Electronic Supplementary Material (ESI) for Journal of Environmental Monitoring

SUPPLEMENTARY MATERIAL (SM)

Manuscript title: Levels, sources and spatiotemporal variation of nutrients and micropollutants in small streams of a Mediterranean River Basin

Authors: Ioannis Karaouzas, Dimitra A. Lambropoulou, Nikolaos T. Skoulikidis, Triantafyllos A. Albanis

Number of pages of the SM section: Eleven

Number of pages of the text: Two

Number of figures: Two

Number of tables: Six

Methods & Materials

Study area

The Evrotas River Basin, a large mid-altitude Mediterranean basin, is located in southeastern Peloponnesus (Fig.1) in the Prefectures of Laconia and Arcadia covering an area of 2,418 km^2. The river discharges into the Laconikos Gulf after crossing 90 km of floodplains and semi-mountainous areas. The basin expands between the mountain complexes of Taygetos (2407 m asl) and Parnon (1940 m asl), where numerous perennial, intermittent and ephemeral streams discharge into the main course. The mountainous area of the basin is formed by Mesozoic-Palaeogene limestones (42% of the basin) and impermeable rocks, such as flysch and schists (29% of the basin), while the lower parts are filled up with Pliocene and Quaternary sediments. The Evrotas basin has a typical Mediterranean climate with mild and cold winters and prolonged hot and dry summers with an average annual temperature of 16^oC. The majority of rainfall occurs during the months of October through March; highest rainfall precipitation being in December and lowest in June.

The vast majority of the river basin is covered by natural and semi-natural areas accounting for 61% of the total river basin followed by agricultural areas that account for 38% while urban areas account for 1%. The main crops are olives and oranges which cover about 90% of the agricultural land and constitute Laconia as one of the main and largest producers of olive oil and orange juice in Greece. The dominant pressures in Evrotas River Basin derive mainly from agricultural activities and include overexploitation of water resources for irrigation, disposal of agro-industrial wastes (mainly from olive oil mills and orange juice processing factories) and agrochemical pollution.

Reagents and materials

High purity (96–99.9%) standards of the selected pesticides were supplied by Riedel-de Häen (Germany). Individual stock solutions were prepared by diluting each analyte solution with methanol. Working solutions were prepared by diluting stock solutions with methanol, for sample fortification, and with ethyl acetate or hexane-dichloromethane (1:1), for GC injection. All stock and working standard solutions were stored in the dark at −20 °C. The standard mixtures were used to prepare the calibration solutions. Pesticide-grade solvents methanol, hexane, dichloromethane and ethyl acetate, used for sample preparation and chromatographic analysis were supplied from Pestiscan (Labscan, Ltd, Dublin, Ireland) and sodium chloride from Merck (Darmstadt, Germany).

