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STUDY ON THE EFFECT OF SEWAGE SLUDGE AMENDMENT ON GROWTH AND YIELD OF SOME WOODY TREES, DYNAMIC OF AM FUNGI AND BIOEXTRACTION–REMEDIATION OF TREES

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ABSTRACT

This study was conducted at the plantation of Solid Disposal Facility, and Compost Production, "N9", Alexandria, Egypt, to pinpoint the effect of treated sludge on the growth, yield of *Acacia saligna*, *Casuarina glauca* and *Eucalyptus camaldulensis* trees aged 10 years and on the dynamic of activity and population of vescicular- arbuscular mycorrhizal fungi. The study aimed also to investigate the bioextractional – remediation role (if any) of trees against sludge pollutants.

Data obtained indicated that, except for height of *A. saligna*, all growth and yield parameters of sludge amended trees (tree height at diameter outside bark at breast height (dodh), commercial volume of wood per tree, yield per feddan, av. of annual yield per feddan and basal area) were significantly higher than those of the control, since the solid dry timber was accounted for 3.76 and 4.76 ton/ feddan in unamended and amended *A. Saligna* with sewage sludge, respectively. At the same order, the yield was 124.81 and 135.47 ton/ feddan in *C. glauca* and 60.6 and 117 ton/ feddan in *E. camaldulensis*. Concentrations of macro-elements, N, P, K and Na and heavy metals (Cd, Zn and Pb) in soil, shoots and woods from bark to pith of sludge-amended and unameneded trees and rhizosphere were increased concentration of macro elements and heavy metals in foliage and wood of trees applied with sewage sludge. However, trunk analysis revealed that the bark displayed the higher concentration of heavy metals followed by heartwood. Specific gravity, either of sapwood or heartwood was significantly higher in unamended trees compared with amended ones due to its increased growth rate.

The data also revealed the presence of clamydospores of AM fungi, namely, *Glomus aggregatum*, *G. fasciculatum* and *G. mossae*

Infection level (%) of feeder roots and population (number of spores per 10 g of dry rhizosphere soil) of clamydospores of AM fungi were affected negatively by sludge amended. It can be concluded that the growth and yield of tree stand studied can best be managed by amendment with sludge to achieve better results. Also, *E. camaldulensis* and *C. glauca* trees displayed potential phytoextraction remediation towards heavy metal pollultants concomitant with positive yield. It is recommended, however, to capitalize on sludge for establishment several tree species and others in afforestation programs in low fertile soils in arid zones which being inadvisable to edible crops and manage native tolerant AM fungi under pollution situations as well.

INTRODUCTION

While sewage effluent disposal is the primary objective in many cases, the need of water for irrigation is becoming more often the driver for using sewage effluent on land. This is particularly true in areas like the Middle East where population growth is resulting in severe water shortages (Stewart, 2008).

The use of wastewater for irrigation is probably an ancient as humankind's cultivation of land. Large-scale, controlled wastewater use for irrigation, however, only dates back to last century when so-called sewage farms were established in parts of Europe, Australia, India and the United States for the purpose of disposing of wastewater and preventing river pollution. In addition, in Egypt, in 1911, plantation (Man made forest) was established in El-Gabal El-Asfar region, about 30 km northeast Cairo, to dispose of the city's sewage water. In the mid-1980s, the forest was shifted to citrus, cereal and vegetable production and extended to be about 1, 260 ha (Braatz and Kandiah, 1996).

Sludge arising during the primary treatment of municipal waste presents a valuable source of organic matter, nitrogen, phosphorus, potassium and some trace elements (Yassen *et al.*, 2006), yet there are also significant risk, notably the presence of toxic heavy metals, viruses, bacteria and parasites which are of important from hygiene, epidemiology and epizootiology standpoints (Venglovsky *et al*, 2002). Sludge applications to forest lands have been in practice for more than 3 decades in several parts of the world. Forest application of sludge provides many advantages, amongst which, its potential health risk is much lower than in agricultural application, because forest products and forest soils and vegetation are often more amenable to sludge application (Muchorel and Richcigl, 1995).

The advantages of capitalize on sewage sludge in plantation or forest implied: established forests are less susceptible to sludge-induced changes in vegetation (e.g. weed growth). Excellent growth response can result from the increased nutrients and forest soil under established forest usually have high C/N ratio resulting in excellent capability to immobile (store) nitrogen for slaw release in future years. Consequently it is often feasible to make an initial heavy application of sludge e. g. 33 tons/acre and achieve tree growth response for up 5 years without subsequent sludge application (Stein *et al.*, 1997).

Ecosystems have been contaminated with heavy or trace metals due to various human and natural activities (Assareh *et al.*, 2008). The sources of metals in the soil are diverse, including burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, sewage sludge amendments, the use of pigments and batteries (Gaur and Adholeya, 2004).

Trace metals are defined as metals that in natural materials are present at levels below 0.1% (Sparks, 1995). Hence, trace metals like cadmium (Cd), copper (Cu) and mercury (Hg) occur only at low concentrations in uncontaminated soils, 0.06– 1.1 µg g^{-1}, 6–80 µg g^{-1} and 0.02–0.41 µg g^{-1} respectively (McBride, 1994). There are few studies regarding the accumulation of heavy metals in wood of tree. Watmough et al.(1999) monitored the labeled Pb in growth ring of sycamore (*Acer pseudoplatanus* L.) to pinpoint the source of its concentration in wood. On the other hand, Hagemeyer.and Weinand (1996) concluded that radial distribution patterns of Pb in Norway spruce stems do not directly reflect changes in soil Pb concentration but depend on several internal, physiological factors.

