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Mycorrhizal inoculum potential of arbuscular mycorrhizal fungi in soils irrigated with wastewater for various lengths of time, as affected by heavy metals and available P

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ABSTRACT

Sewage water is widely used for irrigation in dry countries, but the practice can lead to the accumulation of heavy metals in the soil and consequent poisoning of the soil's micro-organisms. The area irrigated with sewage water in the Mezquital Valley in central Mexico has steadily expanded during the last 100 years, and it provides the opportunity to assess the effects of the practice on the mycorrhiza in particular.

We sampled the topsoil of the two main kinds of soil, Vertisol and Leptosol, in fields irrigated for 5, 35, 65 and 95 years in the Valley. We measured the concentrations of zinc, lead, copper and cadmium, all of which appeared to have increased linearly with time. We also determined the abundances of arbuscular mycorrhizal morphotypes in the soil both at the time of sampling and after incubation in association with *Allium cepa* L. in the greenhouse.

Spores decreased in abundance with increasing duration of irrigation in the Vertisol, whereas in the Leptosol the numbers after only 5 years of irrigation were small, increased after 35 years of irrigation and decreased again thereafter. In the greenhouse the production of spores and sporocarps was maximal in soil irrigated for between 35 and 65 years, as were the intraradical hyphae, spores and vesicles. *Glomus* species dominate the morphotypes, and the spores of *Glomus mosseae* were the most abundant in both soils and after all times.

The total root colonization potential seemed unaffected by duration of irrigation, and was substantially greater in the Leptosol than in the Vertisol. However, species of the *Glomus* genus tend to dominate over other genera as irrigation proceeds, since *Glomus* species spread not only by spores, but also by roots. We conclude that irrigation with sewage water in the Mezquital Valley is decreasing the mycorrhizas' diversity in the long term.

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1. Introduction

Wastes such as sewage sludge and organic residues from the food processing industry are increasingly being spread on agricultural land. The practice recycles nutrients and diminishes the cost of waste disposal. However, some of these wastes contain significant amounts of pollutants, which may accumulate in the soil and find their way into the food chain. Irrigation with wastewater, which is common in many semiarid regions, is one way in which such wastes are spread and with them plant nutrients and pollutants.

In Mexico more than 90,000 ha are irrigated with untreated wastewater from metropolitan Mexico City with its population of some 20 million people. Siebe (1998) showed that the amounts of available phosphorus (P) in particular have increased significantly in soil irrigated for a long time (more than 80 years). Siebe (1995) and Siebe and Fischer (1996) discovered that the amounts of heavy metals had also increased in that time, although their availability to plants is small because they are barely soluble at the prevailing neutral to moderately alkaline pH of the soil. Nevertheless, the transfer of pollutants into plants growing in the soil might be aided (Joner and Leyval, 1997) or inhibited (Kaldorf et al., 1999) by arbuscular mycorrhizal (AM) fungi if they survive or remain effective in the soil. We know that they can play a fundamental role in such transfers in polluted soil (Meharg, 2003; Pawlowska and Charvat, 2002).

There have been few investigations that quantify the effects that these waste materials might have on the fungi. In a previous study (Ortega-Larrocea et al., 2001) we assessed the impact of waste sewage water on the arbuscular mycorrhizas. We found that after 90 years of irrigation there had been a marked decrease in the numbers of AM spores, and that this effect was related in particular to the large concentrations of available P. There were also fairly strong correlations with the concentrations of heavy metals in the soil.

Large amounts of P added to agricultural soil can depress significantly colonization by AM fungi (Sáinz et al., 1998; Bunemann et al., 2006; Covacevich et al., 2006). They can reduce the development of arbuscules, the amount of external mycelium and the number of entry points (Abbott et al., 1984; Smith and Gianinazzi-Pearson, 1988; Amijee et al., 1993; Gryndler et al., 2006). Prolonged fertilization can also lead to a selection of fungi adapted to large concentrations of P (Davis et al., 1984; Jeffries and Barea, 1994). Some scientists (e.g. Weissenhorn et al., 1995; Pawlowska and Charvat, 2004) have shown that large concentrations of pollutants, such as heavy metals, in the soil can also depress colonization by AM fungi.

A reduction of mycorrhizal inoculum could inhibit the formation of mycorrhiza because the amount of inoculum in disturbed soils depends on the concentrations of propagules and their resilience (Brundrett, 1991; Varma, 1998). The relative importance of those depends on the fungal species, and the most important propagules are generally unknown (McGonigle and Miller, 1999). Soil-borne spores have been considered the most important type of inoculum, but their numbers are often poorly correlated with mycorrhizal formation (Bharadwaj et al., 2006). This suggests that other constituents in the soil, such as roots and hyphae, are then the main sources of inoculum (Kabir et al., 1999).

The relative effectiveness of mycorrhizal communities, as well as the total mycorrhizal infectivity of soils, can be inferred from the most probable number (MPM) or bioassays, whereby the potential for the fungi to colonize roots of trap plants is assessed in greenhouse experiments (Brundrett, 1991; Brundrett et al., 1994).

With the above in mind we set ourselves three objectives, as follows.

- To assess the effect of increasing duration of irrigation with wastewater on the abundance of spores in the soil.
- To see whether irrigation with wastewater has modified the mycorrhizal inoculum potential with increasing duration.
- To discover what impact irrigation with wastewater has had on the qualitative diversity of morphotypes in the soil with increasing duration.

To achieve the first objective we sampled the two main kinds of soil (Vertisols and Leptosols) in the Mezquital Valley (see below) of Mexico and counted the numbers of spores in the soil immediately after the maize harvest. For objectives 2 and 3 we grew trap cultures with samples of soil from the same fields in the greenhouse to measure the inoculum potential.

2. Materials and methods

2.1. The Mezquital Valley

The Irrigation District 03, which receives the untreated wastewater from Mexico City, lies in the Mezquital Valley in the state of Hidalgo. It is a vast agricultural area (more than 90,000 ha). Such irrigation has been practised there for more than 100 years, longer than anywhere else in the world in an area of similar size. The District lies between 19°53′ and 20°30′ North and 98°59′ and 99°38′ West. It has a dry climate with a mean annual precipitation of 400 mm in the North and of 700 mm in the South.

Xerophytic shrubs, with *Prosopis laevigata* (mezquite), dominate the natural vegetation. There are two main types of soil: Pellic Vertisols (deep cracking clays) and Rendzic Leptosols (shallow, calcareous soils of loamy texture) (IUSS, 2006). The area of the District has gradually expanded as the volume of wastewater from metropolitan Mexico City has increased, and it is still expanding.

2.2. Soil sampling and quantification of P and heavy metals

We sampled Leptosols and Vertisols in fields that have been irrigated for four lengths of time (5, 35, 65 and 95 years). In each field we sampled the topsoil (from 0 to 30 cm depth) around the stalks of maize immediately after harvest. We expected the maximum density of spores then (Sánchez-Díaz and Honrubia, 1994; Pande and Tarafdar, 2004). Ten subsamples taken in this way were combined into one composite sample for each field. These samples were dried in air and stored at 4 °C until analysis.

'Available' P was extracted with 0.5 M $NaHCO_3$ and determined colorimetrically as blue molybdate–phosphate