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ORIGINAL ARTICLES

Alleviation of Certain Heavy Metals Toxicity on *Zea mays* by Arbuscular Mycorrhiza

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ABSTRACT

Soil pollution with heavy metals causes a lot of hazards especially when they accumulate in plant tissues. Arbuscular mycorrhizal fungi have a magic role in soil remediation that polluted with heavy metals. So, a greenhouse experiment was conducted at the nutrition greenhouse, Soil, Water and Environment institute (SWERI), ARC, Giza, Egypt during summer 2011 to study the interaction of Arbuscular mycorrhizal with certain heavy metals (Zinc and Copper) when soil polluted with three concentrations 0.1, 1 and 5 mM of $ZnSO^4$ or $CuSO^4$, and the impact on metal mobilization and accumulation by Zea mays. Results confirmed the ability of mycorrhizal to increase plant root and shoot lengths, dry weights and yield parameters. The increase of mycorrhizal colonization (%) in roots, the plant content of nitrogen, phosphorous, potassium, crude protein (%) and total carbohydrates were also detected in this study. Increase in soil enzyme activities urease and dehydrogenase were resulted from incorporation of AMF and metals in the soil. Results illustrated that the concentrations of heavy metals in Zea mayze plants were limited according to the actual requirements of plant. Mycorrhizal plants led to the increase of tolerance index against Zn and Cu concentrations and also alleviated the reduction rate in plant. That proved the vital role of AMF to limit heavy metals translocations and gave the possibility to use it as an inoculum in remediation of polluted soil with these fatal metals.

Key words: Arbuscular mycorrhiza, heavy metals, Zinc, Copper, phytobioremediation.

Introduction

It is well known that heavy metals cannot be chemically degraded and need to be physically removed or be immobilized. Traditionally, remediation of heavy metal contaminated soils involves either on-site management or excavation, and subsequent disposal to a landfill site (Prasetyo *et al.,* 2010).

The phytoremediation is used as a promising method to remove and/or stabilize soils contaminated with heavy metals. It has been widely accepted as a cost-effective and environmental-friendly clean up technolology and has recently attracted much attention (Salt *et al.* 1995). There are several approaches of phytoremediation being developed to extract toxic metals from soil such as use of hyperaccumulator plants with exceptional metal accumulating capacity, use of high biomass crops which are only induced to take up large amounts of metals when the mobility of metals in soil is enhanced with chemical treatments; and the use of fast-growing trees (McGrath *et al.,* 2001).

Arbuscular mycorrhizal (AM) fungi can improve plant tolerance to heavy metals and/or enhance plant growth under heavy metal contaminations. Moreover, it has been indicated that AM fungi can colonize plant roots in metal contaminated soil, while their effects on metal uptake by plants are conflicting. In slightly metal contaminated soil, most studies show that AM fungi increased shoot uptake of metals (Vogel- Mikŭs *et al.,* 2005), while in severely contaminated soil, AM fungi could reduce shoot metal concentration and protect plants against harmful effects of metals (Malcova *et al.,* 2003). Therefore, it is recommended to introduce mycorrhizal plants as soil improvers to rehabilitate polluted sites by optimizing the uptake of bioavailable metals due to modification of the root/rhizosphere systems (Khan *et al.,* 2000).

The result of mycorrhizal colonization on clean-up of contaminated soils depends on the plant–fungus–heavy metals combination and is influenced by soil conditions. Furthermore, AM fungi also affect metal uptake by plants from soil and translocation from root to shoot, however, mycorrhizal effects may depend on elements, plant and fungal species/ecotypes (Wang *et al.,* 2005).

The external mycelium of AM fungi provides a wider exploration of soil volumes by spreading beyond the root exploration zone (Malcova *et al.,* 2003), thus providing access to greater volume of heavy metals present in the rhizosphere. A greater volume of metals is also stored in the mycorrhizal structures in the root and in spores. For example, concentrations of over 1200 mg kg^{-1} of Zn have been reported in fungal tissues of *Glomus mosseae* and over 600 mg kg^{-1} in *G. versiforme.* Although Cu is toxic to plant and fungal symbionts at high

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concentrations, AM fungi have been reported to occur widely in Cu mine spoils and Cu contaminated soils. This indicates that there may be some AM fungal species tolerant Cu and suggests their potential use in phytoremediation. It was studied the form and localization of Cu accumulation in the extra- radical mycelium of three AM fungi isolated from the same polluted soil contaminated with Cu (Lins *et al.,* 2006).

Some investigations have indicated that the zinc distribution pattern changed in mycorrhizal compared to non-mycorrhizal roots. In mycorrhizas, zinc was located mainly in the arbuscules and other fungal structures, such as internal hyphae. In contrast, in the corresponding roots, zinc was distributed in the outer layers. Therefore, isolation of indigenous stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants in disturbed ecosystems (Dodd & Thompson, 1994).

Therefore, the aim of this investigation is to study the role of AM-fungi in heavy metals remediation as well as understanding the AM fungal mechanisms during remediation.

Materials and Methods

Isolation of arbuscular mycorrhizal (AM) fungi from polluted regions:

Samples of wild plants with their roots and rhizospheric soil region were collected from 7 different localities polluted with heavy metals in El- Menufia governorate. These localities include Quesna (Industerial region), Drainage channel (Kafr El arab -Tala), Drainage channel (Babel -Tala), Bus station (Shebein El kom), Waste water plant (El Maii – Shebein El kom), Drainage channel (El Salamon - El Shohada) and Waste water plant (El Ghory – Birket - El-Saba).

The rhizospheric soil mass was gently removed from the root system of each plant (250 gm) and suspended in 1 liter tap water and then sieved using wet-sieving and decanting technique. Seven sieves (400, 270, 250, 200, 150, 80, 75 μm-mesh size) were used for extraction of mycorrhizal spores. The 250, 150 and 75 μm fractions were transferred into a glass bottle and diluted with water to give (between 20 and 50 spores/ml). The numbers of spores was estimated by spreading certain volume of mycorrhizal spore suspension onto a gridded filter paper or Petri-dish which was divided into squares from its base and then counting by using a bionuclear microscope (30-50 X) (Daft & Hogarth, 1983).

