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Dry Matter Yield of Sunflower as Affected by EDTA and EDDS Application in Soil Remediation from Ibadan, Metropolis, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Authors EYT and JAIO designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author EYT managed the literature searches, analyses of the study performed the spectroscopy analysis and author EYT managed the experimental process and authors EYT and JAIO identified the species of plant. Both authors read and approved the final manuscript.

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ABSTRACT

Phytoremediation has been found to be cost effective in removing heavy metals (HM) from polluted soils. Enhanced phytoextraction with the use of chelating agents for solubility of metal in soils and their uptake in the aerial parts of plants had also been employed. Phytoextraction efficiency of plants will depend on the metal accumulated in the dry matter yield of plant.
An attempt was made to study the influence of Ethylene diamine tetra acetic acid (EDTA) and ethylene diamine disuccinic acid (EDDS) on the growth and dry matter yield content of sunflower planted in heavy metal polluted soils in a greenhouse pot experiment. Total heavy metal contents of five metals (Cu, Cd, Cr, Pb and Zn) were determined by acid digestion with aqua regia solution. A greenhouse experiment was conducted to assess the phytoextraction potential of sunflower with EDDS and EDTA applied at seven weeks after planting. Treatments were laid out in a completely randomized design with four replicates. Data were collected on metal accumulation in root and shoot, Total metal uptake, shoot and root dry matter yield and analyzed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

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The result shows a significant increase in the overall accumulation of metals in the root and shoot of sunflower and dry matter yield with EDDS except for Zn. However, Zinc only recorded significant increase in shoot uptake at 3 mmol/kg EDDS. The application of EDDS at 3 and 9 mmol/kg did better in total uptake of Cu, Pb and Cd. Ethylene diamine disuccinic acid (EDDS) was more efficient than EDTA in mobilizing metal uptake in sunflower root and shoot and also in increasing total dry matter yield. Translocation factor of Cd, Pb and Cu were enhanced by the addition of EDDS than EDTA application.

Keywords: Heavy metal; sunflower; EDDS and EDTA; dry matter yield; phytoextraction.

1. INTRODUCTION

The presence of heavy metal in soil is a major problem globally. This is because of the difficulty encountered in their removal from soil and their adverse effect on the ecosystem at large. Several attempts have been made by scientist to solve this problem and different technologies have emerged in remediating polluted soils. These methods include physical, Chemical and biological mean, but their application is very expensive except for the biological method of soil remediation which employed the use of plant (Phytoremediation). Phytoremediation has received a wide attention globally because it is cost effective and environmentally friendly [1,2]. Several plants (hyperaccumulators) that can remove metal into their aerial parts have been identified. The largest hyperaccumulating plants families are found in the Brassicaceae [3], others are Fabaceae*, Euphorbiaceae*, *Asteraceae*, *Lamiaceae* and the *Scrophulariacea* [4]. *Brassicajuncea* (Indian mustard) has been reported to have a good ability to move Pb from the roots to the shoots [5]. Most of these plants are characterized with slow growth and low biomass production. This makes them less effective in soil remediation. This explains why recent research focuses mainly on the use of plant with high biomass like Maize (*Zea may*), Oat (*Avenasatina*) and sunflower (*Helianthus annus*) [6-8].

However, the low solubility of metal in soil matrix limits metal absorption through the root system for uptake by plants. Chelating agents have been proposed by Luo et al. [9] to enhance metal solubilization for easy uptake by plants. The uptake mechanisms of metals through plant by chelating agents depend on the following characteristics: its lipophilicity, stability constant to the metals, pKa and complex size. Solute transportation from the external parts of the roots to the central root xylem, takes place through major pathways; the apoplastic (cell wall space between cell membranes) and symplastics crossing many cell membranes along the pathway [10]. Some chelating agents of importance in soil remediation include; ethylene diamine tetraacetic acid (EDTA), n-(2-hyrdoxlyethyl), ethylene diamine triacetic acid (HEDTA), diethylene-triamine-pentaacetic acid (DTPA), ethylenebis-(oxyethylenenitrilo)- tetera acetic acid (EGTA) and ethylene diamine-di (o-hydroxyphenylacetic acid) (EDDHA) [11]. There is paucity of information on the use of chelating agent for soil remediation in Nigeria. Some of these chelating agents are sometimes used as an extractant in the laboratories. Therefore, this study examined the roles of EDDS and EDTA on heavy metal uptake and Dry matter yield of Sunflower planted in polluted soil in a greenhouse pot experiment.

2. MATERIALS AND METHODS

2.1 Description of Study Site

This study was carried out at two locations: Agronomy Department, University of Ibadan and Purdue University, West Lafayette, Indiana State, United State of America. The soil used for this study was collected from Awotan dumpsite in Apete, Ibadan (Oyo State), N07º27.765^1 and E000º50 955^l. The soil was derived from basement complex and belongs to the USDA taxonomy group of the Alfisols order. It has a mean annual rainfall of 1289 mm and mean annual temperature of 26.3°C [12]. The soil was shipped to the United State of America for analysis. Sampling site description is shown in Fig. 1.

