Abdülkadir YILDIZ

INVESTIGATING THE TOXICITY AND GENOTOXICITY OF BÜYÜKÇEKMECE LAKE, ÇORLU AND ERGENE STREAMS

M.S.
2013

FATİH UNIVERSITY

The Graduate School of Sciences and Engineering

Master of Science in
Environmental Engineering

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A thesis submitted to

the Graduate School of Sciences and Engineering

of

Fatih University

in partial fulfillment of the requirements for the degree of

Master of Science

in

Environmental Engineering

January 2013
Istanbul, Turkey

APPROVAL PAGE

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Abdülkadir YILDIZ

M.S Thesis – Environmental Engineering
January 2013

Supervisor: Assist. Prof. Dr. Mustafa PETEK

ABSTRACT

In this study, water samples were collected from Büyükçekmece Lake, Çorlu and Ergene streams after which they were analyzed for the toxic and genotoxic effects of the pollution. Besides these analyses, the samples were also analyzed physically and chemically. As a result of these analyses, the pollution level of Büyükçekmece Lake, an important fresh water source for the megacity of Istanbul as well as the pollution in Ergene and Çorlu basins, one of the important and densely industrialized basins in Turkey have been measured together with the toxic and genotoxic effects of this pollution.

As a result of the analyses, the samples taken from the BL-2 location of Büyükçekmece Lake near the TEM highway bridge have shown an SOS IF value that is greater than 1.2 in two seasons, thus showing genotoxic effects. The TU value at this location is between 0 and 1, and so it can be classified as mildly toxic in all the seasons. In the Çorlu and Ergene streams, toxic and genotoxic effects have been observed especially at locations subject to heavy industrial discharge. At the heavily industrially discharged location CS-5 of the Çorlu Stream, the SOS IF value has been measured between 2.4 and 3.8 while the TU value is between 2.4 and 4.1. This shows the existence of both toxic and genotoxic effects at this location. In the ES-10 location of the Ergene Stream where there is a heavy industrial discharge, both toxic and genotoxic effect have been observed. The SOS IF value at this location is between 0.9 and 2.2, while the TU value is between 1.7 and 2.6.

Keywords: Microtox Toxicity Test, SOS Chromotest Genotoxicity Test, Industrial Pollution, Ergene Stream, Büyükçekmece Lake

BÜYÜKÇEKMECE GÖLÜ, ÇORLU VE ERGENE DERELERİNİN TOKSİSİTESİNİN VE GENOTOKSİSİTENİN ARAŞTIRILMASI

Abdülkadir YILDIZ

Yüksek Lisans Tezi – Çevre Mühendisliği
Ocak 2013

Tez Danışmanı: Yrd. Doç. Dr. Mustafa PETEK

ÖZ

Bu çalışmada Büyükçekmece Gölü, Çorlu ve Ergene derelerinden su numuneleri alınıp, kirliliğin toksik ve genotoksik etkileri araştırılmıştır. Bu analizlerin yanında çeşitli fizikokimyasal analizler de yapılmıştır.. Yapılan bu analizlerle, İstanbul gibi bir megakentin en önemli içme suyu kaynaklarından olan Büyükçekmece Gölü'ndeki ve Türkiye'nin önemli havzalarından olan ve yoğun sanayinin olduğu Ergene Havzası'ndaki Çorlu Deresi, Ergene Deresi ve bu iki derenin birleşmesinden oluşan Ergene Nehri'ndeki kirliliğin sebep olduğu toksik ve genotoksik etkiler incelenmiştir.

Yapılan incelemeler sonucunda Büyükçekmece Gölü'ndeki numunelerden TEM otoyolu köprüsünden alınan BL-2 örnekleme noktasında SOS IF değerinin iki mevsimde 1.2'den büyük olduğu yani genotoksik etkinin görüldüğü saptanmıştır. Bu noktada TU değeri 0-1 arasında görüldüğünden hafif toksik etki her mevsimde görülmüştür. Çorlu ve Ergene derelerinde ise özellikle sanayinin yoğun olduğu noktalarda toksik ve genotoksik etkiler saptanmıştır. Çorlu Dere'sinde sanayi kirliliğine maruz kalmış CS-5 noktasında SOS IF değeri 2.4 ile 3.8 arasında, TU değeri ise 2.4 ile 4.1olarak görülmüştür. Bu da bu noktada hem toksik hem de genotoksik etkinin varlığını göstermektedir. Ergene Dere'sinde ise yine sanayi atıksu girişiminin fazla olduğu ES 10 noktasında hem toksik hem de genotoksik etkiler saptanmıştır. Bu noktada SOS IF değeri 0.9 ile 2.2 arasında, TU değeri ise 1.7 ile 2.6 arasında görülmüştür.

Anahtar Kelimeler: Mikrotox Toksisite Testi, SOS Chromotest Genotoksisite Testi, Endüstriyel Kirlenme, Ergene Deresi, Büyükçekmece Gölü

I lovingly dedicate this thesis to my family, who supported me each step on the way

ACKNOWLEDGMENTS

First and foremost, I am heartily thankful to my supervisor, Assist. Prof. Dr. Mustafa PETEK, whose encouragement, guidance and support from the initial to the final level enabled me to develop an understanding of the subject.

I am also greatful to the gratitude is extended to my committee members Prof. Dr. Mehmet Borat, Assoc. Prof. Dr. Mustafa Fatih Abasıyanık, and Assist. Dr. Sami Gören for their contribution to this study and for their valuable recommendations.

I sincerely wish to thank my dear brother Bahattin Yıldız for his self-sacrifice and being near me whenever I needed. I am also thankful to his family.

I would also like to thank my dear friends; Yüsra Tanrıseven Altun for her infinity assistance, Serkan Akkuş, Muhammed Karaosman, Ramazan Aydemir, Saida Ammi for their interest, help and encouragement during this study.

I would like to express my special thanks to my dear wife, Kadriye Yıldız for her love, friendship, endless support to me and believing, relieving and trusting in me throughout my study and my life.

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SYMBOL/ABBREVIATION

4NQO	4-Nitro-Quinoline-Oxide
β-Gal	β-Galactosidase
AP	Alkaline phosphatase
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
DMSO	Dimethyl Sulfoxide
EC	Efficient Concentration
EPA	Environmental Protection Agency
E. coli	*Escherichia coli*
I0	The initial light levels
IF	Induction Factor
MOAS	Microtox Osmotic Adjustment Solution
NaCl	Sodium chlorur
OD	Optic Density
RF	Reduction Factor
SOSIP	SOS inducing potency
TSS	Total Suspended Solids
TU	Toxicity Unit
UV	Ultraviolet
V. fischeri	*Vibrio fischeri*

CHAPTER 1

INTRODUCTION

The rapidly growing population and the related increase in industrial and agricultural activities are causing pollution in surface waters. The pollution was under control before but now it has come to a level that cannot be controlled anymore. The pollution in surface waters is especially common in regions with increased industrial activities.

Human and industrial activities are at the origin of the discharge of multiple chemical substances in the environment and are the main causes of environmental pollution (White and Rasmussen 1998).

Substances contained in pollution are frequently toxic to aquatic organisms. Except in cases of accidents, their concentrations in surface water are low but the pollutants are present in water environment for long period of time. Under these conditions, the substances can act chronically. Risk of chronic impact of surface water pollution is very often underestimated due to hidden long time action. The impact usually does not manifest markedly by direct death of affected organisms a failure of important vital functions, which decreases organism vitality, is more common. From this reason, a natural balance of ecosystem may be influenced, and finally it can lead to ecosystem destruction (Soldan, 2003).

It is extremely difficult to quantify the risk associated with these chemical pollutants because they usually occur in concentrations too low to allow analytical determination, and putative mutagens, with few exceptions, have never even been identified. Moreover, the composite effects of mixtures cannot be readily assessed via analytical methods. Thus toxicity is often evaluated by means of biological tests, as well as by bacterial genotoxicity tests which do not require a priori knowledge of toxicant identity and/or physical–chemical properties (Aammi, 2010).

Toxicity of a substance can be affected by many different factors, such as the pathway of exposure (is the toxin applied to the skin, ingested, inhaled, injected), the time of exposure (a brief encounter or long term), the number of exposures (a single dose or multiple doses over time), the physical form of the toxin (solid, liquid, gas), the genetic makeup of an individual, an individual's overall health, and many others (Philp, 2000).

Genotoxicity describes a deleterious action on a cell's genetic material affecting its integrity. This includes both certain chemical compounds and certain types of radiation. Genotoxic substances are all those with affinity to interact with DNA - which is not proof of their dangerousness to humans, but does render them potentially mutagenic or carcinogenic (Jha, et al.,2000).

The main purpose in this study is to investigate the effects of the pollution occurring in Büyükçekmece Lake, one of the important fresh water sources of the metropolitan Istanbul area, and Çorlu Stream and Ergene Stream, on the toxicity and DNA’s of the living population.

One of working areas of this study is Büyükçekmece Lake which is one of Istanbul’s main fresh water supplies. Büyükçekmece Lake, which has a drainage area of 620 km2, is the third largest water resources of Istanbul, and is under great risk of contamination. It is located 35 km to the southwest of Istanbul, which is the most significant historic, cultural, and economic center of Turkey (Güyer, 2010).

The other working areas of this work are Ergene and Çorlu streams in Ergene Basin that one of the most prominent basins in Turkey.

Ergene brook and Çorlu Stream rises from Yıldız (Istranca) Mountain ranges in Turkish Thrace region and then they join Meriç River (which is a border river between Greece and Turkey) as Ergene River. The River has 285 km length and maximum 680 cm depth. Quality of water and sediment of Ergene River Basin is deeply affected by numerous factories and agricultural areas settled in Turkish Thrace region (Anonymus, 1989).

Microtox® toxicity testing technology is a biosensor-based measurement system for toxicity. Microtox test systems are based on the use of luminescent bacteria (*Vibrio fischeri*) which produce light as a by-product of normal metabolism.

The SOS Chromotest is a biological assay to assess the genotoxic potential of chemical compounds. The test is a colorimetric assay which measures the expression of genes induced by genotoxic agents in *Escherichia coli*, by means of a fusion with the structural gene for β-galactosidase. The test is performed over a few hours in columns of a 96-well microplate with increasing concentrations of test samples (Mohn, 1981).

In this study, water samples were collected from Büyükçekmece Lake, Çorlu and Ergene streams after which they were analyzed for the toxic and genotoxic effects of the pollution. Water samples from Büyükçekmece Lake were collected from five different points during three seasons. Water samples from Çorlu and Ergene streams however were collected on a regular monthly basis for a whole water year from different locations. The toxicity and genotoxicity analyses were made on these samples. The Microtox Toxicity Test Method was used for the toxicity analyses, while for the genotoxicity analyses the SOS chromotest method was used. Besides these analyses, the samples were also analyzed physically and chemically for their pH, conductivity, chemical oxygen demand (COD), total suspended solids and phenol contents.

As a result of these analyses, the pollution level of Büyükçekmece Lake, an important fresh water source for the megacity of Istanbul as well as the pollution in Ergene and Çorlu basins, one of the important and densely industrialized basins in Turkey have been measured together with the toxic and genotoxic effects of this pollution.

CHAPTER 2

THEORITICAL BACKGROUND

2.1 TOXICITY

Toxicity is a relative property reflecting a chemical's potential to have a harmful effect on a living organism. It is a function of the concentration and composition/properties of the chemical to which the organism is exposed and the duration of exposure. Traditionally, toxicity data have been used in comparing chemical substances or the sensitivities of different species to the same substance. Information about the biological mechanism affected and the conditions under which the toxicant is harmful are also important for this comparison. Toxicity tests are therefore used to evaluate the adverse effects of a chemical on living organisms under standardized, reproducible conditions that permit comparison with other chemicals or species tested and comparison of similar data from different laboratories (Rand, 1995).

Toxicity of a substance can be affected by many different factors, such as the pathway of exposure (is the toxin applied to the skin, ingested, inhaled, injected), the time of exposure (a brief encounter or long term), the number of exposures (a single dose or multiple doses over time), the physical form of the toxin (solid, liquid, gas), the genetic makeup of an individual, an individual's overall health, and many others (Philp, 2000).

Toxicity can be divided into the broad categories; direct and indirect. Direct toxicity results from the toxic agent acting more or less directly at sites of action and/or on organisms; indirect toxicity occurs because of the influence of changes in the chemical, physical and/or biological environment (e.g. changes in the quality and/or biological environment organisms or habitat changes and/or losses). Although most indirect toxicity on a population or community may be tracked back to direct toxicity in a particular group and species, this is not always the case. Most experimental toxicology studies have been concerned with direct toxicity to individual species. The direct toxicity information gained is then used to estimate indirect effects or interpret site-specific situations (Rand, 1995).

The graphic representation describing the response of an enzyme, organism, population, or biological community to a range of concentrations of a xenobiotic is the dose response curve. Enzyme inhibition, DNA damage, death, behavioral changes, and other responses can be described using this relationship (Landis and Yu, 1998).

Two parameters of this curve are used to describe it: (1) the concentration or dose that results in 50% of the measured effect and (2) the slope of the linear part of the curve that passes through the midpoint. Both parameters are necessary to describe accurately the relationship between chemical concentration and effect. The midpoint is commonly referred to as a LD50, LC50, EC50, and IC50. The definitions are relatively straightforward (Landis and Yu, 1998):

- LD50: The dose that causes mortality in 50% of the organisms tested estimated by graphical or computational means.
- LC50: The concentration that causes mortality in 50% of the organisms tested estimated by graphical or computational means.
- EC50: The concentration that has an effect on 50% of the organisms tested estimated by graphical or computational means. Often this parameter is used for effects that are not death.
- IC50: Inhibitory concentration that reduces the normal response of an organism by 50% estimated by graphical or computational means. Growth rates of algae, bacteria, and other organisms are often measured as an IC50.

2.1.1 Microtox Toxicity Test

Microtox® is a standardized toxicity test system which is rapid, sensitive, reproducible, ecologically relevant and cost effective. It is recognized and used throughout the world as a standard test for aquatic toxicity testing. The Procedure employs the bioluminescent marine bacterium (*Vibrio fischeri*) as the test organism. The bacteria are exposed to a range of concentrations of the material being tested (AZUR, 1998).

The reduction in intensity of light emitted from the bacteria is measured along with standard solutions and control samples. The change in light output and concentration of the toxicant produce a dose / response relationship. The results are normalized and the EC50 (concentration producing a 50% reduction in light) is calculated (AZUR, 1998).

