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Effects of municipal sludge and treated waste water on biomass yield and fiber properties of kenaf (*Hibiscus cannabinus* L.)

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ABSTRACT

Kenaf (*Hibiscus cannabinus* L.) was experimentally cultivated with the use of digested, dried sewage sludge (130 t/ha) and water from a municipal Sewage Treatment Plant (STP) in order to assess their potential to replace conventional fertilization (100 kg N/ha, 75 kg P_2O_5/ha and 75 kg K_2O/ha) and irrigation. Tap water and treated wastewater were used for irrigation in quantities corresponding to 6500 m^3/ha. Four different treatment combinations were applied as follows: (a) wastewater irrigation and conventional fertilization, (b) wastewater irrigation and sewage sludge fertilization, (c) tap water irrigation and sewage sludge fertilization, and (d) tap water irrigation and conventional fertilization. The dry plant biomass collected in the final harvest (140 days after plant emergence) from the four treatment plots was 12.3 t/ha, 12.6 t/ha, 12.4 t/ha and 12.8 t/ha respectively. These differences were not statistically significant (ANOVA, $P = 0.05$) and, therefore, it was concluded that the use of municipal wastes had similar effects on dry biomass production with that of conventional fertilization. An earlier harvest (125 days after plant emergence) gave 11.3% lower dry biomass on average in relation to the second harvest, and this difference was statistically significant (ANOVA, $P = 0.05$). Premature harvest may lead to significant biomass losses, so the plant must be collected during its technological maturity stage. There was not any statistically significant difference among the four treatments and between the two harvests in fiber dimensions and derived values (suitability indices for paper manufacture). On the other hand, cellulose and lignin content in the second harvest were significantly higher compared to the first one, whereas no significant differences were detected among the four treatments.

1. Introduction

Kenaf (*Hibiscus cannabinus* L.) is an annual, dicotyledonous, herbaceous plant cultivated for its fibers; it has traditionally been grown throughout the west part of Asia for the manufacture of ropes, nets, carpets and sacks, and was selected by the USDA among 500 other species as one of the most promising source for pulp and paper production (Kugler, 1988). Kenaf pulps are suitable for the manufacture of newsprint and other paper products and are comparable in quality to hardwood and softwood pulps (Ayerza & Coates, 1996).

In view of the shortage of conventional raw materials and the increasing demand for paper products in the European Union, kenaf cultivation has attracted renewed interest, especially in Mediterranean countries like Spain, Italy and Greece. There have been some experimental kenaf cultivations in Greece, which have shown that the plant can successfully adapt to local pedoclimatic conditions and give satisfactory (dry matter) yields ranging from 7 to 23 t/ha (Kosmidou-Dimitropoulou et al., 1991; Kipriotis et al., 1998; Alexopoulou et al., 2000).

In 2012, Greece imported paper pulp and products at a cost of about € 850 million (Globaledge Statistics, 2012). It is, therefore, evident that new, domestic sources of pulp and paper raw materials would not only reduce imports but also would provide an economic incentive to the agricultural and the industrial sector of the country especially in an era of a serious economic crisis.

To our knowledge and despite the fact that a lot of research has been done on the farming practices of kenaf cultivation, little experimentation to grow kenaf by using byproducts (sludge and wastewater) from Sewage Treatment Plants (STP) has taken place (Carlson et al., 1982; Webber, 1992). There are some studies in Greece on the effect of sewage sludge and wastewater use on the

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growth of commercial plants,especially corn (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) (Christodoulakis and Margaris, 1996) as well as cotton (*Gossypium hirsutum* L.), tomato (*Lycopersicon lycopersicum*) (Tsakou et al., 2001; Samaras and Kallianou, 2000; Traka-Mavrona et al., 2000) and olive (*Olea europea* L.) (Menti et al., 2005).

