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ANALYTICAL CHEMISTRY AND PHYTOEXTRACTION OF HEXAVALENT CHROMIUM WITH PORTULACA OLERACEA

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A thesis submitted to the University of Huddersfield in partial fulfilment of the requirements for the degree of Doctor of Philosophy

The University of Huddersfield in collaboration with the American University of Sharjah

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Dedication

To my wife:

Howayda

who gave me her full support and time whilst I was completing this work

Acknowledgment

I would like to express my thanks and recognition to all the people who gave advice and encouragement to complete this work. From those I especially note the contributions of Dr Hassan Tayim, Dr Roger Jewsbury, Dr Paul Humphreys and Dr Mohammad Al-Sayah who were my supervisors. I would like to thank also Mr. Naser Abdo from the American University of Sharjah who was a facilitator to my experiments and analysis and Mr Habib-Alrahman from Ajman Municipality Nursery who granted me the access, the plants and the agricultural tools to carry out the field work.

Abstract

Phytoextraction in the UAE desert soil (sandy, calcareous, less than 0.5% humus, and pH 7.9) has been studied. Twelve suspected polluted sites were investigated for contamination with eight heavy metals and sixteen local plants from the UAE desert were evaluated for their ability to accumulate heavy metals. The soil of Ajman industrial zone demonstrated high amounts of total chromium (1800 mg/kg) and of hexavalent chromium (97 mg/kg) which is a significant environmental threat. Portulaca oleracea (Purslane) has been shown to be the best candidate for Cr(VI) accumulation.

Total chromium concentration exceeded 4600 mg/kg in roots and 1400 mg/kg in stems confirming the role of P. oleracea as a Cr(VI) accumulator. More than 95% of the accumulated Cr(VI) was reduced to the less toxic Cr(III) within the plant.

The uptake of Cr(VI) by this plant has been investigated. The uptake of Cr(VI) increased as its concentration in soil increased between 50 and 400 mg/kg. The highest Cr(VI) uptake was observed at the high pH and low organic matter content of soil confirming the phytoextraction efficiency of P. oleracea in soils found in the UAE. The uptake of Cr(VI) increased in the presence of sulfate anion (suggesting that chromate uses the same carriers of sulfate in root cells) while nitrate and phosphate retarded the uptake. Potassium and ammonium ions, but not sodium ions, enhanced the uptake of Cr(VI) confirming the effect of accompanying cations. EDTA enhanced the translocation factor of chromium from roots to shoots in plants irrigated with either Cr(III) or Cr(VI). HPLC-MS analysis showed that ascorbic acid is the main antioxidant that reduced Cr(VI) to Cr(III) which is then mostly translocated to shoots after chelation with organic acids such as oxalate since glutathione and phytochelatins were not observed at significant levels in the tissues of plants exposed to Cr(VI).

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List of abbreviations

ANOVA	Analysis of Variance
ANRCP	Amarillo National Resource Center for Plutonium
ASA	Ascorbic Acid
ATP	Adenosine Triphosphate
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BDL	Below Detectable Limit
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
DASA	Dehydroascorbic Acid
DC	Dadnah Coast
DEFRA	Department for Environment, Food and Rural Affairs
EDTA	Ethylenediaminetetra-acetic acid
EPA	Environmental Protection Agency
ESI	Electrospray Ionisation
FEA	Federal Environmental Agency
GSH	Glutathione
GSSG	Oxidised Glutathione
HPLC	High Performance Liquid Chromatography
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
MT	Metallothionein
PC	Phytochelatins
TCLP	Toxicity Characteristic Leaching Procedure
TF	Translocation Factor
TI	Tolerance Index
TOF-MS	Time of Flight Mass Spectrometer
UAE	United Arab Emirates
UFLC	Ultra fast liquid chromatography
USGS	United States Geological Survey
VAM	Vascular Arbuscular Mycorrhizas
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Preamble

As human civilization progresses, there is always a price to be paid. When one generation pays the price for health and safety, the hope is that the next will not. This study focuses on the remediation of soil polluted by the toxic and carcinogenic heavy metal chromium (VI). The origin of the problem is the existence of a metallic extrusion factory which mainly uses chromic acid and discharges waste to an open site nearby, a problem aggravated by this polluted site being located within an urban area. This site was discovered by surveying twelve suspected polluted sites. In addition, another survey was carried out on sixteen plants to identify suitable candidate for phytoremediation. It is in brief the attempt to find an accumulator plant which will absorb, translocate, and then accumulate the toxic pollutant in its aerial tissue. The factors which may affect the uptake and the chemistry of the chromium within the plant were also studied.

1.2 Background

Soil pollution or contamination is the mixing of hazardous substances with the natural soil. These pollutants maybe attached to the particles of soil or trapped within them. The sources of soil pollutants, in general, are spilling or burying liquid or solid

industrial wastes such as petroleum hydrocarbons, pesticides, chemical solvents, heavy metals and radionuclides in the soil. Soil pollution can harm plants, animals and humans. Plants may uptake these pollutants which drastically affect the growth of these plants. Pollutants may reach animals and humans through the food chain. Some pollutants can be absorbed through skin and others can be inhaled through airborne dust or small particles of soil.

Sometimes soil contamination can occur naturally because of the existence of natural ores of some heavy metals such as lead, cadmium, mercury and chromium or radionuclides. In this case, mining activities can spread these pollutants or expose them to some factors such as acid rain or water streams that may leach them to the soil.

Historically, the problem of soil contamination has been aggravated by the rupture or damage of underground storage tanks resulting in leaching of pollutants. Improper land filling also resulted in severe pollution of the subsurface soil. In the USA, a federal law for cleaning the contaminated sites, Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), was issued in 1980 [1]. This action increased the awareness of soil pollution, put many restrictions to prevent this offence, and stimulated the efforts to discover and clean up thousands of sites of underground storage of wastes. More than 200,000 polluted sites were identified for remediation.

In the UK, the Department for Environment, Food and Rural Affairs (DEFRA) published (in 1990) the soil guidelines values which determined the maximal accepted

limits for each substance in soil. These regulations were modified and included details for each soil pollutant and how to implement these guidelines such as: improvements to contaminated land guidance and guidance on the legal definition of contaminated land which were issued by DEFRA in 2008. 1n 2009, a scientific report named human health toxicological assessment of contaminants in soil was issued [2].

In UAE, several governmental environmental agencies such as the Federal Environmental Agency (FEA 1993-2009), Ministry of Water and Environment which undertook the competences of FEA since it was closed in 2009, Environment Agency in Abu-Dhabi-1996 and Dubai Municipality were established. In spite of that, the implementation of environmental regulations is still at an early stage especially in the northern Emirates. In the last four decades, the oil and gas industries have prospered in the UAE, and in the absence of implementation of environmental regulations, large quantities of chemical waste have been dumped on and under the ground. Mining and metal industries are expanding in all of the Emirates and many factories and workshops are working without effective waste control. Some are transferring their waste to landfills and others are accumulating their waste in sites beside their factories. In relatively poor emirates such as Sharjah and Ajman, there is no real separation between inhabited areas and the industrial zones. The landfill sites are very close to populated areas and sometimes there is (offensive) overlapping between them. Suspected contaminated sites also exist in the east coast of the UAE. This area is rich in black sand which contains chromite, the major ore of chromium (a mixture of Cr(III) and Fe(II) oxides).

1.2.1 Remediation of contaminated soils

Contaminated soils can be remediated using physical, chemical, or biological techniques. Soil vacuum and soil washing are physical techniques and oxidation, neutralisation, and soil flushing are chemical ones [3]. Both physical and chemical techniques will alter the composition of soil and may stop all of the beneficial biological activities such as the role of bacteria and fungi in soil. Biological techniques of remediation essentially depend on the natural organisms of soil such as bacteria or yeast for the biodegradation of organic pollutants [4].

Remediation techniques may also be classified as ex-situ and in-situ techniques:

- **Ex-situ techniques** involve remediating polluted soil away from the contaminated sites after the excavation and translocation of the polluted soil to prepared sites. Sometimes pre-treatment chemical or biological processes should be applied before the final treatment. Therefore ex-situ techniques are costly in general.
- **In-situ techniques** are relatively cheap since they take place in the same contaminated site. The aim of in-situ remediation is to decrease the concentration of the pollutant to accepted levels by adding liquids or gases to the soil either to enhance the conditions of organisms work or to react directly with pollutants. Oxygen is a good example for both actions [2]. One of the relatively new in-situ biotechniques is

phytoremediation since living cells of plants are the bio-reactors in which the remediation process takes place.

1.2.2 Phytoremediation

Phytoremediation is defined as the use of plants to remediate [5] (contain, remove, or degrade) [6] soil or water pollutants. It is a promising technique for reducing the organic and inorganic pollutants to the accepted levels. Phytoremediation techniques can be thought of as involving three generations or categories. The first is the discovery of accumulators or the plants that can tolerate, absorb, and accumulate a specific pollutant in their tissues. The second is enhancement of phytoaccumulation using chemical reagents such as chelating agents or controlling conditions of soil such as pH, available ions or organic content of soil. The third is enhancement of phytoaccumulation using genetic modification of the accumulator plants, whereby the characteristics of the plant can be modified to increase its potential to tolerate more quantities of pollutants [7]. This type of research is still in its infancy and most of the research work in the field of phytoremediation is in the first and second categories for reasons related to the difficulty of developing equipment and capabilities that are required for third generation.

Phytoremediation process takes place in the rhizosphere [8], the area of soil which contains roots and their activities (tillage area), or inside the plant tissues. Accordingly different phytoremediation techniques have appeared in the literature. The

nature and characteristics of pollutant also have a large effect on the phytoremediation technique since the response of the plant towards the pollutants depends on the nature of the pollutant. The response to organic pollutants differs from the inorganic and the uptake of anions differs from cations or metals uptake.

Phytosequestration or phytostabilization is one of the external mechanisms of phytoremediation. It is the prevention of mobility of the inorganic pollutants [9] such as heavy metals. This can be achieved by the precipitation or immobilization of the pollutants in the rhizosphere. Different causes are suggested for phytosequestration such as Vascular Arbuscular Mycorrhizas (VAM) fungi which reduce the bioavailability of heavy metals by fungi-metal binding. The exudates of the plants like organic anions and hydroxides to the soil alter the pH of soil; therefore the mobility of heavy metal will be affected. Rhizo-degradation is another external mechanism of phytoremediation. It is relevant to the degradation of organic pollutants in rhizosphere area [10, 11] by enhancing the oxygenation of the subsurface soil to initiate the role of the microorganisms in the aerobic biodegradation of these organic pollutants. The roots of the plant play an important role in the oxygenation process. This can take place either physically by aeration of the soil during the growth of roots or chemically by direct secretion of oxygen [11].

Phytodegradation is a mechanism of phytoremediation in which organic pollutants are biodegraded [12] using the specific exudates from roots. It is still a matter of research to determine whether degradation takes place inside or outside the

plant tissue but it is accepted that the fragments of the organic pollutants are being translocated from the roots to the shoots of the plant.

Phytoextraction and rhizofiltration are internal techniques of remediating inorganic pollutants such as toxic metals, metalloids and radionuclides [11]. The first is relevant to the use of plants in the removal of toxic metals from contaminated soils and the second belongs to the removal of heavy metals from wastewater.

1.2.3 Toxic metals and heavy metals

Toxic metals are the group of metals which have a poisonous effect on human health. Many elements can be listed under this category such as beryllium, cadmium, antimony, mercury, lead and bismuth. Heavy metals, defined as metals with a density of more than 5 g/cm^3 [13], represent the majority of the toxic metals. Sixty-five of the known elements fall into this category including iron, the fourth most abundant element in the earth's crust.

Heavy metals can also be defined as a group of metals and metalloids which are associated with pollution and toxicity [14]. However some of these elements are essential for living organisms at low concentrations [15]. Heavy metals can reach the environmental systems of soils and waters from the industrial and mining effluents and from natural resources if the area contains some ores of the heavy metals which may leach to the soil and water through water streams or acid rains.

Not only the total element concentration but also more information about the oxidation state and binding form of the element (speciation) is required because the speciation also gives information on the mobility and therefore availability of the metal to living organisms, and their potential toxicity [16]. Speciation of heavy metals in contaminated soil or wastewater is also necessary for choosing the right technique of its removal or remediation.

1.2.4 Phytoextraction

Phytoextraction is defined as the potential of plant to uptake heavy metal pollutants from soil by the roots and to translocate and accumulate them in the above-ground parts of plant [5, 9] such as shoots, leaves and stems. Normal plants have the ability to exclude and reduce undesired heavy metals up to 100 mg/kg but to uptake the nutrient elements up to 3% of its dry weight. The plant can be classified as a hyperaccumulator if it has high potential to accumulate heavy metals relative to the dry weight of plant, for example more than 0.1% (1000 mg/kg) for chromium, cobalt, copper, and nickel and more than 1.0% of both of zinc and manganese [17, 18**]**. To fulfil the removal process of heavy metals using hyperaccumulators these plants should be harvested after accomplishing the treatment process and safely disposed of by incineration [19]. As a technique of heavy metals removal and compared with other chemical and physical techniques, phytoextraction has the following merits [17, 6]:

- it is easy and cheap compared with other ex-situ techniques which require excavation, transportation, using chemicals and washing, and in some cases returning the remediated soil to the original site,
- phytoextraction technique is very efficient and represents rational solution when it reduces pollutant to below tolerated concentrations,
- the harvested biomass can be used either as bio-energy resource, or for plant fibres production,
- this technique gives a pleasant view for the treated sites since vegetation and remediation are inseparable,
- using phytoextraction, some plant nutrients such as selenium can be translocated from a highly contaminated site to another poor one, and
- using this technique, some precious metals could be extracted from soils containing small non-commercial quantities of them. For example gold was extracted from soil using Brassica juncea (Indian mustard) and Chilopsis linearis (desert willow) [20, 21].

However there are some restrictions for the use of phytoextraction. They include the following:

- it is a slow (long term) technique compared with other physio-chemical techniques [6],
- pollutant concentration should be in the range of plant tolerance since plants cannot grow in severely contaminated sites, therefore, phytoextraction is restricted to sites of moderate contamination[22], and

- in soils of high carbonate content, which have basic pH values, heavy metal cations exist as insoluble forms of metal hydroxide which reduces their uptake by plants.

1.2.5 Ideal plants for phytoextraction

From 250,000 higher plants, only a limited number has been tested for phytoremediation or phytoextraction and among these tested plants only a small number has been founded to be hyperaccumulators. In general, a promising accumulator for a specific heavy metal is not necessarily efficient for another. One of the most efficient hyperaccumulators is Sebertia accuminata. This plant grows on metalliferous soil and can accumulate 260 g of zinc in one kilogram of its dry weight [23]. The Brassica family which includes broccoli and Indian mustard grows very fast producing considerable biomass. It has the ability to accumulate many heavy metals such as Cd, Cr (VI), Cu, Pb, Ni, and Zn more efficiently than many other plants [24, 25]. General characteristics of ideal accumulators can be summarized as follows [26]:

- the ability to uptake heavy metal in roots then translocate it to shoots and accumulate high quantities of it without being severely affected,
- high rate of growth and production of big biomass and ease of harvesting,
- good ability of adapting especially outside its area of collection including resistance to disease and pests [27], and
- production of a profuse and deep root system.

1.2.6 Applicability of phytoremediation in UAE

The severe weather conditions in the Arabian Peninsula may suggest the plants of this environment as strong candidates for phytoextraction. These plants in general can tolerate hard conditions of hot climate, high salinity, high pH, and have exceptional potential for absorbing water from soil. Some plants which are available in the desert of UAE like Prosopis species were investigated in El-Paso, Texas which has similar climate conditions, and were found to yield promising results in accumulating lead and chromium [28, 29]. These results, beside the high tolerance of hard conditions, may form good motivation to investigate desert plants of UAE as accumulators for heavy metals in the soil of UAE.

1.3 Chromium in soil; chemistry and phytoaccumulation

1.3.1 Climatic and geochemical conditions of the soil of UAE

United Arab Emirates (UAE) is one of the fastest developing countries in the Middle East. The total area of the UAE is about 82,880 sq. km [30]. Oil export is the backbone of its economy but industrial activities have increased significantly over the last three decades. In 2008 and according to the Ministry of Finance and Industry the investment in the industrial field was about 77 billion dirham (£14 billion) [31].

Ajman is one of the seven emirates comprising the UAE. It is the smallest Emirate in area - about 260 sq. km - and surrounded to its north, south, and east by the Emirate of Sharjah. Approximately 95% of the population of the emirate of Ajman

reside in the city of Ajman. The population was only 361,000 in 2008 [32] and has grown considerably due to an influx of people from the neighbouring emirates of Dubai, Sharjah, and other countries.

Ajman has an arid subtropical climate, with sunshine all year round. The hottest months are between June and September, when temperatures can soar to 113°F (45°C) and more during the day and humidity levels are very high [33]. Even the sea temperature reaches 104°F (40°C) during the summer months. Temperatures are only slightly more moderate over the rest of the year; the coolest time being between December and April. There is very little rainfall in UAE but when showers do fall it is mainly in the cooler months [34].

The soil of UAE is sandy granular with small amounts of silt and clay. Sand particles (2.0 – 0.05 mm) form about 95-97% of the soil of UAE [35]. The soil is very poor in organic matter content and in most cases organic matter does not exceed (0.5%). So, organic matter should be added frequently to maintain water and to enhance fertility [36]. The Arabian gulf shoreline is a classic carbonate coast (calcareous) [37] where calcium carbonate represents considerable component of the soil of UAE ranging from 25- 42% in the upper surface 10 meters of soil which raises the pH of the soil to 7.9 ± 0.1[36, 38].

The soil of UAE is saline due to frequent planting and irrigation using saline waters. These activities plus the hot climate lead to the accumulation of higher amounts of salt in the soil. Ajman is located adjacent to the sea and the level of soil is

lower than the level of sea. So, sea brackish water flows normally covering the soil forming a salt flat known as sabkha. This sabkha area contains mainly dolomite (calcium and magnesium carbonate) and halite (sodium chloride) [38].

1.3.2 Geochemistry of chromium

Chromium is a solid silvery heavy metal located in group 6 and period 4 of the periodic table. The average atomic mass of chromium is 51.996 a.m.u and its atom contains 24 electrons configured as [Ar] $3d^5 4s^1$. It is a transition element and it can be present in multiple oxidation states ranging from -2 to +6. The most common and stable oxidation states of chromium in the environment are +3 and +6 [39]. Chromium (IV) and (V) are reported to form as unstable intermediates in redox reactions between Cr(III) and Cr(VI) [40].

Chromium is available in different environmental systems. It is the 21^{st} most abundant element in earth's crust with a concentration of 100 mg/kg [41]. The major ore of chromium in earth crust is chromite, $FeCr_2O_4$, which is a mixed metal oxide of Cr(III) and Fe(II) ions [42]. The concentration of chromium in soil ranges from 1 to 1000 mg/kg with an average of 40 mg/kg in the soil of USA [43, 44].

In soils, chromium (III) oxide (Cr_2O_3) and chromium hydroxide ($Cr(OH)_3$) are the most common species of the oxidation state +3 and both of them are sparingly soluble in water [45]. Chromium (III) may be adsorbed by soil particles which prevent its leaching to the groundwater but hexavalent chromium exists as soluble species such as H_2CrO_4 , $HCrO_4^-$, CrO_4^{2-} and dichromate $Cr_2O_7^{2-}$ [46].

Speciation of chromium in soil and water solutions is affected by the presence of organic matter and the inorganic compounds $Fe(OH)_3$, MnO_2, and $CaCO_3$[45 - 48]. Organic matter, iron element, and Fe(II) in soil and usually reduce Cr (VI) to Cr (III). Conversely Mn(III) and Mn(IV) will oxidize Cr(III) to Cr(VI) [40]. Calcium carbonate does not affect Cr(VI) in solution but it decreases the amount of dissolved of Cr(III) by precipitation as $Cr(OH)_3$ [47]. Figure (1-1) shows the oxidation-reduction interactions between chromium, iron, and manganese species in soil [40].

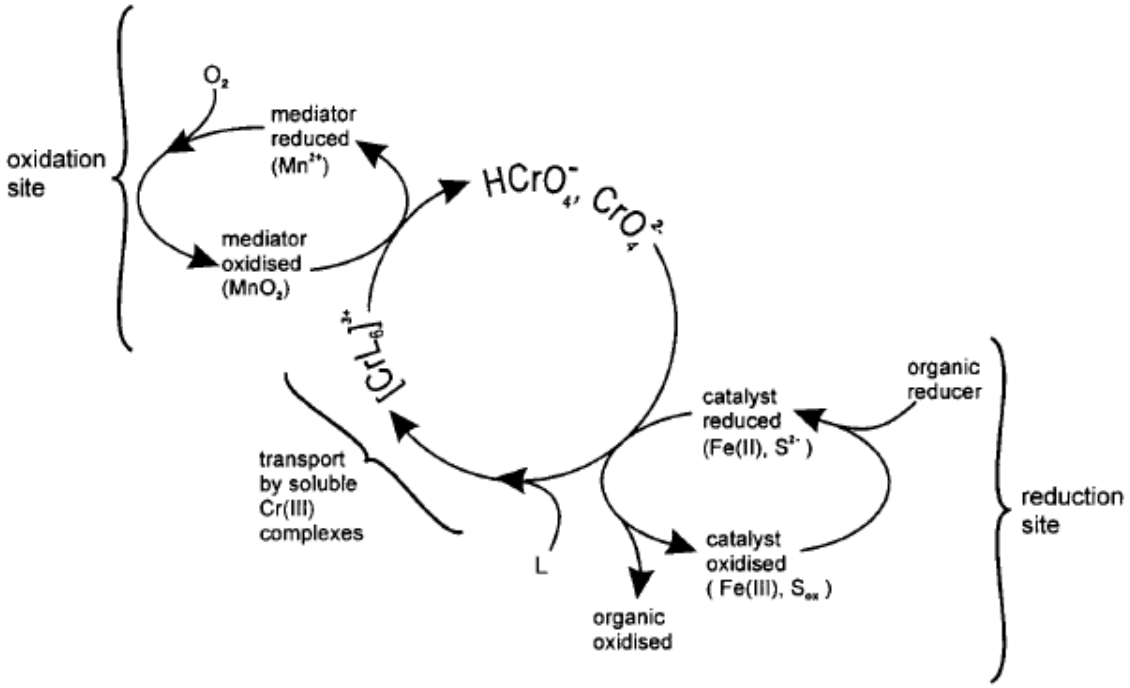


Figure (1-1) Chromium Redox reactions in soil. [40]

The pH value has a strong effect on redox reactions and, consequently, on speciation and mobility of chromium in soil and wastewater. The diagrams of the activities of Eh (half- reaction reduction potential) vs. pH can be very useful in understanding the redox status of a system [48]. Figure (1-2) is a modified diagram of Pourbaix showing the most dominant chromium species at different values of Eh and pH[49].

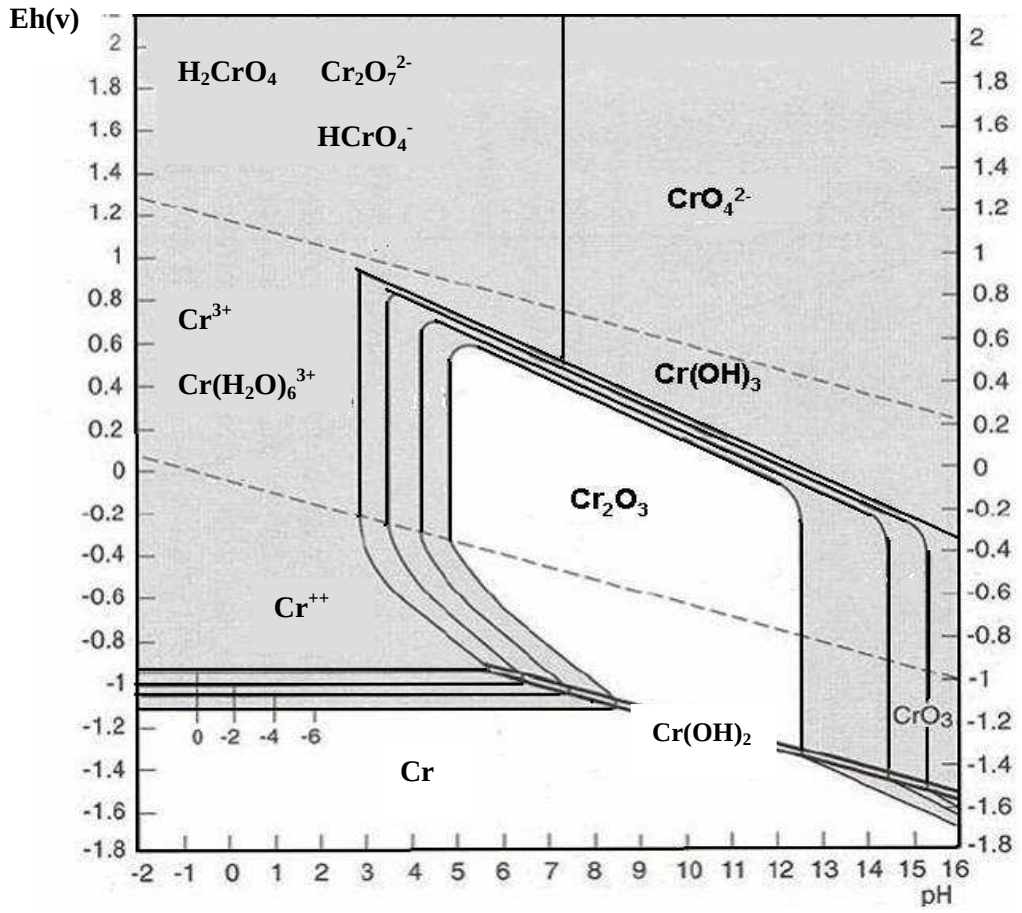


Figure (1-2) Eh/pH modified Pourbaix distribution of Cr species [49].

In acidic soils and solutions (pH<4), chromium(III) mostly exists as hexa-aquachromium(III), $[Cr(H_2O)_6]^{3+}$, which will interchange into trihydroxychromium

$Cr(OH)_3$ as the pH increases [50]. Chromium (VI) may exist as soluble sodium chromate or sparingly insoluble $CaCrO_4$ in neutral-alkaline soils, but in acidic soils $HCrO_4^-$ becomes the dominant form [39, 40]. As the concentration Cr(VI) increases in highly acidic aqueous systems, hydrochromate ion may be converted to dichromate $Cr_2O_7^{2-}$ [39] as illustrated in equation (1-1). Chromium (VI) in acidic solution reveals a very high oxidative behaviour in the presence of electron donors. The reduction of $HCrO_4^-$ is accompanied by the consumption of H^+ as in equation (1-2) but in more basic solutions the reduction of CrO_4^{2-} evolves OH^- as shown in equation (1-3) [38].

$$2HCrO_4^- \longrightarrow Cr_2O_7^{2-} + H_2O \quad (1\text{-}1)$$

$$HCrO_4^- + 7H^+ + 3e^- \longrightarrow Cr^{3+} + 4H_2O \quad (1\text{-}2)$$

$$CrO_4^{2-} + 4H_2O + 3e^- \longrightarrow Cr(OH)_3 + 5OH^- \quad (1\text{-}3)$$

Dissolved oxygen has no direct effect on the oxidation- reduction changes of chromium in the environment but it may oxidize Mn^{2+} to Mn(III) or Mn(IV) which oxidise Cr(III) to Cr(VI) [39, 40].

1.3.3 Chromium in industry

Chromium may reach the environment as waste from metallurgical applications since 80% of chromium produced world- wide is being consumed in this sector of the industry [41]. Chromium has many uses in industry especially in steel, pigments, and refractory industries. It is also common in the field of plating since it gives shiny surface to the plated metals. Chromium sulfate is being used in leather tanning giving

leather the green-blue colour. Chromium is used with copper and arsenic as a wood preservative system resisting the fungi and insects that may induce decay [50]. Chromic acid is commonly used to increase the surface inert layer of the aluminium oxide as protecting technique for aluminium. This anodizing [51] is frequently used in Ajman metal extrusion factory. All of the previous industries form important sources for waste chromium which pose great dangers to the safety of environment.

1.3.4 Toxicity of chromium for humans and plants

Chromium (III) is an essential micronutrient for mammals and humans. It is also believed to be important for the activity of insulin [52]. However, chromium (VI) is classified by World Health Organization (WHO) as a human carcinogen. Studies in Germany, England, and USA concluded that there is a correlation between lung cancer and working in the field of chromate and dichromate production [53]. Cases of gastrointestinal tract cancer were reported among ferrochromium workers and chromium plating industry workers [54]. Chromium (VI) is known to cause damage to respiratory tract tissues [55] and has toxicological effects such as ulcers, corrosive effects on the nasal septum, and harmful effects on kidneys, liver, and skin [53]. The US Environmental Protection Agency (EPA) set a limit of 100 μg/L of total chromium Cr(III) and Cr(VI) in drinking water and 52 $\mu g/m^3$ of the same pollutant in the inhaled air for 8-hour work shifts[56]. EPA issued the Toxicity Characteristic Leaching Procedure (TCLP) in soil which sets the maximum tolerated concentration of

contaminants for toxicity characteristic. The limit for chromium is 5 ppm by this procedure [57].

The toxicity of Chromium (VI) is related to its fast reduction which is associated with the oxidation of components of the living tissues [58] producing reactive intermediates such as Cr(V) and Cr(IV) in addition to reactive oxygen which may react with protein and cause DNA damage [59]. For plants, chromium is not known to be an essential nutrient. Some studies indicate that small concentrations of chromium (III) may stimulate the growth of plants [60], but many other studies suggested the toxic role for both chromium (VI) and (III). Hexavalent chromium compounds are more toxic than chromium (III) due to their solubility and permeability through cell membranes and their ability to oxidise the intracellular proteins and nucleic acids [61]. It has been reported that Cr (VI) is potentially toxic to higher plants at total tissue concentrations of 5 mg/kg dry weight [55]. Symptoms of chromium toxicity are accompanied by insufficient chlorophyll (chlorosis) as a result of the inhibition of translocation of both iron and zinc from roots to shoots [62]. The pH of soil has strong effect on the chromium phyto-toxicity since at low pH (≥5.5) both of Cr(III) and Cr(VI) are available in soil. At higher pH range only Cr(VI) is available and this increases its toxic effect on plants.

1.3.5 Plant nutrients and their transporters

Plants can get their available and soluble nutrients from soil. These nutrients are divided according to their needed amounts for plants into: macronutrients such as nitrogen, potassium, and phosphorus which are essential for the plant to complete life

cycle normally and are needed in considerable quantity [63], or micronutrients which are needed as trace elements such as boron and molybdenum. Table (1-1) shows the plant nutrients and their quantities in dry plant.

Table (1-1) Plant nutrients, their classification, and their quantity in dry weight [63].

Macronutrients	**Micronutrients**
Used in exceptionally large quantities (30-60 mol/kg) dry wt. C, H, and O. Used in moderate quantities (30-1000 mmol/kg) dry wt. N, P, K, Ca, Mg, and S	Used in small quantities (0.001-2.00 mmol/kg) dry wt. B, Cl, Co, Cu, Fe, Mn, Mo, Ni, Si, Na, Zn, and Va.

Nutrient ions can be transported to root cells through specific transporters since root cell membranes prevent ions or charged species passing through. These transporters can be divided into three categories:

- Primary pumps are cell membrane proteins which control the secretion of H^+ out of the cell to regulate the pH of cell and neutralise the charge [63]. These proton pumps (H^+ -ATPase) utilize up to 50% of adenosine triphosphate (ATP) energy of root cell [64] indicating that it may represent the major path for nutrient cations uptake in plants such as K^+, NH_4^+. Electrochemical gradient

controls the flow of ion in or out of the cell membrane. It is the resultant of the effect of two opposite deriving forces, the first is chemical and related to its concentration in cell and the second is the trans-membrane potential which equals 120 mV under ideal conditions.

- Coupled transporters are protein molecules of cell membrane. They transport two types of ions at the same time either in one direction which called symport or in two different directions as antiport. In symport process plant may take up two counter ions (anion and cation) such as nitrate accompanied with proton. Sulfate and phosphate may be taken up the same way. Antiport uptake may include the secretion of ion simultaneously with the uptake of another both of them are the same in charge for example the secretion of OH^- ions when taking up another anion like nitrate or sulfate [63, 64].
- Channels are high selective transport proteins which allow the movement of some specific ions such as K^+ or Ca^{2+}. Sulfate may also be transported using high- affinity transport proteins available at cell membrane [63, 65].

1.3.6 Uptake and accumulation of heavy metals by plants

It is accepted that the total heavy metal content of the soil is not a real indicator for its availability to plants [66]. To study the heavy metal availability in soils, several factors like inorganic salts, pH and organic content of soil have to be taken in consideration. These factors affect the speciation of the heavy metals in the soil. Not only does the bioavailability of a heavy metal affect its uptake by plants but also plant metabolism [67]. Some plants may exclude some heavy metals in spite of their

availability in soil as a phytostabilization process [9]. In conclusion, when evaluating the phytoremediation capability of a plant, it is important to determine the real accumulated quantity of heavy metal in the plant.

Plants uptake metals, in general, as cations but some metals such as Cr(VI) can be taken up as anions. Both cations and anions have different pathways in the uptake and detoxification by the plant tissues. Plants can uptake heavy metal cations either using the primary pumps of H^+ or the coupled transporters (the transporters of the nutrient cations). The secreted protons from the primary pumps may acidify the soil increasing the solubilised cations [68] which enter the roots. The process of heavy metal cations accumulation begins by the bonding between the heavy metal cation and ligands such as organic acids (citrate, oxalate and malate) or sulfur containing proteins forming complexes. Finally, these heavy metal complexes are transported and sequestered in the vacuole [69]. Two types of sulfur-containing low molecular weight proteins were identified as ligands in the accumulator plant tissues: metallothionein (MT) and phytochelatins (PC). Metallothioneins are cysteine-based proteins with low molecular weight ranging between 3500 and 14000 amu and present routinely in animals and fungi as a response to heavy metals toxicity. Metallothioneins have been found in a limited number of plants such as wheat, wall cress, and cotton [70- 72]. They play roles in root development and fruit ripeness [72] but there is no strong evidence confirming their role for heavy metals detoxification in plants.

Phytochelatins are glutathione-based proteins with the general formula $(\gamma Glu\text{-}Cys)_n$-Gly where the repeated unit is (glutamate – cysteine) and n = 2-11 (Figure 1-3). Phytochelatins have been induced as natural ligands in higher plants [73] by different heavy metal cations such as cadmium, lead and mercury. Both metallothioneins and phytochelatins are complexing ligands for heavy metal cations but not anions like chromate and dichromate. Figure (1-4) shows the chelation of cadmium using the phytochelatin PC_3 [74]. Organic acids such as citric acid and oxalic acid also play important role in the sequestration of heavy metals such as zinc and chromium [75].

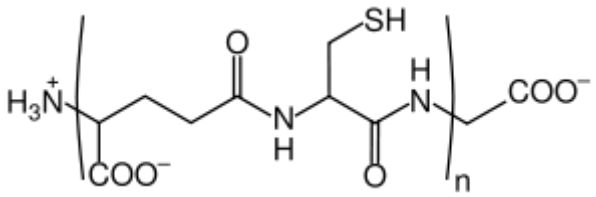


Figure (1- 3) General Formula of Phytochelatins

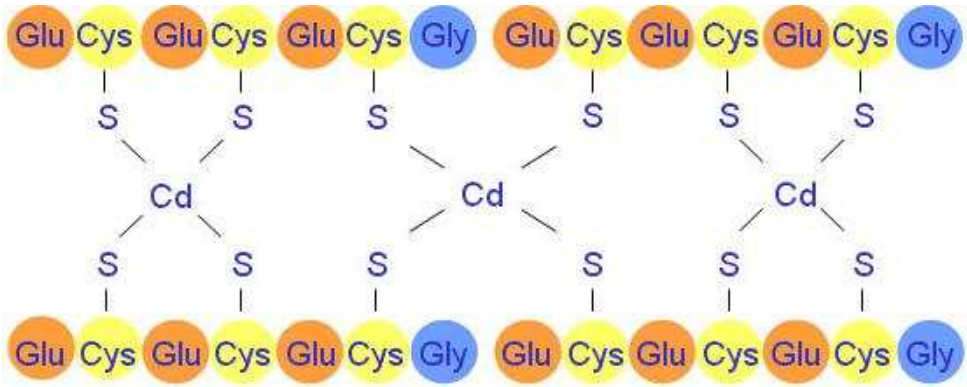
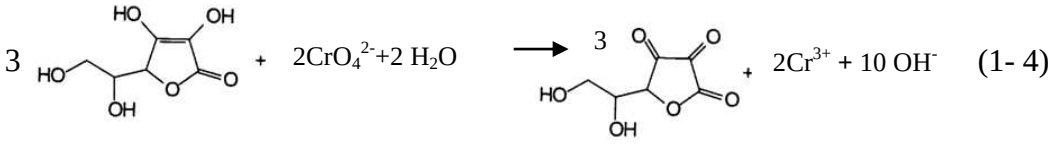


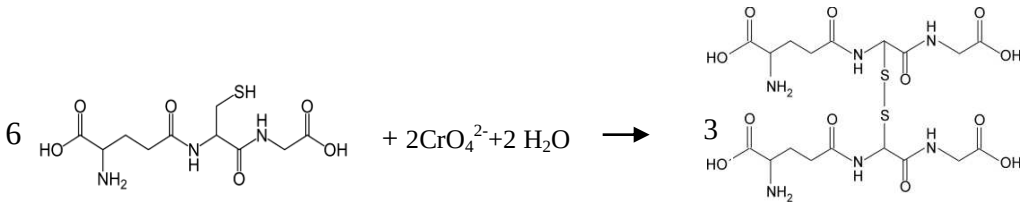
Figure (1-4) Chelating cadmium using PC_3 phytochelatin [74].

It is thought that plants take up the anions that contain heavy metals such as chromate and arsenate in a way similar to the uptake of nutrient anions. Chromate and sulfate are believed to use the same carriers [76] while arsenate may use the phosphate carriers [77]. Arsenate [As(V)] is reduced to As(III) and coordinated by three glutathione molecules as As^{III}-tris-glutathione complex. It is also suggested that glutathione is used as reducing agent for arsenate [78]. The roles of ascorbic acid and glutathione as reducing agents for chromium (VI) have been shown in living cells of both animals and plants [79, 80]. Ascorbic acid is a well known antioxidant found in both animal and plant cells. Glutathione is a simple protein which can be produced by the condensation of the three amino acids: glutamic acid, cysteine and glycine. Equations (1-4) and (1-5) illustrate the reduction of Cr(VI) to Cr(III) using ascorbic acid and glutathione respectively.



(1- 4)

Ascorbic Acid Dehydroascorbic Acid



Glutathione Glutathione oxidised

+ $2Cr^{3+}$ + 10 OH^- (1- 5)

After the reduction of chromate inside the plant tissues, Cr(III) may be chelated using either phytochelatins or organic acid complexes. After Eichornia crassipes was supplied with Cr(VI) in nutrient culture, Cr(VI) was reduced to Cr(III) in roots and cations were subsequently translocated to stems and leaves. The researchers in this study suggested that chromium (III) may form a chelate with oxalate as organic complexing ligand [81] giving the complex ion $[Cr(C_2O_4)_3]^{3-}$. With another plant, Leptospermum scoparium, it was found that most of the absorbed Cr(VI) was accumulated in roots again as tris(oxalato)chromate (III) [82] .

1.3.7 Phytoremediation of chromium

The merits of phytoremediation technique as compared to other techniques of heavy metals removal and the toxicity of chromium have led to research in the field of chromium phytoremediation. Many studies concentrated on rhizofiltration or the removal of chromium from hydroponic systems for the ease of experimental design [83, 84], but other studies used phytoextraction technique in studying the removal of chromium from polluted soils [85 - 87].

Early studies were carried out on chromium uptake by plants but they treated the chromium uptake as a problem of crops and plants contamination. Therefore, most of these studies were using plants of food crops such as wheat, barley and oat [88 -90]. Common vegetables were also investigated for hexavalent chromium absorption and results indicated that total chromium uptake decreased in the order: cauliflower> kale> cabbage> peas> collard> strawberry> lettuce> spinach>chive > celery> onion. These

crops were supplied with 1 mg/ L Cr(VI) and the concentration of total chromium in roots ranged between 500 and 0 mg /kg in dry roots and between 250 and 0 mg/ kg in dry shoots [91].

Many plants were investigated and some of them were classified as hyperaccumulators for chromium (III) but because of the high toxicity of Cr(VI) a small number of plants were classified as Cr(VI) accumulators. Plants are classified as hyperaccumulators if the concentration of chromium exceeds 1000 mg/kg of the dry weight of plant [17, 18]. Two other important factors that should be taken in consideration for accumulator plants are bioaccumulation and translocation factors. Bioaccumulation factor (BAF) (bioconcentration coefficient) is the ratio of concentration of metal in dry roots (mg/kg dry weight) to the concentration of metal in dry soil or wastewater (mg/kg or ppm) [92, 93] while translocation factor, (TF), is the ratio of concentration of metal in dry shoots to its concentration in dry roots. The values of more than 1.0 for both BAF and TF are indication for a promising accumulator plant [92, 94].

Tolerance Index (TI) can give another indication about the growth of the roots of plant in the presence of pollutant. It can be calculated as the ratio of the length of roots of experimental relative to roots of control [95, 96]. In normal cases, it equals the value of 1.0, but when the plant is exposed to stress of toxic pollutants, this ratio is expected to decrease.

1.3.7.1 Chromium (VI) and common accumulators

The high toxicity of chromium (VI) reduced the number of accumulators that may phyto-extract this pollutant. Even the common accumulators for heavy metals of Cd, Ni and Zn, such as willow (Salix spp) [97] and Indian mustard (Brassica juncea) [61], when investigated for their tolerance and uptake of chromium (VI), their results were not encouraging. Only traces of chromium were absorbed and translocated by 20 species of willow. In this experiment, equal concentrations of both Cr(III) or Cr(VI) were used and the results were approximately the same regardless of the chromium species used [98].

Brassica juncea showed a reduction in the total dry mass by 48% when stressed by 20 mg/kg of Cr(VI) in soil compared with plants in control experiments. The concentration of chromium in plant tissues did not exceed 18 mg/kg of dry weight of leaves, stems and roots [61]. Similar results were obtained for the same plant (Brassica juncea) when germinated in 100 mg/kg Cr(VI) soil; the concentration of chromium in shoots was about 20 mg/kg and less than 400 mg/kg in roots [99]. The same plant was investigated for Cr(VI) and Cr(III) accumulation but it did not accumulate more than 1800 mg/kg in roots [100]. Although this concentration is regarded as relatively high compared with other results for the same plant and the same pollutant [61, 98], it is still low compared with other heavy metal pollutants using the same plant (B. juncea can accumulate up to 6,000 mg/kg of lead in its dry weight) [101]. This study also concluded that Brassica juncea is not a good candidate for phytoextraction of Cr(VI) from polluted sites with low concentrations of chromium

(VI) [100]. These discouraging results of willow and Indian mustard with Cr(VI) suggest the need for further search for efficient Cr(VI) accumulators.

1.3.7.2 Phytoextraction and rhizofiltration of Cr (VI)

According to their response to the heavy metal in soil, plants can be divided into three categories:

- excluders which reject the uptake of heavy metal and then keep it in minor quantities in aerial tissues of plant regardless of the concentration of heavy metals in the soil,
- indicators which uptake the heavy metal to a concentration dependent upon the concentration of this metal in soil, and
- hyperaccumulators which are plants that can accumulate the heavy metal in total plant to levels of concentrations far exceeding its concentration in soil [102].

