamarix	24.7	83.3	415.5	83.1
Phoenix	139.4	138.6	481.3	106.4

Figure 17: Mn concentration in plant samples mg/kg.

ranging from 56.4-106.9 mg/kg. The lowest value of Mn concentration recorded in root samples of the study area is *Rhamnus* 13 mg/kg and the highest level recorded is *Phoenix* 139.4 mg/kg. Soil samples collected from study area that showed that the lowest Mn concentration recorded is *Tamarix* 83.3 whereas the highest value is from *Phoenix* 138.6 mg/kg.

Cadmium (Cd): Cadmium is one of the naturally occurring components in the earth's crust and waters. Many elements like: zinc, lead, and copper ores are associated with Cadmium. It is present everywhere in our environment. Cadmium is a poison and is known to cause birth defects and cancer. The pure one is a silvery metal with a bluish tinge to its surface. Cadmium chloride and cadmium sulfate are the soluble form of Cadmium in water. Cadmium is used for the following: rechargeable nickel-cadmium batteries, rods in nuclear reactors, pigments, protecting critical components of aero planes and oil platforms, stabilizers for plastics, nonferrous alloys, photovoltaic devices, and other uses.

From Figure 19, there was no concentration of Cadmium found in study area for both samples plant roots and soil whereas the highest Cd concentration found stored in reference roots samples is of *Tamarix* 5.8 mg/kg. However, the reference soil samples indicate that two plants species *Rhamnus* 0.5 mg/kg and *Tamarix* 0.1 mg/kg had higher Cd concentration. The World Health Organization (WHO) recommended that the maximum level of Cd in medicinal plants is 10 mg/kg.
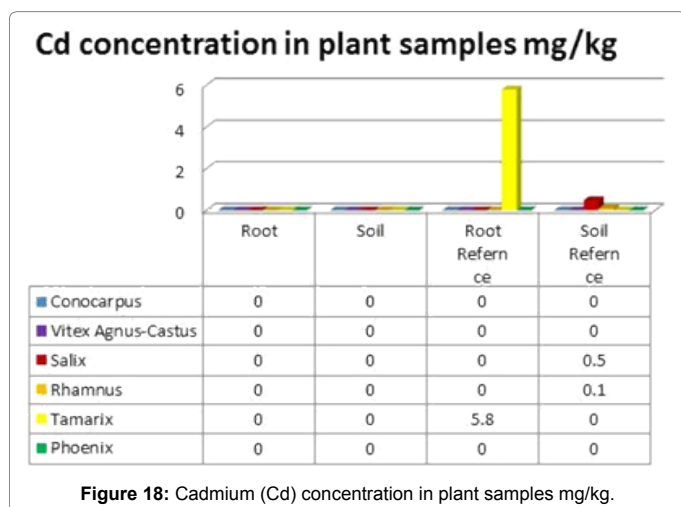

Cd concentration in plant samples mg/kg				
	Root	Soil	Root Reference	Soil Reference
Conocarpus	0	0	0	0
Vitex Agnus-Castus	0	0	0	0
Salix	0	0	0	0.5
Rhamnus	0	0	0	0.1
Tamarix	0	0	5.8	0
Phoenix	0	0	0	0

Figure 18: Cadmium (Cd) concentration in plant samples mg/kg.

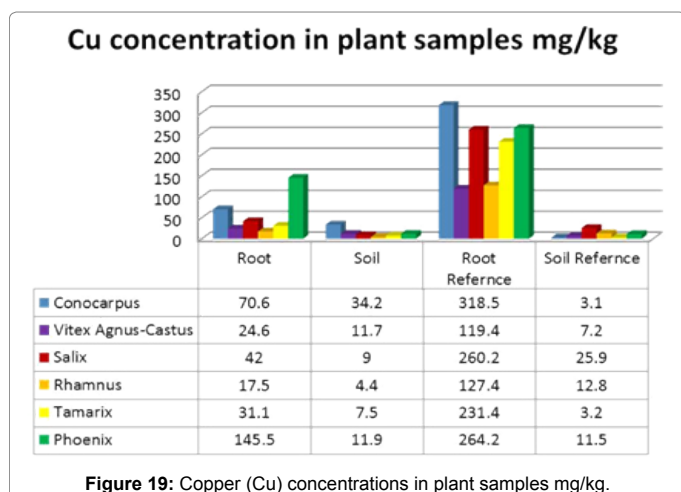| Cu concentration in plant samples mg/kg				
	Root	Soil	Root Reference	Soil Refernce
Conocarpus	70.6	34.2	318.5	3.1
Vitex Agnus-Castus	24.6	11.7	119.4	7.2
Salix	42	9	260.2	25.9
Rhamnus	17.5	4.4	127.4	12.8
Tamarix	31.1	7.5	231.4	3.2
Phoenix	145.5	11.9	264.2	11.5

