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Growth Parameters and Heavy Metal Accumulation in Poplar Tree Cultures (*Populus euramericana*) Utilizing Water and Sludge from a Sewage Treatment Plant

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A dramatic increase of sewage sludge and wastewater followed the construction of numerous secondary treatment sewage treatment plants (STP) after the compliance of many European, maritime cities with the European Union regulations (European Community 1991). Various proposals concerning sludge and water disposal are matter of an unceasing debate.

The effect of sewage sludge application to agricultural soil has been investigated in industrial countries (Towers and Horne 1997). Treated domestic and industrial effluents were also tested on plants (Weir and Allen 1997; Palacios *et al.* 1999; Samaras and Kallianou 2000).

In many Greek cities, sewage is characterized by the lack of heavy metals and toxic substances due to the restriction of the residents activities to agriculture or tourist services. The utilization of the STP products there, in various ways - keeping a reserve for the high microbial load of the otherwise rich in inorganic nutrients sludge - seems unthreatening to the environment. This challenging case was enough to trigger an extensive investigation carried out two Greek cities. The effects of soil amendment with sewage sludge as well as those of plant irrigation with wastewater were studied on plants of commercial interest (Margaris *et al.* 1995, Christodoulakis and Margaris 1996, Tsakou *et al.* 2001a, Tsakou *et al.* 2001b, Tsakou *et al.* 2002). Corn, cotton-plant and flax cultures were closely monitored to give impressive results on the promotion of plant growth, biomass production, seed or fiber yield and priming of flowering and fruition, compared to the same parameters measured in conventionally fertilized and traditionally cultured plants (Christodoulakis and Margaris 1996, Tsakou *et al.* 2001a, Tsakou *et al.* 2001b, Tsakou *et al.* 2002). Poplar trees are fast growing plants of global interest being used by paper producing industries for many purposes. Moreover they are non-edible by humans, a fact greatly considered in this investigation, mainly to line-up with the Council Directive 86/278/ECC (European Community 1986).

The purpose of the investigation presented in this paper is to add a piece of knowledge to the answer of the crucial query about heavy metal bio-accumulation in long-term cultures of trees used as raw materials for the industrial output of widely used products. *Populus euramericana* is a species suitable for this research.

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MATERIALS AND METHODS

Our investigations – including the current one – were worked out at the Sewage Treatment Plant (STP) of Keratea (a small town, east of Athens, Greece). Two-year-aged poplar trees were divided in groups of 12 and planted in the area, keeping the distance between them at 2 meters. Each group had a different treatment during the experiment (Table 1). Physicochemical properties of the local soil used as a basis for the growing substrate, were investigated (Table 2).

Table 1. Groups of trees used in the experiment.

	growing substrate	irrigation with	abbreviation
1	plain soil	tap Water	S + TW
2	plain soil	STP Water	S + STPW
3	soil : sludge 10:1 v/v	tap Water	S:S (10:1) + TW
4	soil : sludge 10:1 v/v	STP Water	S:S (10:1) + STPW
5	soil : sludge 5:1 v/v	tap Water	S:S (5:1) + TW
6	soil : sludge 5:1 v/v	STP Water	S:S (5:1) + STPW

Table 2. Chemical profile of the soil used in the experiment.

Property	Measurement	Property	Measurement
Organic matter %	0.97	Mg (ppm)	363
EC (S/cm)	2.51	Cd (%)	6.33
pH	7.95	Pb (ppm)	2253.48
Total P (ppm)	7	Fe (%)	4.68
Total K (ppm)	105	Cu (ppm)	72.59
Clay %	30.20	Zn (ppm)	1471.84
Silt %	27.60	Mn (ppm)	1862.06
Sand %	42.20	Ni (ppm)	361.029

Table 3. Quality of the STP outlet water (STPW) and tap water (tapW) (average values)

	BOD5	NH_3^+ (ppm)	NO_3^- (ppm)	SS (ppm)	pH	DO	Ca^{+2} (ppm)
STPW	6.25	1.41	6.54	17.21	6.68	3.52	1546.41
tapW		0.003	0.88	22.1	7.88	9.6	46.8

Trees were irrigated daily with a predetermined water quantity. The quality of STP outlet water as well as that of the tap water used for plant irrigation during the experiments is shown in Table 3. The chemical profile of sewage sludge is given in Table 4 (Tsakou et al. 2001a). Soil substrate, sewage sludge and wastewater as well as wood tissues, were analyzed for heavy metals and other elements by means of "EDXRF QuanX Spectrace" Spectrophotometer.

