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Research Article

The Effect of Treatment with SBR Technology in the Central Wwtp Domžale-Kamnik (Slovenia)

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KEYWORDS

Wastewater treatment plant

SBR technology

Domžale-Kamnik Central WWTP

Allium Metaphase Assay

ABSTRACT

The effectiveness of treatment using sequential biological reactor (SBR) technology is demonstrated at the Domžale-Kamnik Central wastewater treatment plant (WWTP). The tests were done with the Allium metaphase test and show the degree of genotoxicity, by observing the aberration of the metaphase chromosomes of the onion plant Allium cepa L. (A. cepa). The results were compared with final treated effluent (FTE) samples and river samples before and after the outflow treated water. The third river sample looks like a negative control (−K), since the sample does not show toxic and genotoxic load. With this, we determined the: (i) effects of treatment at the WWTP and, (ii) as a result, the quality of the Kamniška Bistrica (KB) river after the outflow of water from the WWTP. The lower the level of cytotoxicity and genotoxicity in wastewater, the fewer damaged chromosomes in the chromosome set test plant onion A. cepa, the greater the treatment effect in the WWTP.

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1. Introduction

The toxicity and genotoxicity profile of a complex mixture of waste water (WW) produced by industry and urbanization was tested with the test onion plant *Allium cepa* L. (*A. cepa*) by means of physico-chemical (or chemical) analysis including root length inhibition and malformation (general toxicity) and induction of chromosome aberration in root cells (genotoxicity). In addition, there are significant acute and chronic effects on the ecosystem and public health due to the direct discharge of toxic and genotoxic substances and other contaminants of rising concern into the aquatic ecosystem [1]. Microbial bioremediation, physicochemical treatments and hybrid approaches would all be highlighted as potential means of degradation and removal of xenobiotics [6]. Different remediation procedures can be used to remove toxic and genotoxic substances (xenobiotics). Different combinations of hybrid techniques can be used for their effective removal and/or decomposition [32]. However, a well constructed and well working wastewater treatment plant (WWTP) with various attractive coremediation technologies, can solve these problems.

The WW that flows to the Domžale-Kamnik Central WWTP is also increasingly loaded with synthesized organic substances that we humans use on a daily basis. Domžale-Kamnik Central WWTP represents the world's top technique of simultaneous wastewater treatment. It achieves this with an aerobic biological stage using sequential biological reactor (SBR) technology with anaerobic selectors for the biological removal of phosphorus substances and a new deammonification process. Deammonification is an intensive treatment process for WW containing high concentrations of nitrogen substances [20].

Among others, the *A. cepa* is an excellent in vivo model, suitable for both the toxicity and genotoxicity evaluation [29]. *A. cepa* is considered a good experimental and bioindicator model due to its large chromosomes and low number of chromosomes (2n = 2x = 16). The use of colchicine during chromosome preparation destroys spindle microtubules, allowing for better observation of only metaphase chromosomes [17]. In this regard, the *Allium* test method can be divided into the *Allium* A-T anaphase-telophase (A-T) test without colchicine with defects in the mitotic process, and the *Allium* metaphase (M) test - with colchicine for chromosome and chromatid damage [16].

In addition, *A. cepa* chromosomes exhibit morphological similarities between plant and vertebrates (Firbas and Amon 2021) a feature that provides an excellent correlation between mammalian cell systems to human beings [27,28].

The *A. cepa* root tip chromosome aberration assay has been adopted by the International Program on Plant Bioassay (IPPB) for monitoring or testing environmental pollutants since the late 1990 s (Ma 1999). *A. cepa* is still widely used to determine the cytotoxicity and genotoxicity of water and soil contaminated by pesticides, herbicides, drugs [8], fungicide [3], biosorbents in industrial wastewater treatment [30], endocrine-disrupting chemical such as phenols [31]. For all these reasons, the *A. cepa* test has been the standard test for chemical, drinking water and environmental studies for decades [24,14,34].

2. Material and Methods

2.1. Description of the Water System of the River Kamniška Bistrica

The river Kamniška Bistrica (KB) is a left tributary of the river Sava, which is a right tributary to the Danube river. It is 32.8 km long and has a torrential character from its spring in the Alps to its confluence with the Sava river. The river is spread over 535 km^2 of mountain and plain area. Because of the construction and housing activities the river has been regulated into a moderately narrow channel running through the towns Kamnik and Domžale. In other areas the river is mainly untouched.

