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The Island of Chios (east Mediterranean), Citrus Plantations and the Mercury Nightmare

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Mercury poisoning, as a result of environmental pollution, has become a problem of current interest on a global scale. Natural emissions of mercury form two-thirds of the input; manmade releases form about one-third (Patra and Sarma 2000). Accumulation, toxicity response and mercury distribution seem to affect the plants and differ between plant species. It has been observed that the chlorophyll, protein and nitrogen, phosphorus, potassium contents decrease significantly with increasing Hg concentrations in young plants of *Vallisneria spiralis* (Gupta and Chandra 1998) while the lower the nutrient strength in the culture medium is, the higher the metal concentrations accumulated in the different plant parts (Gothberg et al. 2004). In plants of *Bacopa monieri* grown in high concentrations of Hg, the metal accumulation in the root tissues was about five times more than in the shoot (Sinha et al. 1996) while tobacco plants (*Nicotiana tabaccum*) are less tolerant to heavy metals, including Hg, than sunflower (*Helianthus annuus*) (Ruso et al. 2001). Salix species investigated for heavy metal uptake were found to absorb Cd and Zn at a rate higher than they absorb Ni, Hg, Cu and Pb (Labrecque et al. 1995).

Chios is a Greek island globally known for the mastic trees (*Pistacia lentiscus* var. *chia*) and their products. It is located at the north eastern part of the Aegean Sea, just three and a half miles from the coast of Asia minor. The bed of the main water basin extending below the farmlands of the island, is composed of cinnabar (a mineral containing Hg). During the summer months, excessive pumping for plant irrigation lowers the underground water level by 12–15 cm daily! In a few weeks water starts to turn salty since seawater intrudes the basin. This change induces the release of mercury in the water through the reaction of NaCl with the cinnabar substrate. It is common that mercury concentration, in the basin's water, raises to 7.5 μg Hg/L while the upper limit posed by the European Union directive is 1 μg Hg/L. It seems that the local authorities and the islanders have to deal with a serious problem. Given the water shortage in most Mediterranean islands and the extensive cultures of citrus fruits established on Chios island, a serious approach to the mercury problem has to be made. Although there is a tendency for mercury to accumulate in roots, indicating that roots serve as a barrier to mercury uptake (Patra and Sarma 2000), only after a thorough investigation can leaves and, mainly, the fruits of citrus trees be considered free of mercury contamination.

Materials and Methods

This project was worked out at the farmlands of Chios island. Two farms with citrus trees were picked and the quality of the irrigation water was monitored. In the first farm water is pumped from a well and is literally free of mercury (0.1 μg Hg/L). In the second farm trees are irrigated with water originating from a drill that during the summer months is heavily contaminated with mercury (7.5 μg Hg/L). In each farm six trees from three citrus species were selected and marked. Three branches on each orange (*Citrus sinensis*), lemon (*Citrus limon*) and tangerine tree (*Citrus deliciosa*) were also tagged.

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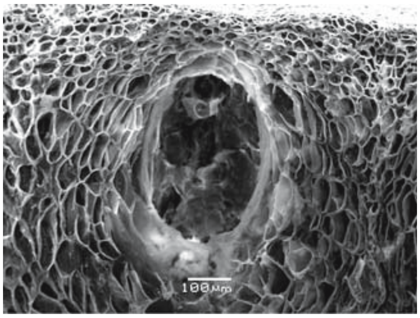
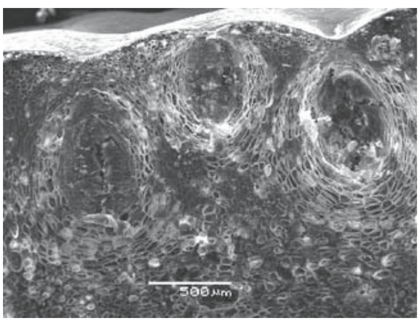