Table 4. Chemical profile of the sewage sludge.

Property	Measurement	Property	Measurement
Organic matter (%)	53.08	Cd (ppm)	9.93
pH	5.80	Zn (ppm)	2307.28
K (%)	0.87	Ni (ppm)	322.23
Fe (%)	3.34	Cu (ppm)	260.40
Ca (%)	14,465	Hg (ppm)	1.99
Mn (ppm)	769.07	Pb (ppm)	817.70

During the experiment plant height and breast diameter (i.e. diameter of trunk at the height of 1, 3 m) were recorded once a week. Stem tissue analysis for heavy metals was conducted after 2 years of cultivation. Analysis of variance was performed with the SPSS software. Plant height, breast diameter of trees and heavy metal concentration were compared using the Mann-Whitney U, Wilcoxon and Kruskall-Wallis tests.

RESULTS AND DISCUSSION

Data of tree height are shown in Figs 1-2. Their statistical analysis revealed that:

All plant groups irrigated with tap water -regardless of their growing substrate- appear to have, statistically, the same growth rate (Fig. 1). This is in agreement with Moffat *et al* (2001) reporting that the use of sewage sludge and STP water for two years on poplar trees had no effect on tree height. This can be attributed to the high pH values of the soil used as growing substrate. High pH values of soil, also recorded in our experiment, are blamed for the immobilization of various elements and heavy metals, restraining them below the ground (Lewandowski *et al.* 1997).

Among plant groups irrigated with STP water (Fig. 2) statistically significant differences (x^2=7.622, $p<0.05$) can be observed in concern to plant height. Greatest height promotion was observed in the low ratio group [S:S (10:1) + STPW]. It seems that either the nutrients of sewage sludge are activated by STP water, being converted into easily absorbed compounds, or the STP water, itself, provides additional nutrients in the appropriate form to be absorbed by the poplar trees or modifies the environment in favor of this absorption. At this point the higher N and Ca concentrations and higher BOD values of STP water - as indicated in Table 3 - might be blamed for this difference. If more sludge is applied [S:S (5:1) + STPW], significantly lower trees are produced which means that high nutrient concentration have an inhibiting effect on the poplar trees.

Comparing groups of Fig. 1 (tap water) to those of Fig. 2 (STP water) we assume that poplar height is not affected by irrigation when trees grow on the same substrate. The only exception - groups with the high ratio sewage sludge [S:S (5:1), arrows in Figs 1 and 2] where the tap water group had significantly higher trees than the STP water one ($U=19.500$, $p=0.002$) - can be explained by the expected growth inhibition when the excessively high in nutrient concentration substrate [S:S (5:1)] is combined with the anyway rich in nutrients STP water. Rawajfih and Gharaibeh (1990) have also reported similar effect on *Triticum durum*.

