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Contents

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Volume 1(15), 2023, Pages 7–15
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The influence of some Ca(II) and Ba(II) with Co(II) compounds on the productivity of the cyanobacteria *Spirulina platensis*

Boris Nedbaliuc, Eduard Coropceanu, Eugeniu Ciobanu, Sofia Grigorcea, Dumitru Ureche, and Lilia Brînză

Abstract. The article includes experimental results related to the productivity of the *Spirulina platensis* (Nordst.) Geitl - CALU - 835 strain, which was cultivated for 8 days on modified nutrient media supplemented with two coordination compound containing Ca and Ba. A greater amount of biomass was recorded when growing spirulina on the Zarrouk culture medium supplemented with these coordination compounds in a concentration of 5 mg/L, accumulating a fresh biomass of about 13,3 g/L and 12,8 g/L, respectively, or about 14,5% and 9,9% higher than that accumulated by the witness.
Keywords: *Spirulina platensis*, trichomes, productivity, stimulation, coordination compound, biomass.

Influenţa unor compuşi ai Ca(II) şi Ba(II) cu Co(II) asupra productivităţii cianobacteriei *Spirulina platensis*

Rezumat. Articolul include rezultate experimentale ce ţin de productivitatea tulpinii *Spirulina platensis* (Nordst.) Geitl – CALU – 835, care a fost cultivată timp de 8 zile pe medii nutritive modificate şi suplinite cu doi compuşi coordinativi cu conţinut de Ca şi Ba. O cantitate mai mare de biomasă s-a înregistrat la cultivarea spirulinei pe mediul de cultură Zarrouk suplimentat cu aceşti compuşi coordinativi în concentraţie de 5 mg/L, acumulând o biomasă proaspătă de circa 13,3 g/L şi 12,8 g/L respectiv, sau cu circa 14,5% şi 9,9% mai mare decât cea acumulată de către martor.
Cuvinte-cheie: Spirulina platensis, trihomi, productivitate, stimulare, compus coordinativ, biomasă.

1. Introduction

Recent international and national biotechnology research has increased interest in obtaining pure cultures of some species of cyanobacteria and microalgae with an increased content of biologically active substances, that can be widely applied in agriculture as biofertilizers, plant growth stimulators, as well as in zootechnics, medicine, food industry, etc. Among the cyanobacteria under investigation there are species from the genera

Spirulina, *Calothrix*, *Nostoc, Anabaena, Cylindrospermum, Phormidium, Synechocystis*, etc., as well as autotrophic protists from the genera *Chlorococcum, Scenedesmus, Chlorella, Dunaliella, Porphyridium*, etc.

Cyanobacteria and microalgae participate in the formation of organismal associations from the composition of aquatic and edaphic biocenoses, that influence the organoleptic qualities of surface basin waters. Enriching them with oxygen, organisms are involved in the process of self-purification of contaminated streams including organic chemicals, heavy metals, radionuclides, etc. Nitrogen-fixing cyanobacteria play an active role in boosting soil fertility. By fixing atmospheric nitrogen, they contribute to the buildup of atmospheric nitrogen in the soil in the form of easily available compounds in plant mineral nutrition [12; 14].

Spirulina platensis (Nordst.) Geitl. is a blue-green trichomed filamentous cyanobacterium. In its natural development, it has spiral trichomes, with more or less regular spirals, with a diameter of 26-36 μ, and a spacing between the spirals of 43-57 μ. When it is cultivated, it loses the ability to form a spiral and transforms into filaments with a linear morphology [1]. Trichomes are able to move along their axis. They don't have any heterocysts. Filaments are solitary but can form clusters and bundles as large as 2-5 mm in diameter. The trichomes are 350-1000 μ long and 6-8 μ wide, with mild strangling between adjoining cells in the region of the transverse walls. The trichomes' heads are rounded. Numerous granules may be seen in the cells along the dividing walls using a photon microscope. These are the gas vacuoles, that appear as darker and brighter spots under the microscope. It grows by fragmenting the thallus and generating hormogons. The life cycle is divided into three major stages: trichome fragmentation with the generation of hormogonia; hormone development and maturation; and trichome elongation [4; 8]. It differs from other *Spirulina* species since it endures high levels of sodium bicarbonate in the growth media, up to 22-25 g/L. It can survive temperature variations of 20-25°C during the day without considerably decreasing production.