Figure 19: Copper (Cu) concentrations in plant samples mg/kg.

Copper (Cu): Copper is a reddish metal that occurs naturally in rock, water, soil, sediment and at low levels in the air with concentration ranging between 8.85-13.44 mg/kg [46]. Copper is found as a pure metal in nature. Pure copper is usually too soft and ductile. Mixing copper with other metals make it strengthened. It is used as a conductor of heat and electricity, as a building material, and as a constituent of various metal alloys. Bronze and brass are the two most familiar alloys of copper. In the earth's crust the average concentration of Cu is about 50 parts copper per million parts soil (ppm) or 50 grams of copper per 1,000,000 grams of soil. Copper is found in all plants and animals. It is a crucial component for all perceived living creatures including people and different creatures at low ranks of consumption. At higher amounts, Cu can be harmful [47]. The concentration of Copper (Cu) in root plant samples of the study area was ranging from 17.5-145.5 mg/kg whereas soil samples of study area ranges from 4.4-34.2 mg/kg (Figure 20). According to Dutch Standards, the soil concentration of Cu that is below the permissible limit is 36 mg/kg. The highest level of Cu concentration in plant roots samples collected from the study area is of *Phoenix* 145.5 mg/kg whereas the lowest one goes to *Rhamnus* 17.5 mg/kg. For the soil sample taken from the study area, the highest level of Cu concentration is of *Conocarpus* 34.2 mg/kg whereas the lowest one goes to *Tamarix* 17.5 mg/kg. This present study indicate that the best plant stored Cu in Its root is *Phoenix*. We can notice the high percentage of Cu metal in reference area is more than the study area and that because the reference sample might be affected by its nearness from sewage pipe.

Iron (Fe): Iron is the most common element on Earth surface and is the fourth most common element in the Earth's crust. It is forming much of Earth's outer and inner core. About 4.6% of igneous rocks and 4.4% of sedimentary rocks contain iron as a regularly occurring metal [48]. Iron involves numerous biological processes. In plants, Iron plays a role in the creation of chlorophyll and is an essential part of hemoglobin in the blood. On the other hand, iron helps keep plants and animals alive. Iron classic concentrations in soils are range between 0.2%-55% [49] and that can differ in localized areas because of soil diversity and availability of other sources.

The present study finds that Iron (Fe) concentration in plant root samples collected from study area ranged between 3241-142.8 mg/kg. The lowest iron concentration is of plant root sample is of *Tamarix* 142.8 mg/kg and the highest one is of *Phoenix* 3241 mg/kg. Also, it indicates

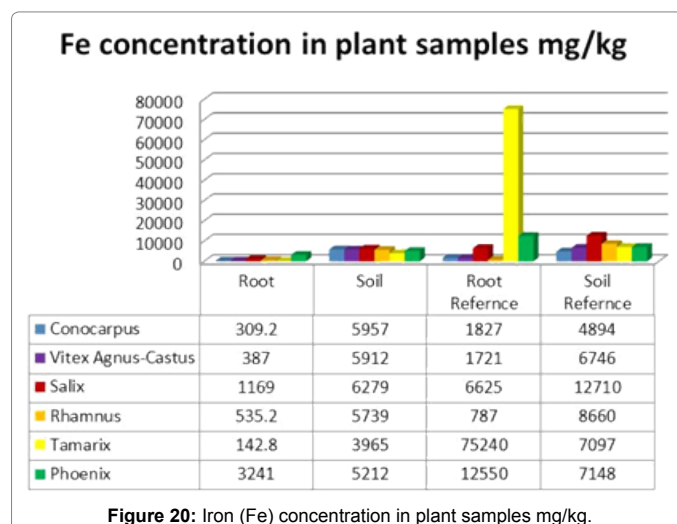| Fe concentration in plant samples mg/kg				
	Root	Soil	Root Refernce	Soil Refernce
Conocarpus	309.2	5957	1827	4894
Vitex Agnus-Castus	387	5912	1721	6746
Salix	1169	6279	6625	12710
Rhamnus	535.2	5739	787	8660
Tamarix	142.8	3965	75240	7097
Phoenix	3241	5212	12550	7148

