INVESTIGATION OF MUTAGENIC EFFECTS ON LIVING ORGANISMS OF TOTAL POLLUTANT IN MARMARA SEA WITH SOS CHROMOTEST KIT, UmuC-GENE

by

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by

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ABSTRACT

The DNA-damaging activity in different stations of Marmara Sea was investigated by the SOS CHROMOTEST (on *E.coli* PQ37) and umuC test (on *Salmonella typhimurium TA 1535 pSK1002*) without metabolic activation. The samples were taken on February 2008 and May 2008 (9:00 to 14:00) from 17 stations in Marmara Sea.

The results was obtained via visual and instrumental analysis. The chromogen solution was used for the visual analysis of both SOS Chromotest and umuC test. The color development was observed for all positive control wells (4NQO). Optic density of test strain was measured with spectrophotometer. The test result showed that both SOS Chromotest and umuC test had different sensitivities against DNA-damaging agents.

The samples were collected from Avsa, Kartal, Izmit Bay, Yalova, Gemlik (two stations), Gölcük, Mudanya, Güzelyali, Dilovasi, Avcilar, Büyükcekmece, Florya, Bakırköy, Yenikapi, Eminönü, Kadıköy. All stations of samples were collected two times to detect seasonal effects

of genotoxic(mutagenic) pollutants on living organisms and tested with SOS Chromotest (*E.coli* PQ37) ve umuC testi (*Salmonella typhimurium TA 1535 pSK1002*) for all stations.

16 samples which were taken on February 2008 were tested with SOS Chromotest and umuC test. According to SOS Chromotest result, three samples (% 17.6) were positive in at least one concentration. The same samples were tested with umuC test. According to test result four samples (25 %) were positive in at least one concentration. 16 samples which were taken on May 2008 were tested with SOS Chromotest, eight (50 %) were positive at one or more concentrations. Induction factors (β-Gal) was equal or bigger than 1.2 at one or more concentrations.

Key words: SOS CHROMOTEST, Salmonella typhimurium pSK1002, umuC Gene, Marmara Sea.

MARMARA DENİZİ'NDEKİ TOPLAM KİRLİLİĞİN CANLILAR ÜZERİNDEKİ MUTAJENİK ETKİLERİNİN SOS CHROMOTEST KİTİ, UMU-C TESTİ İLE ARAŞTIRILMASI

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ÖZ

DNA materyaline hasar vermesi muhtemel aktivite, Marmara Denizi'nin değişik bölgelerinden alınan numuneler ile SOS Chromotest (*E.coli* PQ37) ve umuC testi (*Salmonella typhimurium TA 1535 pSK1002*) ile metabolik aktivasyon olmaksızın (S9) araştırılmıştır. Numuneler Şubat 2008 ve Mayıs 2008 tarihlerinde (9:00 ve 14:00 arasında) Marmara Denizinin 17 noktasından alınmıştır.

Test sonuçları Görsel ve Enstrümental olarak analiz edilmiştir. Kromojen çözeltisi SOS Chromotest ve umuC testi için kullanılmıştır. Renk gelişimi tüm pozitif kontrollerde gözlenmiştir 4NQO. Spektrofotometre ile test suşlarının optik yoğuluğu ölçülmüştür. Test sonuçları SOS Chromotest ve umuC testinin DNA materyaline hasar veren aktivitelere karşı hassasiyetlerinin farklı olduğunu göstermiştir.

Numuneler, Avsa, Kartal, İzmit Körfezi, Yalova, Gemlik, Gölcük, Mudanya, Güzelyalı, Dilovası, Avcılar, Büyükçekmece, Florya, Bakırköy,Yenikapı, Eminönü, Kadıköy istasyonlarından ,mevsimsel olarak iki defa toplanmış,organizma üzerindeki mutajenik

(genotoksik) etkileri SOS Chromotest (*E.coli* PQ37) ve umuC testi (*Salmonella typhimurium TA 1535 pSK1002*) araştırılmıştır

Şubat 2008'de alınan 16 numune SOS Chromotest and umuC testi ile test edilmiştir. SOS Chromotest ile test edilen numuneler içerisinde 3 istasyonda genotoksik aktivite tespit edilmiştir (% 17.6). Şubat 2008'de alınan yine aynı numunelerin umuC testi sonuçlarına göre ise 4 (% 25) noktada genotoksik aktivite tespit edilmiştir. SOS Chromotest sonuçlarından farklı olarak, umuC testi 2 farklı istasyonda genotoksik aktivite tespit etmiştir (Yenikapı ve Yalova). Florya noktasından alınan numune de ise genotoksik aktivite SOS Chromotestin yanında umuC testi ile de tespit edilmiştir. Mayıs 2008'de alınan 16 istasyondan alınan numuneler ise sadece SOS Chromotest ile test edilmiştir. SOS Chromotest ile test edilen numuneler içerisinde 8 istasyonda genotoksik aktivite tespit edilmiştir (% 50). Indüksiyon faktörü (β-Gal değeri), bu numunelerin bir yada daha fazla sayıdaki konsantrasyonlarında 1.20 değerinin üzerinde tespit edilmiştir.

Anahtar Kelimeler: SOS CHROMOTEST , Salmonella typhimurium pSK1002, umuC Gen, Marmara Denizi.

Dedicated to my parents

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LIST OF SYMBOLS AND ABBREVIATION

SYMBOL/ABBREVIATION	
TUIK	Turkey Statistical Establishment
B –Gal	β-galactosidase
AP	Alkaline phosphatase
DMSO	Dimethyl Sulfoxide
pNPP	p-Nitro-Phenyl-Phophate
4NQO	4-Nitro-Quinoline-Oxide
PBS	Phosphate Buffer Salt
UDW	Ultra Deionized Water
SOSIP	SOS inducing potency
OD	Optic Density
CIF	Corrected Induction Factor
IF	Induction Factor
RF	Reduction Factor
EPA	Environmental Protection Agency

CHAPTER 1

INTRODUCTION

Industrial development and uncontrolled urbanization have come with so many environmental problems such as marine pollution in our country like all the world.

Marine environment such as rivers, lakes, pounds and seas have received large quantities of effluents wastewater from industrial plants and domestic sewages. These effluents contain many unknown compounds which are formed by chemical, organic and inorganic matters. Therefore marine pollution can be serious problem for public health and aquatic life.

The high amount production in the industrial plants cause to increase wastewater discharging due to consumption of raw materials. Unfortunately, wastewater contains different types of chemicals and they mix in the receving places with other industrial and domestic wastewater and forms hazardous chemicals such as xenobiotics and its compounds. Unfortunately high rate of wastewater is given to surface waters (river, lake and sea) directly or inderectly, without the determining of dangerous effects of this effluents.

Rasmussen, Houk and White in 1997 reviewed that wastewater which is originated from industries and domestic sources has genotoxic/ mutagenic potency. White et al. İn 1997 also noted that although organic extracts of municipal wastewaters are genotoxic, one must acknowledge that municipal wastewaters are complex mixtures of wastewaters from a variety of sources. Water genotoxicity studies are of interest

because epidemiologic investigations have shown a link between genotoxic drinking water intake and a rise in cancers (Koivusalo et al., 1994, 1995, 1997).

High range discharge of wastewater effects sea product which is rich protein sources for human nutrient. The genetic altering of agricultural products, the chemical matters which are used increasing of hormon vegetal secretion, cause to increase that low value nutrient of animal and vegetal products. This situation have demanding of sea food increased. The sea foods consist on high amount mineral salts and protein when its compared with terrestial food (red and white meat).

1.1 MARMARA SEA

1.1.1 Properties of Marmara Sea

Marmara Sea is a inland sea where all coastal areas inside the one country. This specific situation is unique in the to only our counrty in all the world. Marmara Sea which has 11.500 km² surface area connects Black Sea and Aegean Sea through Bosphorus and Dardanelles straits. Its dimesion are 70 km x 250 km dimension and its maximum depth is 1390 m (Beşiktepe Ş.T.).

High density water coming from the Aegean Sea (salinity ranging 39–39.5 ppt) sink below the surface at the entrance and move along the bottom of the Dardanelles Strait (60–70 m depth) throughout the Marmara Sea, reaching the Black Sea after the Bosphorus Strait (310 km^3 /year). Low density waters (salinity from 20 to 29 ppt) flow at the surface (between 0 and 25 m depth) from the Black Sea to the Aegean Sea (612 km^3/year). Mixing between the layers appears limited (Latif et al., 1991; Besiktepe, 2003).

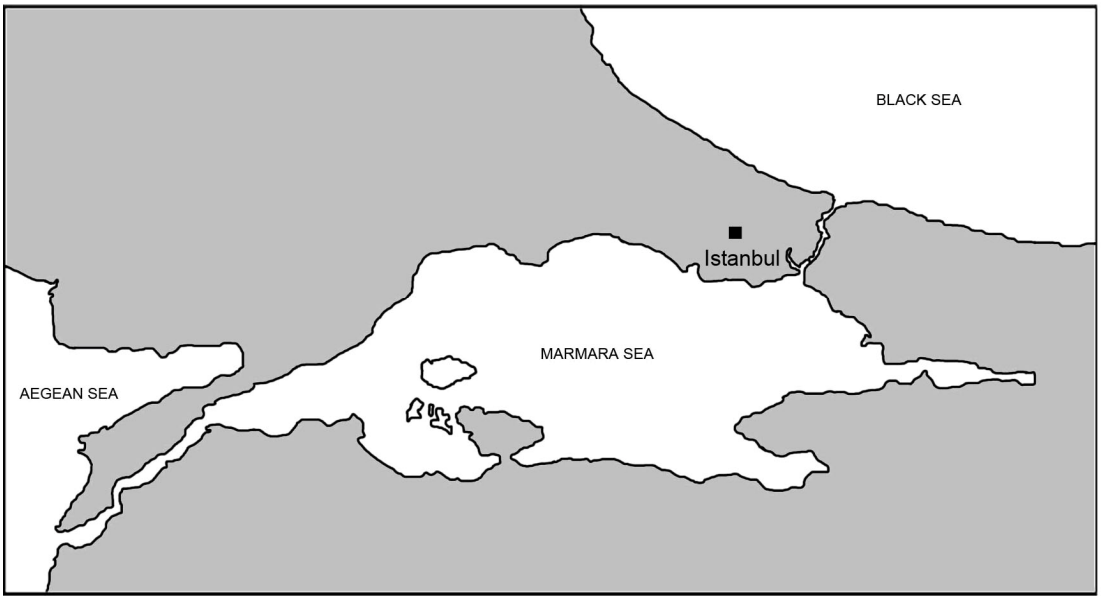


Figure 1.1 . Marmara Sea

1.1.2 Sources of Pollution in Marmara Sea

Marmara Region has received huge amount immigration from other regions of Turkey. This situations cause to increase population and come with uncontrolled urbanization in the cities such as Istanbul. The main problem of uncontrolled urbanization is lack of sewage systems and domestic treatment plants.

Marmara Sea takes an important role for the development of Marmara Region. Marine transportation of raw material to the industrial areas, transportation of passenger, tourism and sea food are main components of economic income from Marmara Sea. The convenience of transportation for both passengers and raw material attracts industrial facilities to the region. Due to this fact the amount of industrial wastewater and its mixture with domestic wastewater increase. The mixing of them may cause the formation of unknown chemical components in the receiving places such as sea and rivers. According to TUIK data, 29 municipalities have made deep sea discharges in Marmara Sea (Istanbul, Bursa,Yalova, Balıkesir, Kocaeli and Sakarya). The reviews were published that genotoxic substances can enter surface waters from industrial and domestic wastewater by V. S. Houk (1991) and Stahl (1992). Houk have indicated genotoxic/mutagenic activity of wastewater from industries of organic chemical manufacturing, pulp and paper, metal refining and founding and petroleum

refining. According to another paper published by White et al, (1996), 42 types of industries release genotoxic wastewaters.

The diffrent pollution source of Marmara Sea is Black Sea. The study indicated that amount pollution carried by the Bosphorus upper flow which come from Black Sea to the Marmara Sea (Okus E., 2007).

The another sources of pollution is that stormwater. Pollution load having approximately 1 x10^9 m^3/year of flowrate from a settlement area of 130,000 ha has been discharged into the Marmara Sea and Strait waters (Sahinoglu D. et al., 1998).

According to the articles, the pollution load of stormwaters resulting from the residential areas within Istanbul's provincial boundaries that results were obtained using US EPA Storm Water Management Model (SWMM) in 1995 and are indicated in Table 1.1. (Okus E. et al., 2007).

Table 1.1 : Diffuse pollution loads resulting from stormwater in residential areas (t/day)

Year	SS	BOD_5	Total-N	Total-P
1995	246	32	10	1.2

The source of pollution of Marmara Sea can be summarized that;

- Increasing of population density in the region.
- Uncontrolled urbanization.
- The different types of industries located in the coastal regions.
- The consumption of raw material in industrial plants which are petrochemical industries, medicine industries, pulp and paper industries etc. have come with releasing high amount industrial wastewater.
- The deficiency of advanced wastewater treatment plants.

According to 2004 TUIK data, the 60.8 % (1.68 billion m^3) of wastewater which was discharge to the sewage system was treated with treatment plant. 39.2 % (1.09

billion m^3) of wastewater was untreated and to released to the sewage system (Total amount 2.77 billion m^3) (Figure 1.2).

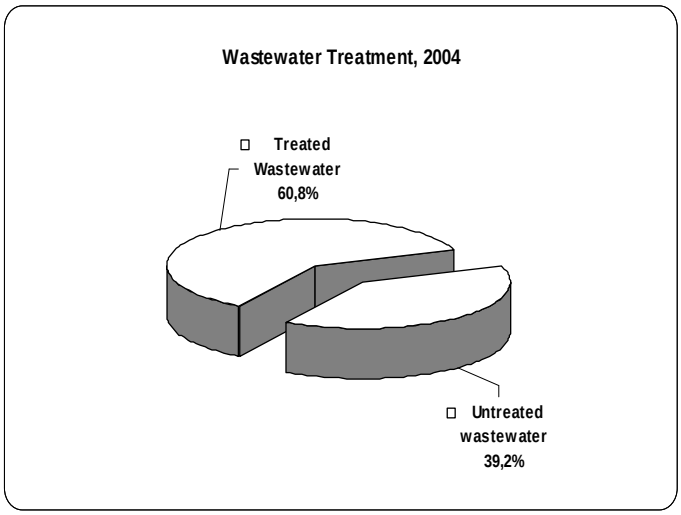


Figure 1.2. The rate of treated and untreated wastewater in Turkey, TUIK 2004

2004 TUIK data indicated that 47 % of 2.77 m^3 wastewater was discharged to the rivers, 39.30 % was discharged to sea, 4 % was discharged to dams, 2 % was discharged to lake and pound, 1 % was discharged to land and 6 % was discharged to other receiving places that shows Figure 1.3.

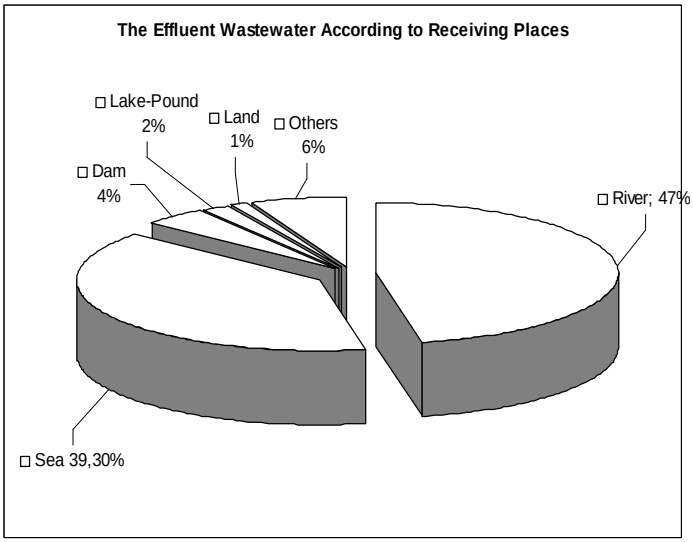


Figure 1.3. Receiving places for waste water in Turkey, TUIK 200

Therefore monitoring of pollution of Marmara Sea is very important to take precautions for protection human health against disease which rise from various pollution. This study is about investigate mutagenic effects on living organisms of total pollutant in Marmara Sea. No paper has been published on the mutagenic activity in Marmaea Sea. In this study, DNA-damaging agents were investigated with two genotoxic assay. The genotoxic assay results have remarkably increased sensitivity of protection marine environment against pollution.

1.2 WHAT IS GENETIC TOXICOLOGY (GENOTOXICITY)

Genetic toxicology is a science field that all kinds of changes to the genetic material of an organism are investigated in order to indentify chemical, physical and biological agents and genetic risk due to certain environmental conditions. Genotoxic/mutagenic agents might be man made or natural origins such as plants which develope have developed chemicals with mutagenic properties.

The field of genetic toxicology began to develope before the biochemical basis of heredity was understood. It was known that physical and chemical agents could cause heritable mutations. The role of radiation in producing heritable changes in a living organism was first reported by Muller (Muller, 1927). Auerbach was the first to report the ability of chemicals to cause mutations (Auerbach et al., 1947).

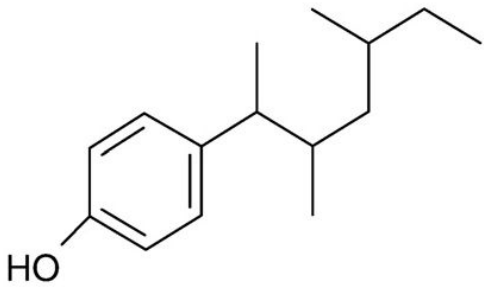
The main important point of genetic toxicology has been determined mechanisms of heredity and study DNA and RNA structure (Cloutier et al., 2001), repair (Hanawalt and Haynes, 1965; Rasmussen and Painter, 1966), and the role of mutation at both the individual (McDiarmid et al., 1995) and population levels (Jacobson-Kram et al., 1993; Robinson et al., 1994).

Mutations more often have detrimental effect for individuals and living organisms. In addition, increased mutations rates, e.g due to environmental pollution, might negative affect on populations (OSPAR Comission 2002). Genotoxic substances are known to be potentially mutagenic or carcinogenic and capable development of tumors.

Mutagenic/genotoxic compounds which may include carcinogens, whether known or unknown, that can damage health and biota (Dearfield K.L. et al., 2002).

The xenobiotics are important example for genotoxic compaunds which not occur natural biological pathway in the body and classified as an endocrine disrupter which can disturb the hormonal system by mimicking the occurrence of natural hormones, blocking their production or by inhibiting or stimulating the endocrine system (Soares A. et al., 2008). They enter the body natural ways such as sea foods.

The nonylphenols (Figure 1.4) are given example for xenobiotics compounds which are manufacture of antioxidants, lubricating oil additives and the production of nonylphenol ethoxylates surfactants which is its major use (65%) (USEPA, 1990) . Nonylphenol ethoxylates are highly cost effective surfactants with exceptional performance and consequently used widely in industrial, institutional, commercial and household applications such as detergents, emulsifiers, wetting and dispersing agents, antistatic agents, demulsifiers and solubilisers (Fiege et al., 2000; Langford and Lester, 2002; Lorenc et al., 2003). When nonylphenols ethoxylates are treated with treatment plants, they biodegrade into several products which include nonylphenol. Because of low solubility and high hydrophobicity ,nonylphenol accumulates in environmental compartments. Nonylphenol is found often in matrices such as sewage sludge, effluents from sewage treatment works, river water and sediments, soil and groundwater (Soares A. et al., 2008).



Molecular formula : $C_{15}H_{24}O$

Figure 1.4 . Nonylphenol

In the early years of genetic toxicology there was a large increase in the number of endpoints used to measure mutagenic damage.

1.2.1 Genotoxicity and Cancer

When potential mutagen/carcinogen enter the cell metabolisms DNA adduct and protein adduct effect can be observed. DNA adduct can be repaired; if its not repaired, mutations occure. Mutations cause two effects those are inherited disoders (Figure 1.5) and cancer (Figure 1.6).

