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RESEARCH PAPER

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Nitrogen fertilizer and EDTA effect on *Cannabis sativa* growth and Phytoextraction of heavy metals (Cu and Zn) contaminated soil

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Abstract

Heavy metals in soil, water and air is a great concern. Toxic heavy metals ultimately affect plant, Animals, and through food chain directly influence human life. In present study, the effect of heavy metals (Cu, Zn), N-fertilizers and EDTA on growth and biomass of cannibus sativa plant was evaluated. The Cu and Zn phytoextraction potential of *Cannabis sativa* plant under various treatments of N-fertilizers and EDTA were investigated. Metals (Cu, Zn) significantly reduced the plant growth and biomass while the fertilizer application increased the plant growth and biomass under metals stress. The application of EDTA alone increased the metals (Cu, Zn) accumulation in root, stem and leaves but reduced the plant height, root length and biomass. The maximum accumulation of Cu in root (75 µg/g DW), stem (55 µg/g DW) and in leaves (45 µg/g DW) was found with EDTA treatment (T3). Maximum Zn concentration in Leaves (155 µg/g DW) and root (148 µg/g DW) was observed in plants treated with EDTA. Conclusively, metals contaminated soil considerably reduced plant growth and biomass while metals in combination with N-Fertilizer the plant growth and biomass was enhanced. Addition of EDTA significantly enhanced phytoaccumulation of metals (Zn, Cu).

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Introduction

Industrial revolution and excessive urbanization have increased the hazardous pollutants concentration in the environment, which consist of different kinds of organic compounds and heavy metals that affect the human health (USEPA, 1997) and wild life (Raskin *et al.,* 1997). Among them soil pollution is one of the most vital environmental problem over the last decades (Raskin *et al.,* 1997). Heavy metal toxicity and its bioaccumulation in the food chain represent one of the major environmental and health problems of our society. Major sources of metals pollution are burning of fuels, mining and smelting of metallic ferrous ores, municipal sewage, fertilizers and pesticides (Ross, 1994). The most common pollutants of the environment are Cadmium, Chromium, Copper, Mercury, Lead, Nickel and Zinc (Lasat *et al.,* 2002). Cu in excess amounts caused chlorosis, reducing of root and shoot growth (Ait Ali *et al.,* 2002). Zn toxicity is associated with the reduction of root elongation, leaf curling by an induced deficiency of Mg or Fe, while in bean plants; it inhibits photosynthesis (Marschner, 1995).

The removal of heavy metals from contaminated soil is a major problem and requires new techniques to enhance the process. Biological processes are being used to decontaminate soils. Some plants are hyper accumulators that actively take up heavy metals and accumulate at in aerial parts with high concentration from soil (Brooks, 1998), such plants can absorb and accumulate about 10,000 µg/g of Zn and 1,000 µg/g of Cu (Turgut *et al.,* 2005). Due to the advancement in technology, Plants are used as green technology or phytoremediation for soil amendments and agronomic practices to remove pollutants from the environment or to minimize its toxicity (Raskin and Ensley, 2000; Jing *et al.,* 2007). Nitrogen fertilizers play a vital role in phytoextraction along with plant growth and enhance heavy metal stress tolerance and absorption (Boroujerdnia and Ansari, 2007).

The aim of this research work is to study the effects of fertilizer (Urea) and EDTA either alone or in combination with heavy metals (Cu and Zn) phytoextraction through *Cannabis sativa* plant.

Materials and methods

Soil Preparation

The soil sample was collected from University of Malakand, sun dried and treated with $CuSO_4$, and $ZnSO_4$ at the ratio of 881mg per kg respectively. The pH of soil samples were adjusted at (7.8) and filled each pot with 2 kg. All the agronomic practices were carried out whenever needed in the glass house.

Seedling transformation

The uniform sized seedlings of *Cannabis sativa* were transferred to pots in replicate and kept up to one week for acclimatization in green house at 35-42ºC.

Treatments to seedlings

Different Treatments of Cu, Zn, 2.5% N-fertilizer (Urea) and EDTA were used during the whole experiment. Control (C) was compared with C1 for Cu and Zn effect on plant growth and biomass, while C1 was compared with all other treatments for Cu and Zn Phytoextraction. Aqueous solutions of nitrogen fertilizer and EDTA were made according to the water holding capacity of soil (2.5%=2.5g /100ml).