2.2 Soil Sampling and Preparation

The soil used for this study (0-20 cm) was randomly sampled with the aid of soil auger and spade. Triplicate soil samples were collected randomly from each location at different points, mixed and bulked to form a composite sample, out of which representative samples were air-dried, crushed and sieved through a 2 mm sieve and for each location after which they were bagged in clean polythene containers to prevent further contamination. Samples were further ground and passed through a 0.5 mm sieve for organic carbon and total nitrogen analysis.

2.3 Laboratory Experiments

All glassware used in this study were pre-washed with HNO_3 and rinsed with deionized water and all the chemicals used were of analytic grade.

2.3.1 Routine soil analysis

Chemical analyses were carried out using the recommended routine soil test procedures for the North Central Region, U.S.A [13]. These include: pH in H_2O, 1:1 (soil: water); total N and organic carbon, available phosphorus (Bray P1), exchangeable cations and exchangeable acidity. Particle size analysis was determined using hydrometer method as outlined by [14].

2.3.2 Total heavy metals (Pb, Cd, Cr, Cu and Zn)

Total heavy metal contents were determined by acid digestion using aqua regia solution (Hydrochloric acid and Nitric acid in ratio 3:1) according to [15]. Three milliliters of distilled water was added to one gram of each of the samples in the glass digestion tubes. Then 12M HCl followed by 14M HNO_3 were added in ratio 3:1 respectively. The samples were then placed in a heating mantle at 85ºC for 19 h. After digestion, the digests were allowed to cool and filtered while the total heavy metal contents were determined by Inductive couple plasma-Mass Spectrophotometer (ICP-MS). The acid digested soil-metal analysis was performed in triplicates.

2.4 Pot Experiment

The Sunflower used for this study is the dwarf type called Sunflower teddy bear. Its maximum height is 61 cm and blooms between 70-75 days. They were obtained from a garden store in Lafayette, USA. Samples of air-dried, homogenized soil (0.25 kg) were treated with fertilizer (NPK 20:15:15) according to soil nutrient content and the fertilizer recommendation for sunflower. The soils were put in pots in the greenhouse. Three seeds of Sunflower were sown in every pot and thinned to 2 plants two weeks after planting. Sunflower plant was chosen as the test plant species because of its high biomass yields and heavy metal tolerance [16,17]. Soil moisture was kept at 60% of water holding capacity (WHC) and watering was done daily with deionized water. Leachates were collected and re-circulated to the system so that mass could be conserved [18].

Biodegradable chelating agents' treatments (3 mmol/kg, 6 mmol/kg and 9 mmol/kg) of EDTA and EDDS were applied as in the batch experiment and were arranged in a completely randomized design to make a factorial combination of 2 chelants and 4 levels of chelant replicated four times. The applications was done at the end of the 7th week and plant were harvested a week after [16]. The control treatment (with only deionized water added) was also included. The parts of the plants harvested for analysis were the shoot and the root. The harvested above-ground biomass were washed carefully using deionized water, dried at 60ºC until constant weight. In order to check the mobility of heavy metals in the pots, the soils were kept moist at 60% field capacity. Two weeks after harvest, 1 g of each of the soil samples was placed in the glass digestion tube, and same procure was repeated as it was done for the total heavy metal analysis described above.

2.4.1 Plant preparations for analysis and translocation factor determination

The plant materials (shoots and roots separately) were analyzed using nitric acid digestion [19] with a little modification. The samples (0.5 g -1 g) were weighed into 50 ml digestion tube and 5 ml concentrated HNO_3 was added and left overnight in a fume cupboard. After that the samples were heated in a temperature controlled digestion block and the temperature was gradually increased to 140°C until 1ml of digest was left. The tubes were brought to room temperature and diluted to the 50 ml mark with distilled water and analyzed by ICP-MS for Pb, Zn, Cr, Cd and Cu. Translocation factor (TF) was determined as a ratio of heavy metal concentration in plant shoot to that in plant root [20].

$$TF = [Metals]_{shoot} / [Metals]_{root}$$

2.5 Statistical Analysis of Data Collected

Statistical analyses were performed using GenStat discovery Edition 4 software.

