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The use of *Allium cepa* L. assay as bioindicator for the investigation of genotoxic effects of industrial waste water

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Abstract: In this study, genotoxic potential of industrial waste water (IWW) samples was investigated using *Allium cepa* assay. The root tips were treated with different IWW samples (A, B and C) for 48 hours. The effects of IWW on cytological effects were determined. It was found that all IWW samples significantly increased the percentage of total abnormality. Mitotic chromosomal abnormalities such as irregular metaphase, stickiness, c-mitosis, micronucleus, vagrant chromosomes and bridges were determined. Furthermore, a significant reduction for the mitotic index that is indicative of cellular toxicity was observed in root tips cells, which were treated with IWW samples. *A. cepa* assay can be used as useful tool for the detection of genotoxic and cytotoxic potential of IWWs.

Introduction

With rapid increase in industrialization, the improvement of living standards, increase in production, and the development and integration of the world economy have led to technological developments. However, more resources were needed and this caused the unconscious and excessive use of natural resources. The destruction of nature and the excessive use of resources have led to the deterioration of the ecological balance over time. The negative effects that disrupt the ecological balance are mostly caused by air, soil and water pollution.

Water pollution, especially waste water pollution, is an important problem all over the world. Waste water is a complex mixture that can contain thousands of distinct contaminants of various origins, like industrial, agricultural, textile and domestic waste. Many of them show cytotoxic and genotoxic effects and are therefore potentially hazardous to humans, animals and the environment (Butt et al. 2005). The use of waste water for irrigation of agricultural areas damages the mitotic division of the plant and the chemical profile of the plants grown in these areas can lead to serious consequences such as allergies, respiratory disorders in childhood, coronary and cancer in the adulthood.

The toxicological effects of waste water exposure can be acute or chronic and occur at all levels of the biological organization. In vitro and in vivo test systems have been developed to measure the genotoxic potential of chemicals and industrial waste water, and the *A. cepa* test system is one of them. The *Allium* test method was first used in 1938 by Levan in the detection of mitotic damage caused by colchicine (Solange and Haywood 2012). In 1985, the importance of *Allium* test method in genotoxicity assessment was demonstrated by Fiskesjö (1985). In 1994, Rank and Nielsen compared the *A. cepa* test system with the Ames test, microscreen test and carcinogenicity tests and reported that the sensitivity of the *A. cepa* test was 82%. The *A. cepa* test is one of the top nine plants used by the US Environmental Protection Agency's (EPA) Gene-tox program to evaluate the genotoxicity of toxic substances due to its high sensitivity. Moreover, the International Chemical Safety Program (IPCS) has been recognized as a standard test for detecting the genotoxicity of environmental pollutants by the World Health Organization and the United Nations Environment Agency (UNEP) (Grant 1994, Liu et al. 1995). Therefore, monitoring the root tip system of plants constitutes a fast and sensitive method for environmental monitoring (Majer et al. 2005). The aim of this study is to investigate the genotoxic effects of waste waters in industrial zone on *A. cepa* L root tip cells.

Material and methods

Test chemicals and treatment

IWW samples used for the present study were randomly collected from industrial units in Kocaeli province of Turkey in 2014 (Fig. 1). They were not diluted with distilled water to investigate their genotoxic effects on *A. cepa*.

Determination of anion, cation and heavy metal levels in water samples

Anion, cation and heavy metal analyzes of the IWW samples analyzes were carried out in Düzce University Scientific and Technological Studies Application and Research Center

Laboratory. Ion chromatography device was used for anion and cation analysis, and ICP-OES device was used for heavy metal analysis. For determination of anions, cations and heavy metals contents in IWW samples, the classification of inland water resources according to the Regulation on the Amendments to the Surface Water Quality Management was carried out by dividing into four categories: I: high quality water; II: lightly contaminated water; III: contaminated water; IV: highly contaminated water (Anonymous 2015).

