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Effect of EDTA on Cadmium and Zinc Uptake by Sugarcane Grown in Contaminated Soil

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Abstract: The effect of EDTA on cadmium and zinc uptake by sugarcane (*Saccarumofficinarum* L.) grown in contaminated soil was investigated. Sugarcane was grown in pots for 1 month and EDTA was added at concentration levels of 0(control), 0.5, 1 and 2 millimole per 1 kilogram of soil. Plants were harvested at 2, 4, 6 and 8 months. Soil samples were analyzed to determined levels of cadmium and zinc. Plants were separated into 5 parts: Leaves, bagasses, underground stem, root and juice, including the phytotoxicity. Moreover, the plants were also analyzed for cadmium and zinc accumulation. This result shows that the concentration of EDTA at 1 millimole per 1 kilogram of soil had the highest cadmium accumulation in the root of sugarcane at 21.87, 44.68, 57.52 and 41.97 mg kg^{-1}, at the contact time, respectively. Furthermore, the root showed the most efficient sugarcane uptake compared to the underground stem, bagasses, leaves and juice (root > undergroundstem > bagasses > leaves > juice). The EDTA concentration at 2 millimoles per 1 kilogram of soil has maximum zinc accumulation in various parts of sugarcane. The harvested time at 2 months showed zinc uptake much higher than for leaves and bagasses, while the maximum accumulation of zinc was found in roots and the underground stem at 4 months.

Keywords: Cadmium, Zinc, EDTA, *Saccarumofficinarum,* Contaminated Soil

Introduction

Heavy metal pollution in soil has become a wide spread global problem, which can threaten ecosystems and human health. In northwest Thailand, cadmium (Cd) contamination in paddy field water and soil has become a growing concern. This affects agricultural products, especially rice, garlic and soybean. Cadmium contamination in soil and plants has increased to such an extent that it does not meet the safety standard set by the codex committee for food additives and contaminants (Codex, 2007). This causes are attributed to mining and human activity which have an impact on human health and the food chain (Luc *et al*., 2012). At presently there are numerous remediation technologies used to clean up heavy metal contamination in water, soil and sedi-ments. These techniques include *in situ* physical and chemical processes (soil flushing, solidi-fication and stabilization), thermal processes, *ex situ* physical and chemical processes (soil washing, chemical reduction and oxidation) and other processes including excavation and off-site disposal (Sampanpanish *et al*., 2006). Thus, alternative energy plants were selected for cultivation in Cd contaminated areas, owing to the energy crisis in Thailand. Sugarcane has been used as a raw material for ethanol production and, for this reason, has replaced rice farming in many areas (Tananonchai, and Sampanpanish, 2014). A chelating agent was proposed to improve the efficiency of conventional phytoremediation of metal polluted soils by solubiliting target metals from the soil (Salt *et al*., 1995) and making them more available for plantuptake and translocation to the shoots and leaves (Lombi *et al*., 2001). EDTA has been used the most widely for phytoremediation because it has strong chelating ability with different metals and increases the bioavailability in soil to plants (Salt *et al*., 1998). The objectives of this study were to determine the effect of EDTA on total Cd and Zn in contaminated soil and leachate at different EDTA application rates as well as determine the efficiency of total Cd and Znuptake by sugarcane grown in contaminated soil.

Science Publications

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Materials and Methods

Soil Preparation

Contaminated soil was collected in Maesot district, Tak province, Thailand from a 0-30 cm surface layer. It was air dried at room temperature before separation through a 2-mm sieve. Soil texture was determined using hydrometer methods (Bouyoucous, 1951). The pH was measured by a pH meter (soil: Water = 1:1) (Thomas, 1996). Cation Exchange Capacity content (CEC) was determined with ammonium saturation and distillation (Hendershot *et al*., 1993). Electrical Conductivity (EC) was measured by a conductance meter (soil: Water = 5:1). Organic Matter content (OM) was determined by the Walkley-Black method (Nelson and Sommers, 1996). Available phosphorus was determined with Bray II sollution (Bray and Kurtz, 1945). Available Potassium was determined by ammonium acetate 1 N pH 7.0 extractions (Tan, 2005). The total Cd and Zn contents in soil were determined by USEPA method 3052 (USEPA, 1996) and analyzed using an Atomic Absorption Spectrometer (AAS). A Perkin Elmer AAs model AAnalyst 800 (Perkin Elmer Instruments LLC, Unberlingen, Germany) was used.

