GENOTOXICITY ASSESMENT OF WASTE WATER IN VARIOUS STAGES OF TREATMENT - CASE STUDY – THE "STAN VIDRIGHIN" WASTE WATER TREATMENT PLANT (WWTP) OF TIMIȘOARA

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Abstract. The quality of wastewater that is discharged from municipal sewerage systems in rivers and channels is very important for the preservation of aquatic fauna and flora, as well as irrigation or supplying water to animals in rural areas. WWTP Timișoara was founded in 1912 and its last refurbishment was carried out in 2011. WWTP Timișoara serves 39 settlements, including the cities of Timișoara and Recaș. The treatment process comprises three main stages: 1st stage – mechanical, 2nd stage – microbiological aerobic, 3rd stage – microbiological anaerobic. In order to assess water quality during the cleaning process, a test was performed for cito/genotocity using a sensitive test plant *Nigella damascena*. Six sampling points were defined at the treatment system: P1 – raw wastewater, P2 – aerobic nitrification, P3 – anoxic denitrification, P4 – sand, P5- sludge, P6 – purified water (output WWTP to Bega Channel) (Fig. 1). The water samples were collected in April 2019. The *N. damascena* L. seeds (100 seeds / Petri dish in 3 replicates) were germinated on water collected from the sampling points mentioned before. Tap water was used for control. The lowest mitotic index was registered in waste water and sludge, with significant diferences in comparison to control. In all experimental variants genomic and chromosomal mutations were observed. The most common aberrations were fragments and bridges, and metabolic changes regarding the degree of compaction of chromatin fibers. Cells with 1-3 micronuclei, binucleated cells and multipolar spindle in mitosis cells were observed. Purified water also presented genotoxic effects indicating decay or that byproducts of the toxic compounds are still in its composition.

Keywords: waste water treatment station, citotoxicity, genotoxicity, *Nigella damascena*.

Rezumat. Evaluarea genotoxicității apelor uzate în diferitele etape ale procesului de epurare – studiu de caz – Stația de Epurare a Apelor Uzate (SEAU) „Stan Vidrighin" Timișoara. Calitatea apei menajere, care se deversează din canalizarea orașelor în râuri și canale este foarte importantă pentru conservarea faunei și florei acvatice, precum și pentru irigații sau adăparea animalelor în zonele rurale. SEAU Timișoara a fost înființată în anul 1912 și ultima retehnologizare a fost realizată în 2011. SEAU Timișoara deservește 39 localități, inclusiv municipiul Timișoara și orașul Recaș. Procesul de epurare cuprinde 3 etape principale: I etapa mecanică, a-II-a etapa microbiologică aerobă, a-III-a etapa microbiologică anaerobă. Pentru a evalua calitatea apei în timpul procesului de curățare a fost efectuat un test de cito/genotocitate folosind o plantă tester sensibilă *Nigella damascena*. Șase puncte de prelevare au fost definite la sistemul de tratare: P1 - ape uzate brute, P2 - nitrificare-aerobic, P3 - denitrificarea anoxică, P4 - nisip, P5 - nămol, P6 - apă purificată (ieșire SEAU pentru Canalul Bega) (Fig. 1). Probele de apă au fost colectate în aprilie 2019. Semințele de *N. damascena* L. (100 semințe / Petri în 3 repetiții) au fost germinate pe ape colectate de la punctele de prelevare menționate anterior. Control a fost utilizată apa de robinet. Cel mai mic indice mitotic a fost înregistrat la apa uzată și nămol, cu diferențe semnificative față de Control. În toate variantele experimentale au fost observate mutații cromosomiale și genomice. Cele mai frecvente aberații au fost fragmentele și punțile, dar și modificări metabolice privind gradul de compactare a fibrelor de cromatină. Au fost observate celule cu 1-3 micronuclei, celule binucleate și mitoze multipolare. Apa epurată a prezentat de asemenea efecte genotoxice indicând faptul că are încă în compoziția sa compuși toxici.

Cuvinte cheie: stație de epurare a apelor uzate, citotoxicitate, genotoxicitate, *Nigella damascena*.

INTRODUCTION

The sustainable use of water resources is a crucial issue that has been discussed worldwide, as a priority, over the last decades. Due to factors such as climate change and resulting drought, population growth, and increased pollution, water shortage will increasingly affect people around the world (PINTILIE et al., 2016). Industrialization is believed to cause all types of pollution problems as the balance in the natural ecosystem is affected by the release of hazardous wastes into the environment, threatening the survival of all living beings (PONDHE et al., 1997). Pollution is causing an imbalance in ecosystems and, nowadays, wastewater is a severe threat to the environment because of its toxic nature (MALATO & ALBA, 2009). Nowadays, in most developing countries, raw and unstabilized sludges are either incinerated or dumped unscientifically in open places and landfills, which disturbs the geochemical cycles and the natural environment (SEN & CHANDRA, 2007).

