Mansoura University
Faculty of Agriculture
Soils Department

Phytoremediation of Some Egyptian Soils Polluted with Heavy Metals

By

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

APCAs	Amino-PolyCarboxylic Acids
Approx.	Approximately
CA	Citric Acid
CDTA	1,2-CyclohexyleneDinitriloTetraAcetic acid
CTAB	cetyl trimethyl ammonium bromide
DGT	Diffusive Gradients in Thin films
EDDHA	EthyleneDiamine-N,N'-bis(2-HydroxyphenylAcetic acid)
EDDS	EthyleneDiamine-N,N'-Di-Succinic acid
EDTA	EthyleneDiamineTetraAcetic acid
EGTA	Ethylene Glycol-bis(β-aminoethyl ether)-N,N,N',N'-TetraAcetic acid
Fed.	Feddan
GA3	Gibberellic Acid
HA	Humic Acid
HEDTA	2-Hydroxyethyl Ethylene-Diamine-Triacetic Acid
IAA	Indole-3-Acetic Acid
MDA	MalonDiAldehyde
NLMWOA	Natural Low Molecular Weight Organic Acids
NTA	NitriloTriacetic acid

1. INTRODUCTION

Over the past century, the byproducts of industrial, agricultural and domestic activities have contaminated many soils with hazardous and harmful chemicals. Nowadays soil pollution is getting greet public attention where the magnitude of this serious problem is growing rapidly. One of the most serious pollutants in the environment, especially agricultural environments, is heavy metals because of their elevated level of durability and toxicity to the biota **(Alkorta *et al.,* 2004)**. Heavy metals tend to be adsorbed very cruelly on the soil matrix, and will not degrade like organics by microbial activity or through chemical oxidation once released to the environment **(Beiergrohslein, 1998)**.

Several human activities such as electroplating, smelting, mining, etc. can lead to contamination of soil with heavy metals. In addition, the use of sewage water for irrigation and sludge in some agricultural practices is also considered as a source of heavy metals in soil. Consequently, it is the media through which heavy metals can be transferred to human through the food chain. Likewise, these metals can be leached to groundwater or it can be transferred to water bodies through runoff and soil erosion, contaminating seas resources and drinking water supplies.

In Egypt, which located in arid and semi-arid regions, water is becoming scarce resource. Rapid increases in population and industrial growth have led to use low quality water such as drainage and wastewater for irrigation. The use of primary and secondary effluent in irrigation can ameliorate soil quality and plant growth because they are considered as natural conditioners through their content of nutrient elements and organic matter.

Primary treated wastewater has been used in agriculture to irrigate about 3 thousand fed. in Al Gabal Al Asfar and Abu Rawash since 1911 and 1944, respectively **(Abd El-Shafy *et al.,* 2008 & Mahjoub, 2016)**. Currently, the cultivated area reached to about 4.5 thousand fed. With increasing the number of sewage water treatment stations, this quality of sewage water were used in many areas in Nile Delta, Assiut, Al-Tebeen, Helwan, Zenein and Bahr El-Baqar causing a great risk to public health **(WHO, 2005; Elewa, 2010 & Abd**

El Lateef *et al.,* 2013). Besides, many industrial facilities discharge their wastewater directly with no treatments into irrigation canals as in El-Mahla El-Kobra, Kafr El-zayat and Shubra El-Kheima causing an accumulation of heavy metals in soil **(Melegy and Paces, 2004; Al Naggar *et al.,* 2014 & Mahmoud and Ghoneim, 2016)**.

In recent years, attention has been paid to remediate heavy metals -polluted soils due to its negative effects on agricultural productivity and human health, where some of these elements are classified as carcinogenic (have a high potential toxicity and high persistence).

The cleanup of contaminated soils is one of the most difficult tasks for environmental engineering, where, soil washing, excavation and landfilling can be used at severely polluted sites but are not viable to large areas and are quite expensive. Immobilization of heavy metals has the advantage of immediately reducing risk of metals, but it considered as temporary alternative. Phytoremediation is a promising new approach, direct use of plants to clean up the soil from heavy metals in situ, environmental friendly, economically cost-effective.

High-biomass and metal-hyper accumulator plant species have a high potential and promising to be used in phytoremediation. Among them the *Brassicaceae* (mustard) and *Asteraceae* families. Availability of heavy metals to plant roots is effective key factor in Phytoremediation. The phytoavailability of heavy metals is affected by numerous soil factors among of them organic matter content. Therefore, addition of amino-poly-carboxylic acids, natural low molecular weight organic acids and humic substances has been proposed.

The objectives of this study were to evaluate the efficiency of some plant species in phytoremediation of metal contaminated soils. Moreover, investigating the effect of synthetic and organic chelators on bioavailability and mobility of heavy metals on one hand, and on leaching behaviors of heavy metals under these chelators on the other hand.

2. REVIEW OF LITERATURE

2.1. Sources of soil contamination by heavy metals:

One of the notable sources participating to raise soil contamination is disposal of municipal wastage, when sewage is used for irrigation. These wastes, although useful as a source of nutrients, are also sources of carcinogens, toxic metals and could include unsafe or excess application of pesticides and fungicides **(Zhen-Guo *et al.*, 2002)**.

Halim *et al.*, (2003) stated that considerable areas of land is polluted with heavy metals because of using sludge or municipal compost, pesticides, fertilizers, and emissions from municipal waste incinerators, vehicle exhausts, residues or metalliferous mines, and smelting industries.

Bridge (2004) outlined that extra potential sources of trace elements include irrigation water contaminated by sewage and industrial effluent leading to metals contaminated soils and vegetables.

Wu *et al.*, (2010) stated that industrial revolution caused more and more hazardous heavy metals releasing to environment. Soils, being the basic and most essential part of the ecological system, are heavily contaminated with these main groups of inorganic contaminants.

2.2. Metal accumulation in plants and its toxicity:

All plants have the capability to accumulate “essential” metals from the soil. Some plants have a capability to collect other “non-essential” trace metals (Al, As, Au, Cd, Cr, Hg, Pb, Pd, Pt, Sb, Te, Tl and U) that have no known biological role **(Djingova and Kuleff, 2000)**.

Baker (1981) studied accumulators and excluders strategies in the response of plants to heavy metals and declared that plants can tolerate the existence of large amounts of metals in their environment by one or more of the following three ways; Exclusion, whereby transport of metals is limited and constant metal concentrations are maintained in

the shoot over a wide range of soil levels. Inclusion, whereby shoot metal concentrations reflect those in the soil solution in a linear relationship. Bioaccumulation, whereby metals are concentrated in the roots and upper parts of plants at both high and low soil concentrations.

Zinc is one of the essential trace elements to all higher plants and animals. Zinc is required in a considerable number of enzymes **(Mengel and Kirkby, 1982)** and plays an essential turn in DNA transcription. Zinc toxicity usually leads to leaf chlorosis **(Cobbett and Goldsbrough, 2002)**.

Assche and Clijsters (1990) revealed that accumulated metals cannot be broken down and when concentrations inside the plant cells above threshold level, it can cause direct toxicity by deteriorating cell structure (due to oxidative stress) and/or blocking a number of cytoplasmic enzymes.

Taiz and Zeiger (2002) reported that accumulated metals can cause indirect toxic effects by replacing essential nutrients at cation exchange sites in plants. On the other hand, **Gardea-Torresdey *et al.*, (2005)** reported that some plant species can grow up and develop in metalliferous soils (near to mining sites). Such plants can be used to clean up trace elements polluted sites.

Schmidt (2003) studied plant accumulation of trace elements and reported that willow (*Salix viminalis L.*), indian mustard (*Brassica juncea L.*), maize (*Zea mays L.*), and sunflower (*Helianthus annuus L.*) have been found to be strongly tolerant to metals.

Lead is a non-essential element in metabolic processes and can become toxic or fatal to numerous organisms even when absorbed in small amounts. **Boonyapookana *et al.*, (2005)** studied Phytoextraction of lead by sunflower, tobacco, and vetiver plants grown in hydroponic solution containing 0.25 and 2.5 mM Pb as $Pb(NO_3)_2$. The results showed that 2.5 mM Pb caused toxic effect to plants including chlorosis, necrosis, stunt growth of root/shoot, and low biomass production on *Helianthus annuus*, *Nicotiana tabacum* and *Vetiveria zizanioides*.

Rylott and Bruce (2008) Performed a research titled with engineering plants for the phytoremediation of explosives and found that Populus species are examples of plants widely used to remediate soils polluted with heavy metal.

Kabata-Pendias and Pendias (2011) in ″trace elements in soils and plants″ book stated that Cu is an essential micronutrient for plants, but it could be toxic at higher concentrations. Copper (Cu) plays important role in a lot of physiological processes in plants involving photosynthesis, respiration, carbohydrate distribution, nitrogen and cell wall metabolism, seed production including also disease resistance. **Baker and Walker (1989)** in ″Ecophysiology of metal uptake by tolerant plants study″ revealed that the higher concentration of Cu may account for the suppressed root growth, leaf chlorosis observed among plants.

Oh *et al.*, (2014) studied the implementation of phytoremediation technology in management and remediation of polluted soils and pointed out that vetiver grass (*Vetiveria zizanioides*) exhibited tolerance to Pb and Zn and it could be used for revegetating Pb/Zn mine tailings.

2.3. Remediation technologies of heavy metals contaminated soil:

2.3.1. Physical remediation:

The physical remediation fundamentally includes soil replacement method and thermal desorption.

Quan and Liu (2000) in overview of development in the soil remediation technologies stated that physical remediation mainly involves soil replacement method which is divided into three types, soil replacement, soil spading and new soil importing. (1) Soil replacement is taking of the polluted soil and placing new soil. This method is appropriate for polluted soil with small area. Besides, the replaced soil should be feasibly processed; otherwise it will incur the second pollution. (2) Soil spading is deeply digging the polluted soil, making the pollutant disseminate into the deep sites and realizing the aim

of diluting and naturally degrading. (3) Land filling is adding lots of clean soil into the polluted soil, covering it at the surface or mixing to reduce the pollutant concentration.

Thermal desorption is based on pollutant's volatility (e.g. Hg, As), where, the polluted soil is heated using steam, microwave, infrared radiation to make the pollutant volatile. Then, collecting the volatized heavy metals using the vacuum negative pressure or carrier gas and realize the goal of removing heavy metals **(Li *et al.*, 2010)**. This technology had been used by a company of mercury collection and service in USA for in-situ remediation and developed commercial service. However, the limited factors, such as the costly devices, long-term time of desorption, restrict its implementation in the soil remediation **(Aresta *et al.*, 2010)**.

2.3.2. Chemical remediation:

2.3.2.1 Leaching:

Chemical remediation is washing the polluted soil using fresh water, reagents, and others fluids or gas. **Ou-Yang *et al.*, (2010)** studied the advance in supercritical CO_2 fluid extraction of pollutants from soil. They found that pollutant can be leached from polluted soil through the ions exchange, precipitation, adsorption and chelation, heavy metals in soil was transferred from solid to liquid phase, and then regained from the leachate. The leachate mainly includes inorganic effluent, chelation agents, and surfactant, etc.

2.3.2.2 Fixation (stabilization):

Chemical fixation refers to adding reagents or materials (clays, metallic oxides, biomaterials, etc) into the polluted soil forming insoluble heavy metals or hard to move, less toxic matters, consequently reducing heavy metals migration to ground water, plant and other environmental media.

Bolan *et al.*, (2003) studied the effect of biosolid compost addition on phytoavailability of cadmium in variable charge soils and outlined that chemical fixation could remediate the soil with low concentration contaminant; however, the bioavailability

of fixed metals may change due to changing the environmental conditions. Besides, the use of conditioning agents could change the soil structure at some degrees and affects the microbes in soil.

2.3.2.3 Electrokinetic remediation:

Electrokinetic remediation is a new remediation technology **(Luo *et al.*, 2004)**, which apply voltage at the two sides of the soil then forming electric field gradient. The pollutant was carried to two poles treatment room via electromigration, electroosmotic flow or electrophoresis and then treated further. The electrokinetic remediation cannot control soil pH value well. It is appropriate for low permeable soil, and has advantages of easily install and operate, low cost and not destroy the original nature environment **(Zhang *et al.*, 2001 and Xu *et al.*, 2006)**.

2.3.2.4 Vitrify technology:

Vitrify technology is heating the polluted soil at temperature of 1400~2000°C, in which process the organic matters volatilize or decompose. The steam produced and pyrolysis product was collected by off-gas treatment system. The resulting melt after cooling form rock shape vitreous, sieges the heavy metals and make it lose by migration. It was announced that the strength of the vitreous is high 10 times than concrete. Concerning the ex-situ remediation, the energy can be supplied by fossil fuel burning or electrode directly heating, and then through arc, plasma and microwave transferring energy. For in-situ remediation, the heat can be through electrodes inserted into the polluted soil. This technology can remove the metals with high efficiency. However, it is complicated and requires lots of energy in the melting, which makes this technology highly cost and limited in implementation **(Fu, 2008)**.

2.3.3. Biological remediation

Biological remediation includes bioremediation, phytoremediation and the combining remediation.

2.3.3.1. Bioremediation:

The microorganisms cannot degrade or destroy the metals, but can affect their migration and transformation through changing their physical and chemical characterizations. **Bosecker (2001)** in a microbial leaching in environmental clean-up programs stated that microbial or biological remediation mechanisms include extracellular complexation, oxidation-reduction reaction, precipitation and intracellular accumulation. Microbial leaching is a simple and effective technique for the extraction of precious metals from low-grade ores and mineral concentrates. So the microbial leaching has some potential for the remediation of mining sites, industrial waste products, detoxification of sewage sludge and for remediation of soils and sediments polluted with metals. However, **Yao *et al.*, (2012)** in the 7th International Conference on Waste (soil polluted by heavy metals) Management and Technology stated that the biological remediation is vulnerable to be affected by different types of factors, such as temperatures, moisture, oxygen and pH value. It is also limited in applications.

Some lower animals can adsorb and migrate the metals and consequently removing and preventing their toxicity. **Wang *et al.,* (2007)** studied earthworm-straw interactions on phytoremediation of soils polluted with Cu using ryegrass. They stated that the treatment of the earthworm-straw mulching combinations enhanced Cu concentration in plant, and the amount increased by it was lower than that of the earthworm treatment but higher than that of straw mulching treatment.

Kou et *al.,* (2008) studied the lead accumulation of earthworm in lead polluted soil. They illustrated that the earthworm could accumulate Pb effectively and the accumulated amount was raised with the Pb concentrations increasing.

2.3.3.2. Phytoremediation:

‘‘Phytoremediation substantially refers to using plants and associated soil microbes to decrease the concentrations or toxic effects of pollutants in the environments’’. Phytoremediation is promising economic effective biotechnology for cleaning up the polluted water and soil from organic and inorganic pollutants **(Hazrat *et al.*, 2013)**.

According to remediation mechanisms, several categories of phytoremediation can be characterized, such as phytoextraction, phytofiltration, phytodegradation, phytostabilization, and phytovolatization **(Ansari *et al.*, 2014)**.

2.4. Techniques/strategies of phytoremediation

- Phytoextraction or phytoaccumulation is the uptake of pollutants from soil or water by plant roots and their accumulation in and translocation to aboveground biomass **(Rafati *et al.*, 2011)**. Translocation of metal to shoots is an efficient phytoextraction because the harvested root biomass is in general not feasible **(Tangahu *et al.*, 2011)**.

- Phytofiltration is the extraction of contaminants from polluted surface waters using plants, contaminants are adsorbed or absorbed and subsequently their movement to underground waters is minimized. Phytofiltration may be blastofiltration (use of seedlings) or rhizofiltration (use of plant roots) or caulofiltration (use of excised plant shoots) **(Mesjasz-Przybylowicz *et al.*, 2004)**.

- Phytostabilization (phytoimmobilization) is using certain plants for stabilizing contaminants in contaminated soils. This technique is used to decrease the mobility and bioavailability of contaminants in the environment, subsequently prohibiting their migration to groundwater or their entrance into the food chain **(Erakhrumen, 2007)**. However, phytostabilization is not a perpetual solution because the metals remain in the soil (only their movement is restricted). In fact, it is a management strategy for potentially inactivating toxic pollutants **(Vangronsveld *et al.*, 2009)**.

- Phytovolatilization is the uptake of soil contaminants using plants, hence transformation to volatile form and subsequent release into the atmosphere. This technique could be used for organic contaminants and some metals like Hg and Se. However, its use is limited by the reality that it does not completely remove the contaminant; only it is transformed from one segment (soil) to another (atmosphere) from where it can be redeposited **(Padmavathiamma and Li, 2007)**.

- Phytodegradation is degrading the organic (heavy metals are nonbiodegradable) contaminants by plants with the help of enzymes like dehalogenase and oxygenase **(Vishnoi and Srivastava, 2008)**.

- ***Rhizodegradation*** refers to using the microorganisms in the rhizosphere to the breakdown of organic pollutants in the soil **(Mukhopadhyay and Maiti, 2010)**.

- Phytodesalination refers to using the halophytic plants to remove salts from salt-affected soils to enable them for supporting normal plant growth **(Sakai *et al.*, 2012)**.

2.5. Advantages and limitations of phytoremediation

Phytoremediation is a novel, efficient, cost-effective, environmental friendly, in situ applicable and solar-driven remediation strategy. The concept of phytoremediation (as phytoextraction) was suggested by **Chaney (1983)**. Vegetation of polluted soils also assists prevent erosion and metal leaching **(Chaudhry *et al.*, 1998)**.

Phytoremediation has a good public acceptance as an alternative to chemical plants and bulldozers **(Pilon-Smits, 2005)**. It is appropriate for application at very large field sites, wherever other remediation techniques are not cost effective or practicable **(Garbisu and Alkorta, 2003)**. Phytoremediation has low installation and maintenance costs as compared to other remediation methods. Where, it costs as less as 5% of alternative clean-up techniques **(Prasad, 2003 and Van Aken, 2009)**.

phytoremediation of metal-contaminated soils suffers from some limitations such as long time required for clean-up, efficiency of most metal hyper-accumulators is usually limited by their slow growth rate and low biomass, low mobility of more tightly bound fraction of metal ions from soil, It is applicable to sites with low to moderate levels of metal pollution because plant growth is not sustained in heavily polluted soils, there is a risk of food chain contamination in case of mismanagement and insufficiency of proper care **(Naees *et al.*, 2011; Ramamurthy and Memarian, 2012)**.

2.6. Strategies for metal phytoextraction and appropriate plants:

There are three main strategies to phytoextract heavy metals from contaminated soils have been used currently: natural hyperaccumulators usage, enhancement of metal uptake by high biomass plants through chemicals addition of (e.g., chelating agents) to soil, and improvement of the phytoextraction capabilities of plants by genetic engineering **(Alkorta *et al.*, 2004)**.

In **1996 Cunningham & Ow** stated that *T. caerulescens* or *B. juncea* should be good sources for genes convenient for phytoremediation.

Krämer and Chardonnens (2001) stated that the rate of contaminant removal using conventional traits and growth conditions is insufficient. Therefore production of novel high biomass plants by transgenic approach is needed for effective phytoremediation.

Hyperaccumulation of heavy metals by higher plants involves several steps, such as, metals transport across the plasma membrane of root cells, xylem loading and translocation, and detoxification and sequestration of metals at the whole plant **(Lombi *et al.*, 2002)**.

Yang *et al.*, (2005) studied the molecular mechanisms of metal hyperaccumulation and phytoremediation and outlined that the first hyperaccumulators characterized were members of the *Brassicaceae* and *Fabaceae* families).

Governments worldwide are establishing research around to select accumulator or hyperaccumulator and decontamination programs. The Canadian Environment has developed database of 775 plants which have the capabilities to hyperaccumulate one or several of 19 key metallic elements. More than 500 vascular plants with metal hyperaccumulating capacities have been undergone to extensive research as regards to their usefulness in phytoremediation. Among them, *Brassica*, *Arabidopsis*, *Thlaspi*, *Helianthus annuus* and *Sedum alfredii H.* have been investigated in more detail **(Lone *et al.*, 2008)**.

Phytoextraction is the main and most useful phytoremediation technique for removal of metals and metalloids from contaminated soils, sediments or water **(Milic *et al.*, 2012)**.

The efficacy of pytoextraction depends on many factors like the bioavailability of metals in soil, soil properties, speciation of heavy metals and plant species concerned.

Appropriate plants for phytoextraction should ideally have the following characteristics: high growth rate, production of high above ground biomass, widely distributed and highly branched root system, more accumulation of heavy metals from soil, translocation of the accumulated metals from roots to shoots, tolerance to the toxic effects of the target heavy metals, good adaptability to prevailing environmental and climatic conditions, resistance to pathogens and pests, easy cultivation and harvest and finally repulsion to herbivores to avoid food chain contamination **(Sakakibara *et al.*, 2011; Shabani and Sayadi, 2012)**.

Ucer *et al.,* (2013) carried out a research on the phytoextraction of heavy metals using *Myriophyllum verticillatum* and inferred that a hyperaccumulators definition as plants that are able to accumulate more than 100 mg kg^{-1} of cadmium (Cd); 1000 mg kg^{-1} of arsenic (As), cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), or Se, or; 10,000 mg kg^{-1} of zinc (Zn) and manganese (Mn) in leaves.

Prescreening of plant species beneficial for phytoremediation of contaminated soils showed that rye grass, tall fescue, sunflower, oat plant, and green gram were eligible to grow under the mixed conditions of contamination. The maximum removal of contaminants from the soil was found in sunflower **(Chirakkara and Reddy, 2015)**.

2.7. Phytoremediation potential of indian mustard (*Brassica juncea L.*):

The oldest reference to *B. juncea* in Sanskrit literature is by the name "Rajika", and its carbonized seeds have been found in the ancient sites of the Harappan civilization (2300-1750 B.C.). Indian mustard is eaten as a leafy vegetable in China but it is grown in India originally for its oil-containing seeds (~ 40% oil) **(Prakash, 1980)**. Indian mustard is an oilseed Brassica crop for which cultivation extends from India through western Egypt and Central Asia to Europe **(Nishi, 1980)**.

Experimental analysis was carried out by **Kumar *et al.*, (1995)** using different *Brassica* species in order to check their ability to resist and accumulate heavy metals. These include: indian mustard (*B. juncea L.*), kale (*B. oleracea L.*), turnip (*B. campestris L.*), black mustard (*B. nigra Koch*) and rape (*B. napus L.*). They found that indian mustard is promising sp. and able to produce biomass of about 18 tons per hectare.

Ebbs and Kochian (1997) studied Zn and Cu toxicity to Brassica species. They stated that although *B. juncea* belongs to the *Brassicaceae* family possess one-third of Zn concentrations in its tissues, it is more efficient to remediate zinc than *T. caerulescens* (known zinc hyperaccumulator) and the reason behind this fact is that the biomass production of *B. juncea* is about ten-times more than *T. caerulescens*.

Salt *et al.*, (1998) revealed that *B. juncea* plants show a high efficacy to remove heavy metals such as Pb and Se. However it needs to be harvested shortly after the plant becomes mature, which causes problems of disposal of obtained biomass.

Gleba *et al.*, (1999) tested the phytoextraction efficacy of a selected line of *B. juncea* plants whose transpiration rate exceeded that of the wild-type plants by 130% in soil (one strategy for raising the efficacy of phytoextraction is to raise the translocation of heavy metal from roots to the shoot by increasing plant transpiration). This high-transpiration line phytoextracted 104% more Pb than the wild-type *B. juncea*, making it a good candidate for phytoremediation.

In **2000**, **Jiang *et al.*,** studied hyperaccumulation of lead by roots, hypocotyls, and shoots of *Brassica juncea*. They declared that *Brassica juncea* is the leading candidate crop to remediate numerous heavy metals from the soil which include: cadmium (Cd), chromium-IV (Cr^{IV}), caesium-137 (^{137}Cs), copper (Cu), nickel (Ni), lead (Pb), uranium (U) and zinc (Zn).

Performance studies with *B. juncea* were held by **Moreno *et al.*, (2005).** They demonstrated that phytovolatilisation of mercury is an effective and economical technique, with maximum extraction yield of 25 g Hg h^{-1}.

Nouairi *et al.,* (2006) conducted a comparative study of cadmium effects on membrane lipid composition of *Brassica juncea* and *Brassica napus* leaves. They found that *B. juncea* exhibits a high potency to uptake and accumulate Cd- mainly in the shoots, where Cd level was 1450 µg Cd/g dry wt. This is three times more than that of *Brassica napus* (555 µg/g dry wt).

Robinson *et al.,* (2009) in a phytomanagement of trace elements in soil study revealed that some species of *Brasicaceae* family possess enormous potential to scavenge heavy metal ions and are thus considered as potential candidates for phytoextraction of metals. Generally, this family is known to scavenge metals like Pb, Cd, Zn and Ni. Before treatment, 19% of the site had Pb concentrations higher than 800 mg/kg, and after three crops, Pb (II) concentrations across the entire site had been brought below 800 mg/kg.

Alcantara *et al.,* (2015) conducted a glasshouse-based screening study which examined the growth of plant species (*Brassica juncea* (indian mustard), *Daucus carota* (carrot), *Lupinus albus* (white lupin), *Beta vulgaris* (sugar beet), *Solanum tuberosum* (potato), and *Manihot esculenta* (cassava)) known for their ability to phytoextract Hg and Au which can grow on substrates consisting of biosolids, Au mine tailings, or different combinations of both. The results of this study demonstrated that indian mustard and carrot could be successfully established in amended with biosolids mine tailings.

Couto *et al.,* (2015) studied the phytoremediation of As and Sb contaminated soil using ryegrass and indian mustard coupled with electrokinetic process and/or phosphate amendment. The results revealed that indian mustard revealed the highest potential to accumulate As and Sb (*approx.* 65% more than ryegrass).

2.8. Phytoremediation potential of sunflower (*Helianthus annuus*):

Nanda-kumar *et al.,* (1995) studied the effect of high metal levels on sunflower growth. Their results pointed out that no signs of toxicity from the high levels of Zn were observed in the shoot. Besides, all zinc concentrations showed positive effects and shoot growth efficiently. The Pb treated sunflower plants at higher concentrations showed weak

growth and decreased leaf expansion. Also, they stated that Pb uptake by shoot amounted by 5.6 ± 1.3 mg Pb g^{-1} and 61.6 ± 3.3 mg Pb g^{-1} by root of *Helianthus annuus* plants.

Kayser (2001) compared *Helianthus annuus, B. juncea* and *S. viminalis* as hyper accumulator in contaminated soil. He outlined that *Helianthus annuus* produced the largest biomass as compared to *B. juncea* and *S. viminalis* in Zn and Cd contaminated soil.

Herrero *et al.,* (2003) studied Zn, Cd, Pb and Cu uptake by *Brassica napus* var. oleifera and *Helianthus annuus* grown on contaminated soils. They revealed that that the shoots had the greatest biomass compared to other plant parts (50% of total mass) and the biomass decreased proportionally to the contamination level (400 mg kg^{-1} soil for Zn; 25 mg kg^{-1} soil for Cu; 350 mg kg^{-1} soil for Pb and 0.135 mg kg^{-1} soil for Cd).

Schmidt (2003) investigated the effect of chemical soil manipulation on mobility and plant accumulation of heavy metals. They found that Sunflower (*Helianthus annuus L.*) show high tolerance to metals (especially Pb, Zn and Cd) and therefore, was used in phytoremediation studies.

Tang *et al.,* (2003) studied the growing response of *Brassica juncea* and *Helianthus annuus* to high CO_2 on Cu contaminated soil. They found that sunflower has a great affinity to accumulate Cu in the upper plant organs.

Boonyapookana *et al.,* (2005) studied the phytoaccumulation of Pb by sunflower (*Helianthus annuus*), Tobacco (*Nicotiana tabacum*), and Vetiver (*Vetiveria zizanioides*). Their results indicated that 80- 87% of the total Pb uptake was found in *H. annuus* roots with only 13-20% translocated to the aboveground parts (shoot) after the fourth week of plantation. They also stated that the plant biomass of *H. annuus* in Pb contaminated nutrient media declined with increasing concentration from 0.25 to 2.5 mM Pb.

Nehnevajova *et al.,* (2005) in sunflower cultivars screening study for the phytoextraction of heavy metal in a contaminated field prior to mutagenesis revealed that sunflower is the fast growing deep rooted industrial oil crop with high biomass production plant species which have the potential to remove heavy metals such as Zn and Cu as well as

several radionuclides. The highest Cu concentration was found in shoot of commercial cultivars of sunflower plants grown on soils contaminated by heavy metals. Sunflower had higher Cd concentrations in their shoots compared to the roots. However, sunflower accumulated relatively very low amounts of heavy metals in flowers and seeds. Sunflower oil may be used for technical lubricants or energy production and metal-enriched biomass can be harvested using standard agricultural methods and smelted to recover the metals.

January *et al.*, (2008) examined efficiency of *H. annuus*, Sundance sunflower, in the removal of multiple heavy metal contaminants. Sunflower were subjected to contaminated solutions containing 3 (Cd, Cr and Ni), 4 (Cd, Cr, Ni and As) or 5 (Cd, Cr, Ni, As and Fe) heavy metals, with and without EDTA. Sunflower exhibited a metal uptake preference of (Cd = Cr > Ni), (Cr > Cd > Ni > As) and (Fe >> As > Cd > Ni > Cr) without EDTA and (Cr > Cd > Ni), (Fe >> As > Cd > Cr > Ni) with EDTA. The most important finding was the ability of Sundance sunflower to achieve hyperaccumulator status for both As and Cd under all conditions studied. Ni hyperaccumulator status was only achieved in the presence of three metals without EDTA.

Kara *et al.*, (2013) studied phytoremediation potential of jojoba (*Simmondsia chinensis*) and sunflower (*Helianthus annuus*) as oilseed plants in hydroponic systems. The results demonstrated that sunflower was more beneficial than oilseed in accumulating Cd and Ni in their tissues.

Lotfy and Mostafa (2014) conducted a phytoremediation trial to study the use of five plant species (Panikum (*Panicum antidotal*), Napier grass (*Pennisetum purpureum*), Squash (*Cucurbita pepo*), cotton (i) and sunflower (*H. annuus*)) to extract Co and Cr out of two different polluted soils. Sunflower shoots and roots exhibited the highest Co and Cr uptake compared to the other tested species at any investigated soil.

2.9. Role of EDTA on heavy metals phytoextraction process:

Availability of heavy metals to plant roots is considered the key factor limiting the phytoextraction efficiency. Heavy metals availability enhancing by adding chelating agents has been utmost in phytoremediation.

In the last decade the use of persistent amino-polycarboxylic acids (APCAs) such as ethylenediaminetetraacetic acid (EDTA), ethylenediamine-N,N'-di-succinic acid (EDDS) and nitrilotriacetic acid (NTA) have been used in many phytoextraction experiments. For more than 50 years, synthetic chelates, including EDTA, have been used in order to supply plants with micronutrients in both soil and hydroponics. In the late 1980s and early 1990s EDTA was suggested as a chelating agent to assist the phytoextraction processes **(Evangelou *et al.*, 2007)**.

Cao *et al.,* (2008) demonstrated the bioavailability and chelate mobilization efficiency of heavy metal in an assisted phytoextraction process. They found that EDTA is able to extract sulphide- and organic-bound metal fractions, which are poorly available to plants in general.

Ethylenediaminetetraacetic acid (EDTA) as organic molecule is able to release heavy metals associated with different soil particles, mostly organic matter and Fe and Mn oxides **(Udovic and Lestan, 2009)**.

Several studies showed that EDTA is the most efficient chelating agents for raising heavy metals solubility in soils and it has been widely used for heavy metals extraction from contaminated soils **(Pociecha *et al.,* 2011; Oh and Yoon, 2013)**. With increasing use of EDTA for remediation techniques, knowledge regarding the processes and factors behind EDTA-enhanced metal phytoextraction is necessary in evaluating and managing the risks related to these techniques.

Kumar *et al.*, (2011) in some traits studied the dielectric constant effect of medium on protonation equilibria of ethylenediamine compounds. They outlined that usage EDTA as a chelator is considered an important breakthrough in chelation therapy. So ethylenediaminetetraacetic acid (EDTA), a member of the polyamino carboxylic acid family, is a hexahydric acid which forms strong complexes with heavy metals through its two amines and four carboxylates groups.

Tandy *et al.,* (2011) studied the influence of ethylenediamine-N,N'-di-succinic acid (EDDS) on heavy metal uptake by *Helianthus annuus* grown hydroponically. They reported that EDTA has a high capability to mobilize nutrients and trace metals in soil. Therefore, EDTA is also used as an extractant in many countries; e.g., Austria, Denmark, France, Finland, Hungary, Ireland, Norway, and Portugal.

Jean-Soro *et al.,* (2012) in a column leaching study of Cr and Ni from a contaminated soil using EDTA and CA revealed that their bounds to those sites have a weaker energy than the metal-EDTA complex. Hence EDTA application increases the exchangeable fraction of metals in soil by forming soluble complexes with them.

Leleyter *et al.,* (2012) in isolation and identification of a citrato-complex trait of Ni from Ni-accumulating plants found that EDTA extracts metals from exchange sites of both organic and inorganic complexes.

2.9.1. Metals availability in soils in relation to EDTA complexation:

Meers *et al.,* (2008) investigated the potential soil amendments for raising the uptake of heavy metals by plants. They reveal that there exists a high binding constant of EDTA for metals (Cd, 18.10; Pb, 19.71; Ni, 20.11; Zn, 18.00; Cu, 20.49 and Co 18.16), allowing the dissolution of heavy metals.

Hadi *et al.,* (2010) studied the role of plant growth regulators (GA3 and IAA) and EDTA alone and in combinations as phytoextraction of Pb improvement way. They outlined that EDTA addition to soil transfers metal sorption and precipitation equilibrium toward enhanced heavy metals dissolution due to forming metal-EDTA complexes.

Ylivainio (2010) studied the effects of iron chelates on heavy metals solubility in calcareous soils. They stated that EDTA increased Cd, Pb, and Ni solubility steadily even in calcareous soils. This increase in plant-available metals mainly corresponds to the exchangeable and carbonate portion of soil.

Zhang *et al.,* (2010) and **Luciano *et al.,* (2013)** explained the mechanisms by which explain the increase in metal mobilization by EDTA. They revealed that at the first step,

EDTA destabilizes the weak bond between oxygen and metal in mineral structures. The second step involves the detachment of metal ions from minerals, thereby raising the mobilization of heavy metal in soils by partly deteriorating the soil minerals and structure.

Gheju *et al.,* (2011) in a comparative study on heavy metal chemical extraction from anaerobically digested biosolids summarized that EDTA is favors for extraction of Cu, Cd, and Pb from a soil contaminated with many metals.

Jean-Soro *et al.,* (2012) conducted a column leaching study of Cr and Ni from a contaminated soil using EDTA and citric acid (CA). They revealed that EDTA is more effective in enhancing heavy metals desorption from the soil solid phase. In most cases, the effect of EDTA was many times greater than that of CA.

Neugschwandtner *et al.,* (2012) studied the chemically enhanced phytoextraction of heavy metals on the performance and metal mobilization over a three year period. They cleared that EDTA bind heavy metals allowing its dissolution in soil solution. However, this dissolution of heavy metals by complexation with EDTA may decrease over time.

Cay *et al.,* (2016) explained that EDTA forms strong complexes with metals through its two amines and four carboxylates groups. Hence, application of EDTA remobilizes metals by forming strong soluble complexes of heavy metals. However, EDTA may cause negative environmental effects when applied to soils. Where, the presence of EDTA may affect the bio-uptake of heavy metals through the formation of metal-EDTA complexes and changes the potential to leach metals below the root zone.

2.9.2. EDTA-enhanced heavy metal uptake by plants:

Huang *et al.,* (1997) studied the role of synthetic chelates in lead phytoextraction. They pointed out that the effectiveness synthetic chelates were in this following order EDTA > HEDTA (2-Hydroxyethyl ethylene-diamine-triacetic acid) > DTPA > EGTA (ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid) > EDDHA **(**ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid)). Pb accumulation in plants took the parallel trend of the chelates effectiveness on Pb desorption from soil to soil solution.

Deram *et al.,* (2000) studied metal extraction by the metal-tolerant grass *Arrhenatherum elatius* supplemented with EDTA to remove Cu, Ni, and Co from a copper ore. They found that the bioaccumulation factor (metal in plant/ore concentration) was significantly larger than 1 only for Cu (i.e., bioaccumulation factor = 40). In this experiment, although EDTA induced metal accumulation relative to the controls, the plants showed necrosis, probably due to the high concentrations of Cu (7500 mg kg^{-1} soil), Ni (1276 mg kg^{-1} soil), and Co (175 mg kg^{-1} soil).

Enhancement of Cr (III) phytoaccumulation studies were done by **Shahandeh and Hossner (2000)**. They found that applying EDTA at 4 mmol EDTA kg^{-1} soil led to a 200-fold increase in Cr concentrations in *Helianthus annuus* and *Brassica juncea* roots and shoots.

Chen and Cutright (2001) found an efficient root to shoot translocation for Cd and Ni after EDTA application, whereas no translocation for Cr could be observed.

Sarret *et al.,* (2001) illustrated that the mechanism and fate of metal accumulation induced by EDTA in *Phaseolus vulgaris* differed between metals. They found that no difference between plants grown in Zn–EDTA and $ZnSO_4$ solutions was found. Furthermore, Zn was predominately precipitated as Zn phosphate in the roots and leaves in both treatments. Contradictory, cerussite was the major form of Pb in the absence of EDTA, whereas in the presence of EDTA, part of the Pb present in the leaves was complexed as Pb–EDTA. It was concluded that metal–EDTA complexes in soil solution could be totally (Zn) or partially (Pb) dissociated when absorbed by *P. vulgaris.* They also revealed that non-toxic metal-EDTA complex breaks down after it enters the plant roots, producing an increase of free ionic heavy metals, which can cause phytotoxicity.

Lead phytoextraction from contaminated soil using high biomass plant species was tested by **Shen *et al.,* (2002)**. They outlined that the effectiveness of stimulating Pb accumulation in cabbage shoots (*B. rapa*) by synthetic chelates was in the order: EDTA > HEDTA > DTPA. They also found that addition of 3.0 mmol EDTA kg^{-1} soil increased Pb

concentrations in shoots and roots, where Pb concentrations in cabbage shoots reached 5010 and 4620 mg kg^{-1} dry matter on days 7 and 14 after EDTA application.

Plant uptake of ^{14}C-EDTA, ^{14}C-Citrate and ^{14}C-Histidine from chelator-buffered and hydroponic solutions was tested by **Bell *et al.,* (2003)**. They found that metal-EDTA complex is easily taken up by most of the plant species.

Phytoremediation of As and Pb contaminated soil using Chinese brake ferns (*Pteris vittata*) and indian mustard (*Brassica juncea*) was studied by **Salido *et al.,* (2003)**. They found that the highest Pb shoot concentration occurred at the highest EDTA concentration (10 mmol kg^{-1} soil) in a soil containing 338 mg Pb kg^{-1}, *B. juncea* plants extract approximately 32 mg/shoot of this metal. While, Pb concentration was only 73 mg Pb kg^{-1} shoot in control.

Wenzel *et al.,* (2003) hypothesized that free protonated EDTA enters the root in soil, subsequently forming metal complexes which enhance metal transport to shoots.

Wu *et al.,* (2003) carried out a pot experiment to study the effects of EDTA and low molecular mass organic acids (citric, oxalic, and malic) on, heavy metals in soil solution in the rhizosphere of *B. juncea* grown on a paddy soil polluted with Cu, Zn, Pb, and Cd. They stated that adding 3 mmol EDTA kg^{-1} soil markedly increased the total concentrations of those metals (31.0, 90.2, 65.8 and 5.63 mg kg^{-1} soil, respectively) in the soil solution. Compared to EDTA, low molecular mass organic acids had a very small effect on metal concentrations in soil solution.

Alkorta *et al.,* (2004) postulated that if EDTA remains in soil after the removal or harvesting of the plants, growth and development of plants grown later on the same soil or land might be inhibited by EDTA salt residues in the soil. They also stated that there is no study, which investigated the effect of EDTA residues on development and growth of the plants grown after (in succession) on the same soil or piece of land that received EDTA in the previous season.

Crist *et al.,* (2004) studied Ion-exchange aspects of Pb uptake by indian mustard and stated that Pb-EDTA is more quickly translocated from a solution through roots and petiole to leaves than Pb^{+2}.

Wu *et al.,* (2004) found that applying EDTA (3 mmol kg^{-1} soil) to pots of a paddy soil polluted with Cu and artificially spiked with Zn, Pb and Cd led to elevate Cu, Zn, Pb and Cd concentrations in the soil solution for about 1 month. Moreover, Rainfall after EDTA application, as simulated by the column leaching experiment, increased Cu, Zn, Pb and Cd concentrations linearly in leachate with increasing EDTA dosage (0–12 mmol kg^{-1} soil). They stated that addition of High EDTA levels (12 mmol kg^{-1} soil) are deleterious or lethal to plants because of high concentrations of free EDTA, and a decrease in the availability of essential nutrients due to losses of soil macronutrients via leaching. However, EDTA raised Cu and Pb concentrations from (15.3 and 5.6 mg kg^{-1} dry weight) to (39.8 and 15.8 mg kg^{-1} dry weight) in *B. juncea* plants growing in the soil, respectively.

Hemidesmus indicus potential for phytoextraction of Pb from industrially contaminated soils was studied by **Sekhar *et al.,* (2005)**. They found that EDTA was several times more effective than CDTA (1,2-Cyclohexylenedinitrilotetraacetic acid) , HEDTA, and DTPA for the uptake of Pb by *Hemidesmus indicus*.

Nowack *et al.,* (2006) in a critical assessment of chelatant-enhanced metal phytoextraction study revealed that EDTA facilitates the diffusion of metals towards plant roots by desorbing heavy metals from soil and subsequently increasing their concentration in soil solution and by decreasing the apparent diffusion coefficient of the metal under the metal-EDTA form. They also found a low efficiency of EDTA to solubilize trace metals in calcareous soils due to the hydrolysis of aquo-complexed metals at higher pH values, besides their low solubility as compared to that of Ca.

Ruley *et al.,* (2006) studied the effect of type and concentration of chelators (EDTA, DTPA, HEDTA, NTA, Citric acid at 0–10 mmol kg^{-1} soil) on Pb accumulation in *Sesbania drummondii* (Rydb.) in soils contaminated with a high concentration of Pb (7.5 g kg^{-1} $Pb(NO_3)_2$). The effect of chelators on the accumulation of Pb in *Sesbania drummondii*

shoot was found to be strongly concentration dependent. The highest Pb uptake was found when EDTA was applied at 10 mmol kg^{-1} soil. Low EDTA rates have been reported to facilitate the breakdown of barriers to the uptake of metals by plants **(Chen *et al.*, 2004)**.

Uptake of EDTA-complexed Pb, Cd and Fe by solution and sand-cultured *B. juncea* were studied by **Schaider *et al.,* (2006)**. They stated that metal–EDTA complexes (Pb-EDTA, Cd-EDTA, and Fe-EDTA) dominate xylem sap metal speciation and the fraction of metal in xylem sap present as metal–EDTA was higher for non-nutrient metals (Pb and Cd) than for the nutrient metal Fe.

Effect, mechanism, toxicity, and fate of chelating agents were assessed by **Evangelou *et al.*, (2007)**. They pointed out that the effect of EDTA on the uptake of metal varied with plant, metal, and soil type between no to 200-fold higher accumulations.

Marques *et al.,* (2007) reported that the addition of EDTA and EDDS at 0.5 g kg^{-1} soil as a solution to contaminated soils increased Zn concentration in *Solanum nigrum* plants. EDTA corroborate an increase in Zn concentration accumulated by *Solanum nigrum* of up to 231% in plant leaves, 93% in the stems, and 81% in the roots, while EDDS addition increased the accumulation in leaves, stems, and roots up to 140%, 124%, and 104%, respectively, with the plants accumulating up to 8267 mg Zn kg^{-1} in the stems.

Johnson *et al.,* (2010) investigated the effect of EDTA, NTA and EDDS chelators on enhancing Cu uptake by *Brassica juncea* and *Lolium perenne* from solution. They revealed that EDTA was more effective than NTA and EDDS for increasing Cu uptake and translocation into the shoots of both species.

Effect of chelating agents in phytoremediation of metals was studied by **Dipu *et al.,* (2012)**. They stated that EDTA enhanced the uptake of heavy metal by plant roots from soils and facilitate metal xylem loading. This increased metals uptake by roots is owing to forming a soluble metal-EDTA complex.

Shahid *et al.,* (2012) reviewed that the neutral charge, metal-EDTA complex are not blocked or attached by carboxyl groups or polysaccharides of rhizoderm cell surface. In this way, EDTA causes the metal to enter directly to the plant roots.

Abbas and Abdelhafez (2013) investigated the impact of different EDTA levels (0, 1.0, 2.5 and 5 mmol kg^{-1} soil) on As uptake by *Zea mays* grown on As-polluted soil (containing 25.36 mg kg^{-1}). They found that applying 2.5 mmol EDTA kg^{-1} decreased significantly the dry weights of both root and shoot where the corresponding reductions were about 25.15% and 13.96%, respectively. The highest EDTA application rate (5.0 mmol kg^{-1}) significantly reduced the roots and shoots dry weight, where the corresponding reductions calculated as percentages of the corresponding control ones were 37.27% and 23.19%, respectively. Values of As uptake (µg pot^{-1}) by shoots increased significantly with increasing the applied EDTA rate. Also a relatively higher uptake of As by roots than shoots for plants grown on soil received no EDTA or received only 1.0 mmol EDTA kg^{-1} soil. On the other hand, As uptake by shoots and roots was almost equal after 2.5 mmol kg^{-1} treatment.

Kanwal *et al.,* (2014) studied the effect of EDTA on the phytoextraction of Pb using rapeseed (*Brassica napus L.*) grown hydroponically in solutions containing Pb (50 and 100 µM) alone or together with 2.5 mM EDTA in the nutrient culture. At higher Pb level (100 µM) the lead concentration significantly increased in root regardless of lead levels followed by stem and leaf. EDTA application significantly increased Pb concentrations in root, stem, and leaf of plants at both Pb levels. Furthermore, the use of EDTA also improved the translocation of Pb from roots to aboveground parts of *Brassica napus*.

Cay *et al.,* (2016) studied the influence of EDTA on the phytoextraction of Cd from artificially contaminated soil (25 mg kg^{-1} soil) by four different ornamental plant species (*Althaea rosea*, *Lonicera japonica*, *Salvia virgata* and *Dahlia hybrid*). The results revealed that addition of 0.5, 1.0 and 1.5 mmol EDTA kg^{-1} soil enhanced Cd contents considerably in all plants in comparison with the control plants. The mean Cd concentrations in different species were significantly differed and they followed the order of *Althaea rosea* > *Lonicera japonica* > *Salvia virgata* > *Dahlia hybrida*. EDTA also shows typical phytotoxic effects to

plants probably due to the increased uptake of metals or due to an imbalance in mineral nutrition as reported by **Neugschwandtner *et al.*, (2009)**.

2.9.3. Mechanism of EDTA-Enhanced Heavy Metal Translocation:

Wallace and Hale, (1962) revealed that the degradation of the Casparian strip by EDTA can be a possible way of increased metal translocation in plant shoot tissues.

Vassil *et al.*, (1998) studied EDTA in relation to Pb transport and accumulation in indian mustard. They stated that the most conventional theory suggests that metal-EDTA complexes are driven from root to shoot by the transpiration stream.

Tandy *et al.*, (2006) investigated the use of Diffusive Gradients in Thin films (DGT) to predict the plant available Cu, Zn and P in agricultural soils. They stated that the metal-EDTA complexes pass through the free space of the roots, which is continuous with the surrounding soil solution. EDTA can increase the root flux of trace metals through the apoplast due to the reduced binding of metal cations to extracellular cation-exchange sites of the apoplast and cell walls.

Effects of insoluble Zn, Cd, and EDTA on the growth and uptake of Zn and Cd in *Vetiveria zizanioides* were studied by **Xu *et al.*, (2009)**. They suggested that EDTA binds metal (metal-EDTA) in the nutrient/soil solution, which is primarily taken up by plant roots through the apoplastic pathway.

Cay *et al.*, (2016) suggested that metal-EDTA complexes in the soil are primarily absorbed by roots through the apoplastic pathway and later translocated to the shoots. EDTA can also increase the root flux of metal through the apoplast into the stele and thus enhances its translocation towards shoots. This happens due to the reduced binding of metal ions to extracellular ion-exchange sites of the apoplast and cell walls, which occurs normally in the absence of chelators.

2.10. Role of Citric Acid (CA) on heavy metals phytoextraction process:

The exudation of organic compounds by roots may influence the solubility of essential and toxic ions both indirectly and directly. Indirectly, through their effects on

microbial activity, rhizosphere physical properties and root growth dynamics and, directly, through acidification, precipitation, chelation and oxidation–reduction reactions in the rhizosphere **(Marschner *et al.*, 1995)**. Most of these compounds, natural low molecular weight organic acids (NLMWOA), such as citric acid, oxalic acid, or malic acid **(Nigam *et al.*, 2001)**.

Lee *et al.,* (1977) in isolation and identification of a citrato-complex of Ni from nickel-accumulating plants study suggested that NLMWOA have an ability to detoxify intracellular metals via binding.

Using organic acids in order to trigger uranium (U) hyperaccumulation in plants was studied by **Huang *et al.,* (1998)**. They outlined that addition of CA (20 mmol kg^{-1}) increased U concentrations in soil solution by 200-fold and in the studied plants (*B. juncea, B. Chinensis, B. narinosa* and *amarath*) 1000-fold as compared to the control. Addition of CA to the contaminated soil transiently decreased the soil pH by 0.5–1.0 unit. CA was also more efficient than EDTA, EDDHA, HEDTA, DTPA and EGTA in raising the soil U desorption.

Chen *et al.,* (2003) studied the role of CA in relation to phytoextraction of metals using *Raphanus sativus*. They showed that application of CA reduced the adsorption of both Pb (1.87%, 4.35%) and Cd (25.25%, 43.89%) and the reduction rate was increased with increasing CA concentration from 1 to 3 mmol l^{-1}. Citric acid also stimulate Pb and Cd transportation from root to shoot by 0.172% to 0.328% and 6.69% to 9.36%, as the ratio of Pb and Cd contents in the leaf to root increased, respectively. So CA application could alleviate the toxicity of Pb and Cd to *Raphanus sativus*.

Wu *et al.,* (2004) found that addition of citric, oxalic or malic acid to soil at the rate of 3 mmol kg^{-1} soil had substantially no effect on metals uptake by *B. juncea*. However, the resulting offtakes were low and a sequence of at least 200 crops would be required to remediate the soil.

Evangelou *et al.,* (2006) investigated the uptake of Cu by tobacco (*N. tabacum*) shoots. They revealed that application of CA, oxalic acid and tartaric acid at the rate of 62.5 mmol kg^{-1} soil had a 2-fold, 0.5-fold, and no increase respectively. Whilst, addition of the NLMWOA in the same trial had no effect on Pb uptake by *N. tabacum*.