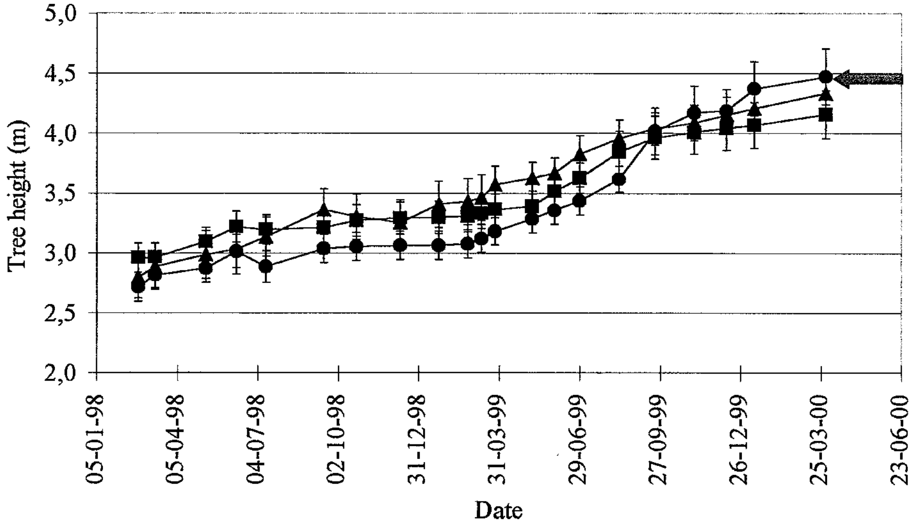

Figure 1. Height of *Populus euramericana* (var. I-214), Tap water [■S, ▲ S:S (10:1), ● S:S (5:1)] n = 12

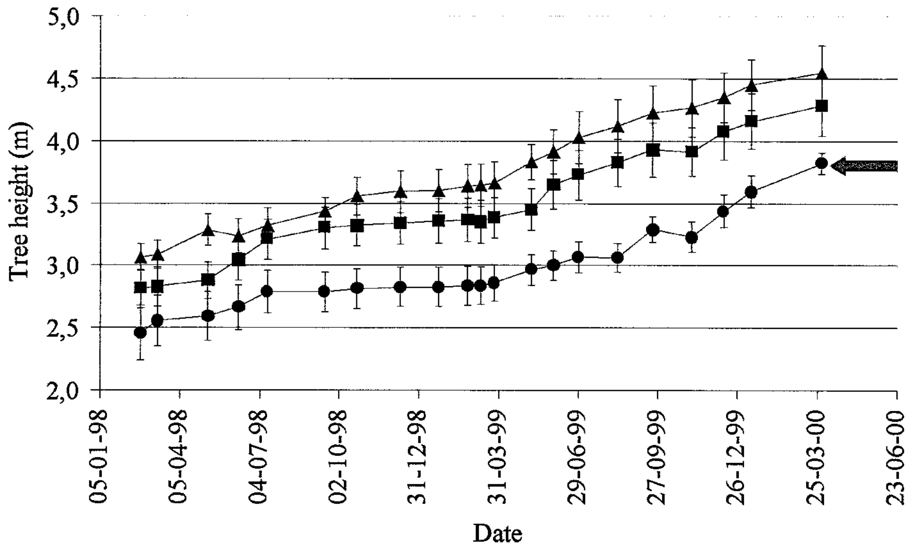

Figure 2. Height of *Populus euramericana* (var. I-214), STP water [■S, ▲ S:S (10:1), ● S:S (5:1)] n = 12

Arrows indicate 2 groups of poplars which are statistically different in tree height ($U=19.500$, $p=0.002$).