The subject of the impacts of heavy metals on the activity of mycorrhizal fungi is being sophisticated. For example, mycorrhizal fungi was not affected by heavy metal contamination and may strongly colonize their partner's roots (Gildon and Tinker, 1983; Dev *et al.*, 1998; Koomen *et al.*, 1990; Weissenhorn *et al.*,1995 and Waschke *et al.*, 2006), whereas some reports pointed out to the negative impacts of heavy metals on mycorrhizal fungi in soil (Gildon and Tinker, 1983; Angle and Heckman, 1986 and Diaz and Honrubia, 1993). Others concluded that there is no relationship between mycorrhizal abundance and degree of metal exposure in soil or inside plant roots (Weissenhorn *et al.*, 1995).

This work aimed to study the response of tree stands of *Acacia saligna*, *Casuarina glauca* and *Eucalyptus camaldulensis* trees and dynamic of mycorrhizal fungi to soil amendments by sludge. This work targeted also to study (if any) phytoremediational potential of these trees towards heavy metal pollutants due to sludge application.

MATERIALS AND METHODS

This work was carried out in summer of 2007 to monitor and analyze the growth and yield of 10 years old three tree species, namely; *Acacia saligna*, *Casuarina glauca* and *Eucalyptus camaldulensis*, planted in man-made forest of solid products of primary treatment of sewage water at site "N9", Solid Disposal Facilities, El-Ameriya, West Alexandria, Egypt, and dynamic of mycorrhizal population both in rhizosphere of stands amended with sludge relative to control one.

The total area of the "N9" station is about 350 feddans and plantation was about 80 feddans in three pure stands of the forementioned the three tree species. About half of the area was amended with composted sewage sludge in two doses: (i) the first dose was about 20 m^3 at time of transplanting (when the transplants aged about one year) and (ii) the second one was about 25 m^3, when the trees attained sapling size (aged 4 years). The physical and chemical properties of the sludge amended are given in Table (1).

Table (1): The average values of the main chemical properties applied sludge of "N9" Station for Sludge Treatment, Soil Disposal Facilities, West Alexandria.

EC.dsm-1	pH	CaCo3 (%)	O.M (%)	N, (ppm)	Total P (%)	Available P (ppm)	K (%)	Mn (ppm)	Zn ppm)	Cu (ppm)	Ni (ppm)	Cd (ppm)	Pb (ppm)
4.12	7.2	12.4	32.5	201	0.41	0.41	0.97	134	744	458	109	9.50	170

Source: Fayed (2009).

2. Sampling:

For each stand species, 3 representative 10 × 10 m blots were randomly selected, as simple random ones. Ten trees were randomly selected for all growth and yield determination. The rules of plant sampling were followed according to Husch *et al.* (1982) to determine height, diameter at breast height out bark (dobh).

2.1. Wood and bark sampling:

Three samples, from the lower 70 cm of tree trunk and branches of wood were taken by increment borer to study some of its physical and chemical properties (from bark to the pith).

2.2. Foliage sampling:

Samples of about 300 g of mature leaves from the same trees selected for growth of *A. saligna* and *E. camaldulensis* and branchlets of *C. glauca* were taken to determine N, P, K, Na and heavy metals.

2.4. Soil sampling:

Soil samples were collected from rhizophere of trees selected before at 50 cm from the trunk to depth at the uppermost 10 cm of the soil to determine N, P,K, Na and heavy metals.

2.5. Total macro element:

Total macro element analysis in soils and plants samples were determined according to FAO (1980).

2.6. Total heavy metals in soils:

Total heavy metals (Cd, Zn and Pb) in soil were determined by wet digestion of 5 g of the ground soil with 25 ml of conc. Nitric acid/ hydrogen peroxide mixture (1:1, v/v) mixture for one hour. Soil extract was filtered and analyzed for elemental composition using Atomic Absorption Spectroscopy Model Perkin-Elmer (Ebbs, et al., 1997).

2.7. Heavy metal in plants:

Exact weight of oven-dried plant tissues were ashed in a muffle at 500 °C for 6 hrs. The ash was dissolved in a mixture of 2 M HCl and 1 M HNO_3 and heavy metal contents of the acid extract were determined by Atomic Absorption Spectroscopy Model Perkin- Elmer (Nanda Kumar et al., 1995).

3. Determinations of specific gravity:

Five g samples of wood of were obtained from the center of sap- and heart-wood, allowed to saturation, weighed, oven dried then weighed again. Specific gravity was calculated according to Smith (1961), using the following equation:

$$\text{Specific gravity} = \frac{1}{\frac{W_s - W_o}{W_o} + \frac{1}{G_{so}}}$$

Where:

W_s = Saturated weight of wood specimen

W_o = Oven-dried weight of wood specimen.

G_{so} = Average density of cell wood substance (using water media) assumed to be 1.53 theoritically.)

4. Mycorrhization Analysis

4.1. Clamydospore isolation, examination and identification:

The soil samples, taken from the rhisosphere of selected tree species to depth of 20 cm, air dried for 10 days and then 10 g samples were sieved in series of sieves from 0.500 to 0.100 mm pore diam. (VEB Mettallweberrei

Neustadi Models) under running water, filtered through Whatmann No. 1. The obtained supernatant was stereomicroscopy examined, then the existed spores were trapped, fixed with glycerin, then identified. The keys of Trappe (1982) for identification were followed for identification of AM fungal species.

To compare the spore number of the rhizosphere of the tree amended with sludge with that of the control, the relative spore number (RSN) was calculated as follos:

$$RSN = \frac{SNC}{SNS}$$

Where,

SNC is spore number of rhizosphere of the control trees and,

SNS is spore number of rhizosphere of the sludge amended trees

4.2. Examination of root system and determination of infection level (%)

Samples of feeder roots were taken from the plants, washed free from debris with tap water then softened, cleaned, stained with 0.1 % trypan blue according to the method described by Phillips and Hayman (1970). Infection level of roots with VAM was determined by measuring the length of actual colonized feeder root segment with arbuscules, vesicules, extramatical hyphae relative to total length of the root in samples.Ten feeder roots of one cm- length were examined in each plant then the average of infection was assessed using the following equation:

$$IL = \frac{FL \quad x\ 100}{TL}$$

Where; IL is an infection level (%), FL is a length of root colonized with AM structures and TL is the total length of the feeder root examined.

