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Growth of Cotton Plants (*Gossypium hirsutum*) as Affected by Water and Sludge from a Sewage Treatment Plant: II. Seed and Fiber Yield and Heavy Metal Accumulation

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Most maritime cities in Greece, are not industrialized. Human activities are limited to farming and various services, particularly touristic. This has an impact in concern to the quality of the untreated sewage of these cities. Sewage has very low to non-detectable concentrations of heavy metals and toxic substances (Margaris et al. 1995). It is also known that alkaline soils, as those of Greece, contribute to heavy metal precipitation making them hardly available to plants (Rawajfih and Gharaibeh 1990). Therefore the type of sewage sludge and wastewater produced by non industrial Greek cities can be used for fertilization and irrigation of crops providing them with macro- and micro-nutrients (Christodoulakis and Margaris 1996; Navarro-Pedreno et al. 1996; Palacios et al. 1999; Weir and Allen 1997) without having any toxic side-effects. Considering these facts as well as the European Community Directives (1986;1991) prompting for thorough investigation on this issue, we launched a series of experiments selecting cotton (*Gossypium hirsutum*) as an ideal plant species for this reason. It naturally grows in alkaline soils. Its main product (fibers), used world-wide, is not edible by humans and by its chemical nature -pure cellulose- can by no means incorporate heavy metals or toxic substances. The objective of this part of the study is to determine the effect that sewage sludge and wastewater have on the yield and quality of plant products (fibers and seeds), to trace any heavy metals along various parts of the plants and to investigate their concentrations.

MATERIALS AND METHODS

For the present investigation, the Secondary 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 Table 1. Pots were divided in groups of 20. Every group had different treatment during the experiment (Table 1). The poor, in nutrients, soil of the STP area was used as a basis for the growing substrate. Grinded plain soil or mixture of it with fertilizer or sludge (dehydrated and digested) was used to fill the pots in all eight groups. The experiments were repeated three times (1996, 1997 and 1998) and the results strongly coincided. In this paper, we give the results of

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the year 1998, when more specimens were used and the statistic approach was far more dependable.

Table 1. Experimental treatments. Group 3 is the reference group because it gives the results that an ordinary farmer would get.

	growing substrate	irrigation with	abbreviation
1	plain soil	+ Tap Water	S + TW
2	plain soil	+ STP Water	S + STPW
3	***soil + fertilizer***	***+ Tap Water***	***S+F + TW***
4	soil + fertilizer	+ STP Water	S+F + STPW
5	soil : sludge 10:1 v/v	+ Tap Water	S:S (10:1) + TW
6	soil : sludge 10:1 v/v	+ STP Water	S:S (10:1) + STPW
7	soil : sludge 2:1 v/v	+ Tap Water	S:S (2:1) + TW
8	soil : sludge 2:1 v/v	+ STP Water	S:S (2:1) + STPW

Soil substrate and sewage sludge, tap water and wastewater were analyzed for heavy metals and other elements by means of "EDXRF QuanX Spectrace" Spectrophotometer. Sample preparation was simple: soil, and sewage sludge were dried (60 ^{o}C), grinded and sieved to minimize particle effects whereas samples of fresh water and wastewater were analyzed without any further precipitation).

At the end of the experiment, dry matter of fibers and seeds was weighed. Leaves and stems from all groups were used for microanalysis of heavy metals and other elements following the same analysis method as described previously for soil and sewage sludge. Fibers were tested for quality in the High Volume Instrument (HVI) of the Hellenic Cotton Board following the ASTM D4605-86 method. Seeds were tested for oil and protein content in a Soxhlet and a Kjeltec-System I instrument respectively.

RESULTS AND DISCUSSION

Results are presented in Tables. In each Table group 3 is marked in bold italics for it is considered a reference group. The yield of this group represents what an ordinary farmer would get if he had carefully followed the advise of an expert for the fertilization of his field.

Fiber and seed yield and quality were measured (Tables 2 and 3). It appears that sewage sludge promoted seed production up to 435.4 % [S:S(10:1)+TW] or 2864.3 % [S:S (2:1)+TW] and fiber production up to 366.4 % [S:S(10:1)+TW] or 2469.5 % [S:S(2:1)+TW]! Samaras and Kallianou (2000) also reported significantly increased cotton yields due to application of sewage sludge. Irrigation with waste water, in addition to sludge application, usually promotes production even more. The quality of cotton fibers from the various groups did not seem to change.

Table 2. Fiber and seed production in the various plant groups of *G. hirsutum* (var. Zeta 2). Highest prices in bold.

Groups	Dry weight - a	Dry weight - b	No. of seeds/group	Average seed weight (g)	Increase % seed - c	Increase % of lintcotton - d	% seeds in seedcotton	% lintcotton in seedctotton
S +TW	1.09	1.16	24	0.05	-62.70	-58.40	51.56	48.44
S +STPW	1.46	1.71	23	0.07	-45.02	-44.27	53.94	46.06
S+F +TW	***2.62***	***3.11***	***69***	***0.05***	***0.00***	***0.00***	***54.28***	***45.72***
S+F +STPW	8.47	10.60	158	0.07	240.84	223.28	55.58	44.42
S:S(10:1)+TW	12.22	16.65	192	0.09	435.37	366.41	57.67	42.33
S:S(10:1)+STPW	25.42	32.95	375	0.09	959.49	870.23	56.45	43.55
S:S(2:1) +TW	**67.32**	**92.19**	**986**	0.09	**2864.31**	**2469.47**	57.80	42.20
S:S(2:1) +STPW	61.65	88.58	765	**0.12**	2748.23	2253.05	**58.96**	41.04

a. fibers/group (g) desiccated for 3 days at 60 °C, b. seeds/group (g) desiccated for 3 days at 60 °, c. seed dry weight compared to group S+F+TW, d: Lintcotton's dry weight compared to group S+F+TW, (highest prices in bold).

