

Determination of Genotoxic Pollution of Ayamama and Haramidere Streams with SOS-Chromotest Method

Ayamama ve Haramidere derelerindeki Genotoksik kirliliğin SOS-Chromotest metodu ile belirlenmesi

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ÖZET

Endüstrilerin ve şehirlerin nüfuslarının artmasıyla birlikte su ekosistemlerinde kirlilik fazlalaşmıştır. Daha önceleri bu kirlilik doğal yaşam tarafından giderilirken sanayi tesislerinin artmasıyla bu kirlilik doğa tarafından yok edilemez derecede boyutlara ulaşmıştır. Artan kirlilik canlı DNA'larında hasara neden olabilmektedir. Bu çalışmada İstanbul'da bulunan Ayamama ve Haramidere nehirlerinden kış ve bahar dönemlerinde örnekler alınarak, SOS Chromotest E-coli PQ37 zinciri kullanılmasıyla bu numulere genotoksik aktivite var mı varsa hangi derecede olduğu gözlemlenmeye çalışılmıştır.

Anahtar kelimeler:Dere,Mutajenite,Genotoksik, SOS chromotest,Ayamama, Haramidere

ABSTRACT

Development of industries and over population creates pollution in aquatic life. In this study genotoxic and mutagenic effects of samples from Ayamama and Haramidere streams which were collected at winter and spring. The scope of this subject is to understand and measure the carcinogenic and mutagenic activities of pollution in these streams. Method of this study can be expressed as taking the sample, preserve them and analyze it with SOS Chromotest. Escherichia coli strain PQ37 was used in this study. 4 samples from Ayamama Stream and 4 samples from Haramidere Stream was taken for research. Result of the study can be expressed as there are any genotoxic activities or not.

Key words:Stream,Genotoxicity ,SOS chromotest,Ayamama,Haramidere,E.Coli PQ37

1. INTRODUCTION

Today's industrial activities and technological improvements made many changes in citizens social life and increase the profit of companies. But one thing is forgotten by us, this is pollution and the harmony between human life and nature. When this harmony is go bad the life areas of animal and plants are start to corrupt. This corruption made changes is DNA chains. These corruptions also affect the future generations animals and plants. Results of these corruptions and pollution can be carcinogenic[6] and also there could be many other different effects which are very difficult to observe. Results can be differ from types of industries and pollution types.[9]

1.1 DNA

Deoxyribonucleic acid (DNA) is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms. The main role of DNA molecules is the long-term storage of information. DNA is often compared to a set of blueprints, since it contains the instructions needed to construct other components of cells, such as proteins and RNA molecules. The DNA segments that carry the genetic information are called genes, but other DNA sequences have structural purposes, or are involved in regulating the use of genetic information.

Chemically, DNA is a long polymer of simple units called nucleotides, with a backbone made of sugars and phosphate groups joined by ester bonds. Attached to each sugar is one of four types of molecules called bases. It is the sequence of these four bases along the backbone that encodes information. This information is read using the genetic code, which specifies the sequence of the amino acids within proteins. The code is read by copying stretches of DNA into the related nucleic acid RNA, in a process called transcription. Most of these RNA molecules are used to synthesize proteins, but others are used directly in structures such as ribosomes and spliceosomes.

Cell division is essential for an organism to grow, but when a cell divides it must replicate the DNA in its genome so that the two daughter cells have the same genetic information as their parent. The double-stranded structure of DNA provides a simple mechanism for DNA replication. Here, the two strands are separated and then each strand's complementary DNA sequence is recreated by an enzyme called DNA polymerase. This enzyme makes the complementary base pairing, and bonding it onto the original strand. Different mechanisms are used to copy the antiparallel strands of the double helix. In this way, the base on the old strand dictates which base appears on the new strand, and the cell ends up with a perfect copy of its DNA.

Within cells, DNA is organized into structures called chromosomes. These chromosomes are duplicated before cells divide, in a process called DNA replication. Eucaryotic organisms such as animals, plants, and fungi store their DNA inside the cell nucleus, while in prokaryotes such as bacteria it is found in the cell's cytoplasm. Within the chromosomes, chromatin proteins such as histones compact and organize DNA, which helps control its interactions with other proteins and thereby control which genes are transcribed.[1]

1.2 Mutations

Mutations are changes to the base pair sequence of the genetic material of an organism. Mutations can be caused by copying errors in the genetic material during the cell division, by exposure to ultraviolet or ionizing radiation, chemical mutagens, or viruses, or can occur deliberately under cellular control during processes such as hypermutation. In multicellular organisms, mutations can be subdivided into germ line mutations, which can be passed on to descendants, and somatic mutations, which cannot be transmitted to descendants in animals. Plants sometimes can transmit somatic mutations to their descendants asexually or sexually.

1.3 Genotoxicity

Genotoxicity describes a deleterious action on a cell genetic material affecting its integrity. Genotoxic substances are known to be potentially mutagenic and carcinogenic, specifically those capable of causing genetic mutation and of contributing to the development of tumors. This includes both certain chemical compounds and certain types of radiation.