2.2. Domžale-Kamnik Central WWTP, Slovenia

Upgrade in year 2016 include construction of a new aerobic biological rate of achievement of tertiary treatment by SBR technology and the construction of the input object for the reception of large quantities of waste water and appropriate mechanical pre-treatment, which will increase operational safety. After upgrading the WWTP fourth largest system for wastewater treatment in Slovenia, and ensuring the quality parameters of treated water for discharge into the watercourse, the river Kamniška Bistrica. The capacity of the upgraded WWTP is 149,000 Population Equivalent (PE), which means that it will accept the waste water of all residents in the reception area and other waste water. PE is a measure of the maximum organic load a sewage treatment plant can process, expressed in terms of a fixed population's contribution to sewage.

2.3. Collection of Water Samples

Natural samples: river fresh water. The study area, which is Kamniška Bistrica (KB) river partially lies between latitude 46° 19'N and 46° 04'N and longitude 14° 34'E and 14° 37'E.

Biotechnological samples: samples for all different stages of WW treatment. *Wastewater*: complex mixtures of municipal, industrial (pharmaceutical, textile, food processing, dyes-paints, timber industry, laundry textile), and rain water so called inflow. Inflow to Domžale-Kamnik central WWTP has a heterogeneous composition composed of municipal, industrial and rain water. *WW treatment after mechanical stage*: sand trapping and solid separation. *Final treated effluent*: treated water from Domžale-Kamnik central WWTP so called outflow.

The detailed description of sampling locations

I. Kamniška Bistrica – Drinov rob (as a negative control in the natural environmental pattern)
II. Kamniška Bistrica – village ŠTUDA (950 m upstream central WWTP – discharge point)
III. WW inflow
IV. WW after solid separation (WW treatment after mechanical stage)
V. FTE outflow from SBR
VI. Kamniška Bistrica – village BIŠČE (1750 m downstream central WWTP) Terms of monitoring and sampling.

Research and sampling took place from 2021 to 2025

We analyzed a total of six samples, three of which were from the Domžale-Kamnik central (DKC) WWTP and three from the Kamniška Bistrica river, and determined the effect of treatment on the WWTP and the resulting impact of the effluent from the DKC WWTP.

At each of these, samples were taken five times. Measurements were carried out in July 2021, September 2022, the third in June 2023, the fourth sampling in August 2024 and the fifth in July 2025. Three experimental areas were selected, namely the Študa area and the Bišče area, which were compared with the Drinov rob locations without the influence of pollution, as a control area.

First sampling: Allium M test and physical analysis on 8. 7. 2021; WW and FTE samples averaged over 24 hours from 8:00 on 7 July 2021 to 8:00 on 8 July 2021 and three environmental samples on the Kamniška Bistrica river.

Second sampling: *Allium* M test and physical analytics 05. 09. 2022; sample WW and FTE is 24 h on average from 8:00 on 04. September 2022 to 8:00 04. September. 2022 and three environmental samples on the Kamniška Bistrica river.

Third sampling: *in vivo Allium* M test and physical analytics 25. 06. 2023; sample WW and FTE is 24 h on average from 24. June to 25. June 2023 and three environmental samples on the Kamniška Bistrica river.

Fourth sampling: *in vivo Allium* M test and physical analysis13. 08. 2024; sample WW and FTE is 24 h on average from 21. August. to 22. August 2024 and three environmental samples on the Kamniška Bistrica river.

Fifth sampling: *in vivo Allium* M test and physical analysis15. 07. 2025; sample WW and FTE is 24 h on average from 15. July to 16. July 2025 and three environmental samples on the Kamniška Bistrica river.

3. Physical and Chemical Analyses

For the measuring of water physical and chemical analyses we collected all six samples. Here three are natural river samples and three biotechnological samples. Samples of river water and biotechnological samples were collected from the sites, stored in bottles with thermostable boxes and transferred to the laboratory. Chemical parameters including: the metal/metalloid and organic component varied depending on the industrial profile. Physical parameters including: temperature, alkalinity (pH), electrical conductivity (EC), total suspended solid (TSS), dissolved oxygen (DO), nutrients (nitrogen, phosphor), Kjeldahl nitrogen (KN), chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) were determined in accordance with standard analytical methods [4].

Water physical parameters such as temperature, pH, EC, DO and TSS were measured *in situ* using HACH electrodes; TSS by (with) gravimetry; Nitrate N and nitrite N by Segmented Flow Analysis (SFA); Ammonia by Ion selective electrode (ISE) or Spectrophotometry; and Kjeldahl N (with the composite method Digestion – Distillation – Titration). Total phosphorus, total nitrogen and COD were determined with spectrophotometry and BOD_5 with Volumetry (VOL) in incubation bottles.