To compare the infection level of feeder roots of the tree amended with sludge with that of the control, relative infection level (RIL) was calculated as follows:

$$RIL = \frac{ILC}{ILS}$$

Where,

ILC is the infection level of the control and,

ILS is the infection level of the feeder roots of sludge amended trees

5. Statistical analysis

For samples taken, complete randomized design was applied, using 10 replicates for growth, yield parameters, mycorrhization characteristics, specific gravity of wood and 3 replicates for chemical analysis. For specific gravity, the data were analyzed using factorial arrangement according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

1. Macro element and heavy metals in soil:

Amendment of soil with sludge of plantation led to an increase of its nutritional value on one hand and deposition of the heavy metals on the other hand. The results of analysis of sludge amended and unamended rhizosphere are given in table (2).

Table (2): The mean values of rhizosphere macro-elements and heavy metals of sludge-amended *A. Saligna* (AS), *C. glauca* (CS) and *E.camaldulensis* (ES) trees and unamended ones; AO, CO and EO, respectively.

Stand species	Na g/ kg	K g/ kg	N g/ kg	P g/ kg	Cd mg/ Kg	Zn mg / Kg	Pb mg / Kg
AO	2.70	2.720	11.025	10.93	0.5	50	42.25
AS	3.51	3.461	18.2	17.18	5.0	75	505
CO	0.98	1.657	11.2	5.63	0.5	50	75.25
CS	2.36	3.461	14.875	6.47	2.5	75	760
EO	3.28	3.27	9.8	6.49	0.5	50	43.25
ES	4.10	3.841	18.025	7.7	7.5	75	725

2. Growth and Yield

Statistical analysis of variance revealed no significant differences between heights of sludge amended *Acacia saligna* tree (AS) and unamended one (AO), whilst the amended *Casuarina glauca* (AS) displayed higher significantly height than that of unamended one (AO) (Table 3). On the other hand, the height of sludge-amended *Eucalyptus camaldulensis* tree (ES) was as much as 2 fold that of unamended one (EO), since it was 10.80 and 5.28 m, respectively.

Table (3): The mean values of growth and yield parameters of sludge amended *A. Saligna* (AS), *C. glauca* (CS) and *E.camaldulensis* (ES) trees and unamended ones; AO, CO and EO, respectively.

	Height (m)	Diameter (cm)	Volume of tree wood (m^3)	Yield/ ton feddan	Av. annual yield (Ton/fed / year)	Basal area
AO	2.29	9.00	0.0092	3.768	0.376	0.00121
AS	2.36	11.13	0.0146	4.764	0.476	0.00185
LCD	0.19	0.05	0.030	0.55	0.0579	0.0003
CO	9.42	2000	0.199	124.81	0.124	0.01105
CS	10.80	20.56	0.216	135.47	0.135	0.111
LSD	0.23	2.3	0.48	8.1	0.063	0.04
EO	5.28	8.8	0.165	60.602	14.77	0.00138
ES	10.80	24.1	0.29	117.425	16.775	0.1139
LSD	2.44	9.21	0.37	29.515	0.78	0.05

As for stand diameters outside bark at breast height (dobh), those of AS, and ES were significantly higher than those of AO and EO. Yet there was no significant differences between AS and AO at dobh. However, dobh of ES was about three folds that of EO (Table 3).

As shown in Table (3), commercial volume of wood of the studied species amended with sludge (AS, CS and ES) were significantly higher than those opposite of unameded ones (AO, Co and EO; at the same order). The net yield of solid wood (based on the oven dry weight) of *A. saligna* was 3.768 and 4.76 ton/ feddan in AO and AS, respectively; whilst that of *C. glauca* was 124.81 and 135.47 ton/ feddan in CO and CS and in 6.606 and 117 ton/ feddan in EO and ES, at the same order. Consequently, the average yield per year (AYY) of the amended studied species was higher than that of unameded ones. However, ES displayed the highest positive response to sludge application, since the AYY of ES was about 3 fold that of EO.

The basal areas (BA) determined in sludge-amended stands of the three species were higher than those of unamended ones (Table 3). This indicated that the applied sludge leads to exaggerate land use of the plantation and ameliorates site quality to a great extent at nutrition stand point (Table 3).

3. Foliar contents of N, P, K and Na

Foliar nitrogen (N) contents of trees (Table 4) revealed significant increases in its concentration in AS and ES relative to those of AO and ES, respectively; whilst no significant differences were detected between CS and CO in N content. On the other hand, the concentrations of foliar phosphorus and potassium in sewage sludge amended tress (AS, CS and ES) were significantly higher than those of unamended ones (AO, CO and EO) respectively.

Table (4): The mean values of foliar macro-elements and heavy metals of sludge amended *A. Saligna* (AS), *C. glauca* (CS) and *E.camaldulensis* (ES) trees and unamended ones; AO, CO and EO, respectively.

	N g/ kg	P g/ kg	K g/ kg	Na g/ kg	Cd mg/ Kg	Zn mg/ Kg	Pb mg/ Kg
AO	36.22	12.95	11.602	6.55	0.02	105	117.0
AS	42.35	14.26	4.017	6.78	5.50	150	1255.5
LSD	1.42	1.33	1.39	1.84	2.22	15.0	25.2
CO	33.60	14.78	5.44	7.82	0.02	115.5	114.0
CS	33.6	16.889	8.833	8.51	5.00	150.2	1230.7
LSD	1.10	1.21	2.29	1.42	2.18	25.3	35.2
EO	35.35	15.57	8.44	9.78	0.02	120.8	127.0
ES	39.9	20.48	14.099	10.81	10.0	160.3	1390.2
LSD	1.43	2.02	2.72	2.02	2.26	20.2	30.7

As for sodium (Table 4) there were no significant differences between its concentrations in leaves of sludge amended and unamended *A. Saligna* and *E.camaldulensis* and branchlets of *C. glauca* trees, i.e, there were no negative impacts of application of sewage sludge on trees at salinity viewpoint.