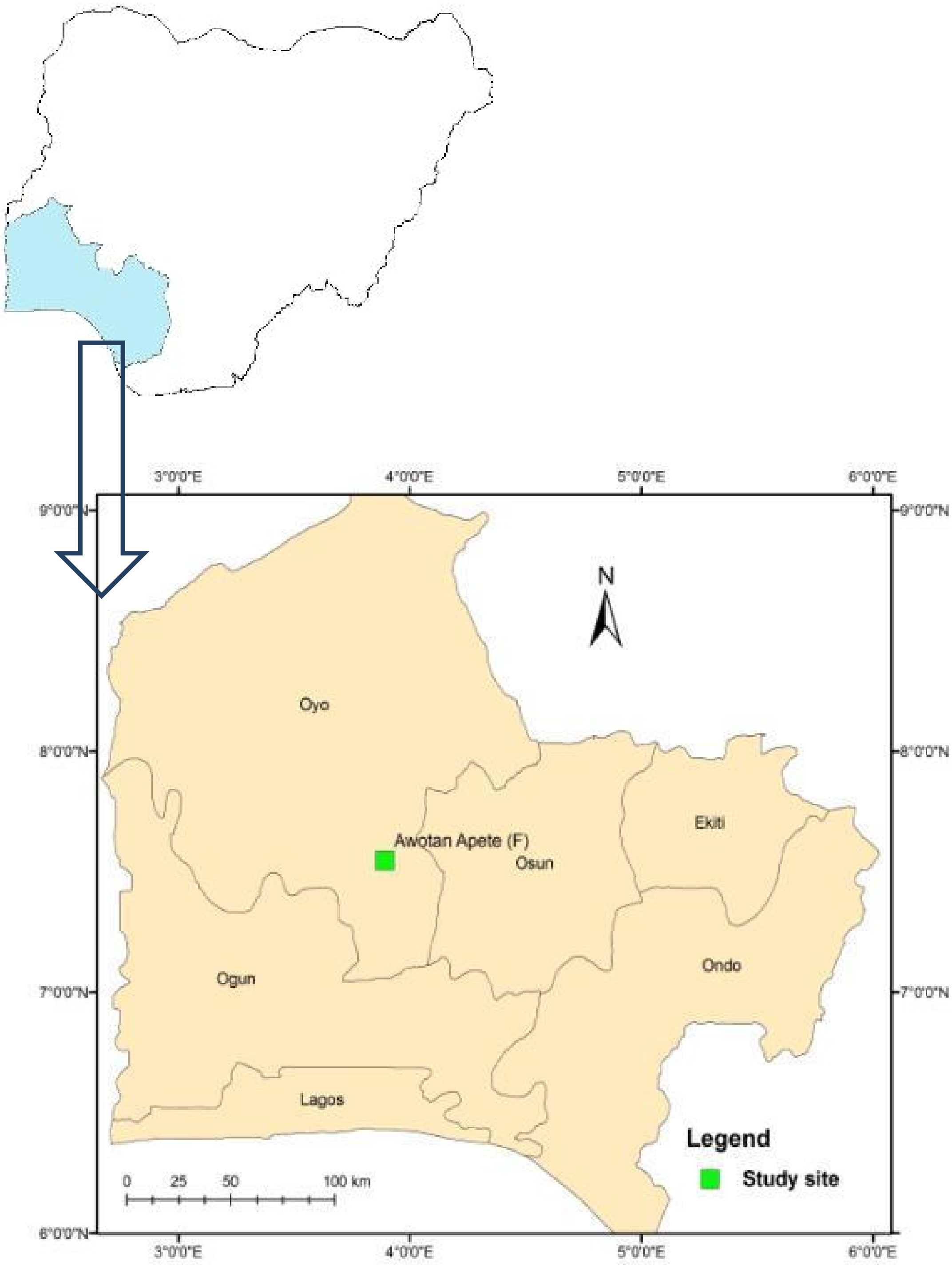

Fig. 1. Map of south-western Nigeria showing polluted soil sample site in Ibadan metropolis

Analysis of variance (ANOVA) with subsequent Duncan Multiple Range Test (DMRT) was performed at the significance level of $p< 0.05$ to compare the means of the dry matter yields and heavy metal concentration of plants under different treatments.

3. RESULTS

3.1 Physicochemical Properties and Total Heavy Metal Concentrations in Experimental Soil

The physical and chemical properties of the soil used for the study are contained in Table 1. The pH of the soil (8.0) was alkaline, Cation exchange capacity (CEC) was 64.6 cmol/kg and soil organic matter was (38 g/kg). It belongs to the sandy loam textural class and total nitrogen for the soil was 3.1(g/kg). The exchangeable bases in the soil varied with potassium (K) value (2.4 cmolkg^{-1}), Calcium (Ca) was (58.8 cmolkg^{-1}) and Mg was (3.5cmol/kg). Available phosphorus (P) in soil E (219 cmolkg^{-1}) was higher than that from soil F (163 cmolkg^{-1}).

The soil was analyzed for the following metals: Cd, Cr, Cu, Pb and Zn as shown in Table 1. Some of their concentrations were higher than the maximum permissible limits in some countries as shown in Table 2. Zinc had the highest concentration (5000 mg/kg)) of all the metal in this soil. The other metal content were in the following concentration: Pb (580.6 mg/kg)), Cu (471 mg/kg)) Cr (136 mg/kg)), and Cd had the least (16.8 mg/kg).