The morphological characteristics of the mixture of extracted mycorrhizal spores were determined according to the key prepared by Trappe (1982). These characteristics included shape, size, color, and distincting wall layer, surface configuration of spores, attached hyphae and sporocarps. The AM spores were identified as *Glomus, Gigaspora, Acaulospora* and *Enterophospora* spp. The spores were propagated and extracted to use as inocula.

The host plant (*Zea mays*) cultivar Giza 321 obtained from the maize department, Agricultural Research Center, Giza, Egypt. These grains were surface sterilized with Clorox solution (0.05 %), then were washed several times with distilled water.

Stock solutions of the tested metals (Zn and Cu) were prepared by dissolving the salts in certain volume of distilled water to get a desired concentration (1M ZnSO4 and 1M CuSO4), and then they were sterilized by membrane filtration (cellulose nitrate, pore size 0.45 μm; whatman, Maidstane, U.K.) and stored for experimentation. An aliquot volume of the stock solution (1M) was diluted and added to the soil to reach a final concentration of 0.1, 1.0 and 5.0 mM ZnSO4 or CuSO4. Then these concentrations were added to the pots and left for 14 days to allow homogeneous distribution of the metal salts within the soil.

The grains were coated with AM spores as described by a modified method obtained by Massoud et.al. (2009). Mixed spores of AM were prepared after propagation and mixed with sterilized vermiculite (20% moist.) as a carrier. Inoculation of AM mycorrhiza fungi was carried out by AM spore coating onto surface sterilized *Zea mays* grains (70 spore/grain) using Arabic gum (40%) as sticker, and air dried for 2h before sowing.

Greenhouse experiment was carried out in the Agricultural Research Center Farm, Giza, Egypt during June, 2011. where the temperature of the greenhouse ranged between 35 °C at day time and 21 °C at night during the period of planting. Each plastic pot contains (5kg) of a clay loam soil which considered as non saline – non alkaline soil where its salinity was 3.4 ds/m and pH was 7.3 (Jackson, 1973). Zinc and copper content in this soil were 3.44 and 3.97 mg/kg soil, respectively.

Three surface sterilized coated grains of *Zea mays* were sown in each pot. After 15 days the seedlings were thinned into two seedlings for each.

The experiment was arranged in 15 treatments with six replicates in randomized block design as follows:

- T1 (control full dose of NPK without AM fungi and heavy metals contamination).
- T2 (inoculation with AM fungi.).
- T3 (0.1 mM zinc sulphate).
- T4 (1 mM zinc sulphate).
- T5 (5 mM zinc sulphate).

- T6 (0.1 mM zinc sulphate + AM fungi).
- T7 (1 mM zinc sulphate + AM fungi).
- T8 (5 mM zinc sulphate + AM fungi).
- T9 (0.1 mM copper sulphate).
- T10 (1 mM copper sulphate).
- T11 (5 mM copper sulphate).
- T12 (0.1 mM copper sulphate +AM fungi).
- T13 (1 mM copper sulphate +AM fungi).
- T14 (5 mM copper sulphate +AM fungi).
- T15 (control without no AM fungi or heavy metals)

The plants were watered to maintain soil moisture at about 40 % water holding capacity by adding dechlorinated tap water during the experimental period (100 days). Two plants in each pot were taken at 3 different intervals (30, 60 and 90 days) of each treatment. Plants with their roots and rhizospheric soil were collected for determination of vegetative, microbiological, physiological, chemical and crop yield parameters.

At sampling time, the lengths of both roots and shoots were determined (cm/plant). Other set of plant roots and shoots were washed twice with distilled water to remove the adhering soil particles. They were oven-dried at 70 °C for 48h, to obtain dry weight (gm /plant) and then grounded to fine powder for chemical analysis.

The mycorrhizal dependency (MD) of the plants was calculated according to Gerdemann (1975) as:

MD = (Dry weight of infected plant at a particular level of metal / Dry weight of non-infected plant at the same level of metal) x 100.

Tolerance indexes (TI) % on infected and non infected plants to heavy metals in soil were determined (Rabie, 2005) as:

TI = (Dry weight of plant in polluted soil at particular level of metal / Dry weight of plant in non-polluted soil at a 0.0 level of metal) x 100

Root infection rate of AM fungi was estimated according to Kormanik *et al*., 1980. Urease and dehydrogenase activity were determined in the rhizospheric plants according to Broadbent *et al.* (1958) and Skujin (1976), respectively.

N, P and K contents in the plant samples were determined in the grounded dried shoot and root samples were wet digested using a sulfuric-perchloric- acids mixture (1:1) (HClO4+H2SO4) according to the procedure of Chapman & Pratt (1961). After digestion, the solution was gently transferred into digested measuring flask (100 ml) and then completed with distilled water to reach 100 ml volume used for various chemical determination. Total P was determined in shoots only using Inductively Coupled Spectrometry Plasma (ICP) (Ultima2- JY Plasma). Total-N in plant samples was determined by Kjeldahl technique while total-K was determined by Flame photometer as described by Jackson (1973).

The translocation factor (TF) for Zn and Cu metals within a plant was determined as described by Stoltz & Greger, 2002. It was expressed as the following:

TF = metal (shoot) / metal (root)

Total carbohydrate was determined in grounded grains according to the method of Dubois *et al.* (1956).

Results:

The results in Table (1) shows that, root and shoot lengths in T1 (the control treatment with full NPK) were higher than T15 (plain unfertilized soil treatment) but lower than that of T2 treatment (AMF). This means that mycorrhizae had a significant stimulatory effect on root and shoot lengths at all time intervals.

