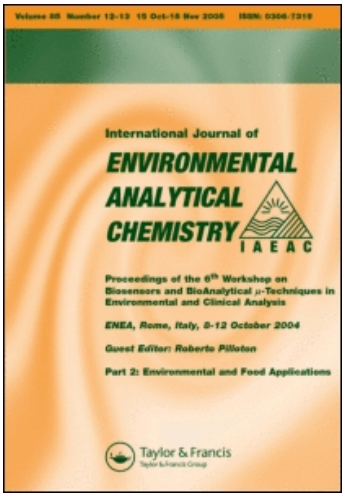

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Spectrophotometric Determination of Phenols and Cyanides After Distillation from Natural Waters

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SPECTROPHOTOMETRIC DETERMINATION OF PHENOLS AND CYANIDES AFTER DISTILLATION FROM NATURAL WATERS

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Total phenols were determined by molecular spectrophotometry, after distillation, complexation with 4-aminoantipyrine and extraction into chloroform. Cyanides were also determined spectrophotometrically after distillation from the acidified samples, and complexation in moderate acidic solution with barbituric acid. The dynamic ranges were 0 – 100 μg L^{-1} for total phenols and 0 – 30 μg L^{-1} for cyanides. The above methods were applied in the analysis of river, lake and stream waters collected from Northern Greece. The seasonal and spatial variation of concentrations was evaluated by two-way ANOVA. Background levels (4 – 12 μg L^{-1} for total phenols and 0.3 – 3 μg L^{-1} for cyanides), were found in almost all surface waters, with some exceptions.

Keywords: Phenols; cyanides; distillation; spectrophotometry; natural waters

INTRODUCTION

The release of phenols and cyanides in the environment, caused by human activities, is of great concern, because both types of compounds have widespread uses in agriculture and industry. Furthermore, many of the phenolic substances and some metal cyanide complexes appear to be very persistent in the aquatic environment, although some phenols are more rapidly biodegraded when trapped in sediments.

Phenols have been used in manufacture of polymers, antioxidants, dyes, drugs, pesticides, petroleum additives, and wood preservatives. In the form of chlorophenols, they are detected in the effluents from sewage chlorination plants, wood

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and paper industries, petroleum refineries, hydrocarbon production units, as well as in a large number of chemical industries [1, 2].

Cyanide compounds have been used in metal finishing processes and during heat treatment process in steel production. They are constituents of wastes from electroplating plants, cooling tower blowdown, gas scrub waters, coke plants, and blast furnace operations [3]. They form stable complexes with Fe, Cd, Cu, which are perhaps more toxic to organisms than equal concentrations of NaCN [4].

The industrial treatment of waste waters for phenol removal, includes ozonation, chlorination, superchlorination, or adsorption in activated carbon. Alkaline chlorination is also the common treatment for cyanide removal from industrial wastewater. By this process, cyanides are easily oxidized by chlorine or ozone to less toxic CNO^-, and furthermore to N_2 [3]. In general, non-substituted phenol, monochlorophenols and alkylphenols are oxidized by molecular oxygen, even in the natural aquatic environment, whereas, highly substituted chlorinated phenols and nitrophenols are more resistant to oxidation under natural environmental conditions [1].

On the other hand, the pollution of drinking water, either of ground or stream origin by cyanides, consists a severe health hazard because of cyanides' toxicity, high solubility, mobility, and similar behavior to Cl^- and NO_3^-. Toxicity of cyanides is due to the well-known reaction of liberation of HCN from acidic media [5].

