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The effect of earthworm and arbuscular mycorrhizal fungi on availability and chemical distribution of Zn, Fe and Mn in a calcareous soil

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ABSTRACT

Micronutrient deficiencies most frequently occur in calcareous soils. In sustainable soil management enhanced soil biological activity has been proposed as a method for improving soil fertility. Therefore, the objective of this study was to evaluate the effect of earthworm and arbuscular mycorrhizal fungi (AMF) on fractionation and availability of Zn, Fe and Mn in a calcareous soil under maize cultivation. For this purpose a greenhouse experiment was conducted with four treatments including control, earthworm addition (E), AMF inoculation (AMF) and both earthworms addition and AMF inoculation (E + AMF). The results showed that the biological treatments (E, AMF and E + AMF) significantly increased Zn, Fe and Mn uptake by maize. Although the presence of earthworms in E + AMF treatment had no significant effect on mycorrhizal colonization but decreased the concentration of Zn and Mn in shoot compared to AMF treatment. Dissolved organic carbon (DOC) and microbial biomass carbon (MBC) increased in all biological treatments. The highest amount of DOC and MBC were found in treatments containing earthworms (E and E + AMF) and E + AMF treatment respectively. The biological treatments significantly decreased bound to Fe-MnO fraction of studied elements compared to control. Due to the slight changes in other fractions, it seems that biological treatments increased the uptake of Zn, Mn and Fe by maize probably with impact on bound to Fe-MnO fractions.

1. Introduction

Micronutrients availability in soil have been identified as one of the main factors affecting crop yield, food quality and human health (Yang et al., 2007; Alloway, 2008), so that deficiency of micronutrients especially Fe and Zn deficiency is very common in most calcareous soils (FAO, 1972). Different factors affect the availability of micronutrients such as soil pH, redox potential, complexing ligands (organic and inorganic), hydrous oxides and clay minerals (Dang et al., 2015). In calcareous soils the presence of calcium carbonate, lack of organic matter and high pH lead to micronutrients deficiency. Many studies show that belowground biotic interactions and soil organic matter have a significant and direct impact on the availability of nutrients (Wardle, 2006; Marschner and Rengel, 2007). Earthworms and arbuscular mycorrhizal (AM) as two important components of macro fauna and microorganism have great effects on bioavailability of micro and macro nutrients in soil. Earthworm's activity and their interactions with microorganism have a great impact in microbial community, degradation of organic matter and nutrient availability (Blouin et al., 2013). Cheng and Wong (2002) noted the positive effects of earthworms on increasing availability of nutrients in natural soils, as well as decreasing availability of heavy metals in contaminated soils was reported in some studies (Wen et al., 2004; Sizmur et al., 2011; Bityutskii et al., 2012). Arbuscular mycorrhizal fungi play an important role in fertility and sustainability of soil ecosystem and have beneficial effects to host plants, including improved nutrient uptake, drought and salinity tolerance stress, and disease resistance (Gosling et al., 2006; Marulanda et al., 2009; Trouvé et al., 2014). These organisms control the transportation of heavy metals and nutrients into plants in contaminated (Huang et al., 2008; Subramanian et al., 2009) and natural soils (Sylvia et al., 2001; Subramanian and Charest, 1997). Although there is much information about beneficial effects of earthworms and arbuscular mycorrhizal fungi specially in soils with low nutrient availability (Sylvia et al., 2001; Cao et al., 2015; Li et al., 2012a; Li et al., 2012b; Milleret et al., 2009) only a few studies have investigated interactions between earthworms and AM fungi on availability of macronutrients especially nitrogen and phosphorus (Li et al., 2012b; Milleret et al., 2009; Laossi et al. 2011), while the interactions between earthworms and AM fungi on bioavailability of micronutrient in calcareous soils are still poorly understood. According to the above, the objective of this study was to investigate the impact of earthworms and arbuscular mycorrhizal interaction on bioavailability and fractionation of Fe, Zn

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and Mn in a non-contaminated calcareous soil and uptake of these elements by maize. In this study have been focused on chemical distribution of Fe, Mn and Zn under the influence of biological treatments using a sequential extraction procedure.

2. Materials and methods

2.1. Plant and soil

A calcareous silty loam classified as Typic Haplocambid (Soil Survey Staff, 2010) was collected from the top layer (0–25 cm) of the campus of Ferdowsi university of Mashhad, Razavi Khorasan province, Iran (36° 18′ 55.77″ N, 59° 31′ 34.11″ E). The studied soil contains 38% sand, 39% silt and 23% clay. Each kilogram of the soil was consisted of 0.51 g total N, 3.5 g organic C, 7.0 mg available P, 151 mg available K, 2.1, 0.61, and 24.4 mg of Zn, Fe and Mn, respectively. Electrical conductivity and pH of studied soil were 2.12 dSm^{-1} and 7.65 respectively. The soil was air-dried, passed through a 2.0 mm sieve and autoclaved for one hour at 121 °C. All treatments received 350 kg ha^{-1} urea, 200 kg ha^{-1} potassium sulfate and one percentage of sterilized manure. Since the activity of AM fungi in soil is affected by soil phosphorus, the amount of phosphorus was supplied in one third of recommendation for field application (50 kg ha^{-1} from mono calcium phosphate monohydrate).