Some studies were carried out on chromium (III) which is very low in toxicity to plants compared with chromium (VI). As a conclusion they made inaccurate generalizations such as discovering hyperaccumulators for chromium without determining if they were for Cr(III) or Cr(VI). Other studies used some natural polluted soils or wastewaters which contain mixtures of chromium (III) and chromium (VI) [103- 105]. These studies measured the total chromium in the polluted source and the total chromium in the plant tissue and finally suggested accumulators for

chromium in general or for chromium (VI) without regard to the proper design of the experiments in their investigations.

A report issued from Amarillo National Resource Center for Plutonium (ANRCP) reviewed some accumulators for chromium. According to that report only a few plant species were identified as chromium hyperaccumulators when grown in high chromium soils [62]. The report mentioned ten plant species as chromium accumulators but did not determine if these ten plant species were accumulators for Cr(III) or Cr(VI). One of these plants is Leptospermum scoparium (Myrtaceae) which can accumulate 20,000 mg/kg of Cr in the ash of the plant. This is a considerable quantity related to ash but when calculated regarding the dry weight of the plant this concentration will decrease by a factor of 30 to 50. This means that chromium in the dry weight of the plant will be about 400- 600 mg/kg which is below the value of 1000 mg/kg. The same report suggested Berkheya coddii as a chromium accumulator even though the concentration of chromium in the dry weight of the plant was 238 mg/kg which is a small amount compared with real accumulators which should fulfil the condition of 1000 mg/kg of dry weight. In the following literature review, well-designed experiments that introduced known concentrations of chromium (VI) in the soil or irrigation solutions are described taking in account; the accumulated amounts of chromium in the different plant tissues, the growth of the plant and the effect of the pollutant on it, the conditions of the experiments especially the tolerated concentration of Cr(VI), and the environment of germination such as soil or hydroponic cultures in each previous study.

Leersia hexandra (Gramineae) Chinese natural plant was investigated for its potential as chromium (VI) and chromium (III) accumulator [83]. Plant seedlings were grown in Hoagland's nutrient solution. Both Cr(III) and Cr(VI) were added in six concentrations ranging from 0 to 60 ppm of Cr(III) and 0 to 30 ppm of Cr(VI). The plant could accumulate up to 3300 mg/kg of dry roots and 2160 mg/ kg of dry leaves at the Cr (VI) concentration of 30 mg/L. No significant decrease of biomass in the leaves of Leersia hexandra was observed and the plant grew rapidly with a great tolerance to chromium in the cultures of Cr (III). However, at the concentration of 20 ppm of Cr (VI) there was a significant decrease in the biomass of the leaves [83]. The pH of the hydroponic system was not mentioned in this study in spite of its importance for Cr (VI) speciation and Cr (III) availability. The researchers in this study avoided calculating the translocation factor from roots to shoots which was below 0.4 in most of their samples. Instead of that they calculated the bioaccumulation coefficient from the nutrient solution to shoots which is normally a higher ratio.

Typha angustinfolia which is a kind of Typhaceae plant species was investigated for Cr(VI) tolerance in contaminated soil [85]. No change in plant growth or height was observed in plants which were exposed to 100 μM and 0 μM Cr(VI) but significant reduction in both height and biomass of the plant at concentrations of 200 – 800 μM Cr(VI) was observed. The highest concentration of total chromium was encountered in the roots of the plant and was 177.5 mg/ kg when the plant was exposed to 800 μM Cr(VI) – about 41.6 ppm- for 30 days [85].

Larra tridentata (Creosote bush grows in south western of North America) showed high ability for chromium (VI) accumulation [84]. Seedlings were grown in hydroponic solution of 520 ppm Cr(VI) and pH of 5.0. Three replicates were performed in this experiment using fluorescent light at room temperature. During 48-hr time period of a flow rate of 2mL/hr of Cr(VI) solution, there was no evidence of chromium toxicity. After the analysis of the dried tissues of the plant, the concentrations of Cr(VI) in roots, stems and leaves were 57400 mg/kg, 14200 mg/kg and 19300 mg/kg, respectively [84]. These concentrations represent the highest among all the investigated plants for chromium (VI) accumulation but the short time of germination is not enough time to evaluate the plant tolerance for the pollutant.

Azolla caroliniana, the small water fern, was investigated for Cr(VI), Cr(III) and Hg(II) accumulation [106]. For Cr(VI) investigation, 0.1, 0.5 and 1.0 mg/L concentrations of Cr(VI) were introduced to the plants as potassium dichromate. The existence of Cr (VI) decreased the growth of biomass of plant by 20-27 % and the highest concentration of chromium in plant tissue was 350 mg/kg [106]. The concentration of pollutant in dry weight of plant is considerable regarding the small concentration of the pollutant in the culture solution (1.0 mg/L) but this small concentration of chromium does not form a real test for Cr(VI) tolerance and accumulation. In spite of the importance of the pH of hydroponic culture, it was not mentioned in this study.

Zea mays (corn) was investigated for its ability to uptake Cr(III) and Cr(VI) [86]. Four replicates were grown in either pure sand (silica quartz) or natural soil with pH of 7.2 and 7.8 respectively. The concentrations of the irrigation solutions ranged between 0.5 -25 ppm. The plant showed an increased chromium accumulation as the concentration of both Cr(III) and Cr(VI) was increased in soil and sand (pure silica). The highest concentration of total chromium 1824 mg/kg was observed in roots when the concentration of Cr(VI) in pure sand was 25.0 ppm. At the same concentration of Cr(VI) in irrigation solution, the total chromium in roots decreased to 580 mg/kg. The concentration of chromium (III) in shoots was more than in roots but conversely in case of Cr(VI). The growing of roots when plants were irrigated by Cr(III) was more than in the plants irrigated by Cr(VI) [86].

In another study, thirty six plants were investigated for chromium (III) and (VI) accumulation [87]. These plants were grown in pots containing contaminated soil either by chromium (III) or chromium (VI). There was a reduction in weight of all plants grown in Cr(VI) but there was no indication of biomass reduction with Cr(III). Among the 36 plants only three plants survived (alkali sacaton, switch grass and Bermuda grass) in soil contaminated with 500 mg/kg of Cr(VI). Chromium concentration in the shoots of all the other plants exceeded the 1000 mg/kg in the dry weight of shoots and all of these plants died [87] apparently due to the high content of Cr(VI) in soil.

A promising hyperaccumulator for Cr(VI) was introduced by J.L. Gardea-Torresdey et al. [107]. Convolvulus arvensis seeds were germinated in an agar-based nutrient mediums which were spiked with concentrations ranged between 0 and 80 ppm of Cr(VI). There was a reduction in the growth of roots and the biomass of the plants as the concentration of Cr(VI) was increased. The accumulated chromium in roots at 20 ppm was about 20,000 mg/kg of dry weight which is extraordinary amount but this amount was to decline to 8600 mg/kg at the concentration of 80 ppm. Convolvulus arvensis accumulated about 2100 mg/kg of chromium(VI) in dry leaves when it was germinated for 15 days in an agar- based nutrient medium of 20 ppm Cr(VI) and this amount was approximately the same in the three concentrations of Cr(VI) [107]. According to the accumulated amounts of Cr(VI), this plant is considered to be a very promising hyperaccumulator for hexavalent chromium. This plant normally grows in Europe and North America and not common in the desert climate of UAE. In addition to that, a useful accumulator would have to be shown to be tolerant to local soil or water rather than an agar based nutrient medium.

In another study [108], six weed plants from Thailand were used and three replicates from each type were performed. The plants were grown for 120 days in three soils with either 100, 200 or 400 mg/kg of Cr(VI). A reduction of growth was observed in all the plants at the three soils. Cynodon dactylon accumulated 1500 mg/kg Cr(VI) in dry weight of roots. The concentration of total chromium in the tissues of the other five plants did not exceed 180 mg/kg at the same concentration of chromium (VI) in soil [108].

In recent study [109], Prosopis laevigata was investigated for its ability for Cr(VI) accumulation. Seeds were germinated in culture tubes containing nutrient solution supplemented with potassium dichromate at pH of 5.8. Their results were very promising since the seedlings accumulated up to 8000 mg Cr/kg of dry root weight and 5000 mg/kg in shoots. In spite of the high accumulation of chromium, translocation factors of chromium using this plant stayed below 0.7. Another Prosopis species was investigated before at pH of 5.3 [28], this calls for further investigations in real soil and at higher pH conditions similar to the UAE where more than one type of Prosopis naturally grow.

1.3.8 Factors affecting chromium (VI) uptake by plants

There are two types of factors that may affect the uptake of chromium (VI) by plants. The first type comprises factors related to the speciation of chromium (VI) in soil such as concentration, pH of soil and the organic content of soil. The second type of factors is related to the accompanying cations or competitive anions of chromate or dichromate.

1.3.8.1 Effect of the concentration of Cr(VI) in soil or wastewater on the uptake of Cr(VI) by plants.

In general as the concentration of the heavy metal in soil increases, its uptake and accumulation in the accumulator plant tissues will increase [110-112]. Regarding Cr(VI) accumulation Zhang et al. [83] suggested this and their results indicated that the uptake of Cr(VI) was increasing in both roots and shoots as the concentration of Cr(VI) in soil increased. The results obtained by Bennicelli et al. [106] and

Sampanpanish et al. [108] are in agreement with this conclusion. The same conclusion was obtained by Shewry and Peterson [89] when they introduced Cr(VI) to barley seedlings. They observed an increase in chromium uptake and translocation as chromium was increased in the nutrient solution. This relation stays consistent till the plant reaches the phytotoxic concentration which varies from accumulator to another. A study with Convolvulus arvensis grown in agar- based medium and irrigated with three concentrations of chromium (80, 40, and 20 ppm) [107] has been reported. The highest uptake of chromium (VI) was observed at the lowest of these concentrations (20 ppm). The authors of this study observed a decline in the uptake of chromium as the concentration of the Cr(VI) was increased [107]. This may be explained by the phytotoxic limit which may reached by plants at high concentrations where the biomass will be reduced and as a result, the total removed amount of the pollutant will decrease. It is very important to determine the concentration at which the best removal of pollutant will be achieved.

1.3.8.2 Effect of pH of soil on the uptake of Cr (VI) by plants

Chromium (VI) is available for plants at a wide range of pH especially in basic medium like the soil of Ajman and UAE in general. It exists as chromate anions and will be neither reduced nor adsorbed but available in the soil and this poses an environmental challenge [88]. In acidic medium, it exists as dichromate which is highly oxidising and toxic to plants. Iron in the soil of UAE exists as Fe_2O_3 [113 -114] and this will not affect Cr(VI), while in the presence of Fe^{2+} (at pH <5), Cr(VI) can be reduced to Cr (III) [115]. All the previous studies which measured the effect of pH on

the uptake of Cr(VI) were carried out on nutrient crops plants[89, 116] or fungi and microorganisms[117, 118]. However very little, or no work, was done on the study of the effect of pH of soil on the uptake of Cr(VI) by non-crop plants as potential accumulators of Cr(VI).

It was found that the uptake of dichromate by barley seedlings from hydroponic culture was doubled when the pH of soil was increased from 3.0 to 6.7 [89]. In this previous study the researchers introduced Cr(VI) as chromate anion which is known to exist as dichromate at this low range of pH. The range of pH in their experiment was limited to the acidic and neutral range (3.0 - 7.7) and it did not cover the high range of pH up to 9.0 to take deeper overview of chromium uptake with regard to different pH values. Cary et al. [116] expanded the range of pH from 5.0 to 8.0 while studying the effect of pH on the uptake of Cr(VI) by wheat. They observed an increment in the uptake as pH increased from 5.0 to 6.0 but they observed a decrease in Cr(VI) uptake from 6.0 to 8.0. Chromium (VI) in this study was introduced as chromate in the hydroponic culture. Both of the two previous studies did not justify their results regarding the change of pH or chromium speciation at this change. In the aquatic fungi Aspergillus foetidus, there was an increase of Cr (VI) uptake when pH was increased over the range 4.0–7.0 [117]. In another study [118], it was found that chromium (VI) was accumulated by microorganisms with highest concentration at pH 9.0 as compared to pH 7.0 or 8.0.

1.3.8.3 Effect of organic content of soil on the uptake of Cr (VI) by plants

The organic content of soil is commonly effective in the reduction of Cr (VI) to Cr (III). Poorly organic soils such as the soil of UAE which contains an organic content of less than 0.5% do not provide an effective reducing environment for Cr(VI). Previous studies investigated the role of the organic content in soil on the reduction and the uptake of chromium (VI) by plants [62, 119 -121]. All of these studies confirmed the essential role of organic content of soil in the reduction of Cr(VI) to the less toxic and less soluble Cr(III). Investigation of the effect of organic content on phytoextraction of Cr(VI) in high pH soil does not appear in the literature.

1.3.8.4 Effect of nutrient anions and accompanying cations on the uptake of Cr (VI) by plants

The mutual effect of anions and accompanying cations on the uptake of each other by the plant was studied specifically for the nutrient ions of plants. The introduction of potassium associated with $H_2PO_4^-$ stimulated its uptake more than in KCl solution in fungi [122]. In barley, the plant accumulated higher amounts of potassium when introduced as KNO_3 as compared to KCl [123]. It is worth pointing out that chloride is not a plant nutrient, unlike phosphate and nitrate anions. The effect of counter ions seems to be important in understanding the relation between the uptake of an anion and its accompanying cation especially in the presence of coupled transporters which may explain the simultaneous uptake (co-transport) of both. Sodium cations efflux from root cells was increased when the plants were grown in solution of K_2SO_4 but decreased with KCl solution [122, 124]. The difference here was the counter anion; it was a nutrient in the first but not in the second case which may

suggest the co-uptake of both potassium and sulfate. The uptake of anions such as nitrate or phosphate will stimulate the formation of organic anions such as malate and oxalate inside the plant tissues or efflux OH^- anions but the uptake of cations such as NH_4^+ will initiate the efflux of H^+ to the nutrient medium around the plant [124 -126] which can be understood according to the role of primary pumps of H^+.

Effect of associated cations on the uptake of nutrient anions was studied in some plants but the effect of cations on the uptake of pollutant anions such as chromate, dichromate or arsenate has not been studied deeply. In a detailed study by John Raven [126], it was suggested that when plant uptakes nitrogen as ammonium cations, hydrogen cations will be produced in response. This produced H^+ will be excreted to the surrounding medium and will react with anions and molecules. These reactions of H^+ consumption or OH^- production will facilitate the uptake of anions such as SO_4^{2-} and SeO_4^{2-}[127]. The effect of common anions such as nitrate, sulfate, chloride and hydrogen carbonate on the uptake of perchlorate anions by lettuce plant was also investigated [128]. A hydroponic system was used and the results of this study indicated that both nitrate and hydrogen carbonate inhibited the uptake of perchlorate. The researchers in this study justified the effect of nitrate as being due to that both anions have the same carrier. The effect of hydrogen carbonate was attributed to the co-transport of H^+/HCO^- across the plasma membrane [128].

Some researchers indicated that the presence of chromium (VI) as dichromate enhanced the concentration of phosphorus in the leaves of citrus plants but not in the roots [129]. The authors of this study did not introduce any explanation to the effect of

Cr(VI) on the uptake of phosphorus [129]. Opposite results were attained in radish plant irrigated by Cr (VI) in pot experiment at 10, 50,100 mg/kg of Cr (VI). The amount of phosphorus decreased in both roots and shoots [130].

In a recent study, the effect of available nitrogen on the removal of both Cr(VI) and Cr(III) from hydroponic system by willow plants was investigated [131]. Nitrogen was introduced as $NaNO_3$ and Cr(VI) as $K_2Cr_2O_7$. No significant variation of Cr(VI) removal was observed between N- free and N- containing nutrient solutions. They used five replicates from each type of solutions and found out that the presence of nitrogen has positive effect on the translocation of chromium from roots to stems and leaves. But the reality is that the uptake in roots was reduced significantly with minor or no observed increase in shoots so when translocation efficiency was calculated (the numerator stays as it and denominator is reduced) they got enhancement in the translocation. The researchers in this study did not indicate anything about the growth of the plants or the biomass during the 8 days of experiment [131].

The effect of chromium (VI) on the growth and development of Arabidopsis thaliana seedlings was also investigated [132]. Small concentrations of chromium (less than 10.4 ppm) were used and no growth of roots was observed. Cr(VI) was introduced as potassium dichromate (at pH 5.7) with or without one of the nutrient anions (nitrate, sulfate or phosphate). The three nutrient anions resumed primary root growth in medium with dichromate. The lengths of roots were of 73%, 83% and 70% with SO_4^{2-}, PO_4^{3-} and NO_3^-, respectively, compared to the lengths in medium free of $Cr_2O_7^{2-}$ [132].

In a recent study, the effect of nutrient anions and cations on the uptake of arsenate anions by the accumulator Pteris vittata was investigated [133]. The experiments were done as four replicates for each type in hydroponic system. It was concluded that both potassium and calcium enhanced the uptake of arsenate as counter anions. This study also indicated that both nitrate and phosphate anions reduced the amount of absorbed arsenate by roots and this may be due to competition between these anions and arsenate. This study did not mention the accompanying cation to arsenate in this investigation and probably did not take it into account, which represents a gap in the experimental design. It seems that the effect of accompanying cations such as Na^+, K^+ and NH_4^+, and the role of some nutrient anions such as nitrate, sulfate and phosphate on the uptake of Cr(VI) by plants need further investigation.

1.3.8.5 Effect of sulfate anions on the uptake of Cr (VI) by plants

It is suggested that chromate is taken up by plants using the same cellular transporters as sulfate in the plant cell membrane [78, 79, 134 -136]. This may be a consequence of the similarity in geometry, charge and size [137, 138] of both sulfate and chromate. Heavy metal accumulation will induce the plant to form thiols (glutathione and phytochelatins) and chromium (VI) may inhibit the uptake of sulfate which is required to produce thiols. Chromium(VI) causes a high stress on the plant, it not only may inhibit the formation of thiols but also, it may oxidise the available of them.

The effect of chromate on the uptake of sulfate by two types of Zea mays grown in hydroponic system was investigated [135]. The authors of this study used

both deprived and supplemented sulfate systems. Chromium (VI) was introduced as potassium chromate (without mentioning the pH of medium). They observed that chromate reduced the uptake of sulfate by the two types of plants. An opposite observation was found by other authors, [129] indicated that the presence of Cr(VI) enhanced the uptake of sulphate by roots of citrus plants.

Others investigated the effect of deprivation of sulfate on the uptake of chromate by wheat [136]. A hydroponic system with nutrient solution was used with pH of 6.0 ± 0.1. Chromate was added as 70 ml of 1 ppm Cr(VI) to both, with and without sulfate solutions. They concluded that sulfate is a strong inhibitor of chromate removal from wastewater [136]. This conclusion seems to be unreliable since they found that the absence of sulfate enhanced the uptake of Cr(VI) but they did not study the effect of different concentrations of SO_4^{2-} on the uptake of Cr(VI). From another point of view, they used a solution of pH 6.0 at which most of the Cr(VI) is available as dichromate not chromate (as they suggested). This will alter the competition between SO_4^{2-} and CrO_4^{2-} and can be classified as defect in the design of the experiments.

Stylosanthes hamata SHST1 gene is a high-affinity sulfate transporter located in the plasma membrane of plant cells. According to Lindblom and his colleagues [76], the initiation of SHST1 in Indian mustard, which is a common hyperaccumulator, increased the accumulated amount of Cr (VI) in roots from 750 mg/kg to 1100 mg/kg and in shoots from 30 mg/kg to 50 mg/kg. The plant was grown in a hydroponic system, where chromium was introduced as potassium chromate with concentration of

5 ppm (the pH of solution was not mentioned). The enhancement of the uptake of chromium after this genetic modification of Brassica juncea may provide the evidence for the role of sulfate in enhancing the uptake of Cr(VI). B. juncea was investigated before for Cr(VI) accumulation and the result was not encouraging enough [61]. The opposite conclusions related to sulfate-chromate uptake by plants call for further investigation

1.3.8.6 Effect of chelating agents on the uptake of Cr(III) and Cr (VI) by plants

Chelating agents such as EDTA (ethylenediaminetetra-acetic acid) and citric acid are commonly used to enhance the uptake of cations of heavy metals and radionuclides by plants [139, 140]. In general, the presence of organic acids such as citric acid and oxalic acid may enhance the translocation of chromium (III) from roots of plants to shoots. Chromium (III), as a heavy metal cation can be affected by chelating agents but few studies were carried out on the uptake of chromium (VI) using chelating agents [141]. In the following text the effect of chelators on the uptake of chromium is reviewed.

Hydroponic systems were used to investigate the uptake and translocation of chromium (III) by tomato plants in the presence of organic acid such as citric and oxalic. Chromium accumulation increased significantly in various tissues of the plant as organic acids were increased [142].

EDTA and citric acid were investigated to enhance the uptake of Cr(III) and Ni(II) by Datura innoxia. An industrial soil contaminated mainly by Cr and Ni was

used. It was observed that citric acid increased the uptake of Cr by the plants and enhanced the translocation factors of Cr between 2 and 3.5 fold relative to the control samples [143].

The effect of oxalic acid, citric acid, and EDTA as chelating agents on phytoextraction of chromium and nickel by Brassica juncea was investigated. Experiments were carried out using soil containing 3100 mg/kg of Cr(III) and 3400 mg/kg of Ni(II) irrigated by solutions of 0.05 and 0.10 mmol/kg dry soil of each chelating agent. As a result EDTA was an efficient chelator in increasing the uptake of Cr and Ni from soil. Significant reduction of plant shoot biomass was observed in the presence of EDTA. The translocation factor (TF) for chromium did not exceed the value of 1 for any chelator or control experiment. Only citric acid enhanced TF from 0.80 in control experiment to 0.95 [140].

The effect of EDTA on the uptake and translocation of Cr(III) by water spinach (Ipomonea aquatic) was investigated. Chromium (III) was introduced in three levels of concentration as $CrCl_3$ in a hydroponic system at pH 6.0. It was found that EDTA significantly enhanced the uptake of chromium by roots but the translocation to shoots decreased at the same conditions. The authors of this study explained these observations by the formation of a Cr-EDTA complex which may enhance the transfer of Cr^{3+} to the root cells while the same complex retarded the translocation from shoots to roots [144].

The effects of EDTA on uptake and translocation of Cr(VI) and Cr(III) by two types of willow plants (hybrid and weeping) were investigated [141]. Hydroponic

solutions were spiked with either potassium chromate or chromium chloride at temperature of 24.0 ± 1°C. The two types of willow tend to uptake Cr(III) by 3 fold more than Cr(VI). Limited or negligible effects on the uptake and translocation of both Cr (III) and Cr (VI) by hybrid willow were observed in the presence of EDTA. In weeping willow, results showed that EDTA did not increase the uptake of Cr (VI) but the translocation of Cr(VI) in the presence of EDTA was possible. The researchers of this study claimed that the limited uptake of Cr(III) in the presence of EDTA may be explained due to the complexation reaction between Cr(III) and EDTA which may take place in the hydroponic solution keeping chromium in the hydroponic system [141].

The effect of organic acids such as citric and oxalic and some amino acids on the uptake and translocation of Cr(III) by tomato plants (Lycopersicum esculentum) was investigated [145]. The plants were grown in pots of sand and soil. Significant increases in Cr(III) accumulation in the treated plants were observed in the presence of organic acids but not with amino acids. The results indicated that Cr(III) may be chelated by organic acids and this may increase its uptake by roots of plant [145]. The conflicting conclusions related to the effect of chelating agents on the uptake of chromium suggest that the understanding of this issue is far from complete.

1.3.9 Re-extraction of chromium from contaminated biomass

One of the challenges that may face the workers in the field of phytoremediation is the safe discarding or reuse of the produced biomass. In most

cases incineration of this biomass is the most probable but, in other cases, the biomass can be reused in the field of the production of bio-fuel.

Heavy metal cation mobility and solubility are pH dependent, and at low pH most of the heavy metal cations are soluble. At high pH range, these metals can be precipitated then removed from wastewater or any aqueous solution. Electrodeposition, adsorption on some active surfaces, and using the living cells are further techniques for heavy- metal removal.

The high carbonate content of the soil of UAE which raises the pH of the soil was used before in the removal of some cations of heavy metals from wastewater [146]. The pH of UAE soil is 7.9 ± 0.1 and from Figure (1-6) it is observed that the lowest solubility of Cr (III) is between 8.0 and 9.0 [147]. Therefore the soil of UAE with its high pH can be used for the precipitation then the removal of Cr(III) cations.

Electrodeposition, or sometimes as it is called electrowinning, depends mainly on using direct current to reduce the cations from their solution to deposit on the cathode. In extracting chromium from electroplating sludge, HCl was used to acidify then mobilise Cr(III) [148]. Both precipitation of Cr(III) as $Cr(OH)_3$ at high pH and electrodeposition may be alternative suggested techniques to the common process of incineration of the dry contaminated biomass after phytoextraction process.

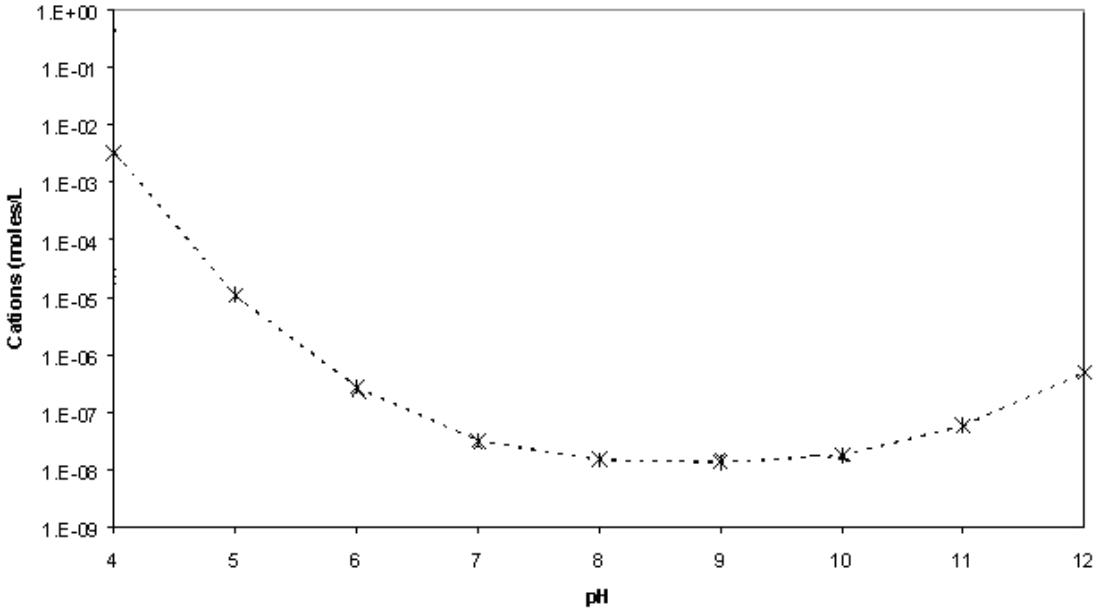


Figure (1-5) Solubility of $Cr(OH)_3$ at different levels of pH (modified from[147]).

1.3.10 Gaps in the previous work

To summarise the previous work reported above the following gaps were found:

- For reasons of designing controllable experiments many previous investigations were carried out using hydroponic systems. So it is logical to conclude that the discovered accumulators in these investigations are appropriate for the phytoremediation of heavy metals from wastewater (rhizofiltration). In some cases, researchers generalised their discovered accumulators for Cr(VI) regardless of the conditions of cultivation (in soil, water or agar) while these plants should be tested under real soil conditions before generalization.

- In some cases researchers used neither soil nor hydroponic systems in their investigations but agar-based nutrient medium and finally they introduced the plant as a promising accumulator neglecting the effects of many factors related the environment of growing. Others used very small concentrations of chromium Cr(VI) (close to the tolerated amount for plant) to study the uptake which cannot be considered as real test for these plants.

- The discouraging uptake of Cr(VI) by the common accumulators of cations such as willow and Indian mustard calls for more investigations in the field of looking for other efficient Cr(VI) accumulators.

- When calculating the concentration of the pollutant in the plant tissue, some researchers regarded the weight of ash of plant not the dry weight. This reduction of weight from dry weight to ash enhanced the calculated uptake 40 fold. When corrected, it can be concluded that the plant has limited accumulation of chromium.

- The contrast between chromium concentration in the contaminated site and the experimental conditions, For example, to use small concentrations in the experimental work while the real concentration of the pollutant in the contaminated soil is very high, so phytoremediation technique is not the best choice of treatment. When such studies recommend plant accumulators, these accumulators are mostly unsuccessful when tested in the real contaminated site.

- In many previous studies, the researchers used contaminated soils or wastewaters which contain a mixture of Cr(VI) and Cr(III) (mostly from industrial effluents) and finally they introduced the discovered plant accumulators as chromium accumulator (in general). Therefore they did not use well- identified species of chromium with known concentration to claim that this accumulator is suitable for either Cr(VI) or Cr(III) phytoextraction.

- Some other researchers did not take into consideration the effect of pH on the speciation of Cr(VI). When they were investigating the competition of sulfate with chromate they used solutions of Cr(VI) with pH of 6.0 or below, and at this pH, Cr(VI) is available as dichromate not chromate.

- The effect of accompanying cations on the uptake of chromate or dichromate by accumulators (not crops plants) has not been investigated. And the effect of the competitive nutrient anions such as sulfate, nitrate, or phosphate on the uptake of Cr(VI) by accumulator plants needs further investigation.

- The relation between chromium (VI) speciation and its uptake by plants was not investigated regarding the interchange between chromate and dichromate in soil at different pH values of soil.

- The effect of organic content of soils with high pH such as Emirates soil on the uptake of Cr(VI) by accumulator plants has not hitherto been investigated.

- Related to the use of chelating agents to enhance the uptake of chromium; only Cr(III) was chelated but not Cr(VI). This is due to the fact that chelating agents do chelate the cations of heavy metals as central ions but not their anionic forms like chromate. Very few investigations considered the effect of chelating agents on the translocation of Cr(III) originating from the reduction of Cr(VI) by plants.

- Antioxidants that may reduce and detoxify Cr(VI) to Cr(III) and the natural ligands that may chelate it are not well identified in the scientific literature and need further investigation.

1.4 Scope of the present work

In the present study the possibility of implementing the phytoextraction technique using native plants of UAE is investigated. To achieve this objective the following questions are addressed:

1- What are the most problematic heavy metal(s) in the soil of the UAE?

2- What are the desert plants that may be promising hyperaccumulators for the problematic heavy metals of the UAE soil?

3- Are the previous investigated plants -which grow in similar conditions like Texas desert - such as Prosopis species suitable for the phytoextraction of some problematic heavy metal(s) in the soil of UAE?

4- What is the effect of Cr(VI) concentration in the soil on the uptake of this pollutant by Portulaca oleracea as a potential accumulator for Cr(VI)?

5- What is the most dominant species of chromium that exists inside Portulaca oleracea tissue?

6- What is the effect of pH of soil on the uptake of Cr(VI) by Portulaca oleracea?

7- What is the effect of organic content in soil on the uptake of Cr(VI) by Portulaca oleracea?

8- What are the effects of nutrient anions such as nitrate, sulfate, and phosphate on the uptake of Cr(VI)

9- What is the effect of sulfate concentration in soil on the uptake of chromate by Portulaca oleracea?

10- What are the effects of accompanying cations such as sodium, potassium, and ammonium on the uptake of Cr(VI) by Portulaca oleracea?

11- What are the effects of chelating agents such as EDTA and citric acid on the uptake of Cr(III) and Cr(VI) by Portulaca oleracea plant?

12- What are the optimum conditions that would maximise the uptake of Cr(VI) by Portulaca oleracea?

13- What is the effect of Cr(VI) in soil on the concentration of ascorbic acid and glutathione as antioxidants for this pollutant inside the plant tissue?

14- What are the expected ligands to chelate Cr(III)) from roots to shoots of P. oleracea?

15- What is the efficiency of filtration using Emirates sand and electrodeposition as alternative suggested techniques for the treatment of polluted plants other than incineration?

CHAPTER 2

EXPERIMENTAL AND METHODOLOGY

2.1 Chemicals and reagents

Table (2-1) Chemicals, purity and suppliers.

Material	Supplier
Standard solutions (1000 ppm) of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn and Na, K, Ca and Mg. Hydrochloric acid (Conc ≥37%, trace analysis grade) Sodium gluconate (≥ 99.0%)	Fluka Chemicals, Gillingham, UK.
Sodium chromate, potassium chromate, ammonium chromate, potassium dichromate, sodium nitrate, sodium sulfate, sodium phosphate, sodium carbonate, citric acid and EDTA (All analytical reagent grade)	Panreac Química S.A.U, Sharjah, UAE
Nitric acid (ACS Reagent ≥ 90.0%), ascorbic acid (reagent grade), dehydroascorbic acid, L-glutathione reduced (>98%) and L-glutathione oxidised (>98%), acetonitrile (HPLC grade), formic acid (HPLC grade), n-butanol (HPLC grade)	Sigma-Aldrich, Gillingham, UK
Phytochelatin 3	Cambridge Bioscience, Cambridge, UK
Sodium tetraborate decahydrate (AR)	Fisher Chemicals, Loughborough,
Silica sand, General purpose	Dubai Sand Purification Co. Jebel Ali, Dubai
Potting soil (70% organic content)	Blumen Erde, Carrefour, Ajman, UAE

2.2 Instruments and equipment

ICP-OES, Sequential Liberty AX (Varian, Victoria, Australia)

Equipped with SPS3 autosampler and used for the measuring heavy metal concentration in soil and plant with the manufacturer recommended conditions.

ICP-OES, iCAP 6300 (Thermo Scientific, Loughborough, UK)

The coolant flow is fixed at 12 L/min and the nebulizer gas is computer controlled from 0 - 1.5 L/min with increments of 0.1 L/min.

The procedure of analysis using ICP-OES is as follows [149]:

(i) Multi-element standard solutions (0.1, 1, 10, 50, 100 mg/L) containing the heavy metals Cd, Cu, Co, Cr , Mn ,Ni, Fe, Pb, Zn were prepared from 1000 ppm standard solutions by sequential dilution.

(ii) The wavelengths of the elements were directly selected by the software. Table (2-5) shows these wavelengths and the detection limit of each element.

Table (2-2) The Characteristic wavelengths of metal cations determined using ICP-OES.

Element	Line (nm)	Detection limit (ppm)
Cadmium (Cd)	214.439	1.5×10^{-3}
Copper (Cu)	324.754	2.0×10^{-3}
Cobalt (Co)	228.615	5.0×10^{-3}
Chromium (Cr)	267.716	4.0×10^{-3}
Manganese (Mn)	257.610	3.0×10^{-4}
Nickel (Ni)	231.604	5.5×10^{-3}
Iron (Fe)	238.204	1.5×10^{-3}
Lead (Pb)	220.353	1.4×10^{-3}
Zinc (Zn)	206.200	9.0×10^{-4}

(iii) The blank solution was prepared by adding 2 mL of 1: 1 (v/v) HNO_3 to 10 mL of 1:1 HCl and then the mixture was diluted by deionised water in 100 mL volumetric flask.

(iv) The standard solutions and the blank solution were analysed to calibrate the instrument before each analysis. The samples were then analysed to determine the concentrations of the metals (Cd, Cu, Co, Cr, Mn, Ni, Fe, Pb, and Zn).

UV- Visible spectrometer, HI 93723 (Hanna Instruments, Bedfordshire, UK).

Used to determine hexavalent chromium in plant and soil according to EPA method (3060A) [150]: The extracted chromium (VI) was reacted with 1, 5-diphenylcarbazide in the presence of sulfuric acid and analysed using UV- spectrometry at the wavelength of 540 nm.

Chromium (VI) was extracted from soil and plant samples according to the following procedure [150]:

(i) The temperature of the hot plate was adjusted so as not to exceed 95 °C, then 1.00g of dried soil or ground plant was placed into a clean and labelled 250 mL digestion vessel.

(ii) Fifty mL ± 1 mL of digestion solution (0.5 M NaOH + 0.28 M Na_2CO_3) were added to each sample using a graduated cylinder, then 400 mg of $MgCl_2$, followed by 0.5 mL of 1.0M phosphate buffer.

(iii) The samples were stirred continuously (unheated) for five minutes using magnetic stirrer. The samples were heated at 90-95 °C for 60 minutes with continuous stirring then gradually cooled to room temperature.

(iv) The contents were filtered through a 0.45µm membrane. The inside of the filter flask and filter pad were rinsed with reagent water and the filtrate and the rinses were transferred to a clean 250-mL vessel.

(v) The pH of the filtrate was neutralized to 7.0-7.5 range using concentrated nitric acid with continuous stirring to eject carbon dioxide from the solution. The neutralized filtrate was made up to 100 mL (in volumetric flask) using deionised water.

Ion Chromatography, 6005 Controller with 616 Pump and 717 Plus autosampler and conductivity detector 432 (Waters Ltd., Hertfordshire, UK)

Used for the determination of sulfate and other anions in plant and soil at the following conditions:

IC-Pak Anion HR 6.4X 75 mm column was used for anions. The number of the efficiency plates of column was 2500 which is recommended by manufacturer for major anions. The flow rate was controlled to be 1.0 mL/min. and running time was 16 minutes for each run.

HPLC Agilent 1100 – diode array detector with autosampler.

Used for the determination of the phytochelatin 3 and glutathione in fresh plant tissues with the following conditions:

The flow rate was adjusted to be 1.0 mL/minute and the column temperature was 30ºC. Twenty µL from either standards or samples were injected automatically into 250 x 4.6 mm Prodigy ODS (octadecyl 3) column. Absorption wavelength of detector was adjusted to 214 nm and the period of running for each sample was 15 minutes.

HPLC-MS, Ultra fast liquid chromatography (UFLC) XB (Shimadzu, Milton Keynes, UK) with Time of Flight Mass Spectrometer Micro TOFQ (Bruker Daltonics, Coventry, UK) with Electrospray Ionisation ESI

Used for the determination of ascorbic acid, dehydroascorbic acid, reduced and oxidised glutathione in fresh plant tissues at the following conditions:

The analytical column was Zorbax SB- C18, 5 µm 4.6 x 150 mm from Agilent. Column temperature was 20 ºC and the flow rate was 1.0 ml/min. The mass spectrometer was operated with endplate and spray tip potentials of 2.8 and 3.3 kV, respectively; in negative ion mode. Nitrogen (drying gas) pressure was kept at 30 psi. Spectra were acquired in the mass/charge ratio (m/z) range of 50- 3000.

Microwave Oven, QLAB 6000 (Questron Technologies Corp, Ontario, Canada)

Used for the digestion of plant and soil using the following procedure:

(i) A volume of 10 mL of 50% (v/v) nitric acid was added to 0.5 g of each dry, ground and sieved plant sample with 10 samples capacity in each run.

(ii) Programmed method for the plant tissue digestion was selected with a temperature of 170 ºC and 15 minutes of running time.

(iii) After samples became cool, filtration and dilution to 100 mL for each sample was carried out.

pH Meter, PerpHecT Basic Benchtop Model Orion 320 (Thermo- Orion, Loughborough, UK)

Used for measuring the pH of soil and irrigation solutions according to the following procedure:

The value of the pH of the soil was measured according to the EPA method (9045D) [151] accredited for measuring pH of soil. The procedure of this method is summarized as follows:

(i) Three composite 100g samples of soil were placed in three polyethylene plastic bottles. The electrode was calibrated using buffers at 4 and 7.

(ii) In a 50-mL beaker 20 mL of deionised water were added to 20 g of each soil sample. The beakers were covered with watch glass, and the produced suspension was continuously stirred for 5 min.

(iii) The soil suspension was allowed to stand for about two hours to allow most of the sand to settle out of the suspension, and then the solution was extracted using suction filtration. The electrode was lowered into the beaker containing the filtrate and immersed deep enough until readings were steady.

2.3 Quality Assurance

2.3.1 Uncertainty

Soil analyses were carried out using standard procedures and are reported with 95% confidence limits. The soil under study is unusual in being predominantly sand and carbonate with very little humus. There is no certified reference material for soil of similar composition. In accordance with current convention, metal analyses of soils are reported as pseudo-totals. However, as a major component of the soils studied is silica rather than silicates, along with calcium carbonate, the totals are likely to be close to actual values. Metal ion binding is likely to be dominated by the large excess of Ca^{2+} ions. Clay minerals are negatively charged and thus binding to anions such as chromate and dichromate will be very limited.

Plant analyses are reported with 95% confidence limits. Whilst replicate experiments with plants were carried out, results will be dependent upon growing conditions and true uncertainties will be greater than quoted statistical limits. This does not detract from any conclusions reached as these depend upon relative results from experiments carried out at the same time. Available reference materials are for rye grass and an aquatic plant, neither of which is similar to the *Portulaca* genus.

2.3.2 Statistical analysis

Statistical analysis was carried out using SPSS software (Version 15, SPSS UK Ltd., Woking, Surrey) with Microsoft Excel (Microsoft UK, Reading, Berkshire) being used for the preparation of the graphs and for some simple statistical operations.

Results are reported in tables with 95% confidence intervals $\overline{X} \pm \frac{t_{n-1}s}{\sqrt{n}}$

(where $\bar{X}$ is the average value, s is the standard deviation, n is the number of replicates which ranged between 3 and 6 in each experiment.)

In studies which involved replicate sets of experiments with a change in one variable, analysis of variance (ANOVA) between the means is used to identify if there are significant differences between means, followed post hoc by a Tukey test to give the significance (p-value) of each pair of values under different conditions. The values of p for each experiment are listed in the appendices.

2.3.3 Experiment design

Experiments were designed to reduce uncertainties as follows:

- Plants were propagated from the same origin by taking cuttings and then stem growing in order to control the genetic variability.
- Only those plants similar in growth (length and vegetation) were selected for each investigation.
- Plants used in each investigation were randomly distributed and grown under the same conditions of irrigation, soil components, temperature, light and nutrients. The only difference was the independent variable (such as sulfate concentration) which was intended to measure the change in the dependent variable which usually was chromium uptake.

- The sufficient amount of water was determined for each size of pot before irrigation with pollutant (Cr(VI)) in order to ensure that all of added solutions remain in the soil of the pot.

- All of irrigation solutions were prepared from chemicals of analytical grade and deionised water following the standard scientific procedure in preparing standard solutions.

2.3.4 Extraction of analytes

- Metaphosphoric acid and EDTA were used when ascorbic acid and protein were extracted from plants; the first can precipitate protein and behaves as antioxidant to ascorbic acid while EDTA forms chelates with heavy metals and deactivates the enzymatic activities which may destroy the protein structure.

- Plant samples were extracted at low temperature and low levels of light and were then frozen in liquid nitrogen and kept in a freezer at below -80ºC until the HPLC analyses.

- Fusion with sodium carbonate was used to extract chromium (III) from chromite since normal acid digestion was not effective.

- For heavy metal extraction using acidic digestion on a hot plate, long neck beakers or conical flasks were used to minimise loss of the digested sample. The temperature was adjusted not to exceed 90ºC to prevent any effervescence or vigorous boiling during the digestion. Heating and adding acid continued

until a clear transparent solution was observed and there was no NO_2 indicating complete digestion.

