© by PSP Volume 30– No. 06/2021 pages 6102-6108 Fresenius Environmental Bulletin

EVALUATION OF CYTOTOXIC AND GENOTOXIC EFFECTS OF HOSAP STREAM GUMUSCAY BIGA CANAKKALE ON *ALLIUM CEPA* L.

Nergis Kaya*

Canakkale Onsekiz Mart University, Biga Vocational School, Food Processing Department, Food Technology Program, Biga, 17200, Canakkale, Turkey

ABSTRACT

Since the Hosap Stream is located near the urban settlement area, water pollution might occur. In this study, the cytotoxic and genotoxic effect of Hosap Stream, which is also used in irrigation of agricultural lands, was investigated at the root tip cells of Allium cepa. The EC50 value in A. cepa roots was determined by exposure to different concentrations of the water sample taken from Hosap Stream. Then, the root tips were treated with EC50 values for 24 h, 48 h and 72 h. The root tips were prepared according to the method of preparing the mitotic preparation and examined under a microscope. It was determined that the water sample taken from Hosap Stream had a cytotoxic and genotoxic effect on A. cepa root tip cells. It was evaluated that the chromosomal aberration index increased statistically significantly depending on the dose in all application periods except for the 24 h application. It was determined that the mitotix index and chromosomal aberration index were higher than the control groups. The lowest mitotix index (2,217±0,625) and the highest chromosomal aberration index (53,153±1,642) was observed with EC50X2 dose at 72h. The prevalent chromosome aberration observed in A. cepa root tip cells were -mitosis, laggard chromosome, polar shifting in anaphase and telophase, equatorial plate shifting, chromosome fragment. According to the results of this study, it can be said that Hosap tea has cytotoxic and genotoxic effects on other living organism.

KEYWORDS:
Allium cepa L., cytotoxicity, genotoxicity, Hosap Stream, water pollution

INTRODUCTION

Rivers, streams, creeks located near urban region are regarded the main water source of the population in the urbanized region. Industrial, domestic and agricultural waste water is discharged into these rivers, streams and creeks causing environmental degradation. Urban, agricultural and industrial wastes can add an important amount of contaminants to sediments and surface water. For the human health and biota, water contamination is a solemn complication that interact with these aquatic ecosystems. Pollution of water resources with toxic compounds is a worldwide complication [1]. Due to the significance of water quality for health, many toxicity tests have been used along with physical and chemical analyzes to assess both environmental and water quality [2]. The water quality is a significant risk factor in cancer and toxic drinking water is assessed to be as a relative risk. The monitored exposing and reaction relationship showed a relevant risk for esophageal cancer, pancreatic cancer and lymphomas in comparison to areas where non-toxic drinking water was used [3]. Cytotoxicity and genotoxicity tests on plant cells have been used to observe complex environmental samplings, for example stream water [4].

Allium cepa test, one of the plant analysis tests, may have more advantages than mammalian cell and microbial tests for environmental observation. Allium cepa test is highly sensitive for environmental contaminants [5]. Furthermore, this test has been used to observe the potential synergistic impacts of contaminant mixtures, including hydrophilic and hydophobic chemicals [6]. In addition to this, test plants can be exposed directly to the laboratory or environmental sampling or in vitro complex mixtures [7]. By the reason of the large size of plants chromosomes, these are suitable for cytological analysis. In addition to this, the replies observed in Allium cepa test are highly correlated with observed in other biological systems such as mammalian test system. So, plant tests are good candidates for assessing toxicity of environmental sampling [8]. Mitotic cells of Allium cepa L. roots can be used to evaluate a variety of cytological and morphological factors that can be used as toxicity indicators for example root morphology and growth, evaluation of mitotic index, factors including micronucleus, aberrant metaphase and anaphase stimulation [9]. In eukaryotic organisms such as Allium cepa, it is thought that the evaluation of water sampling for cell division and its toxic effect on chromosomes might be beneficial for possible side effects that may occur on people consuming agricultural products obtained from this stream-irrigated agricultural areas [10]. Assessment of the reduction in cell division in the

