

International Research Journal of Biological Sciences _ Vol. 2(11), 47-54, November (2013)

Effects of Mycorrhizal Fungi in Phytoremediation of Heavy Zinc-Contaminated Soils by Forage Corn

Hamid Moharami^{1,*}, Mohammad Reza Momayezi² and Mohammad Nasri² ^{1*}Student of Agronomy, Varamin-Pishva Branch, Islamic Azad University, Varamin, IRAN ²Department of Agronomy, Varamin-Pishva Branch, Islamic Azad University, Varamin, IRAN

Available online at: www.isca.in, www.isca.me Received 11th July 2013, revised 25th August 2013, accepted 24th September 2013

Abstract

This study was done aiming at investigating interaction between manure and mycorrhizal fungi on amount of phytoremediation of forage maize in Varamin in summer 2012. It should be noted that sewage water was used for irrigation of this farm in the south of Tehran for many years and then it was irrigated tap water while conducting this research. This study was run in factorial form in the form of a randomized complete block design with three replications with following factors: 1. Mycorrhiza (M) at 3 levels - without the use of mycorrhiza fertilizer (M_0), b. use of mycorrhiza fertilizer as 60 kg per hectare (M_1), c. use of mycorrhiza fertilizer as 120 kg per hectare (M_2) and, manure treatment (F) at 3 levels – a. without use of animal manure (F_0) b – use of 25 tons of manure per ha (F_1), c – use of 50 tons of manure per ha (F_2). Variance analysis results showed that concentration of zinc in leaf is higher than root. The highest zinc concentration with 30.92 mg/kg was observed in aerial organ. According to research, forage corn, presence of mycorrhizal fungi, and animal manure can be used in phytoremediation of zinc.

Keywords: Animal manure, mycorrhizal fungi, forage corn, phytoremediation, heavy elements of zinc.

Introduction

Remediation of soils contaminated with heavy metals is very important because many areas are contaminated with heavy elements which are not suitable for agriculture. Heavy metals may be transferred to plant from soil and may threaten performance and health of food chain, environment for human. Thus replacement and elimination or reduction of their impact in environment on academic and scientific bases is a necessity.

Application of plants with ability of absorbing heavy metals is a low-cost alternative for eliminating soils from heavy metals. Application of plants for remediation of soils and waters contaminated with organic and mineral pollutants is called phytoremediation which is known as a new method for in-situ remediation of contaminated soils.

Phytoremediation technology is using plants for replacement, transfer or stabilization of heavy metals in contaminated soils with low to average contamination in the area of root development. This technique originally was used for groundwater containing contaminated material and then it was applied on contaminated soils and air¹.

Phytomining is a technology with high efficiency for eliminating heavy metals from soils with low to average contamination. In this method, super absorbent plants which are able to absorb high amount o heavy metals by roots and transfer to aerial organs are used. Or other plants which produce high biomass but concentration of the metals are normal in their texture are used (Phytomining)².

Most super absorbent plants produce low biomass or their biomass is reduced over the time due to depletion of nutrients and variety of disease or emergence of stressors such as decreased pH, low air conditioning and other environmental stresses. In addition, soils may be contaminated with various metals, some of which seriously affect plant fertility and since plant species rarely are able to extract more than one metal, duration of contamination reduction is decreased. In condition of lack of zinc, dynamicity and activity of zinc and its transfer to aerial organs of corn and colonized trifolium with mycorrhizal fungi has been shown³. These findings indicate useful role of mycorrhizal coexistence in improvement of zinc nutrition as a micronutrient.

Material and Methods

This study was done aiming at investigating interaction between manure and mycorrhizal fungi on amount of phytoremediation of forage maize in Varamin in summer 2012. This study was run in factorial form in the form of a randomized complete block design with three replications with following factors: 1. Mycorrhiza (M) at 3 levels - without the use of mycorrhiza fertilizer (M₀), b. use of mycorrhiza fertilizer as 60 kg per hectare (M₁), c. use of mycorrhiza fertilizer as 120 kg per hectare (M₂) and, manure treatment (F) at 3 levels – a. without use of animal manure (F₀) b – use of 25 tons of manure per ha (F₁), c – use of 50 tons of manure per ha (F₂).