- When sulfur was determined in plants and soil, closed microwave acidic digestion were used to prevent any loss by volatility of sulfur oxides.

2.3.5 Instrumental analysis

- The environmental analytical methods were standard methods chosen from EPA, RSC etc.

- Standard reagents that used in calibration of analytical instruments were purchased with the purity recommended.

- Standards were freshly prepared just before each analysis and took into account the required conditions especially when preparing the standards of sulfur containing proteins or ascorbic acid which require low temperature and low levels of light which necessitated prior cooling.

- Conditions for instrument (e.g. UV-visible, ICP-OES, HPLC) operation followed the recommendation of manufacturers regarding flow rates of gases, choosing the determinate wavelength, separation columns, pH of solutions, purity of samplers and solutions and the manner of sample introduction.

- Standard addition was used to for calibration of the above instruments, taking in account that the curve should include the analyte concentration. Either

dilution or the preparation of further concentration standards were carried out to achieve this objective.

- Reversed phase conditions with gradient elution were selected in the two HPLC techniques since the eluents were polar (proteins and ascorbic acid) in order to get fast and good resolution.

- Electrospray ionisation technique was used when determination of sulfur containing proteins in the plants since this technique reduces the fragmentation of these proteins and can give real detection to the complete molecule of protein. In addition, this technique is appropriate and compatible with HPLC since the eluents can be aspirated in their liquid phase.

- Detection systems in both HPLC experiments were MS or diode array. The first is very sensitive and gives both qualitative and quantitative information about the eluents while the other gives the opportunity to check the determinands at more than one wavelength to achieve the best UV absorbance.

2.4 Exploring suspected polluted soils

Experiment (1)

Purpose: To find contaminated sites fit for the implementation of phytoextraction as a technique for heavy metal removal.

Steps

(i) Composite samples of soil were collected from tillage area of twelve suspected polluted sites. Most of these sites were located in East Mountains and east coast areas such as Kalba, Muzeera, Manama, Masfoot, Seiji, Bleeda, Dhaid, Bithna, Ajman Desert Masafi, Dadnah, and Ajman industrial zone. Figure 2-1 shows the sampled sites.

(ii) Soil samples were dried, ground, sieved, then digested in concentrated nitric acid and analysed using ICP-OES for the following heavy metals: Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn. Detailed steps are mentioned in section 2.7.1

(iii) To determine chromium in black sand in Dadnah and Kalba coasts, composite soil samples were taken from coasts of Kalba and Dadnah coast (opposite to Zikt chromite mines).

(iv) To extract chromium from chromite, samples were dried and then soda-ash roasted using sodium carbonate to extract chromium as soluble sodium chromate [152]. Figure 2-1 shows a map of the area of the study and illustrates the samples locations in this area.

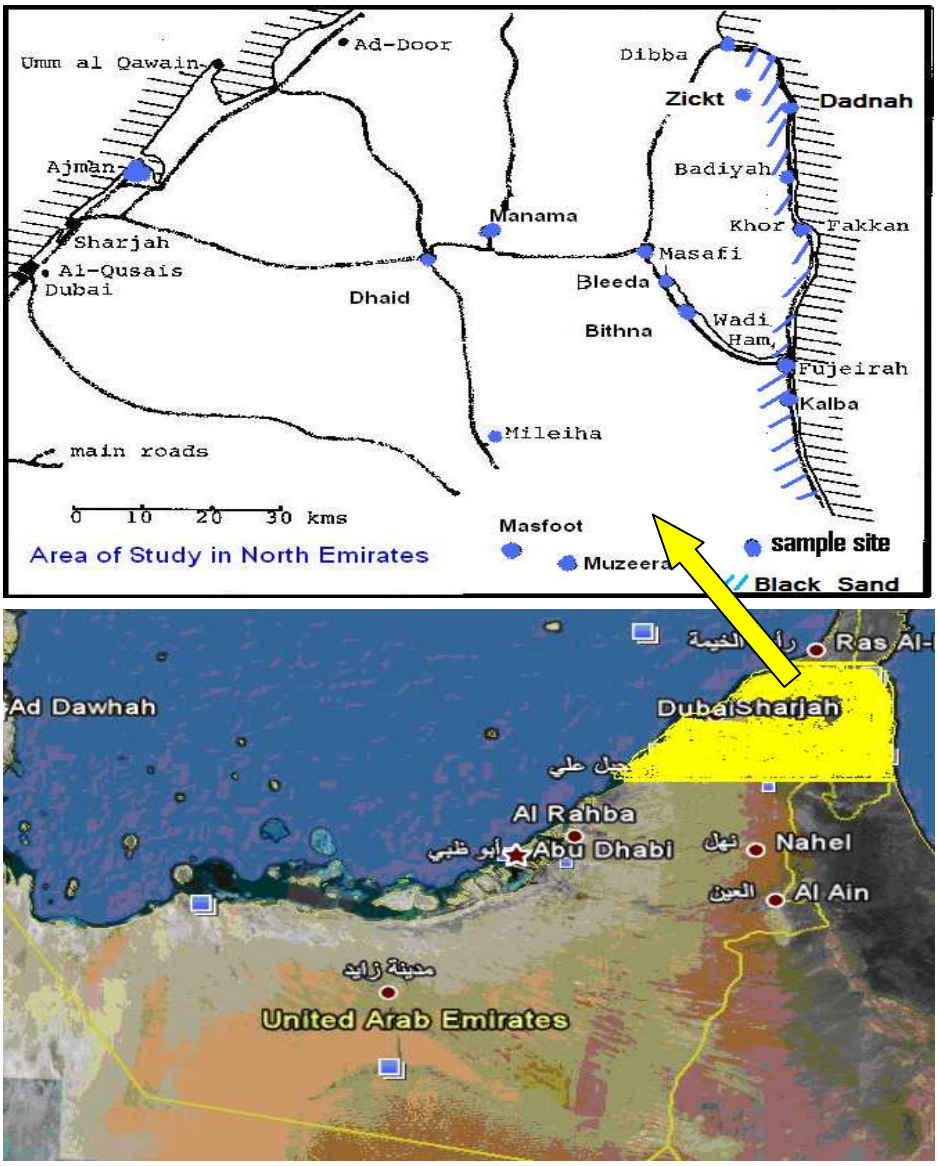


Figure (2-1) Map of location of soil sampling

Determination of the pseudo-total heavy metals in soil samples

During the investigation of the concentrations of the pseudo-total heavy metals the dry samples of soils were digested using concentrated nitric acid according to the following procedures:

Drying of Samples [153-155]

Samples of soil were dried in an oven at 60˚ C for 48 hours, then ground using mortar and pestle, and then passed though a 12-mesh (approximately 2 mm) screen.

Acidic digestion for determination total heavy metals

The following steps were followed in the digestion process of soil or plant samples [155]:

(v) One gram of dried, ground & sieved soil or plant was weighed into a 100 mL tall-form beaker (rinsed with concentrated HNO_3 before using). Nitric acid (30 mL, 1: 1 (v/v), 15 mL water + 15 mL concentrated HNO_3) was added and mixed with the sample.

(vi) The contents of the beaker were boiled gently on a hotplate until the volume was reduced to approximately 5 mL; a magnetic stirrer was used for stirring.

(vii) A further 10 mL of 1: 1 (v/v) HNO_3 were added, and then heating was repeated until a clear transparent solution was obtained. The beaker was then cooled and the contents were filtered through a Whatman no. 541 filter paper. The beaker and the filter paper were washed with successive small portions of 0.25 M HNO_3.

(viii) The filtrate was made up to 100 mL (in volumetric flask) using deionised water, and then analysed using ICP-OES to determine the pseudo-total heavy metals concentrations.

Extraction of chromium from chromite

Since digestion in nitric acid is not efficient in the extraction of chromium from chromite, a fusion technique (Soda-Ash Roasting method) was used. It depends theoretically on transforming the insoluble form of chromium in chromite [Cr (III)] to soluble sodium chromate according to the equation [152, 156]:

$$2FeCr_2O_4 + 4Na_2CO_3 + 3.5O_2 \longrightarrow 4Na_2CrO_4 + Fe_2O_3 + 4CO_2 \qquad (2\text{-}1)$$

Steps

(i) One gram of dried and sieved soil (or black sand) was weighed in clean clay crucible, and then mixed thoroughly with 2.5 g of dry (nonhydrated) sodium carbonate.

(ii) The mixture was heated for four hours in a furnace at 975 ◦C. The contents of the crucible were dissolved in nitric acid then transferred to a 50-mL beaker.

(iii) The residue was removed by washing with nitric acid and transferred to the beaker. The contents of the beaker were filtered and transferred to100-mL volumetric flask. The flask was made up to the mark using deionised water. The extract was analysed by ICP-OES.

Soil analysis of polluted site of Ajman industrial zone

The following analytical methods were used in sampling or analysing the soil of the polluted site of Ajman industrial zone:

(i) Five composite samples, each is about 1.0 kg were composed from 11 subsamples covering the site (from each acre, three subsamples were taken). Samples were taken from depth 0 to 30 cm in the last week of each month during the year.

(ii) Acid digestion for five dried soil samples was carried out using 50% nitric acid. Total chromium, other heavy metals (Fe, Mn, Ni, Co, Cu, and Pb) and major cations (Na, K, Ca, and Mg) were determined using ICP-OES.

(iii) In the aqueous extract of the soil, chromium (VI) was determined using UV-visible spectrophotometry. Concentrations of dissolved anions and metal cations were determined using ion chromatography and ICP-OES respectively. PHREEQC program was used for the prediction of the speciation of chromium (VI) in soil using the generated data.

(iv) Five soil samples of the site, each of 3 g were dissolved in 100 mL of deionised water, then were analysed for the dissolved major anions (Cl^-, NO_3^-, SO_4^{--}, and PO_4^{3-}) and chromate using ion chromatography.

(v) Total carbonate was determined by adding known volume of concentrated HCl to 5 grams of washed soil. The addition was with stirring until the evolution of carbon dioxide ceased. Back titration was carried out for the excess of HCl using sodium hydroxide.

(vi) Organic matter content of soil was determined using the back titration of the excess of added dichromate using ferrous ammonium sulfate.

2.5 Screening of local plants for potential heavy metal phytoextraction activity

Experiment (2)

Purpose: To identify natural accumulators for heavy metals growing in suspected polluted sites.

Steps:

(i) Local plants naturally growing within suspected polluted sites were sampled and analysed in order to recognize natural accumulators within these sites. Samples of shoots of the following 10 plants: Dactyloctenium aegyptium, Heliotropium calcareum, Pluchea arabica, Calotropis procera, Indigofera amblyantha, Asphodelus tenuifolius, Prosopis juliflora, Tamarix aucheriana, Euphorbia larica, and Cyperus conglomerates were washed, dried, weighed, ground, digested in concentrated nitric acid and analysed using ICP-OES for Cd, Co , Cr , Cu , Fe , Pb, Mn , Ni and Zn.

(ii) Additional samples of naturally growing plants were taken from other locations of the suspected area of East Coast (Zikt & Dadnah) which has mining activity of chromium ore (chromite). Further plant samples were taken from Ajman industrial zone site which contains a metal extrusion factory. This factory consumes and wastes chromic acid. Samples were

analysed to determine the concentration of the heavy metals in the plants of these areas.

(iii) Samples of plant species that are naturally growing in both normal clean and polluted areas (e.g. Prosopis juliflora) were sampled for comparison. Detailed analyses were carried out for different parts of Cyperus conglomerates (Stems, Leaves, Seeds, and Roots of) to identify the pollutant distribution in each part of the plant.

Experiment (3)

Purpose: To identify accumulator plants for heavy metals from natural and adapted desert plants.

Steps:

(i) Six plants were selected as satisfying the requirements of high level of vegetation and tolerance of soil salinity and semi-arid climate conditions. These plants were provided by the municipality of Ajman nursery and were: Portulaca oleracea, Bougainvillea spinosa, Atriplex halimus, Iresine herbestii, Pennisetum setaceum, and Azadirachta indica.

(ii) One hundred and twenty six plastic pots of 500-ml volume each were filled with synthesized soil consisting of 85% Ajman washed soil and 15% of potting soil which contained 70% organic matter. The total weight of each pot was 350 ± 30g. Three seedlings of each plant type were grown to get three replicates of each type. Each pot was irrigated with 70 mL of deionised water every 72 hours for a period of one month.

(iii) A blank solution was prepared using deionised water and nitric acid to adjust the pH to 5.5 ± 0.1 for irrigation of control experiments. Pollutant solutions were prepared using the nitrate salts of Pb^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} and Cr^{3+}. A solution of chromium (VI) was prepared using potassium dichromate. The pH of solutions was adjusted at the same pH as that of the blank solution.

(iv) Three replicates of each one of the six plants in the experimental samples were irrigated with the synthesized heavy metals solutions. Each pot received about 700 mL of 50 ppm of one of the above heavy metal solutions in ten doses (10 x 70 mL) during one month. For each plant three pots were irrigated by 700 ml of acidified deionised water as control replicates.

(v) After the one month period of irrigation, plants were harvested, washed, dried, weighed, ground, digested in concentrated nitric acid and analysed using ICP-OES for lead, copper, cobalt, nickel and total chromium.

Experiment (4)

Purpose: To investigate the potential of typical desert plants including Prosopis species as suggested accumulators for lead and chromium (VI) from soil of UAE.

Steps:

(i) Two types of mesquite **(**Prosopis cineraria & Prosopis juliflora) were propagated using stem cuttings.

(ii) Twenty pots were prepared by mixing 85% washed soil from Ajman (Al-Jurf area) and 15% potting soil (70% of its composition is organic matter). The total weight of each pot was about 3000 ±100 gram. Ten replicates of each plant were prepared by growing 3 seedlings from each plant in each pot.

(iii) Plants were irrigated by deionised water for three months where the plants became about 30 centimetres in height. Nine successful pots from each plant were chosen and divided into three groups. The first three were irrigated by deionised water acidified with nitric acid to pH 5.5 ± 0.1 to match the pH of the other heavy metal nitrate solutions. The second group was irrigated by 100 ppm Pb(II) as lead nitrate, and the third one by 100 ppm of Cr(VI) as potassium dichromate (pH also was 5.5± 0.1 for each). Period of irrigation was 30 days for each group.

(iv) For 30 days each pot was irrigated with 7500 mL of either blank (acidified water as control), or Pb(II) or Cr(VI) (each dose = 500 mL of 100 ppm solution). The plants were harvested, rinsed, dried, weighed, ground, digested in concentrated nitric acid and analysed using ICP-OES for lead and chromium.

Determination of total heavy metals in plants

Samples of plants were put in Petri dishes, dried in an oven at 65 ˚C for 48 hours, then ground using mortar and pestle and then sieved and digested in 50% v/v nitric acid as follows:

(i) One gram of dried, ground and sieved plant was weighed into a 100 mL tall-form beaker (rinsed with concentrated HNO_3 before using). Nitric acid (30 mL, 1: 1 (v/v), 15 mL water + 15 mL concentrated HNO_3) was added and mixed with the sample.

(ii) The contents of the beaker were boiled gently on a hotplate until the volume was reduced to approximately 5 mL; a magnetic stirrer was used for stirring.

(iii) Further 10 mL of 1: 1 (v/v) HNO_3 were added, then heating was repeated until a clear transparent solution was obtained. The beaker was then cooled and the contents were filtered through a Whatman no. 541 filter paper. The beaker and the filter paper were washed with successive small portions of 0.25 M HNO_3.

(iv) The filtrate was made up to 100 mL (in volumetric flask) using deionised water, and then analysed using ICP-OES to determine the total heavy metals concentrations.

2.6 Factors that may affect the uptake of Cr(VI) by Portulaca oleracea

The following experimental design aims to investigate the factors that may affect the uptake of Cr(VI) by P. oleracea in order to find out the best conditions that may maximise the uptake of Cr(VI) by this accumulator plant. Concentrations of

Cr(VI) in soil, pH and organic content of soil, nutrient anions of soil, accompanying cations, and chelating agents are investigated.

2.6.1 Investigation of the effect of concentration of chromium (VI) on its uptake by Portulaca oleracea

Experiment (5)

Purpose: To investigate the effect of concentration of Cr(VI) in soil on the uptake of this pollutant by Portulaca oleracea .

Steps:

(i) Forty pots of 85% (v/v) of normal clean soil from Ajman desert in the United Arab Emirates and 15% (v/v) of potting soil (contains 70% organic matter) were prepared. The total mass of soil in each pot was 1500 ±100 g.

(ii) In each pot 5 stems of Portulaca oleracea were propagated by cuttings and irrigated by deionised water.

(iii) Using a stock solution of 10,000 ppm of chromium (VI) (as Na_2CrO_4) and deionised water, solutions with the following concentrations were prepared: 0, 50, 100, 150, 200, 250, 300, 350, and 400 ppm of Cr(VI). The volume of each solution was 5 litres and the pH of each solution was adjusted to 8.0 ± 0.1.

(iv) Once there was considerable vegetation in each pot (usually 60 days of growing) 27 pots were chosen according to the best vegetation. Pots were irrigated by each of the nine concentrations of Cr(VI) in triplicate. After 10

doses of irrigation by pollutant solutions each dose equals 150 mL, the plants were harvested, washed by deionised water and divided into; leaves, stems, and roots.

(v) Samples of plants were dried in an oven at 65 °C for 48 hours, then weighed and ground using mortar and pestle. The samples were digested in two different ways: nitric acid digestion to determine the total chromium and alkaline digestion to determine the Cr(VI) in each sample. The detailed steps of each digestion are described in sections 2.7.4 and 2.7.5 of this chapter.

(vi) Composite soil samples were taken from each pot, dried, sieved, digested and three replicates from each sample were analysed for chromium (VI) and total chromium.

2.6.2 Effect of pH of soil on the uptake of chromium (VI) by Portulaca oleracea

Experiment (6)

Purpose: To determine the most appropriate pH of soil which maximises the uptake of Cr (VI) by Portulaca oleracea.

Steps:

(i) Six groups of pots (each consisting of twelve pots) of different pH soil values were prepared. Table (2-1) shows the composition of each group.

(ii) Values of pH of soil were varied and set at 6.0, 7.0, 7.3, 7.6, 8.0, 9.0 ± 0.1. The total weight of each pot was 1200 ±100g. Buffer solutions were not used in order not to add anions or cations which may alter the uptake of Cr(VI). During the experiment periodic check of soil pH was carried out in order to maintain its constancy.

(iii) Four Seedlings of Portulaca oleracea were grown in each pot and were irrigated by deionised water for three weeks. The temperature ranged from 25ºC at night to 35ºC by day.

(iv) Forty five litres of 200 ppm chromium (VI) were prepared by diluting 10,000 ppm of Cr(VI) as sodium chromate solution which was used as irrigation solution. The thirty litres were divided into six solutions with pH range 6.0, 7.0, 7.3, 7.6, 8.0, and 9.0 matching the pH values of each type of soil (HCl and CaO were used in adjusting the pH of irrigation solutions).

(v) When considerable vegetation and rooting growth were observed at each level of pH, the group of twelve was divided into two six pots groups. The first six were irrigated with deionised water for control and the other six were irrigated with 1200 mL of 200 ppm Cr(VI) at six doses over 12 days (note that each soil was irrigated with Cr(VI) solution of the same pH).

(vi) Each plant was harvested, washed, divided into roots and shoots, and dried at 65°C for 48 hours, then weighed and ground using mortar and pestle.

Sieved and ground samples of plant and soil were digested using 50% HNO_3, then analysed using ICP-OES for total chromium.

(vii) Composite soil samples from each pot were collected, dried and analysed for total chromium.

Table (2-3) Soil composition at different values of pH.

pH of soil	Soil Composition
6.0 ± 0.1	Pure silica + 10% potting soil
7.0 ± 0.1	85 % Pure silica+5 % sand of Ajman(contains 42 % $CaCO_3$) + 10% potting soil
7.3 ± 0.1	80% Pure silica+10 % sand of Ajman(contains 42 % $CaCO_3$) + 10% potting soil
7.6 ± 0.1	75% Pure silica+15 % sand of Ajman(contains 42 % $CaCO_3$) + 10% potting soil
8.0 ± 0.1	90% sand of Ajman (contains 42%$CaCO_3$) + 10% potting soil
9.0 ± 0.1	90% sand of Ajman (contains 42%$CaCO_3$) + 10% potting soil+ > 0.1 % CaO

2.6.3 Effect of organic content of soil on the uptake of chromium (VI) by Portulaca oleracea

Experiment (7)

Purpose: To investigate the effect of organic content of soil on the uptake of Cr(VI) by P. oleracea.

Steps:

(i) Three types of soil were prepared. Table 2-2 shows the components of each type and the organic matter content.

Table (2-4) Organic matter in the soils and the components of each type

No	Components of soil	% organic content
1	50% potting soil(wt/wt) + 50% Clean soil of Ajman	35% ± 0.5%
2	25% potting soil(wt/wt) + 75% Clean soil of Ajman	17.5% ± 0.5%
3	100% Clean soil of Ajman	0.42% ± 0.02%

(ii) The normal clean soil from Ajman, which has no detectable chromium (VI) content, was used either pure or mixed with the potting soil (Blumen Erde – Germany -of total organic content of 70%).

(iii) The organic content (%) in the three types of soil was determined by back titration of the excess of potassium dichromate using ferrous ammonium sulfate. The pH of the three types of soil was measured and was in the same range (7.9 ± 0.1) because of the high content of carbonate in the three types.

(iv) From each type of soil 10 pots of 2 kilograms were prepared. Four seedlings of Portulaca oleracea were grown in each pot. Equal quantities of deionised water were used to irrigate the pots for four weeks until considerable vegetation and rooting were achieved.

(v) Fifteen litres of 200 ppm of Cr(VI) as sodium chromate solution were prepared. Each pot was irrigated with 1.6 litre of chromate solution over a period of ten days (8 doses, each 200 mL). Another 15 pots (5 from each

type of soil) were irrigated with the same quantity of deionised water as control groups.

(vi) The plants were harvested, washed by deionised water, dried at 65º C for 48 hours, weighed, ground using mortar and pestle then sieved. Soil samples were taken from each pot and dried in the same oven at the same temperature.

(vii) Dried samples of about 1.0 g of shoots and 0.4 g of roots were digested using 50% HNO_3. Total chromium in shoots, roots and soil was determined using ICP-OES.

(viii) Chromium (VI) in soil was determined using UV-Visible spectrometry.

Determination of organic content of soil

The total organic matter of soil was determined as reported in [155] with some modifications. The procedures of this method are summarised as follows:

(i) An amount of 0.5g of air-dried and sieved soil was weighed and placed in conical flask. A volume of 10 mL of 0.083 M $K_2Cr_2O_7$ standard solution was added and swirled to be mixed with the sample.

(ii) A volume of 15 mL of concentrated H_2SO_4 was added carefully and drop-wise with gentle swirling to mix and to get rid of the generated heat. The conical flask was connected to the condenser, then cool water was turned on. The open end of the condenser was covered with a small beaker, then the apparatus was fixed on hot plate for 1 hour.

(iii) After cooling, the condenser was rinsed down with deionised water and the water was collected in the flask. The condenser was disconnected from the flask, then 100 mL of deionised water were added. Five drops of ferroin indicator [$FeSO_4.7H_2O$ and 1, 10-phenanthroline monohydrate ($C_{12}H_8N_2.H_2O$) in water] were added.

(iv) The mixture was titrated with ferrous ammonium sulfate. After the colour changed from blue-green to violet-red indicating to the end point, a blank solution (without the soil) was titrated in the same way.

(v) The organic carbon (mg/g), organic carbon (%) and organic matter (%) in soil were calculated from the following equations:

$$\text{Organic Carbon (mg/g)} = \frac{18 \times C \times V \times (1 - V_1/V_2)}{M}$$

Where:

C is the molar concentration of the $K_2Cr_2O_7$ solution (0.083M), while **V** is the added volume of that solution (10 mL). $\mathbf{V_1}$ is the volume of ferrous ammonium sulfate used up in the sample titration (mL), $\mathbf{V_2}$ is the volume of the same titrant used up in the titration of blank (mL), **M** is the sample weight (g).

The organic carbon in % was calculated as:

$$\text{Organic Carbon (\%)} = \frac{\text{Organic Carbon (mg/g)}}{10}$$

(vi) Carbon content represents 58% of the soil organic matter [155], therefore the organic matter (%) was calculated according to the following relation :

$$\text{Organic Matter(\%)} = 1/0.58 \times \text{Organic Carbon(\%)}$$

2.6.4 Effect of nutrient anions on the uptake of chromium (VI) by Portulaca oleracea

Experiment (8)

Purpose: Investigation of the effect of some nutrient anions such as nitrate, sulfate and phosphate on the uptake of Cr(VI) by Portulaca oleracea.

Steps:

(i) Forty identical pots were prepared; each containing 1300 ±100 g of soil with 15% as potting soil (contains 70% as organic matter). The other component was normal sandy soil of Ajman area of pH of 7.9 ± 0.1.

(ii) Three Seedlings of P. oleracea, originally propagated by cuttings, were grown in each pot. After one month of growing and irrigation with deionised water, twenty five pots (5 groups of five replicates) were labelled and irrigated with one of the following solutions:

1- 1600 mL of 100 ppm of Cr(VI) as Na_2CrO_4 (for the control experiment).

2- 1600 mL of 100 ppm of Cr(VI) as Na_2CrO_4 + 0.02M of $NaNO_3$.

3- 1600 mL of 100 ppm of Cr(VI) as Na_2CrO_4+ 0.02M of Na_2SO_4.

4- 1600 mL of 100 ppm of Cr(VI) as Na_2CrO_4+ 0.02M of Na_3PO_4.

5- 1600 mL of deionised water.

(iii) The five groups of 5 replicates each received 8 doses of one of the previous five irrigation solutions (200 mL for each dose) through a period of 24

days. Temperature was in the range of 25 ± 3°C by day and 20 ± 3 °C at night.

(iv) Three other groups (5 pots each) were irrigated with the same quantity (0.02M, and 1600 mL) of nitrate, sulfate or phosphate only as control groups.

(v) After 24 days of irrigation the plants were pulled out of the soil using water stream to remove the wedged soil among the roots. The plants were rinsed with deionised water and the length of the roots was measured for each plant as an indicator of the growth of the plant. The plants were divided into leaves, stems, and roots. Samples of plant were dried in an oven at 65 °C for 48 hours, weighed, ground using mortar and pestle, sieved, digested in 50% nitric acid, then analysed using ICP-OES to determine the total chromium in both shoots and roots.

(vi) Before harvesting, composite soil samples were taken from each pot. Each sample was dried at 65 °C for 48 hours, sieved, digested in 50% nitric acid, then analysed using ICP-OES to determine chromium in soil.

2.6.5 Effect of sulfate on the uptake of chromate by Portulaca oleracea.

The results of the previous investigation (section 2.6.4), especially those of sulfate, called for further investigation. Therefore, detailed experiments of the effect of sulfate ion in soil on the uptake of chromate by Portulaca oleracea were carried out. Two concentrations of Cr(VI) were used, the first is 200 mg/kg of dry soil matching the concentration of pollutant in soil at which the best chromium removal was

achieved. The second concentration of Cr(VI) in soil was 100 mg/kg (half of the first one) matching the concentration of Cr(VI) in the contaminated site of Ajman. The chromium: sulfur ratio stayed constant in the two experiments. Detailed steps of both experiments are reported below in Experiments 9 and 10.

Experiment (9)

Steps:

(i) Thirty identical pots were prepared, each one containing 1500 ±100 g of synthetic soil consisting of 15% v/v potting soil and 85% of normal sand of the emirate of Ajman.

(ii) Chemically pure sodium chromate and sodium sulfate from Panreac were used to prepare 1000 ppm stock solutions of both hexavalent chromium as chromate and sulphur as sulfate which were used in the preparation of the irrigation solutions.

(iii) When considerable vegetation and rooting growth were observed each pot was irrigated with one of the solutions in Table (2- 3) during a period of two weeks at six doses.

Table (2- 5) Concentrations of components of irrigation solutions in the presence of 200 ppm of Cr(VI).

Components and Concentration	Number of Pots	Volume of each dose	Final Volume of each pot
Deionised water (Control)	5	250 mL	1500 mL
200 ppm Cr (VI) as Na_2CrO_4	5	250 mL	1500 mL
200 ppm Cr (VI) as Na_2CrO_4 + 300 ppm sulfate as Na_2SO_4	5	250 mL	1500 mL
200 ppm Cr (VI) as Na_2CrO_4 + 600 ppm sulfate as Na_2SO_4	5	250 mL	1500 mL
200 ppm Cr (VI) as Na_2CrO_4 + 1200 ppm sulfate as Na_2SO_4	5	250 mL	1500 mL
200 ppm Cr (VI) as Na_2CrO_4 + 1800 ppm sulfate as Na_2SO_4	5	250 mL	1500 mL

(iv) The plants were harvested, separated into roots and shoots, washed, dried, and then digested using microwave acid digestion. Ion chromatography was used to determine total sulfur as sulfate, and ICP-OES was used to determine total chromium in each sample of plant.

Experiment (10)

Steps:

(i) Thirty identical pots were prepared, each one containing 1.2 kg of synthetic soil consisting of 15% v/v potting soil and 85% of normal shore sand treated with calcium carbonate to reach the pH of 7.9 ± 0.1.

(ii) Four seedlings of Portulaca oleracea were germinated in each pot and were irrigated with deionised water for four weeks. The temperatures were controlled at 25 ºC at night and at 35ºC at the daytime in an incubator.

(iii) Chemically pure sodium chromate and sodium sulfate (Panreac Spain) were used to prepare 1000 ppm stock solutions of both hexavalent chromium as chromate and sulfur as sulfate, which were used in the preparation of the irrigation solutions.

(iv) When considerable vegetation and root growth were observed, each pot was irrigated with one of the solutions in Table (2- 4) during a period of two weeks at six doses.

(v) The plants were harvested, separated into roots and shoots, washed, dried, then digested using microwave acid digestion. ICP-OES was used to determine total chromium and total sulphur in each sample of plant.

Table(2- 6) Concentrations of components of irrigation solutions in the presence of 100 ppm of Cr(VI).

Components and Concentration	Number of Pots	Volume of each dose	Final Volume for each pot
Deionised water (Controlled Experiments)	5	200 mL	1200 mL
100 ppm Cr (VI) as Na_2CrO_4	5	200 mL	1200 mL
100 ppm Cr (VI) as Na_2CrO_4 + 150 ppm sulfate as Na_2SO_4	5	200 mL	1200 mL
100 ppm Cr (VI) as Na_2CrO_4 + 300 ppm sulfate as Na_2SO_4	5	200 mL	1200 mL
100 ppm Cr (VI) as Na_2CrO_4 + 600 ppm sulfate as Na_2SO_4	5	200 mL	1200 mL
100 ppm Cr (VI) as Na_2CrO_4 + 900 ppm sulfate as Na_2SO_4	5	200 mL	1200 mL

Determination of sulfur as sulfate in plant and soil samples using ion chromatography

To determine the total sulfur as sulfate in plant and soil samples, an ion chromatography machine (6005 Controller and 616 Pump from Waters with 717 Plus autosampler from Waters) was used. Conductivity detector 432 from Waters was used as detecting system for the eluted ions. In the following steps, a brief description of the method is given [157]:

(i) Four standards of sulfur as sulfate of the concentrations 1.0, 10, 50, and 100 ppm were prepared by dilution of 1000 ppm of sulfate stock solution.

(ii) IC-Pak Anion HR 6.4X 75 mm column was used for anions. The number of the efficiency plates of column was 2500 and it was recommended for sulfate anions. Borate /Gluconate mobile phase was used with flow rate of 1.0 mL/min. The running time was 16 minutes for each run.

(iii) The microwave digested samples of both shoots and roots were diluted at 1: 20 dilution factor to reduce the effect of the acidic medium of acid digestion on the stationary phase of column. Sample solutions were filtered, then analysed using the ion chromatography.

(iv) Five samples of 3.0 g of each type of soil were suspended in 100mL of deionised water, then stirred using magnetic stirrer for two hours then filtered. The available sulfate in soil before and after irrigation was determined using the same technique and same conditions of analysis.

Borate /Gluconate mobile phase preparation

Five litres of Borate /Gluconate mobile phase were prepared as reported in [158, 159] with some modifications:

(i) A volume of 100 mL of concentrated borate /gluconate solution was prepared by dissolving 1.8 g of boric acid, 1.6 g of sodium gluconate and 2.5 g of sodium tetra borate decahydrate in 50 mL of ultra pure water.

(ii) A volume of 25 mL of glycerine was added to the solution then the mixture was completed to the mark using ultra pure water.

(iii) Of the previous concentrated borate /gluconate, 100mL solution was added to a 5 litres volumetric flask, then 100 mL of n-butanol HPLC grade was added to the solution.

(iv) A volume of 600 mL of acetonitrile HPLC grade was added, and the flask was completed to the mark using ultra pure water. The mobile phase was then filtered through glass fibre membrane.

2.6.6 Effect of accompanying cations on the uptake of Cr(VI) by Portulaca oleracea

Experiment (11)

Purpose: Investigation of the effect of cations of sodium, potassium and ammonium as accompanying cations on the uptake of Cr(VI) by Portulaca oleracea.

Steps:

(i) Twenty identical pots were prepared, each of 1300 ±100 g of soil containing 15% as potting soil (with 70% of organic matter). The make up component is normal sandy soil of Ajman area of pH of 7.9 ± 0.1. Three Seedlings of Portulaca, originally propagated by cuttings were grown in each pot.

(ii) After one month of growing and irrigation by deionised water, each pot was labelled and irrigated with one of the following solutions.

1- 1600 mL of 100 ppm of Cr (VI) as Na_2CrO_4.

2- 1600 mL of 100 ppm of Cr (VI) as K_2CrO_4.

3- 1600 mL of 100 ppm of Cr (VI) as $(NH_4)_2CrO_4$.

4- 1600 mL Deionised water (for the control).

(iii) The four groups (of 5 replicates each) received 8 doses (of 200 mL each) of one of the previous four irrigation solutions through a period of 24 days. Temperature was in the range of 25 ± 3°C by day and 20 ± 3 °C at night.

(iv) After 24 days of irrigation the plants were pulled out of the soil using water stream to remove the wedged soil among the roots. The plants were rinsed by deionised water and the length of the roots was measured for each plant as an indicator of the growth of the plant. The plants were divided into leaves, stems, and roots.

(v) Samples of plant were dried in an oven at 65 °C for 48 hours, ground using mortar and pestle, sieved, digested in 50% nitric acid then analysed using ICP-OES to determine the total chromium in both shoots and roots. Before harvesting, composite soil samples were taken from each pot. Each sample was dried at 65 °C for 48 hours, sieved, digested in 50% nitric acid then analysed using ICP-OES to determine chromium in soil.

2.6.7 Effect of chelating agents on the uptake of Cr (III) and Cr(VI)

Chelating agents such as EDTA and citric acid may enhance the uptake of heavy metal cations such as Cr(III). Anionic species such as chromate or dichromate

are most unlikely to be chelated in their anionic form but since Cr(VI) can be reduced to Cr(III) in root cells then translocated to shoots as Cr(III) cations, therefore, it is interesting to investigate the effect of chelating agents on the uptake of Cr(VI). In the next two experiments the effect of EDTA and citric acid on the uptake of Cr(III) and Cr(VI) was investigated.

2.6.7.1 Effect of chelating agents on the uptake of Cr (III)

Experiment (12)

Purpose: To investigate the effect of chelating agents like citric acid and EDTA on the uptake of Cr (III) and by Portulaca oleracea.

Steps:

(i) Twelve pots of the same volume, weight and composition were prepared. Each pot was made to contain 85% of washed sand and 15% of potting soil (70% of it is organic matter); the total weight of each pot was 500 ± 50g.

(ii) The pH of soil of each pot was measured and it was 5.5 ± 0.1. Chromium (III) is soluble and available for plants at this relatively low pH.

(iii) Three seedlings of Portulaca oleracea were grown in each pot and irrigated with deionised water for two weeks. The plants were incubated in a special incubator at 12 hours of light and 35 °C; the other 12 hours were controlled at 25°C and darkness. These conditions of relatively high temperature were designed to mimic the hot climate in the Arabian Gulf countries where Portulaca is a native plant.

(iv) Sets of three pots were irrigated with one of the following solutions: 100 ppm of Cr(III) as chromium (III) nitrate, 100 ppm of Cr(III) as chromium (III) nitrate + 0.01 M of EDTA, 100 ppm of Cr(III) as chromium (III) nitrate + 0.01 M of citric acid or deionised water for the control. Each pot was irrigated with 500 mL of one of the previous solutions as 10 doses (50 mL for each) through a period of 20 days.

(v) After this period each plant was harvested, washed, divided into roots and shoots, and dried at 65°C for 48 hours then, ground using mortar and pestle. Samples of plant and soil were digested using 50% HNO_3 and analysed using ICP-OES for total chromium.

2.6.7.2 Effect of chelating agents on the uptake of Cr (VI)

Experiment (13)

Purpose: To investigate the effect of chelating agents such as citric acid and EDTA on the uptake of Cr(VI) by Portulaca oleracea.

Steps:

(i) Twenty pots of the same volume, weight and composition were prepared. Each pot was made to contain 85% of washed shore sand and 15 % of potting soil (70% of it is organic matter); the total weight of each pot was 1200 ± 100g. The pH of soil of each pot was measured and it was 7.8 ± 0.1.

(ii) Three seedlings of Portulaca oleracea were grown in each pot and irrigated by deionised water for two weeks. The plants were incubated for 12 hours of light

at 40 °C and 12 hours at 25°C and darkness. These conditions of relatively high temperature were designed to mimic the climatic conditions of the Arabian Gulf countries where Portulaca was originally grown.

(iii) Every five pots (as group of five replicates) were irrigated with one of the following solutions: 100 ppm of Cr(VI) as sodium dichromate, 100 ppm of Cr(VI) as sodium dichromate + 0.01 M of EDTA, 100 ppm of Cr(VI) as sodium dichromate + 0.01 M of citric acid or acidified deionised water for the control. The pH of the three solutions was adjusted to 5.5 ± 0.1 by dropping small portions of HNO_3 or NaOH.

(iv) Each pot was irrigated with 1200 mL of one of the above solutions for a period of 2 weeks, after that each plant was harvested, washed, divided into roots and shoots , dried at 65°C for 48 hours then ground using mortar and pestle. Samples of plant and soil were digested in microwave using 50% HNO_3 then analysed using ICP-OES for chromium.

2.7 Effect of chromium(VI) on the concentration of sulfur containing proteins and ascorbic acid in P. oleracea.

This part of the investigation is devoted to the understanding of the biochemistry of chromium in P. oleracea tissues, in particular the postulated reducing agents that may reduce Cr(VI) to Cr(III) and the postulated ligand that may transport the complexed chromium(III) from roots to shoots. Glutathione in plants is a reducing agent and at the same time, it is the essential unit in the building of the natural plant ligands (phytochelatins). Ascorbic acid is found in P. oleracea and is a likely Cr (VI)

reductant. It was thus necessary to use two methods of extraction. The first discussed in section 2.7.1 was specifically for the extraction of antioxidants (reducing agents) and the second for the extraction of PC3 phytochelatins in section2.7.2. Since glutathione is essential in the two processes it was determined in both.

2.7.1 Effect of chromium (VI) on the concentration of ascorbic acid and glutathione as antioxidants in Portulaca oleracea

Experiment (14)

Purpose: To investigate the effect of Cr(VI) concentration in soil on the concentration of ascorbic acid and glutathione as antioxidants in Portulaca oleracea tissues.

Steps:

Plants growing:

(i) Pure shore sand was washed and enriched by 15 % as potting soil (contains 70% as organic matter). The pH of the soil was amended to 7.9 ± 0.1 using calcium carbonate to match the soil of UAE.

(ii) Twelve pots were prepared; each containing 1000 ± 50 g of the above synthesised soil. Four seedlings of Portulaca oleracea (20 cm in length and originally propagated by cuttings from the same plant in Ajman municipality nursery, UAE) were grown in each pot. Seedlings were irrigated with deionised water and grown in an incubator at 25ºC in light and 35ºC in darkness for three weeks.

(iii) After considerable vegetation was observed, nine successful pots were selected and divided into three groups of triplicate. The first was irrigated with 50 ppm of chromium (VI) as sodium chromate over a period of two weeks. The chromate solution was introduced to the plants as 5 doses of 200 mL.

(iv) The second group was irrigated with the same volume and doses but with 100 ppm of chromium (VI). And the third group was irrigated with deionised water for control.

(v) After two weeks of irrigation the plants were cleaned from soil using cold water stream, washed with deionised water, divided into shoots and roots.

(vi) The fresh plant tissues were sampled for extraction and the rest of the plant tissues were dried, weighed ground, digested in nitric acid using microwave and analysed using ICP-OES for chromium.

Extraction of antioxidants (reduced and oxidised) from plant tissues

The extraction process was carried out according to the method described in previous studies [160 -161] with some modification as follows:

(i) Fresh plant tissues were weighed (200-300 mg of roots and 500-700 mg of shoots) then frozen in liquid nitrogen.

(ii) One litre of extraction solution (5% (w/v) metaphosphoric acid (MPA) and 1 mM EDTA in 0.1% formic acid) was prepared using ultra pure water then was filtered.

(iii) The frozen samples were ground using cool mortar and pestle. Then 1.0 mL of cool extraction solution added to the ground plant tissue accompanied with 1% (m/v) polyvinyl-polypyrrolidone (PVPP).

(iv) The suspension of ground plant in extraction solution was placed in hard plastic cryovials with stoppers, and then centrifuged at 15,000g for 20 min at 4 °C.

(v) Supernatants were collected, then residues were suspended with 200 –300 µl of the same extraction solution and centrifuged again under the same conditions. The second supernatant obtained was combined with the first and taken to a final volume of 2 ml with extraction solution. The supernatants were filtered through cellulose acetate membrane. The extracted samples were frozen in liquid nitrogen and stored at −80 °C until analysis [160].

HPLC-MS Analysis

(i) Chemicals of standards of glutathione, glutathione oxidised, ascorbic acid and dehydroascorbic acid were purchased from Sigma Aldrich. Solutions of 50 mL volume from the 4 standards were prepared using the same extraction solution used in the above procedures. Concentration of each standard was 1000 ppm which was diluted to prepare different concentrations used in preparing the calibration curve for each standard.

(ii) The mobile phase was prepared for reversed phase using two solvents: A (filtered solution of 0.1% formic acid (HPLC grade) in ultrapure water) and B (0.1% formic acid in acetonitrile). Gradient elution method was programmed

on the machine of ultra fast liquid chromatography, UFLC XB, from Shimadzu.

(iii) The mobile phase composition was changed on a period of 15 minutes according to the following percentages of A and B which was described in a previous study [160]: a linear gradient from 0 to 10% B (0–5 min) was used. Then, to wash the column, the concentration of B was increased linearly from 10 to 50% from 5 to 6 min, and this solvent composition was maintained for 9 min. Finally, to regenerate the column, the solvent was changed linearly to 0% B for 11 min, and then was maintained at 0% B until 15 minutes, when a new sample could be injected.

(iv) The analytical column was Zorbax SB- C18, 5 µm 4.6 x 150 mm from Agilent. Column temperature was 20 ºC and the flow rate was 1.0 ml/min. The detector was a time of flight mass spectrometer micro TOFQ from Bruker Daltonics with electrospray ionisation ESI.