4. Foliar contents heavy metals:

Analysis of statistical variance of the concentrations of foliar heavy metals (Table 4) revealed significant increases in the levels of sludge amended *A. Saligna* (AS), *C. glauca* (CS) and (ES) trees as compared with those of unamended AO, CO and EO, respectively. However, sludge amended trees displayed high levels of foliar lead (Pb), which exceed those of the control when it is compared with cadmium and zinc concentration (Table 4). The availability of some nutritional elements as well as trace elements might be increased owing to the presence of organic materials in sludge. Koo (2001) and Pires *et al.* (2007) detected some organic acids, amongst which, acetic, citric, lactic, and oxalic acids in the rhizosphere of different plant species cultivated in the presence of sewage sludge. Pires *et al.* (2007) found also that there is a positive relationship between the presence of organic acids of low molecular weight in the rhizosphere and sewage sludge-borne trace-elements phytoavailability. On the other hand, liming is increasingly being practical as a management tool to immobilize heavy metals in acid soils, bio-solids and mine tailing, thereby reducing their availability for plant uptake and transport to ground water (Mc-Bride et al., 2000 and Adriano, 2001). Although the last practice is being acceptable at safety-food-production standpoint, yet it would impede phytoremediation potential of trees.

5. Specific Gravity of Wood

As shown in Table (5), except for *E. camaldulensis*, sapwoods and heartwoods of trees amended with sludge displayed specific gravity lower significantly than those unamended one.

Table (5): The mean values of specific gravity (G) of wood and concentrations of heavy metals of sludge amended *A. Saligna* (AS), *C. glauca* (CS) and *E.camaldulensis* (ES) trees and unamended ones, AO, CO and EO, respectively.

	G			Cd mg/ Kg			Zn mg/ Kg			Pb mg/ Kg		
	Sap	heart	LSD	bark	sap	heart	bark	sap	heart	bark	sap	heart
AO	0.70	0.59	0.02	15.0	2. 0	10.0	110	50	100	130.0	129.	95.0
AS	0.52	0.50	0.04	20.0	12.0	13.0	150	130	145	3525	2570	3020
LSD	0.17	0.04		2.5	2.5	2.3	15.5	5.90	25.95	48.29	32. 5	33.1
CO	0.69	0.68	0.02	2.0	2.0.	2.0	25	100	150	155.0	108.	150.
CS	0.65	0.63	0.06	15.0	5.0	8.5	400	120	350	1800	1250	1400
LSD	0.5	0.03		3.2	3.6	0.8	10.55	10.18	22.67	33.97	32.4	30.0
EO	0.59	0.57	0.03	2.0	1.0	1.1	75	50	75	120.0	700.	80.0
ES	0.59	0.51	0.02	20	5.0	8.0	150	100	150	1530	1460	1530
LSD	0.13	0.03		1.1	2.29	0.9	12.75	14.84	32.54	56.26	56.6	42.6

On the other hand, there was no significant difference between sapwood of ES and EO. In most cases, except for AO and ES, there were no significant differences between specific gravity of heartwood and sapwood. The increased specific gravity may be attributed to relative increase of uptake and horizontal translocation potential of nutrients by *E. camaldulensis* tree from sludge compared with the other trees. The decreased specific gravity in heartwood of most cases may ascribed to the presence of juvenile wood or corewood which are characterized by a decrease of cell wall materials and less density. However, any silvicultural treatment, e.g., fertilization and thinning, could lead to increase of corewood portion on the expense of mature wood and may reduce its specific gravity (Haygreen and Bowyer, 1982). Thus, amendment with mineral-rich sludge could be regarded as fertilization.

6. Heavy Metal Contents of Wood

Cadmium, zinc and lead contents of the woods and barks of tree species (Table 5) were increased as a result of amendment of sewage sludge. The bark and heartwood, however, contained heavy metal contents higher than those of sapwood of all species. It is noticeable that the lead contents in wood and bark of all sludge amended species were considerably high relative to the other heavy metals. However some plants displayed clean-up capability of heavy-metal-contaminated soil such as corn plant (Huang and Cunningham, 1996), rye Grass and Fescue (Carlson and Rolfe, 1979), yet there is some risk of accumulated heavy metals toward to human and animals. Furthermore, *Eucalyptus* seedlings displayed bioextractability towards heavy metals (Assareh *et al*., 2008); and also willow (*Salix* sp) short rotation plantation (Lazdi *et al*., 2007).

These findings indicate the ability of the studied trees to extract heavy metals and vertically and horizontally (by aids of vascular ray elements) translocate throughout the wood. Since the accumulation of heavy metals occured inside the heartwood, the dead part of wood tissue has no impacts on tree health and human as well. The amount of heavy metals (. 1) which theoretically could be accumulated annually in wood of plantation can be regarded and considered, particularly Pb. Furthermore, the arrangement of tree in plantation, windbreak, silvopasture, agroforestry and/or shelterbelt, fashion furnishes one more type of phytoremediation throughout the isloaltion of polluted air (Dusty air) and soil conservation, i.e. impede of disperse of pollutants.

The increasing concentration in heartwood relative to the sapwood may reflex the time of absorption of these metals at the time of sludge amendment to the soil earlier to trees at early sapling stage, i.e., when they aged 4 years old.

The increasing concentration of heavy metals in bark might be ascribed to some complicated physiological processes to get rid of such metals via the bark which would peel out after certain time. There is another possible interpretation, that is the uptake of heavy metals trapped by the bark from polluted atmosphere, i.e., not essentially from the soil. Watmough and Hutchinson (2003a), in their dendrochemical studies on sycamore, lime and beech trees, concluded that trees are not simply passive recorders of metal deposition. It possibly explain also the patterns of Cd and Pb found. Furthermore, in another work, Warmouth and Hutchinson (2003b) found that the uptake of heavy metals through bark and foliage may also be possible.