Characteristics of Corn and Mycorrhizal Fungi: KSC704 type was used in this experiment with TKW 7/281 mg and 92%

viability. Its growth time period is about 120 days. Also Glomus mosseae arbuscular will be used as mycorrhizal fungi. This biofertilizer has 120 spores and fungal root length is 6 meters per gram.

Physiochemical Characteristics of Experiment Site's Soil: Before initiation of the work, soil samples were taken in 3 points of the farm in 0-60 cm depth and after mixing samples, they were sent to the laboratory for determining physiochemical characteristics. It should be noted this farm had been irrigated by sewage system water for many years. Obtained results are given in table 1.

Statistical Calculations: Initial data of the experiment were analyzed using Mstat-c software and the results were compared. Averages of each attribute were compared using Duncan's multiple range test at 1% and 5% level with mentioned software.

Results and Discussion

In this work, zinc increased leaf surface because of using mycorrhiza and animal manure, since its application caused more and gradual access to phosphorus. In the step which seed become rich, maximum leaf area index was obtained for photosynthetic production of seeds and then leaf making is reduced and lower leaves gradually begin yellowing. Thus leaf area index is reduced. Up to rapid growth stage, amount of leaf areas was close to each other in different treatments, but since blooming stage, leaf area index in treatments of using mycorrhiza increased considerably compared to control group. These steps are exactly coincides with maximum need to photosynthetic materials for seed production and seed-filling. Therefore, treatment using 120 kg/ha mycorrhiza produced the highest gain. It can be concluded this treatment allocated higher percentage of its activity to production and development of

leaves compared to other treatments, and had the maximum leaf index (5.98) at the end of blooming. Results indicate that usage of mycorrhiza caused increased root making and thus better absorption of other elements needed by the plant including Nitrogen and Zinc. It is done by activating phosphor sorption. Although zinc and phosphor have antagonistic effect, this is due to their competition in the metabolism⁴. However, presence of enough Nitrogen and Zinc and other micronutrient elements leads to higher leaf area development and production of higher photosynthetic materials. As findings in this work showed, zinc affects leaf increase in treatments with use of mycorrhiza, although strong presence of phosphor somehow influenced amount of zinc. However, mycorrhiza coexistence with corn led to increased leaf area index which is observed in increased wet gain of forage too (figure 1).

Results show that treatment of animal manure usage could gain better leaf area index due to having higher mineral elements. Because grow was improved due to providing high and low consumption elements for the bush in this treatment⁵ and the plant could shape its canopy in more effective manner. Doe to having adequate phosphorus and potassium and timely root increase, it has used mineral elements of soil optimally and finally gained higher leaf area index by developing aerial organ. Results indicated that animal manure have effective impact on growth of aerial organs which it can be due to abundance of nutrients accessible for plant and thus its strengthening and vegetative increased growth⁶, while vegetative growth was reduced in the treatment of non-usage of animal manure due to elimination of enough nutrients, and thus leaf area index is reduced. At the end of growth period, the material move from leaves to stem and seed and leaf area index is decreased by falling leaves in some cases. However, in this work which the plant is harvested as forage, this process is not noticeable (figure-2).

Field Soil Profile									
Cd total ppm	Pb total ppm	Zn total ppm	Cd Absorbable ppm	Pb Absorbable ppm	Mn Absorbable ppm	Cu Absorbable ppm	Zn Absorbable ppm	Fe Absorbable	Characteristic
Atomic	Atomic	Atomic	Atomic	Atomic	Atomic	Atomic	Atomic	Atomic	Experiment Method
ND	34	106	ND	2.98	6.06	1.14	1.94	6.38	Soil
Hd -		EC	Ds/m	Ni Absorbable ppm	Ni total ppm	Mn total	ppm Fe total	CO total ppm	Characteristic
PH meter		Conductometer		atomic	atomic	atom	ic atomic	atomic	Experiment Method
7.57		5.02		0.3	26	790	1.67	ND	Soil

Table-1

Leaf area ratio change trend is descending and its highest level was observed early in growth period and it was reduced gradually over the time. At the beginning of the growth, made photosynthetic materials mainly have been spent on the development of photosynthesizing surfaces and then by initiating rapid growth, trend of material distribution changes in favor of stem and sheath and against leaves.

