Bull. Environ. Contam. Toxicol. (1996) 57:300-306

Bulletin of Environmental Contamination and Toxicology

Growth of Corn (*Zea mays*) and Sunflower (*Helianthus annuus*) Plants Is Affected by Water and Sludge from a Sewage Treatment Plant

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Received: 11 June 1995/Accepted: 18 March 1996

The sewage treatment plant in the island of Kos, Greece, designed and built in 1992 under the supervision of I. Kruger AS (Denmark) is considered to be the best functioning unit in Greece and one among the model units in Europe. These installations were the core of a major project for environmental restoration through the use of the sewage treatment products, that is sludge and water. The island of Kos is a resort area, very close to the famous island of Rhodes and the sewage is produced mainly from domestic activities, tourist accommodations and services (stores, restaurants etc.) and a small hospital in the island.

The settling tanks of the sewage treatment plant accumulate a kind of sludge which is removed and dehydrated to reduce its volume to l/IO. This product can be considered chemically stabilized since its total age is more than 24 days. It is a dark brown solid material, rich in nutrients and can be used as fertilizer. Water coming out of the settling tanks, after its chlorination and removal of the residual chlorine is clean with a concentration of inorganic salts not exceeding that of the local tap water.

Therefore, besides the use of water and sludge for a major environmental restoration of the municipal area surrounding the installations (Margaris et al 1994), the by-products of the sewage treatment plant were evaluated for their potential benefits for crop growth against a traditional fertilizer and the local water used by farmers.

Although the use of the by-products from sewage treatment plants falls in an area of intensive research in several industrial countries only few publications are available on the effect of application of sewage sludge containing industrial effluents to agricultural soils (Wild and Jones 1992, Klessa and Desira-Buttigies 1992, Hyll and Nestroy 1993, O'Riordan et al 1994, Smith 1994, Hue and Ranjith 1994). Microbial activity in soils mixed with sludge has been studied (Hirsch et

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al 1993, Obbard et al 1994) and the effects of untreated domestic and industrial effluents (Taghavi and Vora 1994) on plants have been investigated.

MATERIALS AND METHODS

Seeds of Zea *mays* (corn) and *Helianthus annuus* (sunflower) were imbibed and seedlings were moved to black-colored polystyrene pots with upper diameter of 35 cm. These pots were filled with 10 kg of growing substrate prepared as shown below. Pots were divided in groups of 10 pots. Every group had different treatment during the experiment. The sandy, poor in nutrients soil of the installation area was used as a basis for the growing substrate. Grinded plain soil or mixture of it with fertilizer or sludge was used to fill the pots in all eight groups. Corn and sunflower plants undergone the same grouping:

groups of 10 pots:

	group 1: soil only	
	group 2: soil and *Complesal*	**10:1 v/v**
1) tap water	group 3: soil and sludge	**2:1 v/v**
	group 4: soil and sludge	**10:1 v/v**
	group 5: soil only	
2) *water from the STP,*	group 6: soil and *Complesal*	**10:1 v/v**
before chlorination	group 7: soil and sludge	**2:1 v/v**
	group 8: soil and sludge	**10:1 v/v**

The analysis of the soil of the area to a depth of 20 cm gave the following values: *Organic matter* = 0.10%, pH 7.30, *total* N 33.6 ppm, *P* 4 ppm, *K* 95 ppm, *Na* 1400 ppm and *Ca* 4600 ppm. These values are extremely low to support plant growth.

The commercially available fertilizer used on the island by the farmers is *Complesal* (Hoecht) in the grain form (composition N:P:K/12:16:12 plus 0,2 Mg). Following the instructions of the manufacturing company we mixed the fertilizer with the poor soil to produce a rich in nutrients substrate. In the mixture obtained (10:1 v/v) the amount of the fertilizer did not exceed 800 grams per pot. Irrigation of the pots was executed daily with predetermined quantity of water so that fertilizer or sludge could not be washed out of the pot.

During the growing period plant height, as well as length and width of tagged leaves of known age were recorded once a week, along with any other phenological observations. At the end of the experiment, dry weight of the above-ground part, the roots, corns or flower disks and seeds were measured. To insure uniformity of our data, measurements were taken from the 4th, 5th and 6th leaf of the corn plants and the 6th, 7th and 8th leaf for the sunflower plants. Leaves were counted from ground to the apex.

Small pieces from various organs (leaves, stems and roots) were fixed for further anatomical investigations. By the end of the experimental period the leaves were grouped according to the treatment and small pieces from the middle of each leaf blade were incised. These were fixed immediately 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.

RESULTS AND DISCUSSION

The incoming sewage as well as the water at the outlet were monitored for a period longer than one year. The analysis of the contents was executed twice a week by means of a spectrophotometer and the mean values were as in Table 1.