3.1. Test Organisms Used as Bioindicators Plants

Plant: Onion *A. cepa*, (Stuttgarter Riesen). Small onion bulbs of the same uniform size, weighing about 3 – 3.5 g, were denuded by removing the loose outer scales and scraped so that the root primordia were immersed into the different tested liquids. Most studies do not mention the characterization of the onion variety used in cytotoxic testing, except for references to the *A. cepa* variety (Stuttgarter Riesen). Therefore, we can assume that there is no general rule about the genetic material used, for example, variety, clone or cultivar [8].

3.2. Onion Plant as a Cytotoxicity and Genotoxicity Assay Allium M Assay

Five onion bulbs are left to grow in the sample water for 72 hours. Then first the macroscopic morphological parameters are observed – the length of roots (showing the general toxicity and/or cytotoxicity), their shape, number, color and degree of malformation (Figure 1).

The genotoxicity level (GL) on microscopic observation is a general term referring to alteration to the gross structure or content of chromosome damage by exposure to toxic agents [9]. GL is defined by the percentage between all the metaphase cells and the cells with their chromosomes damaged. 200 random chosen metaphase cells (with well-recognized chromosomes) originate from the sample composed of ten root apex cells taken from five onion bulbs - two roots from each onion bulb.

The tests were done with the *Allium* metaphase (M) or *Allium* chromosome damage (CsD) test and show the degree of genotoxicity by observing the aberrations of the exclusively metaphase chromosomes of the plant *A. cepa* that are evoked by genotoxic substances in the polluted water [15,16].

Chromosome preparations were set up from root meristems containing actively growing cells by the following method: developing roots with bulbs were pre-treated with 0.05 % aquatic solution of colchicine for 3 hours at 21 ^{0}C. After washing in distilled water for 20 min the terminal developing roots of 2 mm length were fixed for 1h in methanol:propionic acid mixture (3:1 or 1:1). Then they were macerated and stained in order to obtain a cellular suspension. This sample was stained with 0.5 % aceto-carmine for 4-5 minutes at 60 ^{0}C without hydrolysis, and squashed in aceto-carmine [12]. The optical microscope used in the investigation was the Olympus – BX 41 (Japan) with the photo system PM 10 SP, typical magnifications used were 400x and anisole-immersion 1.000x.

3.3. Karyotypization of Young Onion A. Cepa Chromosomes

Before analyzing chromosome damages, the general characteristics of chromosome morphology should be mentioned, as they allow us to clarify the mechanisms of different chromosome damage origins. The common onion *A. cepa* has 16 monocentric chromosomes (Figure 2). A chromosome is formed by two parallel strands or chromatids of equal length, each representing the longitudinal half of the chromosome. The chromosome has a primary constriction (centromere), dividing the chromosome into long and short arms. Chromosome arms may be of the same length. The ratio between the lengths of the arms gives the form of chromosomes and is the basis for chromosomal karyotypization [25,13].

The possible aberrations seen at metaphase are: chromosome break, chromatide break and centromere break [16]. The cell is called aberrant if at least one chromosome gets damaged (Figure 3A).

Chromosome set damage affects one or two chromosomes, perhaps 3 to 7 or 8 (Figure 3B), and in rare cases up to 12 (Figure 3C). However, all chromosomes in a chromosome set can be damaged (Figure 3D). The higher the level of genotoxicity, the more chromosomes in the set are damaged. Within a set, there can also be multiple different damages to a single chromosome.

The following chromosomal aberrations were observed: the most frequent are chromosome breaks in the primary constriction (centromere region); in a chromosomal set a maximum of two chromosomes is damaged. In positive control samples single-strand and double-strand chromatide breaks were also observed as well as ring and dicentric chromosomes (Figure 4).

The lower the level of cytotoxicity and genotoxicity in wastewater, the fewer damaged chromosomes in the set, the greater the treatment effect in the WWTP [17].

3.4. Statistic Calculation

Statistically established significant differences among the investigated samples are confirmed by the statistical calculation of paired data analysis using the two-way Fisher's exact test, which gives the *p*-value property between pairs of data calculated for a 2x2 contingency table [2].

4. Results and Discussion

The present work has been done in order to evaluate the cytotoxic and genotoxic effects of WW and FTE on different sites in river Kamniška Bistrica using the physicochemical analysis on *Allium* M assay. The results are shown in Tables 1 to 5 and Figures 5 to 11.

4.1. Phytotoxicity Analyses

The results of general toxicity are given in Table 1 to 5 and Figures 5 to 8. General toxicity (phytotoxicity – root length of test plants) does not differ in natural river samples (Sample I, II, VI) and is not statistically significant ($p > 0.05$). All river water samples with the average root length of the test plant *A. cepa* are statistically significantly different from the biotechnological inflow samples (Sample III), the sample after the mechanical cleaning stage (Sample IV), but do not differ from the effluent sample (Sample V). The inflow sample at the CČN (Sample III) has the same root length and does not differ in comparison after mechanical cleaning (Sample IV). The final effluent (Sample V) does not significantly differ from the river samples (Sample I, Sample II, Sample VI).

