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Mycorrhizal Colonisations of Naturally Evolving Weeds at Different Mine Waste Mounds in Central Anatolia–Turkey

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Abstract. Soil and water pollution including heavy metals increased because of the industrialization. Weeds heavy metal uptake and accumulation depend on the density of available metals in waste soils. Arbuscular Mycorrhizal (AM) increases productivity and tolerance of weeds, as well as their resistance to different abio- and biotic stresses. The role of AM in metal uptake remains largely unclear. AM may be beneficial for the metal streesed weeds and several AM fungal strains can be isolated from metal-enriched soils.

In this study AM inoculations on weed populations existing in pastures adjacent to a different waste mounds (mercury, lead, arsenic and cadmium) stocked on open field were surveyed in Central Anatolia. 35 weed species belonging to 16 families were collected from the pastures constituted 60% of total land. AM colonization was recorded in 28 species accounting for 90% of which 35 weeds about 60% covered land. Hyphal, arbuscul and vesicul infection was noticed on the weeds.

Weeds have 70-95% AM root colonization such as *Teucrium* sp. and *Phlomis* sp. (Labiatae), *Trifolium* sp. (Leguminosae), *Echinops* sp. (Compositae) widespreaded in the pasture land. Other species have 10-55% AM root colonization such as *Cirsium* sp. and *Achillea* sp. (Compositae), *Stachys* sp. (Labiatae), *Plantago lanceolata* (Plantaginaceae).

Keywords: Arbuscular mycorrhizal, weed, waste mound, heavy metals.

1. Introduction

Arbuscular mycorrhizal (AM) associations are integral, functioning parts of plant roots and are widely recognized as enhancing plant growth on severely disturbed sites, including those contaminated with heavy metals. Isolation of the indigenous and presumably stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants for successful restoration of degraded ecosystems [1]. AM fungi are of importance as they play a vital role in metal tolerance [2] and accumulation [3] and [4]. External mycelium of AM fungi provides a wider exploration of soil volumes by spreading beyond the root exploration zone [5] and [6] 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* [7]. Another important feature of this symbiosis is that AM fungi can increase plant establishment and growth despite high levels of soil heavy metals [8] due to better nutrition [9], water availability [10] and soil aggregation properties [11] associated with this symbiosis. AM fungus is significant in the ecological improvement of rhizosphere [12].There is considerable evidence that

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colonisation by arbuscular mycorrhizal (AM) fungi can reduce plant uptake and/or phytotoxic effects of soil heavy metals [13]. However, mycorrhizal colonisation represents an energetic cost to the host in the form of carbon supplied to the mycobiont [14]. It has therefore been suggested [15] that selective pressure would act to reduce colonisation unless the symbiosis provided some benefit to the host. Thus, in situations where mycorrhizal colonisation protects plants against heavy metal toxicity, colonisation may be positively correlated with plant-available metal concentrations. There is also evidence, however, that heavy metals can inhibit mycorrhizal spore germination [16], growth of extraradical hyphae and AM colonisation, and that metal tolerance of mycorrhizal fungi differs between fungal species and ecotypes [2].

Pawlowska and Charvat (2004) examined in vitro effects of Cd, Pb, and Zn on critical life stages in metal-sensitive ecotypes of arbuscular mycorrhizal (AM) fungi. Patterns exhibited by *G. intraradices* spore germination, presymbiotic hyphal extension, symbiotic extraradical mycelium expansion, and sporulation under elevated metal concentrations suggest that AM fungi may be able to survive in heavy metal-contaminated environments by using a metal avoidance strategy[17].

Bojarczuk and Kieliszewska-Rokicka (2010) studied the effects of high concentrations of available Cu and Pb in soil originated from the vicinity of a copper foundry in Poland (Cu, 2,585–3,725 mg kg^{-1} d.wt.; Pb, 1,459–1,812 mg kg^{-1} d.wt.) on the growth and chemical constituents of *Betula pendula* seedlings. The heavy metals negatively affected seedling growth, ECM colonization, and soil dehydrogenase activity. A reverse relationship was found between ECM abundance and heavy metal concentrations in birch leaves[18].

