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CYTOGENOTOXIC POTENTIAL OF SURFACE WATER – A CASE STUDY OF ARGEŞ RIVER, ROMANIA

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Abstract
The current study presents the research on the cytogenetic potential of water samples taken from the Argeş River, Romania. The water samples were collected from 7 sites selected dependant on the anthropic impact to which they were subjected. The genotoxic and cytotoxic potentials of the water samples were evaluated using Allium cepa L. as a test organism. The statistical interpretation of results indicated a significant reduction of the mitotic index and an important increase in the frequency of chromosomal and mitotic aberrations for most of the water samples taken for study.

Keywords: aberrations, Allium, mitotic index, surface water.

1. INTRODUCTION

Running waters are the most important resource for the development of human settlements. The global and local growth of the actual human population has exerted a high pressure on the potable water resources, thus leading to extremely ingenious methods for meeting these water requirements. It was reported that the Argeş River is one of the cases in which intensive logging and uncontrolled expansion of agricultural land have determined and accelerated the silting process (Batuca and Jordaan, 2000).

The anthropic impact on the Argeş River was reported under various forms. The ichtyologic studies performed by Truță and Dumitru (2015) in the Budeasa-Goleşti area emphasized the changes in the fish fauna determined by the increase in the number of species, either due to deliberate storage for angling, or due to the biotope changes that favour the development of some species that were identified only accidentally in the past. In the studies focused on the spatial distribution and the monitoring of alkaliphenolic compounds in the Danube River, Micić and Hofmann reported that the highest concentrations of nonylphenols were found in the Argeş River (a tributary of the Danube), in a sample taken near Bucharest (Liška et al., 2008).

Plant biotests, including the *Allium* test are frequently utilized for monitoring the environment (Fiskesjo, 1993; Leme and Marin-Morels, 2009; Pesnya and Romanovsky, 2013) and for evaluating the genotoxic potential of different physical, chemical or biological agents by measuring particular final results such as chromosomal aberrations, micronuclei and disruptions in the mitotic cycle (Grant, 1944; Rank and Nielsen, 1993; Majer et al., 2005; Şuţan et al., 2016).

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In this study, the *Allium cepa* L. test was used to evaluate the cytotoxicity and genotoxicity of particular water samples taken from different locations along the river, which had been subjected to the anthropic impact.

2. MATERIALS AND METHODS

2.1. Area of study

The Argeş River is a stream in the S-SE of Romania, formed by the union of the rivers Capra and Buda, which spring from under the peak of the Făgăraş Mountains, upstream of Vidraru reservoir. According to the Argeş-Vedea water basin administration (the management plan of the Argeş – Vedea hydrographic area), the Argeş River is 350 km in length, with a surface of 12,550 km2 and has 6 main tributaries (Vâlsan, Râul Doamnei, Râul Târgului, Neajlov, Săbar, Dâmboviţa River). Along the Argeş River, 16 power plants and 11 reservoirs were built, of which the biggest is Vidraru Dam, with a capacity of 465 million m^3 (Mititelu, 2010). The water samples were collected along the Argeş River, in 7 different locations with the following GPS coordinates: C1 - sampling station located upstream of Vidraru Dam 45.462663ºN, 24.603219ºE, C2 - sampling station located downstream of Vidraru Dam 45.338349ºN, 24.636401ºE, C3 - sampling station located just before the industrial area of the city of Piteşti 44.889644ºN, 24.848306ºE, C4 - sampling station located before the Goleşti Lake, at the exit from Piteşti, 44.827848ºN, 24.936234ºE, C5 - sampling station located upstream of the wastewater treatment plant 44.839282ºN, 24.912872ºE, C6 - sampling station located downstream of the wastewater treatment plant 44.836221ºN, 24.914987ºE, C7 - sampling station located at the exit of the wastewater treatment plant 44.838149ºN, 24.912965ºE.

2.2. Water sampling and preservation

The water samples were taken from different locations along the Argeş River in April 2016. For sampling we used polyethylene bottles of 2.5 l, previously washed with hydrochloric acid and repeatedly rinsed with tap water, distilled water and then again with tap water to prevent contamination of water samples. The *Allium* test was performed on the samples without prior preservation.

2.3. Evaluation of the cytogenetic potential of the water in the Argeş River

The *Allium cepa* test was used to evaluate the cytogenetic and genotoxic potentials of the water samples taken from different stations along the Argeş River.