The different concentrations of Zn and Cu treatments recorded less roots and shoots lengths putting in consideration *Zea mays* plants negatively affected with the increase of heavy metals concentrations. The mycorrhizal plants plus different concentrations of Zn and Cu showed better shoot and root lengths in comparison to non mycorrhizal ones in addition to controls (plain soil).

Meanwhile, the lengths of roots of non mycorrhizal plants in zinc supplemented soil were higher than that of copper and the reverse was true for shoot. So these results indicated that the mycorrhizal fungi had the ability improve plant viability and growth.

Data presented in (Table, 2) show the reduction rate of roots and shoot, length as affected by mycorrhizal fungi and heavy metals. It was observed that mycorrhizal plants possess reduction (%) lower than that of non mycorrhizal plants. Arbuscular mycorrhizal fungi play an important role in the improvement of plant growth where the increase of heavy metals concentrations decrease plant growth and in turn increase the reduction growth rate.

Table 1: Effect of inoculation with arbuscular mycorrhizal fungi on Root and shoot lengths (cm) of *Zea mays* plants cultivated in soil supplemented with different concentrations of zinc and copper sulphate

Metal	Treatments	Metal conc. (mM)	Growth intervals					
			30 days		60 days		90 days	
			Root	Shoot	Root	Shoot	Root	Shoot
Zn	*NM	0.0	21.00 ± 2.41	40.60 ± 1.39	38.40 ± 1.63	75.10 ± 5.64	48.50 ± 2.03	103.70 ± 3.97
		0.1	14.20 ± 2.83	34.70 ± 1.70	27.60 ± 1.12	69.00 ± 2.20	38.60 ± 0.86	93.20 ± 1.27
		1.0	9.50 ± 2.34	22.90 ± 1.92	20.40 ± 2.47	48.80 ± 3.17	31.10 ± 3.12	71.30 ± 4.49
		5.0	6.30 ± 1.37	15.50 ± 2.78	16.60 ± 2.29	39.30 ± 1.92	25.70 ± 0.96	58.70 ± 1.97
	**M	0.0	30.40 ± 2.78	55.70 ± 2.72	50.70 ± 2.91	96.60 ± 2.16	58.30 ± 3.75	125.50 ± 2.69
		0.1	25.00 ± 3.16	46.20 ± 2.43	45.80 ± 1.55	83.30 ± 1.74	55.60 ± 3.25	111.70 ± 3.73
		1.0	22.20 ± 1.96	41.40 ± 3.27	40.20 ± 1.66	79.00 ± 2.36	49.30 ± 2.19	105.00 ± 2.38
		5.0	19.10 ± 2.09	38.10 ± 2.62	32.30 ± 2.93	73.80 ± 1.53	43.20 ± 3.03	98.50 ± 1.45
Cu	*NM	0.0	21.0 ± 2.41	40.60 ± 1.39	38.40 ± 1.63	75.10 ± 5.64	48.50 ± 2.03	103.70 ± 3.97
		0.1	13.30 ± 2.23	37.60 ± 3.35	25.50 ± 1.84	74.90 ± 1.51	35.20 ± 2.56	99.50 ± 2.63
		1.0	8.20 ± 1.46	25.80 ± 3.13	17.80 ± 1,08	52.50 ± 1.15	27.00 ± 1.95	78.20 ± 1.85
		5.0	5.20 ± 0.49	17.20 ± 1.25	12.40 ± 2.84	44.30 ± 0.92	20.10 ± 2.25	62.10 ± 2.31
	**M	0.0	30.4 ± 2.78	55.70 ± 2.72	50.70 ± 2.91	96.60 ± 2.16	58.30 ± 3.75	125.50 ± 2.69
		0.1	27.00 ± 1.80	50.30 ± 2.06	48.90 ± 2.46	89.80 ± 2.56	56.80 ± 3.05	120.60 ± 2.11
		1.0	24.30 ± 2 78	45.50 ± 1.70	43.70 ± 2.8	84.20 ± 2.27	50.10 ± 2.54	113.20 ± 1.97
		5.0	20.10 ± 3.44	42.40 ± 2.79	35.10 ± 2.87	79.30 ± 2.25	44.30 ± 1.74	108.00 ± 1.87
Control		0.0	7.50 ± 0.66	18.80 ± 2.35	16.90 ± 1.47	46.80 ± 1.37	26.10 ± 3.03	64.4 ± 3.00
LSD 0.05			6.79	7.10	6.34	6.87	6.99	7.45

*NM: non mycorrhizal
**M: mycorrhizal

Table 2: Reduction rate (%) of *Zea mays* root and shoot lengths inoculated with arbuscular mycorrhiza fungi cultivated in zinc and copper sulphate amended soil

Metal	Treatments	Metal conc. (mM)	Reduction rate (%)					
			30 days		60 days		90 days	
			Root	Shoot	Root	Shoot	Root	Shoot
Zn	*NM	0.1	32.38	14.53	28.13	8.12	20.41	10.13
		1.0	54.76	43.59	46.88	35.02	35.88	31.24
		5.0	70.00	61.82	56.77	47.67	47.01	43.39
	**M	0.1	17.76	17.06	9.66	13.77	4.63	11.00
		1.0	26.97	25.67	20.71	18.22	15.44	16.33
		5.0	37.17	31.60	36.29	23.60	25.90	21.51
Cu	*NM	0.1	36.67	7.39	33.59	0.27	27.42	4.05
		1.0	60.95	36.45	53.65	30.09	44.33	24.59
		5.0	75.24	57.64	67.71	41.01	58.56	40.12
	**M	0.1	11.18	9.70	3.55	7.04	2.57	3.90
		1.0	20.07	18.31	13.81	12.84	14.07	9.80
		5.0	33.88	23.88	30.77	17.91	24.01	13.94

*NM: nom mycorrhizal
**M: mycorrhizal

Effect of metal and arbuscular mycorrhizal (AM) fungi on the other plant growth parameters; mycorrhizal dependency and tolerance index:

The results in (Table, 3) show that mycorrhiza significantly stimulate the biomass of root and shoot dry weights in the absence of heavy metals where the highest root, shoot and 100 grains dry weights were observed in T2 (AM fungi treatment). The lowest shoot and 100 grains weight were recorded in T5 (5 mM zinc sulphate treatment). Whereas, the lowest root dry weight was observed in T11 (5 mM copper sulphate treatment) at all intervals. It was also deduced that heavy metals caused a non significant decrease on the dry weight of non mycorrhizal plants at all intervals except for T12 (0.1 mM copper sulphate +AM) shoots at 60 days of planting.