The quality of wastewater that is discharged from municipal sewerage systems into rivers and channels is very important for the preservation of aquatic fauna and flora, as well as irrigation or for supplying water to animals in rural areas. In general, urban wastewaters are composed of a great variety of environmental contaminants which might be toxic and only partially or even not at all eliminated by wastewater treatment systems (RADIĆ et al., 2010; HEMACHANDRA & PATHIRATNE, 2015). Over 700 emerging pollutants, their metabolites and transformation products: pharmaceuticals, hormones and steroids, pesticides, veterinary products, industrial compounds (by-products), food aditives (DULIO & VON DER OHE, 2013) are present in the European aquatic environment. All these chemicals cause adverse ecological and human health effects (high toxicity, carcinogenic and mutagenic effects).

The establishment of appropriate wastewater management systems is very important in order to protect the environment and the water bodies that serve as drinking water sources, especially in the developing countries (LAM et al., 2015). The Wastewater Treatment Plants (WWTP) have the mission to decrease the environmental impact of municipal and industrial wastewater. The WWTP in Timișoara was put into operation in 1912 to accommodate 570 l\s, being available only with mechanical gear. Currently, the treatment plant has a capacity of 3,000 liters. The technological process is formed after a set of mechanical and biological processes. The wastewater and rainfall water are transported through the sewerage to the treatment plant, where they are cleaned before being discharged into the Bega channel. The wastewater reaches the treatment plant through channels following a gravitational flow or by pumping. The wastewater comes from Timișoara and Recaș but also from the nearby communes: Ghiroda, Moșnița Nouă, Sânmihaiu Român, Săcălaz, Șag, Pișchia, Fibiș, Mașloc, Bogda, Remetea Mare, Giarmata, Sânandrei which, together with their villages, cover an operating area of 39 localities.

The potential adverse effects that wastewaters might inflict on wildlife and humans due to the additive, synergistic, or antagonistic interactions between the chemicals present in wastewaters cannot be evaluated by physico-chemical analyses (HEMACHANDRA & PATHIRATNE, 2016). Biomonitoring with the use of different species might be considered an alternative to complement physico-chemical analyses, as test organisms respond to all the compounds in wastewaters (PRASSE et al., 2015). Plants bioassays are very sensitive to a large range of pollutants, metabolizing foreign compounds through a number of mechanisms qualitatively similar to the bio-transformation systems present in the animal. Meristematic and sporogenic tissues of plants generally show patterns of cytotoxic response similar to those of embryogenic and spermatogenic tissues of vertebrates. In order to assess water quality during the cleaning process, a test for cito/genotocity was performed using a sensitive test plant *Nigella damascena*. *N. damascena* L. (commonly known as love-in-a-mist, 2n=12) is an ornamental species and can be used as a test plant due to its large chromosome size and sensitivity to chemical and physical mutagenesis (MOUTSCHEN et al., 1969, 1987; DATTA & SAHA, 2003).

MATERIAL AND METHODS

Wastewater treatment and conditions for sampling points. The treatment process in WWTP comprises three main stages:

- 1st stage – mechanical – the purification step intended for the mechanical removal of coarse material (wood, bottles, wipes, sticks), sand and fats; the 2nd and 3rd stages are biological stages and are composed by separate tanks in active sludge: the 2nd stage is one of biological nitrification, and the 3rd stage is of denitrification.
- 2nd stage – the aerobic nitrification stage presents specialized bacteria of the *Nitrosomonas* and *Nitrobacter* genus which, together with other bacteria in the presence of oxygen 2mg \ l and the ammonium ion, oxidize organic substances and nitrate to nitrite.
- 3rd stage – the anoxic denitrification stage which comprises the step whereby the facultative anaerobic microorganisms degrade specialized nitrate ion to nitrogen and phosphorus. The removal of the biological pathway can be achieved in about 10% of the total cases or by chemical precipitation of iron salts.

Purified water is mechanically inserted into biological sinks, with an equal amount of sludge to create environment for treatments 2 and 3. After the biological processes take place, the water reaches the sedimentation sludge 2 where there is a separation process and is then returned to the sludge treated water. The sludge is collected and re-introduced into the biological basins to an extent of 100%. The excess sludge is waste and must be eliminated. This method is the dehydration in the presence of a cationic polyelectrolyte (remove water and reduce the interstitial volume to about 20%). The dewatered sludge is then dried in a greenhouse (remove water and reduce the interstitial volume to about 20%), the dewatered sludge is then dried in a greenhouse until at least 35% dry matter.