The onion bulbs from a local variety (*Allium cepa* L., 2n = 16), with a diameter of approximately 4 cm, were harvested from a crop without phytosanitary treatments for pest prevention. Rooting was induced in a hydroponic culture system, in containers with a volume of 30 ml. After removing the cataphylls and exposing the root primordia, the bulbs were placed with the discoid stem in contact with the water samples for 72 hours, in the dark, at room temperature (22 ± 2 °C).

The study conducted to determine the cytotoxic and genotoxic potentials of the water samples from the Argeş River consisted in organizing a few experimental samples that were defined by the water sample in which the root genesis took place: C1 (upstream Vidraru Dam), C2 (downstream Vidraru Dam), C3 (entrance Piteşti), C4 (exit Piteşti), C5 (upstream wastewater treatment plant), C6 (downstream wastewater treatment plant), C7 (exit wastewater treatment plant) and Control (potable water).

After the static incubation of the onion root meristems in the 7 water samples for 72 hours, the roots were fixed in a mixture of ethanol (96%) and glacial acetic acid (3:1, v/v) for maximum 12 hours at 4°C, then washed with distilled water and transferred into 70° alcohol for long-term conservation. The attenuated hydrolysis was carried out prior to the coloration of the root meristems induced by their incubation in HCl 1N for 14-15 minutes at 60 °C. From the meristem tips stained with the

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solution of orcein acetic solution (15 minutes at 60 °C) we made microscopic preparations using the squash technique (Șuțan et al., 2016).

2.4. Mitotic index and determination of aberrations frequency

The cytogenotoxic potential of the water samples was evaluated by determining the variations of the mitotic index, of the indices of mitotic division phases and of the frequency of chromosomal aberrations induced in the root meristematic cells of *A. cepa* L.

The mitotic index was calculated using the formula (according to Tedesco and Laughinghouse IV, 2012):

$$MI = \frac{total\ numebr\ of\ mitotic\ cells}{total\ number\ of\ observed\ cells} x100 MI = \frac{total\ numebr\ of\ mitotic\ cells}{total\ number\ of\ observed\ cells} x100$$

The indices of the mitotic division phases were calculated applying the formula:

$$Phase\ index = \frac{total\ number\ of\ cells\ in\ a\ particular\ mitotic\ phase}{total\ number\ of\ observed\ cells} x100$$

Depending on the stage of mitotic division considered in the study, phase indices may be: prophase index, metaphase index, anaphase index or telophase index.

The percentage of cells with chromosomal aberrations and nuclear anomalies was determined by calculating the percentage ratio of the number of abnormal cells to the number of cells in the corresponding phase of mitosis. The microscopic preparations were analyzed and photomicrographs were taken using an Olympus CX-31 microscope with an ocular and objective combination of 400×.

2.5. Statistical analysis

The experiments were performed in triplicate and approximately 3,000 root meristematic cells were analyzed for each experimental sample.

For processing and putting to use the data resulting from the experiments we used the SPSS for Windows (Statistical Package for Social Science) statistical analysis program, version 20.0 (2010), applying the One-Way ANOVA model, the Duncan test (a test for multiple comparisons) and the Ward distance method of hierarchical classification, respectively. The significance of the differences between the effects of the experimental factors or the significance of their interaction, determined using the Duncan test for $p<0.05$, was written in lowercase.

3. RESULTS AND DISCUSSIONS

The statistical interpretation of the results obtained from the analysis of the microscopic preparations indicated a different and significantly different cell proliferation capacity in the root meristems incubated in the 7 samples, compared with the Control. The highest frequency of cells in mitotic division (7.64%) was calculated for the Control sample. The water samples taken upstream of Piteşti and upstream of the water treatment plant had the lowest values of the mitotic index, significantly different from the Control. The mitoinhibitory effect of the two water samples may be explained by the contamination of water with xenobiotics resulted from industrial activity, agriculture and social activity. A percentage of 6.2 of the root meristematic cells incubated in the C6 water sample taken downstream of the water treatment plant were identified in different mitotic phases. The insignificant difference for $p\leq0.05$ between the mitotic index determined for the Control and the C6 sample, respectively indicates the absence of potentially harmful substances in the water once it is directed to the urban circuit (Fig. 1).