The legislation of European Union [6], concerning dangerous substances discharged into the aquatic environment, requires that the maximum admissible concentration of total phenols in drinking water, should be 0.5 $\mu g\ L^{-1}$. In natural and bathing water this level changes to 5 $\mu g\ L^{-1}$. However, concentrations up to 900 $\mu g\ L^{-1}$ have been encountered in heavily polluted rivers [7,8]. In addition, US EPA'S list of priority pollutants includes eleven phenolic compounds, such as 4-chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2-nitrophenol, trichlorophenols (TCP), pentachlorophenols (PCP), etc. [9]. Many studies have concluded that chlorinated phenolic substances are significantly more toxic than non-substituted phenols to most aquatic organisms. For most fishes, the tolerance levels are described by $LD_{50} = 1 - 10\ \mu g\ L^{-1}$ for phenol, and $LD_{50} = 0.01 - 1\ \mu g\ L^{-1}$ for polychlorinated phenols. The bioaccummulation in food chain, as well as the adverse effect of the chlorinated phenols on odor and taste of potable water, have been reported [1]. The permissible level of cyanides in drinking water, according to EU directive is 50 $\mu g\ L^{-1}$ [10].

As a consequence, the environmental pollution studies of natural rivers are increasingly dealing with the analysis of such compounds. A great number of very efficient analytical techniques are available today, either for trace analysis

in the laboratory [11] or for field analysis and monitoring [12, 13]. Many of them are instrumental methods like spectrophotometry [14, 15], liquid chromatography [16 – 23], ion selective electrodes, etc., but very sensitive biosensors have also been introduced and applied for such purpose [17, 22]. The majority of the above methods make use of separation procedures like distillation [23] and preconcentration procedures steps like solid phase extraction [21, 22], in order to eliminate a number of interferences or increase the relative concentration of the analyte [24, 25, 26].

In this work, total recoverable phenols and cyanides after distillation were determined in a number of 29 sampling sites (rivers, streams and lakes) which are located in Northern Greece, in the frame of an extended biannual survey. The analytes were determined by using suitable distillation procedures and subsequent UV-VIS spectrophotometry. The aim of the study was to estimate the profile of these pollutants in surface waters that are used as natural receivers of waste waters, and to investigate their local and seasonal distribution. Two way analysis of variance was used to test the significance of spatial and seasonal variation for each aquatic system separately [27].

EXPERIMENTAL

Sampling procedures

Surface water samples were collected monthly, between February 1997 and December 1998. 29 sampling sites were located in five major rivers (Aliakmon, Axios, Loudias, Strymon, Gallikos), five tributaries (Tripotamos, Koutikas, Arapitsa, Edeseos, Sakolevas), two streams and ditches (Soulou, Ditch No 66), and three lakes (Volvi, Koronia, Doirani), as they are shown in Fig. 1.

The samples for phenol determination were collected in 1 L amber glass bottles, acidified with 4 mL of concentrated H_2SO_4 (18 mol L^{-1}) and stored in the refrigerator (4 °C) not more than 2 days. In some samples obtained from polluted small streams, chlorine was present, thus $FeSO_4$ was added to reduce it, and avoid the partial oxidation of phenols.

The samples for cyanides determination were collected in 1 L plastic bottles and stored in dark not more than 1 day. Sulfides were not present in significant concentrations and so Pb^{2+} ions were not necessary to prevent cyanide conversion to thiocyanates. Traces of free chlorine were detected in some of the small streams and sodium thiosulphate was added in these samples. Medium levels of carbonates were also detected, which produce free CO_2, and eliminate the alka-