As **biological material**, *N. damascena* L. cv Miss Jeckill seeds were used.

Experimental scheme. Six sampling points were defined at the treatment system: P1 – raw wastewater, P2 - aerobic nitrification, P3 – anoxic denitrification, P4 – sand, P5 – sludge, P6 – purified water (output WWTP to Bega Channel) (Table 1; Fig. 1). The water samples were collected in April 2019. The analysis of wastewater and purified water was performed on the same day in WWTP Timișoara.

Table 1. Quality parameters of wastewater and purified water in WWTP Timișoara (April 15th 2019).

Parameters	Wastewater	Purified water
Temperature water (°C)	21	21
pH	7,5	7,1
Material in suspension	138	12
CCOC2 (mg O_2 *dm^{-3})	416	21
NO3 (mg *dm^{-3})	3,4	5,1
NO2 (mg *dm^{-3})	0,58	0,52
NH4 (mg *dm^{-3})	19,9	0,49
PO4 (mg *dm^{-3})	5,2	1,1
Total residue (mg *dm^{-3})	556	364
Stable residue (mg *dm^{-3})	410	350
Volatile residue (mg *dm^{-3})	286	190

The *N. damascena* L. seeds (100 seeds / Petri dish in 3 replicates) were germinated on water collected from the sampling points mentioned before. A 1:10 dilution with tap water was made for sand and sludge. Tap water was used for control. The roots (10 roots/Petri dish) for cytogenetical analysis were harvested 5 days from the start of the experiment, when their length reached 10 mm.

Cytogenetic investigations. The chromosome aberrations were analyzed in the radicular meristematic tissue, on squash preparation type, stained with a Carr solution. The observations were performed with an Olympus optical microscope. 400 -500 cells/root tip, 10 meristematic tip/experimental variant were analysed. The normal and aberrant phases of the mitotic cycle were analyed, as well as the metabolic and structural modifications of the chromosomes and of the mitotic spindle: chromosomal abberations (CA), genomic mutations (GM), micronuclei (MN). The data were statistically analyzed with the STATISTICA 10.0 software, ANOVA/MANOVA, DUNCAN's multiple range test.