Treatments	Denoted
Control (without treatment)	C
control with Cu + Zn	C1
N-fertilizer (Urea)+Cu+ Zn	T1
N-fertilizer (Urea)+100 mg EDTA+ Cu+ Zn	T2
100mg EDTA+ Cu+ Zn	T3

Harvesting and measurement of different parameters

The plants were harvested after eight weeks treatments. The root, stem and leaf length was measured. Plants were separated i.e. roots, stem and leaves. Analytical balance was used for measurement of fresh biomass of the different parts for each plant, and then dried at 80 ºC for 48 hrs in oven. The dried samples were crushed into powdered using mortar and pestle.

Acid digestion and Cu, Zn analysis

The 0.25 g powder sample was taken into 50 ml flask, then 5 ml of Nitric acid (HNO_3), 0.5 ml of perchloric acid ($HClO_4$) and 1 ml sulfuric acid (H_2SO_4) were added (Allen, 1974). The flask was kept on hot plate for 15 minutes at 300 °C until white fumes come out. The sample was cooled, filtered into plastic bottle and the volume of filtrate was raised up to 50 ml by addition of distilled water. The digested samples were then analyzed for the Cu and Zn concentration using atomic absorption spectrometer.

Statistical analysis

The data was subjected to ANOVA and the mean values were compared by using Turkey's Multiple Comparison test, at $P< 0.05$. The data was analyzed using Graph pad prism.

Results and discussions

Effect of Nitrogen fertilizer, Cu, Zn and EDTA on Plant growth and dry biomass

Our results showed that all the treatments had significant affect on plant growth and dry biomass as compared to Control C (Table 1). C1 (Cu + Zn) treated plants showed reduction in plant growth as compared to C. The treatment T1 (N-fertilizer + Cu + Zn) enhanced the plant growth while treatment T2 (N-fertilizer + Cu + Zn + 100 mg EDTA) slightly enhanced growth and biomass except root length as compared to C1. The treatment T3 (Cu + Zn + 100 mg EDTA) showed significant reduction in growth and biomass of plant as compared to C1 (Table 1).

Table 1. Effect of Cu and Zn on growth and biomass of *Cannabis sativa*.

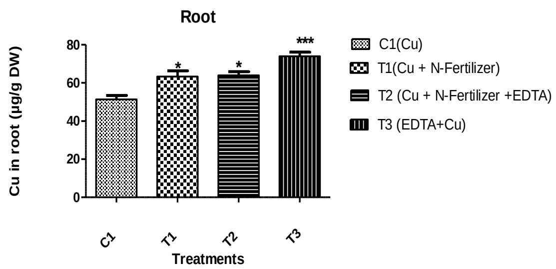
Treatments	Plant Height (cm)	Root Length (cm)	Root Dry Biomass (g)	Stem Dry biomass (g)	Leaves Dry Biomass (g)
C (Without Cu, Zn)	34 ± 0.53^{a}	12 ± 0.57^{a}	0.41 ± 0.05^{a}	0.86 ± 0.11^{a}	1.4 ± 0.10^{a}
C1 (With Cu, Zn)	24 ± 0.61^{c}	11 ± 0.38^{a}	0.36 ± 0.59^{c}	0.42 ± 0.12^{d}	0.66 ± 0.12^{c}
T1(2.5% N- fertilizer + Cu + Zn)	33 ± 0.81^{a}	12 ± 0.25^{a}	0.43 ± 0.17^{b}	0.77 ± 0.12^{b}	1.4 ± 0.23^{a}
T2 (2.5%N- fertilizer + Cu+Zn+100 mg EDTA)	28 ± 1.2^{b}	8.3 ± 0.58^{b}	0.43 ± 0.17^{b}	0.73 ± 0.17^{c}	0.85 ± 0.12^{b}
T3 (100 mg EDTA + Cu+ Zn)	22 ± 0.42^{d}	7.2 ± 0.57^{c}	0.26 ± 0.17^{d}	0.40 ± 0.17^{de}	0.56 ± 0.23^{cd}

Our results are in line with the work of (Mahmood *et al., 2005*). Overall nitrogen fertilizer increased the plant growth along with metals (Cu and Zn) and EDTA. Similar results are also reported by (ShuheWei *et al.,* 2009) that the plant growth, shoot and root biomass increases with the application of fertilizers. The plant length and dry biomass increased as nitrogen fertilizer rate increased to 120 kg N/ha (Boroujerdnia and Ansari, 2007). It revealed that Cu^{2+} and Zn^{2+} significantly reduced the stem and leaves biomass. High levels of heavy metals decrease dry matter content of plants. Our results are also in line with the work of (Hadi *et al.,* 2010).