1.4 SOS-Chromotest

The SOS-Chromotest kit utilizes the cell's own mechanisms for the detection of genotoxicity. All living cells have developed a sensitive system for the detection of lesions in their genetic material so that a complex enzymatic system—the SOS repair system—can be activated to repair the damage. Once a lesion has been detected, a 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 [2].

The advantages and some properties of using SOS chromotest can be expressed as follows;

-SOS Chromotest has an easy processing procedure and designed for his purpose. We don't need special laboratory equipment to do this analysis.

-SOS Chromotest is widely using analyze test to investigate industrial and municipal waste sourced pollution.

-In just a few hours, the kit provides a clear, completely objective measurement of the genotoxicity of a sample by a simple visual appreciation of the colour obtained or by spectrophotometry using a microplate reader.

-The SOS-Chromotest kit has been in use since 1982, has been evaluated by hundreds of compounds and cited over 150 publications.

-It can be completed within 24 hours including the revival of the bacteria.

-The test detects any primary DNA damage caused by genotoxins, and can be used for various kinds of aqueous samples. Therefore the test is particularly suitable for testing of environmental samples.

-The SOS Chromotest kit can be used to detect genotoxic activity in raw materials, cosmetics, pharmaceuticals, foodstuffs, water, soils, sediments.

- The SOS Chromotest allows the examination of multiple samples all processed at once using only one cell tester strain. As was determined by testing more than 100 genotoxic compounds.[2]

1.5 Information about Ayamama and Haramidere Streams

The aim of this study to observe the effects of pollution in these streams looking at genotoxicity perspective. SOS chromotest is the testing procedure of this study. Relation of pollution type and mutagenic results are also going to be discuss.[8] As previously mention, mutagenicity on plants, animals and microorganisms affect also human health. So it is very important to observe the mutagenicity of the streams and water sources in cities especially like İstanbul, Ankara and other metropolitan cities. Because these cities has many different type of industrial activities that creates very high pollution in aquatic systems. Previous studies about mutagenicity of İstanbul streams are examined before this study is began.

Pollution of water resources and aquatic environment are serious and a growing problem. Regardless of its origin, pollution tends to find its way into the aquatic environment. Today population increase rates are very high in developing countries like Turkey. Metropolitan cities have more work sources and job opportunities so immigration from provinces to these cities increase the population of them. This high population form high pollution also. In this study we try to mention the mutagenicity happens because of the pollution of these two streams of İstanbul.[7] These streams are Ayamama and Haramidere. Ayamama streams is flowing in Bakırköy and Küçükçekmece districts. The approximate length of ayamama stream is (with the branch of Sefaköy, Yenibosna and Kaynarca) 50 km. Population living near to Ayamama can be divided as two districts, Bakırköy and Küçükçekmece. As the result of population census at 2000; population of Bakırköy is 203.498 and population of Küçükçekmece is 589,139. [3] Ayamama stream announce it's name with the flood at 1994 that affects the media buildings at İkitelli. Municipalities are try to reform the stream many times but floods happen several times more. Ayamama stream is polluting by domestic and industrial waste water sources.

Haramidere stream is one of the another stream that we are going to mention in İstanbul. There are many industrial activities like textile, construction, petroleum refinery etc are stated near this stream. Pollutants which are deliver to these streams can be observed by studying the samples by SOS Chromotest.

Both these streams are stated the places where the industrial activities are very high. Both of these streams are spilling to Marmara Sea so pollution of these streams means also pollution of Marmara Sea.

2. MATERIALS AND METHODS

2.1 Description of sampling sites



Figure 2.1 Sample 1 taken from circled area in Haramidere Stream[11] [4]



Figure 2.2 Sample 2 taken from circled area in Haramidere Stream[11]



Figure 2.3 Sample 3 taken from circled area in Ayamama Stream[11]

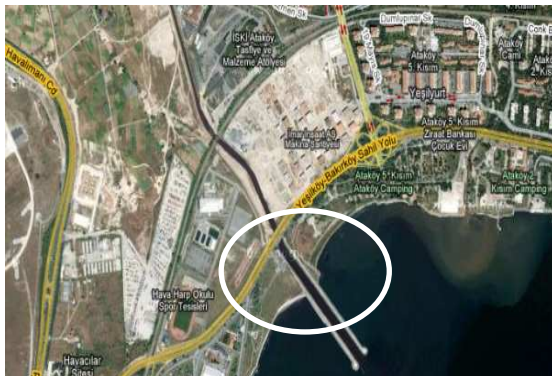


Figure 2.4 Sample 4 taken from circled area in Ayamama Stream[11]

2.2 Sample equipments

As seen at the map the samples are going to take from important sources of pollution because of industrial and domestic wastewaters.

Polyethylene bottles was used for both Ayamama and Haramidere streams material sampling. pH of these samples are decreased to 2 by using H_2SO_4 and samples transferred in glass bottles and stayed until the analysis began.