The reduction in MI may be explained by the blockage of the cell cycle before DNA synthesis, in the G1 stage (Mohandas and Grand, 1972) or by the blockage of cells in mitotic metaphase, as a

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result of the malfunction of the spindle (Darlington and McLeish, 1951). In our study, the calculated mitotic index in the root meristems that was lower and significantly lower compared with the Control was associated with higher and significantly higher frequencies of cells in metaphase.

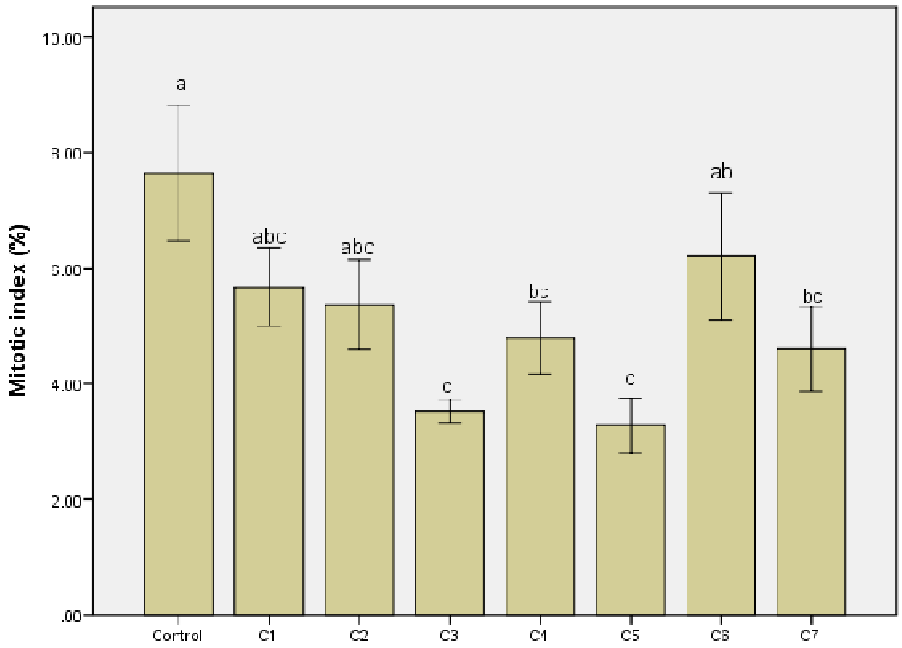

Figure 1. Variation of mitotic index in populations of Allium cepa L. root meristematic cells obtained in water samples taken from the Argeş River (the data are the median values ± SE of triplicates; a, b, c – interpretation of statistical significance and significant differences using the Duncan test, p<0.05)

The statistical analysis of the frequency of cells in different mitotic phases emphasized the presence of a high number of cells in prophase in the root meristems incubated in tap water and a balanced distribution of the other mitotic phases. A significantly lower number of cells in prophase was characteristic to the water samples taken from the Argeş River, with the exception of the C1 sample. The incubation of *A. cepa* L. roots in the water samples taken from stations along the Argeş River induced the increase and significant increase of metaphase frequency up to a maximum of 42.79% in the C7 experimental sample. The highest number of cells in anaphase was induced under the action of the C5 sample and the highest telophase frequency was determined after the incubation of the radicles in the C2 sample. The increase and decrease of the phase indices indicate an alteration of the mitotic cell cycle through the blockage of cells in metaphase or the late completion of the anaphase and telophase, respectively.

Chromosomal aberrations are determined by the action of various factors on DNA synthesis or replication or on nucleoproteins, resulting either in a direct breaking of the chromosomes (clastogenic aberrations), or in the malfunction of the spindle and implicitly an abnormal segregation of chromosomes (aneugenic aberrations). In our study, the most frequent types of chromosomal aberrations observed in the meristematic cells were the sticky and the vagrant chromosomes (Table 1), along with a significantly lower frequency of anaphase chromosomal bridges and laggard chromosomes. The presence of both vagrant and laggard chromosomes in the onion root meristems exposed to a continuous treatment with the water samples under study

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suggests the existence of particular factors causing spindle disturbances. The sticky and vagrant chromosomes were observed with insignificantly different frequencies in the root meristematic cells regardless of the water sample in which root genesis was stimulated while the anaphase chromosomal bridges and the laggard chromosomes were observed especially in the samples taken downstream of Piteşti.