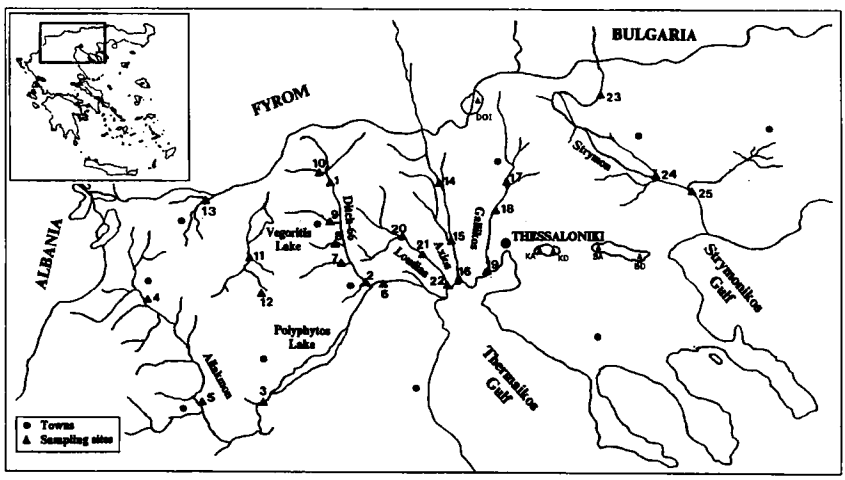

FIGURE 1 Map of Northern Greece, showing the studied area and the locations of the sampling sites

line strength of the NaOH trap. Thus, $Ca(OH)_2$ was added for sample preservation until pH = 12, instead of NaOH, in order to trap CO_3^{2-} as $CaCO_3$. The carbonate precipitate was then filtered, and cyanides were determined in the filtrate. However, by this procedure some kinds of insoluble cyanide substances are excluded from subsequent determination.

Reagents and solutions

All chemicals were of analytical reagent grade (Merck (Darmstadt, Germany), unless stated otherwise. All standard and sample solutions were made up with double de-ionized water.

Stock phenol solution was prepared by dissolving 1 g of accurately weighed phenol in 1 L of freshly boiled and cooled distilled water. From this solution, standard solutions were prepared by appropriate dilutions with the same type of water, with final concentration ranging between 0 and 200 $\mu g\ L^{-1}$.

Buffer solution with pH = 10.0 for phenols, was prepared by dissolving 70 g NH_4Cl in water and adding suitable amount of NH_4OH in 1 L of distilled water. Solutions containing 2.0 g of 4-aminoantipyrine (Sigma) in 100 mL distilled water and 8.0 g $K_3Fe(CN)_6$ (Sigma) in 100 mL distilled water were prepared daily, as complexing reagents for phenol extraction into chloroform and subsequent spectrophotometric determination.

Stock cyanides solution was prepared by dissolving 1 g of accurately weighed potassium cyanide in boiled and cooled distilled water. Dilute standard solutions were prepared by appropriate dilutions of the stock, in order to obtain a series of standards with concentrations ranging between 0 – 50 $\mu g\ L^{-1}$.

The absorber solution in the trap for cyanide collection was 0.04 mol L^{-1} NaOH. The solution of $MgCl_2$ (Riedel de Haan) contained 510 g L^{-1}. Buffer solution with pH = 5.0 was prepared by dissolving 820 g $CH_3COONa{\cdot}3H_2O$ in a small amount of water, adding a suitable amount of glacial CH_3COOH and diluting to 1 L with distilled water. Solutions containing 1.0 g chloramine T (Riedel de Haan) in 100 mL distilled water, and 15.0 g barbituric acid (Sigma) with 75 mL pyridine in 250 mL dilute hydrochloric acid, were prepared fresh daily, as complexing reagents.

Steam – distillation and spectrophotometric determination of phenols

The analytical procedure was similar to that recommended by ASTM [24] and APHA [25] with the modifications described below. An aliquot of 300 mL of the sample was transferred in a round bottom flask and a side condenser was fitted to the flask. The solution was heated by a mantle until 275 mL of distillate were collected. Addition of some 50 mL of distilled water in the flask was followed, to finish the steam-distillation until 300 mL of total distillate volume was collected.

This procedure ensures the maximum distillation of volatile phenols, even of a number of chlorine-substituted ones. The distillate was transferred quantitatively to a separation funnel, 10 mL of the ammonium buffer were added, followed by 2 mL of 4-aminoantipyrine and 2 mL of ferricyanide solution. After exactly 10 min of colour development, the yellow complex formed, was finally extracted into 20 mL of chloroform, by shaking the separatory funnel 20 times twice. The organic layer was dried by passing through anhydrous Na_2SO_4, and the absorbance of the extract was measured at 460 nm using a 4-cm cell against reagent blank, with a Varian DMS 100S double beam UV-VIS spectrophotometer.