Allium cepa assay

Onions selected for the study were equal in size, not germinated and morphologically undamaged. IWW samples taken from three different regions were used as application material and these samples were not diluted with distilled water. Also, distilled water was used as negative control. For each group, five onions were treated with IWW for 48 hours at room temperature. At the end of 48 hours, the onion roots were cut to 1–2 cm and put into 3:1 alcohol:glacial acetic acid, kept at +4° C for 1 night. The roots were kept in 70% alcohol until microscopic examination.

Cytological investigation

For cytological investigation, preparation methods such as fixing (in Carnoy fixative), hydrolyzing (in 1N HCl for 15 min. in oven at 60°C), squashing and staining (in 2% aceto orcein) were carried out as described by Darlington and La Cour (1979) with slight modifications.

To determine the effects of this chemical on mitotic index, 3000 cells were counted in each group and it was calculated for each treatment as a number of dividing cells/100 cells.

Statistical analysis

The data obtained from the investigation were analyzed using one-way analysis of variance (ANOVA) in SPSS 20 for Windows (SPSS Inc., Chicago, IL, USA) and they were showed as mean ± standard error (SE).

Results and discussion

The results of ion chromatography and ICP-OES were given in Table 1. Anions like chlorine, nitrite, sulfate, phosphate, and cation like ammonium in the waste water-B were detected in bigger amount than in other waste water samples. The only heavy metals determined in all IWW samples were arsenic, strontium and antimony. According to Table 1, when the waste water taken from three different regions is evaluated in terms of anion, cation and determined heavy metal values, it is noteworthy that the values are generally close to each other. However, it is seen that waste water-C has lower values.

The chromosomal damages induced in root tip cells treated with IWW collected from three different regions and the numbers of these damages were shown in Table 2. When these data were examined, it was determined that the preparations obtained from IWW samples mostly had chromosomal damages such as irregular metaphase, stickiness, c-mitosis, micronucleus, multipolarity, bridge, fragment, lagging chromosome, vagragant chromosome (Fig. 2 and 3). A significant increase was observed in chromosome abnormalities during mitosis compared to control. The most common mitotic abnormalities were stickiness, irregular metaphase, and C-mitosis. As given in Table 2, total abnormality (%) increased significantly in samples taken from all three stations compared to the control ($p \leq 0.001$). Waste water-C had the highest percentage of total abnormality. Waste water-B-treated root tips showed the lowest percentage of total abnormality. It was seen that the mitotic index decreased significantly compared to the control in IWW samples taken from three different regions ($p \leq 0.001$).

When the relationship between total abnormality and mitotic index is examined, while the percentage of total abnormality increased in onions germinated in IWWs collected from three different stations, it was found that there was a decrease in the mitotic index (Fig. 4). This decrease is statistically significant ($p \leq 0.001$).

Although the use of waste water in agricultural irrigation has some advantages, such as increasing the metabolic activities of soil microorganisms and reducing the need for agricultural fertilizers, the possibility of the presence of contaminants such as pathogens and chemicals are important issues that limit its use. For this reason, chemical analysis of waste waters and investigation of its toxicity on *A. cepa* draw attention of many researchers. El Shahaby et al. (2003) reported that industrial wastewater in Sandub area, Dakahlia Province is highly mutagenic on *A. cepa* due to heavy metals content. The most abnormalities in micronucleus and chromosome aberration methods were found in wastewater samples collected from Shawa and Belgay regions, respectively. It was indicated that both refined and unrefined IWW collected from the organized industrial zone in Manisa province of Turkey (Şık et al. 2009) and petroleum wastewater collected from Ordu province of Turkey (Turkmen et al. 2009) had genotoxic effects on *A. cepa* root tips. Olorunfemi (2011) stated that