4.2. Genotoxic Analyses

Table 1 to 5 and Figures 9 to 11 shows the results of the *Allium* M test analysis for the waste water (WW) and final treated effluent (FTE) from the central Domžale-Kamnik WWTP and the Kamniška Bistrica (KB) river. The *Allium* M results show a higher level of genotoxicity (chromosomal damage in root cells), namely upstream before the effluent from the central WWTP (KB village Študa) 6.51 percentage points (3.00 percentage points in 2024) and downstream after the effluent from the central WWTP (KB village Bišče) 6.42 percentage points (3.00 percentage points in 2024) compared to the location of KB Drinov rob, where the genotoxicity level is 2.00 percentage points (2.00 percentage points in 2024); which means that Kamniška Bistrica is already somewhat polluted at the Študa location, but the difference is not statistically significant ($p = 0.2840 > 0.05$). The genotoxicity level of the influent to the central WWTP (Sample III) after treatment in the SBR decreases from 11.25 percentage points (12.50 percentage points in 2024) through the mechanical treatment stage (Sample IV) to 7.00 percentage points (9.00 percentage points in 2024) ($p = 0.4703 > 0.05$), and by the final treatment stage the effluent from the central WWTP (Sample V) decreases further to 2.72 percentage points (7.00 percentage points in 2024) ($p = 0.3375 > 0.05$). After the mechanical stage, the level of genotoxicity decreases, while the level of phytotoxicity remains more or less constant, which is reflected in the length of the roots of the test plants.

Table 1. United parameters physico-chemical quantities; cytological effects of the investigated samples survey – the genotoxicity level (damage to chromosomes) and general toxicity - phytotoxicity (root length inhibition) on the test onion plant *A. cepa*. First sampling July 2021.

Sample sites		K2 – Drinov rob (Negative control)	K4 – Študa	WW – WWTP	After mechanical step	FTE – WWTP	K4 – Bišče
Parameter	Unit	Physico-chemical analyses					
Water temperature	^0C	7.9	14.3	-	-	-	13.8
pH	-	8.1	8.3	7.8	7.7	7.7	7.4
Electrical conductivity	µS/cm	176	299	1036	985	964	556
Dissolved oxygen	mg/l	11.40	9,83				10.00
To subside 1 h	ml/l	<0.10	<0.10	15	-		<0.10
To subside 2 h	ml/l	<0.10	<0.10	14			<0.10
TSS - 1µm	mg/l					0.97	
Ammonia NH_4—N	mg/l	0.02	0.05	25.2	22.0	3.1	0.027
Kjeldahl N	mg/l			40.8	28.6	8.1	
Nitrate NO_3—N	mg/l	0.41	0.85	0.28	0.25		3.10
Nitrite NO_2—N	mg/l	-	-		-		-
Total N	mg/l	0.85	1.5	34.0	-	6.3	-
Total P	mg/l	0.05	0.05	5.7	3.9	0.93	0.14
COD	mg/l	<5.0	<5.0	494	115	25.1	<5
BOD_5	mg/l	<3.0	<3.0	275	70	7	<3.0
Allium metaphase (M) test							
Phytotoxicity	mm	36.2±2.7	35.8±1.5	12.2±1.2	22.0±1.1	33.6±1.7	35.6±2.4

Genotoxicity	GL – %	2.17±0.3	6.31±0.5	20.76±2.6	12.77±1.3	6.80±0.9	6.33±0.5

Legend. KB: Kamniška Bistrica river, K2, K4: samples area, WW: wastewater, FTE: final treated effluent, WWTP: Domžale-Kamnik Central Wastewater Treatment Plant, mm: length of the root, %: chromosome damage (CsD) per 100 cells, GL: Genotoxicity level, %: CsD per 100 metaphase cells.

Table 2. United parameters physico-chemical quantities; cytological effects of the investigated samples survey – the genotoxicity level (damage to chromosomes) and general toxicity - phytotoxicity (root length inhibition) on the test onion plant *A. cepa*. Second sampling September 2022.