Pot Preparation

The Cd contaminated soil was sepeated into at 10 kg batches and placed in plastic plots (a dry weight soil). These were then covered with plastic bags. Plastic was also placed under the pots to collect drainage water which was then returned to the pots to prevent the loss of metals through leaching. After this, each pot was planted with an underground stem (setts). A month later, the first application of EDTA was conducted. The EDTA was added at concentration levels of 0(control), 0.5, 1 and 2 millimoles per 1 kilogram of soil. The plants were prepared by cutting pieces of mature sugarcane stems (setts), LK 92-11 ecotype, obtained from Kampangpetch province. The USEPA method 3052 was used for the analysis of background cadmium and zinc in plant (setts) samples. The result showed that cadmium and zinc contents in setts were non-detected. Soil, sugarcane and leachate water samples were harvested at 2, 4, 6 and 8 months. An analysis was made to determine levels of cadmium and zinc in the soil and in five plant parts: Leaves, bagasses, underground stem, root and juice.

Sample Preparation and Analysis

Soil was dried at 105°C for 24-48 h to achieve aconstant weight. It was then crushed to pass through a 2-mm sieve and thoroughly mixed to homogenize. To determine the available cadmium and zinc in the soil sample, it was air-dried for 72 h, then crushed to pass through a 2-mm sieve and finally mixed to homogenize before analysis. Sugarcane samples were cleaned and washed with tap water twice and rinsed with deionized water. Next, they were cut into five parts: Leaves, bagasses, underground stem, root and juice. Samples were dried at 105°C for 24-48 h to achieve aconstant weight and dry matter yields were determined. Sugarcane juice was analyzed by digestion in a mixture of 10:1:4 (v/v/v) of HNO_3: H_2SO_4: $HClO_4$ (Jackson, 1973). Available cadmium and zinc in soil were estimated by DTPA extraction method (0.005 M DTPA +0.01 M CaCl2) (Lindsay and Norvell, 1978). Total cadmium and zinc in soil and sugarcane (leaves, bagasses, underground stem, root and juice) were determined using the USEPA method 3052 (USEPA, 1996). Leachate water was analyzed by the USEPA method 3015A (USEPA, 1998). The digested solution was analyzed by an Atomic Absorption Spectrometer (AAS).

Statical Analysis

The variance and significance of cadmium and zinc in soil, sugarcane and leachate water samples were analyzed by Analysis Of Variance (ANOVA). In cases where the data varies, the difference was compared by Duncan's New Multiple Range Test (DMRT). Statistical analysis of data was performed using the Statistical Package for Social Science (SPSS) software.

Results

Soil Properties

The contaminated soil was collected in Maesot district, Tak province, Thailand. The soil background properties were determined and are presented in Table 1. Soil texture is loam with pH value of 7.6 which is considered neutral. The background cadmium and zinc in soil were 136.47 and 4,137.43 mg kg^{-1}, respectively. The contamination levels of heavy metals in soils have been reported for different countries. The USEPA (1990) and Alloway (1995) reported that cadmium contaminated soil at 2 and 12 mg kg^{-1} would be zinc accumulated at 200 and 720 mg kg^{-1}, respectively.

Effect of EDTA on Cadmium and Zinc Accumulation Inleachate Water

Table 2 shows that cadmium and zinc accumulation inleachate water increased when EDTA rates increased. It was found that the highest amounts of cadmium and zinc accumulated in 2 mmol kg^{-1} of soil. Cadmium accumulation was found at 0.17, 0.22, 0.28 and 0.37 mg kg^{-1} and zinc at 1.17, 1.25, 1.62 and 2.00 mg kg^{-1} at harvested time of 2, 4, 6 and 8 months, respectively. The cadmium and zinc concentrations inleachate water increased with the increase in harvesting time.

