Bull. Environ. Contam. Toxicol. (2006) 76:1021–1030

DOI: 10.1007/s00128-006-1020-0

Bulletin of Environmental Contamination and Toxicology

Long-Term Application of Sludge and Water from a Sewage Treatment Plant and the Aftermath on the Almond Trees (*Prunus dulcis*)

J. Menti,[1] M. Roulia,[2] E. Tsadilas,[3] N. S. Christodoulakis[1]

[1] University of Athens, Faculty of Biology, Department of Botany, Athens 157 01, Hellas, Greece
[2] University of Athens, Chemistry Department, Inorganic Chemistry Laboratory, Athens 157 01, Hellas, Greece
[3] Goulandris Natural History Museum, Gaia Center, Laboratory of Soil Ecology and Biotechnology, 100 Othonos St, Athens 145 62, Hellas, Greece

Received: 24 February 2006/Accepted: 20 April 2006

The major problem of disposing sludge and water produced by Sewage Treatment Plants (STP) has been thoroughly investigated. The effect of sewage sludge application to agricultural soils in industrial countries (Raven and Loeppert 1997; Towers and Horne 1997), the effect of the treated domestic and industrial effluents on plants (Hooda et al. 1997; Logan et al. 1997; Palacios et al. 1999; Samaras and Kallianou 2000, Weir and Allen 1997) as well as the significant promotion of plant growth and productivity and the absence of heavy metals from the tissues of stems, leaves, roots and fruits after the long term application of sludge and irrigation with water from treated residential sewage in annual (Tsakou et al. 2001a, Tsakou et al. 2001b, Tsakou et al. 2002) and perennial plants (Tsakou et al. 2003, Menti et al. 2005, Menti et al. 2006) have been clearly demonstrated.

A vast area, surrounding the STP on the island of Kos, Greece, was restored in 1993 (Margaris et al. 1995). Hundreds of trees, traditionally cultured in Mediterranean areas, were planted and flourish there irrigated with water and fertilized with sewage sludge from the installations. Considering the time span of 13 years more than enough to produce effects of bioaccumulation we thoroughly investigated heavy metal concentrations in all tissues of olive (*Olea europaea* L.) (Menti et al. 2005) and lemon trees (*Citrus limon*) (Menti et al. 2006). According to this investigation leaves, young stems and fruits can, by no means, be considered sites of heavy metal accumulation. Wood tissues, even the oldest annual rings, deep in the trunks, did not host any toxic materials or traces of heavy metals.

The seed of the almond fruit is of high nutritional value (Rissolo et al.1991, Ahrens et al. 2005) and is widely used for many purposes in food industry. These seeds are also used as a source of secondary metabolites exhibiting desirable pharmacological properties (Saura-Calixto et al.1984, Malisiova et al. 2004, Ryszardet al. 2005). In this paper, we present the results of our investigation on heavy metal accumulation for the almond trees (*Prunus dulcis*).

MATERIALS AND METHODS

This project was worked out at the Sewage Treatment Plant (STP) of the Municipality of Kos (an island of the southeastern Aegean Sea, Greece). Among the 2000 fruiting trees cultured there are only two almond trees growing. This sample, although small, is of substantial interest thus used for the investigation.

Correspondence to: N. S. Christodoulakis

Plant organs were collected from the almond trees in 2002 and 2003. Leaf primordia, fully unfolded leaves (after 40 days) and mature leaves (after 95 days) were detached from branches of the current vegetative period. Pieces of the branches were also collected for analysis. Leaves from each of the three groups were cut into small pieces, separately, and fixed in phosphate buffered 3% glutaraldehyde (pH 6·8) at 0 °C for 2 hours (Sabatini et al., 1963). Some of the pieces from each group were dehydrated in a graded ethanol series, critical point dried, coated either with carbon or with gold or palladium and viewed with a JEOL JSM-6500F Scanning Electron Microscope. The Energy Dispersive X-ray Microanalysis (EDX) was executed on carbon-coated specimens with the JEOL JSM-6500F using the Oxford Link™ ISIS™ 300 microanalysis system through the Oxford SEM-Quant™ software (statistics and error correction). The accelerating Voltage was 20KV, the beam current 0,5 nA, the beam diameter 2μm and the live time 50 seconds.

