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A statistical evaluation of the potential genotoxic activity in the surface waters of the Golden Horn Estuary

Emel Kocak [a,*], Kaan Yetilmezsoy [a], M. Talha Gonullu [a], Mustafa Petek [b]

[a] Department of Environmental Engineering, Faculty of Civil Engineering, Yildiz Technical University, 34220 Davutpasa, Esenler, Istanbul, Turkey
[b] Fatih University, Faculty of Engineering, Department of Environmental Engineering, 34500 Buyukcekmece, Istanbul, Turkey

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ABSTRACT

The potential genotoxic activity in the surface waters of the Golden Horn Estuary was statistically evaluated utilizing a combination of appropriate parametric and non-parametric tests. The genotoxic activities that were associated with the water samples were determined by the SOS chromotest microplate assay. This assay utilizes β-galactosidase activity, alkaline phosphatase activity, and four different solvent controls, to generate reliable results when corrected induction factors (CIF) are used as quantitative measurements of genotoxic activity. The CIF values were obtained from a total of 384 different genotoxic experiments that were grouped into subsets according to the respective seasons and the selected sampling locations. A total of 160 subsets were statistically compared to assess any possible differences between the pairs of groups, with 95% confidence limits. The findings of this study clearly indicate that some seasonal variations exist in the CIF values at several sampling sites. However, no potentially hazardous impact to the aquatic environment was found in the estuarine system.

1. Introduction

Estuaries are unique semi-enclosed systems, which are transitional zones between rivers and seas. Estuaries are important ecosystems, which are typically rich in biodiversity. The Golden Horn (Halic) is a historic inlet of the Bosphorus, which has played a specific role in Istanbul's culture and tradition for a thousand years. The Golden Horn has been important to the civilizations of Istanbul for a long time. However, its ecosystem has faced increasing amounts of pollution over the centuries. The turning point for the Golden Horn pollution problem occurred during the 1950s and coincided with an increase in the industrialization of Istanbul (Yuksek et al., 2006). Throughout the 1980s, most of the industrial plants that were located along both sides of the Golden Horn were discharging their wastewaters and solid wastes into the estuary. During that period, the Alibeykoy and Kagithane creeks were carrying heavy loads of domestic and industrial pollution into the Golden Horn. Upstream of this area, a 3–4 km stretch of the Golden Horn was filled with debris and organic solids. In addition to the physical blockade, the odors that were produced from the anaerobic decomposition of the organic matter in the sediment created a severe public nuisance (Kinaci et al., 2004).

Considering the complex nature and the dynamic characteristics of estuarine systems, environmental studies of the Golden Horn have remained very superficial. Assessments of the toxicological risks, the associated hazards and the ecological effects of the substances polluting this estuary have been unsatisfactory. Therefore, it is necessary to further examine assay systems that have the ability to evaluate the substantial impact of some of the more persistent pollution sources before attempts are made to characterize of the entire pollution problem. The integration of physical and chemical methods is typically a complementary and useful approach to monitor water quality. However, these techniques are not always capable of detecting the presence of possible mutagenic compounds, recalcitrant substances, and soluble DNA-damaging agents, which may cause harmful effects on the aquatic environment. In such cases, these physicochemical procedures cannot provide sufficient information on the potential toxicity of the various unknown, and often undetermined, substances in a complex mixture. Some of these substances are genotoxic, and many of these substances are suspected to be associated with the increase in cancer rates that have occurred over the last few decades (Jolibois and Guerbet, 2005). Therefore, suitable toxicological tests should be incorporated into the existing water quality monitoring program in an effort to assess the potential risk to the organisms in this unique environment.

In recent years, water genotoxicity studies (Bombardier et al., 2001; Bartos et al., 2005; Cachot et al., 2006; Mansour et al., 2007; Gupta et al., 2006), which monitor environmental water sources for contamination with potentially carcinogenic and mutagenic compounds that represent major concerns for human health,

* Corresponding author. Tel.: +90 212 3835390; fax: +90 212 3835358.
E-mail address: ekocak@yildiz.edu.tr (E. Kocak).

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have become an interesting issue. However, the evaluation of the combined effects of various compounds on water toxicity is a particularly difficult and complicated task. The genotoxic compounds that are typically found in the aquatic environment, have a large chemical diversity and can come from a variety of sources (Jolibois and Guerbet, 2005). Gebel and Koenig (1999) have reported that in vitro test systems are an easier method to assess combined genotoxicity. Therefore, the combined effect of the chemical mixtures and the environmental samples can be rapidly and inexpensively assessed, without detailed knowledge of the chemical constituents in each sample. The SOS chromotest is one of the established in vitro test systems for the assessment of genotoxicity. This test system has been shown to be effective as a test system for bacteria and has been shown to produce results within hours of sample collection. The SOS Chromotest is based on the detection of primary DNA-damaging agents in *Escherichia coli* PQ37 (Jolibois et al., 2003). It was developed by Quillardet et al. (1982) and was miniaturized by Fish et al. (1987) to run in 96-well microplates. It has been recommended for routine use in environmental applications that require the assessment of genotoxic activity (Gonullu et al., 2001). Several studies (Gebel and Koenig, 1999; Legault et al., 1996; Ruiz and Marzin, 1997; Zani et al., 2005) have also reported that the SOS Chromotest is useful for monitoring the genotoxicity of complex environmental matrices.

So far, most of the experimental studies have focused on the use of various, advanced, techniques for water quality improvement. However, these studies have failed to thoroughly integrate toxicity testing into the conventional assay procedures. Over the past 30 years, numerous monitoring studies were undertaken in the Golden Horn Estuary by a wide variety of researchers (Artuz and Korkmaz, 1975; Tuncer et al., 2001; Gonullu et al., 2003, 2005; Aslan-Yilmaz et al., 2004; Tas et al., 2006; Yuksek et al., 2006). However, there are no systematic papers in the literature that are specifically devoted to the investigation and evaluation of the potential genotoxic activity of the surface waters of the Golden Horn Estuary, which used the SOS chromotest microplate assay. Therefore, it is necessary to clarify this issue, in the overall scheme of water pollution control. The use of the SOS chromotest should be considered as a viable method to assess genotoxicity in environmental samples. It is also possible that this test could assist in the development of a sustainable water quality control strategy for the estuarine systems located in industrialized areas. For this reason, the aim of the present study is to fill the gap in this field by focusing our investigation of the genotoxic activity of surface waters on a specific ecological system, the Golden Horn Estuary.