Several heavy metal hyperaccumulating species belonging to the genus *Thlaspi* were reported, among them *Thlaspi caerulescens* hyperaccumulating Zn/Cd [19], *Thlaspi rotundifolium* ssp. *cepaeifolium* hyperaccumulating Pb [20], and *T. praecox* Wulf. Hyperaccumulating Zn [21]. Two predominant grass species *Calamagrostis varia* and *Sesleria caerulea* were selected for phytostabilisation, but a severely reduced seed germination capacity obstructed their use in practice.The mycorrhizal succession showed a gradual replacement of non-mycorrhizal with mycorrhizal plant species. Similar levels of arbuscular mycorrhizal colonisation of a particular plant species may be developed within each growing season regardless of the levels of pollution, with the exception of vesicle/intraradical spore formation. The results suggest that lower overall mycorrhizal colonisation levels and increased vesicle/spore formation may be a part of a mycorrhizal strategy at the most polluted locations [22].

The effectiveness of mycorrhizal colonisation of four fungal isolates of different origin was tested in roots of *Festuca rubra* L. and *Plantago lanceolata* L. cultivated on three zinc waste substrata of different toxicity. F. rubra showed a much beter survival rate than *P. lanceolata*. The highest values of the mycorrhizal parameters were found in roots inoculated with the *Glomus claroideum* strain originating from the industrial waste. Arbuscular richness, assessed in roots stained for viable fungal structures, was demonstrated to be the most sensitive parameter, showing statistically important differences between plants, artificially inoculated and those colonised only by the indigenous mycorrhizal fungi [23]. The consistently heavy AM colonisation of *T. polytrichus* found suggests that these fungi are not inhibited by soil heavy metals at these sites, and that the host derives some benefit from its AM symbiont [24].

Significant hyperaccumulation of Zn, Cd and Pb in field samples of *Thlaspi praecox* Wulf. collected from a heavy metal polluted area in Slovenia was found, with maximal shoot concentrations of 14 590 mg kg^{-1} Zn, 5960 mg kg^{-1} Cd and 3500 mg kg^{-1} Pb. Researchers are said that the first report of Cd hyperaccumulation and AMF colonisation in metal hyperaccumulating *T. praecox* [25]. Plants which appear spontaneously in such places are frequently devoid of mycorrhizal symbiosis and are mostly characterized by poorly developed root and shoot biomass when heavy metals are present [26]**.** The lack of mycorrhiza can hamper the revegetation of the metal-contaminated mine spoil or other degraded sites. The introduction of an AM fungal inoculum into these areas could be a strategy for enhancing the establishment of mycorrhizal herbaceous species. AM fungal isolates differ in their effect on heavy metal uptake by plants [27]. Some reports indicate higher concentrations of heavy metals in plants due to AM [28] whereas others have found a reduced plant concentration; for example, Zn and Cu in mycorrhizal plants [29]. Thus, selection of appropriate isolates could be of importance for a given phytoremediation strategy. AM fungal species can be isolated from areas which are either naturally enriched by heavy metals or are old mine/industry waste sites in origin.

The aims of the present study were (i) to compare plant communities at the different mine areas based on species abundance and arbuscular mycorrhizal colonisation, (ii) to question possible metal pollution effects on mycorrhizal development.

2. Material and Method

AM inoculations on weed populations existing in pastures adjacent from three mine areas were surveyed in Central Anatolia.These mine areas are (A) mercury waste mound (rich in mercury, lead, arsenic and cadmium) stocked on open field in Kursunlu-Konya,(B) the dump site of an old Zn mine located in the Bozkır district of Konya province, (C) the waste sludge of a waste water dam in Seydisehir Al Factory of Konya province. The elemental analysis of the soil samples was carried out by taking extracts in a mixture consisting of 4 acids (HNO_3-$HClO_4$-HF and HCl) and the reading process was performed using ICP-ES and ICP-MS device. These procedures were carried out by Canada Acme Analytical Laboratories. It can be seen in the analysis results of the samples (Table 1). The Pb,Zn,Cr,Co,Cd,As,Al,Ni and Fe contents of the soil were substantially above the acceptable limit values. The weed species identifications of plant samples taken from three different mining areas were made by Bagci.