A part of the tissue was post fixed in 1% osmium tetroxide in phosphate buffer (Ledbetter & Porter, 1963), dehydrated in a graded ethanol series and embedded in Durcupan ACM (Fluka, Steinheim, Switzerland).

Ripe almonds were also collected and prepared, using the same methods, for scanning electron microscopy and microanalysis.

All semi-thin sections of plant tissues were viewed with a Zeiss Axioplan optical microscope. Original light micrographs were recorded digitally using a Nikon D100, 6·31 mega pixels camera. SEM images were digitally recorded as well.

Physicochemical properties of the local soil used as a basis for the growing substrate, were investigated and presented in a previous paper (Menti et al. 2005).

Soil substrate, sewage sludge and wastewater as well as plant tissues, were analyzed for heavy metals and other elements by means of "EDXRF QuanX Spectrace" Spectrometer.

For crosschecking heavy metal accumulations within the plant tissues, Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) was also used. Leaves twigs and seeds were dried at 60 °C for 5 days, grinded, digested with HNO_3 65% in a microwave apparatus (MARS 5 CEM, USA) and filtered through Whatman 41 (20 - 25μm) filters. The extract was injected, with argon plasma, in an Iris Advantage AP/EWR - Duo Option (THERMO JARREL ASH, USA).

RESULTS AND DISCUSSION

Leaves of traditionally cultured plants are thicker (Fig. 1a), with thicker epidermal cells, conductive bundles well protected with mechanical tissue and mucous masses secreted from the upper epidermal cells towards the palisade parenchyma (Fig 1c). Leaves from treated plants (Fig 1b) appear less xeromorphic. Their compact palisade tissue indicates the high productivity of these leaves through the numerous starch granules observed within the elongated cells (Fig 1d).