Nascimento *et al.,* (2006) performed a comparison between synthetic chelates (EDTA and DTPA) and naturally LMWOA (oxalic acid, citric acid, vanillic acid and gallic acid) in enhancing heavy metals phytoextraction by *Brassica juncea* from multi-metal contaminated soils. They reported that the effectiveness of LMWOA were not accompanied by raising the risk of leaching for these metals so unlike EDTA and DTPA, moreover, the lower phytotoxicity of LMWOA, especially Citric and gallic acids, to *Brassica juncea* compared to EDTA.

Physiological and ultrastructural disorders of Cd in *Juncus effusus L.* and its remediation through hexogenous CA were studied by **Najeeb *et al.,* (2011)**. They reported that CA increased plant growth and biomass due to increased nutrient uptake and/or CA-induced chelation of Cd decreasing free metal ions in plants.

The role of CA on Pb phytoextraction was assessed in a field experiment by **Freitas *et al.,* (2013)**. They stated that usage of CA at 40mmol kg^{-1} soil was effective in the Pb solubilization of the soil and in the induction of Pb absorption by Maize (*Zea mays*) and vetiver (*Chrysopogon zizanoides*). Where, CA promoted an increase in Pb concentration in *Zea mays* and *Chrysopogon zizanoides* shoots as compared to the control by a 14-fold and 7.2-fold, respectively.

The role of CA in the phytoremediation of Cd by rapeseed (*Brassica napus L.*) plants was assessed by **Ehsan *et al.,* (2014)**. They found that adding 2.5 mM significantly enhanced Cd uptake and its accumulation in *Brassica napus* roots (37.34% increase), stems (33.22% increase) and leaves (31.58% increase). Hence, CA could alleviate the toxicity of Cu by increasing plant biomass and photosynthetic and growth parameters.

A glasshouse experiment was conducted to assess the role of CA in enhancing Pb phytoextraction by *brassica napus* **(Shakoor *et al.*, 2014)**. The seedlings were grown in hydroponic media and exposed to different rates of Pb (50 and 100 µM) as alone or in combination with CA (2.5mM) for six weeks. The results revealed that Pb-induced deterioration in *B. napus* toxicity was evident from high levels of malondialdehyde (MDA) and H_2O_2 which significantly impeded plant growth, biomass accumulation, leaf chlorophyll contents and gas exchange parameters. In contrast, CA application to Pb-stressed *B. napus* plants restricted lipid membrane deterioration by limiting MDA and H_2O_2 production and by improving antioxidant enzyme activities. In addition, CA significantly raised the Pb uptake by *B. napus* plants. They concluded that CA has a prospect to upgrade the phytoextraction of Pb without deteriorating plant growth.

Zaheer *et al.,* (2015) studied the influence of CA in enhancing Cu uptake by *Brassica napus L.* seedlings under different levels of Cu (0, 50 and 100 mM) alone or with CA (2.5mM) in a nutrient medium for 40 days. The results declared that Cu toxicity reduced plant growth, biomass, photosynthetic pigments and protein contents. Whereas, the negative effects of Cu toxicity can be overturned by CA application. CA application significantly increased plant growth, biomass, and chlorophyll content by reducing oxidative stress and enhancing antioxidant enzyme activities. Addition of CA also increased Cu uptake by *B. napus* plants. So using CA can be a safer choice for increasing the phytoextraction of Cu by *B. napus* and by minimizing Cu toxicity and increasing biomass production.

2.11. Role of humic acid (HA) on heavy metals phytoextraction process:

Chelators can increase the solubility of metal cations, and their bioavailability to plants. However, some chelators such as EDTA are accompanied by negative effects in the soil due to its non-eclectic nature in extracting metals including alkaline earth cations, such as Ca and Mg, which are essential for plant growth **(Barona *et al.*, 2001)**. Moreover, EDTA is not easily biodegradable, and may remain adsorbed on soil particles for a long time **(Wasay *et al.*, 1998)**.

Humic acids are those parts of humic substances which are insoluble in water under acidic conditions, but become soluble and extractable at high pH values. It contains acidic groups and phenolic-OH groups. The major ligand sites for a metal ion in HA are oxygen-containing functional groups. Carboxyl groups are considered to play a prominent role in binding metal ions by soil HA **(Hofrichter and Steinbüchel, 2001)**.

Zhang *et al.,* (2003) stated that the lower stability constant of HA, compared to other synthetic chelates for metals, makes HA an ideal soil amendment for phytoextraction and inhibits the possible movement of metal-HA complexes across the soil profile.

Evangelou *et al.,* (2004) studied the influence of HA as a substitutional to synthetic chelators on the phytremediation of Cd from soil. They found that HA addition raised Cd availability to *Nicotiana tabacum* as Cd concentration in shoots increased from 30.9 to 39.9 mg kg^{-1} dry weight. This enhancement could be attributed to the decrease in pH, resulting in higher cadmium availability. Another possibility taken into account is that plants could take up cadmium complexes with HA fragments which result from microbiological degradation or, self-dissociation.

Turan and Angin (2004) tested organic chelate assisted phytoextraction of B, Cd, Mo and Pb from contaminated soils using *Zea mays L.* and *Helianthus annus L.*. They outlined that HA (at rates of 5 and 10 mmol kg^{-1} soil) was effective natural chelator in enhancing Pb, Cd, Mo and B desorption from soil and increasing their accumulation in *Zea mays L.* and *Helianthus annus L.*.

Angin *et al.,* (2008) studied the effect of HA in increasing B and Pb phytoextraction by vetiver grass (*Vetiveria zizanioides*) from contaminated soils. They found that the efficiency of HA depends on the initial level of Pb and B contamination and the amount of HA applied. Where, addition of HA at a rate of 200 kg ha^{-1} was optimum for maintaining yield at high Pb levels and B uptake was utmost after application of 400 kg HA ha^{-1}.

Wang *et al.,* (2010) studied HA efficiency on accumulating Cd and Cu in *Vallisneria spiralis L.*. They found that increasing HA addition from 3.09 to 7.89 g kg^{-1} soil to Cu and Cd contaminated sediment reduced Cd concentrations in *Vallisneria spiralis* roots and

shoots by 26.4–50.3% and 14.3–33.0%, whereas, copper accumulation was decreased much more with 44.0–77.0% and 35.0-62.7%, respectively. Humic acid clearly reduced Cu and Cd bioavailability and toxicity in *V. speralis* due to forming complexes of HA with metal ions.

The impact of HA on Zn, Cu, Ni, Pb and Cd phytoavailability from sludge applied soil and heavy metal uptake of tobacco plant was examined by **Topcuoğlu (2012)**. The results pointed out that the availability and metals uptake by plants was significantly raised by HA application at 1% and 2%. Whereas, a significant reduction in plant yield were observed especially with HA treatment at 2% without any toxicity symptoms. Results verify the positive role of exogenous HA in raising phytoremediation efficiency of soils contaminated with metals.

Accumulation of Pb and Cd in tobacco leaves grown on four soil types (red, yellow, cinnamon, and paddy soil) as affected by humic acids (humic and fulvic acids) was invistigated by **Zhang *et al.*, (2013)**. They revealed that the effect of HA on Pb and Cd was pH-dependent. Whereas, humic acids at a level of 4 g kg^{-1} soil decreased Pb and Cd accumulation in plant leaves in the acidic red and paddy soils by 17.78% - 48.32% and 8.74% - 32.84% for Pb and Cd, respectively. Whilst, increased leaves concentration in the alkaline (yellow) and calcareous (cinnamon) soils by 11.69% - 37.54% and 14.20% - 46.37% for Pb and Cd, respectively. As a result, humic acids could be used to reduce metal accumulation (stabilization) in plants growing in polluted acidic soils and could be used for metal phytoremediation in alkaline soils.

3. MATERIALS AND METHODS

3.1. Location:

Three pot experiments were conducted in a wired greenhouse at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate (31° 5' 35.93" latitude N and 30°56' 54.33" longitude E) during summer season of (2013) and winter season of (2013-2014).

The experiments aimed to investigate the phytoextraction efficiency of Cu, Zn and Pb from contaminated soils using sunflower (*Helianthus annuus*) (Sakha 53) and indian mustard (*Brassica juncea*) var. Dama (introduced from Canada 1980) under Chelators (EDTA, citric acid (CA) and humic acid (HA)) addition.

3.2. Soil Sampling and analyses:

There are about more than 4.5 thousand fed in Al-Gabal Al-Asfar & Abu Rawash farms are irrigated with sewage water since more than eighty years. This led to metal contamination of these areas. Primary and secondary sewage water treatment (doesn't include advanced treatment which get rid of a large proportion of heavy metals) were established in these areas in Abu Rawash since 1992 and Al-Gabal Al-Asfar Since 1997.

In early spring 2013, the contaminated soils used in this research were collected from the surface layer (0-30 cm) from two heavily polluted sites (Al-Gabal Al-Asfar farm & Abu Rawash farm). Collected soil samples were brought to the wired green house, air-dried for 5-6 days, ground to pass through a 4 mm diameter sieve and mixed thoroughly to achieve homogeneity. Representative sample was ground to pass through a 2 mm sieve, some physical and chemical characteristics were determined including the total and available content of some heavy metals for the tested soils as shown in table (1).

The chemical fractionation of Cu, Zn and Pb metals were determined by the five-steps sequential extraction method based on the work of **Tessier *et al.*, (1979)** and proposed by **Garcia-Delgado *et al.*, (2007)**, and **Sánchez-Martín *et al.*, (2007)** to characterize the partitioning of Cu, Zn and Pb in both studied soils as shown in table (2) and the results were presented in table (3)

Table (1): Some physical and chemical characteristics of the tested soils.

	AR*	GA**
pH (soil paste)	6.61	6.88
EC_e (soil-paste extract), $dS\ m^{-1}$ at 25°C	7.64	1.59
OM (organic matter), %	3.23	2.03
Total carbonates, %	0.40	0.22
Water holding capacity (WHC), %	46.45	35.55
CEC (cation exchange capacity), meq/100g soil.	19.04	17.92
Particle size distribution:		
Sand %	76.79	82.55
Silt %	8.90	5.02
Clay %	14.31	12.43
Texture class	loamy sand	loamy sand
Soluble cations, meq/L (soil-paste extract):		
Ca^{++}	42.68	5.19
Mg^{++}	18.44	3.21
Na^{+}	10.17	6.37
K^{+}	3.47	0.63
Soluble anions, meq/L (soil-paste extract):		
$CO_3^{=}$	--	--
HCO_3^{-}	8.75	6.88
Cl^{-}	14.20	5.76
$SO_4^{=}$	51.81	2.76
Available Macro-nutrients (mg/kg):		
N	84	35
P	60.02	44.39
K	273.75	147.50
Sodium Adsorption Ratio (SAR)	1.84	3.11
Aqua-Regia extracted metals (total), mg kg^{-1} soil:		
Mn	410.00	370.00
Ni	19.60	5.40
Pb	150.00	210.00
Cu	170.00	107.00
Zn	520.00	380.00
DTPA-extractable metal (available), mg kg^{-1} soil:		
Mn	132.00	16.00
Ni	3.60	2.04
Pb	9.44	36.00
Cu	40.00	27.00
Zn	131.00	67.00

* AR= Abu Rawash soil ** GA= Al-Gabal Al-Asfar soil

The total concentrations of Cu, Pb and Zn metals exceeded the threshold values that were legislated by the Ministry of the Environment **(MEF, Finland, 2007)** and have been

applied in an international context for agricultural soils as well **(UNEP, 2013)**. The threshold values for Cu, Pb and Zn in soils are 100, 60 and 200 mg kg^{-1} soil, respectively, indicating the need for remediation actions.

Table (2): Chemical extraction scheme used in this work for metal fractionation*.

Fraction	Reagent conditions**	Shaking time (h)
F1. Exchangeable and water soluble	16 mL 1 M $MgCl_2$ (pH 7.0)	1
F2. Dilute acid-extractable	16 mL 1 M NaOAc adjusted to pH 5 with HOAc	5
F3. Fe-Mn oxide bound	40 mL of 0.175 M $(NH_4)_2C_2O_4$ and 0.1 M $H_2C_2O_4$	4
F4. Organically bound	40 mL of 0.1 M $Na_4P_2O_7$	24
F5. Rxesidual	Aqua regia digestion	

* Sample weight of dried soil was 5 g.

** Extractions F1 through F4 were performed at room temperature for the stated times. Separation between steps was by decantation of the supernatant after centrifugation at 5000 rpm for 20 min.

Table (3): Chemical fractions of Cu, Zn and Pb (mg kg^{-1}) in both studied soils.

Fractions	Abu Rawash soil			Al-Gabal Al-Asfar soil		
	Cu	Zn	Pb	Cu	Zn	Pb
F1	2.62	2.78	3.01	1.85	1.70	2.24
F2	3.67	1.86	3.30	2.17	3.07	3.39
F3	78.40	188.80	14.96	46.40	121.20	17.68
F4	22.41	91.20	77.20	15.44	87.60	118.40
F5	45.60	195.00	61.00	33.38	192.00	59.80
ΣF1-F5	152.70	479.64	159.47	99.24	405.57	201.51
Total	170.00	520.00	150.00	107.00	380.00	210.00
% Recovery*	89.82	92.24	106.31	92.75	106.73	95.96

* % Recovery = (ΣF1-F5/ determined total concentration of metal in soil)*100

3.3. Pot experiments preparation:

Air-dried soil equivalent to 6.5 kg oven dry soil was placed in polyethylene bags which were used to coat the inner walls of crockery pots (25 cm diameter × 40 cm height) in order to avoid water preferential flow. The polyethylene bags and pots were ended with a central hole at the bottom to collect the leached solution. A filter paper and a fine mesh tissue were used in order to prevent soil losses with the leachate solution.

3.4. Performing experiments:

Two hyperaccumulator plants were selected for this study (sunflower and indian mustard) based on the species demonstrated ability to uptake and translocate heavy metals **(Nehnevajova *et al.,* 2005 & Robinson *et al.,* 2009)**.

Two experiments were carried out to study the effect of chelating agents, EDTA, CA and HA, on sunflower (summer season of 2013) and indian mustard (winter season of 2013/2014) phytoextraction efficiency of Cu, Zn and Pb and leaching behavior of these metals from soil.

Third experiment (winter season) was conducted to study the residual effect of agents added to sunflower experiment, without any addition of chelators, on phytoextraction of tested metals by indian mustard and leaching behavior of these metals from soil.

3.5. Experimental Design:

The experiments were conducted in split plot design with three replicates.

The main plots were assigned to chelator type: Disodium ethylenediaminetetraacetic acid (EDTA), Citric Acid (CA) and Humic Acid (HA).

Whereas, the sub-plots were subjected to chelator addition rates (C_0, C_1, C_2 and C_3) as following:

(i) EDTA levels were: 0.0, 1.5, 3 and 4.5 mmol kg^{-1} soil.
(ii) CA levels were : 0, 3, 6 and 9 mmol kg^{-1} soil.
(iii) HA levels were : 0.0, 0.2, 0.4 and 0.6 g kg^{-1} soil.

Each experiment contains 36 pots.

3.6. Cultural practices:

Four seeds of sunflower and indian mustard were sown on June 15^{th} and October 20^{th}, 2013, respectively. The plants were grown under natural daylight in a wired greenhouse. Plants were thinned to one plant per pot after forming the fourth plant leave.

The plants were irrigated as needed in the beginning to enhance germination of seeds and growth of plants. After treatments addition, the irrigation water was added at 100% of water holding capacity (WHC) plus 5% in order to receive the leachate at the end of each pot.

Basal fertilizers were added with irrigation water to all three experiments after thinning as 2.0 fold of Egyptian Ministry of Agriculture recommendation. Potassium sulphate (50% K) was applied at 21 kg (K) fed^{-1} as a source of potassium, phosphoric acid (57% P) was applied at 13 kg (P) fed^{-1} as a source of phosphorus, and Urea (46% N) was applied at 45 45 kg (N) fed^{-1} as a source of nitrogen.

Chelating agents with selected levels were added to the soil in the first and second experiments as two equal doses with irrigation water through rapid growth stage, 30 days interval, after 25 and 45 days of sunflower and indian mustard cultivation, respectively. This could be useful to enhance metal availability to the extent that enhance metal uptake and decrease migration of heavy metals with leachate during the growing seasons at the same time.

In the third experiment, indian mustard were sown on October 20th, 2013, in pot previously planted with sunflower plants without any addition of chelator treatments to study the residual effect of tested chelators on metal accumulation by plants and leaching. The same cultural practices of irrigation and fertilization applied in the first and second experiments were performed in the third experiment.

The leachate solutions collection was started from the bottom of each pot after addition of chelating agents in the first and second experiment up to the end of growing seasons. While in the third experiment the collection of leachate solutions was started after plantation of indian mustard up to the end of growing seasons.

At full maturity of sunflower and indian mustard, plants were totally removed from pots on 15th September (2013) and the 20th of March (2014), respectively.

3.7. Humic acid extraction:

Humic acid used in this study was extracted according to the method described by **Black *et al.*, (1965).** The method depends on adding NaOH 0.5 N to peat or organic rich soil to extract humic and fulvic acids. Then centrifuging the mixture and decanting off the dark colored supernatant. After that adjusting the pH of the solution to about 1 with concentrated HCl to allow the humic acid to settle. Repeating addition of NaOH 0.5 N to the soil and shake the mixture for 1 hour and repeating the centrifuging and decanting procedures then adjust the pH of the solution to 1 using HCl. Siphoning off the excess supernatant to get rid of fulvic acid. Repeating dissolving and precipitating humic acid several times then wash the precipitated humic acid several times with distilled water until it is free of chlorides. Drying the humic acid and grind it to a brown powder.

3.8. Methods of analyses:

3.8.1. Soil Analyses:

The soil properties prior to remediation were determined as following:

- Mechanical analysis: Mechanical analysis of soil samples was carried out by the international pipette method, using sodium hexametaphosphate as a dispersing agent (**Piper, 1950**).
- Soil reaction (**pH**): soil reaction was measured in soil paste using Beckham pH meter **(Page, 1982)**.
- Water holding capacity **(WHC)**: was measured according to **Richards (1954).**
- Cation exchange capacity **(CEC)**: was determined using sodium and ammonium acetate method according to the modified Hissink's methods **(Jackson, 1973)**.
- Total calcium carbonate (%): was measured volumetrically by Collins calcimeter using 2 g of ground (< 0.2 mm) soil and 8 cm3 of 6 M HCl **(Richards, 1954)**.
- Total organic matter (%): was determined by the wet combustion of Walkely and Black method **(Hesse, 1971).**
- Electrical conductivity **(EC)**: (dS m^{-1}) was measured by electrical conductivity meter model Jenway, 4320 at 25˚C in the saturated soil paste extract **Page (1982)**.

- Soluble cations and anions: Ca^{++}, Mg^{++}, K^{+}, Na^{+}, $CO_3^{=}$, HCO_3^{-}, Cl^{-} and $SO_4^{=}$ (meq l^{-1}) were determined in saturated soil paste extract **(Page, 1982)**.
- Available nitrogen was extracted by 1.0 M K_2SO_4 and determined by using the conventional method of micro Kjeldahl method according to **Jackson (1973).**
- Available phosphorus was extracted with 0.5 N sodium bicarbonate **(Olsen *et al.*, 1954)** and determined colorimetrically by ascorbic acid method **(Olsen *et al.*, 1954)**.
- Available potassium: was extracted with ammonium–acetate (pH; 7.0±0.1) and determined using a flame photometer instrument according to the method described by **Jackson (1973)**.
- Total contents of heavy metals: were determined using acid digestion products of soil by aqua regia according to **Cottenie *et al.*, (1982)**.
- Available contents of heavy metals: were extracted by diethylenetriaminepenta-acetic acid (DTPA) **(Lindsay and Norvell, 1978)**.

The total and available amounts of the tested metals were determined using **AAS, PERKIN ELMER 3300**.

3.8.2. Leachate and plant Analyses:

The leachate solutions were collected at the bottom of each pot during the growing seasons after applying chelating agents, filtered through Whatman No. 42 filter paper and analyzed for heavy metals using the Atomic Absorption Spectrophotometer **(AAS, PERKIN ELMER 3300)**.

Dropped leaves of each plant were collected during growing season. At harvesting, the above ground portion of plant was removed, gently brushed using a brush pen for removing the dust and subsequently washed with tap water then distilled water three times. Leaves, stems and seeds were separated, including the collected dropped leaves during the growing seasons.

More attention was paid to clean roots of debris in order to prevent elevated readings due to soil contamination, then roots were rinsed with deionized water.

The root, stems, leaves and seeds samples were oven dried at 70 °C for 72 h, weighted, milled in a stainless steel mill.

For the heavy metal analysis, 0.5 g of each plant part (roots, stems, leaves and seeds) was acid digested by addition of 10 ml of H_2SO_4 and HNO_3 mixture and 1.0 ml $HClO_4$ **(Chapman and Pratt, 1961)**. The heavy metals content of plants were determined by **(AAS, PERKIN ELMER 3300)**.

3.8.3. Statistical Analyses:

All data collected of the two seasons were analyzed according to the methods described by **Snedecor and Cochran (1980)** using the CoStat package program, version 6.311 (cohort software, USA). To compare between treatment means, the Least Significant Differences (LSD) at probability 5% and 1% was used. Statistical analysis was performed.

4. RESULTS AND DISCUSSION

4.1. Effect of chelator types, rates and their interactions on the dry weight of plant organs (g plant^{-1}) grown on the two studied soils:

4.1.1. Sunflower:

Data in Table (4) represent the effect of applied chelators, their addition rates and their interaction on roots, stems, leaves, seeds and total dry weight production of sunflower (*Helianthus annuus L.*) in two contaminated soils.

Regarding to chelators effect on roots dry matter production in Abu Rawash soil, highly significant differences were noticed between chelators treatment means. CA treatment recorded the highest value 31.3 g, 33.76% increase compared to EDTA treatment mean, HA comes in the second order with 26.0 g, 11.11% increase compared to EDTA treatment mean, and EDTA gave the lowest roots weight of 23. 4 g.

In Al-Gabal Al-Asfar soil, roots dry weight took similar trend to that of Abu Rawash soil, but little increase was found due to CA and HA treatments, 6.02 and 12.05 % increases compared to EDTA treatment mean, respectively. EDTA took the latest order with 16.6 g.

The above ground plant organs were also increased due to CA and HA application compared to EDTA addition in both soils. CA increased stems, leaves and seeds by 28.68, 22.73 and 35.44%, respectively, where HA increased the same parts by 12.88, 10.16 and 29.11 % in Abo Rawash soil, respectively. Corresponding values in El-Gabal Al-Asfar soil were 5.23, 3.37 and 29.96% for CA and 9.15, 10.86 and 21.86% for HA. In above ground portion, the highest positive response in each soil was found in seeds.

Data plotted in Table (4) and illustrated in Fig (1) declare that total dry weight of sunflower is highly significant affected by CA and HA compared to EDTA in both soils. Total dry matter increase amounted by 29.46% and 10.02 % as a result of CA addition (means of treatments), whereas, its increase amounted by 15.24% and 12.74% as a result of

HA addition (means of treatments) for Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

Table (4): Effect of chelator types, rates and their interactions on the dry weight of plant organs (g plant^{-1}) of sunflower grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Abu Rawash					Al-Gabal Al-Asfar				
		roots	stems	leaves	seeds	Total	roots	stems	leaves	seeds	Total
EDTA Mmol kg^{-1} soil	0	28.9	75.4	43.1	38.6	185.9	18.4	48.8	28.1	30.2	125.4
	1.5	23.8	69.4	40.8	34.4	168.4	17.7	46.8	28.2	26.1	118.8
	3.0	21.6	61.2	35.8	31.1	149.6	18.0	49.4	27.3	22.3	117.0
	4.5	19.1	54.9	29.8	22.3	126.1	12.4	38.4	23.0	20.1	93.8
Mean		**23.4**	**65.2**	**37.4**	**31.6**	**157.5**	**16.6**	**45.9**	**26.7**	**24.7**	**113.8**
F-test		**	**	**	**	**	**	**	**	**	**
$LSD_{0.05}$		3.6	9.7	6.3	7.5	25.8	2.4	5.0	2.4	2.5	9.9
$LSD_{0.01}$		5.6	15.1	9.9	11.7	40.2	3.8	7.7	3.8	3.8	15.3
CA mmol kg^{-1} soil	0	28.9	75.4	43.1	38.6	185.9	18.4	48.8	28.1	30.2	125.4
	3.0	34.7	101.6	53.2	49.3	238.8	17.4	47.6	26.3	34.4	125.7
	6.0	34.1	90.2	46.6	44.2	215.1	17.0	49.8	28.3	32.9	127.9
	9.0	27.6	68.4	40.5	39.2	175.7	17.5	46.8	27.6	30.9	121.8
Mean		**31.3**	**83.9**	**45.9**	**42.8**	**203.9**	**17.6**	**48.3**	**27.6**	**32.1**	**125.2**
F-test		ns	**	**	**	**	ns	ns	ns	ns	ns
$LSD_{0.05}$			7.4	3.2	3.7	5.9					
$LSD_{0.01}$			11.5	5.0	5.8	9.3					
HA g kg^{-1} soil	0	28.9	75.4	43.1	38.6	185.9	18.4	48.8	28.1	30.2	125.4
	0.2	23.2	73.6	40.2	41.4	178.4	16.0	47.3	26.4	31.9	121.6
	0.4	27.8	74.2	40.2	40.4	182.6	20.1	53.2	31.1	28.1	132.4
	0.6	23.9	71.3	41.2	42.9	179.2	19.9	51.0	32.7	30.3	133.9
Mean		**26.00**	**73.6**	**41.2**	**40.8**	**181.5**	**18.6**	**50.1**	**29.6**	**30.1**	**128.3**
F-test		**	ns	ns	ns	ns	**	**	**	ns	**
$LSD_{0.05}$		2.5					1.7	2.8	2.0		7.2
$LSD_{0.01}$		3.9					2.7	4.3	3.1		11.1
F-test (treatments)		**	**	**	**	**	*	*	*	**	**
$LSD_{0.05}$		**2.9**	**6.1**	**3.7**	**5.5**	**11.0**	**1.7**	**3.4**	**2.4**	**3.2**	**8.8**
$LSD_{0.01}$		**4.4**	**9.3**	**5.6**	**8.4**	**16.6**				**4.9**	**13.4**
F-test (Interaction)		**	**	**	**	**	**	**	**	**	**
$LSD_{0.05}$		**2.5**	**5.3**	**3.2**	**4.8**	**9.5**	**1.4**	**2.9**	**2.1**	**3.2**	**7.6**
$LSD_{0.01}$		**3.8**	**8.0**	**4.9**	**7.2**	**14.4**	**2.2**	**4.4**	**3.1**	**5.2**	**11.6**

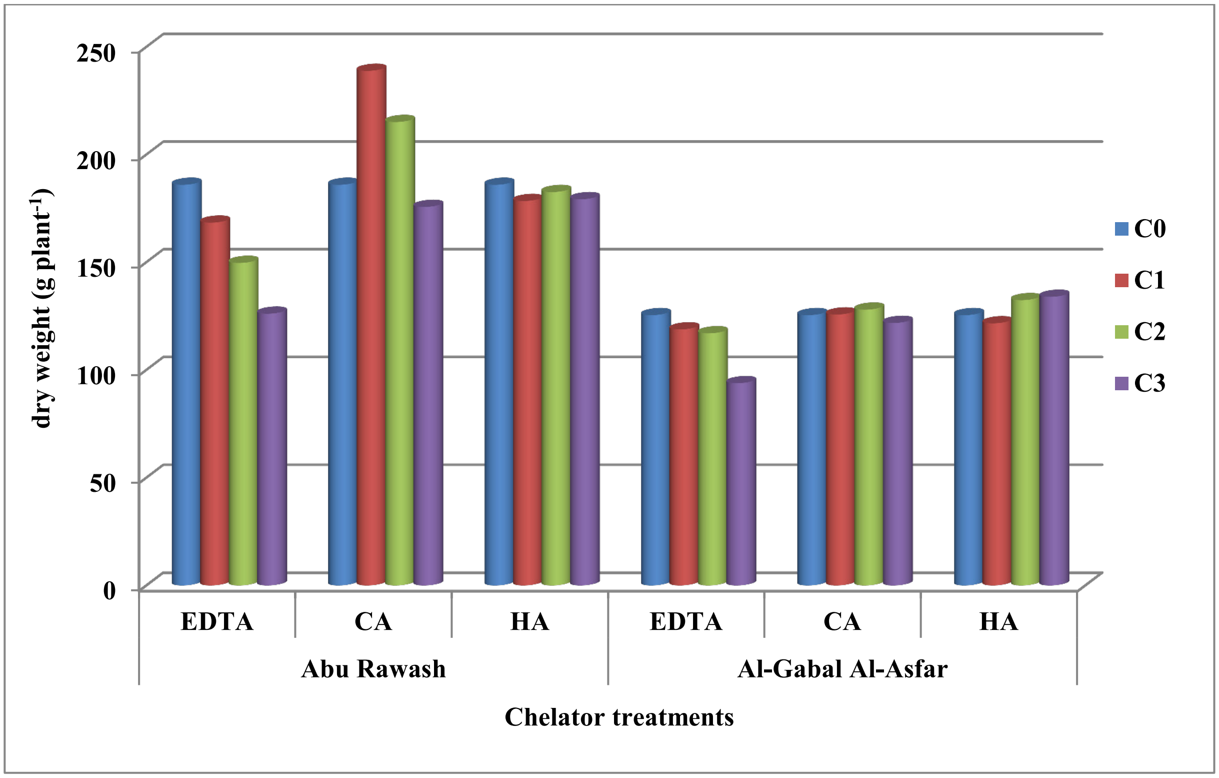


Fig (1): Effect of chelators and their addition rates on total dry weight of sunflower (g plant^{-1}) in Abu Rawash and Al-Gabal Al-Asfar soils.

Data of Table (4) and Fig (2) reveal that EDTA addition in both studied soils led to a significant decrease in dry matter production of sunflower plants as compared to control, whereby, the reduction was increased with increasing EDTA levels up to the highest level used as shown in Fig (2). However, the dry matter reduction for sunflower roots, stems, leaves, seeds and total dry weight were 33.91, 27.19, 30.86, 42.23 and 32.17%, respectively in Abu Rawash soil and the corresponding values of Al-Gabal Al-Asfar soil were 32.61, 21.31, 18.15, 33.44 and 25.20%, due to applying EDTA at a level of 4.5 mmol kg^{-1} soil. In both soils, the highest reduction in plant portion dry matter was found in roots and seeds. These results are in harmony with those of **Abbas and Abdelhafez (2013)**.

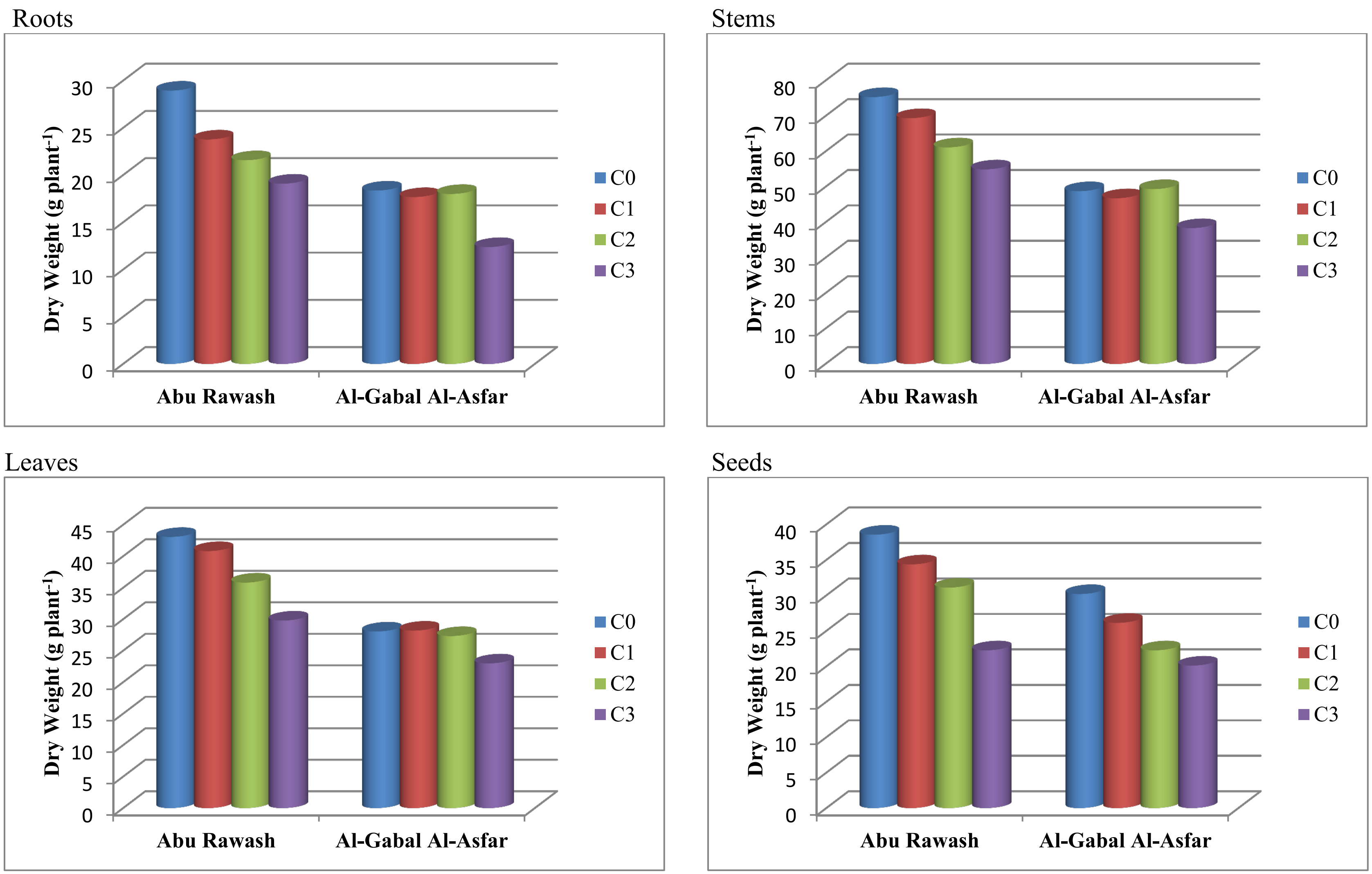


Fig (2): Effect of EDTA application levels on roots, stems, leaves and seeds dry weight of sunflower (g plant^{-1}) in Abu Rawash and Al-Gabal Al-Asfar soils.

Barrier to discuss the obtained results, It should be mentioned that visual symptoms of toxicity were observed in the EDTA treatment especially at high levels (Fig. 3) , such as some chlorosis and necrosis (brown dots) on leaves, particularly in old ones, and the leave became yellow and died slowly. This could be attributed to increasing the accumulation of metals in plants causing toxicity due to EDTA application as shown later (from page 66 to 122). Another reason could be that additions of high EDTA levels are prejudicial or lethal to plants due to high concentrations of free EDTA **(Geebelen *et al.,* 2002; Wu *et al.,* 2004)**.

Data of Table (4) and Fig (4) reveal also that application of CA at levels of 3 and 6 mmol kg^{-1} soil significantly increased sunflower dry weight yield as compared to control in Abu Rawash soil. Where, the highest values for dry weight were recorded using CA at a level of 3 mmol kg^{-1} soil which increased roots, stems, leaves, seeds and total dry weight by 20.07, 34.75, 23.43, 27.72 and 28.46%, respectively, compared with control as shown in Fig (4). In contrary, the highest level of CA (9 mmol kg^{-1} soil) reduced sunflower dry weight organs tested compared with control except for seeds. The reduction amounted by 4.50, 9.28, 6.03 and 5.49% for roots, stems, leaves and total dry weight, respectively. Highest reduction in plant organs tested was found compared to 3 mmol kg^{-1} soil CA treatments, 20.46, 32.68, 23.87, 20.49 and 26.42% for roots, stems, leaves, seeds and total dry weight, respectively.

In Al-Gabal Al-Asfar soil, CA with the used level did not increase or decrease significantly sunflower organs dry weight than that of control.

Data presented in Table (4) show that HA levels did not exhibit any significant effects on sunflower dry weight organs grown on Abu Rawash soil, except for roots, whereas in Al-Gabal Al-Asfar soil significant effects were found between HA treatment means in all the studied traits except for seed dry weight. Application of HA to sunflower in Al-Gabal Al-Asfar soil at a rate of 0.4 g kg^{-1} soil recorded the highest roots and stems dry weight,20.1 g and 53.2g compared with control. HA addition to Al-Gabal Al-Asfar soil at a rate of 0.6 g kg^{-1} soil recorded the highest leaves, seeds and total dry weight, 32.7 g, 30.3g and 133.9 g, respectively.

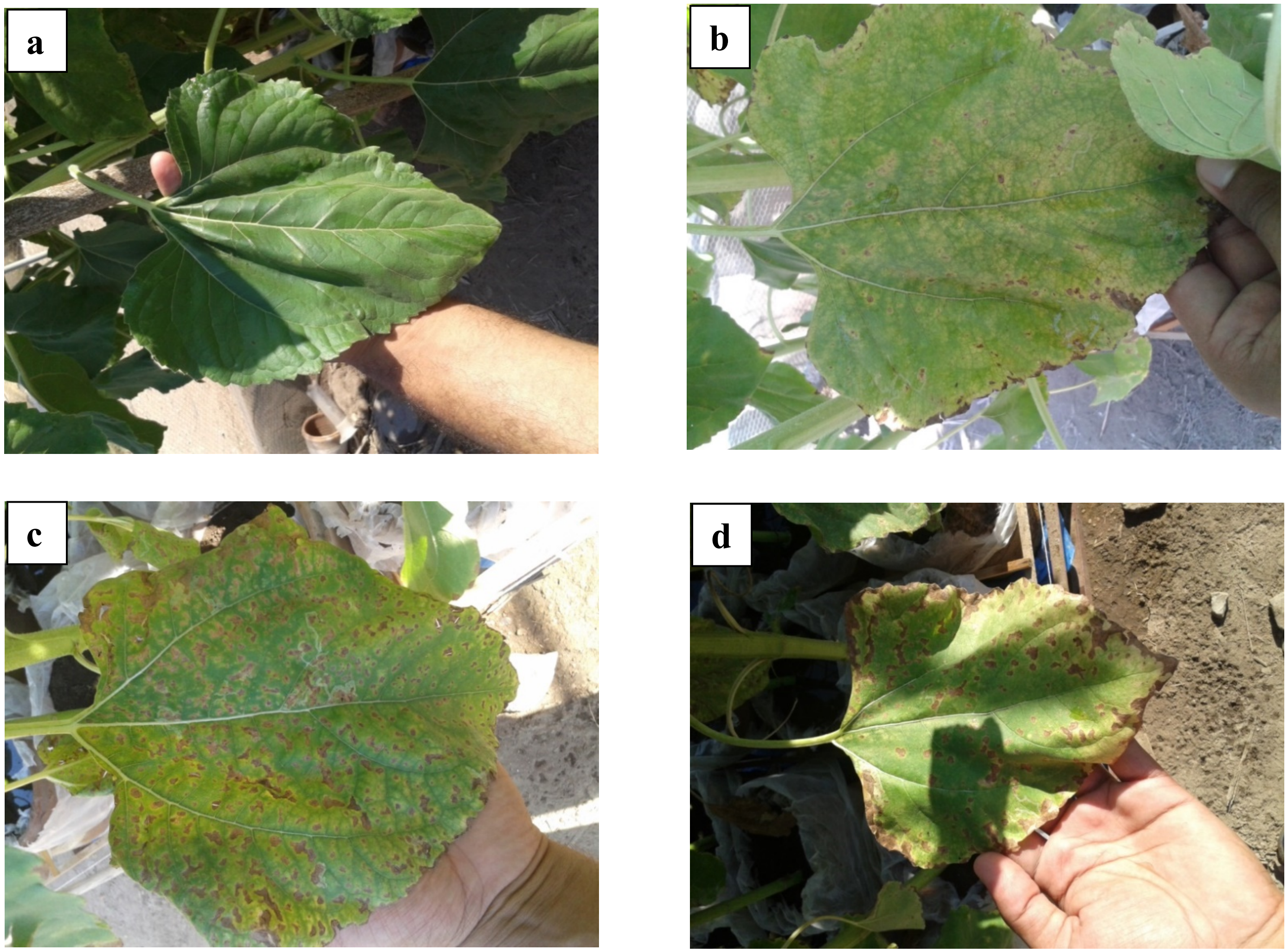


Fig (3): visual symptoms of toxicity on sunflower leaves due to EDTA application levels at: a) 0 (control), b) 1.5 mmol kg^{-1} soil, c) 3.0 mmol kg^{-1} soil and d) 4.5 mmol kg^{-1} soil.

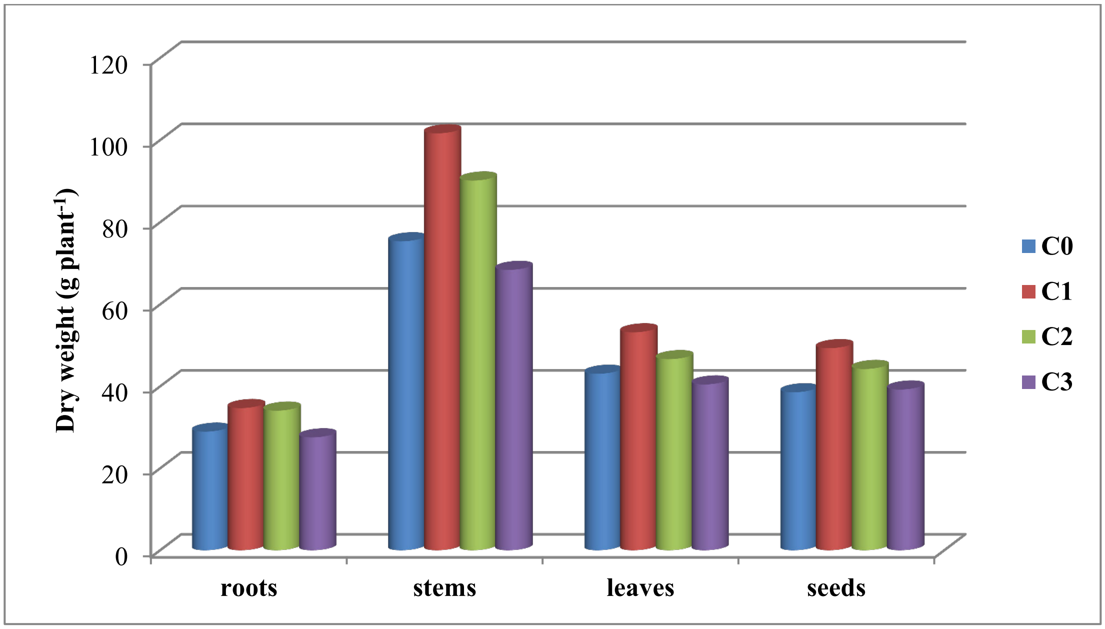


Fig (4): Effect of CA application levels on roots, stems, leaves and seeds dry weight (g plant^{-1}) of sunflower plant grown on Abu Rawash soil.

Significant effects were found due to the interaction between chelator types - chelator levels in both soils regarding to roots, stems, leaves and total dry weight. Whilst, seeds dry weight was significantly affected due to the interaction between chelator types - chelator levels in Abu Rawash soil.

The lowest values of roots, stems, leaves, seeds and total dry weight were achieved with the treatment of EDTA 4.5 mmol kg^{-1} soil in both soils, where the corresponding mean values in Abu Rawash soil were 19.1, 54.9, 29.8, 22.3 and 126.1 g, respectively, and in Al-Gabal Al-Asfar soil were 12.4, 38.4, 23.0, 20.1 and 93.8 g , respectively.

4.1.2. Indian mustard:

Data of Table (5) represents the effect of chelator types, rates and their interaction on roots, stems, leaves, seeds and total dry weight production of indian mustard (*Brassica Juncea*) in Abu Rawash and Al-Gabal Al-Asfar contaminated soils.

Data reveal that chelator types significantly affected the dry weight of different plant parts. All plant parts recorded the highest values due to HA application except for roots dry weight of plants grown on Abu Rawash soil, where the highest value (46.8g) was obtained with CA treatment as shown in Fig (5).

Roots, stems, leaves, seeds and total dry weight of indian mustard plants under HA treatment in Abu Rawash soil were 44.9, 111.1, 55.3, 37.4 and 248.7 g, where the corresponding values in Al-Gabal Al-Asfar soil were 31.6, 79.4, 31.0, 17.2 and 159.2 g. Similar trend was obtained with each of CA and EDTA, where plant parts dry weights in Abu Rawash soil were higher than that of Al-Gabal Al-Asfar soil as shown in Fig (5).

EDTA reduced plant organs dry weight to a large extent compared with CA and HA in both soils, where chelator types ordering in this respect was HA > CA > EDTA.

Table (5): Effect of chelator types, rates and their interactions on the dry weight of plant organs (g plant^{-1}) of indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Abu Rawash					Al-Gabal Al-Asfar				
		roots	stems	leaves	seeds	Total	roots	stems	leaves	seeds	Total
EDTA mmol kg^{-1} soil	0	43.9	93.2	52.7	33.0	222.8	30.0	73.8	29.6	16.4	149.9
	1.5	37.2	87.6	46.2	27.9	198.9	26.9	69.6	26.3	14.0	136.7
	3.0	37.2	83.8	47.3	28.4	196.8	27.1	67.8	26.9	15.0	136.8
	4.5	31.7	80.4	45.1	24.8	182.0	22.5	63.9	22.7	13.6	122.7
Mean		**37.5**	**86.3**	**47.8**	**28.5**	**200.1**	**26.6**	**68.8**	**26.4**	**14.8**	**136.5**
F-test		**	**	ns	**	**	**	**	**	ns	**
$LSD_{0.05}$		4.2	5.7		3.2	17.2	2.5	4.8	2.7		10.2
$LSD_{0.01}$		6.6	8.9		4.9	26.8	3.9	7.5	4.2		15.9
CA mmol kg^{-1} soil	0	43.9	93.2	52.7	33.0	222.8	30.0	73.8	29.6	16.4	149.9
	3.0	48.3	100.3	50.5	34.1	233.2	29.7	74.0	30.2	15.3	149.3
	6.0	47.4	104.4	54.6	35.0	241.3	33.2	81.0	33.3	17.4	164.8
	9.0	47.7	110.7	55.1	35.2	248.7	30.3	75.5	30.0	16.6	152.4
Mean		**46.8**	**102.2**	**53.2**	**34.3**	**236.5**	**30.8**	**76.1**	**30.8**	**16.4**	**154.1**
F-test		ns	**	ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.05}$			8.0								
$LSD_{0.01}$			12.4								
HA g kg^{-1} soil	0	43.9	93.2	52.7	33.0	222.8	30.0	73.8	29.6	16.4	149.9
	0.2	39.3	105.5	54.4	33.7	232.8	29.2	77.2	29.5	17.6	153.6
	0.4	49.6	122.3	58.5	42.0	272.4	32.0	80.6	31.3	16.7	160.6
	0.6	46.9	123.4	55.5	41.0	266.8	35.1	86.0	33.7	17.9	172.6
Mean		**44.9**	**111.1**	**55.3**	**37.4**	**248.7**	**31.6**	**79.4**	**31.0**	**17.2**	**159.2**
F-test		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.05}$											
$LSD_{0.01}$											
F-test (treatments)		**	**	**	**	**	**	**	**	*	**
$LSD_{0.05}$		**4.9**	**7.7**	**4.5**	**2.6**	**20.1**	**1.7**	**2.7**	**2.1**	**1.9**	**6.5**
$LSD_{0.01}$		**7.4**	**12.7**	**6.8**	**4.6**	**30.4**	**2.6**	**3.5**	**3.3**		**9.9**
F-test (interaction)		**ns**	*	**ns**	*	*	**	**ns**	**ns**	**ns**	**ns**
$LSD_{0.05}$			**6.7**		**2.3**	**14.4**	**1.5**				
$LSD_{0.01}$							**2.4**				

Application of HA increased the mean values of root, stems, leaves, seeds and total dry weight of indian mustard plants as compared to the mean values of EDTA by 19.73, 28.74, 15.69, 31.23 and 24.29% in Abu Rawash soil and by 18.80, 15.41, 17.42, 16.22 and 16.63% in Al-Gabal Al-Asfar soils, respectively.

CA treatment increased the mean values of root, stems, leaves, seeds and total dry weight of indian mustard plant by 24.80, 18.42, 11.30, 20.35 and 18.19% in Abu Rawash soil and by 15.79, 10.61, 16.67, 10.81 and 12.89% in Al-Gabal Al-Asfar soils, respectively, as compared to EDTA means. These results are in agreement with that of **Zaheer *et al.*, (2015)** who stated that CA is a safer choice for increasing phytoextraction of Cu by *B. napus* by minimizing Cu toxicity and increasing biomass production.

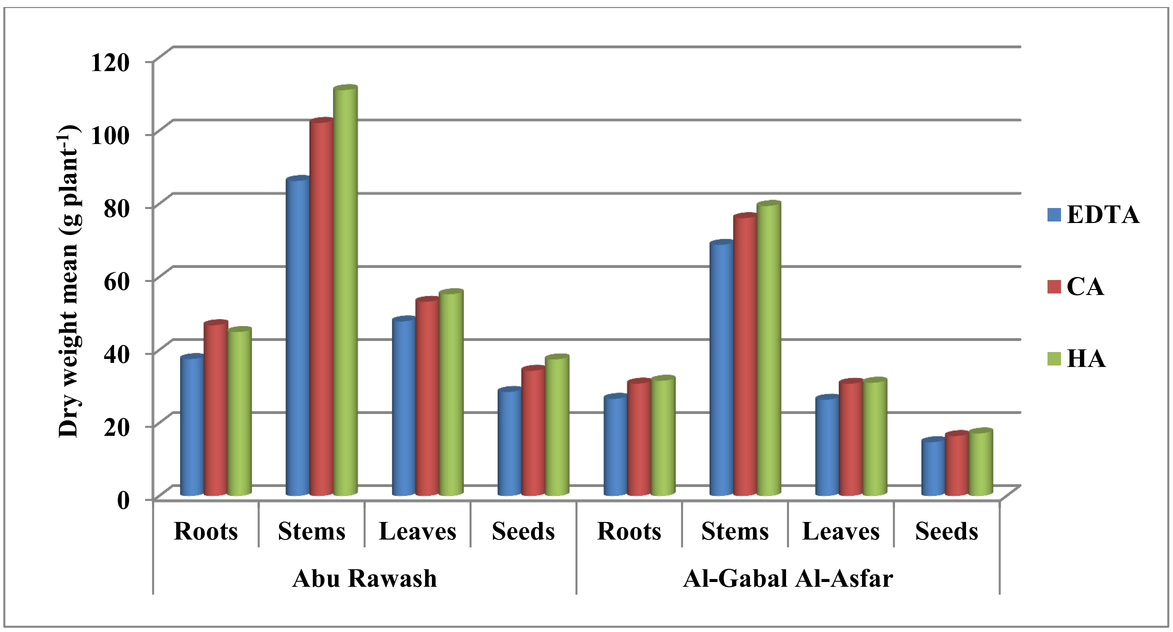


Fig (5): Effect of chelators on roots, stems, leaves and seeds dry weight means (g plant^{-1}) of indian mustard in Abu Rawash and Al-Gabal Al-Asfar soils.