A series of standard solutions was analyzed by exactly the same procedure yielding the calibration curve. The recovery was tested on a mixed sample, which was separated in different portions, and to these portions, known amounts of phenols were added.

Reflux – distillation and spectrophotometric determination of cyanides

The analytical procedure was based on that recommended by ASTM [26] and APHA [25] with some modifications. A volume of 250 mL of the sample was

transferred in round bottom flask with a side thistle tube for reagent additions, and a vertical condenser was fitted to the flask. The absorber trap, with gas dispersion tube inside, was fitted at the top of the condenser and vacuum was applied to the trap. The trap consisted from 15 mL of 0.04 M NaOH.

The solution was heated by a mantle for one hour and the liberated HCN was collected to the alkaline solution. 25 mL of the $MgCl_2$ solution were added as catalyst during the acidic distillation, in order to destroy complex ferrous and other stable metal complex cyanides and to form easily destructible magnesium cyanide. Finally, 25 mL of 9 mol L^{-1} H_2SO_4 were added in the flask to acidify the solution.

The pH of the absorber solution was adjusted to 4.5 – 5.0 by addition of 1 mL acetate buffer. In order to determine even the amenable to chlorination cyanides, chloramine T reagent was added before colour development. Cyanides were determined by development of the violet complex with barbituric acid and pyridine, exactly 8 min after addition of 1 mL of this reagent. The absorbance was measured at 578 nm with a 4-cm cell against reagent blank, using a Varian DMS 100S double beam UV-VIS spectrophotometer. A series of standard solutions was analyzed by the same procedure and the calibration curve obtained was used for the calculation of unknown concentrations. The recovery was tested on a mixed sample, which was separated in different portions, and to these portions, known amounts of cyanides were added.

RESULTS AND DISCUSSION

Determination of total phenols

The calibration curve obtained by a series of aqueous standards of phenol was linear up to 100 μg L^{-1}, although the next curved part showed also good reproducibility. The regression equation was:

$$A = 0.048 + 0.0106[\text{phenol}]$$

and the correlation coefficient was $r = 0.9981$.

Recovery tests were tried on five samples of double distilled water, which were spiked with phenol at final concentration 20 μg L^{-1}. The mean recovery was found to be 91%, while the detection limit, calculated using the 3s criterion, was 1 μg L^{-1}. The repeatability, as expressed by relative standard deviation of these analyses was $s_r = 6.3$ %, and the reproducibility between days was less than 10 %. This precision could be achieved by keeping identical time intervals for mixing of reagents, colour development and solvent extraction.

In Table I, the seasonal means (each seasonal mean value was calculated from 6 months) in each site and also the overall biannual means (from 24 months) were listed. The overall biannual mean value for each sampling station is presented together with the relative standard deviation (s_r) observed. It can be seen that, in all three lakes, the lower concentrations were appeared during autumn. River Tripotamos, which is a tributary of Aliakmon, was found to permanently contain higher concentrations of phenols. The majority of the other studied rivers are found to contain background levels (4 – 12 μg L^{-1}) and the overall relative standard deviation ranged between 5 – 15 %. This low variation proved that the major portion of total phenolic load is of natural origin (e.g. degradation of lignin).