3.2 Heavy Metal Uptake by the Shoot of Sunflower Plants in the Experimental Soil

The accumulation of Cd in the shoot of sunflower ranged from 0.25 -6.68 mg/kg. There is no significant difference in Cd concentration mobilized into plant shoot by the addition of all the treatments as shown in Table 3. However, 3mmol EDDS kg^{-1} recorded the highest Cd concentrations (6.68 mg/kg)). The addition of 3mmol EDTA kg^{-1} gave a significantly higher concentration of Cr (18.03 mg/kg)) in plant shoot than the other treatments. The least significant Cr shoot uptake was obtained with the addition of 9 mmol EDTA kg^{-1}. Application of 6 mmol EDDS kg^{-1} produced significantly higher Cu concentration (182.40 mg/kg)) in plant shoot than all the other treatments at $p<0.05$. The concentration of Pb (28.60 mg/kg)) was significantly higher with 9 mmol EDDS kg^{-1} than those of all the other treatments. Both chelates were relatively more efficient in enhancing Zn uptake through the shoot of the plant. The addition of 6 mmol EDDS kg^{-1} led to a significantly higher uptake of Zn (151.00 mg/kg)) in the plant shoot when compared with the other treatment.

Table1. Some basic physico-chemical properties and total heavy metal content of the experimental soils

Soil properties	Values
pH(1:1 H_2O)	8.0
CEC(cmol/kg)	64.6
OM(g/kg)	38.0
N(g/kg)	3.1
Ca (cmol/kg)	58.8
Mg(cmol/kg)	3.5
K(cmol/kg)	2.4
Available P (mg/kg)	163
Particle size distribution (g/kg)	
Sand	571
Clay	84
Silt	345
Textural class	Sandy loam
Total metal content (mg/kg)	
Cd	16.84±1.58
Cr	136±60
Cu	471±48
Pb	580.61±23.50
Zn	5000±741

3.3 Heavy Metal Uptake in Sunflower Root in the Experimental Soil

The application of the chelants had a significant effect on the concentrations of Cr, Cu, Zn Cd and Pb in the roots of sunflower plants. Cadmium concentrations in the root was not significantly affected by the addition of the chelates, although maximum values were obtained in the root of plant treated with 3 mmol EDTA/kg (2.58 mg/kg)) as shown in Table 4. The control experiment (51.89 mg/kg) and the addition of 3 mmol EDDS/kg (49.19 mg/kg)) had similar Cr root uptake while the least significant Cr concentration (32.62 mg/kg)) in plant root was recorded with the addition of 6 mmol EDTA/kg. The addition of EDDS at 9 mmol kg^{-1} resulted in a significantly higher Cu Concentration (162.30 mg/kg)) in the root of sunflower compared with all the other treatments including the control. The concentration of Pb (129. 00 mg/kg)) with 3mmol EDTA kg^{-1} was significantly higher than those from the other chelant treatments. Pb (1.00 mg/kg)) uptake decreased significantly in the control experiment while the addition of 6 mmol EDDS/kg (90.4 mg/kg)), 6 and 9 mmol EDTA/kg (90.40 and 82.10 mg/kg)) gave similar Pb root uptake. The highest significant concentration of Zn (991.70 mg/kg)) was obtained in sunflower root in pots treated with 3 mmol EDTA /kg. Sunflower pots treated with 9 mmol EDTA/kg, 6 and 9 mmol EDDS/kg had comparable root uptake values for Zn.

3.4 Effects of EDDS and EDTA Treatment on Total Metal Uptake

There was no significant increase in Cd uptake. However, the highest Cd uptake in sunflower plant was obtained when EDTA at 3 mmol/kg was applied. Meanwhile all the EDDS performed better than EDTA treatments at 6 and 9mmol/kg. The control had maximum Cr uptake which was not significantly different from uptake recorded with the addition of the chelants as shown in Table 5. The highest Cu uptake was associated with the application of EDDS at 9 mmol/kg even though this was not significantly different from Cu uptake with the addition of 6 mmol/kg EDDS. It was also observed that there was areduction in Cu uptake from EDTA treatments. Lead uptake in sunflower was increased with both 9 mmol/kg EDDS and 3 mmol/kg EDTA. However Pb uptake from the other treatments was not significant in soil F. There was no significant difference in Zn uptake when all the treatments were applied. However, EDTA at 3 mmol/kg increased Zn uptake than the other treatments and the least Zn uptake was found in the control as shown in Table 5.

3.5 Dry Matter Yield of Plants from Experimental Soil as Influenced by EDDS and EDTA

The shoot, root and total dry matter yield of sunflower grown in the soil are shown in fig 2. There was no significant difference in the shoot dry matter yield from soil F but EDTA at 9 mmol/kg decrease total dry matter yield drastically and affected plant growth, while EDTA at 3 mmol/kg significantly enhanced root and shoot biomass just like EDDS did.

