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Growth of Cotton Plants (*Gossypium hirsutum*) as Affected by Water and Sludge from a Sewage Treatment Plant: I. Plant Phenology and Development

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Complying with the European Union regulations (European Community 1991) maritime cities in Greece with active population of more than 15,000 people will have to run Secondary Treatment Sewage Treatment Plants by the end of the year 2000. Consequently, the increased production of sludge and wastewater, will pose a new problem concerning their disposal and force for alternative solutions for their utilization. Wastewater is known to have a high microbial load and has to be chlorinated prior to any disposal. Sludge commonly contains heavy metals and toxic substances in high concentrations and, although rich in inorganic nutrients, has to be disposed of properly as well. Yet both wastewater and sludge, with certain precautions and under restrictions, can be used for irrigation and soil amendment, respectively (European Community 1986) The effect of sewage sludge application to agricultural soils has been investigated, mostly in industrial countries, but only a few publications are available (Wild and Jones 1992; Klessa and Desira-Buttigies 1992; Hyll and Nestroy 1993; O'Riordan et al. 1994; Smith 1994; Hue and Ranjith 1994; Margaris et al. 1995; Christodoulakis and Margaris 1996). Microbial activity in soils mixed with sludge (Hirsch et al. 1993; Obard et al. 1994) and the effect of untreated domestic and industrial effluents on plants have also been investigated (Taghavi and Vora 1994; Navarro-Pedreno et al. 1996; Weir and Allen 1997; Logan et al. 1997; Hooda et al. 1997; Palacios et al. 1999)

Concerning all these but also having in mind: a) the universal interest in producing organic cotton (no application of chemical fertilizers and pesticides), b) cotton's great value for the economy of several countries (including Greece), and c) the fact that the plant is not used as a food for humans, we selected *Gossypium hirsutum* for our experiments. The objective of the first part of this study is to investigate the impact of sewage sludge and wastewater on the phenology, growth and productivity of cotton plants.

MATERIALS AND METHODS

For the present investigation, the Sewage Treatment Plant (STP) of Keratea (a small town, east of Athens, Greece) was selected. Cottonseeds were imbibed and seedlings were moved to black-colored polystyrene pots with upper diameter of 25 cm. These pots were filled with 10 kg of growing substrate as shown in

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$(NH_3)_3PO_4$ (1.96 g/pot), NH_3NO_3 (0.294 g/pot, 6 times between flowering and plant maturation), atomization with chelate Zn (when plants reached height of 15-30 cm) and root irrigation with chelate Fe. Pots were irrigated daily with predetermined water quantity so that sludge or fertilizer did not wash out of the pot.

Table 4. Chemical profile of the sewage sludge.

Property	Measurement	Property	Measurement
Organic matter (%)	53.08	Zn (ppm)	2307.28
pH	5.80	Ni (ppm)	322.23
K (%)	0.87	Cu (ppm)	260.40
Fe (%)	3.34	Hg (ppm)	1.99
Mn (ppm)	769.07	Pb (ppm)	817.70
Cd (ppm)	9.93		

At the end of the experiment, dry matter of the above-ground parts, roots, fibers and seeds was weighed and leaf areas were measured (Leaf Area Meter, Delta–T Devices LTD). Finally, small pieces of leaves and stems were fixed in phosphate buffered 3% glutaraldehyde at 0 °C. Leaf tissue was post fixed with 1% OsO_4, dehydrated in graded ethanol series and embedded in Durcupan ACM (Fluka). Glass-mounted, semi-thin sections were stained with toluidene blue "O" and observed under a Leitz Light Microscope. Small pieces of leaves were freeze dried and coated for SEM observations (Cambridge S 150 Stereoscan).

RESULTS AND DISCUSSION

An essential point of the investigation is to compare the plants grown with the aid of the conventional fertilizer to those grown on sludge amended soil. In that way we can find out how much can a farmer be favored if he uses sludge to promote his crop. All the results are presented in Figures and Tables. Phenological observations (Figure 1) indicated that in the sludge groups (groups 5, 6, 7, 8) flowering and fruition started 2-3 weeks earlier compared to that of the fertilization group (group 3). It is obvious that priming is very important for countries with temperate climate, like Greece, where the cultivation period of cotton is relatively short and autumn rains may destroy the yield.