Data in Table (5) show that raising EDTA level significantly reduced indian mustard dry weight yield as compared to control in Abu Rawash and Al-Gabal Al-Asfar soils. Moreover, the reduction increased with raising EDTA application rate as shown in Fig (7). Visual symptoms of toxicity were observed on indian mustard leaves similar to that happened with sunflower with EDTA levels treatments, especially at the highest level (4.5

mmol kg^{-1} soil) as shown in Fig (6). Similar results were found by **Wu *et al.,* (2004) and Taalab *et al.,* (2009)**. This could be attributed to the accumulation of metals or EDTA-metal complexes in plants, as suggested by **Chen and Cutright (2001),** causing toxicity to plants and reduction in Dry weight as shown later.

Fig (6): visual symptoms of toxicity on indian mustard leaves due to application of 4.5 mmol kg^{-1} EDTA.

The reductions in roots, stems, leaves, seeds and total dry weight of indian mustard plant as a result of applying EDTA at the rate of 4.5 mmol kg^{-1} soil were 27.79, 13.73, 14.42, 24.85 and 18.31% in Abu Rawash soil and 25.00, 13.41, 23.31, 17.07 and 18.15% in Al-Gabal Al-Asfar soil, respectively, as compared to control.

The differences between EDTA levels treatment means in both soils in each trait, were significant, except leaves means in Abu Rawash soil and seeds means in Al-Gabal Al-Asfar soil.

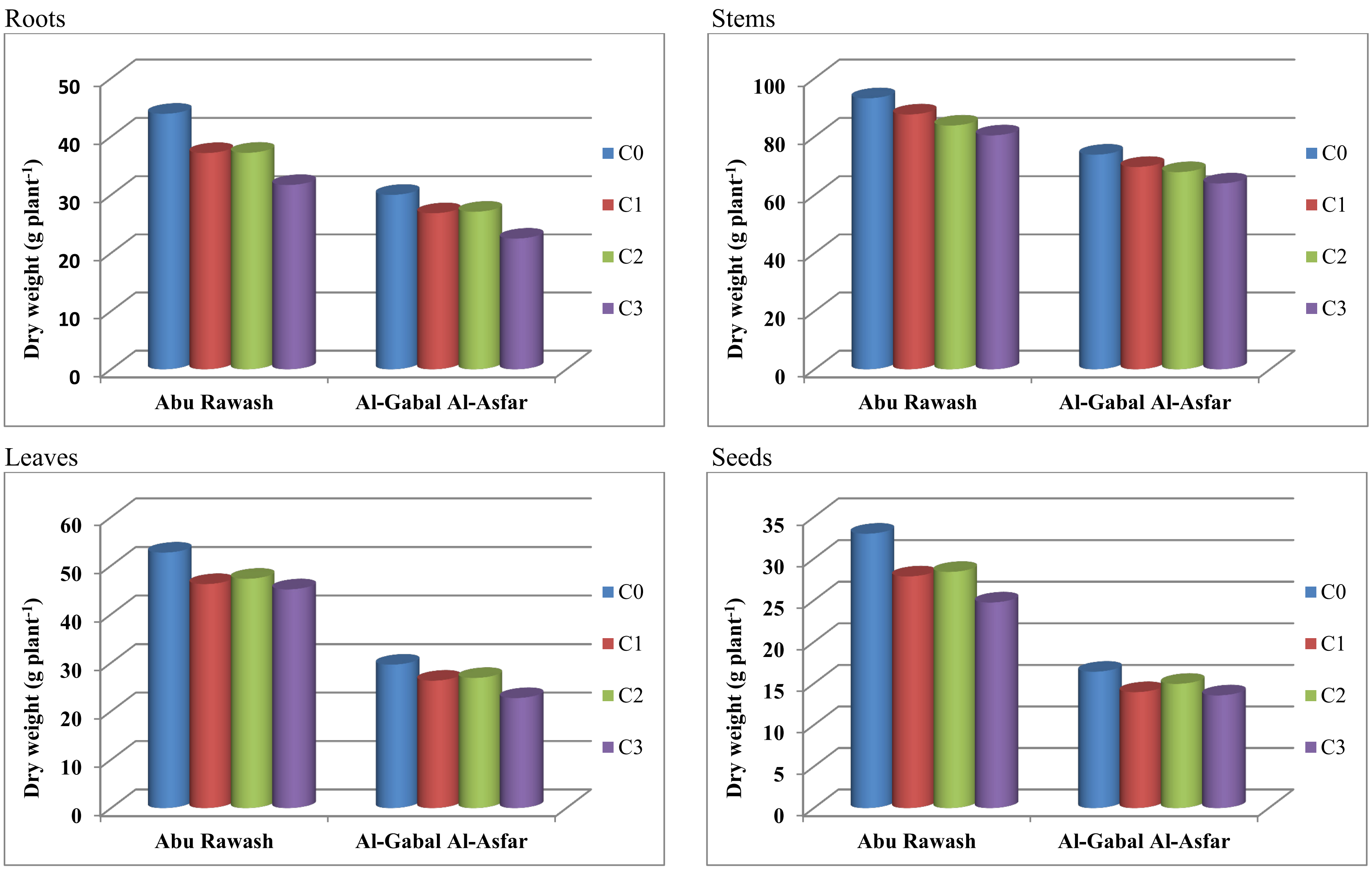


Fig (7): Effect of EDTA application levels on roots, stems, leaves and seeds dry weight (g plant^{-1}) of indian mustard in Abu Rawash and Al-Gabal Al-Asfar soils.

Regarding to CA application rates effects, increasing CA applied rate up to 9.0 mmol kg^{-1} soil and 6.0 mmol kg^{-1} soil in Abu Rawash and Al-Gabal Al-Asfar soil, respectively, increased plant organs and total dry weight but the increment values in each trait were insignificant in both soils; except for stems dry weight in Abu Rawash soil. These increases in plant growth and biomass might be due to increased nutrient uptake and CA-induced chelation of metals decreasing free metal ions in plants as suggested by **Najeeb *et al.*, (2011)**. CA application increased plant growth, biomass, and chlorophyll content by reducing oxidative stress and enhancing antioxidant enzyme activities **(Zaheer *et al.*, 2015)**.

Application of 9 mmol CA kg^{-1} soil in Abu Rawash soil achieved increases by 18.78, 4.55 and 6.67% in stems, leaves and seeds dry weight than that of control, respectively, as shown in Fig (8). Whilst, the high value of root dry weight in Abu Rawash soil were obtained with 3 mmol CA kg^{-1} soil treatment, 10.02% increase compared with control treatment as shown in Fig (8). Hence, the highest value of total dry weight was obtained using 9 mmol CA kg^{-1} soil in Abu Rawash (11.62% increase than that of control).

In Al-Gabal Al-Asfar soil, applying 6 mmol CA kg^{-1} soil achieved the highest values of roots, stems, leaves and seeds and the obtained values represent 10.67, 9.76, 12.50 and 6.10% increase compared with control as shown in Fig (9). Consequently, the highest value of total dry weight was obtained using 6 mmol CA kg^{-1} soil in A Al-Gabal Al-Asfar soil and the obtained values represents 9.94% increase than that of control.

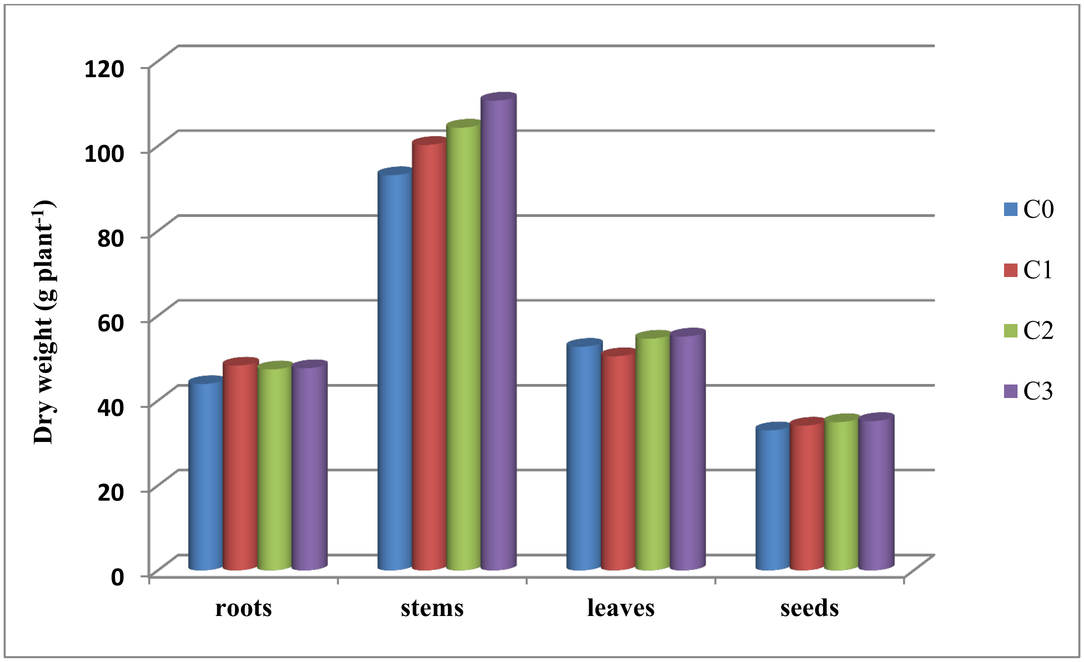


Fig (8): Effect of CA application levels on roots, stems, leaves and seeds dry weight of indian mustard (g plant^{-1}) in Abu Rawash soil.

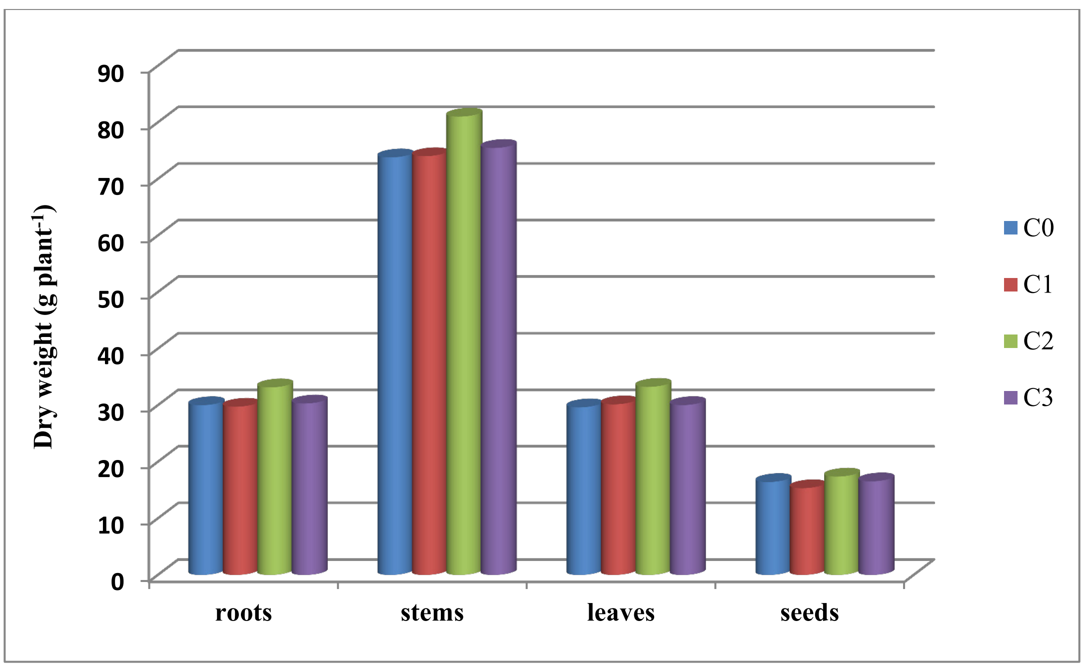


Fig (9): Effect of CA application levels on roots, stems, leaves and seeds dry weight of indian mustard (g plant^{-1}) in Al-Gabal Al-Asfar soil.

Increasing HA applied rates increased indian mustard total dry weight in both soils, but this increase was insignificant as a result of their non-significant effects on plant organs dry weight in both soils.

Application of 0.4 g HA kg^{-1} soil maximized the dry weight of roots, leaves, seeds and total dry weight of indian mustard by 12.98, 11.01, 27.27 and 22.26% in Abu Rawash soil, respectively, as compared to control. Whereas, the maximum value of stems dry weight in the same soil was obtained with 0.6 g HA kg^{-1} soil treatment, 32.40% as compared to control as shown in Fig (10).

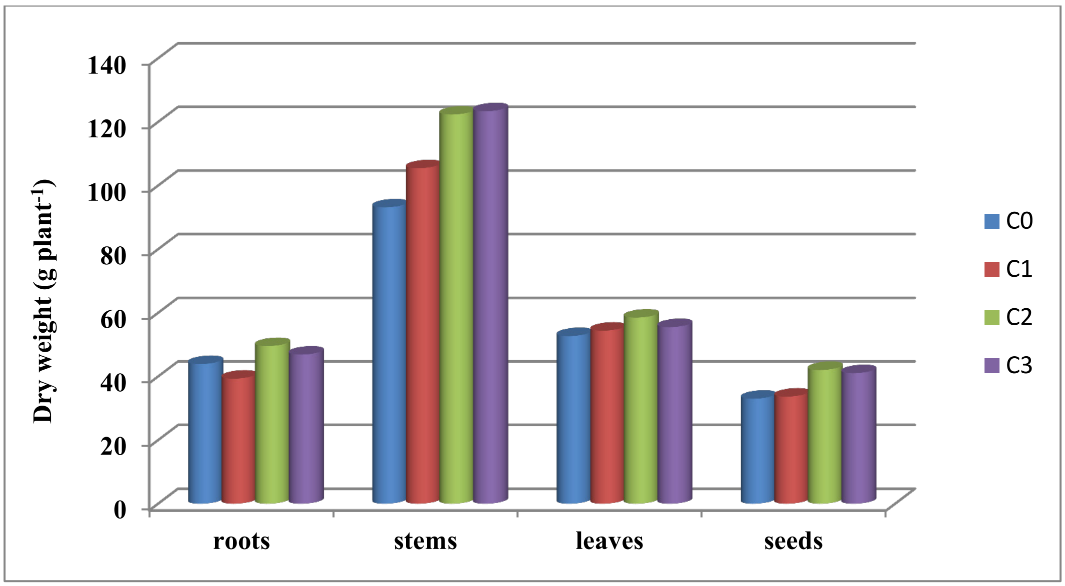


Fig (10): Effect of HA application levels on roots, stems, leaves and seeds dry weight of indian mustard (g $plant^{-1}$) in Abu Rawash soil.

In Al-Gabal Al-Asfar soil, The highest level used of HA (0.6 g HA kg^{-1}) recorded the highest values of indian mustard plant parts dry weight as compared to control as shown in Fig (11), where the increment values of roots, stems, leaves, seeds and total dry weight were 17.00, 16.53, 13.85, 9.15 and 15.14% as compared to control.

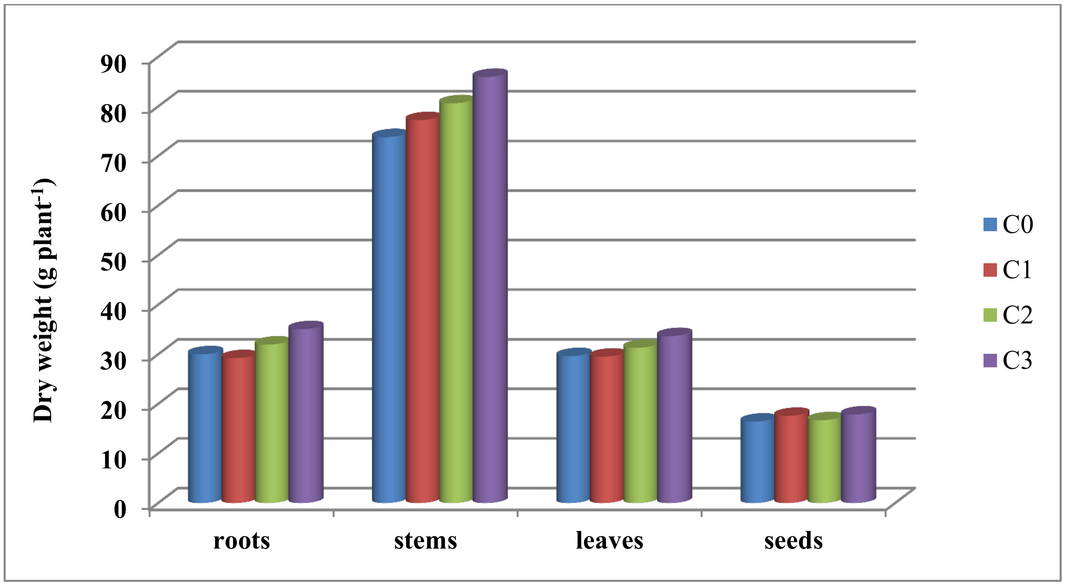


Fig (11): Effect of HA application levels on roots, stems, leaves and seeds dry weight of indian mustard (g plant^{-1}) in Al-Gabal Al-Asfar soil.

Data of Table (5) illustrate that significant effects were found due to the interaction between chelator types and addition rates on stems, seeds and total dry weight of indian mustard in Abu Rawash soil. Where, the highest values of stems dry weight were obtained using HA at 0.4 g kg^{-1} soil, while the highest value of seeds and total dry weight were obtained using HA at 0.6 g kg^{-1} soil.

The roots dry weight was significantly affected by the interaction between chelator types and addition rates, where applying 0.6 g HA kg^{-1} soil had the highest mean value of 35.1 g in Al-Gabal Al-Asfar soil.

4.2. Effect of chelator types, rates and their interactions on Cu concentration (mg kg^{-1} DW) of plant organs grown on the two soils under study:

4.2.1. Sunflower plant:

Data of Table (6) represent the effect of chelator types, rates and their interaction on Cu concentrations of roots, stems, leaves and seeds of sunflower plant grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Concerning the effect of chelator types used, it was noticed that application of different chelators significantly affect Cu concentrations in sunflower plant parts in both soils.

Table (6): Effect of chelator types, rates and their interaction on Cu concentration (mg kg^{-1} DW) of roots, stems, leaves and seeds of sunflower plant in Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Cu concentration (mg kg^{-1} DW)							
		Abu Rawash				Al-Gabal Al-Asfar			
		roots	stems	leaves	seeds	roots	stems	leaves	seeds
EDTA mmol kg^{-1} soil	0	265.9	52.6	45.4	54.2	284.1	52.6	40.5	49.6
	1.5	494.6	79.8	69.5	71.7	502.1	68.1	79.0	68.6
	3.0	752.0	109.5	112.1	81.3	662.8	103.8	120.5	65.0
	4.5	1069.1	130.1	141.5	74.4	1198.2	139.0	158.2	76.9
Mean		**645.4**	**93.0**	**92.1**	**70.4**	**661.8**	**90.9**	**99.6**	**65.0**
F-test		**	**	**	**	**	**	**	**
$LSD_{0.05}$		187.6	20.3	27.5	11.9	101.0	14.7	21.9	10.6
$LSD_{0.01}$		292.2	31.6	42.8	18.5	157.3	23.0	34.1	16.5
CA mmol kg^{-1} soil	0	265.9	52.6	45.4	54.2	284.1	52.6	40.5	49.6
	3.0	355.0	43.7	43.4	60.3	360.8	65.0	42.8	55.5
	6.0	371.7	54.1	50.6	64.3	434.5	67.9	58.8	62.1
	9.0	481.9	72.1	57.5	63.0	344.9	80.3	66.5	67.2
Mean		**368.6**	**55.6**	**49.2**	**60.5**	**356.1**	**66.5**	**52.2**	**58.6**
F-test		**	**	**	ns	ns	**	**	**
$LSD_{0.05}$		47.9	4.9	3.1			6.8	5.4	8.7
$LSD_{0.01}$		74.6	7.6	4.8			10.6	8.3	13.5
HA g kg^{-1} soil	0	265.9	52.6	45.4	54.2	284.1	52.6	40.5	49.6
	0.2	332.2	54.2	52.4	52.7	381.3	62.9	47.9	56.7
	0.4	290.0	57.3	53.7	53.2	298.2	53.7	44.3	55.7
	0.6	358.7	62.9	55.5	57.3	354.0	65.3	49.0	66.2
Mean		**311.7**	**56.8**	**51.8**	**54.4**	**329.4**	**58.6**	**45.4**	**57.1**
F-test		**	**	**	ns	*	*	**	*
$LSD_{0.05}$		34.9	2.8	5.3		69.7	9.5	4.7	14.3
$LSD_{0.01}$		54.3	4.3	8.3				7.4	
F-test (treatments)		**	**	**	**	**	**	**	*
$LSD_{0.05}$		**94.0**	**11.2**	**14.3**	**5.1**	**75.4**	**8.4**	**10.5**	**7.5**
$LSD_{0.01}$		**142.4**	**16.9**	**21.7**	**7.8**	**114.2**	**12.7**	**15.9**	
F-test (Interaction)		**	**	**	**ns**	**	**	**	**ns**
$LSD_{0.05}$		**81.4**	**10.0**	**12.4**		**65.3**	**7.3**	**9.1**	
$LSD_{0.01}$		**123.3**	**14.7**	**18.8**		**98.9**	**11.0**	**13.7**	

EDTA increased Cu concentrations of roots, stems, leaves and seeds as compared to HA treatment by 107.06, 63.73, 77.80 and 29.41% in Abu Rawash soil and by 100.91, 55.12, 119.38 and 13.84% in Al-Gabal Al-Asfar soil, respectively, whereas CA increased Cu concentrations of roots and seeds by 18.25 and 11.21% in Abu Rawash soil as

compared to HA treatment, respectively. No significant differences were found between Cu concentrations means in stems, leaves and seeds as affected by CA and HA treatment.

Cu concentrations in roots, stems, leaves and seeds of sunflower plant was increased by 8.11, 13.48, 14.98 and 2.63%, respectively, of CA treatment compared to HA treatment in Al-Gabal Al-Asfar soil.

Data in Table (6) reveal also that Cu concentrations in different plant parts of sunflower under CA and HA treatments in both Abu Rawash and Al-Gabal Al-Asfar soils were in descending order: roots > stems > seeds > leaves. Under EDTA treatment the descending order where deviated, where Cu concentrations of leaves was higher than that of seeds.

Regarding to chelator rates effect on Cu concentration of sunflower plant parts grown on Abu Rawash and Al-Gabal Al-Aafar soils, data of Table (6) outlined that highly significant effects were found due to raising EDTA level from 0.0 to 4.5 mmol kg^{-1} in both soils. Raising EDTA level from 0.0 to 4.5 increased Cu concentrations of roots from 265.9 to 1069.1, stems from 52.6 to 130.1, leaves from 45.4 to 141.5 and seeds from 54.2 to 74.4 mg kg^{-1} DW in Abu Rawash soil. Similar increases for the same order of plant parts in Al-Gabal Al-Asfar soil were, 284.1 to 1198.2; 52.6 to 139.0; 40.5 to 158.2 and from 49.6 to 76.9 mg kg^{-1} DW, respectively. These increases amounted by 4.02, 2.47, 3.12 and 1.37 folds in Abu Rawash soil and by 4.22, 2.64, 3.91and 1.55 fold in Al-Gabal Al-Asfar soil, respectively as shown in Fig (12).

The high concentrations of Cu in sunflower plants due to application of EDTA levels were accompanied with reduction in dry matter in both soils, where, the dry weight of sunflower plant was reduced with increasing EDTA level up to 4.5 mmol kg^{-1} soil.

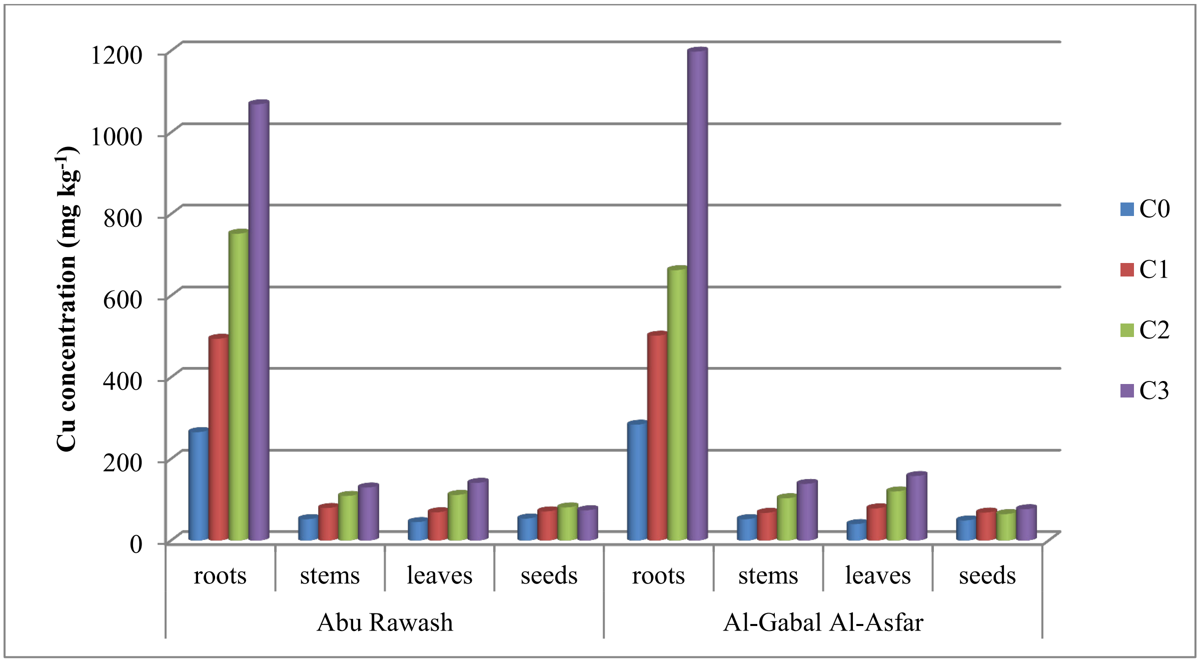


Fig (12): Effect of EDTA application levels on Cu concentrations (mg kg^{-1} DW) in roots, stems, leaves and seeds of sunflower plant in Abu Rawash and Al-Gabal Al-Asfar soils.

The obtained Data also show that increasing CA levels significantly increased Cu concentrations of roots, stems and leaves of sunflower plant, where no significant effect was noticed in Cu concentration of seeds in Abu Rawash soil. Cu concentrations (mg kg^{-1} DW) of roots, stems and leaves were increased with increasing CA addition rates up to 9 mmol kg^{-1} in Abu Rawash soils by 1.81, 1.37 and 1.27 folds as compared to control, respectively, while, CA at a rate of 6 mmol kg^{-1} soil induced the highest value of Cu concentrations in seeds as shown in Fig (13).

In Al-Gabal Al-Asfar soil, applying 9 mmol CA kg^{-1} soil caused an increase of Cu concentration of stems, leaves and seeds of sunflower plant appreciable by 1.53, 1.64 and 1.35 folds over control (0.0 CA treatment), respectively. Highest Cu concentration in roots was achieved by using 9 mmol CA kg^{-1} soil as shown in Fig (13). But the difference between CA levels treatment means in that trait is not significant.

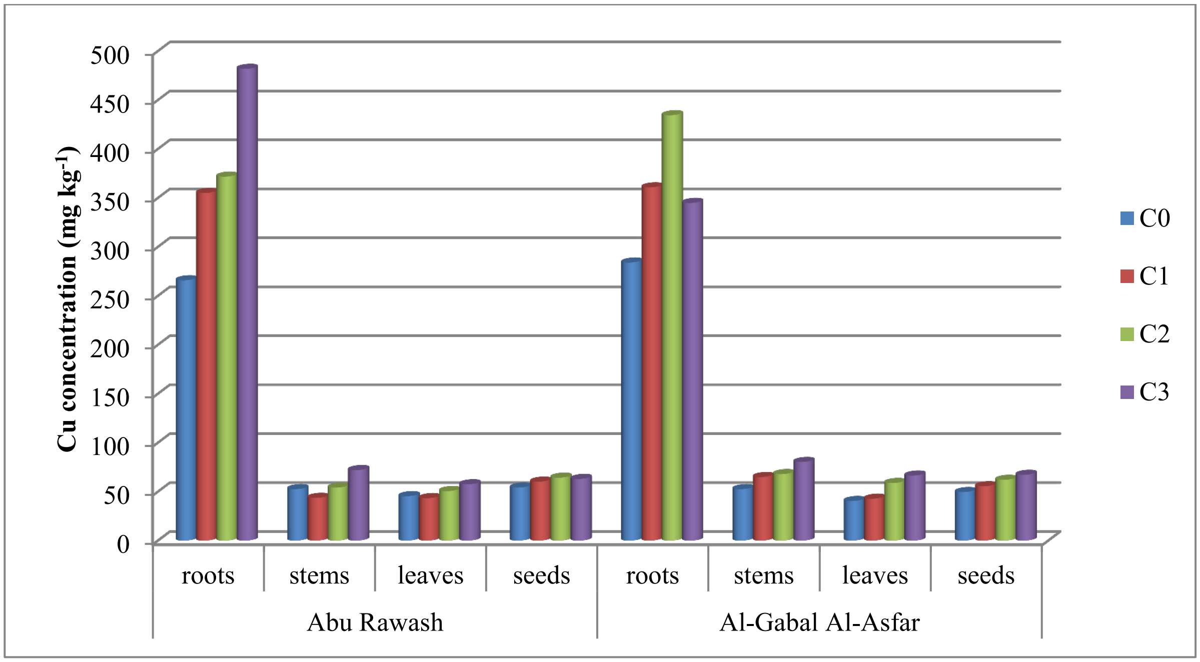


Fig (13): Effect of CA application levels on Cu concentrations (mg kg^{-1}DW) in roots, stems, leaves and seeds of sunflower in Abu Rawash and Al-Gabal Al-Asfar soils.

Regarding to the effect of HA on Cu concentrations in sunflower plant parts, data of Table (6) and Fig (14) point out that HA levels significantly affected roots, stems and leaves Cu concentration in Abu Rawash soil. Where, Cu concentrations (mg kg^{-1} DW) of roots, stems and leaves were increased with increasing HA addition rates up to 0.6 g HA kg^{-1} soil by 34.90, 19.58 and 22.25%, respectively. The effect of HA levels on increasing Cu concentrations in seeds were insignificant in Abu Rawash soil.

In Al-Gabal Al-Asfar soil, Cu concentrations in roots, stems, leaves and seeds were significantly affected by HA levels. Where, Cu concentrations (mg kg^{-1} DW) of roots, stems leaves and seeds were increased with increasing HA addition rates up to 0.6 g kg^{-1} soil by 24.60, 24.14, 20.99 and 33.47%, respectively.

Regarding to chelator type - levels interaction, a significant effect on roots, stems and leaves Cu (mg kg^{-1}) concentrations were found in both soils, while no significant effect on Cu concentrations of seeds was found. However, application of 4.5 mmol EDTA kg^{-1} soil gave the highest concentrations of Cu in sunflower organs in both Abu Rawash and Al-Gabal Al-Asfar soils.

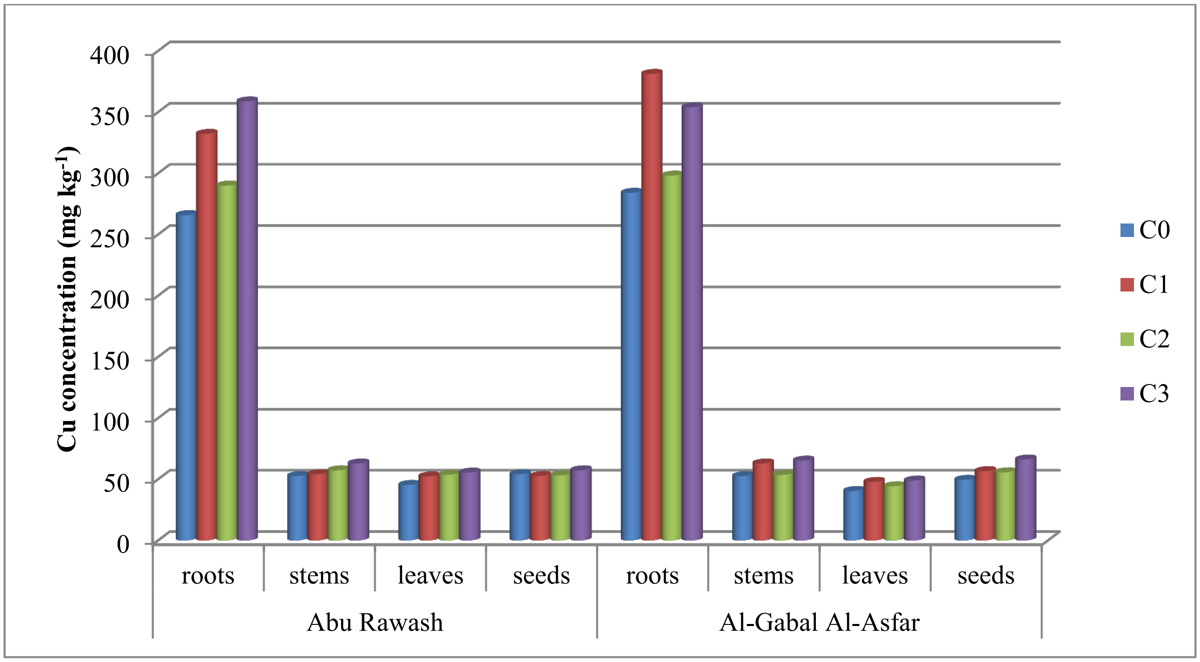


Fig (14): Effect of HA application levels on Cu concentrations (mg kg^{-1} DW) of roots, stems, leaves and seeds of sunflower in Abu Rawash and Al-Gabal Al-Asfar soils.

4.2.2. Indian mustard plant:

Data in Table (7) represent the effect of chelator type, rates and types-rates interactions on Cu concentrations of roots, stems, leaves and seeds of indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Regarding to chelator types effect, significant effects due to application of EDTA, CA and HA on Cu concentrations in indian mustard plant organs in both Abu Rawash and Al-Gabal Al-Asfar soil were found as shown in Fig (15). EDTA gave the highest Cu concentration of indian mustard plant parts in both soils. Whereas, HA application recorded the lowest Cu concentration of the plant parts in both soils as shown in Fig (15).

The concentrations of Cu in each plant part, roots, stems, leaves and seeds of indian mustard plant was increased due to EDTA application compared to HA by 50.52, 101.13, 105.12 and 30.30% in Abu Rawash soil and by 46.78, 100.00, 110.20 and 37.40% in Al-Gabal Al-Asfar soil, respectively as shown in Fig (15). It could be attributed to the addition of EDTA to soil transfers metal sorption and precipitation equilibrium toward enhanced heavy metals dissolution due to forming metal-EDTA complexes **(Hadi *et al.*, 2010)**. CA

treatment increased Cu concentrations of roots, stems, leaves and seeds compared to HA by 24.13, 29.81, 21.26, and 12.63% in Abu Rawash soil and by 25.71, 49.02, 48.98 and 3.25% in Al-Gabal Al-Asfar soil, respectively.

Table (7): Effect of chelator types, rates and their interactions on Cu concentration (mg kg^{-1} DW) of roots, stems, leaves and seeds of indian mustard plant in Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Cu concentration (mg kg^{-1} DW)							
		Abu Rawash				Al-Gabal Al-Asfar			
		roots	stems	leaves	seeds	roots	stems	leaves	seeds
EDTA mmol kg^{-1} soil	0	186.7	30.8	26.7	21.7	163.3	20.8	20.0	13.3
	1.5	228.3	46.7	40.8	25.8	202.5	31.7	35.8	15.8
	3.0	292.5	60.8	64.2	27.5	251.7	50.8	48.3	17.5
	4.5	387.5	75.0	76.7	28.3	312.5	60.0	60.8	20.8
Mean		**273.8**	**53.3**	**52.1**	**25.8**	**232.5**	**40.8**	**41.2**	**16.9**
F-test		**	**	**	ns	**	**	**	**
$LSD_{0.05}$		16.0	4.2	5.8		12.8	3.3	4.2	2.7
$LSD_{0.01}$		24.9	6.6	9.0		20.0	5.1	6.6	4.2
CA mmol kg^{-1} soil	0	186.7	30.8	26.7	21.7	163.3	20.8	20.0	13.3
	3.0	218.3	34.2	28.3	20.8	174.2	27.5	26.7	11.7
	6.0	230.8	34.2	30.0	22.5	202.5	33.3	31.7	11.7
	9.0	267.5	38.3	38.3	24.2	255.8	40.0	38.3	14.2
Mean		**225.8**	**34.4**	**30.8**	**22.3**	**199.0**	**30.4**	**29.2**	**12.7**
F-test		**	ns	**	ns	**	**	**	ns
$LSD_{0.05}$		12.3		4.2		11.7	4.5	4.7	
$LSD_{0.01}$		19.2		6.6		18.2	7.0	7.2	
HA g kg^{-1} soil	0	186.7	30.8	26.7	21.7	163.3	20.8	20.0	13.3
	0.2	198.3	27.5	24.2	22.5	169.2	20.8	20.8	11.7
	0.4	166.7	23.3	25.0	17.5	156.7	20.8	18.3	11.7
	0.6	175.8	24.2	25.8	17.5	144.2	19.2	19.2	12.5
Mean		**181.9**	**26.5**	**25.4**	**19.8**	**158.4**	**20.4**	**19.6**	**12.3**
F-test		**	**	ns	**	*	ns	ns	ns
$LSD_{0.05}$		14.4	3.8		3.5	19.2			
$LSD_{0.01}$		22.4	5.9		5.4				
F-test (treatments)		**	**	**	*	**	**	**	**
$LSD_{0.05}$		**7.8**	**3.1**	**4.9**	**4.1**	**9.8**	**3.3**	**4.9**	**2.1**
$LSD_{0.01}$		**11.9**	**4.7**	**7.4**		**14.9**	**5.0**	**7.5**	**3.2**
F-test (Interaction)		*	**	**	**ns**	**	**	**	**ns**
$LSD_{0.05}$		**6.8**	**2.7**	**4.3**		**8.5**	**2.8**	**4.3**	
$LSD_{0.01}$			**4.0**	**6.4**		**12.9**	**4.3**	**6.5**	

In both studied soils, the highest Cu concentrations were found in roots followed by stems, leaves and seeds. In Abu Rawash soil, stems, leaves and seeds Cu concentration of indian mustard plant represent 19.47, 19.03 and 9.42% of roots Cu concentration under EDTA treatments, corresponding values of Al-Gabal Al-Asfar soil were 17.55, 17.72 and 7.27%, respectively.

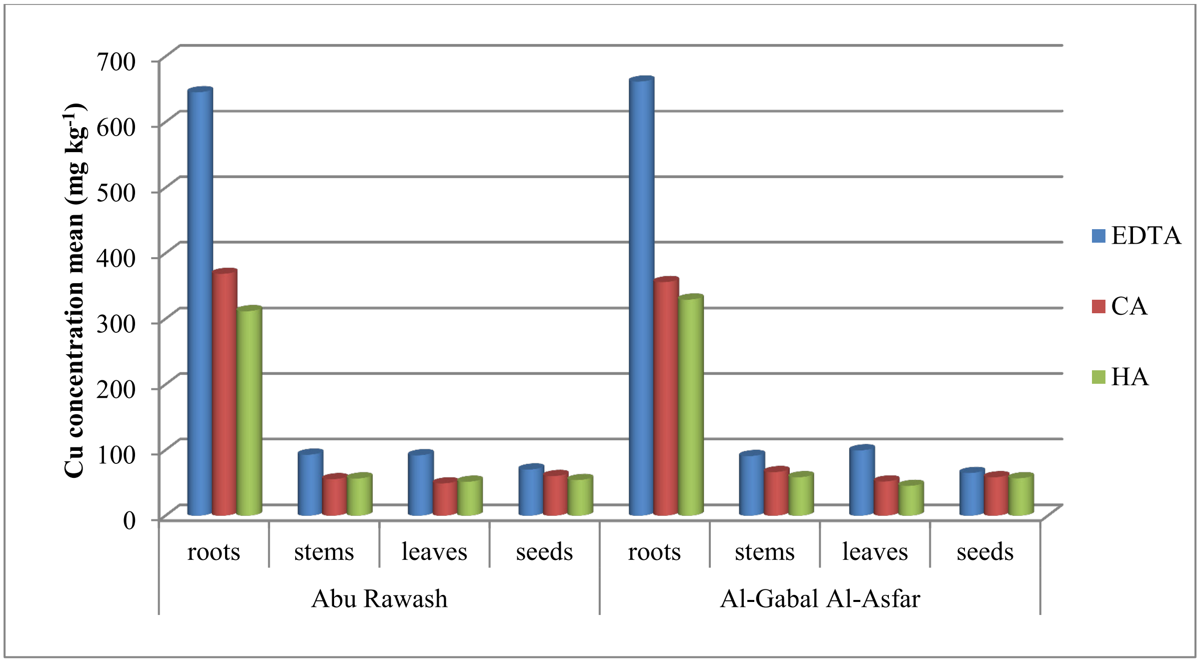


Fig (15): Effect of chelators on Cu concentration means (mg kg^{-1} DW) in roots, stems, leaves and seeds of indian mustard in Abu Rawash and Al-Gabal Al-Asfar soils.

Stems, leaves and seeds Cu concentration of indian mustard grown on Abu Rawash soil represent 15.23, 13.64 and 9.88% of roots Cu concentration under CA treatments, corresponding values of Al-Gabal Al-Asfar soil were 15.28, 14.67 and 6.38 %, respectively.

Cu concentration of indian mustard plant stems, leaves and seeds represent 14.57, 13.96 and 10.89% of roots Cu concentration under HA treatments In Abu Rawash soil, corresponding values of Al-Gabal Al-Asfar soil were 12.88, 12.37 and 7.77 %, respectively.

The concentrations of Cu in indian mustard organs in Abu Rawash soil was higher than that of Al-Gabal Al-Asfar soil due to the higher concentration of Cu in Abu Rawash soil (170 mg kg^{-1} soil) and Al-Gabal Al-Asfar soil (107 mg kg^{-1} soil).

Regarding to chelator rates effect, increasing of EDTA levels high significantly increased Cu concentration of roots, stems, leaves and seeds of indian mustard plant in both

studied soils, with the exception of Cu concentrations in seeds of plant grown on Abu Rawash soil where no significant increase was found.

Cu concentrations (mg kg^{-1} DW) in indian mustard roots, stems, leaves and seeds were increased by 2.08, 2.44, 2.87 and 1.30 folds in Abu Rawash soil and by 1.91, 2.88, 3.04 and 1.56 folds in Al-Gabal Al-Asfar soil, respectively, when EDTA level was increased from 0.0 to 4.5 mmol kg^{-1} soil as shown in Fig (16). Similar trend was found by **Wu *et al.*, (2004)**, where 3 mmol EDTA kg^{-1} soil elevated Cu concentration from 15.3 to 39.8 mg kg^{-1} DW in indian mustard plant grown on a paddy soil polluted with Cu. The neutral charge, metal-EDTA complex are not blocked or attached by carboxyl groups or polysaccharides of rhizoderm cell surface. In this way, EDTA causes the metal to enter directly to the plant roots **(Shahid *et al.*, 2012)**.

The high concentrations of Cu in indian mustard plants due to application of EDTA levels were accompanied with reduction in dry matter in both soils, where, the dry weight of indian mustard plant was reduced with increasing EDTA level up to 4.5 mmol kg^{-1} soil.

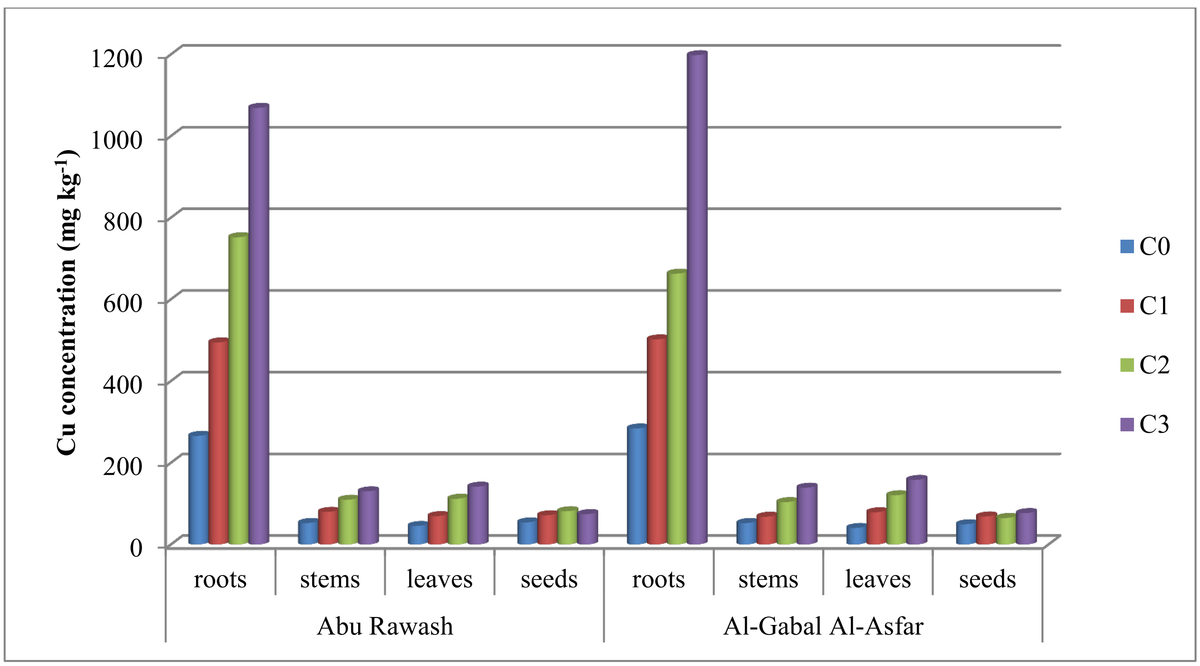


Fig (16): Effect of EDTA application levels on Cu concentrations (mg kg^{-1} DW) in roots, stems, leaves and seeds of indian mustard in Abu Rawash and Al-Gabal Al-Asfar soils.

In case of CA, similar trend to EDTA were found, where CA application rates significantly increased Cu concentrations in plant roots and leaves in Abu Rawash soils. In Al-Gabal Al-Asfar soil, Cu concentrations in indian mustard roots, stems and leaves were significantly increased with increasing CA application levels up to 9 mmol kg^{-1} in both soils. The values of Cu concentration of plant roots, stems, leaves and seeds increased as compared with control by 1.43, 1.24, 1.43 and 1.12 folds in Abu Rawash soil and by 1.57, 1.92, 1.92 and 1.07 folds in Al-Gabal Al-Asfar soil, respectively, when CA was applied at a level of 9 mmol kg^{-1} soil as shown in Fig (17).

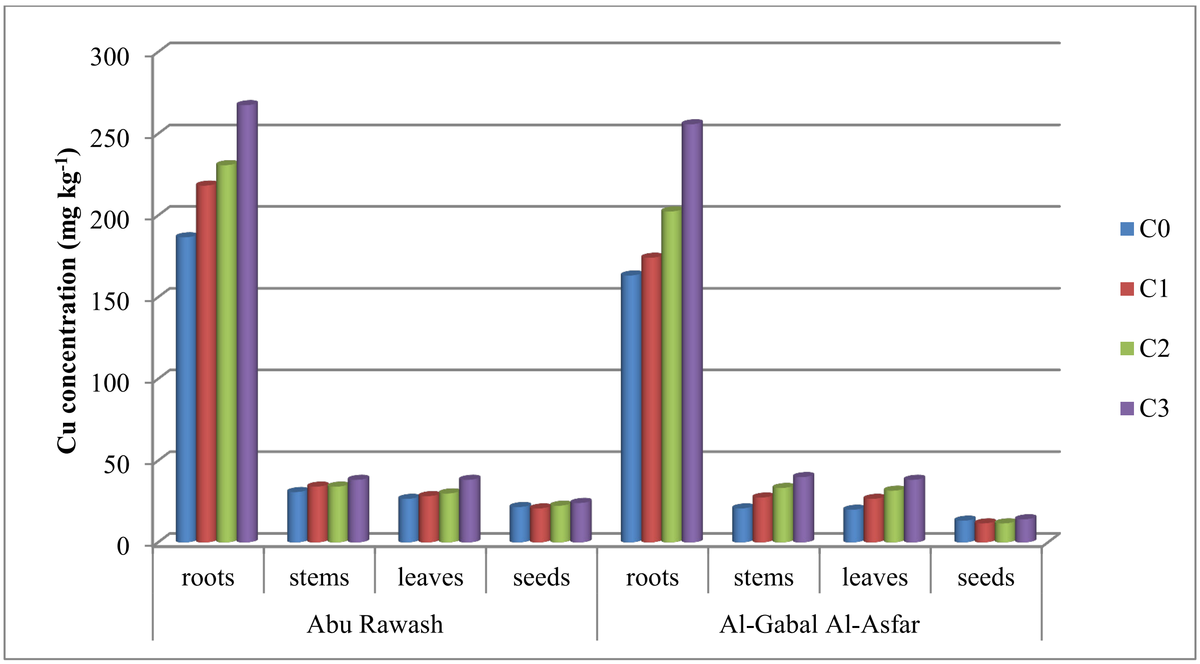


Fig (17): Effect of CA application levels on Cu concentrations (mg kg^{-1} DW) in roots, stems, leaves and seeds of indian mustard in Abu Rawash and Al-Gabal Al-Asfar soils.

In respect of the effect of HA on Cu concentrations in indian mustard plant in the tested soils, the obtained results point out that different applied rates of HA significantly decreased concentrations of Cu in plant parts in Abu Rawash soil, except for leaves was insignificant. The application of 0.4 g kg^{-1} soil recorded the highest reduction of Cu concentrations (mg kg^{-1} DW) in indian mustard roots, stems, leaves and seeds in Abu Rawash soil, and the corresponding reductions calculated as percentages of the corresponding control ones were 10.71, 24.35, 6.37 and 19.35%, respectively. On the other hand, applying different rates of HA insignificantly decreased concentrations of Cu in

indian mustard plant parts in Al-Gabal Al-Asfar soil, except for roots was significant. The highest reduction of Cu concentrations in indian mustard roots and stems (mg kg^{-1} DW) in Al-Gabal Al-Asfar soil were obtained using 0.6 g HA kg^{-1} soil and the corresponding reductions were 11.70 and 7.69% as compared to control, respectively. While application of 0.4 g kg^{-1} achieved the highest reduction of Cu concentration in indian mustard leaves and seeds (mg kg^{-1}) in Al-Gabal Al-Asfar soil and these reductions were amounted by 8.50 and 12.03% as compared to control, respectively. These results are in parallel trend with that of **Wang *et al.*, (2010)**. They found that increasing HA addition from 3.09 to 7.89 g kg^{-1} soil to Cu and Cd contaminated sediment reduced Cd concentrations in *Vallisneria spiralis* roots and stems by 26.4–50.3% and 14.3–33.0%, whereas, Cu accumulation was decreased much more with 44.0–77.0% and 35.0-62.7%, respectively. Humic acid clearly reduced Cu and Cd bioavailability and toxicity in *V. speralis* due to forming complexes of HA with metal ions complexes.