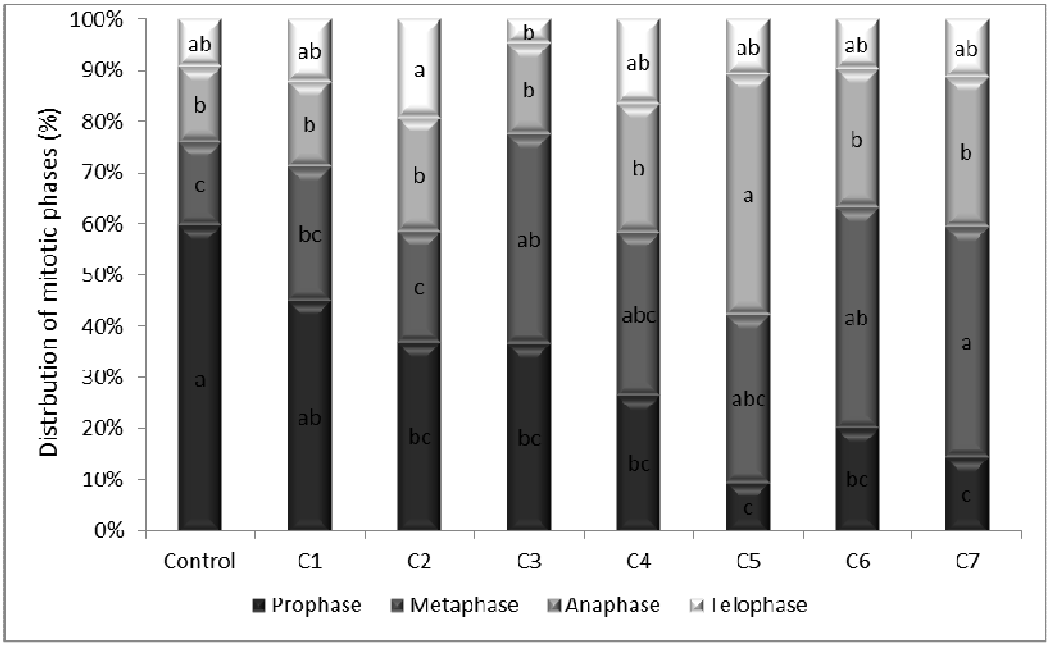

Figure 2. Distribution of mitotic division phases in the Allium cepa L. root meristematic cells obtained in the water samples taken from the Argeş River (the data are the median values ± SE of triplicates; a, b, c – interpretation of statistical significance and significant differences using the Duncan test, p<0.05)

Sticky chromosomes are considered irreversible aberrations that lead to cell death. Following the studies conducted on human lymphocytes, it is believed that the formation of sticky chromosomes is due to a disruption in chromatin condensation during mitosis (Al Achkar et al., 1989; Brangwynne and Marko, 2016). Probably the condensation mechanism works in a similar way in animals and plants because all the involved factors are evolutionarily and functionally conserved (Wolfram and Heinz, 2016). Other authors consider that the mutation of the genes that codify some non-histonic proteins involved in chromosome organization are responsible for the presence of sticky chromosomes (Gulfishan et al., 2010). Anaphase bridging could be explained by the induction of chromosome adhesion, formation of dicentric chromosomes as well as the induction of exchange-type aberrations and unequal or inverse translocations of chromosomal segments (Dulot and Oliviero, 1984; Gömürgen, 2005; Bhat et al., 2007).

Statistical interpretation of data using the Ward distance method of hierarchical classification revealed three clusters (Fig. 3). Cluster 1 includes the water sample used as Control, cluster 2 includes the water samples taken before the exit from Piteşti - C1, C2, C3 and C4, respectively and cluster 3 includes the samples C5, C6 and C7, from the area of the water treatment plant. Each cluster includes those samples defined by similarity parameters.