Sample sites		Drinov rob K2	Študa K4	WW – WWTP	After mechanical step	FTE – WWTP	Bišče K4
Parameter	Unit	Physico-chemical analyses					
Water temperature	^0C	8,3	17,8	-	-	-	15,0
pH	-	8,4	8,3	7,7	8,4	7,8	7,7
Electrical conductivity	µS/cm	219	380	1065	1558	1016	573
Dissolved oxygen	mg/l	12,2	9,9	-	-	-	8,6
To subside 1h	ml/l	0	0	20	1	0	0
To subside 2h	ml/l	0	0	20	1	0	0
TSS - $1\mu m^2$	mg/l	2	4,7	361	132	2,6	2
Ammonia NH_4—N	mg/l	0,04	0,05	35,7	59	0,37	0,17
Nitrate NO_3—N	mg/l	0,59	0,88	0,36	0,34	8,6	3,2
Total N	mg/l	1	2,1	53,3	69,8	9,7	4
Total P	mg/l	0,05	0,13	8,1	7,8	0,75	0,13
COD	mg/l	5	14,7	777	348	22,5	5,8
Allium metaphase (M) test							
Phytotoxixity	mm	38,4	32,6	11,2	12,2	29,8	37,2
Genotoxicity	GL – %	3,55	6,07	25,50	13,63	6,19	5,71

Legend. KB: Kamniška Bistrica river, K2, K4: samples area, WW: wastewater, FTE: final treated effluent, WWTP: Domžale-Kamnik Central Wastewater Treatment Plant, mm: length of the root, %: chromosome damage (CsD) per 100 cells, GL: Genotoxicity level, %: CsD per 100 metaphase cells

Table 3. United parameters physico-chemical quantities; cytological effects of the investigated samples survey – the genotoxicity level (damage to chromosomes) and general toxicity - phytotoxicity (root length inhibition) on the test onion plant *A. cepa*. Third sampling June 2023.

Sample sites		Drinov rob	Študa	WW – WWTP	After mechanical step	FTE – WWTP	Bišče Videm
Parameter	Unit	Physico-chemical analyses					
Water temperature	^0C	7,3	13,0	10,7		18,6	13,5
pH	-	8,4	8,2	7,5	7,6	7,7	8,0
Electrical conductivily	µS/cm	187	305	1096	1310	967	347
Dissolved oxygen	mg/l	11,75	10,67	2,21		4,92	10,28
To subside 1 h	ml/l	0					
To subside 2 h	ml/l	0	<0,1	18	0,5	<0,1	<0,1
TSS - 1µm	mg/l		3,7	393		4,3	4,7
Amonia N	mg/l	0,03	0,05	32,8	35,8	0,46	0,07
Nitrate N	mg/l	0,61	0,97	0,43	0,34	5,78	1,25
Total N	mg/l	1,0	1,26	52,4	46	7,73	1,7
Total P	mg/l	<0,05	0,06	8,4	5,1	0,84	0,11
COD	mg/l	<5	<5	768	300	26,6	7,4
BOD_5	mg/l	<3	<3	395	165	5	<3
Allium metaphase (M) test							

Phytotoxixity	mm	35.0	33.0	10.8	14.0	29.0	32.0
Genotoxicity	GL – %	1.50	5.76	24.0	13.33	8.57	6.66

Legend. KB: Kamniška Bistrica river, K2, K4: samples area, WW: wastewater, FTE: final treated effluent, WWTP: Domžale-Kamnik Central Wastewater Treatment Plant, mm: length of the root, %: chromosome damage (CsD) per 100 cells, GL: Genotoxicity level, %: CsD per 100 metaphase cells.

Table 4. United parameters physico-chemical quantities; cytological effects of the investigated samples survey – the genotoxicity level (damage to chromosomes) and general toxicity - phytotoxicity (root length inhibition) on the test onion plant *A. cepa*. Fourth sampling August 2024.

Sample sites		Drinov rob	Študa	WW – WWTP	After mechanical step	FTE – WWTP	Bišče
Parameter	Unit	Physico-chemical analyses					
Water temperature	^{0}C	9,1	17,2	19,3		21,5	17,1
pH	-	8,3	8,3	7,7	7,8	7,8	7,7
Electrical conductivily	µS/cm	204	336	1116	1161	994	417
Dissolved oxygen	mg/l	11,2	9,3				9,39
To subside 1 h	ml/l	0	0				0
To subside 2 h	ml/l	0	0	14	0,5	<0,1	0
Amonia N	mg/l			33	47	1,1	
Nitrate N	mg/l	0,58	0,97	0,42	0,37	6,3	2,1
Total N	mg/l	<1	1,1	47,6	53,7	<10	2,4
Total P	mg/l	<0,05	<0,05	8,9	5,7	0,95	<0,05
COD	mg/l	<5	<5	608	212	27	<5
BOD_5	mg/l	<3	<3	315	103		<3
Allium metaphase (M) test							
Phytotoxixity	mm	41.0	40.0	12.0	20.0	30.0	40.0
Genotoxicity	GL – %	2.0	3.0	12.50	9.0	7.0	3.0

Legend. KB: Kamniška Bistrica river, K2, K4: samples area, WW: wastewater, FTE: final treated effluent, WWTP: Domžale-Kamnik Central Wastewater Treatment Plant, mm: length of the root, %: chromosome damage (CsD) per 100 cells, GL: Genotoxicity level, %: CsD per 100 metaphase cells.