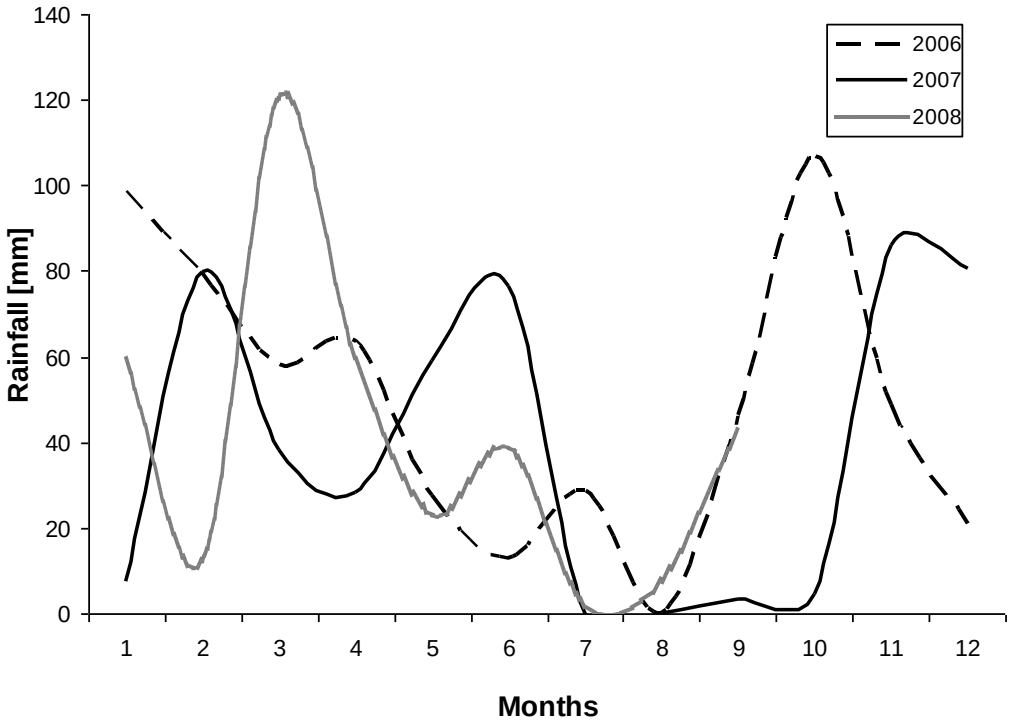

Fig. SM-1. Rainfall precipitation in the Evrotas River Basin for the years 2006 - 2008.

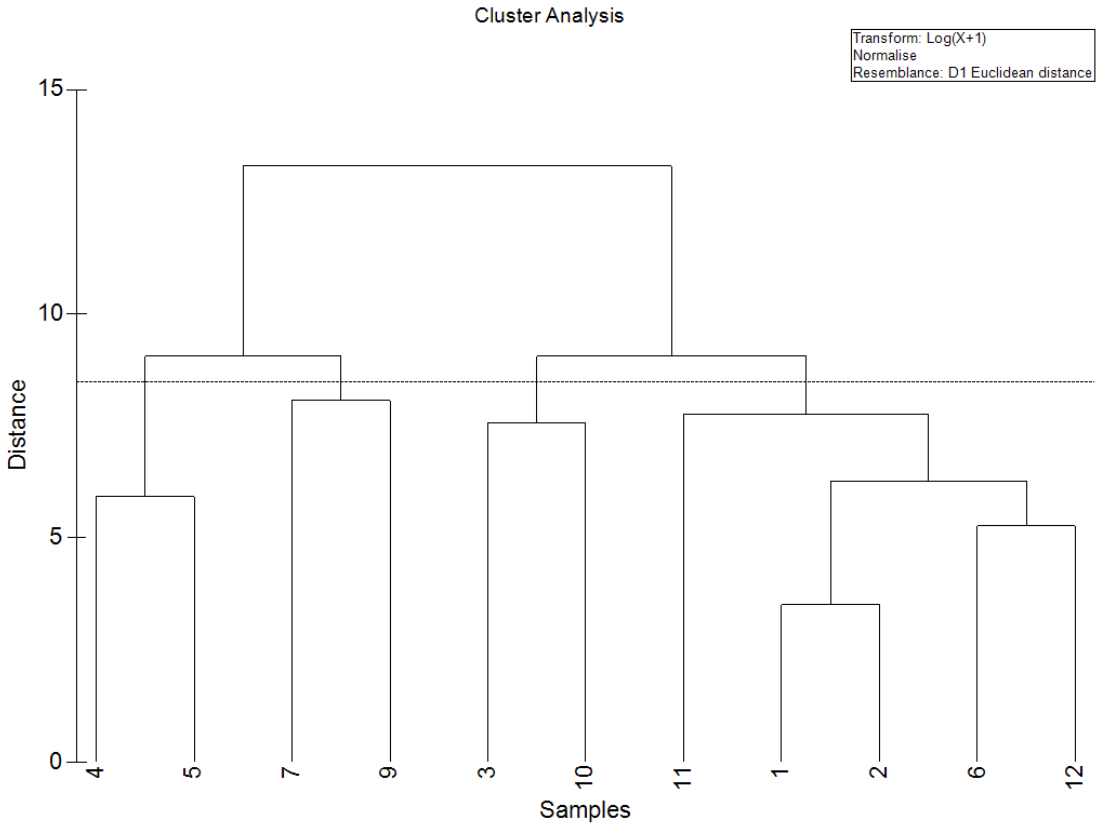

Fig. SM-2. Cluster analysis of chemical and physicochemical data for the 11 sampling sites. Group A: 4, 5; Group B: 7, 9; Group C: 10, 3; Group D: 1, 2, 6, 11, 12.

Table SM-1. Retention time and characteristics ions of all pesticides by GC-MS.