Table 1. Coordinates, altitudes and some heavy metal content of soils taken weed samples

Locations	Coordinate	Altitude (m)	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Fe (%)	Pb (mg kg^{-1})	Ni (mg kg^{-1})	As (mg kg^{-1})	Cd (mg kg^{-1})	Al (%)	Cr (mg kg^{-1})	Hg (mg kg^{-1})	Sb (mg kg^{-1})
A (Hg mine)	38° 10’ 09’’ N 32° 24’ 22’’ E	1278	226	482	2.47	198.4	75.8	485	6.7	5.13	109	>100	4000
B(Zn mine)	36 °58' 24" N 32 °15' 29" E	1994	>10000	910	9.31	>10000	43.7	39	254.7	5.84	101		1.3
C(dam of Al fac.)	37 °45' 90" N 39 °39' 57" E	1154	>10000	796	7.86	>10000	43.1	40	56.4	5.77	91		1.2

2.1. Staining of Mycorrhizal Root

Samples of mycorrhizospheres in roots of 35 weed species growing on different mine areas polluted soils at Konya-Turkey were collected. Weed root clearing and staining was done according to the method described by [30].Entire, cleared with KOH (10%) and stained with Trypan blue (0.05%) with the lacto phenol being changed to lacto glycerol. Naturally dark pigmented roots were cleared with 10% hydrogen peroxide for one hour before the staining with Trypan blue.The percentage of root colonization was calculated by the gridline intersect method, and when the amount of roots was low, by the slide method [31]. The percentage of AM colonisation was calculated as the number of segments infected out of 100 segments that were examined under a light microscope (Nikon ECLİPSE E 100) at 40X and 100X magnification [31].

Statistical analysis: The data obtained through the measurements were statistically analyzed using Minitab software.

3. Result and Discussion

Mycorrhiza colonization status of the weed species taken from three different mining areas were given at Fig. 1. According to colonization levels of weeds were attributed to four listing groups. *Onosma stenolobum* (Boraginaceae), *Acantholimon* sp. (Plumbaginaceae), *Aethionema iberideum* (Cruciferae), *Arabis aubrietioides* (Cruciferae), *Astragalus microcephalus* (Leguminosae), *Fumana aciphylla* (Cistaceae), *Alcea pallida* (Malvaceae), *Erysimum crassipes* (Cruciferae), *Hordeum bulbosum* (Graminea) and *Trigonella spruneriana* (Leguminosae) were not colonized (0%-20%).Low mycorrhizal frequencies of 20%-50% were found in *Achillea tanaceh* (Compositae), *Stachys cretica* ssp.*anatolica* (Labiatae), *Plantago lanceolata* (Plantaginacea), *Cruciata taurica* (Rubiaceae), *Astragalus* sp. (Leguminosae), *Eryngium campestre* var. *virens* (Umbelliferae), *Dianthus crinitus* ssp. *crinitus* (Caryophyllaceae), *Hypericum heterophyllum* (Guttiferae), *Trifolium hirtum* (Leguminosae) and *Crepis* sp. (Compositae). Moderate frequencies of mycorrhizal colonization of 50%-80% were found in *Carduus nutans* ssp.*taygateus* (Compositae), *Euphorbia* sp. (Euphorbiaceae), *Melica persica* ssp. *jacquemontii* (Gramineae), *Echinops* sp. (Compositae), *Phlomis* sp. (Labiatae), *Silene* sp. (Caryophyllaceae) and *Thymus zygioides* (Labiatae).The highest mycorrhizal frequencies 80%-100% were found in *Teucrium* sp. (Labiatae), *Trifolium trigonella*

(Leguminosae), *Astragalus brachypterus* (Leguminosae), *Cistus laurifolius* (Cistaceae), *Verbascum cheiranthifolium* (Scrophulariaceae), *Stipa hohenackeriana* (Gramineae) and *Cardaria draba* (Cruciferae).

Both spore(%) and arbuscul(%) in weed roots were found the highest number in *Teucrium* sp. (Labiatae), *Trifolium trigonella* (Leguminosae) and *Verbascum cheiranthifolium* (Scrophulariaceae).According to other study conducted in the same area, mercury uptake was determined very high in the foliage of *Verbascum cheiranthifolium* (Scrophulariaceae) 5788 mg kg^{-1} and in roots 2097 mg kg^{-1} [32]. Both mycorrhizal inoculation and high Hg uptake of verbascum , it may be advisable for phytoremediation.