(v) Both standards then samples were introduced using the autosampler. From each sample 20-µl were injected. After 1.8 min. from sample injection the exit flow from the column was introduced to the ESI interface of the MS (TOF) apparatus using a T-connector. This precaution was observed to reduce the effect of metaphosphoric acid MPA on the ionisation process since it is being eluted in the first 1.5 minutes.

(vi) The mass spectrometer was operated with endplate and spray tip potentials of 2.8 and 3.3 kV, respectively; in negative ion mode. Nitrogen (drying gas)

pressure was kept at 30 psi. Spectra were acquired in the mass/charge ratio (m/z) range of 50- 3000. The highest molecular weight of required analyte was glutathione oxidised (612.2), but the range of scanning was expanded to 3000 to observe the dimers and polymers of the required analytes.

2.7.2 Effect of chromium(VI) on the formation of PC3 phytochelatins and glutathione and in Portulaca oleracea.

Experiment (15)

Purpose: To investigate the effect of Cr(VI) concentration in soil on the concentration of glutathione and PC3 phytochelatins as sulfur containing proteins in Portulaca oleracea tissues.

Steps:

Plants growing:

Portulaca oleracea was grown and irrigated using Cr(VI) as mentioned in the previous investigation (experiment 14).

Extraction of phytochelatins and glutathione and HPLC method

Detailed steps of the method are mentioned in Hunaiti et al. [162] and in the following they are reported briefly with some modifications

(i) Fresh plant samples (1 g of shoots or 0.5 g of roots) were immersed in liquid nitrogen then ground using a blender. Glutathione and phytochelatins were extracted using 60% perchloric acid (2 mL per g fresh weight).

(ii) Homogeneous mixtures were vortexed then centrifuged at 13,000g for five minutes then the supernatents were filtered through cellulose acetate

membrane. 100 µL of each sample were transferred to autosampler vials of Agilent 1100 HPLC system with diode array detector.

(iii) Reversed phase gradient elution conditions were applied using two solvents as mobile phase (solvents A and B). A consisted of 0.1% trifluoroacetic acid (TFA) and solvent B was 80% of acetonitrile in 0.1% TFA (v/v). The flow rate was adjusted to be 1.0 mL/minute and the column temperature was 30ºC.

(iv) Chemically pure glutathione (GSH) and PC3 phytochelatins were purchased from Sigma Aldrich to prepare the standards. Twenty µL from either standards or samples were injected automatically into 250 x 4.6 mm Prodigy ODS (octadecyl 3) column. Absorption wavelength of detector was adjusted to 214 nm where best absorptions for GSH and PC3 were expected according to the method. The period of running for each sample was 15 minutes.

2.8 Techniques for the treatment of polluted biomass of plants after their use in phytoextraction

Contaminated plant biomasses are used to be incinerated after their use in the remediation process. Alternative techniques, rather than incineration, for re-extracting chromium(III) from the harvested dry P. oleracea such as acidification, electrodeposition, and filtration through the sand of emirates, were investigated.

2.8.1 Extraction and determination of chromium in polluted plants

Experiment (16)

Purpose: To extract chromium from dry plant biomass and to determine the efficiency of this extraction.

Steps:

(i) Fifty four grams of dry ground tissues (mixed shoots and roots) of Portulaca were mixed thoroughly. This amount is the residue of some pre-analysed samples which had been irrigated with chromium (VI) solutions.

(ii) Five samples were taken and each was about 1.0 g. These samples were digested using 50% HNO_3, then analysed using ICP-OES to determine the total chromium.

(iii) About 49 g of dry ground plants were divided into three equal samples, each weighing 16.33 g. Each of the three samples was dissolved in one litre of deionised water in 1 litre beaker.

(iv) Concentrated hydrochloric acid was added as droplets to adjust the pH of the solutions at 5.0 ± 0.1 and 2.0. ± 0.1. The third beaker was left without any acid addition and its pH was measured to be 6.1 ± 0.1.

(v) The two beakers of pH 2.0 and 5.0 were stirred using magnetic stirrer and heated to 80 ºC for two hours. About 30 mL of each solution were filtered and divided into three samples, then chromium (VI) was measured using UV-Visible spectrophotometry. Three replicates of each solution were analysed for total chromium using ICP-OES.

(vi) The pH of the two solutions (of pH 6.1 and 5.0) were acidified using concentrated HCl to the pH of 2.0 ± 0.1 then heated to 80 ºC for two hours.

2.8.2 Removal of chromium from the dry biomass of P. oleracea

Experiment (17)

Purpose: To remove Cr(III) from the extract using UAE sand and electrodeposition.

Steps:

(i) An amount of exactly 400 g of sand of Ajman was washed using deionised water, dried, and then was placed in a cylindrical glass column with diameter of 5cm and length of 60 cm. This column was open from the top and fitted with valve from the bottom.

(ii) The sand was analysed for pH, some heavy metals and total carbonate. The two litres of the extract solution (pH 2.0) were filtered though plastic tiny net, then added gradually from the upper inlet of the column and passed through the sand. The filtrate solution was analysed for pH and total chromium using ICP-OES.

(iii) The third litre of extract solution (pH 2) was divided into four equal volumes and each volume was placed in 400 mL beaker. Two cylindrical graphite electrodes (15 cm in length and 0.4 radius) were immersed in each solution then were connected to 9V battery for 12, 24 and 36 hours. After each period the solution was analysed for pH and total chromium using ICP-OES.

2.9 Sampling of soil

Composite soil samples were taken as follows [163]:

(i) A systematic sampling grid was planned for the area of sampling and from each 10 acres area six subsamples were taken (Plastic sampling containers, 500-mL in volume and a steel spade were used. A V-shaped hole six inches deep was excavated. A 1-inch slice from one side of the hole was taken. The sides of slice were trimmed, leaving a 1-inch strip on the spade. Then these strips were transferred to a clean plastic container.)

(ii) All soil samples were taken from a depth from 0 to 6 inches which is known as the tillage depth since most of root activities are restricted to this depth in most plants.

(i) These subsamples were mixed thoroughly in a plastic container, and about 1 kilogram of this mixture was saved for analysis as a composite soil sample.

2.10 PHREEQC program

PHREEQC (Version 2) is a computer program available from the U.S. Geological Survey (USGS, Denver, Colorado, USA) which is designed to perform a wide variety of aqueous geochemical calculations including the speciation of soluble ions [164]. This programme has capability to model almost any chemical reaction that is recognized to influence rain, soil, ground and surface water quality [165].

Soluble ions were extracted from soil using deionised water. Dissolved metal cations were determined using ICP-OES and ion chromatography was used to determine dissolved anions. Generated data from the program were then used to predict the actual species of chromium in the soil of Ajman industrial zone.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Exploring suspected polluted soils

The main objective of this project is the implementation of phytoextraction to remediate polluted soil of UAE. Thus, twelve suspected polluted sites in northern emirates (Figure 3-1) were chosen on the recommendation of the environmental laboratory of Ajman municipality (personal communication). Some of these sites contained industrial waste, old landfill or mining activities for chromite like the east coast area of UAE. Composite soil samples of these sites were analysed for the following heavy metals: Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn. The results of the analysis of soil samples of these sites are listed in Table 3-1.

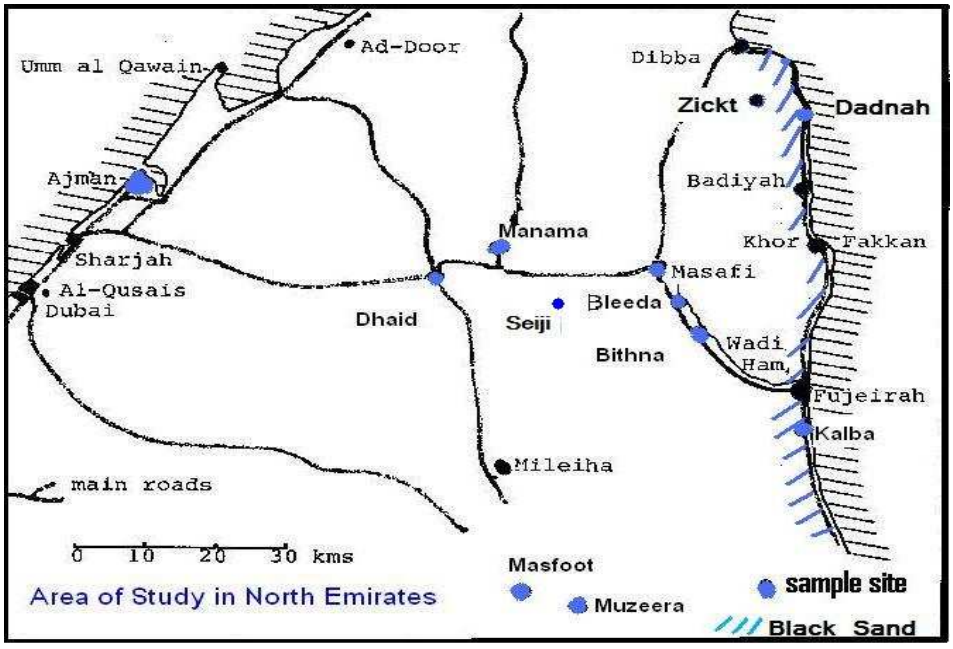


Figure (3-1) The area of the sampled sites in the northern emirates.

Table (3-1) Concentration of heavy metals in soil samples of different sites in northern emirates of UAE.

*Conc. mg/kg / Site	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Kalba	20.5 ± 3.3	135 ± 20	15.6 ± 2.1	(1.6 ± 0.1) x10^4	290 ± 40	590 ± 70	11.1 ± 2.1	46 ± 6
Muzeera	<0.5	110 ± 16	15.2 ± 2.5	(1.5 ± 0.1) x10^4	380 ± 30	520 ± 60	6.2 ± 1.1	33 ± 5
Manama	18.2 ± 2.4	105 ± 17	9.2 ± 1.8	(1.2 ± 0.1) x10^4	320 ± 30	420 ± 50	5.50± 0.68	24 ± 4
Masfoot	14.2 ± 2.1	105 ± 20	12.9 ± 1.9	(1.3 ± 0.1) x10^4	340 ± 40	430 ± 60	3.40± 0.42	40 ± 6
Seiji	10.4 ± 1.8	79 ± 11	14.2 ± 1.5	(1.2 ± 0.1) x10^4	300 ± 40	340 ± 40	5.00± 0.61	30 ± 4
Bleeda	11.2 ± 1.8	86 ± 13	41.6 ± 5.7	(1.3 ± 0.1) x10^4	420 ± 50	240 ± 30	10.5 ± 1.8	50 ± 6
Dhaid	12.3 ± 2.0	115 ± 18	12.8 ± 2.1	(1.3 ± 0.1) x10^4	260 ± 30	460 ± 60	2.20 ± 0.25	20 ± 3
Bithna	24.0 ± 4.3	202 ± 31	19.5 ± 2.9	(1.6 ± 0.2) x10^4	400 ± 40	680 ± 80	80.2 ± 11.6	48 ± 7
Ajman Desert	1.8 ± 0.3	145 ± 23	118 ± 15	(4.8 ± 0.5) x10^3	370 ± 40	100 ± 20	21.9 ± 3.3	110 ± 10
Masafi	11.1± 2.0	118 ± 16	10.9 ± 1.7	(1.2 ± 0.1) x10^4	300 ± 40	410 ± 50	4.4 ± 0.5	28 ± 4
Dadnah	22.5 ± 4.3	155 ± 30	17.6 ± 2.1	(1.9 ± 0.2) x10^4	320 ± 40	680 ± 80	12.5 ± 1.9	63 ± 9
Ajman Industrial Zone	1.01±0.03	1300 ± 150	6.7 ± 0.9	(4.7 ± 0.2) x10^3	160 ± 20	30 ± 5.0	3.4 ± 0.4	90 ± 20

*** Concentration is expressed in mg of heavy metal per kg of dry soil (mg/kg) (Samples were analysed in February 2006 for triplicates)**

It was believed that the level of chromium in black sand of Dadnah and Kalba is much higher than the value determined in table 3-1 using acidic digestion (155 and 135 mg/kg) because it contains chromite $FeCr_2O_4$ as major component. Thus, fusion with Na_2CO_3 (Soda-Ash Roasting) was carried out for black sand of Dadnah and Kalba and the concentration of Cr(III) in soil was found to be 3890 mg/kg and 25000 mg/kg, respectively (Table 3-2). This confirms that it is unavailable to plants under normal conditions.

Table (3-2) Concentration of total chromium in east coast black sand, Kalba and Ajman industrial zone.

Site	Concentration of Cr (mg/kg)
Dadnah Coast	$(2.5 \pm 0.2) \times 10^4$
Kalba Coast	$(3.9 \pm 0.5) \times 10^3$
Ajman Industrial Zone	$(1.3 \pm 0.2) \times 10^3$

The dominant heavy metal in the investigated sites is iron which ranged from 4,700 to 19,000 mg/kg. Iron in soil does not represent environmental challenge at this limit according to U.S. Geological Survey (USGS) which indicated that the median of Fe in soil is 26,000 mg/kg [44]. Nickel and manganese are relatively high in different sites (more 300 mg/kg) especially in the sites which include high content of iron (such as Dadnah, Kalba, Manama, Masfoot, Bithna, Bleeda, and Muzeera), but both Mn and Ni concentrations are still below the maximum allowed limit according to USGS which is 7000 mg/kg for Mn and 700 mg/kg for Ni [44].

It is observed that the most problematic heavy metal in the main polluted sites of UAE is chromium. These sites are Dadnah coast, Kalba coast and Industrial Zone of Ajman. The highest concentration of chromium was found in the black sand of Dadnah cost which reached 25,000 mg/kg followed by Kalba sand up to 3900 mg/kg. Chromium (III) in the soil of Kalba and Dadnah is unlikely to be extracted to soil since chromite is a very stable compound and chromium was extracted from it in laboratory only using soda ash diffusion at 950 ºC. Therefore, chromium in black sand will not form a real environmental problem since its content of chromium is unavailable chromium (III), which is a less harmful form of chromium. According to USGS, in soils, chromium ranged between 3-300 mg/kg with a median of 54 mg/kg [44]. Except Ajman industrial zone, chromium in all investigated sites ranged between 79- 202 mg/kg which falls within the range of USGS. Moreover the UAE soil has a pH 7.9 ± 0.1, at that value chromium (III) is mostly insoluble as $Cr(OH)_3$. So if a small amount of it was extracted, it would not be available to plants at this pH.

The average concentration of total chromium in Ajman industrial zone is 1300 ± 150 mg/kg. The risk associated with this quantity of chromium is its high content of hexavalent chromium (Cr(VI)). This site is a part of the city of Ajman and it is surrounded with schools and other civil establishments. The concentration of chromium in this site stays within the limit that some plants can tolerate, so phytoremediation may be the technique of choice for the remediation of this site. Detailed investigation for the soil of Ajman industrial zone was carried out. The results are reported and discussed in the next section.

3.1.1 The polluted site of Ajman industrial zone

As a result of surveying twelve suspected sites for heavy metal contamination, Ajman industrial zone (Figure 3-2) was identified as polluted with chromium to an extent that calls for immediate remediation. Detailed chemical analyses of the soil of the site were carried out to measure the anions, cations, carbonate content, organic matter and pH. (The results are shown in table 3-3). Hexavalent chromium in the soil of the site was measured from October 2008 to June 2009 and the results are shown in Table 3-4. PHREEQC software from USGS [165] was used to predict the speciation of Cr(VI) in the soil extract (Tables 3-5) and Cr(III) in soil (Table 3-6).

Figure (3-2) Satellite photograph and detailed sketch for the polluted site in Ajman Industrial Zone

Table (3.3) Concentrations of the cations and anions and the pH of soil of the site of Ajman industrial zone.

Variable	Measured Value	Median in USGS (mg/kg)
pH	7.9 ± 0.1	na
Total Chromium	1760 ± 120 mg/kg	54
Chromium (VI)	97.3 ± 14.8 mg/kg	na
Total Carbonate	42% wt/wt ± 3.6 %	na
Total Organic Matter	0. 42 % wt/wt ± 0.02 %	0.5-100% wt/wt
Nitrate	< 0.01 mg/kg	na
Sulfate	55.3 ± 8.8 mg/kg	na
Chloride	1300 ± 100 mg/kg	na
Phosphate	< 0.01 mg/kg	na
Sodium	730 ± 80 mg/kg	12,000
Potassium	500 ± 50 mg/kg	15,000
Calcium	14700 ± 400 mg/kg	24,000
Magnesium	6100 ± 300 mg/kg	9,000
Iron	4700 ± 150 mg/kg	26,000
Manganese	170 ± 20 mg/kg	550
Copper	7.5 ± 1.1 mg/kg	25
Zinc	95.3 ± 11.6 mg/kg	60
Lead	3.6 ± 0.4 mg/kg	19
Cobalt	1.0 ± 0.13 mg/kg	9.1

na = not available.

Table (3.4) Concentrations of total and hexavalent chromium in the soil of the site from October 2008 to June 2009.

Month	Total Chromium (mg/kg)	Chromium (VI) (mg/kg)
October 2008	1710 ± 120	97.3 ± 14.8
December 2008	1740 ± 120	65.7 ± 16.4
February 2009	1770 ± 130	52.8 ± 11.5
April 2009	1820 ± 130	68.2 ± 13.7
June 2009	1860 ± 140	74.6 ± 14.5

Table (3-5) Predicted chromium (VI) species in the soil extract of the polluted site using PHREEQC program.

Chromium(VI) Species	Predicted concentration (mg/kg)
CrO_4^{-2}	88
$KCrO_4^-$	3.4
$NaCrO_4^-$	2.4
$HCrO_4^-$	2.0

Table (3-6) Predicted chromium (III) species in soil of the polluted site using PHREEQC program.

Chromium(III) Species	Predicted concentration (mg/kg)
$Cr(OH)_3$	1400
$Cr(OH)_2^+$	280
CrO_2^-	3.2
$Cr(OH)^{+2}$	2.5
$Cr(OH)_4^-$	1.2

Analyses of composite soil samples indicate that the pH of the soil of 7.9 ± 0.1, which reflects the high carbonate content (42%) (Table 3-3). At this pH, chromium (VI) is available as chromate anions CrO_4^{2-}, which was confirmed by the PHREEQC program as the major Cr(VI) species (Table 3-5). Most of the chromium in the soil of the site exists as Cr(III) since the total chromium was 1800 mg/kg while Cr(VI) was 97.3 mg/kg. According to EPA Toxicity Characteristic Leaching Procedure (TCLP), the maximum allowed concentration of chromium in soil is 5 ppm [57]. So 97 mg/kg of chromium (VI) in soil represents a direct threat to the environmental systems in the area of the site.

Meanwhile, the existence of iron in soil as Fe(III) [113, 114] does not alter the oxidation state of Cr(VI) leaving it available for living organisms (plants or microorganisms). The amount of organic matter content may contribute to the reduction of Cr(VI) to Cr(III) but its effect stays limited due to its small amount (<0.42% Table 3-3). Finally the decrease of Cr(VI) in winter months (Figure 3-3) is suggested to be due to the seasonal rains which fall in UAE within winter and it may leach soluble Cr(VI) to the ground water.

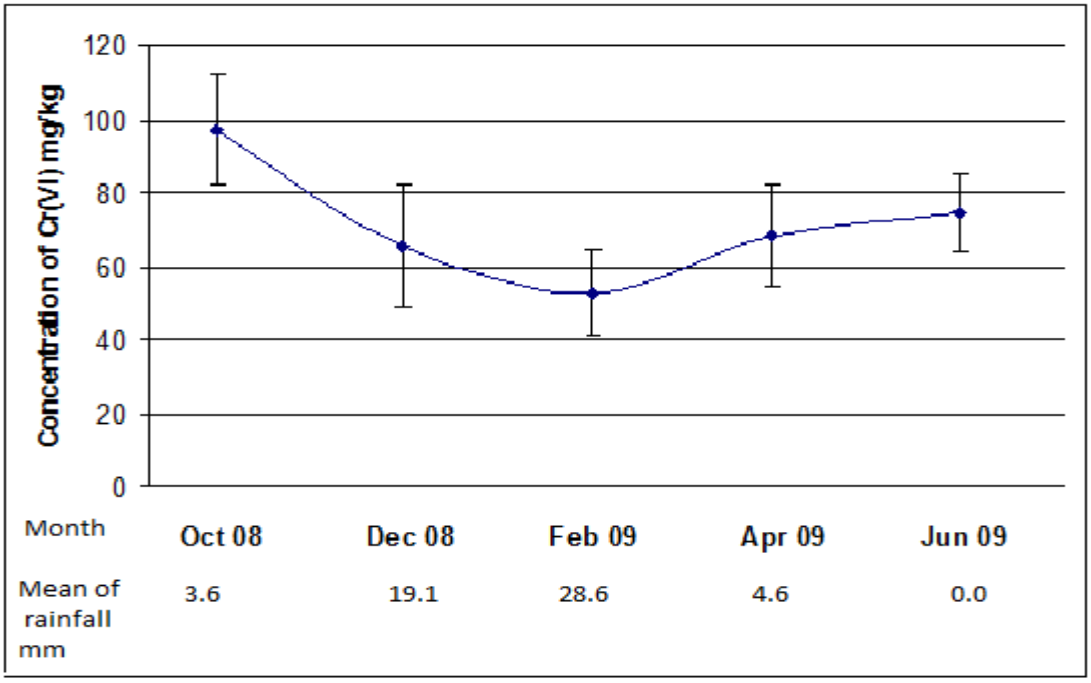


Figure (3-3) Concentration of chromium (VI) in the soil of Ajman Industrial Zone and the mean of rain fall [166] in 2008-2009.

The concentration of Cr(VI) observed ranged between 53 and 97 mg/kg in the polluted Ajman industrial zone and this concentration is relatively moderate and may be below the phytotoxic limit of Cr(VI) towards certain plants, since some plants (e.g.

Tamarisk and Prosopis) were thriving in the site. In such sites with moderate pollution, phytoextraction technique is well suited for soil remediation.

3.2 Screening of local desert plants for potential heavy metal phytoextraction activity

Three approaches were used to identify accumulators for heavy metals including chromium (VI):

1- Investigation of natural plants growing in the suspected polluted sites to find natural accumulator(s) for one or more heavy metals.

2- Investigation of six recommended plants (by environmental municipality of Ajman) with respect to their tolerance of the harsh desert environment.

3- Detailed investigation of two species of mesquite (Prosipis) plant which were reported in the literature as promising accumulators for both Pb and Cr(VI) [28, 29].

3.2.1 Investigation of natural plants growing in the suspected polluted sites

Parallel to the analysis of the soils of polluted sites for 8 heavy metals, ten local desert plant species naturally growing within these sites were sampled and analysed for the same 8 heavy metals in order to find natural accumulator(s) growing in these sites. Cadmium in these plants was also analysed since it is highly toxic. The results are shown in Table 3-7. The major heavy metal encountered in the investigated plants

was iron which reached 1900, 1700 and 1500 mg/kg in Tamarix aucheriana grown in Ajman industrial zone, Dactyloctenium aegyptium and Heliotropium calcareum growing in Kalba, respectively. The second heavy metal taken up by plants was manganese which reached 252 mg/kg in Euphorbia larica grown in DC. Both iron and manganese are plant nutrients, so the uptake of these two metals does not represent an environmental threat and the concentration of manganese in Euphorbia larica is not considerable (Table 3-7).

Cyperus conglomerates from Dadnah coast (DC) showed specific response towards cadmium (Table 3-7) therefore, a detailed analysis which included soil and different parts of this plant was carried out and the results are shown in (Table 3-8). Bioconcentration factors BCF(s) were calculated as concentration of heavy metal in whole dry plant (mg/kg) / concentration of heavy metal in dry soil mg/kg. Bioconcentration factors ($\geq$ 1.0) for promising plants growing in these sites are shown in Table 3-9. Among the fourteen plants analysed, the most promising was Tamarix aucheriana growing in Ajman industrial site. It demonstrated a relatively high uptake for most of the measured heavy metals with bioconcentration factors greater than 2.0 for Pb, Cu and Co (Table 3-9). Total chromium in Tamarix aucheriana growing in the same site is 28.5 mg/kg. This is a small amount compared to the amount of chromium in soil which is 1800 mg/kg suggesting that Tamarix is chromium excluder.

Table (3-7) Analysis of dry plants growing in suspected polluted sites for some heavy metals uptake.

Concentration (mg/kg) / Plant/ Site	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Dactyloctenium aegyptium/ Kalba	< 0.15	1.85±0.23	8.29±0.00	8.30±0.23	1700±230	40.5±12.5	50.0±17.5	< 0.14	15.7±2.8
Heliotropium calcareum/ Kalba	< 0.15	1.30±0.17	7.80±1.63	10.8±2.3	1500±194	50.1±18.6	39.3±11.1	0.75±0.12	12.5±2.4
Pluchea Arabica/ Muzeera	< 0.15	< 0.5	0.90±0.13	4.30±1.19	164± 34	52.1±14.7	3.10±0.65	6.10±1.22	22.5±0.4
Calotropis procera/ Masfoot	< 0.15	1.40±0.20	0.90±0.11	9.20±2.63	93.2±23.8	78.3±22.6	6.20±1.6	13.7±2.3	41.5±5.8
Indigofera/ Bithna	< 0.15	< 0.5	1.30±0.24	6.40±1.28	167±32	52.7±15.5	5.70±1.2	2.00±0.26	26.3±4.4
Calotropis procera/ Bithna	< 0.15	1.10±0.13	0.90±0.14	3.40±1.11	81.2±20.1	77.6±21.4	4.30±0.93	0.30±0.08	24.4±3.6
Asphodelus tenuifolius/ Bithna	< 0.15	< 0.5	1.70±0.26	6.30±1.56	58.3±16.3	20.9±3.5	9.20±1.9	0.50±0.10	40.5±6.2
Prosopis juliflora/ Ajman Desert	< 0.15	< 0.5	2.50±0.46	12.5±2.6	< 0.15	< 0.03	8.60±2.0	< 0.14	< 0.1
Calotropis procera/ Ajman Desert	< 0.15	1.05±0.17	2.80±0.51	6.50±1.90	< 0.15	< 0.03	2.70±0.82	8.15±2.34	< 0.1
Tamarix aucheriana/ Ajman I. Zone	< 0.15	2.30±0.32	28.5±6.23	28.4±4.2	1900±280	139±30	28.2±4.15	11.5±2.9	191±39
Prosopis juliflora/ Dadnah coast	< 0.15	< 0.5	1.18 ± 0.19	4.83 ± 0.45	148 ± 16.5	40.8 ± 6.2	6.40 ± 0.56	0.42 ± 0.06	30.8 ± 5.2
Prosopis juliflora/ Masfoot	< 0.15	< 0.5	1.83 ± 0.33	11.2 ± 2.1	132 ± 15.8	39.6± 5.3	3.23 ± 0.38	0.95 ± 0.14	36.0 ± 6.6
Euphorbia Larica / Dadnah Coast	< 0.15	2.75 ± 0.48	1.04 ± 0.21	3.51 ± 0.45	72.8 ± 10.3	252 ± 37.5	6.66 ± 0.75	0.75 ± 0.09	14.4 ± 1.8
Cyperus conglomerates / Dadnah	0.86 ± 0.14	< 0.5	0.99 ± 0.16	2.37 ± 0.35	175 ±19.9	79.2 ± 10.5	4.10 ± 0.5	0.90 ± 0.11	5.03 ± 0.65

(Number of replicates =3)

Table (3-8) Concentration of cadmium in different parts of Cyperus conglomerates naturally growing in Dadnah Coast (DC).

Cyperus conglomerates plant tissue	Concentration of cadmium (mg/kg)	Translocation factor (TF)
Stems	0.50 ± 0.08	0.8 ± 0.2
Leaves	0.76 ± 0.14	1.2 ± 0.3
Flowers and seeds	1.12 ± 0.20	1.7 ± 0.4
Roots	0.65 ± 0.13	

Table (3-9) Bioconcentration Factor (BCF) for some investigated naturally growing plants (* BCF < 1.0).

Heavy metal / Plant	Pb	Cu	Co	Cd
Tamarix aucheriana	3.4 ± 0.5	4.4 ± 0.6	2.3 ± 0.2	*
Cyperus conglomerates stem	*	2.6 ± 0.3	*	26 ± 4.5
Cyperus conglomerates flower& seeds	*	2.9 ± 0.4	*	59 ± 5.8
Cyperus conglomerates leaves	*	1.3 ± 0.1	*	40 ± 5.3
Cyperus conglomerates roots	*	2.6 ± 0.4	*	34 ± 4.8

Bioconcentration factors (Table 3-9) of cadmium in Cyperus conglomerates exceeded the value of 26 and reached 59 in flowers and seeds. These results are very important since cadmium is classified as one of the most toxic pollutants in soil and water - with concentration of 1.0 mg/kg for toxicity [57] and these BCF values are very promising for such pollutant. The translocation factor of cadmium (Table 3-8) from roots to leaves exceeded the value of 1.0 (1. 2) and in flowers and seeds reached 1.7 which confirms the role of this plant in the accumulation of cadmium.

In most plant samples the uptake of heavy metals was limited. This can be related to the limited number of accumulators in nature and the high pH of investigated soils (7.9 ±0.1). Most of the heavy metal cations are insoluble and unavailable to the plants at this pH. Total chromium in Dactyloctenium aegyptium, Heliotropium calcareum and Cyperus conglomerates, naturally growing in Kalba and Dadnah (Tables 3-7, 3-8), did not exceed 10 mg/kg although the soils of these sites are very rich in chromite. This confirms the conclusion that Cr(III) in black sand is not bioavailable for plants. The results suggest that none of the fourteen investigated plants reported in table 3-7 is a chromium accumulator.

3.2.2 Investigation of recommended desert plants

Six plant species either local or exotic, but well adapted to the environment, were chosen and propagated by stem cuttings. These plants were recommended by Ajman municipality nursery due to their potential to tolerate high temperature, soil salinity, and high pH of soil. The plants were irrigated either with acidified deionised water for control or heavy metal nitrate solution (Co, Pb, Cr(III), Cu, Ni)

while chromium (VI) was introduced as $K_2Cr_2O_7$. The plants were analysed for the six heavy metal ions. Figure 3-4 shows the concentration of heavy metals in the dry plants, BCF(s) were calculated and are shown in Table 3-10.

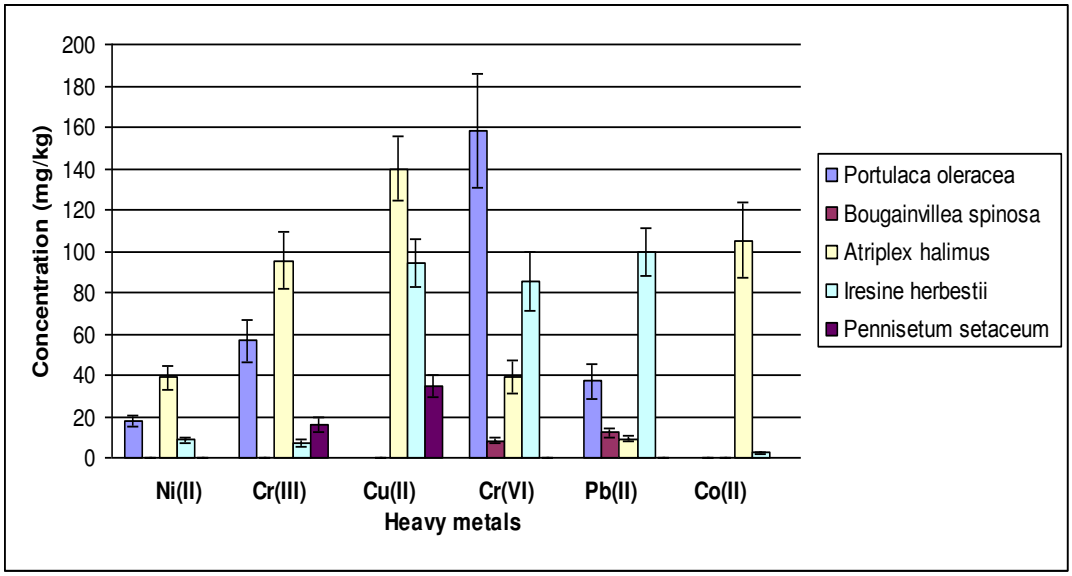


Figure (3-4) Uptake of heavy metals by a range of local plants. (Mean of triplicates)

Table (3-10) Bioconcentration factors (BCF) for some heavy metals (* BF < 1.0)

Heavy Metal / Plant	Cr(III)	Cr(VI)	Pb(II)	Cu (II)	Co(II)
Portulaca oleracea	*	1.6 ± 0.1	*	*	*
Atriplex halimus	1.0 ± 0.1	*	*	1.4 ± 0.1	1.0± 0.1
Iresine herbestii	*	*	1.0 ± 0.1	1.0 ± 0.10	*

Portulaca oleracea demonstrated the greatest uptake of chromium (VI) accumulating 158 mg/kg of Cr (VI) giving a BCF of 1.6 which is the highest among the six investigated plants (Figure 3-3 and Table 3-10). P. oleracea can be

classified as a promising plant for the phytoextraction of Cr(VI) since the BCF exceeded the value of one which means that the concentration of pollutant in the dry plant tissue is more than its concentration in the dry soil. There was no significant difference between the dry weight of P. oleracea in control and experimental samples in the presence of Cr(VI) ($P > 0.05$ using ANOVA Post hoc test "Tukey"). This suggests that P. oleracea may accumulate concentrations of Cr(VI) higher than the 158 mg/kg value.

Iresine herbestii accumulated lead and copper with a BCF value of 1.0 for these two heavy metals. Atriplex halimus showed the capability to accumulate copper with a BCF of 1.4 and Cr(III) and Co(II) with a value of 1.0. These two plants have shown capability to solubilise and uptake heavy metal cations even from soil of high pH (7.9) which is in agreement with previous study [86]. In this study Cr(III) was introduced to Zea mays in sand and soils of pH 7.2 and 7.8 respectively and observed considerable chromium uptake by plant at these pH values.

For the three plants (Portulaca oleracea, Iresine herbestii and Atriplex halimus), no significant difference ($P > 0.05$ using ANOVA Post hoc test "Tukey") between the dry biomass of the experimental and the control plants was observed in the presence of the five heavy metals. This normal growth of plants in the presence of heavy metals may give these plants special characteristics of heavy metal phytoextraction in soils of high pH like the soil of UAE.

Bougainvillea spinosa and Pennisetum setaceum did not show significant absorption of any heavy metal among the six metal ions investigated in the

experiment. No significant difference was observed in the dry biomass between plants grown in control and experimental conditions ($p > 0.05$ using ANOVA Post hoc test "Tukey"). These two plants can be classified as excluders for the six heavy metal ions. The sixth plant Azadirachta indica did not give sufficient vegetation when exposed to the five heavy metals. This indicates that these small concentrations of pollutants are phytotoxic to Azadirachta indica as demonstrated by limited growth, yellowish leaves and deterioration of roots.

3.2.3 Investigation of mesquite species for the accumulation of lead and hexavalent chromium

According to previous studies [28, 29] mesquite (Prosopis juliflora) demonstrated high ability to uptake Cr(VI) and Pb(II). Those studies were carried out in El-Paso Texas which has desert climatic conditions similar to those of the UAE. The potential of Prosopis species for phytoextraction of lead and chromium (VI) from soil of UAE was investigated in the present work. Two types of mesquite **(**Prosopis cineraria and Prosopis juliflora) were used in this investigation. In this experiment, the two plants were irrigated with either Cr(VI) or Pb(II) or deionised water for control. The concentrations and the BCF values for Pb (II) and Cr (VI) are shown in Tables 3-11 and 3-12 respectively.

Table (3-11) Uptake of Pb (II) , Cr(VI) by two types of mesquite plants of UAE. (Mean of triplicates of whole plant)

Plant	Pb(II) mg/kg	Cr (VI) mg/kg
Prosopis cineraria	12.4 ± 3.13	26.4 ± 5.37
Prosopis juliflora	8.46 ± 1.52	11.4 ± 2.12

Table (3-12) Bioconcentration factors for lead and Cr(VI) in mesquite (Prosopis species).

Plant	Bioconcentration of Pb (**II**)	Bioconcentration of Cr (**VI**)
Prosopis cineraria	0.05	0.11
Prosopis juliflora	0.03	0.05

The two types of Prosopis showed limited accumulation of either Pb (II) or Cr (VI) (Table 3-11). These results contradict those of previous studies on the same plants and pollutants [28, 29]. This difference is likely to be due to the different conditions used in these two studies. Agar paste and hydroponics at pH 5.3 were used in the El-Paso studies resulting in chromium (VI) being available as dichromate. In the present study, although the pH of the irrigation solution of dichromate was 5.5, the high carbonate content in soil (42%) which fixed the pH at 7.9 would made Cr(VI) available as chromate. The difference in the uptake of Cr(VI) may be due to the introduction of chromium as different species of Cr(VI) at different pH values and in different nutrient media. In El-Paso, Pb (II) was introduced to the plants at pH of 5.3, where it will be soluble and available to the plant. In the present study, the soil pH of 7.9 would result in most of the lead being precipitated as $Pb(OH)_2$ and not available to the plants.

The results of analysis of Prosopis juliflora in four different locations of UAE (Tables 3-7, and 3-11) did not give any indication of heavy metal accumulation. This plant is very common in the UAE and it was sampled from different locations in the northern emirates hoping to find some high ability of accumulating any pollutant but the results were negative and inconsistent with the

previous studies [28, 29]. Nevertheless, this observation is very important in the Emirates since Prosopis plant is the essential food of camels around UAE, so its insignificant accumulation of polluting metals would make it nontoxic to animals at UAE soil conditions.

Since the concentrations of heavy metals in all the plants were found to be less than 1000 mg/kg in dry plant tissue, it could not be concluded that a hyperaccumulator was discovered. However, the bioconcentration factors for Portulaca, Atriplex, and Irisine ($\geq$1.0) indicate that the concentration of the heavy metal in the dry plant is more than its concentration in the soil suggesting that these plants have phytoextraction potential.

Ajman industrial zone which is the polluted site suitable for phytoextraction contains chromium (VI) as the problematic heavy metal. Concentration of total chromium in the site increased from 1300 ±150 mg/kg in 2006 to 1850 ±140 mg/kg in 2009, which confirms the continuity of discharging chromium (VI) wastes to the site by, for example chromic acid. This acid is routinely used in the factory of aluminium extrusion nearby the site.

The plant that demonstrated potential to accumulate chromium (VI) is Portulaca oleracea since it has the highest bioconcentration factor for chromium (VI). It is also a succulent plant and can absorb significant quantities of water and this may enhance the phytoextraction process. P. oleracea grows in the UAE and its optimum season is in the hot months from April to November; so it is a perfect plant for Ajman industrial zone site which has the highest concentration of Cr(VI) in the summer season.

3.3 Factors that may affect chromium (VI) uptake by P. oleracea

As a result of the previous investigations, chromium (VI) was identified as the problematic heavy metal in Ajman industrial zone and P. oleracea was the best option for its phytoextraction. In order to optimize chromium accumulation efficiency by P. oleracea, factors that may affect such efficiency were investigated. These factors include: concentration of pollutant in soil, pH of soil, organic content in soil, competitive anions, accompanying cations, and chelating agents.

3.3.1 Effect of the concentration of chromium (VI) in the soil on its uptake by P. oleracea

The effect of the concentration of Cr(VI) in soil on its uptake by some accumulators was previously investigated using different plant species, however conflicting results were reported [106-108]. The results of the present study may hopefully contribute to the solution of this disagreement at least with respect to P. oleracea. Plants were irrigated with 9 different levels of Cr(VI) as sodium chromate where each group consisted of three replicates in addition to a control, which was irrigated with deionised water. Plants were harvested and analysed for total chromium and chromium(VI). Chromium (III) was calculated by subtraction of chromium (VI) from total chromium in roots, leaves and stems.

3.3.1.1 Concentration and speciation of chromium in plant tissues

The concentrations of total chromium, (Cr (VI) and Cr (III)) in leaves of P. oleracea were calculated and plotted in Figure 3-5. The concentration of total chromium in leaves increased from 99.5 mg/kg to 1067 mg/kg when Cr(VI) in

irrigation solution increased from 50 to 350 ppm. Chromium (VI) in leaves increased from 3.3 to 30 mg/kg over the same range. Total chromium in stems increased from 72 mg/kg to 1404 mg/kg when chromium (VI) in the irrigation solution increased from 50 to 350 ppm (Figure 3-6). Chromium (VI) ranged from 2.3 to 43 mg/kg in dry stems of the plant. Total chromium in roots increased from 404 mg/kg of dry roots to 4624 mg/kg at the same level of increasing Cr(VI) in the irrigation solution (Figure 3-7). Chromium (VI) ranged from 32 to 135 mg/kg in dry roots of the plant.

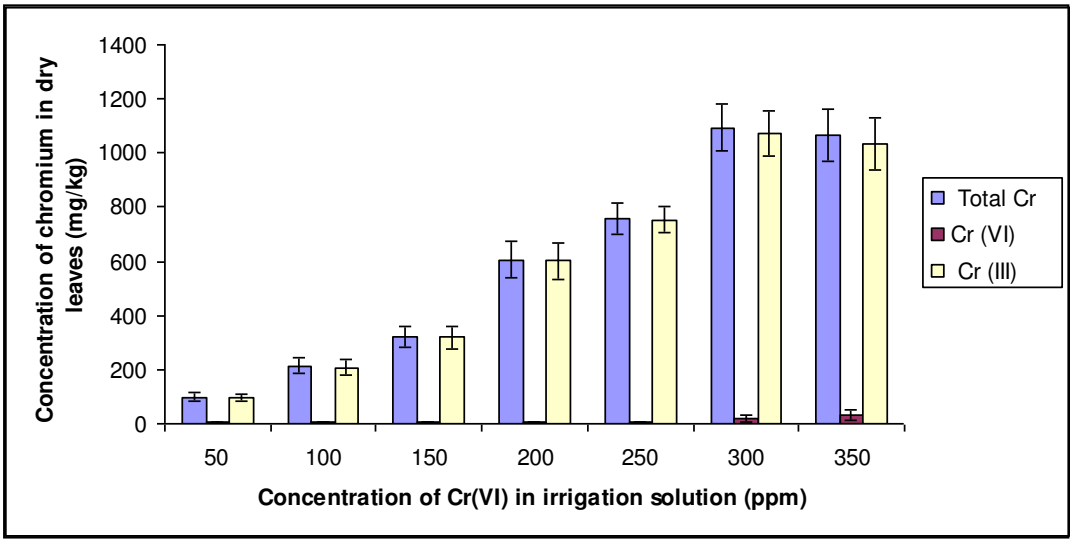


Figure (3-5) Concentrations of total chromium, Cr (VI) and Chromium (III) in leaves of Portulaca oleracea at different concentrations of Cr (VI) in irrigation solution. (Mean of triplicates)

When comparing mean values of concentration of chromium in the leaves, stems and roots, total chromium is observed to increase significantly in all plant parts as the concentration of the Cr (VI) in irrigation solution increases from 0 to 300 ppm in increments of 100 ppm ($p<0.01$ using ANOVA Post hoc test "Tukey" , Figure 3-8). This increase tends to be linear in roots, stems and leaves.