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Effect of EDTA on Cadmium and Zinc Accumulation in Soils

Figure 1 shows the effect of EDTA concentration levels at 0(control), 0.5, 1 and 2 mmol kg^{-1} on cadmium and zinc accumulation in soil. The results tend to suggest that EDTA decreased cadmium and zinc concentrations in soil slightly because of its uptake to accumulate in plant tissues. Cadmium and zinc accumulation in soil was lowest at 8 months. The accumulation of cadmium in soil at 8 month was 102.36, 102.05, 96.08 and 98.34 mg kg^{-1} and zinc were 3,200.76, 3,116.97, 3,031.52 and 3,005.63 mg kg^{-1} for EDTA applied at the rate of 0(control), 0.5, 1 and 2 mmol kg^{-1} of soil, respectively. The concentrations of total cadmium and zinc in soil were lowest at 0.5, 1 and 2 millimole per 1 kilogram of soil as compared to non-EDTA soil (0 mmol kg^{-1} of soil). Madrid *et al*. (2003) also found EDTA to be highly effective at mobilizing metals in soil making it easy for uptake by plants.

Effect of EDTA on Availability of Cadmium and Zinc in Soil

Availability or bioavailability is the proportion of total metals that are available for incorporation into biota or taken up by plants (John and Leventhal, 1995). This study shows that the level of available cadmium and zinc tended to decrease when havesting time increased (Fig. 2). However, statistical analysis by ANOVA indicated that there was no significanted difference in the available cadmium and zinc concentration in soil throughout the harvesting time and when adding EDTA of 0(control), 0.5, 1 and 2 mmol kg^{-1} of soil. The highest available cadmium concentrations in the soil were 27.81, 29.31, 30.11 and 30.34 mg kg^{-1} for EDTA applied at the rate of 0(control), 0.5, 1 and 2 mmol kg^{-1} of soil, respectively at 2 months. While, available zinc concentration highest at 2 months were 78.02, 81.77, 85.25 and 102.05 mg kg^{-1} for EDTA applied at the rate of 0(control), 0.5, 1 and 2 mmol kg^{-1} of soil, respectively.