The Microtox® system has been used as an aquatic toxicity test since the early 1980's and is accepted as a standard test in a number of countries including Australia. In the United States it is a recognized test method in a number of federal programs (AZUR, 1998).

Overall, the Microtox system seems to be a good method of determining the toxicity of a substance. It seems to have very few disadvantages and many advantages. It can be used for a variety of applications, and is relatively quick, easy, and cheap. A greater number of organisms can be used in the test since bacteria are used, as compared to fish, which take up much more space. A larger sample size is preferred for statistical reasons. Since this test is widely used, there is more information available on it, and results between different studies can be compared. The Microtox system seems like a good choice for measuring the toxicity of sample (Qureshi et al.,1998).

One disadvantage of the Microtox system is that some investigators found it to be too sensitive, while others found it to not be sensitive enough, although most found it to have the right amount of sensitivity. It is more sensitive to some organic chemicals that can easily cross the cell walls of the bacteria. It is less sensitive to metals because of salt present in the medium. Another disadvantage of the Microtox system is that it gives quantitative results when sometimes it only needs to be known whether a sample is toxic or not toxic, not specifically how toxic. There can also be inconsistencies in results with different temperatures, improper handling of the bacteria, inaccurate dilutions, different exposure times, and other human errors in following the procedure (Qureshi et al., 1998).

2.1.1.1 Vibrio fischeri

The marine bacterium *V. fischeri* exists naturally either in a free-living planktonic state or as a symbiont of certain luminescent fish or squid (Ruby & McFall-Ngai, 1999). The bacteria colonies specialized light organs in the fish or squid, which cause them to bioluminescent. Luminescence in the fish or squid is thought to be involved in the attraction of prey or even as camouflage. During the nocturnal feeding of the squid *Euprymna scolopes*, luminescence from the light organ is directed towards the sea floor and is modulated by the squid to match the intensity of moonlight, therefore preventing a shadow of the squid on the sea floor (Visick & McFall-Ngai, 2000). The source of the luminescence is the bacteria themselves. In the marine environment, the bacteria only luminescence when colonizing the light organs and do not emit light when in the free-living state. It makes good evolutionary sense for the bacteria to tightly regulate bioluminescence as the mechanism by which light is produced is very energy intensive. Research carried out to answer the question of how *V. fischeri* regulates bioluminescence led to the discovery of bacterial quorum sensing via N-acyl-L-homoserine lactones (AHLs) (Nottingham, 2011).

2.2 GENOTOXICITY

Genotoxicity or genetic toxicology refers to the capability of substances to damage DNA and/or cellular components regulating the fidelity of the genome such as the spindle apparatus, topoisomerases, DNA repair systems and DNA polymerases and includes all adverse effects on genetic information. These potentially harmful effects on genetic material may be mediated directly or indirectly and are not necessarily associated with mutagenicity. Genotoxicity is therefore a broader term than ‘mutagenicity’, which refers to the capacity to give rise to mutations (Eastmond, et al. 2009).

2.2.1 DNA Repair System

If chemical damage to the DNA is repaired correctly before cell division, then the mutation will be corrected and no effects will be seen.

After chemicals or carcinogenic induce DNA damage, a complex system of DNA repair is in place in mammals to correct DNA adducts, damaged bases or other mutagenic lesions before DNA replication sets the change permanently. As illustrated in figure 2.1, it is the interplay of all of these protective factors that determines the degree to which damage is translated into mutation and disease. Recently a large effort has gone into characterizing variation in DNA repair genes (William, et al., 2003).

Detoxication systems, then the consequences of high risk metabolic genotypes may be less significant. DNA repair enzymes maintain the integrity of the genetic code by minimizing replication errors caused by damaged or rearranged DNA templates and by removing damaged DNA segments. DNA damage may be a consequence of normal cellular function (e.g. replication errors, oxidative metabolism) or of environmental factors such as radiation or xenobiotic chemicals (William, et al. 2003).

Because DNA repair is a multi-path, multi-step, multi -enzyme process, and routine functional assays are not available, understanding how polymorphism in repair genes affects repair function and disease is a difficult technical problem. A large number of these enzymes depend on direct protein–protein interaction to form large repair complexes. Therefore, the fidelity of these systems can potentially be affected by polymorphisms that alter either the enzymatic activity or the ability of a component protein to bind a necessary protein partner. It is known that individuals lacking in various elements of DNA repair are predisposed to tumors, and changes in DNA repair activity may also affect a tumor's sensitivity to alkylating agents or cisplatin (Merrill, et al. 2001).

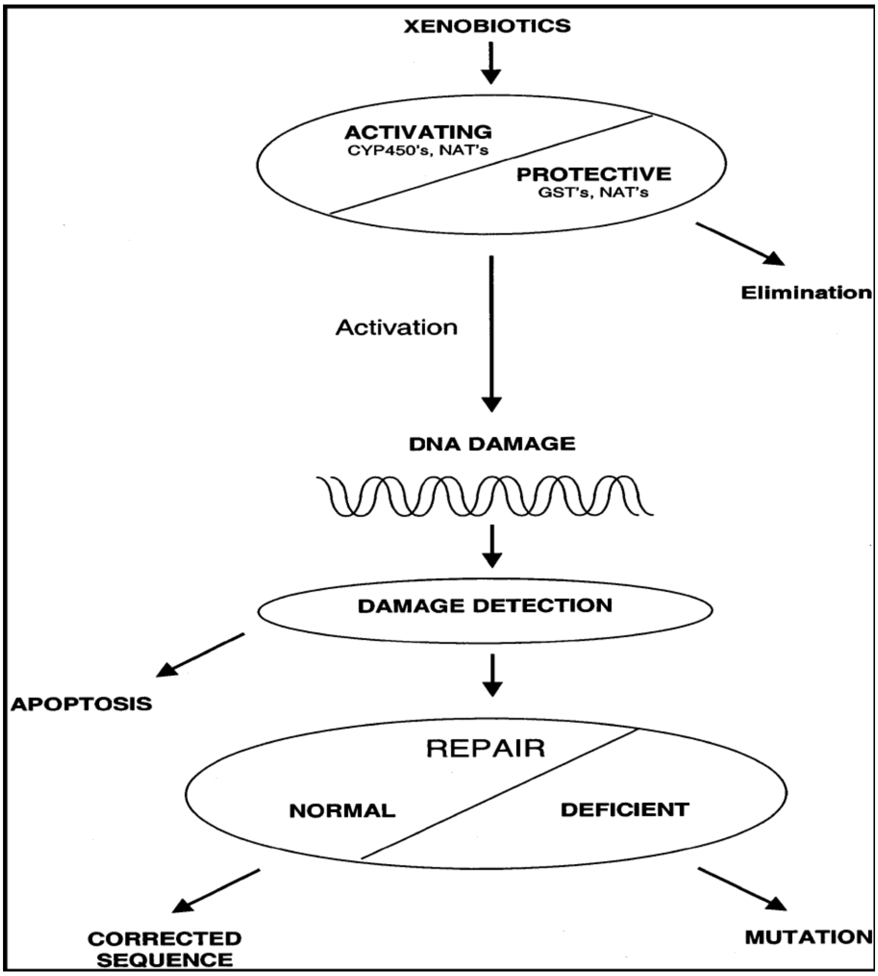

Figure 2.1 Cell responses after entrance of xenobiotics (Merrill, et al. 2001).

2.2.2 SOS Chromotest

The SOS Chromotest is a colorimetric assay of enzymatic activities that employs the error-prone DNA-repair pathway of *E. coli* strains PQ37, also known as the SOS response, a complex regulatory network that is induced by DNA-damaging substances (Neidhardt, et al 1987). The strain used in this study was *E. coli* PQ 37, which is constitutive for alkaline-phosphatase synthesis. This strain exhibits sfiA:lacZ fusion and has a deletion of the normal lac region, so β-galactosidase activity is strictly dependent on sfiA expression. This test involves incubation of the bacteria with the sample under investigation and subsequent determination of β-galactosidase (β-gal) activity (Quillardet and Hofnung, 1985).

The SOS-CHROMOTEST kit utilizes the cell's own mechanisms for the detection of genotoxicity. All living cells have developed a sensitive system for the detection of lesions in their genetic material so that a complex enzymatic system - the SOS repair system - can be activated to repair the damage. Once a lesion has been detected, an SOS promoter is induced to start the transcription of the SOS genes. This is the basis for the dependability and sensitivity of the SOSCHROMOTEST: even limited repairable damage to the genetic material will be detected by the SOS-CHROMOTEST, before the cell's repair system has had the chance to handle the emergency. The SOS-CHROMOTEST bacterial strain has been especially engineered to detect DNA damage: The strain's own repair system was altered by a series of mutations so that even limited damage to the DNA will not be repaired (SOS Chromotest Version 6.3, 2008).

The outer membrane of the cell was modified to increase permeability to many materials.

The SOS promoter does not activate the SOS system; instead it induces the synthesis of a readily detectable enzyme, which when it comes in contact with a chromogenic substrate catalyses the formation of color. The amount of color produced in the SOS-CHROMOTEST is a direct measure of the genotoxic damage to the DNA of the SOS-CHROMOTEST bacterial strain (SOS Chromotest Version 6.3, 2008).

2.2.3 *E.coli PQ 37* Strain

E. coli PQ37, whose genetic markers and biomolecular mechanisms has been described by Quillardet and Hofnung (1985). To describe the procedure briefly: *E. coli* PQ37s genetic map has been altered so that the β -galactosidase (β -gal) gene (lacZ) is fused to the sulA gene. This latter gene is part of the so-called error-prone SOS repair system (Walker, 1987). When bacterial DNA comes under the attack of genotoxins, the suZA gene is derepressed, causing β -gal to be synthesized. Since the normal lac region has been removed, the detection of significant amounts of, β -gal therefore can be linked to DNA lesions. Cell viability or cytotoxicity is monitored through the activity of alkaline phosphates (AP), which is constitutively expressed in E. *coli* PQ37 (Legault, et al., 1996).

This strain exhibits sfiA: lacZ fusion and has a deletion of the normal lac region, so that β-galactosidase activity is strictly dependent on sfiA expression.

An uvrA mutation renders the strain deficient in excision repair and accordingly increases the response to certain DNA damaging agents. An rfa mutation renders the strain lipopolysaccharide deficient and allows better diffusion of certain chemicals into the cell.

Two genes play a key role: lexA encodes a repressor for all the genes of the system and recA encodes a protein able to cleave the lexA repressor upon activation by an SOS inducing signal.

So once a lesion has been detected in the genetic material, an SOS promoter is induced to start transcription of the SOS genes .This the basis for their dependability and sensitivity of the SOS Chromotest (Petta, et al., 2004).

2.3 BÜYÜKÇEKMECE LAKE

Drinking and usage water of Istanbul is obtained from surface water sources which are lakes and dams in service. Büyükçekmece Lake which has 620 km2 drainage area and 100.0 million m3 annual capacity is located in European side and is the number 3 water source. Büyükçekmece Dam Lake is located near side of Istanbul. The watershed is affected by zone demand of Istanbul that is an expanding city day by day. The watershed and around of it are also an attractive area for industry because of its low gradient, nearness to the Ambarli Harbor and work power of city. Büyükcekmece Lake is an old valley gate which firstly was occupied by sea and became a bay and after that shore lake. In 1985, after the gates of Büyükçekmece Dam were closed, dam lake was formed by separating it from the sea. Lagoon was separated from the sea by constructing the body of dam inside the lake and submerged ground (IMP DYG, 2006).

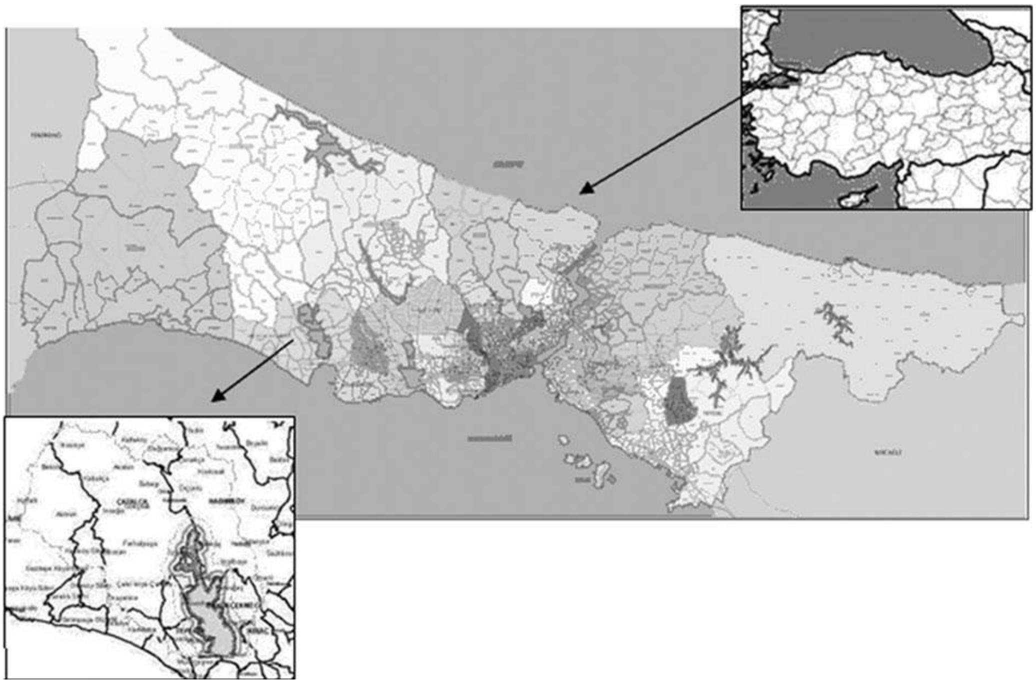

Figure 2.2 Location of Büyükçekmece Lake (Güyer and İlhan, 2010).

Maintaining and controlling fresh water sources that support big cities is possible with evaluating and controlling possible polluting factors that can reach the fresh water source through different channels. So far, in maintaining the fresh water sources for the city of Istanbul. In the global literature, there have been a lot of studies that show that poisonous and heavy metals such as chrome, lead, nickel, cadmium, cupper, arsenic, and mercury can be carried through the atmosphere into the fresh water sources and can be stored in substantial amounts in the terrestrial and aquatic media by the dry and wet sedimentation (Samura, et al., 2003).

When considering the metals that can be carried through the air to the Büyükçekmece Lake, chrome stands out as a significant ingredient. Because there are several sources in the region that can emit substantial amounts of chrome (Başak and Alagha 2004).

The rapid increase of Büyükçekmece Watershed population, urbanization, industrialization and unconscious and excess use of fertilizers in agricultural areas coming with environmental pollution destroy the quality of water resources (İlhan, 2009).

