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Statistical analysis techniques for the assessment of the toxicity of raw surface water intended for human consumption—a case study

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ABSTRACT

During a seven-year time period (2000–2006), the data obtained by the monthly toxicological analyses of raw (untreated) water from the main reservoirs supply sources of Athens, the Greek capital with more than 4 million inhabitants, had been registered using a bioluminescence test, which is based on the correlation between toxicity of the water sample and its effects on the light intensity of marine bacteria *Vibrio fischeri*, measured by the bioluminometer Microtox®. The statistical analysis of the water toxicity over a long time period can provide important and useful information for the management and quality control of the water resources. However, due to the inherent characteristics of the water quality data, sophisticated statistical techniques for their analysis may be required. In this study, the available data were subjected to exhaustive statistical analysis by the usage of specialized nonparametric statistical methods. A small amount of autocorrelation was observed for each time series implying that corrective actions should be made in the statistical analyses. The overall performance of the raw waters was apparently nontoxic. The study of seasonality for each reservoir resulted in no statistical significance. Trend analysis resulted in no statistically significant upward or downward trends. Moreover, no statistically significant differences of the central tendency measures between the reservoirs were observed.

Keywords: Nonparametric statistical methods; Trend analysis; Seasonality; Autocorrelation; Microtox® test.

1. Introduction

Surface water quality data may exhibit high variability for many parameters. Therefore, a larger sample size, based on the aggregation of many years of monitoring results, increases the probability that the distribution of a water quality parameter is appropriately represented, while reducing the influence of anomalous observations. When calculating descriptive statistics or testing for differences using data from routine monitoring programs, it is recommended that

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three to five years of data be aggregated (pooled) [1]. However, in this case, it is assumed that there have been no major changes in the water quality parameter within the years that are being aggregated. Generally, it is acceptable to aggregate three to five years of data. Trend analysis should be used to confirm the absence of a trend if longer periods of data are aggregated.

During the recent decades, there has been constantly increasing concern for the quality of surface water indented for human consumption. Ecotoxicological monitoring is playing an increasingly important role in the evaluation of water quality [2–5]. Ecotoxicology deals with potentially harmful effects of man-made chemicals, released into biosphere, on organisms in the receiving environments. Traditionally the necessary tests involve the use of fish. However, the use of fish in toxicity testing is expensive and time-consuming, so alternatives are required. Microbial tests have similar complex biochemical functions to those of higher organisms. Generally, it is assumed that there is a good correlation between the toxicity determinations made by using *Vibrio fischeri* and those made by using higher organisms such as *Daphnia* and rainbow trout [6].

The Water framework Directive 2000/60/EC refers to the determination of ecological quality of surface waters in addition to the chemical profile. Some European countries, such as Italy and Spain, have legislations that ask for ecotoxicological analyses also [7]. In Greece, only the Athens Water Supply & Sewerage Company (EYDAP SA) systematically measured the toxicity of water during the time period 2000–2006. The Athens Water Supply & Sewerage Company (EYDAP SA) is the water supplier of Athens, the Greek capital with more than 4 million inhabitants. The main supply sources of raw water are the Mornos and Marathon reservoirs (artificial lakes).

The aim of this study was to study the toxicity of raw (untreated) water in the aforementioned reservoirs for a long time period. Particularly, differences in population (differences between sites), monotonic trends, and seasonality were examined. Since the distribution of surface water quality data is frequently skewed by outliers, the assumptions of parametric statistical tests are often violated. Therefore, more sophisticated techniques including the consideration of nonparametric statistical approaches are required [8–10]. Nonparametric statistics do not assume a particular form of distribution (i.e. normal distribution), and they can handle outliers that are common in water quality data. In this study, the exploration of the specific characteristics of the available data imposed the appropriate selection and adjustment of the statistical methods that were used in order to gain the ultimate information.

In recent years, there has been increased interest in analyzing trends in water quality parameters. In the scientific literature, there are several studies on surface waters e.g. [11–14]. However, to the best of our knowledge, studies are mainly limited to physicochemical and chemical parameters of water, whereas data obtained by toxicological analysis have not been explored so far. Moreover, analysis of trends in any water quality parameters of Greek surface waters intended for human consumption has not been implemented so far.

2. Materials and methods

2.1. Data description

Raw water samples from the reservoirs had been collected on a monthly basis, over a seven-year period (2000–2006). The samples were collected from the water supply towers of the Mornos and Marathon reservoirs. The data obtained by the toxicological analyses had been registered using a bioluminescence test, which is based on the correlation between toxicity of the water sample and its effects on the light intensity of marine bacteria *V. fischeri* (former *Photobacterium phosphoreum*), measured by the bioluminometer Microtox®. The bacteria *V. fischeri* have been used in many tests for the toxicity of surface waters (e.g. [4,15–17]). Toxic substances causing a disturbance to the normal metabolism of the bacterium result in a reduction of light output. The degree of toxicity is proportional to the measured light loss.