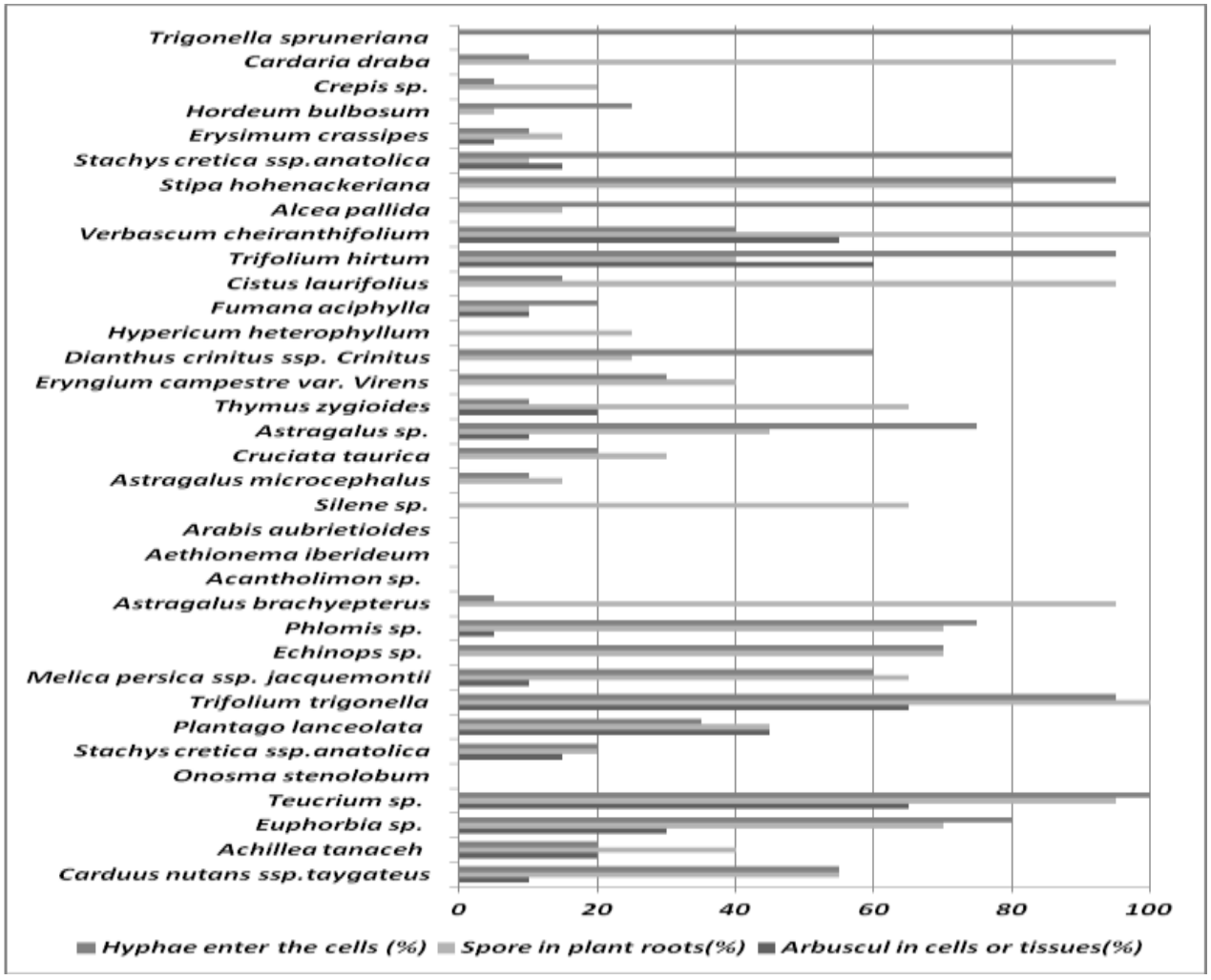

Fig. 1: Mycorrhiza hyphae, arbuscul and spore number (%) in roots of different weed species in reseach areas

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