Based on the above-mentioned facts, the specific objectives of the present study were as follows: (1) to use the SOS chromotest microplate assay to investigate the genotoxic activities in the surface waters of the Golden Horn Estuary during various seasons and throughout selected sampling locations; (2) to statistically appraise the potential genotoxic activities of the obtained samples using several, appropriate, parametric tests (unpaired and matched-pair *t* tests), non-parametric tests (the Mann–Whitney *U* and the Kruskal–Wallis *H* tests) and precondition analyses (the Shapiro–Wilk *W* and the Levene's tests); and (3) to improve the evaluation of the multivariate nature of a specific ecosystem by incorporating a genotoxicological test into the conventional physicochemical analyses.

2. Materials and methods

2.1. Description of the study area

The Golden Horn Estuary is a small, intercontinental, basin that is located southwest of the Strait of Istanbul (Bosphorus). It serves as a transitional zone that connects the Alibeykoy and Kagithane Rivers to the Bosphorus. The Golden Horn forms a deep, natural, harbor for the peninsula, which it encloses together with the Sea of Marmara. This horn-shaped body of water is 7.5 km long, 200–900 m wide, and the surface area covers about 2.6 km^2. The maximum depth is 36 m at the mouth and slopes to <1 m near tributary inflows (Coleman et al., 2009). A small portion (2%) of the area is deeper than 30 m. More than one third (38%) of the area is <10 m. The average width of the estuary is 370 m; the width of the estuary ranges between 293 m (Galata) and 685 m (Kasimpasa). Its northeast coastline is longer (7934 m) than the southeast coastline (6684 m). The main freshwater source to the Golden Horn is rainfall. However, uncontrolled anthropogenic discharges also contribute freshwater, but to a lesser extent than rainfall (Sur et al., 2002). In the Golden Horn, the particular characteristics of the circulation are governed by the volume and rate of flow of freshwater (runoff–rainfall), brackish water (from the Black Sea), and saline water (from the Mediterranean Sea) entering the estuary. The circulation can also be influenced by the size and shape of the basin and the effects of the prevailing winds (Balkis et al., 2009). Moreover, today the estuary is spanned by four bridges, which have an important influence on the water circulation (Alpar et al., 2003).

2.2. Sampling

In the present study, 8 sampling locations (Karakoy (P1), Galata Bridge (P2), Unkapani (P3), Kasimpasa (P4), Valide Sultan Bridge/Old Galata Bridge (P5), Halic Bridge (P6), Sutluce (P7), and Front of the Adalar (P8)) were selected to design a sampling network that covered a wide range of determinants along the Golden Horn Estuary (Fig. 1). The exact coordinates of the sampling locations were determined by using a GPS device (Garmin GPSMAP® 76CS) with a coded coordinate program (WGS 84). The surface water samples were each collected on site, at all of the sampling locations, to investigate the potential genotoxic variations that occurred during the study period (November 2007–June 2008).

Because genotoxic effects decrease during rainy weather (Jolibois et al., 2002), all of the samples were collected during dry weather conditions. This allowed us to eliminate the effect of the rain water on the genotoxic activity analyses. The collected samples were placed in 1 L polyethylene bottles and put into ice-bag containers for transport back to the laboratory on the same day as the collection. Prior to conducting the genotoxic experiments, the samples were filtered through cellulose acetate filters (0.45 μm pore diameter). Filtered samples were stored in sterile, polyethylene, bottles at 4 °C for no longer than seven days prior to the genotoxicity assessments. During the study period, four seasonal samplings were performed in specific months (November, February, May, July), and a total of 384 samples were collected.

2.3. SOS chromotest procedure

The SOS chromotest is a colorimetric assay of the enzymatic activities that occur after incubating the test strain of bacteria in the presence of various amounts of experimental sample (Quillardet et al., 1982). The test utilizes a genetically engineered bacterium, *E. coli* PQ37, to detect DNA-damaging agents. In this assay, the β-galactosidase (β-gal) gene (*lacZ*) of the *E. coli* PQ37 tester strain is fused to the bacterial *sfiA* SOS operon. Thus, *lacZ* is concomitantly expressed during the bacterial SOS response, and this gene expression can be photometrically determined by the induction of β-gal. The amount of β-gal induction is indicative of the extent of SOS induction and bacterial genotoxicity. Bacterial alkaline phosphatase (AP) activity was used to determine the range of bacterial cytotoxicity. The ratio of β-gal/AP activity