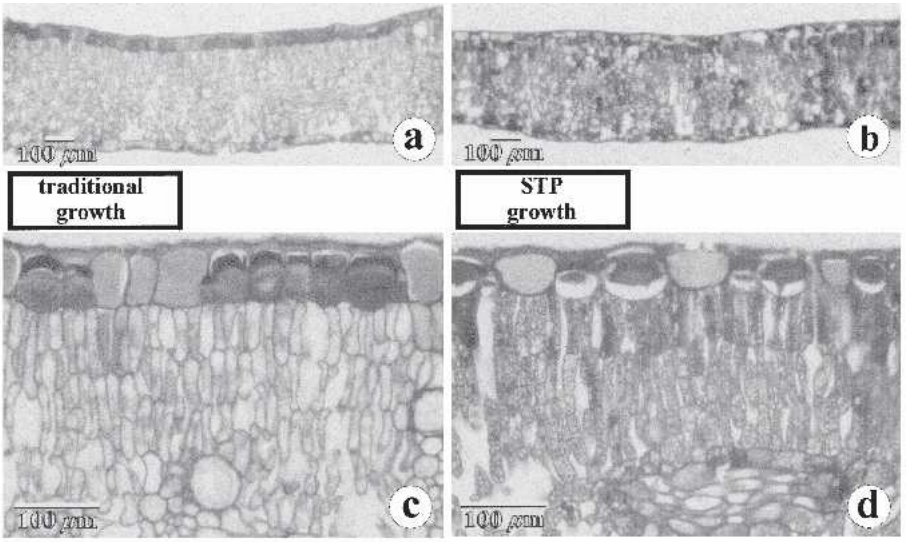


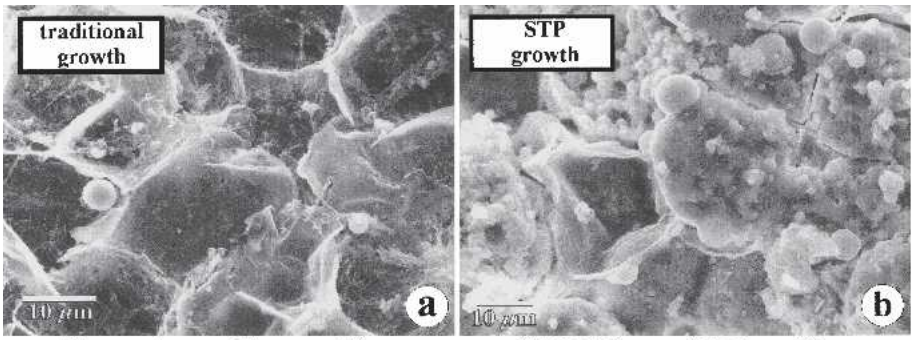
Figure 1. Cross-sections of leaves from traditionally grown (left) and STP grown (right) plants. Leaves from trees treated within the STP appear thinner, more compact and far more productive as the numerous starch grains within their mesophyll cells indicate. Mucus masses can be distinguished below epidermal cells of the leaves from traditionally grown trees.

Palisade parenchyma is equally developed in both leaf types occupying about 50% of the mesophyll. The differences in the xeromorphic characteristics between the two leaf types are probably due to the rich in nutrients soil and the irrigation that treated plants regularly receive with STP water.

Energy Dispersive X-ray Microanalysis (EDX) performed on leaf tissue (epidermis and mesophyll) as well as within the cotyledons of the almond seed (Figures 2). Heavy metals were either absent (negative values) or present in absolutely inconsiderable quantities, as the analysis printouts indicate.

Microanalysis data, although encouraging for a long-term use of water and sludge, are quantitative and have to be checked further more. The "EDXRF QuanX Spectrace" Spectrometer was so far used for all our investigations on heavy metal accumulation (Tsakou et al. 2001a, Tsakou et al. 2001b, Tsakou et al. 2002, Tsakou et al. 2003). Keeping the advantage of a direct comparison of any new data to our previous measurements integrates the whole investigation. Inductively Coupled Plasma Atomic Emission Spectrometry, a challenging and extremely precise method, remained our second choice.

Heavy metal concentrations, as measured in young (y), fully expanded (fe) leaves and mature (m) leaves detached from the two coetaneous almond trees, growing in the same area, are presented in Table 1. Mean values of these two specimens are compared to the mean values of the heavy metal analysis of the leaves from six almond trees growing outside the installations, on a field never exposed to sludge.



Element		Type	%	Element		Type	%
Na	K	ED	- 0.59	Na	K	ED	- 0.85*
Mg	K	ED	0.14	Mg	K	ED	0.92
Ca	K	ED	0.21	Ca	K	ED	0.03*
Cr	K	ED	0.00*	Cr	K	ED	0.12*
Zn	K	ED	0.00	Zn	K	ED	0.24*
Co	K	ED	0.01*	Co	K	ED	- 0.06*
Ni	K	ED	- 0.01*	Ni	K	ED	0.08*
Cu	K	ED	0.00*	Cu	K	ED	0.68
As	K	ED	- 0.51*	As	K	ED	- 1.64*
Pb	K	ED	0.03*	Pb	K	ED	0.30*

Figure 2. Scanning electron micrographs of the parenchymatic tissue within the almond seed cotyledons. Large almond-oil droplets are demonstrated. The printout of the heavy metal microanalysis, focused within the cells and the oil droplets, is given below each picture.

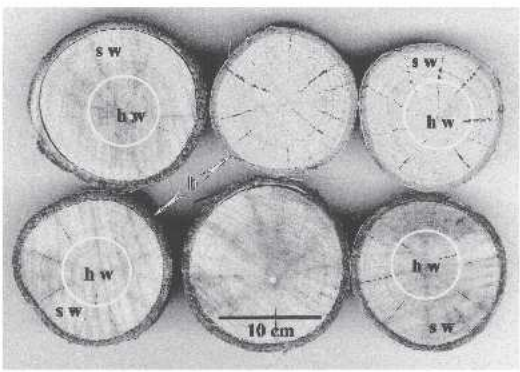


Figure 3. Trunk slices from ten to eleven years of age almond trees (*Prunus dulcis*). Annual rings are clearly visible.

Heartwood (h w) is indicated with the circles. The externally located sapwood (sw) is a younger tissue and is surrounded by bark. (b).