Maize seeds (K SC 260) were sterilized with a 10% (v/v) solution of H_2O_2 for 10 min, rinsed thoroughly with deionized water, then placed on autoclaved filter papers, soaked with sterile distilled water and incubated at 25 °C for 24 h (Li et al., 2012b). Six seeds were planted in each pot.

2.2. Earthworm and AMF

Ten earthworms (*Eisenia Fetida*) with well visible clitellum and similar in fresh weight (0.5 ± 0.06 g) and lengths (6 ± 0.74 cm) were used for each pot. Selected earthworms were washed with distilled water and kept in sterilized glass vessels for 24 h to minimize the number of mycorrhizal propagules associated with their surfaces or gut contents (Li et al., 2012b). The AMF inoculums (*Funneliformis Mosseae)* were prepared from the Tooran biotech company (Science and Technology Park of Semnan Province, Iran).

2.3. Experimental design

The experiment was performed in a completely randomized design with three replications. Experimental treatments included soil without earthworm and mycorrhizal addition (control), earthworm addition)E(, AMF inoculation (AMF) and both earthworms addition and AMF inoculation (E + AMF). The pots (19.5 cm top diameter, 10.5 cm bottom diameter and 24 cm height) were filled with 4.9 kg sieved (< 2 mm) and sterilized calcareous soil. Ten grams per pot mycorrhizal inoculum and sterilized mycorrhizal inoculum were added to AMF and non-AMF treatments, respectively in about 5 cm of soil surface. After the emergence of the third leaf, only two plants were kept and ten earthworms were added to each pot. All pots were arranged randomly in greenhouse and irrigated daily with deionized water to maintain the moisture content at approximately 70% of the soil water holding capacity. The plants were grown under 25°/20 °C temperature (day/night), 16 h/8h (light/dark) photoperiod, and 35% relative humidity.

2.4. Sampling and analysis

Root and shoot of maize and soil samples were collected 60 days after planting. Dry weight of the shoots roots biomass was determined after oven drying at 65 °C for 48 h. In order to estimate mycorrhizal colonization, collected roots were cut into 1 cm pieces, cleared in 10% KOH, acidified with 1% HCl and stained with 1% Fushin acid in Lactic acid (Kormanik et al., 1980). Mycorrhizal colonization was estimated using a line intersect method (Tennant, 1975). Fifty random pieces of each root sample were used for analysis.

The soil availability of Zn, Fe and Mn was determined by Linsay and Norvel method (1978). Soil pH and electrical conductivity (EC) were determined in a 1:5 soil/solution ratio (Rayment and Higginson, 1992). Organic carbon was determined using Walkely-Black method (1934). DOC was measured after soil extraction by 0.5 $molL^{-1}$ K_2SO_4 solution according to the method provided by Herbert and Bertsch (1995). Soil microbial biomass carbon (MBC) was determined through fumigation-incubation method (Horwath and Paul, 1994). The Tessier fractionation method (Tessier et al., 1979) was used to determine the distribution of chemical fractions of Zn, Fe and Mn in soil. In this method the fractions were specified as exchangeable (Exch), bound to carbonates (CB), bound to iron–manganese oxides (Fe-MnO), bound to organic matter (Org.B) and residual fractions (R).

Analysis of variance was carried out using the JMP software version 8 (developed by SAS institute). Tukey's multiple range was used to show significant differences between treatment means at the 5% level.

3. Results

3.1. Mycorrhizal colonization

The results showed that AMF inoculation in AMF and E + AMF treatments significantly caused successful root colonization. The highest percentage of root colonization was observed by 60% in AMF treatment that had significant difference compared to the E and control treatments but not with E + AMF treatment (Table 1). In other words the earthworm's activities had no effect on root colonization by arbuscular mycorrhizal. In non-AMF treatments (control and E treatments) the plants root were colonized lees than 10% due to non-sterile conditions in greenhouse (Table 1).

3.2. Shoot dry weight and Zn, Mn and Fe concentration in shoot

Shoot dry weight of maize and Zn, Mn and Fe concentration in shoot significantly increased in E, AMF and E + AMF treatments compared to the control (Table 1). The results showed that E, AMF and E + AMF treatments increased shoot dry weight by 1.46, 1.55 and 1.44 times compared to control. Due to the non significant differences in shoot dry weight between E, AMF and E + AMF treatments, the Zn, Mn and Fe

Table 1
Effect of earthworms and arbuscular mycorrhizal on root colonization, shoot dry weight and Zn, Mn and Fe concentration in maize shoot.

Treatment	Root colonization %	Shoot dry weight	Zn	Mn	Fe
		g $plant^{-1}$	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Control	1.6	7.20 ± 0.24b[†]	11.95 ± 0.67 c	146.55 ± 4.87 d	125.35 ± 6.80 b
Earthworm	3.3	10.52 ± 0.92 a	12.2 ± 0.45 c	170.21 ± 4.40 c	277.21 ± 7.12 a
AMF	60	11.18 ± 0.13 a	24.01 ± 0.78 a	236.93 ± 6.65 a	260.48 ± 7.81 a
Earthworm + AMF	52	10.42 ± 0.52 a	19.06 ± 0.75 b	214.95 ± 4.67 b	255.35 ± 6.56 a

[†] Values with the same lower case letters in a column are not significantly different at $P < 0.05$.