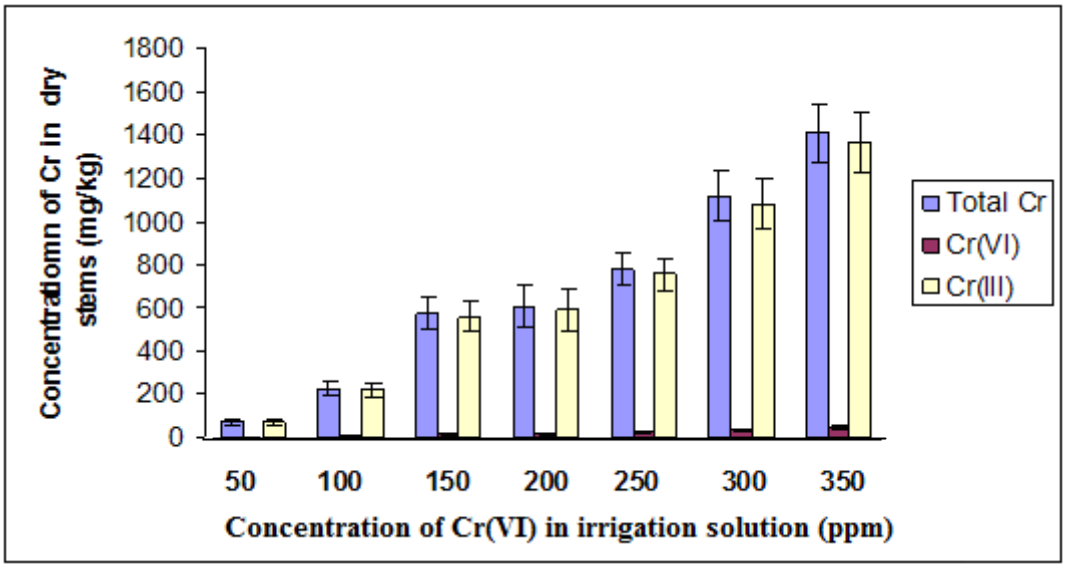


Figure (3-6) Concentrations of total chromium, Cr (VI) and Chromium (III) in stems of Portulaca oleracea at different concentrations of Cr (VI) in irrigation solution. (Mean of triplicates)

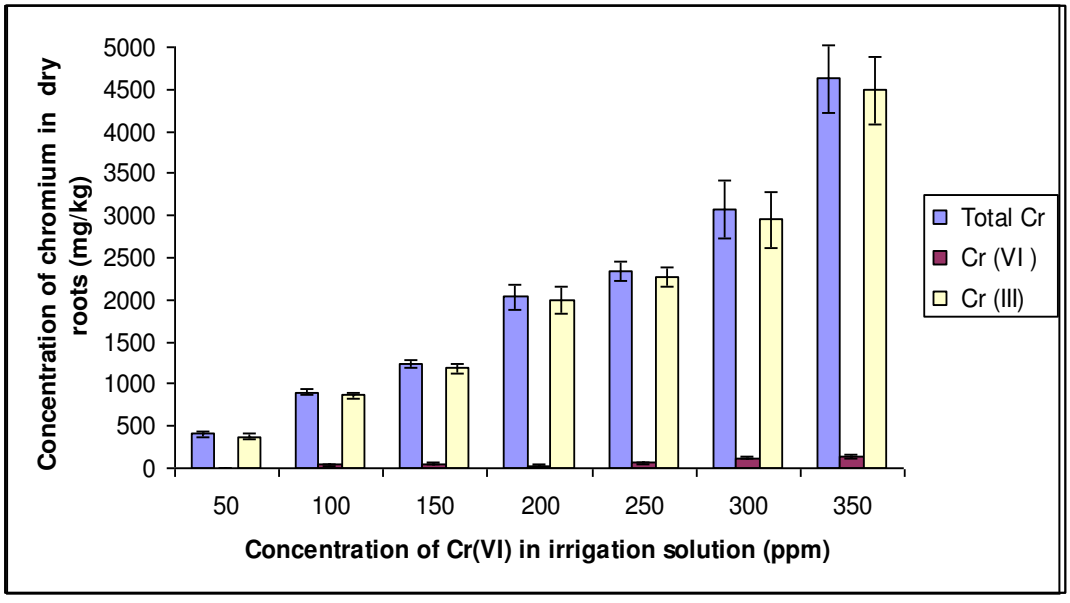


Figure (3-7) Concentrations of total chromium, Cr (VI) and Chromium (III) in roots of Portulaca oleracea at different concentrations of Cr (VI) in irrigation solution. (Mean of triplicates)

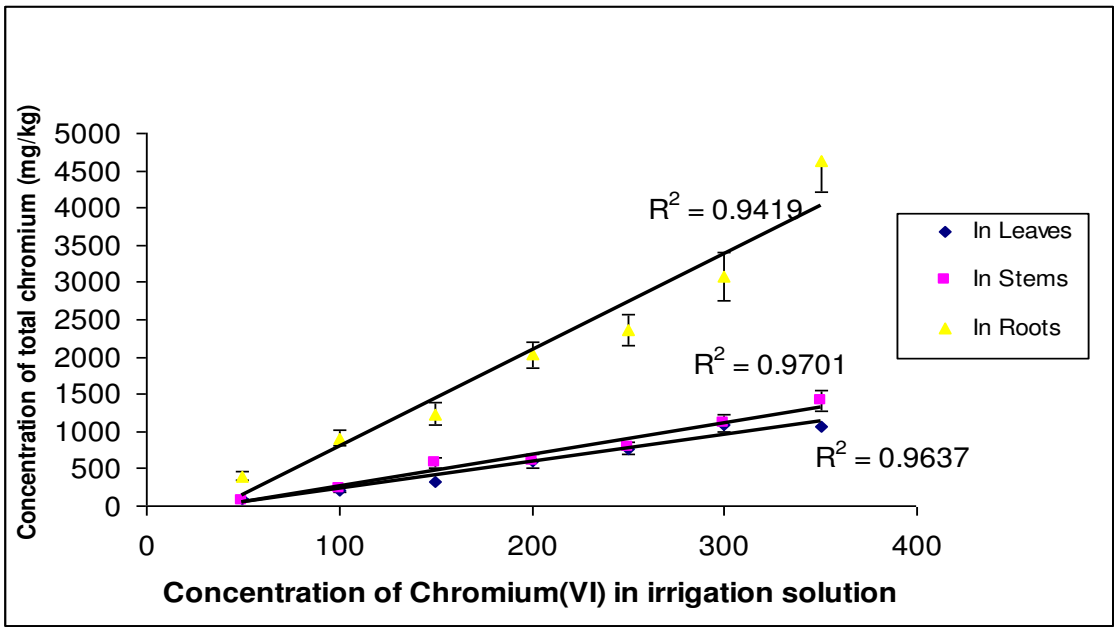


Figure (3-8) Relation between the concentration of chromium in irrigation solution and the total chromium in roots, stems, and shoots of Portulaca oleracea. (Mean of triplicates)

These results agree with some previous investigators who studied the uptake of chromium by plants other than P. oleracea such as Leersia hexandra [83], Typha angustinfolia [85], barley seedlings [89], Azolla caroliniana [106], and Cynodon dactylon [108]. The reports of these studies came to the same conclusion that the uptake of chromium by plants increases as the introduced chromium (VI) increased. The results of this study differ from the results reported in another study [107] which introduced Cr(VI) to Convolvulus arvensis in three concentrations 80, 40, and 20 ppm and found that the highest uptake by roots was observed at the lowest concentration of Cr(VI) (20 ppm). The roots of C. arvensis began to be severely affected by Cr(VI) at concentrations higher than 20 mg/kg so the uptake of Cr(VI) decreased at the higher concentrations.

The total chromium in leaves of Portulaca increased from 99.5 to 1067 mg/kg and in stems from 72 to 1404 mg/kg (Figures 3-5, 3-6) exceeding the barrier of the 1000 mg/kg of pollutant in both parts of the plant which indicates that a hyperaccumulator for Cr (VI) is at hand. The concentration of chromium in the roots broke the barrier of 1000 to reach 4600 mg/kg (Figure 3-7). Since Portulaca oleracea has the ability to uptake and accumulate these amounts of chromium(VI), it can be classified as a promising accumulator of chromium(VI). When comparing P. oleracea with the other accumulators of Cr(VI) such as Leptospepermum scoparium [62], Typha angustinfolia [85], Zea mays [86], and Leersia hexandra [93], it can be concluded that P. oleracea may be the best among them in achieving maximum concentration of chromium in roots and the second regarding chromium in shoots (Table 3- 13).

Table (3-13) Concentration of chromium in dry plant tissues of Cr(VI) accumulators.

Chromium(VI) accumulator	Cr concentration in dry roots (mg/kg)	Cr concentration in dry shoots (mg/kg)
Leptospepermum scoparium [62]	na	400-600
Leersia hexandra [93]	3300	2160
Typha angustinfolia [85]	177.5	5.6
Zea mays [86]	1824	494
Portulaca oleracea	4600	1067-1400

The percentage of reduced chromium [(concentration of Cr (III) / Concentration of total chromium) x 100] was calculated (Table 3-13) and ranged between 92% and 99% in roots, 96 and 97% in stems and from 96% to 99% in

leaves. So almost all Cr(VI) that was accumulated by P. oleracea was reduced to Cr(III) inside the plant tissues.

Table (3-14) Percentages reduced chromium (VI) in roots, stems and leaves of P. oleracea at different concentrations of Cr (VI) in the irrigation solution.

Concentration of Cr(VI) in Irrigation solution (ppm)	percentage of reduced Cr in roots %	percentage of reduced Cr in stems %	percentage of reduced Cr in leaves %
50	92.2	96.8	96.7
100	95.7	96.9	97.2
150	96.0	97.3	98.3
200	98.5	97.2	99.1
250	97.3	96.8	99.4
300	96.1	96.9	98.0
350	97.0	96.9	96.9

The major chromium species in both shoots and roots was Cr (III) by percentage up to 99.4% in shoots and over 98.5% in roots, whilst soil analysis after harvesting confirmed that Cr (VI) was still the major species in soil. This confirms that most of chromium was absorbed as Cr(VI) then was reduced with a high efficiency in plant tissues (Table 3-14). The percentage of reduction indicates that most of chromium (VI) was reduced in roots before reaching shoots and this may explain the degradation of roots at the high concentration of 400 ppm of Cr(VI) as compared to stems and shoots. These results are in agreement with results previously reported for other plants [79, 80, 83] and exactly what would be expected as chromate would be expected to oxidise the plant material.

3.3.1.2 Bioaccumulation Factors and Translocation Factors

Accumulators can be assessed using two factors, bioaccumulation and translocation factors. Bioaccumulation factor (BAF) is the ratio of concentration of heavy metal in roots to its concentration in soil while translocation factor (TF) is the ratio of concentration of heavy metal in shoots to its concentration in roots. For promising accumulators, the two factors have to exceed the value of 1.0. The values of BAF and TF were calculated and are shown in Table (3-15).

Table (3-15) Bioaccumulation and translocation factors to leaves and stems at different concentrations of chromium (VI) in irrigation solutions.

Conc. of Cr in Irrigation (ppm)	Bioaccumulation factors BAF	Translocation Factor TF for leaves	Translocation Factor. TF for stems
50	10.12 ± 1.15	0.25 ± 0.07	0.18 ± 0.04
100	10.25 ± 1.14	0.24 ± 0.05	0.25 ± 0.08
150	9.64 ± 1.11	0.26 ± 0.05	0.46 ± 0.07
200	10.51 ±1.16	0.30 ± 0.05	0.30 ± 0.05
250	10.47 ± 1.20	0.32 ± 0.07	0.33 ± 0.06
300	12.21 ± 1.58	0.36 ± 0.06	0.36 ± 0.06
350	15.36 ± 1.94	0.23 ± 0.05	0.31 ± 0.05

The calculated bioaccumulation factors (Table 3-15) were around the value of 10.0 within the range of 50 to 250 ppm of Cr(VI) in irrigation solution indicating that P. oleracea accumulated Cr(VI) in roots in constant ratio within this range of Cr(VI) concentration. Bioaccumulation factors increased as the concentration of Cr(VI) increased over 250 ppm to reach 15.0 at 350 ppm of Cr(VI). Bioaccumulation factor values of 10 to 15 confirm the potential of P. oleracea as

an efficient hyperaccumulator for Cr (VI). No significant difference was observed in mean values of translocation factors which ranged between 0.24 and 0.35 for leaves and between 0.18 and 0.46 for stems. Translocation factors of chromium (VI) using other plants did not reach the value of 0.7 [83, 109] which is in agreement with the results of this study. The low translocation factors observed in P. oleracea are likely to be due to the stress of highly oxidative Cr(VI) species which would cause severe damage to plant tissues, especially roots.

3.3.1.3 Plant growth and total removed chromium

No significant difference was observed between means of roots dry weight in the level of 0-150 ppm of Cr(VI) and in shoots in level 0-100 ppm ($p>0.05$) Figure (3-9). This means that plants were not affected significantly by Cr(VI) at these concentrations which indicate that P. oleracea can grow normally in Ajman industrial zone which has similar content of Cr(VI). Significant decrease in dry wt. of plants was observed at levels higher than 200 ppm of Cr(VI) ($p<0.01$). Phytotoxicity symptoms were also noticed since leaves became yellow and inflated at concentrations higher than 300 ppm. When harvesting the roots of the plants at 400 ppm of Cr (VI), they were degraded. This significant decline is an indication of the severe stress caused by Cr(VI) which will oxidize the bioorganic materials especially the protein of the cells. Several previous studies have indicated the reduction of other plants biomass as the concentration of Cr(VI) increases in the nutrient medium [79, 81, 87]. When the total chromium in both roots and shoots is calculated, the total amount removed by 5 plants grown in one pot was increased

from 0.90 ± 0.15 mg at 50 to 3.00 ± 0.34 mg at 200 ppm then it declined as the chromium in irrigation solution was increased above 200 ppm (Figure 3-10).

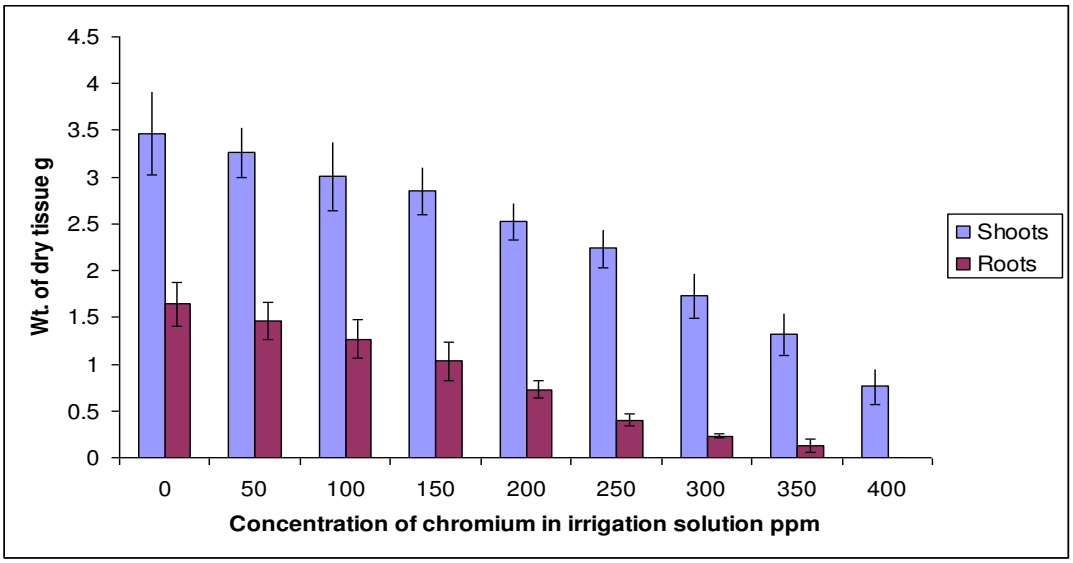


Figure (3-9) Dry weight of both shoots and roots of Portulaca oleracea at different concentration of Cr (VI) in irrigation solution.

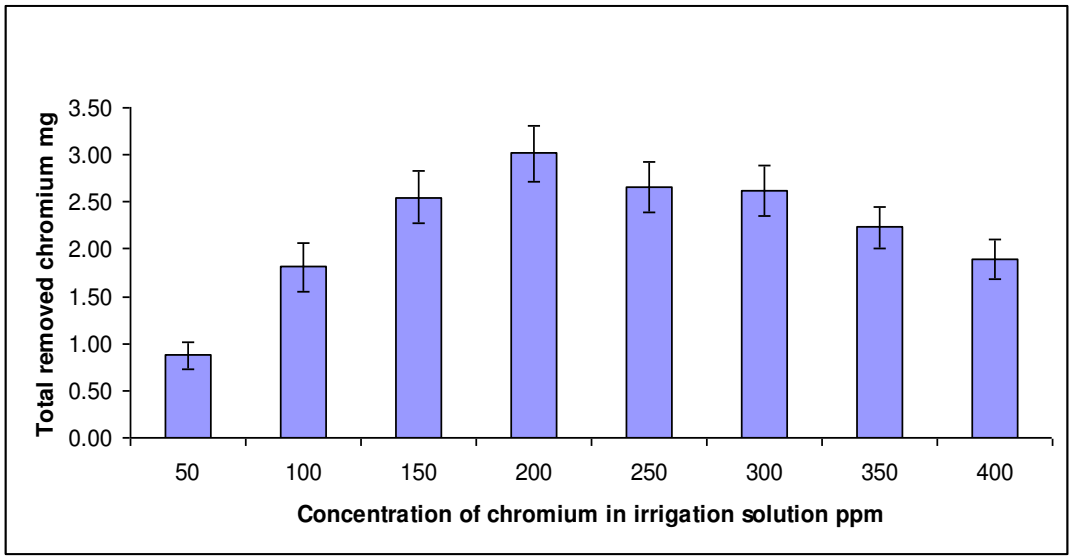


Figure (3-10) The total removed chromium by the whole dry tissue of 5 seedlings of Portulaca oleracea germinated in one pot at different concentrations of Cr(VI) in irrigation solution.

3.3.2 Effect of soil pH on the uptake of chromium by Portulaca Oleracea

Chromium speciation in soil or water is pH-dependent as reported by previous studies on the effect of pH on the uptake of Cr(VI) by crop plants or microorganisms [98, 116-118]. No research was done before on using pH of soil to enhance the uptake of Cr(VI) by potential non-crop accumulators of Cr(VI) like P. oleracea. The present work reports on the investigation of the effect of pH of soil on the uptake of Cr(VI) by P. oleracea as accumulator for this pollutant.

Six types of soil were prepared with different six levels of pH (6.0, 7.0, 7.3, 7.6, 8.0, 9.0 ± 0.1). Pure silica, sand of Ajman (which is very rich in carbonate content), and calcium oxide were used with specific percentages to obtain the different pH levels. P. oleracea seedlings were grown and irrigated either with deionised water or with 200 ppm of Cr(VI) as sodium chromate. Another six groups were irrigated with deionised water for control. Dry plants were analysed for total chromium in both roots and shoots.

Concentrations of total chromium in both roots and shoots at the six different pH levels were determined and are shown in Figures 3-11 and 3-12, respectively. Bioaccumulation factors (BAF) from soil to roots of Portulaca and translocation factors (TF) from roots to shoots were calculated and the results are shown in Figure 3-13. Weight of dry biomass of plants in both control and experimental were calculated and the results are shown in Figure 3-14 and the total removed chromium was calculated in the total dry weight of the whole plants in each pot and the results are shown in Table 3-16.

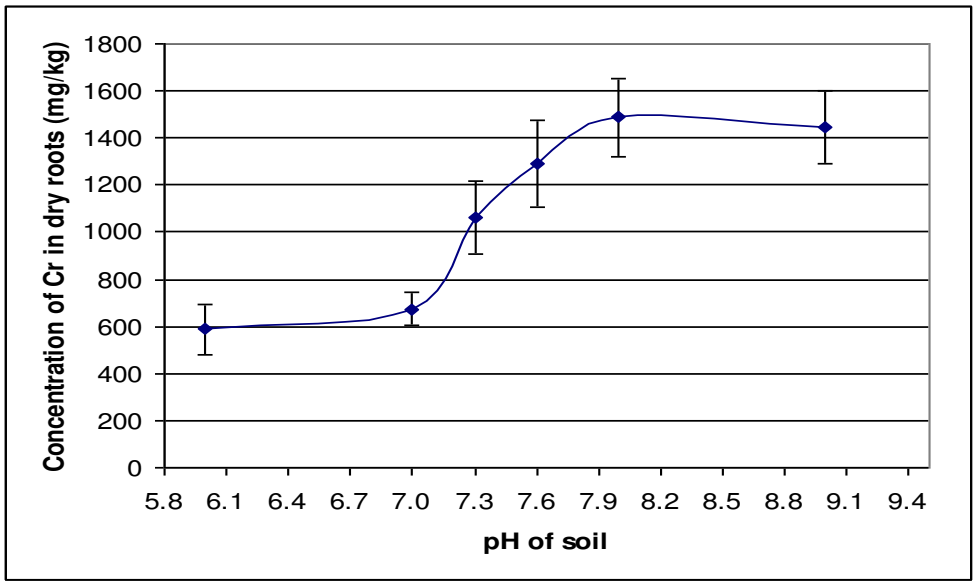


Figure (3-11) Concentrations of chromium in the roots of Portulaca oleracea at different values of pH of Soil. (Mean of six replicates)

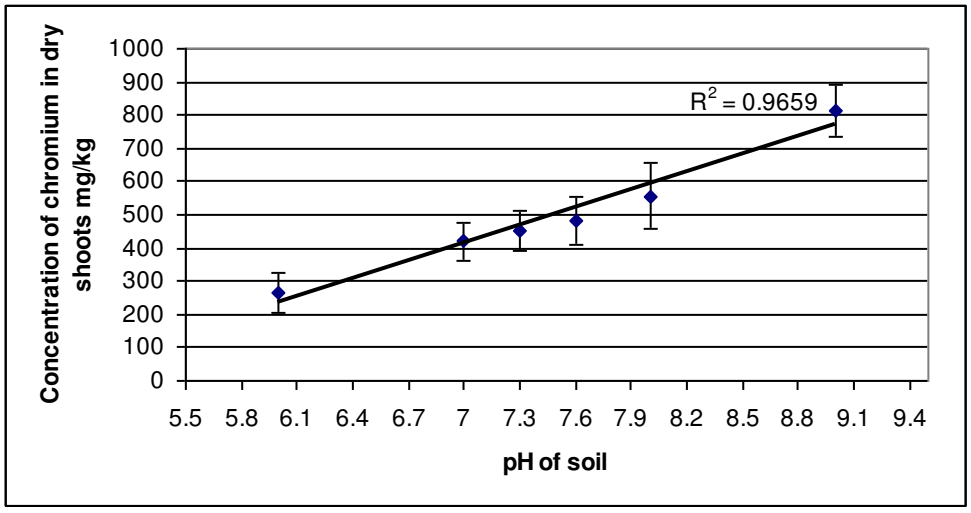


Figure (3-12) Concentrations of chromium in the shoots of Portulaca oleracea at different values of pH of Soil. (Mean of six replicates)

The results indicate that as the pH of soil increases, the uptake of chromium (VI) in roots increases (Figure 3-11). Significant increments between the mean values of chromium in roots were observed when compared at the three levels of

pH (6.0, 7.3, 9.0) using ANOVA, Tukey test ($p<0.01$). In shoots, at the same three pH levels, chromium increases significantly as pH of soil increases (Figure 3-12). The increase of chromium in shoots is mostly a result to its increase in roots. These results are in agreement with two previous studies [117, 118], which investigated the effect of pH on the uptake of Cr(VI) from hydroponics using fungi and microorganisms. Researchers found that the accumulation of chromate by the aquatic fungi Aspergillus foetidus increased as pH of nutrient medium increased from 4-7 [117]. In another study, it was found that between the 3 values of pH; 7.0, 8.0, 9.0; at pH 9 chromium (VI) was accumulated by microorganisms with highest concentration [118].

Concentration of Cr(VI) in roots of Portulaca at a pH range of 6.0 –7.0 was 587 to 675 mg/kg of the dry roots with no significant difference ($p>0.05$ using ANOVA Post hoc test "Tukey") (Figure 3-12). At this relatively low range of pH, chromium (VI) mostly exists in soil as $Cr_2O_7^{2-}$ species. At pH range of 7.6 – 9.0, the concentration of Cr(VI) in roots of Portulaca jumped to 1300-1500 mg/kg of the dry roots with no significant difference ($p>0.05$ using ANOVA Post hoc test "Tukey"). At this range of pH, chromium (VI) exists as CrO_4^{2-} species. Those two observations reflect the effect of chromium(VI) speciation (which is pH-dependent) on the uptake of Cr (VI) by P. oleracea. The high uptake of CrO_4^{2-} at pH levels above 7.0 confirms that Portulaca oleracea prefers to accumulate CrO_4^{2-} rather than $Cr_2O_7^{2-}$, which is available at the lower range of pH (below 7). According to previous studies, [76, 79, 134, 136]; it has been suggested that chromate anions use the carriers of sulfate (as an essential nutrient) in their uptake

by root cells due to the structural similarity between the two anions in charge, shape and size [137, 138].

The results of the present study are in disagreement with two previous studies carried out on the crop plants, barley and wheat [89, 116]. The investigators of those studies found that both barley and wheat accumulated higher amounts of Cr(VI) at the low levels of pH (below 6.1) which indicates that both barley (Hordeum vulgare) and wheat (Triticum aestivum) tend to take up Cr(VI) as dichromate which is the most dominant at this low pH range.

The concentration of chromium in the shoots of Portulaca shows an increase from 262 mg/kg at pH 6 to 813 mg/kg at pH 9. This increase seems to be linear at the six levels of pH (Figure 3 -12). The increase in the uptake of Cr(VI) as the pH of soil increases supports the role of P. oleracea as accumulator for Cr(VI) from soils like Ajman industrial site, which is contaminated with Cr(VI) and has pH of 7.9 .

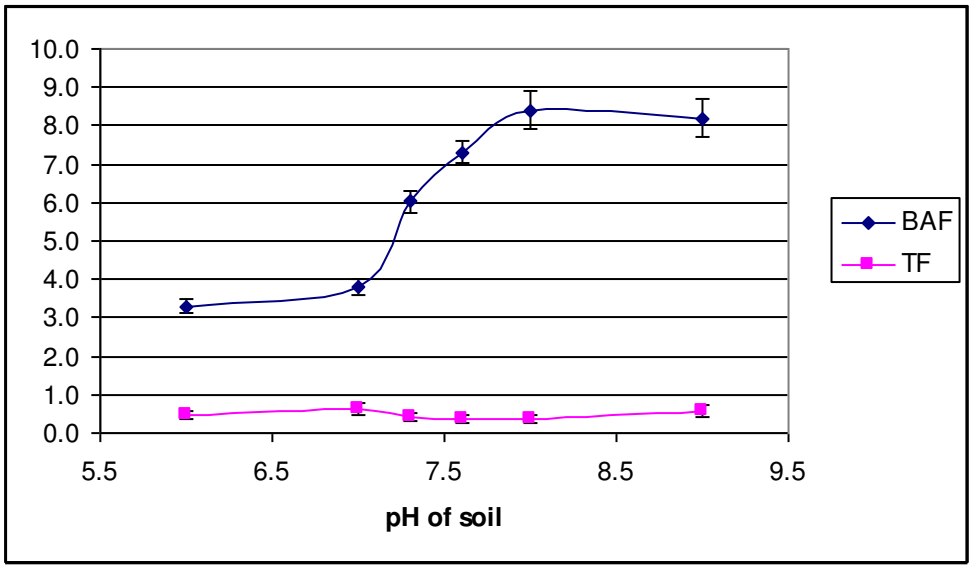


Figure (3-13): Bioaccumulation and Translocation Factors of chromium using Portulaca oleracea at different pH values of soil.

The increase in bioaccumulation factor (Figure 3- 13) as the pH increases reflects the increase of the uptake of chromium in roots. Bioaccumulation factors ranged from 3.3 at pH 6.0 to 8.4 at pH 8.0, which was the best value of accumulation factor among the six levels of pH. The translocation factor ranged from 0.37 to 0.62 (Figure 3- 13). It seems to be independent of the pH of soil. This may be due to the reduction of Cr(VI) in roots to Cr(III) which suggest its translocation as chelated cation [65, 60].

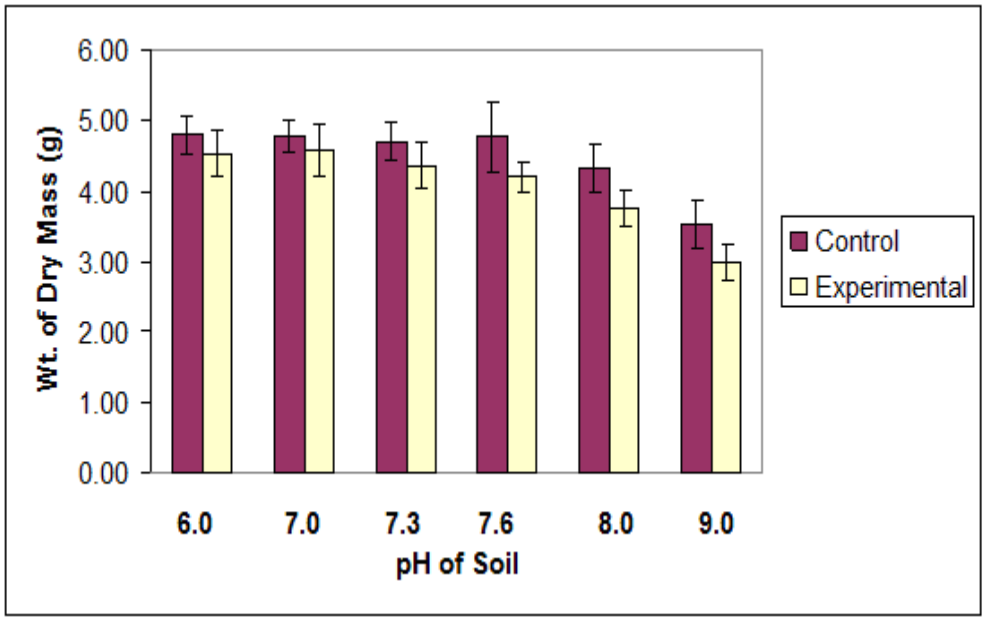


Figure (3- 14) Weight of dry biomass of Portulaca oleracea at controls and in presence of Cr(VI) at different values of pH of soil. (Mean of six replicates)

When comparing the means of the total dry biomass at the first 5 values of pH (6.0-8.0) in the control (Figure 3-14), no significant difference was observed ($p>0.05$ using ANOVA Post hoc test "Tukey"). This means that there is no significant effect of the pH of soil on the growth of the plant at the range of 6.0-8.0. A significant reduction in the weight of control samples was observed at pH 9.0

compared with other pH levels. No significant reduction in dry biomass was observed in the experimental samples (irrigated with Cr(VI)) at the range of 6.0-8.0). But at the highest levels of pH (9.0) significant reduction in dry biomass of plants was observed ($p < 0.05$ using ANOVA Post hoc test "Tukey"). This reduction may be due to the effect of high pH rather than the accumulated chromium since similar amounts of chromium were accumulated in roots at pH 8.0 and 9.0 without significant reduction in dry biomass.

Table (3-16) The total removed chromium in the total dry weight of the whole 4 plants in each pot at different levels of pH of soil. (Mean of six replicates)

pH of Soil	Wt. of total dry biomass in each pot (g)	Wt. of Removed Cr (mg) using the 4 plants of one pot (in 14 days)
6.0 ± 0.1	4.54 ± 0.43	1.46 ± 0.12
7.0 ± 0.1	4.58 ± 0.45	2.15 ± 0.17
7.3 ± 0.1	4.37 ± 0.36	2.43 ± 0.21
7.6 ± 0.1	4.21 ± 0.25	2.52 ± 0.22
8.0 ± 0.1	3.77 ± 0.29	2.63 ± 0.20
9.0 ± 0.1	3.00 ± 0.31	2.66 ± 0.24

When calculating the total amounts of chromium in the total dry weight of plants, it was observed that the highest amount removed by 4 seedlings was at the pH 8.0 -9.0, which was 2.63 – 2.66 mg respectively (Table 3-16). This result supports the conclusion that P. oleracea should be used in phytoextraction of chromium (VI) from soils of high pH such as that of Ajman polluted site (pH = 7.9 ± 0.1).

3.3.3 Effect of organic content of soil on the uptake of chromium (VI) by Portulaca oleracea.

The role of the organic content of the soil in the reduction of Cr(VI) to Cr(III) was previously investigated [62, 119-121]. High organic content of soil is mostly associated with low pH of soil so the investigation of the effect of organic content on phytoextraction of Cr(VI) in high pH soil such as Ajman industrial zone does not appear in the literature. To investigate the effect of organic content of soil on the uptake of Cr(VI) by Portulaca oleracea, three types of soil with different organic matter content (0.42%, 17.5% and 35.0 %) were prepared. P. oleracea was grown in the three types (5 replicates each) and irrigated with the same quantity of Cr (VI) as sodium chromate. Another batch of three groups of 5 replicates was irrigated with deionised water for control. The plants were harvested and analysed for total chromium (Figure 3-15). Soils were sampled and analysed for Cr(VI) and total chromium.

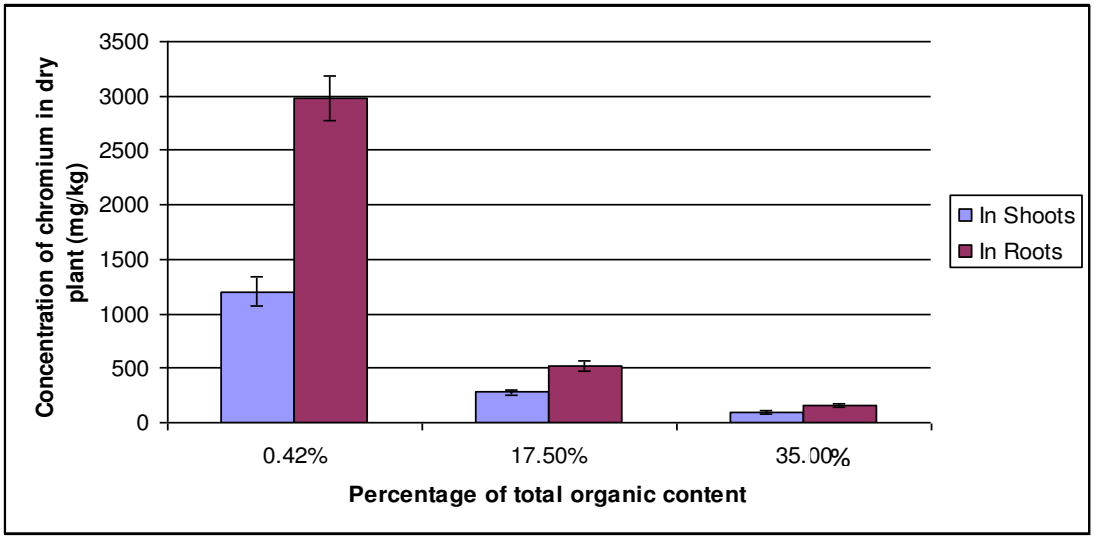


Figure (3-15) Concentrations of total chromium in both shoots and roots of Portulaca oleracea at different organic matter content of soil. (Mean of five replicates)

Significant decrease of the uptake of chromium (VI) by Portulaca oleracea was observed in both roots and shoots as the organic matter content of soil increased ($p<0.01$ using ANOVA Post hoc test "Tukey") (Figure 3-15). P. oleracea could accumulate 3000 mg/kg Cr in dry roots in the presence of 0.42% of organic content of soil. This amount represents the highest value compared to other investigations in this study carried out using similar amounts of chromium in soil (150 – 200 mg/kg) but in the presence of 15% organic content. Chromium concentration in roots decreased to 500 mg/kg at 17.5% of organic content in soil and to below 160 mg/kg at 35% of organic content. The quantity of hexavalent chromium available in soil was measured and the results are shown in Figure 3-16. These results indicate that Cr(VI) decreases as the percentage of organic matter in soil increases. This relation seems to be linear since organic matter behaves as a reducing agent for soluble Cr(VI) to the less soluble Cr(III), which is consequently less available to the plant, especially at high pH range. These results confirm that the effect of organic content of soil on the uptake of Cr(VI) by plants is indirect since it reduces Cr(VI) in soil which will result as deficiency in its uptake by P. oleracea. These results are in agreement with previous studies which used the organic matter for soil amendment to reduce Cr (VI) to Cr (III) [62, 119-121].

Bioaccumulation factors (BAFs) were calculated for the total chromium and the available Cr(VI), results are shown in Figure 3-17. Translocation factor (TF) was calculated for total chromium and the results are shown in Table 3-17. Bioaccumulation factor for total chromium using P. oleracea was 20 at 0.42% organic matter and jumped to 28 when calculated for Cr(VI) at the same percentage of organic matter. At 35.0% organic matter in soil BAF of Cr(VI) decreased to 5.0

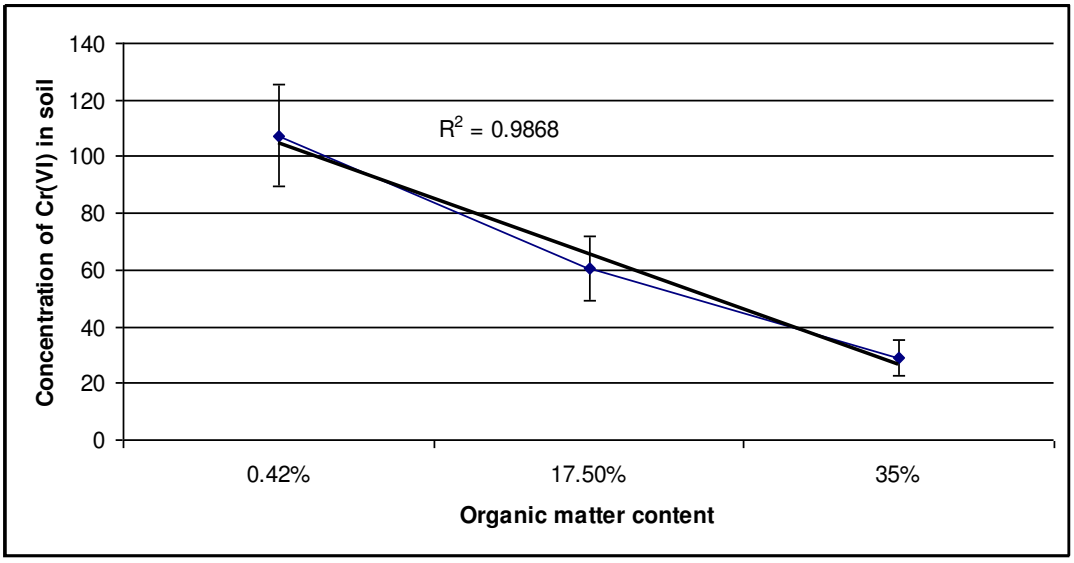


Figure (3-16) Concentration of hexavalent chromium in soil at different percentages of organic matter in soil

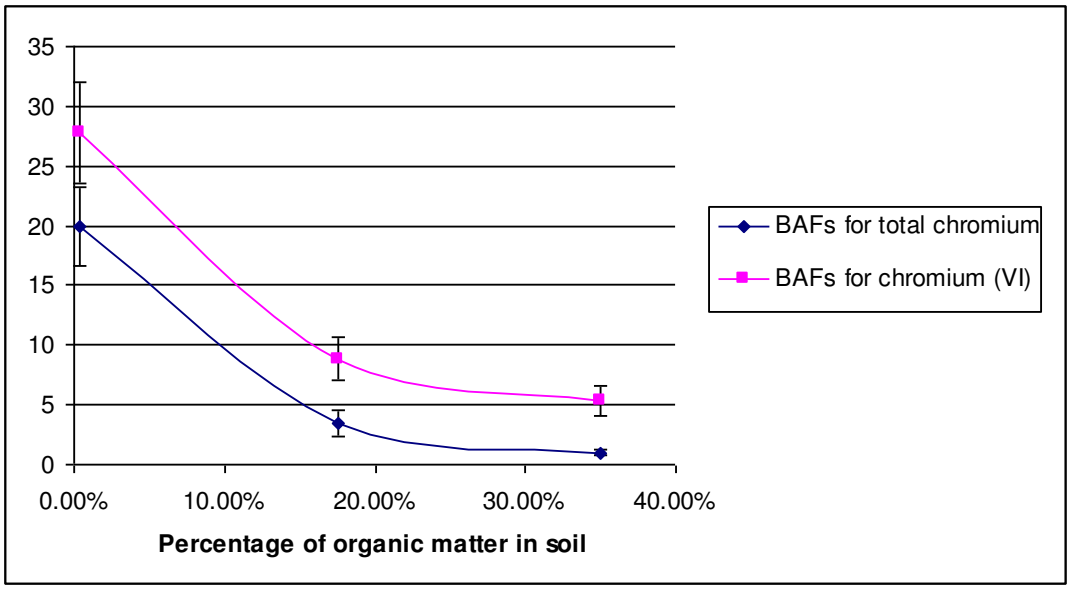


Figure (3-17) Bioaccumulation factors of total chromium and chromium (VI) at different levels of organic content in soil.

confirming the role of Portulaca as hyperaccumulator for chromium (VI) even in soils that contain considerable organic matter. The value of BAF for Cr(VI) at 0.42% of organic content of soil is 28 which is the highest achieved value in this study. This high BAF value confirms the role of P. oleracea in accumulation

Cr(VI) from soils of low organic content and high pH such as the soil of Ajman industrial zone.

Table (3-17) Translocation Factors of total chromium using Portulaca oleracea at different organic matter content of soil.

% Organic Matter	Translocation Factor
0.42%	0.40 ± 0.05
17.5%	0.54 ± 0.04
35.0%	0.60 ± 0.06

There was significant decrease in the weight of dry shoots of plants in the controls (irrigated with deionised water) as organic matter content decreased which is expected ($p < 0.05$ using ANOVA Post hoc test "Tukey"). In the experimental plants, (in the presence of Cr(VI)), significant decrease was also observed at 17.5% and 0.42% of organic content when compared with dry shoots at 35% organic matter ($p < 0.05$ using ANOVA Post hoc test "Tukey") (Figure 3-18). This is suggested to be due to the accumulated chromium (VI) in plant since as Cr(VI) accumulation increases, biomass reduction increases (3000 mg/kg at 0.42% and 500 mg/kg at 17.5% while it was 160 mg/kg at 35%). No significant difference was observed in biomass of plants irrigated with Cr(VI) solution at the level of 35% organic matter compared with control of the same organic content (Figure 3-18). This is being due to the small accumulated amount of Cr(VI) (160 mg/kg). At this level of high organic content (35%) most of Cr(VI) is being reduced to Cr(III) which is unavailable to the plant at pH of 7.9±0.1.

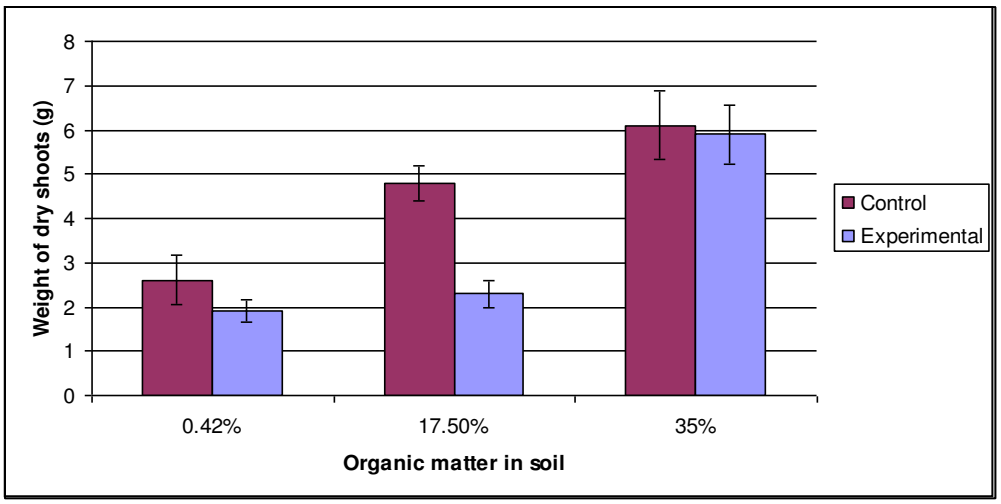


Figure (3-18) Weight of dry shoots of Portulaca oleracea at different levels of organic matter in soil in both control and experimental samples.