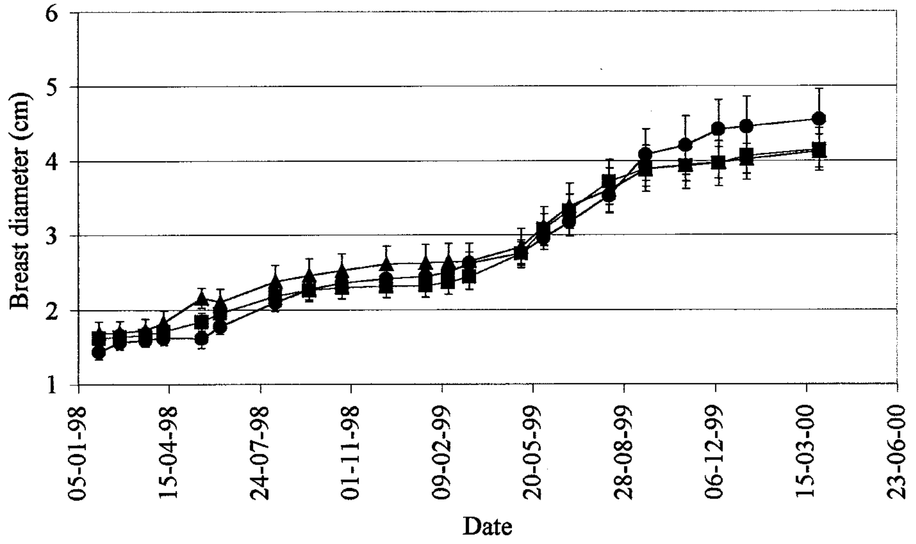

Figure 3. Breast diameter of *Populus euramericana* (var. I-214), Tap water [■S, ▲ S:S (10:1), ● S:S (5:1)] n = 12

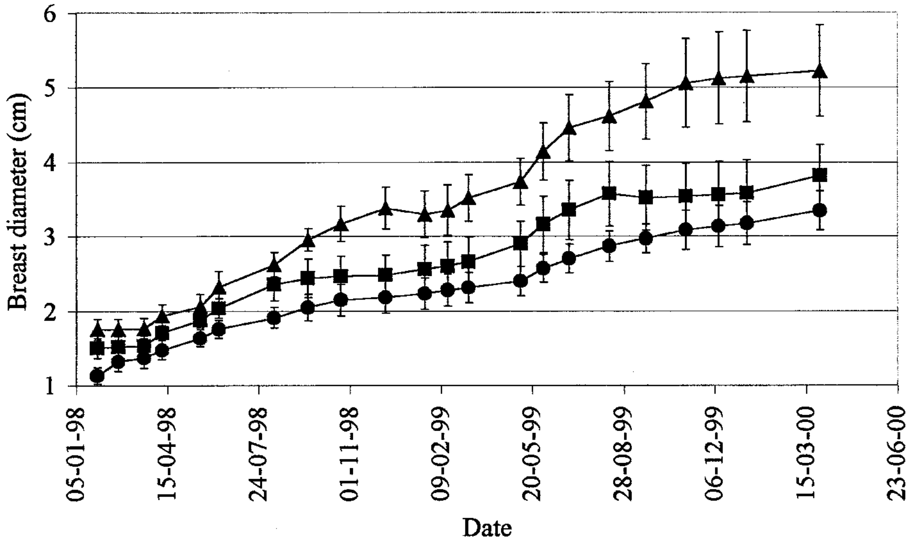

Figure 4. Breast diameter of *Populus euramericana* (var. I-214), STP water [■S, ▲ S:S (10:1), ● S:S (5:1)] n = 12

When it comes to breast diameter (Figs 3 and 4) it seems that curve extrapolation leads to a similar, as with tree height, speculation. Yet, statistical analysis indicates that - in contrast to height - breast diameter is not significantly affected from application of STP products. Since trunk diameter is increased as a result of the seasonal activation of cambium (secondary meristem) and since cambial meristematic activity has to follow the time limits of the cell life-cycle, it seems that the environmental impact on this tree feature is more or less limited.

Data from the EDXRF QuanX Spectrace, Spectrophotometer (Table 5) indicate that concentration levels of K, C, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Pb, Br, Hg, Cd and Ba in the wood tissue are not affected by the application of STP products. Low heavy metal concentrations in plants growing in sludge amended soil or irrigated by STP water were expected as a result of low concentrations of these elements in the sewage of an area where residents are mainly occupied in agricultural activities. Two other reasons probably contributing to these low concentrations of heavy metals are: a) the high pH values of the soil known to immobilize heavy metals keeping them below ground and b) the response of the specific poplar clone used. Differences among the varieties of poplar trees, concerning their ability to absorb and accumulate heavy metals, were reported by Moffat *et al.* (2001). It can, therefore, be explained why our results are in contrast with those of Lee and Woo (1998) who reported that during application of industrial sewage on poplar cultures, trees did accumulate heavy metals and other toxic elements in their tissues.

