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5. SUMMARY

An experiment was carried out in the greenhouse at Sabahia Horticultural Research Station, Alexandria, Egypt, to study the response of five tree seedlings species and their symbionts (VA mycorrhizal fungi) to irrigation with primary, secondary wastewater treatments or by freshwater. Five species of timber trees seedlings that are most common in Egypt were used in this study:

1. *Albizzia lebbek*
2. *Melia azedarach*
3. *Pongamia glabra*
4. *Taxodium distichum*
5. *Tipuana speciosa*

- Calcareous virgin soil, from New Borg El-Arab City (60 km South West of Alexandria) was used. The Seed were grown by direct seeding in April 1st 2002 in polyethylene bags containing one kg loamy sand soil. The seedlings were irrigated by freshwater (tap water). After two months from sowing, homogenous seedlings were randomly selected for the experimented study. The seedlings were transplanted into fired clay pots (40cm diameter and 40m depth) filled with 18 kg of the calcareous soil. The soil was air-dried and passed through 2mm sieve. One seedling was grown in each pot. Treated wastewater was obtained from the stabilization ponds, at New Borg El-Arab city.

- Split-plot design in randomized complete block design (RCBD) was used. The experiment layout with three replicates for each treatment, six pots for each species for each treatment.

- The irrigation treatments were: (i) Freshwater (Control), (ii) primary treated wastewater and, (iii) secondary treated wastewater. The wastewater used in

irrigation was derived from industrial and municipal sources. Irrigation was carried out three times every week in summer and two times in winter with 500 ml for each pot.

- Treatments were applied beginning from May 1st 2002 to June 2nd 2003 (first period); from May 1st 2002 to July 2nd 2004 (second period) and from May 1st 2002 to November 2nd 2004 (third period). The effect on the growth was represented by height and stem diameter of the tree seedlings which were measured every four months, until the end of the experiment.

The sampling

- Calcareous soil samples were collected from under the five tree seedlings species for each treatment. The soils were prepared properly for biological and chemical analysis according to standard procedures.
- Samples of freshwater irrigation and treated wastewater were collected according to standard procedures for biological and chemical analysis.
- Seedlings tree samples were collected, washed by tap water then with distilled water and the fresh weights were measured. The seedlings samples were dried in an oven at 70 °C for 48 hrs, and the oven-dried weights were measured. These oven-dried plants materials were finely ground using steel mill and stored for chemical analysis.

One gm fresh roots were stained to determine roots infection percent by VA mycorrhizal fungi.

The analysis:

1) **Soil:** Chemical analysis of the soil samples was carried out to determine pH, EC, water soluble ions, organic matter (O.M.), available N, P, K, Cu, Mn, Zn, Pb, Ni and Cd.

Microbiological analysis of the soil samples was carried out to identify the prevalent VA mycorrhizae spores.

2) **Water:** Chemical analysis of the water was carried out to determine the pH, EC, soluble ions, N, P, K and heavy metals.

Microbiological properties of the water were determined for biochemical oxygen demand (BOD₅), chemical oxygen demand (COD) and dissolved oxygen (DO).

3) **Litters:** Litters were collected from the five tree seedlings species at January 2003 and January 2004, dried and finely ground to determine N, P, K, Cu, Mn, Zn, Pb, Ni and Cd.

4) **Plants:** The oven-dried seedling materials were wet digested and the amounts of N, P and K were measured by the proper methods.

The amounts of Cu, Mn, Zn, Ni, Pb and cadmium were measured by atomic absorption spectrophotometer.

Statistical analysis of the obtained data was calculated for the least significant difference (L.S.D).

The following paragraphs summarize the obtained results in this study.