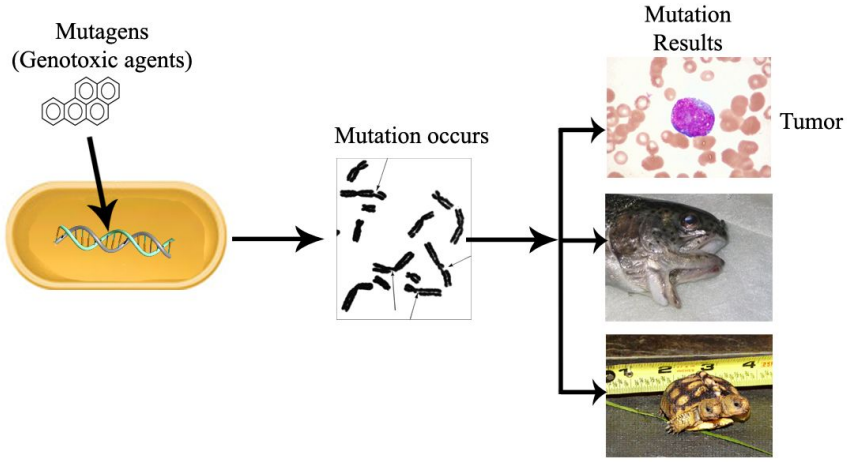


Figure 1.5 . The effects of genotoxic agents

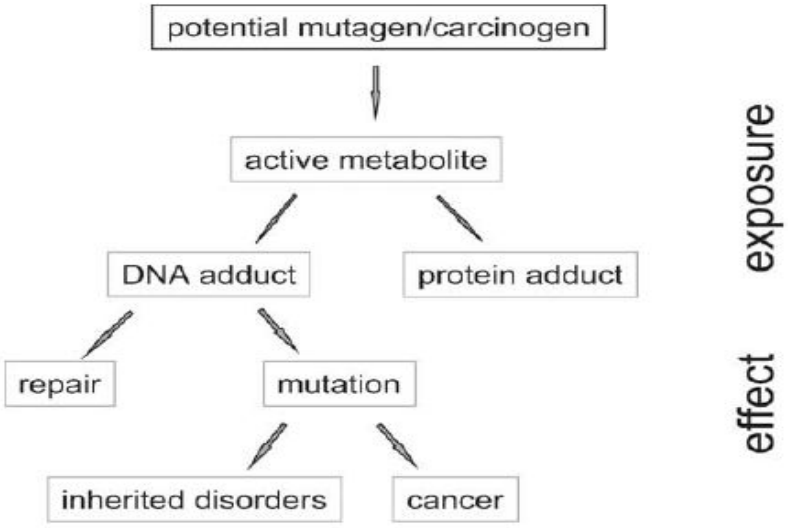


Figure 1.6 . Genotoxic carcinogenesis (Farmer P.B. et al., 2008)

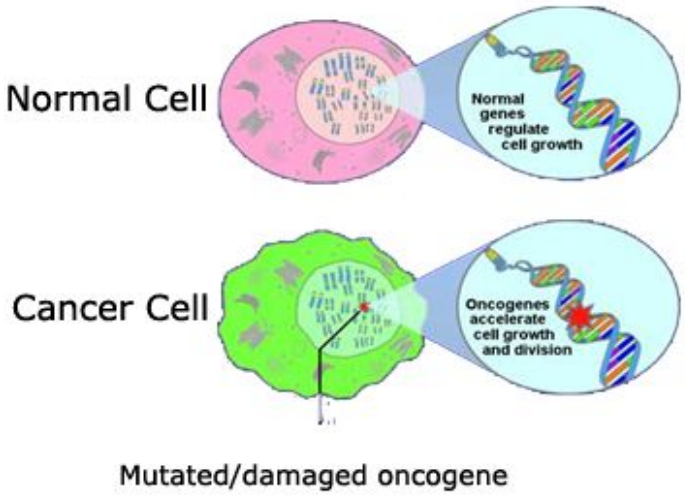


Figure 1.7 . Normal cells and Cancer cells (National Cancer Institute, USA)[1]

[1] National Cancer Institute, USA / www.web-books.com

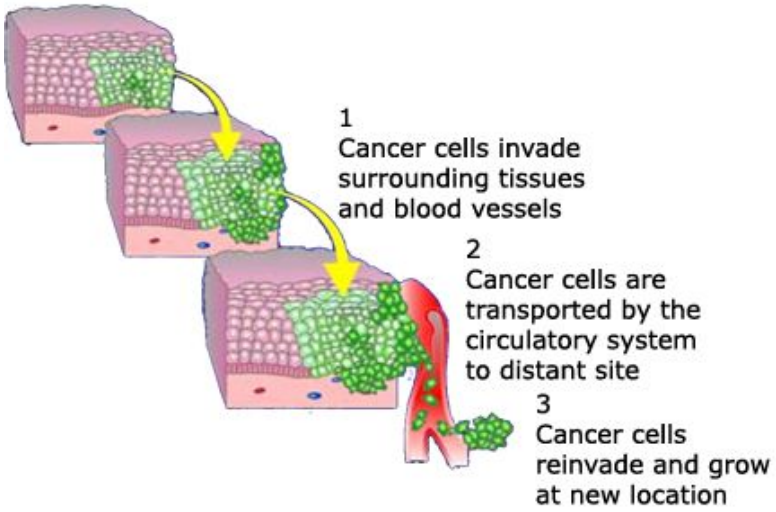


Figure 1.8 . The transportation of cancer cell (National Cancer Institute, USA)

Cancer cells attack to surrounding tissue and may enter blood system. They can occur only special tissue or transfer other tissue with blood vessel and occur other tissue. When they enter circulatory system, they are transpoted to a distant site. Reproduction of cancer cells damage or stop tissue function. Depending on the to type of tissue which cancer cells locate, various types of cancer occure.

Patterns of the cancer disease are not fully explained by known risk factors. Virtually all cancers arise from an accumulation of genetic mutations and the more recent recognition of the role of inflammation and the tissue microenvironment, in particular for hormonedependant cancers. However, most genetic mutations that contribute to cancer are not inherited, and thus must be attributable to accumulation of somatic mutations and epigenetic changes, from as yet poorly understood environmental factors (Davis D.L et al., 1998).

Table 1.2 indicates workplace, agricultural, pharmaceutical and other chemicals for which the US National Toxicology Program finds experimental and epidemiological evidence of carcinogenicity, many of which are not directly regulated as carcinogens.

Table 1.2 : National Toxicology Program's cancer bioassays[1]

Chemical solvent	Benzene 1,1-Dichloroethane 1,2-Dichloropropane Methylene chloride Nitromethane (also used in rocket and engine fuels) 1,2,3-Trichloropropane
Chemicals used or formed in the manufacturing of dyes	C.I. acid red 114 C.I. basic red 9 2,4-Diaminotoluene 3,3′-Dimethlybenzidine dihydrochloride 3,3′-Dimethoxybenzidine dihydrochloride 2,4-Dinitrotoluene Hydrazobenzene *o*-Nitrotoluene *o*-Toluidine hydrochloride
Chemical used or formed in the manufacturing of rubber, vinyl, polyurethane foams or neoprene	Benzene (rubber manufacturing) 1,3-Butadiene (rubber manufacturing) Chloroprene (neoprene manufacturing) 2,4-Diaminotoluene (polyethylene manugacturing) 1,2-Dichloroethane (vinyl chloride manufacturing) Glycidol (vinyl manufacturing) *o*-Nitrotoluene (rubber manufacturing) 2,4-2,6-Toluene di isocyanate (polyethhylene foam manufacturing)
Chemical intermediates	Ethylene oxide (anti-freeze products) Isoprene (formed during ethylene production)
Flame reterdants	2,2-Bis (bromomethyl)-1,3-propanediol 2,3-Dibbromo-1-propanol
Food additive Fumigants and pesticides	Methhyleugenol (flavoring) Clonitralid (molluscicide) 1,2-Dibromoethane (also called ethylene dibromide) 1,2-Dibromo-3chloropropane (soil fumigant) 1,2-Dichloroethane (soil/grain fumigant) 1,2-Dichloropropane (soil/grain fumigant) Dichloropropane (insecticide) Sulfallate (herbicide)
Gasoline additives	Benzene 1,2-Dibromoethane (lead scavenger) 1,2- Dichloroethane (lead scavenger)
Microelectronics Mycotoxin Pharmaceutical drugs	Indium phosphide (used semiconductors) Ochratoxin A (toxin produced by molds) Acronycine (anti-cancer drug) Furosemide (diuretic) Hydrazobenzene (used making phenylbutazone, an antiarthritic drug) Isophosphamide (anti-cancer drug) Nitrofurazone (anti-bacterial agent) Phenesterin (anti-cancer drug) Procarbazine hydrochloride (anti-cancer drug) Reserpine (anti-hypertension drug)
Sterilizing agents for medical instruments Research chemical Riot control/tear gas	Ethylene oxide 5-Nitroacenaphthenol 2-Chloroacetophenone

[1] http://ntp.niehs.nih.

1.3 DNA REPAIR

1.3.1 DNA REPAIR SYSTEM

The genome is constantly exposed to mutagenic agents. If the genome is not repaired by the DNA repair mechanisms , the genome would be unable to survive the multitude of lesions that form throughout the cell cycle. Therefore a range of molecular mechanisms has evolved that ensures that damaged DNA is effectively dealt with. In mammalian cells, more than 150 different proteins have been described that are involved in the response to DNA damage. These proteins coordinate the repair of DNA lesions and the stalling of the cell cycle to allow repair to occur (Lord CJ et al., 2006) (Hoeijmakers JH 2001).

DNA repair system can be divided into three categories (Beth A. Montelone, 1998):

1. **Damage reversal**--simplest; enzymatic action restores normal structure without breaking backbone
2. **Damage removal**--involves cutting out and replacing a damaged or inappropriate base or section of nucleotides
3. **Damage tolerance**--not truly repair but a way of coping with damage so that life can go on

The cell then suffers one of three possible fates:

1. An irreversible state of dormancy, known as senescence
2. Cell suicide, also known as apoptosis or programmed cell death
3. Cancer

Most cells in the body become senescent. Then, after irreparable DNA damage, apoptosis occurs. In this case, apoptosis functions as a "last resort" mechanism to prevent a cell from becoming cancerous and endangering the organism.

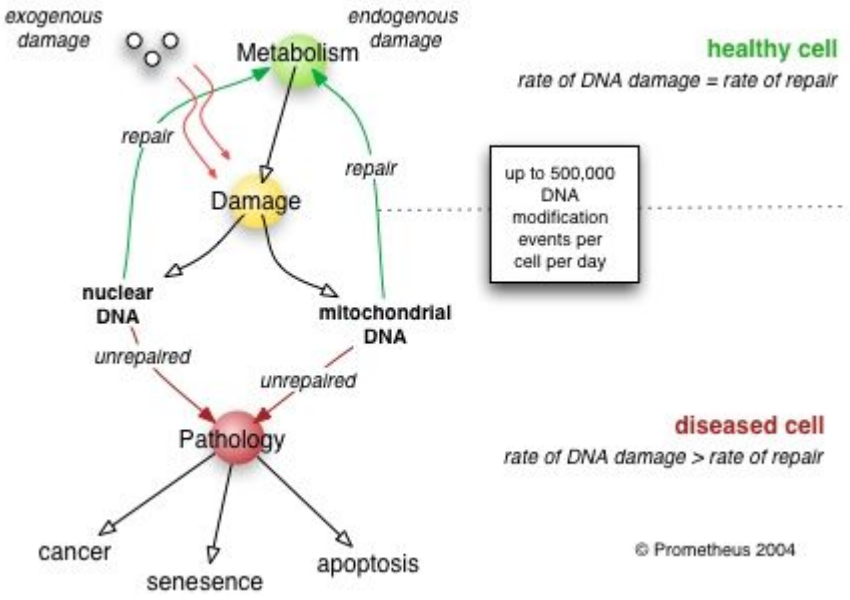


Figure 1.9 . DNA Repair functions[1]

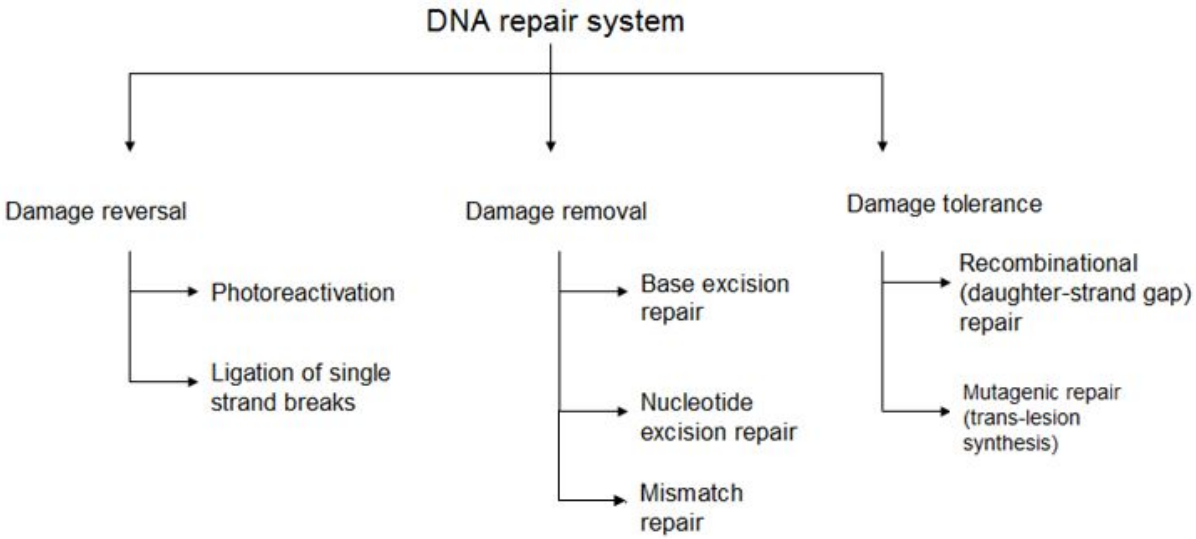


Figure 1.10 . DNA Repair System

[1]DNA damage. http://www.nationmaster.com/encyclopedia/DNA-damage

1.3.1.1 Damage Reversal

1.3.1.1.1 Photoreactivation

This is one of the simplest and perhaps the oldest repair systems: it consists of a single enzyme which can split pyrimidine dimers (break the covalent bond) in presence of light.

The photolyase enzyme catalyzes this reaction; it is found in many bacteria, lower eukaryotes, insects, and plants. It seems to be absent in mammals (including humans). The gene is present in mammals but may code for a protein with an accessory function in another type of repair.

1.3.1.1.2 Ligation of single strand breaks

X-rays and some chemicals like peroxides can cause breaks in backbone of DNA. Simple breaks in one strand are rapidly repaired by DNA ligase. Microbial mutants lacking ligase tend to have high levels of recombination since DNA ends are recombinogenic (very reactive). The rare hereditary disease Bloom syndrome also somehow is involved with DNA ligase deficiency (although the Bloom syndrome protein is a DNA helicase); patients' cultured cells have high levels of chromosome aberrations and spontaneous mutation.

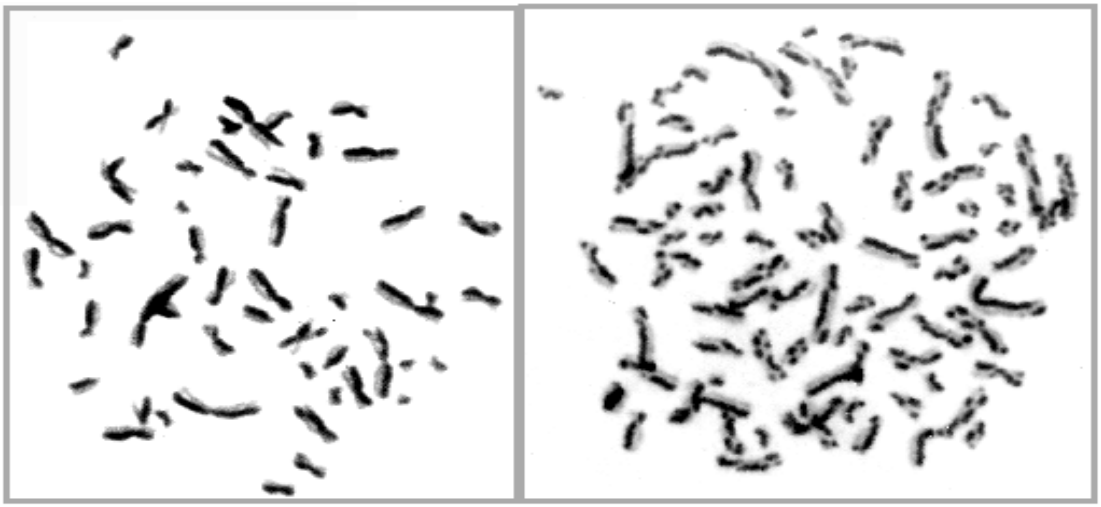


Figure 1.11. The sister chromatid exchange in a normal subject (left) and in a Bloom syndrome patient (right) (from: Mounira Amor-Guéret).

1.3.1.2 Damage Removal

1.3.1.2.1 Base excision repair

The damaged or inappropriate base is removed from its sugar linkage and replaced. These are glycosylase enzymes which cut the base-sugar bond. example: uracil glycosylase--enzyme which removes uracil from DNA. Uracil is not supposed to be in DNA--can occur if RNA primers not removed in DNA replication or (more likely) if cytosine is deaminated (this is potentially mutagenic). The enzyme recognizes uracil and cuts the glyscosyl linkage to deoxyribose. The sugar is then cleaved and a new base put in by DNA polymerase using the other strand as a template. Mutants lacking uracil glycosylase have elevated spontaneous mutation levels (C to U is not fixed, which leads to transitions) and are hyper-sensitive to killing and mutation by nitrous acid (which causes C to U deamination).

There are other specific glycosylases for particular types of DNA damage caused by radiation and chemicals.

1.3.1.2.2 Mismatch repair

DNA mismatch repair is a system for recognising and repairing erroneous insertion, deletion and mis-incorporation of bases that can arise during DNA replication and recombination (Iyer R., 2006). Examples of mismatched bases include a G/T or A/C pairing .The damage is repaired by excising the wrongly incorporated base and replacing it with the correct nucleotide. Usually, this involves more than just the mismatched nucleotide itself, and can lead to the removal of significant tracts of DNA[1].

1.3.1.2.3 Nucleotide excision repair

Although base excision repair is clearly important, it is insufficient to deal with all types of damage. For a given type of damage to be corrected by base excision repair, there must be a DNA glycosylase capable of recognizing that specific damage. The huge variety of DNA-reactive chemicals in our environment combined with the huge variety of alterations that can be produced by radiation and by oxidative and free radical attack on DNA can generate so many types of damage that coping with all types of

damage by evolutionary development of damage-specific DNA glycosylases would be difficult if not impossible. Fortunately, a different, more flexible damage repair mechanism has evolved in living organisms, nucleotide excision repair (NER), which recognizes damaged regions based on their abnormal structure as well as on their abnormal chemistry, then excises and replaces them[1].

In all organisms, NER involves the following steps:

1. Damage recognition.
2. Binding of a multi-protein complex at the damaged site.
3. Double incision of the damaged strand several nucleotides away from the damaged site, on both the 5' and 3' sides.
4. Removal of the damage-containing oligonucleotide from between the two nicks.
5. Filling in of the resulting gap by a DNA polymerase.
6. Ligation.

[1] DNA Repair. http://asajj.roswellpark.org/huberman/DNA_Repair/ner.html

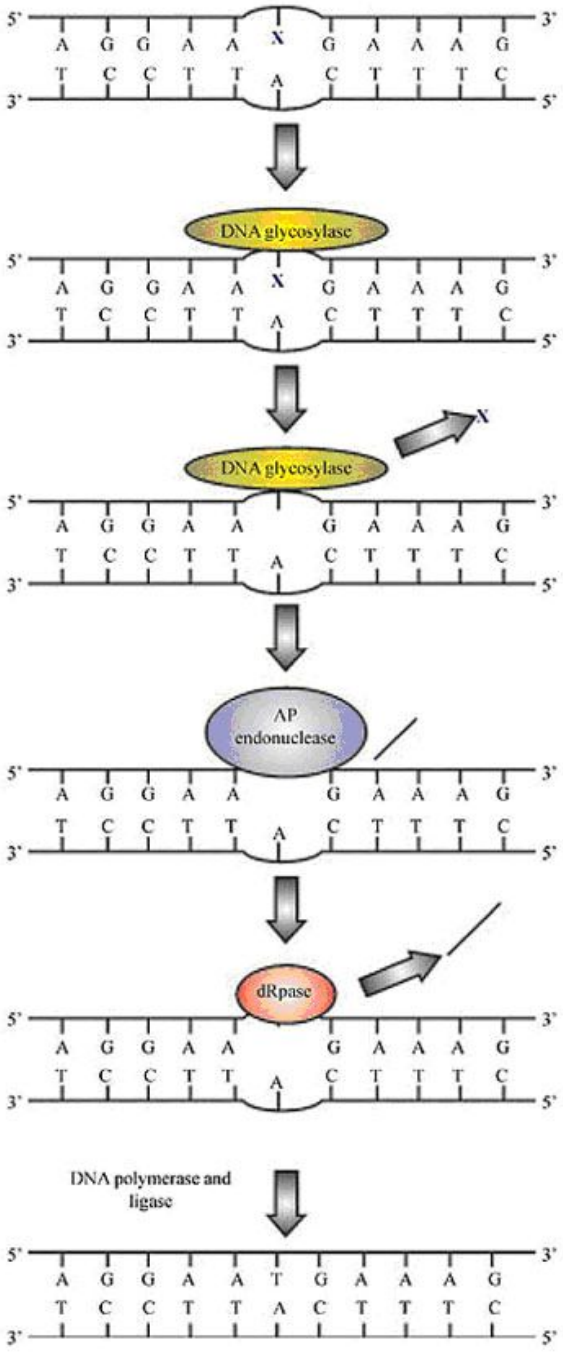


Figure 1.12 . Nucleotide excision repair[1]

[1] Pinto L.A., et al, 2002

1.3.1.3 DNA damage Tolerance

Not all DNA damage is or can be removed immediately; some of it may persist for a while. If a DNA replication fork encounters DNA damage such as a pyrimidine dimer it will normally act as a block to further replication.

However, in eukaryotes, DNA replication initiates at multiple sites and it may be able to resume downstream of a dimer, leaving a "gap" of single-stranded unreplicated DNA. The gap is potentially just as dangerous if not more so than the dimer if the cell divides. So there is a way to repair the gap by recombination with either the other homolog or the sister chromatid--this yields two intact daughter molecules, one of which still contains the dimer.

1.3.1.3.1 Recombinational (daughter-strand gap) repair

A repair mechanism which the dimers that are opposite DNA daughter-strand gaps are no longer subject to excision, since this process requires an intact complementary strand. (Jansz HS et al., 1963). Only after the gaps are filled by sister-strand exchanges will the dimers again be subject to excision repair.