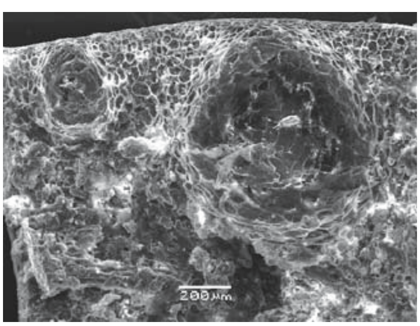
Elmt		Spect. Type	Element %
•Na	K	ED	-0.08*
•Mg	K	ED	-0.02*
•Al	K	ED	0.42
•S	K	ED	0.05*
•Cr	K	ED	0.06*
•Co	K	ED	0.10*
•Ni	K	ED	0.08*
•Sr	L	ED	-0.02*
•Cd	L	ED	0.18*
•Hg	**M**	**ED**	**-0.17***
•Pb	M	ED	-0.12*

Fig. 1 Lemon fruit (pericarp) originating from the "clean" plantation. In the middle the essential oil producing cavity. Microanalysis to the right of the picture. (* = <2 Sigma)



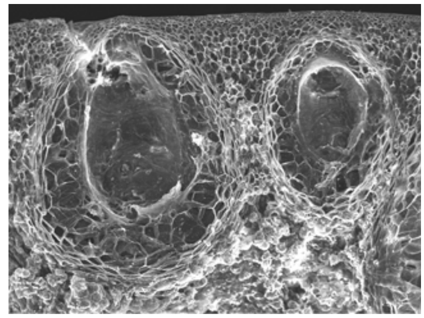
Elmt		Spect. Type	Element %
•Na	K	ED	-0.16*
•Mg	K	ED	-0.08*
•Al	K	ED	0.08*
•S	K	ED	0.14
•Cr	K	ED	0.01*
•Co	K	ED	0.09*
•Ni	K	ED	-0.22*
•Sr	L	ED	-0.00
•Cd	L	ED	-0.07*
•Hg	**M**	**ED**	**-0.20***
•Pb	M	ED	-0.26*

Fig. 2 Lemon fruit (pericarp) originating from the contaminated plantation. Three oil producing cavities can be seen. Microanalysis to the right of the picture. (* = <2 Sigma)



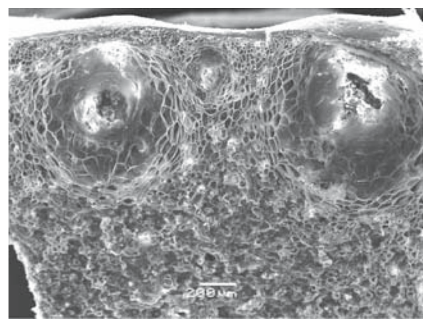
Elmt		Spect. Type	Element %
•Na	K	ED	-0.01*
•Mg	K	ED	-0.02*
•Al	K	ED	0.06*
•S	K	ED	0.13
•Cr	K	ED	0.03*
•Co	K	ED	-0.02*
•Ni	K	ED	-0.10*
•Sr	L	ED	-0.05*
•Cd	L	ED	0.08*
•Hg	**M**	**ED**	**-0.20***
•Pb	M	ED	-0.01*

Fig. 3 Tangerine fruit (pericarp) originating from the "clean" plantation. Two oil producing cavities can be seen. Microanalysis to the right of the picture. (* = <2 Sigma)



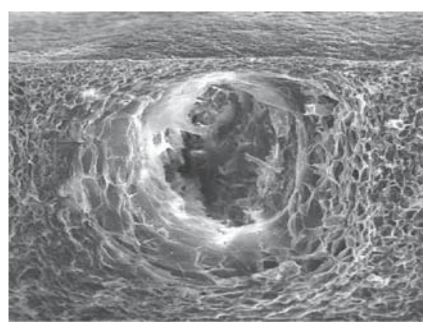
Elmt		Spect. Type	Element %
•Na	K	ED	-0.01*
•Mg	K	ED	0.06*
•Al	K	ED	0.00*
•S	K	ED	0.11
•Cr	K	ED	0.08*
•Co	K	ED	-0.08*
•Ni	K	ED	-0.08*
•Sr	L	ED	-0.02*
•Cd	L	ED	-0.05*
•Hg	**M**	**ED**	**-0.06***
•Pb	M	ED	-0.05*

Fig. 4 Tangerine fruit (pericarp) originating from the contaminated plantation. Two oil producing cavities can be seen. Microanalysis to the right of the picture. (* = <2 Sigma)