Values of heavy metals detected with the "EDXRF QuanX Spectrace" Spectrometer have no correlation to leaf age.

Heavy metals within the leaves were also investigated using Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES). This data, given in Table 2, confirm that heavy metal concentrations are very low, sometimes close to the resolution limits of the instrument and do not correlate to leaf age as well.

Values of heavy metals given in Tables 3 (EDXRF) and 4 (ICP-AES) were detected in recently sprouted (young) branchlets collected in April or in older branchlets collected in September (mature), from the same almond trees. These stems are considered young organs. Heavy metal concentrations in all stems are very low, most of the times close to the resolution limits of the instruments.

Table 1. Values of heavy metals detected within almond tree leaf tissues using "EDXRF Quan X Spectrace" Spectrometer (EDXRF Spectrometry).

metal	specimen 1			specimen 2			mean values			*tradit. cultured*		
	y	fe	m	y	fe	m	y	fe	m	y	fe	m
Cd mg/Kg	3.40	ND	7.40	0.60	2.30	0.40	**1.90**	**1.10**	**3.90**	*1.40*	*0.20*	*3.60*
Cr mg/Kg	ND	ND	0.30	9.10	ND	ND	**4.50**	**ND**	**0.10**	*0.30*	*ND*	*2.90*
Cu mg/Kg	13.5	17.9	ND	8.00	7.70	2.50	**10.8**	**12.8**	**1.30**	*9.70*	*16.1*	*8.80*
Mn mg/Kg	43.4	9.80	66.8	38.9	12.3	51.8	**41.2**	**11.1**	**5.90**	*24.8*	*5.90*	*38.4*
Ni mg/Kg	ND	ND	7.70	ND	6.20	8.60	**ND**	**3.10**	**8.10**	*4.40*	*12.6*	*ND*
Pb mg/Kg	10.5	ND	12.6	9.50	0.20	ND	**10.0**	**0.20**	**6.30**	*5.30*	*5.60*	*ND*
Zn mg/Kg	52.7	36.9	15.9	55.7	25.1	12.5	**54.2**	**31.0**	**14.2**	*37.8*	*8.70*	*16.8*
As mg/Kg	ND	0.40	4.40	ND	ND	ND	**ND**	**0.20**	**2.20**	*0.20*	*ND*	*1.20*
Co mg/Kg	0.30	ND	6.60	1.30	ND	ND	**0.80**	**ND**	**3.30**	*ND*	*ND*	*2.60*
Ca %	1.70	1.80	3.70	1.60	1.90	3.70	**1.60**	**1.80**	**3.70**	*1.60*	*2.40*	*3.50*
K %	1.80	0.80	1.00	1.58	0.80	1.10	**1.70**	**0.80**	**1.00**	*1.70*	*6.00*	*3.10*

(y = young leaf, y = fully expanded leaf, m = mature leaf)

Table 2. Values of heavy metals detected within almond tree leaf tissues using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP – AES).

metal	specimen 1			specimen 2			mean values			*tradit. cultured*		
	y	fe	m	y	fe	m	y	fe	m	y	fe	m
Cd mg/Kg	ND	0.10	0.30	0.10	ND	0.10	**0.05**	**0.05**	**0.20**	*0.30*	*0.10*	*ND*
Cr mg/Kg	1.70	3.40	ND	1.60	2.10	ND	**1.65**	**2.75**	**ND**	*1.40*	*1.20*	*0.20*
Cu mg/Kg	8.00	8.20	3.20	9.20	7.70	3.50	**8.6**	**7.95**	**3.35**	*13.6*	*8.00*	*4.50*
Mn mg/Kg	42.1	59.8	67.9	46.2	95.0	44.9	**44.1**	**77.4**	**56.4**	*18.4*	*41.2*	*38.9*
Ni mg/Kg	2.20	6.30	5.30	4.00	9.80	9.00	**3.1**	**8.05**	**7.15**	*2.10*	*ND*	*ND*
Pb mg/Kg	0.40	ND	ND	ND	ND	ND	**0.2**	**ND**	**ND**	*1.00*	*ND*	*ND*
Zn mg/Kg	51.3	31.0	17.0	67.7	21.5	9.00	**59.5**	**26.2**	**13.0**	*39.3*	*ND*	*23.0*
As mg/Kg	ND	ND	ND	ND	ND	ND	**ND**	**ND**	**ND**	*ND*	*ND*	*ND*
Co mg/Kg	ND	ND	ND	ND	ND	ND	**ND**	**ND**	**ND**	*ND*	*ND*	*ND*
Ca %	13.9	26.7	38.2	17.2	17.3	37.4	**15.5**	**22.0**	**37.8**	*10.8*	*10.2*	*23.3*
K %	20.4	13.8	10.2	17.2	13.8	11.4	**18.8**	**13.8**	**10.8**	*16.2*	*38.4*	*17.0*