meristemic cells of A. cepa L. roots is a trusted method for rapidly determining the presence of toxic substances in the environment. Allium cepa test is used to evaluate pollution levels and water pollution levels in natural environments. The results of this test may indicate the presence of cytotoxic, genotoxic or mutagenic submodalities in the environment that compromise the survival of living organisms. Allium cepa test is routinely used in laboratories around the world. It is accepted as an important tool in determining environmental pollution by chemicals [11]. Many hazardous organic chemicals have been identified in wastewater, and some of them- polychlorinated dibenzo-p-dioxins and furans, polychlorinated biphenyls, nitrosamines and polychromatic hydrocarbons-are known to cause DNA damage [12, 13]. Genotoxic substances change the stability of cell processes, particularly cell division, causing chemical changes in DNA. Its effects are mainly determined by changes in the growth and reproduction of living organisms. In addition to that, they are generally cytotoxic when they interfere with cell viability [10]. Wastewater discharge of stream and regular pollution by humans pose a danger both fauna and flora in it. In addition to this, this situation poses a danger to animals and humans along the food chain. Because, plants watered with this polluted water is the primary producers of the river [10].

Canakkale, Biga, Gumuşçay, Hosap Stream is used for irrigation of the agricultural areas in the region and for the discharge of city wastes and sewage. There haven't been a study on the cytotoxic and genotoxic effects of water sample made from Hosap Stream on any eukaryotic organism. The purpose of this research is to examine the effects of water sample taken from stream on mitotic index and chromosomal aberration index of A. cepa root tips.

MATERIALS AND METHODS

Materials. Allium cepa L. was used as test material. Surface water sample was collected from Hosap Stream in Gumuscay Municipality, Biga District of Canakkale Province, Turkey.

Methods. Treatment with Water Samples of Root Tips. Firstly, water sample was diluted and applied to A. cepa root tips. Dilutions were prepared as follows: 3%, 6%, 12.5%, 25%, 50%, 75%, 100% (not diluted). The control group was prepared with pure water. A. cepa L. root tips were treated with these prepared water samples. Ten onions were used for each concentration. Root tip lengths were measured. This measurements were used to calculate the EC50 value, which is the concentration [14] that reduces root elongation by 50% compared to control. In this way, EC50 (effective concentration) (25%) was determined.

The onions were placed in test tubes filled with water and left at room temperature (20±2°C). When the roots of Allium cepa L. reach 1.5-2 cm in 5 days, they were treated with 12.5% (EC50/2), 25% (EC50) and 50%(EC50X2) dilutions of water samples for 24, 48, 72 hours. Ten onions were used for group.

Preparation of Root Tips. Root tips were cut and placed in a farmer fixative. It is hydrolyzed assessed with Souguir et al [15]. Samples were prepared by preparing a mash preparation with 2% acetocarmin (w/v). At least 5000 cells were counted for all application groups. The cells in the mitotic division stage were monitored with microscope at 1000X objective.

Evaluation of Cytotoxicity. Cytotoxicity was evaluated by determining the mitotic index (MI). Mitotic index is generally accepted as the number of mitosis in 100 cells. MI = Number of dividing cells/Total number of cells calculated by formula X100. In this research, 1000 cells were counted for each application group in light microscope. MI was calculated by evaluating the cells that undergoing mitosis within 1000 cells and their division phases.

Evaluation of Genotoxicity. 1000 cells were counted for each application group. The undergoing mitosis cells were identified by counting the phases of mitosis in which they were found and the mitotic aberrations monitored in these phases by light microscop. In this way, the chromosomal aberration index (CAI) was calculated. CAI = Number of cells with chromosomal aberrations/Total number of cells was calculated by formula X100.

Statistical analysis. Repeated measurement ANOVA with two factors and TUKEY multiple comparison test was used.

RESULTS

It was observed that the mitotic index decreased generally due to the dose increase in all application periods. There was no statistically significant difference between the EC50 and EC50x2 doses at the 24 h hour application. The mitotic index decreased significantly in all treatment groups compared to the control group, except for between EC50/2 and control applications in 24 hours application. It was determined that the chromosomal aberration index increased statistically significantly depending on the dose in all application periods except for the 24 h application period. It was demonstrated that the chromosomal aberration index was significantly higher compared to the control groups. Mitotic phases, % chromosomal aberrations monitored at root tips after treatment of stream samplings at doses EC50/2, EC50, EC50X2 for 24, 48 and 72 hours were evaluated with statistically. In addition, %

mitotix index and % chromosomal aberration index were evaluated with the mean and standard errors (Table 1).

Non-aberrant cells monitored in A. cepa root tips were indicated in Figure 1.