Samples were adjusted to contain 2% w/v NaCl, which provides osmotic protection for the marine bioassay organism. The inhibitory effect of the test sample after the contact time of 30 min (H_{30}) is an expression of toxicity. This expression is given by the following formula:

$$H_t = [(I_{ct} - I_{Tt})/I_{ct}] \times 100$$

where H_t is the inhibitory effect of the sample after the contact time of 30 min in %, I_{Tt} is the luminescence intensity of sample after the contact time of 30 min in relative luminescence units, and I_{ct} is the initial luminescence intensity of the control suspension in relative luminescence units.

All the tests were carried out according to standard protocols described in the Microtox Acute Toxicity Users' Guide [18]. The duplicate basic test procedure was used. In duplicate testing, data quality is improved through cross comparison. The average value from the two measurements is used in subsequent calculations.

There is not an upper limit set by legislation for the inhibitory effect of the raw water samples. However, some authors consider that if it is higher than 20% the water sample might be toxic to some extent [19,20]. In that case, a further test that is based on the determination of the sample concentration producing a 50% (EC50 value) decrease in luminescence compared to the control sample can be used for confirmation of the initial screening test.

Every effort was made to ensure that samples would be representative for the overall monthly toxicity of water. The water supply towers have served as the sampling points throughout the seven-year period, while sampling dates and conditions were representative of each month.

Even the sheer number of observations and the timescale of the survey may cancel out the effect of possibly outlying samples. Moreover, statistical techniques were used in this research to smooth out the impact of outliers. Unfortunately, sampling of water reservoirs includes field trips even in unfavorable conditions; therefore, more frequent sampling is not always an option. Should more frequent sampling data be available, average or median values could be used as a measure of the monthly toxicity status of water.

Sampling was conducted on a monthly basis during the seven-year period with consecutive samples taken approximately 30 days apart during late morning hours, between 09:00 am and 12:00 pm. Only trivial deviations from that sampling scheme were allowed, mainly as minor adjustments to guarantee that the date of sampling would be representative of each month.

The sample size ($n = 84$) is big enough in order to make safe conclusions for the period under examination and conduct trend analysis.

The initial concentration during toxicity testing was the same during all the tests. Samples were prepared at nominal 100% solution (actually 91% after Microtox Osmotic Adjustment Solution and finally 82% after addition in the reagent solution) in sets of two in series.

2.2. Statistical analyses

Nonparametric methods are considered as "recognized practice" in studies for detection trends in water quality parameters [9,10,21]. Non-parametric methods are proposed to be used systematically for detecting trends in water quality parameters, whereas the parametric methods (linear regressions) are proposed to be used only when their use, in terms of distributional assumptions, is fully justified [10,21]. Besides, the nonparametric tests, based on ranks, can be used without prior knowledge about the kind of the trend (linear or nonlinear) [22,23].

The nonparametric Mann–Kendall test for trends involves computing a statistic S and its variance Var(S). Gilbert (1987) [24] notes that a minimum sample size of 10 is normally required for the application of Mann–Kendall test.

Even for sample size $n = 10$, under the null hypothesis of the randomness of data against trend, the quantity z, computed by the following equation, is approximately standard normally distributed [23].

$$Z = \begin{cases} \dfrac{S-1}{\sqrt{Var(S)}} & , \quad \text{if } S>0 \\ 0 & , \quad \text{if } S=0 \\ \dfrac{S+1}{\sqrt{Var(S)}} & , \quad \text{if } S<0 \end{cases}$$

The existence of positive autocorrelation in the data can lead to the rejection of the null hypothesis of no trend, while the null hypothesis is actually true [25]. In order to eliminate the effect of autocorrelation, methods such as "pre-whitening" [25,26] and the most advanced "trend-free pre-whitening" [27] have been proposed. In the case of seasonal data, the seasonal Kendall test (a modified version of the Mann–Kendall test) has been proposed [28]. The seasonal Kendall Test requires a minimum of three years of monthly data or 36 data points [24]. Simulation studies have shown that when the data do not present seasonality, the seasonal Kendall test is less powerful than the Mann–Kendall test [29,30]. Therefore, the data were tested for the existence of autocorrelation and seasonality as well. Seasonality was investigated by the nonparametric Kruskall–Wallis test (nonparametric equivalent to one-way ANOVA) [31] as well as the plots of the sample autocorrelation functions. The "Kruskall–Wallis" test requires the homogeneity of variances assumption as well as the assumption of independence "within" and "between" groups.