Water qualities of the streams of Büyükçekmece Lake are classified as 3rd and 4th class according to phosphorus parameter. The high phosphorus concentrations can be attributed to the transportation of fertilizers from agricultural zones and domestic wastewater discharge from residential areas around sampling stations (İlhan, 2009).

Büyükçekmece Watershed can be used as a source of drinking and usage water but note that Büyükçekmece Lake and its watershed are under risk of over pollution. Uncontrolled extend of settlement, industrial and agricultural areas inside the watershed are causing contamination and pollution (İlhan, 2009).

Aammi (2009) has measured genotoxicity of the samples she has taken from the Buyukcekmece Lake by using the SOS chromotest method. As a result of these studies, it has been observed that the Summer samples taken from the sea side of the Buyukcekmece Bridge as well as those taken from the regions around the Hezarfen village have shown genotoxic effects.

Güyer and İlhan (2010) have investigated the water quality parameters of samples collected from five different stations from Büyükçekmece Lake and its watersheeds. In this work, it was observed that the COD values of these samples were greater than 25, the upper limit for first class water quality. The water quality is second class with respect to the organic parameters (COD, BOD5, TKN). The TSS values vary between 28,33 mg/L and 93,33 mg/L. According to the physical parameters of water quality, it was observed as second class in the unrefined water, and in the other stations it was observed to be 3rd and 4th class.

2.4 ERGENE BASIN

The Ergene Basin is located in the middle of the Trakya region and is enclosed by the Northern Marmara Basin, The Meriç Basin and the Bulgarian border. The Ergene Basin is one of the 13 sedimentary basins in Turkey (EHÇDP, 2002).

The most important over ground water source for the basin is the Ergene River and its branches. Cutting right through the basin, the Ergene River starts as a stream from the sources close to the Taşpınar Hill, a 312 meter above the sea level hill in the Yıldız Mountains located in the northern part of Saray town of Tekirdağ. It then joins the the Çorlu Stream that comes from Çerkezköy, making up the Ergene River, with a continuous west-bound flow. The river converges with the Meriç River around the Meriç-Adasarhanlı village located 40 kilometers southwest of the Uzunköprü town.

Figure 2.3 Location of Ergene River Basin (ERPAP, 2012).

The Çorlu Stream starts from the east of Çerkezköy and arrives at Çerkezköy after coalescing with Boyacıçatağı, Lefka and Paşaderesi streams. After passing from the north of Çorlu, it takes on the Marmaracık and Kütüklü streams and joins the Ergene on the North of Muratlı. Its water gathering basin is 1319 km2 and its length is 62 km. (Topal, 2000).

Güneş et.al. have evaluated the industrial and soil based toxicity of the Ergene River in 2007. In this work, 3 out of the 4 locations chosen on the Ergene River have shown toxic effects. At these locations, the TU values have been measured to be between 2.04 and 7.67.

The water of Ergene River is polluted severely, especially regarding color, organics, total chromium, total sulfide and ammonium concentration The highly polluted sites were determined those located near the most urbanized and industrialized area, Çorlu and Çerkezköy. Uncontrolled landfill leachates are important pollution sources in the area as well (Güneş, et al., 2007).

Ergene River Basin has effluent-dependent and effluent-dominated streams and due to rapid urbanization and industrialization, presents a drastic environmental deteriotion example. A total number of about 1500 industries which use technology-based effluent limits in the region discharge their effluents with or without treatment into the river and streams. Some branches of the river have intermittent flow in dry seasons. Mostly as these streams receive little or no upstream dilution effluents comprise the majority of stream flows. A percentage of 80–85 % of the total flows in Ergene River comprise wastewaters (Güneş and Talınlı, 2013).

Many municipalities and industrial plants discharge wastewater directly or indirectly to Ergene River, while landfill leachate and fertilizers from cultivated land drain toward the river. A recent ecological assessment at Ergene River concluded the severe toxicity on *Vibrio fisheri* and the high COD concentrations (over 300mg/l) and therefore, water quality of the river was classified as “bad”, based on Turkish Water Quality Regulation (Nikolaou, et al., 2008).

Sakçalı et al. (2009) have studied the water pollution of the Ergene River in this work. According to this study, the average pH value is observed to be 7.47, the COD value is 94.5 mg/L, the BOD value is 33.46 mg/L, ($SO_{4)}$ value is 177.7 mg/L, Total Nitrogen value is 9.71 mg/L, dissolved oxygen value is 3 mg/L, Copper value is 0.019 mg/L, Zinc value is 0.01 mg/L, Iron value is 0.222 mg/L and Chrome value is measured to be 0.022 mg/L.

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Sampling

3.1.1.1 Sampling of Büyükçekmece Lake

Samples were collected from five different locations in Büyükçekmece Lake during three seasons in order to observe seasonal changes. The sampling locations for Büyükcekmece Lake are given in Figure 3.1. The samples were kept at +4 degrees.

Figure 3.1 The sample points of Büyükçekmece Lake.

3.1.1.2 Sampling of Çorlu Stream

The Çorlu Stream is heavily exposed to industrial wastes. Samples were collected from 7 different locations, starting from the stream bed in Çerkezköy (CS1), where there was no industrial discharge, up to regions where the discharge is more dense. The samplings were made on a regular seasonal basis during a whole water year. The collected samples were kept at +4 degrees.

3.1.1.3 Sampling of Ergene Stream

The Ergene Stream is also exposed to heavy industrial discharge much like the Çorlu Stream. Samples were collected from three different locations starting from region (ES8) where there is no discharge. Samples were collected on a regular monthly basis from this stream as well and the samples were kept at +4 degrees.

3.1.1.4 Sampling of Ergene River

The Ergene River is formed from the confluence of the Çorlu and Ergene streams. Samples were collected from the region right after the confluence point (ER11). Samples were collected for a year and kept at +4 degrees.

The sampling locations for the Çorlu and Ergene streams are given in figure 3.2.

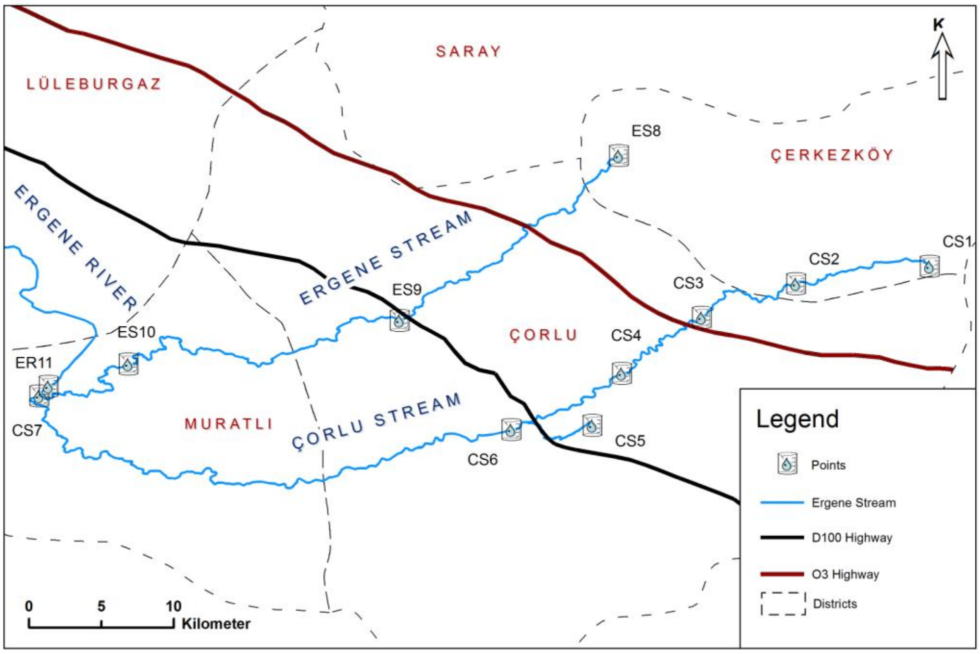

Figure 3.2 The sample points of Çorlu and Ergene Streams.

3.1.2 Laboratory Equipments

3.1.2.1 Spectrophotometer

The HACH DR 5000 UV-Visiable spectrophotometer instrument was used with hach test kits in chemical analyses such as COD, Phenol and zinc content. The instrument is seen in figure 3.3.

Figure 3.3 HACH DR 5000 UV-Visiable Spectrophotometer.

3.1.2.2 Microtox Analyzer

Microtox Analysis: Toxicity of the samples was determined using a Microtox Model 500 Analyzer (Figure 3.4), which utilizes freeze-dried luminescent bacteria (*V. fisheri*) as test organisms.

Figure 3.4 Microtox Model 500 Analyzer.

3.1.2.3 pH meter

A WTW pH 330i model (Figure 3.5) portable Ph meter was used to measure the Ph levels of the water samples.

Figure 3.5 WTW pH 330i model portable pH meter.

3.1.2.4 Conductivity meter

An EUTEC Cybersacn Con 11 model (Figure 3.6) portable conductivity meter was used to measure the conductivity of the water samples.

Figure 3.6 EUTEC Cybersacn Con 11 model portable conductivity meter.

3.2 METHODS

3.2.1 Microtox Toxicity Test Method

The Microtox Basic Test was carried out using the following protocol from the manufacturer (Microbics Corporation, Carlsbad, USA). Briefly, a range of culture filtrate dilutions from 45% to 0.56% was made in solvent supplied by the manufacturer. For the reactions, freezedried *V. fischeri* were reconstituted with 0.01% sodium chloride and 10 ml was mixed with 500 ml of each culture filtrate dilution. A Microtox_ Model 500 Analyser (AZUR Environmental, Carlsbad, CA, USA) using to measure the luminosity from the reconstituted bacteria after 5 and 15 min of exposure to culture filtrate. The luminescence inhibition after a 15 min exposure was taken as the endpoint (Kaiser, 1998; Froehner et al., 2000). A 2% sodium chloride solution was used for bacterial regeneration sample dilution and control. The osmotic control was made using OAS (Osmotic Adjusting Solution) of 22% sodium chloride. In the Microtox_ test, the inhibition of light emission was measured in relative units of luminescence. The data were used to calculate the EC50, which is the mean sample concentration that causes a 50% reduction in bacteria bioluminescence (Chen and Que Hee, 1995; Guzzella et al., 1996). The behavior of the bacteria was tested with reference toxins ZnSO4$7H2O and phenol, according to normative AFNOR T90-320 (AFNOR, 1991). Toxicity values are the average of four replicates of each filtrate sample, expressed as EC50-15 min with 95% confidence limits (Alba et al., 2009).

3.2.1.1 Microtox Toxicity Test Reagents

Microtox Reagent; It's freeze – dried culture of a specially developed strain of the marine bacterium *Photobacterium phosphoreum*. A vial of reagent contains roughly one hundred million test organisms. The reagent has a shelf – life of at least one year when stored in a freezer it minus 20°C. The sensitivity of the reagent is essentially unchanged for 3 – 4 hours after reconstitution.

Microtox Reconstitution Solution is specially prepared distilled water, free of toxic material. It's used only to reconstitute the reagent. It may be stored at room temperature.

Microtox Diluent; It's specially prepared 2 percent NaCl solution, toxicity free used for diluting the sample and the reagent. It may be stored at room temperature.

Microtox Osmotic Adjustment Solution (MOAS); It's specially prepared 22 percent solution NaCl, toxicity free, used to adjust the osmotic pressure of the sample to approximately 2 percent NaCl. The marine bacterium in the reagent requires osmotic protection and the rest is normally run in 2 percent NaCl. To adjust a sample somatically, one part of MOAS is added to pen parts of sample.

Microtox Cuvettes; Microtox cuvettes are used to contain samples, controls, and Microtox Reagent during testing. They are toxicity – free and disposable. The cuvettes are flat – bottomed test tubes. Their shape provides good heat – transfer characteristics in the Microtox Analyzer incubation block. Used cuvettes cannot reliably be cleaned for reuse. Traces of detergent or other contaminants interfere with later tests. The risk of contamination is unacceptably high.

3.2.1.2 Basic Test Procedure

200 µl of reconstituted reagent is transferred to 1800 µl Microtox diluents in Read Well and mixed thoroughly 15 – 20 times using 900 µl micropippetor. 100 µl of the reagent / diluent mixture (from the Read Well) is transferred to cuvettes B5 and B1.

They are allowed to stand for 15 minutes for 15°C temperature equilibrium. After the equilibrium period, the B5 cuvette is transferred into the turret (Read Well) and the set button is pressed to calibrate the instrument. The initial light levels (I0) are read by cycling the cuvettes through turret in the following order: B5, B4, B3, B2, and B1 by pressing the read button every 10 – 20 seconds.

The timer is set for 20 minutes and without removing the cuvettes from the incubator wells, bacteria challenge is started by removing 900 µl of each sample dilution and adding into the cuvettes containing 100 µl reagent / diluent as follows:

- 900 µl dilution blank is pipetted from A5 – B5 (Reagent Blank).
- 900 µl sample dilution is pipetted from A4 – B4.
- 900 µl sample dilution is pipetted from A3 – B3.
- 900 µl sample dilution is pipetted from A2 – B2.
- 900 µl sample dilution is pipetted from A1 – B1.

Transfers are made in the order of increasing concentration specified above.

5 minutes after the first bacterial challenge, the cuvettes are cycled through the turret in the order of increasing concentration (B5, B4, B3, B2, and B1) to obtain (I5) light levels.

The cycling of cuvettes is repeated for obtaining 15 minutes (I15) light readings.

3.2.1.3 EC50

EC50 is the sample concentration that reduces the reagent light output by 50 percent. This is obtained by calculating (or observing) the point at which gamma equals 1.0 on the dose – response curve. The EC50 is seen on the plot of the data where the dotted lines meet.

The Microtox System can calculate other ECs from the same stored data. For example, EC20, or even EC05 provide measures of greater sensitivity. An increase in the 95 percent Confidence Range may EC20 and EC05 values, depending on the observed responses to the test concentrations of the sample.

The effect measured by the Microtox System, light loss, is rate of biological activity, rather than a count of organisms affected (quantal data). The light is a by – product of the organism's respiration, and reflects change in respiration. The measurement of light integrates the response of about a million individual organisms per exposure to toxicant or control. Microtox data are subject to data linearization over a wide concentration span.

Microtox does not produce quantal data (yes / no, dead or alive). Quantal data (e.g. fish mortality data) can be treated statistically only when concentration levels both above and below the LC50 (Lethal concentration to 50 percent of the organisms) are tested. Confidence levels for quantal tests are greatest for a median result, such as LC50. This is not the case for Microtox, which generates quantitative data.