Table 2. Comparative heavy metals concentration (mg/Kg) in soil samples with similar works reported in the literature and the maximum permissible limits in some countries

Metals	(mg/kg)[21]	*Great Britain(mg/kg)	**USEPA(ppm)
Pb	216.93	400	300
Cu	54.13	100	501
Zn	118.06	300	200
Fe	ND	NA	NL
Cr	44.70	50	400
Ni	NA	50	50
Cd	0.55	3	3
Mg	NA	NA	NA
Mn	ND	NA	80
Al	ND	NA	NA
Cu	ND	NA	NA

** Maximum permissible limits of metals (mg/Kg) in soil in Great Britain*
***Maximum permissible limit of metals (ppm) in soil by USEPA, ND: Not Determined Source: [22]*

Table 3. Effect of chelating agents on shoot uptake of metals (mg/kg) by sunflower plant

Treatment	Rates (mmol/kg)	Metals (mg/kg)				
		Cd	Cr	Cu	Pb	Zn
Control	0	0.24b	5.88cd	11.00e	3.1d	95.90e
EDDS [a]	3	6.68a	7.32c	111.40c	8.6c	108.20d
	6	5.56a	8.58c	182.40a	17.2bc	151.00a
	9	3.68ab	12.42b	165.00b	28.60a	122.80c
EDTA [b]	3	3.0ab	18.03a	21.50d	20.7a	135.70b
	6	3.24ab	7.46c	23.10d	5.40d	130.20bc
	9	1.84b	3.53c	15.70e	3.60d	124.80e
SE		1.78	0.92	4.99	4.36	4.29

Means with same letters in each column are not significantly different at $p < 0.05$ with Duncan Multiple Range Test. [a]EDDS = Ethylene diamine disuccinic acid, [b] EDTA= Ethylene diamine tetra acetic acid, SE- Standard error

3.6 Effect of EDDS and EDTA on the Translocation Factors (TF) of Cu, Cr, Cd, Pb, and Zn in the Experimental Soil

All the EDDS treatments had TF value greater than 1 for Cadmium with the highest (3.43) recorded in the 3 mmol EDDS kg^{-1} treatment in the experimental soil (Fig. 3). The least TF value 0.10 was found in the control. The pot with 3 mmol EDTA kg^{-1} had the highest (TF) value for Chromium (0.29). The lowest 0.15 was found with 3 mmol EDTA kg^{-1}. For Copper the highest (TF) value (1.55) was found with the addition of 6 mmol EDDS kg^{-1}, followed by that of 9 mmol EDDS kg^{-1}. All the TF values from the EDTA treatments were below one. The highest TF value for Pb (6.59) was recorded in the control experiment. The next is (0.65) from 6 mmol EDDS kg^{-1} treatment and the least (0.04) was found with 9 mmol EDDS kg^{-1}. Zinc highest TF (0.85) was obtained in the 6 mmol EDDS kg^{-1}, the next was found with the application of 6 mmol EDTA kg^{-1}. It was observed that the addition of 3 and 9 mmol EDDS kg^{-1} gave 0.51 and 0.62 respectively which were more than (TF) values (0.13 and 0.21) from 3 and 9 mmol EDTA kg^{-1} treatments.

3.7 Heavy Metal Concentrations in Soil after Sunflower Harvest

All the metals concentrations were significantly reduced with the addition of EDDS at all levels of application than the EDTA treatments. The only exception was Cu when EDTA was applied at 9 mmol/kg. It was evident that the least reduction in metal concentration after sunflower harvest was observed in the control experiment.

4. DISCUSSION

The results of chemical and physical analysis showed that Awotan Apete dumpsite soil was alkaline. The solubility and hydrolysis of metal sorption was largely influenced by the soil pH. Several adsorption sites like Fe and Mn oxides, organic matter and clay minerals edges are pH dependent [23,24].

Table 4. Effect of treatment on root uptake of metals (mg/kg) by sunflower plant

Treatments	Rates (mmol/kg)	Metals(mg/kg)				
		Cd	Cr	Cu	Pb	Zn
Control	0	1.74b	51.89c	103.50d	1.00e	403c
EDDS[a]	3	2.30ab	49.19c	120.90c	47.4c	417.90c
	6	1.84b	37.78d	118.00cd	90.40b	521.40b
	9	2.36ab	66.68a	162.30a	37.80d	519.10b
EDTA[b]	3	2.58a	65.91ab	139.80b	129.00a	991.70a
	6	1.04c	32.62e	64.90e	90.40b	163.30d
	9	1.87ab	61.22b	138.00b	82.10b	539.80b
SE		0.33	2.41	8.03	4.07	13.82

Means with same letters in each column are not significantly different at p <0.05 with Duncan Multiple Range Test. [a]EDDS = Ethylene diamine disuccinic acid, [b] EDTA= Ethylene diamine tetra acetic acid.SE- Standard error

Table 5. Heavy metal concentration (mg/kg) in soil ARD (Awotan refuse dump site) after sunflower harvest

Treatments	Rates (mmol/kg)	Metals(mg/kg)				
		Cd	Cr	Cu	Pb	Zn
Control	0	2.67**c**	116.91a	399.5a	256.00a	1743a
EDDS[a]	3	2.71**bc**	72.48e	341.3bcd	198.1ab	1620c
	6	2.90**b**	81.01d	354.65bc	214.0ab	1698b
	9	2.63**c**	73.32e	332.6cde	231.0a	1673b
EDTA[b]	3	3.10**a**	82.41c	358.4b	195.0ab	1676b
	6	2.83ab	86.69c	315.5e	202.0ab	1643c
	9	2.76bc	94.91b	327.5de	130.2b	1732a
SE		0.06	3.11	10.54	27.9	40.0