Regarding to the interaction between chelator types and their addition rates, significant effects on roots, stems, leaves and seeds Cu concentration (mg kg^{-1} DW) of indian mustard were found in both soils. No significant effect on Cu concentrations in seeds was found. However, application of 4.5 mmol EDTA kg^{-1} soil achieved the highest concentrations of Cu in plant organs in both Abu Rawash and Al-Gabal Al-Asfar soils.

4.3. Effect of chelator types, rates and their interaction on roots, stems, leaves, seeds and total plant uptake of Cu (mg $plant^{-1}$) by plants grown on the two soils under study:

4.3.1. Sunflower plant:

Data of Table (8) represent the effect of chelator types, rates and their interaction on the uptake of Cu (mg $plant^{-1}$) by roots, stems, leaves, seeds as well as the total uptake of Cu (mg $plant^{-1}$) by sunflower plant in Abu Rawash and Al-Gabal Al-Asfar soils.

Chelator types significantly affected Cu uptake by each plant part consequently a significant effect was appeared on total uptake of Cu by sunflower in both Abu Rawash and Al-Gabal Al-Asfar soils.

Application of EDTA recorded the highest values of Cu uptake by roots, stems and leaves; hence the whole plant uptake of Cu was the highest. Compared with HA treatment, EDTA increased roots, stems, leaves and the whole plant Cu uptake (mg plant^{-1}) by 73.25, 38.61, 51.42 and 51.39% in Abu Rawash soil and by 67.66, 37.88, 91.79 and 52.37% in Al-Gabal Al-Asfar soil, respectively. Similarly, using CA increased roots, stems, leaves and total uptake (mg plant^{-1}) as compared with HA treatment by 43.00, 9.11, 5.66 and 25.97% in Abu Rawash soil and by 11.39, 9.22, 7.46 and 10.22% in Al-Gabal Al-Asfar soil, respectively.

Table (8): Effect of chelators, their addition rates and their interaction on the uptake of Cu by roots, stems, leaves, seeds and total plant (mg plant^{-1}) by sunflower grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Cu uptake (mg plant^{-1})									
		Abu Rawash					Al-Gabal Al-Asfar				
		roots	stems	leaves	seeds	total uptake	roots	stems	leaves	seeds	total uptake
EDTA mmol kg^{-1} soil	0	7.68	3.96	1.95	2.09	15.69	5.19	2.56	1.13	1.49	10.38
	1.5	11.75	5.54	2.83	2.46	22.59	8.87	3.19	2.23	1.78	16.07
	3.0	16.22	6.68	3.99	2.53	29.42	11.78	5.10	3.29	1.44	21.62
	4.5	19.77	6.95	4.05	1.58	32.34	14.79	5.30	3.62	1.54	25.24
Mean		**13.86**	**5.78**	**3.21**	**2.17**	**25.01**	**10.16**	**4.04**	**2.57**	**1.57**	**18.33**
F-test		**	**	**	**	**	**	**	**	*	**
LSD$_{0.05}$		1.25	0.31	0.19	0.37	0.82	0.62	0.35	0.46	0.19	1.33
LSD$_{0.01}$		1.94	0.49	0.30	0.57	1.28	0.96	0.54	0.72		2.07
CA mmol kg^{-1} soil	0	7.68	3.96	1.95	2.09	15.69	5.19	2.56	1.13	1.49	10.38
	3.0	12.14	4.42	2.31	2.97	21.83	6.26	3.09	1.13	1.91	12.39
	6.0	12.66	4.88	2.36	2.84	22.74	7.37	3.38	1.66	2.04	14.44
	9.0	13.26	4.93	2.33	2.47	22.98	8.16	3.75	1.83	2.06	15.81
Mean		**11.44**	**4.55**	**2.24**	**2.59**	**20.81**	**6.75**	**3.20**	**1.44**	**1.88**	**13.26**
F-test		**	**	**	**	**	**	**	**	**	**
LSD$_{0.05}$		0.82	0.25	0.12	0.35	0.96	0.66	0.22	0.13	0.16	0.85
LSD$_{0.01}$		1.28	0.39	0.19	0.55	1.50	1.03	0.34	0.20	0.26	1.32
HA g kg^{-1} soil	0	7.68	3.96	1.95	2.09	15.69	5.19	2.56	1.13	1.49	10.38
	0.2	7.69	3.99	2.10	2.18	15.95	6.07	2.97	1.26	1.79	12.10
	0.4	8.06	4.25	2.16	2.16	16.63	5.94	2.85	1.37	1.56	11.73
	0.6	8.57	4.48	2.28	2.46	17.80	7.03	3.32	1.60	1.98	13.93
Mean		**8.00**	**4.17**	**2.12**	**2.22**	**16.52**	**6.06**	**2.93**	**1.34**	**1.71**	**12.03**
F-test		ns	**	**	ns	**	**	*	**	*	**
LSD$_{0.05}$			0.21	0.09		0.84	0.81	0.41	0.08	0.29	1.34
LSD$_{0.01}$			0.33	0.15		1.31	1.27		0.12		2.09
F-test (treatments)		**	**	**	*	**	**	**	**	**	**
LSD$_{0.05}$		**0.88**	**0.25**	**0.13**	**0.41**	**0.86**	**0.53**	**0.29**	**0.34**	**0.11**	**0.99**
LSD$_{0.01}$		**1.33**	**0.37**	**0.20**		**1.30**	**0.81**	**0.44**	**0.51**	**0.17**	**1.51**
F-test (Interaction)		**	**	**	**	**	**	**	**	**ns**	**
LSD$_{0.05}$		**0.76**	**0.21**	**0.11**	**0.36**	**0.74**	**0.46**	**0.25**	**0.29**		**0.86**
LSD$_{0.01}$		**1.15**	**0.32**	**0.17**	**0.54**	**1.12**	**0.70**	**0.38**	**0.44**		**1.31**

In respect to Cu uptake by seeds, application of CA and HA treatments increased its Cu uptake compared with EDTA by 19.35% and 2.30% in Abu Rawash soil and by 19.75% and 8.92% in Al-Gabal Al-Asfar soil, respectively.

Regarding to chelator levels effect, each of EDTA levels and CA levels significantly affect the uptake of Cu by roots, stems, leaves and seeds of sunflower plant. Total Cu uptake (mg plant^{-1}) significantly increased as well in both Abu Rawash and Al-Gabal Al-Asfar soils. HA levels did not induce significant effect on roots and seed Cu uptake of sunflower plant in Abu Rawash soil.

In both soils, Cu uptake by roots, stems, leaves and whole plant were significantly increased with increasing EDTA addition rates up to 4.5 mmol kg^{-1} soil. The highest value of Cu uptake by seeds (2.53 mg plant^{-1}) was obtained with 3.0 mmol EDTA kg^{-1} soil in Abu Rawash soil, whenever in Al-Gabal Al-Asfar soil the highest value (1.78 mg plant^{-1}) of Cu uptake by seeds was obtained with 1.5 mmol EDTA kg^{-1} soil.

The highest values of roots, stems, leaves and total Cu uptake (mg plant^{-1}) as compared to control were 2.57, 1.76, 2.08 and 2.06 folds in Abu Rawash soil and 2.85, 2.07, 3.20 and 2.43 folds in Al-Gabal Al-Asfar soil, respectively, when EDTA was applied at a level of 4.5 mmol kg^{-1} soil. Whilst, the highest values of Cu uptake by seeds (mg plant^{-1}) as compared to control were 1.21 folds of control in Abu Rawash soil due to using 3 mmol EDTA kg^{-1} soil. In Al-Gabal Al-Asfar soil, the highest values of Cu uptake by seeds (mg plant^{-1}) as compared to control were 1.9 folds compared to control due to using 1.5 mmol EDTA kg^{-1} soil.

In Abu Rawash soil, increasing CA level up to 9.0 mmol kg^{-1} soil significantly increased Cu uptake by roots, stems and the whole plant (mg plant^{-1}) and the corresponding increases were 1.73, 1.24 and 1.46 folds as compared to control, respectively. While leaves and seeds Cu uptake were increased with increasing CA level up to 6.0 mmol kg^{-1} soil and slightly decreased with increasing CA level from 6.0 mmol kg^{-1} soil to 9.0 mmol kg^{-1} soil. In Al-Gabal Al-Asfar soil, Cu uptake by roots, stems, leaves, seeds and whole plant were significantly increased with level increasing CA up to the highest level used (9 mmol kg^{-1}

soil) by 1.57, 1.46, 1.62, 1.38 and 1.52 folds compared to control. These results are in a contradictory trend with that obtained by **Wu *et al.*, (2004)**. They stated that addition of CA to the soil at a rate of 3.0 mmol kg^{-1} soil had substantially no effect on Cu uptake by indian mustard plants.

No constant trend was found regarding to HA effect on Cu uptake by sunflower plant parts in the both soils. The data reveal that application of HA significantly increased Cu uptake (mg $plant^{-1}$) of stems and leaves and did not affect Cu uptake by roots and seeds in Abu Rawash soil. In Al-Gabal Al-Asfar soil, data reveal that increasing HA applied rate significantly increased Cu uptake (mg $plant^{-1}$) by roots, stems, leaves and seeds of sunflower plant.

The highest HA applied rate (0.6 g kg^{-1} soil) slightly increased roots, stems, leaves, seeds and whole plant uptake of Cu (mg $plant^{-1}$) as compared to control by 1.12, 1.13, 1.17, 1.18 and 1.13 folds in Abu Rawash soil and 1.35, 1.30, 1.42, 1.33 and 1.34 in Al-Gabal Al-Asfar soil, respectively. These results are in coincidence with that of **Topcuoğlu (2012)** who pointed out that the availability and uptake of Cu by tobacco plants were significantly raised by HA application at 1% and 2%.

The total uptake of Cu by sunflower plants in Abu Rawash soil at any level of applied chelators was always higher than that of Al-Gabal Al-Asfar soil, as shown in Fig (18). This could be attributed to the higher concentration of Cu in Abu Rawash soil (170 mg kg^{-1} soil) than that of Al-Gabal Al-Asfar soil (107 mg kg^{-1} soil).

Data of Table (8) declare the significant interaction between chelator types and their addition rates on Cu uptake of sunflower plant parts and total uptake of Cu (mg $plant^{-1}$) in both soils, except for cu uptake of sunflower seeds in Al-Gabal Al-Asfar soil (no significance). EDTA at 4.5 mmol kg^{-1} soil achieved the highest Cu uptake of roots, stems, leaves and whole plant of sunflower in both studied soils. CA at a rate of 6.0 and 9.0 recorded the highest values (2.84 and 2.06 mg $plant^{-1}$) of Cu uptake by seeds of sunflower plant in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

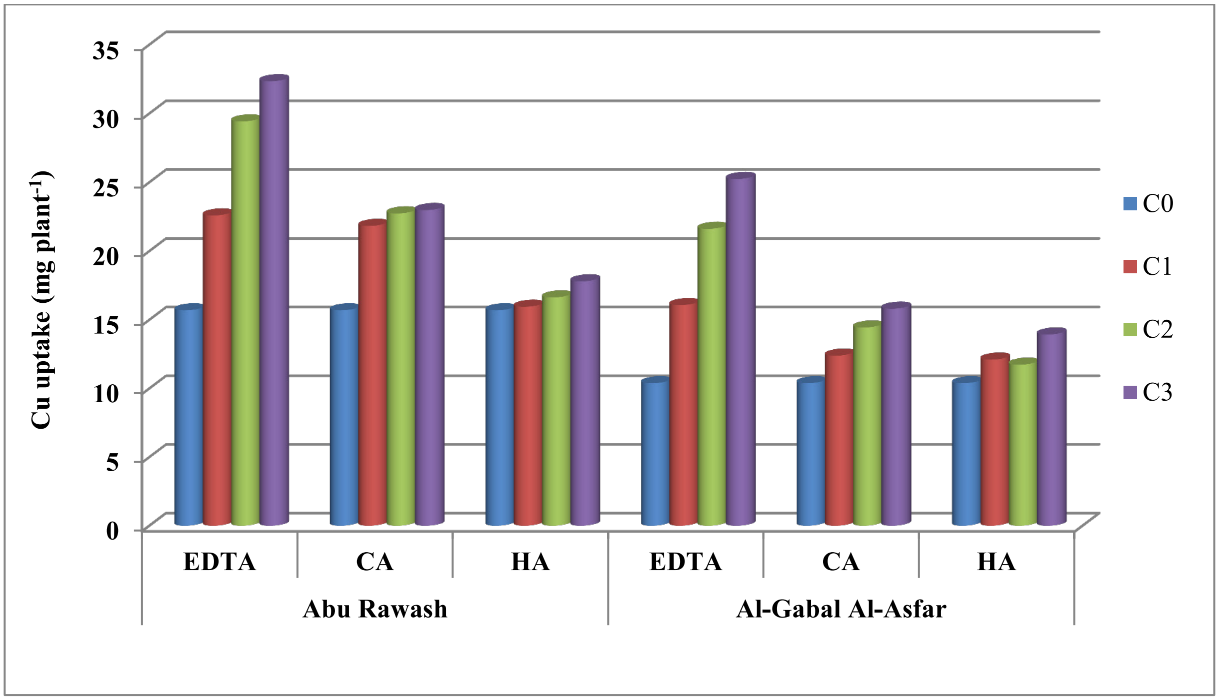


Fig (18): Effect of chelators and their addition rates on total uptake of Cu (mg plant^{-1}) by sunflower grown on Abu Rawash and Al-Gabal Al-Asfar soils.

4.3.2. Indian mustard plant:

Data of Table (9) represent the effect of chelator types, rates and their interaction on the uptake of Cu by roots, stems, leaves, seeds and whole plant (mg plant^{-1}) by indian mustard grown on in Abu Rawash and Al-Gabal Al-Asfar soils.

Concerning the effect of chelator types used, it was noticed that Cu uptake by root, stems, leaves, whole plant significantly differ in indian mustard plant due to chelator type effect, where Cu uptake by seeds did significantly differ due to the same reason in Abu Rawash soil. In Al-Gabal Al-Asfar soil, Cu uptake by root, stems, leaves, seeds and whole indian mustard plant significantly differ due to chelator type. Data of Table (9) reveal that Cu uptake by different parts of indian mustard plant was in the order: roots > stems > leaves > seeds.

HA recorded the lowest mean values of Cu uptake by plant organs, where the Cu uptake by roots was 8.08 and 4.96 mg; stems was 2.89 and 1.61 mg; leaves was 1.40 and

0.60 mg; seeds was 0.73 and 0.21 mg and whole plant was 13.13 and 7.39 mg plant^{-1} in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

Table (9): Effect of chelators, their addition rates and their interaction on the uptake of Cu by roots, stems, leaves, seeds and total plant (mg plant^{-1}) by indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Cu uptake (mg plant^{-1})									
		Abu Rawash					Al-Gabal Al-Asfar				
		roots	stems	leaves	seeds	total uptake	roots	stems	leaves	seeds	total uptake
EDTA mmol kg^{-1} soil	0	8.19	2.87	1.40	0.72	13.18	4.90	1.54	0.59	0.22	7.24
	1.5	8.48	4.09	1.88	0.72	15.18	5.43	2.20	0.94	0.22	8.79
	3.0	10.89	5.09	3.03	0.78	19.78	6.82	3.45	1.30	0.26	11.83
	4.5	12.26	6.03	3.47	0.71	22.46	7.02	3.83	1.38	0.28	12.52
Mean		**9.95**	**4.52**	**2.45**	**0.73**	**17.65**	**6.04**	**2.75**	**1.05**	**0.25**	**10.10**
F-test		**	**	**	ns	**	**	**	**	*	**
LSD 0.05		0.98	0.41	0.40		1.66	0.46	0.30	0.09	0.04	0.51
LSD 0.01		1.53	0.64	0.62		2.59	0.72	0.47	0.14		0.80
CA mmol kg^{-1} soil	0	8.19	2.87	1.40	0.72	13.18	4.90	1.54	0.59	0.22	7.24
	3.0	10.54	3.43	1.43	0.70	16.10	5.18	2.04	0.80	0.18	8.20
	6.0	10.95	3.55	1.64	0.79	16.92	6.71	2.69	1.04	0.20	10.65
	9.0	12.75	4.26	2.11	0.85	19.98	7.76	3.01	1.14	0.23	12.15
Mean		**10.61**	**3.53**	**1.64**	**0.77**	**16.54**	**6.14**	**2.32**	**0.89**	**0.21**	**9.56**
F-test		**	**	**	ns	**	**	**	**	ns	**
LSD 0.05		0.94	0.70	0.24		1.44	0.65	0.35	0.06		0.83
LSD 0.01		1.47	1.10	0.37		2.25	1.02	0.54	0.10		1.29
HA g kg^{-1} soil	0	8.19	2.87	1.40	0.72	13.18	4.90	1.54	0.59	0.22	7.24
	0.2	7.77	2.86	1.30	0.75	12.69	4.92	1.61	0.61	0.20	7.35
	0.4	8.26	2.85	1.46	0.74	13.31	5.01	1.67	0.58	0.19	7.44
	0.6	8.21	2.97	1.43	0.71	13.32	5.03	1.63	0.64	0.22	7.52
Mean		**8.08**	**2.89**	**1.40**	**0.73**	**13.13**	**4.96**	**1.61**	**0.60**	**0.21**	**7.39**
F-test		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
LSD 0.05											
LSD 0.01											
F-test (treatments)		**	**	**	**ns**	**	**	**	**	**	**
LSD 0.05		**0.80**	**0.29**	**0.20**		**1.07**	**0.26**	**0.16**	**0.12**	**0.02**	**0.18**
LSD 0.01		**1.21**	**0.44**	**0.30**		**1.62**	**0.40**	**0.25**	**0.18**	**0.04**	**0.27**
F-test (Interaction)		*	**	**	**ns**	**	**	**	**	**ns**	**
LSD 0.05		**0.69**	**0.25**	**0.17**		**0.93**	**0.23**	**0.14**	**0.10**		**0.16**
LSD 0.01			**0.38**	**0.26**		**1.40**	**0.34**	**0.21**	**0.16**		**0.24**

EDTA increased Cu uptake by indian mustard roots, stems, leaves, seeds and whole plant (mg plant^{-1}) as compared to HA by 23.14, 56.40, 75.00, 0.00 and 34.42% in Abu Rawash soil and 21.77, 70.81, 75.00, 19.05 and 36.67% in Al-Gabal Al-Asfar soil, respectively, whereas, CA treatment increased that traits by 31.31, 22.15, 17.14, 2.66 and

25.97% in Abu Rawash soil and 23.79, 44.10, 48.33, 0.00 and 29.36% in Al-Gabal Al-Asfar soil, respectively as shown in Fig (19).

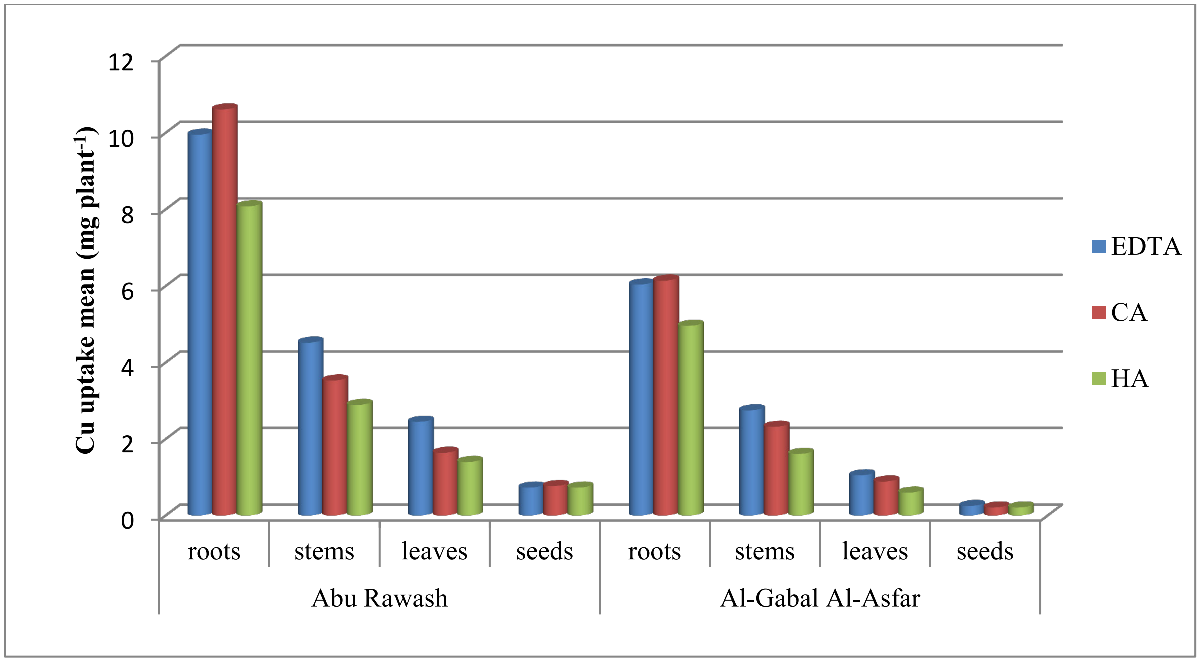


Fig (19): Effect of chelator types on Cu uptake means (mg plant^{-1}) by roots, stems, leaves and seeds of indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Data presented in Table (9) and illustrated Fig (20) reveal that increasing EDTA addition rates up to 4.5 mmol kg^{-1} soil significantly increased Cu uptake by indian mustard plant roots, stems, leaves and consequently total uptake in Abu Rawash soil. Whereas, in Al-Gabal Al-Asfar soil, Cu uptake by roots, stems, leaves, seeds and consequently total uptake were significantly increased with increasing EDTA level up to the highest level used.

Cu uptake by roots, stems, leaves, seeds and consequently total plant (mg plant^{-1}) accompanied with 4.5 mmol EDTA amounted by 12.26, 6.03, 3.47, 0.71and 22.46 mg in Abu Rawash soil, corresponding values in Al-Gabal Al-Asfar soil were 7.02, 3.83, 1.38, 0.28 and 12.52 mg.

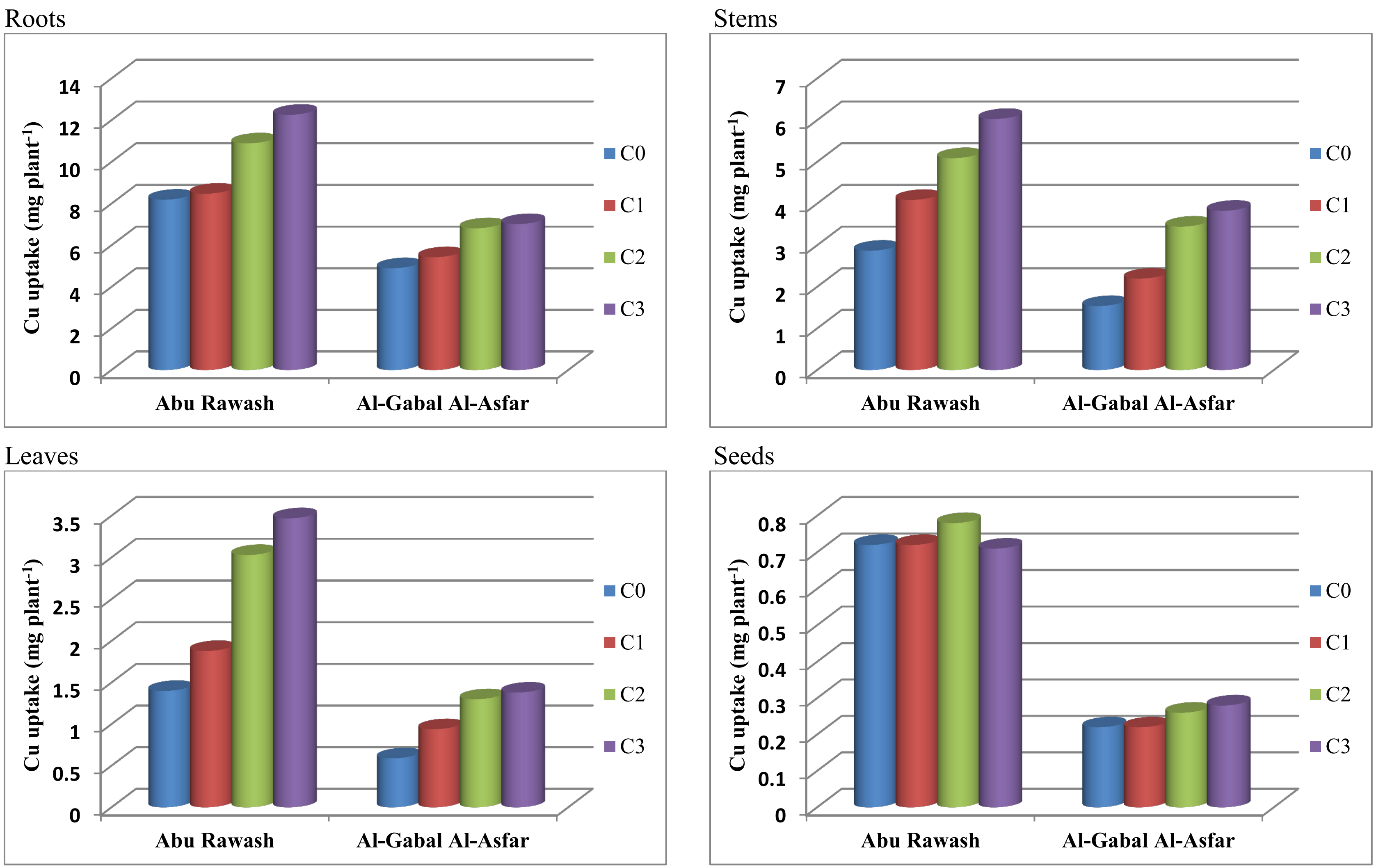


Fig (20): Effect of EDTA application levels on Cu uptake (mg plant^{-1}) by roots, stems, leaves and seeds of indian mustard in Abu Rawash and Al-Gabal Al-Asfar soils.

The highest EDTA applied rate (4.5 mmol kg^{-1} soil) increased indian mustard roots, stems, leaves and total uptake of Cu (mg $plant^{-1}$) by 1.50, 2.10, 2.48 and 1.70 folds compared with control in Abu Rawash soil, while no significant effect on seeds were found due to the same applied rate. Likewise, 4.5 mmol EDTA kg^{-1} soil increased indian mustard roots, stems, leaves, seeds and total uptake of Cu (mg $plant^{-1}$) by 1.43, 2.49, 2.34, 1.27 and 1.73 folds compared to control in Al-Gabal Al-Asfar soil, respectively.

Data of Table (9) and Fig (21) reveal that CA levels significantly affect Cu uptake by indian mustard plant parts in both soils.

Cu uptake by roots, stems, leaves and seeds were increased from 8.19, 2.87, 1.40 and 0.72 mg $plant^{-1}$ to 12.75, 4.26, 2.11 and 0.85 mg $plant^{-1}$, respectively, due to CA level increasing from 0.00 to 9.0 mmol kg^{-1} soil in Abu Rawash soil. Corresponding values in Al-Gabal Al-Asfar soil were increased from 4.90, 1.54, 0.59 and 0.22 mg $plant^{-1}$ to 7.76, 3.01, 1.14 and 0.23 mg $plant^{-1}$. CA at 9 mmol kg^{-1} soil increased total uptake of Cu (mg $plant^{-1}$) by indian mustard plant compared with control by1.52 and 1.68 folds in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

These results are in agreement with that of **Zaheer *et al.*, (2015)**, where the negative effect of Cu toxicity was overturned by CA application, hence plant growth was increased due to reducing oxidative stress and enhancing antioxidant enzyme activities.

Regarding the effect of HA on Cu uptake by tested plant, the obtained results demonstrated that no significant effects were found on Cu uptake (mg $plant^{-1}$) by different plant parts due to HA application.

The total uptake of Cu by indian mustard plants in Abu Rawash soil at any level of applied chelators was always higher than that of Al-Gabal Al-Asfar soil. This could be attributed to the higher concentration of Cu in Abu Rawash soil (170 mg kg^{-1} soil) than that of Al-Gabal Al-Asfar soil (107 mg kg^{-1} soil).

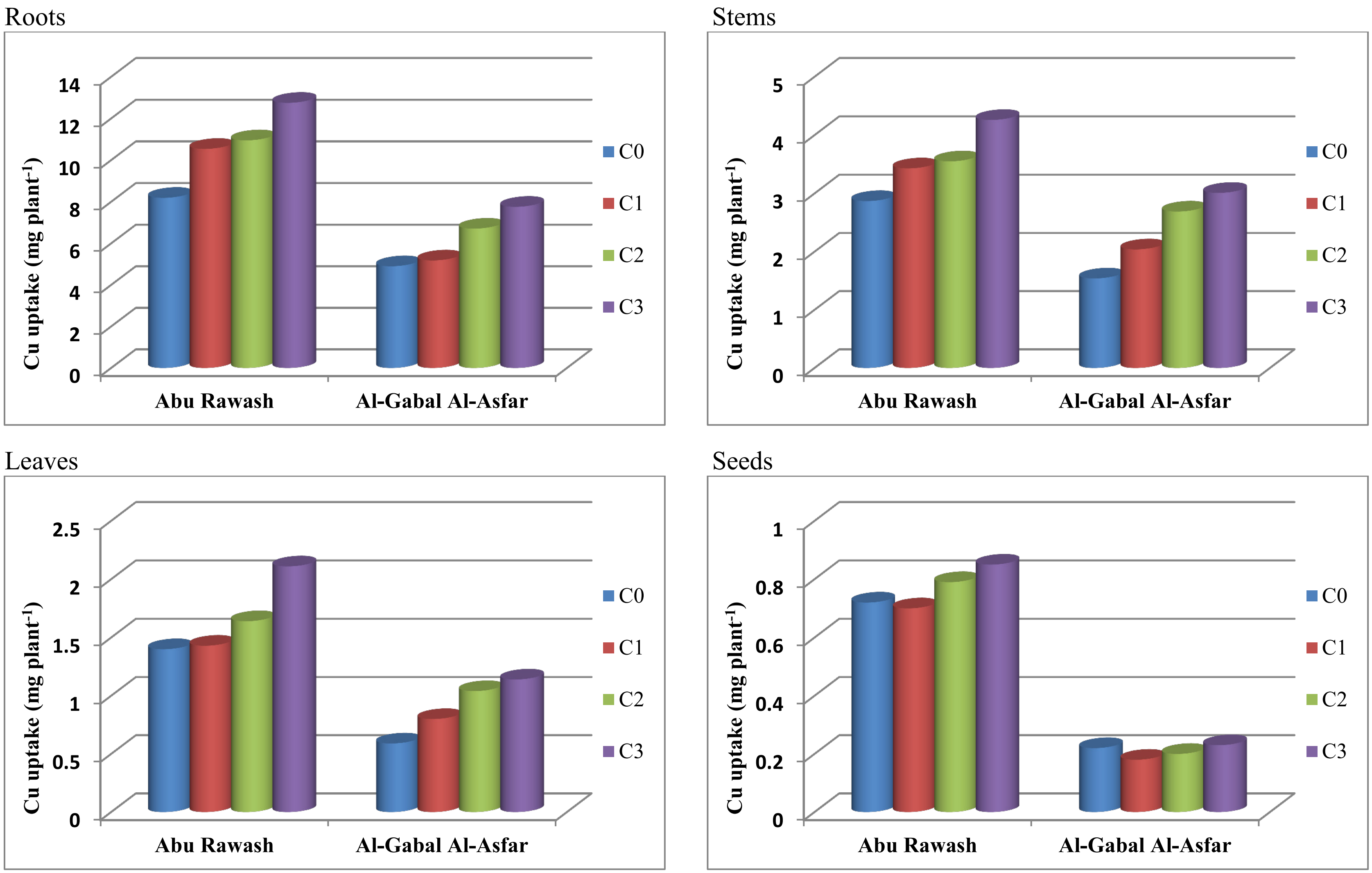


Fig (21): Effect of CA application levels on Cu uptake (mg plant^{-1}) by roots, stems, leaves and seeds of indian mustard in Abu Rawash and Al-Gabal Al-Asfar soils.

Concerning chelator - chelator levels interaction, a significant effects on roots, stems, leaves and total uptake of Cu (mg plant^{-1}) by indian mustard plant parts in both tested soils were found, where, Application of 4.5 mmol EDTA kg^{-1} soil in Abu Rawash soil recorded the highest values of Cu uptake by roots, stems, leaves and whole plant, 12.26, 6.03, 3.47 and 22.46 mg plant^{-1}, respectively. Application of 4.5 mmol EDTA kg^{-1} soil in Al-Gabal Al-Asfar soil recorded the highest values of Cu uptake by roots, stems, leaves and whole plant, 12.26, 6.03, 3.47 and 22.46 mg plant^{-1}, respectively. While no significant effect on Cu uptake by seeds of both soils was found.

The overall results demonstrate that sunflower plant had the ability to accumulate Cu in its different plant parts than indian mustard plant. Where, the mean values of Cu total uptake by sunflower was higher than that of indian mustard when EDTA, CA and HA chelators were applied by 41.70, 25.82 and 25.82% in Abu Rawash soil and by 81.49, 38.70 and 62.79% in Al-Gabal Al-Asfar soil, respectively.

4.4. Effect of chelator types, rates and their interactions on Zn concentration (mg kg^{-1} DW) of plant organs grown on the two soils under study:

4.4.1. Sunflower plants:

Data in Table (10) represent the effect of chelator types, rates and their interactions on Zn concentrations of roots, stems, leaves and seeds of sunflower grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Concerning the effect of chelator types used, it was noticed that Zn concentrations in the different sunflower plant parts, except for seeds, were significantly differ due to application of different chelator types in both soils.

The mean values of Zn concentrations in plant roots were 1189.0, 1125.2, and 1089.0 mg kg^{-1} DW when EDTA, CA and HA was applied in Abu Rawash soil. While, the mean values of Zn concentrations in roots in Al-Gabal Al-Asfar soil took the same manner with a lesser values, 931.7 for EDTA, 877.7 for CA and 855.0 mg kg^{-1} DW for HA. Zn concentration of stems in Abu Rawash soil varied from 576.7 mg kg^{-1} DW for EDTA to 496.3 mg kg^{-1} for CA, wherever in Al-Gabal Al-Asfar soil varied from 419.6 mg kg^{-1} DW

for EDTA to 319.6 mg kg^{-1} DW for HA. Zn concentration of leaves in Abu Rawash soil varied from 712.1 mg kg^{-1} for EDTA to 617.1 mg kg^{-1} for HA whenever in Al-Gabal soil varied from 505.9 for EDTA to 412.9 mg kg^{-1} DW for HA. Zn concentration of seeds in Abu Rawash soil varied from 142.7 for EDTA to 130.4 mg kg^{-1} DW for CA whenever in Al-Gabal Al-Asfar soil varied from 117.6 with EDTA to 112.8 mg kg^{-1} DW with CA.

Table (10): Effect of chelator types, rates and their interaction on Zn concentration (mg kg^{-1} DW) of roots, stems, leaves and seeds of sunflower plant in Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Zn concentration (mg kg^{-1} DW)							
		Abu Rawash				Al-Gabal Al-Asfar			
		roots	stems	leaves	seeds	roots	stems	leaves	seeds
EDTA mmol kg^{-1} soil	0	1070.0	460.0	604.2	135.0	829.2	281.7	401.7	111.7
	1.5	1165.8	529.2	665.8	135.0	892.5	374.2	464.2	112.5
	3.0	1227.5	623.3	735.8	145.8	960.0	470.8	536.7	119.7
	4.5	1292.5	694.2	842.5	154.8	1045.0	551.7	620.8	126.7
Mean		**1189.0**	**576.7**	**712.1**	**142.7**	**931.7**	**419.6**	**505.9**	**117.7**
F-test		**	**	**	ns	**	**	**	**
$LSD_{0.05}$		70.9	54.5	64.7		28.2	26.8	29.9	7.5
$LSD_{0.01}$		110.4	84.9	100.8		43.9	41.7	46.5	11.7
CA mmol kg^{-1} soil	0	1070.0	460.0	604.2	135.0	829.2	281.7	401.7	111.7
	3.0	1101.7	466.7	632.5	126.7	844.2	325.8	414.2	109.2
	6.0	1152.5	505.0	659.2	133.3	889.2	383.3	443.3	115.4
	9.0	1176.7	553.3	748.3	126.7	948.3	465.0	485.8	114.9
Mean		**1125.2**	**496.3**	**661.1**	**130.4**	**877.7**	**364.0**	**436.3**	**112.8**
F-test		**	**	**	ns	**	**	**	ns
$LSD_{0.05}$		34.9	36.2	22.1		25.9	18.2	29.9	
$LSD_{0.01}$		54.3	56.5	34.5		40.4	28.4	46.5	
HA g kg^{-1} soil	0	1070.0	460.0	604.2	135.0	829.2	281.7	401.7	111.7
	0.2	1084.2	496.7	611.7	133.3	839.2	300.8	403.3	114.9
	0.4	1080.0	551.7	624.2	132.5	881.7	333.3	438.3	115.0
	0.6	1121.7	542.5	628.3	135.8	870.0	362.5	408.3	116.7
Mean		**1089.0**	**512.7**	**617.1**	**134.2**	**855.0**	**319.6**	**412.9**	**114.6**
F-test		ns	**	ns	ns	**	**	ns	ns
$LSD_{0.05}$			29.9			21.0	23.0		
$LSD_{0.01}$			46.6			32.7	35.8		
F-test (treatments)		**	**	**	**ns**	**	**	**	**ns**
$LSD_{0.05}$		**49.0**	**48.7**	**32.9**		**11.2**	**14.8**	**28.0**	
$LSD_{0.01}$		**74.3**	**73.8**	**49.8**		**17.0**	**4.1**	**42.4**	
F-test (Interaction)		**	**	**	*	**	**	**	**ns**
$LSD_{0.05}$		**42.5**	**42.2**	**28.4**	**12.5**	**9.7**	**12.8**	**24.2**	
$LSD_{0.01}$		**64.4**	**63.9**	**43.1**		**14.7**	**1.2**	**36.7**	

It is worthy to note that Zn concentration in plant parts of sunflower grown on Abu Rawash soil was higher than those grown in Al-Gabal Al-Asfar soil. This could be

attributed to the higher concentration of Zn in Abu Rawash soil (520 mg kg^{-1} soil) than that of Al-Gabal Al-Asfar soil (380 mg kg^{-1} soil).

Fig (22) shows that using EDTA increased Zn concentrations of roots, stems, leaves and seeds of sunflower plant than that of HA treatment by 9.18, 12.48, 15.39 and 6.33% in Abu Rawash soil and by 8.97, 31.29, 22.52 and 2.71% in Al-Gabal Al-Asfar soil, respectively. On the other hand, the treatment of CA increased Zn concentrations of roots and leaves of sunflower by 3.32% and 7.13% in Abu Rawash soil than that of HA treatment, respectively. No significant difference of Zn concentrations in stems and seeds was found. Whilst, using CA in Al-Gabal Al-Asfar soil increased Zn concentrations in roots and stems than that of HA by 2.65% and 13.89%, respectively. No significant difference in leaves and seeds Zn concentrations were found.

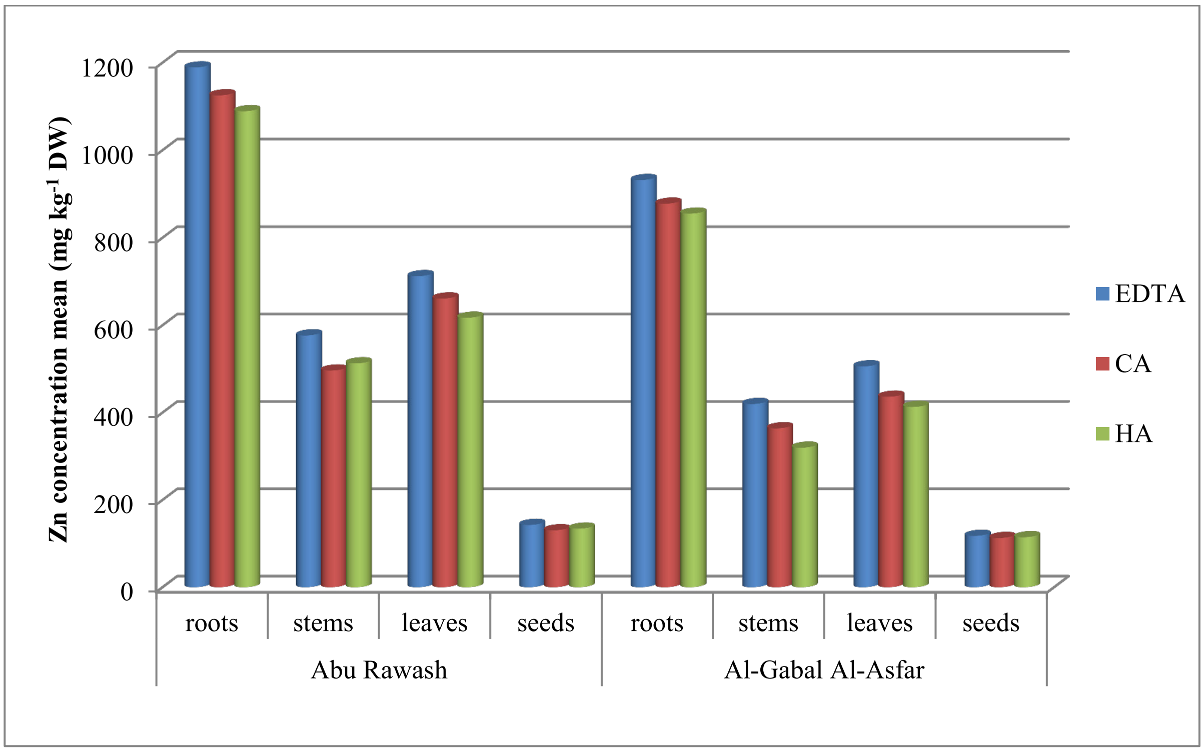


Fig (22): Effect of chelators on Zn concentration means (mg kg^{-1} DW) in roots, stems, leaves and seeds of sunflower grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Regarding the effect of chelator levels, Table (10) and Fig (23) show that Zn concentration was significantly increased with increasing EDTA addition rate up to 4.5

mmol kg^{-1}. Accordingly, the concentrations of Zn (mg kg^{-1} DW) in plant roots, stems and leaves was increased as compared to control by 1.21, 1.51and 1.39 folds in Abu Rawash soil and by 1.26, 1.96 and 1.55 folds in Al-Gabal Al-Asfar soil, respectively. The concentrations of Zn in seeds was increased with increasing EDTA levels up to 4.5 mmol kg^{-1} soil by 1.15 and 1.13 folds over control in both Abu Rawash and Al-Gabal Al-Asfar soils, respectively. However, this effect was insignificant in Abu Rawash soil.

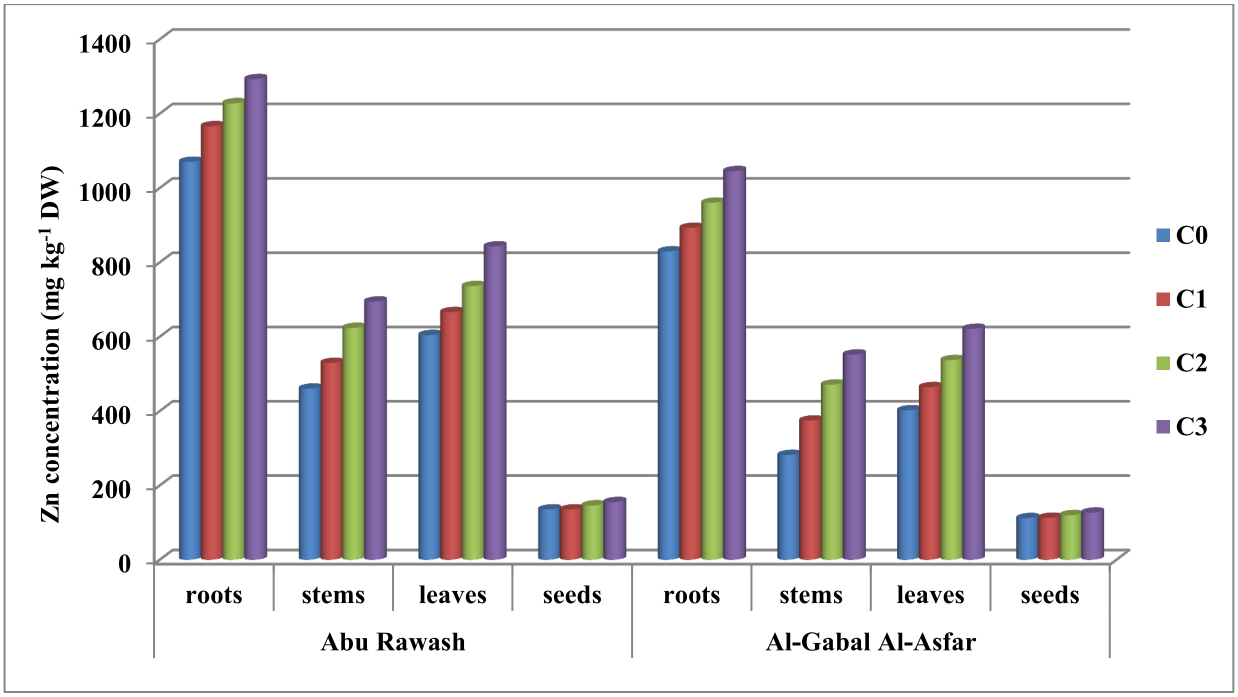


Fig (23): Effect of EDTA application levels on Zn concentrations (mg kg^{-1} DW) in roots, stems, leaves and seeds of sunflower grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Increasing Zn concentration of sunflower plant parts with EDTA application is a normal result due to its effect on Zn availability in soil. These results are in a consistency with that of **Wu *et al.*, (2003)** who outlined that 3 mmol EDTA kg^{-1} soil increased Zn concentration in soil solution. **Cay *et al.*, (2016)** explained that EDTA forms strong complexes with metals through its two amines and four carboxylates groups. Hence, application of EDTA remobilizes metals by forming strong soluble complexes of heavy metals.

CA levels significantly increased Zn concentrations in plant roots, stems and leaves in both soils as shown in Fig (24). On the other hand, did not significantly affect Zn concentrations of seeds. Moreover, the values of Zn concentration of roots, stems and leaves were increased by 9.97, 20.28 and 23.85% in Abu Rawash soil and by 14.36, 65.07 and 20.94% in Al-Gabal Al-Asfar soil, respectively, due to CA was applied at a rate of 9 mmol kg^{-1} soil compared with control, 0.0 CA addition.

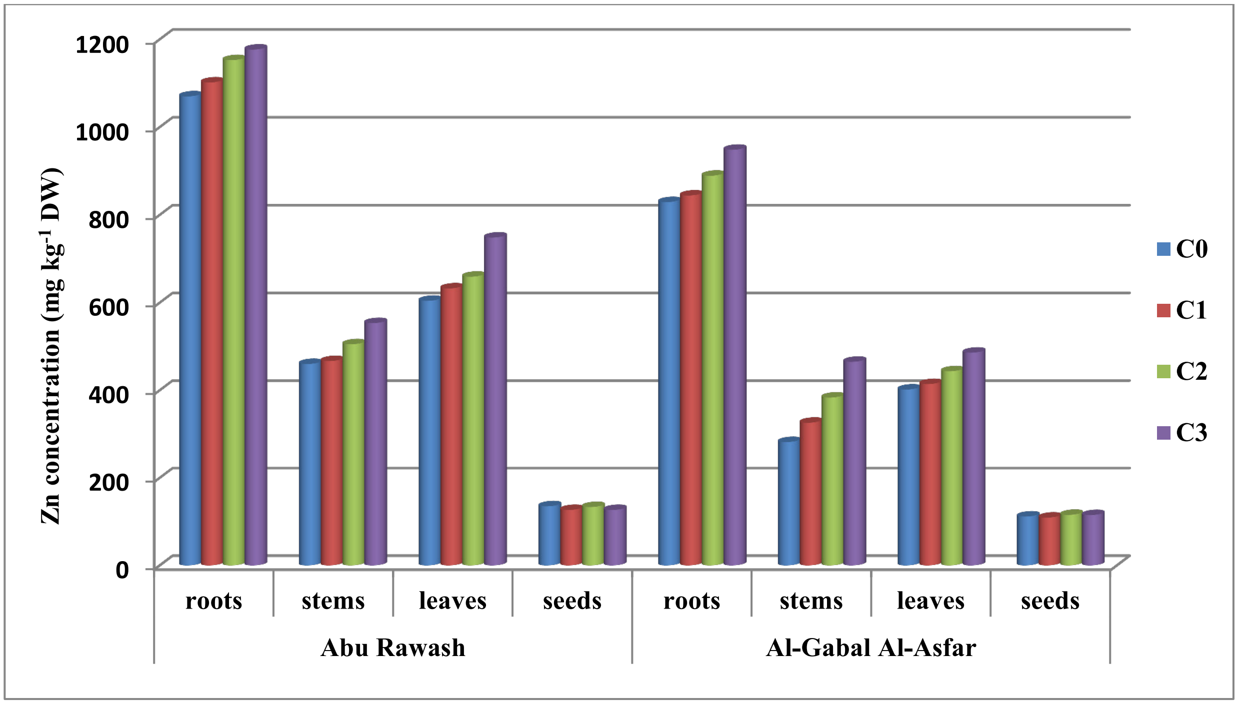


Fig (24): Effect of CA application levels on Zn concentrations (mg kg^{-1} DW) in roots, stems, leaves and seeds of sunflower in Abu Rawash and Al-Gabal Al-Asfar soils.

Regarding to HA effect of on Zn concentrations of sunflower plant parts in the tested soils, the results stated that application of HA significantly increased Zn concentration (mg kg^{-1} DW) of stems in Abu Rawash soil. Where, Zn concentration in stems increased by 19.93% with increasing HA application rate up to 0.4 g kg^{-1} soil. However, this effect was significant for roots and stems in Al-Gabal Al-Asfar soil. The highest values of Zn concentration of roots (881.7 mg kg^{-1}) was achieved using 0.4 g HA kg^{-1} in Al-Gabal Al-Asfar soil which revealed an increase amounted by 6.33% over control, While, application of 0.6 g kg^{-1} soil achieved the highest increase in Zn concentrations of sunflower stems (28.68% increase over control) in Al-Gabal Al-Asfar soil.

Zn concentration of leaves and seeds did not significantly affect by HA application in both soils.

Significant interaction effects were found between chelator types and rates on roots, stems and leaves Zn concentration (mg kg^{-1} DW) of sunflower in both soils. While the significant effect of chelator types and rates interaction on Zn concentration of seeds was found in Abu Rawash soil only.

However, application of 4.5 mmol EDTA kg^{-1} soil recorded the highest concentrations of Zn in plant parts in both soils. Zn concentration of roots, stems, leaves and seeds were 1292.5, 694.2, 842.5 and 154.8 mg kg^{-1} DW in Abu Rawash soil, corresponding values of the same parts in Al-Gabal Al-Asfar soil were 1045.0, 551.7, 620.8 and 126.7 mg kg^{-1} DW, respectively.