Table 5. United parameters physico-chemical quantities; cytological effects of the investigated samples survey – the genotoxicity level (damage to chromosomes) and general toxicity - phytotoxicity (root length inhibition) on the test onion plant *A. cepa*. Fifth sampling July 2025.

Sample sites		Drinov rob	Študa	WW – WWTP	After mechanical step	FTE – WWTP	Bišče
Parameter	Unit	Physico-chemical analyses					
Water temperature	^{0}C	10,7	17,1	20,7	23,6	20,1	17,3
pH	-	8,2	8,2	7,4	7,4	7,6	7,7
Electrical conductivily	µS/cm	207	356	1.065	1.172	954	450
Dissolved oxygen	mg/l	12,34	11,19				12,13
To subside 1 h	ml/l	0	0				0
To subside 2 h	ml/l	0	0	19	1	0	0
Amonia N	mg/l			29,3	33,7	0,95	
Nitrate N	mg/l	<0,23	<0,23	0,42	0,43	1,9	0,57
Total N	mg/l	<1	1,4	56,6	47,1	5,6	2,7
Total P	mg/l	<0,05	<0,05	8,6	7,6	0,66	<0,05
COD	mg/l	1,4	6,3	839	427	35	5,3
BOD_5	mg/l	<3	<3	420	195	11	<3
Allium metaphase (M) test							
	mm	35	32	10	10	32	33
	GL – %	2.00	6.15	11.25	7.00	2.72	5.38

Legend. KB: Kamniška Bistrica river, K2, K4: samples area, WW: wastewater, FTE: final treated effluent, WWTP: Domžale-Kamnik Central Wastewater Treatment Plant, mm: length of the root, %: chromosome damage (CsD) per 100 cells, GL: Genotoxicity level, %: CsD per 100 metaphase cells.

Figure 1. Examples of series of onions (*A. cepa*) cultivated for 72 hour in different concentration MMS: 100 ppm (Figure 1a), 10 ppm (Figure 1c), 1 ppm (Figure 1e), 0.1 ppm (Figure 1f), 0.01 ppm (Figure 1d) and 0.001 ppm (Figure 1b).

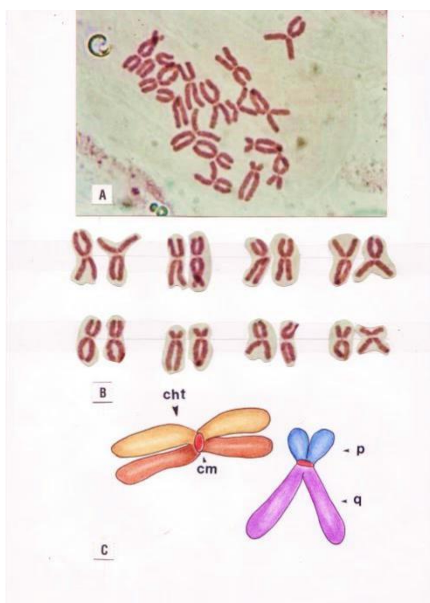

Figure 2. (A) Diploid metaphase chromosome; (B) its karyotype from the root cell of onion (*A. cepa*), containing 2n of 16 (2n = 16) with 6m, 8sm and 2st chromosomes; (C) metaphase chromosome with centromere (cm) dividing the chromosome into long (p) and short (q) arm. Arms may be of the same length.

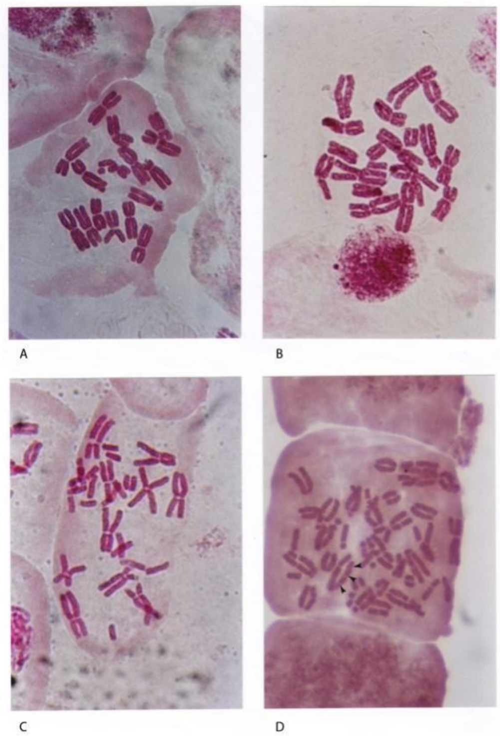

Figure 3. Different number chromosome damage in metaphase cells obtained from the meristeme root-type cells of onion (*A. cepa*): one damaged chromosome (3A), four damaged chromosome (3B), eight damaged chromosome (3C), and whole chromosome set is damaged (3D)

Figure 4. Damage chromosome: ring chromosome (left) and dicentric chromosome (right).