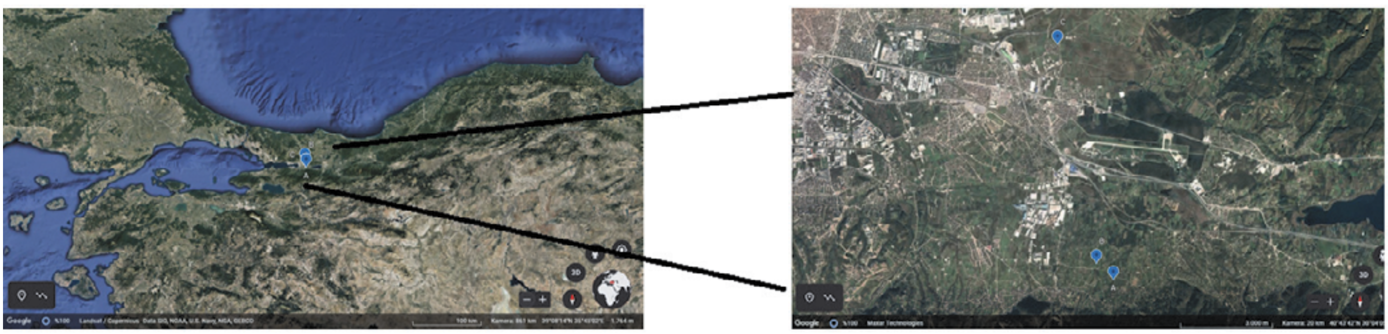

Fig. 1. Map of the study area showing sampling sites in Kocaeli province of Turkey (Site A: Derbent; Site B: Suadiye; Site C: Uzunbey)

Table 1. Anions, cations and heavy metals contents in IWW samples and classification of inland water resources according to the amount of metal they contain

		Waste water-A	Waste water-B	Waste water-C
Anions (mg/L)	Fluorine	1.08 (I)	1.04 (I)	0.19 (I)
	Chlorine	136.12 (II)	137.52 (II)	70.23 (II)
	Nitrite nitrogen	0.09 (II)	0.17 (III)	0.02 (I)
	Bromine	n.a.	n.a.	0.12
	Nitrate nitrogen	2.32 (I)	1.83 (I)	2.01 (I)
	Sulfate	192.77 (I)	214.14 (I)	83.19 (I)
	Phosphate	0.24 (II)	0.28 (II)	0.08 (II)
Cations (mg/L)	Lithium	n.a.	n.a.	n.a.
	Sodium	n.a.	n.a.	n.a.
	Ammonium nitrogen	192.44 (IV)	209.65 (IV)	96.60 (IV)
	Potassium	n.a.	n.a.	n.a.
	Magnesium	n.a.	n.a.	n.a.
	Calcium	16.63*	16.97*	25.23*
Heavy metals (mg/L)	Aluminum (Al)	n.a.	n.a.	n.a.
	Scandium (Sc)	n.a.	n.a.	n.a.
	Vanadium (V)	n.a.	n.a.	n.a.
	Chromium (Cr)	n.a.	n.a.	n.a.
	Manganese (Mn)	n.a.	n.a.	n.a.
	Iron (Fe)	n.a.	n.a.	n.a.
	Cobalt (Co)	n.a.	n.a.	n.a.
	Nickel (Ni)	n.a.	n.a.	n.a.
	Copper (Cu)	n.a.	n.a.	n.a.
	Zinc (Zn)	n.a.	n.a.	n.a.
	Arsenic (As)	5.70 (I)	5.60 (I)	5.50 (I)
	Selenium (Se)	n.a.	n.a.	n.a.
	Strontium (Sr)	538.00*	562.00*	613.00*
	Molybdenum (Mo)	n.a.	n.a.	n.a.
	Silver (Ag)	n.a.	n.a.	n.a.
	Cadmium (Cd)	n.a.	n.a.	n.a.
	Stannum (Sn)	n.a.	n.a.	n.a.
	Antimony (Sb)	0.37*	0.40*	0.25*
	Barium (Ba)	n.a.	n.a.	n.a.
	Mercury (Hg)	n.a.	n.a.	n.a.
	Lead (Pb)	n.a.	n.a.	n.a.
	Bismuth (Bi)	n.a.	n.a.	n.a.

n.a.: Measurements is below limits; *: not in the list.
I: High quality water; II: Lightly contaminated water; III: Contaminated water; IV: Highly contaminated water (Anonymous 2015)

Table 2. The mitotic division abnormalities by IWW samples collected from three different industrial regions

