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APPLICATION OF THE ALLIUM TEST FOR EXAMINATION THE QUALITY OF WATER FROM DIFFERENT LOCATIONS

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Abstract: *All waters, whether of natural or anthropogenic origin, have certain qualities or characteristics, and due to various pollutions, they lose their quality and change their characteristics. Today, there are numerous chemical, physical-chemical and biological analyzes for determining the quality of water. Biological methods are based on the application of test organisms–bioindicators. The Allium test is a well-known and simple bioassay, and it can be used to determine total toxicity, cytotoxicity and genotoxicity. Therefore, the aim of the research is to determine the differences in the quality of different types of water using red onion (Allium cepa L) as a bioindicator. During the investigated period, parameters were monitored: dry and fresh matter, root length and antioxidant activity. Based on the observed parameters, it was noticed that there are differences in water quality between control water and water from other different localities. The bulbs growing in the sample from the Fiskija source had the longest roots, which was 3.05 cm, while the bulbs growing in the distilled water sample had the smallest roots, with a root length of 0.75 cm. The moisture percentage of the bulbs ranges from 86.46% to 90.31%, with the lowest percentages recorded in the bulbs that grew in the Dabar source, while the highest percentage of moisture was in the bulbs that grew in the source Hašimovića bunar. The antioxidant activity of bulbs in all water samples ranges from 14.94% to 55.23%, where the highest percentages (55.23%) were recorded in bulbs that grew in the sample from the Sana River from Sanski Most, while the lowest percentages (14.94 %) were observed in the bulbs that grew in the distilled water sample. Onion root that grew in the water sample from Fiskija source had the highest percentage (37.36 %) of antioxidant activity, while the lowest percentage (13.42 %) was recorded in the sample of the Sana River from Prijedor.*

Key words*: water quality, Allium test, root length, fresh and dry weight, antioxidant activity*

Introduction

Water represents the basic condition for the survival of the living world because it enters into the composition of cells, builds intercellular fluid, forms a means of transport for nutrients and protective substances and transports metabolic products intended for elimination from the body. It can be found in nature as surface, underground and atmospheric. Each water has its own unique characteristics and qualities. Chemically pure water does not exist in nature, because it contains various substances that affect its properties. Water is characterized by its microflora and fauna, content of dissolved oxygen, assimilating ability to receive pollution, factors that affect the power of self-purification, etc. The quality of surface water depends on several factors, most of all on atmospheric precipitation, erosion, population and industrial development, temperature changes during the seasons, as well as the mixing of different types of water. Degradation of water quality mainly causes the arrival of various pollutants in watercourses, either naturally (eutrophication) or by anthropogenic action. The origin of water pollution can be: physical, chemical and biological. It is considered that the main water polluters are: waste water from urban and industrial sewage systems and uncontrolled landfills of solid waste, and agricultural lands due to the use of artificial fertilizers and pesticides, traffic surfaces, polluted air, etc. Monitoring of chemical and physical-chemical parameters of surface waters is a very important factor in quality control and water management. Numerous monitoring methods of surface water, have been developed, which include systematic and organized monitoring change with the aim of timely detection of negative consequences in a certain time and space [1]. Chemical and physical analyzes are not sufficient to detect the effects of all potentially toxic substances in water. By applying biological analyzes on model organisms, it is possible to indicate the potential toxic effect caused by the action of mixtures of pollutants. The Allium test is one of the most reliable biological tests. The Allium test has many advantages such as: low price, simple execution, availability of a large number of cells on one microscopic preparation, which contributes to the reliability of the results obtained, and high agreement of the results with the results obtained in some other tests [2]. It is used in testing of acute toxicity of drinking water, as well as water that contains organic and inorganic compounds or effluents from the chemical industry. The tested samples are not required sterilization or pre-treatment [3]. Among the various species of the Allium genus, *Allium cepa* L. (common onion) has proven to be the most useful model organism. Different root growth responses of the test plant (*Allium cepa* L.) are general indicators of sample quality and toxicity. The growth of roots shows that the tested waters are suitable for life. The longer the roots, the better the quality of the sample and the shorter the roots, the worse the quality of the sample. The length of the rootstock is a useful indicator of the toxicity of various substances as well as the growth and development of the plant. Toxic substances inhibit root growth. Toxic substances can also cause changes in the morphology of onion roots, such as wavy roots, tips of the roots twisted in the form of a hook, and thickened or broken tips of the roots [4]. The test takes place in two phases. In the first phase, the water quality is determined in such a way that, if the onion roots in the water have grown less in a certain time, the degree of water pollution is higher, and vice versa, in unpolluted water, and at the same time, the roots will grow longer. In the second phase, with the help of a microscope, in the process of mitosis at the growing tips of the roots, damage to onion chromosomes is monitored [5].The aim of the research was to determine the differences in the quality of different types of water using red onion (Allium cepa L) as a bioindicator, and in the investigated period the following parameters were monitored: dry and fresh matter, root length and antioxidant activity.