Due to the heterogeneity of variances, as an alternative to the common nonparametric Mann–Whitney test (nonparametric equivalent to independent-measures t-test; it assumes that the shape of the data distribution is the same in each group), the nonparametric "unequal variance t-test on the ranks of the data" was used to test for differences in central tendencies between the two reservoirs [32].

Table 1
Descriptive statistics for the H_t data per reservoir

	Marathon	Mornos
Number of samples	84	84
Minimum value	−34	−33
Maximum value	20	20
Median	3.36	1.05
Arithmetic mean	2.54	−0.35
Standard deviation	8.55	10.51
Skewness	−1.004	−0.224
Standard error of Skewness	0.263	0.263
Kurtosis	3.447	0.151
Standard error of Kurtosis	0.52	0.52

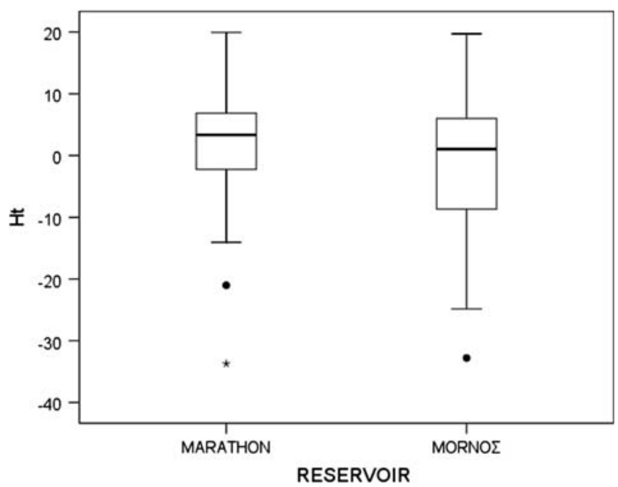

Fig. 1. Box plots of H_t per reservoir.

3. Results and discussion

3.1. Preliminary data analysis

Descriptive statistics for the H_t data per reservoir are presented in Table 1. The existence of negative values in the data can be attributed in the phenomenon of hormesis [33]. Hormesis is a biological phenomenon whereby an adaptive beneficial effect results from exposure to a low dose of a chemical agent or environmental factor that is damaging at higher doses. Therefore, in the case that the samples contain pollutants in very low concentrations, excitation of the *photobacterium* is caused, resulting in the increase of the luminescence intensity [34]. Herein, the overall performance of the raw waters under examination is apparently nontoxic.

In order to eliminate the effect of outliers (Fig. 1) and/or nonnormality, nonparametric techniques were selected for further statistical analysis. Nonparametric methods are less sensitive to outliers compared to parametric methods.

3.2. Autocorrelation and seasonality

The time series plots of the H_t values per reservoir (Fig. 2) did not present powerful clues for seasonality since they do not have obvious circular patterns. However, the existence or non existence of seasonality was further examined.

In Fig. 3, the sample autocorrelation functions of the data per reservoir are presented. The sample autocorrelation function (ACF) provides important information on the correlation between the pairs of observations that are k units of time (lags) apart. Generally, the existence of positive autocorrelation in the twelfth lag of monthly water quality data declares a circular behavior which is repeated each 12 months

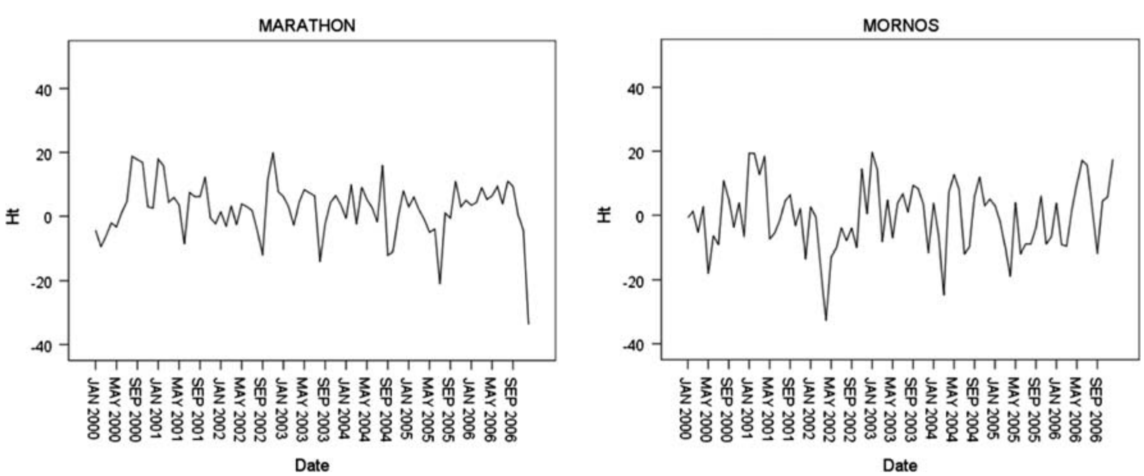

Fig. 2. Time series plots for H_t per reservoir.