3.2.1.4 Toxicity Units (TU)

Some regularity agencies require that toxicity be expressed in Toxicity Units, as defined by the U.S. Environmental Protection Agency (EPA). To convert EC50s into EPA TU, divide 100 by the EC50.

TU = 100 / EC50

Toxicity Units avoid the confusion created by the inverse relationship: lower EC50s indicate higher toxicity (the lower the EC50 value the greater the toxicity is), whereas higher TU values indicate higher toxicity, another benefit of working with TU is that they simplify the comparability of data; a 20 TU (EC50 =5) sample is twice as toxic as a 10 TU (EC50 = 10) sample.

3.2.2 SOS Chromotest Genotoxicity Test

3.2.2.1 The SOS Chromotest kit components

The SOS Chromotest kit contains:

- Growth Media for the SOS-Chromotest Bacteria (5 units)
- The SOS-Chromotest bacteria (1 unit)
- Saline Solution (1 unit)
- Standard Genotoxic Solution – 4NQO (1 unit)
- Blue Chromogen Solution (2 units)
- Diluent for Alkaline phosphatase Substrate (1 unit)
- Dried Alkaline Phosphatase Substrate (1 unit)
- Stop Solution (1 unit)
- DMOS solution for dissolving water insoluble materials (1 unit)
- 96 Well Micro-Plates for assays (2 units)

3.2.2.2 The SOS Chromotest Test Procedure

SOS Chromotest was performed according to The SOS Chromotest Kit version 6.3 (Quillardet and Hofnung, 1985) with modification described by Aiub et al. (2003).

The same day of bacteria growth was used for test all samples in order to optimize the result. Using aseptic technique lyophilized bacteria was mixed with medium and incubated at 37°C in approximately 4 hours.

When this method is used the bacteria still in log phase growth and the color development, when exposed to a genotoxin, will occur within an hour or so. After that the following steps were applicated to complete the SOS Chromotest procedure.

The end of 4 hours, the bacterial suspension and measure the OD (at 600 nm) of the suspension against a fresh medium blank in a 1 cm light path cuvette suspension. Calculate the volume of suspension required to obtain 10mL of bacterial suspension with a final OD600 of 0.05 as outlined in the protocol. OD of bacteria was measured given 0.06 to 0.065 and 100µl of bacteria was transferred to 96 well micro plate which was consist in diluted samples, positive and negative controls.

Once the correct OD (600) of the bacteria has been completed, dispense 100µl of the diluted bacteria to each well of the micro-plate that will be used in the assay.

The 96 micro plate was incubated at 37°C during 2 hours. During this time, the bacteria are exposed to the material which contains the suspected genotoxins.

Blue chromogen solution (bottle F) was transferred to the dried alkaline phosphatase substrate (bottle H) and mixed 30 second.100µl of mixing solution was added immediately into each well of the plate.

After 25 minutes, absorbance of 96 well plates was measured at 405nm with ELISA –READER to determine alkaline phosphatase (AP) activity and then incubation continued.

According to OD of bacteria the plate was incubated at 37°C for 60 to 90 minutes until a blue color develops.

Finally, the absorbance of 96 well plate was measured at 620nm with ELISA –REDAER to determine and β-Gal activity and alkaline phosphatase activity. 50µl of the stop solution in bottle was added to each well of the plates.

Table 3.1 According to OD value at 600 nm, color development time.

OD^{600} of bacteria	Color development time (β-Gal activity)
0.05	1.5 hrs
0.06	1 hrs
0.07	0.5 hrs

Each sample was tested at two concentrations (100% and 50%) and repeated two times in order to make result clear. Positive control was prepared at eight concentrations to determine each step of β-Gal activity of bacteria and negative control was tested two times to check non-genotoxic conditions (SOS Chromotest Version 6.3, 2008).

During the experiment in the laboratory some instruments were necessary: Micropipettes were used for being disposable tips and in the range of 10 to 200 micro-liters; incubator 37 ºC.

Spectrophotometer or a photometer equipped with 600nm filter and using 1 cm light-path rectangular cuvettes (for preparation of the bacterial suspension).

and; for quantitative analysis of the results a micro plate reader (= "ELISA Reader") Spectrophotometer to measure optic density of bacteria; Micro-centrifuge (+"Microfuge"); Calculation of SOS Inducing Potency (SOSIP).

As defined before SOSIP is the slope of the linear portion of the plot and is given in the following equation:

$$\text{SOSIP} = \frac{10x(OD_3 - OD_1)}{C_3 - C_1} \tag{3.1}$$

OD ⇨ Optic density; C ⇨ Concentration;

$$\text{C} = \frac{ConcxVol}{MW} \tag{3.2}$$

where:

Conc. : Concentration of tested material in µg/mL,

Vol : Volume of the tested material solution in the well expressed in micro-liters, and

MW : Molecular weight of the tested material.

Calculation of induction factor IF:

The induction factor for a concentration c of the sample is given in the following equation:

$$\text{IF} = \text{Rc/R0} \tag{3.2}$$

where:

RC: The genotoxic activity for a concentration c of the sample RC= β gal/AP

R0: The ratio measured in the solvent control R0= β gal/AP

CHAPTER 4

RESULTS and DISCUSSIONS

4.1 TOXICITY

The Microtox_ Basic Test was carried out using the following protocol from the manufacturer (Microbics Corporation, Carlsbad, USA). Briefly, a range of culture filtrate dilutions from 45% to 0.56% was made in solvent supplied by the manufacturer. For the reactions, freezedried *V. fischeri* were reconstituted with 0.01% sodium chloride and 10 ml was mixed with 500 ml of each culture filtrate dilution. A Microtox_ Model 500 Analyser (AZUR Environmental, Carlsbad, CA, USA) was used to measure the luminosity from the reconstituted bacteria after 5 and 15 min of exposure to culture filtrate. The luminescence inhibition after a 15 min exposure was taken as the endpoint (Kaiser, 1998; Froehner et al., 2000). A 2% sodium chloride solution was used for bacterial regeneration sample dilution and control. The osmotic control was made using OAS (Osmotic Adjusting Solution) of 22% sodium chloride. In the Microtox test, the inhibition of light emission was measured in relative units of luminescence. The data were used to calculate the EC50, which is the mean sample concentration that causes a 50% reduction in bacteria bioluminescence (Chen and Que Hee, 1995; Guzzella et al., 1996). The behavior of the bacteria was tested with reference toxins ZnSO4$7H2O and phenol, according to normative AFNOR T90-320 (AFNOR, 1991). Toxicity values are the average of four replicates of each filtrate sample, expressed as EC50- 15 min with 95% confidence limits (Alba et al., 2009).

Some regularity agencies require that toxicity be expressed in Toxicity Units, as defined by the U.S. Environmental Protection Agency (EPA). To convert EC50s into EPA TU, divide 100 by the EC50.

TU=100/EC50

The TU results have been evaluated according to the classification done by Persone et. al. (1993): TU=0 non-toxic; 0<TU<1 moderated toxic; 1<TU<10 toxic; 11<TU<100 very toxic.

4.2 GENOTOXICITY

A same day culture of *E.coli* PQ37 strain was used for testing 4NQO, ultra Deionized Water and stream water samples. Different concentration (% 100 and % 50) of test samples and controls were dissolved in 10%DMSO and transferred to 96 well plate. The color development was measured at 405 nm and 620 nm to determine respectively Alkaline Phosphate activity and β-Gal synthesize for all test material. Absorbance results were calculated according to SOS Chromotest protocol (Table 4.1).

Eight concentration of 4NQO (positive control) was prepared in 96 well micro plate for this study and the absorbance of β-Gal and Alkaline phosphate activity AP was measured with ELISA READER.

The maximum and minimum absorbance value showed experiments were successful and results were suitable for SOS Chromotest protocol. SOS Inducing Potency (SOSIP), Alkaline Phosphate activity, β-Gal synthesize and Induction Factor (IF) were calculated for 4NQO (positive control) and for each test samples.

Accordingly to Quillardet and Hofnung (1985); a compound was classified as genotoxic if the IF max exceeded 1.2 and increased with increasing compound concentration. Therefore 4-Nitroquinoline-1-oxide is a genotoxic compound.

Table 4.1 Results of SOS Chromotest analysis of %100 and %50 concentrations.

	Spring		Summer		Autumn		Winter	
	100	**50**	**100**	**50**	**100**	**50**	**100**	**50**
CS- 1	0,014	0,01	0,083	0,079	0,248	0,238	0,794	0,854
CS- 2	0,238	0,214	0,392	0,512	0,776	0,748	0,942	0,986
CS- 3	0,794	0,854	1,192	1,052	**1,331**	**1,321**	0,932	1,064
CS- 4	**1,294**	**1,51**	0,864	0,984	**3,295**	**3,189**	0,846	0,806
CS- 5	**3,428**	**3,024**	**2,49**	**2,402**	**3,845**	**3,799**	**2,613**	**2,511**
CS- 6	**1,386**	1,102	**2,03**	**1,876**	**2,411**	**2,421**	**1,417**	**1,447**
CS- 7	1,056	1,148	**1,397**	**1,245**	**1,626**	**1,606**	1,087	0,969
ES-8	0,092	0,104	0,12	0,128	0,223	0,245	0,427	0,423
ES-9	0,134	0,152	0,142	0,13	0,122	0,128	0,741	0,787
ES-10	0,937	0,905	**2,228**	**2,218**	**1,726**	**1,742**	**1,26**	**1,204**
ER-11	**1,224**	**1,24**	**1,357**	**1,327**	**1,733**	**1,711**	**1,364**	**1,484**
BL-1	0,959	0,925	0,97	0,994	1,124	1,1	-	-
BL-2	**1,265**	1,163	0,726	0,726	**1,288**	1,198	-	-
BL-3	0,846	0,81	0,12	0,128	0,335	0,349	-	-
BL-4	0,728	0,736	0,63	0,618	0,637	0,611	-	-
BL-5	0,134	0,114	0,017	0,031	0,136	0,148	-	-

4.2.1 Positive Control of SOS Chromotest System with Genotoxicity of 4-Nitroquinoline-1-oxide

The genotoxin 4NQO was obtained from EBPI (Environmental bio-detection products). A stock solution was diluted with distilled water before the test.

The genotoxic properties of 4NQO were studied using SOS Chromotest. Results showed (Figure 4.1, 4.2, 4.3 and 4.4) that 4NQO was strong inducer of the SOS–repair system in strain *E-coli PQ37*. The maximum value of the IF coefficient was respectively during the four sampling periods: 4.2, 4.3, 5.1 and 5.2 at a dose of 10µg/ml. The, β-galactosidase activity was significantly increased compared to the negative control and the induction factor showed a clear dose-effect relationship.

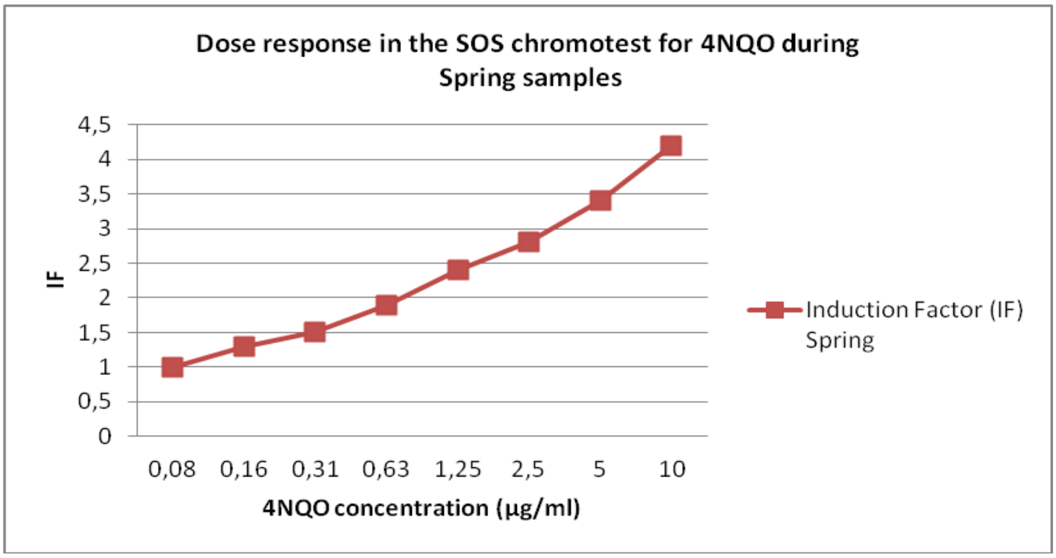

Figure 4.1. Dose response in the SOS chromotest for 4NQO during Spring samples.

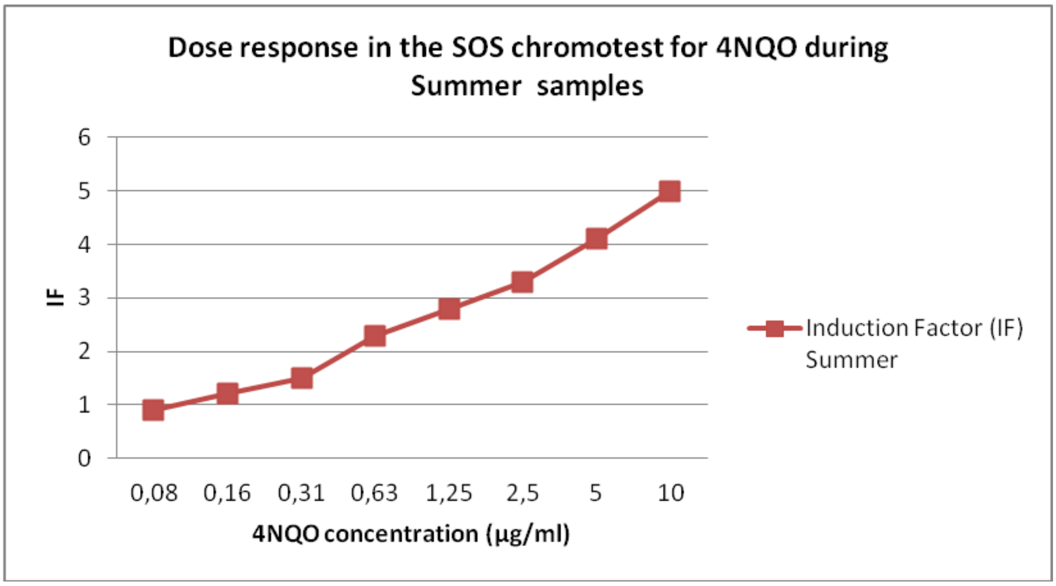

Figure 4.2. Dose response in the SOS chromotest for 4NQO during Summer samples.