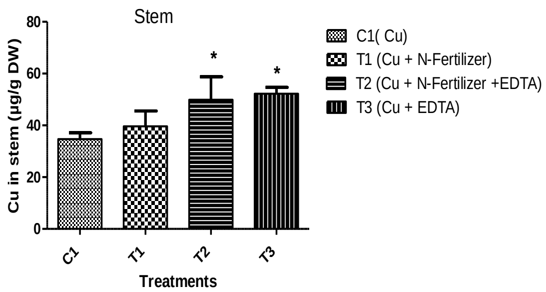
The effect of heavy metals on stem and leaves biomass was more adverse than root as compared to control C1 (Table 1). Such results have been reported by (Sun *et al.,* 2009) that the addition of EDTA and Cu inhibit the plant growth and dry biomass. The results indicated that EDTA treatments significantly reduced plant height, root length and biomass as compared with C1 (Cu + Zn). EDTA led to a severe yield reduction in the biomass across the treatments. severe reduction in the growth was attributed to the combination of heavy metal concentration and the addition of chelators that exceed the capacity of plants to activated defense systems (Chen *et al.,* 2000; Sun *et al.,* 2009).

Phyto-accumulation of Cu and Zn in different parts

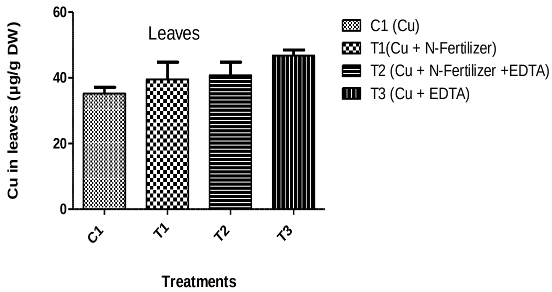
The effect of different treatments on the accumulation of Cu and Zn in the roots and its translocation into the stem and leaf tissues were evaluated. The level of Cu and Zn ($\mu g\ g^{-1}$) content in the roots stems, and leaf tissues are presented in (Fig 1A–C) and (Fig 2 A-C) respectively. Our results indicated that N-fertilizer and EDTA alone and in combination played different roles in the accumulation and translocation of Cu and Zn into different parts of the plant. The accumulation of Cu and Zn in roots, stem and leaves significantly increased with all treatments as compared to control C1(Fig 1A-C) and (Fig 2A-C) respectively. The only EDTA treatment (T3) significantly increased the accumulation of Cu and Zn content in root, stem leaves followed by the treatment T2 and T1 as compared with C1. Similar results were also reported by (Boroujerdnia and Ansari, 2007; Jordan *et al.,* 2002; Barocsi *et al.,* 2003; Hernandez-Allica *et al.,* 2003; Reinhard *et al.,* 2007) that nitrogen fertilizer increased the accumulation of Cu and Zn in different parts of plant.



A

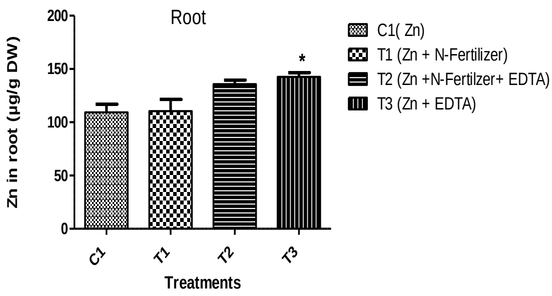


B

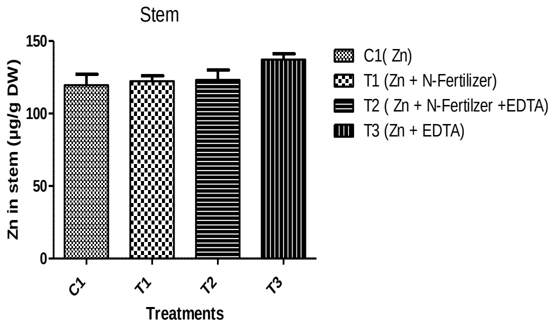


C

Fig. 1. Accumulation of Copper (Cu) in roots (A), stems (B), and leaves (C)



A



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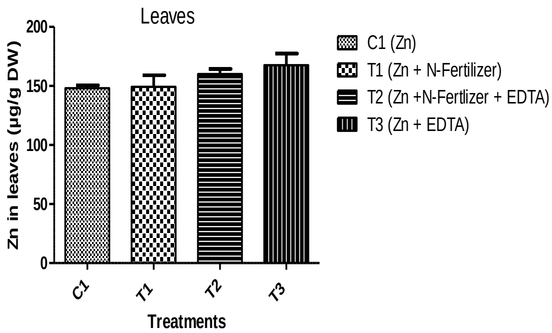

C

Fig. 2. Accumulation of Zinc (Zn) in (A) roots, (B) stems, and (C) leaves

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