In addition to organic matter content, iron and manganese may alter the oxidation state of chromium in soil. These two elements were analysed in the soil of the polluted site and results are shown in Table 3-18. Previous studies of metal speciation in soil of different sites in the Emirates indicated that iron is present as Fe_2O_3 or Fe^{3+} [113, 114] and not Fe^{2+}. According to Iron E-pH (Pourbaix diagram) at pH 8 (pH of soil) the most dominant iron species is Fe^{3+}. The presence of iron as Fe^{3+} will keep chromium in (VI) oxidation state. Manganese will not affect the reduction of chromium (VI). It may oxidise Cr(III) to Cr(VI) if it is available as Mn(IV) but at the relatively small concentration detected (Table 3-18) it is unlikely to have an impact. Thus organic content is the most potential reducing agent that may affect the concentration of Cr(VI) in the soil.

Table (3-18) Iron and manganese in the polluted soil of Ajman industrial zone.

Element	**Concentration in soil of polluted site (mg/kg)**
Fe	4700 ± 150
Mn	170 ± 20

The organic matter content of the soil at the polluted site is < 0.5%. Soil with such a low organic content that is too low to reduce chromium (VI) to Cr(III), and in the absence of other reducing agents, would be highly favourable for phytoextraction of Cr(VI).

3.3.4 Effect of anionic nutrients on the uptake of Cr(VI) by Portulaca oleracea

The effects of the anions such as nitrate, sulfate and phosphate on the uptake of Cr(VI) by certain plants have been reported in the literature. Most of the previous work concentrated on chromate and sulfate [76, 79, 134-136], but nitrate and phosphate were investigated to a lesser extent [129-1132]. In both cases conflicting results were obtained for the effect of these anions on the uptake of Cr(VI) by plants. The effect of these three anions (nitrate, sulfate and phosphate) on the uptake of Cr(VI) by Portulaca oleracea has been investigated in the present work. Eight groups of pots each of five replicates were prepared. Each pot contained three seedlings of P. oleracea. Plants were irrigated with 100 ppm Cr(VI) as Na_2CrO_4 alone or accompanied with 0.02M of $NaNO_3$, Na_2SO_4, Na_3PO_4 or deionised water. The other three groups were irrigated with $NaNO_3$, Na_2SO_4 or Na_3PO_4 (without chromate) for control. The conditions were identical for all groups and the dependant variable was the added anion. Plants were analysed for total chromium.

Root lengths in the presence of nutrient anions with chromate are shown in Figure 3-19. No significant difference in root lengths was observed between experimental and control in plants irrigated with nitrate and phosphate in addition

to Cr(VI) or nitrate and phosphate only ($p>0.05$ using ANOVA Post hoc test "Tukey") (Figure 3-19). A significant reduction in root lengths was observed in plants irrigated with either chromate plus sulfate or chromate only compared with plants irrigated with chromate accompanied with nitrate or phosphate ($p<0.01$ using ANOVA Post hoc test "Tukey"). These observations are supported by the chromium uptake in roots which indicated that the highest concentration of accumulated chromium was achieved in the presence of sulfate followed by Cr(VI) only while the lowest uptake was achieved in the presence of nitrate and phosphate.

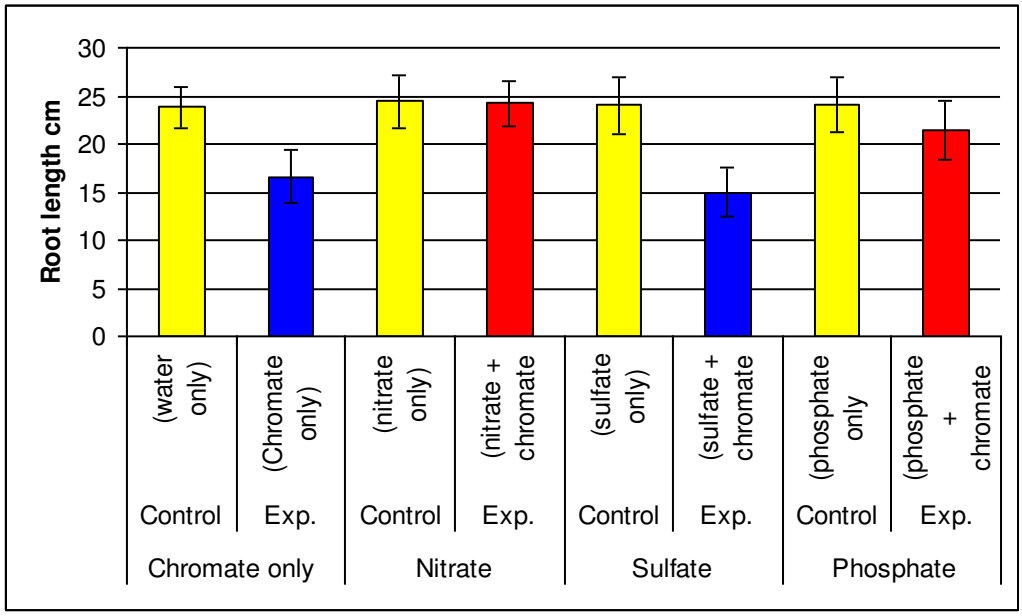


Figure (3-19) Average length of the roots in the presence of nutrient anions beside chromate. (Mean of five replicates)

Tolerance indices TI (length of roots in experimental / Length of roots in the control) were calculated and are shown in Figure 3-20. The values of TI of Cr(VI) in the presence of nitrate, phosphate, chromate only and sulfate were 0.99, 0.88, 0.70, and 0.62 respectively. The high values of TI in the presence of both

nitrate and phosphate were accompanied by the lowest accumulated amounts of Cr(VI) in roots (Figure 3-21). This may suggest that both nitrate and phosphate may have an inhibitory effect on the uptake of chromate by P. oleracea which may prefer to uptake these macro-nutrients at the expense of chromate.

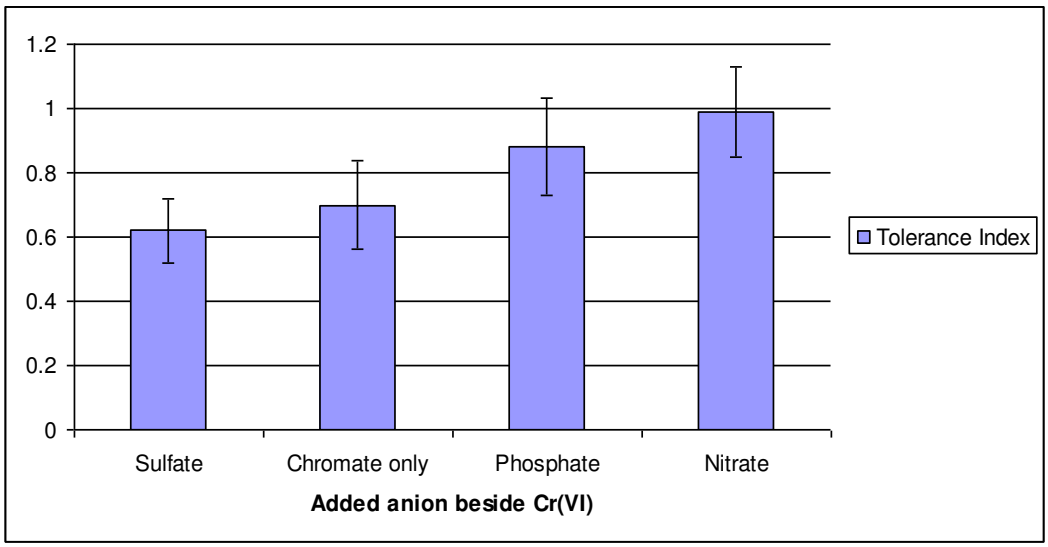


Figure (3-20) Tolerance indexes of chromium (VI) in the presence of nutrients anions.

In a previous study [132], TI of chromium (VI) in Arabidopsis thaliana in the presence of SO_4^{2-}, PO_4^{3-} and NO_3^- was 0.73, 0.83 and 0.70, respectively. When comparing these results with the results obtained in the current study (Figure 3-20), the values of TI of chromium in the presence of phosphate were very close (0.88 and 0.83). However, the higher concentration of chromium used in the current investigation (100 mg/kg) indicates that P. oleracea has a higher chromium (VI) tolerance than Arabidopsis thaliana which could not develop roots in concentrations above 10.4 mg/kg. The chromium TI of A. thaliana was about 0.60 at 10.4 mg/kg of Cr(VI) only where as P. oleracea has a TI of 0.70 at 100 mg/kg of

Cr(VI) in dry soil. This value gives P. oleracea a preference for the phytoextraction of Cr(VI).

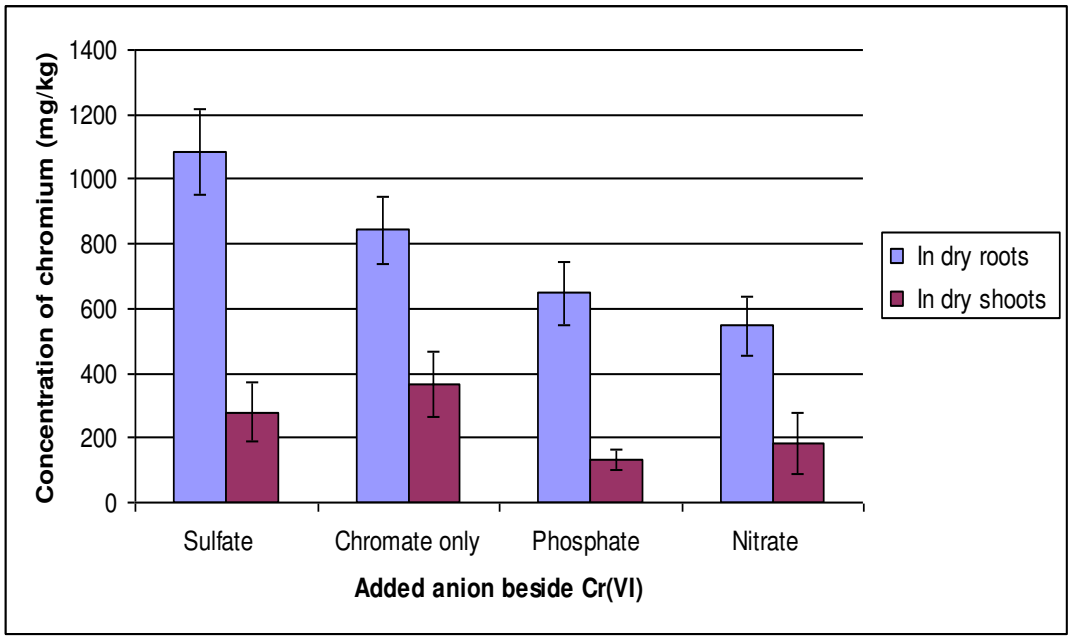


Figure (3-21) Concentration of Chromium in roots and shoots of P. oleracea using different nutrient anions beside Cr(VI). (Mean of five replicates)

The existence of sulfate anions significantly enhanced the uptake of Cr (VI) from 860 mg/kg in roots of plants irrigated with Cr(VI) only to 1140 mg/kg in the roots of Portulaca irrigated with Cr(VI) + Na_2SO_4 (Figure 3-21) ($p < 0.05$ using ANOVA Post Hoc Tukey test). This significant increase in the uptake of Cr (VI) in the presence of sulfate could be due to the similar pathway of uptake of CrO_4^{2-} and SO_4^{-2} as micronutrient [79, 134-136]. Chromium (VI) in the soil of Emirates with a pH of 7.9 will stay as chromate [CrO_4^{2-}] which is very similar to sulfate ion in charge, geometry, and size [137, 138]. Chromate anions seem to be taken up by root cells through the activities of sulfate carriers (transporters) [136]. These transporters are protein molecules on root cell's membrane and mostly are initiated

by the presence of sulfate [167], but they can also uptake chromate due to the similarity between the two anions.

No significant difference in chromium uptake was observed by roots of plants irrigated with nitrate and phosphate ($p > 0.05$ using ANOVA Post hoc test "Tukey"). But there was significant decrease in chromium uptake by roots of *P. oleracea* irrigated with Cr (VI) and nitrate compared with Cr (VI) only ($p<0.01$ using ANOVA Post hoc test "Tukey", Figure 3-20). The uptake of Cr (VI) in roots decreased from 840 mg/kg in the presence of Cr (VI) only to 550 mg/kg of dry roots of plants irrigated with Cr(VI) + nitrate. These results differ from the results of a study on willow [131] which indicated that there is no significant effect of nitrogen as nitrate on the uptake of Cr(VI) by hydroponically grown willow plants. In another study, carried out on the uptake of arsenate anions by the accumulator *Pteris vittata*, [133], the results confirmed the inhibitory effect of nitrate in the uptake of arsenate anions, which agrees with the result of the current study (taking into consideration the similarity between chromate and arsenate ions).

In shoots, the uptake of chromium in plants irrigated with Cr(VI) only was significantly greater than its uptake in those irrigated with Cr(VI) accompanied with nitrate or phosphate (Figure 3-21) ($p < 0.05$ Using ANOVA Post hoc Tukey test). No significant difference was observed between chromium in shoots of plants irrigated with Cr(VI) + nitrate compared with plants irrigated with Cr(VI) accompanied with sulfate or phosphate ($p > 0.05$ using ANOVA Post hoc test "Tukey"). Chromium in shoots was: 182.5 mg/kg in the presence of nitrate, 132.3 mg/kg in the presence of phosphate and 336.3 mg/kg for Cr (VI) only.

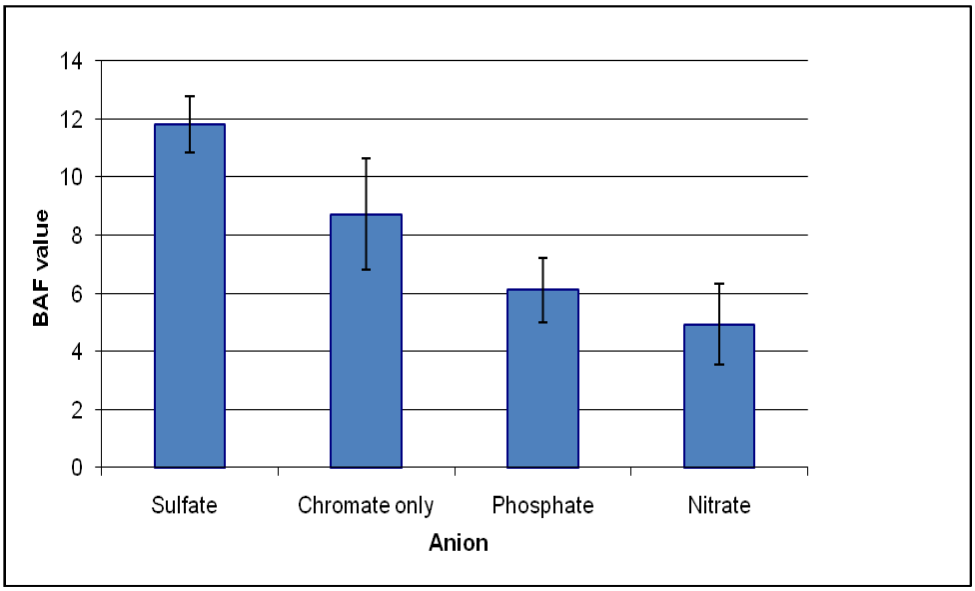


Figure (3 -22) Bioaccumulation factors (BAF) for chromium (VI) in P. oleracea in the presence of nutrient anions.

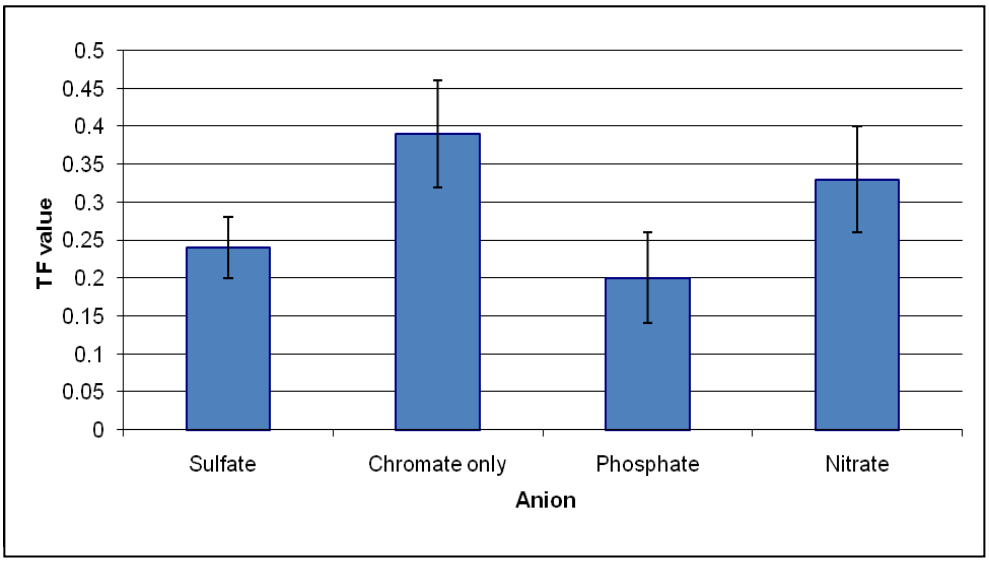


Figure (3 -23) Translocation factors (TF) for chromium in P. oleracea in the presence of nutrient anions.

Bioaccumulation factor (BAF) of chromium from soil to roots decreased in the order: in presence of sulfate> in chromate only> in presence of phosphate> in

presence of nitrate (Figure 3-22). Bioaccumulation factor was 8.72 in the presence of Cr (VI) only but in the presence of sulfate, it increased significantly to 11.8 which confirm the role of sulfate in enhancing chromium (VI) uptake by P. oleracea. Translocation factor (TF) values were 0.39, 0.33, 0.20 and 0.24 corresponding to irrigation with Cr(VI) only, Cr(VI) + nitrate, Cr(VI) + phosphate and Cr(VI) + sulfate, respectively (Figure 3-23). It seems that the presence of sulfate and phosphate significantly decreased the translocation of chromium to shoots.

3.3.5 Effect of sulfate ions on the uptake of chromate by P. oleracea

In the previous investigation, it was observed that the use of 0.02 M sulfate in irrigation solution significantly enhanced the uptake of chromium (VI) by P. oleracea. A detailed investigation of the effect of sulfate on the uptake of chromate by P. oleracea was therefore undertaken. Two experiments were carried out to study this effect. Chromium (VI) was introduced as 200 ppm in the first experiment. At this concentration of chromium, the highest removal was observed in a previous investigation (section 3.3.1). In the second experiment Cr(VI) was 100 ppm to match the concentration of Cr(VI) in the polluted site of Ajman. The ratio of sulfate to chromate stayed constant in the two experiments. Sulfate was introduced at five different concentrations (0, 300, 600, 1200, 1800 ppm + 200 ppm of Cr(VI) in each solution) into five groups of identical pots each containing 4 seedlings of P. oleracea. Each group consisted of five replicates. The sixth group was irrigated with deionised water as control. It is believed that the uptake of chromate takes place through the sulfate carriers in root cells. In order to determine

the sulfate concentration at which secretion of these carriers is being optimised, the second experiment was carried out using the sulfate concentrations of (0, 150, 300, 600, 900 ppm + 100 ppm of Cr(VI) in each solution). The plants were harvested and analysed for total chromium using ICP-OES, and for total sulfur using ion chromatography. Concentrations of chromium in roots and shoots of P. oleracea at different levels of sulfate in the irrigation solution are shown in Figures 3-24, 3-25. Concentrations of sulfur in shoots and roots are shown in Figures 3-26, 3-27.

Concentration of chromium in roots increased significantly ($p<0.01$ using ANOVA Post hoc test "Tukey") as the concentration of sulfate increased from 0.0 ppm to 300 ppm. The same trend was observed in the results of the two experiments (200 and 100 mg/kg of Cr(VI) Figures 3-24, 3-25). From 300-600 ppm of sulfate the uptake of Cr(VI) stayed without significant difference ($p>0.05$), however when the concentration of sulfate exceeded the value of 600 ppm, chromium in roots decreased significantly ($p<0.01$ using ANOVA Post hoc test "Tukey" Figures 3-24, 3-25). This could be explained as being due to the role of sulfate in initiating sulfate carriers in the plant thus allowing chromate to get into the roots. But the increase of sulfate in the rhizosphere initiates a competition with chromate

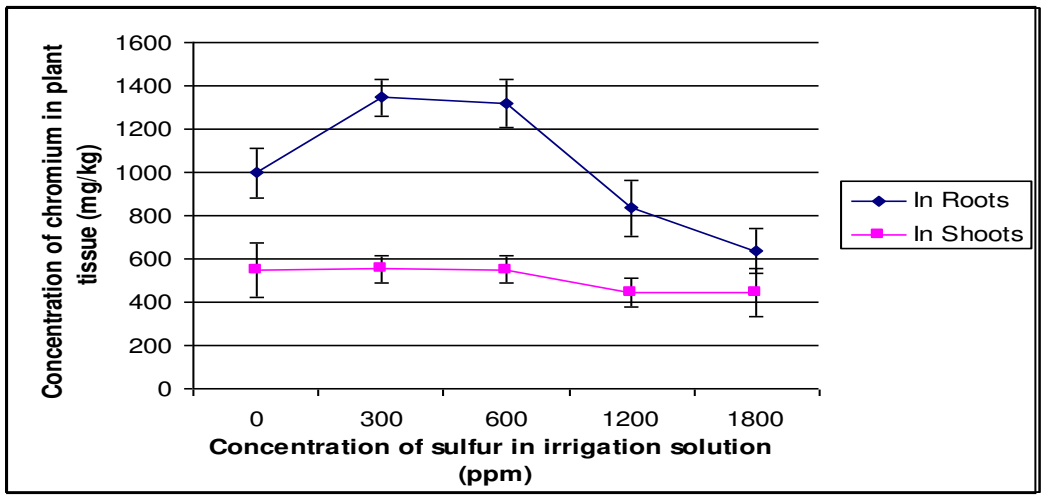


Figure (3-24) Concentrations of chromium in roots and shoots of P. oleracea irrigated with 200 ppm of chromium (VI) at different concentrations of sulfate. (Mean of five replicates)

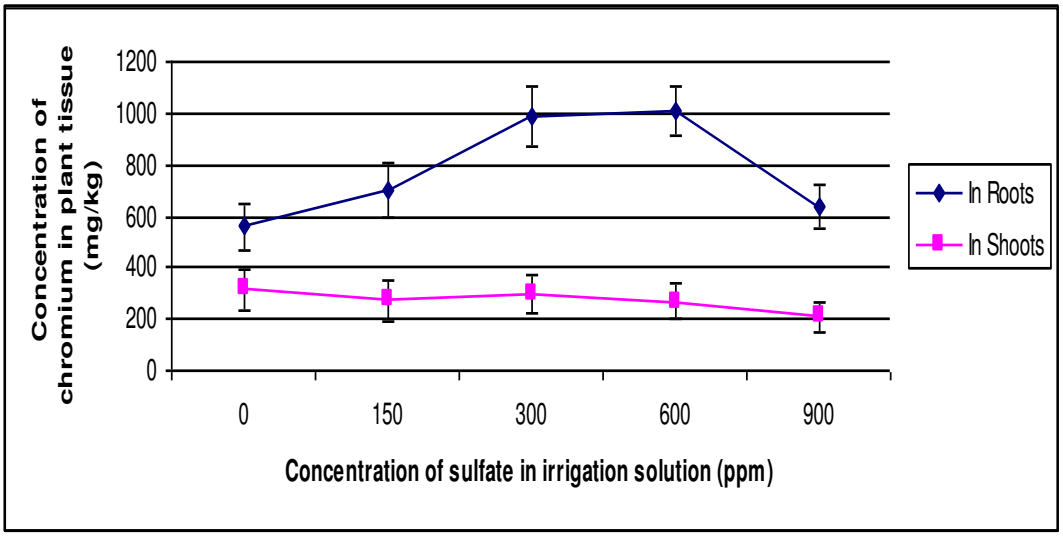


Figure (3-25) Concentration of chromium in roots and shoots of P. oleracea at the different levels of sulfate in irrigation solution (half concentrations). (Mean of five replicates)

which explains the significant lowering of the uptake of chromate at the high levels of sulfate (over 600 ppm in the two experiments) since sulfate has the priority to be taken up due to its high concentration in soil. Similar impacts have been reported in previous study using wheat plants (Triticum aestivum) [136].

No significant difference in mean chromium concentrations in shoots was observed over the range of 0 to 1800 ppm of sulfate at 200 ppm of Cr (VI) ($p>0.05$ using ANOVA Post hoc test "Tukey", Figure 3-24). The same trend was observed in the second experiment at 100 ppm of Cr(VI) (Figure 3-25). Concentration of chromium in shoots approximately remained constant in each experiment. This may indicate that the translocation of chromium to shoots depends on the concentration of plant carrier ligands not the concentration of chromium in roots which differs. These ligands are proposed to be organic acids such as oxalate and not phytochelatins which are found to increase as sulfur in soil increases.

It can be observed that the concentrations of sulfur in roots and shoots increased significantly ($p<0.01$ using ANOVA Post hoc test "Tukey") as the concentration of sulfate in soil increases at the levels 0, 600, 1200 (Figures 3-26) and the levels 0, 300, 600 (Figures 3-27). These significant increments are expected for a required nutrient as sulfate.

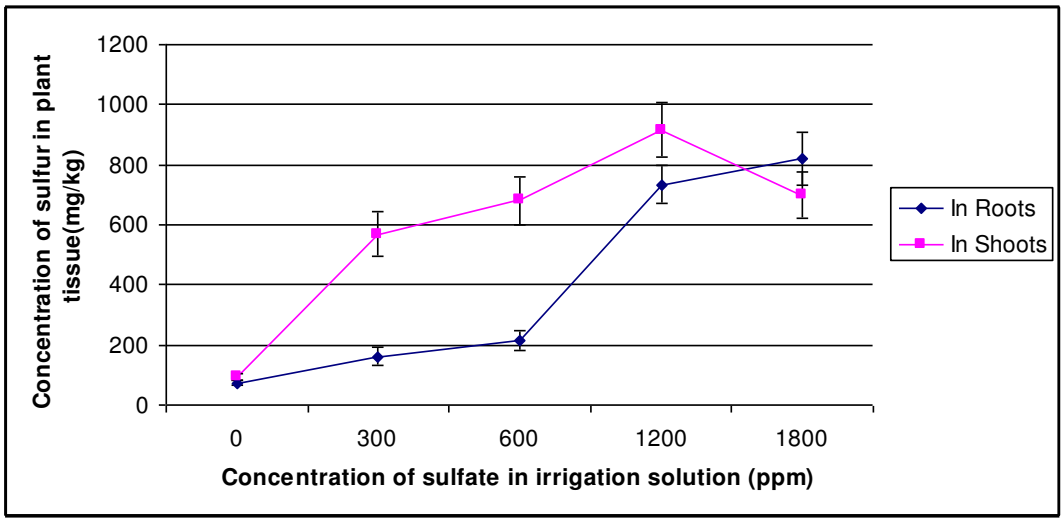


Figure (3- 26) Concentrations of sulfur in roots and shoots of P. oleracea irrigated with 200 ppm of chromium (VI) at different concentrations of sulfate. (Mean of five replicates)

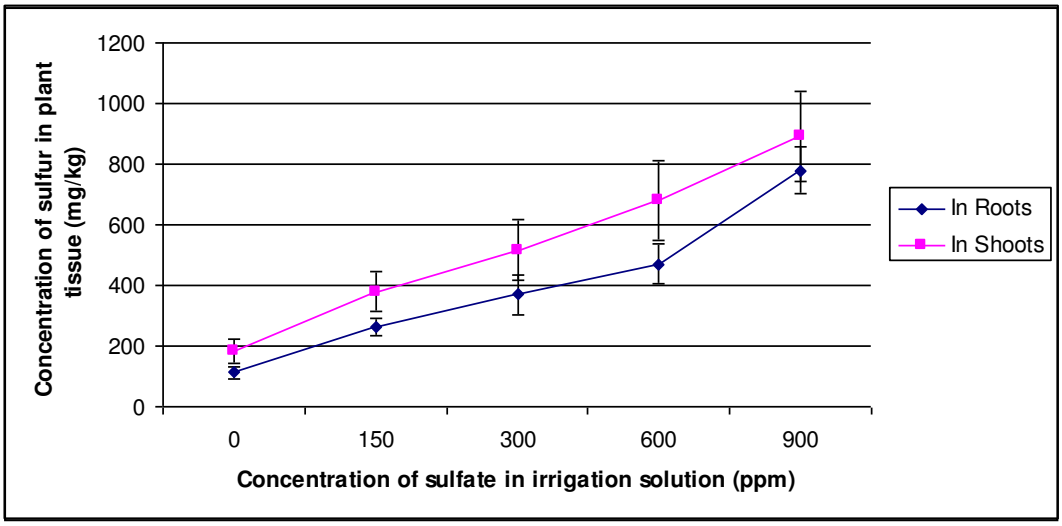


Figure (3-27) Concentration of sulfur in roots and shoots of P. oleracea at the different levels of sulfate in irrigation solution (half concentrations).

Bioaccumulation factors and translocation factors were calculated for chromium and sulfur (at different concentrations of sulfate) and they are shown in Table 3-19. The bioaccumulation factors of chromium in Portulaca oleracea are greater than the bioaccumulation factors of sulfur using the same plants, even when the concentration of sulfur was greater than chromium in the samples irrigated by 1200 and 1800 ppm of sulfate. This can be attributed to the relatively low concentration of sulfur in roots since most of it is being translocated to shoots. Sulfate is reduced to sulfide (S^{2-}) - either in roots or shoots [168] - because it is needed at low oxidation states for building sulfur- containing amino acids (thiols) which are mostly synthesised in leaves [169], while Cr(VI) is mostly reduced in roots to Cr(III). It seems that chromium and sulfur have different pathways of translocation in spite of their similar suggested pathway of take up by the same plants.

Table (3-19) Bioaccumulation and translocation factors for chromium and sulphur in P. oleracea at different concentration of sulfate.

Concentration of sulfate in irrigation solution (ppm)	Bioaccumulation factor for		Translocation factor for	
	chromium	sulfur	chromium	sulfur
0.0	5.2±1.1	1.3±0.2	0.55±0.13	1.3±0.2
300	7.2±1.4	1.4±0.2	0.41±0.11	3.5±0.6
600	7.2±1.3	1.2±0.2	0.42±0.12	3.2±0.5
1200	4.6±1.0	2.5±0.3	0.53±0.14	1.2±0.2
1800	3.4±0.9	2.1±0.3	0.70±0.16	0.9±0.1

Figure 3-28 shows the bioaccumulation factors for sulphur and chromium and Figure 3-29 shows the translocation factors for the two elements at different concentrations of sulfur. The maximum bioaccumulation factor for chromium can be observed at 300-600 ppm of sulfate in irrigation solution, and, at the same range of sulfate in irrigation solution, the bioaccumulation of sulfur was the minimum. This observation may reflect the competitive relationship between sulfate and chromate in the uptake by roots, and suggest that both anions are being taken up within the same carriers when getting into the plant. The translocation factor was at its minimum value for chromium at 300-600 ppm of sulfate but it attained the maximum value for sulfur at the same level of sulfate.

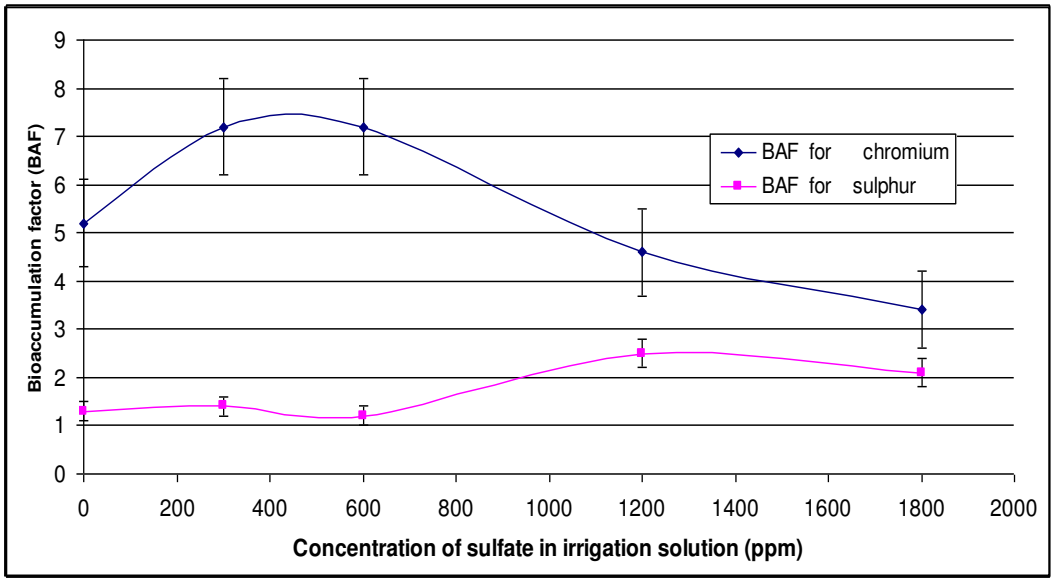


Figure (3-28) Bioaccumulation factors of sulfur and chromium in the roots of P. oleracea at different concentrations of sulfate.

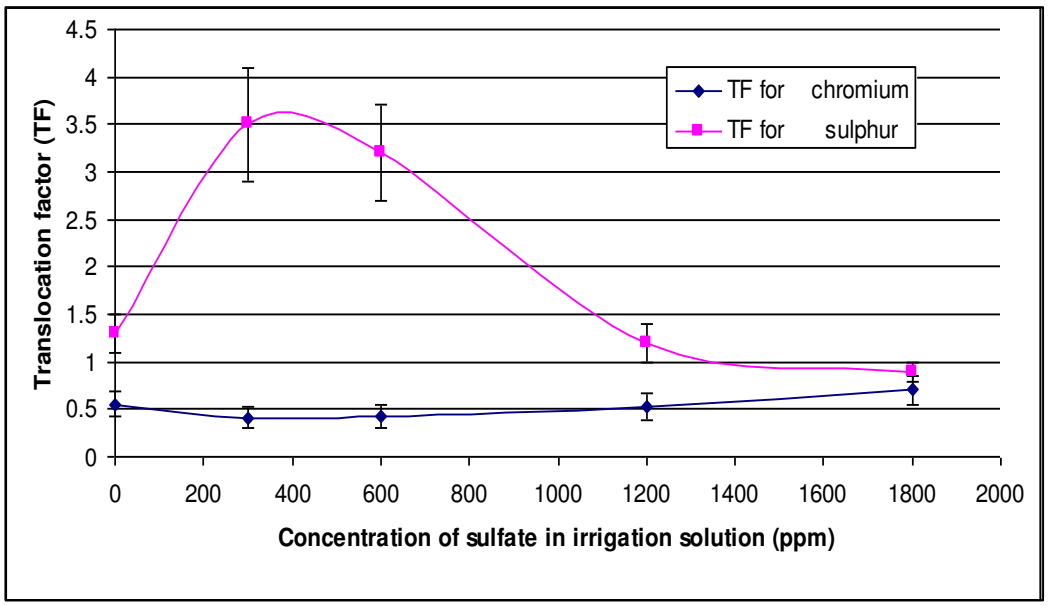


Figure (3-29) Translocation factors of sulfur and chromium from roots to shoots of P. oleracea at different concentrations of sulfate

To evaluate the efficiency of phytoextraction process at the different levels of sulfur in irrigation solution, the total dry weight of four P. oleracea seedlings in each pot and the amounts of chromium removed by these plants were calculated.

The results are shown in Table 3-20. No significant differences between the weights of dry biomass of plants were observed ($p > 0.05$ using ANOVA Post hoc test "Tukey"). This means that in the presence of sulfate, the effect of chromium (VI) on the vegetation of the plant remained limited. The total amount of chromium removed by the total dry weight of plant was the highest when the plant was irrigated with 300 and 600 ppm of sulfate solution (Table 3-20). This confirms the previous conclusion that the presence of sulfate in small amounts enhances the overall uptake of chromium (VI).

Table (3-20) Total amount of chromium removed by the dry weight of 4 seedlings of P. oleracea grown in one pot.

Concentration of sulfate in irrigation solution (ppm)	Mean weight of total dry plant (g)	weight of total Cr in 4 seedlings (mg)
0	3.00 ± 0.15	1.79 ± 0.14
300	3.26 ± 0.19	2.06 ± 0.17
600	3.21 ± 0.21	2.00 ± 0.16
1200	3.49 ± 0.23	1.68 ± 0.13
1800	3.45 ± 0.219	1.59 ± 0.13

The sums of the concentrations of chromium and sulfur (mol/kg of dry roots) were calculated. The results are shown in Table (3-21). It can be observed that at the concentration of sulfate 300 ppm in irrigation solution or greater, the sum of both chromium and sulfur in roots ranged between 0.031 and 0.037 mol/kg of dry roots at the half concentrations investigation (from 0 to 900 ppm sulfate). The same sum ranged between 0.031 and 0.038 mol/kg at the double concentration (from 0 to 1800 ppm sulfur). These close concentrations at sulfate levels 300 ppm

and above (300, 600, 900, 1200 and 1800 ppm) may indicate that sulfate carriers are initiated in roots to their maximum level above 300 ppm of sulfate and are being produced in root cells with specific concentration regardless of the concentration of sulfate in soil which supports the role of sulfate carriers in the uptake of chromate in plants.

Table (3-21) Sums of concentrations of both chromium and sulphur in roots of Portulaca at different levels of sulfate in irrigation solution.

Con. Of sulfate (ppm)	Concentration in Roots (mol/kg)		Sum of S and Cr (mol/kg)	Con. Of sulfate (ppm)	Concentration in Roots (mol/kg)		Sum of S and Cr (mol/kg)
	Sulfur	Chromium			Sulfur	Chromium	
0	0.004	0.011	0.014	0	0.002	0.019	0.021
150	0.008	0.013	0.022	300	0.005	0.026	0.031
300	0.012	0.019	0.031	600	0.007	0.025	0.032
600	0.015	0.019	0.034	1200	0.023	0.016	0.039
900	0.024	0.012	0.037	1800	0.026	0.012	0.038

The results of this investigation suggest that chromate is taken up by plants using the same cellular carriers of sulfate in the plant cell membrane which is in agreement with previous studies carried out using different plants [76, 79, 134-136]. The effect of chromate on the uptake of sulfate by Zea mays was investigated [135]. Chromium (VI) was introduced as potassium chromate. The reporters of that study observed that the presence of chromate reduced the uptake of sulfate by the plants, which agrees with the conclusion of the current study related to the competitive relationship between chromate and sulfate at high concentrations of sulfate. The results of the present investigation suggest that when Cr(VI)-contaminated soils are remediated using phytoextraction technologies, the concentration of sulfate in soil must be taken in account.

3.3.6 Effect of accompanying cations on the uptake of Cr(VI) using Portulaca oleracea

The effect of counter ions seems to be important in understanding the relation between the uptake of an anion and its accompanying cation by plants [63, 65] especially when regarding the role of coupled transporters of both. Effect of associated cations on the uptake of nutrient anions such as nitrate or phosphate was studied in some crops plants [122- 127] but the effect of cations on the uptake of pollutant anions such as chromate, dichromate or arsenate has not been studied deeply. The present work looked into the effect of accompanying cations such as sodium, potassium and ammonium on the uptake of Cr(VI) by P. oleracea. Four groups of plants were irrigated with Cr(VI) either as Na_2CrO_4, K_2CrO_4, $(NH_4)_2CrO_4$ or with deionised water for control. Plants were analysed for total chromium. Tolerance indexes (TI) were calculated as indicators of chromium (VI) tolerance and the results are shown in Figure 3-30.

There was significant difference between TI values in the different groups including control except between the two groups irrigated with potassium and ammonium chromate ($p<0.01$, using ANOVA Post hoc test "Tukey") (Fig 3-30). This confirms the effect of accompanying cation of chromate on the plant growth and root developing. The plants in the two groups irrigated with potassium and ammonium chromate demonstrated significant close reduction in root growth. This can be explained as being due to the higher amounts of chromium (VI) accumulated in roots in the presence of potassium and ammonium (Figure 3-31).

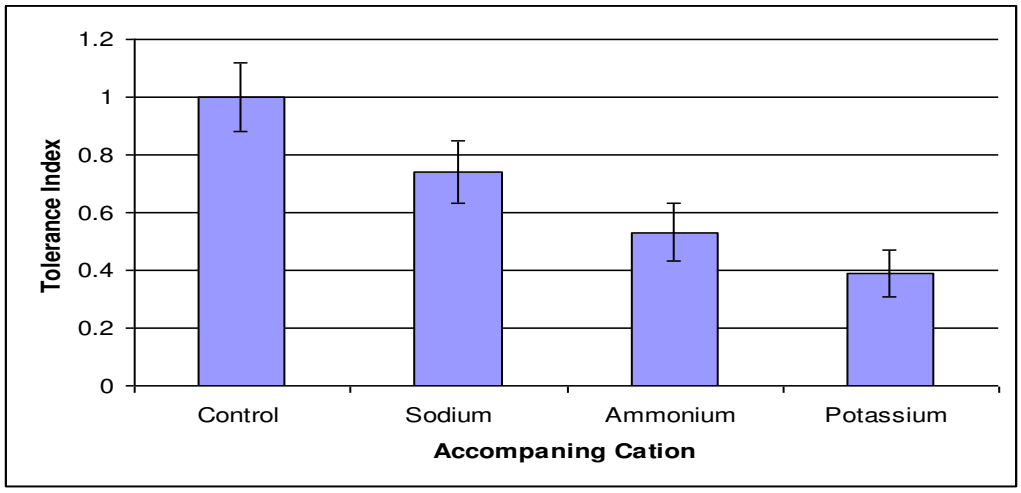


Figure (3-30) Tolerance Indexes of chromium in P. oleracea in presence of accompanying cations of chromate.