A decade ago, it was estimated (Kouloumbis et al., 2000) that by the year 2010 there would be over 300 wastewater treatment plants in Greece, producing about 2 Mm^3 wastewater/day and this is what is actually happening currently (Stamatis et al., 2010; Panikkar, 2010). The use of treated municipal wastewaters in agriculture is the most environmentally sound management practice as it improves soil properties, provides plants with nutrients and saves valuable water resources in countries with dry climates (Asano & Levine, 1995). This is crucially important for Greece, where there is a decreasing rainfall trend and wasteful usage of water for irrigation purposes (Galanis et al., 2000). On the other hand, sewage sludge yield is expected to rise to over 300,000 tds/year by 2010, and restrictions set by the EU for landfill will render agricultural application the principal disposal method of sewage sludge in Greece (Kouloumbis et al., 2000). The relatively high content of sewage sludge in nutrients (N, P, K), trace elements and organic matter increases soil fertility and reduces fertilizer use and subsequently cultivation costs. Thus, the use of STP byproducts for agricultural purposes is important both in environmental and economic terms. This is especially true for non-food crops such as kenaf, where there is no risk of sludge heavy metal accumulation in the plant tissues (Webber, 1992).

The objectives of this study were to determine the suitability of using digested sewage sludge and treated wastewater from a STP to grow kenaf and to compare yield components with kenaf grown by using conventional fertilizer and tap water. In addition, the effect of applying sludge and treated wastewater along with maturity stages on basic fiber properties and α-cellulose and lignin content was examined.

2. Material studied, area descriptions, methods and techniques

2.1. Experimental site and layout

The experiment was conducted at Keratea, a small town, about 50 km southeast of Athens (latitude 37° 48′ 10″ N, longtitude 24° 00′ 23″ E and 130 m altitude), within the premises of the local STP. The Plant offers primary, secondary and tertiary wastewater treatment (removal of nitrates, phosphates and chlorine disinfection), and serves a total population of 15,000 residents producing about 0.6 tds sewage sludge/day. Due to the fact that soils in the STP area are poor (Tsakou et al., 2001), fresh sandy clay loam soil was transported from elsewhere, and, separate, experimental plots were laid by filling ditches of 4.2 m^2 (3.5 m × 1.2 m) and 0.8 m depth each.

2.2. Soil, sludge and water chemical analyses

Soil was analyzed for texture (Bouyoukos hydrometer), pH (1:1 v/v distilled water), electrical conductivity, N content (Kjeldahl method), available P (Olsen method), K and Na (flame photometry). Heavy metal concentration in sewage sludge was obtained by X-ray fluorescence spectrometry using an EDXRF QuanX Spectrace spectrometer (Tsakou et al., 2001), and in STP water by AAS using a BUCK Scientific 210VGP AA spectrophotometer and suitable standards. Fifteen samples (5 samples for 3 different days) each week were analyzed.

2.3. Kenaf cultivation

Seeds of the Everglades 71 cultivar were sown in late May in rows 0.5 m apart with a plant-to-plant gap of 10 cm (Ali et al., 1994; Manzanares et al., 1997) to reach a final density of about 150,000 plants/ha (Paschalidis et al., 1996; Mc Millin et al., 1998). Seedling emergence was observed within 7 days from the date of planting. The experimental design was a randomized block with four plots for each of the four replicated treatment combinations (Mc Millin et al., 1998): one with treated STP water and conventional fertilization (STPW CF), one with treated STP water and sewage sludge (STPW SS), one with tap water and sewage sludge (TW SS) and, finally, one with tap water and conventional fertilization (TW CF).

Conventional fertilization (100 kg N/ha, 75 kg P_2O_5/ha and 75 kg K_2O/ha) was applied on soil surface in two equal doses (Manzanares et al., 1997): the first, 30 days after seed germination, and the second, 40 days after the first application to ensure nutrient availability before the flowering stage (Paschalidis, 1997). For the rest of the treatment combinations, digested, stabilized dry sewage sludge was applied on soil surface at a rate corresponding to 130 t/ha in order to provide the same amount of essential nutrients (P, K) with the conventional fertilizer and in two equal doses as above. The sludge was previously screened using a handmade 15 mm mesh screen to facilitate nutrient dilution and absorption by the plants.

The plants were watered using a drip irrigation method during the early morning hours to avoid severe evaporation water losses (Alexopoulou et al., 2000). A network of pipes fitted with an automated irrigation system was adjusted to supply to each plot a total amount of water corresponding to 6500 m^3/ha (650 mm), which covers the typical plant needs in the dry Mediterranean climate (Paschalidis et al., 1996).