Additionally, AM fungi inoculation and also the presence of heavy metals had a non significant influence on root.

Shoot and grains dry weight however, the dry weight of mycorrhizal plants was higher than that of non mycorrhizal ones and the increase of heavy metal concentration caused a decrease in dry weight at all intervals. It was observed that the root dry weight of non mycorrhizal plants in zinc supplemented soil was higher than that of non mycorrhizal ones in copper supplemented soil, and the reverse were true for shoot and grain dry weights.

Mycorrhizal dependency (MD) in copper treatments was higher than that of zinc treatments, and the evidence from the data indicated that mycorrhizal dependency for plant dry mass increased by raising Zn and Cu concentrations in soil. The results also showed that the tolerance index (TI) was markedly increased in the presence of mycorrhizal fungi more than in the absence of these fungi.

Table 3: Effect of arbuscular mycorrhizal fungi on root and shoot dry weights (gm), mycorrhizal dependency % (MD), tolerance index % (TI) and grains weight (g) of *Zea mays* plants cultivated in soil supplemented with different concentrations of zinc and copper sulphate

Metal	Treatments	Metal conc. (mM)	Growth intervals								
			30 days		60 days		90 days				Harvest
			Root	Shoot	Root	Shoot	Root	Shoot	MD	TI	100 grains
Zn	*NM	0.0	3.33	12.90	11.32	45.48	21.83	73.49	-	-	19.09
		0.1	2.05	8.58	10.98	31.56	14.83	53.00	-	142.50	12.21
		1.0	1.68	5.54	7.88	18.66	10.58	40.23	-	106.74	8.16
		5.0	1.31	3.19	5.39	12.35	7.39	29.76	-	78.04	5.44
	**M	0.0	5.10	25.67	21.54	61.00	33.19	84.46	-	-	25.63
		0.1	3.86	16.51	14.54	51.22	25.37	77.00	150.92	215.06	22.51
		1.0	3.15	13.31	13.22	42.43	22.46	65.21	172.55	184.18	20.06
		5.0	2.62	10.12	11.73	36.85	19.07	60.82	215.05	167.84	17.08
Cu	*NM	0.0	3.33	12.90	11.32	45.48	21.83	73.49	-	-	19.09
		0.1	1.87	10.86	9.84	37.00	13.15	61.00	-	155.78	15.29
		1.0	1.49	7.84	6.96	21.89	9.03	45.27	-	114.08	10.25
		5.0	1.11	4.85	4.03	14.00	6.37	33.48	-	83.71	6.61
	**M	0.0	5.10	25.67	21.54	61.00	33.19	84.46	-	-	25.63
		0.1	4.11	18.45	14.77	57.21	27.66	81.25	146.88	228.80	24.06
		1.0	3.75	15.76	13.88	49.00	24.68	75.00	183.57	209.41	22.49
		5.0	3.13	12.68	12.08	41.47	20.48	66.46	218.17	182.65	18.32
Control		0.0	1.36	12.90	11.32	45.48	21.83	73.49	-	-	19.09
LSD 0.05			1.66	6.28	5.52	8.34	7.00	8.96	-	-	7.43

*NM: non mycorrhizal
**M: mycorrhizal

Moreover, it was decreased with the increase of soil metal concentration in both mycorrhizal and non mycorrhizal plants.

Table 4: Reduction rate (%) of root, shoot and grain dry weights of *Zea mays* plants inoculated with arbuscular mycorrhiza fungi and cultivated in soil supplemented with different concentrations of zinc and copper sulphate

Metal	Treatments	Metal conc. (mM)	Reduction rate (%)						
			30 days		60 days		90 days		Harvest
			Root	Shoot	Root	Shoot	Root	Shoot	Grains
Zn	*NM	0.1	38.44	33.49	3.00	30.61	32.07	27.88	36.03
		1.0	49.55	57.05	30.39	58.97	51.53	45.26	57.26
		5.0	60.66	75.27	52.39	72.85	66.15	59.50	71.50
	**M	0.1	24.31	35.68	32.50	16.03	23.56	8.83	12.17
		1.0	38.24	48.14	38.63	30.44	32.33	22.79	21.73
		5.0	48.63	60.58	54.46	39.59	42.54	27.99	33.36
Cu	*NM	0.1	43.84	15.81	13.07	18.65	39.76	38.40	46.31
		1.0	55.26	39.22	38.52	51.87	58.63	38.40	46.31
		5.0	66.67	62.40	64.40	69.22	70.82	54.44	65.37
	**M	0.1	19.41	28.13	31.43	6.21	16.66	3.80	6.13
		1.0	26.47	38.61	35.56	19.67	25.64	11.20	12.25
		5.0	38.63	50.60	56.08	32.02	38.29	21.31	28.52

*NM: non mycorrhizal
**M: mycorrhizal

The results in (Table, 4) also proved that mycorrhiza alleviated the reduction rate of plant and grain dry weights in polluted soils. However, the reduction rate increased with the increase of heavy metal concentrations

in soil. The presence of heavy metals in soil decreased the growth of non mycorrhizal plants more than that of mycorrhizal ones leading to the increase of reduction rate.