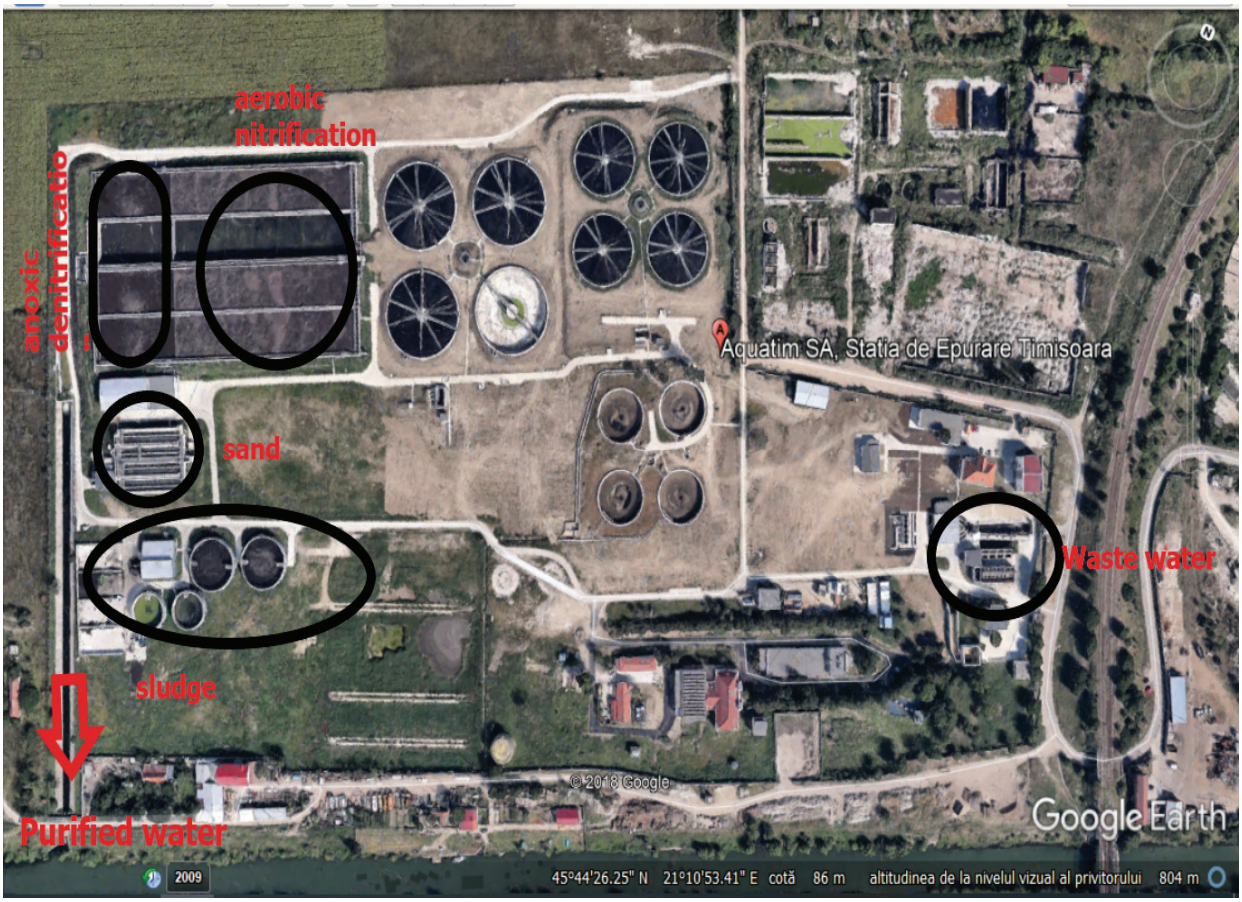

Figure 1. The sampling points from WWTP Timisoara (source Google Earth).

RESULTS AND DISCUSSIONS

Cytotoxicity evaluation in mitotic division cycle.

The Mitotic Index (MI), characterized by the total number of dividing cells, reported to the total number of observed cells, is an important parameter, due to the fact that the mitotic cycle can be disturbed by different toxic chemicals. The analysis of variance showed that the effect of water samples composition is very significant on MI (Table 2).

MI significantly increases in the P2 sample (aerobic nitrification), while the lowest value (12.4%) is registered in wastewater (P1) compared to Control (Table 3). A dramatic decrease of MI was seen in the next stages of water treatment. These results show that, during the various stages of purification of water and decomposition of pollutants, the resulting products were cytotoxic, either alone or in combination. Although the results of physicochemical analyses show that all the analysed parameters dropped in terms of quantity, the evaluation of the mitotic index shows that other cytotoxic products were formed. These findings are in agreement with the results of HEMACHANDRA & PATHIRATNE (2016). Genotoxicity can be determined by the decrease or increase in mitotic index (FERNANDES et al., 2007).

In general, heavy metals induce a decrease of the prophase index, a slight increase of the metaphase and anaphase index, while the telophase index is considerably increased (STAYKOVA et al., 2005). The physico-chemical analysis provided by AQUATIM SA for April 2019 (***. https://www.aquatim.ro/parametri-de-calitate-ai-apei-epurate-2798.html) shows that the level of heavy metals is below the detection limits. The explanation for the variability in MI values lies in other toxic compounds, new formed or some pharmaceudical by-products or hormones. HOWELL et al.

(2007) proved that a low concentration in the plant steroidal hormone, 24-epibrassinolide can induce a significant increase in the number of dividing cells.

Table 2. The effect of water samples on mitotic division.

Analysis of Variance Marked effects (bold) are significant at p <0.05000		
Experimental variant	F	P
Mitotic Index %	**114.2164**	**0.000000**
Prophase %	**242.3356**	**0.000000**
Metaphase %	**187.0600**	**0.000000**
Anaphase %	**8.8257**	**0.000420**
Telophase %	**71.0106**	**0.000000**

Table 3. Mitotic index (MI) in *Nigella damascena*, in different experimental variants (significant differences in comparison with Control are marked in bold).

Variant	**Total analyzed cells**	**Mitotic index %**		**Division phases %**							
				Prophase		**Metaphase**		**Anaphase**		**Telophase**	
		Average	**SE**	**Average**	**SE**	**Average**	**SE**	**Average**	**SE**	**Average**	**SE**
C	4784	18,10	0,23	48,70	0,17	15,20	0,06	11,90	0,17	24,20	0,06
P1	5298	**12,40**^{000}	0,21	**58,43*****	0,09	**11,20**^{000}	0,10	11,80	0,40	**18,57**^{000}	0,35
P2	5358	**22,15*****	0,55	**61,80*****	0,35	**7,90**^{000}	0,46	11,50	0,46	**18,80**^{000}	0,58
P3	4174	**13,55**^{000}	0,09	**45,05**^{000}	0,09	**15,95***	0,20	12,55	0,20	26,12	0,41
P4	4304	**13,79**^{000}	0,02	**56,00*****	0,06	**12,85**^{000}	0,09	12,85	0,49	**18,30**^{000}	0,61
P5	4424	18,05	0,32	**53,85*****	0,20	**10,30**^{000}	0,12	**9,55**^{000}	0,29	26,30	0,06
P6	4356	18,37	0,43	**51,20*****	0,87	**12,70**^{000}	0,06	11,60	0,35	24,50	0,58