Pest management included precautionary application of Thiodan 35 EC (Endosulfan—0.75 ml a.i./l of water) for locusts and ants in early July, and application of Confidor 200 SL (Imidacloprid—60 ml a.i./ha) to control aphide infection in early September (Giannopolitis, 1997). Weed control was regularly done by hand hoeing.

2.4. Dry biomass measurements

Plant dry biomass was measured for two harvests. The first one (Harvest 1) in early October, 125 days after emergence (DAE), and the second one (Harvest 2) 140 DAE (late October) to estimate quantitative crop performance as influenced by harvest date (Mambelli and Grandi, 1995). During each harvest, plants from 1 m^2 of each plot were collected. All plants were dried in a SELECTA (model 2000207) oven at 70 °C for 48 h, and weighted on a top-load Sartorius balance to obtain dry weights (Mc Millin et al., 1998).

2.5. Fiber dimensions

Five randomly selected stalks were obtained from each plot and harvest. In order to get more representative results, three samples from each stalk were taken at 10% (base), 50% (middle) and 90% (top) of its height, an approach similar to that followed by Paraskevopoulou (1987). For fiber length determination, small slivers were obtained and macerated with 10 ml of 67% HNO_3 and boiled in a water bath (100° ± 2 °C) for 10 min (Ogbonnaya et al., 1997). The slivers were then washed, placed in small flasks with 50 ml distilled water and the fiber bundles were separated into individual fibers using a small mixer with a plastic end to avoid fiber breaking. The macerated fiber suspension was finally mounted on a slide (standard, 7.5 × 2.5 cm) using a medicine dropper (Han et al., 1999). For fiber diameter, lumen diameter and cell wall thickness determination, cross sections were obtained from the same height as above and were stained with 1:1 anilin sulphate–glycerine

Table 1
Physicochemical properties of soil and STP sludge and water.

Parameter	Soil	STP sludge	STP water	Concentration limits of heavy metals in soil (Directive 86/278 EEC)
pH	7.95	5.36	–	
Conductivity (mmhos/cm)	3.60	–	–	
Nitrogen (N) % — (ppm)	0.08	2.66	–	
Phosphorus (P) — (ppm)	34.70	244.60	–	
Potassium (K) — (ppm)	240	480	–	
Sodium (Na) — (ppm)	600	240	–	
Zinc (Zn) — (ppm)	–	2307.20	0.5	2500–4000
Lead (Pb) — (ppm)	–	817.70	ND[a]	750–1200
Cadmium (Cd) — (ppm)	–	9.90	ND[a]	20–40
Copper (Cu) — (ppm)	–	260.30	ND[a]	1000–1750
Nickel (Ni) — (ppm)	–	322.20	ND[a]	300–400

[a] Non detectable.

Table 2
Fiber dimensions and derived values (indices for papermaking suitability) of kenaf bark for all treatments and harvests. ±Refers to standard deviation.

			Treatment combination (irrigation & fertilization)			
			STPW CF	STPW SS	TW SS	TW CF
Fiber dimensions(BARK)	Length (mm)	H1	2.16 ± 0.21	2.20 ± 0.17	2.11 ± 0.18	2.09 ± 0.20
		H2	2.18 ± 0.19	2.21 ± 0.20	2.14 ± 0.16	2.13 ± 0.18
	Diameter (μm)	H1	21.30 ± 3.90	21.20 ± 3.80	20.80 ± 3.81	20.70 ± 3.89
		H2	21.40 ± 4.10	21.60 ± 4.00	21.00 ± 3.95	20.90 ± 3.79
	Lumen (μm)	H1	11.90 ± 3.21	11.60 ± 3.16	11.50 ± 2.90	11.80 ± 3.12
		H2	11.90 ± 3.12	11.50 ± 3.24	11.60 ± 3.10	11.70 ± 3.18
	Cell wall (μm)	H1	3.95 ± 0.8	4.02 ± 0.5	4.00 ± 0.9	3.88 ± 0.8
		H2	4.00 ± 0.7	4.05 ± 0.8	4.02 ± 0.6	4.10 ± 0.7
Indices	Slenderness ratio	H1	101.4	103.8	101.5	101.0
		H2	101.9	102.3	102.0	101.9
	Flexibility ratio	H1	56	55	56	57
		H2	56	53	55	56
	Runkel ratio	H1	0.66	0.69	0.70	0.64
		H2	0.67	0.70	0.69	0.72