Although material needed be corn bush was provided at the beginning of the growth period due to using animal manure, and vegetative increased growth was increased considerably, total dry matter was increased and leaf area ratio had descending trend over the time and because of increased age of plant and allocating more photosynthetic materials to the non-leaf organs (figure 3).

The highest ratio belongs to beginning of growth season with seed inoculation with mycorrhiza. Coexistence of mycorrhiza with corn root causes increase absorption of elements including zinc and phosphor so that insoluble phosphorus in soil is provided as soluble phosphor for the plant. Considering role of phosphor and other absorbed micronutrients early at growth process, the plant has higher leaf area, but over the time assimilates produced from photosynthesis are spent on material making and thus leaf area is decreased gradually, while the plant has reduced growth from the beginning in treatment of non-use of mycorrhiza due to not providing developing factors of vegetative growth, and subsequently photosynthesis production is lower and leaf area ratio is also decreased. In work by D'Angelo et al.⁷ on oat it is stated that early in growth process, the plant spends its energy for producing leaf to reach to appropriate level for production and storing energy before reproductive growth, hence there is high leaf area ratio. However, later in blooming stage some changes occur in various organs of the plant so that competition is developed between vegetative and reproductive organs for absorption of nutrients and increasing dry weight and photosynthesized materials move considerably toward reproductive organ in blooming stage and leaf area ratio is reduced, which is consistent with findings in the current work (figure 4).



Figure-2 Leaf area index change trend in treatment of animal usage



Figure-3 Leaf area index change trend in treatment of animal manure usage





Figure 5 indicates trend of changes in net absorption in different treatments with animal manure. Treatment of animal manure use had better net absorption speed at the beginning due to increased leaf area, but the speed of net absorption was reduced over the time due to shadowing of leaves and lowering received radiation. The highest net absorption speed was related to treatment of non-use of animal manure in initial stage of vegetative growth (12-day) with 8.11 g/m² per day. However, although leaf area index was high in days 62 and 85, because of high extinction coefficient efficiency of light consumption was low and material produced by photosynthesis was decreased in the leaf. Therefore reduction was observed in net absorption speed. It should be noted that though the highest net absorption speed at the beginning of the growth was for treatment of nonuse of animal manure, reduction of net absorption speed in this treatment showed higher slope in the middle of growth process compared to two other treatments of animal manure usage, which can be attributed to production of processed material resulting from photosynthesis in both treatments.

Figure 6 shows change trends in absorption speed in different mycorrhiza treatments. Speed of NAR changes had equal trend in treatments of mycorrhiza usage; however, in treatment of non-use of mycorrhiza though it was similar to two other treatments initially, descending trend of net absorption speed was higher over the time. It should be noted that in treatment of mycorrhiza usage, net absorption speed showed a trivial increase in the time of forage corn harvest, which can be due to reduced shadowing by leaves and increased material making level in these treatments. Because mineral materials were provided better in these treatments due to better absorption and higher cooperation with the root and increased root branches, and net absorption speed was increased in harvest time by increasing assimilates produced in leaf area. This finding is consistent with finding by Zahran⁸. He concluded plant growth speed and its net absorption may increase suddenly at the end of growth season. It was stated that it is because of sudden increase in seed demand for assimilate. In fact activity of leaves is increased because of increased seed demand leading to increasing plant growth speed and its net absorption. The

highest net absorption speed was for treatment of using 60/ha mycorrhiza with 5.35 g/m² per day. Given its optimal canopy condition, it could preserve this situation until the end of growth period. Net absorption speed had stable trend in this treatment. The lowest net absorption speed was related to treatment of non-use of mycorrhiza in day 85 with 3.37 g/m² per day.

Results suggest that specific leaf area is increased by

mycorrhiza usage; however it is decreased over the time. In calculating specific leaf area, time and nutrition play significant role in determining this attribute. In treatments with mycorrhiza usage, leaf area increased along with leaf weight during growth period; although leaf's dry weight increase was higher than leaf area index in blooming stage. Therefore specific leaf area is reduced in this stage which is clear in the current work (figure 7).