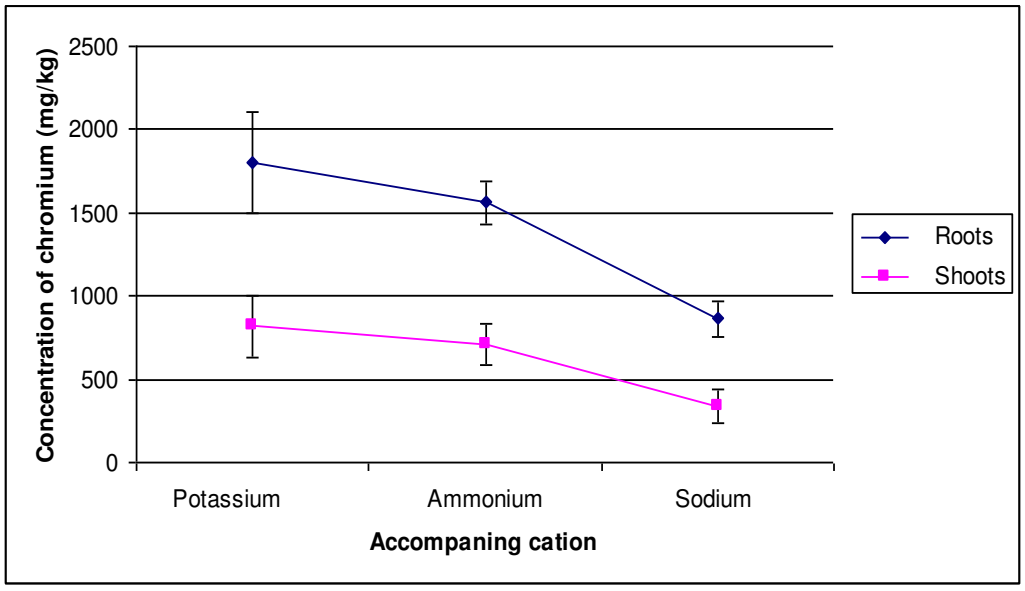


Figure (3 -31) Concentration of chromium in dry tissues of P. oleracea irrigated with chromate accompanied with different cations. (Mean of five replicates)

There was significant reduction in the uptake of chromium by roots and shoots of plants irrigated with sodium chromate compared to those irrigated with potassium and ammonium chromate ($p<0.01$ using ANOVA Post hoc test "Tukey"). Chromium content in both roots and shoots was in the order of the

accompanying cation as: $K^+ \geq NH_4^+ > Na^+$. No significant difference was observed between chromium uptake in roots or shoots of plants irrigated with potassium and ammonium chromate (Figure 3-31) ($p > 0.05$ using ANOVA Post hoc test "Tukey").

The highest uptake of chromium was observed in the presence of K_2CrO_4 which was around 1802 mg/kg in roots and 820 mg/kg in shoots followed by $(NH_4)_2CrO_4$ with 1560 mg/kg in roots and 709 mg/kg in shoots (Figure 3-31). The lowest concentration of chromium in Portulaca tissues was observed when Na_2CrO_4 was used for irrigation which was around 862 mg/kg in roots and 336 mg/kg in shoots.

The high uptake of Cr (VI) in the presence of both potassium and ammonium may be due to the counter cation effect since the plant requires both of ammonium and potassium cations as primary plant macronutrients. Plants may uptake both of potassium and ammonium cations using coupled transporters [65] which may suggest that chromate may be coupled with these counter cations through passing the root cell membrane. These results confirm the role of the accompanying nutrient cations in enhancing the uptake of counter anions by plants. These results are in agreement with the results of a previous study carried out using the accumulator Pteris vittata [133]. The workers of this study concluded that both of potassium and calcium enhanced the uptake of arsenate as counter anions using P. vittata.

Table (3-22) Bioaccumulation factors and Translocation factors of chromium in P. oleracea in the presence of accompanying cations of chromate

Irrigation Solution	Bioaccumulation Factor	Translocation Factor
K_2CrO_4	20.0 ± 2.4	0.46 ± 0.11
$(NH_4)_2CrO_4$	17.3 ± 2.1	0.46 ± 0.10
Na_2CrO_4	8.7 ± 1.2	0.39 ± 0.10

Both bioaccumulation and translocation factors (BAF and TF) were calculated and tabulated in Table 3-22. Bioaccumulation factors were 20.0 and 17.3, respectively, for chromium(VI) when potassium chromate and ammonium chromate were used in irrigation solutions. When sodium chromate was used the bioaccumulation factor was reduced to 8.7, reflecting the fact that sodium is not an essential element for plants. This clearly reflects the priority need of plant for both nitrogen and potassium and illustrates the effect of accompanying cation on the uptake of chromium (VI) anions. These results suggest that the enrichment of a polluted site with potassium and ammonium cations would enhance the phytoextraction of chromate.

No significant difference was observed in the mean values of the translocation factors of chromium from roots to shoots of P. oleracea (Table 3-22). The values of TF in the presence of ammonium chromate, potassium chromate and sodium chromate were 0.46, 0.46 and 0.39 respectively. These results may suggest that the translocation of chromium in P. oleracea is independent of ammonium, potassium or sodium cations as expected since translocation takes place inside the plant tissues.

3.3.7 Effect of chelating agents on the uptake of chromium by P. oleracea

Investigation of the effect of chelating agents on the uptake of cations such as Cr(III) occurs in the scientific literature of phytoextraction but it is rarely to find investigation on such effect for Cr(VI). It was concluded previously (section 3.3.1) that most of Cr(VI) was reduced to Cr(III) in roots of P. oleracea. It may seem reasonable to claim that chelating agents may affect the translocation of this cation since it may chelate and translocate it to shoots. It has thus been decided to investigate the effect of chelating agents such as citric acid and EDTA on the uptake of Cr(III) and Cr(VI) by Portulaca oleracea.

3.3.7.1 Effect of chelating agents on the uptake of chromium(III) by P. oleracea

Twelve pots of soil with pH of 5.5 ± 0.1 were prepared. At this relatively low pH, chromium (III) is soluble and available for plants. Three seedlings of Portulaca oleracea were grown in each pot. Sets of three pots were irrigated with one of the following solutions: chromium (III) nitrate, chromium (III) nitrate + 0.01 M of EDTA, chromium (III) nitrate + 0.01 M of citric acid in addition to a fourth set irrigated with deionised water for the control. Plants were harvested then analysed for total chromium.

Concentrations of chromium in roots and shoots of Portulaca were measured and the results are shown in Figure (3-32). Table (3-23) shows bioaccumulation and translocation factors of chromium in Portulaca oleracea in the presence of citric acid and EDTA as typical chelating agents.

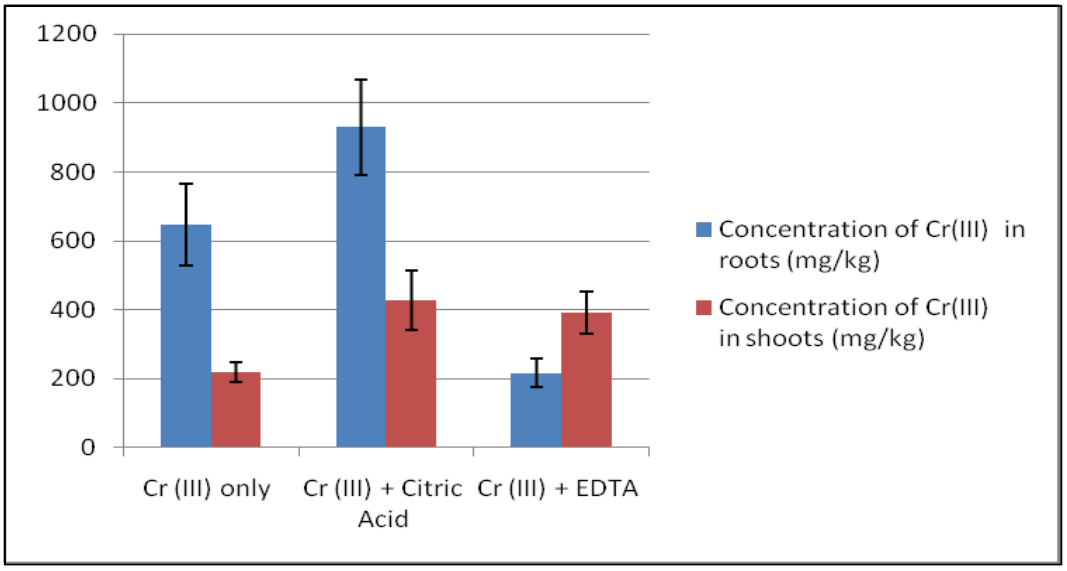


Figure (3-32) Uptake of Chromium (III) in roots and shoots of Portulaca in the presence of EDTA and citric acid. (Mean of triplicates)

Table (3-23) Bioaccumulation and translocation factors of chromium (III) in Portulaca in the presence of chelating agents

Irrigation Solution	Bioaccumulation Factor BAF	Translocation Factor TF
Cr (III) only	7.2 ± 2.1	0.34 ± 0.08
Cr (III) + Citric Acid	10.3 ± 2.8	0.46 ± 0.11
Cr (III) + EDTA	2.3 ± 0.4	1.8 ± 0.44

The highest concentration of chromium in roots was observed in plants irrigated with citric acid at 930 mg/kg followed by plants irrigated with Cr(III) only which reached 650 mg/kg. This significant increment in the presence of citric acid (Figure 3- 32, $p< 0.05$ using ANOVA Post hoc test "Tukey") confirms its effect in enhancing the uptake of Cr (III) by P. oleracea. Citric acid occurs naturally in Portulaca plants [170], so it is easily accepted by the plants while it chelates Cr(III). Bioaccumulation factor was the highest with citric acid (10.3) followed by

BAF for Cr(III) only with 7.2. When comparing the quantities of accumulated chromium in the whole plant (both roots and shoots), it can be concluded that the presence of citric acid gave P. oleracea the best chromium accumulation among the plants in control and in EDTA, which is in line with literature reports on Brassica juncea [140] and Lycopersicum esculentum [142, 145].

Significant decrease in the uptake of Cr(III) was observed in roots in the presence of EDTA compared to Cr(III) only ($p<0.05$ using ANOVA Post hoc test "Tukey") indicating the role of EDTA in retarding the uptake of Cr(III). When calculating the total chromium in plant (in roots and shoots), it was found that its amount in the presence of EDTA may exceed its amount in the presence of chromium only. This can be explained due to the enhancement of translocation of Cr(III) in the presence of EDTA which should be taken in consideration. This interpretation is supported by the enhanced translocation factor of Cr(III) from roots to shoots from 0.34 in control to 1.8 (Table 3-23) in the presence of EDTA which explains the low concentration of chromium in roots. The current results are in line with the results of a previous study which investigated the effect of citric acid and EDTA on the uptake of Cr(III) by Datura innoxia [143]. The researchers in that study found that citric acid enhanced the uptake of Cr(III) compared to EDTA but EDTA enhanced the translocation of Cr(III) compared to citric acid. In another study, the role of EDTA on the uptake of chromium (III) by willow trees was investigated [141]. The investigators of this study reported the decrease of chromium in roots and the elevation of translocation factor in the presence of EDTA. Their explanation of this behaviour was that EDTA may keep Cr (III) in the

nutrient medium which seems strange with the high translocated amount of this element to shoots which explains its decrease in roots.

The result of the current study is in disagreement with the result of recent study investigated the effect of EDTA on the uptake of Cr(III) by roots and shoots of water spinach (Ipomonea aquatic) [144]. They found that EDTA significantly enhanced the uptake of chromium by roots but inhibited its translocation to shoots. They explained their observations due to the formation of Cr-EDTA which enhanced the transfer of Cr^{3+} to the root cells and retarded the translocation from shoots to roots. This explanation seems unlikely since EDTA is commonly used to enhance translocation of cations of heavy metals such as Cd, Pb and Ni [139, 140, 171-173].

The highest concentration of chromium in shoots was observed in plants irrigated with citric acid which was 426 mg/kg, followed by plants irrigated with Cr(III) plus EDTA at 391 mg/kg (Figure 3-32). These significant increments of chromium in shoots in the presence of the two chelating agents compared with shoots in the presence of Cr(III) only confirm the role of the two chelating agents in enhancing the translocation of Cr(III) (Table 3- 23).

There was no significant difference in the weight of the dry biomass between plants irrigated with Cr(III) with and without citric acid or EDTA ($p > 0.05$). This may be due to the low toxicity of chromium (III) on the root cells on the one hand and to the relatively small amounts of accumulated chromium which may not reach the phytotoxic limit on the other (Fig 3- 33).

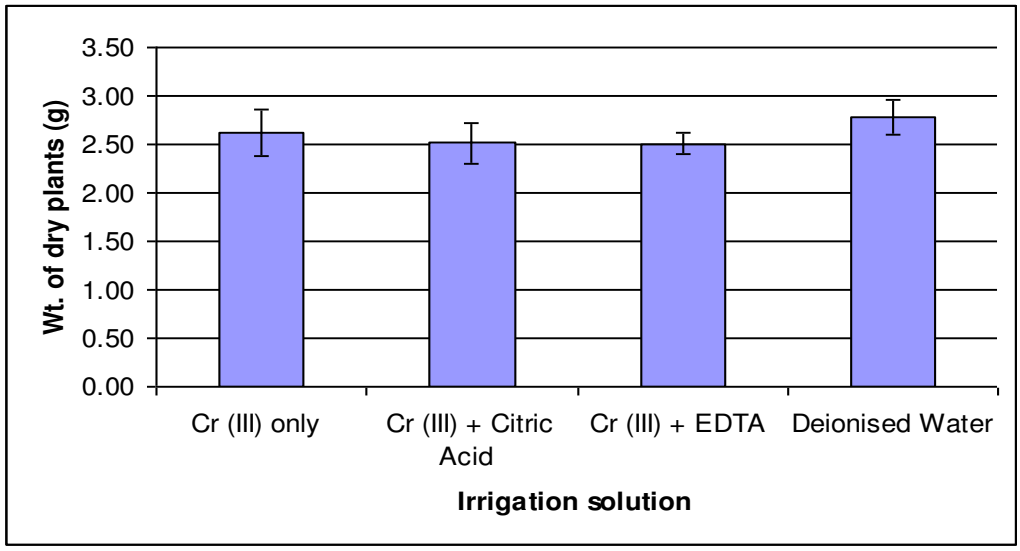


Figure (3-33) Weight of whole dry plants in one pot at different chelating agents added with chromium (III).

3.3.7.2 Effect of chelating agents on the uptake of chromium(VI) by P. oleracea.

Portulaca oleracea demonstrated the highest quantity of accumulated Cr(III) in the presence of citric acid, while EDTA confirmed significant enhancement for Cr(III) translocation using the same plant. Since Cr(VI) is likely to be reduced and translocated as Cr(III), it is interesting to investigate EDTA and citric acid for their effect on the phytoextraction of Cr(VI). To investigate the effect of the two chelating agents on the uptake of Cr(VI) by P. oleracea, four groups of five replicates were irrigated with one of the following solutions: sodium dichromate, sodium dichromate + 0.01 M of EDTA , sodium dichromate + 0.01 M of citric acid, or acidified deionised water for the control. Each plant was harvested then analysed for total chromium. Table (3-24) shows the uptake of chromium (VI) in roots and shoots of Portulaca in the presence of EDTA and citric acid and Table

(3-25) shows bioaccumulation and translocation factors of chromium(VI) in the presence of these two chelating agents. Figure (3- 34) show the mean weights of dry whole plants in one pot of Cr(VI).

Table (3-24) Uptake of chromium (VI) in roots and shoots of Portulaca in the presence of EDTA and citric acid. (Mean of triplicates)

Irrigation Solution Components	Concentration of chromium in Root (mg/kg)	Concentration of chromium in Shoot (mg/kg)
Cr(VI)	740 ±70	250 ± 46
Cr(VI) + EDTA	730 ±120	400 ± 60
Cr(VI) + Citric acid	450 ± 90	110 ± 30

Table (3-25) Bioaccumulation and translocation factors of chromium (VI) in Portulaca in the presence of EDTA and citric acid

Irrigation solution	Bioaccumulation factor (BAF)	Translocation factor (TF)
Cr(VI)	7.4 ± 1.8	0.34 ± 0.07
Cr(VI) + EDTA	7.3 ± 2.1	0.54 ± 0.11
Cr(VI) + Citric acid	4.5 ± 1.2	0.25 ± 0.07

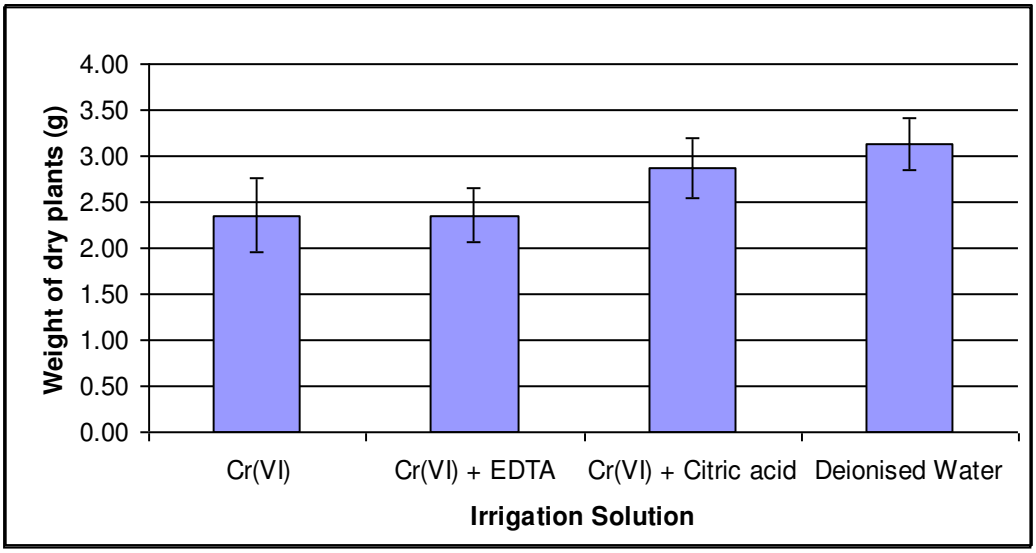


Figure (3-34) Weight of whole dry plants in one pot at different chelating agents added with chromium(VI).

No significant difference was observed between mean concentrations of chromium in the roots of plants irrigated with Cr(VI) only and with Cr(VI) + EDTA ($p>0.05$ using ANOVA Post hoc test "Tukey", Table 3- 24). This means that EDTA has no significant effect on the uptake of Cr(VI) which is expected since chromium(VI) is available as chromate anion and most unlikely to be chelated with EDTA. The uptake of Cr(VI) by roots in the presence of citric acid decreased significantly compared with its uptake in the presence of Cr(VI) only (Table 3-24). It is possible that citric acid was oxidised by dichromate in the acidic solution which means that Cr(VI) in irrigation solution was decreased which explain the negative effect of citric acid on the uptake of Cr(VI).

The highest concentration of chromium in shoots was 394 mg/kg and it was achieved in the presence of Cr(VI) accompanied with EDTA. The presence of EDTA significantly enhanced the translocation factor of Cr (III) (originated from

reduced Cr(VI) ($p<0.05$ using ANOVA Post hoc test "Tukey") from 0.34 to 0.54 but this enhancement is still small compared to the value of TF in the presence of EDTA for Cr(III) which was 1.8. The role of EDTA in enhancing the translocation of Cr(VI) (after its reduction to Cr(III) in roots) is in agreement with the results of a previous study carried out on two types of willow plants (Salix matsudana and Salix babylonica) [141]. No significant differences in weight of dry plants was observed between the plants irrigated with Cr(VI) only, Cr(VI) + EDTA or Cr(VI) + citric acid ($p>0.05$ using ANOVA Post hoc test "Tukey"). This may be due to the relatively small amounts of chromium in the plants (Figure 3- 34).

In conclusion, EDTA has a significant effect in increasing the translocation factor of both Cr(III) and Cr(VI) in Portulaca oleracea but it has no significant effect on the bioaccumulation of the chromium. This confirms the role of EDTA inside the plant and specifically from roots to shoots which means that the chelation of Cr(III) takes place inside the plant tissues. Citric acid enhanced the uptake of Cr(III) but did not show such enhancement with Cr(VI).

3.3.8 Maximising the uptake of Cr(VI) using P. oleracea

As a comprehensive evaluation of the factors that affect the uptake of Cr(VI) using P. oleracea, it can be observed that the values of bioaccumulation factor (BAF) represent a conclusive indicator of the effect of each investigated factor on enhancing the uptake process. Regarding the pH of soil, it was concluded that at pH 8.0 and above, the BAF values ranged from 8.5 to 10 but these values were achieved in the presence of 15% organic content in soil. When the organic content decreased to 0.42% (the real percentage of organic matter in Ajman

industrial zone) the value of BAF increased to 20.0 and jumped to 28.0 when calculated regarding available Cr(VI) in soil. Meanwhile, the effect of nutrient anions on BAF exhibited an increase from 7.0 in control to 12.0 in the presence of sulfate and while the effect of cations showed the highest BAF in the presence of potassium (17.0) and ammonium (20.0). In conclusion, the best bioaccumulation for Cr(VI) using P. oleracea can be achieved at: pH of soil 7.9 and organic content 0.42% where both conditions are already available in the polluted site of Ajman. The best bioaccumulation of Cr(VI) also can be achieved in the presence of 0.02 M of sulfate and 0.002 M of potassium or ammonium.

The translocation factor (TF) of chromium using P. oleracea ranges from 0.25 to 0.45 but this value was enhanced in the presence of 0.02 M sulfate and in the presence of 0.002 M potassium or ammonium (0.5). A translocation factor value of 0.6 was achieved in the presence of 35% organic content and pH of 7.0 but these results were achieved in experimental work and far from the real conditions in the polluted site of Ajman industrial zone.

3.4 Effect of chromium(VI) on the concentration of sulfur containing proteins and ascorbic acid in P. oleracea.

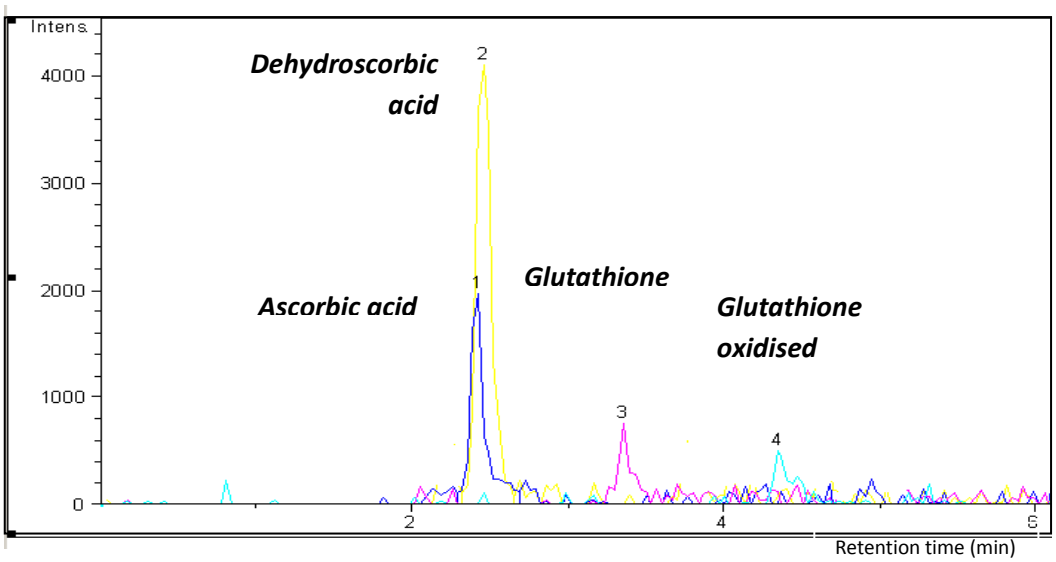
Glutathione is a sulfur containing simple protein and it is the basic building block of phytochelatins which are thought to chelate cations through the translocation process from roots to shoots. Chromium (VI) is mostly reduced in roots to chromium(III) cations then chelated either with phytochelatins or organic acids such as oxalate. Both glutathione and ascorbic acid are thought to reduce chromium(VI) in root tissues. In this section, two investigations were carried out. Glutathione and ascorbic acid in P. oleracea were investigated as Cr(VI)

antioxidants firstly. In another investigation glutathione and the phytochelatin PC3 were determined as sulfur containing proteins. This investigation was designed to find the most probable antioxidant for Cr(VI) and the suggested ligand to chelate it to shoots after its reduction as Cr(III).

3.4.1 Investigation of the antioxidants of chromium(VI) in Portulaca oleracea.

The effect of sulfate in enhancing the uptake of chromate has been confirmed in the present study. Sulfate is being reduced, then used in the synthesis of sulfur-containing amino acids or thiols such as glutathione which are common antioxidants in plants. Ascorbic acid is a natural component in P. oleracea and it may act as antioxidant. Both glutathione and ascorbic acid were investigated for their probable participation in the reduction of chromium (VI) in P. oleracea. The effect of Cr(VI) on the concentration of these two antioxidants in P. oleracea was also investigated. Plants were grown in identical soils then irrigated with three different concentrations of Cr(VI); 0, 50 and 100 ppm. The fresh shoots and roots of P. oleracea were analysed using HPLC-MS for ascorbic acid (ASA), dehydroascorbic acid (DASA), glutathione (GSH) and oxidised glutathione (GSSG). The four compounds of ASA, DASA, GHS, and GSSG were eluted at retention times of 2.4, 2.5, 3.4, 4.4 min. respectively in standards and samples with m/z 175.1, 173.1, 306.2, 611.2.

Figure (3- 35) shows the chromatogram of the four compounds extracted from shoots of Portulaca oleracea irrigated with 50 ppm of chromium(VI). Figure (3-36) shows the mass spec (MS) peaks of ASA and DASA. Figures (3-37) and (3-38) show the MS peaks of, GSH and GSSG. Table (3-26) shows the concentrations of the four compounds in roots and shoots of Portulaca oleracea. Total thiols (sum of concentration of glutathione and oxidised glutathione) and total ascorbic (sum of ascorbic acid and dehydroascorbic acid) were calculated and the results are shown in Figures (3-39) and (3-40). Concentration of total chromium in shoots and roots were calculated at the different levels of chromium in irrigation solution and are shown in table (3-27).



Figure(3-35) Mass Spec. Chromatogram showing the retention time and intensity of ascorbic acid, dehydroascorbic acid, glutathione and glutathione oxidised in shoots of Portulaca irrigated with 50 ppm of chromium(VI).

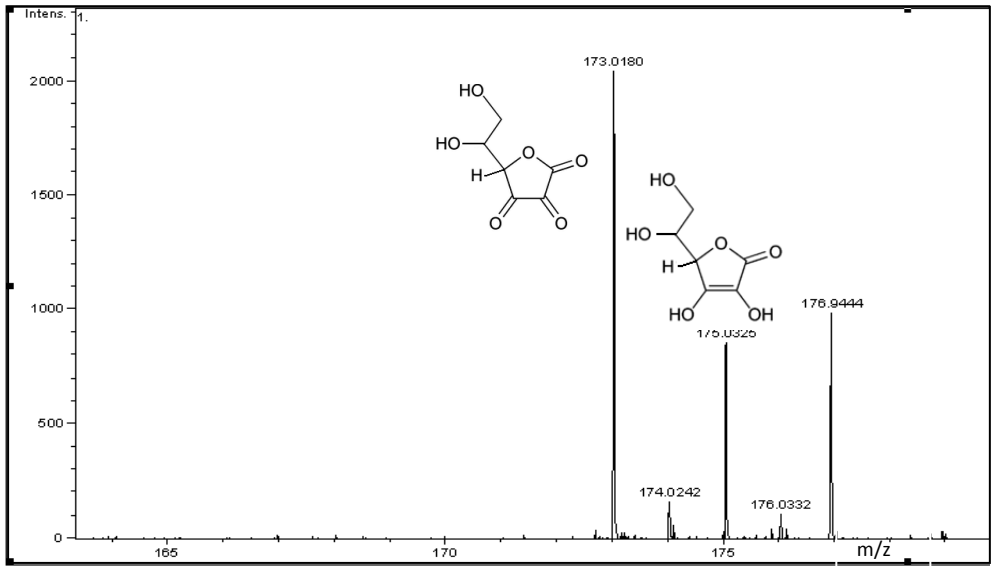


Figure (3-36) Ascorbic acid (M-1= 175) and dehydroascorbic acid (M-1 = 173) in shoots of Portulaca irrigated with 50 ppm of Cr(VI).

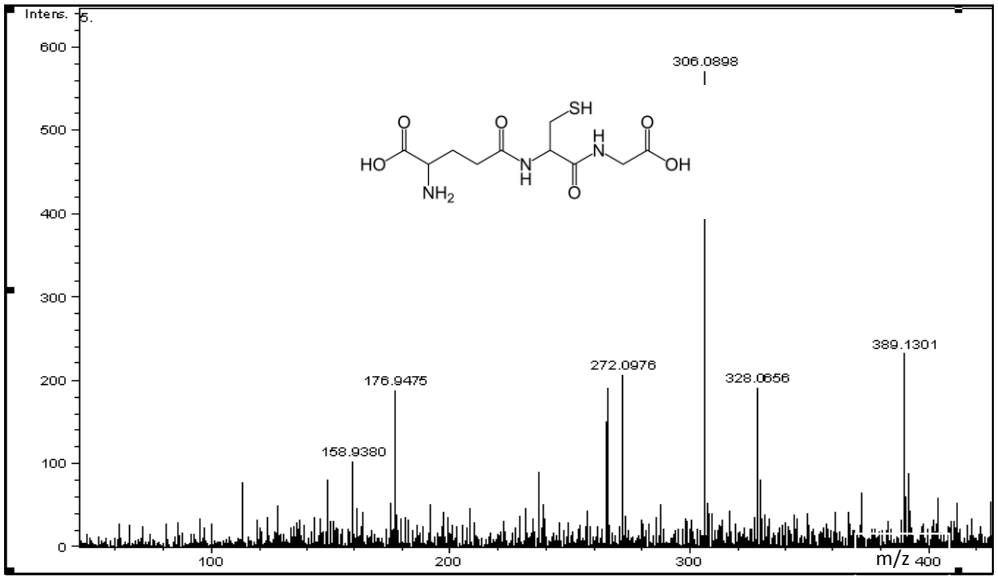


Figure (3-37) Glutathione reduced (M-1 = 306.08) in shoots of Portulaca irrigated with deionised water (control).

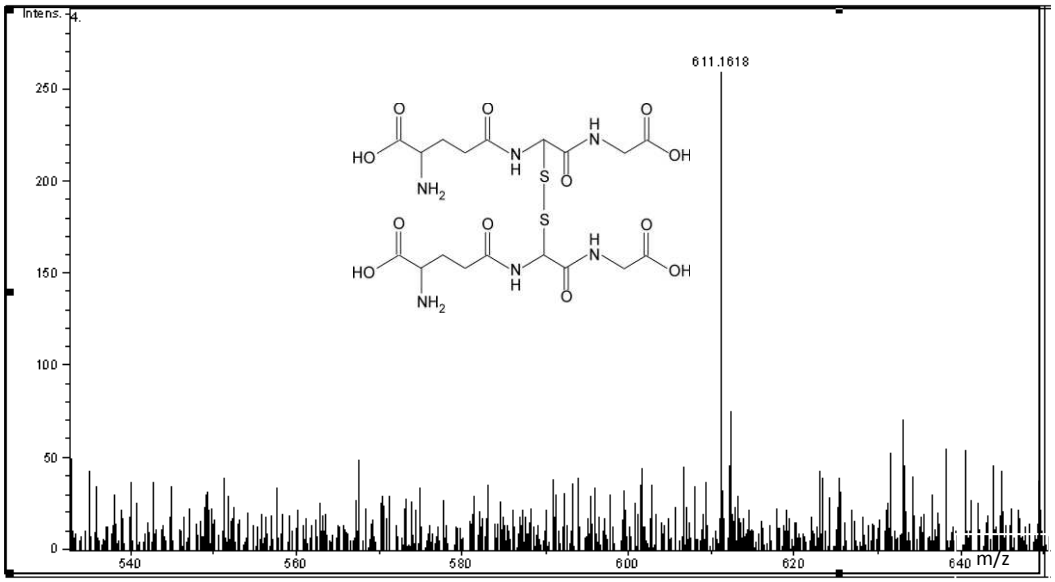


Figure (3-38) Oxidised glutathione (M-1= 611.16) in shoots of Portulaca irrigated with 50 ppm of Cr(VI).

Table (3-26) Concentration of ascorbic acid ASA, dehydroascorbic acid DASA, glutathione GSH and oxidised glutathione GSSG in fresh tissues of Portulaca at different concentrations of Cr(VI) in irrigation solution.

Plant tissue and concentration of Cr(VI)	ASA (mg/kg)	DASA (mg/kg)	GSH (mg/kg)	GSSG (mg/kg)
0 ppm root	134 ± 37	99 ± 26	3.4 ± 0.2	1.8 ± 0.3
50 ppm root	254± 49	780 ± 120	< 0.01	< 0.01
100 ppm root	< 0.1	1387 ± 153	< 0.01	< 0.01
0 ppm shoot	251 ± 52	173 ± 32	4.1 ± 0.8	2.5 ± 0.6
50 ppm shoot	212 ± 47	743 ±134	2.7 ± 0.3	3.4 ±1.5
100 ppm shoot	< 0.1	914.0 ±140.6	< 0.01	1.5 ±1.3

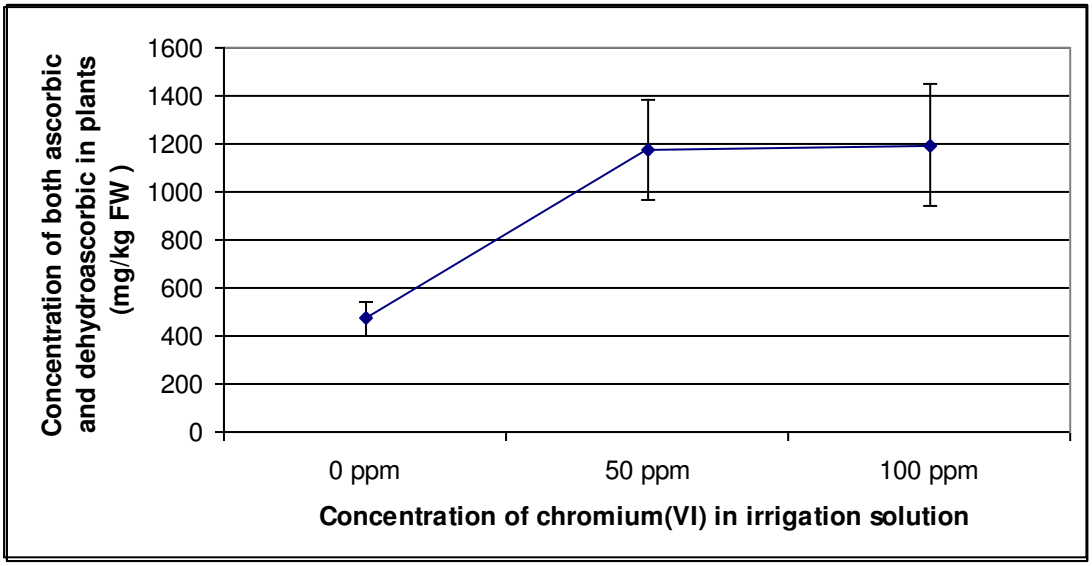


Figure (3-39) Concentration of total ascorbic acid (sum of ascorbic and dehydroascorbic acid in the fresh weight (FW) of the whole plant of Portulaca oleracea at different levels of Cr(VI) in irrigation solution. (Mean of triplicates)

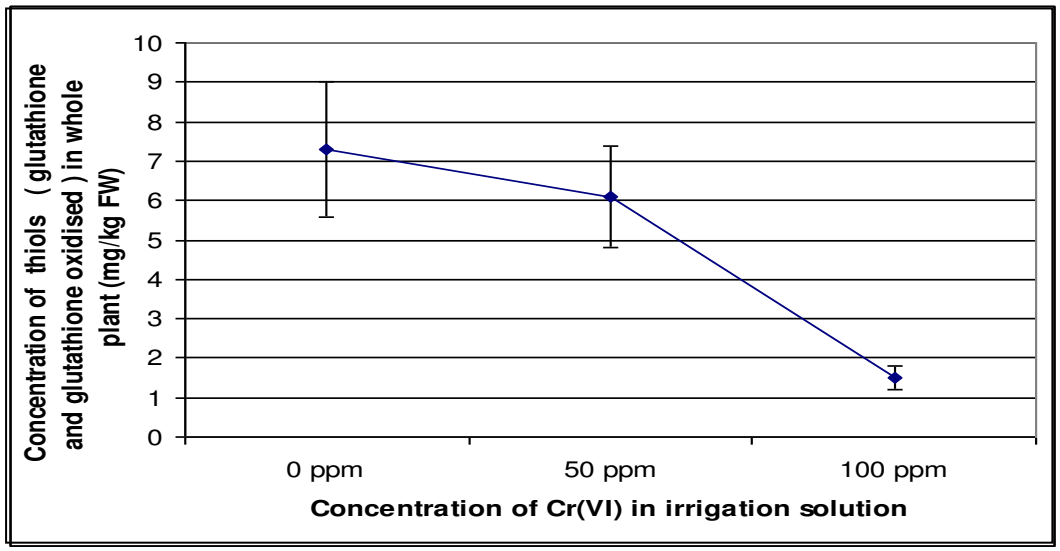


Figure (3-40) Concentration of total thiols (sum of glutathione and oxidised glutathione) in the fresh weight (FW) of the whole plant of Portulaca oleracea at different levels of Cr(VI) in irrigation solution. (Mean of triplicates)

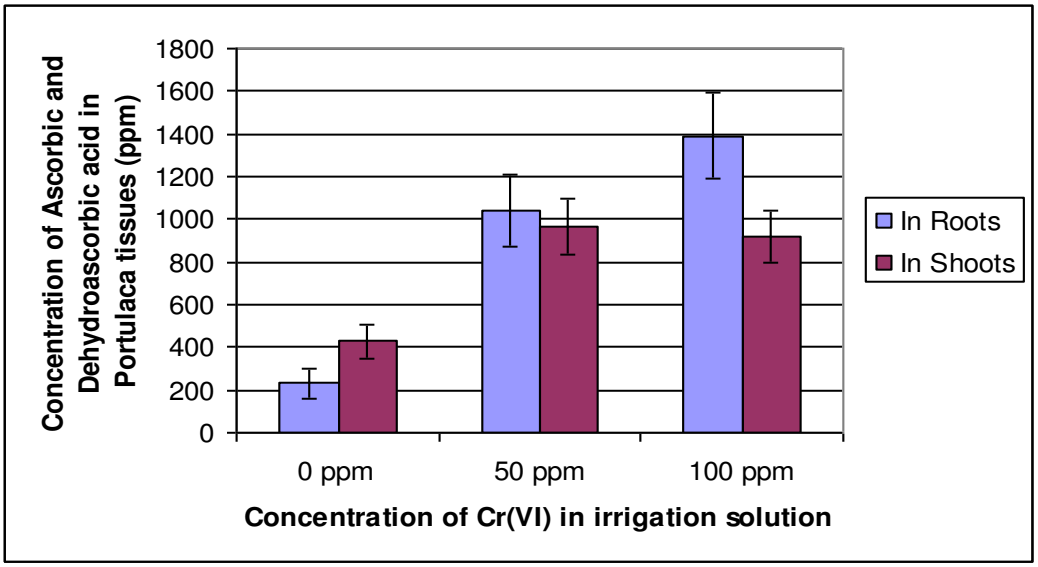


Figure (3-41) Concentration of total ascorbic acid (sum of ascorbic and dehydroascorbic acid) in roots and shoots of Portulaca oleracea at different levels of Cr(VI) in irrigation solution.

Table (3-27) Concentration of total chromium in roots and shoots of Portulaca irrigated with 50, 100 ppm of hexavalent chromium.

Cr(VI) in irrigation solution (ppm)	Cr in Roots (mg/kg)	Cr in Shoots (mg/kg)
50	452 ± 87	257 ± 54
100	675 ± 116	304 ± 62

A significant difference was observed between the mean values of ascorbic acid ASA in roots of Portulaca ($p < 0.01$ using ANOVA Post hoc test "Tukey")). Concentration of ASA increased in roots from 134 mg/kg of fresh roots in control to 254 mg/kg then declined to below detectable limit (BDL) at 100 ppm of Cr(VI) (Table 3-26). This implies that the plant increased the amount of ascorbic acid

produced as a response to the stress of Cr(VI) but when Cr(VI) increased to 100 ppm in irrigation solution the amount of ASA decreased to BDL which means that this entire amount was oxidised by Cr(VI). In shoots the concentration of ASA decreased from 251 mg/kg of fresh shoots of the controls to 212 mg/kg at 50 ppm of Cr(VI) then declined to BDL at 100 ppm of Cr (VI). The decrease of ASA in shoots may be due to the mobility of this material from shoots to roots as response to the increased amounts of Cr(VI) in roots. This explanation is supported by the increase of the dehydroascorbic acid (DASA) in both roots and shoots of plant. This increase was significant ($p < 0.01$ using ANOVA Post hoc test "Tukey") in both roots and from 0 to 50 ppm of Cr(VI) in shoots. In roots DASA increased from 99 in the controls to 1387 mg/kg of fresh roots at 100 ppm of Cr(VI). In shoots, DASA increased from 173 mg/kg in control to 914 mg/kg in the presence of 100 ppm of Cr(VI). These increased amounts of DASA in both roots and shoots are in line with the increase of Cr(VI) in the irrigation solution from 0 to 100 ppm. A previous study also interpreted the decrease of ascorbic acid due to its consumption in reducing chromium (VI) [174].

Comparing the amounts of total thiols (sum of glutathione and oxidised glutathione) which were less than 8.0 mg/kg with the total ascorbic acid (sum of ascorbic and dehydroascorbic acid) which increased from 500 mg/kg of fresh weight to 1200 mg/kg, strongly suggests that chromium(VI) reduction in P. oleracea is due to the use of ascorbic acid as antioxidant. This conclusion can explain the increase of dehydroascorbic acid in the plant tissue from 99 mg/kg in fresh roots of control to 8 fold as the chromium (VI) in irrigation solution was increased to 50 ppm which confirms the effect of Cr(VI) in increasing the

formation of ascorbic acid by the plant as response to this stress. These results are in agreement with the results of Shanker et al. [175] who confirmed the priority of ascorbic acid as antioxidant versus the glutathione in mung bean (Vigna radiate) when irrigated with 50 µM of Cr(VI).

3.4.2 Effect of chromium(VI) on the concentration of glutathione and PC3 phytochelatins in Portulaca oleracea

The current study confirmed the role of P. oleracea in the reduction of chromium (VI) in roots to chromium (III) cations (section 3.3.1). These cations then formed chelates either with phytochelatins or organic acids such as oxalate. This investigation aims to find the most probable ligand to chelate Cr(III) in roots. HPLC reversed phase was used to separate and identify glutathione and the phytochelation PC3. Standards of glutathione were eluted at a retention time of 4.7 min and PC3 at 6.2 min. In the samples only glutathione and at low concentrations was detected to be 1.2 ± 0.7 mg/kg fresh wt. (irrigated with 50 ppm of Cr(VI)). Phytochelatin PC3 was not detected in any sample. The lack of detection of phytochelatins in the presence of Cr(VI) may suggest that the chromium(III) - which originated from reduced Cr(VI) in roots – may be translocated to shoots of P. oleracea using the organic acid ligands such as oxalate. These results are in line with the results of a previous study [176] carried out on tomato plants. This study reported that no phytochelatins were detected when tomato was irrigated with Cr(VI) suggesting that chromium is chelated by organic acids which agree with previous suggestions [81, 82].

The results of previous investigation of the current study, carried out to determine the concentration of ascorbic acid and glutathione using HPLC-MS, confirmed the priority to the role of ascorbic acid as antioxidant. Only small concentrations of glutathione were detected (less than 5 mg/kg fresh wt) which supports the results of this investigation. It seems that Cr(VI) is being reduced to Cr(III) using ascorbic acid and then translocated as chelated cation with organic acids such as oxalate.