Figure 20: Iron (Fe) concentration in plant samples mg/kg.

that Iron (Fe) concentration in soil samples collected from study area ranged between 6279-3965 mg/kg. The lowest Fe concentration of soil goes for *Tamarix* 3965 mg/kg yet; the highest one is *Salix* 6279 mg/kg (Figure 21). The concentration of Fe is between 20000-250000 mg/kg in dry plant tissue.

Nickel (Ni): Nickel is a shiny white transition metal. Pure nickel is hard and ductile. According to McGrath [50] nickel appears naturally in soils due to parent rock weathering. Nickel has many properties that make it extremely attractive to be alloyed with different metals like: iron, copper, chromium and zinc. These combinations are used for making metal coins and in industry. Most nickel is utilized to make stainless steel. Nickel is considered as corrosion resistant because of its slow rate of oxidation. Nickel concentration in soil is ranging from 0.7 to 259 mg/kg [45].

In our study area the Ni in roots samples is ranging from 3.3-136.6 mg/kg whereas the soil sample is ranging from 12.5-19.3 mg/kg. The root samples of *Phoenix* contributed the best in storing Nickel in both reference and study area as it is shown in Figure 21.

Vanadium (V): Vanadium is a rare, soft, ductile gray-white element found combined in certain minerals and is widely distributed in the earth's crust. Vanadium occurs naturally and has many forms. Pure vanadium is a bright white, soft and ductile metal. Unintentionally discharged of coal bi-products may cause Vanadium Pollution of soil [36]. Vanadium is abundant in variable amounts in soils and it is taken up by plants at different levels. There are different ideas about the mean content of Vanadium in plants. Smith [51] mentioned that Vanadium concentration is lower than 0.5 mg/kg while others have referred concentrations ranges from 0.5-22 mg/kg. However, Cannon [52] mentioned that Vanadium concentration is from 0.8- 2.7 mg/kg.

As shown in Figure 22, the root samples analysis of the study area show that the range of Vanadium is between 0.6-12 mg/kg whereas reference samples range between 4.2-19.2 mg/kg. The least level recorded is of *Vitex Angus-Castus* 0.6 mg/kg in study area root samples yet; the highest one is of *Conocarpus* 12 mg/kg. Surprisingly, the soil samples for both study and reference area showed higher Vanadium amount [53-56]. The range of soil samples in the study area is 17.7-27 mg/kg whereas reference area samples showed a range of Vanadium concentration between 13.9-21.6 mg/kg. Moreover, the soil samples

in study area had the least level in *Tamarix* 17.7 mg/kg whereas the highest V recorded is of *Vitex Angus-Castus* 27 mg/kg.

Zinc (Zn): Zinc is a bluish-white, glittery, diamagnetic metal. It is one of the most common elements in the Earth's crust. It can be found in different places such as in air, water, and soil in addition to food. Metallic zinc has many uses in industry. It is most commonly used as an anti-corrosion agent. Metallic zinc is mixed with other metals such as bronze to form alloys. It is also used to produce dry cell batteries [57,58]. Zinc sulfide and zinc oxide is usually utilized in manufacturing such as white paints, ceramics, and other products. According to Emsley 2001, soil contains 5-770 mg/kg of zinc with an average of 64 mg/kg whereas seawater has only 30 mg/kg zinc. The permissible limit of Zinc in human consumption is 10-50 [37].

This present study shows that the least zinc concentration in root samples is in *Salix* at 16.2 mg/kg whereas the highest one is in *Conocarpus* plant species at 49.5 mg/kg. The concentration of Zinc in soil samples collected from study area ranged between 27.5-163.1 mg/kg. In another hand, the least level of Zn concentration stored in soil is of *Rhamnus* 27.5 mg/kg and the highest one is of *Conocarpus* 163.1 mg/kg (Figure 23).