The prevalent chromosomal aberrations monitored in roots were C-mitosis, laggard chromosome, polar shifting in anaphase and telophase, equatorial plate shifting, chromosome fragment. The common chromosomal aberrations observed was in Figure 2.

TABLE 1
Mitotic phases %, mitotic index, chromosomal aberrations % and chromosomal aberration index in A. cepa roots

Application time (h)	Concentration (g/l)	Mitotic Phases (%)				Mitotic index % (Mean±Std. Error)	Chromosomal aberrations (%)						Chromosomal aberration index % (Mean±Std. Error)
		Prophase	Metaphase	Anaphase	Telophase		C-Mitosis	Equatorial Plate Shifting	Polar Shifting	Laggard chromosome	Chrl Fragment	Polipl oidi	
24	Control	37,56	23,36	19,82	18,72	16,25± 0,92Aa	12,09	18,02	17,82	19,62	4,02	0,00	0,342±0,321Cb
	EC50/2	40,57	26,36	17,09	9,73	15,126±0,52Aa	23,73	22,09	16,53	27,84	7,20	0,84	17,532±0,431Bc
	EC50	50,32	21,76	13,73	14,82	9,053±0,321Ba	39,26	16,02	21,30	19,54	10,28	0,00	18,521±0,683Bc
	EC50X2	51,57	42,53	15,36	15,34	9,021±0,182Ba	13,21	15,87	34,42	28,04	16,29	0,50	24,651±0,091Ac
48	Control	41,90	26,53	21,52	19,03	19,042±0,282Aa	25,04	22,81	12,09	0,00	0,05	0,00	1,009±0,312Da
	EC50/2	48,34	36,43	19,65	13,82	8,626±0,621Bb	32,82	19,82	0,00	0,00	8,92	0,00	25,430±0,216Cb
	EC50	57,92	39,63	25,72	21,73	8,127±0,532Bb	40,52	16,73	7,05	21,45	7,65	0,00	36,069±1,004Bb
	EC50X2	44,42	48,52	16,73	22,13	5,821±0,432Cb	50,62	21,34	15,65	29,54	12,63	0,00	40,128±0,213Ab
72	Control	39,82	21,76	21,65	12,83	18,627±0,234Aa	29,04	8,92	14,43	0,91	0,52	0,00	1,490±0,312Da
	EC50/2	46,33	35,27	29,78	10,92	6,261±0,431Bc	28,01	15,92	0,00	12,56	5,82	2,34	32,003±0,621Ca
	EC50	52,84	21,19	17,82	15,82	6,009±0,642Bc	51,92	17,82	24,80	18,45	3,98	0,00	41,210±1,271Ba
	EC50X2	25,89	38,92	26,83	14,72	2,217±0,625Bc	43,83	21,62	38,92	31,28	5,73	0,00	53,153±1,642Aa

The difference between the concentrations stated in different capital letters during the same time is significant
The difference between application time stated in different small letters at the same concentration is significant

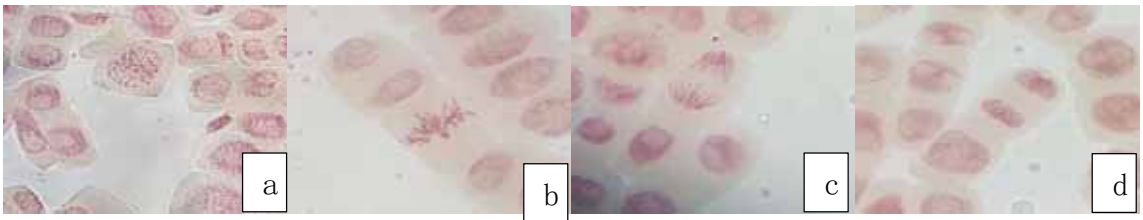

FIGURE 1
Cells with non-chromosomal aberration in the division phase
a) Prophase, b) Metaphase, c) Anaphase, d) Telophase