These gaps in the daughter strands, which average 1000 nucleotides in length, (Iyer VN et al., 2006) are subsequently repaired in recombination-proficient strains by transferring the appropriate sections of DNA from the parental strands into the daughter strands. This transfer of parental strands into daughter strands has been confirmed by direct measurement.

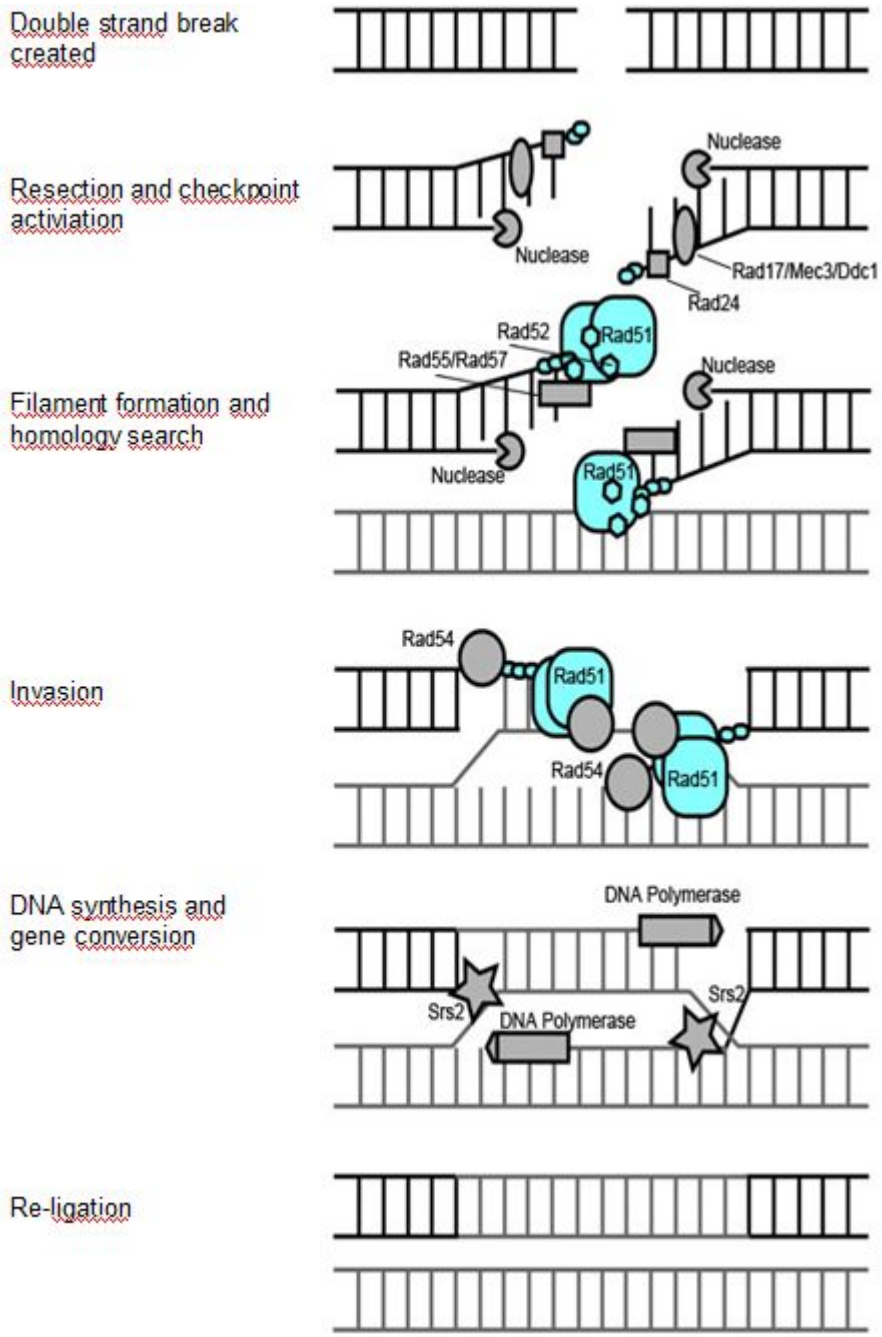


Figure 1.13 . Recombinational (daughter-strand gap) repair[1]

[1] http://www.biomedcentral.com/1471-2199/8/24/figure/F2?highres=y

1.3.1.3.2 Mutagenic repair (trans-lesion synthesis)

An alternative scenario for a DNA polymerase blocked at a dimer is to change its specificity so that it can insert any nucleotide opposite the dimer and continue replication ("mutate or die" scenario).

1.3.2 DNA repair systems and genetic toxicology

The discovery of DNA repair mechanisms has changed the notion of genetic risk of environmental agents, or of particular life styles, because the genotoxic impact is dependent on the efficacy of many DNA repair systems. Not only do the error-free constitutive and inducible DNA repair activities decrease the impact of the initial DNA modifications, but also the activities of inducible error-prone DNA replication (tolerance) systems determine the final genetic consequences of such damage. Whereas error-free DNA repair systems increase the effective dose of genotoxic agents, the error-prone systems decrease the effective dose. Error-free systems act to increase cell survival and decrease the genotoxic outcome, error-prone lead also to an increase in survival but at the expense of increased induced mutation or recombination rates. Hence, the mutant organisms deficient in error-free repair (e.g., NER and BER) are sensitive to genotoxic agents and are hypermutable, whereas mutants in error-prone repair are sensitive but non-mutable (Radman M. , 2006.).

Furthermore, some repair systems cause the cell killing by specific lesions, e.g., the mismatch repair system activity kills bacterial and mammalian cells treated with simple alkylating agents (methylation or *cis*-platin). Mismatch repair, and specifically the level of the MutL, controls the frequency of chromosomal deletions mediated by homologous recombination between directly repeated sequences (M. Elez, I. Matic and M. Radman, unpublished). Mismatch repair can also become saturated by a critical level of certain kind of DNA damage (e.g., base analogs, alkylations, etc.) having a generalised indirect genomic genotoxic effect resulting from the reversible mismatch repair deficient phenotype. Some extremophile organisms, e.g., *Deinococcus radiodurans* have an exceedingly efficient error-free recombination repair system, but lack error-prone, mutagenic, DNA polymerases resulting in an extraordinary resistance to genotoxic agents with little or no mutagenic consequence (Radman M. , 2006.).

These are examples of known DNA repair systems which profoundly modify the genotoxic consequences of DNA damaging agents. Thus, the risk assessment must include the diagnostic of the DNA repair systems active in the relevant organism. In other words, risk assessment will be one day individualised just like the general medicine (Radman M. , 2006.).

1.3.3 THE SOS RESPONSE

Despite having multiple repair system, sometimes the damage to an organism's DNA is so great that the normal repair mechanisms just described cannot repair all damage. As a result, DNA synthesis stops completely. In such situations, a global control network called the SOS response is activated.

The SOS response, like recombinational repair, is dependent on the activity of the RecA protein. Rec A binds to single-or double-standed DNA breaks and gaps generated by cessation of DNA synthesis. RecA binding initiates recombinational repair. Simultaneously, RecA takes on a proteolytic function that destroys a repressor protein called LexA. LexA negatively regulates the function of many genes involved in DNA repair and synthesis. Destruction of LexA increases transcription of genes for excision repair and recombinational repair, in particular. The first genes to be transcribed are those that encode the Uvr proteins needed for nucleotide excision repair (Figure 1.12). Then genes involved in recombinational repair are further upregulated.To give the cell time to repair its DNA, the protein sfiA is produced; sfiA blocks cell division. Finally, if the DNA has not been fully repaired after about 40 minutes, a process,DNA polimerases IV (also known as dinB)and V (umuCD) synthesize DNA across gaps and other lections (e.g., thymine dimers) that had stopped DNA polimerase III. However, because an intact template does existi these SOS response polimerases often insert incorrect bases. Furthermore, thay lack proofreading activity. Therefore even though DNA synthesis continues, it is highly error prone and results an the generation of numerous mutations.

The response is so named because it is a response made in a life-or-death situations.The response increases the likehood that some cells will survive by allowing

DNA sythesis to continue.For the cell , the risk of dying because of failure to replicate DNA is greater than risk posed by mutations generated by this error-prone process

The SOS response to DNA damage was first described in *Escherichia coli* as a system promoting DNA repair, cell cycle control, and recombination.16 In bacteria, single- stranded DNA generated during the course of DNA damage and repair interacts with and activates a protease, leading ultimately to derepression of at least 20 genes involved in DNA repair, replication, and cell survival (Figure 1.14).

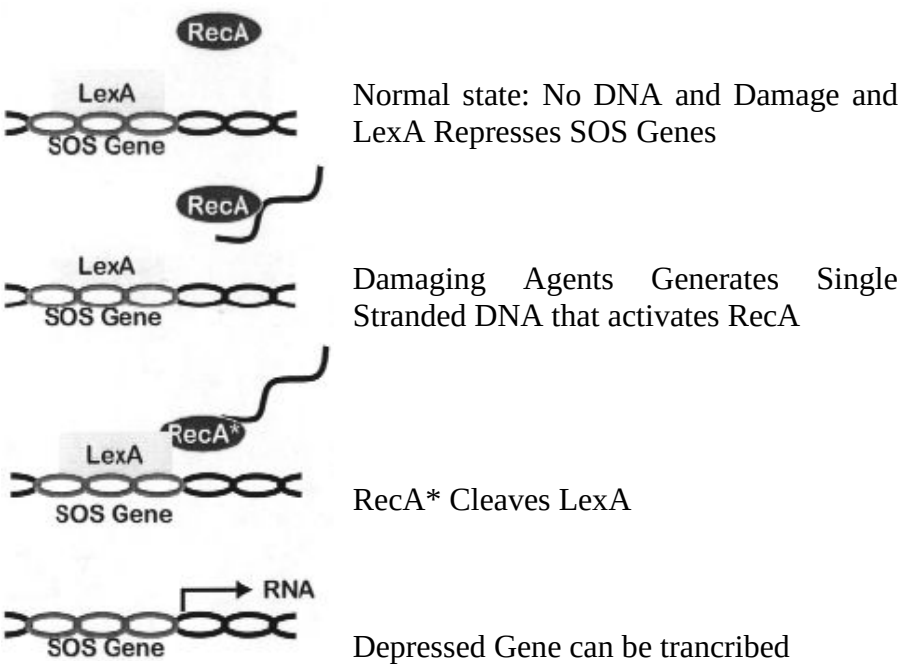


Figure 1.14 . Single-stranded DNA induces expression of SOS genes in bacteria.

The LexA protein normally represses genes that are induced as part of the SOS response. After DNA damage, single-stranded DNA is generated by the excision repair process and at stalled replication forks. This single-stranded DNA interacts with and activates the RecA protease (designated as *RecA*)* which then cleaves the LexA protein, de-repressing and inducing the transcription of the SOS response genes.

Another feature of the SOS response is bypassing the block on DNA replication induced by DNA damage. Induction of two genes, *UmuC* and *UmuD,* allows DNA polymerase to replicate a damaged template by relaxing normal Watson-Crick base-pairing requirements. This repair is error-prone but immediately beneficial for cell

survival, even though it increases the risk of mutations that may affect cellular function in subsequent generations. Indeed, it has been postulated that error-prone repair of the ultraviolet-irradiated bacterial genome is evolutionarily beneficial because it encourages the appearance of mutations, some of which are likely to improve cell function or confer better survival in the presence of the environmental DNA-damaging agent.

1.4 BACTERIAL TEST METHODS

All bacterial test methods have some common characteristics. Most tester strains contain mutations which increase sensitivity to genotoxins. The *rfa* mutation for example causes a partial loss of cell wall and therefore increases permeability to larger molecules such as benzo[a]pyrene. The *uvrB* mutation of most Ames-tester strains deletes a gene coding for the DNA excision repair system and therefore hinders the repair of DNA damage. Often a test battery of several tester strains is applied in order to characterise specific genotoxic spectra or get hints on the origin of genotoxins. As bacteria do not possess the metabolic capacity of eucaryotes the tests are usually performed in the absence and the presence of S9 liver homogenate (supernatant of rat liver extract centrifuged at 9000 g) (OSPAR Commission, 2002).

1.4.1 Bacterial Genotoxicity Tests

Millions of animals are raised in the United States each year for routine toxicology tests, exposed to compounds in food additives, cosmetics, and industrial products, and then studied for ill effects. This is a time-honored way of identifying human health risks, but it can be an imprecise science. It's also expensive and increasingly under attack by animal-rights activists as wasteful. Now, according to researchers who gathered at a high-powered summit this month, toxicology may be on the verge of changing the way it collects raw data--adopting a process that could reduce animal use and improve test results **(**Richard A. Lovett, 2000**).**

The new approach, called "toxicogenomics," grows out of the human genome project. Rather than using animal pathology to identify illnesses, it probes human or animal genetic material printed on plates, called DNA arrays. Cancer researchers have already been using such arrays for several years to compare gene expression in healthy

and diseased cells (*Science*, 15 October 1999, p. 444). Toxicologists are using the same technology to profile gene expression in cells exposed to test compounds (Richard A. Lovett).

The advantages of these DNA tests are legion: They are fast, efficient, and reduce live-animal expenses, which can range as high as $3000 per week, per animal, when nonhuman primates are used. Some of the biggest gains may come in cancer toxicology: New tests may be able to spot the metabolic precursors of slow-developing diseases without holding up research for the months or years it takes for tumors to develop. If adapted for use in tissue cultures, these tests might even eliminate the need to sacrifice animals (Richard A. Lovett, 2000).

1.4.1.1 Umu C-Test

The umuC-assay was originally developed by Oda *et al*. in 1985. A microplate version of the test is available (Reifferscheid *et al*. 1991). The assay is based on the use of a genetically modified *Salmonella typhimurium* strain TA 1535 that contains the plasmid pSK1002. Here the umuC gene, as a part of the SOS system, is fused in a reporter gene, lacZ, that encodes for ß-galactosidase. If genotoxins induce the SOS function, the reporter gene is also activated and the formation of ß-galactosidase is quantified photometrically at 420 nm by its ability to form a yellow-coloured metabolite (Oda *et al*. 1985). The test is carried out with and without S9. Bacterial growth is measured as turbidity at 600 nm and biomass factors are considered in the test results. A reduction of cell growth by more than 50% is considered as a toxic effect and ß-galactosidase should not be evaluated for those wells. National (DIN 38415-4: 1996) as well as international standards (ISO 13829: 2000) exist.

Practical experience with the umu-test is available on extracts of bleached kraft mill effluents in Canada (Rao *et al*. 1995). In Switzerland and Germany hospital, municipal and various industrial waste waters have been investigated (Fenn and Popp 1996, Giuliani *et al*. 1996, Miltenburger 1997, Zipperle 1997, Hartmann *et al*. 1998, Siersdorfer *et al*. 1998, Hartmann *et al*. 1999, Gartiser 2000, Gartiser *et al*. 2001). The test method has been introduced for routine regulatory testing of chemical and

pharmaceutical effluents (Wastewater Ordinance of Germany). Extracts from suspended particulate matter of river water have also been tested (Vahl *et al*. 1997). Recently several other tester strains which overexpress specific activation enzymes (acetyltransferase, nitroreductase) have been developed in order to increase the sensitivity against specific genotoxins like nitroarenes and/or aromatic amines (Oda *et al*. 1992, Oda *et al*. 1993, 1995). But these tester strains have been applied to environmental samples only in a few studies (Ohe 1996, 1997). The application of a fluorometric *umu*-test system has been developed in order to increase the sensitivity of the test for the detection of genotoxic compounds in surface water (Reifferscheid and Zipperle 2000).

1.4.1.2 SOS Chromotest

The SOS chromotest originally was developed by Quillardet *et al*. (1982, 1985). The test detects induction of the SOS genes, which are involved in DNA repair in *Escherichia coli* K12 bacteria. The principle is similar to that of the umuC-test (SOS genes are fused in the *lacZ* reporter gene). There is some evidence that the umuC test detects lower genotoxic responses than the SOS chromotest for two reasons: firstly, the outer wall of the *Salmonella* tester strain used is made more permeable to genotoxins, and secondly, the umuC reporter gene is placed on a multicopy plasmid while in the SOS chromotest it is placed on a single bacterial chromosome (De Maagd 2000). But there are only few comparative studies about the sensitivity of tests. Waste water studies using the SOS chromotest were performed in Canada (Legault *et al*. 1996, White *et al*. 1996a, White *et al*. 1996b, White and Rasmussen 1998, White *et al*. 1998b, White *et al*. 1998a), Austria (Helma *et al*. 1996), Finland (Suominen *et al*. 1998), and Germany (Janz *et al*. 1990). Sorption of genotoxins to effluent suspended particulate or detection of genotoxic substances in bivalve molluscs has also been studied (White *et al*. 1996b, White *et al*. 1997).

CHAPTER 2

MATERIAL AND METHOD

2.1 Sampling of Marmara Sea

Out of total 32 samples were collected from coastal region of Marmara Sea. During the taking of samples clean side of coastal sites were selected to not allow sediment and solid particles in the samples. Especially sampling areas were away from harbours to take homogenous samples which did not included oil spills

The samples were collected into the 2 ml sterile eppendorf tubes and stored at + 4 °C untill they were tested.

Table 2.1: Sampling stations in Marmara Sea

	Stations	**sampling**	**Stations**	**sampling**
1	Güzelyalı	28.03.2008	Güzelyalı	18.05.2008
2	Mudanya	28.03.2008	Mudanya	18.05.2008
3	Gemlik	28.03.2008	Gemlik	18.05.2008
4	Gemlik (Canal)	28.03.2008	Gemlik (Canal)	18.05.2008
5	Gölcük	28.03.2008	Gölcük	18.05.2008
6	Yalova	28.03.2008	Yalova	18.05.2008
7	Dilovası	28.03.2008	Dilovası	18.05.2008
8	İzmit (Bay)	28.03.2008	İzmit (Bay)	18.05.2008
9	Kartal	28.03.2008	-	
10	Mimarsinan	28.03.2008	Mimarsinan	18.05.2008
11	Avcılar	28.03.2008	Avcılar	18.05.2008
12	Florya	28.03.2008	Florya	18.05.2008
13	Bakırköy	28.03.2008	Bakırköy	18.05.2008
14	Yenikapi	28.03.2008	Yenikapi	18.05.2008
15	Eminönü	28.03.2008	Eminönü	18.05.2008
16	Kadiköy	28.03.2008	Kadiköy	18.05.2008
17	-		Avşa	18.05.2008

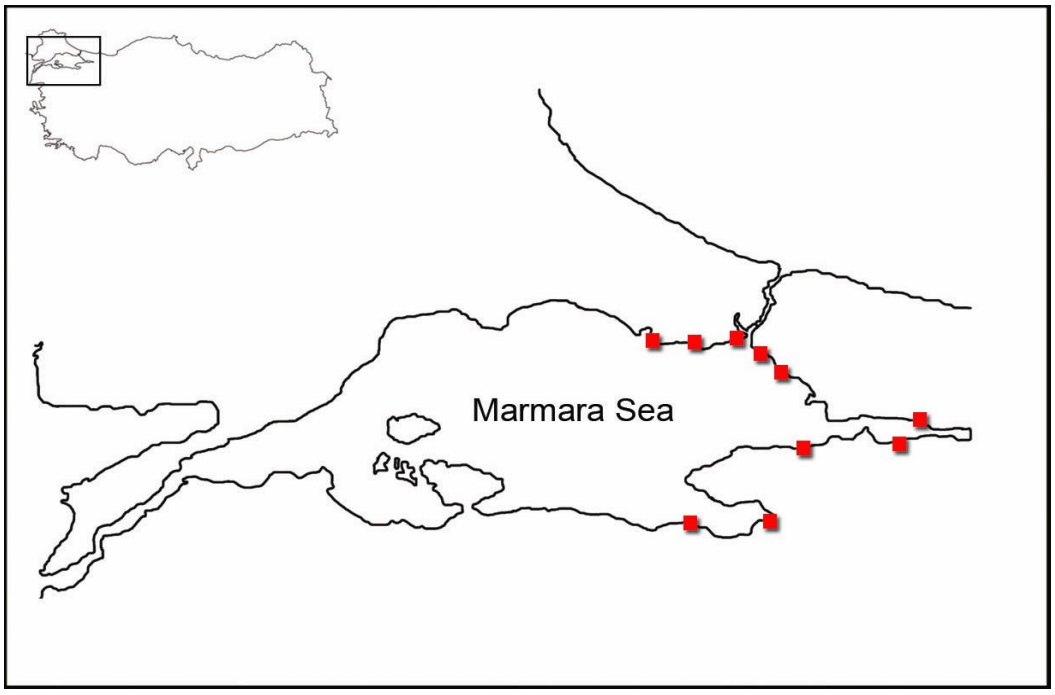


Figure 2.1 . Marmara Sea

2.2. Bacterial Short-Term Genotoxicity Tests

2.2.1 SOS Chromotest

The EBPI SOS-CHROMOTEST is a convenient approach for the detection of genotoxic activity and genotoxic materials in environmental water, sediment, air, chemicals, food components, cosmetics and biological fluids. Genotoxic materials may be hazardous due to their ability to induce mutations and cancerous transformation of normal cells (SOS Chromotest Version 6.0).

E.coli PQ37 is used to detect DNA-damaging in SOS Chromotest that 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 SOS-CHROMOTEST: 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.
- 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 colour. The amount of colour 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 6.0).