In Figures 2 - 6, a percentage is given for each group of plants. This indicates promotion or retarding (negative sign) of growth, always compared to No 3 group of plants which is supposed to give the results that a conventional farmer would get. Asterisks in Figures 4-6 indicate significant differences ($P<0.05$) among plant roots, stems or leaf surface of the same group due to the irrigation water. Use of sewage sludge results in significantly higher (H=41.170, p<0.001), more robust plants with faster development (Fig. 1) and significantly greater stem biomass (H=43.026, p<0.001), and root biomass (H=31.038, p<0.001) production. On the other hand, irrigation with wastewater instead of tap water

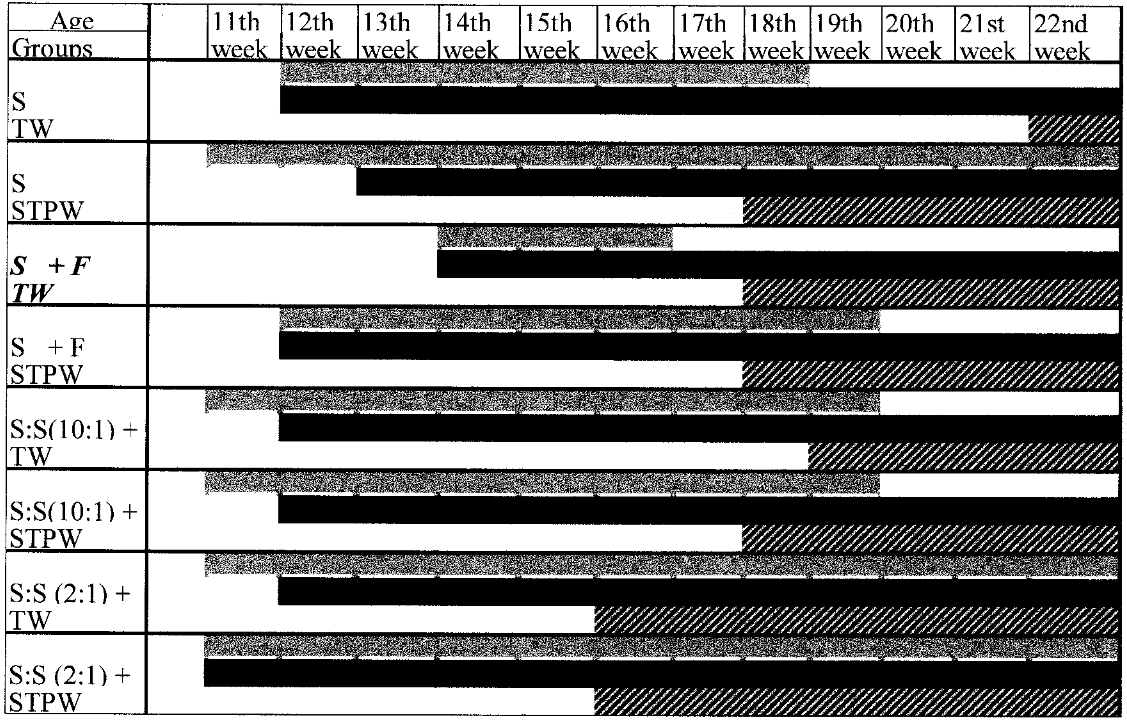

Figure 1. Phenological observations of *Gossypium hirsutum* (var. Zeta 2) during the growing period

Flowers
Green bolls (fruition,)
Open bolls (open capsules)