Fig. 1-A

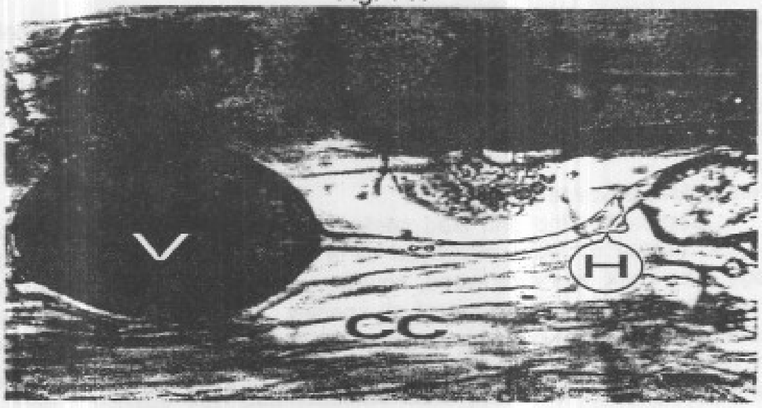

Fig. 1- B

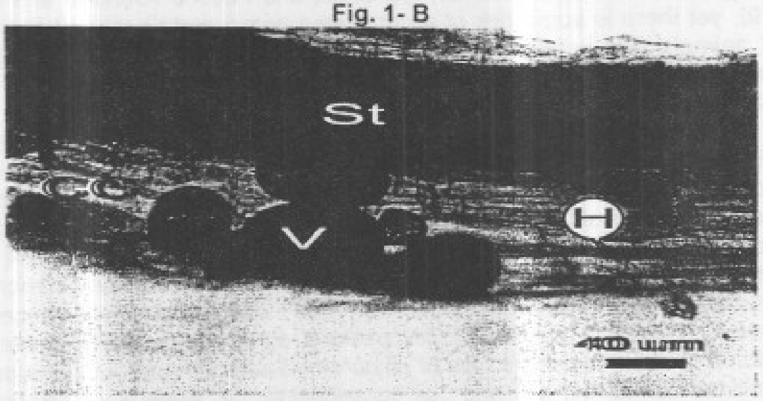

Fig. 1- C

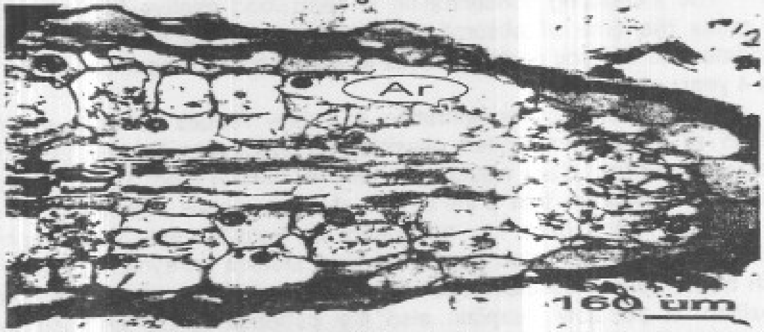

Fig.1A, 1B and 1C: Feeder root of *Eucalyptus camaldulensis*, *Casuarina glauca* and *A. salign* infected with AM fungus. V: vesicule, H: internal hyphae of the fungus, CC: cortex cells, St: stele and Ar: arbuscule.

This may interpret the accumulation of heavy metals in foliages and woods of unamended trees with sludge in this work. There are some trees can be employed in the dendrochemical studies, notably, fir (Watmough and Hutchinson, 1999) and *Araucaria columnaris* (Medeiros *et al.*, 2008) while some others unsuitable for such studies, amongst which, beech (*Fagus sylvatica*) trees (Hagemeyer *et al.*, 1994). This interesting aspect would merit more research, using several native species, labeled heavy metals and more advanced techniques to precisely pinpoint the source, amounts and time of pollutants absorption and deposition in wood.

7. Dynamic of Native Vesicular Arbuscular Mycorrhizal Fungi

7.1. Infection level (%) of feeder roots

Microscopic examination of feeder roots of all tree species revealed the formation of the most common structures of vesicular-arbuscular mycorrhizal (AM) fungi (Fig.1). Infection levels (IL) (%) of feeder roots of all sludge- amended tree species by AM fungi were less than those of unamended ones (Table 6). However, relative infection levels (RIL) of *A. sligna*, *C. glauca* and *E. camaldulensis* were 2.22, 2.9 and 2.12 (Fig. 2). . i.e.. infection levels of AO and EO were about two fold those of AS and EO, respectively. Furthermore, IL of sludge-amended *C. glauca* was about one third that of unamended one (Table 6).

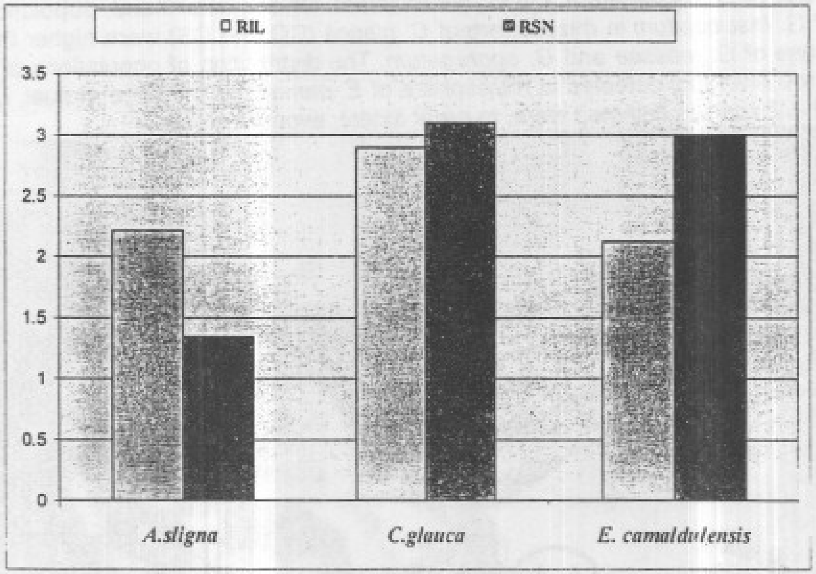

Fig. 2: Relative infection level (RIL) of feeder roots with AM fungi and relative spore number (RSN) of AM fungi in rhizosphere.