Material and methods

Material

Water samples from different locations

Water samples were taken from several different places, after which they were transported in portable refrigerators to the Sanitary Chemistry Laboratory at the College of Health Sciences Prijedor, where they were subjected to further processing and testing. The samples were taken from the following waters: Zdena (from the source and estuary), Dabar river (from the source and estuary), Sana river (from the area of Sanski Most and Prijedor), from the source of Hašimović bunar, the source of Fiskija and the natural source from Mlječanica. Distilled water and tap water were used as a control sample.

„Apardžika“ bulbs

For the purposes of this research, „apardžika“ bulbs (seed onion, Allium cepa) were used, which were purchased in an agricultural pharmacy. For each water sample, 5 bulbs were used, and placed in the tested water in the region of root growth.

Reagents and devices

Reagents: 2,2-diphenyl, 1-picrylhydrazyl (DPPH) in methanol.

Devices:

- analytical balance (Adventurer pro TYPE, Ohaus corporation, Pine Brook, NJ USA)
- magnetic stirrer (Heating magnetic stirrer, Velp Scientifica, Ser. No: 73685)
- centrifuge (SIGMA 2-6 Laboratory Centrifuges, Osterode, Germany)
- Spectrophotometer (Jenway 6305 UV/VIS, 190-1000nm, UK)

Methods

Determination

The first stage is based on the observation of root length, which gives toxicity results. 24 hours before setting up the experiment, tap water, which represented the control, was left to stand in a wide container, in order to allow chlorine to evaporate from the water, which can otherwise negatively affect root growth. The temperature of all water must be approximately equal to room temperature before setting up the experiment, which is achieved by keeping the water at room temperature for at least 2 hours before use. For each sample of water, at least 5 bulbs are selected, placed in individual test tubes, marked with a marker and placed in a dark place, uniform room temperature. Healthy, fresh bulbs (seed onion, Allium cepa), of equal size, are used. The scalpel is used to remove the surface layer in the area of the roots, taking care not to damage the parts of the bulbs. After 24 hours, the water is changed.

Inhibition of root growth

After 72 hours, the lengths of all roots are measured with a ruler. The measured values are recorded in a table from which statistical calculations are made later. Based on the obtained root growth values, percent of inhibition is calculated and the level of toxicity of the investigated water is determined:

$$\% \text{ inhibition} = 100\% - \% \text{ root growth compared to control}$$

Determination of dry matter

The onion bulb and root were washed with distilled water and dried, and the mass of fresh material was weighed and expressed as g plant^{-1}. The dry mass of the bulbs was obtained by drying for 24 hours at 105°C. Dry matter is determined according to the following formula:

$$\text{The water content in the sample } (\%) = \frac{\text{m (before drying)} - \text{m(after drying)}}{\text{m (before drying)}} * 100$$

Where are:

m (before drying) - the mass of the sample before drying
m (after drying) - mass of the sample after drying