4.4.2. Indian mustard plant:

Data in Table (11) represent the effect of chelator types, rates and their interactions on Zn concentrations in roots, stems, leaves and seeds of indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Concerning the effect of chelators types used, it was noticed that Zn concentrations in different parts of indian mustard, except for seeds, were significantly affected due to chelators types application in both soils.

The mean values of Zn concentrations in plant roots were 853.3, 800.0, and 748.6 mg kg^{-1} DW for EDTA, CA and HA in Abu Rawash soil. In Al-Gabal Al-Asfar soil, the mean values of Zn concentrations in indian mustard roots took the same manner with a lesser values, 642.5, 588.5 and 559.2 mg kg^{-1} DW For EDTA, CA and HA, respectively. Zn concentration of stems in Abu Rawash soil varied from 334.8 for EDTA to 252.5 mg kg^{-1} DW for HA, wherever, in Al-Gabal Al-Asfar soil varied from 185.2 mg kg^{-1} DW for EDTA to 134.6 mg kg^{-1} with HA. The mean values of Zn concentration in leaves varied from 532.9 mg kg^{-1} DW for EDTA to 408.3 mg kg^{-1} DW for HA in Abu Rawash soil, wherever, in Al-Gabal Al-Asfar soil varied from 318.1 mg kg^{-1} DW with EDTA to 269.0 mg kg^{-1} DW

with HA. Zn concentration means in plant seeds in Abu Rawash soil varied from 115.8 mg kg^{-1} DW for EDTA to 104.4 mg kg^{-1} DW for HA, whenever, in Al-Gabal Al-Asfar soil varied from 84.5 mg kg^{-1} DW with CA to 75.5 mg kg^{-1} DW with HA.

Table (11): Effect of chelator types, rates and their interaction on Zn concentration (mg kg^{-1} DW) of roots, stems, leaves and seeds of indian mustard plant in Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Zn concentration (mg kg^{-1} DW)							
		Abu Rawash				Al-Gabal Al-Asfar			
		roots	stems	leaves	seeds	roots	stems	leaves	seeds
EDTA mmol kg^{-1} soil	0	745.0	233.3	398.3	107.3	533.3	127.5	235.8	74.2
	1.5	838.3	260.0	454.2	113.3	580.8	170.0	280.0	78.3
	3.0	889.2	399.6	574.2	118.3	691.7	200.0	325.0	79.2
	4.5	940.8	446.3	705.0	124.2	764.2	243.3	431.7	82.5
Mean		**853.3**	**334.8**	**532.9**	**115.8**	**642.5**	**185.2**	**318.1**	**78.6**
F-test		**	**	**	**	**	**	**	ns
$LSD_{0.05}$		34.6	28.9	26.0	5.4	24.5	16.1	19.0	
$LSD_{0.01}$		53.9	45.0	40.5	8.4	38.1	25.1	29.7	
CA mmol kg^{-1} soil	0	745.0	233.3	398.3	107.3	533.3	127.5	235.8	74.2
	3.0	779.2	257.5	413.3	105.8	560.8	138.3	253.3	78.2
	6.0	821.7	285.0	443.3	107.6	602.5	152.5	280.0	85.8
	9.0	854.2	331.8	515.0	109.2	657.5	166.7	325.0	99.9
Mean		**800.0**	**276.9**	**442.5**	**107.5**	**588.5**	**146.3**	**273.5**	**84.5**
F-test		**	**	**	ns	**	**	**	**
$LSD_{0.05}$		34.9	19.5	20.6		20.9	14.2	15.6	9.5
$LSD_{0.01}$		54.4	30.3	32.1		32.6	22.1	24.3	14.7
HA g kg^{-1} soil	0	745.0	233.3	398.3	107.3	533.3	127.5	235.8	74.2
	0.2	750.0	250.0	402.5	103.5	544.2	128.3	255.0	77.3
	0.4	739.2	262.5	416.7	102.5	560.8	134.2	270.8	75.0
	0.6	760.0	264.2	415.8	104.2	598.3	148.3	314.2	75.3
Mean		**748.6**	**252.5**	**408.3**	**104.4**	**559.2**	**134.6**	**269.0**	**75.5**
F-test		ns	ns	ns	ns	**	**	**	ns
$LSD_{0.05}$						21.0	7.8	23.7	
$LSD_{0.01}$						32.7	12.2	36.9	
F-test (treatments)		**	**	**	**	**	**	**	*
$LSD_{0.05}$		**31.2**	**19.8**	**18.7**	**5.1**	**16.9**	**8.4**	**16.2**	**8.2**
$LSD_{0.01}$		**47.2**	**30.1**	**28.4**	**7.7**	**25.6**	**12.7**	**24.5**	
F-test (Interaction)		**	**	**	**ns**	**	**	**	**
$LSD_{0.05}$		**27.0**	**17.2**	**16.2**		**14.6**	**7.3**	**14.0**	**7.1**
$LSD_{0.01}$		**40.9**	**26.0**	**24.6**		**22.1**	**11.0**	**21.3**	**10.8**

It is worthy to note that all plant parts of indian mustard (*Brassica juncea*) which is grown on Abu Rawash soil contains a higher Zn concentration than that of the same plant parts which is grown in Al-Gabal Al-Asfar soil as shown in Fig (25). This could be attributed to the higher concentration of Zn in Abu Rawash soil (520 mg kg^{-1} soil) than that of Al-Gabal Al-Asfar soil (380 mg kg^{-1} soil).

EDTA increased the roots, stems, leaves and seeds Zn concentration means (mg kg^{-1} DW) by 13.99, 32.59, 30.52 and 10.92% as compared to HA in Abu Rawash soil and by 14.90, 37.59, 18.25 and 4.11% in Al-Gabal Al-Asfar soil, respectively as shown in Fig (25). Whereas, application of CA treatment increased the mean values of Zn concentration (mg kg^{-1} DW) in roots, stems, leaves and seeds by 6.87, 9.66, 8.38 and 2.97% compared to HA treatment in Abu Rawash soil and by 5.24, 8.69, 1.67 and 11.92% in Al-Gabal Al-Asfar soil, respectively.

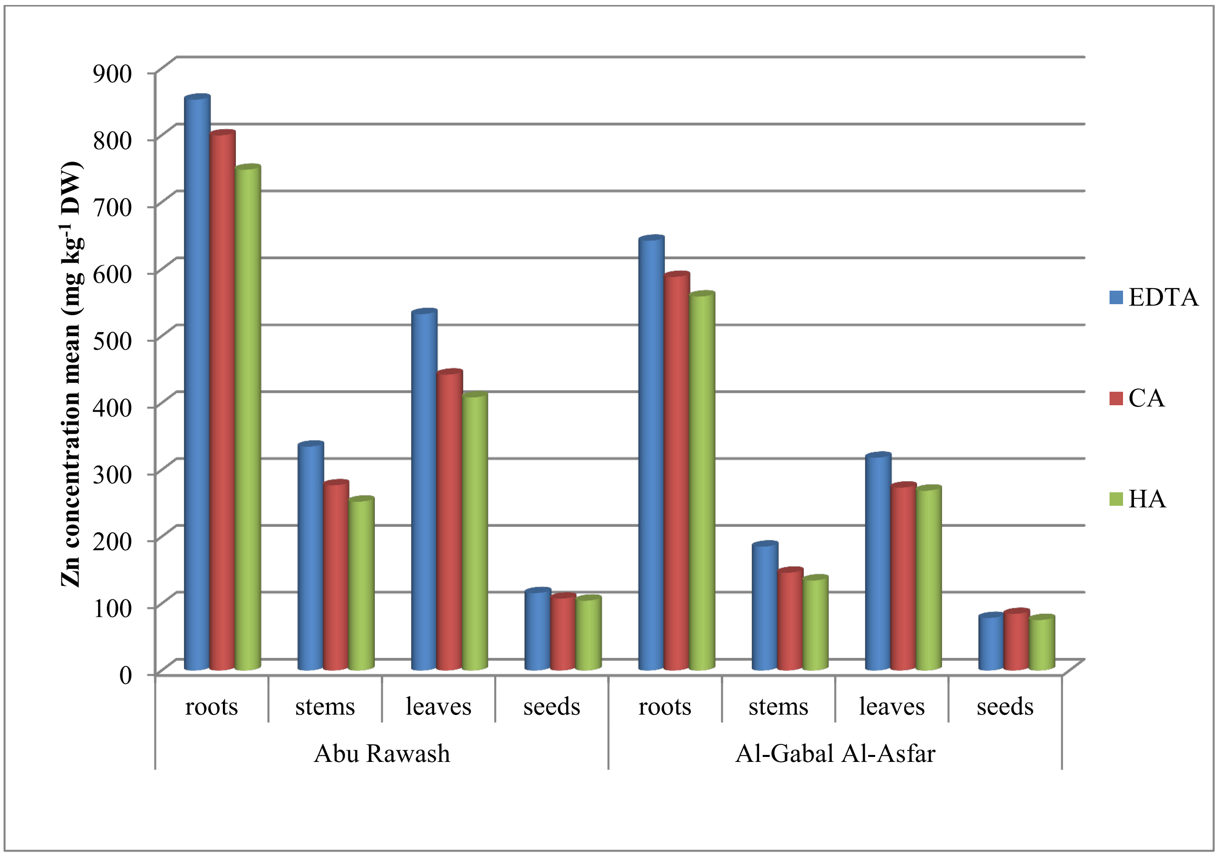


Fig (25): Effect of chelators on Zn concentration means (mg kg^{-1} DW) in roots, stems, leaves and seeds of indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Data in Table (10) and Fig (26) show that Zn concentration of each plant part was significantly increased with increasing EDTA addition rate up to 4.5 mmol kg^{-1} soil in both soil, except for seeds in Al-Gabal Al-Asfar soil. Accordingly, the concentrations of Zn (mg kg^{-1} DW) in roots, stems, leaves and seed were increased as compared to control by 26.28,

91.30, 77.00 and 15.75% in Abu Rawash soil. In Al-Gabal Al-Asfar soil, addition of 4.5 mmol EDTA kg^{-1} soil significantly increased Zn concentrations of roots, stems and leaves by 43.30, 90.82 and 83.08% increases as compared to control, respectively. The concentrations of Zn of seeds was insignificantly increased with increasing EDTA levels up to 4.5 mmol kg^{-1} by 11.19 % increase compared to control in Al-Gabal Al-Asfar soils.

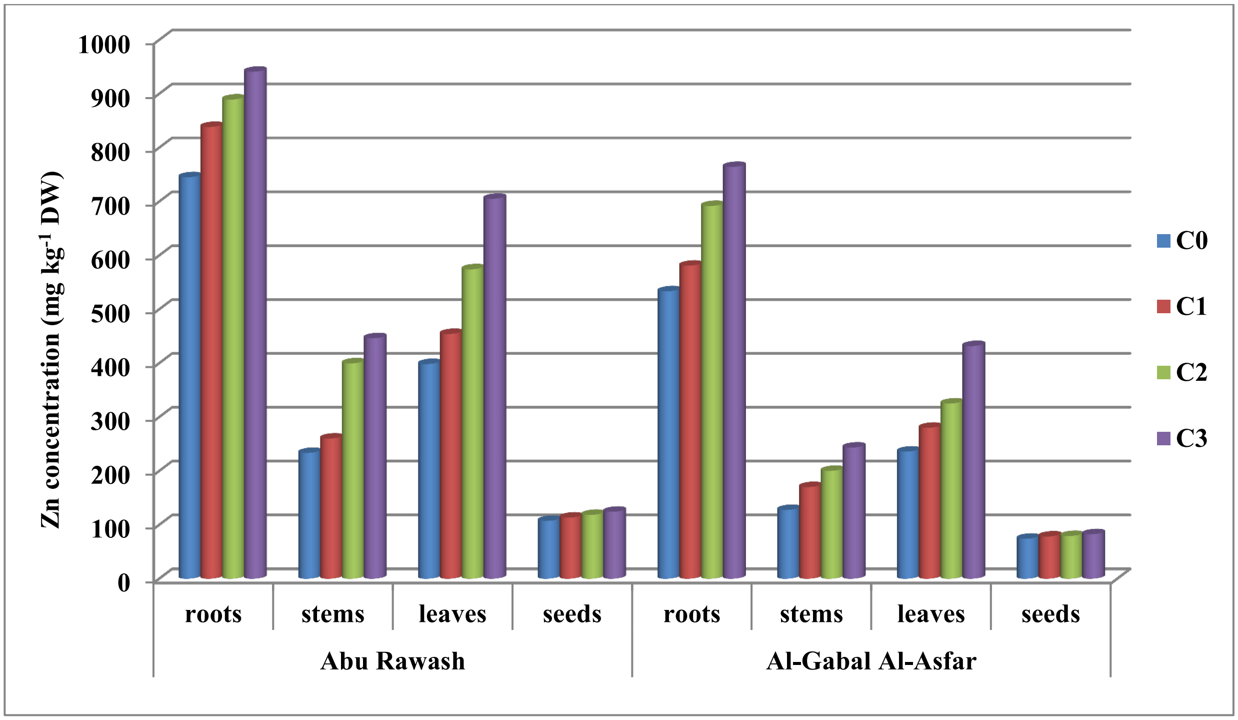


Fig (26): Effect of EDTA application levels on Zn concentrations (mg kg^{-1} DW) in roots, stems, leaves and seeds of indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

The obtained results also reveal that Zn concentrations in indian mustard plant parts were increased by increasing CA levels in both soils as shown in Fig (27). Moreover, Zn concentrations in plant parts were significantly increased with increasing CA level up to 9 mmol kg^{-1} soil in both soils, except for seeds in Abu Rawash soil. These results are in Harmony with those of **Najeeb *et al.*, (2009).** They stated that CA induced chelation of metals decreasing free ions in plant, hence CA promote plant for more metal uptake with no negative effect.

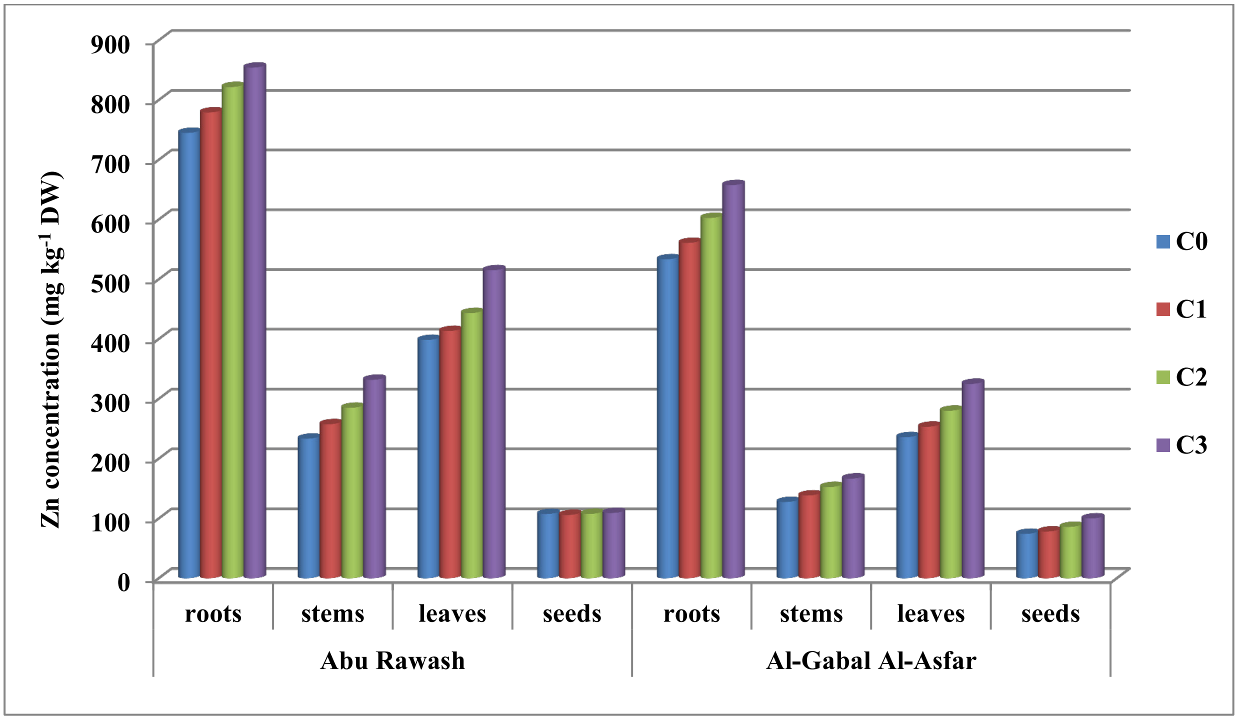


Fig (27): Effect of CA application levels on Zn concentrations (mg kg^{-1} DW) in roots, stems, leaves and seeds of indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Application of 9.0 mmol CA kg^{-1} soil significantly increased Zn concentrations of roots, stems and leaves that represent 1.15, 1.42 and 1.29 folds in Abu Rawash soil and 1.23, 1.31 and 1.38 folds in Al-Gabal Al-Asfar soil compared with control, respectively.

Zn concentration of seeds was significantly increased by 34.72% compared to control in Al-Gabal Al-Asfar soil due to applying 9.0 mmol CA kg^{-1} soil, while no significant effect on seeds Zn concentration in Abu Rawash soil were found due to application of the same level of CA.

Regarding to HA levels effect on Zn concentrations of indian mustard plant parts in the studied soils, slightly increase in Zn concentrations in roots, stems and leaves (1.02, 1.13 and 1.04 folds comparing to control) and slightly decrease in Zn concentration in seeds were found due to increasing HA level from 0.0 to 0.6 g kg^{-1} in Abu Rawash soil and the increase and decrease were insignificant.

Application of different HA levels revealed significant increases in Zn concentration in different plant parts in Al-Gabal Al-Asfar soil, except for seeds. The highest values of Zn concentration of roots, stems and leaves (598.33, 148.33, 314.17 and 77.33 mg kg^{-1} DW) were increased as compared to control by 12.19, 16.31 and 33.25%, respectively, due to increasing HA level up to 0.6 g kg^{-1} soil.

. The highest values of Zn concentration of roots, stems and leaves were accompanied with the highest level used of HA and the highest values of Zn concentration of seeds were accompanied with 0.2 g HA kg^{-1} soil treatment.

In respect to the interaction between chelator types and their addition rates, significant effects on Zn concentration in roots, stems, leaves and seeds (mg kg^{-1} DW) in both soils were found, except for seeds in Abu Rawash soil. Whereas, application of 4.5 mmol EDTA kg^{-1} soil gave the highest concentrations of Zn in different plant parts in both soils.

Data show also that the Zn roots had the highest Zn concentration as compared to other plant parts, where, Zn concentrations in the different plant parts were in the order: roots > leaves > stems > seeds in both Abu Rawash and Al-Gabal Al-Asfar soils.

Significant interaction effects were found between chelator types and their addition rates on roots, stems and leaves Zn concentration (mg kg^{-1} DW) in both soils. While the significant effect of chelator types and their rates interaction on Zn concentration of seeds was found in Al-Gabal Al-Asfar soil only.

However, application of 4.5 mmol EDTA kg^{-1} soil recorded the highest concentrations of Zn in plant roots, stems and leaves in both Abu Rawash and Al-Gabal Al-Asfar soils. Zn concentration in roots, stems and leaves were 940.8, 446.3 and 705.0 mg kg^{-1} DW in Abu Rawash soil. Corresponding values of the same parts in Al-Gabal Al-Asfar soil were 764.2, 243.3 and 431.7 mg kg^{-1} DW, respectively.

The highest concentrations of Zn in plant seeds in both Abu Rawash and Al-Gabal Al-Asfar soils were 124.2 and 99.9 mg kg^{-1} DW accompanied with 4.5 mmol EDTA and 9.0 mmol CA kg^{-1} soil, respectively.

4.5. Effect of chelator types, rates and their interaction on roots, stems, leaves, seeds and total plant uptake of Zn (mg plant^{-1}) by plants grown on the two soils under study:

4.5.1. Sunflower plant:

Data of Table (12) represent the effect of chelator types, rates and their interaction on roots, stems, leaves, seeds and total uptake of Zn (mg plant^{-1}) by sunflower plant grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Table (12): Effect of chelators, their addition rates and their interaction on the uptake of Zn by roots, stems, leaves, seeds and total plant (mg plant^{-1}) by sunflower grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Zn uptake (mg plant^{-1})									
		Abu Rawash					Al-Gabal Al-Asfar				
		roots	stems	leaves	seeds	total uptake	roots	stems	leaves	seeds	total uptake
EDTA mmol kg^{-1} soil	0	30.91	34.71	26.01	5.21	96.84	15.22	13.73	11.26	3.37	43.58
	1.5	27.71	36.73	27.15	4.64	96.23	15.76	17.51	13.08	2.94	49.29
	3.0	26.47	38.09	26.33	4.52	95.40	17.25	23.16	14.64	2.66	57.72
	4.5	24.48	37.63	24.70	3.39	90.20	12.93	21.14	14.25	2.54	50.85
Mean		**27.39**	**36.79**	**26.05**	**4.44**	**94.67**	**15.29**	**18.89**	**13.31**	**2.88**	**50.36**
F-test		**	ns	ns	ns	ns	**	**	**	**	**
$LSD_{0.05}$		3.19					1.96	1.68	0.83	0.33	3.93
$LSD_{0.01}$		4.97					3.06	2.62	1.29	0.51	6.12
CA mmol kg^{-1} soil	0	30.91	34.71	26.01	5.21	96.84	15.22	13.73	11.26	3.37	43.58
	3.0	38.17	47.27	33.61	6.24	125.30	14.66	15.51	10.91	3.75	44.82
	6.0	39.26	45.50	30.72	5.89	121.37	15.09	19.05	12.49	3.80	50.42
	9.0	32.42	37.85	30.27	4.96	105.51	16.60	21.77	13.39	3.55	55.31
Mean		**35.19**	**41.33**	**30.16**	**5.58**	**112.25**	**15.39**	**17.51**	**12.01**	**3.62**	**48.53**
F-test		**	**	**	**	**	ns	**	**	ns	**
$LSD_{0.05}$		4.66	3.14	1.47	0.54	5.24		0.64	0.61		1.63
$LSD_{0.01}$		7.25	4.89	2.30	0.84	8.15		0.99	0.95		2.55
HA g kg^{-1} soil	0	30.91	34.71	26.01	5.21	96.84	15.22	13.73	11.26	3.37	43.58
	0.2	25.20	36.49	24.57	5.51	91.77	13.44	14.23	10.66	3.66	41.98
	0.4	29.98	40.94	25.09	5.34	101.35	17.67	17.70	13.65	3.22	52.24
	0.6	26.85	38.67	25.86	5.82	97.19	17.30	18.46	13.34	3.53	52.64
Mean		**28.24**	**37.70**	**25.38**	**5.47**	**96.79**	**15.91**	**16.03**	**12.23**	**3.45**	**47.61**
F-test		**	**	ns	ns	**	**	**	**	ns	**
$LSD_{0.05}$		2.53	3.12			5.52	1.35	0.84	1.40		2.64
$LSD_{0.01}$		3.95	4.86			8.58	2.10	1.31	2.18		4.11
F-test (treatments)		**	*	**	**	**	**ns**	**	**	**	*
$LSD_{0.05}$		**2.71**	**3.81**	**1.75**	**0.62**	**6.52**		**0.82**	**1.08**	**0.42**	**2.74**
$LSD_{0.01}$		**4.11**		**2.65**	**0.95**	**9.88**		**1.24**	**1.64**	**0.63**	
F-test (Interaction)		*	**	**ns**	*	**	**	**	**	**ns**	**
$LSD_{0.05}$		**2.35**	**3.30**		**0.54**	**5.65**	**1.17**	**0.71**	**0.94**		**2.54**
$LSD_{0.01}$			**5.00**			**8.56**	**1.77**	**1.08**	**1.42**		**3.85**

Data presented in Table (12) outlined that Zn uptake (mg plant^{-1}) by stems represent the highest values among different plant parts of sunflower plant in both soils. Meanwhile, values of Zn uptake by roots comes after and lowest values were that taken by seeds.

Significant effects were found in Zn uptake (mg plant^{-1}) by sunflower plant parts due to chelator type effect as shown in Fig (28). Concerning to chelator efficiency, CA in Abu Rawash soil maximize Zn uptake by different plant parts, as well as total Zn uptake by plants.

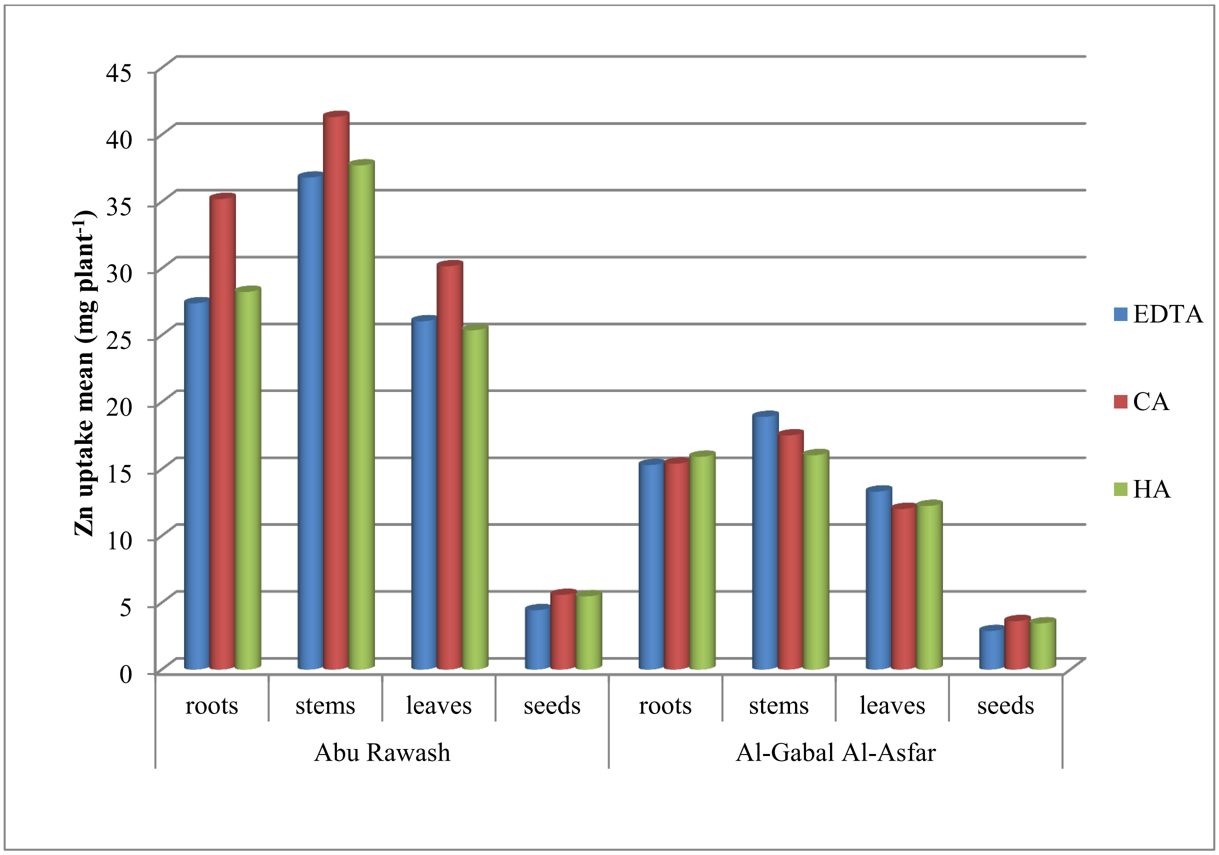


Fig (28): Effect of chelators on Zn uptake means (mg plant^{-1}) by roots, stems, leaves and seeds of sunflower grown on in Abu Rawash and Al-Gabal Al-Asfar soils.

Application of CA increased Zn uptake of roots, stems, leaves, seeds and total uptake (mg plant^{-1}) as compared to EDTA treatment by 28.48, 12.34, 15.78, 25.68 and 18.57%, respectively, in Abu Rawash soil. Whereas, HA treatment increased roots, stems, leaves and total uptake (mg plant^{-1}) of Zn as compared to EDTA treatment by 3.10, 2.47, 23.20

and 2.24%, respectively. No significant differences in Zn uptake by leaves was found between HA and EDTA treatment.

Application of CA and HA increased total uptake of Zn (mg $plant^{-1}$) by sunflower as compared to EDTA in Abu Rawash soil, where a contrary trend was found in Al-Gabal Al-Asfar soil where EDTA increased total uptake of Zn (mg $plant^{-1}$) as compared to CA and HA as shown in Fig (29).

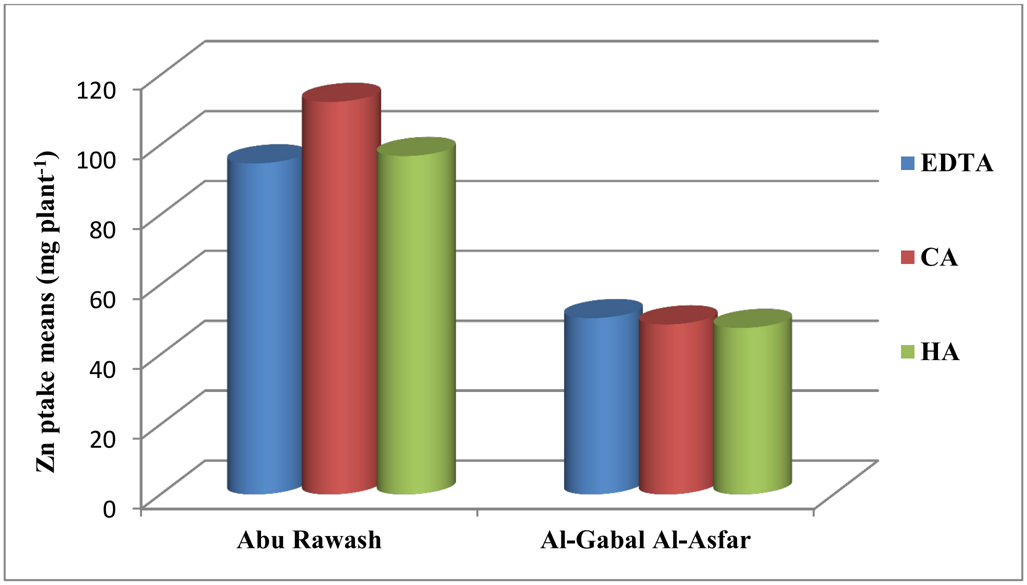


Fig (29): Effect of chelators on total uptake of Zn (mg $plant^{-1}$) by sunflower plant grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Regarding to the effect of EDTA levels on Zn uptake by sunflower, the results showed that even no significant effects were found on Zn uptake by plant parts and total uptake of Zn (mg $plant^{-1}$) in Abu Rawash soil, except for root which its Zn uptake significantly decreased with increasing EDTA levels. Where application of 4.5 mmol EDTA kg^{-1} soil decreased Zn uptake of roots by 20.80% as compared to control. The total uptake by plant was less than control due to the high decrease in plant dry weight, especially the above ground portion; with increasing EDTA level (4.5 mmol kg^{-1} reduced sunflower dry weight by 32.17%) as shown in Fig (30).

On the other hand, increasing EDTA level in Al-Gabal Al-Asfar soil significantly increased Zn uptake by plant parts than that its reduction effect on dry matter production. Accordingly, the highest values of total uptake (mg plant^{-1}) in Al-Gabal Al-Asfar soil was 1.32 fold of that of control was obtained with 3 mmol EDTA kg^{-1} treatment as shown in Fig (30).

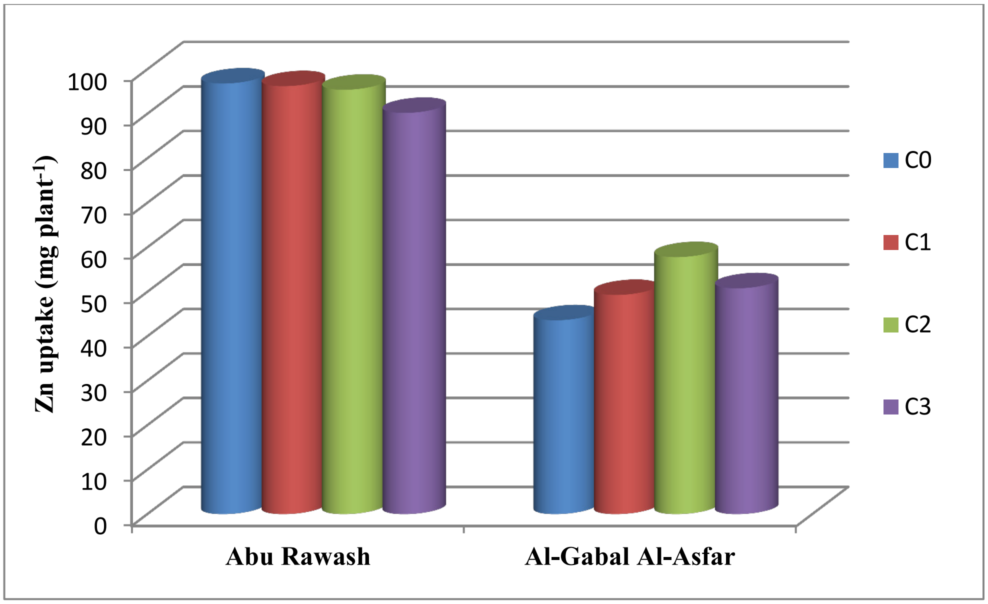


Fig (30): Effect of EDTA levels on total uptake of Zn (mg plant^{-1}) by sunflower grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Data of Table (12) reveal that in Abu Rawash soil Zn uptake by roots significantly increased by increasing CA level up to 6.0 mmol kg^{-1} soil then decreased after that. Where stems, leaves, seeds and total uptake of Zn were increased with increasing CA level from 0.0 to 3.0 mmol kg^{-1} soil then decreased by increasing CA level from 3.0 to 6.0 and 9.0 mmol kg^{-1} soil. In Al-Gabal Al-Asfar soil, the highest values of Zn uptake (mg plant^{-1}) by plant parts were noticed with 9.0 mmlo kg^{-1} soil treatment as shown in Fig (31). Zn uptake by roots, stems, leaves and seeds were 16.60, 21.77, 13.39 and 3.55 mg plant^{-1}. Whereas, the highest total uptake of Zn (mg plant^{-1}) by sunflower in the same soil was 55.31 mg plant^{-1}, which represent 26.92% increase over control.

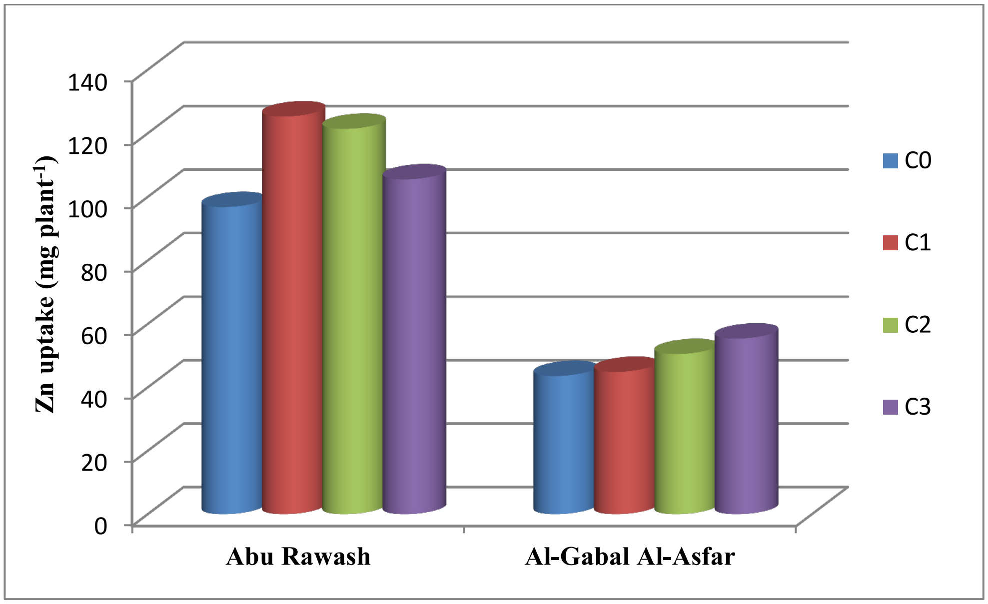


Fig (31): Effect of CA levels on total uptake of Zn (mg plant^{-1}) by sunflower grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Data plotted in Table (12) show that increasing HA level in Abu Rawash soil significantly decreased Zn uptake by roots (mg plant^{-1}), while significant increase in stems and total uptake of Zn as compared to control was noticed due to increasing HA addition rate up to 0.4 g kg^{-1} soil then decreased due to increasing HA level from 0.4 to 0.6 g Kg^{-1} soil.

No significant effect was found on plant leaves and seeds uptake of Zn (mg plant^{-1}) due to applying HA at different rates in Abu Rawash soil.

HA levels significantly affect roots, stems, leaves and total uptake of Zn (mg plant^{-1}) in Al-Gabal Al-Asfar soil, while no significant effect on seeds were found. Accordingly, using 0.6 g HA kg^{-1} soil recorded the highest values of total uptake of Zn (mg plant^{-1}) by plants which was significantly increase by 20.79% over control.

Data also illustrate that significant interactions were found between chelator types and their addition rates on Zn contents of plant parts and total uptake of Zn (mg plant^{-1}) in both soils, except for leaves in Abu Rawash soil and seeds in Al-Gabal Al-Asfar soil (no significance). Where, application of 3 mmol CA kg^{-1} soil had the highest values of total

uptake of Zn by sunflower grown on Abu Rawash soil, while application of 3.0 mmol EDTA kg^{-1} soil had the highest uptake of Zn in Al-Gabal Al-Asfar soil.

4.5.2. indian mustard plant:

Data of Table (13) represent the effect of chelator types, rates and their interactions on roots, stems, leaves, seeds and total uptake of Zn (mg plant^{-1}) by indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Table (13): Effect of chelators, their addition rates and their interaction on the uptake of Zn by roots, stems, leaves, seeds and total plant (mg plant^{-1}) by indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Zn uptake (mg plant^{-1})									
		Abu Rawash					Al-Gabal Al-Asfar				
		roots	stems	leaves	seeds	total uptake	roots	stems	leaves	seeds	total uptake
EDTA mmol kg^{-1} soil	0	32.74	21.75	21.01	3.54	79.03	16.01	9.41	6.98	1.21	33.61
	1.5	31.19	22.79	21.01	3.16	78.15	15.63	11.84	7.36	1.09	35.92
	3.0	33.09	33.55	27.19	3.36	97.20	18.76	13.56	8.75	1.18	42.25
	4.5	29.78	35.93	31.75	3.07	100.53	17.19	15.50	9.81	1.12	43.63
Mean		**31.70**	**28.51**	**25.24**	**3.28**	**88.73**	**16.90**	**12.58**	**8.22**	**1.15**	**38.85**
F-test		ns	**	**	**	**	ns	**	**	ns	**
LSD$_{0.05}$			4.34	2.75	0.26	10.40		0.99	0.90		3.07
LSD$_{0.01}$			6.77	4.28	0.41	16.20		1.54	1.41		4.78
CA mmol kg^{-1} soil	0	32.74	21.75	21.01	3.54	79.03	16.01	9.41	6.98	1.21	33.61
	3.0	37.61	25.81	20.86	3.61	87.89	16.69	10.25	7.64	1.20	35.78
	6.0	38.89	29.77	24.21	3.76	96.62	19.99	12.37	9.35	1.49	43.19
	9.0	40.69	36.68	28.45	3.84	109.65	19.91	12.62	9.74	1.64	43.92
Mean		**37.48**	**28.50**	**23.63**	**3.69**	**93.30**	**18.15**	**11.16**	**8.43**	**1.39**	**39.13**
F-test		**	**	**	ns	**	**	**	**	**	**
LSD$_{0.05}$		2.24	2.84	3.11		6.47	1.87	1.86	1.55	0.13	4.67
LSD$_{0.01}$		3.49	4.42	4.85		10.08	2.91	2.89	2.42	0.20	7.28
HA g kg^{-1} soil	0	32.74	21.75	21.01	3.54	79.03	16.01	9.41	6.98	1.21	33.61
	0.2	29.35	26.25	21.95	3.46	81.01	15.89	9.89	7.50	1.35	34.64
	0.4	36.62	32.08	24.38	4.32	97.39	17.92	10.81	8.46	1.25	38.43
	0.6	35.58	32.47	23.06	4.27	95.39	20.95	12.75	10.54	1.35	45.58
Mean		**33.57**	**28.14**	**22.60**	**3.90**	**88.21**	**17.69**	**10.72**	**8.37**	**1.29**	**38.07**
F-test		*	**	ns	*	*	**	**	**	ns	**
LSD$_{0.05}$		6.36	4.30		0.73	14.36	1.73	0.99	0.48		2.86
LSD$_{0.01}$			6.70				2.70	1.54	0.75		4.46
F-test (treatments)		*****	**ns**	*****	******	**ns**	******	******	**ns**	******	**ns**
LSD$_{0.05}$		**4.54**		**2.50**	**0.20**		**1.05**	**0.81**		**0.07**	
LSD$_{0.01}$					**0.31**		**1.59**	**1.23**		**0.11**	
F-test (Interaction)		**ns**	******	******	**ns**	**ns**	*****	*****	******	*****	*****
LSD$_{0.05}$			**2.74**	**2.17**			**0.91**	**0.70**	**0.74**	**0.06**	**2.03**
LSD$_{0.01}$			**4.15**	**3.28**					**1.13**		

Data in Table (13) reveal that chelator types significantly affect Zn uptake by different parts of indian mustard plant grown on the two studied soils, except Zn uptake by stems in Abu Rawash soil and Zn uptake by leaves in Al-Gabal Al-Asfar soil.

It is worthy to mention that no obvious trend among different chelators used on increasing or decreasing Zn uptake by different plant parts in both soils. However, application of EDTA and CA treatments increased Total uptake of Zn (mg $plant^{-1}$) by plants as compared to HA treatment by 0.59% and 5.77% in Abu Rawash soil and by 2.05% and 2.78% in Al-Gabal Al-Asfar soil, respectively as shown in Fig (32).

Regarding to the effect of different EDTA application rates on Zn uptake by different plant parts, increasing EDTA level in both soils did not significantly affect Zn uptake by plant roots, in spite of that slightly decrease, from 32.74to 29.78 mg $plant^{-1}$, was found in Abu Rawash soil due to increasing EDTA application rate from 0.0 to 4.5 mmol kg^{-1} soil. A contrary trend was found in Al-Gabal Al-Asfar soil, where Zn uptake by roots was increased from 16.01 to 17.19 mg $plant^{-1}$.

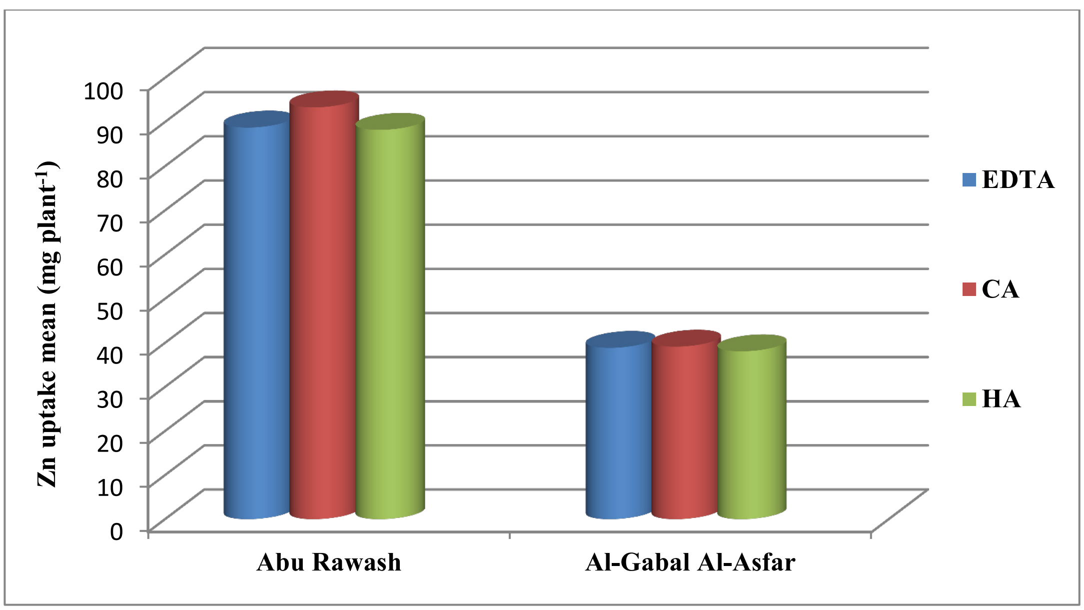


Fig (32): Effect of chelators on total uptake of Zn (mg $plant^{-1}$) by indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

indian mustard plant stems, leaves and total uptake of Zn (mg plant^{-1}) were high significantly affected by increasing EDTA levels in both Abu Rawash and Al-Gabal Al-Asfar soil. Applying 4.5 mmol EDTA kg^{-1} soil increased stems, leaves and total uptake of Zn by 65.20, 51.12 and 27.20% in Abu Rawash soil and 64.72, 40.54 and 29.81% in Al-Gabal Al-Asfar soil, respectively, than that of control (0.0 mmol EDTA kg^{-1}). This indicates that EDTA was efficient at enhancing Zn uptake by indian mustard as shown in Fig (33).

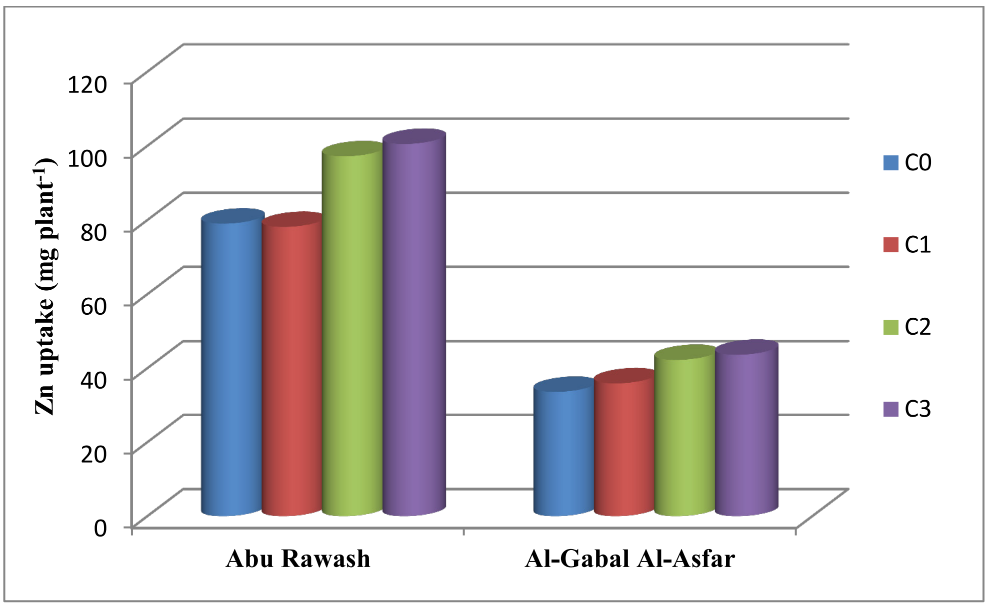


Fig (33): Effect of EDTA levels on total uptake of Zn (mg plant^{-1}) by indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Zn uptake by seeds were decreased due to application of different EDTA levels in both soils but the differences between Zn mean uptake by seeds under different EDTA levels are not significant. Zn uptake by seeds was decreased from 3.54 to 3.07 mg plant^{-1} and from 1.21 to 1.12 mg plant^{-1} due to increasing EDTA level from 0.0 to 4.5 mmol kg^{-1} soil in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

Application of different CA rates showed a deviated trend than that of EDTA, where, Zn uptake of different plant parts were increased with increasing CA application rate up to the highest level used (9 mmol kg^{-1} soil). This indicates that increasing CA application rates enhanced the total uptake of Zn by indian mustard plants as shown in Fig (34).

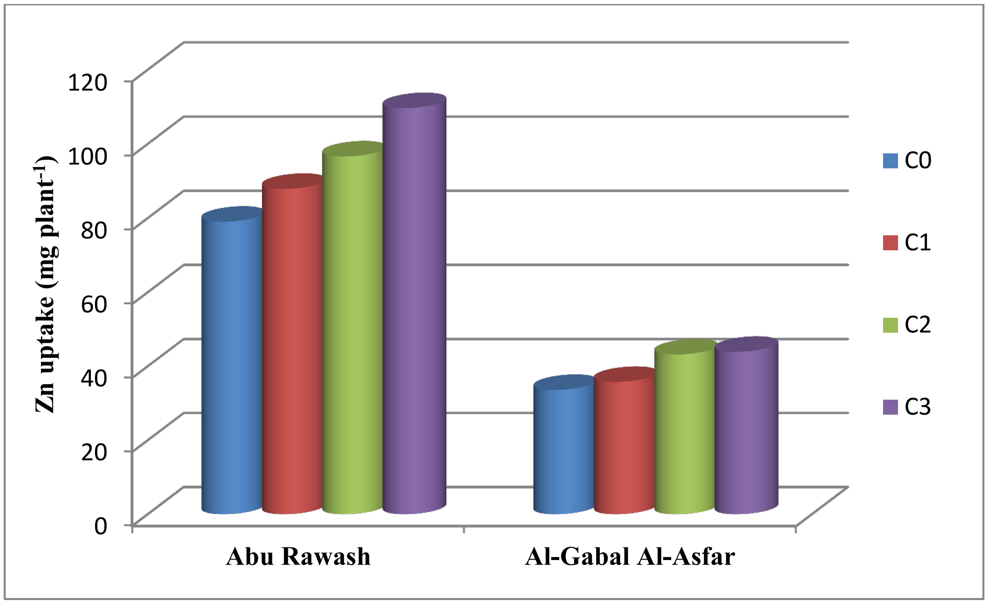


Fig (34): Effect of CA levels on total uptake of Zn (mg plant^{-1}) by indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

The obtained Zn uptake values of roots, stems, leaves, seeds and total uptake (mg plant^{-1}) of plants accompanied with 9 mmol CA kg^{-1} soil application were increased as compared to control (0.0 mmol CA kg^{-1}) by 24.28, 68.64, 35.41, 8.47 and 38.74% in Abu Rawash soil and by 24.36, 34.11, 39.54, 35.54 and 30.68% in Al-Gabal Al-Asfar soil, respectively, .

Concerning the effect of HA levels on Zn uptake by indian mustard plant in Abu Rawash soil, the results revealed that increasing HA addition rate up to 0.4 g kg^{-1} soil increased the uptake (mg plant^{-1}) of Zn, then slightly decreased with increasing HA from 0.4 to 0.6 g kg^{-1} soil as shown in Fig (35). However, these increases and decreases were significant except for leaves in Abu Rawash soil. Application of 0.4 g HA kg^{-1} soil increased Zn uptake by roots, stems, seeds and whole plant by 11.85, 47.49, 22.03 and 23.23% compared to 0.0 g HA Kg^{-1} soil.