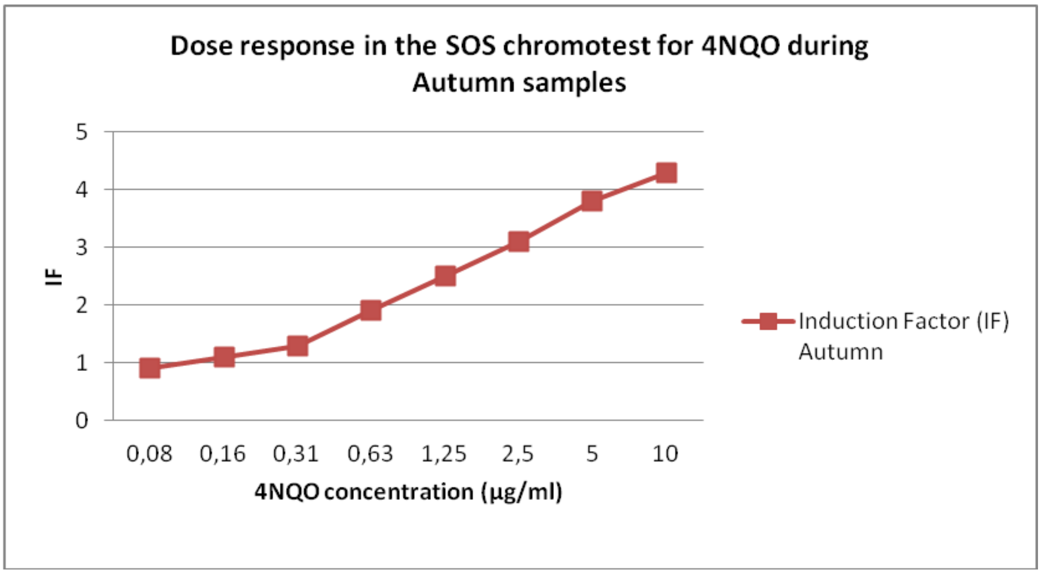

Figure 4.3. Dose response in the SOS chromotest for 4NQO during Autumn samples.

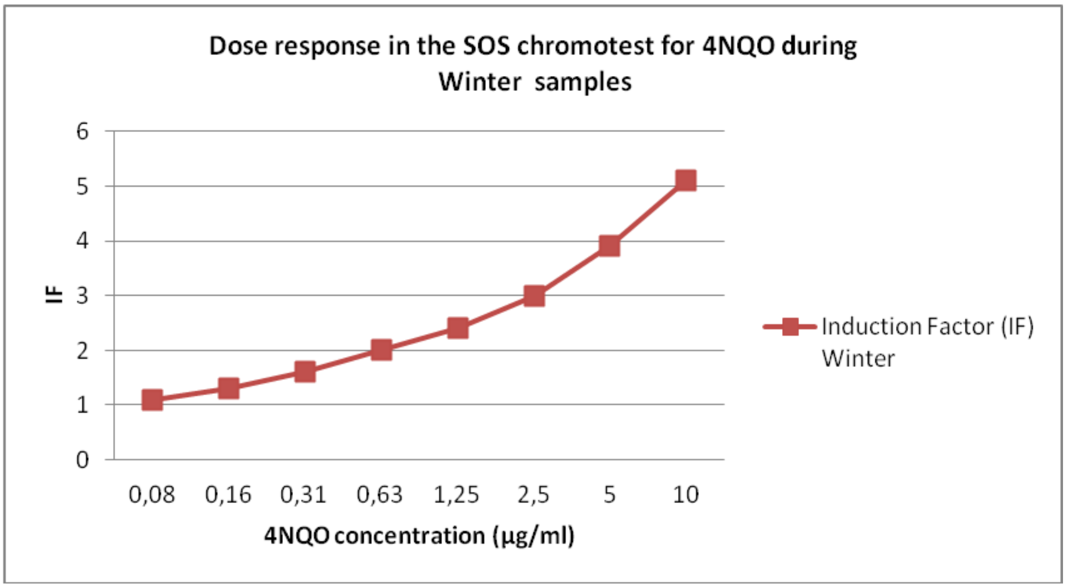

Figure 4.4. Dose response in the SOS chromotest for 4NQO during Winter samples.

4.3 RESULTS of ÇORLU STREAM

The seasonal water samples collected from the Çorlu Stream were tested for such physicochemical analyses as the TSS, The COD and phenol content to determine the level of the water pollution. Later the toxicity and genotoxicity that might be caused by this pollution were analyzed by the Microtox Toxicity Test and SOS Chromotest methods, respectively. The seasonal results of these analyses are given in Tables 4.2, 4.3, 4.4 and 4.5.

Table: 4.2 Autumn 2011 season results of Çorlu Stream.

	Sample points	Date:	ph	Conductivity	TSS	COD	Phenol	T.U	SOS IF
Çorlu Stream Autmn 2011	CS- 1	12.10.2011	7,75	155	68	82	0,699	-	0,243
	CS- 2	12.10.2011	8	1473	92	103	0,532	0,152	0,762
	CS- 3	12.10.2011	8,35	3030	84	175	0,934	0,9864	1,326
	CS- 4	12.10.2011	8,97	2880	236	211	0,867	2,4578	3,242
	CS- 5	12.10.2011	9,63	6530	136	240	2,09	4,1876	3,822
	CS- 6	12.10.2011	8,82	2880	216	189	0,901	2,7842	2,416
	CS- 7	12.10.2011	8,12	1760	668	116	0,66	1,7562	1,616

Table: 4.3 Winter 2012 season results of Çorlu Stream.

	Sample points	Date:	ph	Conductivity	TSS	COD	Phenol	T.U	SOS IF
Çorlu Stream Winter 2012	CS- 1	19.01.2012	8,3	174	60	50,9	0,477	-	0.824
	CS- 2	19.01.2012	8,7	3450	80	279	1,52	0,4297	0,964
	CS- 3	19.01.2012	8,4	5300	120	189	1,36	1,232	0,998
	CS- 4	19.01.2012	8,3	4760	420	190	1,57	0,5566	0,826
	CS- 5	19.01.2012	9,3	6040	320	371	3,12	3,144	2,562
	CS- 6	19.01.2012	8,6	4660	440	300	1,81	2,4322	1,432
	CS- 7	19.01.2012	8,2	4600	230	166	1,22	1,8412	1,028

Table: 4.4 Spring 2012 season results of Çorlu Stream.

	Saple points	Date:	ph	Conductivity	TSS	COD	Phenol	T.U	SOS IF
Çorlu Stream Spring 2012	CS- 1	26.04.2012	8,40	360	16	69,5	0,503	-	0,012
	CS- 2	26.04.2012	8,00	4870	84	348	1,73	0,4876	0,226
	CS- 3	26.04.2012	8,30	6110	148	257	2,16	0,5169	0,824
	CS- 4	26.04.2012	8,20	5480	132	294	2,23	2,596	1,402
	CS- 5	26.04.2012	8,90	5580	336	313	1,8	4,127	3,226
	CS- 6	26.04.2012	8,50	5670	440	323	2,05	1,997	1,244
	CS- 7	26.04.2012	8,00	5390	48	179	1,08	1,222	1,102

Table: 4.5 Summer 2012 season results of Çorlu Stream.

	Saple points	Date:	ph	Conductivity	TSS	COD	Phenol	T.U	SOS IF
Çorlu Stream Summer 2012	CS- 1	17.07.2012	8,1	891	196	122	0,344	-	0.081
	CS- 2	17.07.2012	9,48	5740	204	295	0,721	0,6896	0,452
	CS- 3	17.07.2012	8,26	6380	36	251	1,27	1,2424	1,122
	CS- 4	17.07.2012	8,3	6030	172	359	1,43	0,7426	0,924
	CS- 5	17.07.2012	9,34	7800	380	537	1,45	2,4624	2,446
	CS- 6	17.07.2012	8,5	8080	308	335	1,2	2,0846	1,953
	CS- 7	17.07.2012	8,12	5620	116	229	1,1	1,7887	1,321

The Çorlu Stream is heavily exposed to industrial discharges. The level of discharge is increasing, starting from the stream bed (CS-1). The CS-5 location is a branch joining the stream and it consists entirely of industrial waste water. As seen from Tables 4.2, 4.3, 4.4 and 4.5, the pollution level is highest at this point. The toxicity and genotoxicity values are also higher at this point compared to the other locations. As for the other locations, the pollution level is increasing parallel to the level of industrial discharge. No toxic or genotoxic effects have been observed at CS-1 location for a year. But toxic and genotoxic effects have been seen at other locations.

4.3.1 The Seasonal Changes in the results from the Çorlu Stream

4.3.1.1 Toxicity

The seasonal changes in the toxicity levels of the Çorlu Stream are given in figure 4.5. According to these results, the highest toxicity value is given as 4.2, which is observed in the sample collected during the Autumn season from location CS-5. No toxic effects were observed during any of the seasons in location CS-1. In location CS-2, the TU value has not exceeded 1 during any of the seasons. Thus in all the season, a moderated toxic level has been observed. As can be seen from the figure, the highest toxic values were observed in the CS-5 location in all the seasons. This location is followed by CS-6 and CS-4 in toxicity.

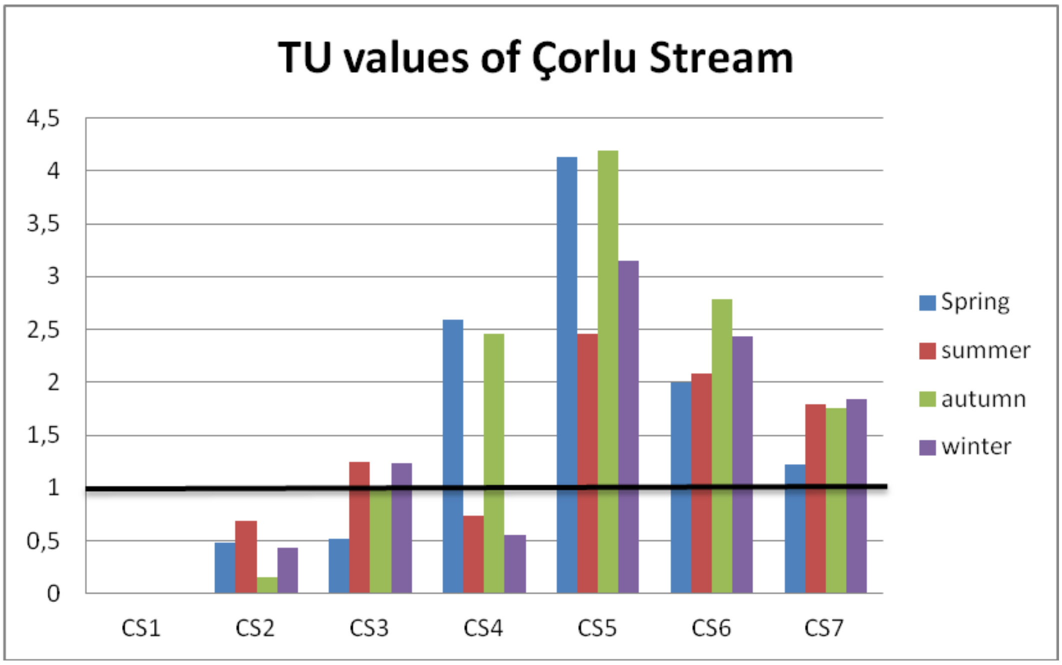

Figure 4.5 The seasonal changes in the toxicity levels of the Çorlu Stream.

4.3.1.2 Genotoxicity

The seasonal changes in the genotoxicity levels of the Çorlu Stream are given in figure 6. According to these results, the highest SOS chromotest IF value is observed at 3.8 in the samples collected from location CS-5 during the Autumn season. No genotoxic effects have been observed at locations CS-1 and CS-2. The highest IF value has been observed in the samples collected from location CS-5. As can be seen from the figure, the genotoxic effect in the Autumn season is higher.

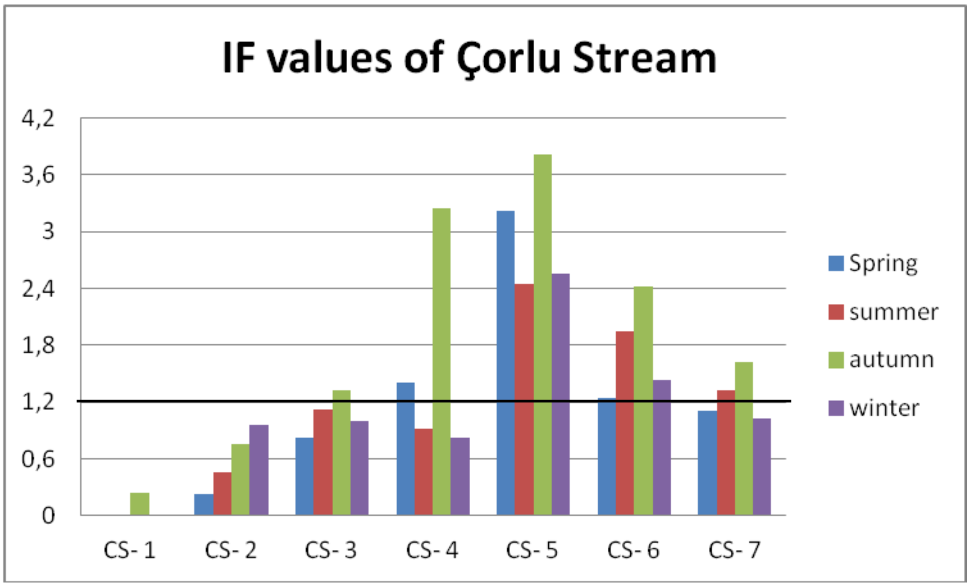

Figure 4.6 The seasonal changes in the genotoxicity levels of the Çorlu Stream.

4.3.1.3 Physicochemical Analyses

The seasonal changes in the physicochemical analyses performed on samples from the Çorlu Stream are given in figure 4.7, 4.8, 4.9 and 4.10.

Figure 4.7 shows the seasonal changes in the conductivity parameter. According to this, the highest conductivity parameter is observed to be 8080 µS, in the samples collected from location CS-6 during Summer.