The criterias that were given under mentioned based on for optimization of SOS Chromotest:

- β-galactosidase activity ⇨ The indicator of SOS gene induction,
- Alkaline phosphatase activity ⇨ The indicator of cytotoxicity,
- Two genotoxicity measurement end points ⇨ Minimum genotoxic concentration and maximum induction factor

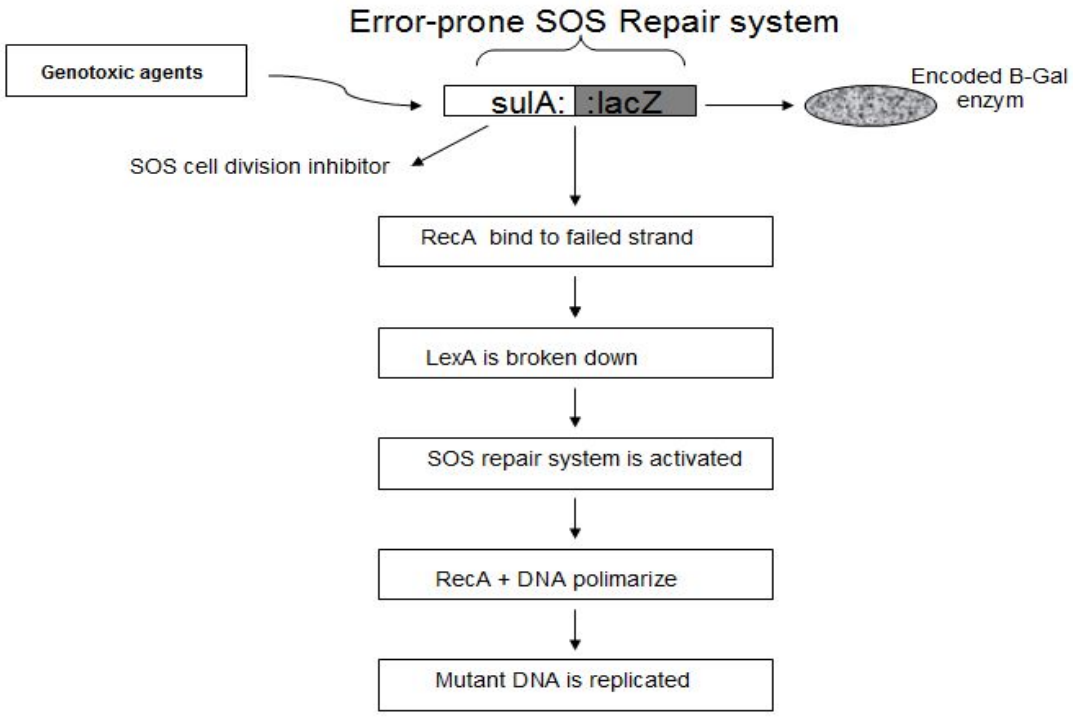


Figure 2 .2 . The function of error-prone SOS Repair system

2.2.2. UmuC Test

In vitro genotoxicity tests are useful to detect DNA-damaging agents. One of them that the umu-test is based on the use of the genetically engineered bacteria *Salmonella thyphimurium* TA 1535 pSK1002 (gram negative, facultative anaerobic enterobacteriaceae).

The umu-test can be used as reference test system for the analysis of different chemical categories of compounds (including heavy metals) because extremely high toxicity ranges were not observed. Especially complex mixtures (industrial effluents) can be analysed with this test system in a high sensitivity range (Wittekindt et al., 1999). The umu-test was selected to detect primary damage processes caused by mono substances and complex environmental contamination because this bacterial test system is used as a reference test in many studies. Bacterial genotoxicity test systems showed high correlation in comparative studies of inter-species bacterial genotoxicity assays (Nakamura et al., 1987; McDaniels et al., 1990).

The umu-test has been standardised and validated by German DIN (DIN 38 415 T3) and on the international level by ISO (ISO/DIS 13829, 2000). High sensitivity to genotoxins (mono substances, environmental samples and food) have been described by Oda et al, 1985, Oda et al, 1988; Reifferscheid et al, 1991and Ono et al, 1992.

2.3. Chemicals

All chemicals which ready-to-use bottled were purchased from EBPI (Brampton, Ontario, Canada) and they were convenient according to SOS Chromotest Version 6.0 to detect genotoxic activity in liquid samples.

Table 2 .2 : Chemical Table which was used SOS Chromotest

Chemical	Commercial Label	Bottle Label	Volume-Concentration	Contains
10 % DMSO	Dimethyl Sulfoxide in saline	C-Bottle	12 mL	
-	(X-Gal) Blue chromogen solution	F- Bottle	12 mL	**A variety of cell lysis agents to release cytoplasmic β-gal was mixed with 12 mL of Tris buffer
pNPP	p-Nitro-Phenyl-Phophate	H-Bottle		
4NQO	4-Nitro-Quinoline-Oxide	D-Bottle	110 µg/mL (MW*: 190.16)	

*Molecular Weight
**P.A.White , Richard Legault , C.Blake and S.Trottier

2.3.1 Solvent

The dilutions of all test material (Test samples, positive control and negative control) were used 10 % DMSO. PBS (Phosphate Buffer Salt, pH :7.4 Sigma P-3813) could be used to prepare 10% DMSO in which 1 packed PBS is dissolved in deionized water. 100 mL of PBS solutions are mixed with 900 mL pure DMSO and autoclaved at 121 °C in 15 min.

The commercial bottle of 10 % DMSO (C-bottle) was used for tests.

2.3.2 Positive and Negative Control

Positive control wells were prepared on each microplate to confirm that chemical's mutagenity could be properly detected (A.Yamamato ,et al.,2001). 4NQO (4-Nitro-Quinoline-Oxide) is a standart genotoxic chemicals were used positive control to check bioreaction of cell culture.

Negative control wells were also prepared on each microplate to check Alkaline Phosphate (AP) activity and confirm non-genotoxicty. Ultra Deionized Water (UDW) was used for negative control and two replicates were for each microplate.

Table 2.3: Dilution rate and concentration of 4NQO (4-Nitro-Quinoline-Oxide)

4NQO		
Percentage	**Dilution Rate**	**Concentration (µg/mL)**
1:1	Undiluted (Undiluted)	10
1:2	% 50	5
1:4	% 25	2,5
1:8	% 12.5	1,25
1:16	% 6.25	0,625
1:32	%3.13	0,313
1:64	%1.56	0,1565
1:128	%0.78	0,0781

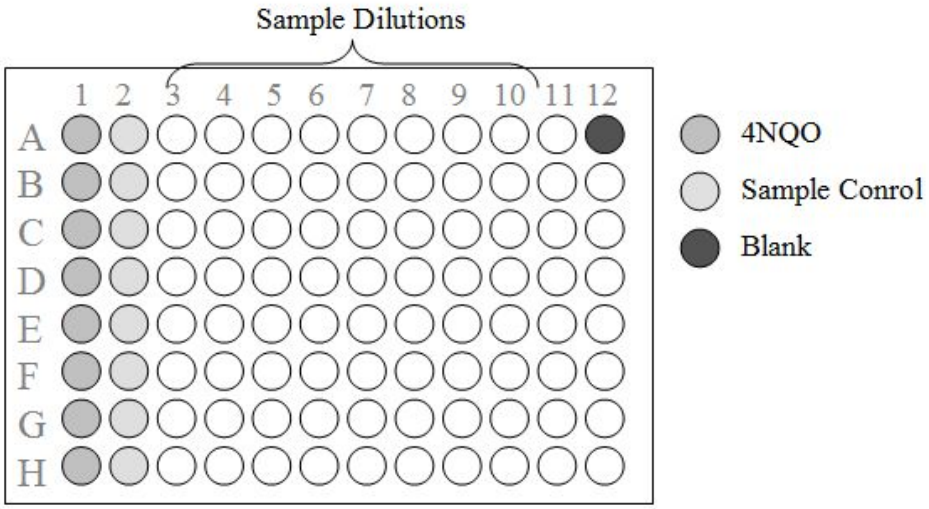


Figure 2.3. Schematic delivery of samples on 96 well plate

2.4 The SOS inducing potency (SOSIP)

The assay is quantitative and dose– response curves present a linear region. The slope of the linear region allows estimation of the SOS inducing potency (SOSIP), which representing the increase in induction factor tested. (Hofnung and Quillardet, 1986; Mersch-Sundermann et al., 1998; Quillardet and Hofnung, 1985, 1993).

2.5. Bacterial tester strains

Freeze-dried bacterial reagents of *E.coli* PQ37 (SOS Chromotest) and *S. typhimurium* TA1535/pSK1002 (umuC test) were purchased from EBPI

(Environmental Bio-Detection Products Inc., Brampton, Ontario, Canada). Genetic characteristic of strains used are listed in Table 2.4.

2.5.1. *E.coli* PQ37

The genetic maps of *E.coli* PQ37 and *S. typhimurium* TA1535/pSK1002 has been altered to detect DNA-damaging. The genetic markers and biomolecular mechanisms of *E.coli* have been described by P.Quillardet and Hofnung (1985). *E. coli* PQ37 that is constitutive for alkaline phosphatase synthesis. 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 (Quillardet and Hofnung, 1985). 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 (Isidori, M. et al., 2004).

2.5.2. *S. typhimurium* TA1535/pSK1002

Salmonella typhimurium TA1535/pSK 1002 carrying a fused *umu*C-*lac*Z gene by transformation of *S. typhimurium* TA1535 with the plasmid, pSK1002 (Oda et al., 1985) was kindly donated by Dr. Oda, Osaka, Japan.

Table 2.4 : Specific properties of species which are used for this study

Species	Strains	Relevant genotype	Genetic endpoint	Source
E. coli	PQ37 (SOS Chromotest)	*F-thr leu his-4 pyrD thy galE galK(galT)lac169 rl300::Tn10 rpo B rpsL uvrA rfa trp::MUC sulA::Mud(AP,lac) c-ts*	Genetic mutation	P.Quillardet and Hofnung [1985]
S. typhimurium	TA1535/pSK1002 (umu test)	*hisG46 rfa ΔuvrB Δ lacZ umuC'-'lacZ*	Primary DNA damage*	Y.Oda [Oda et al., 1985]

ΔuvrB, deletion in excision repair; rfa, deep rough mutation (LPS); hisD3052 and other his mutations, description of the substitution or deletion in the specific his gene; umuC-lacZ, umuC-lacZ gene fusion.

* Primary DNA damage, which leads to increasing nuclear amounts of DNA single strands and oligonucleotides: O- and N-alkylation, adducts, depurinisation, depyrimidation, deamination oxidative damage of DNA and DNA dimers.

2.6 Nutrient Medium

Lyophilized *E.coli* PQ37 was growth in LB media which is included 10 gr tryptone, 5 gr yeast extract and 5 gr NaCl in 1L of deionized water. It's supplemented with 20 µg/mL of ampicillin.

Lyophilized *Salmonella typhimurium* TA1535/pSK1002 was also growth in TGA medium [1% trypton, 0.5% sodium chloride, 0.2% D-glucopyranoside, and 20 µg. mL^{-1} ampicillin (aminobenzylpenicillin)].

During the experiment ready-to-use bottled of commercial mediums were used for both strain *E.Coli* PQ37 and *Salmonella typhimurium* TA1535/pSK1002 to growth them.

2.7 Test procedures

2.7.1 The procedures of SOS Chromotest

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).

For test of all samples, same day growth was used according to optimisation of test result. Using aseptic technique lyophilized bacteria was re-hydrated with medium and incubated at 37 °C in approximately 4 hrs.

- The end of the 4 hours, OD (optic density) of bacteria reached to 0.05-0.052 and 100 µL was transferred to 96 well plate which was consist on diluted samples, positive and negative controls.
- 96 well plate was incubated 2 hours.
- Blue chromogen (bottle "F") was transferred to the pNPP (bottle "H") and mixed 30-40 min. 100 µL of mixing was transferred immediately on the 96 well plate.
- After 25 min, absorbance of 96 well plate was measured 405 nm with ELISA READER, to determine AP activity and then incubation continued.
- According to OD of bacteria, 96 well plate was incubated 60 to 90 min.
- The end of the this process, absorbance of 96 well plate was measured 620 nm with ELISA READER to determine β-Gal activity.

Table 2.5: 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

All samples were tested at four concentration and for two times. Positive control were prepared at eight concentrations to determine each step of β-Gal activity of bacteria; and negative control was tested two times to chech non-genotoxic conditions.

2.7.2 The procedures of umuC Test

The umu assay (Oda et al., 1985) for measuring primary DNA damage by means of SOS induction was performed according to Reifferscheid et al. UmuC Test procedures are similar to SOS Chromotest procedures. However 96 well absorbance was measured at 420 nm and 550 nm to calculate alkaline phosphate activity and β-Gal activity.

2.8 Required Instruments

For this study,

- Micropipettors using disposable tips in the range of 10 to 200 micro-litres
- 37°C incubator.
- 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 microplate reader (= "ELISA Reader")
- Spectrophotometer to measure optic density of bacteria
- Micro-centrifuge (+"Microfuge").

were used during the expriments in the labratory.

2.9 Calculation

Calculation of SOS Inducing Potency (SOSIP)

$$SOSIP = \frac{10x(OD_3 - OD_1)}{C_3 - C_1} \quad \text{..... (1)}$$

OD ⇨ Optik density
C ⇨ Concentration

$$C = \frac{ConcxVol}{MW} \quad \text{..... (2)}$$

where:

Conc ⇨ Concentration of tested material in μg/mL,

Vol ⇨ Volume of the tested material solution in the well expressed in micro-litres, and

MW ⇨ Molecular weight of the tested material.

AP reduction factors (RF), β -gal induction factors (IF), and corrected induction factors (CIF) were computed for each tested concentration using the formulae given below. RFs must be determined to compensate for any (sub)lethal effects on exposed test cultures owing to sample toxicity. CIFs are then determined to normalize for cell viability. If Corrected Induction Factor exceed 1.2 or equall, this result is considered genotoxic.The calculation was described by Legault et al.(1996):

$RF = \bar{x}\ OD_{405}t / \bar{x}\ OD_{405}c$

$IF = \bar{x}\ OD_{620}t / \bar{x}\ OD_{620}t$

CIF = IF / RF

where $\bar{x}$ is the mean of eight OD readings and ***t*** and ***c*** are referred to as sample dilution(s) and control, respectively.

CHAPTER 3

RESULTS

4NQO, ultra deionized water and sea water samples were tested with *E.coli* PQ 37 strain and *Salmonella typhimurium* TA1535/pSK1002 without metabolic activation (S9). Different concentration of test samples were dissolved in DMSO and transferred to 96 well plate. Color development was measured at 405 nm and 620 nm to determine Alkaline Phosphate activity and β-Gal synthesize for all test material for SOS Chromotest.

Color development was measured at 420 nm and 550 nm to determine Alkaline Phosphate activity and β-Gal synthesize for all test material for umuC test. Absorbans results were calculated according to SOS Chromotest and umuC test protocol.

Ultra deionized water (negative control) showed that AP activity was occured for E.Coli PQ 37 strain.Color development of ultra deionized water was observed and measured with ELISA READER.

The absorbans result of β-Gal and Alkaline phosphate for 4NQO was measured with ELISA READER and eight different concentrations of 4NQO was prepared for 96 well microplate. The maximum and minimum absorbance values proved that the experiments were successful and results were suitable for SOS Chromotest protocol. SOS Inducing Potency (SOSIP), Alkaline Phosphate activity, β-Gal synthesize and Correction Induction Factor (CIF) (Figure 3.4) were calculated for 4NQO (positive control) and each test samples. The results proved that biochemical reaction between E.Coli PQ 37 and test samples were appropriate for test protocol (SOS Version 6.0 or Quillardet). β-Gal synthesize of 4NQO is given in Figure 3.2 and Figure 3.3.

Test samples were not found to be cytotoxic at any concentration of samples. For each concentration AP activity was calculated and color development was observed in the absence of the cytotoxic effects of the test samples

3.1. SOS Chromotest Result

3.1.1 Bacterial Incubation

The same day bacterial growth culture was used to detect genotoxic activity in the test samples. The freeze-dried bacterial strain was mixed with medium and incubate 4 hrs. The optic density of bacteria was 0.05-0.052 for *E.coli* PQ37 strain at the end of the 4 hrs.

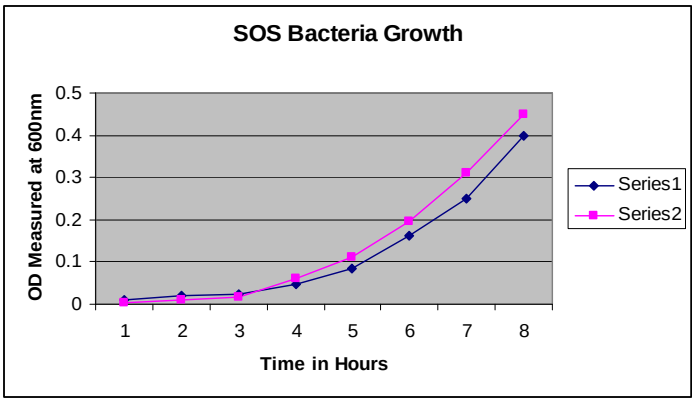


Figure 3.1. E.coli PQ37 growth curve

3.1.2. Positive and negative control

The positive and negative control test result exhibited that alkaline phosphate activity and β-Gal activity were convenient for test procedure. When the concentration of genotoxic matter was increased, induction reaction were also increased. The absorbance result of negative control were between 0.25 and 0.3 that showed test samples were not cytotoxic.

SOS Inducing Potency showed that increasing of induction factor was occured correctly and maximum value of SOSIP was 122.

February 2008

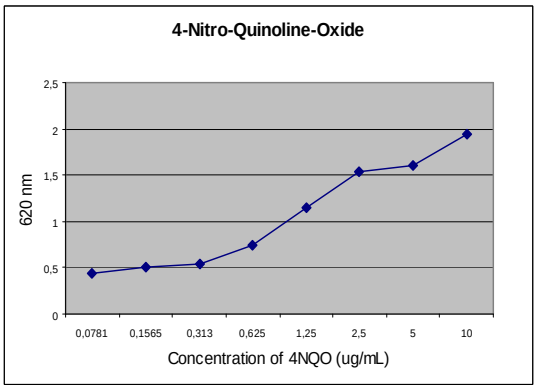


Figure 3.2. Absorbans (620 nm)- 4NQO concentration curve

May 2008

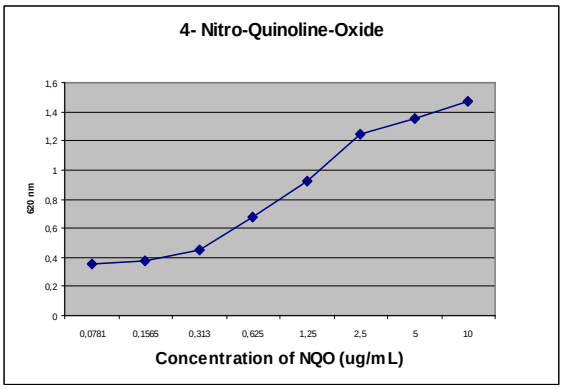


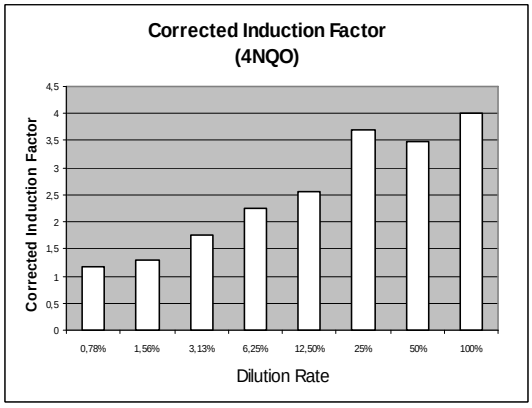
Figure 3.3. Absorbans (620 nm)- 4NQO concentration curve

SOSIP value was 122 which was calculated with absorbans curve at 620 nm- 4NQO concentration curve for February 2008 samples and also SOSIP value was 96 for May 2008 samples (Figure 3.2 and Figure 3.3).

The result of bioreaction between 4NQO and bacterials strain genotoxic reaction was occured and Corrected Induction Factor was maximum 5.

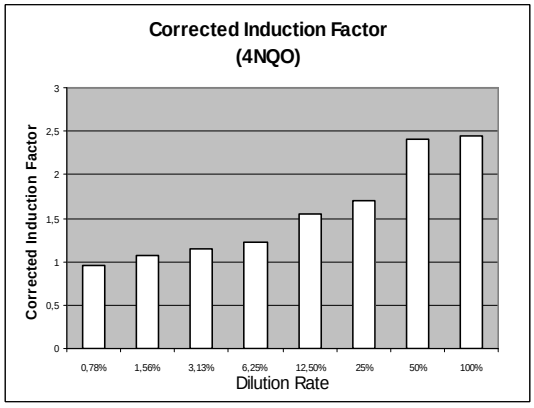
Because of in fact that decreasing of 4NQO concentration, CIF rate decreased.This means that bacterial culture reacted with 4NQO and β-Gal activity was occured in the 96 well plate.

February 2008



(a)

May 2008



(b)

Figure 3.4. (a) CIF of 4NQO according to Dilution Rate (b) CIF of 4NQO according to Dilution Rate

3.1.3 Genotoxic Activity in Test samples

According to results of samples the cytotoxic effect was not determined for any sample. Alkaline phosphate activity was evaluated and color development was observed in the all wells.

A corrected induction factor of sample was 1.2 or more; it was considered genotoxic. A corrected induction factor of sample was below 1.2, it was considered non-genotoxic (Legault et al. 1996). For this study, the samples of 33 stations were tested at four concentration and two times. Totaly 128 concentrations were prepared to be tested.

3.1.3.1 The Result of February 2008 samples

16 samples were tested (February2008 samples) with SOS Chromotest and 3 (% 17.6) samples were induced a significant (CIF $\geq$ 1.20) SOS response at one or two concentrations.