Research on the optimal chemical parameters of nutrient medium for the cyanobacteria culture under laboratory circumstances can serve as a benchmark for the development of new technologies for their intense cultivation. By improving their nutritional circumstances, cyanobacteria boost their daily biomass growth rate in a very short period of time (8-10 days). Thus, *Spirulina platensis* strains are also used as microbiological objects to evaluate the activity of various chemical agents that can either stimulate or hinder the development and accumulation of their biomass. Thus, *Spirulina platensis* strains are also used as microbiological objects for evaluating the activity of various chemical agents that can either stimulate or hinder the development and accumulation of their biomass.

Coordination compounds are complex molecules that contain both inorganic cations of some bioelements and an organic component which is made up of various functional groups containing electron-donating atoms and can increase the properties of influence on physiological systems through a synergistic effect [5]. Previous studies have shown that certain coordinating chemicals have a favorable effect on biosynthetic processes in specific microbes [2; 5-7].

The goal of this study was to investigate how coordination compounds with Ca and Ba content affected the productivity of the strain *Spirulina platensis* (Nordst.) Geitl - CALU - 835.

2. Materials and Methods

The investigations were carried out in the "Ecological Biotechnologies" scientific laboratory of the "Ion Creangă" State Pedagogical University in Chisinau. The cyanobacteria strain *Spirulina platensis* (Nordst.) Geitl - CALU - 835, which was taken from the "Vasile Şalaru" scientific research laboratory in Algology at the State University of Moldova, served as the object of research. For cultivation, the Zarrouk liquid nutrient medium was used with the following chemical composition (g/L): $NaHCO_3$ – 16,8; $NaNO_3$ – 2,5; $K_2HPO_4 \cdot 3H_2O$ – 0,5; K_2SO_4 – 0,1; NaCl – 1,0; $MgSO_4 \cdot 7H_2O$ – 0,2; $CaCl_2 \cdot 6H_2O$ – 0,04; $FeSO_4$ – 0,01; EDTA – 0,08; solution of trace elements – 1 mL/L (H_3BO_3 – 2,86; $MnCl_2 \cdot 4H_2O$ – 1,13; $ZnSO_4 \cdot 7H_2O$ – 0,222; $NaMoO_4 \cdot 5H_2O$ – 0,39; $Co(NO_3)2 \cdot 6H_2O$ – 0,049; $CuSO_4 \cdot 5H_2O$ – 0,079), pH – 9,5 [9]. Part of the experimental variants, from the assembled experience, were supplemented with the coordination compound containing Ca – $[CaL_3][Co(NCS)_4]$ (1), and another part of the experimental variants – with the coordinating compound containing Ba – $[BaL_3\text{-}\mu\text{-}(NCS)_2\text{-}Co(NCS)_2]$ (2), where L – pyridine-2,6-dimethyldicarboxylate. The coordination compounds were synthesized according to the method described in [3].

Cultivation was carried out in Erlenmeyer flasks with the 100 mL volume, in which 75 mL of Zarrouk nutrient medium with *Spirulina platensis* suspension (starting biomass 0,09 g/75 mL) was poured. Over 2 days, the spirulina stem, from these flasks, was treated with coordinating compounds 1 and 2 and cultivated for another 8 days at an artificial lighting of about 4000 lx and at a temperature of about 27°C (Fig. 1). Chemical compounds 1 and 2 were administered in concentrations of 50 mg/L, 10 mg/L, 5 mg/L, 1 mg/L and 0,5 mg/L. The samples grown in the absence of these chemical compounds, respecting the other cultivation parameters, were considered control samples.

Figure 1. Erlenmeyer flasks with the inoculated culture of *Spirulina platensis* on Zarrouk nutrient medium supplemented with coordinating compounds of Ca and Ba.

The data collected were statistically analyzed using the "STATISTICA 7" computer software, and the standard error of the average was calculated. Spirulina productivity was calculated using the current approach [10, 11].

3. Results and Discussions

It is important to provide macro- and microelements to *Spirulina platensis* when cultivating it in the laboratory. For the synthesis of the main cellular components, spirulina requires macroelements such as N, P, K, Mg, S, Ca, etc., as well as microelements such as Fe, Mn, B, Sr, Cu, Zn, Ba, Ti, Mo, etc., which are present in varying concentrations in the Zarrouk liquid nutritional medium [15].