Determination of antioxidant activity

Extraction is carried out in 80% methanol in a ratio of 1:10 (plant mass: methanol). The samples are then heated to a temperature of 55°C and mixed on a magnetic stirrer for 30 min. After that, the homogenates are centrifuged for 10 minutes at 4000 rpm (revolutions per minute), and the supernatants are used for further analyses. Determination of the antioxidant activity by the DPPH (2,2-diphenyl, 1-picrylhydrazyl) test was done spectrophotometrically, using a modified method according to Blois [6]. A solution of DPPH in methanol was prepared, with a concentration of 74 mg/l. Then the DPPH solution is added in the amount of 1800 µl to 200 µl of the tested solution (A1), everything is shaken and left to stand for 30 min in the dark at room temperature. After 30 min of staying in a dark place, the absorbance of the solution is measured at a wavelength of 517 nm. After setting the baseline, the absorbance of the blank sample,was measured, i.e. the DPPH solution itself (A0). The reduction in DPPH absorption is expressed in % and is calculated using the following formula:

$$\% \text{ antioxidant activity (AA) at 517 nm} = (A0 - A1) \times 100 / A0$$

All concentrations are done in triplicate.

Statistical analysis

Statistical processing of data and their graphic display were carried out using the computer program Excel 2016 and the Microsoft Office package. The mean value of the samples and measures of dispersion (standard deviation and coefficient of variation) were calculated.

Results and discussion

Root growth in the observed samples

The average length of the roots, i.e. the inhibition of the roots' growth, is the first indicator, on the basis of which it can be estimated whether certain types of water affect its growth. After 72 hours of immersing the bulb in certain waters (the Zdena river - from the source and estuary, the Dabar river - from the source and estuary, the Sana river - from Sanski Most and Prijedor, the Hašimović bunar source, the Fiskija and Mlječanica source), the roots are measured which grew during that time, and measurement was made in centrimetres (cm). For the control sample, a sample of tap water was taken.

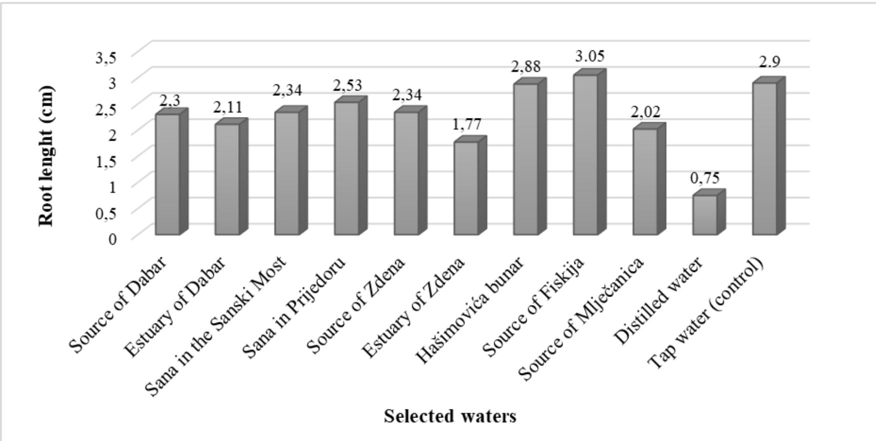

Figure 1. Mean value of bulb root length (cm) in selected waters

According to the results (Figure 1), the longest root length (3.05 cm) was found in the bulbs growing in the water sample from the Fiskija source. Tap water was the control sample, and the bulbs growing in the control water had a root length of 2.9 cm. Only in the case of the source of Fiskija, the result is higher than the control sample, and the bulbs that grew in the other samples had a shorter root length compared to the control sample. The smallest root length (0.75 cm) was measured in bulbs grown in a distilled water sample. Different responses of root growth of the tested plant (Allium cepa L.) are general indicators of the quality of the sample and its toxicity. Plant growth, among other things, shows to what extent the tested waters are suitable for life. It has been proven that if onion roots in water (in a certain time) have grown less, the degree of water pollution is higher, and vice versa, in unpolluted water the roots are longer [4]. The obtained results agree with this statement, with the exception of the results related to the Sana River. Namely, the assumption is that the results (root growth) would be better in the water sample taken in Sanski Most compared to the samples taken in Prijedor, given that Prijedor is located downstream from Sanski Most. There are various factors that can influence this result, and one of the possible factors is that the Sana water sample in Prijedor was taken a day later. There is a possibility that the structure and composition of the water changed during that period. Understandably, the best root growth results were in a source of water. Toxic substances can also cause changes in the morphology of onion roots, such as wavy roots, tips of the roots twisted in the shape of a hook, and thickened or broken tips of the roots [4].