Lead (Pb): Lead is a bluish to gray or bluish-gray metal that occurs naturally in the Earth's crust. It is a heavy metal with high density. Lead has the highest atomic number of any non-radioactive element. Yet, it is easily discovered naturally as a metal and it is easily mined and refined so it is not considered to be a rare element. It is corrosion
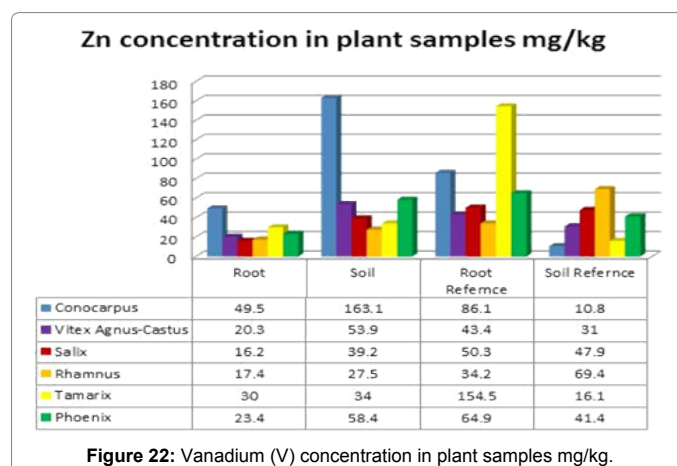

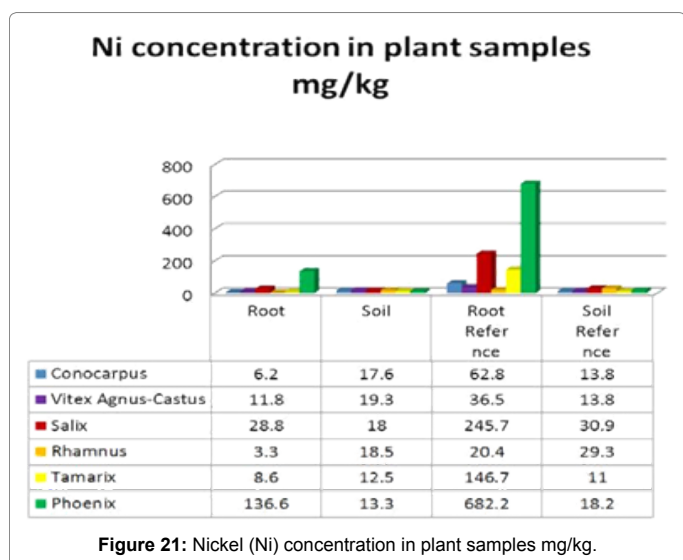**Figure 22:** Vanadium (V) concentration in plant samples mg/kg.

	Root	Soil	Root Refernce	Soil Refernce
Conocarpus	49.5	163.1	86.1	10.8
Vitex Angus-Castus	20.3	53.9	43.4	31
Salix	16.2	39.2	50.3	47.9
Rhamnus	17.4	27.5	34.2	69.4
Tamarix	30	34	154.5	16.1
Phoenix	23.4	58.4	64.9	41.4

Figure 21: Nickel (Ni) concentration in plant samples mg/kg.

	Root	Soil	Root Refernce	Soil Refernce
Conocarpus	6.2	17.6	62.8	13.8
Vitex Agnus-Castus	11.8	19.3	36.5	13.8
Salix	28.8	18	245.7	30.9
Rhamnus	3.3	18.5	20.4	29.3
Tamarix	8.6	12.5	146.7	11
Phoenix	136.6	13.3	682.2	18.2
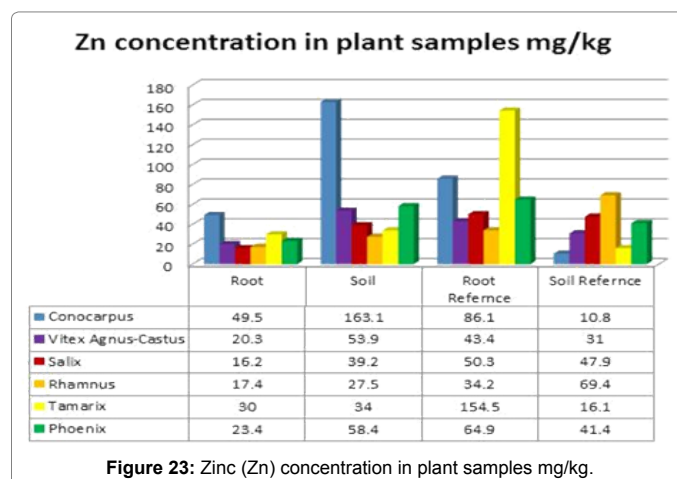
Figure 23: Zinc (Zn) concentration in plant samples mg/kg.