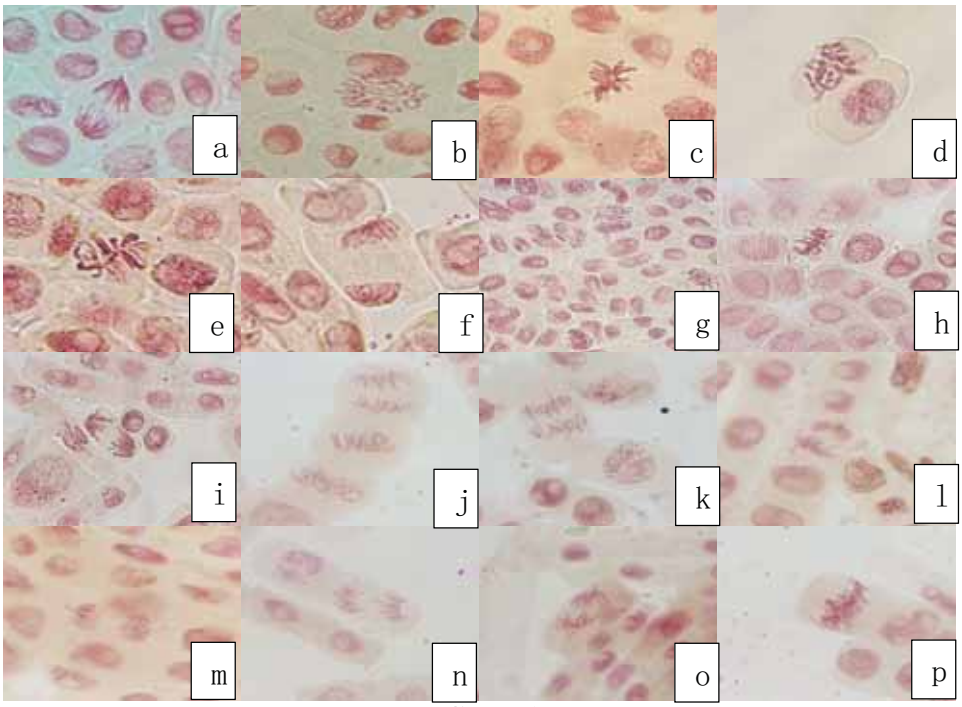

FIGURE 2
Cells with chromosomal aberration in the division phase
a) Polar shifting in telophase, b) C-mitosis, c)sticky chromosome, d) C-mitosis, e) Equatorial plate shifting in metaphase, f) Polar shifting in telophase, g) Chromosome break, h) Equatorial plate shifting in metaphase, i) Polar shifting in anaphase, j) Polar shifting in anaphase, k) laggard chromosome and multipolar anaphase, l) Polar shifting in anaphase, m) Laggard chromosome, n) Polar shifting in anaphase, o) Polar shifting and laggard chromosome in anaphase, p) Poliploidy

Mitotic index is the indicator of cytotoxicity and chromosomal aberration index is the indicator of genotoxicity. Among the assays used to assess water property, plant test systems are considered sensitive biomonitors of toxic impacts of environmental chemicals and can be used for evaluation of cytotoxic and genotoxic effects [16]. Plant assays have been thought useful tools for assessing genotoxicity [17, 18]. A. cepa seedlings have been shown to be extremely sensitive in evaluating the genotoxic effects of environmental water samples. In addition to this, it have been reported to be the first tool for hazard identification and prediction for environmental health [19]. The Allium cepa test is an easy and rapid genetic test to demonstrate the cytotoxic and genotoxic effects of contaminated water and other chemicals on mitosis and chromosome structure in A. cepa cells [20, 21]. Liman et al [22] stated that the Allium cepa test is more sensitive than the Ames test. Surface water comprise of variable levels of organic matter, such as humic acid, that is the main source as potential toxic [23]. Tabrez et al [24] suggested that the Allium cepa test be used as the first bioassay for the assessment of surface water mutagenicity. Ferraro [25] stated that the relationship between the number of nuclear aberrations, genotoxic aberration index and morphological changes (micronuclei, erythrocytic nuclear abnormalities and micronucleated cells) might show the effects of chemicals found in the ecosystems studied.

Leme and Marin-Morales [26] was stated that mitotic index was used as a parameter to evaluate the cytotoxicity of some agents. The reduction in mitotic index may remark the effects on the growth and development of organisms exposed to chemicals. Also, the increase in cell division, which can be detrimental to cells, can be used to determine the cytotoxic effects of pollutants. It has been stated that both the increase and decrease of mitotic index can be useful indicators of pollution in the environmental observing. Chromosomal aberrations are useful for detecting the genotoxic effects of toxic agents. It also provides evaluation of clastogenic and aneugenic activity [19].