STPW CF = Sewage treatment plant water & conventional fertilization.
STPW SS = Sewage treatment plant water & sewage sludge.
TW SS = Tap water & sewage sludge.
TW CF = Tap water & conventional fertilization.
H1 = Harvest 1 (125 DAE).
H2 = Harvest 2 (140 DAE).

Table 3
Fiber dimensions and derived values (indices for papermaking suitability) of kenaf core for all treatments and harvests. ±Refers to standard deviation.

			Treatment combination (irrigation & fertilization)			
			STPW CF	STPW SS	TW SS	TW CF
Fiber dimensions (CORE)	Length (mm)	H1	0.70 ± 0.08	0.72 ± 0.07	0.69 ± 0.08	0.71 ± 0.06
		H2	0.71 ± 0.07	0.73 ± 0.06	0.70 ± 0.07	0.73 ± 0.08
	Diameter (μm)	H1	22.30 ± 3.90	21.90 ± 4.00	21.60 ± 3.96	21.40 ± 3.88
		H2	22.50 ± 3.95	22.10 ± 3.86	21.80 ± 4.01	21.70 ± 3.76
	Lumen (μm)	H1	12.75 ± 3.20	13.00 ± 2.95	12.90 ± 3.10	13.10 ± 3.00
		H2	12.70 ± 3.30	12.90 ± 3.00	12.94 ± 3.15	12.98 ± 2.90
	Cell wall (μm)	H1	4.22 ± 0.7	4.12 ± 0.8	4.00 ± 0.7	4.05 ± 0.6
		H2	4.25 ± 0.6	4.15 ± 0.6	4.05 ± 0.8	4.15 ± 0.7
Indices	Slenderness ratio	H1	31.40	32.90	31.90	33.20
		H2	31.30	33.00	32.10	33.20
	Flexibility ratio	H1	57	59	60	61
		H2	57	58	59	60
	Runkel ratio	H1	0.66	0.63	0.62	0.62
		H2	0.67	0.64	0.63	0.67

STPW CF = Sewage treatment plant water & conventional fertilization.
STPW SS = Sewage treatment plant water & sewage sludge.
TW SS = Tap water & sewage sludge.
TW CF = Tap water & conventional fertilization.
H1 = Harvest 1 (125 DAE).
H2 = Harvest 2 (140 DAE).

mixture to enhance cell wall visibility (cell walls retain a characteristic yellowish color). All fiber samples were viewed under a calibrated ZEISS microscope; a total of 25 random fibers were measured from each sample at each stalk height, for a total of 75 fiber measurements from each stalk, and 375 measurements for each of the four fiber dimensions. Measurements were made for bark/core kenaf fibers for every plot and harvest.

Table 4
Kenaf dry biomass, α-cellulose and Klasson lignin for all treatments and harvests. ±Refers to standard deviation.

		Treatment combination (irrigation & fertilization)			
		STPW CF	STPW SS	TW SS	TW CF
Biomass t/ha	H1	10.89 ± 0.9^a	11.09 ± 1.0^a	11.19 ± 0.8^a	11.29 ± 0.9^a
	H2	12.31 ± 1.0^b	12.60 ± 1.1^b	12.40 ± 0.9^b	12.81 ± 0.8^b
α-Cellulose%	H1	36.80 ± 2.7^c	38.18 ± 2.9^c	37.93 ± 3.0^c	37.85 ± 2.6^c
	H2	40.60 ± 3.4^d	41.12 ± 3.0^d	41.44 ± 3.1^d	41.54 ± 3.3d
Klasson lignin%	H1	13.00 ± 1.1^e	13.31 ± 1.2^e	13.47 ± 1.3^e	13.77 ± 1.0^e
	H2	14.81 ± 1.5^f	14.53 ± 1.4^f	15.49 ± 1.5^f	14.79 ± 1.3^f