3.5 Techniques for the removal of chromium from the polluted dry biomass of P. oleracea.

Incineration is the most common way to treat the harvested contaminated plants (after their use in the phytoextraction process). In this investigation, two alternative proposed techniques for extraction and removal of chromium from the dry Portulaca plants were investigated. Heavy metal cations mobility and solubility are pH- dependent and at low pH most of the heavy metal cations are soluble thus the suspension of ground plant was acidified using hydrochloric acid to pH 6.1, then divided into two volumes of low different values of pH (2.0 ± 0.1and 5.0 ± 0.1) by further acidification. The two solutions were analysed for chromium (VI) and chromium (III) then were treated either by passing through the calcareous sand of Emirates or by electrodeposition.

Concentration of extracted chromium at different pH values is shown in Table (3-28). Table (3-29) shows the concentration of chromium, the pH of solutions before and after treatment using the sand, and the time for each litre to be leached out. Table (3-30) shows the concentration of chromium before and after

electrodepositing and the pH of the solution after the electrodepositing process. The percentages of removal (BOR) were calculated from the following relation:

$$BOR = \frac{(\text{concentration of Cr before} - \text{concentration of Cr after}) \times 100}{\text{Concentration of chromium before}}$$

The results are included in Tables 3-29 and 3-30.

Table (3-28) The concentration of extracted chromium at different pH values

pH	6.1	5.0	2.0
Concentration of total extracted chromium (ppm)	1.0	4.0	6.8
% Extraction	12.3%	52.3%	88.5%

Table (3-29) the concentration of chromium before and after treatment using the sand of the emirates.

Extract Solution	**Concentration of Chromium (ppm)**	**% removal**	**pH of solution**	**Time of treatment (min.)**
Extracted at pH 2 (before passing through sand)	6.80 ± 0.40		2.0	
The First litre (after passing through sand)	0.20 ± 0.03	97.5%	7.8	74
The Second litre (after passing through sand)	0.70 ± 0.05	89.2%	7.8	92

Table (3-30) The concentration of chromium before and after different periods of time of implementing electrodeposition technique.

Solution	Concentration of chromium (ppm)	% Removal	pH
Extracted at pH 2	6.8 ± 0.4		2.0
after 12h	3.0 ± 0.3	56.1%	2.4
after 24h	1.3 ± 0.2	80.8%	2.5
after 36h	1.2 ± 0.2	82.3%	2.6

The amount of chromium (VI) in the whole samples of the extracted chromium was below the detection limit and this confirms the previous conclusion which indicated that most of the chromium(VI) absorbed by P. oleracea was reduced to chromium(III). When the dissolved chromium (III) in the acidified ground plant suspension was filtered through the calcareous sand of the Emirates, most of the Cr(III) was precipitated as $Cr(OH)_3$. The percent removal of chromium ranged between 89 to 97% and the amount of chromium in the filtrate was reduced to below 1.0 ppm. This concentration meets the accepted limits of chromium in the effluent wastewater according to Dubai municipality and the percentage of removal of chromium in this work is in agreement with the results of removal of some heavy metals using the same sand [146].

The use of the sand of the Emirates with high carbonate content (42%) is an efficient and cheap technique for the removal of Cr(III) cations. When comparing the two techniques, of using sand and using electrodepositing it is clear that the first one is faster, more efficient in BOR and the pH of the effluent solution was 7.8 which occurs within the accepted range (6-9) of many environmental organizations

whereas effluent solutions in electrodeposition process are high acidic, pH < 2.6 which is still far from the accepted pH range.

As conclusion, these experiments show that the filtration of extracted Cr(III) solution through calcareous sand of Emirates is more efficient and gives effluents within the accepted range of pH compared with electrodeposition, therefore, it may represent suggested alternative technique other than incineration.

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

From this study, it can be concluded that:

- Of the twelve sites investigated, Ajman industrial zone demonstrated the highest pollution with chromium at 1800 mg/kg of which 97 mg/kg is chromium(VI), classified as carcinogen by the world health organization [177]. This moderate pollution calls for the implementation of a technique such as phytoextraction to remove this heavy metal from soil.

- Black sand in the east coast of the Emirates does not represent real threat to the environment in spite of its huge content of chromite and Cr(III) which is unlikely to be extracted and it is unavailable to the plants growing in the same area (Kalba and Dadnah).

- Of more than twenty plants investigated, Portulaca oleracea demonstrated the highest potential for accumulating Cr(VI). Atriplex halimus showed the capability to accumulate Cu(II), Cr(III) and Co(II), and Cyperus conglomerates demonstrated selective cadmium accumulation with bioconcentration factors ranging from 20 to 50, which is very high for such a toxic element.

- Prosopis juliflora did not demonstrate any potential for the accumulation of chromium(VI) or lead, either in experimental or in natural plants growing in polluted sites. This conclusion is important in the UAE since Prosopis is a major food for camels and other animals in Emirates.

- Portulaca oleracae can be classified as a promising Cr(VI) hyperaccumulator since it could accumulate high amounts of this pollutant exceeding 4600 mg/kg in dry roots and 1500 mg/kg in dry stems. The accumulated chromium increased linearly in all parts of P. oleracea as the concentration of Cr(VI) increased in the soil. Bioaccumulation factors for Cr(VI) in P. oleracea exceeded the value of 28 in some experiments which means that the concentration of chromium in the plant tissue exceeded its concentration in soil by 28 fold. These merits put P. oleracea in a favourable position compared to some other known Cr(VI) accumulators like Leptospepermum scoparium, Leersia hexandra, Typha angustinfolia and Zea mays.

- P. oleracea has the ability to reduce Cr(VI) to Cr(III) with percentages ranging from 95 to 99%, which indicated the high ability of this plant to change the toxic and highly oxidative form of Cr(VI) to the safer, nontoxic Cr(III).

- Chromium(VI) phytoextraction by P. oleracea can be enhanced by elevating the pH of soil above 7.5 which reflects the effect of chromium(VI) speciation on its uptake by P. oleracea. This plant accumulated high quantities of Cr(VI) as the pH of soil increased which

means that P. oleracea absorbed Cr(VI) as chromate species which is much more abundant at this high range of pH.

- The organic content of soil decreased the available Cr(VI) which resulted as a lack of its uptake by P. oleracae. This is due to the ability of organic content of soil to reduce Cr(VI) to Cr(III) which is mostly unavailable to the plants at the high pH levels like the soil of Emirates (pH 7.9) At this level of pH most of Cr(III) is unavailable as insoluble $Cr(OH)_3$.

- The highest Cr(VI) uptake using P. oleracea was achieved in the presence of the smallest amount of organic content of soil (0.42% in soil of Ajman). This gives Portulaca a preference in accumulation Cr(VI) from soils of small organic content and high pH such as soil of Emirates.

- The presence of nutrient anions such as nitrate and phosphate reduced the uptake of chromate by P. oleracea which may be due to the need of the plant to absorb these nutrient anions at the expense of chromate uptake.

- The presence of sulfate in specific concentrations (300-600 ppm) significantly enhanced the uptake of chromate by P. oleracea. Chromate is analogous to sulfate anion since they are similar in charge, geometry, and size. So chromate is likely to be taken up by P. oleracea using sulfate transporters (carriers) in roots. It can be also concluded that at concentration of sulfate above 300 ppm these transporters are being formed to the maximum extent.

- At high concentrations of sulfate, there is a competitive relationship between the uptake of chromate and sulfate using P. oleracea. It was observed that chromate decreased as sulfate increased in the roots of P. oleracea. In shoots the plant translocated sulfate in larger amounts than chromate since it is one of the macronutrients that is required for the plant.

- The presence of ammonium or potassium cations significantly enhanced the uptake of chromate by P. oleracea reflecting the effect of accompanying cation on the uptake of the anions. Since both of ammonium and potassium are being taken up by coupled transporters (in root cell membrane) it is suggested that chromate may be taken up coupled with these nutrient cations. The effect of counter cation on the uptake of accompanying anions needs future investigation.

- When evaluating the effect of chelating agents on the uptake of chromium by P. oleracea, it was found that EDTA in the soil enhanced the translocation of both Cr(III) and Cr(VI) (after its reduction). This plant demonstrated a high potential for accumulating Cr(III) in the presence of citric acid, while it did not show this potential in the uptake of Cr(VI) in the presence of the same chelating agent.

- The translocation factor of Cr(VI) (after its reduction in roots), using P. oleracea or other Cr(VI) accumulators is still below the value of 1.0. The enhancement of the translocation factor of Cr(VI) still needs further investigation.

- Ascorbic acid is the most dominant reducing agent for Cr(VI) to Cr(III) inside P. oleracea tissues, while glutathione has a minor effect in the reduction of Cr(VI). The effect of sulfate in enhancing the role of glutathione in the reduction of Cr(VI) needs more investigation.

- P. oleracea demonstrated considerable ability to increase the production of ascorbic acid as the concentration of Cr(VI) in the irrigation solution increased, which indicates the strong adaptability of this plant under the stress of such oxidative pollutant.

- Phytochelatin PC3 was not detected in P. oleracea grown in the presence of Cr(VI) which may suggest that chromium(III) - which originated from reduced Cr(VI) in roots – may be translocated to shoots of P. oleracea using the organic acid ligands such as oxalate.

- The extraction of Cr(III) from dried plant tissue is most efficient after acidification of a suspension of ground plant material. The use of calcareous sand in the filtration of Cr(III) is a very efficient technique. The percentage of removal of chromium using this technique ranged between 89 to 97% and the amount of chromium in the filtrate was reduced to <1.0 ppm. This concentration meets the accepted limits of chromium in the effluent wastewater according to Dubai environmental regulations. The pH of the effluent solution was (7.8) which lies within the accepted range (6.0-9.0) of many environmental organizations.

4.2 Recommendations

- Chromium (VI) in the soil of Ajman industrial zone represents a serious threat and should be removed urgently since this contaminated area contains many civilian establishments such as schools and workshops.

- Phytoremediation seems to be promising, cheap, and easily implemented in the remediation of the soil of UAE and this study recommends that more research is carried out in this field not only with heavy metals but also with organic pollutants since the oil industry is common in UAE.

- Desert plants may be of interest in the field of phytoremediation research since they have exceptional potential to tolerate the hard conditions of desert. Among these plants Iresine herbestii and Atriplex halimus which have shown capability to solubilise and uptake heavy metal cations such as cobalt, copper, chromium(III) and lead from soil of Emirates with the high pH (7.9).

- The environmental regulations should be developed and applied in the field especially in the northern emirates whereas many factories and workshops do not implement these regulations.

- This study suggests the need to carry out further investigations on the effect of sulfate on the role of glutathione in the reduction of Cr(VI) and the effect of counter cation on the uptake on accompanying pollutant anions such as chromate.

- Designing experiments in the field of phytoremediation and phytoextraction needs more control of especially the pH of the nutrient medium which strongly affects heavy metal speciation and availability. Plant nutrients (cations and anions) may enhance or inhibit the uptake of heavy metal, so they must be taken in account when designing experiments. Researchers should be more careful when declaring that a plant is an accumulator to specific heavy metal e.g. a plant may be efficient accumulator for Cr(III) but not necessary be efficient for Cr(VI).

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Appendices

Appendix (1)

Numerical data for section 3.2

Uptake of heavy metals by a range of local plants.

Heavy metal (mg/kg) / Plant	Co (II)	Pb(II)	Cr (VI)	Cu (II)	Cr (III)	Ni(II)
Portulaca oleracea	<0.5	37 ± 8	158 ± 27	<0.2	60 ± 10	20 ± 3
Bougainvillea spinosa	<0.5	12 ± 2	8 ± 1	<0.2	<0.4	<0.6
Atriplex halimus	110 ± 20	9 ± 2	40 ± 8	140 ± 15	95 ± 14	40 ± 6
Iresine herbestii	2.4 ± 0.3	100 ± 10	90 ± 14	94 ± 11	7 ± 2	9 ± 1
Pennisetum setaceum	<0.5	<0. 14	<0.4	35 ± 5	16 ± 4	<0.6

Concentration of total added chromium in soil and the measured concentration at harvesting time.

Experiment (Number and details)	Total added chromium or other heavy metal in soil (mg/kg)	Measured quantity at the harvest (mg/kg)
(3)	100	90 ± 5
(4)	250	230 ± 10

Appendix (2)

Numerical data for section 3.3.1

Total added concentration of chromium in soil and the measured at harvesting time.

Concentration of Cr(VI) in irrigation solution (ppm)	Total added chromium in soil (mg/kg)	Measured quantity at the harvest (mg/kg)
50	50	40 ±5
100	100	90 ±7
150	150	130 ±10
200	200	190 ±5
250	250	220 ±10
300	300	250 ±15
350	350	300 ±15
400	400	360 ±15

Comparing the means of concentration of Cr in roots at 50, 150, 250 and 350 ppm levels of chromium in irrigation solution.
Dependent Variable: Concentration of total chromium in roots (SPSS- Tukey HSD)

Conc. of Cr(VI) in irrigation soln. (ppm)	Conc. of Cr(VI) in irrigation soln. in groups of comparison (ppm)	Mean chromium concentration in roots for each group (mg/kg)	Significance (p value)
50	150	1200	0.01
	250	2300	<0.001
	350	4600	<0.001
150	50	400	0.01
	250	2300	0.003
	350	4600	<0.001
250	50	400	<0.001
	150	1200	0.003
	350	4600	<0.001
350	50	400	<0.001
	150	1200	<0.001
	250	2300	<0.001

Comparing the means of concentration of Cr in roots at 100, 200, 300 and 350 ppm levels of chromium in irrigation solution
Dependent Variable: Concentration of total chromium in roots (SPSS- Tukey HSD)

Conc. of Cr(VI) in irrigation soln. (ppm)	Conc. of Cr(VI) in irrigation soln. in groups of comparison (ppm)	Mean chromium concentration in roots for each group (mg/kg)	Significance (p value)
100	200	2000	0.005
	300	3100	<0.001
	350	4600	<0.001
200	100	900	0.005
	300	3100	0.009
	350	4600	<0.001
300	100	900	<0.001
	200	2000	0.009
	350	4600	0.001
350	100	900	<0.001
	200	200	<0.001
	300	3100	0.001

Multiple Comparisons of means of concentration of Cr in leaves at 50, 150, 250 and 350 ppm levels of chromium in irrigation solution
Dependent Variable: Concentration of total chromium in leaves (SPSS- Tukey HSD)

Conc. of Cr(VI) in irrigation soln. (ppm)	Conc. of Cr(VI) in irrigation soln. in groups of comparison (ppm)	Mean chromium concentration in leaves for each group (mg/kg)	Significance (p value)
leaf 50	leaf 150	320	0.031
	leaf 250	760	<0.001
	leaf 350	1100	<0.001
leaf 150	leaf 50	100	0.031
	leaf 250	760	0.001
	leaf 350	1100	<0.001
leaf 250	leaf 50	100	<0.001
	leaf 150	320	0.001
	leaf 350	1100	0.005
leaf 350	leaf 50	100	<0.001
	leaf 150	320	<0.001
	leaf 250	760	0.005

Multiple Comparisons of means of concentration of Cr in leaves at 100, 200, and 300 ppm levels of chromium in irrigation solution
Dependent Variable: Concentration of total chromium in leaves (SPSS- Tukey HSD)

Conc. of Cr(VI) in irrigation soln. (ppm)	Conc. of Cr(VI) in irrigation soln. in groups of comparison (ppm)	Mean chromium concentration in leaves for each group (mg/kg)	Significance (p value)
leaf 100	leaf 200	610	0.003
	leaf 300	1100	<0.001
leaf 200	leaf 100	210	0.003
	leaf 300	1100	0.001
leaf 300	leaf 100	210	<0.001
	leaf 200	610	0.001

Multiple Comparisons of means of concentration of Cr in stems at 100, 200, 350 and 350 ppm levels of chromium in irrigation solution

Dependent Variable: Concentration of total chromium in stems (SPSS- Tukey HSD)

Conc. of Cr(VI) in irrigation soln. (ppm)	Conc. of Cr(VI) in irrigation soln. in groups of comparison (ppm)	Mean chromium concentration in stems for each group (mg/kg)	Significance (p value)
stem 100	stem 200	600	0.008
	stem 350	1400	<0.001
stem 200	stem 100	220	0.008
	stem 350	1400	<0.001
stem 350	stem 100	220	<0.001
	stem 200	600	<0.001

Multiple Comparisons of means of concentration of Cr in stems at 100, 150, and 300 ppm levels of chromium in irrigation solution

Dependent Variable: Concentration of total chromium in stems (SPSS- Tukey HSD)

Conc. of Cr(VI) in irrigation soln. (ppm)	Conc. of Cr(VI) in irrigation soln. in groups of comparison (ppm)	Mean chromium concentration in stems for each group (mg/kg)	Significance (p value)
stem 100	stem 150	570	0.004
	stem 300	1100	<0.001
stem 150	stem 100	220	0.004
	stem 300	1100	<0.001
stem 300	stem 100	220	<0.001
	stem 150	570	<0.001

Multiple Comparisons of means of concentration of Cr in stems at 50, 150, and 300 ppm levels of chromium in irrigation solution

Dependent Variable: Concentration of total chromium in stems (SPSS- Tukey HSD)

Conc. of Cr(VI) in irrigation soln. (ppm)	Conc. of Cr(VI) in irrigation soln. in groups of comparison (ppm)	Mean chromium concentration in stems for each group (mg/kg)	Significance (p value)
stem 50	stem 150	570	0.001
	stem 300	1100	<0.001
stem 150	stem 50	70	0.001
	stem 300	1100	<0.001
stem 300	stem 50	70	<0.001
	stem 150	570	<0.001

Appendix (3)
Numerical data for section 3.3.2

Concentration of total chromium in shoots and roots of P. oleracea at different pH values of soil

pH	Concentration of Chromium in Roots of P. oleracea	Concentration of Chromium in shoots of P. oleracea
6.0 ± 0.1	590 ± 100	260 ± 60
7.0 ± 0.1	680 ± 70	420 ± 60
7.3 ± 0.1	1060 ± 150	450 ± 60
7.6 ± 0.1	1290 ± 180	480 ± 70
8.0 ± 0.1	1490 ±170	60 ± 100
9.0 ± 0.1	1450 ± 160	800 ± 70

Total added concentration of chromium in soil and the measured at harvesting time at different pH values of soil.

Experiment (6) pH of soil	Total added chromium or other heavy metal in soil mg/kg	Measured quantity at the harvest mg/kg
6.0	200	180 ±10
7.0	200	180 ±10
7.3	200	180 ±10
7.6	200	175 ±15
8.0	200	177 ±15
9.0	200	175 ±15

Multiple Comparisons of means of concentration of Cr in shoots at different values of soil pH.
Dependent Variable: Concentration of total chromium in shoots (SPSS- Tukey HSD)

pH of soil	pH of soil of compared groups	Mean Concentration of total chromium in shoots (mg/kg)	Significance (p value)
6.0	7.0	420	<0.001
	7.3	450	<0.001
	7.6	480	<0.001
	8.0	560	<0.001
	9.0	810	<0.001
7.0	6.0	260	<0.001
	7.3	450	0.95
	7.6	480	0.83
	8.0	560	0.01
	9.0	810	<0.001
7.3	6.0	260	<0.001
	7.0	420	0.95
	7.6	480	1.00
	8.0	560	0.08
	9.0	810	<0.001
7.6	6.0	260	0.00
	7.0	420	0.83
	7.3	450	1.00
	8.0	560	0.17
	9.0	810	<0.001
8.0	6.0	260	<0.001
	7.0	420	0.01
	7.3	450	0.08
	7.6	480	0.17
	9.0	810	<0.001
9.0	6.0	260	<0.001
	7.0	420	<0.001
	7.3	450	<0.001
	7.6	480	<0.001
	8.0	560	<0.001

Multiple Comparisons of means of concentration of Cr in shoots at three different values of soil pH.
Dependent Variable: Concentration of total chromium in shoots (SPSS- Tukey HSD)

pH of soil	pH of soil of compared group	Mean Concentration of total chromium in shoots (mg/kg)	Significance (p value)
6.0	7.3	450	<0.001
	9.0	810	<0.001
7.3	6.0	260	<0.001
	9.0	810	<0.001
9.0	6.0	260	<0.001
	7.3	450	<0.001

Multiple Comparisons of means of concentration of Cr in roots at three different values of soil pH.

Dependent Variable: Concentration of total chromium in roots (SPSS- Tukey HSD)

pH of soil	pH of soil of compared group	Mean Concentration of total chromium in roots (mg/kg)	Significance (p value)
6.0	7.3	1100	<0.001
	9.0	1500	<0.001
7.3	6.0	600	<0.001
	9.0	1500	<0.001
9.0	6.0	600	<0.001
	7.3	1100	<0.001

Multiple Comparisons of means of concentration of Cr in roots at different values of soil pH.

Dependent Variable: Concentration of total chromium in roots (SPSS- Tukey HSD)

pH of soil	pH of soil of compared group	Mean Concentration of total chromium in roots (mg/kg)	Significance (p value)
6.0	7.0	700	0.81
	7.3	1100	<0.001
	7.6	1300	<0.001
	8.0	1500	<0.001
	9.0	1500	<0.001
7.0	6.0	600	0.81
	7.3	1100	<0.001
	7.6	1300	<0.001
	8.0	1500	<0.001
	9.0	1500	<0.001
7.3	6.0	600	<0.001
	7.0	700	<0.001
	7.6	1300	0.03
	8.0	1500	<0.001
	9.0	1500	<0.001
7.6	6.0	600	<0.001
	7.0	700	<0.001
	7.3	1100	0.03
	8.0	1500	0.08
	9.0	1500	0.23
8.0	6.0	600	<0.001
	7.0	700	<0.001
	7.3	1100	<0.001
	7.6	1300	0.08
	9.0	1500	0.99
9.0	6.0	600	<0.001
	7.0	700	<0.001
	7.3	1100	<0.001
	7.6	1300	0.23
	8.0	1500	0.99

Appendix (4)

Numerical data for section 3.3.3

Concentration of total chromium in shoots and roots of P. oleracea at different concentrations of organic content of soil

% Total Organic Matter Content	Concentration of Chromium in Shoots (mg/kg)	Concentration of Chromium in Roots (mg/kg)
35%	93 ± 14	160 ± 16
17.5%	280 ± 23	520 ± 40
0.42%	1200 ± 140	3000 ± 210

Concentration of chromium(VI) in soil at different concentrations of organic content of soil.

% Organic Matter	Concentration of Chromium (VI) in soil (mg/kg)
35% org	30 ± 6
17.5% org	60 ± 10
0.42% org	110 ± 18

Multiple Comparisons of means of concentration of Cr in roots in the presence of different concentrations of organic content of soil. (SPSS- Tukey HSD).
Dependent Variable: Concentration of total chromium in roots .

Percentage of organic content	Per. of organic content of compared groups	Mean Concentration of total chromium in roots (mg/kg)	Significance (p value)
.042%	17.5%	500	<0.001
	35%	200	<0.001
17.5%	.042%	3000	<0.001
	35%	200	<0.001
35%	.042%	3000	<0.001
	17.5%	500	<0.001

Appendix (5)

Numerical data for section 3.3.4

Concentration of Chromium in roots and shoots of P. oleracea using different nutrient anions beside Cr(VI).

Companying Ion to Cr(VI) and Plant Tissue	Concentration of Cr in dry roots (mg/kg)	Concentration of Cr in dry shoots (mg/kg)
NO_3^-	550 ± 90	180 ± 90
SO_4^{2-}	1100 ± 130	280 ± 90
PO_4^{3-}	650 ± 100	130 ± 30
Cr (VI) only	840 ± 100	400 ± 100

Average length of the roots in each type of investigated plants and tolerance index

Cr(VI) only		With nitrate		With sulfate		With phosphate	
Control	**Exp.**	**Control**	**Exp.**	**Control**	**Exp.**	**Control**	**Exp.**
23.8 ± 2.2	16.6 ± 2.7	24.4 ± 2.7	24.2 ± 2.4	24.0 ± 3.0	15.0 ± 2.5	24.1 ± 2.8	21.4 ± 3.1
Tolerance Index	0.70 ± 0.14		0.99± 0.14		0.62 ± 0.10		0.88± 0.15

Added chromium in soil and concentration measured at harvesting time.

Irrigation Solution	Added chromium (mg/kg)	Measured at harvesting time (mg/kg)
Na_2CrO_4 + 0.02M of $NaNO_3$	120	110 ± 5
Na_2CrO_4+ 0.02M of Na_3PO_4	120	105 ± 5
Na_2CrO_4only	120	95 ± 10
Na_2CrO_4+ 0.02M of Na_2SO_4	120	90 ± 10

Multiple Comparisons of means of concentration of Cr in roots in the presence of different nutrient anions in the irrigation solution. (SPSS- Tukey HSD)
Dependent Variable: Concentration of total chromium in roots of P. oleracea

Anions in irrigation solution	Compared groups	Mean Concentration of total chromium in roots (mg/kg)	Significance (p value)
Cr(VI) only	Cr(VI) + Nitrate	550	<0.001
	Cr(VI) + Sulfate	1100	0.02
	Cr(VI) + Phosphate	650	0.06
Cr(VI) + Nitrate	Cr(VI) only	840	<0.001
	Cr(VI) + Sulfate	1100	<0.001
	Cr(VI) + Phosphate	650	0.49
Cr(VI) + Sulfate	Cr(VI) only	840	0.02
	Cr(VI) + Nitrate	550	<0.001
	Cr(VI) + Phosphate	650	<0.001
Cr(VI) + Phosphate	Cr(VI) only	840	0.06
	Cr(VI) + Nitrate	550	0.49
	Cr(VI) + Sulfate	1100	<0.001

Multiple Comparisons of means of concentration of Cr in shoots in the presence of different nutrient anions in the irrigation solution. (SPSS- Tukey HSD)
Dependent Variable: Concentration of total chromium in shoots.

Anions in irrigation solution	Compared groups	Mean Concentration of total chromium in roots (mg/kg)	Significance (p value)
Cr(VI) only	Cr(VI) + Nitrate	180	<0.001
	Cr(VI) + Sulfate	280	0.13
	Cr(VI) + Phosphate	130	<0.001
Cr(VI) + Nitrate	Cr(VI) only	400	<0.001
	Cr(VI) + Sulfate	280	0.10
	Cr(VI) + Phosphate	130	0.59
Cr(VI) + Sulfate	Cr(VI) only	400	0.13
	Cr(VI) + Nitrate	180	0.10
	Cr(VI) + Phosphate	130	0.01
Cr(VI) + Phosphate	Cr(VI) only	400	0.00
	Cr(VI) + Nitrate	180	0.59
	Cr(VI) + Sulfate	280	0.01

Appendix (6)

Numerical data for section 3.3.5

Concentration of sulfur and chromium in roots and shoots of Portulaca at different levels of sulfate in the irrigation solution (at half concentration of both elements)

In irrigation solution (mg/kg)		**In Roots (mg/kg)**		**In Shoots (mg/kg)**	
Sulfur	**Chromium**	**Sulfur**	**Chromium**	**Sulfur**	**Chromium**
0	100	112 ± 21	562 ± 90	182 ± 39	315 ± 80
150	100	262 ± 37	701 ± 107	380 ± 72	272 ± 81
300	100	370 ± 72	990 ± 117	561 ±102	294 ±74
600	100	470 ± 70	1010 ± 94	679 ± 138	270 ± 67
900	100	779 ± 78	635 ± 82	891 ±152	208 ± 61
Control (Irrigated by deionised water		173 ± 40	<0.5	288 ± 56	<0.5

Multiple Comparisons of means of concentration of Cr in roots in the presence of different concentrations of sulfur in the irrigation solution.
Dependent Variable: Concentration of total chromium in roots (SPSS- Tukey HSD)

Conc. of sulfur in irrigation soln. (mg/kg)	Compared groups	Mean Concentration of total chromium in roots (mg/kg)	Significance (p value)
S0	S300	1350	<0.001
	S600	1300	<0.001
	S1200	830	0.06
	S1800	640	<0.001
S300	S0	990	<0.001
	S600	1300	0.99
	S1200	830	<0.001
	S1800	640	<0.001
S600	S0	990	<0.001
	S300	1350	0.99
	S1200	830	<0.001
	S1800	640	<0.001
S1200	S0	990	0.06
	S300	1350	<0.001
	S600	1300	<0.001
	S1800	640	0.02
S1800	S0	990	<0.001
	S300	1350	<0.001
	S600	1300	<0.001
	S1200	830	0.02

Multiple Comparisons of means of concentration of Cr in shoots in the presence of different concentrations of sulfur in the irrigation solution. (SPSS- Tukey HSD)
Dependent Variable: Concentration of total chromium in shoots.

Conc. of sulfur in irrigation soln. (mg/kg)	Conc. of sulfur in compared groups	Mean Concentration of total chromium in shoots (mg/kg)	Significance (p value)
S0	S300	550	1.00
	S600	550	1.00
	S1200	450	0.19
	S1800	440	0.18
S300	S0	450	1.00
	S600	550	1.00
	S1200	450	0.15
	S1800	440	0.14
S600	S0	450	1.00
	S300	550	1.00
	S1200	450	0.17
	S1800	440	0.16
S1200	S0	450	0.19
	S300	550	0.15
	S600	550	0.17
	S1800	440	1.00
S1800	S0	450	0.18
	S300	550	0.14
	S600	550	0.16
	S1200	450	1.00

Appendix (7)

Numerical data for section 3.3.6

Average length of the roots and tolerance indexes for chromium(VI) in the presence of different cations.

Irrigation solution	Average Length of Roots (cm)	Tolerance Index TI
Na_2CrO_4	17 ± 2.2	0.74 ± 0.11
K_2CrO_4	9.1 ± 1.5	0.39 ± 0.08
$(NH_4)_2CrO_4$	12.1 ± 2.1	0.53 ± 0.10
Control	23.1 ± 3.1	

Concentration of Chromium in dry tissues of Portulaca oleracea irrigated by chromate accompanied with different cations.

Accompanying cation to Cr(VI)	Concentration of Cr mg/kg	
	In Dry Roots	In Dry Shoots
Na^+	860 ±100	340 ± 100
K^+	1800 ± 300	820 ± 190
NH_4^+	1560 ±130	710 ± 120

Added chromium in soil and measured at harvesting time

Irrigation Solution	Added chromium (mg/kg)	Measured at harvesting time (mg/kg)
Na^+	120	100 ± 5
K^+	120	90 ± 10
NH_4^+	120	90 ± 10

Multiple Comparisons of means of concentration of Cr in roots in the presence of different cations in the irrigation solution (SPSS- Tukey HSD).
Dependent Variable: Concentration of total chromium in shoots.

Cation	Cations of compared groups	Mean Concentration of total chromium in roots (mg/kg)	Significance (p value)
sodium	potassium	-1300	<0.001
	ammonium	-10000	<0.001
potassium	sodium	1300	<0.001
	ammonium	240	0.08
ammonium	sodium	1000	<0.001
	potassium	-240	0.08

Appendix (8)

Numerical data for section 3.3.7

Uptake of chromium (III) in roots and shoots of Portulaca in the presence of EDTA and citric acid.

Irrigation Solution Components	Concentration in Root (mg/kg)	Concentration in Shoots (mg/kg)
Cr (III) only	650 ±110	220 ± 30
Cr (III) + Citric Acid	930 ± 130	430 ± 190
Cr (III) + EDTA	220 ± 40	390 ± 60

Added chromium in soil and measured at harvesting time

Irrigation Solution	Added chromium (mg/kg)	Measured at harvesting time (mg/kg)
Cr (III) only	100	90 ± 5
Cr (III) + Citric Acid	100	90 ± 5
Cr (III) + EDTA	100	94 ± 5

Multiple Comparisons of means of Uptake of chromium (III) in of Portulaca in the presence of EDTA and citric acid using SPSS, Tukey- HSD.
Dependent Variable: Concentration of total chromium in roots.

Chelating agent added to chromium	Compared groups	Mean Concentration of total chromium in roots (mg/kg)	Significance (p value)
Cr only	citric	930	0.004
	EDTA	220	0.001
citric	Cr only	650	0.004
	EDTA	220	.<0.001
EDTA	Cr only	650	0.001
	citric	930	<0.001

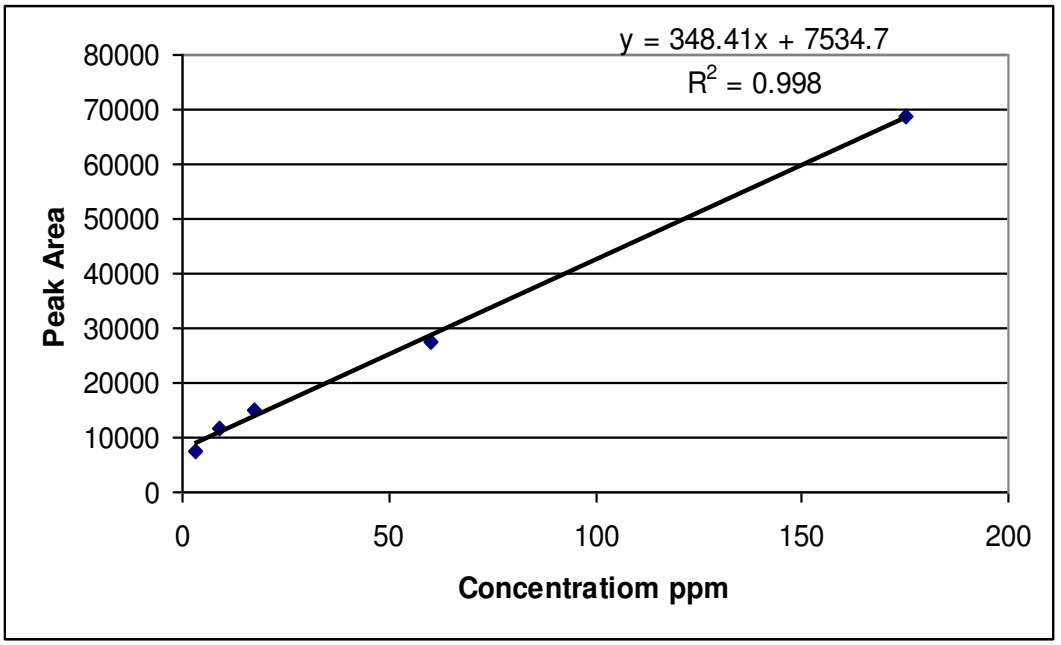
Multiple Comparisons of means of Uptake of chromium (VI) in of Portulaca in the presence of EDTA and citric acid using SPSS, Tukey- HSD.
Dependent Variable: Concentration of total chromium in roots.

Chelating agent added to chromium	Compared groups	Mean Concentration of total chromium in roots (mg/kg)	Significance (p value)
Cr only	citric	450	<0.001
	EDTA	730	0.091
citric	Cr only	740	<0.001
	EDTA	730	<0.001
EDTA	Cr only	740	0.091
	citric	450	<0.001

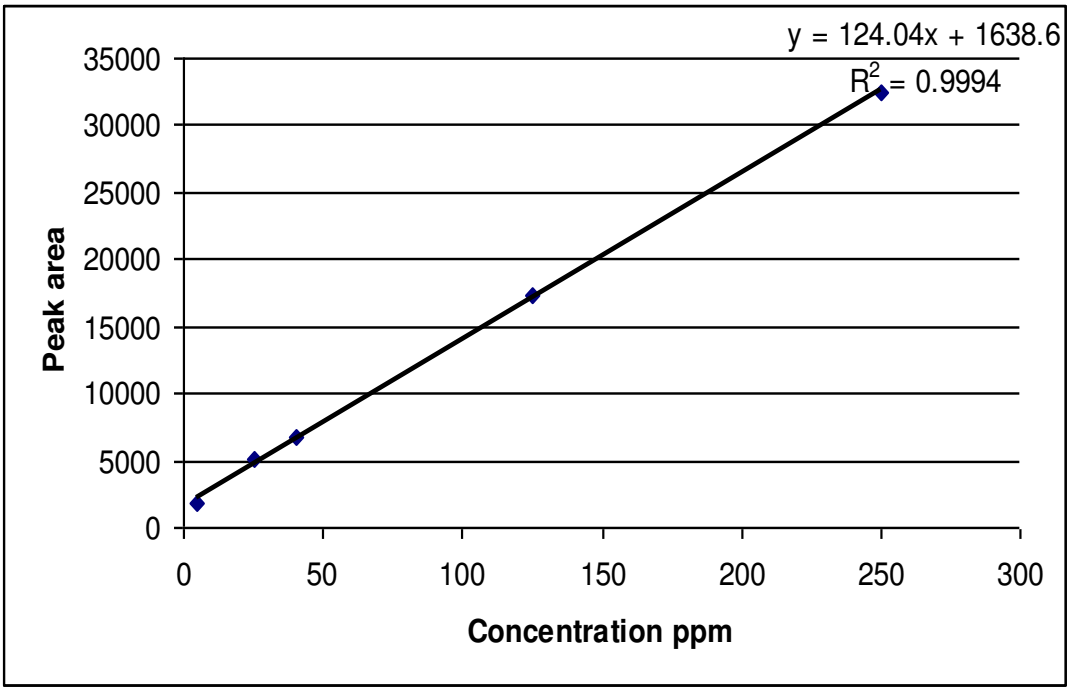
Appendix (9)

Numerical data for section 3.4.1

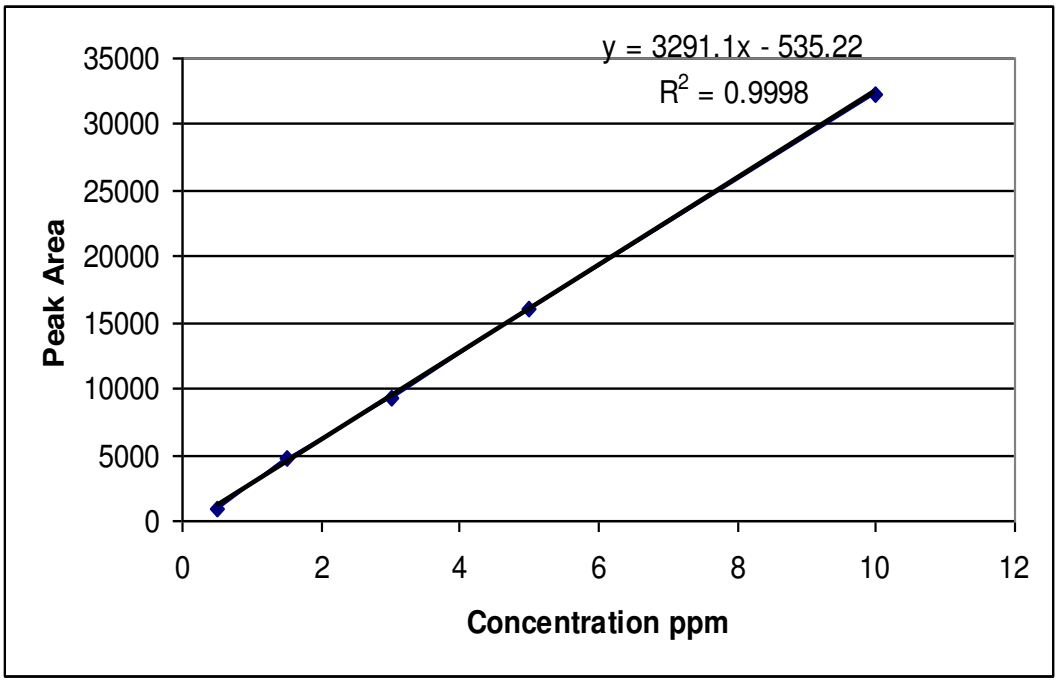
Calibration curves of ascorbic acid, dehydroascorbic acid, glutathione, glutathione oxidised using HPLC-MS.



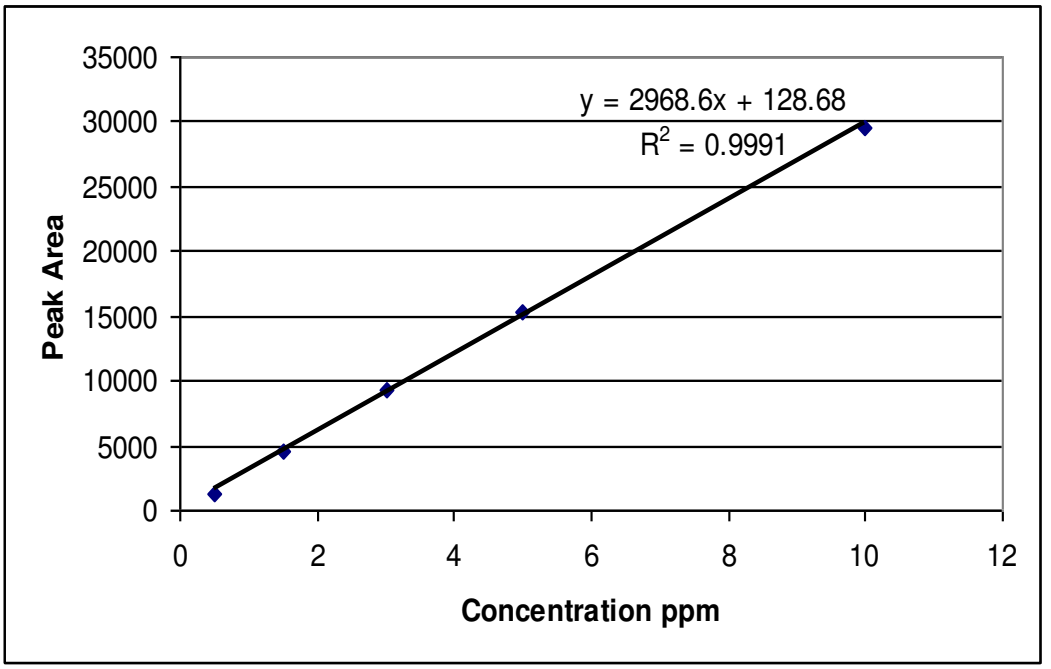
Calibration curve of ascorbic acid



Calibration curve of dehydroascorbic acid



Calibration curve of glutathione



Calibration curve of oxidised glutathione

Appendix (10)

Portulaca oleracea

Portulaca oleracea or Purslane (Figure 1) is a succulent plant that grows naturally in the coast line of the UAE. The growth of this plant is mostly between March and December or in the warm to hot seasons of UAE. The plant is being used for nutrient and medical purposes. In Arabian countries, it is used in salad since it is rich in vitamins such as A and C and omega-3 fatty acids. It has both laxative and diuretic effect and it is used for treatment of burns and as an anti-scorbutic. The whole plant is effective as an antibacterial in bacterial dysentery. The plant can be reproduced either by seeds which are tiny and black or by cuttings of the stem of the plants which are much branched [1]. The plant can spread vertically up to 16 inches and horizontally between 2-3 feet.

Figure(1) Portulaca oleracea or Purslane.

[1] M.V.D Jongbloed, Wildflowers of the United Arab Emirates, Environmental Research and Wildlife Development Agency (ERWDA), 2003.

Appendix (11)

Portulaca oleracea grown in the incubator in Huddersfield laboratory

Appendix (12)

Names and pictures of some investigated plants in the present study.

Prosopis cineraria

Calotropis procera

Euphorbia Larica

Prosopis juliflora

Cyperus conglomerates

Tamarix aucheriana

Portulaca oleracea

Atriplex halimus

Bougainvillea spinosa

Iresine herbestii

Pennisetum setaceum

Azadirachta indica