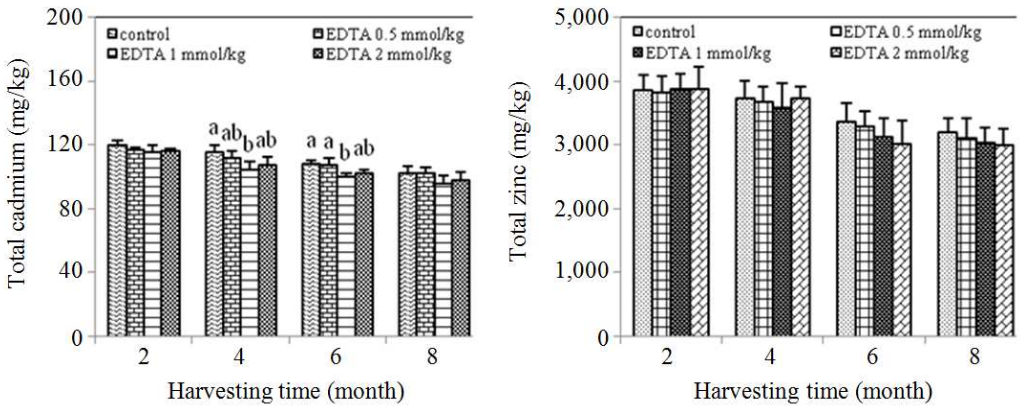

Fig. 1. Effect of EDTA on cadmium and zinc accumulation in soil

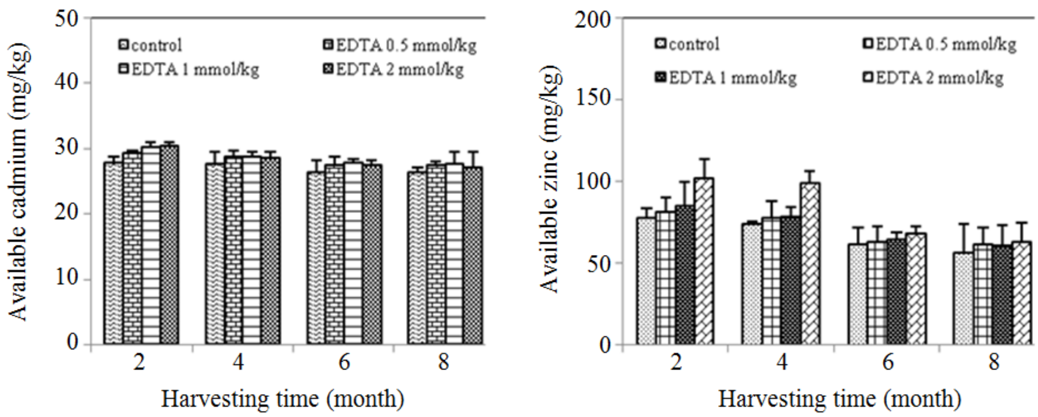

Fig. 2. Effect of EDTA on availability of cadmium and zinc in soil
Note: Differences on each bar mean statistical difference at significant level of 95% in the same concentration but different duration according to Duncan's new multiple range test

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Table 1. Soil properties for the experiment

Parameters	Content
Sand (%)	43.00
Silt (%)	43.40
Clay (%)	22.60
Soil texture	Loam
Ph	7.60
Cation Exchange Capacity (c $mol_{(+)}kg^{-1}$)	7.50
Electrical Conductivity (ds m^{-1})	0.19
Organic Matter (%)	3.00
Total Nitrogen (%)	0.15
Available Phosphorus (ppm)	7.00
Available Potassium (ppm)	62.00
Total Cd (mg kg^{-1})	136.47
Total Zn (mg kg^{-1}) 4,137.43	

Table 2. Effect of EDTA on Cadmium and Zinc accumulation inleachate water

EDTA concentration (mmol/kg)	Cd accumulation (mg/kg)				Zn accumulation (mg/kg)			
	2 months	4 months	6 months	8 months	2 months	4 months	6 months	8 months
0	0.14±0.05*	0.18±0.02	0.23±009	0.27±0.06	0.73±0.28	0.81±0.72	1.40±1.10	1.76±0.12
0.5	0.14±0.06	0.18±0.03	0.24±0.02	0.29±0.07	0.75±0.31	0.83±0.52	1.36±0.77	1.72±1.17
1	0.16±0.04	0.20±0.05	0.28±0.05	0.30±0.01	0.78±0.33	0.83±0.41	1.38±0.65	1.93±0.48
2	0.17±0.04	0.22±0.03	0.28±0.03	0.37±0.02	1.17±0.31	1.25±0.06	1.62±0.38	2.00±0.95

Note: *Mean ± Standard deviation (n = 3)

Effect of EDTA on Cadmium Accumulation in Different Parts of Sugarcane

The effect of EDTA on cadmium accumulation in different parts of sugarcane is illustrated in Fig. 3. The result shows that EDTA at 1 mmol kg^{-1} of soil produced maximum cadmium accumulation in different parts of sugarcane. The highest cadmium accumulation in leaves was 7.21, 8.24, 12.45 and 11.16 mg kg^{-1} for EDTA rates of 0(control), 0.5, 1 and 2 mmol kg^{-1} of soil, respectively, at 4 months (Fig. 3a) and 9.71, 10.40, 14.13 and 12.75 mg kg^{-1} in bagasses, respectively at 4 months (Fig. 3b). At the same time, the highest cadmium accumulation in underground stems was 11.07, 11.59, 16.76 and 15.76 for EDTA rate, respectively (Fig. 3c). The highest cadmium accumulation in roots was 49.09, 50.65, 57.52 and 52.27 mg kg^{-1} for EDTA applied rate, respectively at 6 months (Fig. 3d). Moreover, this result shows lower cadmium contamination in juice among 0.15-0.23 mg kg^{-1} (Fig. 3e). In their study, Chen and Cutright (2001) found EDTA at 0.5 g kg^{-1} tended to increase cadmiumin the shoot of *Helianthus annuus* from 34 to 115 mg kg^{-1}, demonstrating the total removal efficiency at 59 µg/plant. The cadmium accumulation in different parts of sugarcane were highest in roots followed by underground stems>bagasses>leaves> juice. This result conforms with the research of Segura *et al*. (2006) who noted that highest cadmium accumulation was in the root of sugarcane (*Saccharum* spp.) at 0.23 mg kg^{-1}, followed by stems and leaves which was equal to 0.20 and 0.13 mg kg^{-1}, respectively.