Table 1: Contents of inlet and outlet water at the sewage treatment plant.

in mg/lit	water at the inlet (x_{mean} ± Standard Error of the Mean for 95%)		water at the outlet	
Cl-	592	± 117	6,3	± 0,8 (before chlorination)
NO_3-	12.2	± 1.3	9.1	± 0.9
total sulfur	84.1	± 6.7	17.9	± 0.5
oils	9.8	± 3.3	0.0	± 0
detergents	6.9	± 0.1	0.5	± 0

Sludge physical and chemical properties were also monitored weekly for a period longer than one year. Dry matter was determined by incubating sludge in a desiccator at 80 °C for 48 hours while various elements were traced in different sludge dilutions by means of a spectrophotometer. The results were:

Dry matter 16.60%, *humidity* 83.40%
organic matter 60.14%, *ash* 39.86%
specific weight 0.125 gr/cm^3, *pH* 6.83
total N 2.76%, *P* 1.05%, *K* 0.17%, *Ca* 5.47%,

From the data we present it appears that adding sludge to the soil promotes plant growth significantly more than when commercial fertilizer is added. Plant height increased in corn individuals by 77% in the sludge amended treatment compared to 25% in the case of the commercial fertilizer amendment. Leaf area in sunflower increased more than 150% when sludge was used, compared to 76% with the fertilizer. Watering with the outlet water prior to its chlorination has a slight inhibitory effect, probably because of the high microbial load of the water.

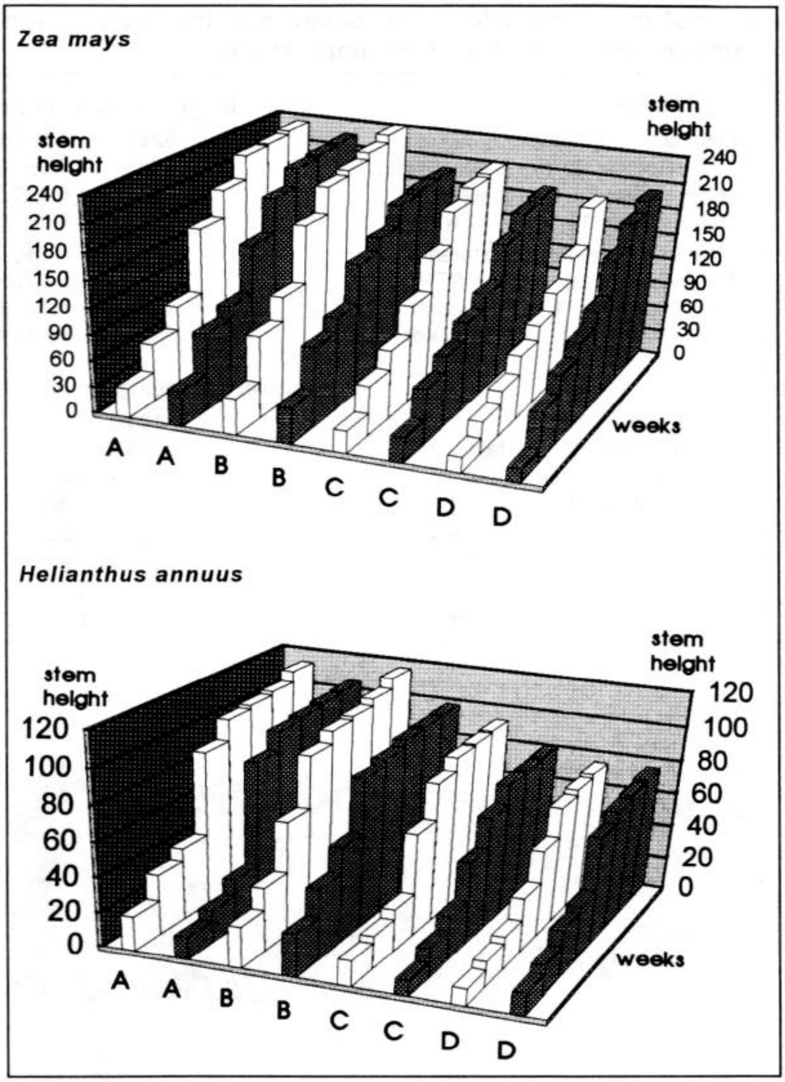

Figure 1. Plant height for corn and sunflower plants under different growing conditions. White bars = tap water, Gray bars = Sewage Treatment Plant water.
A = soil + sludge 10 : 1, **B** = soil + sludge 2 : 1, **C** = soil + Complesal, **D** = soil only.