Concentration (ppm)	Total abnormality (%)		Abnormal Metaphase				Abnormal Anaphase				Mitotic Index (%)
		Irregular metaphase	Stickiness	C-Mitosis	Micronucleus	Multipolarity	Bridge	Fragment	Lagging Chromosome	Vagragant Chromosome	
Negative Control	5.22±0.45	1.15±0.08	1.03±0.03	1.27±0.12	0.15±0.06	0.32±0.11	0.37±0.17	0.00±0.00	0.42±0.13	0.50±0.22	9.08±0.23
Waste water-A	23.00±5.14***	7.60±2.52	8.20±1.39	2.00±0.89	1.00±0.63	0.80±0.37	1.80±0.37	0.00±0.00	0.80±0.58	0.80±0.58	5.11±0.26***
Waste water-B	21.83±5.70***	8.16±2.41	9.83±2.21	2.16±0.87	0.16±0.16	0.33±0.21	0.50±0.50	0.16±0.16	0.16±0.16	0.33±0.33	5.74±0.30***
Waste water-C	27.20±2.67***	8.40±1.93	12.60±2.06	2.60±1.02	0.20±0.20	0.00±0.00	1.00±0.44	0.00±0.00	1.60±1.12	0.80±0.37	4.82±0.21***

Data are reported as mean value ± SE; *P≤0.05; **P≤0.01; ***P≤0.001.

genotoxicity on *A. cepa* root tip cells result from chemicals in IWWs in Benin City of Nigeria and wastewater from bottling facility was the most toxic. It has been shown that olive oil wastewater was more toxic than milk wastewater (Aksoy et al. 2011), water samples taken from two different stations where domestic and industrial wastes are discharged have genotoxic and cytotoxic effects on onion (Kanev et al. 2017) and textile wastewater collected from Kanpur, India caused chromosomal abnormalities (Khan et al. 2019) in the genotoxicity studies conducted on *A. cepa*. The results of previous studies which