(y = young leaf, y = fully expanded leaf, m = mature leaf)

The heartwood is the oldest, non-living tissue of a plant compared to the sapwood which is a younger, alive and mostly functioning tissue (Figure 3). These wood types as well as the bark of the trunk were carefully separated, grinded and undergone investigations with both methods.

Table 3. Values of heavy metals detected within almond tree branchlets using "EDXRF Quan X Spectrace" Spectrometer (EDXRF Spectrometry).

metal	specimen 1		specimen 2		mean values		*tradit. cultured*	
	y	m	y	m	y	m	*y*	*m*
Cd mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Cr mg/Kg	1.10	0.90	1.30	1.10	**1.20**	**1.00**	0.90	*1.00*
Cu mg/Kg	13.01	11.90	14.30	13.20	**13.65**	**12.55**	11.90	*13.20*
Mn mg/Kg	15.10	14.90	14.60	15.80	**14.85**	**15.35**	*14.70*	*16.20*
Ni mg/Kg	0.89	0.97	ND	0.83	**0.44**	**0.90**	*1.10*	*0.79*
Pb mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Zn mg/Kg	39.70	40.10	44.30	42.70	**42.00**	**41.4**	*45.10*	*47.30*
As mg/Kg	ND	ND	ND	ND	**ND**	**ND**	ND	ND
Co mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Ca %	1.92	1.95	2.03	2.12	**1.97**	**2.03**	*2.32*	*1.99*
K %	1.56	1.23	1.65	1.39	**1.60**	**1.31**	*1.61*	*1.23*

(y = young branch, m = mature branch)

Table 4. Values of heavy metals detected within almond tree branchlets using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP – AES).

metal	specimen 1		specimen 2		mean values		*tradit. cultured*	
	y	m	y	m	y	m	*y*	*m*
Cd mg/Kg	0.90	1.20	1.70	1.10	**1.30**	**1.15**	*0.90*	*1.10*
Cr mg/Kg	0.10	0.10	ND	ND	**0.05**	**0.05**	*0.10*	*ND*
Cu mg/Kg	8.10	9.40	7.80	8.30	**7.95**	**8.85**	*9.90*	*10.20*
Mn mg/Kg	12.10	14.70	13.30	15.80	**12.70**	**15.25**	*14.70*	*16.10*
Ni mg/Kg	1.50	1.70	1.30	1.40	**1.40**	**1.55**	*1.80*	*1.80*
Pb mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Zn mg/Kg	43.50	47.30	47.10	49.80	**45.30**	**48.50**	*46.20*	*50.70*
As mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Co mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Ca %	12.40	14.80	16.90	17.90	**14.65**	**16.30**	*16.30*	*18.40*
K %	10.50	11.10	13.20	9.90	**11.85**	**10.50**	*13.20*	*10.90*

(y = young branch, m = mature branch)

Heavy metal concentrations in heartwood and sapwood are presented in Table 5 (EDXRF Spectrometry) and Table 6 (ICP – AES). Through these two Tables we can have a "view" of the heavy metal dispersal and accumulation, within the plant body, versus time. It seems that heartwood, although the oldest tissue of the tree, falls short in heavy metal concentration, in all metal cases, than the sap wood, the younger and water conducting wood tissue of the plant. Finally we can say that wood, regardless of its age, is literally free of heavy metals.