	Root	Soil	Root Refernce	Soil Refernce
Conocarpus	49.5	163.1	86.1	10.8
Vitex Angus-Castus	20.3	53.9	43.4	31
Salix	16.2	39.2	50.3	47.9
Rhamnus	17.4	27.5	34.2	69.4
Tamarix	30	34	154.5	16.1
Phoenix	23.4	58.4	64.9	41.4

resistant material used in building construction, lead-acid batteries, bullets and shot, weights and as a radiation shield. In another hand, Lead is poisonous to animals and humans.

Pb concentration in plant samples is below detection limit except in root samples of with 1 mg/kg for *Phoenix* species. The study area sample has 7.3 mg/kg in the *Conocarpus* soil sample [59-61]. The increase of reference sample Pb concentration might be due to its nearness to a sewage pipe. For all samples, *Phoenix* species has the highest level of Pb concentration (Figure 24).

Percentage of total petroleum hydrocarbons (TPH) and trace metals absorbed in plants and soil

TPH: This section will show the percentage of TPH absorbed by the roots and soil of the six plants species collected from the study area and the reference samples collected from Sabah Al -Salem area. The study showed that the highest TPH for both reference and study area samples is storing in the roots 92% (Figure 25).

Trace metals: Figure 26, showed that the highest trace metal that was absorbed is iron (94%) for both reference and study area samples. Chromium is the second absorbed trace metal (2%) whereas zinc, manganese, nickel and copper all absorbed at the same percentage (1%).
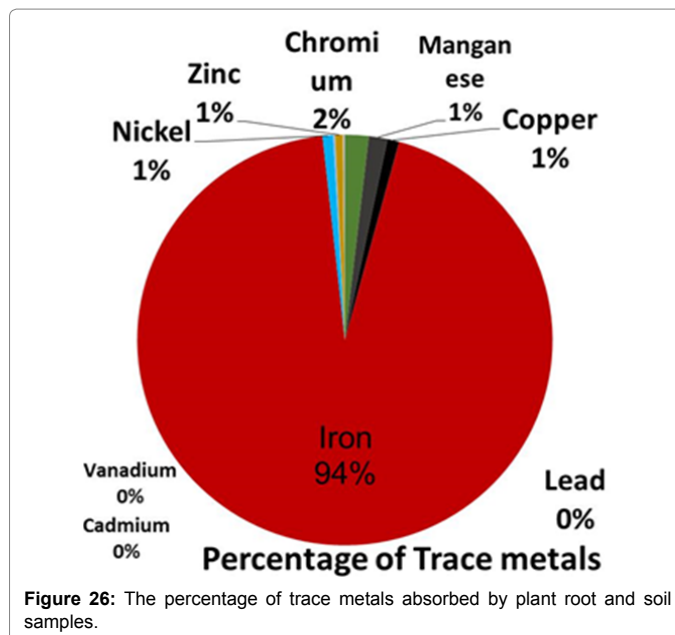

Figure 24: Lead (Pb) concentration in plant samples mg/kg.

	Root	Soil	Root Refernce	Soil Refernce
■ Conocarpus	0	7.3	0.7	1.3
■ Vitex Agnus-Castus	0.1	2.9	0.5	3.3
■ Salix	0.2	2.7	24.3	22.9
■ Rhamnus	0	2	1.4	18.3
■ Tamarix	0	1.8	6.3	3.4
■ Phoenix	1	3.4	72.2	106.9

Figure 25: The percentage of TPH absorbed by plant root and soil samples.