Effect of metals on mycorrhizal infection (%) of Zea mays plants:

Data observed in (Table.5) showed the mycorrhizal colonization (%) of *Zea mays* plants cultivated in soil supplemented with different concentrations of zinc (Zn) or copper (Cu) after 30 and 60 days of planting. The highest value of mycorrhizal colonization in *Zea mays* roots reached 90 % and 100 % at both intervals of planting, respectively, while the lowest effect being 20 % obtained at both growth intervals in (5 mM copper sulphate treatment).

The mycorrhiza caused a significant increase in root colonization rate at both intervals. But, the metals has non significant effect on non mycorrhizal plants at all intervals except for (0.1 mM zinc sulphate), (1 mM copper sulphate) and (5 mM copper sulphate). Root colonization was reduced by the presence of heavy metals in soil where mycorrhizal infection rate at both 30 and 60 days of planting decreased with the increase of heavy metal concentrations in soil.

Table 5: Mycorhizal colonization (%) of *Zea mays* roots cultivated in soil amended with different concentrations of zinc and copper

Metal	Treatments	Metal concentration (mμ)	Growth Intervals	
			30 d	60d
Zn	NM	0.0	70±2.6	70±2.0
		0.1	50±1	50±0.0
		1.0	40±2	40±1.53
		5.0	30±1.53	30±2.52
	M	0.0	90±1.53	100±0.0
		0.1	80±5.0	80±5.77
		1.0	70±2.89	70±3.61
		5.0	60±3.46	60±2.52
Cu	NM	0.0	70±2.65	70±2.0
		0.1	40±1.35	40±3.06
		1.0	30±2.0	30±0.53
		5.0	20±2.0	20±1.53
	M	0.0	70±2.89	70±3.61
		0.1	80±1.53	90±2.0
		1.0	70±3.06	80±2.0
		5.0	60±0.53	70±2.89

*NM: Non Mycorhizal treatment
**M: Mycorhizal treatment

Generally, in mycorrhizal plants treated with copper, the colonization rate was higher than that treated with zinc while the reverse was true in non mycorrhizal plants. Whereas, colonization rate in (control with full NPK treatment) was higher than (control plain unfertilized soil treatment).

On the other hand, there was variations in the density of mycelia, arbuscules and vesicles. So, it was found that the superior infection was presented in mycorrhizal plants of non supplemented soil at 60 days of planting whereas the lowest percentage was recorded in (5 mM zinc sulphate treatment) at both intervals. This meant that the increase of heavy metal concentration led to an inhibition effect on mycorrhizal infection and fungal structures.

Effect of metal concentration on nitrogen (N), phosphorus (P), potassium (K) uptake, crude protein and total carbohydrate (%) of Zea mays plants:

Data obtained in (Tables 6& 7) showed nitrogen (N), phosphorus (P) and potassium (K) uptake (mg/gm dry weight) of shoot and grains, crude protein and total carbohydrates (%) of *Zea mays* grains cultivated in soil supplemented with different concentrations of zinc (Zn) and copper (Cu) in the presence and absence of AM mycorrhiza at different growth intervals. With respect to the nutrient composition of plant tissues, *Zea mays* responded differently to Zn and Cu stresses, in agreement with the results of growth parameters.

The highest content of N, P and K and the percent of crude protein and total carbohydrates in shoot and grains at all growth intervals was observed in T2 (AM fungi treatment) while the lowest value was recorded at T5 (5 mM zinc sulphate) where heavy metals caused a non significant decrease on non mycorrhizal plants at all intervals. The increase of Zn and Cu concentrations in soil caused a significant decrease in shoot and grains contents of all nutrients except for the total carbohydrates (%) in the presence of mycorrhiza especially at T6 and T12.

Table 6: Effect of inoculation with arbuscular mycorrhiza fungi on NPK (mg/ gm) of shoot and grains of *Zea mays* plants cultivated in soil supplemented with different concentration of zinc and copper sulphate

Metal	Treatments	Metal conc. (mM)	Growth intervals											
			30 days			60 days			90 days			Harvest		
			N	P	K	N	P	K	N	P	K	N	P	K
Zn	*NM	0.0	22.11	2.04	22.00	25.01	3.48	19.25	15.51	1.63	12.12	18.23	2.64	20.31
		0.1	19.03	1.51	18.76	21.46	2.68	15.21	11.14	1.01	7.90	16.91	1.86	17.53
		1.0	15.78	0.94	13.23	17.55	1.84	11.92	9.91	0.63	4.56	14.63	1.27	15.59
		5.0	12.01	0.63	11.77	14.94	1.23	8.31	7.17	0.35	1.12	12.48	0.78	13.40
	**M	0.0	27.62	2.91	29.30	29.11	5.12	25.77	21.54	2.34	18.45	26.71	4.51	27.52
		0.1	25.61	2.48	25.43	27.71	4.11	21.10	18.81	1.96	15.55	22.51	3.51	23.51
		1.0	23.54	2.11	23.51	25.95	3.64	19.78	15.57	1.67	12.28	19.46	2.93	21.53
		5.0	21.75	1.83	21.48	23.46	3.13	17.66	13.65	1.48	10.11	17.07	2.34	19.34
Cu	*NM	0.0	22.11	2.04	22.00	25.01	3.48	19.25	15.51	1.63	12.12	18.23	2.64	20.31
		0.1	20.53	1.86	20.64	22.01	2.75	17.14	12.31	1.21	8.19	17.65	2.16	18.34
		1.0	16.06	1.03	15.55	18.11	2.09	12.56	10.85	0.72	5.83	15.13	1.57	16.38
		5.0	13.34	0.74	12.03	15.92	1.48	9.21	9.38	0.53	2.23	13.59	0.95	14.71
	**M	0.0	27.62	2.91	29.30	29.11	5.12	25.77	21.54	2.34	18.45	26.71	4.51	27.52
		0.1	26.66	2.68	28.58	28.33	4.52	23.41	20.24	2.17	17.98	23.61	4.09	25.77
		1.0	24.81	2.32	25.35	26.36	3.84	21.97	16.13	1.89	13.77	22.64	3.34	23.33
		5.0	22.61	2.07	23.86	24.97	3.31	19.67	14.92	1.58	11.34	18.51	2.93	21.18
Control		0.0	14.71	0.86	13.13	16.34	1.54	10.34	9.51	0.60	3.66	14.04	1.08	15.47
LSD 0.05			4.41	0.34	4.49	5.79	0.20	3.35	4.68	0.26	2.84	5.38	0.29	4.63