Elmt		Spect. Type	Element %
•Na	K	ED	-0.04*
•Mg	K	ED	-0.05*
•Al	K	ED	0.64
•S	K	ED	0.08*
•Cr	K	ED	-0.04*
•Co	K	ED	-0.04*
•Ni	K	ED	0.03*
•Sr	L	ED	-0.03*
•Cd	L	ED	-0.13*
•Hg	**M**	**ED**	**-0.05***
•Pb	M	ED	-0.03*

Fig. 5 Orange fruit (pericarp) originating from the "clean" plantation. Two oil cavities can be seen. Microanalysis to the right of the picture. (* = <2 Sigma)



Elmt		Spect. Type	Element %
•Na	K	ED	-0.05*
•Mg	K	ED	-0.00*
•Al	K	ED	0.38*
•S	K	ED	0.16
•Cr	K	ED	0.07*
•Co	K	ED	0.01*
•Ni	K	ED	0.06*
•Sr	L	ED	-0.04*
•Cd	L	ED	0.14*
•Hg	**M**	**ED**	**-0.21***
•Pb	M	ED	0.12*

Fig. 6 Orange fruit (pericarp) originating from the contaminated plantation. An oil producing cavity can be seen in the middle. Microanalysis to the right of the picture. (* = <2 Sigma)

Fruits were collected in December 2004 and December 2005, always from the same, tagged branches. Small pieces from the ectocarp and the juicy mesocarp were cut and fixed in phosphate buffered 3% glutaraldehyde (pH 6.8) at 0°C for 2 h (Sabatini et al. 1963). They 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 SEMQuant™ software (statistics and error correction). The accelerating voltage was 20 KV, the beam

Table 1 Mercury concentrations within the parenchyma and pericarp of non-contaminated fruits detected using "EDXRF Quan X Spectrace" spectrometer

		Spec 1	Spec 2	Spec 3	Spec 4	Spec 5	Spec 6
Citrus limon							
Hg (μg/kg)	Parenchyma	ND	ND	ND	0.010	ND	ND
Hg (μg/kg)	Pericarp	0.007	ND	0.012	ND	ND	ND
Citrus deliciosa							
Hg (μg/kg)	Parenchyma	0.212	ND	ND	0.106	ND	ND
Hg (μg/kg)	Pericarp	ND	0.110	ND	ND	0.123	ND
Citrus sinencis							
Hg (μg/kg)	Parenchyma	0.135	ND	0.010	ND	ND	ND
Hg (μg/kg)	Pericarp	ND	0.021	ND	ND	0.098	D

All three species are presented. Year 2004

Table 2 Mercury concentrations within the parenchyma and pericarp of contaminated fruits detected using "EDXRF Quan X Spectrace" spectrometer

		Spec 1	Spec 2	Spec 3	Spec 4	Spec 5	Spec 6
Citrus limon							
Hg (μg/kg)	Parenchyma	0.028	ND	ND	0.311	ND	0.025
Hg (μg/kg)	Pericarp	0.173	0.092	0.025	ND	0.156	ND
Citrus deliciosa							
Hg (μg/kg)	Parenchyma	ND	0.034	0.025	ND	ND	ND
Hg (μg/kg)	Pericarp	0.021	ND	0.098	ND	ND	ND
Citrus sinencis							
Hg (μg/kg)	Parenchyma	ND	0.129	0.162	ND	0.067	ND
Hg (μg/kg)	Pericarp	ND	0.101	ND	0.034	ND	0.087

All three species are presented. Year 2004

Table 3 Mercury concentrations within the parenchyma and pericarp of non-contaminated fruits detected using "EDXRF Quan X Spectrace" spectrometer

		Spec 1	Spec 2	Spec 3	Spec 4	Spec 5	Spec 6
Citrus limon							
Hg (μg/kg)	Parenchyma	ND	0.009	ND	ND	ND	ND
Hg (μg/kg)	Pericarp	ND	ND	ND	ND	ND	ND
Citrus deliciosa							
Hg (μg/kg)	Parenchyma	ND	ND	ND	ND	0.032	ND
Hg (μg/kg)	Pericarp	ND	ND	0.112	0.232	0.464	ND
Citrus sinencis							
Hg (μg/kg)	Parenchyma	ND	ND	ND	ND	0.145	ND
Hg (μg/kg)	Pericarp	ND	0.165	0.037	0.004	ND	0.011

All three species are presented. Year 2005

current 0.5 nA, the beam diameter 2 μm and the live time 50 s.