Pesticides	**Time**	**M_r**	**Diagnostic**	
	(min)		***m/z* ions**	
Dichlorvos	14.34	220	109	185
EPTC	17.52	189	128	43
Captafol	18.15	349	79	150
Mevinphos	19.49	224	127	192
Captan	21.48	299	79	107
Molinate	22.48	187	126	
Propachlor	24.12	211	120	176
Ethoprophos	24.87	243	158	126
Ethafluralin	25.21	316	292	276
Trifluralin	25.61	335	264	306
Monocrotophos	26.06	223	192	127
Dimethoate	26.95	229	87	93
Simazine	27.40	202	201	186
Atrazine	27.55	215	200	173
Diazinon	28.49	304	137	179
Pyrimethanil	28.59	198	102	129
Phosphamidon	30.16	264	127	72
M.Parathion	30.84	269	160	188
Alachlor	31.01	263	263	109
Prometryne	31.51	241	241	184
Fenitrothion	32.18	277	277	125
Malathion	32.79	330	173	125
Metolachlor	32.78	283	162	238
Fenthion	33.37	278	278	169
E.Parathion	33.55	291	291	109
Dicofol	33.95	368	139	251
Penconazole	35.57	248	192	157
Phenthoate	36.29	274	79	125
Triadimenol	36.55	168	112	128
Methidathion	37.20	302	85	145
α-Endosulfan	38.15	404	195	241
Fenamiphos	39.16	303	303	154
Muchlobutanil	40.79	288	179	150
Oxyfluorfen	41.19	361	252	331
Endrin	41.90	317	263	281
β-Endosulfan	42.48	404	195	339
Ethion	43.00	231	203	97
Benalaxyl	43.75	325	148	91
Carbofenothion	43.85	342	157	199

Table SM-2. Properties and physicochemical characteristics of the pesticides detected in Evrotas River Basin.

Pesticide Name	*Molecular Mass (g/mol)*	*Solubility* (mg/L)*	*Solubility in water***	*t1/2 in Water (Days)*	*t1/2 in Soil (Days)*	*Koc (mg/L)*	*BCF*
Triadimenol	295.76	72	Moderate	n/a	250	273	21
Penconazole	284.18	73	Moderate	>706	138	2205	320
Alachlor	269.77	240	Moderate	n/a	8	124	39
Metolachlor	283.8	530	High	97	15-70	200	68.8
Dimethoate	229.26	39800	High	8	20	30	n/a
Malathion	330.36	148	Moderate	< 1 wk	1-25	217	103
Fenthion	278.33	4.2	Low	2 wks	4-6 wks	1500	226
Carbofenothion	342.9	0.34	Low	n/a	>6 months	50000	1000
Monocrotophos	223.16	818000	High	n/a	7	19	n/a

*Solubility in water at 20ºC
t1/2: Half-life of a compound.
** Solubility in water - <=50 low; 50-500 moderate; >500 high.
Koc: Organic-carbon sorption constant.
BCF: Bio-concentration factor. According to REACh chemicals are "very bioaccumulative" if their BCF is larger than 5,000 and "very persistent" if their half-life in marine water or fresh water exceeds 60 days or their half-life in marine or freshwater sediment exceeds 180 days.
Note: Data were assembled by the Pesticide Properties Database (http://sitem.herts.ac.uk/aeru/footprint/en/index.htm) and The Extension Toxicology Network (http://extoxnet.orst.edu).

Table SM-3. Residual levels of pesticides (μg/L) detected in river water samples

Site	*Sampling period*	*Triadimenol*	*Penconazole*	*Alachlor*	*Metolachlor*	*Dimethoate*	*Malathion*	*Fenthion*
1	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7	Feb-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Jun-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Nov-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mar-08	n.d.	**0.222**	n.d.	n.d.	n.d.	n.d.	n.d.
8	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9	Feb-07	n.d.	**0.748**	n.d.	n.d.	n.d.	n.d.	n.d.
	Jun-07	n.d.	**0.198**	n.d.	n.d.	n.d.	n.d.	n.d.
	Nov-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mar-08	**0.098**	n.d.	n.d.	n.d.	**5.288**	n.d.	n.d.
10	Feb-07	n.d.	**0.546**	**0.124**	**0.314**	n.d.	**0.548**	**0.06**
	Jun-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Nov-07	n.d.	**0.312**	n.d.	n.d.	n.d.	n.d.	n.d.
	Mar-08	n.d.	**0.071**	n.d.	n.d.	n.d.	n.d.	n.d.
11	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