Yenikapı

IF was 1.2 ±0.2 in undiluted sample of Yenikapı. But this stations CIF value was 1.06±0.3. STDEV values of Yenikapı were 0.0 and 0.2. According to procedure, test result indicated that this station was considered to be non-genotoxic (Legault et al. 1996).

Eminönü

CIF values for fourth concentration was 1.18 ±0.1 and IF value was 1.24 ±0.2 for second concentration. The test result indicated that this station was considered to be non-genotoxic (Legault et al. 1996).

Avcılar

IF values for first and fourth concentration were 1.23 ±0.3 and 1.22 ±0.3 . IF value of the other concentrations were below 1.20. CIF value was 1.06±0.0. The test result indicated that this station was considered to be non-genotoxic (Legault et al. 1996).

Florya

IF value of Florya was 1.20±0.2 at first concentration and CIF value was 1.19±0.0 at fourth concenration. According to this result that the sample was considered to be genotoxic (Legault et al. 1996).

Gölcük

CIF value of Gölcük sample was 1.22±0.4 at second concentration. According to this result that the sample was considered to be genotoxic. CIF value and IF value were below 1.20 for other concentrations (Legault et al. 1996) .

Mudanya

IF value of Mudanya sample was 1.20±0.3 at second concentration. According to this result that the sample was considered to be genotoxic. CIF value and IF value were below 1.20 for other concentrations (Legault et al. 1996).

The stations of Mimarsinan, Gemlik (Canal), Kadıköy, Gemlik, Kartal, Bakırköy, Dilovası, Körfez, Yalova and Güzelyalı samples were considered to be non-genotoxic according to IF and CIF results. β-Gal activities were below 1.20 for these samples (Legault et al. 1996).

3.1.3.2 The Result of May 2008 samples

Out of 16 samples were tested (May 2008 samples) with SOS Chromotest and 9 (% 56.2) samples were induced a significant (IF ≥ 1.20) SOS response in one or two concentrations. The all samples were tested at four concentrations and for two times.

Eminönü

IF value of Eminönü was 1.28±0.2 at second concentration. According to this result that the sample was considered genotoxic. CIF value and IF value were below 1.20 for other concentrations. STDEV value was 0.2-0.1 in Eminönü sample.

Avcılar

For all concentration of Avcılar samples, CIF values was exceed 1.20. IF value of Avcılar was 1.19±0.0 at second concentration According to this result that the sample was considered to be genotoxic. Reduction Factor (RF) value was low, when it compared with RF factor of other regions. STDEV value was 0.0-0.2 in Avcılar sample.

Florya

In the first and second concentration of Florya samples, CIF values was exceed 1.20.IF value was 1.32±0.0 at second concentration. According to this result that the sample was considered genotoxic. STDEV value was 0.0-0.2 in Avcılar sample.

Mimarsinan

IF value of Mimarsinan was 1.17±0.1. CIF was 1.22±0.1 at fourth concentration. According to this result that the sample was considered genotoxic.

Gemlik (Canal)

In the second and fourth concentration of Gemlik(Canal) samples, CIF values was was exceed 1.20. According to this result that the sample was considered genotoxic. STDEV value was 0.1 for each sample.

Gölcük

At first and second concentration of Gölcük samples, CIF values were that 1.35±0.1 and 1.23±0.0. CIF value was 1.17±0.1 at fourth concentration. According to this result that the sample was considered genotoxic.

Kadıköy

In the first and second concentration of Kadıköy samples, CIF values were that 1.22±0.0 and 1.27±0.1. According to this result that the sample was considered genotoxic.

Gemlik

In the first concentration of Gemlik samples, CIF values were that 1.20±0.1. According to this result that the sample was considered genotoxic.

Bakırköy

In the first concentration of Gölcük samples, IF values were that 1.21±0.0 and CIF value was that 1.18±0.0. According to this result that the sample was considered genotoxic.

The samples of Yenikapı, Avşa, Dilovası, Mudanya, Körfez, Yalova and Güzelyalı stations were not genotoxic according to IF and CIF results. β-Gal activities were below 1.20 for samples of May2008.

Table 3.1:AP ,β-Gal activity and Corrected Induction Factor of test samples which were taken on February 2008

		February 2008		
		405 nm (25 min)	620 nm (60-90 min)	
	Sample Dilution Rate	RF AP Activity[2]	IF β-Gal Activity	CIF
Yenikapi	1:1	1,056 ± 0,1	1,205 ± 0,2	1,069 ± 0,3
	1:4	0,921 ± 0,3	1,110 ± 0,0	1,025 ± 0,3
	1:16	0,878 ± 0,0	0,947 ± 0,2	1,050 ± 0,2
	1:128	1,138 ± 0,0	1,186 ± 0,2	1,037 ± 0,2
Eminönü	1:1	1,025 ± 0,0	1,146 ± 0,2	1,150 ± 0,2
	1:4	1,039 ± 0,1	1,165 ± 0,2	1,181 ± 0,1
	1:16	0,961 ± 0,1	0,961 ± 0,1	0,956 ± 0,2
	1:128	1,151 ± 0,0	1,242 ± 0,2	1,098 ± 0,2
Avcilar	1:1	1,373 ± 0,3	1,235 ± 0,3	1,060 ± 0,0
	1:4	0,905 ± 0,2	1,134 ± 0,2	1,121 ± 0,4
	1:16	0,965 ± 0,0	1,015 ± 0,2	1,024 ± 0,2
	1:128	0,935 ± 0,2	1,226 ± 0,3	1,128 ± 0,1
Florya	1:1	1,264 ± 0,0	1,205 ± 0,2	0,970 ± 0,2
	1:4	0,996 ± 0,4	1,077 ± 0,1	0,901 ± 0,4
	1:16	1,077 ± 0,1	0,883 ± 0,1	0,886 ± 0,0
	1:128	0,796 ± 0,0	0,974 ± 0,0	1,192 ± 0,0
Mimarsinan	1:1	1,388 ± 0,4	0,997 ± 0,3	0,880 ± 0,0
	1:4	0,968 ± 0,1	0,961 ± 0,1	0,937 ± 0,1
	1:16	1,203 ± 0,0	0,938 ± 0,1	0,799 ± 0,1
	1:128	0,891 ± 0,2	1,100 ± 0,2	1,102 ± 0,0
Gemlik (Canal)	1:1	1,336 ± 0,0	1,000 ± 0,2	0,752 ± 0,2
	1:4	0,972 ± 0,3	0,969 ± 0,0	0,857 ± 0,2
	1:16	1,008 ± 0,1	0,925 ± 0,1	0,877 ± 0,2
	1:128	1,047 ± 0,1	1,087 ± 0,1	1,113 ± 0,2
Gölcük	1:1	1,036 ± 0,0	0,972 ± 0,2	0,941 ± 0,2
	1:4	0,59 ± 0,3	0,921 ± 0,0	1,228 ± 0,4
	1:16	1,025 ± 0,1	0,928 ± 0,1	0,868 ± 0,2
	1:128	0,935 ± 0,0	1,125 ± 0,1	1,176 ± 0,1
Kadiköy	1:1	1,248 ± 0,1	1,060 ± 0,1	0,908 ± 0,0
	1:4	0,692 ± 0,3	0,919 ± 0,0	1,091 ± 0,4
	1:16	0,904 ± 0,0	0,979 ± 0,2	1,070 ± 0,2
	1:128	1,043 ± 0,1	1,070 ± 0,0	0,990 ± 0,0

		February 2008		
		405 nm (25 min)	620 nm (60-90 min)	
	Sample Dilution Rate	RF[1] AP Activity[4]	IF[2] β-Gal Activity[5]	CIF[3]
Gemlik	1:1	1,101 ± 0,1	1,161 ± 0,0	1,058 ± 0,1
	1:4	1,004 ± 0,0	1,040 ± 0,0	1,038 ± 0,1
	1:16	1,053 ± 0,0	1,017 ± 0,1	0,967 ± 0,0
	1:128	1,016 ± 0,1	0,993 ± 0,0	0,979 ± 0,0
Kartal	1:1	1,202 ± 0,0	1,145 ± 0,0	0,953 ± 0,0
	1:4	0,981 ± 0,0	1,020 ± 0,0	1,042 ± 0,1
	1:16	1,073 ± 0,0	1,030 ± 0,0	0,961 ± 0,0
	1:128	0,962 ± 0,1	0,942 ± 0,0	0,980 ± 0,0
Bakırköy	1:1	1,163 ± 0,0	1,152 ± 0,0	0,991 ± 0,1
	1:4	0,964 ± 0,0	0,988 ± 0,0	1,025 ± 0,0
	1:16	1,059 ± 0,0	0,985 ± 0,0	0,931 ± 0,0
	1:128	0,940 ± 0,0	0,935 ± 0,0	0,995 ± 0,0
Dilovası	1:1	1,144 ± 0,0	1,162 ± 0,0	1,017 ± 0,0
	1:4	1,027 ± 0,0	1,002 ± 0,0	0,976 ± 0,0
	1:16	1,160 ± 0,1	0,991 ± 0,0	0,856 ± 0,0
	1:128	1,003 ± 0,0	0,939 ± 0,1	0,936 ± 0,1
Mudanya	1:1	1,376 ± 0,3	1,145 ± 0,1	0,850 ± 0,1
	1:4	1,329 ± 0,4	1,663 ± 0,9	1,203 ± 0,3
	1:16	1,112 ± 0,1	1,021 ± 0,0	0,922 ± 0,1
	1:128	1,041 ± 0,1	0,966 ± 0,1	0,939 ± 0,2
Körfez	1:1	1,295 ± 0,2	1,078 ± 0,0	0,838 ± 0,1
	1:4	1,132 ± 0,0	1,058 ± 0,0	0,935 ± 0,0
	1:16	1,083 ± 0,0	1,048 ± 0,1	0,967 ± 0,0
	1:128	0,902 ± 0,0	0,970 ± 0,1	1,076 ± 0,1
Yalova	1:1	1,279 ± 0,0	1,095 ± 0,0	0,857 ± 0,0
	1:4	1,112 ± 0,1	1,056 ± 0,0	0,951 ± 0,0
	1:16	1,097 ± 0,0	1,034 ± 0,0	0,943 ± 0,0
	1:128	0,966 ± 0,1	0,930 ± 0,1	0,966 ± 0,1
Güzelyalı	1:1	1,237 ± 0,1	1,074 ± 0,1	0,871 ± 0,0
	1:4	1,056 ± 0,1	1,006 ± 0,0	0,955 ± 0,1
	1:16	1,184 ± 0,2	1,029 ± 0,0	0,881 ± 0,2
	1:128	1,111 ± 0,2	0,900 ± 0,2	0,830 ± 0,3

1 – Alkaline Phosphate Activity Standart Deviation 2-- β – Galactosidase Activity Standart Deviation, 3- Corrected Induction Factor Standart Deviation, 4 –AP Reduction Factor, 5-- Corrected Induction Factor

Table 3.2: AP ,β-Gal activity and Corrected Induction Factor of test samples which were taken on May 2008

		May 2008		
		405 nm (25 min)	620 nm (60-90 min)	
	Sample Dilution Rate	RF[1] AP Activity[2]	IF[2] β-Gal Activity[5]	CIF[3]
Yenikapi	1:1	0,891 ± 0,0	1,042 ± 0,0	1,170 ± 0,0
	1:4	0,900 ± 0,0	1,015 ± 0,0	1,127 ± 0,0
	1:16	0,973 ± 0,1	1,093 ± 0,1	1,135 ± 0,2
	1:128	0,968 ± 0,1	1,093 ± 0,1	1,143 ± 0,2
Eminönü	1:1	0,900 ± 0,0	1,040 ± 0,0	1,157 ± 0,1
	1:4	0,844 ± 0,1	1,080 ± 0,1	1,287 ± 0,2
	1:16	0,935 ± 0,1	1,028 ± 0,1	1,111 ± 0,2
	1:128	1,025 ± 0,1	1,102 ± 0,1	1,090 ± 0,2
Avcilar	1:1	0,845 ± 0,0	1,115 ± 0,0	1,320 ± 0,0
	1:4	0,814 ± 0,0	1,192 ± 0,0	1,466 ± 0,1
	1:16	0,909 ± 0,0	1,096 ± 0,0	1,206 ± 0,0
	1:128	0,949 ± 0,0	1,167 ± 0,1	1,230 ± 0,1
Florya	1:1	0,796 ± 0,1	1,065 ± 0,1	1,340 ± 0,0
	1:4	0,862 ± 0,1	1,167 ± 0,1	1,355 ± 0,0
	1:16	0,926 ± 0,1	1,073 ± 0,1	1,162 ± 0,0
	1:128	1,164 ± 0,2	1,326 ± 0,0	1,149 ± 0,2
Mimarsinan	1:1	0,949 ± 0,3	0,612 ± 0,3	0,718 ± 0,5
	1:4	0,933 ± 0,0	1,049 ± 0,0	1,126 ± 0,0
	1:16	0,968 ± 0,0	1,078 ± 0,1	1,116 ± 0,1
	1:128	0,961 ± 0,0	1,171 ± 0,1	1,222 ± 0,1
Gemlik (Canal)	1:1	0,971 ± 0,0	1,013 ± 0,1	1,042 ± 0,1
	1:4	0,862 ± 0,1	1,011 ± 0,0	1,176 ± 0,1
	1:16	1,010 ± 0,1	1,027 ± 0,0	1,020 ± 0,1
	1:128	0,880 ± 0,0	1,120 ± 0,1	1,273 ± 0,1
Gölcük	1:1	0,793 ± 0,0	1,072 ± 0,1	1,353 ± 0,1
	1:4	0,810 ± 0,0	1,000 ± 0,1	1,235 ± 0,0
	1:16	1,042 ± 0,1	1,132 ± 0,0	1,094 ± 0,1
	1:128	0,961 ± 0,0	1,128 ± 0,0	1,177 ± 0,1
Kadiköy	1:1	0,880 ± 0,0	1,080 ± 0,0	1,227 ± 0,0
	1:4	0,901 ± 0,1	1,140 ± 0,0	1,275 ± 0,1
	1:16	0,921 ± 0,0	0,982 ± 0,0	1,068 ± 0,1
	1:128	0,993 ± 0,1	1,082 ± 0,0	1,100 ± 0,2

		May 2008		
		405 nm (25 min)	620 nm (60-90 min)	
	Sample Dilution Rate	RF[1] AP Activity[4]	IF[2] β-Gal Activity[5]	CIF[3]
Gemlik	1:1	0,966 ± 0,0	1,163 ± 0,1	1,203 ± 0,1
	1:4	0,958 ± 0,1	0,966 ± 0,1	1,009 ± 0,0
	1:16	1,023 ± 0,1	1,003 ± 0,0	0,983 ± 0,1
	1:128	1,065 ± 0,1	0,855 ± 0,0	0,804 ± 0,0
Avşa	1:1	0,966 ± 0,0	1,141 ± 0,1	1,182 ± 0,0
	1:4	1,052 ± 0,0	1,009 ± 0,0	0,961 ± 0,1
	1:16	1,057 ± 0,1	0,956 ± 0,0	0,905 ± 0,0
	1:128	1,070 ± 0,1	0,848 ± 0,0	0,795 ± 0,1
Bakırköy	1:1	1,030 ± 0,1	1,216 ± 0,0	1,184 ± 0,1
	1:4	1,057 ± 0,1	0,996 ± 0,0	0,945 ± 0,1
	1:16	1,083 ± 0,0	0,986 ± 0,1	0,911 ± 0,1
	1:128	1,096 ± 0,0	0,906 ± 0,0	0,826 ± 0,0
Dilovası	1:1	0,975 ± 0,0	1,061 ± 0,1	1,087 ± 0,1
	1:4	1,035 ± 0,0	0,995 ± 0,0	0,961 ± 0,0
	1:16	1,135 ± 0,1	0,953 ± 0,0	0,840 ± 0,0
	1:128	1,081 ± 0,0	0,860 ± 0,1	0,796 ± 0,0
Mudanya	1:1	0,916 ± 0,1	1,006 ± 0,2	1,095 ± 0,1
	1:4	1,000 ± 0,0	1,004 ± 0,0	1,005 ± 0,0
	1:16	1,163 ± 0,1	0,956 ± 0,1	0,822 ± 0,0
	1:128	1,096 ± 0,0	0,854 ± 0,0	0,779 ± 0,0
Körfez	1:1	0,963 ± 0,0	1,087 ± 0,0	1,131 ± 0,1
	1:4	1,013 ± 0,0	1,036 ± 0,0	1,024 ± 0,0
	1:16	1,091 ± 0,0	1,033 ± 0,1	0,947 ± 0,1
	1:128	1,073 ± 0,1	0,934 ± 0,0	0,871 ± 0,0
Yalova	1:1	0,975 ± 0,0	1,058 ± 0,1	1,084 ± 0,0
	1:4	1,037 ± 0,0	0,951 ± 0,0	0,917 ± 0,0
	1:16	1,122 ± 0,0	0,961 ± 0,0	0,858 ± 0,0
	1:128	1,044 ± 0,1	0,875 ± 0,0	0,842 ± 0,1
Güzelyalı	1:1	0,983 ± 0,0	1,030 ± 0,0	1,048 ± 0,0
	1:4	1,037 ± 0,0	0,980 ± 0,1	0,946 ± 0,1
	1:16	1,083 ± 0,0	0,859 ± 0,0	0,794 ± 0,0
	1:128	1,169 ± 0,1	0,807 ± 0,0	0,693 ± 0,1

1 – Alkaline Phosphate Activity Standart Deviation 2-- β – Galactosidase Activity Standart Deviation, 3- Corrected Induction Factor Standart Deviation, 4 –AP Reduction Factor, 5-- Corrected Induction Factor

3.1.4 The Seasonal Comparision of Test Results

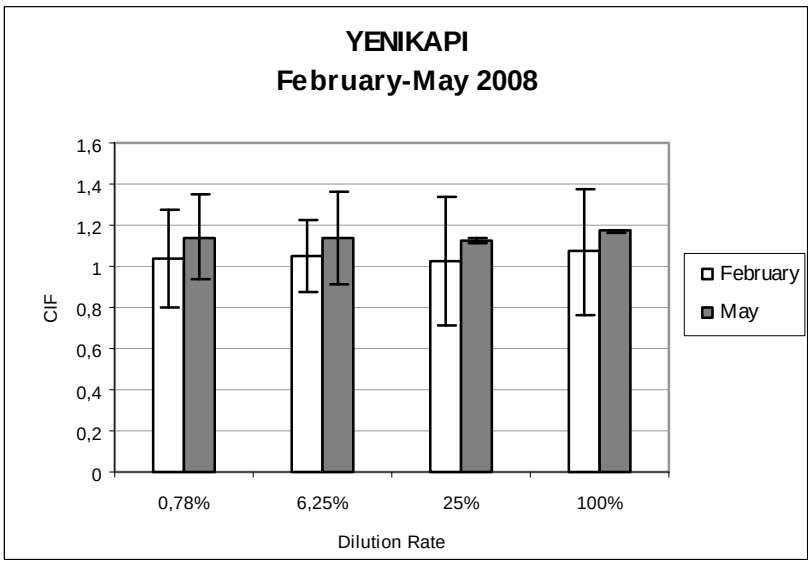


Figure 3.5. Yenikapı (February-May 2008)

Yenikapi samples were considered non-genotoxic for both seasons. But CIF result indicated that β-Gal synthesis increased for May 2008 samples (Figure 3.5).

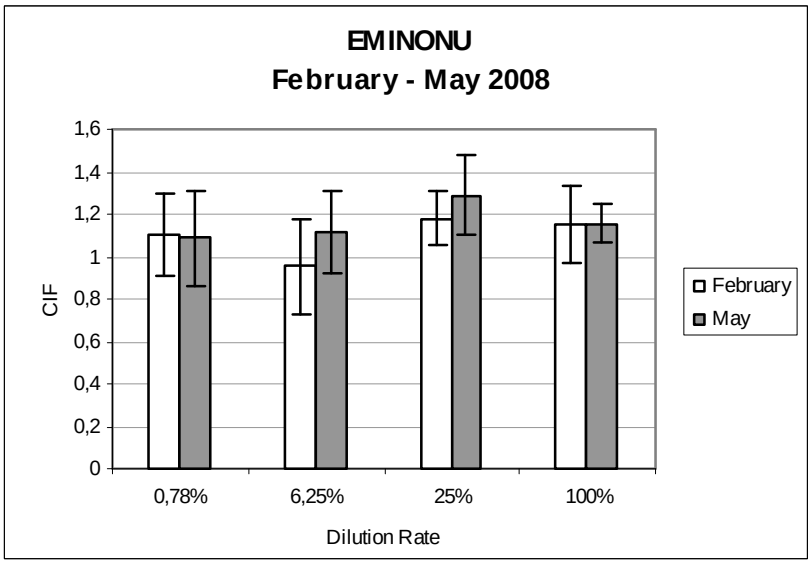


Figure 3.6. Eminönü (February-May 2008)

CIF value of Eminönü exceeded 1.2 for second dilution on May2008. Besides that CIF value was near 1.2 for second dilution (% 25) on February 2008 sample. This result can be indicated that on May 2008, β-Gal synthesis increased in Eminönü (Figure 3.6).