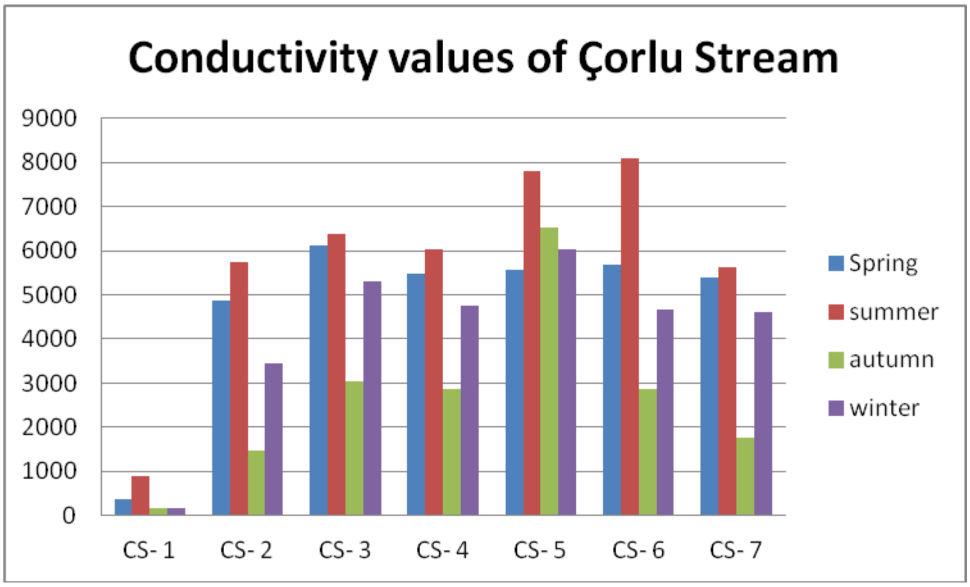

Figure 4.7 The seasonal changes in the conductivity levels of the Çorlu Stream.

Figure 4.8 shows the seasonal changes in the TSS parameter. Accordingly, the highest TSS value is measured to be 668 mg/L, observed in the samples collected from the location CS-7 during Autumn.

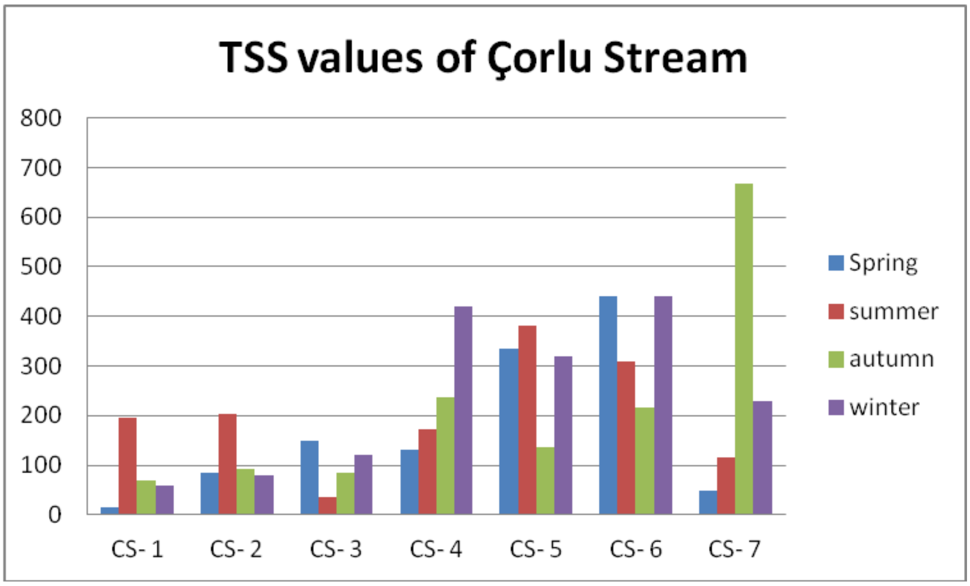

Figure 4.8 The seasonal changes in the TSS levels of the Çorlu Stream.

Figure 4.9 shows the seasonal changes in the COD parameter. The figures suggests that the highest COD value is 537 mg/L, observed in the samples collected from CS-5 during the Summer.

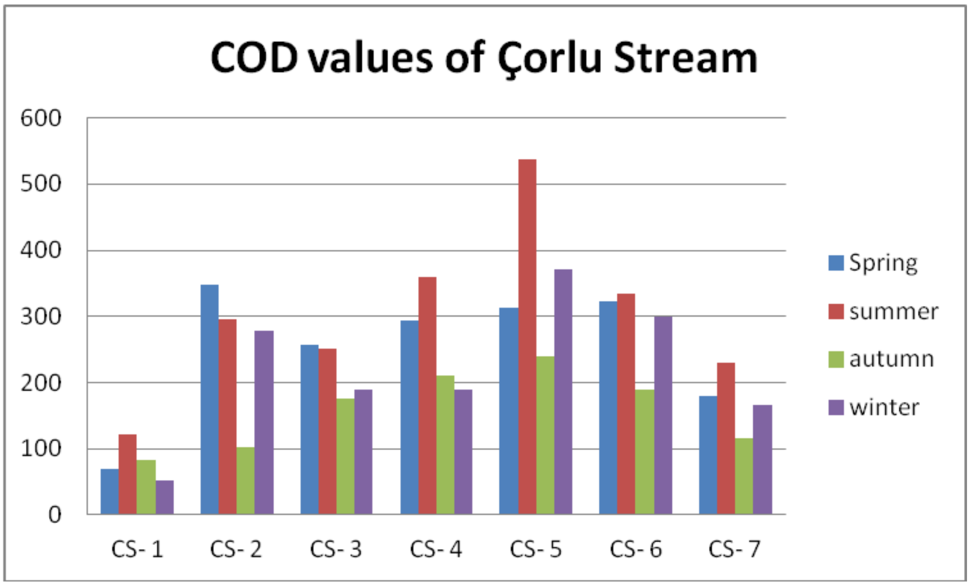

Figure 4.9 The seasonal changes in the COD levels of the Çorlu Stream.

The seasonal changes in the Phenol parameter are given in figure 4.10. According to the results we see that the highest Phenol value is 3.12 mg/L, which was observed in the samples collected from CS-5 during the Winter.

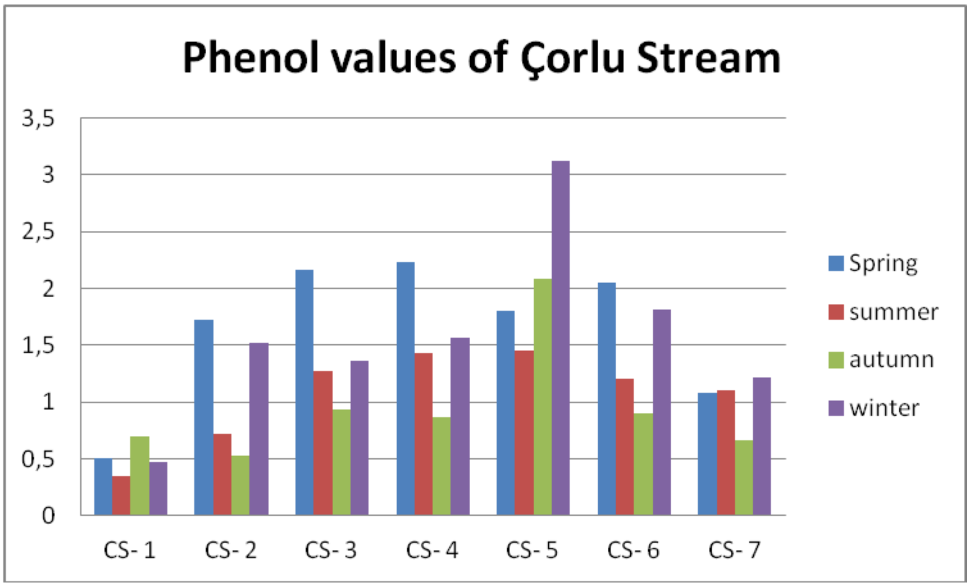

Figure 4.10 The seasonal changes in the Phenol levels of the Çorlu Stream.

4.4 RESULTS of ERGENE STREAM

The seasonal water samples collected from the Ergene Stream were tested for such physicochemical analyses as the TSS, The COD and phenol content to determine the level of the water pollution. Later the toxicity and genotoxicity that might be caused by this pollution were analyzed by the Microtox Toxicity Test and SOS Chromotest methods, respectively. The seasonal results of these analyses are given in Tables 4.6, 4.7, 4.8 and 4.9.

Table 4.6 Autumn 2011 season results of Ergene Stream.

Ergene Stream Autmn 2011	Sample points	Date:	ph	Conductivity	TSS	COD	Phenol	T.U	SOS IF
	ES-8	12.10.2011	7,84	475	180	74,4	0,251	-	0,234
	ES-9	12.10.2011	7,84	380	104	71,1	0,316	-	0,125
	ES-10	12.10.2011	8,4	1173	140	119	0,457	1,8742	1,734
	ER-11	12.10.2011	8,15	1672	592	238	0,782	2,1549	1,722

Table 4.7 Winter 2012 season results of Ergene Stream.

	Sample points	Date:	ph	Conductivity	TSS	COD	Phenol	T.U	SOS IF
Ergene Stream Winter 2012	ES-8	19.01.2012	7,53	444	12	33,4	0,276	-	0,425
	ES-9	19.01.2012	7,68	581	32	79,8	0,233	-	0,764
	ES-10	19.01.2012	8,1	2520	120	144	0,572	2,6892	1,232
	ER-11	19.01.2012	8	3300	240	166	0,919	2,4264	1,424

Table 4.8 Spring 2012 season results of Ergene Stream.

	Sample points	Date:	ph	Conductivity	TSS	COD	Phenol	T.U	SOS IF
Ergene Stream Spring 2012	ES-8	26.04.2012	7,6	378	88	30,4	0,159	-	0,098
	ES-9	26.04.2012	7,5	450	140	85	0,166	-	0,143
	ES-10	26.04.2012	7,9	2220	320	89,6	0,552	1,78	0,921
	ER-11	26.04.2012	7,5	3460	240	116	0,775	2,1462	1,232

Table 4.9 Summer 2012 season results of Ergene Stream.

	Sample points	Date:	ph	Conductivity	TSS	COD	Phenol	T.U	SOS IF
Ergene Stream Summer 2012	ES-8	17.07.2012	7,99	692	24	56,6	0,09	-	0,124
	ES-9	17.07.2012	7,6	992	46	63,6	0,11	-	0,136
	ES-10	17.07.2012	8,1	4920	120	274	1,42	2,1986	2,223
	ER-11	17.07.2012	8,1	4960	98	313	1,11	2,6842	1,342

The Ergene Stream is also exposed to industrial discharge much like the Çorlu Stream. The ES-8 location is a discharge-free location. The ES-9 location is not exposed to industrial discharge but there is a flow of domestic waste water to it. The ER-11 location is the point where the Ergene and Çorlu Stream merge to make up the Ergene River. As seen from Tables 4.6, 4.7, 4.8, and 4.9 there is no pollution and toxic or genotoxic effects at the locations ES-8 and ES-9 where there is no industrial discharge. However toxic and genotoxic effects are observed at the locations ES-10 and ER-11.

4.4.1 The Seasonal Changes in the Ergene Stream

4.4.1.1 Toxicity

The seasonal changes in the toxicity values observed in the Ergene Stream are given in Figure 4.11. According to these results, the highest toxicity value is measured at 2.689, in the samples collected from the ES-10 location during the Winter. No toxic effects have been observed in the samples collected from the ES-8 and ES-9 locations in any of the seasons.

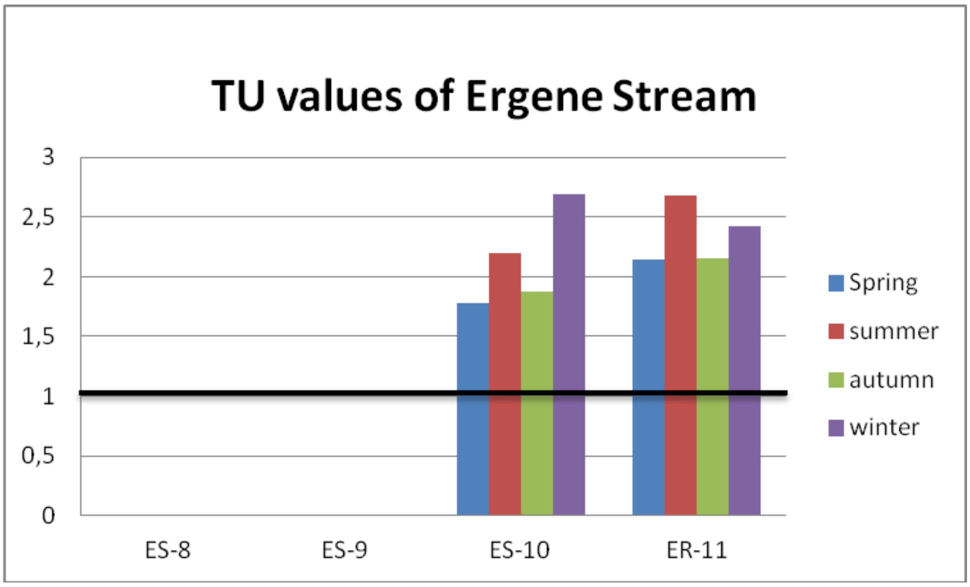

Figure 4.11 The seasonal changes in the toxicity levels of the Ergene Stream.

4.4.1.2 Genotoxicity

The seasonal changes in the genotoxicity levels of the Ergene Stream are given in figure 4.12. According to these results, the highest SOS chromotest IF value is observed at 1.73 in the samples collected from location ES-10 during the Autumn season. No genotoxic effects have been observed at locations ES-8 and ES-9. The highest IF value has been observed in the samples collected from location ER-11 and the genotoxic effect has been observed in each season samples in this point. As can be seen from the figure, the genotoxic effect in the Autumn season is higher.