Table 1. Degree of inhibition of root growth (Allium cepa L.) after 72 hours of growth in selected waters.

Selected water samples	Degree of inhibition of root growth (%)
The source of Dabar	33.69
Estuary of Dabar	33.76
River Sana in Sanski Most	33.68
River Sana in Prijedor	33.61
The source of the Zdena river	33.68
Estuary of Zdena	33.87
Hašimovića bunar	33.49
Fiskija	33.43
source of Mlječanica	33.79
Distilled water	34.22

The degree of inhibition of root growth (%) is shown in Table 1. In the Allium test, inhibition of root growth and the appearance of stunted roots indicate cytotoxicity, while wilting of roots indicates toxicity caused by pollutants [7]. The obtained results show that the degree of inhibition of root growth ranges from 33.43% to 34.22%. Bulbs growing in the sample of distilled water had the highest degree of inhibition of root growth (34.22 %), and bulbs growing in the sample of Fiskija source had the lowest (33.43 %). From the results, it can be concluded that there is no big difference in the degree of inhibition between all observed samples.

Fresh and dry matter of the onion bulb

The fresh and dry matter of the onion bulb after 72 hours of growth in the selected waters is shown in Tables 2 and 3.

Table 2. Fresh mass of onion samples

	Fresh bulb mass (g)	
Samples	**x ± SD**	**Cv**
Source of Dabar	3.90 ± 0.873	0.224
Estuary of Dabar	4.12 ± 0.327	0.079
river Sana in Sanski Most	4.01 ± 0.792	0.198
river Sana in Prijedor	3.81 ± 0.886	0.233
Source of Zdena	3.77 ± 1.142	0.303
Estuary of Zdena	3.95 ± 0.850	0.215
Hašimovića bunar	4.15 ± 0.697	0.168
Source of Fiskija	3.12 ± 0.746	0.239
Source of Mlječanica	2.94 ± 0.781	0.266
Distilled water	2.81 ± 0.934	0.332
tap water (control)	2.85 ± 0.513	0.180

The fresh mass of the bulb ranges from 2.81 to 4.15 g. It can be seen that the bulbs growing in the sample from the source of the Hašimović bunar had the highest fresh mass (4.15 g), and the bulbs growing in the distilled water sample had the smallest weight (2.81 g). The dry matter of the bulb ranges from 0.292 g to 0.528 g (Table 3). Based on this Table, it can be seen that the highest dry matter was in the bulbs that grew in the sample from the Dabar source (0.528 g), and the lowest in the bulbs that grew in the sample from the Mlječanica source (0.292 g).

Table 3. Dry matter of onion samples

	Bulb dry matter (g)	
Samples	**x±SD**	**Cv**
Source of Dabar	0.528 ± 0.088	0.166
Estuary of Dabar	0.474 ± 0.014	0.029
River Sana in Sanski Most	0.43 ± 0.094	0.219
River Sana in Prijedor	0.392 ± 0.097	0.253
Source of Zdena	0.446 ± 0.169	0.379
Estuary of Zdena	0.488 ± 0.115	0.236
Hašimovića bunar	0.402 ± 0.064	0.159
Source of Fiskija	0.382 ± 0.082	0.212
Source of Mlječanica	0.292 ± 0.086	0.295
Distilled water	0.310 ± 0.125	0.403
tap water (control)	0.306 ± 0.076	0.248

Based on Tables 2 and 3, the bulb moisture percentage was calculated. The percentage of bulb moisture ranges from 86.46 to 90.31%. Bulbs growing in the sample from the Dabar source had the lowest percentage of moisture (86.46%), while bulbs growing in the sample from the Hašimović bunar had the highest percentage (90.31%). Based on the difference between the fresh and dry mass of onion samples with roots, the percentage of moisture (water) in the samples was determined (Figure 2). The results show that there is no significant difference between individual samples in relation to this parameter.