The Effect of EDTA on Zinc Accumulation in Different Parts of Sugarcane

The effect of EDTA on zinc accumulation in different parts of sugarcane is reported in Fig. 4. The result shows the highest zinc accumulation in leaves at 62.11, 66.20, 79.66 and 87.18 mg kg^{-1} at 2 month for EDTA rate of 0(control), 0.5, 1 and 2 mmol kg^{-1} of soil, respectively (Fig. 4a), while EDTA at 2 mmol kg^{-1} in soil produced maximum zinc accumulation in the leaves: 87.18, 36.65, 35.09 and 26.67 mg kg^{-1}, at harvest time, respectively. The highest zinc accumulation in bagasses was 111.58, 281.30, 320.00 and 378.16 mg kg^{-1}, respectively at 2 months (Fig. 4b). EDTA at 2 mmol kg^{-1} of soil produced maximum zinc accumulation in the bagasses of 378.16, 144.78, 115.70 and 65.90 mg kg^{-1}, at harvest time, respectively. Figure 4c shows that the highest cadmium accumulation in underground stems was 114.40, 116.06, 125.00 and 151.12 for EDTA applied at the rate of 0(control), 0.5, 1 and 2 mmol kg^{-1} of soil, respectively at 4 months, while EDTA at 2 mmol kg^{-1} of soil produced maximum zinc accumulation in the underground stems at 130.21, 151.12, 89.27 and 69.95 mg kg^{-1}, of harvest time, respectively. The highest zinc accumulation in roots was 1,003.62, 1,069.34, 1,165.75 and 1,169.98 mg kg^{-1} for EDTA applied at the rate of 0(control), 0.5, 1 and 2

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mmol kg^{-1} of soil, respectively at 4 months. However, the maximum zinc accumulation in the root at EDTA 2 mmol kg^{-1} was 849.56, 1,169.98, 762.15 and 538.33 mg kg^{-1}, at harvest time, respectively (Fig. 4d). Moreover, this study found that the EDTA had produced zinc accumulate in juice among 10.91-12.88 mg kg^{-1} (Fig. 4e). Note that the result shows zinc accumulation in different parts of sugarcane tends to be highest in roots followed by bagasses> underground stems> leaves> juice. Weihong *et al*. (2009) reported that with EDTA at 0.8 mmol kg^{-1}, shoot and root of *Vetiveriazizanioides* tend produce an increase of zinc accumulation at 7.3 and 37.4%, respectively. Kabata-Pendias and Pendias (2000) reported that roots tend to contain more zinc than shoots and leaves, particularly if plants are grown in zinc-rich soil. Our findings were similar to the results obtained by Oprea *et al*. (2010) and Ranjan *et al*. (2012).