*NM: Non Mycorhizal treatment
**M: Mycorhizal treatment

Table 7: Effect of inoculation with arbuscular mycorrhiza fungi on crude portion and total carbohydrates (%) in grains of *Zea mays* plants cultivated in soil supplemented with different concentration of zinc and copper sulphate

Metal	Treatments	Metal concentration (mM).	Grains	
			Crude protein (%)	Total carbohydrate (%)
Zn	*NM	0.0	11.93 ± 1.44	45.36 ± 1.71
		0.1	10.57 ± 1.08	38.50 ± 1.43
		1.0	9.14 ± 1.09	33.18 ± 1.75
		5.0	7.80 ± 0.76	25.65 ± 3.91
	**M	0.0	16 96 ± 1.43	50.99 ± 1.57
		0.1	14.06 ± 0.74	47.53 ± 1.65
		1.0	12.16 ± 0.84	44.93 ± 2.55
		5.0	10.67 ± 1.64	41.81 ± 1.68
Cu	*NM	0.0	11.39 ± 1.44	45.36 ± 1.71
		0.1	11.03 ± 1.33	40.28 ± 1.49
		1.0	9.45 ± 1.44	35.71 ± 1.76
		5.0	8.49 ± 1.38	27.41 ± 1.71
	**M	0.0	16.96 ± 1.43	50.99 ± 1.57
		0.1	14.75 ± 0.92	48.90 ± 2.91
		1.0	14.15 ± 1.37	46.36 ± 2.41
		5.0	11.56 ± 0.99	43.21 ± 1.30
Control			8.77 ± 1.41	29.12 ± 1.40
LSD 0.05			3.36	6.57

*NM: Non Mycorhizal treatment
**M: Mycorhizal treatment

Shoot nutrients of mycorrhizal plants at different concentrations of heavy metals were significantly higher than that of non mycorrhizal ones. This means that mycorrhiza played an important role in the improvement of nutrient contents of shoots in *Zea mays* plants even at high concentrations of heavy metals as in phosphorous content of T14 grains at harvest.

Effect of metals on dehydrogenase and urease activities in the presence and absence of AM mycorrhiza:

Data presented in (Table 8, 9) showed urease (µg urea hydrolysed/ g dry soil/ day)and dehydrogenase (µg TPF /g dry soil /Day) activities in soil supplemented with different concentrations of zinc (Zn) and copper (Cu) added to *Zea mays* soil in the presence and absence of AM mycorrhiza at different growth intervals. Soil enzymes varied among the different treatments being studied where urease and dehydrogenase activities were

significantly enhanced by mycorrhiza as in T2 (AM fungi treatment) which had the highest enzymes activities at all intervals. As a result both enzymes activities of mycorrhizal plants were higher than that of non mycorrhizal plants and there was a non significant decrease of both enzymes activities with the increase of zinc and copper concentrations in mycorrhizal treatments except for T12 (0.1 mM copper sulphate +AM fungi) and T13 (1.0 mM copper sulphate +AM fungi).

Table 8: Urease (µg urea hydrolysed/ g dry soil/ day) and dehydrogense (µg TPF/ g dry soil /Day) activities in soil supplemented with different concentration of zinc sulphate (ZnSO4) and copper sulphate (CuSO4).

Metal	Treatments	Metal conc. (mM)	Growth intervals					
			30 days		60 days		90 days	
			Urease	Dehydrogenase	Urease	Dehydrogenase	Urease	Dehydrogenase
Zn	*NM	0.0	309.80 ± 8.81	25.99 ± 4.09	302.66 ± 4.05	30.98 ± 3.51	269.33 ± 3.38	36.57 ± 3.44
		0.1	293.15 ± 2.52	19.93 ± 2.14	286.05 ± 2.08	26.55 ± 2.81	255.83 ± 2.54	31.00 ± 2.54
		1.0	274.50 ± 3.13	14.37 ± 3.17	264.55 ± 3.22	19.84 ± 3.82	232.23 ± 4.23	24.85 ± 2.46
		5.0	251.41 ± 2.31	11.61 ± 1.56	247.68 ± 1.47	13.96 ± 2.75	219.66 ± 4.07	18.84 ± 2.28
	**M	0.0	336.65 ± 2.07	39.41 ± 4.33	328.48 ± 4.02	43.59 ± 3.04	302.42 ± 3.40	48.45 ± 4.27
		0.1	320.19 ± 3.15	32.53 ± 2.77	310.95 ± 1.69	34.24 ± 1.13	286.06 ± 2.19	40.19 ± 2.38
		1.0	311.32 ± 3.11	27.56 ± 0.65	305.85 ± 3.08	31.03 ± 4.55	273.69 ± 1.17	37.00 ± 2.89
		5.0	301.88 ± 3 71	23.73 ± 2.76	294.33 ± 1.82	27.95 ± 3.85	266.76 ± 2.26	34.45 ± 2.60
Cu	*NM	0.0	309.80 ± 8.81	25.99 ± 4.09	302.66 ± 4.05	30.98 ± 3.51	2.69.33 ± 3.38	36.57 ± 3.44
		0.1	285.78 ± 2.07	15.78 ± 2.68	279.89 ± 2.67	22.39 ± 1.93	250.65 ± 3.10	29.61 ± 2.43
		1.0	254.76 ± 1.70	12.13 ± 1.49	256.14 ± 2.47	16.60 ± 2.09	225.52 ± 1.95	21.93 ± 4.09
		5.0	234.09 ± 2.27	11.03 ± 2.57	240.44 ± 2.05	12.30 ± 1.56	210.43 ± 3.46	18.11 ± 3.33
	**M	0.0	336.65 ± 2.07	39.41 ± 4.33	328.48 ± 4.02	43.59 ± 3.04	302.42 ± 3.40	48.45 ± 4.27
		0.1	330.61 ± 2.67	36.77 ± 2.88	320.09 ± 3.79	39.23 ± 3.45	295.45 ± 3.02	44.47 ± 3.55
		1.0	323.78 ± 1.83	31.48 ± 1.99	311.78 ± 4.43	34.25 ± 2.70	286.15 ± 1.65	41.49 ± 3.81
		5.0	314.38 ± 3.54	27.42 ± 2.97	299.97 ± 3.82	32.06 ± 2.05	271.69 ± 6.35	36.08 ± 2.07
Control		0.0	253.77 ± 4.13	11.97 ± 2.62	254.80 ± 1.54	15.00 ± 2.78	220.34 ± 3.31	20.61 ± 1.30
LSD 0.05			7.97	7.97	8.97	8.45	9.58	8.72