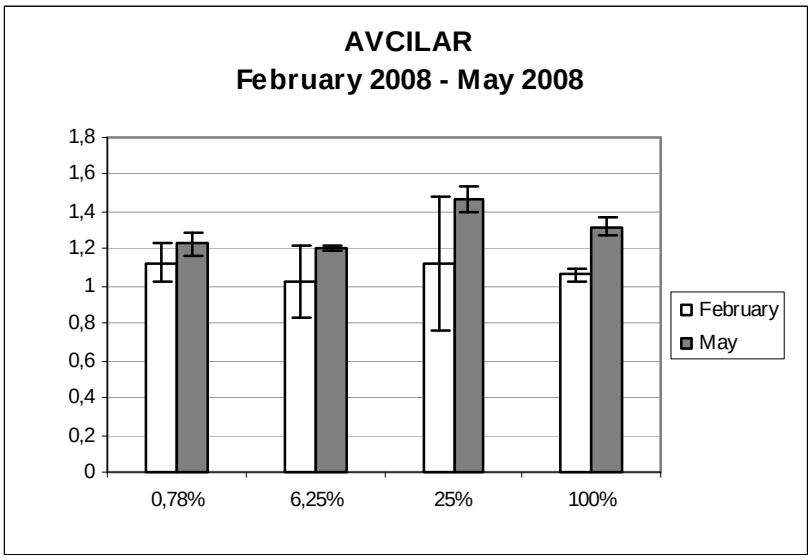


Figure 3.7. Avcılar (February-May 2008)

CIF value of Avcılar sample exceeded 1.2 both seasons for this station. The variability of CIF value, according to different dilution range proved that β-Gal synthesized because of DNA-damaging agents. It was considered genotoxic (Figure 3.7).

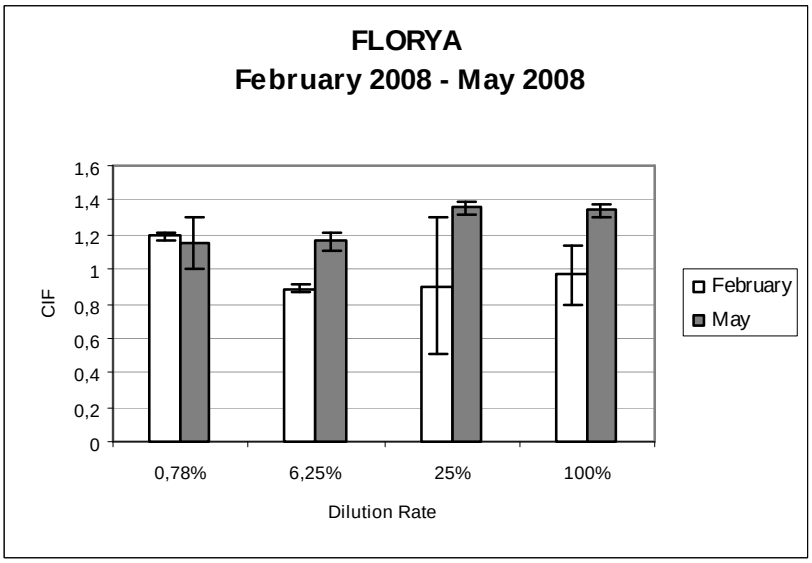


Figure 3.8. Florya (February-May 2008)

CIF value of Florya sample exceeded 1.2 on May 2008. This result showed that this station β-Gal synthesized and the synthesis increased May 2008 because of DNA-damaging agents. (Figure 3.8).

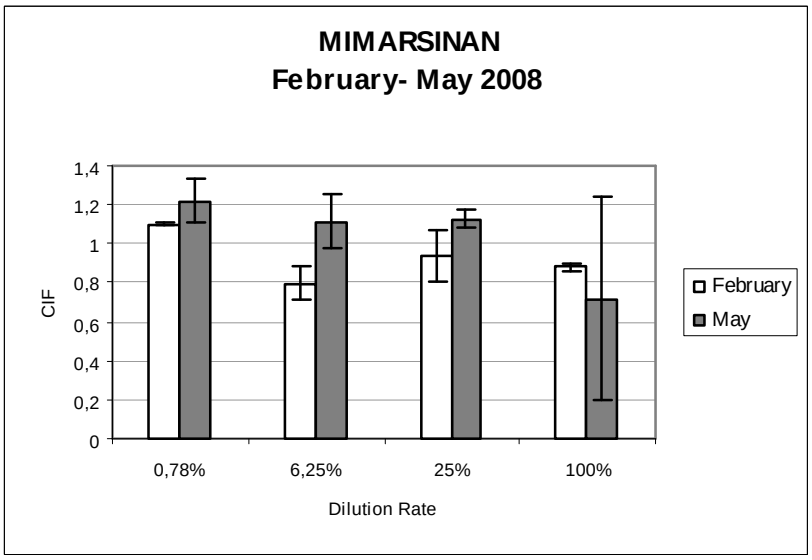


Figure 3.9. Mimarsinan (February-May 2008)

In spite of CIF value of Mimarsinan samples exceeded 1.2 value only fourth dilution rate (%0.78) on May2008, the other dilutions of samples on both February 2008 and May 2008 were below 1.2. According to this result that β-Gal synthesized and the synthesis increased on May2008 because of DNA-damaging agents (Figure 3.9).

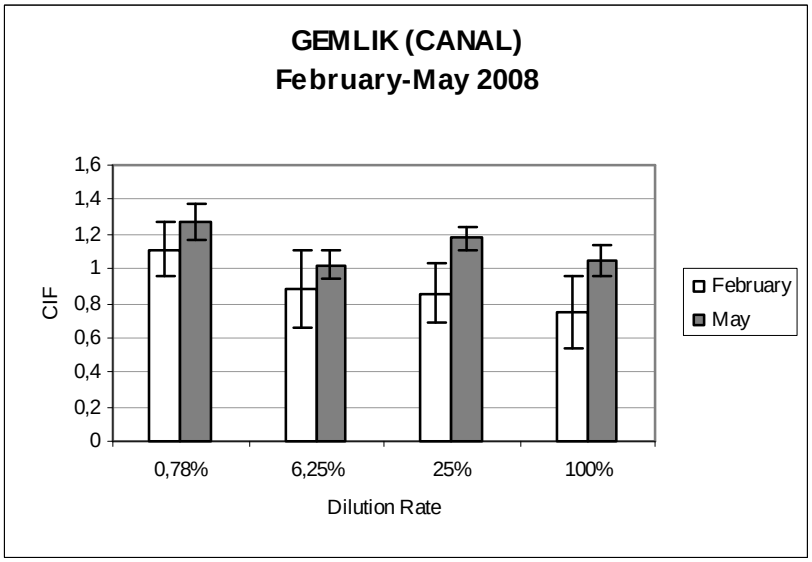


Figure 3.10. Gemlik (Canal) (February-May 2008)

In spite of CIF value of Gemlik (Canal) sample exceeded 1.2 for fourth dilution rate (%0.78) on May 2008, the other concentration of May 2008 sample and dilutions of February 2008 sample were below 1.2 (Figure 3.10).

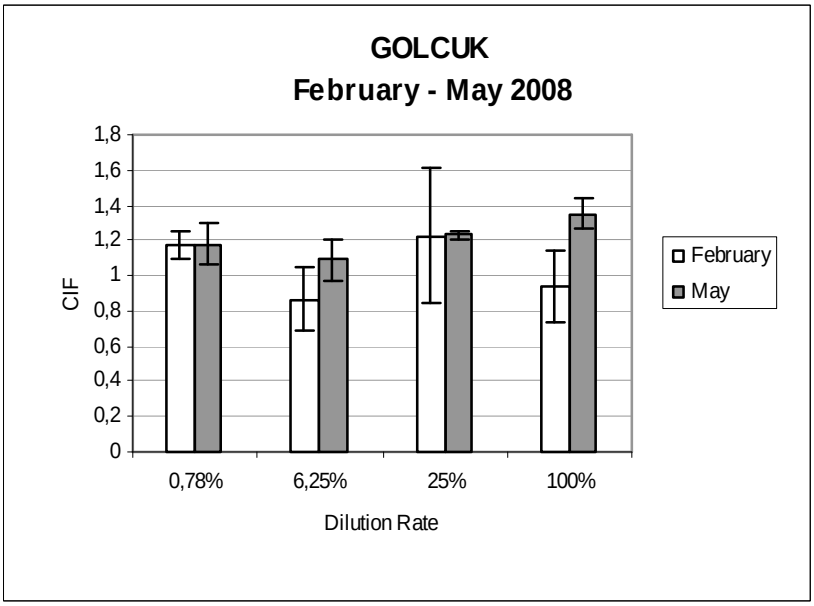


Figure 3.11. Gölcük (February-May 2008)

In Gölcük sample, CIF value exceeded 1.2 for only second concentration (25 %) of sample for February 2008 sample. But CIF value exceeded 1.2 for the undilution and second dilution rate of sample for May2008 sample (Figure 3.11).

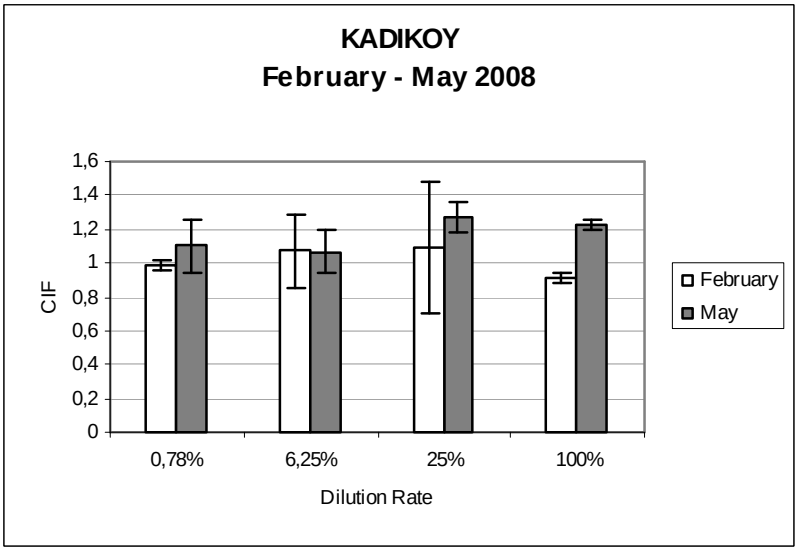


Figure 3.12. Kadıköy (February-May 2008)

In Kadıköy sample, the undilution and second dilution rate of sample, CIF value exceeded 1.2 for May 2008 sample. CIF value of sample for each dilutions was below 1.2 on February2008. According to this result, β-Gal synthesis increased for May 2008 sample for this station (Figure 3.12).

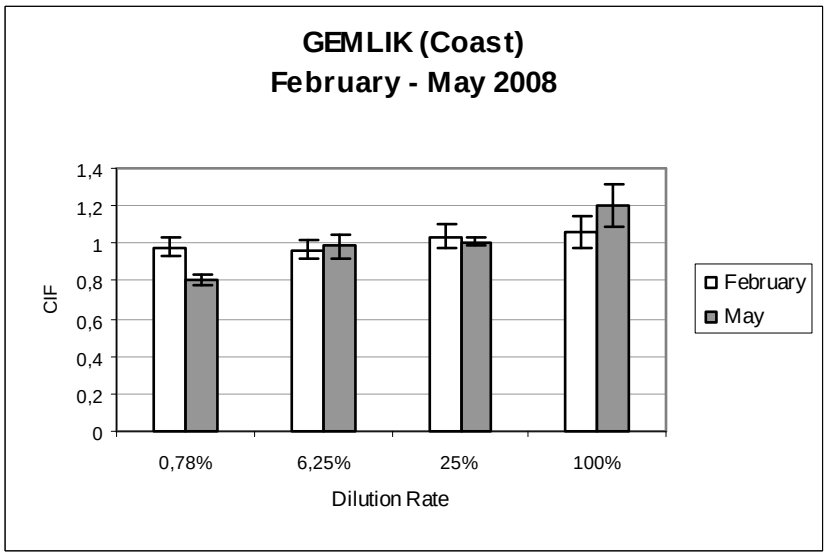


Figure 3.13. Gemlik (Coast) (February-May2008)

In Gemlik (Coast) sample, CIF value was 1.2 on May2008 in the undiluted concentration. β-Gal synthesis increased on May 2008 for this station (Figure 3.13).

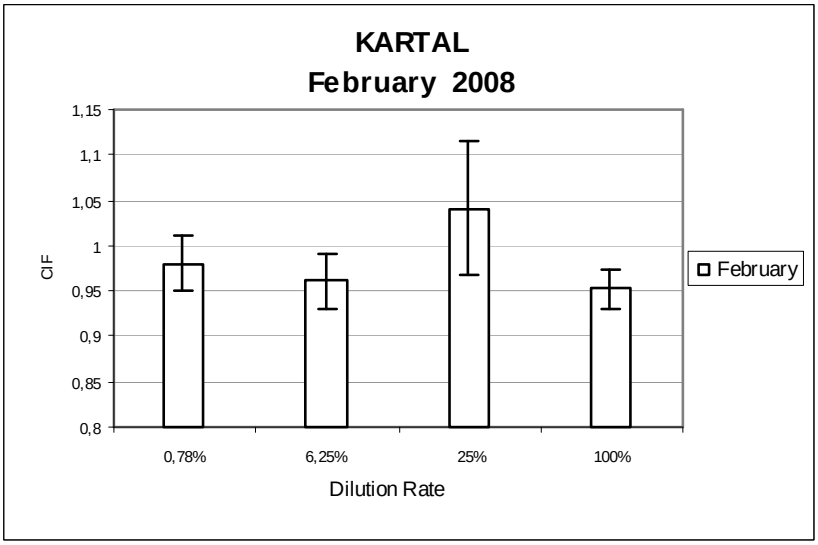


Figure 3.14. Kartal (February 2008)

In Kartal sample was tested for only one season. According to result, CIF values of the all concentrations were below 1.2 so that β-Gal synthesis was low range on February 2008 sample (Figure 3.14).

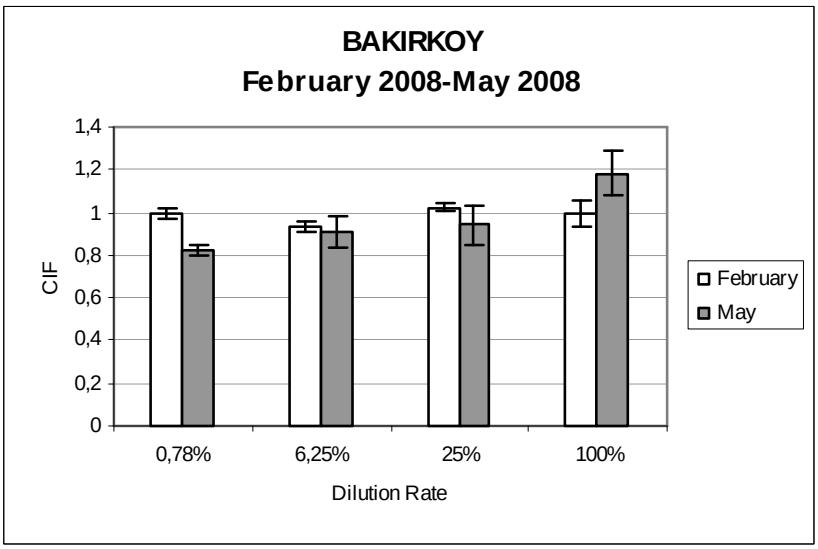


Figure 3.15. Bakırköy (February-May 2008)

In Bakırköy sample, CIF value was near 1.2 for February and May 2008 samples. But β-Gal synthesis was low range (Figure 3.15).

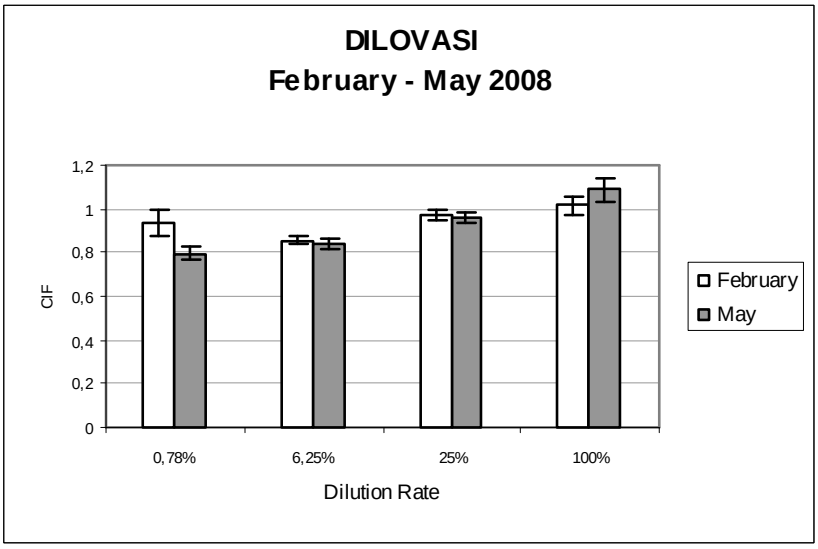


Figure 3.16. Dilovası (February-May 2008)

In Dilovası sample, CIF value was near 1.2 for February and May 2008 samples. But β-Gal synthesis was low range (Figure 3.16).

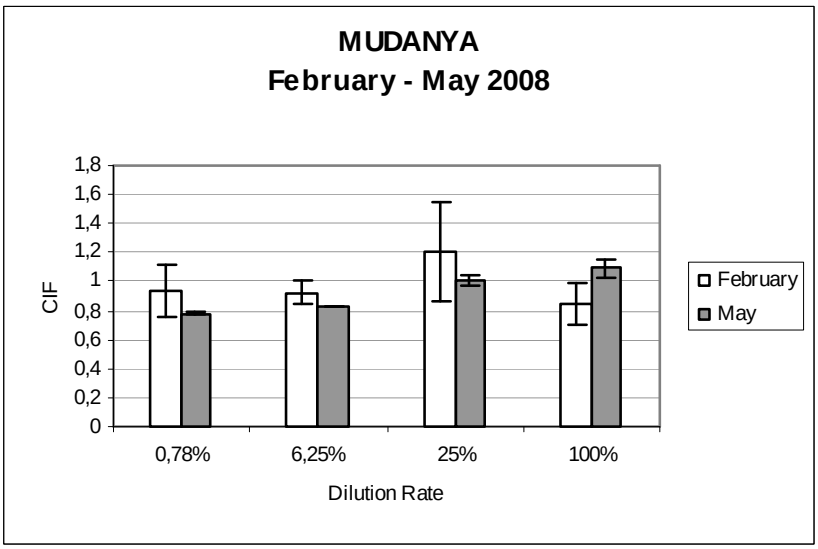


Figure 3.17. Mudanya (February-May 2008)

In Mudanya sample, the second concentration of sample, CIF value was 1.2 on February 2008. CIF value of sample for each dilutions was below 1.2 on May2008. For this stations, β-Gal synthesis of February sample is higher than May 2008 sample (Figure 3.17).

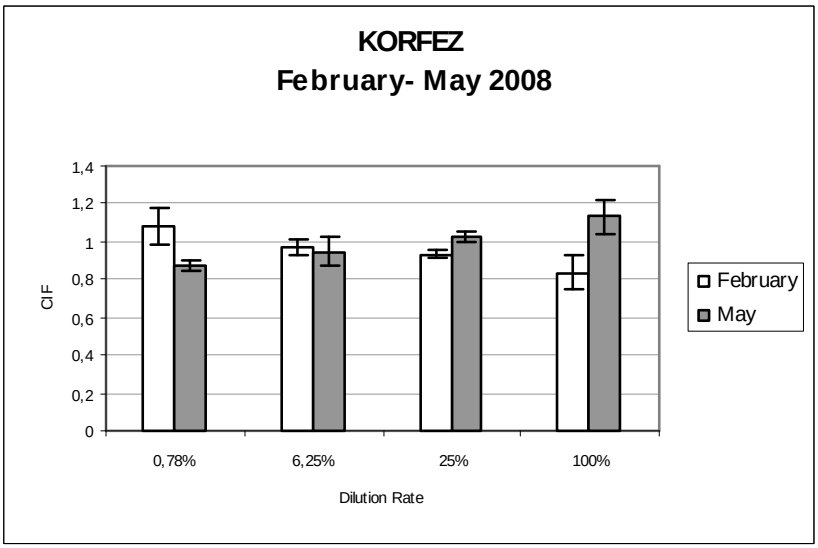


Figure 3.18. Körfez (February-May 2008)

In Körfez sample, CIF values of the all concentrations were below 1.2. β-Gal synthesis was low range on both February 2008 and May 2008 (Figure 3.18).

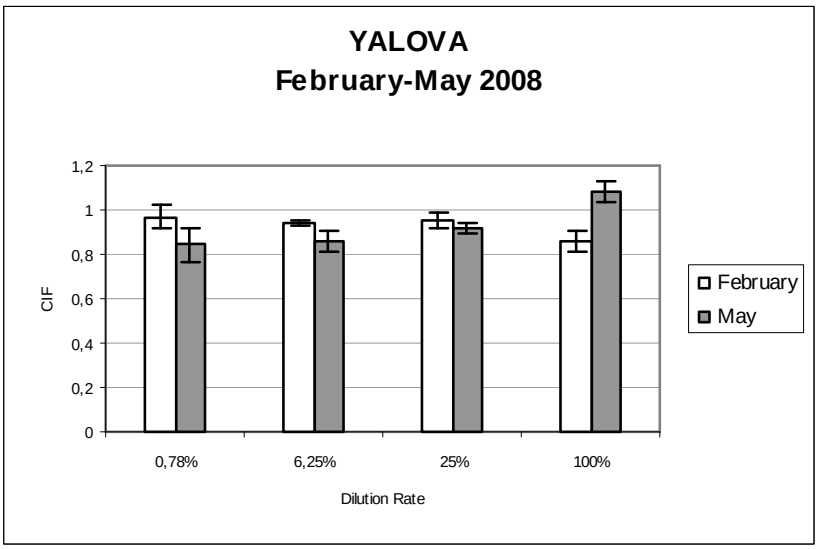


Figure 3.19. Yalova (February-May 2008)

In Yalova sample, CIF value of the all concentration were below 1.2. β-Gal synthesis was low range on both February 2008 and May 2008 (Figure 3.19).