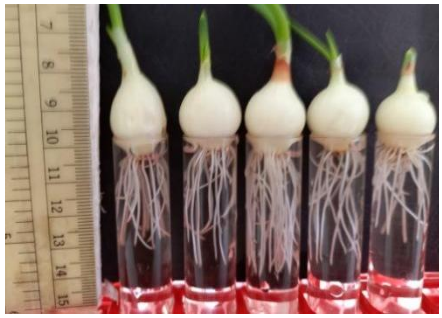

Figure 5. General toxicity (growth inhibition and root malformations) in the test plant *A. cepa* with the lokation Drinov rob of the river Kamniška Bistrica as an negative control (–K).

Figure 6. General toxicity (growth inhibition and root malformations) in the test plant *A. cepa* with the WW samples (left) and samples after mechanical step (right).

Figure 7. General toxicity (growth inhibition and root malformations) in the test plant *A. cepa* with the final treated effluent (FTE) samples.

Figure 8. General toxicity (growth inhibition and root malformations) in the test plant *A. cepa* with the location Študa before of the WWTP (left) and location Bišče after WWTP (right).

Figure 9. Undamaged chromosomes of *A. cepa* in the meristeme root cell.

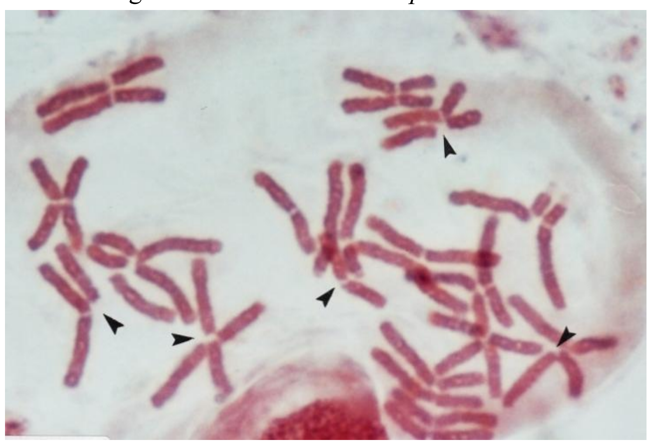

Figure 10. Several chromosomes are damaged (arrow).

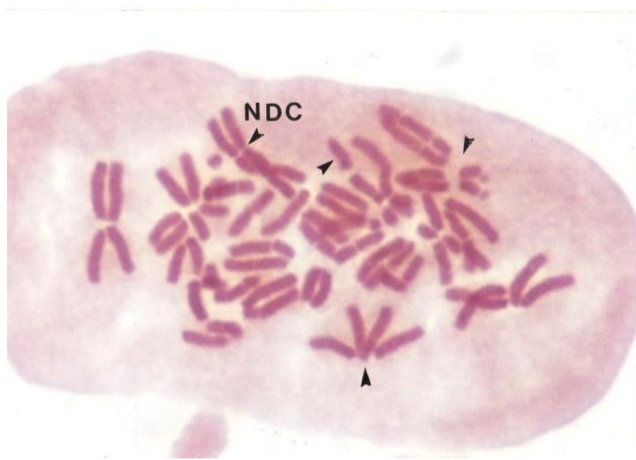

Figure 11. All chromosomes in the set are damaged, except one (no damaged chromosome – NDC).

WW treatment is an important process of considerable significance for the environment. In the eco-genotoxicology the samples are subjected to the physical and chemical analysis as well as to the genotoxicity tests [18,22]. As the water leaves the WWTP it flows into the river and again becomes an integral part of the ecosystem [36]. Our results show how important is the effectiveness of this WWTP along with the continual monitoring including the study of all necessary parameters [7].

Domžale-Kamnik central WWTP after the upgrade in 2016 represents the superb state of the technology of modern wastewater treatment in the world and high-quality clean waste water and achieves a high cleaning effect. The modernization also included additional system comprises three main process blocks: (i) a new inlet that complements the existing mechanical stage, (ii) a new aerobic biological stage with advanced SBR technology with anaerobic selector for partial biological phosphorus removal and a new de-ionization process, (iii) the existing anaerobic biological stage with the production of biogas and cogeneration [35].