TABLE I Seasonal (3 months × 2 years) and overall (24 months) mean values of phenols (μg L^{-1}) in sampling stations

code	*Name / Type*	*Seasonal mean*				*Overall*	
		Winter	*Spring*	*Summer*	*Autumn*	*Mean*	*100 s_r*
1	Ditch 66 / Stream	11.1	10.5	10.1	11.3	10.8	8.6
2	Ditch 66 / Stream	15.4	10.4	12.2	11.2	12.2	11.1
3	Aliakmon / River	9.1	7.3	9.6	8.0	8.5	10.8
4	Aliakmon / River	13.4	9.8	15.8	14.1	13.3	11.7
5	Aliakmon / River	12.2	11.4	12.4	9.4	11.3	10.5
6	Aliakmon / River	12.4	9.6	12.7	10.2	11.2	14.0
7	Tripotamos / River	18.2	26.4	21.7	12.2	19.4	13.9
8	Koutikas / River	9.3	7.3	12.8	11.1	10.2	18.0
9	Arapitsa / River	13.1	10.1	13.6	11.6	12.0	16.3
10	Fdeseos / River	11.2	12.9	13.6	9.9	11.9	12.5
11	Soulou / Stream	10.2	9.5	15.8	14.8	12.6	15.0
12	Soulou / Stream	13.5	11.5	15.3	13.0	13.3	8.9
13	Sakolevas / River	10.8	12.1	11.9	9.5	11.1	9.5
14	Axios / River	10.9	13.0	8.2	9.3	10.4	11.3
15	Axios / River	9.8	11.7	12.9	8.1	10.7	12.0
16	Axios / River	11.8	10.0	13.9	9.2	11.2	14.4
17	Gallikos / River	11.3	9.4	–	9.9	10.2	10.7
18	Gallikos / River	11.6	7.2	20.8	13.8	11.9	14.0

code	*Name / Type*	*Seasonal mean*				*Overall*	
		Winter	*Spring*	*Summer*	*Autumn*	*Mean*	*100 s_r*
20	Loudias / River	10.6	13.5	15.6	11.1	12.8	11.9
21	Loudias / River	13.1	13.0	20.4	13.0	15.0	13.1
22	Loudias / River	10.1	12.6	16.9	9.7	12.4	9.9
23	Strymon / River	10.5	6.8	14.9	14.7	11.7	12.9
24	Strymon / River	12.4	11.1	12.0	11.6	11.8	9.2
25	Strymon / River	17.4	9.4	13.2	12.6	13.2	8.5
BA	Volvi / Lake	11.8	9.3	7.9	6.3	8.2	16.7
BD	Volvi / Lake	11.6	8.7	6.3	4.1	6.9	15.9
DOI	Doirani / Lake	13.9	13.3	4.5	5.9	8.0	22.4
KA	Koronia / Lake	18.2	12.3	17.0	8.2	13.2	16.1
KD	Koronia / Lake	11.5	12.5	15.3	6.2	12.3	15.4

Correlation analysis was applied separately for each group of sampling sites. The correlation matrix of the raw data was consisted from all Pearson's correlation coefficients calculated between the sampling sites of each specific sampling region. It was proved that not good correlation exists between the sampling sites, even in the same river. Poor correlation ($r = 0.7 - 0.9$) was observed only in three cases, namely Aliakmon, Loudias and Axios. However it is worth to mention, that these rivers have sufficiently high flow rate throughout the year, thus many chemical parameters show significant correlation along the river. This is not true for tributaries and streams.

The results of two-way analysis of variance (ANOVA) performed for each group of sampling sites are listed in Table II. The columns corresponded to seasonal means and the rows to the sampling sites along the specified group. The experimental values of *F*ratio (F_{exp}), were compared to critical ones (F_{crit}), for 95 % significance level. Not significant effect is expressed with minus symbol (-) while significant effect is symbolized with the plus symbol (+). The results, showed that, for rivers Aliakmon and Loudias, both seasonal and local variations were significant. For the majority of other sampling sites no significant variation was observed. However, in almost all sampling regions, the seasonal variation was an order of magnitude greater than the spatial variation. This is caused to the fact that these two rivers receive increased amounts of land drainage, thus a significant variation of phenolic compounds is expected.