Calcium, for example, is administered only in low concentrations and represents an essential chemical element necessary for the normal growth and development of all autotrophic organisms, including cyanobacteria. Calcium ions contribute to the stabilization of cell membrane structures, and serve as a messenger in many physiological processes of growth and development of microalgae and cyanobacteria, allowing the cells to absorb anions and cations from the nutrient environment [13]. This element favors shifting the pH of the environment towards alkaline values, inhibits the release of potassium from senile cells, etc., creating suitable conditions for the normal growth and development of the *Spirulina platensis* culture [9].

In the case of testing the coordination compound with Ca content, it was established that its effect on the *Spirulina platensis* culture depends on the concentration added to the Zarrouk nutrient medium, as well as on its period of action. Thus, on the 8th day

of cultivation, a greater amount of biomass of the researched crop was recorded in the experimental version with a coordination compound with a Ca content administered in a concentration of 5 mg/L, which produced 13,37 g/L, or 14,5% more than the control variant, as well as the variants with coordination compound 1 with concentrations of 10 mg/L and 1 mg/L, accumulating respectively 13,01 g/L and 13,09 g/L, or with 11,4% and 12,1% more than the control variant (Tab. 1; Fig. 2).

Table 1. The fresh biomass accumulated by the *Spirulina platensis* strain when treated with the compound $[CaL_3][Co(NCS)_4]$.

Nr.	Variant		Fresh start biomass g/1000 mL	Fresh biomass after 8 days of cultivation, g/L		Δ
				x±mx	σ	
1.	control		0,9	11,67±0,4	0,8	-
2.	$[CaL_3][Co(NCS)_4]$	50 mg/L	0,9	11,91±1,5	2,1	2,0
3.		10 mg/L	0,9	13,01±0,8	1,6	11,4
4.		5 mg/L	0,9	13,36±0,7	1,4	14,5
5.		1 mg/L	0,9	13,09±1,0	2,0	12,1
6.		0,5 mg/L	0,9	11,82±0,5	0,6	1,2

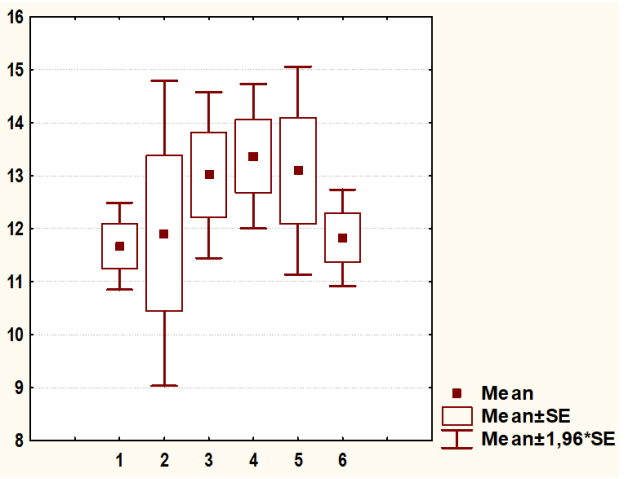

Figure 2. The fresh biomass accumulated by the *Spirulina platensis* strain when treated with the compound $[CaL_3][Co(NCS)_4]$ (g/L); 1. – control; 2. – 50 mg/L; 3. – 10 mg/L; 4. – 5 mg/L; 5. – 1 mg/L; 6. – 0,5 mg/L.

The concentrations of 50 mg/L and 0,5 mg/L of administered complex 1, proved to be ineffective because the productivity of the spirulina strain was almost equal to that of the control - 11,91 g/L and 11,82 respectively g/L. Concentrations of coordinating compound 1 higher than 50 mg/L have a negative effect on the strain of *Spirulina platensis*, the productivity being lower than in the control variant.

The findings of the eighth day of the *Spirulina platensis* strain cultivation in Zarrouk liquid nutritional medium supplemented with the chemical compound [BaL_3-μ-$(NCS)_2$-$Co(NCS)_2$] showed that its effect is highly dependent on the concentration provided. Thus, compound 2 provided at the concentration of 5 mg/L revealed to be the most appropriate for the investigated culture, ensuring a spirulina production of about 12,83 g/L, or 9,9% more than the collected biomass by the control variant. Coordination compound 2 enhanced the production of the spirulina strain, collecting 14 times more biomass in 8 days than was initially supplied (Tab. 2; Fig. 3).

Table 2. The fresh biomass accumulated by *Spirulina platensis* during 8 days when treated with the coordinating compound containing Ba.