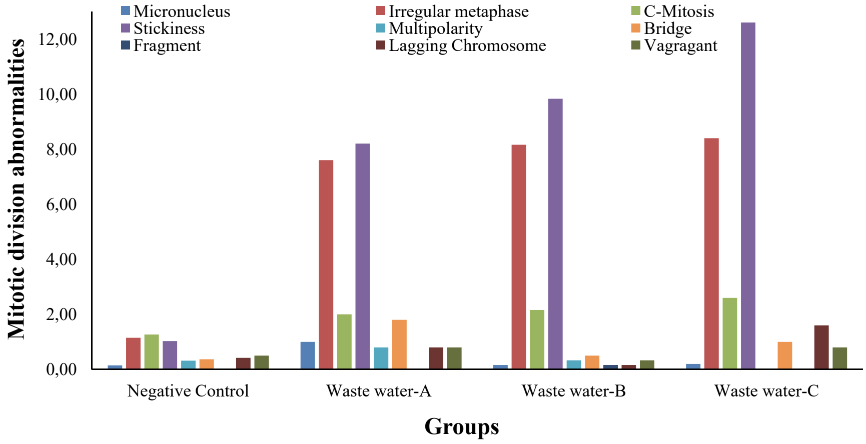

Fig. 2. Mitotic division abnormalities in *A. cepa* treated with IWWs for 48 h

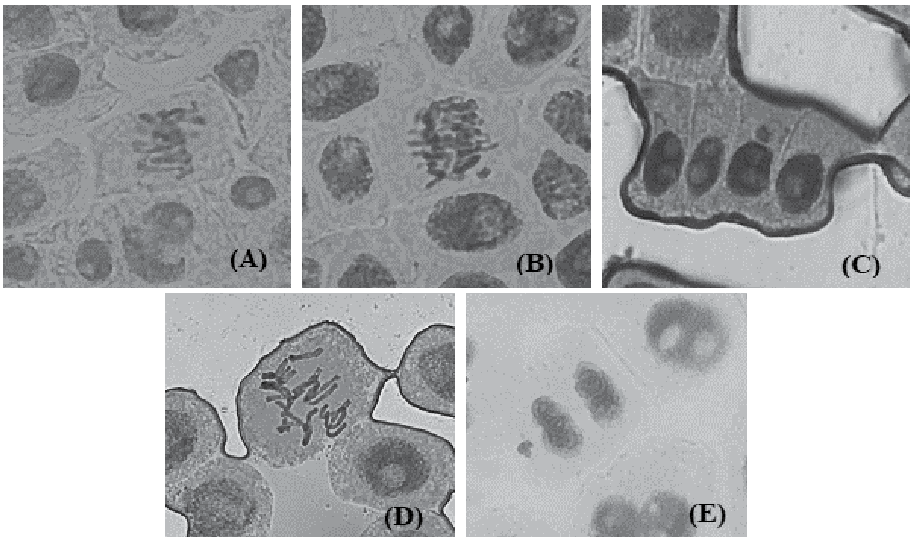

Fig. 3. Mitotic division abnormalities determined in the root tips of *A. cepa* L. treated with IWW samples (A) Irregular metaphase; (B) Stickiness and fragment; (C) Micronucleus; (D) C-Mitosis; (E) Vagragant Chromosome

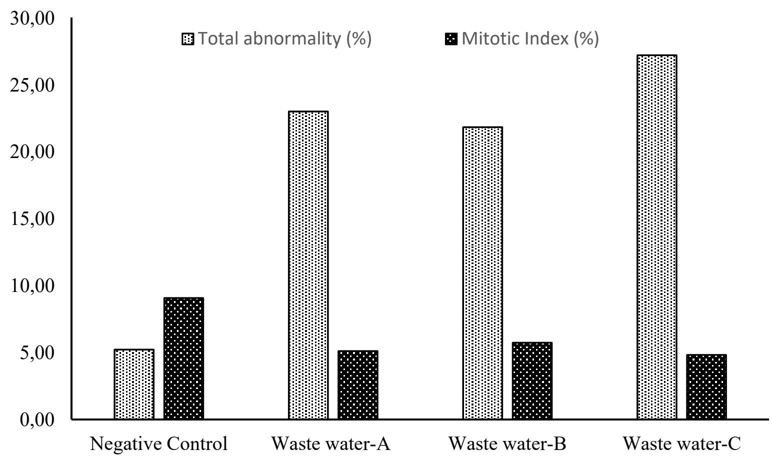

Fig. 4. The relationship between total abnormality (%) and mitotic index (%) in *A. cepa* treated with IWWs for 48 h ($p \leq 0.001$)

observed genotoxic effect after waste water treatment support the results of our study. Similarly, genotoxic, embryotoxic and mutagenic effects of IWWs such as textile, petroleum, olive oil on root tip cells of *Triticum vulgare* (Aybeke et al. 2000) and *Vicia faba* (El Hajjouji et al. 2007, Cavusoglu et al. 2010), on cultured human lymphocytes (Turkez et al. 2009), on fishes (Bianchi et al. 2015, Babic et al. 2017), on mice bone marrow cells (Oliveira Júnior et al. 2013), on *E. coli* and *Salmonella* tester strains (Khan et al. 2019) have been reported in the literature. One of the most important causes of environmental pollution of air, soil and water are heavy metals due to their toxic effects and accumulations (Rasgele et al. 2013). The mechanisms of cytotoxic and genotoxic effects caused by different heavy metals are also different and each element is distinctive (Tchounwou et al. 2012). Heavy metals can directly damage the genetic material, cell walls and organelles, or they can also be indirectly effective on genetic material by disrupting the structures of enzymes involved in DNA repair and cell division control mechanisms, causing an increase in oxidative stress (Squibb and Fowler 1981, Beyersman and Hartwig 2008).

Conclusions

The results showed that IWW samples decreased MI because of its cytotoxicity and increased total abnormality in mitotic cell division. This study also showed that the *A. cepa* L. test material could be used as a suitable indicator to determine the toxic effects of wastewater pollution.