TABLE II Results of Analysis of variance for phenols at a significance level of 95%

Aquatic system	*Source of Variation*	F_{exp}	F_{crit}	*Result*
Ditch 66 stream	Spatial	2.100152	10.12796	–
	Seasonal	1.240053	9.276619	–
Aliakmon river	Spatial	11.13435	3.862539	++
	Seasonal	5.396285	3.862539	+
Soulou stream	Spatial	0.459957	10.12796	–
	Seasonal	3.617377	9.276619	–
Axios river	Spatial	0.209714	5.143249	–
	Seasonal	1.259411	4.757055	–
Loudias river	Spatial	5.56936	5.143249	+
	Seasonal	19.37355	4.757055	++
Strymon river	Spatial	0.411902	5.143249	–
	Seasonal	2.006382	4.757055	–
Volvi lake	Spatial	6.139679	10.12796	–
	Seasonal	36.4186	9.276619	++
Koronia lake	Spatial	3.023247	10.12796	–
	Seasonal	7.259073	9.276619	–

Determination of total cyanides

The dynamic range of the calibration curve obtained by a series of aqueous standards of cyanides was linear up to 30 μg L^{-1}. The regression equation was:

$$A = 0.033 + 0.0687[\text{tot } CN^-]$$

and the correlation coefficient was $r = 0.9996$.

Recovery tests were tried on six samples of double distilled water, which were spiked with cyanide at final concentration 10 μg L^{-1}. The mean recovery was found to be 94 %, and the detection limit (3s criterion) was 0.2 μg L^{-1}. The repeatability was good ($s_r = 6.5$ %) and the reproducibility between days was less than 8 %.

In Table III, the seasonal means in each site, the overall biannual means, and the relative standard deviation for cyanides, were also calculated as described in the case of phenols. The calculation for the seasonal means in each site showed that in river Aliakmon lower concentrations were observed in comparison to its tributaries: Tripotamos, Koutikas, Arapitsa and Edeseos.

TABLE III Seasonal (3 months × 2 years) and overall (24 months) mean values of cyanides (μg L^{-1}) in sampling stations

code	*Name / Type*	*Seasonal mean*				*Overall*	
		Winter	*Spring*	*Summer*	*Autumn*	*Mean*	*100 s_r*
1	Ditch 66 / Stream	2.7	3.1	2.9	3.4	3.0	11.0
2	Ditch 66 / Stream	4.2	9.3	7.0	5.0	6.5	14.2
3	Aliakmon / River	1.1	2.4	1.1	1.3	1.5	24.0
4	Aliakmon / River	2.6	0.9	1.9	1.4	1.7	14.7
5	Aliakmon / River	3.2	1.3	3.4	2.8	2.6	17.7
6	Aliakmon / River	2.0	3.0	3.9	2.4	2.8	12.1
7	Tripotamos / River	2.7	5.1	5.3	10.8	6.2	22.6
8	Koutikas / River	2.0	5.8	3.2	6.7	4.5	18.0
9	Arapitsa / River	3.4	2.9	4.5	5.2	4.0	11.5
10	Fdeseos / River	4.0	10.9	5.0	5.5	6.2	17.7
11	Soulou / Stream	13.8	21.7	16.5	8.8	15.2	11.9
12	Soulou / Stream	31.4	26.7	23.1	11.1	22.7	20.1
13	Sakolevas / River	1.8	4.9	3.6	3.1	3.4	19.1
14	Axios / River	5.9	4.4	2.2	6.5	4.8	25.4
15	Axios / River	1.7	1.5	2.4	3.1	2.2	21.4
16	Axios / River	1.3	1.4	2.4	1.7	1.7	11.2
17	Gallikos / River	4.9	8.5	–	5.4	6.6	31.8
18	Gallikos / River	1.3	3.7	1.9	1.6	2.2	18.2
20	Loudias / River	4.2	3.6	3.6	6.1	4.4	11.6
21	Loudias / River	4.4	3.8	2.8	5.4	4.1	12.4
22	Loudias / River	5.1	4.0	5.9	4.5	4.8	13.8
23	Strymon / River	1.6	1.1	0.8	0.7	1.0	17.0
24	Strymon / River	1.4	3.8	1.8	6.7	3.4	22.4
25	Strymon / River	3.5	4.9	2.4	6.4	4.3	12.8
BA	Volvi / Lake	1.0	0.9	1.0	1.9	1.2	18.3
BD	Volvi / Lake	0.7	1.1	0.8	1.8	1.2	16.7
DOI	Doirani / Lake	1.2	0.8	1.0	1.4	1.1	17.3
KA	Koronia / Lake	1.3	10.2	1.6	1.2	4.7	74.0
KD	Koronia / Lake	0.5	1.1	1.5	2.3	1.3	19.2