Calcium (Ca) is a unique exception since its concentration seems to depend on the STP products used. Application of sewage sludge with simultaneous irrigation with tap water causes a statistically significant increase of Ca concentration in the wood tissue of poplar trees ($x^2=7.449,\ p=0.002$). On the contrary, irrigation with STP water keeps off calcium concentration from increasing significantly.

Among groups cultured on the same growing substrate but with different types of irrigation water [3 pairs of groups: S, S:S (5:1) and S:S (10:1)] statistically significant differences are observed only between tree groups growing on plain soil ($U=0.000,\ p=0.014$). In this case irrigating with STP instead of Tap water results in a statistically significant increase of Ca concentration in wood tissue.

It is known that Ca plays an important role in protecting plants against the disruptive effects of high sodium concentration in soil (Epstein 1998). Therefore it seems likely that high Ca concentration in the wood of poplar trees can be attributed to the high Na concentrations in the sewage sludge and STP water, which might have activated physiological mechanisms of poplars to sustain high salt concentrations, eventually increasing Ca concentration in wood tissues of the trees.

Concluding, we might say that the use of STP products - even if it is not aiming to the tremendous growth promotion as that reported in the cases of cotton and flax (Tsakou *et al.* 2001a; Tsakou *et al.* 2001b; Tsakou *et al.* 2002) - remains environmentally safe and is not – in concern to heavy metals - dangerous to humans, if residential (household) sewage is not mixed with industrial effluents. It also seems that an investigation on heavy metal mobility and their accumulation within edible fruits, after long term tree cultures using STP products, would be of great interest and importance for further planning on sludge and STP water disposal.

Table 5. Heavy metal concentrations in *P. euramericana* wood from the various plant groups.

	S TW		**S STPW**		**S:S (10:1) TW**		**S:S (10:1) STPW**		**S:S (5:1) TW**		**S:S (5:1) STPW**	
	AV	**SE**	**AV**	**SE**	**AV**	**SE**	**AV**	**SE**	**AV**	**SE**	**AV**	**SE**
K (%)	0.05	0.01	0.05	0.00	0.06	0.01	0.08	0.02	0.08	0.01	0.06	0.01
Ca (%)	**0.11**	**0.00**	**0.14**	**0.00**	**0.17**	**0.02**	**0.16**	**0.02**	**0.16**	**0.01**	**0.13**	**0.01**
Fe (ppm)	18.45	6.04	14.09	1.61	30.71	11.08	40.26	12.10	21.35	2.91	26.89	8.67
Zn (ppm)	13.96	1.31	14.26	3.23	13.42	1.84	14.81	2.61	16.64	2.14	16.04	1.34
Ba (ppm)	9.93	2.31	22.34	12.62	13.11	3.40	16.64	5.14	43.20	34.46	10.13	1.71
Mn (ppm)	3.77	1.47	4.76	0.83	4.76	0.56	5.54	1.25	8.32	1.32	6.60	1.49
Pb (ppm)	3.54	2.43	0.97	0.45	0.90	0.09	2.05	0.70	2.96	1.52	0.30	0.04
Cr (ppm)	2.93	0.87	3.39	0.96	1.79	0.98	3.97	1.54	1.40	1.01	2.41	1.63
Cu (ppm)	2.64	1.97	2.27	0.81	3.28	0.92	3.35	0.56	2.55	0.80	3.08	0.83
Ni (ppm)	1.91	1.10	2.26	0.71	1.44	0.97	2.04	1.41	2.85	0.74	1.63	1.46
Hg (ppm)	1.64	0.50	*1.78*		*2.84*		0.28	0.11	1.83	0.20	ND	
Co (ppm)	1.53	0.47	*0.95*		0.52	0.21	*0.19*		1.64	0.05	0.97	0.20
Cd (ppm)	1.31	0.67	2.21	0.99	*0.28*	ND			*1.25*		0.89	0.35
Br (ppm)	1.00	0.68	1.04	0.28	1.12	0.65	1.49	0.48	*0.96*		0.82	0.25

n = 4, ND = Not Detected (*Values in italics represent a sole value from just one sample and not the average of 4 samples. Therefore there is no standard error next to them*).

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