Figure-6 Net absorption speed changes in different treatments of mycorrhiza



Figure-7 Specific leaf area changes in different treatments of mycorrhiza

At the beginning of the growth period which plant needs high nutrients in vegetative growth for shaping its canopy considerable difference is observed, but this differences reduces by approaching to reproductive growth period and increasing thickness of Cuticle, higher production and increasing leaf weight, and it reduces in a stable trend so that difference between three treatments is not observable at the end of growth period (figure 8).

According to obtained results, no significant difference was observed in different treatments of animal manure and non-sue of it in different growth stages and all treatments increased in a stable trend. In treatments of manure usage bushes had appropriate leaf area due to provision of nutrients in vegetative growth period and optimal canopy formation, and it was continued until the end of growth period, however, in treatment of non-use of animal manure it was reverse, thus by reduced leaf area in this treatment, leaf specific weight was higher in this treatment compared to others which was not significant statistically (figure 9).

Conclusion

In this work, effects of coexistence between mycorrhiza and animal manure in soil phytoremediation in forage corn are observed. Mutual effects of mycorrhiza and animal manure show that they have positive and direct impact on zinc absorption in the soil so that usage of 60 kg/ha mycorrhiza and 50 tons of animal manure could have the highest zinc absorption in leaf which had 85% increase in absorption in comparison with minimum treatment. This increase trend is also observed in stem and aerial organ. But stability of zinc in root is less than leaf and stem. It seems that zinc by its specific mechanisms, is absorbed in root by consistence of mycorrhiza. Then it reaches to plant's aerial organs and sometimes to seed in seeded plants through transforming to organic materials. High endurance of zinc in plant's foliage is because of seclusion and separation in vacuoles9. Various mechanisms occur for inactivating zinc in vacuole which include sedimentation as Phytic acid combined with zinc and attaching it to organic acids with low molecular weight¹⁰.



Figure-8 Specific leaf area in different treatments of manure



Figure-9 Specific leaf weight changes in different manure treatments

Since zinc is one of necessary material for the human, its accumulation in leaf and seed, which either directly or through livestock and poultry is consumed by human, can decrease considerably need of human to this vital element. Especially its absorption by such plants as corn also leads to soil remediation. The important point in this work is transformation of total zinc which is not accessible by the plant to absorbable zinc, which occurs in mycorrhizal hyphae. Of course role of animal manure should not be overlook in soil remediation, since absorbable phosphor is increased by application of animal manure and mycorrhiza. These two elements have antagonistic relationship, but coexistence of these elements is as a mechanism for increasing plant endurability toward zinc¹¹. In soils polluted by zinc, mycorrhiza fungi causes balancing nutrients in the plant by increased phosphor absorption and plant's biomass as well as its durability to higher amounts of zinc is increased. In noncontaminated soils, zinc is transferred through granules of poly phosphate in hyphae. However, mechanism of zinc transfer in hyphae is not clear in polluted soils. Phosphor may lead to sedimentation of zinc in hyphae and roots and directly cause sequestration of zinc in the plant through formation of Phytic acid molecules and reduce its toxicity¹². Christie et al.¹² have contrast idea. They stated zinc concentration increase in aerial organs of mycorrhiza plants is lower than non- mycorrhiza plants and it is higher concerning roots. High zinc concentration in root of mycorrhiza plants compared to non- mycorrhiza ones shows that fungi store zinc metal at Mycelia surface or within them. In the current work, although not high amount of zinc was observed in the root, the highest amount of absorbed zinc as 30.92 mg/kg was observed in aerial organ of M₁*F₁ treatment and such accumulation was not observed in the root. The highest amount of absorbed zinc in the root was also related to the same treatment as 17.49 mg/kg. considering interactions it was found that the lowest zinc accumulation in leaf, stem, aerial organ and root as 6.02 ppm belonged to $M_0 * F_0$ treatment in the root. It suggested zinc movement from soil to the root and then to aerial organ so that in corn plant inoculated with mycorrhiza fungi, zinc concentration and absorption, phytomining efficiency, zinc transfer to plant's aerial organ was different. Inoculation of plant had effective role in zinc increase in aerial organ and root of corn in comparison with non-inoculated plants.