Hyphenated values after each chelating agent are concentrations in mmol/kg.Means with same letters in each column are not significantly different at p < 0.05 with Duncan Multiple Range Test.SE = Standard Error

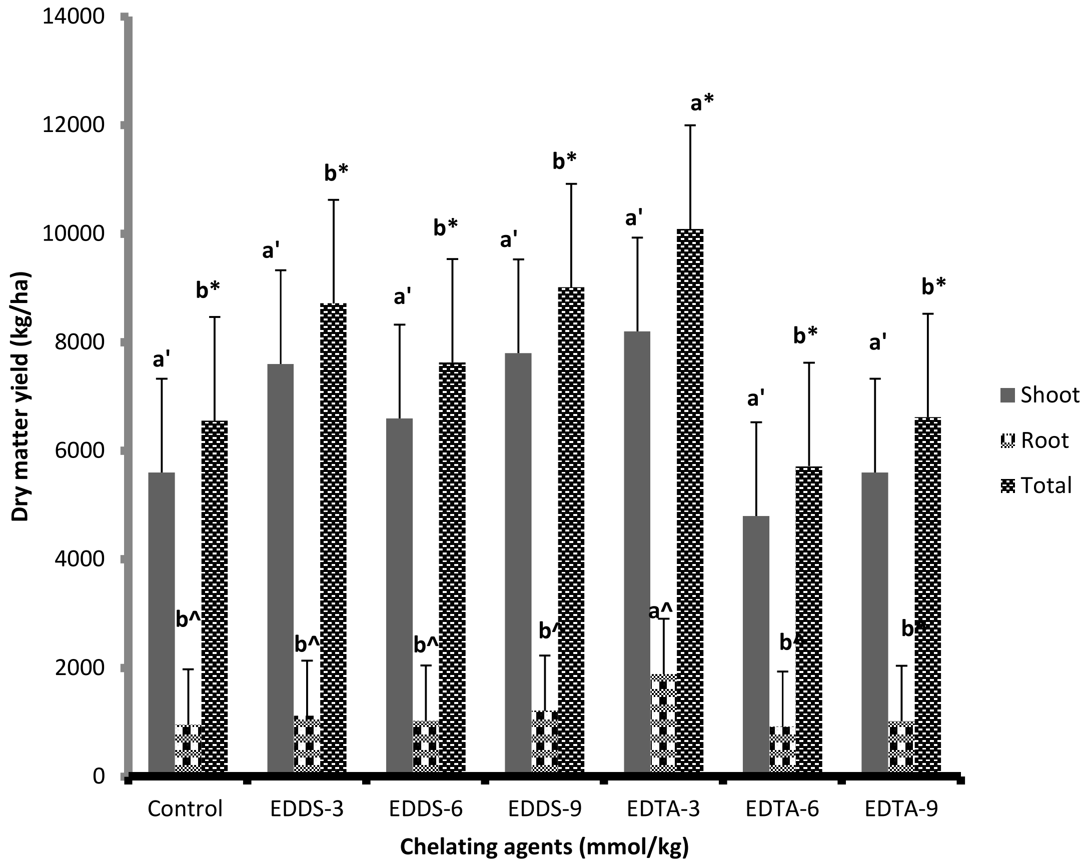

Fig. 2. Effect of EDDS and EDTA on dry matter yield of shoot, root and the total (shoot +root) of Sunflower grown in experimental soil. Different letters among treatments showed significant difference according to Duncan's multiple range test at P<0.05)

EDDS -Ethylene diamine disuccinic acid, EDTA - Ethylene diamine tetra acetic acid

The organic matter contents of the soil were generally high. Soil organic matter consists of humic substances or humus and non humic substances. The interaction between humic acids and metals ions by complexation and adsorption result in water soluble and insoluble complexes which can bring about an increase ora decrease of metal solubility [25]. Textural analysis showed that the soil is sandy. Sandy soils are known to have poor retention capacity for water and metals [26]. The high CEC of the soil accounts for a greater ability to retain metal. The high P values in the soil could be as a result of the high organic matter content in the soil. Soil Organic matter and phosphorus have a positive significant correlation according to the findings of [27]. [28] also asserted that organic matter is an important source of N and P. Excess K would promote luxury consumption and could inhibit the absorption of other nutrient elements. There could be a depression of heavy metal uptake in the presence of high Ca concentrations in the study as earlier reported by [29].

The high concentration of the following metals: Pb, Zn, Cr, and Cu when compared to the normal and critical range of heavy metal in soil according to [30,31] could be attributed to the dumping of waste from different sources over time. This ascertains the fact that metal accumulation in soils builds up with time [32]. Industrial soils and their dumpsites are major sources of Cd pollution in soils. Also, Nickel - cadmium batteries, cadmium pigments, ceramics, glasses, paints and enamels, cadmium coated ferrous and non - ferrous products, cadmium stabilized polyvinyl chloride (pvc) products, cadmium alloys, cadmium electronics or electronic compounds are among anthropogenic sources of Cd in the environment [33].