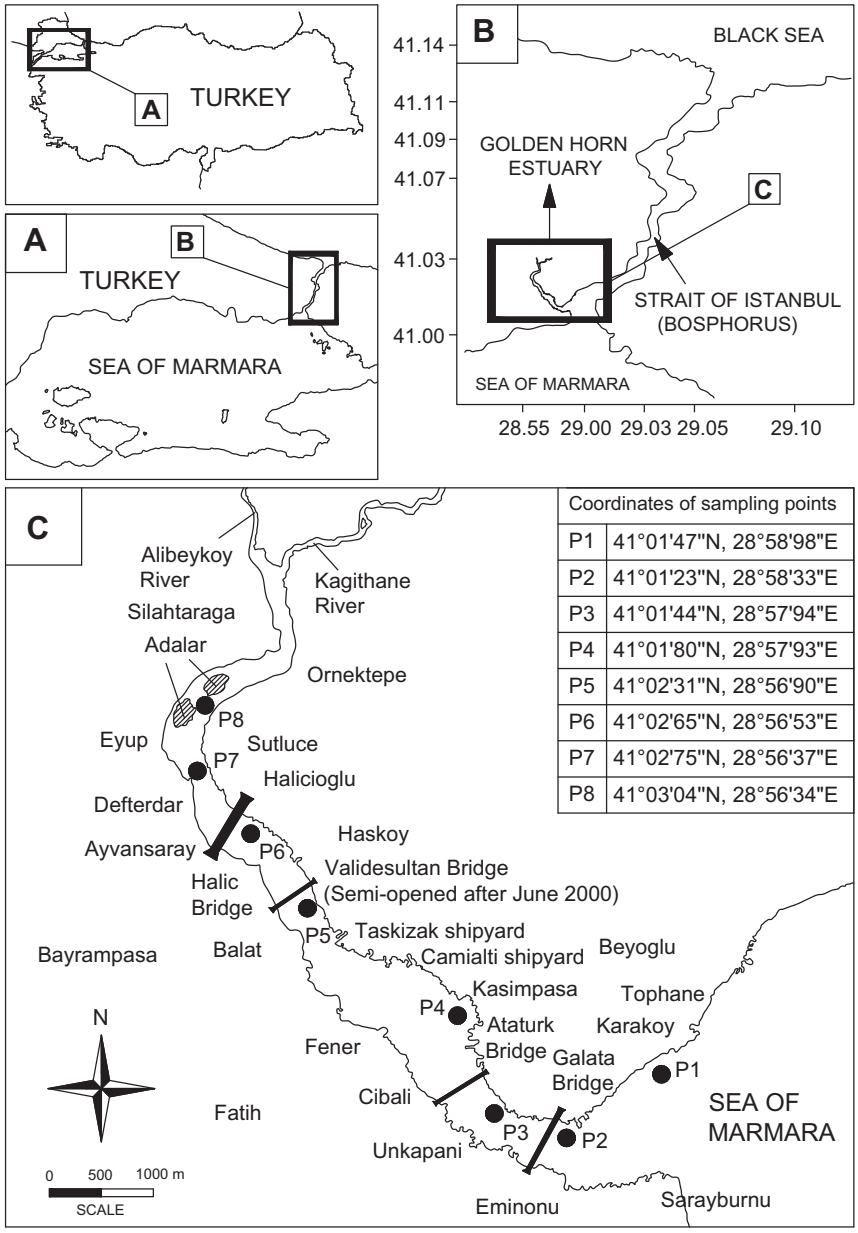

Fig. 1. Map of the study area showing the location of the sampling stations (closed circles) on the Golden Horn Estuary.

was defined as the induction factor (IF), and this ratio was used to indicate the extent of the SOS induction for the tested compounds (Gebel and Koenig, 1999). The test is available as a test kit, which includes all of the necessary materials. No special measuring devices, with the exception of a plate reader, were needed to complete this assay. This test can also be used as a qualitative test, based on the use of a color scale. The assay can be completed within 24 h, including the revival of the bacteria. The test detects any primary DNA damage that is caused by genotoxins, and the test can be used for various kinds of aqueous samples (Dulger et al., 2008).

In this study, the SOS chromotest was performed, without metabolic activation, as described by Quillardet and Hofnung (1985). The *E.coli* PQ37 tester strain was kindly provided by Environmental Bio-Detection Products Inc. (Brampton, Ontario, Canada). We tested four different dilutions (100%, 50%, 6.25%, and 0.781% v/v.) for eight sampling points, in triplicate, and the testing began with a 100 mL sample that was equivalent for each cuvette. The test was performed at 37 °C, and the cuvettes were read after 2 h with a spectrophotometer. For the direct assay, the negative control was composed of a 10% DMSO (dimethyl sulfoxide) solution in sterile, ultrapure, water, and the positive control was 4-nitro-quinoline-oxide (4NQO).

2.4. Determination of genotoxic activity

The SOS Chromotest involves incubating the bacteria with the experimental sample and assessing the β-galactosidase (β-gal) activity (i.e. the level of SOS induction). Alkaline phosphatase (AP) activity is also measured and serves as a control for toxicity (Bombardier et al., 2001). AP reduction factors (RF), β-gal induction factors (IF), and corrected induction factors (CIF = IF/RF) were calculated as described by Legault et al. (1996):

$$RF = \frac{(OD_{405})_{mean,\,t}}{(OD_{405})_{mean,\,c}} \quad (1)$$

$$IF = \frac{(OD_{620})_{mean,\,t}}{(OD_{620})_{mean,\,c}} \quad (2)$$

$$CIF = \frac{RF}{IF} \quad (3)$$

where $(OD_{405})_{mean}$ and $(OD_{620})_{mean}$ are the means of the optical density (OD) readings that were taken at 620 nm (β-gal) and 405 nm (AP), and t and c refer to the test and the control dilutions, respectively. Bombardier et al. (2001) have reported that the RF and IF values account for the background activity of the control. The ratio of IF to RF units yields an estimate of β-gal activity that is corrected for toxicity. The criterion that was used to consider a sample as "positive" in the SOS chromotest differs between authors (Jolibois and Guerbet, 2005; Cachot et al., 2006; Gebel and Koenig, 1999; Legault et al., 1996). Table 1 summarizes the genotoxic activity levels and the corresponding threshold values that were defined in the different studies. In the present study, significant genotoxic activity was defined as having a corrected induction factor that was equal to or greater than 1.5, as suggested by most of the previously published studies (Jolibois and Guerbet, 2005; Mersch-Sundermann et al., 1992; Margulis et al., 2003).

2.5. Analytical procedure

All SOS chromotest analyses were carried out according to the EBPI (Environmental Bio-Detection Products Inc.) protocols (EBPI, 2008). The experimental equipment (i.e. micropipettes, Eppendorf pipettes) were autoclave sterilized at 121 °C and 10.6 bar for 15 min (Nuve OT 032). A minishaker that had an orbit of 4.5 mm and a speed range of 0–2500 rpm (IKA Labortechnik, Staufen, Germany) was used to centrifuge the bacteria at a fixed agitation speed of 1500 rpm. The bacteria were grown at a stable temperature of 37 °C in a temperature-controlled incubator (Memmert, Germany). This incubator was also used for the development of the enzymatic activities. The bacteria cultures were grown, and the optical density values (600 nm) were measured using an UV–VIS-Spectrophotometer (Shimadzu, UV-1202, UV–VIS) with a special quartz cuvette that allowed for a light path length of 1 cm. ATP and β-gal activities were measured, using a Biotek PowerWave XS Microplate ELISA Reader (BioTek Instruments Inc., Winooski, VT, USA) with data analysis software (Gen 5), at 405 nm (OD_{405}) and 620 nm (OD_{620}), respectively. The pH values of the samples were measured with a pH meter (Jenway 3040 Ion Analyzer) and a pH probe (HI1230, Hanna Instruments, USA). Deionized and sterile ultrapure water was used in the experiments, and the water was supplied from a TKA-GenPure water purification system (Niederelbert, Germany). The physicochemical analyses of the surface water samples were conducted by the procedures described in the Standard Methods (APHA, 1995).