Table 5. Values of heavy metals detected within trunks of almond tree plants using "EDXRF Quan X Spectrace" Spectrometer (EDXRF Spectrometry).

metal	specimen 1		specimen 2		mean values		*tradit. cultured*	
	h w	**s w**	**h w**	**s w**	**h w**	**s w**	***h w***	***s w***
Cd mg/Kg	ND	ND	0.12	ND	**0.06**	**ND**	*MA*	*ND*
Cr mg/Kg	13.50	19.37	13.35	13.98	**13.42**	**16.67**	15.33	*19.11*
Cu mg/Kg	6.71	6.70	5.25	9.65	**5.98**	**8.17**	10.6	*10.50*
Mn mg/Kg	3.71	9.85	4.24	10.51	**3.97**	**10.18**	4.47	*11.39*
Ni mg/Kg	5.98	10.38	5.5	5.86	**5.74**	**8.12**	12.41	*ND*
Pb mg/Kg	ND	4.94	ND	2.86	**ND**	**2.77**	3.64	*2.45*
Zn mg/Kg	35.26	36.33	35.12	38.70	**35.19**	**37.51**	32.76	*34.16*
As mg/Kg	ND	1.47	3.26	0.94	**1.63**	**1.20**	1.51	ND
Co mg/Kg	ND	ND	ND	ND	**ND**	**ND**	ND	*ND*
Ca %	0.21	0.54	0.21	1.17	**0.21**	**0.85**	0.21	*1.29*
K %	0.07	0.12	0.07	0.20	**0.07**	**0.16**	0.07	*0.25*

(hw = heart wood, sw = sap wood)

Table 6. Values of heavy metals detected within trunks of almond tree plants using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP – AES).

metal	specimen 1		specimen 2		mean values		*tradit. cultured*	
	h w	**s w**	**h w**	**s w**	**h w**	**s w**	***h w***	***s w***
Cd mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Cr mg/Kg	3.40	8.90	6.70	9.90	**5.05**	**9.40**	*6.80*	*8.60*
Cu mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Mn mg/Kg	7.10	14.10	8.00	10.60	**7.55**	**12.35**	*5.70*	*9.60*
Ni mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Pb mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Zn mg/Kg	21.00	48.70	11.20	48.70	**16.10**	**48.70**	*23.60*	*39.40*
As mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Co mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Ca %	2.80	3.70	4.20	6.40	**3.50**	**5.05**	*4.20*	*5.70*
K %	14.13	13.17	15.1	14.9	**14.61**	**14.03**	*17.90*	*12.10*

(hw = heart wood, sw = sap wood)

In Tables 7 and 8 data for the heavy metal concentrations within the fruit of the almond tree are presented. The edible and commercially valuable part of the almond fruit, the seed with its sized cotyledons, was investigated. As in all other cases, concentrations of heavy metals within the seed tissue remain close to the resolution limits of both instruments.

Table 7. Values of heavy metals detected within the edible seed of the almond tree using "EDXRF Quan X Spectrace" Spectrometer (EDXRF Spectrometry).

metal	specimen 1		specimen 2		mean values		*tradit. cultured*	
	im	**m**	**im**	**m**	**im**	**m**	***im***	***m***
Cd mg/Kg	ND	0.34	ND	0.67	**ND**	**0.51**	*ND*	*0.46*
Cr mg/Kg	3.68	1.65	ND	1.53	**1.84**	**1.59**	*1.88*	*0.48*
Cu mg/Kg	9.04	19.04	14.15	15.78	**11.59**	**17.41**	*3.81*	*9.23*
Mn mg/Kg	12.74	28.55	16.34	32.7	**14.54**	**30.63**	*13.79*	*10.88*
Ni mg/Kg	3.56	ND	ND	3.04	**1.78**	**1.52**	*7.47*	*5.78*
Pb mg/Kg	ND	ND	1.52	ND	**0.76**	**ND**	*ND*	*ND*
Zn mg/Kg	36.0	44.37	38.18	50.89	**37.09**	**47.63**	*38.23*	*19.84*
As mg/Kg	2.28	ND	ND	ND	**1.14**	**ND**	*0.17*	*0.55*
Co mg/Kg	1.24	6.42	ND	ND	**0.62**	**3.21**	*ND*	*ND*
Ca %	0.35	0.57	0.45	0.59	**0.4**	**0.58**	*0.51*	*0.35*
K %	1.70	1.59	2.33	1.62	**2.02**	**1.61**	*2.57*	*1.81*