Fruit pieces, from both ectocarp and mesocarp, were dried, grinded and analyzed for heavy metals and other elements by means of an "EDXRF QuanX Spectrace" Spectrophotometer. Chemical properties of the water (for mercury and other heavy metals) were also investigated using the same method.

Analysis of variance was performed with the SPSS software. Mercury concentrations were compared using the Mann–Whitney *U*, Wilcoxon and Kruskall–Wallis tests.

Table 4 Mercury concentrations within the parenchyma and pericarp of contaminated fruits detected using "EDXRF Quan X Spectrace" spectrometer

		Spec 1	Spec 2	Spec 3	Spec 4	Spec 5	Spec 6
Citrus limon							
Hg (μg/kg)	Parenchyma	0.099	ND	0.224	0.461	0.152	0.083
Hg (μg/kg)	Pericarp	0.240	0.117	0.030	0.015	0.180	ND
Citrus deliciosa							
Hg (μg/kg)	Parenchyma	ND	ND	0.025	ND	ND	ND
Hg (μg/kg)	Pericarp	ND	ND	0.183	ND	0.098	ND
Citrus sinencis							
Hg (μg/kg)	Parenchyma	ND	ND	0.199	ND	ND	0.004
Hg (μg/kg)	Pericarp	ND	0.153	ND	ND	ND	ND

All three species are presented. Year 2005

Results and Discussion

The essential point of this investigation is to compare the fruits of the plants grown in a field irrigated with practically "clean" water to those originating from the plants grown in the field irrigated with highly contaminated water. We have to trace mercury till the terminal destination of the plant vascular system and investigate the extend of mercury leaching within the fruit tissues. This information is crucial for the farmers who face a strong argument about the quality of their crop. Potential fruit contamination could result to a total collapse of the agricultural economy of the island.

Energy Dispersive X-ray Microanalysis (EDX) was performed on the fruit tissue (pericarp and the juicy mesocarp). The results indicated that mercury is literally absent (negative values), as the analysis printouts presented to the right of the micrographs indicate (Figs. 1, 2, 3, 4, 5, 6). Hg values are highlighted. EDX microanalysis can trace heavy metals and point out their presence on any surface yet gives no quantitative information.

Microanalysis data, although encouraging, had to be checked further more. Spectrometry using an "EDXRF QuanX Spectrace" Spectrometer was executed primarily because it is a nondestructive method and samples could be kept for further investigation. Moreover all our investigations on heavy metal accumulation were so far conducted with this method and comparisons could be carried out.

Data on mercury concentration are presented in Tables 1, 2, 3 and 4. It becomes obvious that mercury is either non detectable or appears in extremely law concentrations. We have to point out that directive EUROPEAN COMMUNITY 466/2001 (2001) of the European Union has the limit of 0.5 mg/kg (fresh weight) for most fishery products and 1 mg/kg (fresh weight) for certain, fatty fishes (swordfish, eel, tuna fish, leather belt, shark etc). Values as high as 0.464 μg/kg (dry weight) measured in non-contaminated or 0.461 μg/kg (dry weight) measured in contaminated fruits, seem negligible! It also seems that there are not any significant differences, concerning mercury concentration, between the pericarp (skin) and the juicy mesocarp (edible part of the fruit).

Complicated statistical tests worked out (see "Materials and methods") are not presented in Tables 1 and 4 to avoid producing "bulky" and difficult-to-read images. Taking into account (a) statistical analysis and (b) the fact that minor variations, as those recorded, are usually expected for natural specimens measured by "pushing" the instrument at the limits of it's resolution, we may assume that mercury is practically absent from the tissue of lemons, tangerines and oranges.

Therefore, since this dangerous heavy metal seems to stand clear from the production (fruits), consumption by humans can be considered safe.

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