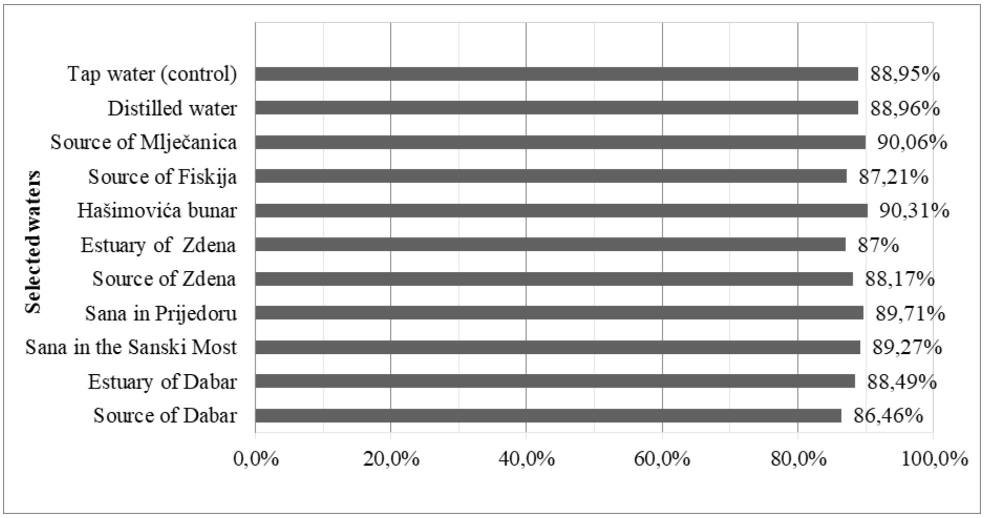

Figure 2. Bulb moisture (%) calculated after drying the samples

Antioxidant activities of bulb and onion root samples using the DPPH method

The results of the measurement of onion absorption (*Allium cepa* L) after growing for 72 hours in selected waters are shown in Table 4.

Table 4. Results of absorption measurements of onions (Allium cepa L) after 72 hours of growth in selected waters

Samples	x ± SD	CV
Source of Dabar	0.199 ± 0.002	0.011
Estuary of Dabar	0.191 ± 0.002	0.011
River Sana in Sanski Most	0.188 ± 0.002	0.010
River Sana in Prijedor	0.258 ± 0.008	0.030
Source of Zdena	0.162 ± 0.003	0.020
Estuary of Zdena	0.172 ± 0.019	0.110
Hašimovića bunar	0.177 ± 0.005	0.027
Source of Fiskija	0.171 ± 0.009	0.050
Source of Mlječanica	0.178 ± 0.006	0.032
Distilled water	0.173 ± 0.005	0.031
Tap water (control)	0.247 ± 0.009	0.035

The absorption value of onion root ranges from 0.162 (Zdena source) to 0.258 (Sana Prijedor).

Table 5 shows the results of measuring absorption of onion roots after growing for 72 hours in selected waters. It can be seen that the onion bulbs growing in the sample of the Sana River from Prijedor had the highest absorption (0.248), and the onion bulbs growing in the water sample from the source of the Hašimović bunar (0.012) had the lowest absorption.

Table 5. Results of absorption measurements of onion roots after 72 hours of growth in selected waters

Samples	X ± SD	CV
Source of Dabar	0.126 ± 0.002	0.017
Estuary of Dabar	0.191 ± 0.110	0.577
River Sana in Sanski Most	0.124 ± 0.003	0.021
River Sana in Prijedor	0.248 ± 0.015	0.061
Source of Zdena	0.193 ± 0.051	0.262
Estuary of Zdena	0.136 ± 0.046	0.337
Hašimovica bunar	0.012 ± 0.008	0.067
Source of Fiskija	0.123 ± 0.002	0.018
Source of Mlječanica	0.198 ± 0.007	0.007
Distilled water	0.222 ± 0.085	0.384
Tap water (control)	0.234 ± 0.020	0.020

Based on the results of measuring the absorption of onions and onion roots (Tables 4 and 5), their antioxidant activity expressed in % was also calculated (Figure 3).