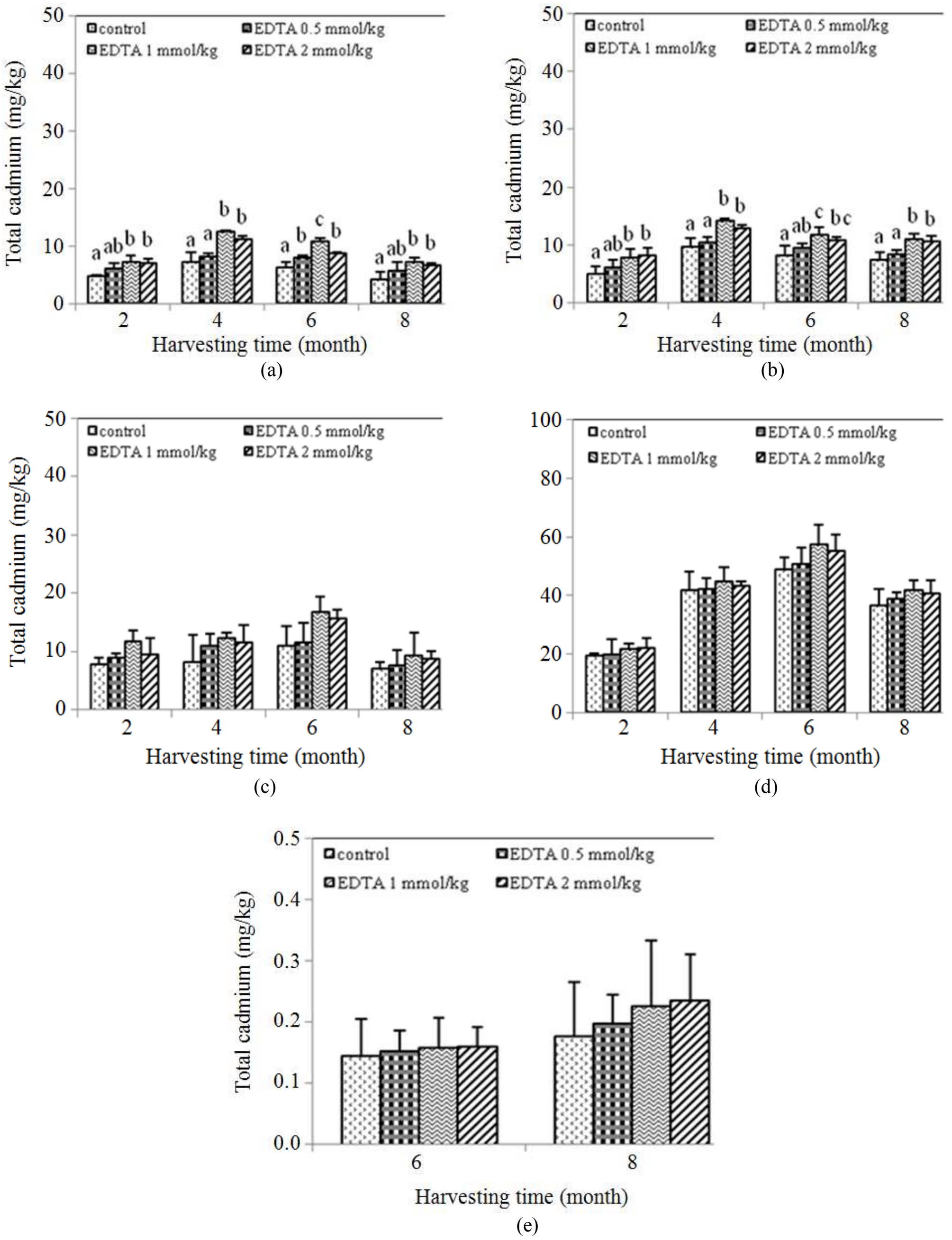

Fig. 3. Concentration of total cadmium in different parts of sugarcane (a) Leave, (b) Bagasses, (c) Underground Stem, (d) Root and (e) Juice