1) **Effect of treated wastewater irrigation on tree seedlings growth**

- The obtained results showed that the height, height increment, mean monthly increment and stem diameter of the seedlings grown on calcareous soil and irrigated with primary or secondary treated wastewater were significantly higher than those grown in soil irrigated with fresh water during the three periods of the experiment. However, height, height increment and mean monthly increment did not significantly differ between the secondary wastewater treatments and freshwater treatments after twenty six months. *Tipuana speciosa* seedlings recorded the highest growth at June 2003 (118.45cm). However, *Albizia lebbek* and *Pongamia glabra* seedlings showed the highest growth at July 2004 (165.5 and 145.45 cm, respectively). *Tipuana speciosa*, *A. lebbek* and *P. glabra* seedlings gave the highest growth (250.45, 246.23 and 246.12 cm, respectively) at November 2004, and they are

statistically equal as compared with *Melia azedarach* and *Taxodium distichum* (160.78 and 106.34 cm, respectively).

- Total fresh weights of the seedlings irrigated with primary treated wastewater were significantly higher than those irrigated with fresh water after thirty months. *Albizzia lebbek*, *T. speciosa* and *P. glabra* gave the highest fresh weight (154.64, 153.12 and 152.21 gm, respectively) at June 2003. However, *A. lebbek* gave the highest total fresh weight (423.4 and 659.82 gm, respectively) at July 2004 and November 2004 as compared with the other tree seedlings species.
- Total dry weight of the seedlings did not significantly differ between the primary and the secondary wastewater treatments; however, there were significant differences between the primary wastewater treatments and fresh water at July 2004 and November 2004. At November 2004, the total dry weight of the seedlings did not significantly differ between the irrigation with the secondary treated wastewater and fresh water. *Albizzia lebbek* seedlings showed the highest dry weight at the three periods (76.41, 224.46 and 377.15 gm, respectively) as compared with the other tree species, at the different periods of the experiment.
- Shoot/root ratio differed significantly between the treatments at June 2003, and November 2004. Shoot/root ratio was higher under irrigation with the primary and secondary wastewater treatment than fresh water. *Albizzia lebbek* seedlings gave the lowest shoot/root ratio (1.10) at June 2003. However, *T. speciosa* seedlings gave the highest ratio (2.44) at November 2004 as compared with the other tree seedlings species.

2) Effect of treated wastewater irrigation on the minerals contents of the tree seedlings

A. Macronutrient elements contents

The N, P and K contents were determined in the leaves, stems and roots of the seedlings and as concentration, uptake and translocation from roots to leaves (leaves/root ratio) and from roots to stem (stem/root ratio).

The amounts of nitrogen, phosphorus and potassium of the five tree seedlings species grown in calcareous soil and irrigated with primary treated wastewater were significantly higher than the other seedlings which were irrigated with freshwater. For example, for the third period (November 2004), total nitrogen uptake was 7.31 g g^{-1} with the primary treated wastewater. However, with the secondary treated wastewater it was 5.34 g g^{-1} and with freshwater it was 3.25 g g^{-1} . Total phosphorus uptake was 0.24 g g^{-1} with the primary treated wastewater. However, with the secondary treated wastewater it was 0.167 g g^{-1} , and with freshwater it was 0.11 g g^{-1} . Total potassium uptake was 3.09 g g^{-1} with the primary treated wastewater while, with the secondary treated wastewater it was 2.31 g g^{-1} , and with freshwater it was 1.46 g g^{-1} . However, potassium concentration in the leaves, stems and roots at June 2003 did not significantly differ between the primary treated wastewater and fresh water.

The same trends were found in the seedlings which were irrigated with the secondary wastewater and freshwater, but, potassium concentration at June 2003 did not significantly differ between them.

B. Heavy metals

There were significant increases in the amounts of Cu, Mn, Zn, Ni and Pb in the seedlings which were irrigated with primary treated wastewater compared with the seedlings which were irrigated with fresh water.

- The seedlings which were irrigated with primary treated wastewater contained the highest total uptake of Cu, Mn, Zn, Ni and Pb (4703.9 , 7255 , 36736 , 1015.2 and $1669.6 \text{ } \mu\text{g g}^{-1}$, respectively) compared with the seedlings

which were irrigated with freshwater (1265.3, 1723, 15040, 445.9 and 723.7 $\mu\text{g g}^{-1}$, respectively, at the third period (November 2004).