Nr.	Variant		Fresh start biomass g/1000 mL	Fresh biomass after 8 days of cultivation, g/L		Δ
				x±mx	σ	
1.	control		0,9	11,67±0,4	0,8	-
2.	[BaL_3-μ-$(NCS)_2$-$Co(NCS)_2$]	50 mg/L	0,9	11,31±1,0	1,4	-3,0
3.		10 mg/L	0,9	11,74±0,3	0,7	0,5
4.		5 mg/L	0,9	12,83±0,9	1,7	9,9
5.		1 mg/L	0,9	12,74±1,3	2,5	9,1
6.		0,5 mg/L	0,9	12,15±0,3	0,4	4,1

An obvious stimulatory effect was also observed in the experimental version with a concentration of 1 mg/L, where the cyanobacterium strain generated 12,74 g/L of biomass, 9,1% more than the control. Concentrations of coordination compound 2 of 10 mg/L and 0,5 mg/L resulted in a 0,5% and 4,1% increase in strain production, respectively, which was slightly higher than that produced by the control variant. At the same time, higher concentrations of coordinating chemical 2 of 50 mg/L, as well as higher amounts provided to the Zarrouk culture medium, hinder *Spirulina platensis* strain growth and development.

During the first 2-3 days of cultivation, the *Spirulina platensis* strain goes through the phase of latency and growth acceleration. In the microscopic preparations, numerous

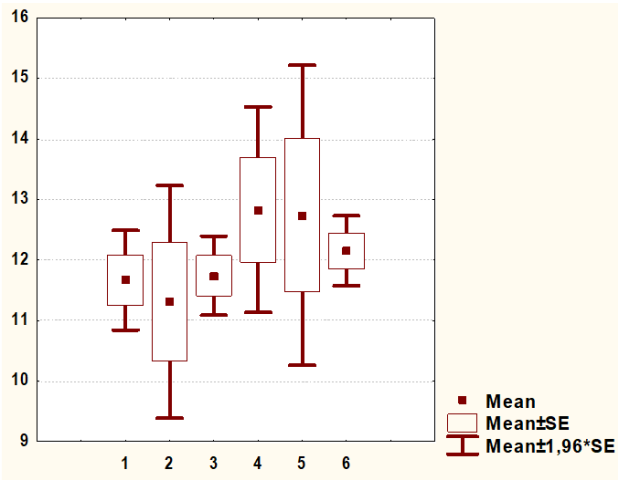

Figure 3. The fresh biomass accumulated by the *Spirulina platensis* strain when treated with the compound [BaL_3-μ-$(NCS)_2$-$Co(NCS)_2$] (g/L); 1. – control; 2. – 50 mg/L; 3. – 10 mg/L; 4. – 5 mg/L; 5. – 1 mg/L; 6. – 0,5 mg/L.

fragments of trichomes (hormogons) with variable lengths from 45 μ to 120 μ and with a thickness of 4,1-5,4 μ were highlighted. At the same time, trichomes exceeding the length of 500 μ were recorded. On the 3rd-4th day of cultivation, the phase of exponential multiplication of the culture is established, which is manifested by rapid growth (binary division of cells) and maturation of the trichomes. Starting from the 10th day, the logarithmic growth phase of the culture decreases obviously, and the produced biomass decreases. Under the microscope, most of the trichomes exceeded the length of 500 μ, being highlighted cells devoid of content, from which the thallus fragmented forming hormogons.

4. Conclusions

(1) The coordination compounds with a Ca and Ba content of 5 mg/L significantly increased the productivity of spirulina, registering after 8 days of action a fresh biomass of about 13,3 g/L and 12,8 g/L, being higher about 14 times and 12 times compared to the initial biomass administered in the Erlenmeyer flasks and about 14,5% and 9,9% higher than that accumulated by the control variant.

(2) The productivity of the *Spirulina platensis* culture decreases as the concentration of coordination compounds increases, and concentrations more than 50 mg/L have an inhibitory effect.

(3) The concentrations of the tested compounds of 0,5 mg/L resulted in an insignificant increase in the productivity of the strain of just 0,5% and 4,1%, respectively, being slightly higher than that produced by the control.