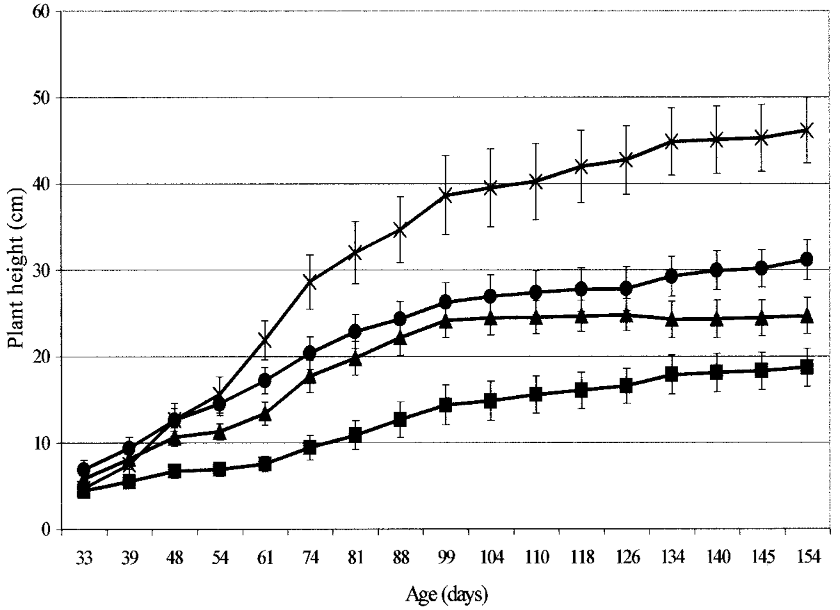

Figure 2. Height of *G.hirsutum* (var. Zeta 2), Tap water [▪S, ▲S + F, •S:S (10:1), ∗S:S (2:1)], n =20

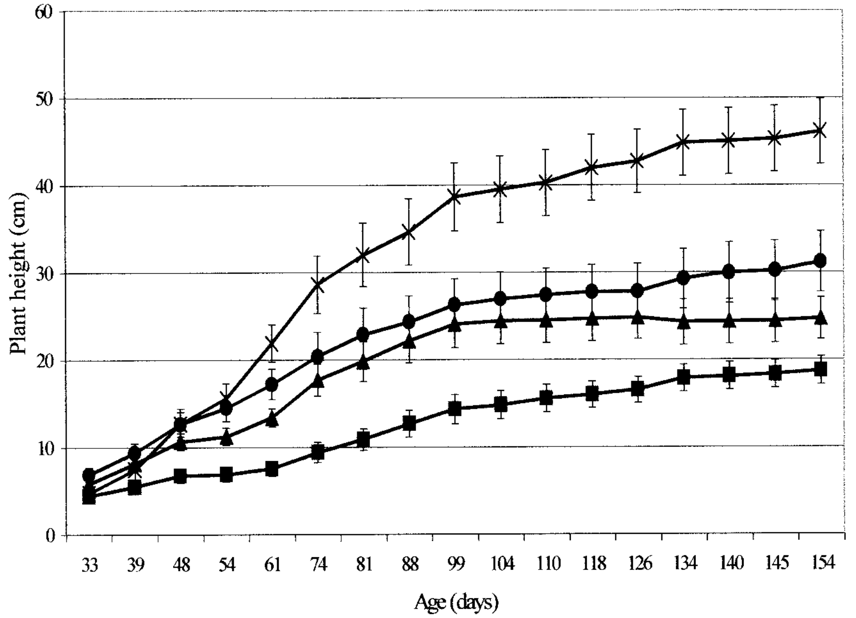

Figure 3. Height of *G.hirsutum* (var. Zeta 2), STP water [▪S, ▲S + F, •S:S (10:1), ∗S:S (2:1)] n = 20

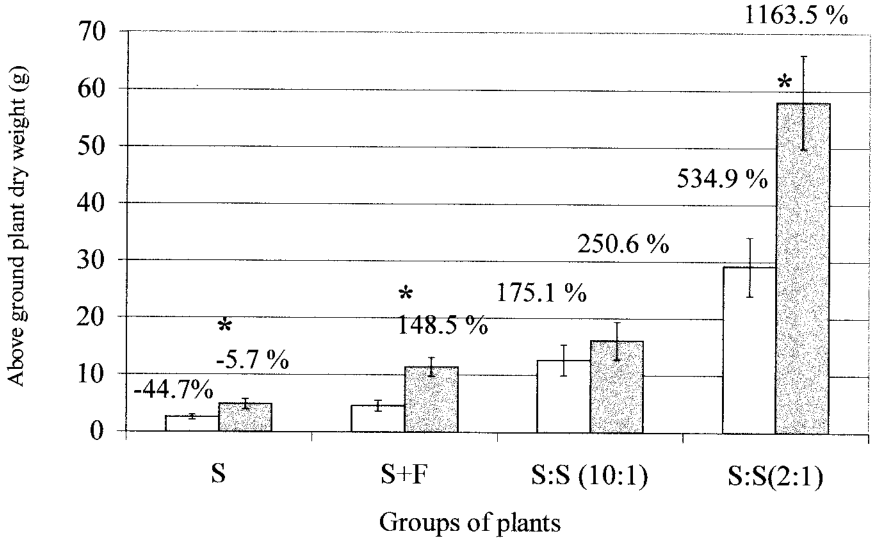

Figure 4. *Gossypium hirsutum* (var. Zeta 2), Above ground plant's dry weight (g), 1998 (white bars = tap water, gray bars = STP water) n = 20.