The calculated correlations prove that a small percentage of MI indicates that cells remain arrested in the prophase, other phases of division being reduced (Table 4). This phenomenon is due to the mitotic spindle inhibition or some metabolic disorders in G2, which induce delays in the chromatin fibers condensation.

Table 4. Correlation matrix between the MI and divison phases (r marked in bold is significant for p<0.0500).

	MI %	**Prophase%**	**Metaphase %**	**Anaphase %**	**Telophase %**
MI %	1.0000	0.2683	**-0.5197**	**-0.4359**	0.1269
	p= ---	p=0.240	**p=.016**	**p=0.048**	p=0.584
Prophase%	0.2683	1.0000	**-0.8929**	-0.1935	**-0.7949**
	p=0.240	p= ---	**p=0.000**	p=0.401	**p=.000**
Metaphase %	**-0.5197**	**-0.8929**	1.0000	**0.4795**	0.4614
	p=.016	**p=0.000**	p= ---	**p=0.028**	p=0.035
Anaphase %	**-0.4359**	-0.1935	**0.4795**	1.0000	-0.3962
	p=0.048	p=.401	**p=0.028**	p= ---	p=0.075
Telophase %	0.1269	**-0.7949**	**0.4614**	**-0.3962**	1.0000
	p=0.584	**p=0.000**	**p=0.035**	**p=0.075**	p= ---

Genotoxicity evaluation in mitotic division cycle. Most of the compounds from wastewater have mutagenic potential. They can act at the genes level by inhibiting the syntesis of some histonic proteins and inducing metabolic disorders, or at the chromosome level, producing chromosomal aberations, or they can affect the genome, inducing polyploidy or aneuploidy. In the samples collected from WWTP Timisoara all types of mutations mentioned before were observed. The highest percent of aberrant divisions, as well as metabolic disorders were observed in the sample with wastewater (P1- 14.7%), followed by anoxic denitrification (P3 -10.8%) (Table 5). In these samples, also the mitotic index was at the lowest values.

The metabolic disorders (MD) observed in the analysed samples are: the alteration of the condensation degree of chromatine fibers (premature chromosome condensation - PCC or delay in chromosome condensation - DCC), parallel disposal of the chromatine fibers with obvious euchromatine and heterochromatine bands (Fig. 2e), the suprachromosomal organisation of the genetic material in prophase, due to the links between telomers (sticky chromosomes), the chromatine fiber depolimerisation in metaphase and anaphase, a.o (Fig 2a, b, c). Most of the MD were observed in metaphase being represented by chromosome depolimerisation or agglutination. The highest percent of metaphase with MD was registered in variant P3 (38.6%), but also in variant P6 (20% - purified water). All experimental variants (with an exception P2) presented very significant positive differences in comparison with control.

Chromosomal aberrations (CA) are produced as a consequence of the action of free radicals and can be highlighted in the metaphase, but especially in anaphase and telophase: chromosome breakage and reunion of broken ends (ring chromosomes, dicentric chromosomes – bridges, arches), changes in chromosome structure or loss of genetic material (acentric fragments, minutes) (Fig 2d, f, g, h). The highest percent of fragments and bridges was recorded in variant P1 (38.3% in metaphase and 40.0 % in anaphase (Table 5). A high percent of CA was also observed in purified water (P6) in all division phases with a significant positive difference in comparison with Control.