(n.d = Not detected)

Table SM- 4. Residual levels of pesticides (ng/g-dw) detected in river sediment samples (n.d. Not detected)

Site	*Sampling period*	*Triadimenol*	*Penconazole*	*Metolachlor*	*Monocrotophos*	*Malathion*	*Carbofenothion*	*Fenthion*
1	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3	Feb-07	**32.918**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Jun-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Nov-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mar-08	**162.714**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	Feb-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Jun-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Nov-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mar-08	**238.484**	n.d.	n.d.	**296.547**	n.d.	n.d.	n.d.
5	Feb-07	n.d.	**905.937**	**335.975**	n.d.	n.d.	**83.936**	**818.062**
	Jun-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Nov-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mar-08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7	Feb-07	**65.96**	n.d.	**166.098**	n.d.	**308.761**	n.d.	n.d.
	Jun-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Nov-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mar-08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10	Feb-07	**51.944**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Jun-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Nov-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mar-08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
11	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12	All dates	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table SM-5. Pesticide toxicity data for different aquatic organisms and trophic levels.

	Fish		***Aquatic invertebrates***			***Algae***
	Oncorhynchus mykiss		*Daphnia magna*		*Chironomus riparius*	*Pseudokirchneriella subcapitata*
Pesticide Name	Acute 96 hour LC_{50} (µg/L)	Chronic 21 day NOEC (µg/L)	Acute 48 hour EC_{50} (µg/L)	Chronic 21 day NOEC (µg/L)	Acute 24 hour EC_{50} (µg/L)	Acute 72 hour EC_{50} (µg/L)
Triadimenol	21300	3130	51000	100	-	9600
Penconazole	1130	320	6750	60	-	2000
Alachlor	1800	190	10000	220	27500	966
Metolachlor	3900	-	23500	707	-	57100
Dimethoate	30200	400	2000	40	481*	90400
Malathion	18	91	0.7	0.15	0.34	13000
Fenthion	800	-	5.7	-	5.62	1790
Carbofenothion	56	6.3	-	-	-	-
Monocrotophos	7000	-	23	-	-	-

***** Acute 48 hour EC_{50}
NOEC: No observed effect concentration
Note: Data were assembled by the Pesticide Properties Database (http://sitem.herts.ac.uk/aeru/footprint/en/index.htm), The Extension Toxicology Network (http://extoxnet.orst.edu) and the PAN Pesticide Database (http://www.pesticideinfo.org)

Table SM-6. PCA results of all chemical and physicochemical variables. For clarity reasons only high loadings are presented.

PC Axis	*Eigenvalues*	*%Variation*	*Cum.%Variation*
1	15.9	42.9	42.9
2	4.9	13.2	56.1
3	4.32	11.7	67.8
Variable	PC1	PC2	PC3
DO	**0.23**	0.081	-0.064
T	**-0.193**	0.138	0.087
Conductivity	**-0.195**	**0.217**	0.091
COD	**-0.198**	**-0.232**	-0.01
BOD_5	**-0.197**	**-0.233**	-0.013
TSS	**-0.188**	**-0.249**	0.062
Ca	-0.067	**0.369**	0.108
Na	**-0.23**	-0.031	-0.019
K	**-0.188**	**-0.21**	0.097
HCO_3	**-0.189**	**0.21**	0.036
Cl	**-0.202**	-0.002	-0.092
NO_3	**-0.186**	0.209	0.032
NO_2	**-0.197**	-0.019	0.233
NH_4	**-0.197**	0.016	-0.019
PO_4	**-0.202**	0.006	-0.029
Total N	**-0.216**	0.058	0.031
Total P	**-0.175**	**-0.25**	-0.065
N-TOTAL	**-0.202**	0.178	0.038
Water Hardness	-0.119	**0.312**	0.149
Total Phenols	-0.022	**-0.329**	-0.104
Water Discharge	-0.06	**-0.233**	0.145
Cr	0.166	0.015	**0.223**
Mn	**0.233**	-0.052	-0.005
Co	**0.205**	-0.074	0.152
Ni	**0.178**	-0.022	**0.203**
Cu	0.103	-0.063	**0.427**
Zn	0.022	-0.138	**0.431**
As	**0.218**	-0.04	0.143
Ba	-0.055	**-0.213**	-0.017
Mo	0.027	0.07	**0.413**
Pb	-0.017	-0.111	**0.324**