(im = immature fruit, m = mature fruit)

Table 8. Values of heavy metals detected within the edible seed of the almond tree using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP – AES).

metal	specimen 1		specimen 2		mean values		*tradit. cultured*	
	im	**m**	**im**	**m**	**im**	**m**	***im***	***m***
Cd mg/Kg	0.20	0.10	0.10	0.10	**0.15**	**0.10**	*ND*	*0.30*
Cr mg/Kg	0.80	1.80	1.20	1.20	**1.00**	**1.50**	*0.80*	*1.50*
Cu mg/Kg	11.40	13.30	16.60	14.50	**14.00**	**13.90**	*5.70*	*8.50*
Mn mg/Kg	12.60	24.60	15.50	23.20	**14.05**	**23.90**	*7.40*	*14.80*
Ni mg/Kg	0.20	ND	1.30	ND	**0.75**	**ND**	*1.00*	*2.70*
Pb mg/Kg	0.10	0.9	1.00	0.20	**0.55**	**0.55**	*0.50*	*0.20*
Zn mg/Kg	26.60	11.00	35.50	33.20	**31.05**	**22.10**	*39.70*	*41.20*
As mg/Kg	ND	0.10	ND	0.20	**ND**	**0.15**	*ND*	*ND*
Co mg/Kg	ND	ND	ND	ND	**ND**	**ND**	*ND*	*ND*
Ca %	2.68	4.54	3.60	4.33	**3.14**	**4.43**	*1.99*	*3.72*
K %	17.89	14.45	27.54	13.75	**22.71**	**14.1**	*16.25*	*20.70*

(im = immature fruit, m = mature fruit)

Concluding we might say that differently standardized, very sensitive instruments, functioning almost at the limits of their resolution, provided numerous data convincing that heavy metals are hardly detectable even within the oldest tissue of the almond trees, the heart wood, ageing ten years at the time of our experiment. The same is true for the leaf tissue, branchlets, trunks and fruit of *Prunus dulcis* (Tables 1-8). Both EDXRF and ICP analysis indicate that heavy metal

concentrations in untreated plants are quite comparable to those of the treated samples for all metals examined. Among the nine heavy metals investigated it seems that only cadmium (Cd), chromium (Cr) and lead (Pb) appear in slightly raised concentrations within the mature, edible fruit in treated plants. This was detected with both methods yet concentrations of Cd and Pb, in all samples of treated plants investigated, remain far lower than the strict limits (0.1 – 0.2 mg/kg of fresh tissue in fruits) of the Directive 466/2001 of the European Committee. Nickel (Ni) concentration was also detected in lower values than within the untreated plant fruits.

Concentrations of Cu and Mn appear increased within the fruit tissues of treated plants. The concentration values for these two elements are significantly higher compared to the corresponding values in lemon tree fruits (*Citrus limon* - Menti et al. 2006) as well as in all olive tree (*Olea europaea L.* - Menti et al. 2005), cotton (*Gossipium hisrsitum*) and flax (*Linum usitatissimum*) tissues reported previously (Tsakou et al. 2001b, Tsakou et al. 2002). They are also twice as high as those detected in fruits of traditionally cultured trees. Yet, concentrations of Cu and Mn in fresh tissue of fruits are not specified in the Directive 466/2001 of the European Committee.

Acknowledgments. We thank the General Secretariat of Research and Technology (Ministry of Development) and FAMAR S.A. for financial assistance through project 01ED29. We also thank the Municipality of Kos – Greece for providing the STP facilities and travel expenses and Mr. E. Michaelidis for technical assistance.