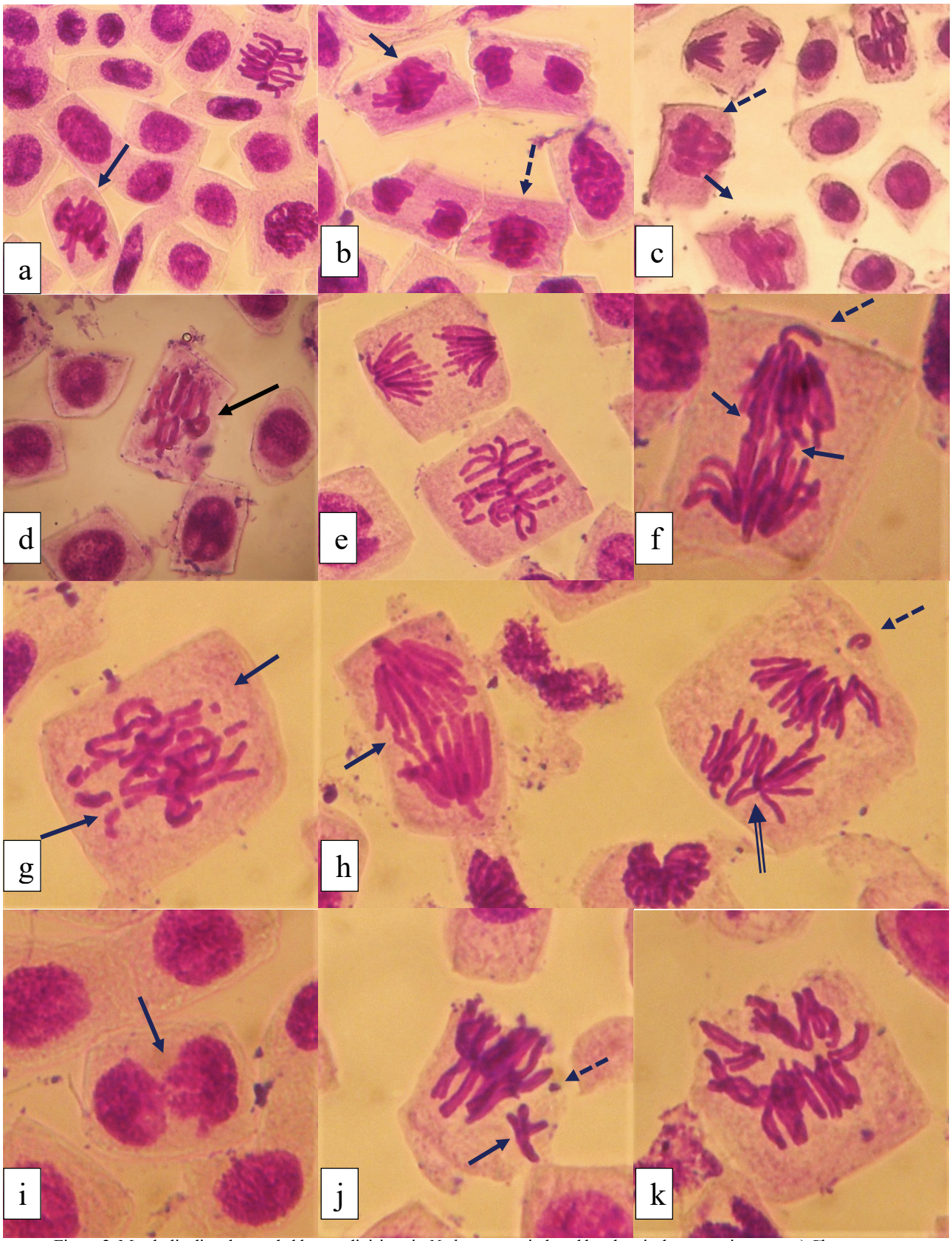

Figure 2. Metabolic disorders and abberant divisions in *N. damascena* induced by chemicals present in water: a) Chromosome depolimerisation in metaphase (P5) (arrow); b) Chromosome depolimerisation in metaphase (arrow) and in anaphase (dot arrow) (P5); c) Chromosome depolimerisation in metaphase (arrow) and in anaphase (dot arrow) (P4); d) Ring chromosome in metaphase (arrow) (P3); e) Banded chromosomes in anaphase (up) and in metaphase (down) (P3); f) Multiple bridges in anaphase (arrow) and a laggard chromosome (dot arrow) (P4); g) Fragments in metaphase (arrow) (P4); h) Multiple bridges in anaphase -left (arrow) and anaphase with a chromosome with centromere inactivation at one pole (dot arrow) and asincrony in migration on the other pole (double arrow) (P1); i) Binucleated cell with a chromatine bridge (arrow) (P6); j) Centromere inactivation (arrow) and a minute (dot arrow) in metaphase (P1); k) C-metaphase (P1).

Table 5. The percent of metabolic disorders, aberrant divisions and micronuclei in *Nigella damascena* L., in different experimental variants.