Figure 26: The percentage of trace metals absorbed by plant root and soil samples.

Conclusion and Recommendations

From the study area in Al-Wafra (14Km south of Kuwait), the Total Petroleum Hydrocarbons (TPH) and Trace Metals analysis for the six types of plant tree species collected (*Conocarpus, Tamarix, Phoenix, Rhamnus, Vitex Agnus-Castus and Salix*) shows that the concentration of Total Petroleum of Hydrocarbons is very high. Moreover, the concentration of conducted trace metals analysis in plant and soil depicted sometimes-high variation than the permissible limits of many trace metal elements [62-64].

Total Petroleum Hydrocarbons analysis (TPH) in this research study shows that the best part to absorbed and store the contaminants is the roots; the same conclusion was reached by Srinivas et al. [37] and Lotfinasabasl [32]. From the study area *Tamarix* species roots got the highest storage of TPH value of 7600 mg/kg while the lowest is *Phoenix* roots with TPH value of 350 mg/kg. However, TPH analysis of soil samples collected from the study area showed that *Conocarpus* species is the best plant for absorbing the contaminants from soil and has the highest value of TPH 1900 mg/kg.

The results and interpretation of trace metals (Cr, Mn, Cd, Cu, Fe, Ni, V, Zn and Pb) analysis in this research study show that the highest value of Trace metals in the study area was absorbed by *Phoenix* (4053.7 mg/kg) and the highest trace metal absorbed by all plants is iron.

The following recommendations should be followed after this research study:

Accordingly, roots show the most important part of plants in the process of phytoremediation.

1. Further study of the fruits of *Phoenix* should be conducted to detect accumulate of TPH and Trace Metals.

2. Research on rhizoremediation should be encouraged and understood to reduce soil contamination.
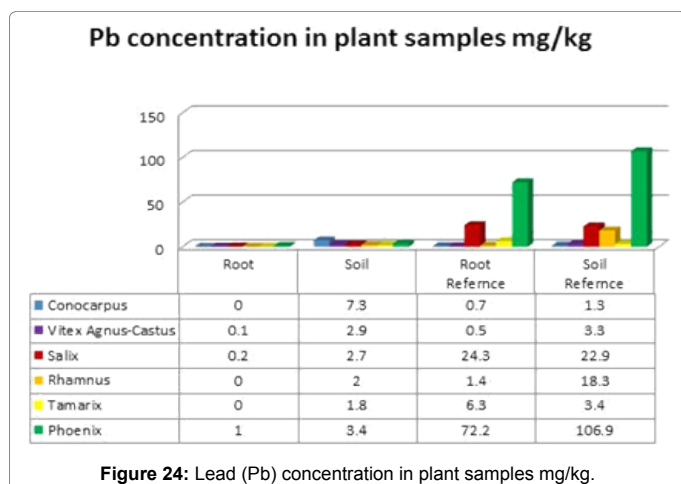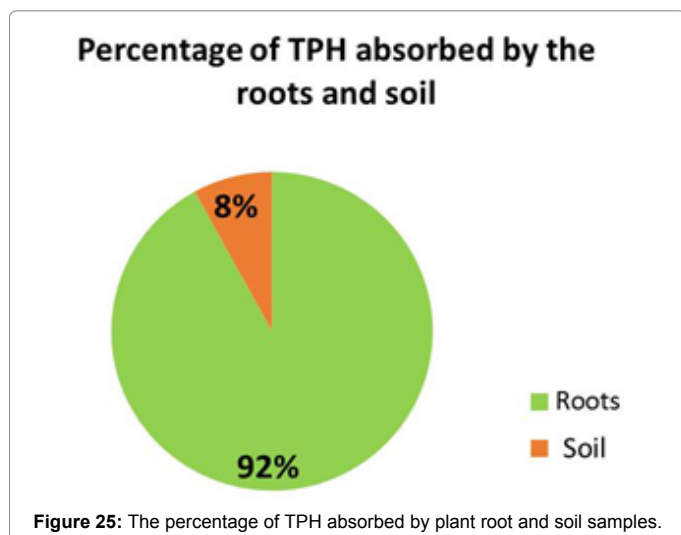
to Florida International University, Department of Earth and Environment, United States of America for their technical guidance.

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