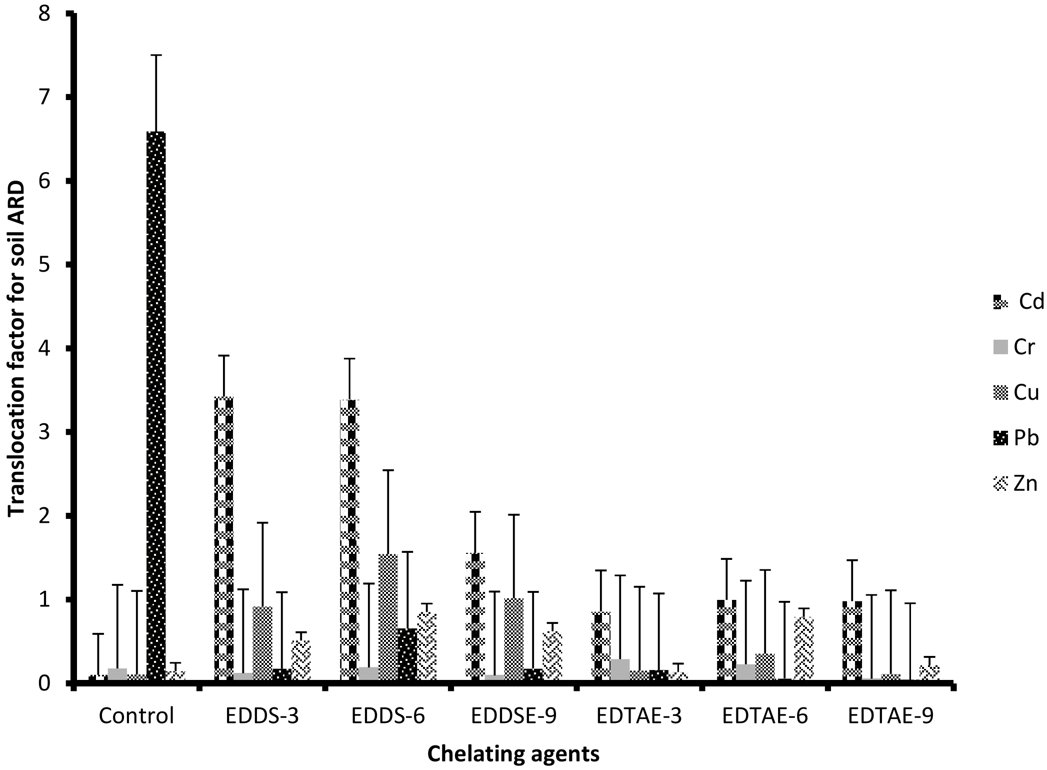

Fig. 3. Effect of chelating agents on translocation factors of Cu, Cr, Cd, Pb and Zn in ARD soil hyphenated values after each chelating agent are concentrations in mmol/kg
EDDS -Ethylene diamine disuccinic acid, EDTA - Ethylene diamine tetra acetic acid

[34] reported that automobiles and electronic waste introduced metallic Cr into the soil. That could inform the high Cr content found in the soil. Chromium is also a major component of paints and pigments used. The reason for the elevated Cu concentration in the soil could be due to indiscriminate disposal of Cu containing waste from the industries or even municipal waste. The high concentration of Zn in the soil could be attributed to the fact that Zn is a major component of industrial and municipal waste.

Total Zn and Cd by 3 mmol/kg EDTA is in line with the findings of [8] that reported a significant boost in uptake of zinc after the application of EDTA in, barley, Indian mustard and oat, also anincreased in the concentration of Zn up to 231%, 93% and 81% were observed in the leaves, stems and the roots of *Solanum nigrum*, respectively [35]. Although, the stability complex of EDTA with Pb is higher than that of EDDS-Pb complex, EDDS aided Pb uptake than EDTA, Pb uptake in the presence of EDDS is most likely in the form of Pb –EDDS complex through apoplastic pathway as suggested by [18]. Uptake of Zn by EDTA could be as a result of higher stability constant other than completion of other metals in soil solution. Maximum uptake of Pb and Cu recorded with the EDDS treatments may be due to competition of EDTA with other cations in solution “most especially Ca” which is very high in the experimental soil.

The application of EDTA to soil except at 3mmol/kg had an adverse effect on sunflower plant shoot and root by causing a reduction in their dry matter yield. Dry matter yields of plant root and shoot decreased in heavy metal polluted soils when treated with chemical chelator EDTA, whereas total dry matter yield was enhanced by the EDDS treatments as described by [36]. This is consistent with [18]. They reported that sunflower plant that received EDDS treatments did not showed phytoxicity symptoms which could be due to the fact that EDDS only had a slight reduction in total dry matter yield of sunflower when compared with the plant under EDTA treatments. Also, [37,38] reported that

EDTA at higher concentration is detrimental or lethal to plant because of free EDTA that hamper plant growth. Furthermore, very high concentration of heavy metals in soils was observed with the high concentration of Pb (5000 µg/g) in the experimental soil can adversely affect the growth of plant. [39-41,7,9,38] reported that the presence of free EDTA is toxic to plants because it can negatively affect the balance of minerals, e.g., Zn, Cu, Fe and Ca, leading to disturbances in cell metabolism and destabilization of biological membranes which could affect plant growth.