As the environmental discharge standards are getting more advanced, the traditional (continuous flow-based) WW treatment process faces severe challenges. It has become inevitable to include tertiary treatment units for nutrient removal from WW. SBRs due to its operational flexibility and excellent process control possibility are being extensively used for the treatment of WW which nowadays is fast becoming contaminated with newer and more complex pollutants [26]. Some WWTP with SBR may use additional steps such as nitrogen or phosphorus removal as well as biological nutrient removal. This third and last step in the basic wastewater management system consists mainly of removing phosphates and nitrates. Nitrogen and phosphorus have become the key factors leading to eutrophication of receiving water. While achieving simultaneous nitrogen and phosphorous removal, biological methods play an important role in treating municipal and/or industrial wastewater such as SBRs [5].

The character of WWs effluents varies greatly, depending on the nature of the specific industry involved, both in terms of the likely BOD_5 loading of any organic components and the type of additional contaminants which may also be present. Accordingly, the chemical industry may offer WWs with high COD and rich various toxic compounds as another high BOD_5 that effluent contains [31]. Parameters of the COD, BOD_5 and TSS properties are the key parameters for the standardized monitoring of the cleaning process in the WWTP and at the same time they show how much the environmental picture gets modified after mixing with the effluent of the WWTP. The correlation between COD, BOD_5 and TSS properties is linearly proportional to the results obtained from the genotoxicity tests [15].

Test systems need to be developed on the basis of criteria that allow a realistic assessment of GL and are of major ecological importance in environmental screening and monitoring at the cell, organism, population and ecosystem levels. Many toxic and potentially toxic chemical substances, some of natural origin and others due to human activities, are released into the environment daily [37]. It has been shown that the chemical analysis alone is not enough to assure that the effluent water is really clean. To protect human and ecosystem health, it is necessary to perform also the biological analysis and to develop sensitive assays and to identify responsive cells and species and their life stages [38].

Very little of *Allium* test studies are focused on clastogenic and/or aneugenic effects, thus, chromosome damage and chromosome number changes in the chromosome set [19]. The use of colchicine during chromosome preparation destroys microtubules, but influencing the chromosome movement, increased frequency of metaphase with arranged condensed chromosomes and reduced transition from metaphase to anaphase, allowing a better observations of the exclusively metaphase chromosome. However this research strategy is provided by the *Allium* metaphase (M) test. Chromosome preparation is a key and crucial step in all cytogenetic techniques [23], and cytogenetic assay are classical method to detect chromosome damage (aberration, anomalies).

The metaphase chromosome anomalies as detected in the *Allium* M test procedure are not excluded to occur also in the human chromosomes when exposed to similar pollutants. Plant cytogenetic, using *Allium* M test, identifies same chromosome damages as they are identified in human cytogenetic such as: chromosome and chromatid damages, dicentric chromosomes, aneuploidy, euploidy and translocation [16,33]. Mentioned chromosome aberrations cause clinical defects on human body [21,10]. The study of DNA damage at the chromosome level is an essential part of genetic toxicology because chromosomal mutation is an important event in carcinogenesis [11].

Enclusion

Waste valorization involves reusing and recycling waste materials to create useful products such as wastewater. The treated water is then returned to the receiving watercourse in the environment.

The purpose of wastewater treatment plants (WWTP) is to effectively treat municipal, industrial and stormwater wastewater, regardless of changing environmental conditions. This important process is of considerable importance for the environmental-economic and social aspect of sustainability. Existing wastewater treatment plants are constantly being upgraded to better handle changes in wastewater flow and composition, reduce operating costs, and implement newer and more stringent regulatory standards regarding wastewater discharge restrictions. In environmental monitoring biomarkers are biochemical and cellular responses measured in native organisms that show evidence of exposure and/or effects to pollutants. Ecotoxicological bioassays test the toxicity (cytotoxicity, genotoxicity) of environmental samples according to strictly standardized procedures, using sensitive species and life stages representative of the ecosystems to be protected. Alcality, salinity, sulfide or ammonia are confounding factors that may cause positives in the bioassays. With physico-chemical and chemical analysis, we identify a diverse set of chemicals and physico-chemical properties of the aquatic environment. The ecotoxicological strategy of bioassays gives us the effect of xenobiotics in the test organism (e.g. chromosomal damage in the test organism), or organisms living in a particular biotope. So, physico-chemical analyses determine the quality of the environmental sample, while biological tests determine the possible toxic or genotoxic effect in the organism.

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Data Availability

Photos are the property of the author, physicochemical analyses were performed in the laboratory of Central Domžale-Kamnik WWTP.

Conflicts of Interest

No potential conflict of interest was reported by the authors

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