Exp. Var.	Total cells in mitosis %					MD%			Aberrant Divisions %									MN/ 1000 cells
	N	MD	Aberrant Divisions			P	M	A	Metaphase			Anaphase			Telophase			
			Tot	CA	GM				Tot	CA	GM	Tot	CA	GM	Tot	CA	GM	
C	97.9	0.7	1.4	1.2	0.2	0.5	3.1	0	0	0	0	9.8	7.8	2.0	2.9	2.9	0	0.5
P1	85.3	4.6	10.1	4.9	5.2	3.1	17.6	2.5	38.3	5.9	32.4	40.0	25.0	15.0	6.5	6.5	0	4
P2	95.3	0.3	4.4	2.4	2.0	0.3	2.1	0	26.0	21.7	4.3	22.0	19.1	2.7	0.9	0.9	1.8	3
P3	89.2	5.8	5.0	3.2	1.8	0	38.6	2.8	11.4	6.8	4.6	25.0	16.7	8.3	2.8	2.8	0	4
P4	93.0	2.5	4.5	1.0	3.5	1.4	17.0	0	4.9	0	4.9	31.6	7.9	23.7	1.9	0.9	1.0	2
P5	92.6	2.0	5.4	2.4	3.0	1.2	10.5	0	18.4	0	18.4	18.4	18.4	0	3.7	3.7	0	7
P6	91.4	3.0	5.6	5.1	0.5	0.5	20.0	4.3	8.0	8.0	0	25.5	23.4	2.1	7.6	6.5	1.1	1

N = normal; MD = metabolic disorders; P = Prophase; M = Metaphase; A = Anaphase; CA = chromosomal aberrations; GM = genomic mutations; MN = micronuclei.

Genomic mutations that were recorded in the analysed samples suggest that water samples contain heavy metals with an affinity to centromere and/or kinetocor inducing inactivation (Fig. 2h, j), like Ni or Cd (LEME & MARIN-MORALES, 2009), and compounds causing the inhibition or inactivation of the mitotic spindle, having as a result characteristic aspects of C-mitosis or binucleated cells (Fig 2i, k). The highest percent of GM were observed in variant P1 (wastewater -5.2%) and in sludge and sand samples.

All these genotoxic effects were mentioned in the last years in many research papers using the *Allium* test and are in agreement with our findings (FISKESJÖ, 1993; ATEEQ et al., 2002; LEME & MARIN-MORALES, 2009; BONCIU et al., 2018; SANTOS et al., 2019).

CONCLUSIONS

The lowest mitotic index was seen in waste water and sludge, with significant diferences in comparison with Control. Genomic and chromosomal mutations were observed in all experimental variants. The most common aberrations were fragments and bridges, and metabolic changes regarding the degree of compaction of chromatin fibers. Cells with 1-3 micronuclei, binucleated cells and multipolar spindle in mitosis cells were observed.

The purified water discharged into the Bega Channel presented a high genotoxic effect indicating the decay or the presence of by-products of the toxic compounds. The genotoxic effects were also observed in other test species, suggesting that physical and chemical analyses performed in wastewater treatment plants should be complemented by bioassays.

REFERENCES

ATEEQ B., FARAH M. A., ALI M. N., AHMAD W. 2002. Clastogenicity of pentachlorophenol, 2, 4-D and butachlor evaluated by Allium root tip test. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. University Press. London. **514**(1-2): 105-113.

DATTA A. K. & SAHA A. 2003. Cytomorphological studies and seed protein characterization of *Nigella sativa* L. and *Nigella damascena* L. *Cytologia*. The Japan Mendel Society Press. Tokyo. **68**(1): 51-60.

BONCIU E., FIRBAS P., FONTANETTI C. S., WUSHENG J., KARAISMAILOĞLU M. C., LIU D., MENICUCCI F., PESNYA D. S., POPESCU A., ROMANOVSKY A. V., SCHIFF S. 2018. An evaluation for the standardization of the Allium cepa test as cytotoxicity and genotoxicity assay. *Caryologia*. Taylor & Francis Press. New York. **71**(3): 191-209.

DULIO V. & VON DER OHE P. C. 2013. NORMAN Prioritization Framework for Emerging Substances. NORMAN Association Network of Reference Laboratories and Related Organisations for Monitoring and Bio-monitoring of Emerging Environmental Substances. *Working Group on Prioritisation of Emerging Substances, NORMAN Association Press*. Verneuil en Halatte Publisher. Paris: 212-234.

FERNANDES T. C. C., MAZZEO D. E. C., MARIN-MORALES E. A. 2007. Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa*, exposed to trifluralin herbicide. *Pesticide Biochemistry and Physiology*. Elsevier. Paris. **88**: 252-259.

FISKESJÖ G. 1993. The *Allium* test in wastewater monitoring. *Environmental toxicology and water quality*. Wiley Press. New York. **8**(3): 291-298.