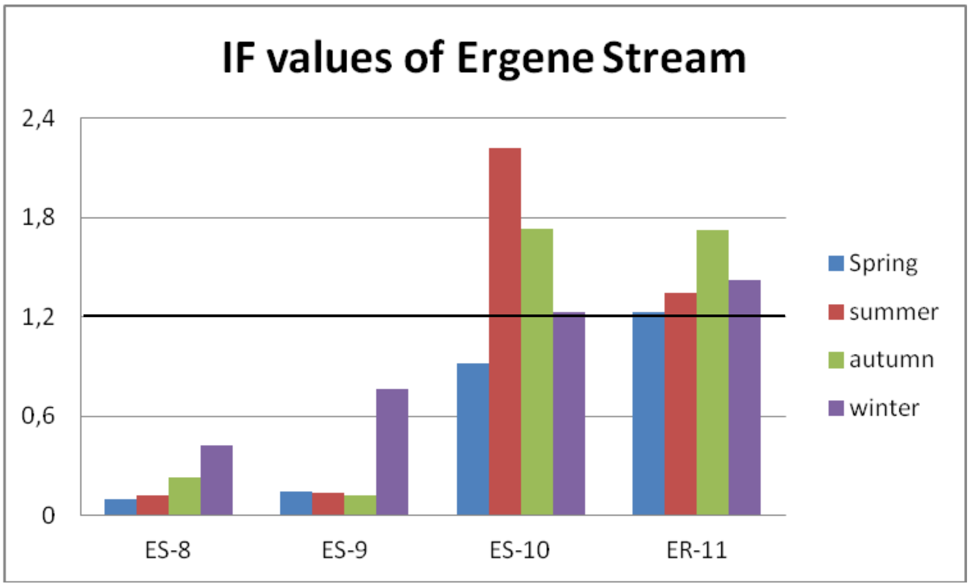

Figure 4.12 The seasonal changes in the genotoxicity levels of the Ergene Stream.

4.4.1.3 Physicochemical Analyses

The seasonal changes in the physicochemical analyses performed on samples from the Çorlu Stream are given in figure 4.13 - 4.16.

Figure 4.13 shows the seasonal changes in the conductivity parameter. According to this, the highest conductivity parameter is observed to be 4960 µS, in the samples collected from location ER-11 during Summer.

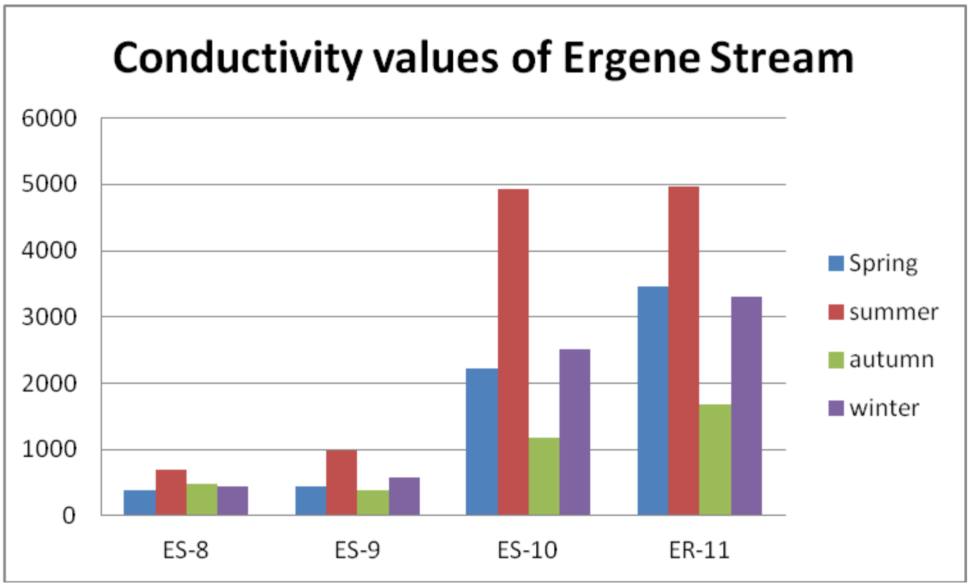

Figure 4.13 The seasonal changes in the conductivity levels of the Ergene Stream.

Figure 4.14 shows the seasonal changes in the TSS parameter. Accordingly, the highest TSS value is measured to be 592 mg/L, observed in the samples collected from the location ER-11 during Autumn.

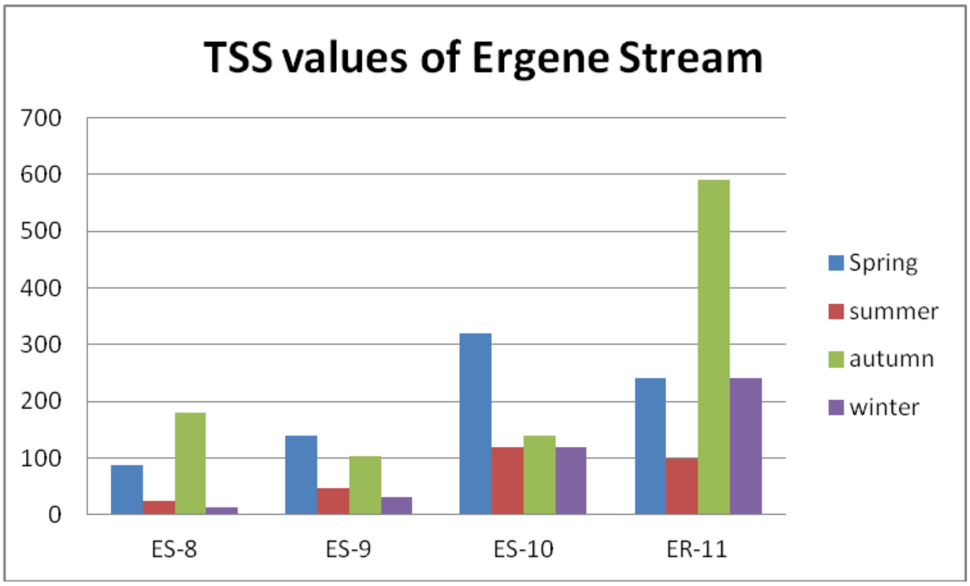

Figure 4.14 The seasonal changes in the TSS levels of the Ergene Stream.

Figure 4.15 shows the seasonal changes in the COD parameter. The figures suggests that the highest COD value is 313 mg/L, observed in the samples collected from ER-11 during the Summer.

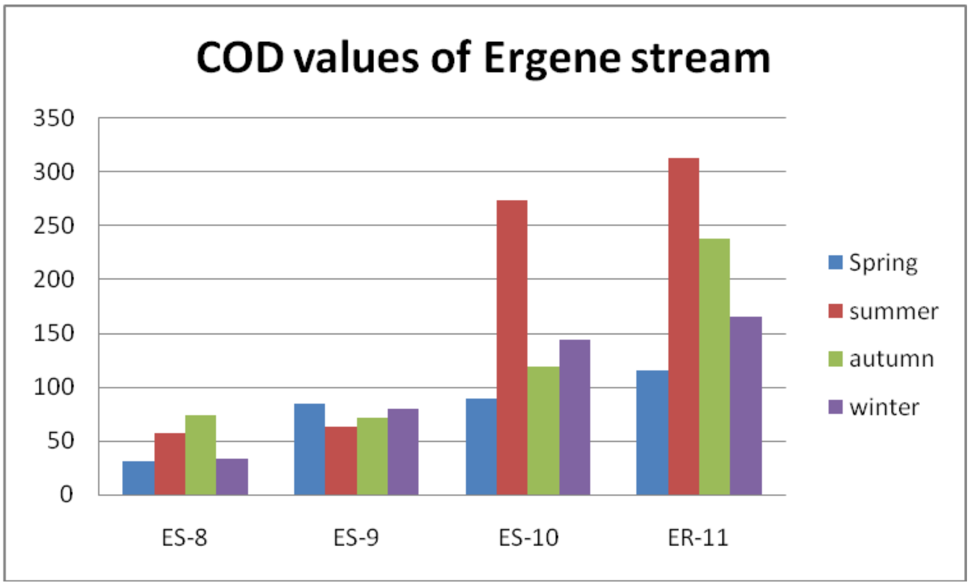

Figure 4.15 The seasonal changes in the COD levels of the Ergene Stream.

The seasonal changes in the Phenol parameter are given in figure 4.16. According to the results we see that the highest Phenol value is 1.42 mg/L, which was observed in the samples collected from ES-10 during the Summer.

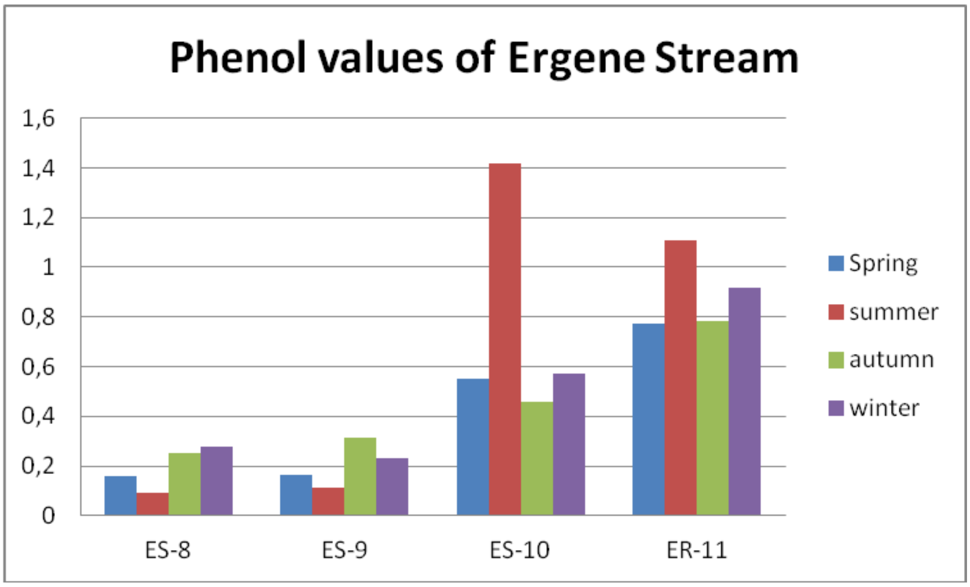

Figure 4.16 The seasonal changes in the Phenol levels of the Ergene Stream.

4.5 RESULTS of BÜYÜKÇEKMECE LAKE

The seasonal water samples collected from the Büyükçekmece Lake were tested for such physicochemical analyses as the TSS, The COD and phenol content to determine the level of the water pollution. Later the toxicity and genotoxicity that might be caused by this pollution were analyzed by the Microtox Toxicity Test and SOS Chromotest methods, respectively. The seasonal results of these analyses are given in Tables, 4.10, 4.11 and 4.12.

Table 4.10 Summer 2012 season results of Büyükçekmece Lake.

	Sample points	Date:	ph	Conductivity	TSS	COD	Phenol	T.U	SOS IF
Büyükçekmece Lake Summer 2012	BL-1	24.07.2012	7,7	480	80	64	0,245	-	0,942
	BL-2	24.07.2012	7,9	542	82	82	0,35	0,278	1,214
	BL-3	24.07.2012	7,9	431	58	78	0,087	-	0,828
	BL-4	24.07.2012	7,3	524	64	74	0,104	-	0,732
	BL-5	24.07.2012	7,1	230	46	56	0,03	-	0,124

Table 4.11 Autumn 2012 season results of Büyükçekmece Lake.

Büyükçekmece Lake Autmn 2012	Sample points	Date:	ph	Conductivity	TSS	COD	Phenol	T.U	SOS IF
	BL-1	16.10.2012	7,8	524	80	90	0,233	0,231	0,982
	BL-2	16.10.2012	7,8	450	75	80	0,212	0,124	0,726
	BL-3	16.10.2012	7,6	342	38	75	0,192	-	0,124
	BL-4	16.10.2012	7,5	420	42	86	0,201	-	0,624
	BL-5	16.10.2012	7,7	270	28	60	0,024	-	0,024

Table 4.12 Winter 2012 season results of Büyükçekmece Lake.

Büyükçekmece Lake Winter 2012	Sample points	Date:	ph	Conductivity	TSS	COD	Phenol	T.U	SOS IF
	BL-1	19.12.2012	7,8	480	120	78	0,324	0,245	1,112
	BL-2	19.12.2012	8,1	542	132	84	0,421	0,345	1,243
	BL-3	19.12.2012	7,7	431	124	58	0,124	-	0,342
	BL-4	19.12.2012	8,3	524	112	60	0,187	-	0,624
	BL-5	19.12.2012	7,2	230	60	46	0,12	-	0,142

The Büyükçekmece Lake is a fresh water source used for drinking purposes. In this work, the toxic and genotoxic effects caused by the water pollution in Büyükçekmece Lake have been analyzed. As seen from the date tabulated in Tables 4.10, 4.11 and 4.12 out of the samples collected from five different locations during different seasons, the samples collected from BL-1 and BL-2 locations show mildly-toxic effects. The Summer and Winter samples from BL-2 location also show genotoxic effects. These two locations are the locations where there is a heavy agricultural activity and there are TEM highway bridges.

Thus the cause of the pollution at these locations could be the result of a flux of agricultural chemicals into the lake. A traffic-related pollution due to the TEM highway could also have an effect. No toxic or genotoxic effects have been observed at the other locations of the lake. The pollution level at the BL-5 location is lower compared to the other locations. This location is very close to the drinking water treatment facility. That is why the low level of pollution at this location is relieving. This location is far from agricultural lands as well as the domestic wastes coming from residential areas.

4.5.1 The seasonal changes in the results obtained from Büyükçekmece Lake

4.5.1.1 Toxicity

No toxic effects have been observed in the sample collected from Büyükçekmece Lake during any of the seasons. The seasonal changes in the toxicity results of Büyükçekmece Lake are given in Figure 4.17. According to this, the highest toxicity values are measured at 0.345, in the samples collected from BL-2 location during the Winter. But since this value is between 0 and 1, it can be classified as moderated toxic.

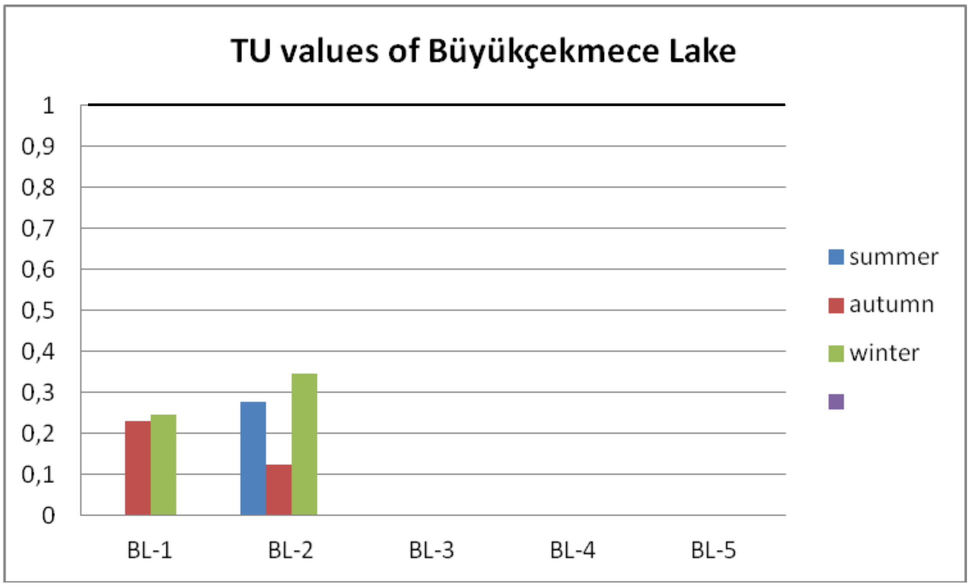

Figure 4.17 The seasonal changes in the toxicity levels of the Büyükçekmece Lake.