Table 3. Quality characters of lintcotton and cottonseed.

	Mic	Str	Len	Unf	SFI	Elg	CG	Rd	+b	% cottonseed protein	% cottonseed oil
S +TW	-	-	-	-	-	-	-	-	-	-	-
S +STPW	-	-	-	-	-	-	-	-	-	-	-
S+F +TW	-	-	-	-	-	-	-	-	-	-	-
S+F +STPW	3.7	22.6	28.9	52.8	<3.5	7.0	21-2	81.7	7.2	16.86	20.89
S:S(10:1)+TW	3.0	22.2	30.6	47.6	<3.5	6.7	31-2	80.7	7.4	20.84	15.76
S:S(10:1)+STPW	3.0	22.9	28.8	52.3	<3.5	6.2	21-2	80.2	8.1	20.79	19.19
S:S(2:1) +TW	3.8	22.6	29.9	52.6	<3.5	6.5	21-2	80.4	7.8	23.71	16.47
S:S(2:1) +STPW	3.8	24.5	31.5	49.5	<3.5	6.7	31-1	78.8	8.0	23.92	19.57

Mic: Micronaire, **Str:** Fiber strength (g/tex), **Len:** Fiber length (mm), **Unf:** Uniformity of fiber length, **SFI:** % of short fibers, **Elg:** Fiber elongation, **CG:** Cotton Color Grade (based on Universal Standards for Grades of American Upland Cotton), **Rd:** Fiber Reflectance, **+b:** Fiber Yellowness, **-** : not enough fibers were obtained (for this analysis) from this group of plants.

Table 4. Heavy metal concentrations in *Gossypium hirsutum* (var. Zeta 2) shoot from the various plant groups, ND = Not Detected

Group	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cr (ppm)	Ni (ppm)	Cu (ppm)	Pb (ppm)	Cd (ppm)	As (ppm)	Hg (ppm)	Co (ppm)
1	124	17.5	26.5	4.58	8.7	20.4	1.7	ND	ND	ND	0.4
2	111	24.5	25.2	3.87	6.0	20.6	2.7	ND	ND	1.3	1.8
3	***132***	***23.5***	***21.9***	***2.48***	***5.6***	***14.4***	***ND***	***0.6***	***0.05***	***ND***	***ND***
4	120	21.0	19.8	2.97	9.9	14.5	4.9	ND	ND	0.7	4.2
5	127	32.8	20.3	3.41	1.2	6.6	3.2	1.8	1.0	0.5	ND
6	116	26.0	34.8	2.31	11.8	14.3	2.4	1.9	0.4	ND	1.3
7	90	29.1	18.9	2.02	4.4	14.2	1.4	0.8	0.8	2.3	5.9
8	56	18.5	22.1	2.60	3.3	19.1	ND	0.7	0.6	ND	ND

Table 5. Heavy metal concentrations in *Gossypium hirsutum* (var. Zeta 2) leaves from various plant groups, ND = Not Detected

Group	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cr (ppm)	Ni (ppm)	Cu (ppm)	Pb (ppm)	Cd (ppm)	As (ppm)	Hg (ppm)	Co (ppm)
1	1057.3	86.3	46.4	15.8	11.8	8.1	7.5	1.3	1.4	6.2	ND
2	685.6	97.5	33.6	9.0	9.3	8.3	8.1	2.2	ND	ND	ND
3	***870.7***	***112.4***	***46.4***	***14.4***	***4.7***	***3.0***	***8.8***	***2.3***	***0.9***	***0.2***	***0.2***
4	992.9	94.1	35.9	21.2	12.9	4.8	11.5	1.4	ND	ND	2.0
5	1250.5	132.7	34.9	23.8	17.2	4.6	9.3	0.08	2.9	ND	ND
6	1000.1	91.4	42.2	18.7	24.0	ND	14.1	1.5	ND	6.2	ND
7	841.9	104.3	27.2	13.9	20.3	5.8	ND	ND	4.1	ND	ND
8	459.8	108.3	25.7	7.3	7.3	10.9	11.6	ND	0.25	ND	1.4

Measurements of oil concentration in the seeds from the various groups showed that sewage sludge and wastewater do affect oil and protein content of seeds: they increase seed protein and decrease seed oil concentration (Table 3).

Heavy metal tracing (Tables 4, 5) indicated that four elements (Co, As, Hg and Cd) were detected in very low or non definable concentrations. The other seven (Fe, Mn, Cr, Ni, Cu and Pb) were not significantly higher in plants of the sludge treated groups compared to those of plain soil (groups 1, 2) and soil + fertilizer (group 3) and by no means beyond the limits posed in the EU directives. Furthermore, no toxic effect was observed on the plants of any treatment. Therefore, the use of the plants and their products for animal or human food is within the European Community (1986; 1991) regulations. Cotton fibers, in particular, consisting of pure cellulose, can be used in any case.

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