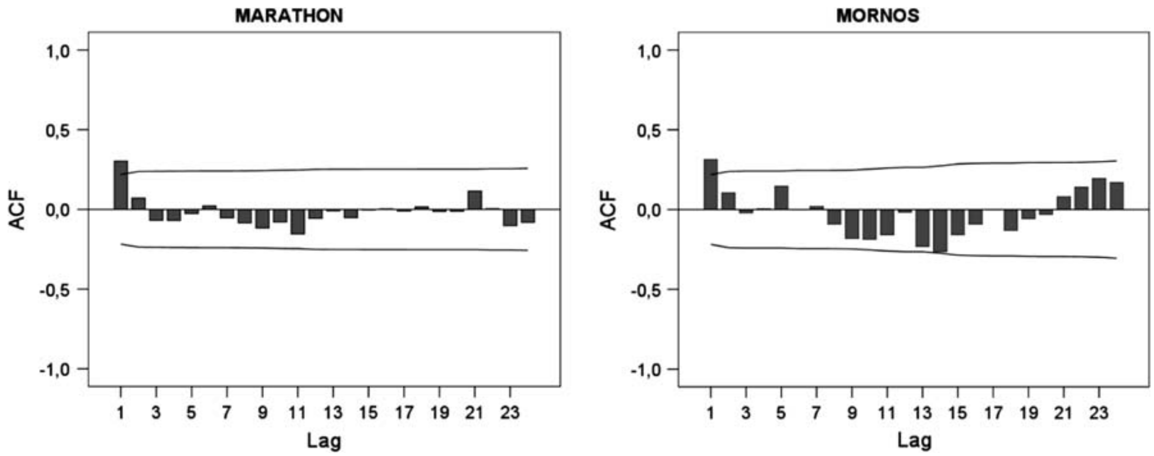

Fig. 3. ACFs for H_t per reservoir.

(seasonality) [10]. Moreover, the existence of negative autocorrelation in the sixth lag indicates an opposite correspondence with data six months apart and also declares one 12 month (seasonal) circle. Herein, the form of the ACFs per reservoir (Fig. 3) implied the lack of seasonality.

However, the diagrams of ACF on the data per reservoir indicated the existence of relatively small positive autocorrelation at the first lag. Consequently, each observation is correlated with the previous one. This means that a part of the information that is transferred by an observation has already been transferred by the previous observation. Thus, the assumption of independence is violated.

Due to the existence of even a small amount of autocorrelation in the data, the nonparametric Kruskal–Wallis test for the exploration of seasonality was carried out for the pre-whited time series data, ($X'_t = X_t - r_1 X_{t-1}$ where X_t is the observation at time t and X_{t-1} is the observation of the previous time period and r_1 is the autocorrelation at lag 1) for each reservoir. The pre-whitening procedure was used in order to eliminate the effect of autocorrelation and achieve independency between groups. The values of the sample autocorrelation function at the first lag were 0.303 for Marathon and 0.314 for Mornos. The Levene's test for the homogeneity of variances was not significant at $\alpha = 0.05$ (Table 2). The Kruskall–Wallis tests were not statistically significant (Table 3). Thus, for each reservoir, the null hypothesis of the equality of the monthly medians is not rejected at $\alpha = 0.05$. The lack of seasonality of the water toxicity might be attributed to probably low values of the parameters that could affect it and their possible slight variation in the annual cycle.

Table 2
Levene's test per reservoir (grouping variable: month)

Reservoir	Levene statistic	df1	df2	*p*-value
Marathon	1.030	11	72	0.430
Mornos	1.071	11	72	0.396

Table 3
Summary of the Kruskal–Wallis test per reservoir (grouping variable: month)

	Marathon	Mornos
Chi-Square	6.114	13.781
df	11	11
p-value	0.866	0.245

3.3. Trend analysis

Despite the existence of autocorrelation, the Mann–Kendall tests, without any prior correction for autocorrelation, were not statistically significant for both the reservoirs (Table 4).