In the experimental soil samples, plant TF values for Cd were greater than 1 with the application of all the EDDS than the EDTA treatments. The lower rates 3 and 6 mmol/kg caused higher TFs for Cd than high rate (9 mmol/kg). The observed low TF values (<1) forCr with the application of all chelants and control indicated relatively limited mobility of the metal to the shoot of sunflower in line with the findings of [42]. The addition of EDDS at 6 and 9 mmol kg^{-1} to the soil resulted in plants TF value of 1.55 and 1.02 'respectively' for Cu while other treatment showed a TF <1. In the control experiment in Awotan Apete dumpsite soil (F), the TF of Pb is very high (6.59) while those of the other treatment were less than 1. All the TF values for Zn were lower than 1 in the soil. This is an indication that in Soil F, EDDS treatments enhanced a higher root to shoot metal translocation in sunflower at all rates for Cd and 9 mmol/kg for Cu. [43] had earlier reported that EDDS was more effective than EDTA in stimulating the translocation of metals from roots to shoots "especially for Cu, Cr and Zn". [9] had also reported that the application of EDTA and EDDS increases the root-to-shoot ratios of the metals Cu, Pb, Zn and Cd in "*Zea mays*" and "*Phaseolus vulgaris*".

The significant reduction in metal concentration in soil after harvest implied the effectiveness of the combination of EDDS treatments and sunflower in the removal of the metals from the soil than EDTA treatments. EDTA is known to have a very high stability constant for metals but its effectiveness can be hampered in the presence of competing cations like, Ca^{2+}, Al, Fe and others. Decrease in Pb sorption in the presence of complexing ligands and competing cations have been demonstrated by [44]. Another reason could also be the possibility of some metal to be highly mobile. An example is Cd in this. Also the performance of the EDDS treatment in Zn reduction could also be attributed to the high metal stability constant of EDDS for Zn according to the findings of [9] that high EDDS stability constant was responsible for the reduction of Zn in the soil than EDTA.

5. CONCLUSION

The pollution of soil by heavy metal is a major problem across the globe and the clean- up is usually a difficult task. Enhanced phytoextraction of heavy metal from contaminated soil through the use of high biomass plants was proposed as an effective method because it is cost effective and does not affect the soil texture and structure. However, the main drawback of this method is that some heavy metals have low motility and are not readily bioavailable. An increase in metal solubility has been enhanced by the use of chelating agents through complexation of these metals, thereby promoting heavy metal translocation from root to the shoot of plants.

In this study, the concentrations of seven heavy metals in soils were determined. The normal levels of metals in soils which are not harmful are: Cd (1 mg/kg), Cr (34 mg/kg), Cu (20-30 mg/kg), Pb (50 mg/kg) and Zn (50 mg/kg). Their concentrations in the soils sampled exceeded normal levels of metals in soils [45,46], thus, making them to be termed contaminated soils. Therefore, there is a strong need to remove heavy metals from the soils to make them safe for agricultural activities. Phytoextraction with sunflower has been reported to be very efficient in removing metals from polluted soil but due to the low availability of these metals, chelating agents are applied to soils to enhance their uptake by plants.

Two chelating agents were used to evaluate their potentials to remove these metals from the contaminated soils. These were EDDS (Ethylene Diamine Disuccinic Acid) and Ethylene diamine tetra acetic acid (EDTA) at the following concentrations: 3, 6 and 9 mmol/kg. EDDS and EDTA were efficient in mobilizing metals in soil for plant uptake via the root into the shoot. The combination of sunflower and the selected chelating agents were effective in removing heavy metals from the contaminated soil samples, while EDDS compared favorably with EDTA in metal uptake by sunflower.

Total uptake of Cr from the experimental soil increased with the addition of EDDS at all rates than the EDTA treatments while the EDTA rate at

9 mmol/kg performed better than EDDS. Cadmium uptake from soils was greatly impacted significantly by all the EDDS rates applied, while EDDS at 3 mmol/kg only increased Cd. EDDS at all rates of application drastically enhanced total Cu uptake. Lead uptake greatly increased with the application of EDTA at 9 mmol/kg. Zinc uptake was generally enhanced by the addition of EDTA at 3mmol/kg. Total Dry matter yield in some of the control experiment was drastically reduced due to high concentration of some of the metal examined "particularly Pb. EDDS "however" enhanced total dry matter yield while reduction in dry matter yield were observed in sunflower grown in pots treated with EDTA.

The following metals Cd, Cu and Pb had a higher translocation factor under EDDS treatments than EDTA except Cd and Zn at 6 mmol/kg EDTA. EDDS and sunflower significantly reduced heavy metal concentration in the experimental soil.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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