2.6. Statistical analysis

Each genotoxic test was performed in triplicate, and repeated at least three times, to observe the reproducibility of each test. The StatsDirect (version 2.7.2, Copyright© 1990–2008 StatsDirect Ltd.) software package was used for the statistical analysis of the experimental data. In all calculations, spreadsheets were generated using Microsoft Excel® 2000 or DataFit® scientific software (version 8.1.69, Copyright© 1995–2005 Oakdale Engineering), and these spreadsheets were used as ODBC (Open Database Connectivity) data sources that ran under Microsoft Windows®. An alpha (α) level of 0.05 (or 95% confidence) was used to determine the statistical significance in all of the analyses. Various descriptive statistics (such as the ranges of CIF values, minimum and maximum values, medians, means, standard deviations, lower and upper 95% confidence limit of the means, variances, variance coefficients, standard errors of means, geometric means, skewness and kurtosis values, and lower and upper quartiles for each subset) were analyzed to characterize the selected groups and to identify the extreme values.

The CIF values that were used as a quantitative measurement of genotoxic activities were statistically evaluated utilizing several appropriate parametric (unpaired or two-sample and matched-pair *t* tests) and non-parametric tests (the Mann–Whitney *U* (or the Wilcoxon rank-sum) test and the Kruskal–Wallis test with the Dwass–Steel–Chritchlow–Fligner method). Prior to applying parametric tests, the Shapiro–Wilk *W* and the Levene's tests were consecutively conducted, as preconditions, to evaluate whether the experimental subsets had either a normal or a non-normal distribution, and to evaluate whether the variances (or standard deviations) of the paired groups were either homogeneous or unequal. Because the CIF values were not normally distributed, non-parametric tests were applied. The results were assessed with various descriptive statistics, such as two-tailed *p* values, *t*-statistics, combined (or pooled) standard errors (se_p), and power values (or probability of detecting a true effect), to reflect the statistical significance between the paired groups ($\alpha = 0.05$).

3. Results and discussion

3.1. Surface water quality

Table 2 reports the average, seasonal, water quality parameters, which indicates the physicochemical characteristics of the surface

Table 1
Genotoxic activity levels and the corresponding threshold values defined in different studies.

Genotoxic activity levels	Corrected induction factors (CIF)	Reference and region
Genotoxic	>2.0	Isidori et al. (2004), South Italy Cenci et al. (2008), Italy Ilinskaya et al. (2004), Russia
Genotoxic	>1.5	Guzzella et al. (2006), Italy
β-galactosidase activity significantly increases compared with the solvent control	>1.5	Jolibois and Guerbet (2005), France
High genotoxic	>2.0	Cachot et al. (2006), France Gebel and Koenig (1999), Germany
Moderate genotoxic	1.5–2.0	
Not genotoxic	<1.5	
β-galactosidase activity is higher than the negative control	>2.0	Isidori et al. (2006), Italy
SOS response	>1.2	Legault et al. (1996), Canada
Genotoxic	⩾2.0	Ortolan and Ayub (2007), Brazil
Not genotoxic	=1.0	
Genotoxic	⩾1.5	Mersch-Sundermann et al. (1992), Germany Margulis et al. (2003), Russia

Table 2
Physicochemical characteristics (seasonal average values) of the surface waters of the Golden Horn Estuary during different periods of the present study.