*NM: Non Mycorhizal treatment
**M: Mycorhizal treatment

Table 9: Reduction rate (%) of ureease and dehydrogenase activities in soil treated with different levels of n and Cu.

Metal	Treatments	Metal conc. (mM)	Reduction rate (%)					
			30 days		60 days		90 days	
			Urease	Dehydrogenase	Urease	Dehydrogenase	Urease	Dehydrogenase
Zn	*NM	0.1	5.15	23.32	5.49	14.30	5.01	15.23
		1.0	11.39	44.71	12.59	37.12	13.77	32.05
		5.0	18.85	55.33	18.16	54.94	18.44	48.48
	**M	0.1	4.89	17.46	5.34	21.45	5.41	17.05
		1.0	7.52	30.07	6.89	28.81	9.50	23.63
		5.0	10.33	39.79	10.40	35.88	11.79	29.90
Cu	*NM	0.1	7.75	39.28	7.52	27.73	6.94	19.03
		1.0	17.77	53.33	15.37	46.42	16.27	40.03
		5.0	24.44	57.56	20.52	60.30	21.87	50.48
	**M	0.1	1.79	6.70	2.55	10.00	2.30	8.21
		1.0	3.82	20.12	5.08	21.43	5.38	14.37
		5.0	6.62	30.42	8.68	26.45	10.16	25.53

*NM: Non Mycorhizal treatment
**M: Mycorhizal treatment

Also, there was a non significant decrease in non mycorrhizal plants treatments at all intervals. Therefore, it was found that the lowest enzymes activities were detected in T11 (5 mM copper sulphate treatment) soil at all intervals. Also, N.P.K. amendment plays an important role in enzyme activities in T1 (control with full NPK treatment) soil which was higher than that of T15 soils. So, it was postulated that the presence of Arbuscular

mycorrhiza increase of soil enzyme activities that enhanced soil microorganisms' activities and diversity and consequently affect positively on plant growth. There was a decrease in the reduction rate of enzyme activities of mycorrhizal soil compared with that of non mycorrhizal ones (Table 8, 9). Arbuscular mycorrhizal fungi enhance enzyme activities in soil and in turn decrease of reduction rate. Whereas heavy metals suppress enzymes activities leading to the increase of the reduction rate, although high concentrations of metals in soils increase the reduction rate of both mycorrhizal and non mycorrhizal soil

Discussion:

Zea mays has the ability to extract a considerable amount of metals from contaminated soils such as Pb (Huang & Cunningham, 1996), and Zn (Wenger *et al.,* 2002). In the same time it is characterized by its high mycorrhizal colonization which plays an important role in the phytoextraction process of heavy metals (Wang *et al.,* 2006). The contribution of arbuscular mycorrhiza in heavy metal accumulation by *Zea mays* in contaminated soil discussed as follow: after planting, the germination of *Zea mays* grains wasn't influenced by the presence of Zn^{+2} or Cu^{+2} in soil in all treatments. The increase of Zn or Cu concentrations had no effect on germination that might be due to the consumption of nutrient food components stored in grains which was not influenced by the presence of metal ions in soil.

These findings are concerned and discussed by Mahmood *et al.* (2005) who indicated that the germination of corn wasn't affected by toxic levels of zinc and copper through the first 15 days of sowing.

The results demonstrated that inoculation with AM fungi can increase root and shoot lengths, dry weights and weight of grains of *Zea mays* more than the non inoculated plants in all tested Zn and Cu levels over the three intervals. It can also decrease the reduction rate in mycorrhizal plants more than that of non mycorrhizal plants. The explanation of that is attributed to the ability of AM fungi to play an important role in the establishment of plants in soil contaminated with heavy metals. This was in agreement with that found by Al-Garni (2006) who indicated the increased heavy metal tolerance of cowpea plants by dual inoculation of an arbuscular mycorrhizal fungi and symbiotic nitrogen fixing Rhizobium improving plant growth and yield. With respect to non inoculated plants under Zn and Cu stresses, it was found that the presence of high concentrations of Cu^{+2} in the soil showed a marked decrease of roots growth than in shoots, whereas, the reverse was true for Zn^{+2}.

Generally, there was reduction in length and dry weight of roots in Cu treatments more than that of Zn, whereas, there was reduction in length and dry weight of shoots in Zn treatments more than that of Cu due to some preventive mechanisms for reduced translocation of Cu^{+2} from root to shoot (Nishizono *et al.,* 1989). The distribution of the two metals between roots and shoots was different. Cu^{+2} seemed to be concentrated in the roots while Zn appeared to be more diffusible to shoot than Cu (Mahmood *et al.*, 2005).