References

Aksoy, O., Erbulucu, T. & Vatan, E. (2011). Effects of wastewater from olive oil and milk industry on growth and mitosis in *Allium cepa* root apical meristem. *Journal of Applied Biological Sciences,* 5, 3, pp. 75–78.

Anonymous, (2015). Regulation on the Amendments to the Surface Water Quality Management Regulation (Yüzeysel Su Kalitesi Yönetimi Yönetmeliğinde Değişiklik Yapilmasina Dair Yönetmelik), https://www.resmigazete.gov.tr/eskiler/2015/04/20150415-18.htm date of access: 22.05.2021

Aybeke, M., Olgun, G., Sidal, U. & Kolankaya, D. (2000). The effect of olive oil mill effluent on the mitotic cell division and total protein amount of the root tips of *Triticum aestivum* L. *Turkish Journal of Biology,* 24, pp. 127–140.

Babic, S., Barisic, J., Visic, H., Klobucar, R.S., Popovic, N.T., Strunjak-Perovic, I., Coz-Rakovac, R. & Klobucar, G. (2017). Embryotoxic and genotoxic effects of sewage effluents in zebrafish embryo using multiple endpoint testing. *Water Research,* 115, pp. 9–21. DOI: 10.1016/j.watres.2017.02.049

Beyersmann, D. & Hartwig, A. (2008). Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms, *Archieves of Toxicology*, 82, pp. 493–512. DOI: 10.1007/s00204-008-0313-y

Bianchi, E., Goldoni, A., Trintinaglia, L., Lessing, G., Silva, C.E.M., Nascimento, C.A., Ziulkoski, A.L., Spilki, F.R. & Silva, L.B. (2015). Evaluation of genotoxicity and cytotoxicity of water samples from the Sinos River Basin, southern Brazil. *Brazilian Journal of Biology*, 75, 2, pp. 68–74. DOI: 10.1590/1519-6984.1913

Butt, M.S., Sharif, K., Bajwa, B.E. & Aziz, A. (2005). Hazardous effects of sewage water on the environment: Focus on heavy metals and chemical composition of soil and vegetables. *Management of Environmental Quality: An International Journal*, 16, pp. 338–346. DOI 10.1108/14777830510601217

Cavusoglu, K., Yapar, K., Kinalioglu, K., Turkmen, Z., Cavusoglu, K. & Yalcin, E. (2010). Protective role of *Ginkgo biloba* on petroleum wastewater-induced toxicity in *Vicia faba* L. (Fabaceae) root tip cells. *Journal of Environmental Biology,* 31, pp. 319–324.

Darlington, C.A. & La Cour L.E. (1979). The Handling of Chromosomes. 6th ed. Allen and Unwin, London 1979.

El Hajjouji, H., Pinelli, E., Guiresse, M., Merlina, G., Revel, J.C. & Hafidi, M. (2007). Assessment of the genotoxicity of olive mill waste water (OMWW) with the *Vicia faba* micronucleus test. *Mutation Research.* 634, pp. 25–31. DOI: 10.1016/j.mrgentox.2007.05.015

El-Shahaby, O.A., Abdel Migid, H.M., Soliman, M.I. & Mashaly, I.A. (2003). Genotoxicity screening of industrial wastewater using the *Allium cepa* chromosome aberration assay. *Pakistan Journal of Biological Sciences,* 6, 1, pp. 23–28. DOI: 10.3923/pjbs.2003.23.28

Fiskesjö, G. (1985). The Allium test as a standard in environmental monitoring. *Hereditas*, 102,1, pp. 99–112. DOI: 10.1111/j.1601-5223

Grant, W.F. (1994). The present status of higher plant bioassays for the detection of environmental mutagens. *Mutation Research*, 310, pp. 175–185. DOI: 10.1016/0027-5107(94)90112-0

Kanev, M.O., Ozdemir, K. & Gokalp, F.D. (2017). Evaluation of genotoxic effects on onion (*Allium cepa* L.) root tip cell of ergene river water. *Marmara Journal of Pure and Applied Sciences*, 3, pp. 111–117. DOI: 10.7240/marufbd.311079