REFERENCES

Ahrens S, Venkatachalam M, Mistry AM, Lapsley K, Sathe SK (2005). Almond (*Prunus dulcis* L.) protein quality, Plant Foods Human Nutr, 60:123-128

Amorowicz R, Troszynska A, Shahidi F (2005). Antioxidant activity of almond seed extract and its fractions, J Food Lipids 12: 344-358

EUROPEAN COMMUNITY (2001) Council Directive 466/2001/ECC No L77. Official Journal of European Communities, 08/04/2001, Brussels, Belgium

Hooda PS, McNulty D, Alloway BJ, Aitken MN (1997) Plant availability of heavy metals in soils previously amended with heavy applications of sewage sludge. J Sci Food Agric 73: 446-454

Ledbetter MC, Porter KR.(1963) A "microtubule" in plant cell fine structure. J. Cell Biol.19: 239-250

Logan TJ, Lindsay BJ, Aitken MN (1997) Field assessment of sludge metal bioavailability to crops: sludge rate response. J Environ Qual 26:534-550

Malisiova F, Hatziantoniou S, Dimas K, Kletstas D, Demetzos C (2004). Liposomal formulations from phospholipids of Greek almond oil. Properties and biological activities. Zeitschrift fur Naturforschung 59: 330-334

Margaris NS, Christodoulakis NS, Giourga C (1995) Waste management and water use in the island of Kos, Greece. Insula 3: 36-39

Menti J, Roulia M, Stamatiadis S, Christodoulakis NS (2005). Aftermath of the Long-term Application of Sludge and Water from a Sewage Treatment Plant to

an Olive Tree (*Olea europaea* L.) Plantation. Bull Environ Contam Toxicol 75: 57-66

Menti J, Roulia M, Tsandilas E, Christodoulakis NS (2006). Aftermath of the Long-term Application of Sludge and Water from a Sewage Treatment Plant to an Lemon Tree (*Citrus limon*) Plantation. Bull Environ Contam Toxicol 76: 33-43

Palacios G, Carbonell-Barrachina A, Gomez I (1999) The influence of organic amendment and nickel pollution on tomato fruit yield and quality. J Environ Sci Health B34: 133-150

Raven KR, Loeppert RH (1997) Heavy metals in the environment. Trace element composition of fertilizers and soil amendments. J Environ Qual 26: 551-557

Reynolds ES (1963) The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J Cell Biol 17: 208-212

Rissolo A, Baldo C, Polesello A (1991). Application of high performance liquid chromatography to the analysis of niacin and biotin in Italian almond cultivars, J Chromatog 553: 187-192

Sabatini DD, Bensch K, Barrnett BJ (1963) Cytochemistry and Electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J Cell Biol 17: 19-58

Samaras V, Kallianou C (2000) Effect of sewage sludge application on cotton yield and contamination of soils and plant leaves. Commun Soil Sci Plant Anal 31: 331-343

Saura-Calixto F, Canellas J and Raso-Garcia A, (1984). Gas chromatographic analysis for sugars and sugar-alcohols in the mesocarp, endocarp and kernel of almond fruit. J Agric Food Chem 32: 1018-1020

Towers W, Horne P (1997) Sewage sludge recycling to agricultural land: the environmental scientist's perspective. J Chart Inst Water E: 126-132

Tsakou A, Roulia M, Christodoulakis NS (2001a) Growth of cotton plants (*Gossypium hirsutum*) as affected by water and sludge from a sewage treatment plant. I. Plant phenology and development. Bull Environ Contam Toxicol 66: 735-742

Tsakou A, Roulia M, Christodoulakis NS (2001b) 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. Bull Environ Contam Toxicol 66: 743-747

Tsakou A, Roulia M, Christodoulakis NS (2002) Growth of flax plants (*Linum usitatissimum*) as affected by water and sludge from a sewage treatment plant. Bull Environ Contam Toxicol 68: 56-63

Tsakou A, Roulia M, Christodoulakis NS (2003) Growth Parameters and Heavy Metal Accumulation in Poplar Tree Cultures (*Populus euramericana*) Utilizing Water and Sludge from a Sewage Treatment Plant. Bull Environ Contam Toxicol 71: 330-337

Weir CC, Allen JR (1997) Effects of using organic wastes as soil amendments in urban horticultural practices in the District of Columbia. J Environ Sci Health A32: 323-332