Seasons	Sampling locations	Temperature (°C)	Salinity (%)	Conductivity (mS/cm)	pH	TSS (mg/L)	DO (mg/L)	NO_3–N (mg/L)	TP (mg/L)	TOG (mg/L)	Total sulfur (mg/L)	Cd (mg/L)	Pb (mg/L)	Cu (mg/L)	Cr (mg/L)	Zn (mg/L)	Fe (mg/L)	Mn (mg/L)	Al (mg/L)
Autumn	P1	13.85	1.91	24.25	8.14	25.00	6.78	<0.10	0.09	0.40	–	0.000	0.000	0.000	0.001	0.006	0.398	0.020	0.037
	P2	13.78	1.91	24.10	8.10	25.00	6.04	<0.10	0.11	<0.30	–	0.000	0.000	0.000	0.001	0.002	0.030	0.058	0.009
	P3	13.68	1.90	23.88	8.10	25.00	4.70	<0.10	0.15	<0.30	–	0.000	0.000	0.000	0.001	0.004	0.043	0.061	0.000
	P4	13.30	1.86	23.24	7.96	25.00	5.05	<0.10	0.18	<0.30	–	0.000	0.000	0.000	0.002	0.000	0.058	0.033	0.037
	P5	13.90	1.82	23.13	7.80	35.00	1.55	<0.10	0.22	0.40	–	0.000	0.000	0.000	0.003	0.001	0.097	0.053	0.039
	P6	13.60	1.76	22.26	7.75	35.00	1.68	<0.10	0.32	0.50	<0.1	0.000	0.000	0.000	0.005	0.000	0.104	0.071	0.036
	P7	13.50	1.73	21.95	7.70	35.00	1.00	<0.10	0.40	0.60	0.10	0.000	0.000	0.000	0.005	0.004	0.106	0.078	0.052
	P8	13.50	1.57	20.03	7.65	35.00	0.95	<0.10	1.00	1.20	0.40	0.000	0.000	0.005	0.008	0.010	0.164	0.120	0.096
Winter	P1	7.10	1.90	20.34	8.05	30.0	10.60	<0.10	<0.03	<0.3	–	0.000	0.000	0.000	0.002	0.020	0.031	0.001	0.048
	P2	7.00	1..85	19.75	8.05	25.0	9.72	0.23	0.07	<0.3	–	0.000	0.000	0.000	0.004	0.007	0.055	0.007	0.071
	P3	7.40	1.69	18.40	7.90	25.0	7.82	0.27	0.16	0.3	–	0.000	0.000	0.002	0.005	0.007	0.065	0.020	0.086
	P4	6.50	1.72	18.30	7.90	25.0	8.49	0.23	0.13	0.7	–	0.000	0.000	0.003	0.005	0.006	0.112	0.019	0.254
	P5	7.80	1.31	14.70	7.80	25.0	4.58	0.70	0.46	0.9	–	0.000	0.000	0.010	0.014	0.025	0.185	0.070	0.220
	P6	6.50	1.42	15.25	7.90	30.0	4.85	0.69	0.38	1.0	<0.1	0.000	0.000	0.007	0.011	0.013	0.148	0.065	0.179
	P7	7.20	0.98	11.05	7.80	35.0	3.60	1.22	0.85	2,2	<0.1	0.000	0.000	0.018	0.025	0.028	0.325	0.130	0.381
	P8	7.50	0.69	8.01	7.80	45.0	3.50	1.45	1.22	3.3	<0.1	0.000	0.000	0.025	0.037	0.045	0.508	0.177	0.590
Spring	P1	14.49	1.92	24.66	8.22	35.0	7.29	<0.10	0.23	<0.3	–	–	–	–	–	–	–	–	–
	P2	14.73	1.93	24.97	8.33	35.0	10.83	<0.10	0.20	<0.3	–	–	–	–	–	–	–	–	–
	P3	15.13	1.92	25.04	8.40	40.0	12.81	<0.10	0.22	<0.3	–	–	–	–	–	–	–	–	–
	P4	15.00	1.92	24.99	8.38	40.0	11.73	<0.10	0.34	<0.3	–	–	–	–	–	–	–	–	–
	P5	15.96	1.85	24.60	8.52	50.0	13.80	<0.10	0.14	<0.3	–	–	–	–	–	–	–	–	–
	P6	15.81	1.84	24.46	8.49	55.0	13.18	<0.10	0.20	<0.3	0.2	–	–	–	–	–	–	–	–
	P7	16.03	1.82	24.26	8.35	50.0	11.32	<0.10	0.33	<0.3	0.2	–	–	–	–	–	–	–	–
	P8	16.12	1.80	24.18	8.23	50.0	6.23	<0.10	0.34	<0.3	0.1	–	–	–	–	–	–	–	–
Summer	P1	22.61	1.92	29.43	7.98	30.0	7.43	<0.10	0.14	<0.3	–	0.000	0.000	0.000	0.000	0.000	0.011	0.002	–
	P2	23.75	1.90	29.74	8.27	25.0	9.82	<0.10	0.11	<0.3	–	0.000	0.000	0.000	0.000	0.002	0.026	0.009	–
	P3	24.34	1.88	29.89	8.40	25.0	9.79	<0.10	0.17	<0.3	–	0.000	0.000	0.000	0.000	0.001	0.048	0.016	–
	P4	24.17	1.88	29.85	8.40	30.0	10.85	<0.10	0.08	<0.3	–	0.000	0.000	0.000	0.000	0.001	0.044	0.022	–
	P5	24.39	1.82	29.05	8.42	40.0	7.01	<0.10	0.27	<0.3	–	0.000	0.000	0.000	0.001	0.001	0.160	0.040	–
	P6	24.03	1.82	28.88	8.36	50.0	9.11	<0.10	0.28	<0.3	<0.1	0.000	0.000	0.000	0.000	0.003	0.151	0.040	–
	P7	24.26	1.79	28.63	8.37	70.0	8.17	<0.10	0.26	<0.3	0.3	0.000	0.002	0.000	0.001	0.004	0.231	0.054	–
	P8	24.71	1.73	27.96	8.29	80.0	3.02	<0.10	0.36	<0.3	0.6	0.000	0.000	0.004	0.001	0.007	0.294	0.090	–

waters of the Golden Horn Estuary, during different periods of the present study. During the study period, surface water quality data indicated that the temperature ranged from 6.5 to 24.7 (±6.1) °C, and the mean and median temperature values fell between 15.1 and 14.2 °C. The mean temperature values were about 13.6 (±0.2), 7.1 (±0.46), 15.4 (±0.64) and 24 (±0.64) °C in autumn, winter, spring and summer, respectively. The salinity values ranged from 0.69% to 1.93%, and the mean and median salinity values ranged between 1.74% and 1.83%. The highest variation in conductivity was recorded in the winter (8.01–20.34 mS/cm), which was probably due to the intense hydrodynamic conditions that dominated the estuary during the winter months. The pH values of the samples that were collected ranged from 7.62 to 8.52, and the mean pH value was 8.12 (±0.26).

Cadmium (Cd) was not detected in the samples that were collected. However, we detected lead (Pb), copper (Cu), chromium (Cr), zinc (Zn), iron (Fe), manganese (Mn) and aluminum (Al) in low quantities. Higher concentrations of Mn, Zn, Cd, and Cu were observed during the winter, and lower concentrations were observed in the summer. This observation was probably due to the coprecipitation of these elements with other compounds during the spring and summer months. We observed a similar pattern for Cr, Fe and Al, which reached their maximum values during the winter and autumn.

3.2. SOS chromotest responses

In this study, the CIF values of all of the collected samples were observed within the 0.80–1.21 range. When the CIF for any of the test concentrations reached 1.5, the test substance was scored as significantly genotoxic. However, the SOS chromotest results clearly indicated that no genotoxic effects were found in the surface waters of the Golden Horn Estuary. We have presented a detailed set of descriptive statistics that are associated with the seasonal genotoxic activities in Table 3. All of the calculated CIF values were determined to be below the level that is considered to be the 1.5 threshold level. However, a comparison of the pairs of groups by the means of both seasonal and regional genotoxic activities is an important task for the interpretation of the experimental findings that were obtained from the present genotoxicological investigation. Therefore, an extensive statistical analysis was also conducted, as a further step, to examine CIF variations associated with seasonal and regional basis. This extensive analysis provides a better evaluation of the genotoxicity profile in the estuarine system.