HEMACHANDRA C. K. & PATHIRATNE A. 2015. Assessing toxicity of copper, cadmium and chromium levels relevant to discharge limits of industrial effluents into inland surface waters using common onion, *Allium cepa* bioassay. *Bulletin Environmental Contamination Toxicology*. Springer. Berlin. **94**(2): 199-203.

HEMACHANDRA C. K. & PATHIRATNE A. 2016. Combination of physico-chemical analysis, *Allium cepa* test system and *Oreochromis niloticus* erythrocyte based comet assay/nuclear abnormalities tests for cytogenotoxicity assessments of treated effluents discharged from textile industries. *Ecotoxicology and Environmental Safety*. Elsevier. Paris. **131**: 54-64.

HOWELL W. M., KELLER G. E., KIRKPATRICK J. D., JENKINS R. L., HUNSINGER R. N., MCLAUGHLIN E. W. 2007. Effects of the plant steroidal hormone, 24-epibrassinolide, on the mitotic index and growth of onion (*Allium cepa*) root tips. *Genetics Molecular Research*. Ribeirao Publisher. Madrid. **6**(1): 50-58.

LAM L., KURISU K., HANAKI K. 2015. Comparative environmental impacts of sourceseparation systems for domestic wastewater management in rural China. *Journal of Cleaner Production*. Elsevier. Paris. **104**: 185-198.

LEME D. M. & MARIN-MORALES M. A. 2009. *Allium cepa* test in environmental monitoring: a review on its application. *Mutation Research/Reviews in Mutation Research*. University Press. London. **682**(1): 71-81.

MALATO S. & ALBA A. R. F. 2009. Decontamination and disinfection of water by solar photocatalysis: recent overview and trends. *Catalysis Today*. Elsevier. Paris. **147**: 1-59.

MOUTSCHEN J., MOUTSCHEN-DAHMEN M., WOODLEY R., GILOT J. 1969. The Relative Biological Effectiveness of Different Kinds of Radiations on Chromosome Aberrations in *Nigella damascena* Seed. *International Journal of Radiation Biology and Related Studies in Physics, Chemistry and Medicine*. Martin Published. New York. **15**(6): 525-540.

MOUTSCHEN J., GILOT-DELHALLE J., MOUTSCHEN-DAHMEN M. 1987. Clastogenic effects of benzodiazepines in a Nigella damascena seed test. *Environmental and Experimental Botany*. Elsevier. Paris. **27**(2): 227-231.

PINTILIE L., TORRES C. M., TEODOSIU C., CASTELLS F. 2016. Urban wastewater reclamation for industrial reuse: an LCA case study. *Journal of Cleaner Production*. Elsevier. Paris. **139**: 1-14.

PONDHE G. M., PAWAR N. J., PATIL S. F., DHEMBANE A. J. 1997. Impact of sugar mill effluent on the quality of ground water. *Pollution Research*. Springer. Berlin. **16**: 191-195.

PRASSE C., STALTER D., SCHULTE-OEHLMANN U., OEHLMANN J., TERNES T. A. 2015. Spoilt for choice: a critical review on the chemical and biological assessment of current wastewater treatment technologies. *Water Research*. Elsevier. Paris. **87**: 237-270.

RADIĆ S., STIPANIČEV D., VUJČIĆ V., RAJČIĆ M. M., ŠIRAC S., PEVALEK-KOZLINA B. 2010. The evaluation of surface and wastewater genotoxicity using the *Allium cepa* test. *Science of the Total Environment*. Elsevier. Paris. **408**(5): 1228-1233.

SANTOS FILHO R. D., VICARI T., SANTOS S. A., FELISBINO K., MATTOSO N., SANT'ANNA-SANTOS B. F., CESTARI M. M., LEME D. M. 2019. Genotoxicity of titanium dioxide nanoparticles and triggering of defense mechanisms in *Allium cepa*. *Genetics and Molecular Biology*. AHEAD Press. London: 28-34.

SEN B. & CHANDRA T. S. 2007. Chemolytic and solid-state spectroscopic evaluation of organic matter transformation during vermicomposting of sugar industry wastes. *Bioresource of Technology*. Elsevier. Paris. **98**: 1680-1683.

STAYKOVA T. A., IVANOVA E. N., VELCHEVA I. G. 2005. Cytogenetic effect of heavy-metal and cyanide in contaminated waters from the region of southwest Bulgaria. *Journal of Cell and Molecular Biology*. Springer. Berlin. **4**(1): 41-46.

***. https://www.aquatim.ro/parametri-de-calitate-ai-apei-epurate-2798.html (Accessed February, 2019).

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