Concerning the effect of HA levels on Zn uptake by plant parts in Al-Gabal Al-Asfar soil, the results revealed that increasing HA addition rates increased the uptake (mg plant^{-1}) of Zn up to the highest level used 0.6 g Kg^{-1} soil as shown in Fig (35). Hence, applying 0.6 g HA kg^{-1} soil significantly increased roots, stems, leaves and total uptake of Zn by 30.86,

35.49, 51.00 and 35.61% over control in Al-Gabal Al-Asfar soil, respectively. No significant effect of HA level treatments on Zn uptake by seeds were found.

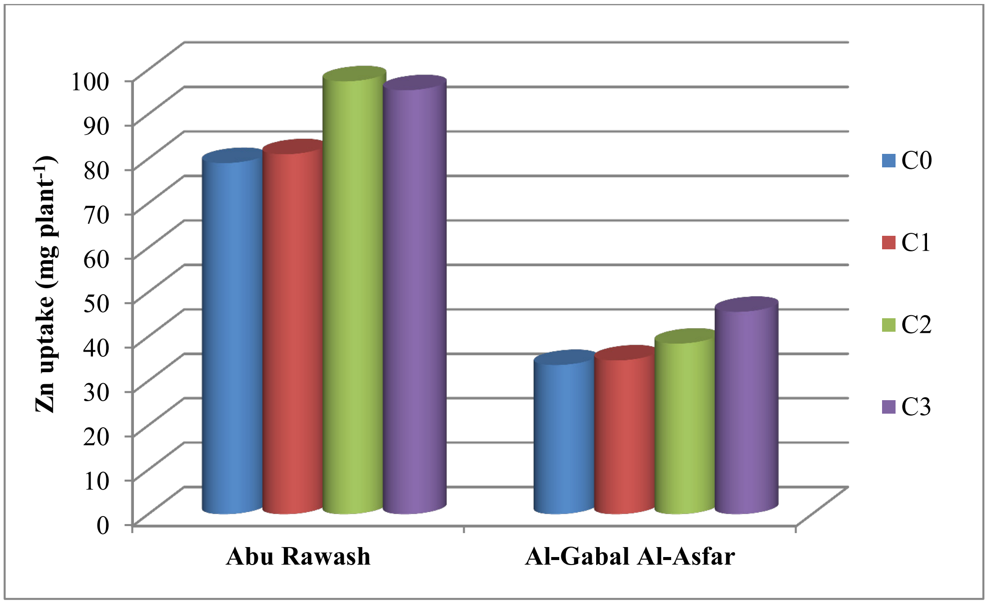


Fig (35): Effect of HA levels on total uptake of Zn (mg plant^{-1}) by indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Regarding to chelator types and their rates interaction on Zn uptake by indian mustard plant parts, the results declared that significant effects were found on the uptake of Zn (mg plant^{-1}) by stems and leaves in Abu Rawash soil, while no significant effect on Zn uptake by roots, seeds and total uptake were found. The highest values of stems uptake of Zn (36.68 mg plant^{-1}) was achieved using 9 mmol CA kg^{-1} soil, while the highest values of leaves uptake of Zn (31.75 mg plant^{-1}) were achieved using 4.5 mmol CA kg^{-1} soil.

In Al-Gabal Al-Asfar soil, significant interaction between chelator types and their rates were found on roots, stems, leaves, seeds and total uptake of Zn. The highest value of roots, leaves and total uptake of Zn, 20.95, 10.54 and 45.58 mg plant^{-1}, were achieved using 0.6 g HA kg^{-1} soil and the highest values of stems uptake of Zn, 15.50 mg plant^{-1} was achieved using 4.5 mmol EDTA kg^{-1} soil. Application of 9 mmol CA kg^{-1} soil achieved the highest Zn uptake by seeds, 1.64 mg plant^{-1}, in Al-Gabal Al-Asfar soil.

In general, application of EDTA and CA had the highest effect on increasing Zn concentration in sunflower and indian mustard plants in both soils. However, the efficiency

of phytoextraction (uptake mg plant^{-1}) was higher for CA than EDTA in all conditions due to the reduction of plants dry weight under EDTA treatment as previously discussed. These results are in harmony with those obtained by **Nascimento *et al.*, (2006)** and **Marques *et al.*, (2007)**. While, these results were contrary to those found by **Wu *et al.*, (2004)** who found that EDTA had no effect on Zn mobility and phytoavailability.

The overall results demonstrate that sunflower was more efficient than indian mustard in the phytoextraction of Zn from polluted soils. Where, the mean values of Zn total uptake by sunflower was higher than that of indian mustard when EDTA, CA and HA chelators were applied by 6.73, 20.36 and 9.74% in Abu Rawash soil and by 29.73, 24.20 and 25.03% in Al-Gabal Al-Asfar soil, respectively.

4.6. Effect of chelator types, rates and their interactions on Pb roots concentration (mg kg^{-1} DW) of sunflower and indian mustard plants grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Data of Table (14) and Figs (36 and 37) show the effect of chelator types, rates and their interactions on Pb concentrations in roots of sunflower (*Helianthus annuus L.*) and indian mustard (*Brassica juncea*) grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Data reveal that chelator types significantly affected Pb concentration of sunflower roots as shown in Fig (36). Whereas, the average concentrations of Pb in roots were 328.8, 224.2 and 202.1 mg kg^{-1} DW in Abu Rawash soil, corresponding values in Al-Gabal Al-Asfar soil were 556.8, 415.5 and 378.9 mg kg^{-1} DW accompanied with EDTA, CA and HA, respectively.

EDTA and CA increased Pb concentrations in sunflower roots compared to HA treatment by 62.69% and 10.94% in Abu Rawash soil and by 46.95% and 9.66% in Al-Gabal Al-Asfar soil, respectively.

Tabulated Data in Table (14) and Fig (37) also prove that Pb concentrations in indian mustard roots were affected by chelator types used in this study, where the average concentrations of Pb in roots were 351.7, 201.5 and 190.2 mg kg^{-1} DW in Abu Rawash soil,

corresponding values in Al-Gabal Al-Asfar soil were 517.9, 342.9 and 328.3 mg kg^{-1} DW accompanied with EDTA, CA and HA, respectively.

Table (14): Effect of chelator types, rates and their interactions on Pb roots concentration (mg kg^{-1} DW) of sunflower and indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Pb-roots concentration (mg kg^{-1} DW)			
		Sunflower		Indian mustard	
		Abu Rawash	Al-Gabal Al-Asfar	Abu Rawash	Al-Gabal Al-Asfar
EDTA mmol kg^{-1} soil	**0**	207.8	395.4	203.3	342.5
	1.5	260.0	474.4	328.3	449.2
	3.0	354.8	565.0	370.0	559.2
	4.5	492.5	792.3	505.0	720.8
Mean		**328.8**	**556.8**	**351.7**	**517.9**
F-test		**	**	**	**
$LSD_{0.05}$		63.3	66.3	48.8	64.7
$LSD_{0.01}$		98.7	103.2	76.0	100.8
CA mmol kg^{-1} soil	**0**	207.8	395.4	203.3	342.5
	3.0	210.8	402.0	186.7	351.7
	6.0	208.5	439.1	188.3	320.0
	9.0	269.5	425.3	227.5	357.5
Mean		**224.2**	**415.5**	**201.5**	**342.9**
F-test		**	ns	ns	ns
$LSD_{0.05}$		25.9			
$LSD_{0.01}$		40.3			
HA g kg^{-1} soil	**0**	207.8	395.4	203.3	342.5
	0.2	205.0	396.3	215.0	347.5
	0.4	190.7	365.0	165.0	319.2
	0.6	205.0	358.8	177.5	304.2
Mean		**202.1**	**378.9**	**190.2**	**328.4**
F-test		ns	ns	ns	ns
$LSD_{0.05}$					
$LSD_{0.01}$					
F-test (treatments)		**	**	**	**
$LSD_{0.05}$		**34.3**	**52.0**	**40.9**	**34.1**
$LSD_{0.01}$		**52.0**	**78.7**	**62.0**	**51.6**
F-test (Interaction)		**	**	**	**
$LSD_{0.05}$		**29.7**	**44.1**	**35.4**	**29.5**
$LSD_{0.01}$		**45.1**	**68.2**	**53.7**	**44.7**

EDTA and CA increased Pb concentrations in indian mustard plant roots as compared to HA treatment, these increases was appreciated by 84.91% and 5.94% in Abu Rawash soil and by 57.74% and 4.44% in Al-Gabal Al-Asfar soil, respectively.

Raising EDTA levels up to the highest level used (4.5 mmol kg^{-1} soil) significantly increased Pb concentrations in both sunflower and indian mustard plant roots in both soils used. Where, Pb concentrations in sunflower roots increased by 2.37 and 2.00 folds than that of control in Abu Rawash and Al-Gabal Al-Asfar soils, respectively. Likewise, Pb concentrations in indian mustard roots were increased by 2.48 and 2.10 folds than that of control in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

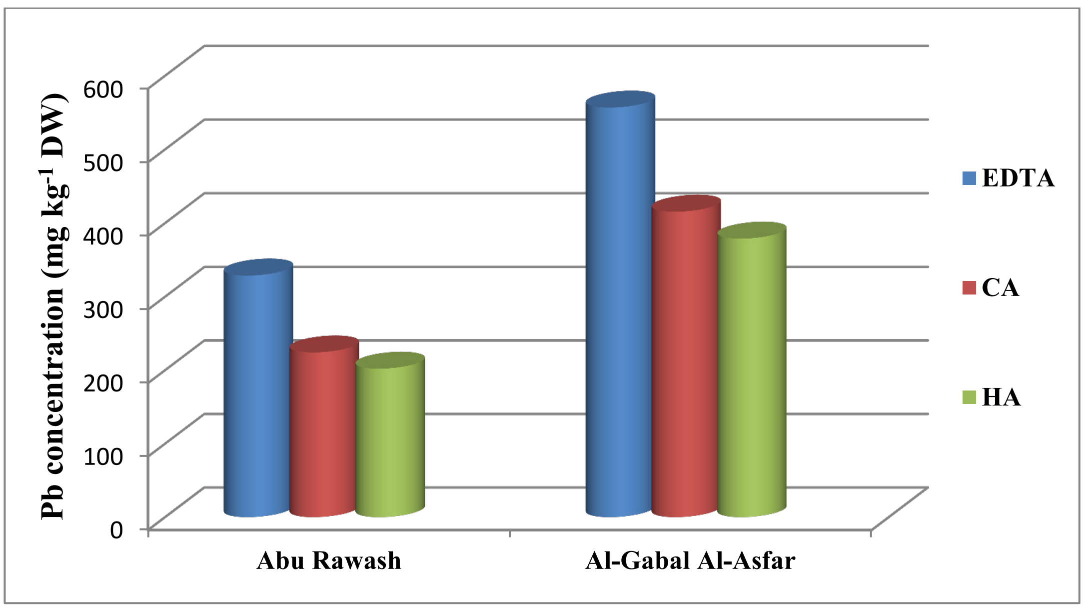


Fig (36): Effect of chelators on Pb concentration means (mg kg^{-1}) in roots of sunflower plant grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Raising CA levels up to 9 mmol kg^{-1} soil significantly increased Pb concentration of sunflower plant roots as compared to control in Abu Rawash soil. Accordingly, the highest Pb concentration of roots was appreciated by 1.30 fold of that control in Abu Rawash soil, despite of that CA levels in Al-Gabal Al-Asfar soil increased Pb concentration in roots but this increase was insignificant. These results are in parallel trend with that of **Freitas *et al.*, (2013)**.

Increasing CA levels up to 9 mmol kg^{-1} soil did not significantly affect Pb concentration of indian mustard plant roots in both Abu Rawash and Al-Gabal Al-Asfar soil, whenever the highest Pb concentration of roots, 227.5 and 357.5 mg kg^{-1} were obtained with 9 mmol kg^{-1} soil in Abu Rawash and Al-Gabal Al-Asfar soil, respectively.

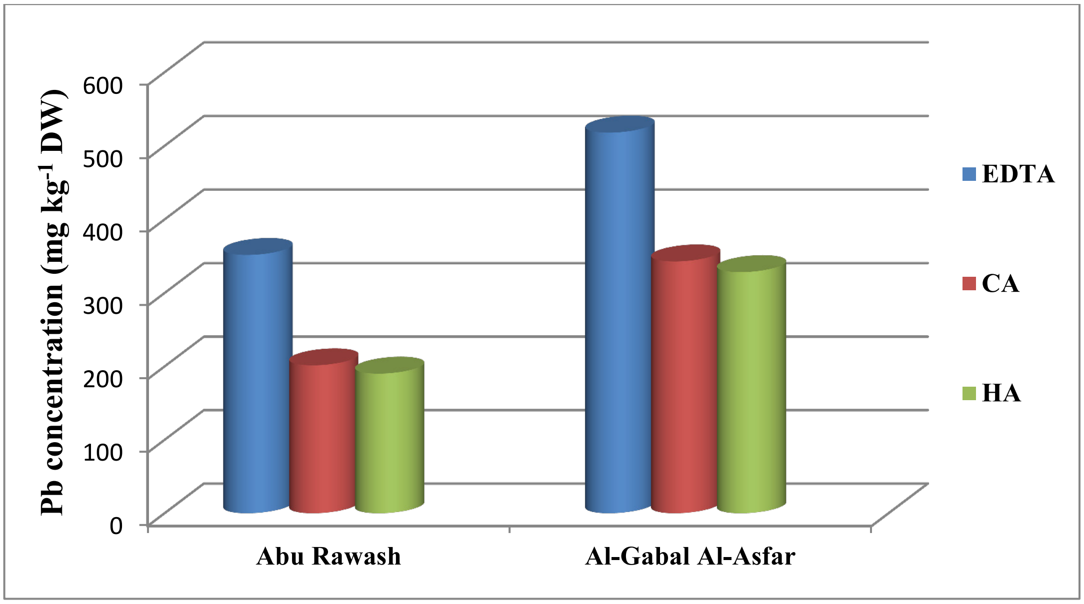


Fig (37): Effect of chelators on Pb concentration means (mg kg^{-1}) in roots of indian mustard plant grown on Abu Rawash and Al-Gabal Al-Asfar soils.

On the other hand, no significant effect was found due to increasing HA level in both soils and on both plant roots. However, a slight decrease in Pb concentrations in both plant roots were found in two soils under study as shown in Table (14), which are slightly acidic soils as shown in table (1). These results are in harmony with that of **Zhang et *al.*, (2013)** who revealed that the effect of HA effect on Pb and Cd was pH-dependent. Whereas, humic acid at a level of 4 g kg^{-1} soil decreased Pb and Cd accumulation in plant leaves in the acidic red and paddy soils by 17.78% - 48.32% and 8.74% - 32.84% for Pb and Cd, respectively. Whilst, increased leaves concentration in the alkaline (yellow) and calcareous (cinnamon) soils by 11.69% - 37.54% and 14.20% - 46.37% for Pb and Cd, respectively. As a result, humic acids could be used to reduce Pb accumulation (stabilization) in plants growing on polluted acidic soils (as in Abu Rawash and Al-Gabal Al-Asfar soils) and could be used for metal phytoremediation in alkaline soils.

Data also show that the interaction between chelator types and their application rates significantly affected Pb concentrations in sunflower roots, where the highest values, 492.5 and 792.3 mg kg^{-1}, of Pb concentration of roots were obtained using 4.5 mmol EDTA kg^{-1} soil in both Abu Rawash and Al-Gabal Al-Asfar soils, respectively. These results are in agreement with that of **Ylivainio (2010)** who stated that EDTA increases Cd, Pb, and Ni solubility steadily even in calcareous soils. This increase in plant-available metals mainly corresponds to the exchangeable and carbonate portion of soil.

Highly significant interaction effect was found too between chelator types and their application rates on Pb concentrations of indian mustard roots, where the highest values, 505.00 and 720.83 mg kg^{-1}, of Pb concentration in roots were obtained using 4.5 mmol EDTA kg^{-1} soil in both Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

It is worthy to mention that there were neither any perceptible concentrations of Pb in different plant parts of both sunflower and indian mustard species except for roots in both soils. Even with different chelating agents' levels, no effect on Pb translocation and uptake by the aerial parts of both plants had happened. In this respect, **Cunningham & Berti1 (2000) and Padmavathiamma and Li (2007)** reported that because Pb is very strongly bound in almost all types of soil, its phytoextraction is rather limited. When Pb is taken up by plants, its translocation to above-ground parts is very poor. The major proportion of Pb concentrated in root tissues. Thus, the Pb translocation from roots to tops is greatly limited, and as **Zimdahl (1975)** described, only 3% of the Pb in roots is translocated to shoots. The main process responsible for Pb accumulation in root tissue is its deposition, especially as Pb-pyrophosphate, along the cell walls (**Meyers *et al.,* 2008**).

4.7. Effect of chelator types, rates and their interaction on Pb roots uptake (mg $plant^{-1}$) by sunflower and indian mustard planted on Abu Rawash and Al-Gabal Al-Asfar soils:

Data of Table (15) and Figs (38 and 39) show the effect of chelator types, rates and their interaction on Pb net uptake by roots (mg $plant^{-1}$) of sunflower and indian mustard planted on Abu Rawash and Al-Gabal Al-Asfar soils.

Table (15): Effect of Chelator types, rates and their interaction on Pb roots uptake (mg plant^{-1}) of sunflower (*Helianthus annuus L.*) and indian mustard (*Brassica Juncea*) in Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Pb-roots uptake (mg plant^{-1})			
		Sunflower		Indian mustard	
		Abu Rawash	Al-Gabal Al-Asfar	Abu Rawash	Al-Gabal Al-Asfar
EDTA mmol kg^{-1} soil	0	5.99	7.24	8.89	10.26
	1.5	6.18	8.39	12.21	12.06
	3.0	7.62	10.10	13.76	15.12
	4.5	9.27	9.78	15.96	16.18
Mean		**7.26**	**8.88**	**12.70**	**13.41**
F-test		**	**	**	**
$LSD_{0.05}$		**1.08**	**0.94**	**1.71**	**1.34**
$LSD_{0.01}$		**1.67**	**1.47**	**2.67**	**2.09**
CA mmol kg^{-1} soil	0	5.99	7.24	8.89	10.26
	3.0	7.29	6.99	9.01	10.43
	6.0	7.10	7.43	8.88	10.57
	9.0	7.42	7.40	10.83	10.84
Mean		**6.95**	**7.26**	**9.41**	**10.52**
F-test		*	ns	**	ns
$LSD_{0.05}$		**1.02**		**0.96**	
$LSD_{0.01}$				**1.49**	
HA g kg^{-1} soil	0	5.99	7.24	8.89	10.26
	0.2	4.75	6.35	8.09	10.16
	0.4	5.29	7.31	8.17	10.20
	0.6	4.91	7.13	8.31	10.61
Mean		**5.23**	**7.01**	**8.36**	**10.31**
F-test		**	**	ns	ns
$LSD_{0.05}$		**0.53**	**0.47**		
$LSD_{0.01}$		**0.83**	**0.74**		
F-test (treatments)		**	**	**	**
$LSD_{0.05}$		**0.83**	**0.73**	**1.51**	**0.88**
$LSD_{0.01}$		**1.25**	**1.11**	**2.29**	**1.34**
F-test (Interaction)		**	*	**	**
$LSD_{0.05}$		**0.72**	**0.63**	**1.31**	**0.76**
$LSD_{0.01}$		**1.09**		**1.99**	**1.16**

Regarding to sunflower, application of different chelator treatments significantly affected Pb contents in plant roots, where the average roots net uptake of Pb took the increasing order: EDTA > CA > HA in both soils as shown in Fig (38). Applying EDTA and CA increased Pb uptake by plant roots as compared to HA by 38.81% and 32.89% in

Abu Rawash soil and by 26.68% and 3.57% in Al-Gabal Al-Asfar soil, respectively. Consequently, EDTA had the highest effect on increasing Pb uptake by plant roots, and this effect increased with increasing EDTA application rate. Hence, the highest values of Pb net uptake by roots as compared to control were 1.55 fold in Abu Rawash soil and 1.40 fold in Al-Gabal Al-Asfar soil due to EDTA application at levels of 4.5 mmol kg^{-1} and 3 mmol kg^{-1}, respectively.

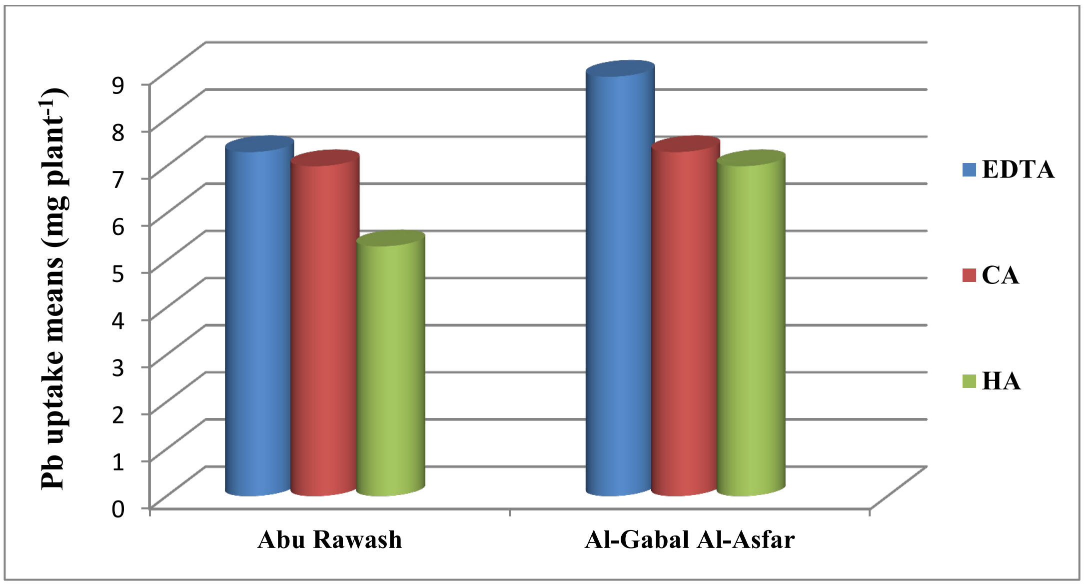


Fig (38): Effect of chelators on roots uptake means of Pb (mg $plant^{-1}$) by sunflower plant grown on Abu Rawash and Al-Gabal Al-Asfar soils.

In case of CA, despite applying different levels of CA increased Pb uptake by roots, this effect was significant in Abu Rawash soil and insignificant in Al-Gabal Al-Asfar soils. Where, the highest obtained values of Pb net uptake by roots were higher than control by 23.87% fold in Abu Rawash soil and 2.62% in Al-Gabal Al-Asfar soil when applying 9 and 6 mmol CA kg^{-1} soil, respectively. Contradictory, the application of HA significantly decreased Pb net uptake by sunflower roots (mg $plant^{-1}$) as compared to control in both soils, where the highest reduction were recorded using 0.2 g HA kg^{-1} soil which revealed decreases in Pb content in roots as compared to control by 20.70% and 12.29% in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

The uptake of Pb by sunflower roots were significantly affected by the interaction between Chelator treatments and their addition rates, where the highest values of Pb uptake

by roots were obtained using 4.5 and 3 mmol EDTA kg^{-1} soil in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

In case of indian mustard, different chelator treatments also significantly affected Pb net uptake by plant roots (mg $plant^{-1}$). Where, application of EDTA and CA increased Pb uptake by roots (mg $plant^{-1}$) in both soils as shown in Fig (39). Hence, the uptake of Pb by plant roots due to chelators application were in the order: EDTA > CA > HA in both Abu Rawash and Al-Gabal Al-Asfar soils. In this respect, using EDTA and CA increased Pb uptake by plant roots as compared to HA by 51.91% and 12.56% in Abu Rawash soil and by 30.07% and 2.04% in Al-Gabal Al-Asfar soil, respectively.

EDTA had the highest effect on increasing Pb uptake by indian mustard roots. Moreover, Pb net uptake by roots was significantly increased with increasing EDTA levels up to 4.5 mmol kg^{-1} soil. Hence, Pb uptake by roots were significantly increased by 1.80 and 1.58 folds over control due to application of 4.5 mmol EDTA kg^{-1} soil in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

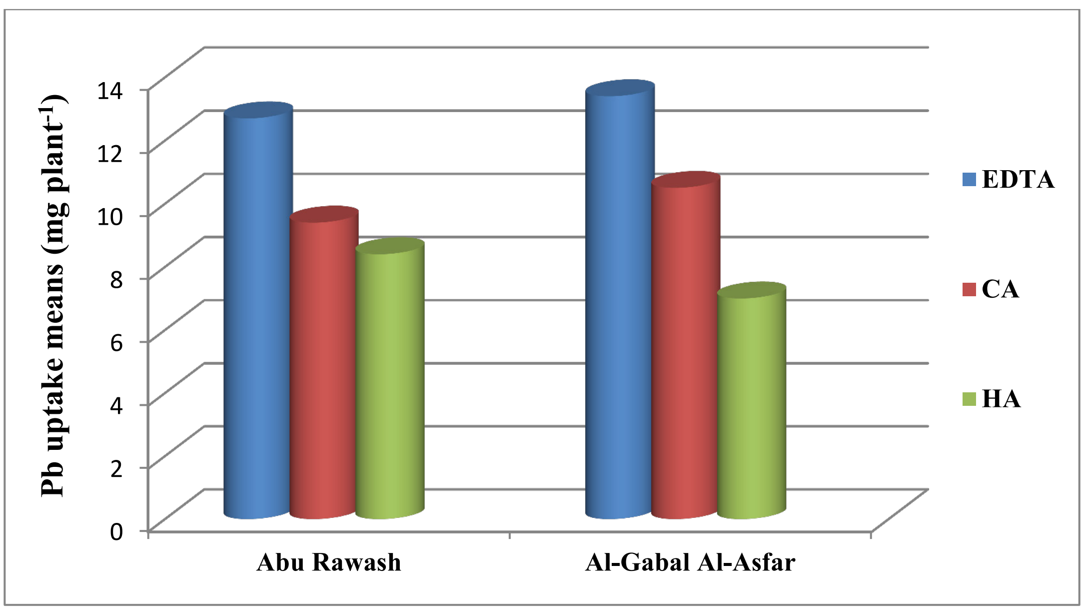


Fig (39): Effect of chelators on roots uptake means of Pb (mg $plant^{-1}$) by indian mustard plant grown on Abu Rawash and Al-Gabal Al-Asfar soils.

In case of CA, application of different rates significantly increased Pb uptake by indian mustard roots in Abu Rawash soil, where the highest value of Pb net uptake by roots (mg plant^{-1}) were recorded using CA at level of 9 mmol kg^{-1} soil which revealed increases in Pb uptake by roots by 21.82% over control. Despite application of different CA rates increased Pb uptake by roots in Al-Gabal Al-Asfar soil, especially at 9 mmol kg^{-1} which revealed increase in Pb uptake by roots (mg plant^{-1}) by 5.65% over control, this increase was insignificant.

No significant effect in Pb content in roots was found due to HA application in both soils.

Significant effects were found due to the interaction between chelator types and their addition rates on Pb uptake by indian mustard roots. The highest values of Pb uptake by roots in both Abu Rawash and Al-Gabal Al-Asfar soils roots were realized by addition of 4.5 mmol EDTA kg^{-1} soil.

4.8. Effect of chelator types, rates and their interactions on Cu, Zn and Pb leaching (mg pot^{-1}) under cultivation of Abu Rawash and Al-Gabal Al-Asfar soils:

4.8.1. Sunflower plant:

Data in Table (16) illustrate the effect of studied chelator types, their addition rates and their interactions on Cu, Zn and Pb leaching (mg pot^{-1}) under the cultivation of sunflower on Abu Rawash and Al-Gabal Al-Asfar soils.

The results show that chelate types significantly affected the leached amount of Cu, Zn and Pb in both soils under sunflower cultivation. EDTA have abnormal effect on Cu, Zn and Pb leaching than that of CA and HA in both soils as shown in Fig (40).

Table (16): Effect of chelator types, rates and their interactions on Cu, Zn and Pb leaching (mg pot^{-1}) under sunflower cultivated on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Metal leaching (mg pot^{-1})					
		Abu Rawash			Al-Gabal Al-Asfar		
		Cu	Zn	Pb	Cu	Zn	Pb
EDTA mmol kg^{-1} soil	0	0.99	8.13	0.20	0.57	4.38	0.76
	1.5	9.36	119.03	1.73	5.53	54.13	6.68
	3.0	26.26	263.72	5.57	18.49	116.96	21.03
	4.5	52.91	457.57	12.22	26.11	169.97	41.59
Mean		**22.38**	**212.12**	**4.93**	**12.68**	**86.36**	**17.51**
F-test		**	**	**	**	**	**
$LSD_{0.05}$		5.43	42.16	1.71	3.63	17.29	3.55
$LSD_{0.01}$		8.46	65.67	2.66	5.66	26.93	5.53
CA mmol kg^{-1} soil	0	0.99	8.13	0.20	0.57	4.38	0.76
	3.0	1.31	10.91	0.20	0.52	6.91	0.76
	6.0	3.74	30.39	0.21	1.07	17.52	0.79
	9.0	5.80	50.97	0.24	2.22	29.00	0.82
Mean		**2.96**	**25.10**	**0.21**	**1.10**	**14.46**	**0.78**
F-test		**	**	ns	**	**	ns
$LSD_{0.05}$		0.87	11.05		0.29	5.94	
$LSD_{0.01}$		1.35	17.22		0.45	9.25	
HA g kg^{-1} soil	0	0.99	8.13	0.20	0.57	4.38	0.76
	0.2	1.02	8.99	0.17	0.55	5.45	0.72
	0.4	0.93	8.10	0.22	0.47	4.74	0.77
	0.6	0.90	8.50	0.19	0.51	4.12	0.72
Mean		**0.96**	**8.43**	**0.19**	**0.53**	**4.67**	**0.74**
F-test		ns	ns	ns	ns	ns	ns
$LSD_{0.05}$							
$LSD_{0.01}$							
F-test (treatments)		**	**	**	**	**	**
$LSD_{0.05}$		**2.72**	**23.44**	**0.88**	**2.57**	**9.34**	**1.53**
$LSD_{0.01}$		**4.12**	**35.52**	**1.33**	**3.90**	**14.15**	**2.32**
F-test (Interaction)		**	**	**	**	**	**
$LSD_{0.05}$		**2.36**	**20.30**	**0.76**	**2.23**	**8.09**	**1.32**
$LSD_{0.01}$		**2.57**	**30.76**	**1.15**	**3.38**	**12.25**	**2.01**

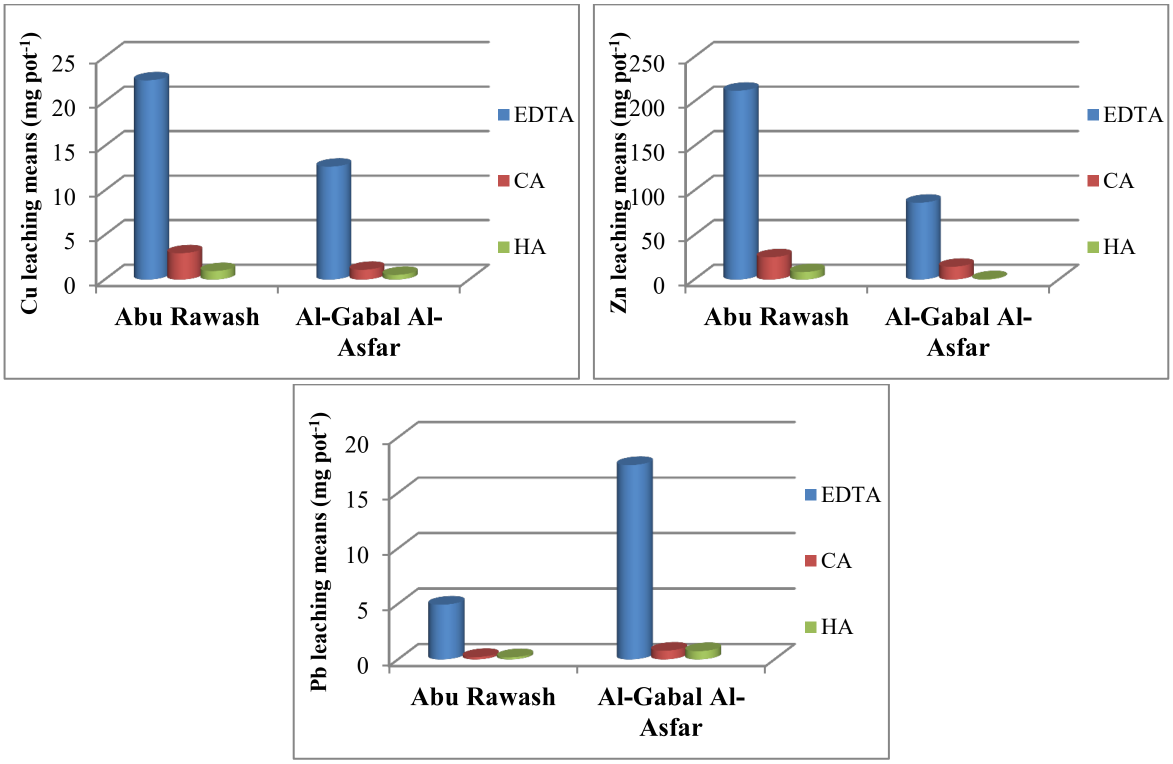


Fig (40): Effect of chelators on metals leaching means (mg pot^{-1}) under sunflower cultivated on Abu Rawash and Al-Gabal Al-Asfar soils.

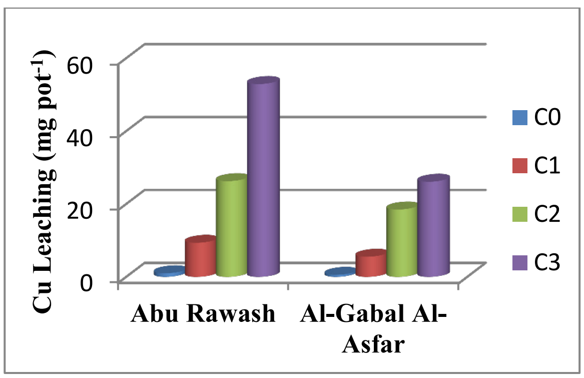
Application of EDTA increased Cu, Zn and Pb leaching as compared to HA treatment by 23.31, 25.16 and 25.95 folds in Abu Rawash soil and by 23.92, 18.49 and 23.66 folds in Al-Gabal Al-Asfar soil, respectively. Whilst, CA treatment increased Cu, Zn and Pb leaching as compared to HA treatment by 3.08, 2.98 and 1.11 folds in Abu Rawash soil and by 2.08, 3.10 and 1.05 folds in Al-Gabal Al-Asfar soil, respectively.

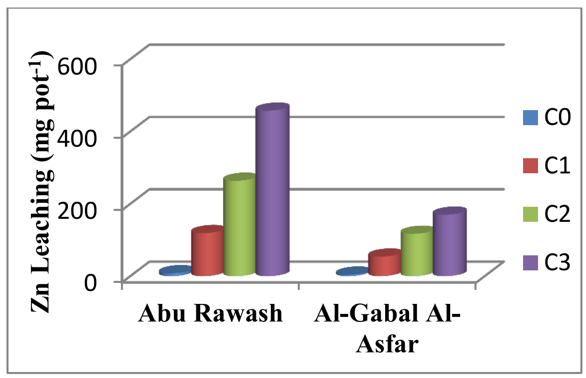
These results are in harmony with that of **Jean-Soro et *al.*, (2012**) who found that EDTA is more effective in enhancing heavy metals desorption from the soil solid phase. In most cases, the effect of EDTA was many times greater than that of CA.

Highly significant effects were found on leached amount of Cu, Zn and Pb due to EDTA levels application in both soils. On the other hand, highly significant effects were found on leached amount of Cu and Zn due to CA, while the leached amount of Pb was not significantly affected due to CA levels application in both soils. HA levels did not significantly affect any of metal leaching studied in both soil used.

Drastically increase in Cu, Zn and Pb leaching amount were found with increasing EDTA level up to 4.5 mmol kg^{-1} soil as shown in Fig (41). EDTA level of 4.5 mmol kg^{-1} soil increased the leaching amount of Cu, Zn and Pb (mg pot^{-1}) compared to the corresponding control ones by 53.44, 56.28 and 61.10 folds in Abu Rawash soil and by 45.81, 38.81 and 54.72 folds in Al-Gabal Al-Asfar soil, respectively.

These results are in conformity with **Wu et *al.*, (2004),** they found that rainfall after EDTA application, as simulated by the column leaching experiment, increased Cu, Zn, Pb and Cd concentrations linearly in leachate with increasing EDTA dosage (0–12 mmol kg^{-1} soil). Also **Alkorta et *al.*, (2004)** found that although EDTA application enhanced phytoavailability of metals, this was associated with enormously increased metal concentrations in the leachates collected below the root zone.





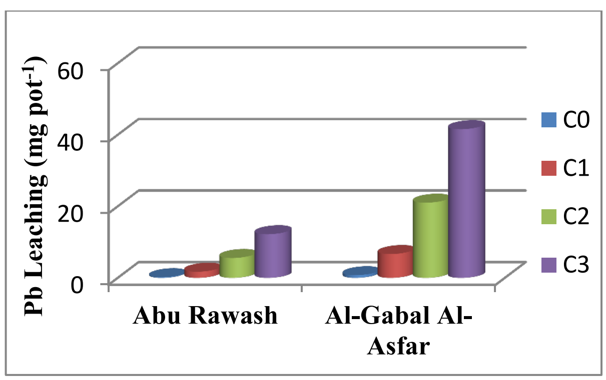


Fig (41): Effect of EDTA levels on Cu, Zn and Pb leaching (mg pot^{-1}) under sunflower cultivated on Abu Rawash and Al-Gabal Al-Asfar soils.

On the other hand, CA levels effect is Similar to EDTA levels effect with a lesser degree. The leaching of Cu, Zn and Pb increased with increasing CA level up to 9 mmol kg^{-1} soil as shown in Fig (42). Accordingly, application of 9 mmol CA kg^{-1} soil increased Cu, Zn and Pb amount in the leachate (mg pot^{-1}) as compared to the corresponding control by 5.86, 6.27 and 1.20 folds in Abu Rawash soil and by 3.89, 6.62 and 1.08 folds in Al-Gabal Al-Asfar soil, respectively.

It is worth to mention that no significant effect was found on tested metals in leachate due application of different levels of HA in both soils.

Significant effects were found due to the interaction between chelator types-rates interaction on the leaching of Cu, Zn and Pb (mg pot^{-1}) under sunflower cultivated in both soils. The highest values of Cu, Zn and Pb leaching (mg pot^{-1}) were obtained using EDTA at a level of 4.5 mmol kg^{-1} soil in both soils.

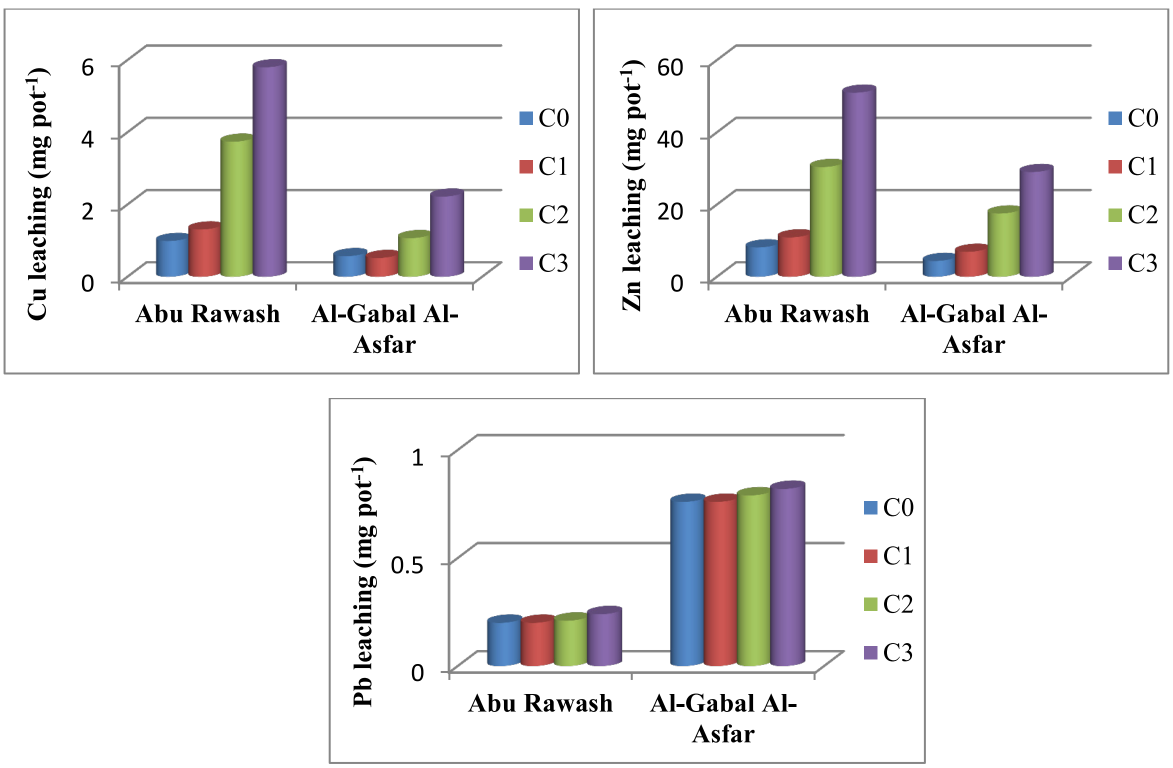


Fig (42): Effect of CA levels on Cu, Zn and Pb leaching (mg pot^{-1}) under sunflower cultivated on Abu Rawash and Al-Gabal Al-Asfar soils.

4.8.2. Indian mustard plant:

Data of Table (17) show the effect of chelator types, rates and their interactions on Cu, Zn and Pb leaching (mg pot^{-1}) under indian mustard cultivated on Abu Rawash and Al-Gabal Al-Asfar soils.

Table (17): Effect of chelator types, rates and their interactions on Cu, Zn and Pb leaching (mg pot^{-1}) under indian mustard cultivated on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Metal leaching (mg pot^{-1})					
		Abu Rawash			Al-Gabal Al-Asfar		
		Cu	Zn	Pb	Cu	Zn	Pb
EDTA mmol kg^{-1} soil	0	1.23	11.27	0.21	0.66	5.51	0.90
	1.5	12.76	123.11	2.10	6.96	57.12	8.09
	3.0	39.87	273.66	7.38	24.60	119.23	29.10
	4.5	62.52	499.57	15.71	40.81	211.02	56.63
Mean		**29.09**	**226.90**	**6.35**	**18.26**	**98.22**	**23.68**
F-test		**	**	**	**	**	**
$LSD_{0.05}$		8.21	40.31	1.99	3.87	17.61	1.38
$LSD_{0.01}$		12.80	62.78	3.10	6.03	27.43	2.15
CA mmol kg^{-1} soil	0	1.23	11.27	0.21	0.66	5.51	0.90
	3.0	2.58	16.60	0.22	0.84	9.25	0.98
	6.0	5.12	37.26	0.24	1.42	15.38	0.97
	9.0	7.26	62.36	0.26	2.89	34.05	1.05
Mean		**4.05**	**31.87**	**0.23**	**1.45**	**16.05**	**0.98**
F-test		**	**	ns	**	**	ns
$LSD_{0.05}$		0.90	11.41		0.46	6.66	
$LSD_{0.01}$		1.41	17.77		0.72	10.38	
HA g kg^{-1} soil	0	1.23	11.27	0.21	0.66	5.51	0.90
	0.2	1.10	10.89	0.16	0.67	5.40	0.87
	0.4	1.07	11.54	0.31	0.64	5.29	0.91
	0.6	1.03	9.34	0.21	0.59	6.03	0.96
Mean		**1.11**	**10.76**	**0.22**	**0.64**	**5.56**	**0.91**
F-test		ns	ns	ns	ns	ns	ns
$LSD_{0.05}$							
$LSD_{0.01}$							
F-test (treatments)		**	**	**	**	**	**
$LSD_{0.05}$		**4.27**	**26.51**	**0.80**	**2.09**	**5.53**	**0.23**
$LSD_{0.01}$		**6.48**	**40.17**	**1.21**	**3.17**	**8.38**	**0.35**
F-test (Interaction)		**	**	*	**	**	**
$LSD_{0.05}$		**3.70**	**22.96**	**0.69**	**1.81**	**4.79**	**0.20**
$LSD_{0.01}$		**5.61**	**34.79**		**2.74**	**7.26**	**0.31**

Data reveal that chelator types significantly affected Cu, Zn and Pb in both Abu Rawash and Al-Gabal Al-Asfar soils. The mean values of the leached Cu, Zn and Pb (mg pot^{-1}) due to applying EDTA are 29.09, 226.9 and 6.35 mg pot^{-1} in Abu Rawash soil and 18.26, 98.22 and 23.68 (mg pot^{-1}) in Al-Gabal Al-Asfar soil; but the mean values of Cu, Zn and Pb leached are 4.05, 31.87 and 0.23 (mg pot^{-1}) in Abu Rawash soil and 1.45, 16.05 and 0.98 mg pot^{-1} in Al-Gabal Al-Asfar under CA treatment. The mean values of the above metals are 1.11, 10.76 and 0.22 (mg pot^{-1}) in Abu Rawash soil and 0.64, 5.56 and 0.91 (mg pot^{-1}) in Al-Gabal Al-Asfar soil due to applying HA treatment.

Application of EDTA treatment increased Cu, Zn and Pb leaching than that of HA treatment by 26.21, 21.09 and 28.86 folds in Abu Rawash soil and by 28.53, 17.67 and 26.02 folds in Al-Gabal Al-Asfar soil, respectively, as shown in Fig (43). Whilst, using CA increased Cu, Zn and Pb leaching than that of HA treatment by 3.65, 2.96 and 1.05 folds in Abu Rawash soil and by 2.27, 2.89 and 1.08 folds in Al-Gabal Al-Asfar soil, respectively.

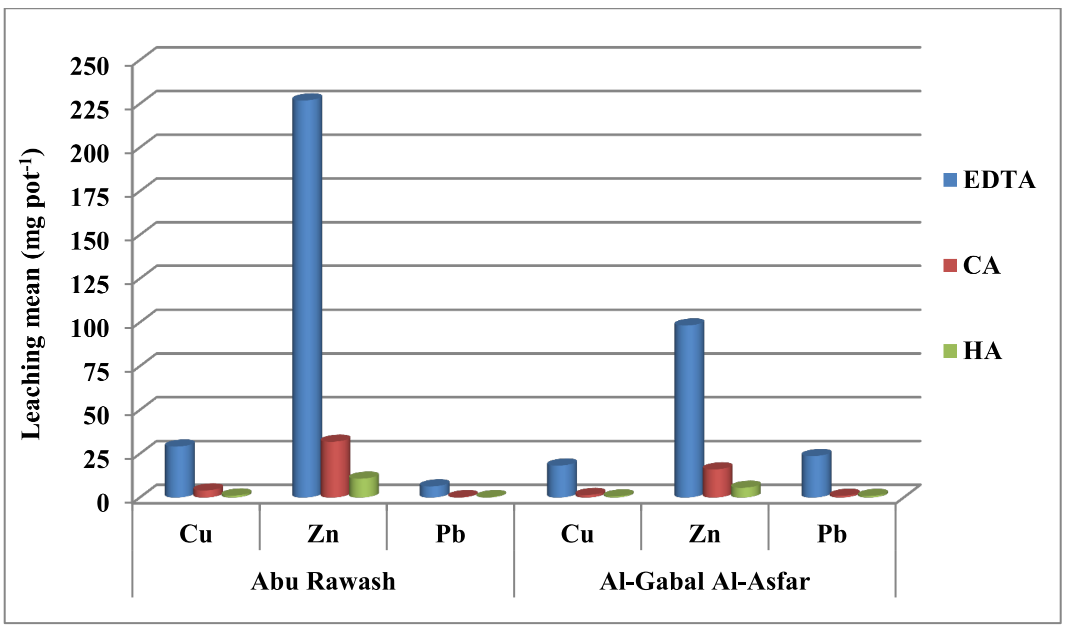


Fig (43): Effect of chelators on metals leaching means (mg pot^{-1}) under indian mustard cultivated on Abu Rawash and Al-Gabal Al-Asfar soils.

The previous results show that EDTA application had a high significant and multiplier effects on increasing the studied metals in the leachate under indian mustard cultivation in both soils. These results are in agreement with **Nascimento *et al.*, (2006)** who

compared synthetic chelates (EDTA and DTPA) with LMWOA (oxalic acid, citric acid, vanillic acid and gallic acid) in enhancing heavy metals phytoextraction by indian mustard from multi-metal contaminated soils. They reported that the effectiveness of LMWOA were not accompanied by raising the risk of leaching for these metals so unlike EDTA and DTPA.

Concerning chelator levels effect on leached studied metals, increasing EDTA level up to 4.5 mmol kg^{-1} soil linearly increased Cu, Zn and Pb in the leachate (mg pot^{-1}) as compared to the control by 50.83, 44.33 and 74.81 folds in Abu Rawash soil and by 61.83, 38.30 and 62.92 folds in Al-Gabal Al-Asfar soil, respectively, as shown in Fig (44).

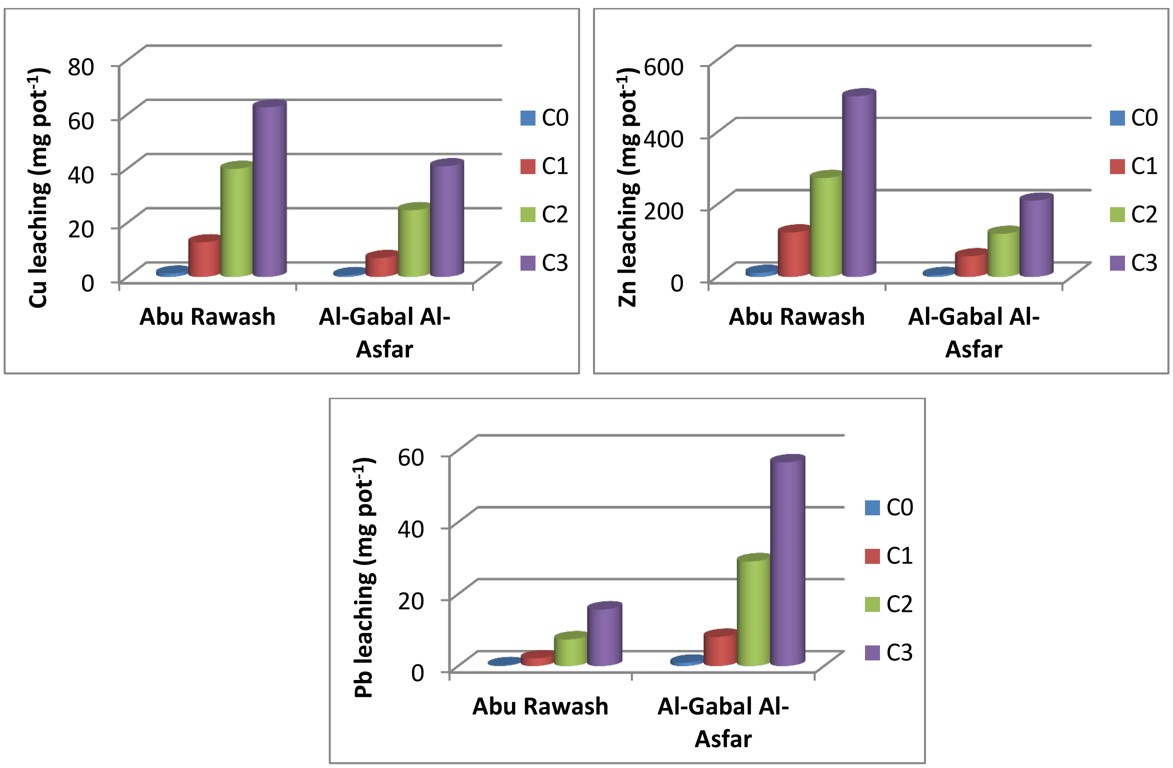


Fig (44): Effect of EDTA levels on Cu, Zn and Pb in the leachate (mg pot^{-1}) under indian mustard cultivated on Abu Rawash and Al-Gabal Al-Asfar soils.