2.3 SOS-Chromotest Work Procedure

We are going to perform the activities in the presented order after taking the sample.

Morning of the test or night prior to the test [2]

1. Re-suspend dried bacteria in bottle B with medium in bottle A.
2. Incubate at $37^\circ C$ for 4-5 hours or for 16-18 hours (3.1). Write the date and time.

Day of the test:

1. Check bacteria grown for existence of turbidity. If turbid – proceed.
2. Dissolve samples and dilute; make serial dilutions of controls.
3. Dispense $10 \mu L$ aliquots of sample and control dilutions into appropriate wells of the assay micro-plate .
4. Prepare bacterial suspensions for testing by dilution of bacterial suspension grown overnight with growth medium from bottle B to obtain $10 mL$ of bacterial suspension of 0.05 .
5. Dispense $100 \mu L$ of the bacterial suspension into the appropriate wells of the micro-plate .
6. Incubate at $37^\circ C$ for two hours. Write the time.
7. Proceed with colour development by either one of (a) for machine reading or (b) for visual analysis:
 - a. Rehydrate the dry alkaline phosphatase chromogen in bottle H with the liquid β -galactosidase chromogen in bottle F. Add $100 \mu L$ of the mix to all wells. Incubate at $37^\circ C$ for 60 to 90 minutes until green colour develops. Stop with $50 \mu L$ of reagent I (optional). Measure corrected for reagent blank at $615 nm$ for genotoxic activity and at 405 for viability.
 - b. Add $100 \mu L$ of the liquid β -galactosidase chromogen in bottle F to all the wells. Incubate at $37^\circ C$ for 60 to 90 minutes until blue colour develops. If no colour develops in the test samples, perform viability check by adding $50 \mu L$ /well of the rehydrated alkaline phosphatase chromogen, made from rehydrating bottle H with diluent G. Incubate 30 to 60 minutes at $37^\circ C$, until yellow colour develops in the controls and stop with $50 \mu L$ of stop solution I.
8. Analyze results, visually or with a plate reader, following the guidelines.[2]

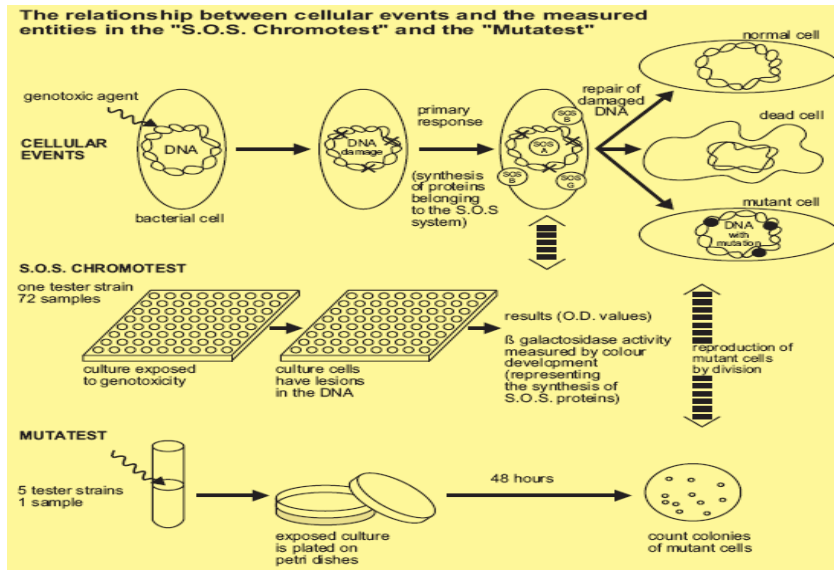


Figure 2.6 The cell incidents and genotoxic measurements[2]

2.4 Analysis of SOS-Chromotest Results

Check the blue colour density appearing in the wells of your test materials and continuously compare with the diluent-only wells. Start checking from the highest concentration of the test material to the lowest. High concentrations may not induce any positive response due to acutely toxic concentrations in which the cells are killed outright. As the material is diluted out, toxicity is reduced and a positive reaction (deep blue colour) may then appear indicating chronic genotoxicity. The colour density will finally be gradually reduced as concentrations are diluted below genotoxic levels depending on the range of dilutions tested.

If no positive blue colour is obtained, check the yellow colour density appearing in the wells of your test materials, following the addition of the alkaline phosphatase and subsequent incubation, and continuously compare with the diluent-only wells. Since the diluent-only wells also contain bacteria but no test material they should show a good yellow colour development. The yellow colour is a measure of bacteria viability by the alkaline phosphatase reaction. If the yellow colour appearing in the wells of the test material is similar to the background (diluent-only wells), the material was not toxic and not genotoxic. If the diluent control is yellow and the test material wells are not this means that the test material was toxic to the SOS-CHROMOTEST bacteria. Try higher dilutions (lower concentrations) of test material so that inherent acute toxicity will not be expressed.[2]

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