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Table 1. Frequency of chromosomal aberrations in root meristems of Allium cepa L. obtained in water samples taken from the Argeş River (the data are the median values ± SE of triplicates; a, b, c – interpretation of statistical significance and significant differences using the Duncan test, p<0.05)

Chromosomal aberrations	Vagrants	Laggards	Stickiness	Bridges anaphases
Control	18.79±9.57 ab	1.39±1.39 b	11.27±7.5 a	2.78±2.78 b
C1	8.91±2.23 b	13.09±7.24 a	NO	NO
C2	32.62±7.55 ab	1.75±1.75 b	34.92±13.78 a	5.92±3.62 ab
C3	17.14±8.83 ab	NO	13.4±5.16 a	NO
C4	7.39±2.1 b	NO	17.96±3.31 a	NO
C5	40.58±10.3 a	0.85±0.85 b	9.55±6.71 a	7.31±2.63 ab
C6	26.41±8.97 ab	3.03±3.03 b	44.09±33.99 a	13.03±3.49 ab
C7	31.09±7.04 ab	NO	18±7.73 a	8.1±4.23 ab

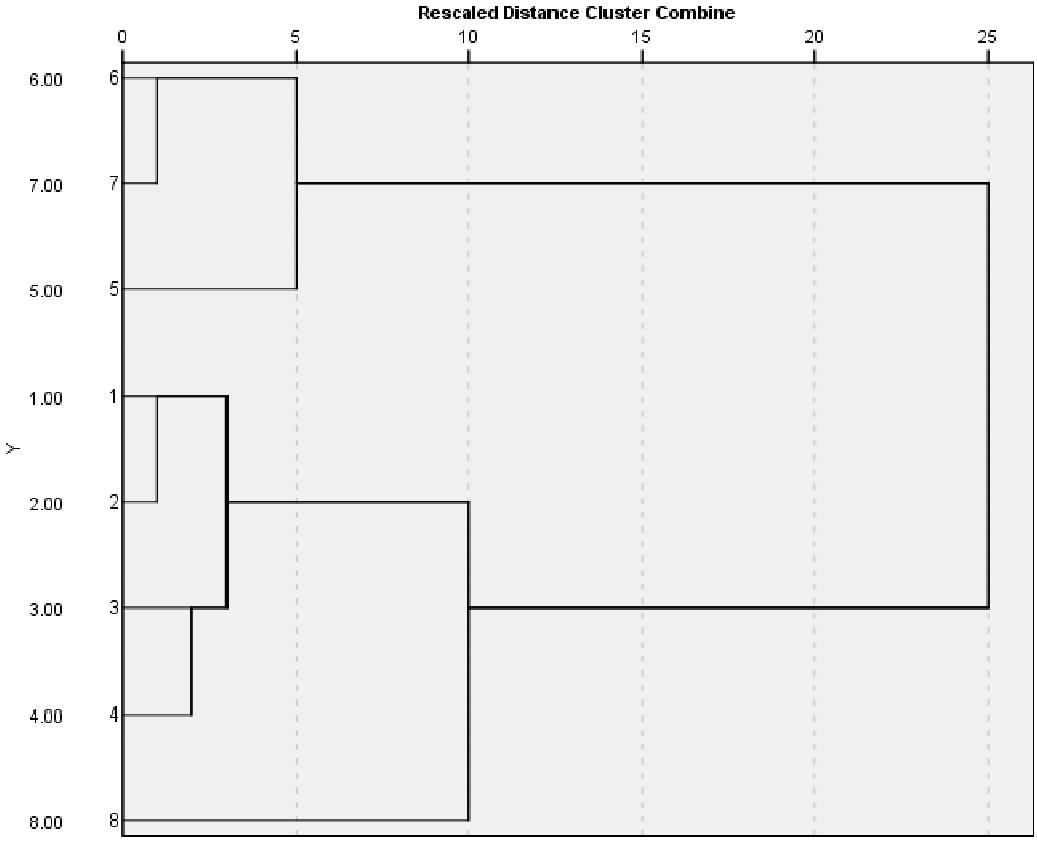

Figure 3. Hierarchical dendrogram for linkage of 5 parameters

4. CONCLUSIONS

The highest value of the mitotic index corresponded to the water sample taken downstream of the water treatment plant; for all the other samples, MI was significantly lower, which shows the impairment of water quality under the anthropic impact. The blockage of cells in metaphase was higher in the experimental samples containing the water taken near Piteşti. The most frequent

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chromosomal aberrations were the sticky and vagrant chromosomes found in the sampling stations downstream of Piteşti and of the water treatment plant.

5. FUNDING

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6. CONFLICT OF INTERESTS

The authors declare no conflict of interest.