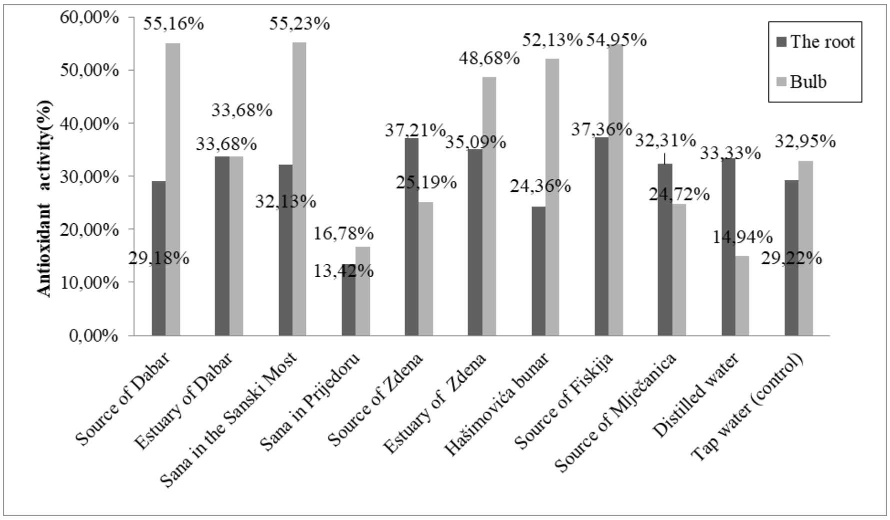

Figure 3. Antioxidant activity (%) of onion roots and bulbs (Allium cepa L) after 72 hours of growth in selected waters

The antioxidant activity of onion samples ranges from 14.94 to 55.23%. The change in antioxidant activity was most pronounced in onion samples that grew in the sample of the Sana River from Sanski Most (55.23 %), the source of Dabar (55.16 %) and the source of Fiskija (54.95 %), while the least changes have bulb onions that have grew in the sample of distilled water (14.94 %). The antioxidant activity of onion root ranges from 13.42 to 37.36%. The onion root that grew in the sample of the source of Fiskija had the most pronounced change in antioxidant activity (37.36%), while the smallest change was in the onion root that grew in the sample of the Sana River in Prijedor (13.42%). Other samples are located in some approximate interval. Plants grow in a dynamic environment and constantly face stressful conditions, including oxidative stress [8]. Oxidative stress can lead to numerous cell damage or death, as well as wilting of the plant [9]. Plants possess effective antioxidant systems to protect themselves from oxidative stress, which increase can be caused by organic and inorganic pollutants present in water samples. Oxidative stress occurs when the production of free radicals overpowered the antioxidant system [10].

Conclusion

The water samples that were taken from the observed sources (Fiskija, Hašimov bunar, Dabar, Zdena, Mlječanica) did not significantly affect the growth of onion roots compared to the control (tap water), while a slightly slower growth of roots was observed in samples from river flows. This leads to the conclusion that there was no overall toxic effect of the tested samples on the growth of onion roots.
Inhibition of the growth of onion roots as well as changes in the morphology of the roots, can be used as indicators of total contamination of water samples. From the results, it can be seen that none of the tested water samples inhibited the growth of onion roots and that there is no great difference in the degree of inhibition between the individual observed samples. Antioxidant activity of onion samples ranges from 14.94% (distilled water) to 55.23% (Sana River in Sanski Most). The onion root that grew in the sample of Fiskija source had the most pronounced change in antioxidant activity (37.36%), while it was the least in the sample of the Sana River from Prijedor (13.42%). In general, the bioindicator test cannot replace physical and chemical measurements of the degree of pollution, but it can certainly contribute to a better interpretation of them.