Similarly Joner and Leyval¹³ compared mycorrhiza and nonmycorrhiza treatments of trifolium and corn in soil contaminated with zinc and concluded zinc had higher accumulation in mycorrhiza plants. Jamal et al.¹⁴ reported in soils contaminated with zinc, zinc was higher in soya and lentil plants inoculated with Arbuscular mycorrhizal fungi compared to control treatments. Results of previous works in this regard show role of Arbuscular mycorrhizal fungi in heavy metals becoming organic inside soil and roots. They suggest the fact that Arbuscular mycorrhizal fungi play effectively in plant stabilization and the best option for this purpose are indigenous fungi originated from contaminated soils¹⁵. According to this work, it was found that using forage corn is an appropriate option for remediation of zinc-contaminated soils. By producing high biomass, corn could absorb considerable amount of zinc from the soil and presence of phosphor reduced its toxicity in the plant and thus its amount was balanced both in the soil and plant.

References

- Khan A.G., Kuek C., Chaudhry T.M., Khoo C.S. and Hayes W.J., Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation, *Chemosphere*, 41(1), 197-207 (2000)
- 2. Krämer U., Phytoremediation: novel approaches to cleaning up polluted soils, *Current opinion in biotechnology*, **16**(2), 133-141 (2005)
- **3.** Chen B.D., Li X.L., Tao H.Q., Christie P. and Wong M.H., The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc, Chemosphere, **50(6)**, 839-846 (**2003**)
- **4.** Mengel K. and Kirkby E.A., Principles of plant nutrition (No. Ed. 4), International Potash Institute (**1987**)
- **5.** Wang M.C. and C.H. Yang, Effect of paddy upland crop rotation with various fertilization of soil physical and chemical properties, Research Paper, 17, WCSS. Thailand. 64-69 (**2002**)
- 6. Najm A., Effect of Integrated management of nitrogen fertilizer and manure on qualitative and quantitative characteristics of potato. MA thesis (agriculture field). College of Agriculture, Islamic Azad University of Rudehen (2009)
- 7. D'Angelo G.H., Postulka E.B. and Ferrari L., Infrequent and intense defoliation benefits drymatter accumulation and persistence of clipped Arrhenatherum elatius, *Grass and Forage Science*, **60(1)**, 17-24 (**2005**)
- 8. Zahran, H.H. Rhizobium –legume symbiosis and nitrogen fixation under severe conditions and in an aria climate, *Micro. Mol. Biol.*, 63(1),968-989(2007).
- Lasat M.M., Pence N.S., Garvin D.F., Ebbs S.D. and Kochian L.V., Molecular physiology of zinc transport in the Zn hyperaccumulator Thlaspi caerulescens, *Journal of Experimental Botany*, 51(342), 71-79 (2000)
- Tone S., Effect of temperature on growth, flower bud differentiation and flower stalk development of leaf lettuce, Bulletin of the Yamaguchi Agricaltural Experiment Station, 40(3), 34-43 (1988)
- Chen B., Shen H., Li X., Feng G. and Christie P., Effects of EDTA application and arbuscular mycorrhizal colonization on growth and zinc uptake by maize (Zea mays L.) in soil experimentally contaminated with zinc., *Plant and soil*, 261(1-2), 219-229 (2004)

- Christie P., Li X. and Chen B., Arbuscular mycorrhiza can depress translocation of zinc to shoots of host plants in soils moderately polluted with zinc, *Plant and Soil*, 261(1-2), 209-217 (2004)
- **13.** Joner E. and Leyval C., Time-course of heavy metal uptake in maize and clover as affected by root density and different mycorrhizal inoculation regimes, *Biology and Fertility of Soils*, **33(5)**, 351-357 (2001)
- 14. Jamal A., Ayub N., Usman M. and Khan A.G., Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by soybean and lentil, *International Journal of Phytoremediation*, 4(3), 205-221 (2002)
- **15.** Göhre V. and Paszkowski U., Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation, *Planta*, **223**(6), 1115-1122 (**2006**)