STPW CF: Sewage treatment plant water & conventional fertilization.
STPW SS: Sewage treatment plant water & sewage sludge.
TW SS: Tap water & sewage sludge.
TW CF: Tap water & conventional fertilization.
H1 = Harvest 1 (125 DAE).
H2 = Harvest 2 (140 DAE).
Different letters (a,b), (c,d) and (e,f) indicate statistically significant differences between harvests (ANOVA, $P = 0.05$).

2.6. Derived values (indices)

Three derived values were also calculated using fiber dimensions: Slenderness ratio as fiber length/fiber diameter, flexibility coefficient as (fiber lumen diameter/fiber diameter) × 100, and Runkel ratio as (2 × fiber cell wall thickness)/lumen diameter (Saikia et al., 1997; Ogbonnaya et al., 1997). These values are a useful indication of the suitability of plant raw materials for paper manufacture as fiber dimensions are closely correlated with the mechanical properties of the pulp (Ververis et al., 2004).

2.7. α-Cellulose and lignin determination

Kenaf stalks were analysed for α-cellulose and acid insoluble (Klason) lignin; α-cellulose was determined using a colorimetric method with the anthrone reagent. 0.3 g (dry weight) ground (0.5 mm) samples were treated and boiled (at 100 °C) with a mixture of nitric/acetic acid (1:8 v/v) for 1 h; the solution was centrifuged to remove lignin, hemicelluloses and xylosans, and diluted with 67% H_2SO_4 (v/v). α-Cellulose was then determined at 620 nm using cold anthrone reagent (Updegraff, 1969). The method is suitable for analysing a large number of samples and has been used to determine α-cellulose in other plant materials (Aguiar, 2001). For each stalk, three samples (small cylindrical pieces), one from the base, one from the middle and one from the top, were analysed for each stalk for a total of 5 stalks (15 samples) for every plot and harvest. Klason lignin was determined using the APPITA P11s-78 method. The same number of samples as in α-cellulose determination was used.

2.8. Statistical analysis

Data collected was subjected to analysis of variance (ANOVA, $P = 0.05$), using appropriate (SAS/STAT) statistical software. ANOVA is the most appropriate method when many different means are to be compared (Snedecor and Cochran, 1980).

3. Results and discussion

Results from soil, sludge and physicochemical water analysis are presented in Table 1. Although relatively poor in nitrogen, the soil used for kenaf cultivation (sandy clay loam) had satisfactory phosphorus and potassium levels to support the plant's growth (Manzanares et al., 1997). Heavy metal concentration in sewage sludge was within the acceptable limits set by the 86/278 EEC Directive, and practically non-detectable in treated wastewaters (except for Zn at low concentration).

3.1. Kenaf biomass yield

Table 4 presents the effects of irrigation/fertilization combinations on kenaf biomass. The final yield was between 12.3 and 12.8 t/ha and is comparable to yields reported by Ayerza and Coates (1996), Kipriotis et al. (1999) and Shakhes et al. (2012). Under more favorable pedoclimatic conditions or using more productive kenal cultivar, higher yields have been reported in Greece by Alexopoulou et al. (2000) and Kosmidou et al. (1991). The highest yield was recorded for the plot with conventional fertilization and tap water (TWCF), and the lowest for the plot with conventional fertilization and STP water (STPW CF). The differences, however, were not statistically significant (ANOVA, $P = 0.05$). This practically means that conventional fertilization and irrigation can be replaced by STP sludge and water without losses in biomass yield, but with lower costs and significant savings on water resources. Carlson et al. (1982) reported a 25% average kenaf biomass increase, when they applied 94 t/ha sewage sludge in comparison to conventional fertilization; in a similar study, Webber (1992) found a slight (not statistically significant) increase in biomass yield when 24 t/ha of sewage sludge was applied in comparison to conventional fertilization. Samaras and Kallianou (2000) have also reported an 11% increase in cotton biomass when they used sewage sludge (77 t/ha) in comparison to conventional fertilization. In the present study, sewage sludge contained comparable P and K quantities with those of the fertilizer, but about 20 times higher N content (Table 1). This excessive N in the sludge did not result in higher biomass yield, either due to partial absorption or probably because kenaf does not usually respond to excessive N application (Muchow, 1979; Webber, 1996; Manzanares et al., 1997).