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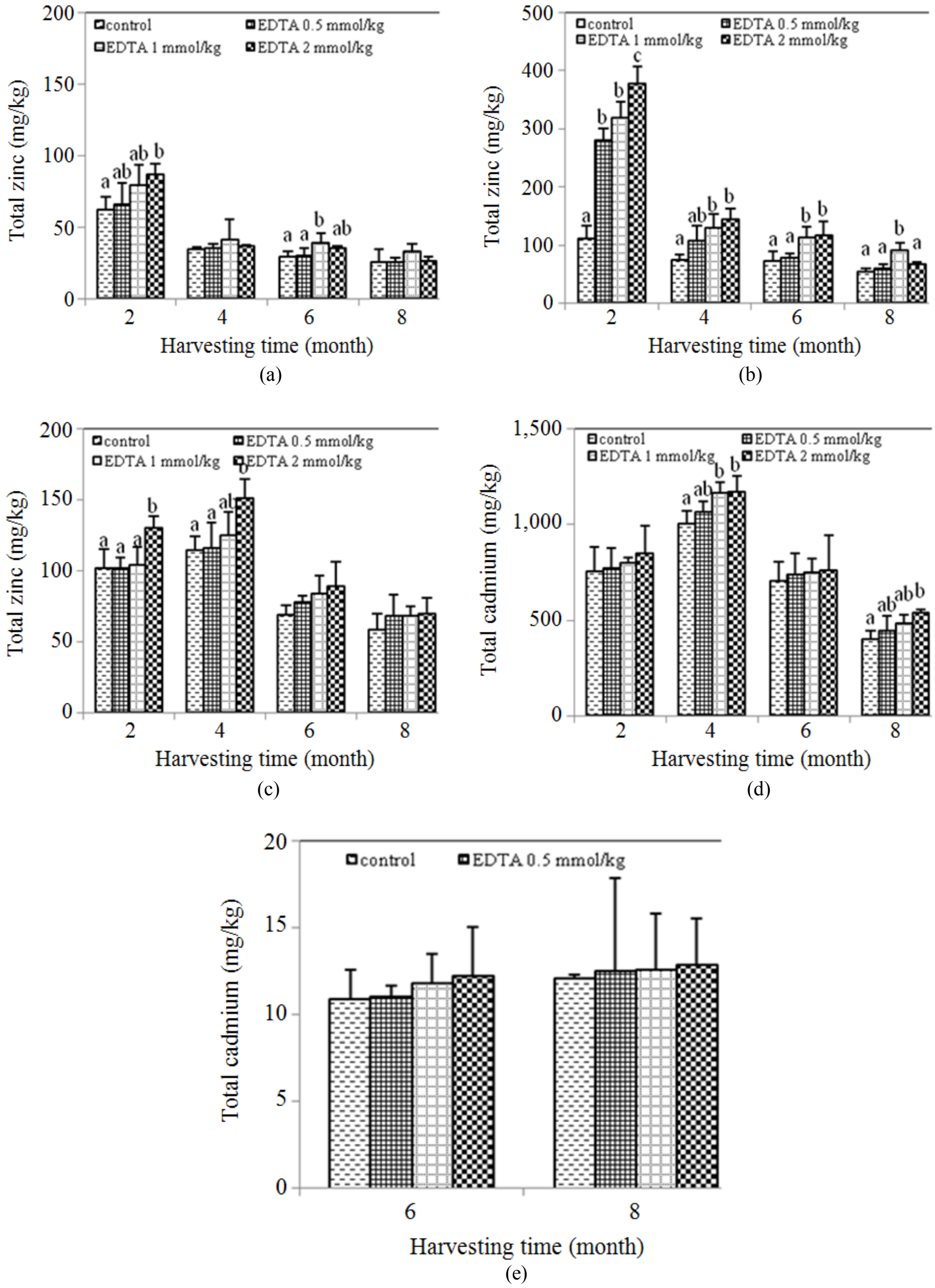

Fig. 4. Concentration of total zinc in different parts of sugarcane (a) Leave; (b) Bagasses; (c) Underground Stem; (d) Roots and (e) Juice

Conclusion

The effect of EDTA on cadmium and zinc uptake by sugarcane (*Saccarumofficinarum* L.) grown in contaminated soil was investigated. This result shows that soil cadmium and zinc contamination tends to decrease when harvesting times increase. In contrast, at increased harvesting time, cadmium and zinc accumulation increasing in plants while EDTA did not affect sugarcane growth. However, the results show that EDTA at 1 mmol kg^{-1} in soil produced the highest cadmium accumulation in various parts of sugarcane. Two mmol/kg of EDTA in soil resulted in maximum zinc accumulation in various parts of sugarcane. Thus, the EDTA increased cadmium accumulation of sugarcane in the order: Root > underground stem >

bagasses> leaves> juice, while EDTA produced an increase in zinc accumulation in sugarcane as follows: Root> bagasses> underground stem > leaves > juice.

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Author's Contributions

This article is original research paper. Authors participated in all experiments, coordinated the data-analysis and contributed to the written and read of this manuscript. Authors give final approval of the version to be submitted this journal.

Ethics

The authors declare no conflicts of interest and confirm that the manuscript has been submitted solely to this journal and is not published, in press, or submitted elsewhere.

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