The *p*-values of the tests were: 0.985 for Marathon and 0.74 for Mornos. Therefore, there was no sufficient

Table 4
Summary statistics of the Mann–Kendall test for H_t per reservoir

	Marathon	Mornos
Statistic (S)	−6	87
Standard deviation of S	258.856	258.849
Z	−0.019	0.332
p-value*	0.985	0.74

*H_0: there is no trend vs. H_1: there is a trend.

evidence to reject the null hypothesis of no trend for both reservoirs at any of the ordinary levels of significance. Moreover, there was no need to further investigate the effects of autocorrelation on the Mann–Kendall test [35]. Further examination of the structure of autocorrelation, as a possible cause of an apparent trend, would be necessary only in the case of rejection of the null hypothesis of the Mann–Kendall test.

3.4. Differences between reservoirs

Due to a small amount of autocorrelation in the data for each reservoir, observations within each group are not fully independent. Serial correlation may affect the results of tests for differences in measures of central tendencies between the groups due to the underestimation of the variations [36]. Therefore, any test for differences in measures of central tendencies between the reservoirs should be performed for the pre-whited time series. Prior to the application of such a test to the pre-whited time series, the Levene's test for the homogeneity variances was used. Levene's test was significant at the $\alpha = 0.05$ level of significance (p-value = 0.001). Therefore, alternative to the Mann–Whitney test, the "unequal variance t-test" was carried out for the ranked pre-whited data. This test is nonparametric and overcomes the violation of the homogeneity of variances assumption which is required by the Mann–Whitney test [33]. The p-value of the test was found to be equal to 0.190. Therefore, at the $\alpha = 0.05$ level of significance, the inhibitory effect is not statistically significant different between the reservoirs.

3.5. Discussion

This study comprises a first attempt to following up the effect of raw water, intended for human consumption, on *V. fischeri* for a seven-year period. The characteristics of the *V. fischeri* results are also revealed.

Toxicity tests using only a kind of organism indicate the effect of the sample in this particular organism. The assessment of toxicity on other organisms belonging to different levels of the food chain will allow a more comprehensive assessment of it. However, only the monitoring and recording of data using the same indicator organism allows comparison of values [37]. Through the toxicological analyses, the combined effect of toxic substances is detected. In the case of elevated levels of toxicity, chemical analyses are necessary to determine the problem. In order to ensure the quality of raw water from the reservoirs and the excellent water quality reaching consumers, after the suitable treatment, the Quality Control Department of EYDAP SA, complying with the guidelines set by the European Council, has also been registering systematically the physicochemical, chemical, and microbiological parameters regarding the quality of water. Toxicological analyses are used as a complementary approach to ensure water quality.

This study presents a framework for the exploration of the particular characteristics of the *V. fischeri* results for reservoirs' water, intended for human consumption, as well as an integrated statistical analysis by means of selected appropriate nonparametric statistical methods which can effectively handle particular problems such as outliers and/or violation of distributional assumptions (limitations regarding the normality of data) for parametric ones, lack of the homogeneity of variances assumption and moreover they can be adjusted to overcome lack of data independency due to autocorrelation. The methodology described in this study can also be used for the assessment of other water quality parameters, systematically measured, in the reservoirs. However, when it comes to the cases of reservoirs and/or water quality parameters that are affected by conditions that induce seasonality, the method for trend analysis should be suitably modified. In any case, the frequency of sampling scheme should also be taken under consideration as it may induce autocorrelation, implying special remedies that have to be undertaken.

4. Conclusions

The statistical analysis of the raw water toxicity over an extended time period can provide important and useful information for the management, quality control, and improvement of the water resources. However, due to the inherent characteristics of the water quality data, sophisticated statistical techniques for their analysis might be required. In this study, the data that were obtained from the monthly toxicological analysis (monitoring time period: 2000–2006) of the raw water from two reservoirs that supply Athens were subjected to exhaustive statistical analysis by the usage of specialized nonparametric statistical methods. A small amount of autocorrelation was observed for each time series implying that corrective remedies should be used in the statistical analyses.

Toxicity tests conducted on raw water samples using the Microtox® system resulted in no evidence of toxicity to the test organism *V. fischeri*. The overall performance of the raw water from the two reservoirs was apparently nontoxic and comparable to this one usually observed in drinking water after treatment [38].

The study of seasonality (based on Kruskal–Wallis test) of the data for each reservoir resulted in no statistical significance. Trend analysis (based on Mann–Kendall test) resulted in no statistical significant upward or downward trends for both the reservoirs. In addition, there was not observed statistically significant difference at the measures of central tendency of inhibitory effect between the two reservoirs.

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