Table 2. Leaf dimensions for corn leaves and the area of sunflower leaves (xmean± SEM for 95% of the population).

treatments	length and width in cm	
plain soil + tap water	52±4	4.3±0.9
soil + Complesal 10 : 1 + tap water	65±5	4.8±0.6
soil + sludge 2 : 1 + tap water	82±7	6.4±0.9
soil + sludge 10 : 1 + tap water	59±4	6.0±0.7
plain soil + Sewage Treatment water	57±6	4.1±0.7
soil + Complesal 10 : 1 + STP water	63±9	5.0±0.6
soil + sludge 2 : 1 + STP water	71±7	6.1±0.8
soil + sludge 10 : 1 + STP water	68±8	5.8±0.8
	sunflower leaf area given in cm^2	
plain soil + tap water	104.9 ± 23	
soil + Complesal 10 : 1 + tap water	185.4 ± 62	
soil + sludge 2 : 1 + tap water	265.2 ± 51	
soil + sludge 10 : 1 + tap water	228.0 ± 52	
plain soil + Sewage Treatment water	132.1 ± 44	
soil + Complesal 10:1 + STP water	136.4 ± 60	
soil + sludge 2 : 1 + STP water	255.7 ± 71	
soil + sludge 10 : 1 + STP water	172.0 ± 46	

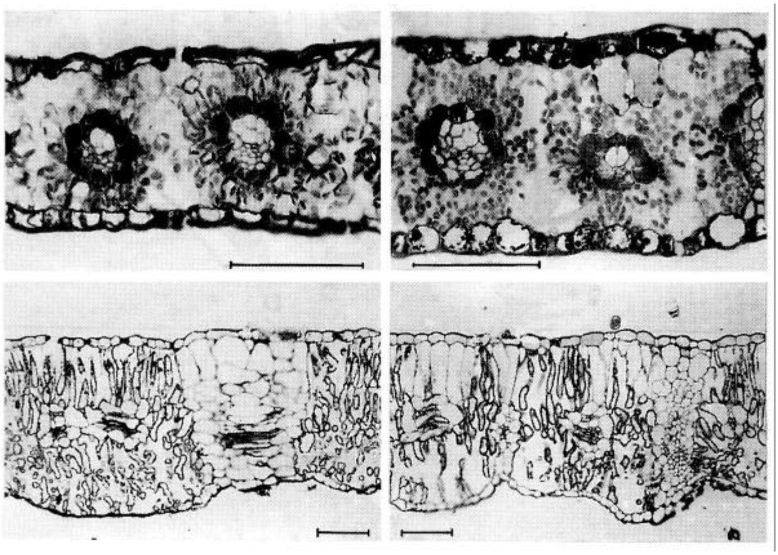

Figure 2. A) and B) Cross sections of leaves from corn plants. Groups 1 and 5 respectively.
C) and D) Cross sections of leaves from sunflower plants. Groups 1 and 5 respectively. All bars on micrographs: 100 µm.

To investigate this effect a parallel experiment was conducted. A group of *Zea mays* plants was irrigated from the outlet of the STP but after chlorination (all microorganisms died) and removal of the residual chlorine, and tested against a group of plants irrigated with tap water.

Plants of both groups had statistically insignificant variations in their development. These results strongly favor our hypothesis that water micro-organisms, mainly coliforms, compete with plants for the same nutrients, thus inhibiting plant growth. An interesting contradiction is observed in *soil-only* pots (groups 1 and 5) irrigated with STP water. It has to do with the increased growth rate of these plants although irrigated with STP water which was proved to slightly inhibit growth. This probably has to do with the higher, compared to tap water, nutrient content of the outlet water which promotes growth and compensates for microbial competition only when used to irrigate plants growing in the nutrient deficient, sandy substrate of the area.

Light microscope observations indicate that the thin, compact leaves of *Zea mays* present minor structural differences between the various groups. Since all leaf sections were cut from the middle of the leaf blades we can easily observe that the longer the leaf blade grows the thicker it becomes. The long leaves in plants of group 3 are 20% thicker than those of group 1. Thicker corn leaves also possess thicker epidermal cells and appear more productive since chloroplasts are numerous in the mesophyll cells (Figure 1 A and 1B). Stomatal index and frequency, on both leaf surfaces, present no statistically significant variations.

The loose leaves of *Helianthus annuus* seem to disregard the various nutritional conditions. The structure of these leaves has no differences in all details examined. (Figure 1, C and D).

Our observations indicate, that although leaf structure, in the plants of all groups, is slightly affected by the varying conditions of the experiment, the differences observed for the leaf and plant magnitude in the different groups are impressive and contribute to higher primary productivity. The availability of nutrients seems to be hardly able to interfere with leaf structure and provoke major alternations to characters readily affected by other environmental factors as light, water, temperature, wind etc. A reason for this probably is the low genetic potential of the plants. Environmental pressure or alternation of the environmental factors usually causes changes in major morphological characteristics only in plants having the ability to pick up and develop various features from a rich genetic pool.

On the basis that sewage of the island is free of industrial or other toxic effluents and taking into account that no structural changes, indicating stress or other environmental pressure, occur on the plants, we may assume that using sludge instead of -commercial fertilizers for agricultural purposes seems environmentally safe and economically favorable. Sludge enhances productivity and natives, overcoming the hesitations of the first months, now compete for its use.

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