6. REFERENCES

Al Achkar, W., Sabatier, L., Dutrillaux, B. (1989). How are sticky chromosomes formed? Ann Genet, 32(1)**,** 10-15.

Bath, T.A., Parveen, S., Khan, A.H. (2007). Meiotic studies in two varieties of *Vicia faba* L. (Fabaceae) after EMS treatment. *Asian J Pl Sci*, 6**,** 51-55.

Batuca, D.G., Jordaan, J.M.(Jr). (2000). Silting and desilting of reservoirs. A.A. Balkema, Rotterdam, Netherlands.

Brangwynne, C.P., Marko, J.F. (2016). Cell division: A sticky problem for chromosomes. *Nature*, 535**,** 234-235.

Darlington, C., McLeish, J. (1951). Action of maleic hydrazide on the cell. *Nature*, 167 (4245)**,** 407–408.

Dulot, F.N., Oliviera, O.A. (1984). Anaphase-telophase analysis of chromosomal damage induced by chemicals. *Environ Mutagen*, 6(3)**,** 299-310.

Fiskesjo, G. (1993). The *Allium cepa* test in wastewater monitoring. Environ Toxicol Water Qual, 8(3), 291–298.

Gömürgen, AN. (2005). Cytological effect of the potassium metabisulphite and potassium nitrate food preservative on root tip of *Allium cepa* L. Cytologia, 70**,** 119–128.

Grant, W.F. (1994). The present status of higher plant bioassays for the detection of environmental mutagens. *Mutat Res*, 310(2)**,** 175-185.

Gulfishan, M., Khan, A.H., Bhat, T.A. (2010). Studies on cytotoxicity induced by DES and SA in *Vicia faba* var. major. *Turk J Bot*, 34**,** 31-37.

Leme, DM, Marine-Morales, MA. (2009). *Allium cepa* test in environmental monitoring: a review on its applications. *Mutat Res*, 682(1)**,** 71–81.

Liška, I., Wagner, F., Slobodnik, J. (2008). Joint Danube survey 2. Final scientific report, Vienna, p. 242.

Mićić, V., Hofmann, T. (2015). Alkalyphenolic compounds in the Danube River. In I. Liska, ed, Barceló D., Kostianoy A.G. (Series Editors), *The Danube River Basin, The Handbook of Environmental Chemistry 39* (p. 198-215). Springer-Verlag Berlin Heidelberg,

Mititelu, L.A. (2010). Impactul amenajărilor hidrotehnice asupra mediului pe Valea Argeşului (până la Goleşti). Retrieved December 2015, from http://www.limnology.ro/water2010/Proceedings/27.pdf

Majer, B.J., Grummt, T., Uhl, M., Knasmüller, S. (2005). Use of plant bioassays for the detection of genotoxins in the aquatic environment. *Soil Air Water*, 33(1)**,** 45-55.

Mohandas, T., Grant, W. (1972). Cytogenetic effects of 2,4-D and amitrole in relation to nuclear volume and DNA content in some higher plants. *Can J Genet Cytol*, 14(4)**,** 773–783.

Pesnya, DS, Romanovsky, AV. (2013). Comparison of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phone GSM 900 radiation in the *Allium cepa* test. *Mutat Res*, 750(1–2)**,** 27–33.

Planul de management al spatiului hidrografic Argeş – Vedea,
http://www.rowater.ro/daarges/SCAR/Planul%20de%20management.aspx

Rank, J., Nielsen, M.H. (1993). A modified *Allium* test as a tool in the screening of the genotoxicity of complex mixtures. *Hereditas*, 118**,** 49-53.

Wolfram, A., Heinz, N. (2016). Chromosome condensation and decondensation during mitosis. *Curr Opin Cell Biol*, 40**,** 15-22. https://doi.org/10.1016/j.ceb.2016.01.013

Şuţan, N.A., Fierăscu, I., Fierăscu, R.C., Manolescu, D.Ş., Soare, L.C. (2016). Comparative analytical characterization and *in vitro* cytogenotoxic activity evaluation of *Asplenium scolopendrium* L. leaves and rhizome extracts prior to and after Ag nanoparticles phytosynthesis. Ind Crops Prod, 83**,** 379–386.

Truţă, A.M., Dumitru, D.D. (2015). Research on Argeş River fish fauna in Budeasa-Goleşti area. *Current Trends in Natural Science*, 4(8)**,** 95-105.

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