References

[1] Pereza J. Procjena Kvalitete vode rijeke Riječine *Allium Cepa* test. Diplomski rad, Medicinski fakultet, Sveučiliste u Rijeci. 2019.
[2] Kokotović I, i Radić Brkanac S. Primjena Allium-testa u nastavi biologije, Educatio biologiae. 2019; (5): 87-90 https://doi.org/10.32633/eb.5.7.
[3] Kokotović I. Procjena toksičnosti vode rijeke Save upotrebom običnog luka (*Allium cepa L.*) kao modelnog organizma. Diplomski rad, PMF, Sveučilište u Zagrebu, Zagreb. 2019.
[4] Fiskesjö G: The Allium test as a standard in environmental monitoring. Hereditas. 1985; 102 (1): 99-112. doi:10.1111/j.1601-5223.1985.tb00471.x.

[5] Križanec O. Analiza izbranih pokazateljev kvalitete površinskih in odpadnih vod Maribora in okolice, Mladi za napredek Maribora « 32. srečanje, Maribor. 2015.
[6] Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 1985; 181:1199-1200. http://dx.doi.org/10.1038/1811199a0.
[7] Khanna N, Sharma S: *Allium Cepa* Root Chromosomal Aberration Assay: A Review. Indian Journal of Pharmaceutical and Biological Research. 2013; (1):105-119.
[8] Schmidt RR, Weits DA, Feulner CFJ, van Dongen JT. Oxygen Sensing and Integrative Stress Signaling in Plants. Plant Physiology. 2018; (176)2: 1131-42.
[9] Baek KH, Skinner D: Production of reactive oxygen species by freezing stress and the protective roles of antioxidant enzymes in plants. Journal of Agricultural Chemistry and Environment. 2012; 1(1): 34-40 doi:10.4236/jacen.2012.11006.
[10] Perrone S, Tataranno ML, Stazzoni G, Buonocore G. Oxidative stress and free radicals related diseases of the newborn. Advances in Bioscience and Biotechnology. 2012; 3(7): 1043-50.

PRIMJENA ALLIUM TESTA ZA ISPITIVANJE KVALITETA VODE SA RAZLIČITIH LOKALITETA

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Sažetak*: Sve vode bilo da su prirodnog ili antropogenog porijekla imaju svoje određene kvalitete ili karakteristike, a zbog različitih zagađenja one gube svoj kvalitet i mijenjaju karakteristike. Danas postoje brojne hemijske, fizičko-hemijske i biološke analize za utvrđivanje kvaliteta voda. Biološke metode zasnivaju se na primjeni test organizama–bioindikatora. Allium test je dobro poznat i jednostavan biotest, a pomoću njega se može odrediti ukupna toksičnost, citotoskičnost i genotoksičnost. S toga, cilj istraživanja je utvrditi razlike u kvalitetu različitih vrsta voda primjenom crvenog luka (Allium cepa L.) kao biondikatora. Za vrijeme ispitivanog perioda praćeni su parametri: suha i svježa materija, dužina korijena i antioksidativna aktivnost. Na osnovu posmatranih parametara uočeno je da postoje razlike u kvalitetu vode između kontrolne vode i voda sa drugih različitih lokaliteta. Najduži korijen imale su lukovice koje su rasle u uzorku iz izvora Fiskije koji je iznosio 3,05 cm, dok su najmanji korijen imale lukovice koje su rasle u uzorku destilovane vode, sa dužinom korijena od 0,75 cm. Procenat vlage lukovica kreće se u rasponu od 86,46 % do 90,31 %, pri čemu su najmanji procenti zabilježeni u lukovicama koje su rasle u izvoru Dabar, dok najveći procenat vlage su imale lukovice koje su rasle u izvoru Hašimovića bunar. Antioksidativna aktivnost lukovica u svim uzorcima vode kreće se u rasponu od 14,94 % do 55,23 %, gdje su najveći procenti (55,23 %) zabilježeni u lukovicama koje su rasle u uzorku iz rijeke Sane iz Sanskog Mosta, dok su najmanji procenti (14,94 %) uočeni kod lukovica koje su rasle u uzorku destilovane vode. Korijen luka koji je rastao u uzorku vode iz izvora Fiskija imao je najviši procenat (37,36 %) antioksidativne aktivnosti, dok je najmanji procenat (13,42 %) zabilježen u uzorku koji je uzet iz rijeke Sane u Prijedoru.*

Ključne riječi*: kvalitet vode, Allium test, dužina korijena, svježa i suha masa, antioksidativna aktivnost*