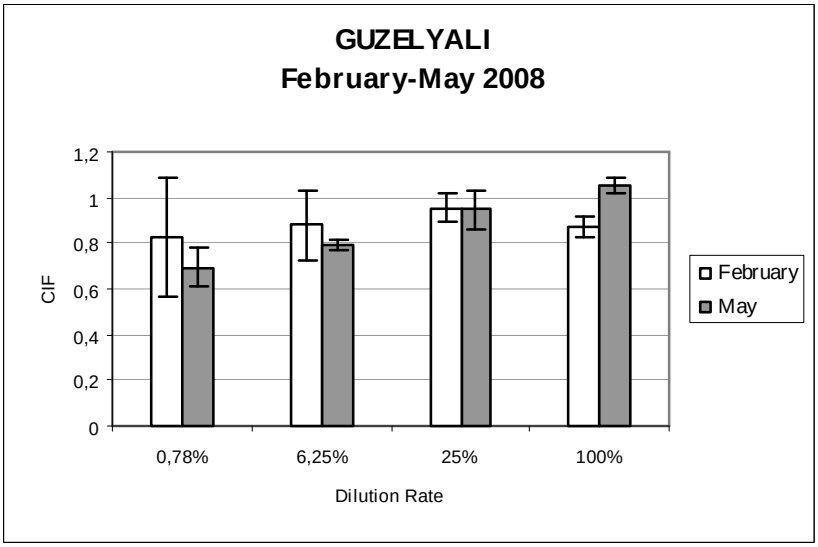


Figure 3.20. Güzelyalı (February-May 2008)

In Güzelyalı sample,the CIF values of the all concentrations were below 1.2. β-Gal synthesis was low range on both February 2008 and May 2008 (Figure 3.20).

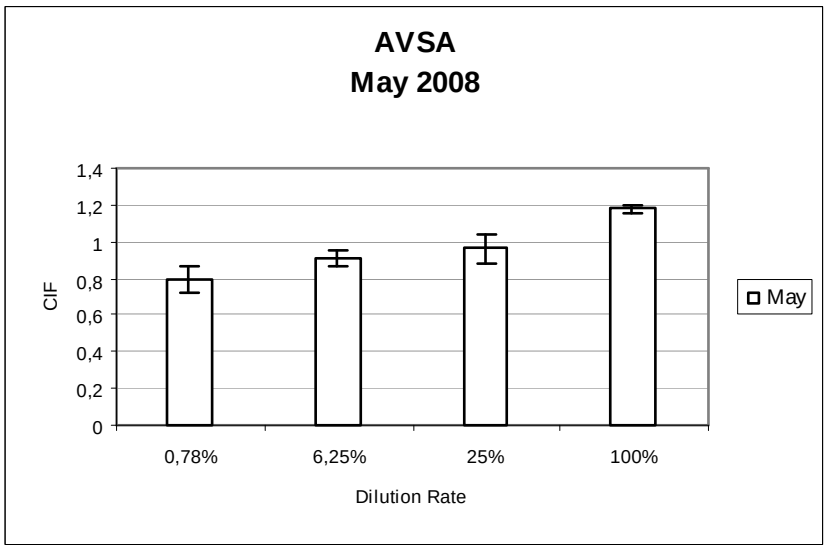


Figure 3.21. Avşa (May 2008)

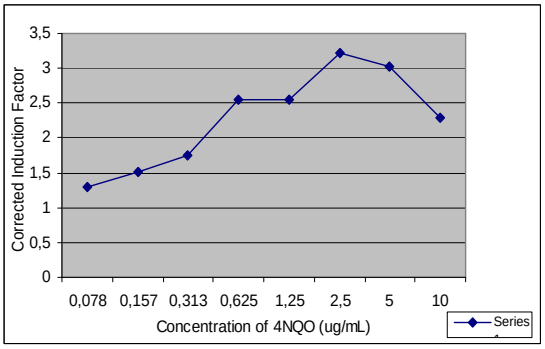
In Avşa sample was tested for only one season.According to result, CIF values of the all concentrations were below 1.2 so that β-Gal synthesis was low range on May 2008 (Figure 3.21).

3.2. UmuC Test Results

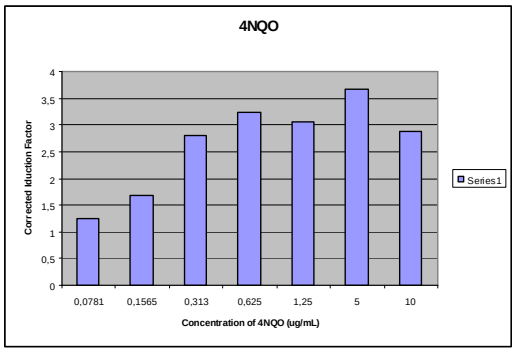
Primary DNA damage in the umuC test expressed with Induction Factor (IF) that was found maximum 1.33±0.1(for Yalova sample) in Marmara Sea water. *Salmonella typhimurium* TA1535/pSK1002 strain used to detect DNA damage. No toxic effect was detected with umuC test. Toxic and genotoxic effects were quantified and evaluated in the same way as in the case of the SOS Chromotest (Bartos T.,Skarek M., Pavel C.,Kosubova P., Holoubek I.).

3.2.1 Bacterial Incubation

OD of becteria was measured at 600 nm like that SOS Chromotest.But Alkaline Phosphate Activity was measured 420 nm and β-Gal was measured 550 nm with ELISA READER (Oda *et al.*,1985,1995).



(a)



(b)

Figure 3.22. (a) Concentration – CIF Line curve **(b)** Concentration – CIF Column Graph

4NQO was used to positive control for umuC test . Figure 3.22 above mentioned that Dose – CIF curve for *Salmonella typhimurium* TA1535/pSK1002 strain.

3.2.2 The Results of February 2008 samples

Yenikapı

In Yenikapı sample, CIF value exceed 1.2 for second (25 %) dilution of umuC test. According to this result, this sample was considered genotoxic.

Avcılar

In Avcılar sample, CIF value exceed 1.2 for fourth (0.78 %) dilution of umuC test. According to this result, this sample was considered genotoxic.

Florya

In Florya sample, CIF value exceed 1.2 for fourth (0.78 %) dilution of umuC test. According to this result, this sample was considered genotoxic.

Yalova

In Yalova sample, CIF value exceed 1.2 for fourth (0.78 %) dilution of umuC test. According to this result, this sample was considered genotoxic.

The samples of Eminönü , Mimarsinan , Gemlik, Gölcük, Kadıköy, Gemlik, Kartal, Bakırköy, Dilovası, Mudanya, Körfez, Güzelyalı stations were not genotoxic according to IF and CIF results. β-Gal activities were below 1.20 for samples of February 2008.

Table 3.3: AP ,β-Gal activity and Corrected Induction Factor of test samples which were taken on February 2008

		February 2008		
		405 nm (25 min)	620 nm (60-90 min)	
	Sample Dilution Rate	AP Activity[1]	β-Gal Activity[2]	CIF[3]
Yenikapi	1:1	0,998 ± 0,4	0,897 ± 0,0	0,952 ± 0,3
	1:4	0,821 ± 0,2	0,981 ± 0,1	1,249 ± 0,4
	1:16	0,939 ± 0,3	0,731 ± 0,1	0,823 ± 0,3
	1:128	0,889 ± 0,3	0,878 ± 0,2	1,061 ± 0,5
Eminönü	1:1	0,951 ± 0,4	0,953 ± 0,1	1,115 ± 0,6
	1:4	0,902 ± 0,3	0,863 ± 0,1	0,989 ± 0,2
	1:16	0,992 ± 0,3	0,856 ± 0,0	0,900 ± 0,2
	1:128	1,022 ± 0,4	1,089 ± 0,1	1,149 ± 0,4
Avcilar	1:1	0,932 ± 0,2	1,010 ± 0,0	1,125 ± 0,3
	1:4	0,922 ± 0,4	0,863 ± 0,2	1,052 ± 0,6
	1:16	1,004 ± 0,3	1,068 ± 0,3	1,185 ± 0,7
	1:128	1,022 ± 0,2	1,178 ± 0,4	1,204 ± 0,6
Florya	1:1	0,941 ± 0,2	1,076 ± 0,1	1,190 ± 0,4
	1:4	0,904 ± 0,3	1,020 ± 0,1	1,184 ± 0,3
	1:16	1,020 ± 0,4	0,904 ± 0,0	0,942 ± 0,3
	1:128	1,026 ± 0,3	1,211 ± 0,2	1,218 ± 0,2
Mimarsinan	1:1	0,839 ± 0,2	0,849 ± 0,1	1,058 ± 0,3
	1:4	0,772 ± 0,2	0,824 ± 0,1	1,091 ± 0,2
	1:16	1,005 ± 0,3	0,798 ± 0,1	0,811 ± 0,1
	1:128	1,047 ± 0,3	0,967 ± 0,0	0,957 ± 0,3
Gemlik (Canal)	1:1	0,974 ± 0,3	0,887 ± 0,1	0,969 ± 0,4
	1:4	0,903 ± 0,4	0,843 ± 0,1	0,983 ± 0,2
	1:16	1,045 ± 0,5	0,923 ± 0,0	0,983 ± 0,4
	1:128	1,038 ± 0,4	1,122 ± 0,0	1,145 ± 0,4
Gölcük	1:1	0,904 ± 0,3	0,934 ± 0,0	1,114 ± 0,4
	1:4	0,878 ± 0,3	0,932 ± 0,0	1,133 ± 0,4
	1:16	1,024 ± 0,4	0,981 ± 0,1	1,009 ± 0,3
	1:128	1,072 ± 0,4	1,100 ± 0,0	1,095 ± 0,4
Kadiköy	1:1	0,799 ± 0,2	0,821 ± 0,0	1,050 ± 0,2
	1:4	0,935 ± 0,3	0,912 ± 0,0	1,048 ± 0,4
	1:16	1,013 ± 0,3	0,981 ± 0,1	1,025 ± 0,4
	1:128	1,093 ± 0,2	1,056 ± 0,1	0,999 ± 0,3

		February 2008		
		405 nm (25 min)	620 nm (60-90 min)	
	Sample Dilution Rate	AP Activity[1]	β-Gal Activity[2]	CIF[3]
Gemlik	1:1	0,956 ± 0,0	1,049 ± 0,0	1,099 ± 0,0
	1:4	0,905 ± 0,0	1,020 ± 0,0	1,127 ± 0,0
	1:16	0,960 ± 0,0	0,998 ± 0,1	1,044 ± 0,2
	1:128	0,937 ± 0,1	1,042 ± 0,1	1,121 ± 0,2
Kartal	1:1	0,909 ± 0,0	0,991 ± 0,0	1,090 ± 0,0
	1:4	0,964 ± 0,1	1,116 ± 0,0	1,160 ± 0,0
	1:16	0,967 ± 0,1	1,019 ± 0,1	1,052 ± 0,1
	1:128	1,033 ± 0,1	0,969 ± 0,1	0,950 ± 0,2
Bakırköy	1:1	0,928 ± 0,0	1,088 ± 0,0	1,173 ± 0,0
	1:4	0,951 ± 0,1	0,981 ± 0,0	1,037 ± 0,1
	1:16	0,949 ± 0,1	1,100 ± 0,1	1,157 ± 0,1
	1:128	1,006 ± 0,1	0,932 ± 0,1	0,936 ± 0,2
Dilovası	1:1	0,903 ± 0,0	0,991 ± 0,0	1,099 ± 0,0
	1:4	0,961 ± 0,1	1,058 ± 0,0	1,107 ± 0,1
	1:16	0,942 ± 0,0	0,978 ± 0,1	1,038 ± 0,1
	1:128	0,982 ± 0,1	0,841 ± 0,1	0,871 ± 0,2
Mudanya	1:1	0,984 ± 0,1	0,991 ± 0,0	1,010 ± 0,1
	1:4	0,933 ± 0,1	0,808 ± 0,0	0,873 ± 0,1
	1:16	1,011 ± 0,1	0,957 ± 0,1	0,946 ± 0,1
	1:128	1,092 ± 0,5	0,622 ± 0,1	0,639 ± 0,3
Körfez	1:1	1,001 ± 0,1	0,991 ± 0,0	0,993 ± 0,1
	1:4	1,007 ± 0,2	0,905 ± 0,0	0,906 ± 0,1
	1:16	1,012 ± 0,0	1,039 ± 0,1	1,026 ± 0,1
	1:128	1,094 ± 0,1	0,914 ± 0,1	0,845 ± 0,2
Yalova	1:1	0,971 ± 0,1	0,932 ± 0,0	0,965 ± 0,1
	1:4	0,988 ± 0,0	1,020 ± 0,0	1,032 ± 0,0
	1:16	1,014 ± 0,0	1,019 ± 0,1	1,003 ± 0,1
	1:128	1,030 ± 0,1	1,371 ± 0,1	1,330 ± 0,1
Güzelyalı	1:1	0,927 ± 0,1	1,010 ± 0,0	1,098 ± 0,1
	1:4	0,974 ± 0,2	0,943 ± 0,0	0,980 ± 0,1
	1:16	0,922 ± 0,0	1,019 ± 0,1	1,105 ± 0,1
	1:128	1,127 ± 0,3	0,951 ± 0,1	0,878 ± 0,0

1 –Alkaline Phosphate Activity , 2 - β – Galactosidase Activity ,3- Corrected Induction Factor

3.2.3 The Comparision of SOS Chromotest Result and Umu-C Test Result for February Samples

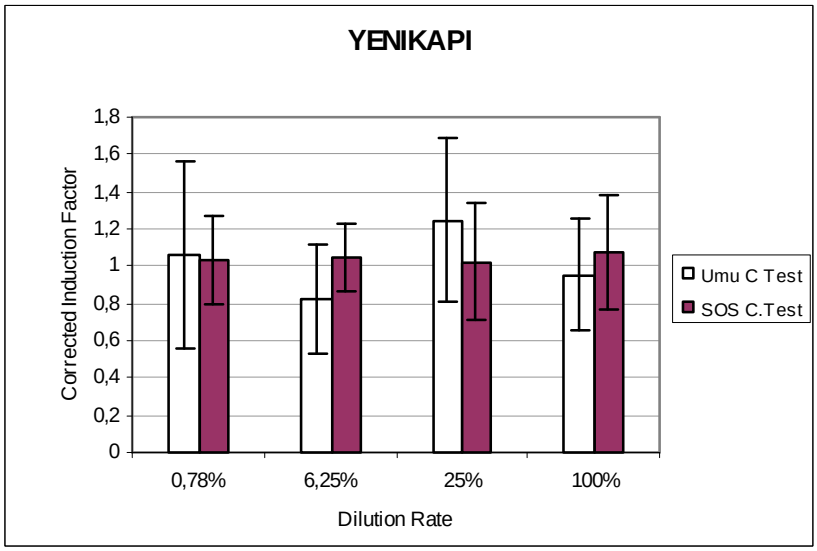


Figure 3.23. Yenikapı

In Yenikapı sample, CIF value exceed 1.2 for second dilution of umuC test. But according to SOS Chromotest CIF value was below the 1.2 for same sample (Figure 3.23).

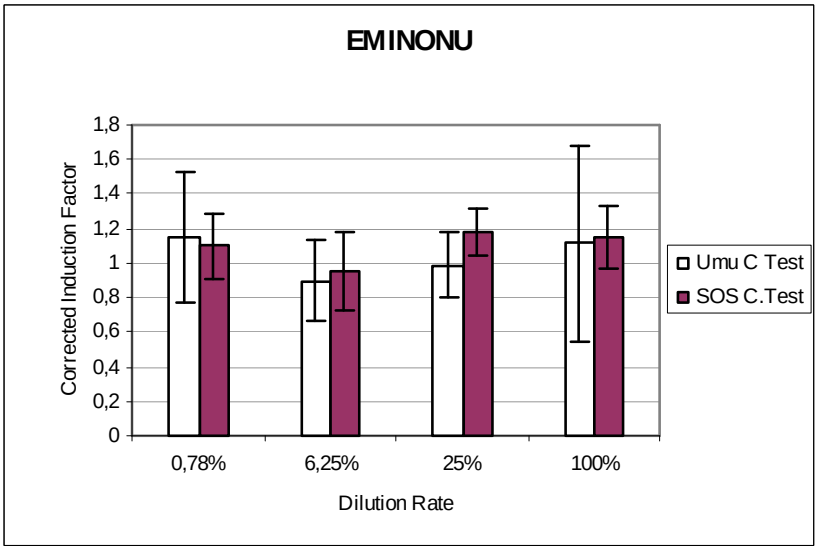


Figure 3.24. Eminönü

In Eminönü sample, the all sample CIF value was below 1.2 for each dilution rate. β-Gal synthesis was low range in both umuC test and SOS Chromotest (Figure 3.24).

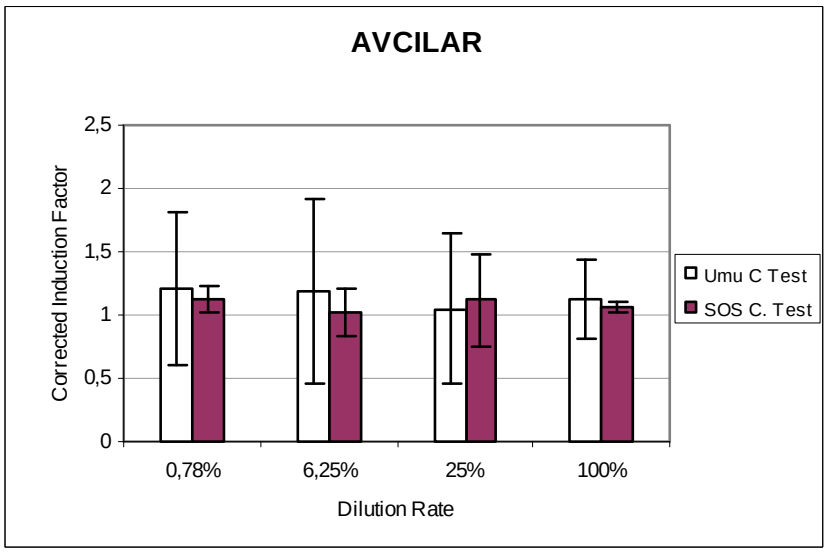


Figure 3.25. Avcılar

In Avcılar sample; umuC test detected high range β-Gal synthesis for fourth dilution which CIF value 1.2 exceeded. But according to SOS Chromotest CIF value was below the 1.2 for same sample (Figure 3.25).

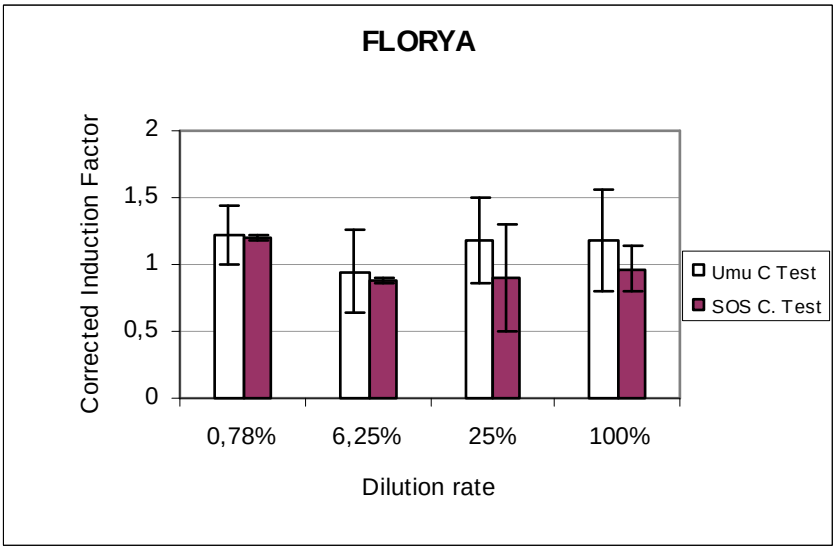


Figure 3.26. Florya

In Florya sample; umuC test detected high range β-Gal synthesis for first and fourth dilution which CIF value 1.2 exceeded.Besides that in second dilution CIF value was 1.18±0.3 for umuC test. But according to SOS Chromotest CIF value was 1.2 for only fourth dilution (Figure 3.26).

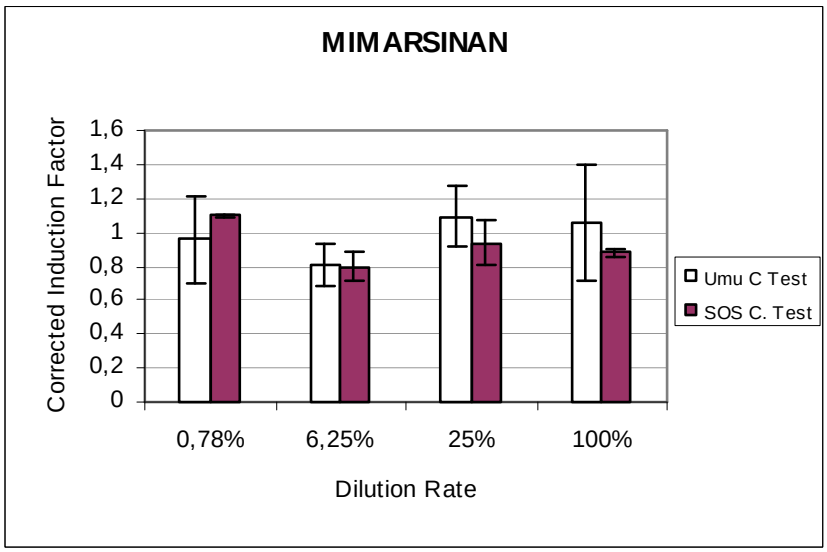


Figure 3.27. Mimarsinan

In Mimarsinan sample, CIF values of the all concentrations were below 1.2. β-Gal synthesis was low range in both umuC test and SOS Chromotest (Figure 3.27).