Long-term application of sewage sludge in agriculture needs to be controlled (Shulz and Romheld, 1997); it is well known that excessive heavy metal accumulation in soils may damage both the plants and the soil microbial community (Pahlsson, 1989; Kelly et al., 1999). Furthermore, continuous sludge application may alter the soil pH and adversely affect productivity or cause nitrate pollution of the groundwater reservoirs (Goda et al., 1986; Min-Jian, 1997; Wang, 1997; Tsadila et al., 2009). However, in the case of our study, municipal wastes contained heavy metals in acceptable concentrations (Table 1). Table 4 also presents the difference in kenaf biomass between the two successive harvests. There is a statistically significant biomass increase of 11.3% in the second harvest (140 DAE) compared to that of the first (125 DAE). This shows that premature harvest may lead to significant biomass losses and thus it is best to harvest the plant at full maturity. Shakhes et al. (2012) report a 20% increase in biomass comparing the 105 DAE and 135 DAE harvests. Similar results have been also published by Webber (1992), Mambelli and Grandi (1995) and Webber and Bledsoe (2002).

3.2. Fiber dimensions and derived values (indices)

Bast and core fiber dimensions and the derived values (indices) are presented in Tables 2 and 3 for all the treatments and harvests. There were not any statistically significant differences in fiber dimensions and indices neither among the plants of the different treatments nor between the two harvests. Our findings are in agreement with those of Francois et al. (1992), who reported that changes in cultivation conditions do not affect fiber dimensions, and Ogbonnaya et al. (1998), who found that only severe water stress can affect fiber dimensions and their derived indices. The two harvests were 15 days apart, so the plant had already reached a stage of maturity, when fiber development probably slowed down (Cutter, 1978). In a similar study, Shakhes et al. (2012) reported a non-statistically significant increase in fiber length between the 105 DAE and 135 DAE harvests. From the derived values point of view, it is evident that the plant is equally suitable for paper production even from the first harvest (125 DAE). However, the significant biomass loss of the premature harvest should be taken into account when pulp production is planned.

3.3. α-Cellulose and lignin content

Table 4 also presents α-cellulose and lignin content for all treatments and harvests. It is clear that the different fertilization and irrigation regimes did not affect lignocellulosic content of kenaf plants. However, there was a statistically significant difference in α-cellulose and lignin content between the two harvests. Plants of the second harvest present a higher lignocellulosic concentration as a result of metabolic accumulation during the plant's maturity process. Mambelli and Grandi (1995) have also reported similar results, but Morrison et al. (1999) found that lignin content does not significantly change from the 90th DAE onwards. So, the possible advantage of a lower lignin content in an early harvest is counteracted by the significant biomass loss and the lower α-cellulose content, which may result to a mechanically weaker pulp. Thus, the decision of when to harvest will be dictated by the installed equipment, operating costs and requirements of the individual papermaking facility, but we recommend that the plant should not be collected before its full maturity.

4. Conclusion

Experimental cultivation of kenaf using sewage and water from a STP showed that conventional fertilization can be replaced by STP by-products without biomass yield losses. Thus, dried, digested sludge and treated wastewater can contribute to minimize the use of chemical fertilizers and valuable irrigation water resources. In addition, the application of treated wastes in non-food crops is a promising way of waste by-product management, especially for countries like Greece. The application of sewage sludge and the use of STP wastewater resulted in comparable kenaf fiber dimensions, their derived values (indices) of suitability for papermaking, and lignocellulosic content. Based on these indices, pulp properties of kenaf grown with STP by-products are expected to be similar with those of kenaf cultivated with conventional fertilization and irrigation regimes. Premature harvest of kenaf leads to a significant biomass loss and a lower α-cellulose content. It is, therefore, preferable to collect the plant when its full maturity stage is reached.

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