Likewise EDTA, application of CA up to 9 mmol kg^{-1} soil significantly increased the leaching of Cu and Zn under indian mustard cultivation in both Abu Rawash and Al-Gabal Al-Asfar soil, Whilst, it did not significantly increase Pb in the leachate in both soils as shown in Fig (45). Therefore, application of 9 mmol CA kg^{-1} soil increased Cu, Zn and Pb amount in leachate (mg pot^{-1}) by 5.90, 5.53 and 1.24 folds in Abu Rawash soil and by 4.38,

6.18 and 1.17 folds in Al-Gabal Al-Asfar soil, respectively compared with control. Contradictory, application of different levels of HA did not induce any significant effect on the leaching of tested metals in both soils.

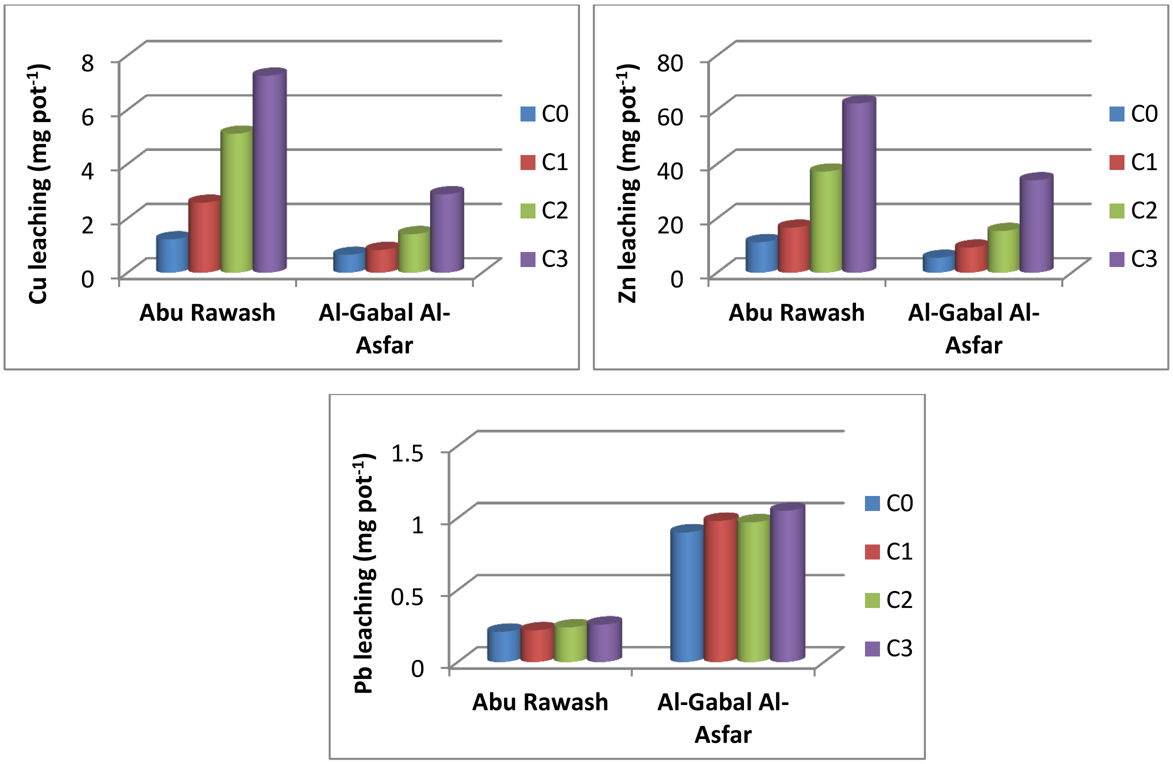


Fig (45): Effect of CA levels on Cu, Zn and Pb in the leachate (mg pot^{-1}) under indian mustard cultivated on Abu Rawash and Al-Gabal Al-Asfar soils.

Concerning the effect of the interaction between chelator type and rates on leached Cu, Zn and Pb, highly significant effect was found on these metals leaching (Cu, Zn and Pb) (mg pot^{-1}) under indian mustard cultivation on both soils. Where, the highest values of Cu, Zn and Pb leaching (62.52 and 40.81 mg pot^{-1}), (499.57 and 211.02 mg pot^{-1}) and (15.71 and 56.63 mg pot^{-1}) were obtained using EDTA at the level of 4.5 mmol kg^{-1} soil in both soils.

The leached amount of Cu and Zn metals in Abu Rawash soil was higher than that of Al-Gabal Al-Asfar soil, while, the leached amount of Pb was higher in Al-Gabal Al-Asfar soil. These results are proportionate with Cu, Zn and Pb concentrations in Abu Rawash

(170, 520 and 150 mg kg^{-1} soil, respectively) and Al-Gabal Al-Asfar soils (107, 380 and 210 mg kg^{-1}, respectively).

Commonly, application of EDTA to the soil markedly increased total amount of Cu, Zn and Pb in the leachate by too many folds under sunflower and indian mustard cultivaltion on both soils. Hence, EDTA is inappropriate for use in enhanced phytoextraction because application of higher dose of EDTA to multi-contaminated soils may be of environmental concern because of the increased risk of groundwater contamination via metal leaching. These results are in conformity with **Meers et *al.,* (2005); Wu et *al.,* (2004) and Cay *et al.,* (2016)**. **Cay *et al.,* (2016)** explained that the presence of EDTA may affect the bio-uptake of heavy metals through the formation of metal-EDTA complexes and changes the potential to leach metals below the root zone.

Compared to EDTA, CA had a small effect on metal concentrations in leachate as its effectiveness was not accompanied by increasing the risk of leaching for these metals so unlike EDTA. These results are in agreement with **Wu et *al.,* (2003); Nascimento *et al.,* (2006); Hadi *et al.,* (2010) and Miao *et al.,* (2012)**.

Application of different HA levels did not affect metal leachabilty under sunflower and indian mustard cultivation on both soils. This result was in agreement with **Halim *et al.,* (2003)** who signified that while humic substances are potentially useful to increase plant-availability of heavy metals in soil, they concomitantly reduce the environmental mobility of these contaminants.

4.9. Residual effect of chelator types, rates and their interactions on indian mustard plant parts and total dry weight (g $plant^{-1}$) grown on Abu Rawash and Al-Gabal Al-Asfar:

Data of Table (18) represent the applied chelator types, rates and their interaction effects on roots, stems, leaves, seeds and total dry weight production of indian mustard (*Brassica juncea*) in two contaminated soils.

Regarding to the residual effect of chelator types on dry matter production of plants, the obtained results demonstrated that no significant or perceptible differences in dry

weight of any plant parts were found due to previous application of EDTA, CA and HA treatments in both soils.

Table (18): Residual effect of chelator types, rates and their interactions on plant parts dry weight (g plant^{-1}) of indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Abu Rawash					Al-Gabal Al-Asfar				
		roots	stems	leaves	seeds	Total	roots	stems	leaves	seeds	Total
EDTA mmol kg^{-1} soil	0	41.3	88.5	48.9	30.5	209.2	27.4	71.7	25.9	14.6	139.6
	1.5	39.9	87.4	45.6	27.8	200.7	27.7	68.4	27.3	14.0	137.4
	3.0	39.6	89.9	44.2	28.3	202.0	28.9	68.3	23.2	14.5	134.9
	4.5	38.0	85.8	43.6	28.1	195.5	28.3	67.8	23.7	14.5	134.3
Mean		**39.7**	**87.9**	**45.6**	**28.7**	**201.9**	**28.1**	**69.1**	**25.0**	**14.4**	**136.6**
F-test		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.05}$											
$LSD_{0.01}$											
CA mmol kg^{-1} soil	0	41.3	88.5	48.9	30.5	209.2	27.4	71.7	25.9	14.6	139.6
	3.0	40.8	88.6	49.7	30.5	209.6	29.4	71.2	27.3	13.8	141.7
	6.0	39.6	95.3	47.4	30.7	213.0	29.0	68.3	25.3	13.1	135.7
	9.0	39.4	91.1	47.1	29.2	206.8	32.2	70.3	27.9	14.4	144.8
Mean		**40.3**	**90.9**	**48.3**	**30.2**	**209.7**	**29.5**	**70.4**	**26.6**	**14.0**	**140.5**
F-test		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.05}$											
$LSD_{0.01}$											
HA g kg^{-1} soil	0	41.3	88.5	48.9	30.5	209.2	27.4	71.7	25.9	14.6	139.6
	0.2	38.5	94.1	45.8	27.7	206.1	26.6	63.1	24.3	14.1	128.1
	0.4	40.0	88.8	48.6	28.3	205.7	27.9	68.0	25.8	14.3	136.0
	0.6	39.3	90.5	47.1	29.5	206.4	27.0	68.2	26.2	14.5	135.9
Mean		**39.8**	**90.5**	**47.6**	**29.0**	**206.9**	**27.2**	**67.8**	**25.6**	**14.4**	**134.9**
F-test		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.05}$											
$LSD_{0.01}$											
F-test (treatments)		**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
$LSD_{0.05}$											
$LSD_{0.01}$											
F-test (Interaction)		**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
$LSD_{0.05}$											
$LSD_{0.01}$											

The previous application of different rates of any chelator (EDTA or CA or HA) did not reveal any significant effect on the dry weight of indian mustard plants in both Abu Rawash and Al-Gabal Al-Asfar soils.

Concerning the interaction between chelator types and their addition rates effects, there is no any residual significant effect of previous treatments on dry weight of different plant parts was found in both Abu Rawash and Al-Gabal Al-Asfar soils.

4.10. Residual effect of chelator types, rates and their interaction on Cu concentration (mg kg^{-1} DW) in indian mustard plant parts grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Data in Table (19) show the residual effect of tested chelator types, rates and their interaction on Cu concentrations of roots, stems, leaves and seeds of indian mustard (*Brassica Juncea*) in Abu Rawash and Al-Gabal Al-Asfar soils.

Data pointed out that Cu concentration in different plant parts of the plants grown on both soils was not significantly affect by chlator types, except for Cu concentration of stems in Abu Rawash soil and leaves in Al-Gabal Al-Asfar soil.

Table (19): Residual effect of chelator types, rates and their interactions on Cu concentration (mg kg^{-1} DW) in indian mustard plant parts grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Cu concentration (mg kg^{-1} DW)							
		Abu Rawash				Al-Gabal Al-Asfar			
		roots	stems	leaves	seeds	roots	stems	leaves	seeds
EDTA mmol kg^{-1} soil	0	173.3	25.8	23.3	19.2	155.8	19.2	20.8	12.5
	1.5	181.7	26.7	26.7	21.7	159.2	20.0	20.8	13.3
	3.0	190.0	30.0	28.3	21.7	158.3	21.7	27.5	14.2
	4.5	213.3	33.3	31.7	20.8	170.8	24.2	29.2	14.2
Mean		**189.6**	**29.0**	**27.5**	**20.9**	**161.0**	**21.3**	**24.6**	**13.6**
F-test		**	**	ns	ns	ns	ns	**	ns
$LSD_{0.05}$		14.5	3.8					5.02	
$LSD_{0.01}$		22.5	5.9					7.82	
CA mmol kg^{-1} soil	0	173.3	25.8	23.3	19.2	155.8	19.2	20.8	12.5
	3.0	176.7	26.7	22.5	20.0	147.5	19.2	20.0	14.2
	6.0	183.3	25.0	25.0	19.2	152.5	20.0	23.3	15.8
	9.0	188.3	26.7	25.8	20.8	142.5	20.0	24.2	13.3
Mean		**180.4**	**26.1**	**24.2**	**19.8**	**149.6**	**19.6**	**22.1**	**14.0**
F-test		ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.05}$									
$LSD_{0.01}$									
HA g kg^{-1} soil	0	173.3	25.8	23.3	19.2	155.8	19.2	20.8	12.5
	0.2	185.0	24.2	24.2	21.7	160.8	21.7	20.8	14.2
	0.4	180.8	25.8	24.2	21.7	154.2	20.8	21.7	15.0
	0.6	181.7	25.0	24.2	20.0	156.7	20.0	20.8	13.3
Mean		**180.2**	**25.2**	**24.0**	**20.7**	**156.9**	**20.4**	**21.0**	**13.8**
F-test		ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.05}$									
$LSD_{0.01}$									
F-test (treatments)		**ns**	*	**ns**	**ns**	**ns**	**ns**	*	**ns**
$LSD_{0.05}$			**3.22**					**2.59**	
$LSD_{0.01}$									
F-test (Interaction)		**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
$LSD_{0.05}$									
$LSD_{0.01}$									

Tabulated data demonstrate that previous application of EDTA led to increases in Cu concentrations in roots, stems, leaves and seeds of indian mustard by 5.22, 15.08, 14.58 and 0.97% as compared to HA treatment in Abu Rawash soil. Whereas, previous application of EDTA increased Cu concentrations in roots, stems and leaves of the plants by 7.62, 8.67 and 11.31% as compared to CA treatment in Al-Gabal Al-Asfar soil. Regarding to seeds, no perceptible difference in Cu concenrations in indian mustard seeds were found due to previous application of EDTA, CA or HA treatments in Al-Gabal Al-Asfar soil.

Data of Table (19) reveal that Cu concentration in plant parts were increased with increasing previous EDTA addition rates up to 4.5 mmol kg^{-1} soil in both soils as shown in fig (46). This effect was significant for Cu concentrations of roots and stems of plants grown on Abu Rawash soil and was only significant for Cu concentrations in leaves of plants grown on in Al-Gabal Al-Asfar soil. In spite of that Cu concentrations of roots, stems, leaves and seeds of the plants were increased by 23.08, 29.07, 36.05 and 8.33% compared to control in Abu Rawash soil and by 9.63, 26.04, 40.38 and 13.60% in Al-Gabal Al-Asfar soil, respectively, when EDTA was applied at 4.5 mmol kg^{-1} soil in the previous season. This could be due to that EDTA can persist in soil for long periods of time because of its low biodegradability **(Tandy *et al.,* 2004)**. Also, **Alkorta *et al.,* (2004)** postulated that if EDTA remains in soil after the removal or harvesting of the plants, growth and development of plants grown later on the same soil or land might be affected by EDTA salt residues in the soil.

On the other hand no significant residual effect was found on Cu concentrations in different plant parts due to previous application of different levels of CA and HA in both Abu Rawash and Al-Gabal Al-Asfar soil. This could be attributed to the rapid biodegradability of CA by the indigenous microbes thereby limiting its efficacy as reported by **Elkhatib *et al.,* (2001)**, who studied the effect of various organic complexing agents (malic acid, citric acid, cetyl trimethyl ammonium bromide (CTAB) and EDTA) on the capacity of sunflower plants to accumulate Cd from two cadmium (Cd) contaminated soils.

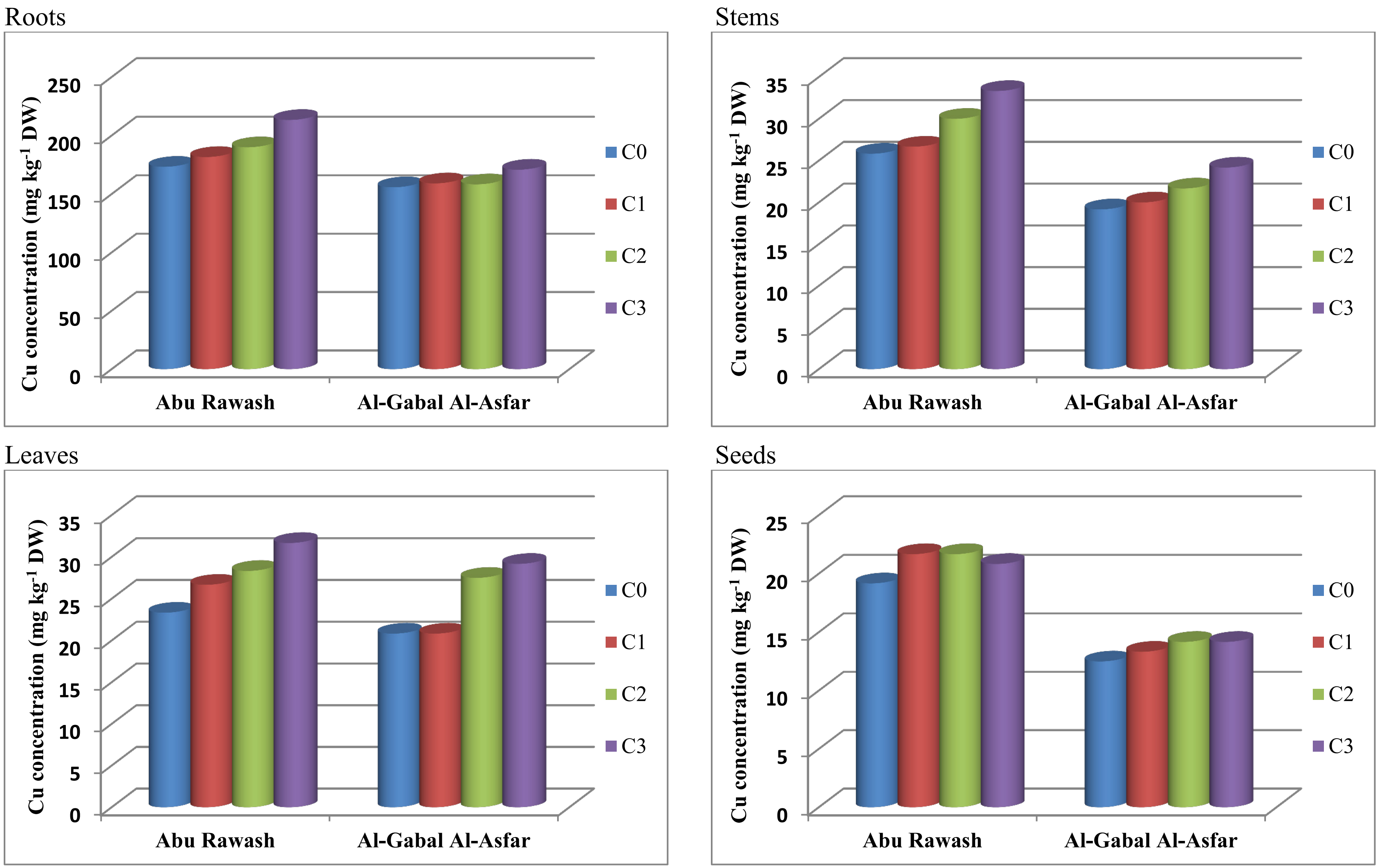


Fig (46): Residual effect of EDTA application levels on Cu concentations (mg kg^{-1} DW) in roots, stems, leaves and seeds indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

It is worthy to mention that no interaction effect was found between chelator types and their addition rates on Cu concentration in plants neither in Abu Rawash soil nor in Al-Gabal Al-Asfar soil due to previous applications of chelators. However, the highest concentrations of Cu in plant organs were obtained when EDTA was previously applied at 4.5 mmol kg^{-1} soil in both Abu Rawash and Al-Gabal Al-Asfar soils.

4.11. Residual effect of chelator types, rates and their interaction on Cu uptake (mg $plant^{-1}$) by indian mustard plant parts grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Data in Table (20) represent the residual effect of tested chelator types, rates and their interactions on roots, stems, leaves, seeds and total uptake of Cu (mg $plant^{-1}$) by indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

The results indicate that Cu uptake by each plant parts and Cu total uptake of plants vary with a narrow extent due to previous applications of EDTA, CA and HA even it is significant in some cases like Cu uptake by roots and total uptake of Cu (mg $plant^{-1}$) in both soils and Cu uptake by stems in Abu Rawash soil.

Previous application of EDTA increased roots, stems, leaves and total uptake of Cu (mg $plant^{-1}$) by plants by 5.04, 11.89, 9.73 and 6.55% in Abu Rawash soil as compared to HA treatment and by 6.12, 5.80, 13.21 and 6.45% in Al-Gabal Al-Asfar soil, respectively.

No tangible differences in seeds uptake of Cu (mg $plant^{-1}$) due to previous application of EDTA, CA and HA treatments. The differences in Cu uptake by plant due to previous application of CA and HA treatments were imperceptible.

Regarding to EDTA, Cu uptake (mg $plant^{-1}$) by indian mustard plant parts was increased with increasing EDTA levels up to 4.5 mmol kg^{-1} soil in both soils, except for seeds as shown in fig (47). Where, previous application of 4.5 mmol EDTA kg^{-1} soil significantly increased roots, leaves and total uptake of Cu (mg $plant^{-1}$) by 13.29, 20.18 and 15.70% compared with control in Abu Rawash soil and by 12.91, 27.78 and 15.59% in Al-Gabal Al-Asfar soil, respectively. Cu uptake by stems was significantly increased by 25% in Abu Rawash soil due to application of 4.5 mmol EDTA kg^{-1} soil, while the application

of the same level increased Cu uptake of stems by 18.98% compared with control in Al-Gabal Al-Asfar soil but this increase was insignificant.

Table (20): Residual effect of chelator types, rates and their interactions on Cu uptake (mg plant^{-1}) by indian mustard plant parts grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Cu uptake (mg plant^{-1})									
		Abu Rawash					Al-Gabal Al-Asfar				
		roots	stems	leaves	seeds	total uptake	roots	stems	leaves	seeds	total uptake
EDTA mmol kg^{-1} soil	**0**	7.15	2.28	1.14	0.58	11.15	4.26	1.37	0.54	0.18	6.35
	1.5	7.23	2.33	1.21	0.60	11.37	4.40	1.36	0.56	0.19	6.50
	3.0	7.51	2.69	1.25	0.61	12.07	4.57	1.47	0.63	0.20	6.88
	4.5	8.10	2.85	1.37	0.58	12.90	4.81	1.63	0.69	0.20	7.34
Mean		**7.50**	**2.54**	**1.24**	**0.59**	**11.87**	**4.51**	**1.46**	**0.60**	**0.19**	**6.77**
F-test		**	**	**	ns	**	**	ns	**	ns	**
$LSD_{0.05}$		0.21	0.18	0.12		0.31	0.26		0.07		0.25
$LSD_{0.01}$		0.33	0.28	0.19		0.49	0.40		0.10		0.40
CA mmol kg^{-1} soil	**0**	7.15	2.28	1.14	0.58	11.15	4.26	1.37	0.54	0.18	6.35
	3.0	7.17	2.36	1.11	0.61	11.25	4.31	1.37	0.54	0.19	6.41
	6.0	7.24	2.37	1.18	0.59	11.37	4.40	1.35	0.59	0.20	6.54
	9.0	7.41	2.42	1.22	0.61	11.65	4.51	1.40	0.66	0.19	6.77
Mean		**7.24**	**2.36**	**1.16**	**0.60**	**11.36**	**4.37**	**1.37**	**0.58**	**0.19**	**6.52**
F-test		ns	ns	ns	ns	ns	ns	ns	*	ns	ns
$LSD_{0.05}$									0.08		
$LSD_{0.01}$											
HA g kg^{-1} soil	**0**	7.15	2.28	1.14	0.58	11.15	4.26	1.37	0.54	0.18	6.35
	0.2	7.11	2.26	1.11	0.60	11.09	4.25	1.36	0.51	0.20	6.32
	0.4	7.16	2.29	1.16	0.61	11.21	4.29	1.41	0.56	0.21	6.47
	0.6	7.13	2.26	1.13	0.59	11.11	4.21	1.36	0.54	0.19	6.30
Mean		**7.14**	**2.27**	**1.13**	**0.59**	**11.14**	**4.25**	**1.38**	**0.53**	**0.20**	**6.36**
F-test		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.05}$											
$LSD_{0.01}$											
F-test (treatments)		*	*	**ns**	**ns**	**	*	**ns**	**ns**	**ns**	**
$LSD_{0.05}$		**0.27**	**0.18**			**0.32**	**0.24**				**0.25**
$LSD_{0.01}$						**0.48**					**0.38**
F-test (Interaction)		**	*	**ns**	**ns**	**	**ns**	**ns**	**ns**	**ns**	*
$LSD_{0.05}$		**0.24**	**0.16**			**0.26**					**0.22**
$LSD_{0.01}$		**0.36**				**0.42**					

Regarding to the residual effect of CA levels on Cu uptake by plants, the results declare that Cu uptake by plant parts was increased with increasing CA levels up to 9 mmol kg^{-1} soil in both soils. However, this effect was insignificant for all plant parts in both soils, except for leaves in Al-Gabal Al-Asfar soil. In this respect, previous application of CA at 9 mmol kg^{-1} soil increased roots, stems, leaves, seeds and total uptake of Cu (mg

plant^{-1}) compared with control by 3.64, 6.14, 7.02, 5.17 and 4.48% in Abu Rawash soil and by 5.87, 2.19, 22.22, 5.56 and 6.61% in Al-Gabal Al-Asfar soil, respectively.

Previous application, in the first season, of HA levels did not induce any residual effect on Cu uptake by indian mustard plant parts neither in Abu Rawash soil nor in Al-Gabal Al-Asfar soil.

Concerning chelator types and their addition rates interaction, significant residual effects on roots, stems and total uptake of Cu (mg plant^{-1}) by plant parts in Abu Rawash soil, while only a significant residual effect on Cu total uptake (mg plant^{-1}) by plant parts in Al-Gabal Al-Asfar was found. By any way, previous application of 4.5 mmol EDTA kg^{-1} soil recorded the highest uptake of Cu by the plant organs in both soils.

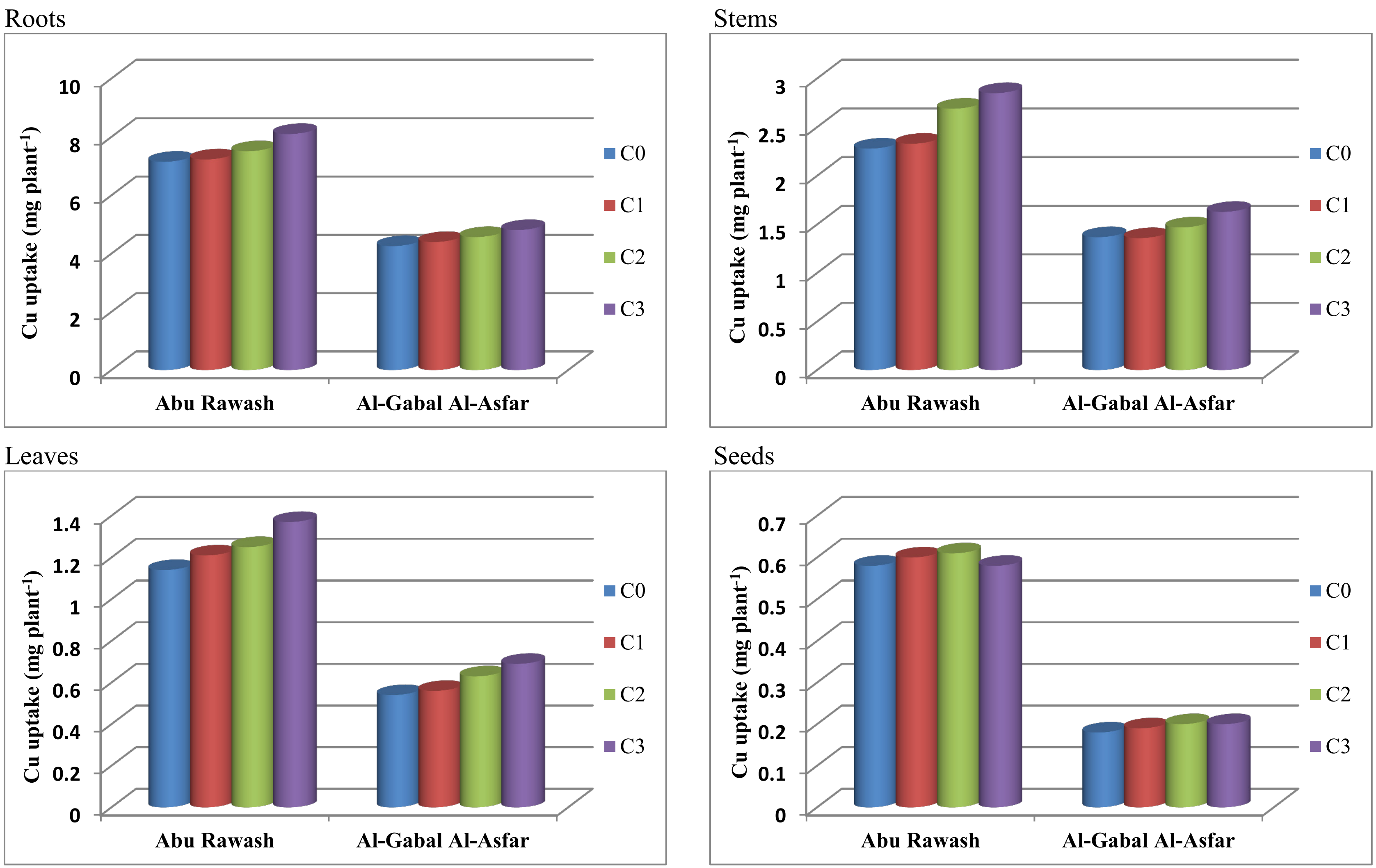


Fig (47): Residual effect of EDTA application levels on Cu uptake (mg plant^{-1}) by roots, stems, leaves and seeds of indian mustard plant grown on Abu Rawash and Al-Gabal Al-Asfar soils.

4.12. Residual effect of chelator types, rates and their interaction on Zn concentration (mg kg^{-1} DW) of indian mustard plant parts grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Data of Table (21) show the residual effect of tested chelator types, rates and their interaction on Zn concentrations of roots, stems, leaves and seeds of indian mustard cultivated in Abu Rawash and Al-Gabal Al-Asfar soils.

Table (21): Residual effect of chelator types, rates and their interactions on Zn concentration (mg kg^{-1} DW) of indian mustard plant parts grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Zn concentration (mg kg^{-1} DW)							
		Abu Rawash				Al-Gabal Al-Asfar			
		roots	stems	leaves	seeds	roots	stems	leaves	seeds
EDTA mmol kg^{-1} soil	0	738.3	217.5	392.5	96.7	473.3	102.5	238.3	70.0
	1.5	773.3	224.2	440.8	108.3	472.5	107.5	223.3	75.8
	3.0	790.0	222.5	471.7	106.7	463.3	112.5	296.7	69.2
	4.5	820.0	240.8	497.5	109.2	489.9	118.3	298.3	72.5
Mean		**780.4**	**226.3**	**450.6**	**105.2**	**474.8**	**110.2**	**264.2**	**71.9**
F-test		*	*	**	ns	ns	ns	**	ns
$LSD_{0.05}$		55.3	16.6	44.8				42.0	
$LSD_{0.01}$				69.8				65.4	
CA mmol kg^{-1} soil	0	738.3	217.5	392.5	96.7	473.3	102.5	238.3	70.0
	3.0	734.2	220.0	384.2	96.7	446.7	100.8	221.7	77.5
	6.0	775.8	203.3	415.8	98.3	466.7	108.3	247.5	80.0
	9.0	782.5	210.8	415.0	105.0	422.5	105.0	230.0	73.3
Mean		**757.7**	**212.9**	**401.9**	**99.2**	**452.3**	**104.2**	**234.4**	**75.2**
F-test		ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.05}$									
$LSD_{0.01}$									
HA g kg^{-1} soil	0	738.3	217.5	392.5	96.7	473.3	102.5	238.3	70.0
	0.2	781.7	201.7	414.2	105.0	496.7	115.0	251.7	72.5
	0.4	775.8	218.3	405.8	104.2	478.3	109.2	247.5	71.7
	0.6	786.7	212.5	414.2	100.0	495.8	107.5	243.3	70.8
Mean		**770.6**	**212.5**	**406.7**	**101.5**	**486.0**	**108.6**	**245.2**	**71.3**
F-test		ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.05}$									
$LSD_{0.01}$									
F-test (treatments)		**ns**	**ns**	*****	**ns**	**ns**	**ns**	*****	**ns**
$LSD_{0.05}$				**33.5**				**20.8**	
$LSD_{0.01}$									
F-test (Interaction)		**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
$LSD_{0.05}$									
$LSD_{0.01}$									

Concerning the residual effect of chelator types used, the results show that previous applications of EDTA treatment increased Zn concentrations in plant as compared to CA

and HA treatments in Abu Rawash soil. This effect was significant for only plant leaves. In this respect, previous application of EDTA led to increase Zn concentrations in roots, stems, leaves and seeds compared with CA treatment by 3.00, 6.29, 12.12 and 6.05% in Abu Rawash soil, respectively. Whereas, previous application of HA increased Zn concentrations of roots, leaves and seeds by 1.71, 1.19 and 2.32% as compared with CA treatment, respectively.

In Al-Gabal Al-Asfar soil, Zn concentration means in different plant parts did not took a clear trend due to previous application of different chelators.

Regarding to EDTA levels effect, previous application of EDTA increased Zn concentration in most indian mustard plant parts and this effect was increased with increasing EDTA addition rates up to 4.5 mmol kg^{-1} soil in both soils. This effect was significant for Zn concentrations in roots, stems and leaves in Abu Rawash soil and was only significant for Zn concentrations in leaves in Al-Gabal Al-Asfar soil. Zn concentrations of roots, stems, leaves and seeds were increased compared to control by 11.07, 10.71, 26.75 and 12.93% in Abu Rawash soil and by 3.51, 15.41, 25.18 and 3.57% in Al-Gabal Al-Asfar soil.

The obtained results also stated that no significant residual effect on Zn concentration in indian mustard plant parts were found due to previous application of CA or HA in both Abu Rawash and Al-Gabal Al-Asfar soil.

Concerning the interaction effect between chelator types and their addition rates, no significant residual effect on Zn concentration in the plants were found in both Abu Rawash and Al-Gabal Al-Asfar soils. However, previous application of 4.5 mmol EDTA kg^{-1} soil achieved the highest concentrations of Zn in the plant parts, except for roots and seeds in Al-Gabal Al-Asfar soil where the highest values were achieved due to previous application of 0.2 g HA kg^{-1} soil and 6 mmol CA kg^{-1} soil, respectively.

4.13. Residual effect of chelator types, rates and their interactions on Zn uptake (mg plant^{-1} DW) of indian mustard plant parts grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Data of Table (22) show the residual effect of tested chelator types and their addition rates on roots, stems, leaves, seeds and total uptake of Zn (mg plant^{-1}) by indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

Table (22): Residual effect of chelator types, rates and their interaction on Zn uptake (mg plant^{-1}) by indian mustard plant parts grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Zn uptake (mg plant^{-1})									
		Abu Rawash					Al-Gabal Al-Asfar				
		roots	stems	leaves	seeds	total uptake	roots	stems	leaves	seeds	total uptake
EDTA mmol kg^{-1} soil	**0**	30.45	19.22	19.15	2.95	71.77	12.95	7.34	6.14	1.01	27.45
	1.5	30.76	19.59	20.02	3.00	73.38	13.04	7.33	5.96	1.06	27.39
	3.0	31.26	19.98	20.83	3.02	75.08	13.37	7.68	6.83	1.00	28.87
	4.5	31.15	20.63	21.58	3.05	76.42	13.79	8.02	7.08	1.03	29.92
Mean		**30.91**	**19.85**	**20.40**	**3.01**	**74.16**	**13.28**	**7.59**	**6.50**	**1.03**	**28.40**
F-test		ns	*	**	ns	**	ns	ns	**	ns	**
$LSD_{0.05}$			0.91	0.74		1.76			0.20		0.86
$LSD_{0.01}$				1.16		2.74			0.31		1.34
CA mmol kg^{-1} soil	**0**	30.45	19.22	19.15	2.95	71.77	12.95	7.34	6.14	1.01	27.45
	3.0	29.84	19.47	18.98	2.94	71.22	13.01	7.16	5.98	1.05	27.20
	6.0	30.64	19.31	19.64	3.01	72.60	13.41	7.35	6.23	1.03	28.03
	9.0	30.82	19.14	19.54	3.06	72.56	13.38	7.37	6.34	1.04	28.14
Mean		**30.44**	**19.28**	**19.33**	**2.99**	**72.04**	**13.19**	**7.31**	**6.17**	**1.03**	**27.70**
F-test		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.05}$											
$LSD_{0.01}$											
HA g kg^{-1} soil	**0**	30.45	19.22	19.15	2.95	71.77	12.95	7.34	6.14	1.01	27.45
	0.2	30.09	18.92	18.93	2.90	70.84	13.14	7.24	6.04	1.01	27.45
	0.4	30.83	19.37	19.48	2.93	72.61	13.30	7.41	6.31	1.02	28.03
	0.6	30.89	19.19	19.42	2.94	72.44	13.31	7.33	6.30	1.02	27.96
Mean		**30.57**	**19.18**	**19.24**	**2.93**	**71.92**	**13.17**	**7.33**	**6.20**	**1.02**	**27.72**
F-test		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.05}$											
$LSD_{0.01}$											
F-test (treatments)		**ns**	**ns**	**	**ns**	*	**ns**	**ns**	*	**ns**	**ns**
$LSD_{0.05}$				**0.75**		**1.92**			**0.22**		
$LSD_{0.01}$				**1.14**							
F-test (Interaction)		**ns**	*	**ns**	**ns**	**ns**	**ns**	**ns**	**	**ns**	**
$LSD_{0.05}$			**0.79**						**0.19**		**0.86**
$LSD_{0.01}$									**0.28**		**1.31**

The results reveal that Zn uptake by plant as a residual effect of types of chelators used, previous applications of EDTA treatments increased Zn uptake (mg plant^{-1}) by plants

as compared to CA and HA treatments in both soils and this effect was significant for leaves and total uptake of Zn by plants in Abu Rawash soil and for leaves uptake of Zn in Al-Gabal Al-Asfar soil.

Previous application of EDTA increased roots, stems, leaves, seeds and total uptake of Zn (mg plant^{-1}) by indian mustard plants compared with HA treatment by 1.11, 3.49, 6.03, 2.73 and 3.11% in Abu Rawash soil and by 0.84, 3.55, 4.84, 0.98 and 2.45% in Al-Gabal Al-Asfar soil, respectively.

No perceptible differences in Zn uptake by indian mustard plants between previous application of CA and HA treatment means were found in both soils.

The obtained results also point out that the residual effect of EDTA on Zn uptake by indian mustard plant parts (mg plant^{-1}) were increased with increasing EDTA levels up to 4.5 mmol kg^{-1} soil in both soils. This effect was significant for leaves and total uptake of Zn by plants in both soils and stems in Abu Rawash soil. In Abu Rawash soil, the highest values of stems, leaves, seeds and total uptake of Zn (mg plant^{-1}) compared with control were 7.34, 12.69, 3.39 and 6.48% increase, respectively, when EDTA was previously applied at 4.5 mmol kg^{-1} soil. Whilst, previous application of 3 mmol EDTA kg^{-1} soil recorded the highest value of Zn uptake by roots as compared with control (2.66% increase over control).

Previous application of 4.5 mmol EDTA kg^{-1} soil in Al-Gabal Al-Asfar soil recorded the highest values, too, of roots, stems, leaves and total uptake of Zn (mg plant^{-1}), where these values were higher than control by 6.49, 9.26, 15.31 and 9.00%, respectively. Whereas, the highest value of Zn uptake by seeds was 4.95% higher than control, when EDTA was previously added by 1.5 mmol EDTA kg^{-1} soil.

It is clear that a significant residual effect of previous EDTA applications on Zn uptake by plants in both soils. In this respect, the values of total uptake of Zn were increased with increasing EDTA levels applied in the first season up to 4.5 mmol kg^{-1} soil as shown in Fig (48).

No significant residual effect on Zn uptake by different plant parts was found due to previous application of CA and HA different levels.

Concerning the interaction between chelator types and their addition rates, significant residual effects on shoot uptake of Zn (mg plant^{-1}) by the plant in Abu Rawash soil. Whereas, significant residual effects on leaves and total uptake of Zn (mg plant^{-1}) by indian mustard plant in Al-Gabal Al-Asfar was found. The highest uptake of Zn by the plant organs were obtained due to previous application of 4.5 mmol EDTA kg^{-1} soil, except for roots in Abu Rawash soil and seeds in Al-Gabal Al-Asfar soil.

Previous application of 3 mmol EDTA kg^{-1} soil recorded the highest value of Zn uptake by roots in Al-Gabal Al-Asfar, while the highest value of Zn uptake by seeds in Al-Gabal Al-Asfar soil were obtained when EDTA was previously added at a level of 1.5 mmol kg^{-1} soil.

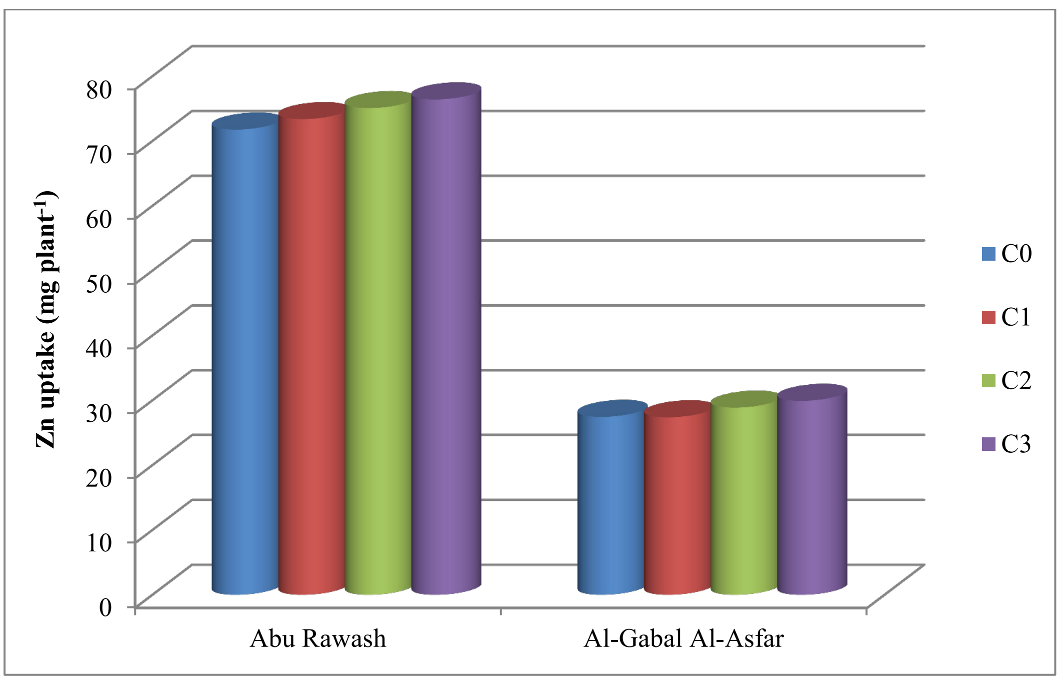


Fig (48): Residual effect of EDTA levels on total uptake of Zn (mg plant^{-1}) by indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils.

4.14. Residual effect of Chelator types, rates and their interactions on Pb root concentration (mg kg^{-1} DW) of indian mustard plant grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Data of Table (23) show the residual effect of tested chelator types, rates and their interaction on Pb concentration of indian mustard plant roots (mg plant^{-1}) in Abu Rawash and Al-Gabal Al-Asfar soils.

Table (23): Residual effect of chelator types, rates and their interaction on Pb root concentration (mg kg^{-1} DW) of indian mustard plant grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Pb-roots concentration (mg kg^{-1})	
		Abu Rawash	**Al-Gabal Al-Asfar**
EDTA mmol kg^{-1} soil	**0**	149.3	260.0
	1.5	157.3	276.7
	3.0	171.8	262.5
	4.5	179.8	268.3
Mean		**164.6**	**266.9**
F-test		ns	ns
$LSD_{0.05}$			
$LSD_{0.01}$			
CA mmol kg^{-1} soil	**0**	149.3	260.0
	3.0	147.1	240.0
	6.0	154.0	245.8
	9.0	147.8	233.3
Mean		**149.6**	**244.8**
F-test		ns	ns
$LSD_{0.05}$			
$LSD_{0.01}$			
HA g kg^{-1} soil	**0**	149.3	260.0
	0.2	158.1	262.5
	0.4	155.9	251.7
	0.6	156.7	258.3
Mean		**155.0**	**258.1**
F-test		ns	ns
$LSD_{0.05}$			
$LSD_{0.01}$			
F-test (treatments)		**ns**	**ns**
$LSD_{0.05}$			
$LSD_{0.01}$			
F-test (Interaction)		**ns**	**ns**
LSD0.05			
$LSD_{0.01}$			

The results illustrate that previous application of EDTA treatment increased the mean values of Pb concentrations in plant roots (mg plant^{-1}) compared with CA and HA treatments in both studied soils. However, this effect was insignificant in both soils. In this respect, previous application of EDTA and HA treatments increased Pb concentrations in the plant roots by 10.03 and 3.61% in Abu Rawash soil and by 9.03 and 5.43% in Al-Gabal Al-Asfar soil compared with HA treatment, respectively.

Regarding to EDTA levels, previous application of EDTA levels led to increases in Pb concentrations in plant roots up to the highest level used in Abu Rawash soil, but these increases were insignificant. EDTA was previously added at 4.5 mmol kg^{-1} soil in Abu Rawash soil increased Pb concentration of roots by 20.43% compared with control (0.0 EDTA level). EDTA previously added at 1.5 mmol kg^{-1} soil in Al-Gabal Al-Asfar soil recorded the highest increase of roots Pb concentration, 6.42% increase compared with control (0.0 EDTA level).

So, previous application of different CA or HA levels did not prove any significant or obvious effect on Pb concentration of indian mustard plant roots in both Abu Rawash and Al-Gabal Al-Asfar soils.

Concerning the interaction effect between chelator types and their addition rates, no significant residual effects on roots Pb concentration in the plant neither in Abu Rawash soil nor in Al-Gabal due Al-Asfar soil due to previous applications of different treatments. However, the highest concentrations of Pb in plant roots were obtained when EDTA was previously applied at 4.5 mmol kg^{-1} soil in both Abu Rawash, 179.8 mg kg^{-1} DW, and 1.5 mmol kg^{-1} soil in Al-Gabal Al-Asfar soils, 276.7 mg kg^{-1} DW.

4.15. Residual effect of chelator types, rates and their interactions on Pb uptake (mg plant^{-1}) by root of indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Data of Table (24) and Fig (57) show the residual effect of tested chelator types, rates and their interactions on Pb uptake by indian mustard plant roots (mg plant^{-1}) in Abu Rawash and Al-Gabal Al-Asfar soils.

Table (24): Residual effect of chelator types, rates and their interactions on Pb uptake (mg plant^{-1}) by roots of indian mustard grown on Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Pb-roots uptake (mg plant^{-1})	
		Abu Rawash	Al-Gabal Al-Asfar
EDTA mmol kg^{-1} soil	0	6.14	7.09
	1.5	6.25	7.64
	3.0	6.79	7.58
	4.5	6.82	7.53
Mean		**6.50**	**7.46**
F-test		ns	ns
$LSD_{0.05}$			
$LSD_{0.01}$			
CA mmol kg^{-1} soil	0	6.14	7.09
	3.0	5.96	7.00
	6.0	6.05	7.06
	9.0	5.82	7.37
Mean		**5.99**	**7.13**
F-test		ns	ns
$LSD_{0.05}$			
$LSD_{0.01}$			
HA g kg^{-1} soil	0	6.14	7.09
	0.2	6.08	6.93
	0.4	6.14	7.01
	0.6	6.17	6.94
Mean		**6.14**	**6.99**
F-test		ns	ns
$LSD_{0.05}$			
$LSD_{0.01}$			
F-test (treatments)		*	**ns**
$LSD_{0.05}$		0.38	
$LSD_{0.01}$			
F-test (Interaction)		**ns**	**ns**
$LSD_{0.05}$			
$LSD_{0.01}$			

Data indicate that the mean values of Pb uptake by indian mustard roots (mg plant^{-1}) were higher in soils previously treated with EDTA than that of soils treated with CA and HA in both studied soils, and this effect was significant in Abu Rawash soil and insignificant in Al-Gabal Al-Asfar soil.

Previous application of EDTA and HA treatments increased Pb uptake by roots (mg plant^{-1}) in Abu Rawash soil compared with CA treatment by 8.51 and 2.50%, respectively. Whilst, Pb uptake by plant roots was increased due to previous application of EDTA and CA compared with HA by 6.72 and 2.00% in Al-Gabal Al-Asfar soil, respectively.

Previous application of EDTA, CA and HA levels in any soil did not prove any significant effect on Pb uptake by plant roots. Even that previous application of EDTA levels increased Pb uptake by roots (mg plant^{-1}) and the highest values, 6.82 and 7.64 mg plant^{-1}, of Pb uptake by roots were obtained using EDTA at 4.5 mmol kg^{-1} soil in Abu Rawash soil and 1.5 mmol kg^{-1} soil in Al-Gabal Al-Asfer, 11.07 and 7.76%, increase compared with control (0.0 EDTA).

It is worthy to mention that no significant effect was found between chelator types and their addition rates interaction on Pb uptake by plant roots in both soils.

4.16. Resedial effect of chelator types, rates and their interaction on Cu, Zn and Pb leaching (mg pot^{-1}) under indian mustard plant cultivated in Abu Rawash and Al-Gabal Al-Asfar soils:

Data of Table (25) and Fig (49) shows the residual effect of studied chelator types, rates and their interaction on Cu, Zn and Pb leaching (mg/pot) under cultivation of indian mustard in Abu Rawash and Al-Gabal Al-Asfar soils.

Tabulated data and Fig (49) reveal that previous application of different chelates significantly affected Cu, Zn and Pb leaching in both Abu Rawash and Al-Gabal Al-Asfar soils. Previous application of EDTA increased Cu, Zn and Pb in the leachate compared with HA treatment. Leached Cu, Zn and Pb amounted by 3.24, 3.46 and 2.06 folds in Abu Rawash soil and by 2.33, 3.16 and 2.34 folds as compared to HA treatment in Al-Gabal Al-Asfar soil, respectively.