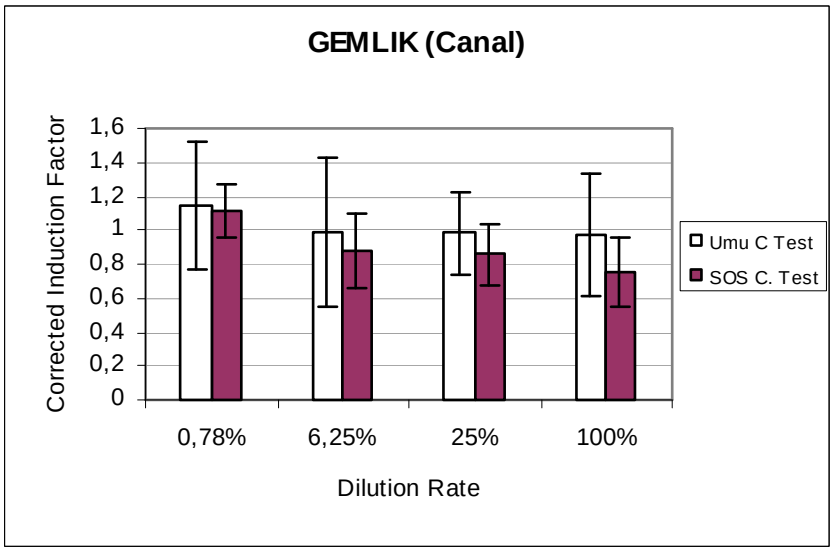


Figure 3.28. Gemlik (Canal)

In Gemlik sample, CIF values of the all concentrations were below 1.2. β-Gal synthesis was low range in both umuC test and SOS Chromotest (Figure 3.28).

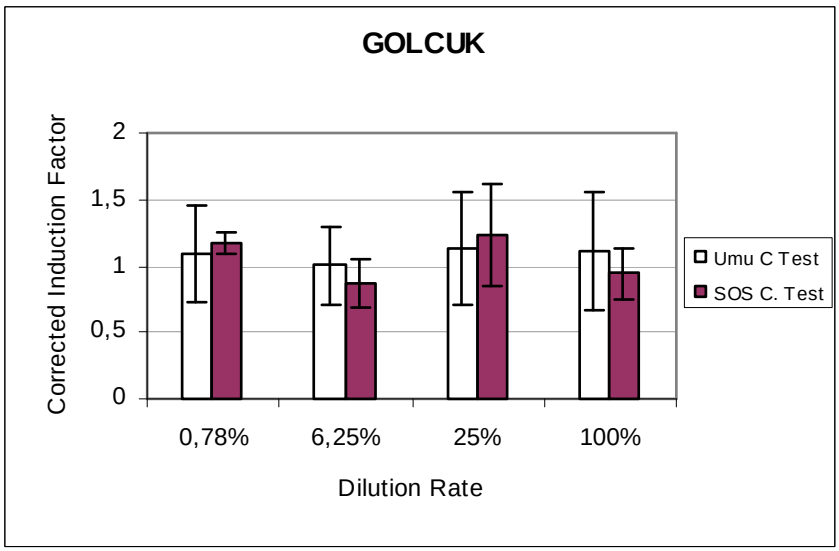


Figure 3.29. Gölcük

In Gölcük sample, according to umuC CIF values of the all concentrations were below 1.2 .But in second dilution, CIF value exceeded 1.2 according to SOS Chromotest (Figure 3.29).

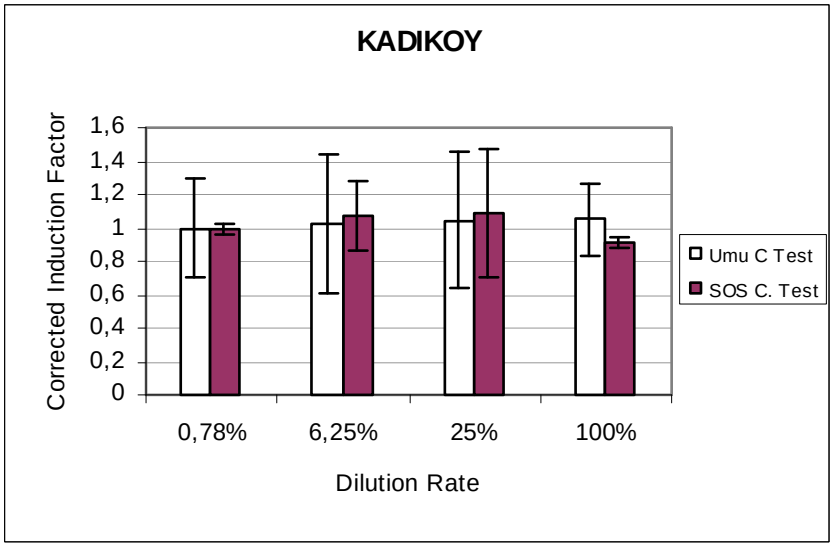


Figure 3.30. Kadıköy

In Kadıköy sample, CIF values of the all concentrations were below 1.2. β-Gal synthesis was low range in both umuC test and SOS Chromotest (Figure 3.30).

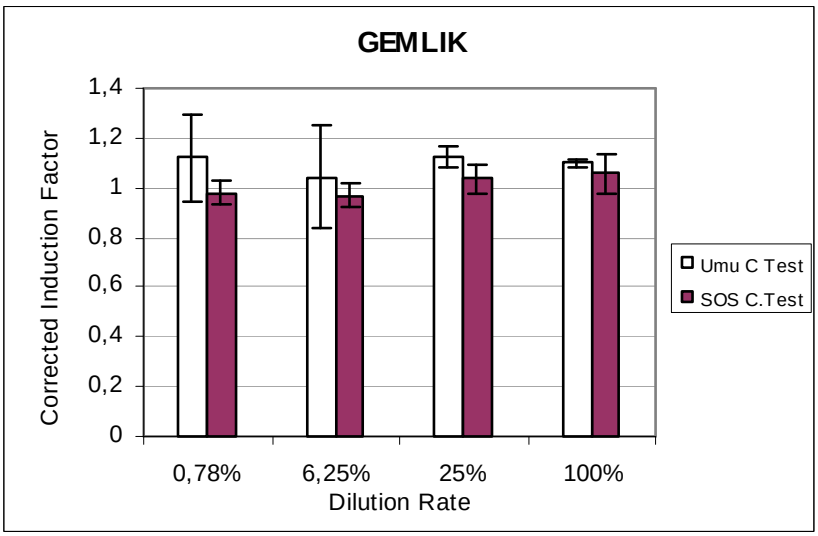


Figure 3.31. Gemlik

In Gemlik sample, CIF values of the all concentrations were below 1.2. β-Gal synthesis was low range in both umuC test and SOS Chromotest. According to result, β-Gal synthesis was high range for umuC test (Figure 3.31).

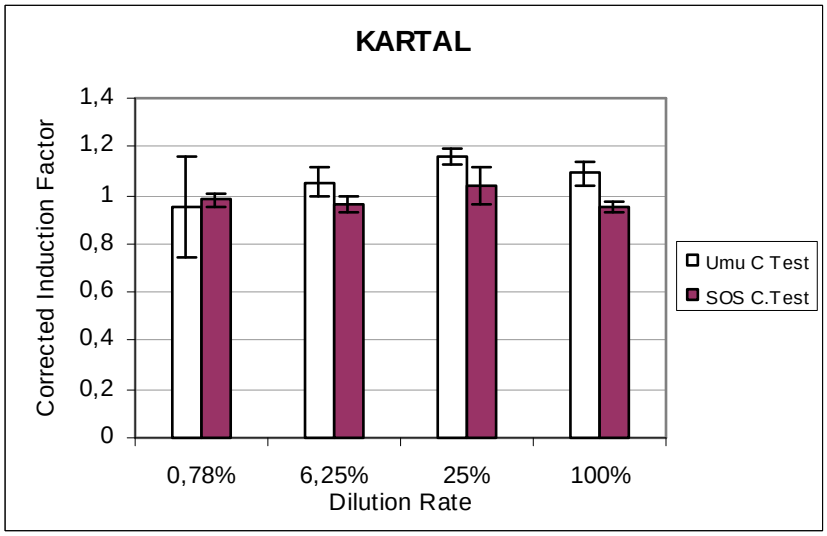


Figure 3.32. Kartal

In Kartal sample, CIF values of the all concentrations were below 1.2. β-Gal synthesis was low range in both umuC test and SOS Chromotest. According to result, β-Gal synthesis was high range for umuC test (Figure 3.32).

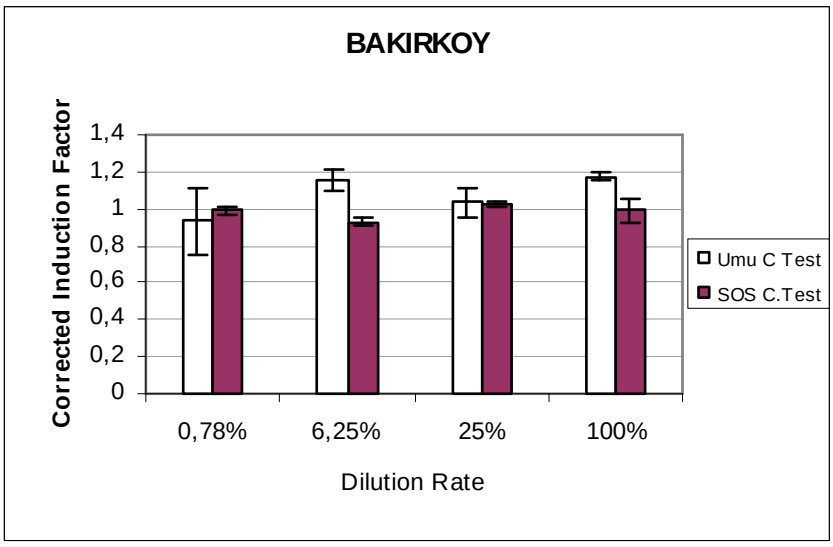


Figure 3.33. Bakırköy

In Bakırköy sample, CIF values of the all concentrations were below 1.2. β-Gal synthesis was low range in both umuC test and SOS Chromotest. According to result, β-Gal synthesis was high range for umuC test (Figure 3.33).

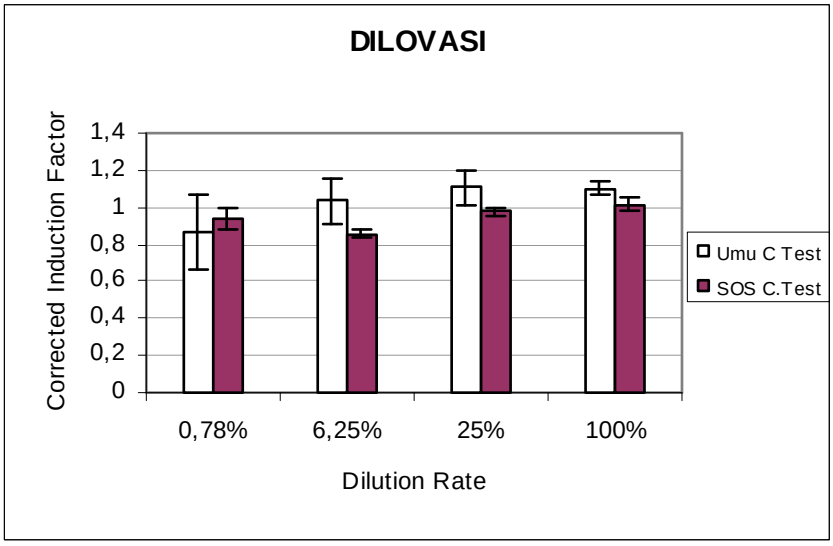


Figure 3.34. Dilovası

In Dilovası sample, CIF values of the all concentrations were below 1.2. β-Gal synthesis was low range in both umuC test and SOS Chromotest (Figure 3.34).

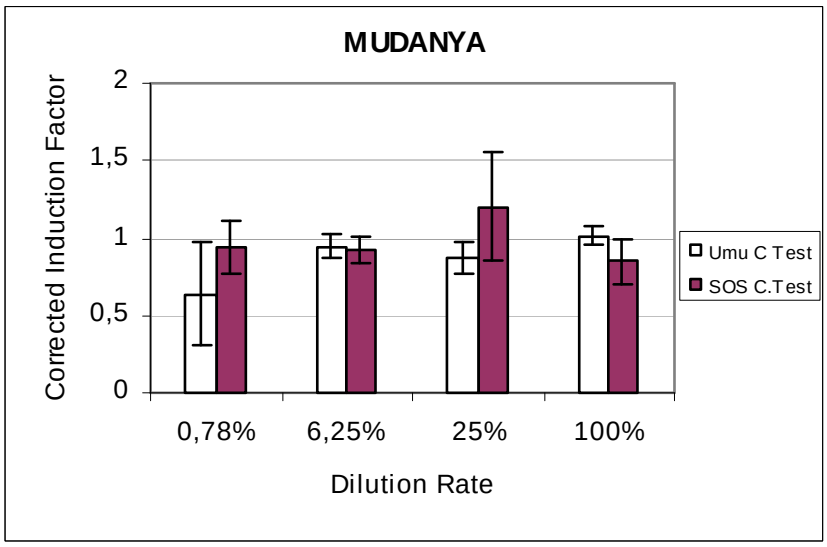


Figure 3.35. Mudanya

In Mudanya sample, CIF values of the all concentrations were below 1.2. β-Gal synthesis was low range in both umuC test and SOS Chromotest (Figure 3.35).

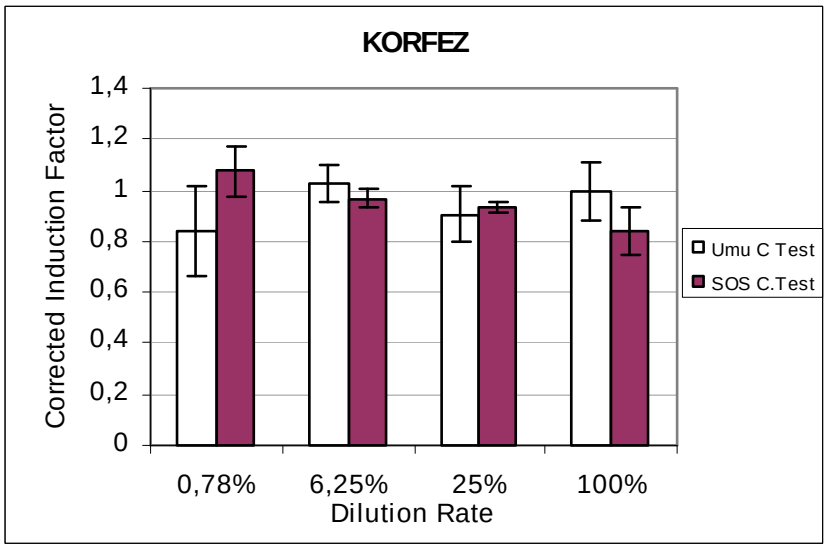


Figure 3.36. Körfez

In Körfez sample, the CIF values of the all concentrations were below 1.2. β-Gal synthesis was low range in both umuC test and SOS Chromotest (Figure 3.36).

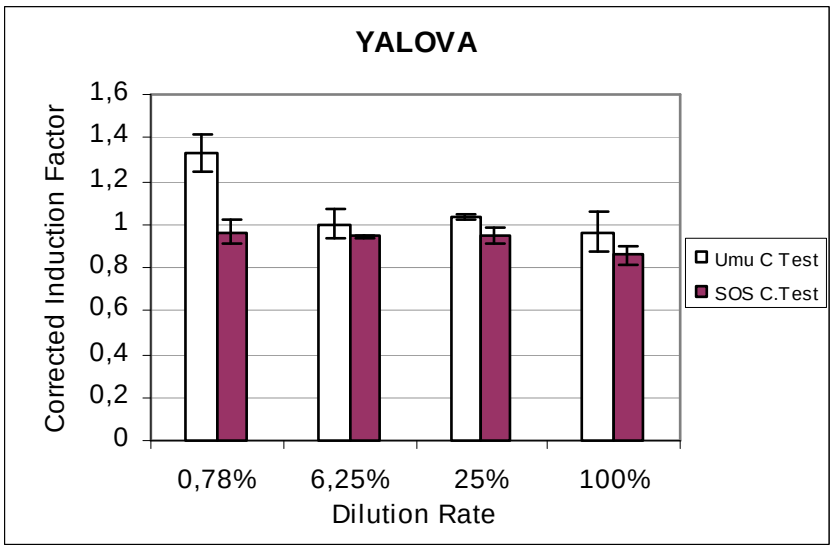


Figure 3.37. Yalova

In Yalova sample, CIF value exceeded 1.2 for fourth dilution (0.78 %).The other dilutions β-Gal synthesis was low range in both umuC test and SOS Chromotest (Figure 3.37).

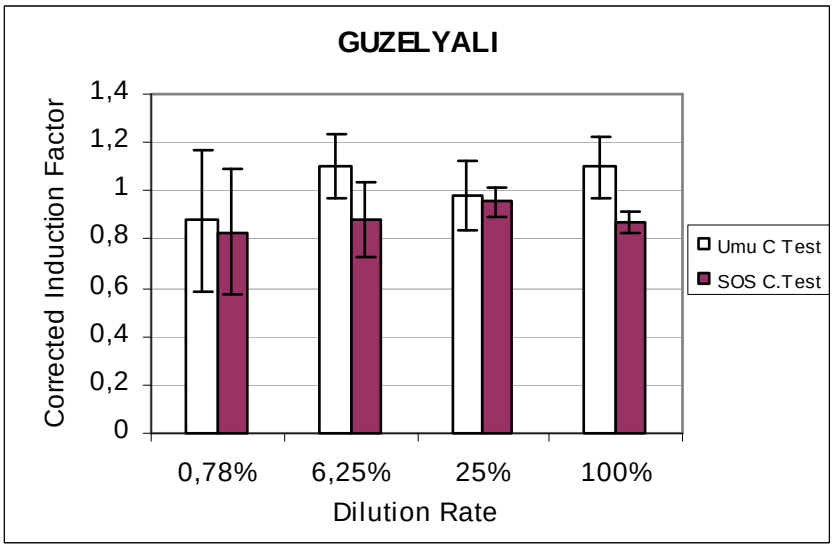


Figure 3.38. Güzelyalı

In Güzelyalı sample, CIF values of the all concentrations were below 1.2. β-Gal synthesis was low range in both umuC test and SOS Chromotest (Figure 3.38).

The result of the in vitro mutagenity tests are summarized in Table 3.4.

Table 3.4 : The all results of SOS Chromotest and umuC test.

	Stations	SOS Chromotest		umuC test
		February 2008	May 2008	February 2008
1	Güzelyalı	-	-	-
2	Mudanya	+	-	-
3	Gemlik	-	+	-
4	Gemlik (Canal)	-	+	-
5	Gölcük	+	+	-
6	Yalova	-	-	+
7	Dilovası	-	-	-
8	İzmit (Bay)	-	-	-
9	Kartal*	-		-
10	Mimarsinan	-	+	-
11	Avcılar	-	+	+
12	Florya	+	+	+
13	Bakırköy	-	-	-
14	Yenikapi	-	-	+
15	Eminönü	-	+	-
16	Kadiköy	-	+	-
17	Avşa*		-	-

Table: (+)Genotoxicity detected in one more concentration ; (-) Genotoxicity non detected * This stations were tested only one season

CHAPTER 4

DISCUSSION AND CONCLUSION

This study evaluated the in vitro genotoxic activity in sea water. The SOS Chromotest and umuC test with *E.coli* and *Salmonella typhimurium* TA1535/pSK1002 showed weak mutagenity in sea water samples. The formation of compaunds with mutagenic activity is usually dependent on various water parameters (Zani C. et al., 2005). The SOS Chromotest and The umuC test exhibited a higher sensitivity against the genotoxic compounds which present in sea water. In the SOS Chromotest and umuC test, cytotoxicity were not found in any samples test results the probably because of in fact that *E.coli* and *Salmonella typhimurium* TA1535/pSK1002 are more resistant to toxicity because of cell wall which genetic map modified to detect DNA-damaging agents.

This study showed that different sensitivity of E.coli and *Salmonella typhimurium* TA1535/pSK1002 same sample because of the end-points of DNA-damage. Besides that for sea water concentration of sample was decreased with 10% DMSO, β-Gal synthesis increased for some stations probably due to sea water characteristics and inhibition effect of 10% DMSO which was react with unknown DNA-damage agents.

The comparing of February 2008 test and May 2008 test, it was clear that genotoxic activity increased on May 2008. The SOS Chromotest result of February 2008 samples indicated that 3 stations were genotoxic. But May 2008 samples indicated that 8 stations were genotoxic (Table 3.4). The reasons of these results may be explained as;

- The increasing of temperature on May2008
- The seasonal changes in pH of sea water

- The increasing of effluent water which include unknown chemical substances from Industrial Plants.

This paper reports the first data from study investigating genotoxicity in Marmara Sea. It was shown that the different types of pollutans released to Marmara Sea. These pollutants have genotoxic effects for microorganisms. The results presented in this study emphasized that genotoxic studies should be increased in Marmara Sea because of various reasons previously mentioned.

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