Table 4 summarizes the results of some of the experimental data that have been associated with the comparison of various experimental techniques, which have been used for the assessment of genotoxic compounds in various types of water and wastewater samples. The performance data reveal that a wide range of CIF values have been observed, and the range of CIF values depends on the characteristics and complex nature of the water and wastewater matrices. High genotoxic activity values are probably due to the presence of several mutagenic and carcinogenic agents, which includes persistent components, soluble DNA-damaging products, recalcitrant substances, and other undesirable impurities that are present in the collected water samples. It is apparent from the previous studies that various chemical compounds have been widely used in numerous industrial and environmental applications. However, relatively few genotoxicological investigations are available in the literature. Therefore, additional studies that use the genotoxicological data, in addition to the contaminant monitoring data, will be necessary to identify the sources of the toxicants and to ensure that more environmental risk assessments can be verified. Both of these points have been suggested by other individuals (Jolibois and Guerbet, 2005; Guzzella et al., 2006).

3.3. Statistical comparison of seasonal genotoxic activities

A total of 112 subsets were compared to assess possible statistical differences in the mean seasonal genotoxic activities that existed between pairs of groups. Statistically significant differences were recorded for nine subsets that were part of the autumn data. However, in these cases, the p values were found to be less than the chosen α level of 0.05. Therefore, the null hypothesis (H_0) was rejected in favor of an alternative hypothesis (H_a). The statistical analysis of these nine subsets indicated that the two-tailed p values, t-statistics, combined (or pooled) standard errors (se_p), and power values (PW) for each ranged between 0.0019–0.0453, 2.133–3.527, 0.0145–0.0172 and 81.73–99.73%, respectively ($\alpha = 0.05$). However, based on results that were generated from the unpaired t test analysis of the remaining 19 subsets that were part of the autumn data, no sufficient evidence was generated to support a significant difference Therefore we rejected the H_a. The CIF values for the winter data were also compared. This analysis concluded that there was sufficient evidence to support a significant difference between six subsets; however, there were no significant differences between the remaining 22 subsets that composed the winter data. The statistical results indicated that the two-tailed p values, t-statistics, se_p, and PW values ($\alpha = 0.05$) for the statistically significant subsets in the winter data ranged between 0.0016–0.0321, 2.466–3.601, 0.0166–0.0232 and 91.44–99.8%, respectively.

The statistical evaluation of the experimental findings for two of the subsets that were obtained as part of the spring data collection indicated that there were significant differences that reject the H_0. However, no significant differences were observed in the remaining 26 subsets from the spring data. The differences in the CIF values for the Unkapani (P3)–Valide Sultan Bridge (P5) and Valide Sultan Bridge (P5)–Sutluce (P7) data, which were part of the winter subset, were noticeable ($p = 0.0175$ and $p = 0.0368$, respectively). Parametric and non-parametric test results indicated that there was sufficient evidence to support significant differences between nine subsets that were part of the present summer data. This finding was similar to the results found for the autumn data. The statistical results indicated that the two-tailed p values, t-statistics, se_p and PW values ($\alpha = 0.05$) for these subsets each ranged between 0.0005–0.0333%, 2.271–4.075%, 0.0153–0.0346% and 86.57–99.9%, respectively.

Although all calculated CIF values were found to be below the 1.5 threshold genotoxic activity level, seasonal variations in the CIF values were observed at several sampling sites. The statistical analysis concluded that about 23.21% of the total mutual comparisons ($n = 112$), by means of seasonal genotoxic activities, were observed to be statistically noticeable ($p < 0.05$). For the spring subset, the CIF variations were recorded, and these variations were found to be much less (only 7.14% of the total spring data, $n = 28$) than those of the other subsets. On the other hand, the CIF variations were higher for the autumn and summer subsets (about 32.14% of the total autumn or summer data), compared to the CIF variations observed for other seasons. Following the autumn and summer subsets, the winter subset had the next highest amount of CIF variations (21.43% of the total spring data, $n = 28$). Therefore, the differences in the results can be ascribed to the fact that both thermodynamic and meteorological conditions (i.e. temperature, pressure, wind) have a greater impact on the biochemical characteristics of the surface waters during hot and cold seasons, as compared to milder periods of the year (Table 2). Similarly, Alpar et al. (2003) have reported that the Golden Horn area is affected by two distinct seasonal climatic regimes. During the winter, the weather is dominated by an almost continuous passage of cyclonic systems. During the summer, NE winds that come from the Black Sea are dominant. When the wind is not blowing from

Table 3
Detailed descriptive statistics of the present genotoxic activity data for different periods of the present study.