The inoculation with AM fungi would increase Zn and Cu tolerance indices of *Zea mays* plants comparing with that non inoculated ones that grew in Zn and Cu polluted soil emphasizes that AM fungi are potentially effective in protecting plants exposed to heavy metal stress (Burleigh *et al.,* 2003). AM fungi can increase tolerance indix (TI) of plants grown in polluted soil with the increase of heavy metals concentrations in this soil.

Increasing Zn and Cu concentrations in the soil would increase the mycorrhizal dependence of *Zea mays* plants exposed to high concentrations of both metals as reported by Gonzalez-Chavez *et al.* (2002) who suggested that mycorrhiza application to the soil may play an intelligible main role in a synergistic interaction.

In addition the percentage of AM infection was slightly reduced in inoculated plants with the increase of Zn and Cu concentrations in soil and yet the AM fungi active in incidence colonization of *Zea mays* plants grown in Zn and Cu polluted soil. These results were obtained because the tested concentrations of Cu in the soil were not harmful to heavy metal tolerance AM fungi (Wang *et al.,* 2007) however, high levels of Cu inhibited spore germination and development and mycorrhizal colonization (Lins *et al.,* 2006).

AM fungi plays a remarkable role in nutrient (nitrogen, potassium and phosphorous) aquesition for plants where AM fungi had an intensive ability to increase N, P and K in all mycorrhizal plants especially those untreated with heavy metals. The mycorrhizal plants treated with heavy metals showed the more content of NPK than the non mycorrhizal ones, this is attributed to mycorrhizal mycelium which provides an increased surface area for nutrient uptake and in turn improves the nutrient aquesition of the host plants. The hyphae are also able to penetrate small microsites that are incessible, to the much coarser plant roots.

Active uptake of poorly mobile nutrients such as phosphorous lead to the formation of nutrient depleted volumes of soil around roots which the mycorrhizal hyphae are able to bridge. According to the findings that discussed by Smith & Read (2008), AM fungi had high ability to improve and increase plant nutrients uptake even if there were high concentrations of heavy metals in soil. On the other hand, plant nutrients aquesition decreased in non AM inoculated plants where heavy metals had an inhibitory effect on plant nutrients uptake. As a result there was an increase in the reduction rate of plant nutrients in non mycorrhizal plants more than that in mycorrhizal ones. There was a damage of plasmalemma of root cells by heavy metals causing loss of ions such as K+ and other solutes and decrease in the level of N (Dhillon *et al.* (1987).

The effect of AM fungi on some of the growth parameters of corn plants was investigated and the physiological activity of mycorrhizal plants was found to be better than that of non mycorrhizal plants. The most important indicators of physiological activity were the estimation of crude protein and total carbohydrate in grains. Since AM are a dominant aspect of plant's environment, evidence of their effects on expression of genetic differences in host plants also suggested a role in seed physiology. The crude protein was in a close relationship with nitrogen percent where the higher the nitrogen content the higher the crud protein and vice versa. These findings are in occurrence with that indicated by Velasco-Velasco *et al*. (2001) who indicated that this effect may be because arbuscular mycorrhiza helps absorb ammonia, which could increase crude protein. AM hyphae improve the capacity of higher plants to acquire inorganic nitrogen. Uptake and transport of nitrogen through AM external hyphae have been reported by Johansen *et al.* (1992).

In this study, in soil supplemented with heavy metals, the contents of total carbohydrate compounds of grains of mycorrhizal plants at harvest were usually higher than those of non mycorrhizal plants however total carbohydrate in the grains of the mycorrhizal plants in the absence of heavy metal was the highest. Phosphorus also plays the most important role during the breakdown of carbohydrates and/or synthesis of polysaccharides. In particular, phosphorus is very effective in the synthesis of starch from glucose. As AM fungi increase the uptake of phosphorus, they may also increase the synthesis of carbon compounds (Graham, 2000). Therefore, it is clear that when the phosphorus concentration of plants increases, the photosynthetic rate and its substances also increase and this positively affects the plants (Jacobsen, 1991) and in turn grains.

The reduction rate of total carbohydrates in mycorrhizal plants was lower than that in non mycorrhizal ones. The symbiotic interactions in AM associations are based on the exchange of carbohydrates and mineral nutrients between the plant and the fungus. It was demonstrated that AM fungal colonization stimulated the rate of photosynthesis sufficiently to compensate for the carbon requirement of the fungus and to eliminate growth reduction of the autotroph (Wright *et al.,* 1998).

AM inocula enhanced urease and dehydrogenase activities in the soil contaminated with Zn and Cu where urease is closely related to N mineralization potential because it is required to break down urea to liberate the nitrogen into usable form for plant. On the other hand dehydrogenase provides correlative information on the biological activities and microbiological population in soil (Caravaca *et al.,* 2003, 2004). It was suggested that AM fungi had an important direct and indirect role in the enhancement of soil urease and dehydrogenase activities where AM propagules themselves synthesize soil enzymes as mentioned by Gracia- Garrido *et al.* (2000).

Mycorrhizal plants may release more root exudates containing enzymes more than that of non mycorrhizal ones because mycorrhizal plants contain larger root system that help in nutrition improvement and resistance to stresses Rao & Tak (2001).

Heavy metals affect enzyme activities by modifying the protein conformation due to interaction with enzymes' protein active group and by inhibiting enzyme synthesis (Ruggiero *et al.,* 1996). However, the indirect effects are also possible because changes in microbial communities of soil can modify the behavior of enzyme activities. Kanadeler *et al.* (2000) revealed that urease activity decreased in particle size fraction in soil contaminated with different levels of Cu, Zn, Ni, V and Cd.

Generally, the increases in urease and dehydrogenase activities by AM fungal inocula proved that AM fungi can play an important role in improvement of heavy metal contaminated soils. Thus, soil enzymes are useful indicators of potentially beneficial effects of AM fungi on soil quality and ecosystem.

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