Table (25): Resedial effect of chelator types, rates and their interactions on Cu, Zn and Pb in the leachate (mg pot^{-1}) under the cultivation of indian mustard plant in Abu Rawash and Al-Gabal Al-Asfar soils:

Treatments		Metal leaching (mg pot^{-1})					
		Abu Rawash			Al-Gabal Al-Asfar		
		Cu	Zn	Pb	Cu	Zn	Pb
EDTA mmol kg^{-1} soil	0	0.80	6.04	0.16	0.31	3.44	0.78
	1.5	1.83	14.58	0.36	0.44	7.28	1.21
	3.0	2.29	23.75	0.37	0.97	13.44	1.81
	4.5	5.43	38.00	0.53	1.38	21.41	3.40
Mean		**2.59**	**20.59**	**0.35**	**0.77**	**11.39**	**1.80**
F-test		**	**	**	**	**	**
$LSD_{0.05}$		0.89	4.31	0.11	0.24	4.67	0.58
$LSD_{0.01}$		1.38	6.72	0.17	0.37	7.27	0.91
CA mmol kg^{-1} soil	0	0.80	6.04	0.16	0.31	3.44	0.78
	3.0	0.94	7.61	0.19	0.31	5.55	0.91
	6.0	1.05	11.45	0.20	0.42	6.30	0.80
	9.0	1.15	14.96	0.21	0.56	8.22	0.85
Mean		**0.99**	**10.02**	**0.19**	**0.40**	**5.88**	**0.83**
F-test		**	**	**	**	**	ns
$LSD_{0.05}$		0.18	3.83	0.03	0.10	3.44	
$LSD_{0.01}$		0.28	5.97	0.05	0.15	5.55	
HA g kg^{-1} soil	0	0.80	6.04	0.16	0.31	3.44	0.78
	0.2	0.74	5.98	0.17	0.35	4.06	0.77
	0.4	0.82	6.69	0.16	0.37	3.31	0.76
	0.6	0.84	5.09	0.18	0.31	3.58	0.78
Mean		**0.80**	**5.95**	**0.17**	**0.33**	**3.60**	**0.77**
F-test		ns	ns	ns	ns	ns	ns
$LSD_{0.05}$							
$LSD_{0.01}$							
F-test (treatments)		**	**	**	**	**	**
$LSD_{0.05}$		**0.46**	**2.10**	**0.08**	**0.12**	**2.77**	**0.18**
$LSD_{0.01}$		**0.70**	**3.18**	**0.12**	**0.19**	**4.19**	**0.28**
F-test (Interaction)		**	**	**	**	**	**
$LSD_{0.05}$		**0.40**	**1.82**	**0.07**	**0.11**	**2.40**	**0.16**
$LSD_{0.01}$		**0.60**	**2.76**	**0.11**	**0.16**	**3.63**	**0.24**

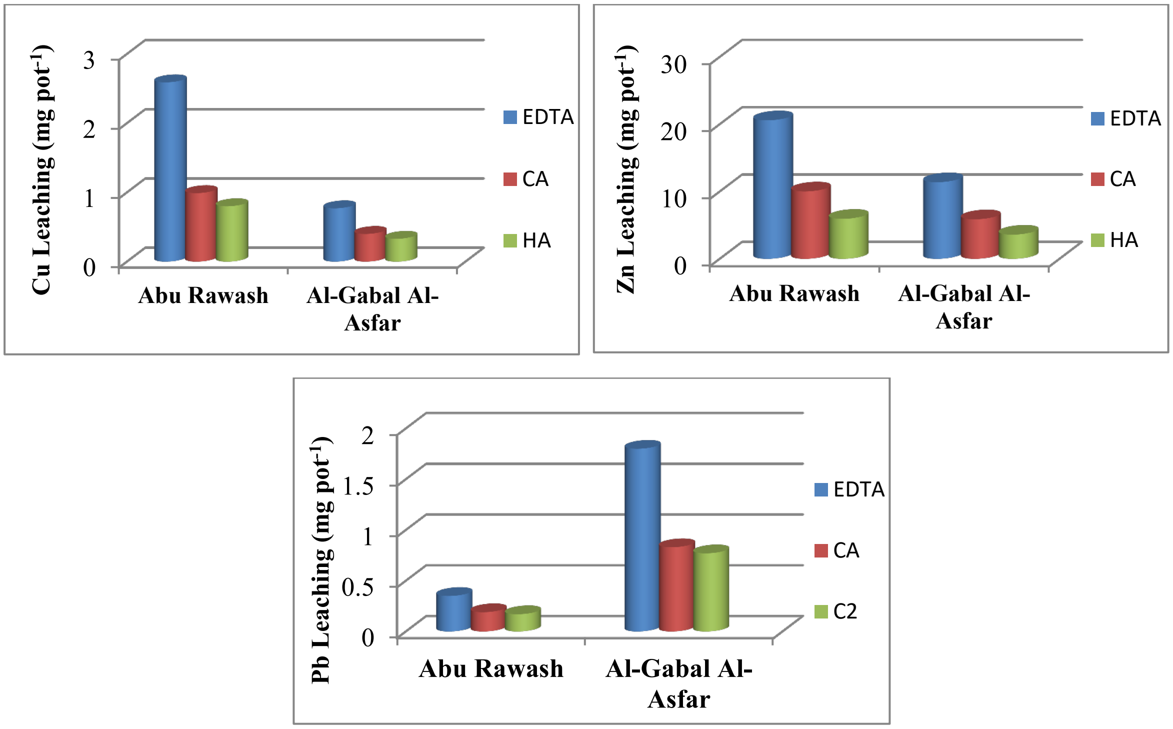


Fig (49): Residual effect of chelators on the mean values of Cu, Zn and Pb in the leachate (mg pot^{-1}) under the cultivation of indian mustard plant in Abu Rawash and Al-Gabal Al-Asfar soils.

Data of Table (25) and Fig (50) also indicate that the previous application of different EDTA levels had high significant effects on leaching of the three studied metals in both soils, where leached metals were increased with increasing EDTA level up to 4.5 mmol kg^{-1} soil. Leaching of Cu, Zn and Pb (mg pot^{-1}) were increased as compared to the corresponding control ones by 6.79, 6.29 and 3.31 folds in Abu Rawash soil and by 4.45, 6.22 and 4.36 folds in Al-Gabal Al-Asfar soil, respectively.

Due to its low biodegradability, EDTA may remain absorbed on soil particles. Its prolonged presence in the soil, and its non-selective nature, dramatically increase the leaching risk of heavy metals as reported by **Grčman *et al.*, (2003)**. This explains the previous residual effect of EDTA on the amount of Cu, Zn and Pb in the leachate of in both soils.

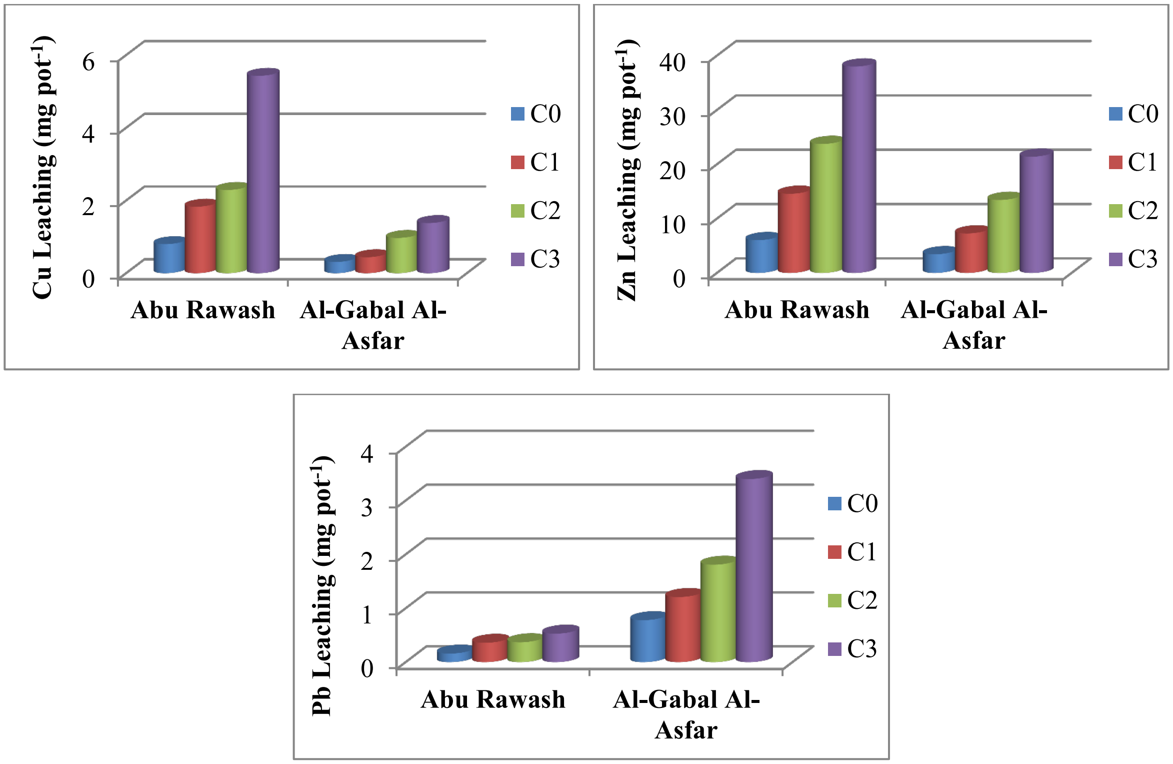


Fig (50): Effect of EDTA levels on the amount of Cu, Zn and Pb in the leachate (mg pot^{-1}) under the cultivation of indian mustard plant in Abu Rawash and Al-Gabal Al-Asfar soils.

Previous application of different CA levels in the first season also significantly increased the leached metals in both Abu Rawash and Al-Gabal Al-Asfar soils as shown in Fig (51); except for Pb leaching in Al-Gabal Al-Asfar soil (insignificant increase was found). Accordingly, application of 9 mmol CA kg^{-1} soil significantly increased Cu and Zn concentration in leachate (mg pot^{-1}) as compared to the corresponding control by 1.44 and 2.48 folds in Abu Rawash soil and by 1.81 and 2.39 folds in Al-Gabal Al-Asfar soil, respectively. Whilst, the highest values of the leached Pb were achieved due to previous application of CA at 9 mmol kg^{-1} soil in Abu Rawash soil and 3 mmol kg^{-1} soil in Al-Gabal Al-Asfar soil; corresponding increases were 1.31 and 1.17 folds over control, respectively.

It is worth mentioning that no significant effect was found on tested metals in leachate due to previous application of HA levels at the first season in both soils.

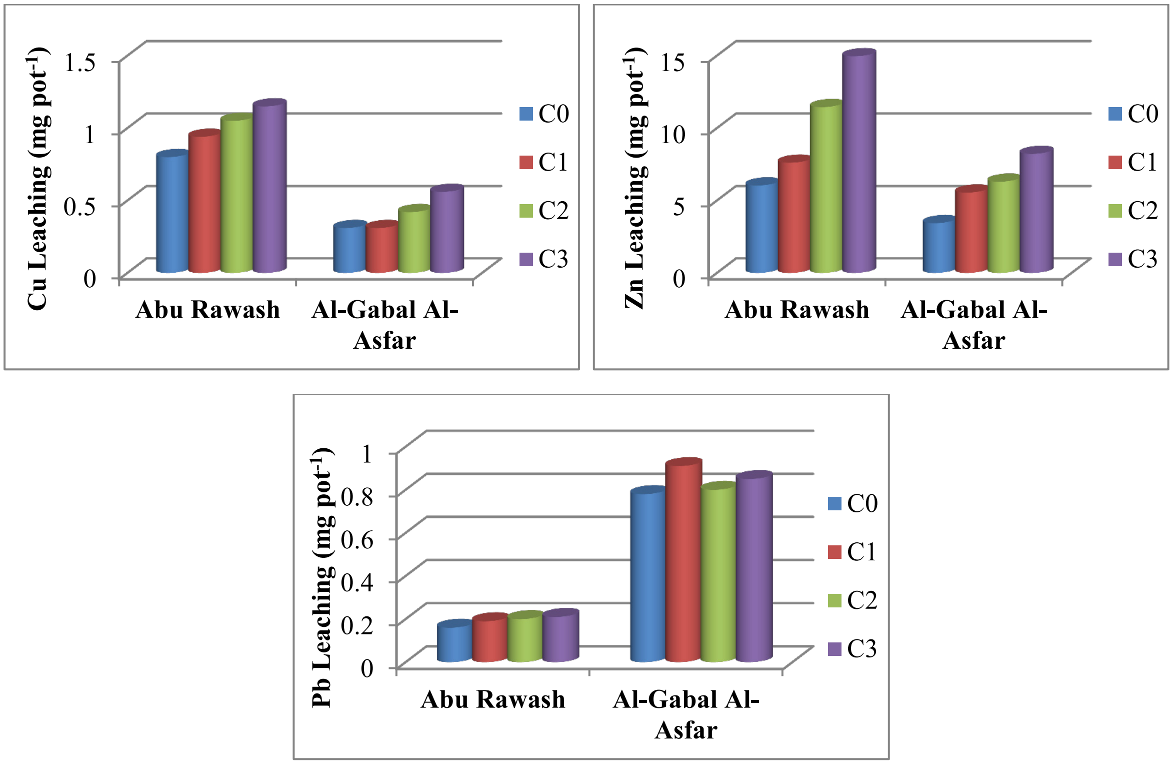


Fig (51): Effect of CA levels on the amount of Cu, Zn and Pb in the leachate (mg pot^{-1}) under cultivation of indian mustard plant in Abu Rawash and Al-Gabal Al-Asfar soils.

Significant residual effects were found due to the interaction between chelator types and their rates on the amount of Cu, Zn and Pb (mg pot^{-1}) in the leachate under the cultivation of indian mustard plant in both soils. The highest values of Cu, Zn and Pb leached amount (mg pot^{-1}) were obtained when EDTA was previously added at a rate of 4.5 mmol kg^{-1} soil in Abu Rawash and Al-Gabal Al-Asfar soils.

5. SUMMARY AND CONCLUSION

Three pot experiments were conducted in a wired greenhouse at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate. The experiments aimed to investigate the phytoextraction efficiency of Cu, Zn and Pb from contaminated soils using sunflower (*Helianthus annuus*) and indian mustard (*Brassica juncea*) under chelators addition. Two experiments were carried out to study the effect of chelating agents, EDTA (0.0, 1.5, 3.0 and 4.5 mmol kg^{-1} soil), citric acid (CA) (0, 3, 6 and 9 mmol kg^{-1} soil) and humic acid (HA) (0.0, 0.2, 0.4 and 0.6 g kg^{-1} soil), on the phytoextraction efficiency of Cu, Zn and Pb by sunflower (summer season of 2013) and indian mustard plants (winter season of 2013/2014) and leaching behavior of these metals from Abu Rawash and Al-Gabal Al-Asfar contaminated soils. Third experiment (winter season 2013/2014) was conducted to study the residual effect of chelating agents added to sunflower experiment, without any addition of chelators, on phytoextraction of tested metals by indian mustard and leaching behavior of these metals from studied soils.

The main results can be summarized as follow:

5.1. Effect of chelators on the dry weight (g $plant^{-1}$) of sunflower and indian mustard plants grown on the two studied soils:

The plant organs of sunflower were increased due to CA and HA application (especially at 3 mmol kg^{-1} soil and 6 mmol kg^{-1} soil, respectively) compared to EDTA addition in both soils. Where, EDTA addition in both studied soils led to a significant decrease in dry matter production of sunflower plant as compared to control. In this respect, visual symptoms of toxicity were observed in the EDTA treatment especially at high levels.

The highest value of indian mustard dry weight were recorded due to CA and HA application, but this effect was higher for HA treatment. While, using EDTA reduced plant dry weight yield as compared to control in both soils. Besides, visual symptoms of toxicity were observed on indian mustard similar to that happened with sunflower.

5.2. Effect of chelators on Cu concentration (mg kg^{-1} DW) in plant organs of sunflower and indian mustard plants grown on the two soils under study:

Application of EDTA and CA increased Cu concentrations of sunflower and indian mustard plant organs as compared to HA, but this effect was higher for EDTA. Application of 4.5 mmol EDTA kg^{-1} soil gave the highest concentrations of Cu in roots, stems, leaves

and seeds of both plants as compared to control in both studied soils. The increments for sunflower were 4.02, 2.47, 3.12 and 1.37 folds in Abu Rawash soil and were 4.22, 2.64, 3.91 and 1.55 fold in Al-Gabal Al-Asfar soil, respectively. While, the increments for indian mustard were 2.08, 2.44, 2.87 and 1.30 folds in Abu Rawash soil and were 1.91, 2.88, 3.04 and 1.56 folds in Al-Gabal Al-Asfar soil, respectively.

5.3. Effect of chelators on Cu uptake (mg plant^{-1}) by sunflower and indian mustard plants grown on the two soils under study:

Each of EDTA and CA treatments significantly increased the uptake of Cu by roots, stems, leaves, seeds of sunflower plant and total uptake (mg plant^{-1}) by sunflower and indian mustard plants in both studied soils. Where, Cu uptake by sunflower and indian mustard plants as affected by using different chelator treatments was in the order: EDTA > CA > HA in both soils. No constant trend regarding to the effect of HA on Cu uptake by both plants in the both soils. EDTA at 4.5 mmol kg^{-1} soil achieved the highest values of Cu total uptake by plants in both studied soils. Where, the corresponding values increased as compared to control by 2.06 and 2.43 folds in case of sunflower and by 1.70 and 1.73 folds in case of indian mustard in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

5.4. Effect of chelators on Zn concentration (mg kg^{-1} DW) in plant organs of sunflower and indian mustard plants grown on the two soils under study:

Application of EDTA, CA and HA increased Zn concentrations in plant organs of sunflower and indian mustard plants. However, the order of different chelators on increasing Zn concentrations in plants was: EDTA > CA > HA in both studied soils. The concentrations of Zn in different plant parts increased with increasing EDTA and CA levels up to the highest level used (4.5 and 9.0 mmol kg^{-1} soil, respectively). Application of 4.5 mmol EDTA kg^{-1} soil recorded the highest concentrations of Zn in both plant parts in both soils as compared to control. These increases amounted by 1.21, 1.51, 1.39 and 1.15 folds in Abu Rawash soil and by 1.26, 1.96, 1.55 and 1.13 folds in Al-Gabal Al-Asfar soil, respectively, for sunflower plant. Whereas, these increases amounted by 26.28, 91.30, 77.00 and 15.75% in Abu Rawash soil and by 43.30, 90.82, 83.08 and 11.19% in Al-Gabal Al-Asfar soil, respectively, for indian mustard plant.

5.5. Effect of chelators on Zn uptake (mg plant^{-1}) by sunflower and indian mustard plants grown on the two soils under study:

Application of CA and HA treatment increased Zn uptake of sunflower plants (mg plant^{-1}) as compared to EDTA treatment in Abu Rawash soil. Whilst, a contrary trend was found in Al-Gabal Al-Asfar soil where EDTA increased Zn uptake (mg plant^{-1}) as compared to CA and HA. Application of 3 mmol CA kg^{-1} soil had the highest values of total uptake of Zn by plant in Abu Rawash soil (29.39% increase over control), while application of 3 mmol EDTA kg^{-1} soil had the highest uptake of Zn by sunflower in Al-Gabal Al-Asfar soil (32.45% increase over control).

Regarding to indian mustard plant, application of chelator treatments significantly increased the total uptake of different plant parts in both soils. However, application of EDTA and CA treatments increased total uptake of Zn (mg plant^{-1}) by plants as compared to HA treatment. Increasing the applied rate of EDTA and CA treatments up to the highest levels used (4.5 and 9 mmol kg^{-1} soil, respectively) resulted in increasing Zn uptake by indian mustard plants in both studied soils.

5.6. Effect of chelators on Pb roots concentration (mg kg^{-1} DW) of sunflower and indian mustard plants grown on the two soils under study:

Application of EDTA and CA treatments increased Pb concentrations in roots of both plants compared to HA treatment in both soils (the order was: EDTA > CA > HA). However, Pb concentrations in both plants roots increased with increasing EDTA and CA levels in both soils. The highest values of Pb concentration in roots of both plants were obtained due to using 4.5 mmol EDTA kg^{-1} soil in both studied soils, respectively. The increments for sunflower were 2.37 and 2.00 folds compared to control in Abu Rawash and Al-Gabal Al-Asfar soils, and the increments for indian mustard were 2.48 and 2.10 folds compared to control in Abu Rawash and Al-Gabal Al-Asfar soils.

5.7. Effect of chelators on Pb roots uptake (mg plant^{-1}) of sunflower and indian mustard plants grown on the two soils under study:

The uptake of Pb (mg plant^{-1}) by both plant roots due to EDTA and CA treatments application were increased compared to HA treatment in both soils. The uptake of Pb (mg plant^{-1}) by roots of both plant species due to application of chelator treatments were in the order: EDTA > CA > HA in both soils. The highest values of Pb uptake by indian mustard

and sunflower roots in both studied soils were realized by addition of 4.5 mmol EDTA kg^{-1} soil. Where, the increments in Abu Rawash and Al-Gabal Al-Asfar soils were 1.55 and 1.40 folds for sunflower roots and 1.80 and 1.58 folds for indian mustard roots as compared to control respectively.

5.8. Effect of chelators on Cu, Zn and Pb leaching (mg pot^{-1}) under cultivation of sunflower and indian mustard plants in the two soils under study:

EDTA had abnormal effects on Cu, Zn and Pb leaching than that of CA and HA in both soils, causing serious environmental threat due to the leaching of metals towards groundwater. CA also has a similar effect to that of EDTA, but to a lesser extent, on the leaching of these metals. HA levels did not affect any of metal leaching studied in both soil used. The highest values of Cu, Zn and Pb leaching (mg pot^{-1}) under cultivation of both plants were obtained using EDTA at a level of 4.5 mmol kg^{-1} soil. The corresponding increases were 53.44, 56.28 and 61.10 folds over control in Abu Rawash soil and 45.81, 38.81 and 54.72 folds over control in Al-Gabal Al-Asfar soil, respectively, under sunflower cultivation. Whereas, the corresponding increases were 50.83, 44.33 and 74.81 folds over control in Abu Rawash soil and 61.83, 38.30 and 62.92 folds over control in Al-Gabal Al-Asfar soil, respectively, under indian mustard cultivation.

5.9. Residual effect of chelators on indian mustard dry weight (g $plant^{-1}$) grown on the two studied soils:

the obtained results demonstrated that no significant or perceptible differences in dry weight of any indian mustard plant parts were found due to previous application of EDTA, CA and HA treatments in both soils.

5.10. Residual effect of chelators on Cu concentration (mg kg^{-1} DW) in plant organs and Cu uptake (mg $plant^{-1}$) by indian mustard grown on the two soils under study:

Previous application of EDTA has significant residual effects on Cu concentrations in some indian mustard organs in both studied soils. On the other hand no significant residual effect was found on Cu concentrations in different plant parts due to previous application of different levels of CA and HA in both soils. The highest concentrations of Cu in indian mustard organs were obtained when EDTA was previously applied at 4.5 mmol kg^{-1} soil in both soils, where the corresponding increases percentage for roots, stems, leaves and seeds

compared to control were 23.08, 29.07, 36.05 and 8.33% compared to control in Abu Rawash soil and by 9.63, 26.04, 40.38 and 13.60% in Al-Gabal Al-Asfar soil, respectively.

Previous application of EDTA significantly increased Cu uptake by indian mustard (mg $plant^{-1}$) in both tested soils. Anyway, previous application of 4.5 mmol EDTA kg^{-1} soil recorded the highest values of total Cu uptake (mg $plant^{-1}$) by plants in both soils, where the corresponding increases percentage compared to control were 15.70 and 15.59% in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

5.11. Residual effect of chelators on Zn concentration (mg kg^{-1} DW) in plant organs and Zn uptake (mg $plant^{-1}$) by indian mustard grown on the two soils under study:

Previous applications of EDTA treatment increased Zn concentrations in plants as compared to CA and HA treatments, and this effect was increased with increasing EDTA addition rates up to 4.5 mmol kg^{-1} soil in both soils. While, no significant residual effect on Zn concentration in plant parts were found due to previous application of CA or HA in both soils. However, previous application of 4.5 mmol EDTA kg^{-1} soil achieved the highest concentrations of Zn in plant parts in both soils, except for roots and seeds in Al-Gabal Al-Asfar soil.

Regarding to Zn uptake (mg $plant^{-1}$) by indian mustard, the highest values of total uptake of Zn by plants were obtained due to previous application of 4.5 mmol EDTA kg^{-1} soil and the corresponding increases as compared to control were 6.48 and 9.00% in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

5.12. Residual effect of chelators on Pb roots concentration (mg kg^{-1} DW) and Pb roots uptake (mg $plant^{-1}$) of indian mustard plant grown on the two soils under study:

Previous application of EDTA treatment increased the mean values of Pb concentrations in plant roots (mg $plant^{-1}$) compared with CA and HA treatments in both studied soils. However, this effect was insignificant in both soils. Previous application of different CA or HA levels did not prove any significant or obvious effect on Pb concentration in roots in both soils. The highest concentrations of Pb in plant roots were obtained when EDTA was previously applied at 4.5 mmol kg^{-1} soil in Abu Rawash soil (20.43% increase over control) and 1.5 mmol kg^{-1} in Al-Gabal Al-Asfar soil (6.42% increase over control).

Previous application of EDTA increased Pb uptake by roots (mg plant^{-1}) in both soils as compared with CA and HA treatments, but this effect was insignificant in both studied soil. Previous application of CA and HA levels in any soil did not prove any significant or perceptible effect on Pb uptake by plant roots.

5.16. Resedial effect of chelators on Cu, Zn and Pb leaching (mg pot^{-1}) under indian mustard cultivation in the two soils under study:

Previous application of EDTA and CA increased Cu, Zn and Pb in the leachate compared with HA treatment. The leaching of Cu, Zn and Pb due to previous applications of EDTA was many times higher than that of CA. The highest values of Cu, Zn and Pb leaching (mg pot^{-1}) were obtained when EDTA was previously added at the rate of 4.5 mmol kg^{-1} in both soils, where the corresponding increases as compared to control were 6.79, 6.29 and 3.31 folds in Abu Rawash soil and were 4.45, 6.22 and 4.36 folds in Al-Gabal Al-Asfar soil, respectively.

It can be concluded that:

- EDTA had the highest efficacy on enhancing Cu and Zn absorption by sunflower and indian mustard plants. Application of CA also increased Cu and Zn absorption by both plant species, while HA was only efficient to increase Zn absorption by both plant species. The high concentrations of Cu and Zn in the plant organs due to applying EDTA compared to the other chelators induced toxicity, so reduced the dry matter of these organs.

- The overall results demonstrated that sunflower was more efficient than indian mustard in the phytoextraction of Cu and Zn from polluted soils. Indian mustard had the ability to accumulate Pb in its roots than sunflower plant.

- Using CA for the phytoremediation of Cu as well as CA and HA for the phytoremediation of Zn is favorable than EDTA despite the high efficiency of EDTA, due to either its harmful effect of high rates on plant growth or its increment effect of groundwater contamination risk via metal leaching.

- Phytoextraction of Pb was failed even with EDTA, CA and HA chelators addition, where there were neither any perceptible concentrations of Pb in the above ground portion of indian mustard plant parts.

- EDTA can persist in soil for long periods of time because of its low biodegradability compared to CA and HA. Consequently, EDTA can enhance the phytoremediation of metals from contaminated soil in two successive seasons after addition once in the first season. However, its prolonged presence in the soil, and its non-selective nature, dramatically increase the leaching risk of heavy metals.

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جامعـة المنصـورة
كليــة الزراعـة
قسم الأراضـي

علاج بعض الأراضي المصرية الملوثة بالعناصر الثقيلة بإستخدام بعض النباتات المجمعة لهذه العناصر

رسالة مقدمة من

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ماجستير علوم زراعية (علوم الأراضي) - كلية الزراعة - جامعة كفر الشيخ - ٢٠٠٩

كجزء من المتطلبات للحصول على

درجة دكتوراه الفلسفة فى العلوم الزراعية

(علوم أراضي)

قسم الأراضي- كلية الزراعة- جامعة المنصورة

تحت إشراف

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الدكتور

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كلية الزراعة - جامعة المنصورة

٢٠١٦

جامعة المنصورة
كلية الزراعة
قسم الأراضي

لجنة الإشراف

عنوان الرسالة:

علاج بعض الأراضي المصرية الملوثة بالعناصر الثقيلة باستخدام بعض النباتات المجمعة لهذه العناصر.

اسم الباحث: محمد سعد عبد الستار رمضان

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رئيس القسم	وكيل الكلية للدراسات العليا	عميد الكلية
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جامعـة المنصـورة
كليــة الزراعـة
قسم الأراضي

قرار لجنة الحكم والمناقشة

عنوان الرسالة: علاج بعض الأراضي المصرية الملوثة بالعناصر الثقيلة باستخدام بعض النباتات المجمعة لهذه العناصر.

اسم الباحث: محمد سعد عبد الستار رمضان

لجنة الاشراف:

م	الإســم	الوظـيفة	التوقيع
١	الأستاذ الدكتور **محمد وجدى محمد العجرودى**	أستاذ علوم الأراضي كلية الزراعة - جامعة المنصورة	
٢	الأستاذ الدكتور **فاروق إبراهيم زين**	رئيس بحوث - بمعهد بحوث الأراضي والمياه والبيئة - مركز البحوث الزراعية	
٣	أستاذ مساعد دكتور **جمعة لبيب أحمد**	أستاذ علوم الأراضي المساعد كلية الزراعة - جامعة المنصورة	

لجنة الحكم و المناقشة:

م	الإســم	الوظـيفة	التوقيع
١	الأستاذ الدكتور **محمد وجدى محمد العجرودى**	أستاذ علوم الأراضي كلية الزراعة - جامعة المنصورة	
٢	الأستاذ الدكتور **السيد عوض محمد عوض**	أستاذ علوم الأراضي كلية الزراعة - جامعة الزقازيق	
٣	أستاذ مساعد دكتور **جمعة لبيب أحمد**	أستاذ علوم الأراضي المساعد كلية الزراعة - جامعة المنصورة	
٤	أستاذ مساعد دكتور **طارق محمد الزهيري**	أستاذ علوم الأراضي المساعد كلية الزراعة - جامعة المنصورة	

تاريخ المناقشة: / / ٢٠

رئيس القسم	وكيل الكلية للدراسات العليا	عميد الكلية
الأستاذ الدكتور **أيمن محمد الغمرى**	**الأستاذ الدكتور** **ياسر محمد شبانة**	**الأستاذ الدكتور** **ياسر مختار الحديدى**

٢٠١٦

٧. الملخص العربي

أجريت ثلاث تجارب أصص في صوبة سلكية في محطة البحوث الزراعية بسخا - محافظة كفر الشيخ بهدف دراسة كفاءة استخلاص النحاس والزنك والرصاص من التربة الملوثة باستخدام نباتي عباد الشمس والخردل الهندي تحت تأثير بعض المخلبيات المضافة . أجريت تجربتان لدراسة تأثير بعض المواد المخلبية [حامض الستريك (CA) وحامض الهيوميك (HA) وأيثيلين دأي أمين تيترا أسيتيك أسيد (EDTA)] على كفاءة استخلاص النحاس والزنك والرصاص بواسطة نبات عباد الشمس (موسم صيف ٢٠١٣) ونبات الخردل الهندي (موسم شتاء ٢٠١٤/٢٠١٣) من ناحية، وعلى غسيل هذه المعادن من التربة من ناحية أخرى. وقد أجريت التجربة الثالثة (موسم الشتاء ٢٠١٤/٢٠١٣) لدراسة الأثر المتبقي من المواد المخلبية المضافة إلى تجربة عباد الشمس (في الموسم الاول) -دون إضافة مواد اخري-على كفاءة استخلاص المعادن موضع الدراسة بواسطة الخردل الهندي وسلوك الغسيل لهذه المعادن من التربة.

ومن أهم النتائج المتحصل عليها ما يلي:

١. تأثير المخلبيات المضافة على الوزن الجاف لنباتات عباد الشمس والخردل الهندي المنزرعة في الأراضي موضع الدراسة:

إضافة كل من حامضي الستريك والهيوميك (خصوصاً بتركيزات ٣ ملليمول / كجم تربه و٦ ملليمول / كجم تربه علي التوالي) أدي الي زيادة الوزن الجاف للأجزاء النباتية لنباتات عباد الشمس المختلفة مقارنة بالـEDTA في كلا الأرضين. في حين أن إضافة الـEDTA أظهرت انخفاضاً في الوزن الجاف لنباتات عباد الشمس في كلا الأرضين مقارنة بالكنترول بالإضافة الي ظهور بعض علامات التسمم على النباتات النامية خصوصا في التركيزات العالية من الـEDTA.

إضافة كل من حامضي الهيوميك والستريك أدي الي زيادة الوزن الجاف لنباتات الخردل الهندي في كلا الأرضين، ولكن ذلك التأثير كان أقوى في حالة الهيوميك. بينما استخدام الـEDTA أدي الي خفض الوزن الجاف للنبات مع ظهور بعض علامات التسمم على النباتات. استخدام الهيوميك بمعدل ٠,٦ جم/كجم تربة أدي الي الحصول على أعلى قيمة للوزن الجاف للخردل الهندي.

٢- تأثير المخلبيات المضافة على تركيز النحاس في الأجزاء النباتية المختلفة لنباتات عباد الشمس والخردل الهندي المنزرعة في الأراضي موضع الدراسة:

إضافة كلا من الـEDTA وحامض الستريك أدي الي زيادة تركيزات النحاس في الأجزاء النباتية المختلفة لنباتات عباد الشمس والخردل الهندي مقارنة بمعاملات حامض الهيوميك في كلا الأرضين وذلك بزيادة

معدل الإضافة، ولكن كان ذلك التأثير أقوي في حالة الـEDTA. أعلى تركيزات للنحاس في الأجزاء النباتية المختلفة لعباد الشمس تم الحصول عليها بإضافة الـEDTA بمعدل ٤,٥ ملليمول / كجم تربة، حيث أدي الي زيادة تركيزات النحاس في الجذور والسيقان والأوراق والبذور مقارنة بالكنترول بمعدل ٤,٠٢ و ٢,٤٧ و ٣,١٢ و ١,٣٧ ضعف في أرض أبو رواش وبمعدل ٤,٢٢ و ٢,٦٤ و ٣,٩١ و ١,٥٥ ضعف في أرض الجبل الأصفر على التوالي. أيضاً إضافة الـEDTA بمعدل ٤,٥ ملليمول/كجم تربه أدي الي الحصول على أعلى تركيزات للنحاس في الأجزاء النباتية المختلفة للخردل الهندي، حيث أدي الي زيادة تركيزات النحاس في الجذور والسيقان والأوراق والبذور مقارنة بالكنترول بمعدل ٢,٠٨ و ٢,٤٤ و ٢,٨٧ و ١,٣٠ ضعف في أرض أبو رواش وبمعدل ١,٩١ و ٢,٨٨ و ٣,٠٤ و ١,٥٦ ضعف في أرض الجبل الأصفر على التوالي.

٣- تأثير المخلبيات المضافة على امتصاص النحاس (مجم/نبات) بواسطة نباتات عباد الشمس والخردل الهندي المنزرعة في الأراضي موضع الدراسة:

إضافة كل من معاملات الـEDTA وحامض الستريك أدي الي زيادة امتصاص النحاس بواسطة الأجزاء النباتية المخلفة والامتصاص الكلي للنبات والمزال من النحاس بواسطة الأجزاء الهوائية (مجم/نبات) لنباتات عباد الشمس والخردل الهندي في كلا الأرضين. لم يكن هناك تأثير واضح للهيوميك على امتصاص النحاس بواسطة كلا النوعين من النباتات في كلا الأرضين. إضافة الـEDTA بمعدل ٤,٥ ملليمول/كجم تربة أدي الي الحصول على أعلى قيم الامتصاص الكلي للنبات (مجم/نبات) في كلا الأرضين، حيث أدي الي زيادة الامتصاص الكلي (مجم/نبات) مقارنة بالكنترول بمعدل ٢,٠٦ و ٢,٤٣ ضعف لنبات عباد الشمس وبمعدل ١,٧٠ و ١,٧٣ ضعف لنبات الخردل الهندي في أراضي أبو رواش والجبل الأصفر على التوالي.

٤- تأثير المخلبيات المضافة على تركيز الزنك في الأجزاء النباتية المختلفة لنباتات عباد الشمس والخردل الهندي المنزرعة في الأراضي موضع الدراسة:

إضافة كل من الـEDTA وحامض الهيوميك وحامض الستريك أدي الي زيادة تركيزات الزنك في الأجزاء النباتية المختلفة لنباتات عباد الشمس والخردل الهندي في كلا الأرضين. ترتيب المواد المخلبية المختلفة من خلال التأثير على زيادة تركيزات الزنك في النباتات المنزرعة في كلا الأرضين كان على النحو التالي: EDTA > حامض الستريك > حامض الهيوميك. زأدت تركيزات الزنك في نباتات عباد الشمس والخردل الهندي بزيادة المعدل المضاف من الـEDTA وحامض الستريك الي أعلى معدل مضاف (٤,٥ و ٩ ملليمول/كجم تربة على التوالي). أعلى تركيزات للزنك في الأجزاء النباتية المختلفة لكلا النباتين تم

الحصول عليها بإضافة الـEDTA بمعدل ٤,٥ ملليمول / كجم تربة، حيث أدي الي زيادة تركيزات الزنك في الجذور والسيقان والأوراق والبذور الخاصة بنباتات عباد الشمس مقارنة بالكنترول بمعدل ١,٢١ و ١,٥١ و ١,٣٩ و ١,١٥ ضعف في أرض أبو رواش وبمعدل ١,٢٦ و ١,٩٦ و ١,٥٥ و ١,١٣ ضعف في أرض الجبل الأصفر على التوالي. إضافة الـEDTA بمعدل ٤,٥ ملليمول / كجم تربة أدي الي زيادة تركيزات الزنك في الجذور والسيقان والأوراق والبذور الخاصة بنباتات الخردل الهندي مقارنة بالكنترول بمعدل ٢٦,٢٨ و ٩١,٣٠ و ٧٧,٠٠ و ١٥,٧٥% في أرض أبو رواش وبمعدل ٤٣,٣٠ و ٩٠,٨٢ و ٨٣,٠٨ و ١١,١٩% في أرض الجبل الأصفر على التوالي.

٥- تأثير المخلبيات المضافة على امتصاص الزنك (مجم/نبات) بواسطة نباتات عباد الشمس والخردل الهندي المنزرعة في الأراضي موضع الدراسة:

أدي إضافة كل من معاملات حامضي الستريك والهيوميك الي زيادة امتصاص الزنك بواسطة نباتات عباد الشمس (مجم/نبات) مقارنة بالـEDTA في أرض أبو رواش. بينما في أرض الجبل الأصفر كان هناك اتجاه مخالف، حيث أن إضافة الـEDTA أدي الي زيادة امتصاص الزنك بواسطة نباتات عباد الشمس (مجم/نبات) مقارنة بمعاملات حامضي الستريك والهيوميك. تم الحصول على أعلى قيم الامتصاص الكلي للزنك (مجم/نبات) بواسطة نباتات عباد الشمس باستخدام حامض الستريك بمعدل ٣ ملليمول/كجم تربة في أرض أبو رواش (زيادة عن الكنترول بمقدار ٢٩,٣٩%) والـEDTA بمعدل ٣ ملليمول/كجم تربة في أرض الجبل الأصفر (زيادة عن الكنترول بمقدار ٣٢,٤٥%).

بالنسبة لنباتات الخردل الهندي، أدت إضافة المركبات المخلبية الي زيادة امتصاص الزنك بواسطة الأجزاء النباتية المختلفة لنباتات الخردل الهندي. ومع ذلك إضافة كل من الـEDTA وحامض الستريك أدي الي زيادة الامتصاص الكلي للزنك (مجم/نبات) بواسطة نباتات الخردل مقارنة بحامض الهيوميك، وذلك بزيادة المعدل المضاف من الـEDTA وحامض الستريك الي أعلى معدل مضاف (٤,٥ و ٩ ملليمول على التوالي).

٦- تأثير المخلبيات المضافة على تركيز الرصاص في جذور نباتات عباد الشمس والخردل الهندي المنزرعة في أراضي أبو رواش والجبل الأصفر:

إضافة كل من الـEDTA وحامض الستريك أدي الي زيادة تركيزات الرصاص في جذور نباتات عباد الشمس والخردل الهندي مقارنة بحامض الهيوميك في كلا الأرضين. ترتيب المواد المخلبية المختلفة من خلال التأثير على زيادة تركيزات الرصاص في جذور النباتات في كلا الأرضين كان على النحو التالي: EDTA > حامض الستريك > حامض الهيوميك. أعلى تركيزات للرصاص في جذور عباد الشمس

والخردل الهندي في كلا الأرضين تم الحصول عليها بإضافة الـEDTA بمعدل ٤,٥ ملليمول / كجم تربة، حيث أدي الي زيادة تركيزات الرصاص مقارنة بالكنترول في أرض أبو رواش بمعدل ٢,٣٧ و ٢,٤٨ ضعف في جذور عباد الشمس والخردل الهندي على التوالي، وفي أرض الجبل الأصفر بمعدل ٢ و ٢,١٠ ضعف في جذور عباد الشمس والخردل الهندي على التوالي.

٧- تأثير المخلبيات المضافة على امتصاص الرصاص (مجم/نبات) بواسطة جذور نباتات عباد الشمس والخردل الهندي المنزرعة في أراضي أبو رواش والجبل الأصفر:

إضافة معاملات الـEDTA أدي الي زيادة امتصاص الرصاص بواسطة جذور كلا من عباد الشمس والخردل الهندي مقارنة بحامض الهيوميك في كلا الأرضين. ترتيب المواد المخلبية المختلفة من خلال التأثير على امتصاص الرصاص بواسطة جذور النباتات في كلا الأرضين كان على النحو التالي: EDTA > حامض الستريك > حامض الهيوميك. أعلى قيم امتصاص الرصاص بواسطة جذور عباد الشمس والخردل الهندي في كلا الأرضين تم الحصول عليها بإضافة الـEDTA بمعدل ٤,٥ ملليمول / كجم تربة. حيث أدي الي زيادة امتصاص الرصاص (مجم / نبات) مقارنة بالكنترول في أرض أبو رواش بمعدل ١,٥٥ و ١,٨٠ ضعف في جذور نباتات عباد الشمس والخردل الهندي على التوالي، وفي أرض الجبل الأصفر بمعدل ١,٤٠ و ١,٥٨ ضعف في جذور نباتات عباد الشمس والخردل الهندي على التوالي.

٨- تأثير المخلبيات المضافة على رشح النحاس والزنك والرصاص من التربة (مجم/نبات) في ظل زراعة نباتات عباد الشمس والخردل الهندي في أراضي أبو رواش والجبل الأصفر:

كان للـ EDTA تأثير عالي جدا على رشح وغسيل النحاس والزنك والرصاص من التربة مقارنة بمعاملات حامضي الستريك والهيوميك في كلا الأرضين، مسببة بذلك مخاطر بيئية شديدة نتيجة رشح هذه المعادن الي الماء الأرضي. كان أيضا لحامض الستريك تأثير مشابه للـEDTA ولكن بدرجة أقل من حيث التأثير على زيادة رشح المعادن موضع الدراسة خلال التربة. لم يكن هناك أي تأثير لحامض الهيوميك على غسيل المعادن موضع الدراسة خلال قطاع التربة في ظل زراعة نباتات عباد الشمس والخردل الهندي. أعلى قيم غسيل المعادن المدروسة من التربة في ظل زراعة النباتات في كلا الأرضين تم الحصول عليها بإضافة الـEDTA بمعدل ٤,٥ ملليمول / كجم تربة. حيث أدي الي زيادة رشح النحاس والزنك والرصاص مقارنة بالكنترول بمقدار ٥٣,٤٤ و ٥٦,٢٨ و ٦١,١٠ ضعف في أرض أبو رواش وبمقدار ٤٥,٨١ و ٣٨,٨١ و ٥٤,٧٢ ضعف في أرض الجبل الأصفر على التوالي في ظل زرعة نباتات عباد الشمس، وبمقدار ٥٠,٨٣ و ٤٤,٣٣ و ٧٤,٨١ ضعف في أرض أبو رواش وبمقدار ٦١,٨٣ و ٣٨,٣٠ و ٦٢,٩٢ ضعف في أرض الجبل الأصفر على التوالي في ظل زراعة نباتات الخردل الهندي.

٩- التأثير المتبقي للمخلبيات المضافة على الوزن الجاف لنبات الخردل الهندي المنزرع في أراضي أبو رواش والجبل الأصفر:

اوضحت النتائج أنه لم يكن هناك أي تأثير متبقي معنوي او ملموس على المحصول الجاف لنباتات الخردل الهندي نتيجة الإضافات السابقة لكل من الـEDTA أو حامض الستريك أو حامض الهيوميك في كلا الأرضين موضع الدراسة.

١٠- التأثير المتبقي للمخلبيات المضافة على تركيز النحاس في الأجزاء النباتية المختلفة وامتصاص النحاس (مجم/نبات) بواسطة نباتات الخردل الهندي المنزرعة في الأراضي موضع الدراسه:

وجد أن للـEDTA تأثير متبقي معنوي على زيادة تركيزات النحاس في بعض الأجزاء النباتية المختلفة للخردل الهندي. على النقيض، لم يكن هناك أي تأثير معنوي على تركيزات النحاس في الأجزاء النباتية المختلفة لنبات للخردل الهندي نتيجة الإضافات السابقة من حامضي الستريك والهيوميك بمستوياتهم المختلفة في أراضي أبو رواش والجبل الأصفر. الإضافات السابقة من الـEDTA بمعدل ٤,٥ ملليمول / كجم تربة أدت الي الحصول على أعلى تركيزات للنحاس في جذور وسيقان والأوراق والبذور مقارنة بالكنترول بمعدل ٢٣,٠٨ و ٢٩,٠٧ و ٣٦,٠٥ و ٨,٣٣% في أرض أبو رواش وبمعدل ٩,٦٣ و ٢٦,٠٤ و ٤٠,٣٨ و ١٣,٦٠% في أرض الجبل الأصفر على التوالي.

اوضحت النتائج أن الإضافات السابقة من الـEDTA كان لها تأثير متبقي معنوي على زيادة امتصاص النحاس بواسطة نباتات الخردل الهندي (مجم/نبات) في كلا الأرضين. حيث أن الإضافات السابقة من الـEDTA بمعدل ٤,٥ ملليمول / كجم تربة أدت الي الحصول على أعلى امتصاص للنحاس بوسطة النباتات، حيث أدت الي زيادة الامتصاص الكلي للنبات (مجم/نبات) مقارنة بالكنترول بمعدل ١٥,٧٠ و ١٥,٥٩% في أراضي أبو رواش والجبل الأصفر على التوالي.

١١- التأثير المتبقي للمخلبيات المضافة على تركيز الزنك في الأجزاء النباتية المختلفة وامتصاص الزنك (مجم/نبات) بواسطة نباتات الخردل الهندي المنزرعة في الأراضي موضع الدراسه:

أوضحت النتائج أن الإضافات السابقة من الـEDTA أدت الي زيادة تركيزات الزنك في الأجزاء النباتية المختلفة لنباتات الخردل الهندي، وذلك بزيادة مستوي الـEDTA المضاف في الموسم السابق الي ٤,٥ ملليمول / كجم تربة. في حين أنه لم يكن هناك أي تأثير متبقي معنوي على تركيزات الزنك في الأجزاء النباتية المختلفة لنبات الخردل الهندي نتيجة الإضافات السابقة من حامضي الستريك والهيوميك. الإضافات السابقة من الـEDTA بمعدل ٤,٥ ملليمول / كجم تربة أدت الي الحصول على أعلى تركيزات للزنك في الأجزاء النباتية المختلفة لنباتات الخردل الهندي، فيما عدا الجذور والبذور في الجبل الأصفر.

بالنسبة لامتصاص الزنك بواسطة النباتات، الإضافات السابقة من الـEDTA بمعدل ٤,٥ ملليمول / كجم تربة أدت الي الحصول على أعلى امتصاص للزنك بوسطة نباتات للخردل الهندي، حيث أدت الي زيادة الامتصاص الكلي للنبات (مجم/نبات) مقارنة بالكنترول بمعدل ٦,٤٨ و ٩% في أراضي أبو رواش والجبل الأصفر على التوالي.

١٢- التأثير المتبقي للمخلبيات المضافة على تركيز الرصاص وامتصاص الرصاص (مجم/نبات) بواسطة جذور نباتات الخردل الهندي المنزرعة في أراضي أبو رواش والجبل الأصفر:

أوضحت النتائج وجود تأثير متبقي للـEDTA حيث أدت الإضافات السابقة الي زيادة تركيزات الرصاص في جذور نباتات الخردل الهندي، لكن ذلك التأثير كان غير معنوي في كلا الأرضين. في حين أن لم يكن هناك أي إختلاف محسوس في تركيزات الرصاص في جذور نباتات الخردل نتيجة الإضافات السابقة من حامضي الستريك والهيوميك. الإضافات السابقة من الـEDTA في الموسم الأول بمعدل ٤,٥ ملليمول / كجم تربة أدت الي الحصول على أعلى تركيزات للرصاص في جذور نباتات الخردل الهندي في أرض أبو رواش (٢٠,٤٣% زيادة عن الكنترول)، في حين أن الإضافات السابقة من الـEDTA بمعدل ١,٥ ملليمول / كجم تربة أدت الي الحصول على أعلى تركيزات للرصاص في جذور نباتات الخردل الهندي في أرض الجبل الأصفر (٦,٤٢% زيادة عن الكنترول).

الإضافات السابقة من الـEDTA أدت الي زيادة امتصاص الرصاص بواسطة جذور نباتات الخردل الهندي وذلك بزيادة مستويات الـEDTA المضافة في كلا الأرضين ولكن هذه الزيادات غير معنوية. في حين لم يكن هناك أي تأثير متبقي على امتصاص الرصاص بواسطة جذور النبات نتيجة الإضافات السابقة من حامضي الستريك والهيوميك.

١٣- التأثير المتبقي للمخلبيات المضافة على رشح النحاس والزنك والرصاص من التربة (مجم/إصيص) في ظل زراعة الخردل الهندي في أراضي أبو رواش والجبل الأصفر:

أوضحت النتائج وجود تأثير متبقي للـEDTA وحامض الستريك على رشح المعادن الثقيلة من التربة في كلا الأرضين، حيث أدت الي زيادة غسيل المعادن موضع الدراسة من التربة مقارنة بحامض الهيوميك الذي لم يكن له أي اثر متبقي على حركية وغسيل العناصر من التربة. وجد أيضا أن التأثير المتبقي الخاص بالـEDTA كان أعلى بكثير من التأثير المتبقي لحامض الستريك. الإضافات السابقة من الـEDTA بمعدل ٤,٥ ملليمول / كجم تربة أدت الي الحصول على قيم رشح النحاس والزنك والرصاص من التربة في كلا الأرضين، حيث زادت مقارنة بالكنترول بمعدل ٦,٧٩ و ٦,٢٩ و ٣.٣١ ضعف في أرض أبو رواش وبمعدل ٤,٤٥ و ٦,٢٢ و ٤,٣٦ ضعف في أرض الجبل الأصفر على التوالي.