Table (6): Infection levels (%) of feeder roots and number of clamdospores/ 10 gm in rhizosphere of sludge amended *A. Saligna* (AS), *C. glauca* (CS) and *E.camaldulensis* (ES) trees and unamended ones, AO, CO and EO, respectively.

Species	Infection level (%)	No of spores/ 10 g of dry soil	Notes
AO	32.2	224.3	*G. mossae* more abundant.
AS	14.5	166.4	Viability of spore high (Light colored spores)
LCD	7.9	38.0	
CO	18.3	115.4	*G. mossae* less abundant, *G. fasciculatum* more abundant.
CS	6.3	37.0	Viability of spore is low (dark colored spores)
LCD	9.0	25.2	
EO	22.8	135.8	*G. mossae* and *G. fasciculatum* intermediate.
ES	10.4	45.5	Viability of spores intermediate.
LCD	8.9	36.3	

7.2. Population of clamydospores in rhizosphere

Microscpoic examination of isolated spores indicated that the most AM species native in rhizosphere of trees studied were *Glomus aggregatum* (Fig.3), *G. fasciculatum* (Fig.4 and 5) and *G. mossae* (Fig.4 and 6). However, the populations of *G. mossae* of rhizosphere of AO and AS were higher than those of *G. aggregatum* and *G. fasciculatum*. On the other hand, populations of *G. fasciculatum* in rhizosphere of *C. glauca* (CO and CS) were higher than those of *G. mossae* and *G. aggregatum*. The distribution of populations of all three AM fungi detected in rhizosphere of *E. camaldulensis* were mutual, i.e., all AM species detected were, to great extent, evenly existed.

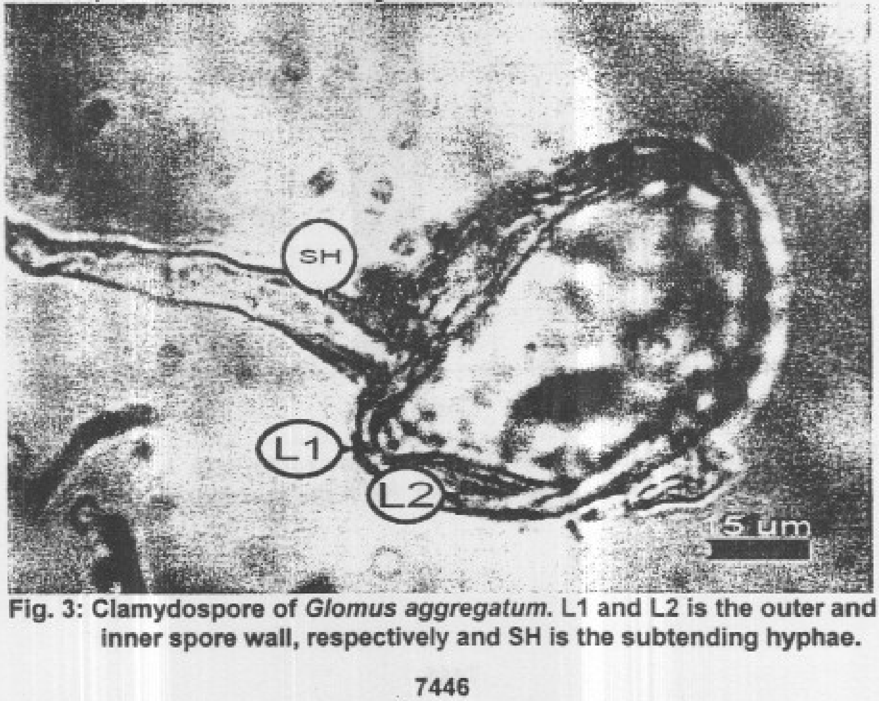

Fig. 3: Clamydospore of *Glomus aggregatum*. L1 and L2 is the outer and inner spore wall, respectively and SH is the subtending hyphae.

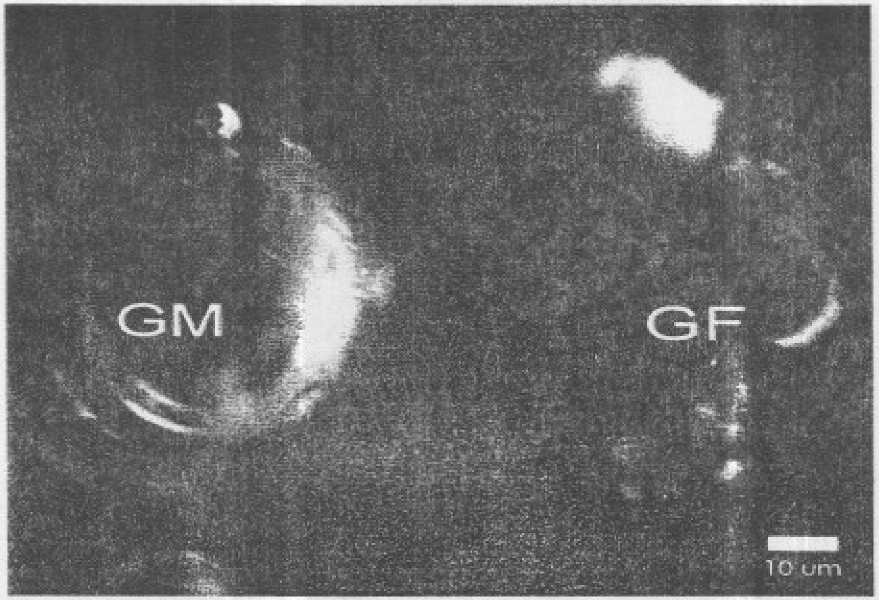

Fig. 4: Stereo shape of the clamydospore of *Glomus mossae (GM)* and *G. fasciculatum.*

The dominance of *G. fasciculatum* in rhizosphere of *C. glauca* (the harshest rhizosphere) relative to the other mycorrhizal species detected, may vindicate its highly reluctance nature. Such reluctance is possibly owing to its thick wall, which composed of three layers (Fig. 5) or may be attributed to its genetic characteristics.