Seasons	Sampling locations	Mean	Variance	Standard deviation	Variance coefficient	Standard error of mean	Upper 95% CL of mean	Lower 95% CL of mean	Geometric mean	Skewness	Kurtosis	Maximum	Upper quartile	Median	Lower quartile	Minimum	Range
Autumn	P1	0.99	0.001	0.036	0.036	0.010	1.01	0.97	0.99	0.15	2.23	1.05	1.02	0.99	0.97	0.93	0.12
	P2	1.04	0.002	0.044	0.042	0.013	1.07	1.01	1.04	0.70	2.39	1.13	1.07	1.03	1.01	0.99	0.14
	P3	0.98	0.001	0.036	0.037	0.010	1.01	0.96	0.98	−0.87	2.47	1.02	1.01	1.00	0.96	0.91	0.11
	P4	1.00	0.001	0.033	0.033	0.010	1.02	0.98	1.00	0.00	2.47	1.06	1.03	1.00	0.98	0.94	0.12
	P5	1.02	0.002	0.042	0.041	0.012	1.05	1.00	1.02	−0.60	2.39	1.08	1.05	1.04	1.00	0.94	0.14
	P6	1.02	0.001	0.039	0.038	0.011	1.04	1.00	1.02	0.27	1.81	1.08	1.05	1.01	0.98	0.97	0.11
	P7	0.99	0.001	0.032	0.032	0.010	1.01	0.96	0.98	−0.07	1.81	1.03	1.01	0.99	0.96	0.94	0.09
	P8	0.98	0.002	0.040	0.041	0.012	1.01	0.96	0.98	−0.04	2.21	1.05	1.01	0.98	0.96	0.91	0.14
Winter	P1	1.03	0.014	0.118	0.114	0.034	1.11	0.96	1.03	−1.03	2.95	1.16	1.12	1.06	1.00	0.80	0.36
	P2	1.06	0.005	0.067	0.063	0.019	1.11	1.02	1.06	−0.60	2.08	1.14	1.12	1.07	1.02	0.94	0.20
	P3	1.02	0.002	0.042	0.042	0.012	1.04	1.00	1.02	−0.06	2.79	1.10	1.04	1.03	1.00	0.95	0.15
	P4	1.01	0.001	0.032	0.032	0.010	1.03	0.99	1.01	0.02	2.15	1.06	1.03	1.02	0.99	0.96	0.10
	P5	1.04	0.002	0.045	0.043	0.013	1.07	1.01	1.04	0.04	1.94	1.12	1.08	1.05	1.00	0.98	0.14
	P6	1.04	0.004	0.060	0.060	0.020	1.08	1.00	1.03	0.11	2.08	1.14	1.09	1.03	0.99	0.93	0.21
	P7	0.99	0.001	0.036	0.036	0.010	1.01	0.96	0.98	0.04	1.74	1.04	1.02	0.99	0.95	0.93	0.11
	P8	1.00	0.002	0.044	0.044	0.013	1.03	0.98	1.00	0.82	3.00	1.10	1.03	1.00	0.97	0.95	0.15
Spring	P1	1.02	0.005	0.072	0.071	0.021	1.07	0.97	1.02	0.97	2.53	1.16	1.06	1.00	0.97	0.95	0.21
	P2	1.02	0.005	0.072	0.071	0.021	1.07	0.97	1.02	0.41	2.16	1.16	1.08	1.02	0.96	0.93	0.23
	P3	0.97	0.005	0.070	0.072	0.020	1.02	0.93	0.97	0.62	2.74	1.12	1.01	0.97	0.92	0.88	0.24
	P4	1.02	0.003	0.055	0.054	0.016	1.05	0.98	1.02	0.11	1.76	1.11	1.07	1.01	0.97	0.94	0.17
	P5	1.03	0.002	0.050	0.048	0.014	1.06	1.00	1.03	1.11	3.00	1.13	1.05	1.02	1.00	0.98	0.15
	P6	1.00	0.001	0.028	0.028	0.008	1.02	0.99	1.00	0.12	1.91	1.05	1.03	1.01	0.98	0.96	0.09
	P7	0.99	0.002	0.040	0.041	0.012	1.01	0.96	0.99	0.44	2.62	1.07	1.01	0.99	0.96	0.93	0.14
	P8	1.02	0.004	0.063	0.062	0.018	1.06	0.98	1.02	0.17	2.39	1.14	1.05	1.03	0.96	0.93	0.21
Summer	P1	1.00	0.006	0.076	0.076	0.022	1.05	0.95	1.00	0.23	2.01	1.13	1.07	0.98	0.95	0.88	0.25
	P2	1.01	0.005	0.070	0.070	0.020	1.06	0.97	1.01	0.44	2.29	1.15	1.06	1.01	0.95	0.92	0.23
	P3	1.03	0.008	0.092	0.089	0.026	1.09	0.97	1.02	0.01	1.82	1.17	1.09	1.04	0.94	0.89	0.28
	P4	0.98	0.004	0.062	0.063	0.018	1.02	0.94	0.97	0.78	2.58	1.10	1.01	0.97	0.94	0.90	0.20
	P5	1.03	0.001	0.032	0.031	0.009	1.05	1.01	1.03	−0.70	2.28	1.07	1.06	1.04	1.01	0.97	0.10
	P6	1.08	0.009	0.093	0.086	0.027	1.14	1.02	1.08	−1.11	4.24	1.21	1.14	1.09	1.04	0.85	0.36
	P7	0.98	0.002	0.043	0.044	0.012	1.00	0.94	0.97	−0.59	2.31	1.02	1.00	0.98	0.95	0.89	0.13
	P8	0.95	0.005	0.068	0.072	0.020	0.99	0.90	0.94	0.05	1.76	1.05	1.01	0.95	0.89	0.85	0.20

Table 4
Comparison of different process typologies on genotoxic assessment of various types of water and wastewater samples.

Sample type	Potential genotoxic hazards	CIF levels	Reference and region
Domestic and industrial effluent samples (organic and inorganic chemical plants, metallurgical plants, pulp and paper mills, and municipal wastewater treatment plants)	Soluble DNA-damaging agents	1.56–1.88	Legault et al. (1996), Canada
Extracts of a medicinal plant *Thuya occidentalys* tictures	Presence of the activating mixtures	0.47–1.42	Valsa and Felzenszwalb (2001), Brazil
Surface water samples	Mutagenic compounds	2.94–7.45	Isidori et al. (2004), South Italy
Surface drinking water treated with chlorine and alternative disinfectants	NaClO, ClO_2, peracetic acid (PAA)	Moderate and high levels defined in the study	Guzzella et al. (2004), Italy
Hospital wastewater	Possible causative agents, anticancer drugs, antimicrobial agents	0.92–1.90	Jolibois et al. (2003), France
Industrial, hospital and domestic wastewater	Anticancer drugs (mitomycin, adriamycin, bleomycin, daunomycin, etc.)	1.01–2.15	Jolibois and Guerbet (2005), France
Surface water before and after various potabilization steps	Pre-disinfectant residues, ClO_2	1.00–2.00	Zani et al. (2005), Italy
Sediment samples	Polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and metals	0.80–8.96	Cachot et al. (2006), France
Untreated hospital effluent	Several chemicals, remnants of medicine, disinfectants, antineoplastic drugs,	1.02–6.54	Ortolan and Ayub (2007), Brazil

the NE direction, it is most often blowing from the SW. Northerly winds are dominant from May to October and occur with a frequency of 60%. The winds are southerly 20% of the time, and these winds occur mainly during the winter months. Hence, the possible effects of meteorological conditions on the variation of genotoxic activities in the surface waters of the Golden Horn Estuary were proven with a series of statistical tests that were performed in the present study. Furthermore, differences in the variation of genotoxic activity may also be attributed to the water level fluctuations, the effect of surface water flow, topographic and hydraulic factors, and to the experimental conditions (i.e. bacteria intensity, dilution ratios) that were considered in this study.