Athanásio et al. [19] indicated that Pedras stream caused a decrease in mitotic index and an increase in micronuclei, indicating potential cytotoxicity and mutagenicity. Egito et al [27] evaluated the Pitimbu river genotoxicity with the Allium cepa test. Surface water from the Pitimbu River has been reported to contain toxic and genotoxic compounds that could potentially affect this aquatic ecosystem. It was observed fragments, C-mitosis, chromosome bridges, multipolar anaphase, stickness, chromosome break in mitotic cells. De Campos Júnior et al [28] investigated the genotoxic potential of water taken from Mumbuca stream in Brazil. The results showed an increase in genotoxic activity. The increasing of genotoxic activity was evaluated with an increase in the frequency of micronucleus formation. Athanásio et al [19] found that water samples col-

lected from three rivers in an urban area of a municipality in the south of Brazil showed higher values in terms of total chromosomal aberration compared to negative control. It has also been shown that downstream sites also show higher chromosomal aberration values than upstream sites. For this reason, it has been stated that the water collected from this region can be said to have a genotoxic potential. Şık et al [29] applied waste water in concentrations of 10, 25, 50 and 100% to the root tip of A. cepa. They observed that the rate of mitotic division decreased and chromosomal aberrations occurred depending on the concentration. Bianchi et al [30] investigated the effects of domestic and industrial waste in the Monjolinho River. It was reported that chromosome aberrations, micronucleus, cell death and inhibition of mitotic index were observed in water samples. They reported that excessive metal accumulation was mainly responsible for the effects observed in A. cepa cells. Phosphated compounds showed that the pollution of the river was caused by organic matter discharge. Ju´nior et al [31] evaluated the genotoxicity of water samples collected from domestic sewage Estaˆncia Velha stream of southern Brazil. When the collected samples were evaluated with the Allium cepa test, it was revealed that they showed high toxicity. A significant inhibition in root growth and micronucleated cells were observed in A. cepa root treated with water samples. Matsumoto et al [32] evaluated water samples collected from the Córrego dos Bagres stream in the Franca using the Allium cepa test. They were stated that the most common chromosomal anomalies at root tips were found to be C-metaphase, stick chromosome, chromosome break and chromosome losses, bridged anaphase, multipolar anaphase, and micronucleated and binucleated cell.

Omar et al [33] and Wierzbicka [34] stated that metals exhibit genotoxic capacity, and induce the formation of various nuclear changes by interfering with cell division mechanisms. It was remarked that metals might indicate the presence of toxic substances in the environment.

Akınboro et al [35] was stated that contaminated waters have unwanted negative effects on both fauna and flora. This effects can also be transferred to all living organisms through with the food chain. It was showed that water samples taken from Sungai Dua River inhibited mitotic index and root growth in A. cepa root tips. Nevertheless, the inhibition was not determined to be dose dependent. It was found that there was no chromosomal aberration at undiluted water sampling. These results showed that water sampling taken from Sungai Dua River have weak genotoxic and mitodepressive effects on A. cepa tip cells. It has been reported that water samples taken from the river and the control group induce different chromosomal aberrations such as disturbed spindles, chromosome lag, sticky chromosomes, anaphase bridges and chromosome fragmentation. It was demonstrated that these chromosomal aberrations were not dose dependent, but were significantly different from the control groups.

This study is the first to assess the cytotoxicity and genotoxicity of environmental sample from Hosap Stream in Gumuscay, Biga, Canakkale. Our results indicate that Hosap Stream has cytotoxic and genotoxic potential.

CONCLUSIONS

This study is important as it is the first study evaluating the cytotoxicity and genotoxicity of the surface water sample taken from Hosap Stream. According to the results of the research, it was determined that Hosap Stream has a cytotoxic and a genotoxic potential in certain concentrations on Allium cepa test system. Hosap Stream is used for irrigating the surrounding lands. Chemical substances, which might be present in the stream, have a mutagenic effect on plants and can be transferred to the plants by irrigation. Clearing these waters from chemical and biological contaminants may be a good solution to reduce potential mutagenicity and toxicity on plants. Agricultural biotechnology can be used to both reduce the amount of pesticides and increase yield by transferring genes that create resistance to diseases and pests. Thus, the tolerance of plants to pollution sources can be increased.

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Received: **07.01.2021**
Accepted: **20.02.2021**

CORRESPONDING AUTHOR

Nergis Kaya
Food Processing Department,
Food Technology Program,
Biga Vocational School,
Canakkale Onsekiz Mart University,
Biga Canakkale 17200 – Turkey

e-mail: nergiskaya@comu.edu.tr