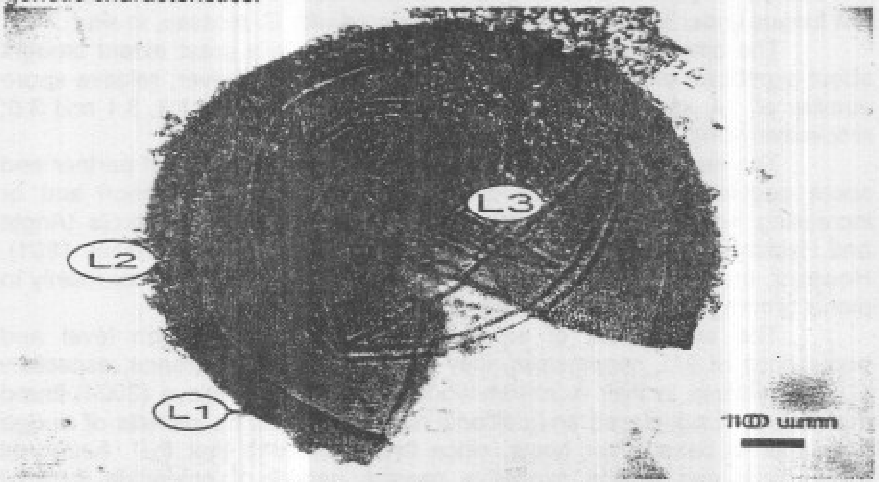

Fig. 5: Clamydospore of *Glomus fasciculatum*. L1, L2and and L3 is the outer, middle and inner spore wall, respectively.

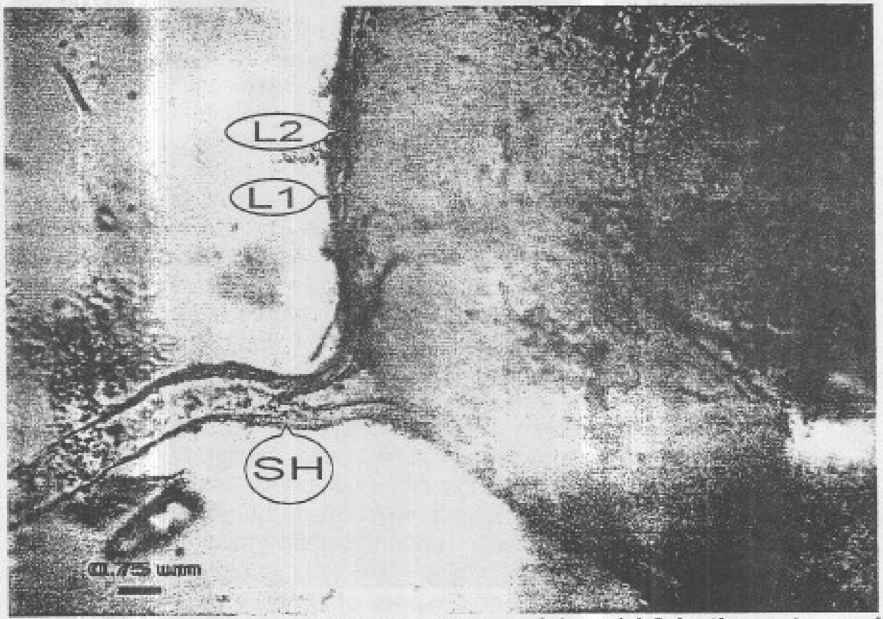

Fig. 6: Clamydospore of *Glomus mossae*. L1 and L2 is the outer and inner spore wall, respectively and SH is the subtending hyphae.

Coral del Val *et al.* (1999) similarly identified one tolerant species from polluted site, with sewage-sludge heavy metal, namely, *G. claroideum* relative to *G. mossae*, *in situ*. Pawlowska *et al.* (2000) pointed out to the dominance of dark colored and thick walled clamydospores of unidentified AM fungus under stressed conditions compared with *G. mossae*, *in situ*.

The amendment by sludge, generally, has to a great extent brought about significant drop of AM populations (Table 6). However, relative spore number of *A. sligna*, *C. glauca* and *E. camaldulensis* was 1.3, 3.1 and 3.0; respectively (Fig. 2).

The negative changes of mycorrhizal activity (infection of partner and spore population) as a result of sludge amendment, fertilization and or increasing heavy metal contents were reported by several workers (Angle and Heckman,1986; Safir *et al.* ,1990 and Lambert and Weidensaul, 1991). However, most authors reported extensive colonization could occur mainly in plants growing in soils of low fertility (Turk *at al.*, 2006).

The amendment of sewage sludge reduced infection level and populations of AM, nonetheless, they displayed superb tolerance, especially *G. fasciculatum.* In their extensive works, Gaur and Adholeya (2004) found that the AM fungi played an additional role under extreme impacts of sludge pollutants in behalf their hosts, since they concluded that the functional mycorrhizas can reduce excessive passive uptake of potentially harmful elements through the roots while maintaining an adequate supply of the other elements like N and P through active hyphal uptake. Upon the evidences of positive role of AM adapted with extreme conditions, it is of important to select the highly tolerant ones and manage it in afforestation programs using effluent water or sewage sludge.