3.4. Statistical comparison of regional genotoxic activities

A total of 48 subsets were statistically compared, based on their regional genotoxic activities, to determine differences between different pairs of groups. Statistical analysis of the seasonal genotoxic data from Karakoy (P1) showed that there was sufficient evidence to suggest that a significant difference in genotoxic activity existed between the autumn and winter subsets ($p = 0.0246$). Based on this finding, we rejected the H_0. Furthermore, results from the paired t test analysis revealed that there were significant differences between two subsets from Galata Bridge (P2) (winter–spring ($p = 0.0052$) and winter–summer ($p = 0.0046$)), which resulted in our rejection of the H_0. In a similar manner, results from the paired t test analysis revealed that there were significant differences between two subsets from Unkapani (P3) (winter–spring ($p = 0.0092$) and autumn–winter ($p = 0.0084$)). In addition to P2 and P3, two subsets from Kasimpasa (P4) (winter–summer ($p = 0.0394$) and spring–summer ($p = 0.0031$)) were also found to be statistically different. Based on these findings, we rejected the null hypothesis in favor of the alternative hypothesis ($\alpha = 0.05$). However, both parametric and non-parametric tests concluded that no sufficient evidence was generated to suggest that a significant difference in CIF values exists across any of the comparison groups from Valide Sultan Bridge (P5).

As seen in Table 5, the paired t test results indicated that significant differences were only found between the spring and the summer subsets from both Halic Bridge (P6) and Sutluce (P7)

Table 5
Results of appropriate parametric or non-parametric tests presenting only statistically significant subsets by means of the regional genotoxic activities.

Sampling location	Pairs of groups	Homogeneity of variances (Levene's test)	Test type	Statistical outputs[c]
Karakoy (P1)	Autumn–Winter	$F = 5.54640$; $p = 0.0279$[b]	Non-parametric (Mann–Whitney or Kruskal–Wallis)	p(MW) = 0.0246, p(KW) = 0.0257
Galata Bridge (P2)	Winter–Spring	$F = 0.3044$; $p = 0.5867$	Paired t test	se_p[a] = 0.012939, $p = 0.0052$
	Winter–Summer	$F = 0.1023$; $p = 0.7521$	Paired t test	$se_p = 0.015042$, $p = 0.0046$
Unkapani (P3)	Autumn–Winter	$F = 0.1248$; $p = 0.7272$	Paired t test	$se_p = 0.010833$, $p = 0.0092$
	Winter–Spring	$F = 1.8446$; $p = 0.1882$	Paired t test	$se_p = 0.013787$, $p = 0.0084$
Kasimpasa (P4)	Winter–Spring	$F = 2.2373$; $p = 0.1489$	Paired t test	$se_p = 0.014267$, $p = 0.0394$
	Spring–Summer	$F = 0.0134$; $p = 0.9087$	Paired t test	$se_p = 0.010589$, $p = 0.0031$
Halic Bridge (P6)	Spring–Summer	$F = 4.1548$; $p = 0.0537$	Paired t test	$se_p = 0.024659$, $p = 0.0099$
Sutluce (P7)	Spring–Summer	$F = 0.0604$; $p = 0.8081$	Paired t test	$se_p = 0.008299$, $p = 0.0413$
Adalar (P8)	Autumn–Winter	$F = 0.0000$; $p > 0.9999$	Paired t test	$se_p = 0.008424$, $p = 0.026$
	Autumn–Spring	$F = 1.2049$; $p = 0.2842$	Paired t test	$se_p = 0.012401$, $p = 0.0166$
	Autumn–Summer	$F = 3.2400$; $p = 0.0856$	Paired t test	$se_p = 0.014794$, $p = 0.0339$
	Winter–Summer	$F = 2.7815$; $p = 0.1095$	Paired t test	$se_p = 0.019153$, $p = 0.012$
	Spring–Summer	$F = 0.2235$; $p = 0.6411$	Paired t test	$se_p = 0.016535$, $p = 0.0013$

[a] Combined (or pooled) standard error.
[b] p Values <0.05 shows that variances are heterogeneous.
[c] p Values <0.05 were considered to be significant.

($p = 0.0099$ and $p = 0.0413$, respectively). Statistical analysis of the regional genotoxic activities also revealed that the highest CIF variations were observed for Adalar (P8), compared to the other sampling locations. For this sampling location (P8), five of the individual comparisons (about 83.33% of the total subsets from P8) showed significant differences, with 95% confidence, in paired *t* test. Besides the hydrodynamic and meteorological conditions, differences in the genotoxic responses were probably due to the topographic heterogeneity of the sampling site, which gave rise to regional CIF variations in the estuarine system. The results of the statistical analysis suggest that about 29.17% of the total mutual comparisons ($n = 48$), which were based on regional genotoxic activities, were observed to be statistically noticeable ($p < 0.05$). Similar to the observations made for the seasonal genotoxic activities, the statistical comparisons of the regional genotoxic activities clearly indicate that CIF values show some regional variations during several periods of the year.

4. Conclusions

The SOS chromotest procedure performed remarkably well as an early assay to screen for potential genotoxic activities in the surface waters of the Golden Horn Estuary. The success of this assay was, at least in part, due to its simplicity and rapidity. The SOS chromotest results clearly indicated that there were no potential genotoxic impacts, in terms of CIF values, found in the surface waters of the Golden Horn Estuary. All of the calculated CIF values were determined to be below the 1.5 threshold level. However, the extensive statistical analysis of the experimental data, which utilized several appropriate parametric and non-parametric tests, confirmed with 95% certainty that the CIF values can be influenced by seasonal variations at several sampling sites. During the spring period, the CIF variations were much lower than CIF variations that were observed during the autumn and summer months. These variations possibly depend on the thermodynamic and meteorological conditions that dominate the region. The statistical findings from the analysis of the regional genotoxic activities also revealed that the highest CIF variations were observed for the Adalar location. The present study also showed that incorporating a genotoxicological test into the conventional physicochemical analyses provided a better evaluation of the surface water characteristics of specific estuarine systems. However, the work described here is the first report from an integrated study investigating genotoxicity in the Golden Horn Estuary. Although the SOS chromotest responses indicated that the surface waters of the Golden Horn Estuary were not found to have any genotoxic effects on the aquatic environment, further investigations will be conducted to better characterize the nature and extent of the pollution. Further investigations will also assess the level of genotoxicity at different depths (from the upper parts to the lower parts and the sediments) of the estuarine system.

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