Khan, S., Anas, M. & Malik, A. (2019). Mutagenicity and genotoxicity evaluation of textile industry wastewater using bacterial and plant bioassays. *Toxicology Reports,* 6, pp. 193–201. DOI: 10.1016/j.toxrep.2019.02.002

Levan, A. (1938). The effect of colchicine on root mitoses in *Allium. Hereditas*, 24,4, pp. 471– 486. DOI: 10.1111/j.1601-5223.1938.tb03221.x

Liu, D., Jiang, W., Wang, W. & Zhai, L. (1995). Evaluation of metal ion toxicity on root tip cells by the *Allium* test, *Israel Journal of Plant Sciences*, 43: 125–133. DOI: 10.1080/07 929978.1995.10676598

Majer, B.J., Grummt, T., Uhi, M. & Knasmuller, S. (2005). Use of plant assays for the detection of genotoxins in the aquatic environment. *Acta of Hydrochemistry and Hydrobiology,* 33, pp. 45–55. DOI: 10.1002/aheh.200300557

Oliveira Júnior, H.M., Sales, P.T.F., Oliveira, D.B., Schimidt, F., Santiago, M.F. & Campos, L.C. (2013). Characterization and genotoxicity evaluation of effluent from a pharmacy industry. *AmbiAgua, Taubaté*, 8,2, pp. 34–45. DOI: 10.4136/ambi-agua1107

Olorunfemi, D., Ogieseri, U.M. & Akinboro, A. (2011). Genotoxicity screening of industrial effluents using onion bulbs (*Allium cepa* L.). *J. Appl. Sci. Environ. Manage*, 15,1, pp. 211–216.

Rank, J. & Nielsen, M.H. (1994). Evaluation of the *Allium* anaphase-telophase test in relation to genotoxicity screening of industrial wastewater. *Mutation Research*, 312,1, pp. 17–24. DOI: 10.1016/0165-1161(94)90004-3

Rasgele, P.G., Kekecoglu, M. & Muranli, F.D.G. (2013). Induction of micronuclei in mice bone marrow cells by cobalt and copper chlorides. *Archives of Environmental Protection,* 39, 1, pp. 75–82. DOI: 10.2478/aep-2013-0007.

Solange, B.T. & Haywood, D.L. (2012). Bioindicator of Genotoxicity: The Allium cepa Test, Environmental Contamination, Jatin Kumar Srivastava, IntechOpen, Available from: https://www.intechopen.com/books/environmental-contamination/bioindicator-of-genotoxicity-the-allium-cepa-test. DOI: 10.5772/31371.

Squibb, K.S. & Fowler, B.A. (1981). Relationship between metal toxicity to subcellular systems and the carcinogenic response.

Environmental Health Perspectives, 40, pp. 181–188. DOI: 10.1289/ehp.8140181

Şık, L., Acar, O. & Aki, C. (2009). Genotoxic effects of industrial wastewater on *Allium cepa* L. *African Journal of Biotechnology,* 8, 9, pp. 1919–1923.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. & Sutton, D.J. (2012). Heavy metal toxicity and the environment. *Molecular, Clinical and Environmental Toxicology*, [In:] Luch, A. (ed.), Vol 1: Molecular Toxicology (Experientia Supplementum), Birkhäuser, Berlin, 2012. 133–164. DOI: 10.1007/978-3-7643-8340-4_6

Turkez, H., Sisman, T., Incekara, U., Geyikoglu, F., Tatar, A. & Keles, M.S. (2009). The genotoxic and biochemical effects of wastewater samples from a fat plant in Erzurum. *Journal of Balıkesir University Institute of Science and Technology,* 11, 2, pp. 55–63.

Turkmen, Z., Cavusoglu, K., Cavusoglu, K., Yapar, K. & Yalçin, E. (2009). Protective role of Royal Jelly (honeybee) on genotoxicity and lipid peroxidation, induced by petroleum wastewater, in *Allium cepa* L. root tips. *Environmental Technology*, 30,11, pp. 1205–1214. DOI: 10.1080/09593330903179757