Conclusions

Amendment by sewage sludge has positive effects on fertility of soil, growth parameters (tree height and dobh and commercial volume of wood), and yield of solid wood. Tree species studied revealed its phytoremediation potential, particularly *C. glauca* and *E. camaldulensis*. *Acacia saligna* stand fixed about 0.006, 0.06 and 1.3 kg/feddan/ year of Cd, Zn and Pb in its wood, respectively; whereas *C. glauca* fixed 0.8, 3.1 and 17.9 and *E.camaldulensis* about 0.08, 1.7 and 17.5 kg/ feddan/ year, at the same order. Heavy metal concentrations in bark and heartwood were more than those of sapwood. Specific gravity was relegated owing to the positive impact of sludge on growth rate of tree. *Glomus. fasciculatum* was more abundant in rhizosphere of *C. glauca*, whilst *G. mossae* in the rhizosphere of *A. saligna* and both of them were mutually throve under *E. camaldulensis*' rhizosphere. Clamydospore populations of AM fungi and infection levels (%) roots of their partners, to certain extent, were dropped when sewage sludge amended, yet the fungi displayed considerable tolerance towards sludge pollutants and may to allelopathic effect of the trees (*C. glauca* and *E.camaldulensis*). Dendrochemical aspects merit more advanced research to pinpoint historical deposition of trace element with more aovanced technology. It is recommended to capitalize on sewage sludge in fertilization of timber trees and use the later to remediate polluted soil as well and select and utilize AM fungi adaptable with harsh or polluted sites in afforestation program concomitant with sludge and/ or sewage water management.

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تأثير معاملة بعض الأشجار الخشبية بالحمأة وديناميكية فطريات الميكوريزا الداخلية ودور الأشجار فى معالجة ملوثات الحمأة بالإستخلاص البيولوجى

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****قسم الملوحة و القلوية- معهد بحوث الاراضى و المياه و البيئة- مركز البحوث الزراعية- مصر**

تمت الد راسة فى الغابة الصناعية فى محطة معالجة النواتج الصلبة للصرف الصحى "ن٩"، وقد درس تأثير إضافة الحمأة بمعدل ٤٥ م^{٣}/ فدان على نمو ومحصول أشجار الأكاسيا ساليجنا *Acacia saligna* والكازوارينا البيضاء *Casuarina glauca* والكافور البلدى *Eucalyptus camaldulensis* عمرها ١٠ سنوات. وكذلك درست ديناميكية نمو فطريات الميكوريزا النامية المحلية على جذوع الأشجار وعشائر جراثيمها الكلاميدية فى محيط الجذور، كذلك تمت دراسة دور الأشجار فى معالجة التربة المضاف لها حمأة من العناصر الثقيلة بما يعرف بالاستخلاص البيولوجي bioextraction remediation وقد أوضحت النتائج، باستثناء النمو الطولى في أشجار الأكاسيا ساليجنا تفوقت الأشجار المسمدة بالحمأة فى كل من صفات النمو الطولى وقطر الساق عند مستوى الصدر وحجم الخشب التجارى/ شجرة ومحصول الخشب ومتوسط المحصول فى السنة والمساحة القاعدية، وقد وجد أن المحصول الخشبى فى الأشجار المسمدة وغير المسمدة كان ٣,٧٦، ٤,٧٦ طن للفدان فى الأكاسيا وفى الكازوارينا كان ١٢٤,٨١، ١٣٥ طن للفدان وفى الكافور ٦٠,٦ و١١٧ طن للفدان على التتابع. وقد تم تقدير تركيز النيتروجين والفوسفور والبوتاسيوم والصوديوم في الأوراق والعناصر الثقيلة، الكادميوم والزنك والرصاص كذلك فى الأوراق والتربة والخشب، وقد تمت دراسة تركيز العناصر الثقيلة من القلف للنخاع فى الخشب. وفى معظم الأحوال وجد أن هناك زيادة معنوية في تركيز العناصر الكبرى والثقيلة فى أوراق الأشجار المسمدة بالحمأة مقارنة بغير المسمدة، أما أعلى نسبة فى العناصر الثقيلة فكانت في عنصر الرصاص في الأشجار ووجد أن القلف يتركز به أعلى تركيز يليه خشب القلب ثم الخشب العصارى. وسواء فى الخشب العصارى أو خشب القلب، لوحظ أن هناك انخفاض معنوى فى الثقل النوعى للخشب (باستثناء الخشب العصارى فى الكافور) نتيجة التسميد بالحمأة، وذلك لزيادة معدل النمو. تم فحص التربة لمعرفة أنواع الميكورايزا المستوطنة وعينات من جذور عوائلها لدراسة أثر الحمأة عليها نوعا و كما. وقد أكتشفت أنواع محتملة للظروف القاسية من جنس جلومص وأهمها: *Glomus aggregatum*, و *G. fasciculatum* و *G. mossae* ووجد أن نسبة إصابة جذور الأشجار بفطريات الميكوريزا المحلية وكذلك عشائر الجراثيم فى حيز الجذور قد انخفضت بسبب إضافة الحمأة ولكن تعتبر تلك الأنواع محتملة لحد ما لتأثير الحمأة خصوصا مع أشجار الأكاسيا ساليجنا والكافور. وقد استنتج من الدراسة أن للحماة أثر إيجابى على المحصول الحشبى وإقصار دورة القطع وكذلك إمكانية الحد من انتشار الملوثات بالعناصر الثقيلة بالاستخلاص الحيوى للأشجار الخشبية ويوصى باستخدام الحمأة فى مشاريع التشجير الصناعى فى المناطق الجافة وفى الأراضى غير الصالحة للمحاصيل التقليدية وكذلك الاستفادة من أنواع الميكوريزا المتكافلة على جذورها والمحتملة فى مثل هذه الظروف.