These tributaries as well as Ditch 66 receive industrial wastewater's from neighboring medium sized industries. The group of Aliakmon sampling sites showed low concentrations near to the origins of the river (sampling sites 3 and 4). On the other hand, Soulou stream, which receives effluents from a large power station, was found to permanently contain higher concentrations of cyanides ranging between 8 – 32 $\mu g\ L^{-1}$. Rivers Axios, Loudias and Strymon had the same levels of cyanides (< 5 $\mu g\ L^{-1}$) as well as the three lakes studied. The biannual relative standard deviation was high, ranging between 10 – 35 %. Concentration levels for total cyanides between 0.5 – 3 $\mu g\ L^{-1}$are considered as background levels.

The correlation analysis of the raw data, among the sampling sites of each specific region, proved that all Pearson's correlation coefficients were not significant ($r < 0.6$).

TABLE IV Results of Analysis of variance for cyanides at a significance level of 95%

Aquatic system	*Source of Variation*	F_{exp}	F_{crit}	*Result*
Ditch 66 stream	Spatial	9.17061	10.12796	–
	Seasonal	1.114721	9.276619	–
Aliakmon river	Spatial	2.516492	3.862539	–
	Seasonal	0.505525	3.862539	–
Soulou stream	Spatial	5.552209	10.12796	–
	Seasonal	3.626352	9.276619	–
Axios river	Spatial	6.978468	5.143249	+
	Seasonal	0.810181	4.757055	–
Loudias river	Spatial	0.603126	5.143249	–
	Seasonal	1.432943	4.757055	–
Strymon river	Spatial	5.912339	5.143249	+
	Seasonal	2.530087	4.757055	–
Volvi lake	Spatial	0.739726	10.12796	–
	Seasonal	16.36986	9.276619	++
Koronia lake	Spatial	0.901715	10.12796	–
	Seasonal	0.871974	9.276619	–

The results of two-way analysis of variance performed for each group of sampling sites are listed in Table IV. The experimental ratios of estimated variances

(F_{exp}) were tested to critical values (F_{crit}), for significance level 95 %. The results listed in Table IV, showed that for rivers Axios and Strymon the local variations were significant, although the seasonal ones were not. For the majority of other rivers, streams and lakes no significant variation was observed. In addition, a rough estimation of the spatial variations was greater than the seasonal ones for each aquatic system.

CONCLUSIONS

The ASTM recommended spectrophotometric methods for determination of total phenols and cyanides were used, modified for lower samples volumes, with very good performance for river, stream and lake water samples. The level of total phenol concentration was low to medium (< 15 $\mu g\ L^{-1}$) in all sampling regions, with minor exceptions. These levels are common at non-polluted rivers and lakes. The effect of the season was greater than that of the site, along the rivers or lakes, although in the majority of the studied regions, this effect was not significant at 95 % probability. The level of cyanide concentration was relatively low (< 7 $\mu g\ L^{-1}$) in almost all sampling regions, with one exception, and the effect of the sampling site was greater than that of the season. The level of total cyanides were similar to these reported from not heavily polluted areas.

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