4.5.1.2 Genotoxicity

The seasonal changes in the genotoxicity levels of the Büyükçekmece lake are given in figure 4.18. According to these results, the highest SOS chromotest IF value is observed at 1.24 in the samples collected from location BL-1 during the Autumn season. No genotoxic effects have been observed at locations BL-2, BL-3 and BL-5.

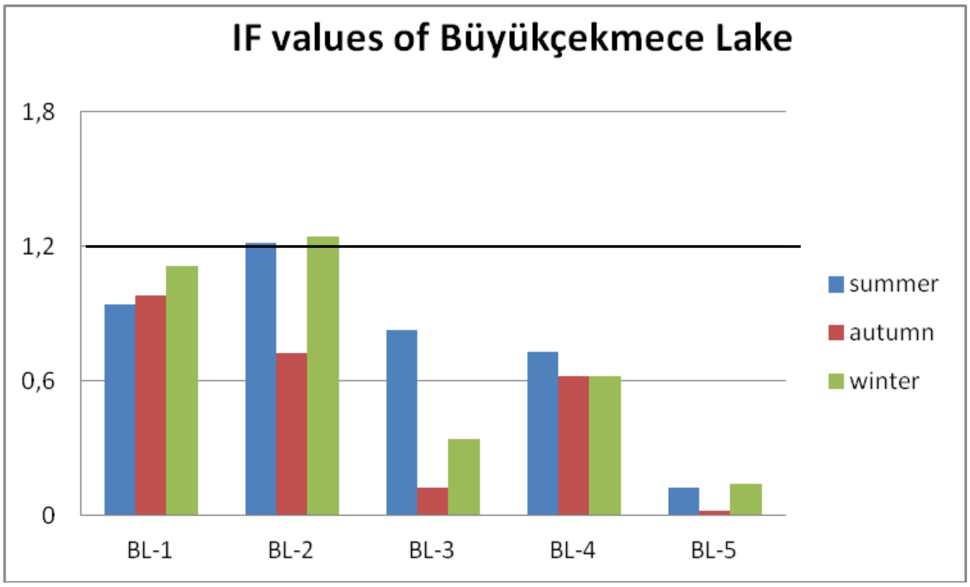

Figure 4.18 The seasonal changes in the genotoxicity levels of the Büyükçekmece Lake.

4.5.1.3 Physicochemical Analyses

The seasonal changes in the physicochemical analyses performed on samples from the Büyükçemece Lake are given in figure 4.19 - 4.21.

Figure 19 shows the seasonal changes in the conductivity parameter. According to this, the highest conductivity parameter is observed to be 542 µS, in the samples collected from location BL-2 during Summer.

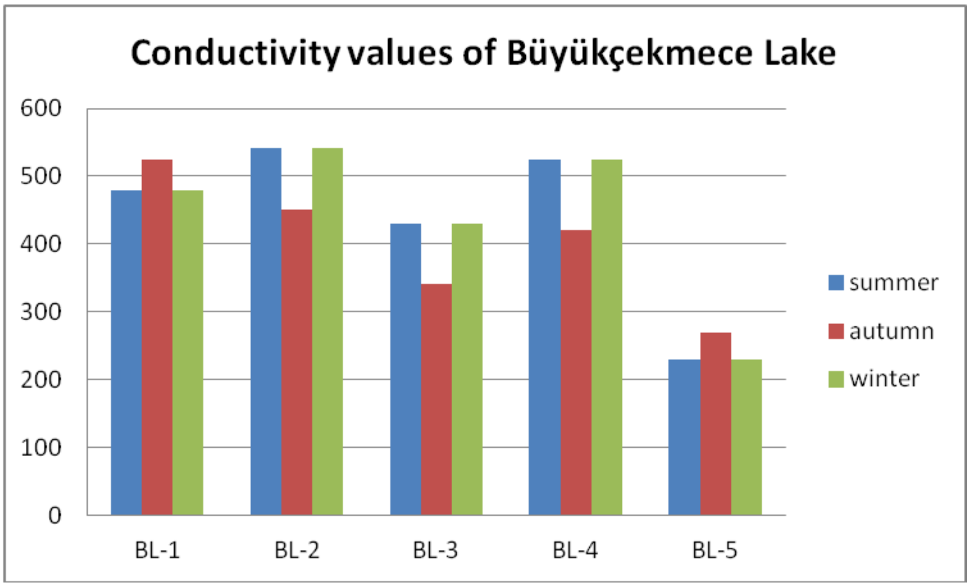

Figure 4.19 The seasonal changes in the conductivity levels of the Büyükçekmece Lake.

Figure 20 shows the seasonal changes in the TSS parameter. Accordingly, the highest TSS value is measured to be 132 mg/L, observed in the samples collected from the location BL-2 during Winter.

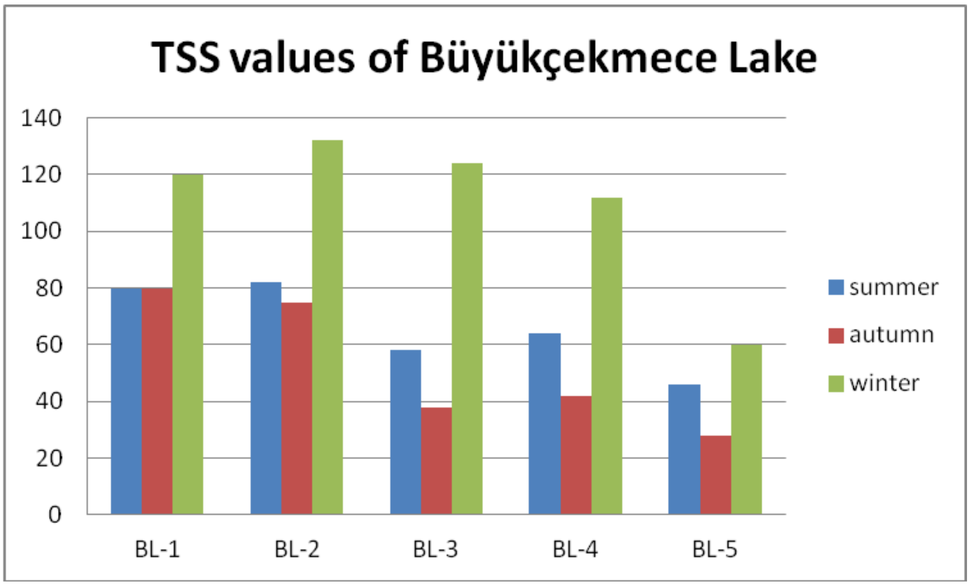

Figure 4.20 The seasonal changes in the TSS levels of the Büyükçekmece Lake.

Figure 21 shows the seasonal changes in the COD parameter. The figures suggest that the highest COD value is 90 mg/L, observed in the samples collected from BL-1 during the Autumn.

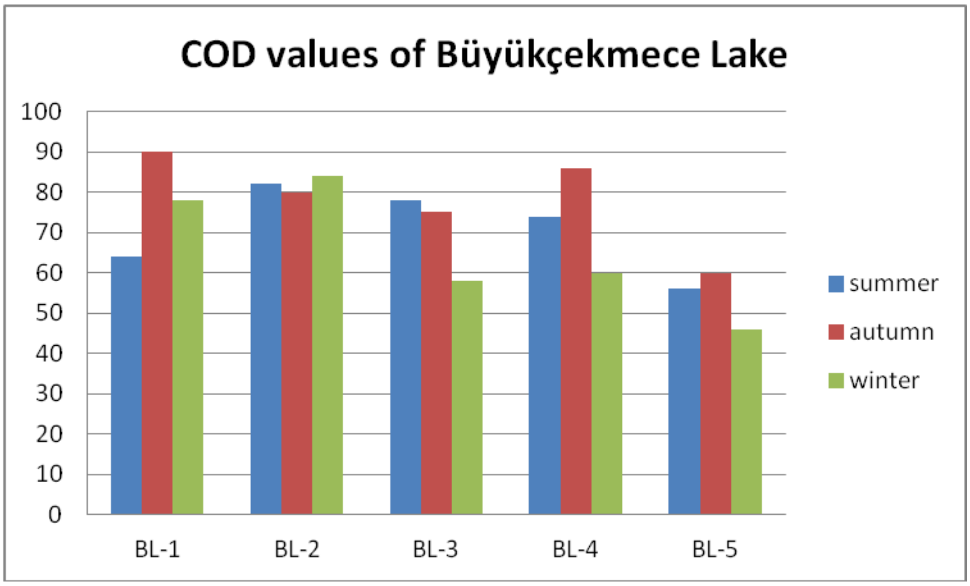

Figure 4.21 The seasonal changes in the COD levels of the Büyükçekmece Lake.

The seasonal changes in the Phenol parameter are given in figure 4.22. According to the results we see that the highest Phenol value is 0,42 mg/L, which was observed in the samples collected from BL-2 during the Winter.

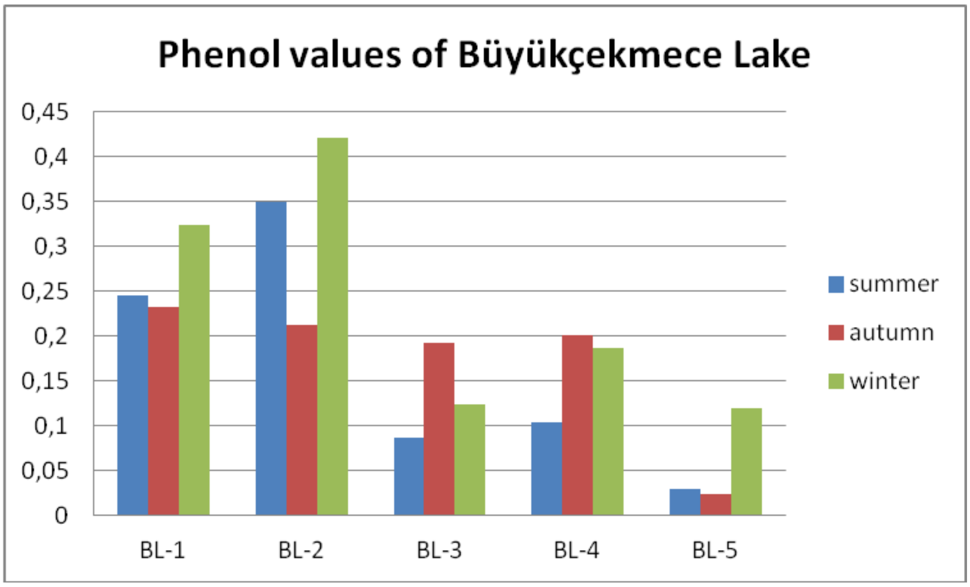

Figure 4.22 The seasonal changes in the Phenol levels of the Büyükçekmece Lake.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

The Çorlu and Ergene Streams merge to make up the Ergene River. These water bodies make up a major water source for the Ergene Basin, one of the leading agriculture basins of Turkey. Since 1980, a substantial number of industrial facilities have emerged in the basin and this number is rapidly growing. Currently, there are 2037 industrial facilities in the basin. The increasing number of these industrial plants has caused serious pollution in the basin, especially in the water sources. The Ergene River is one of the most affected water bodies by this pollution.

In this work, seasonal samples have been collected regularly from the Ergene River, the Ergene Stream and Çorlu Stream for a year. As a result of the analyses on these samples, the level of pollution and the toxic and genotoxic effects caused by the pollution have been determined. The results suggest that the level of pollution in these water bodies has reached to a high level and toxic and genotoxic effects have been observed at some locations.

The water quality in the streambed CS-1 location of the Çorlu Stream is second class (according to the COD parameter) and no toxic or genotoxic effects have been observed. However, at the other locations where there is industrial discharge, the water quality drops to fourth class and toxic and genotoxic effects have been observed at some locations.

Similarly, the water quality in the discharge-free first two locations of the Ergene Stream is second class while in the heavily exposed ES-10 location it drops to fourth class. Moreover, toxic and genotoxic effects are observed at this point.

The sample was collected from ER-11 (Ergene River) location is also heavily polluted because of heavily exposed confluence of Çorlu and Ergene Streams. The water quality at this location is also fourth quality and toxic and genotoxic effects can be seen.

As suggested from these results, we can say that the pollution of the Ergene Basin caused by the industrial plants has reached to significant levels. When looking at the water quality, we see that it has dropped to fourth class in such an important agricultural basin and it is well known that fourth class water is not usable in agriculture.

The pollution seen in these waters not only makes these waters unusable but it also has negative effects on the aqua life in these waters. When pollution is caused by industrial discharges, it causes a lot of chemicals to accumulate. These chemicals have toxic and genotoxic effects on living organisms. A lot of water organisms start to die from poisoning caused by these effects. Moreover, the genotoxic effects also cause some deformations in the genetic structure of organisms, resulting in different mutations.

The water pollution in the Ergene Basin, a leading agricultural basin in Turkey, needs to be put under control to keep these waters usable and to preserve the water life. Some permanent solutions need to be found to preserve these waters and abate the pollution.

There are 2037 industrial plants in the region. It is extremely difficult to oversee and inspect the waste waters of all these plants. Thus some common treatment facilities must be formed with support from the state and these facilities must be equipped with advanced purification techniques.

The Büyükçekmece Lake makes up one of the significant fresh water sources of the metropolitan area of Istanbul. That is why it is under protection by the Istanbul's Main Mayoral Office as one of the protected fresh water basins. However, despite this the number of residences around the lake has been increasing. Naturally, there is a lot of domestic discharge of wastes to the lake from these residences.

In this work, the toxic and genotoxic effects of the pollution were studied. As seen from our analyses, toxic and genotoxic effects have not been observed in the samples collected at the locations close to the drinking water treatment facility. As for the other locations, mild toxic and genotoxic effects have been observed at the locations close to the agricultural lands and highway bridges of the TEM motorway.

Uncontrolled extend of settlement, industrial and agricultural areas inside the lake basin are causing contamination and pollution. Because of those facilities, in the near future the Büyükcekmece Lake may not be the source of drinking water.

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