- The same trend was found between secondary wastewater and fresh water. Sometimes, they did not show significant differences.
- There was a reduction in the concentration and uptake of the heavy metals in the leaves as compared with the roots (at June 2003).
- In general, *A. lebbek* seedlings contained the highest heavy metals uptake, followed by *P. glabra*, *T. speciosa*, *M. azedarach* and *T. distichum*.
- There was a reduction in the amounts of Mn, Zn, Pb and Ni at November 2004 more than at June 2003 and July 2004.
- There was a reduction in the concentration of cadmium in all parts of the seedlings. So, it could not be determined by Atomic absorption spectrophotometer at the three periods.

3) Effect of treated wastewater irrigation on the minerals contents in the litters

There were significant increases in the amounts of N, P, K, Cu, Mn, Zn, Pb and Ni in the litters of the seedlings irrigated with primary treated wastewater as compared with the seedlings irrigated with the secondary treated wastewater and freshwater, in January 2003 and January 2004.

- There was a reduction in the amounts of Mn, Zn and Pb in the litters in January 2004 than at January 2003.

4) Effect of treated wastewater irrigation on the root infection percent by VA mycorrhizae and the role of VAM in the heavy metals bioremediation

The root infection percent did not significantly differ between the seedlings which irrigated with primary (91.0%) or secondary wastewater treatments (87.43%) and freshwater (89.34%) at November 2004. Also, the root infection

percent did not significantly differ between the five tree seedlings species at the three periods.

- A reduction was noticed in the concentration of Cu, Mn, Zn, Ni and Pb in the leaves compared with the roots at June 2003. Also, there were reductions in the concentration of Mn, Zn and Pb at July 2004. However, the concentration of Pb in the leaves was lower than in the roots, at November 2004. It is clear that the VA mycorrhizae played an important role in accumulation of heavy metals in the roots and bioremediation. On the other hand, arbuscular mycorrhizae can depress the translocation of heavy metals to the shoots of the host plants in the moderately soils with heavy metals.
- The prevalent mycorrhizal species found in the soil were: *Glomus convolutum*, *Glomus monosporum* and *Glomus pallidum*.
- Significant correlation coefficients matrix between the root infection percent and total lead uptake were found at June 2003, also for manganese uptake in the leaves, total manganese uptake, nickel uptake in the roots and total nickel uptake at July 2004.
- It is demonstrated that at high soil heavy metals concentration, arbuscular mycorrhizal infection reduced the concentrations of Zn, Cd and Mn in the tree leaves. Also, AM infection increased total uptake of lead, total manganese and nickel, and nickel uptake in the roots.

5) Effect of treated wastewater irrigation on the soil chemical properties

Irrigation with treated wastewater significantly increased pH, EC, O.M. content, available N, P, K and heavy metals (Cu, Mn, Zn, Pb and Ni) of the cultivated calcareous soil. However, at November 2004, there were reductions in the amount of cadmium in the soil; so, it could not be determined by atomic absorption spectrophotometer.

Recommendation

For accumulation of the heavy metals and biomass production the tree seedlings species can be arranged in the following descending order: *Albizzia lebbek*, *Tipuana speciosa*, *Pongamia glabra*, *Melia azedarach* and *Taxodium distichum*. So, the importance of selecting species and their VAM symbioses on the basis of not only growth but also nutrient accumulation to optimize renovation of wastewater and phytoremediation by tree plantations.

It is also clear that Pb, Ni and Cd contents, in the five tree seedlings species irrigated with the primary or secondary treated wastewater were lower than the excessive or toxic levels, therefore they did not affect seedlings growth.

So, we can reuse treated wastewater not only as a source of water but also as a source of nutrients and organic matter. Also, polluted and virgin soils can be used to cultivate hyperaccumulation trees species.