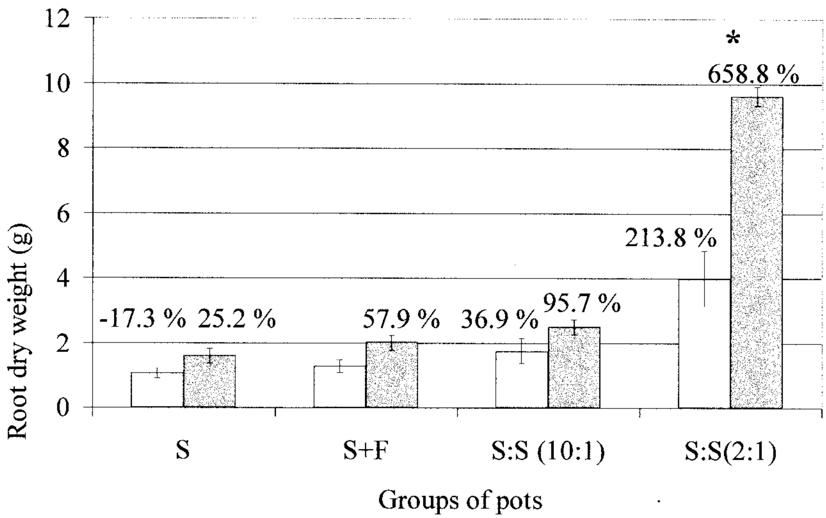

Figure 5. *Gossypium hirsutum* (var. Zeta 2), Root dry weight (g), 1998 (white bars = tap water, gray bars = STP water) n = 20

results in significant differences in root biomass (U=18.000 p=0.004) and leaf surface (U=20.000, p=0.006) in combination with high ratio of sewage sludge. It also produces significantly greater stem biomass in groups S (U=29.000 p=0.043), S+F (U=1.000 p=0.004) and S:S(2:1) (U=21.000 p=0.009).

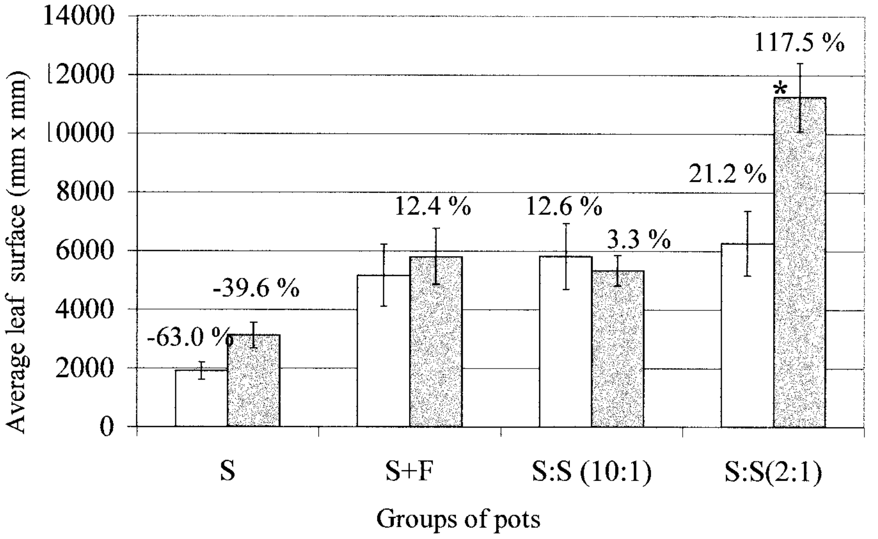

Figure 6. *Gossypium hirsutum* (var. Zeta 2), Average leaf surface (mm x mm), 1998 (white bars = tap water, gray bars = STP water) n = 20

L. M. observations indicated that leaves from the plants of the various groups present minor structural differences. Since all leaf sections were cut from the middle of the leaf blade we can easily observe that the larger the lamina grows, the thicker it becomes. Observations of the leaf surface under the scanning electron microscope showed that sludge and wastewater increased the leaf's glandular trichomes. These trichomes are suspected for producing *gossypol* - a secondary metabolite of cotton plants - that has a role as insect repelant. (McAuslane et al. 1997). This is of great significance because it may lead to a reduction of the insecticides used in the field.

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