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(Boris Nedbaliuc, Eduard Coropceanu, Eugeniu Ciobanu, Sofia Grigorcea, Lilia Brînză) "Ion Creangă" State Pedagogical University of Chisinau
E-mail address: nedbaliuc.boris@upsc.md

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Revisiting the paleolithic reindeer of Moldova: an in-depth analysis

Roman Croitor

Abstract. This study examines reindeer remains from Paleolithic sites in Moldova, including re-description and reclassification of antler findings, and identifies a previously undocumented complete antler from Cosăuți. The antler from Duruitoarea Veche is determined to be from red deer (*Cervus elaphus*), consistent with the site's fauna. Using a t-test on talus samples, the study investigates body size variations between an older reindeer form from Duruitoarea Veche and Brînzeni 1, and the younger *R. tarandus constantini* from Cosăuți. Results show no statistically significant differences in body size, but confirm the larger cheek teeth in *R. tarandus constantini*. Reindeer from Rașcov 7 are reclassified as *R. tarandus* cf. *constantini*, with relatively large cheek teeth.
Keywords: reindeer, *Rangifer tarandus*, t-test, Late Paleolithic, southeastern Europe.

Reevaluarea renului paleolitic din Moldova: o analiză detaliată

Rezumat. Acest studiu examinează resturile de ren de pe siturile paleolitice din Moldova, incluzând redescrierea și reclasificarea descoperirilor anterioare de coarne și identificarea unui corn complet nedocumentat anterior de la Cosăuți. Cornul de la Duruitoarea Veche este determinat că provine de la cerbul comun (*Cervus elaphus*), în concordanță cu fauna sitului paleolitic. Prin utilizarea unui test t pe eșantioane de astragal, studiul investighează variațiile dimensiunilor între o formă mai veche de ren descoperită la Duruitoarea Veche și Brînzeni 1, și subspecia mai tânără *R. tarandus constantini* găsită la Cosăuți. Rezultatele arată că aceste diferențe în dimensiuni nu sunt semnificative din punct de vedere statistic. Totuși, testul t confirmă semnificația taxonomică a dimensiunilor dinților, deoarece aceștia sunt comparativ și relativ mai mari la *R. tarandus constantini* din Cosăuți. Un alt rezultat interesant este dimensiunea relativ mare a dinților observată la renii de la Rașcov 7, care sunt reclasificați în acest studiu ca *R. tarandus* cf. *constantini*.
Cuvinte-cheie: ren, *Rangifer tarandus*, test t, paleolitic târziu, Europa de sud-est..

1. Introduction

Reindeer played a significant role as a faunal component during the Late Pleistocene period in the East-Carpathian region, emerging as a crucial game animal for Paleolithic hunters. The size of the reindeer's body was optimal, being large enough to attract the interest of Paleolithic hunters yet small enough to allow for the transportation of the entire

prey carcass to the butchering site. Consequently, all skeletal elements of Late Pleistocene reindeer are well-represented in archaeological assemblages. However, this specific type of accumulation of reindeer osteological material also led to the extreme fragmentation of reindeer remains found at Paleolithic sites, predominantly representing discarded kitchen refuse. Previous studies have extensively examined reindeer remains from late Paleolithic sites in Moldova [1, 2]. A biometric analysis was conducted, enabling the identification of three distinct forms of reindeer within the territory of the Republic of Moldova. The first form, presumably a forest reindeer, was found in "cave fauna" complexes dating back to 100-21 thousand years ago. The second form, relatively small in size, was discovered at Rașcov 7, approximately 19,300 years ago. Lastly, the third form was identified as the reindeer subspecies of eastern origin, characterized by relatively large cheek teeth, and attributed to the Siberian Paleolithic subspecies *Rangifer tarandus constantini* Flerov [1].

Investigations into sexual dimorphism among postcranial reindeer remains from various Paleolithic sites have revealed distinct demographic patterns. These patterns were interpreted as a consequence of diverse hunting strategies influenced by environmental conditions and the ecological characteristics of reindeer populations [2].

This article presents a comprehensive investigation comprising the description of new fossil remains, a critical reassessment of certain questionable specimens, and a statistical study focusing on selected reindeer remains. The primary objective is to validate the previously proposed conclusions and affirm observations pertaining to the biometric characteristics of reindeer samples obtained from diverse Paleolithic sites in Moldova. Through this rigorous analysis, we aim to enhance our understanding of the taxonomical diversity of fossil reindeer from Moldova, enabling us to elucidate the hunting practices and population dynamics of reindeer during the Late Pleistocene in the region.

2. Research Methods and Material

The fossil material examined in this study is housed in the Paleontological collection of the Institute of Zoology at the State University of Moldova, located in Chisinau. To ensure the reliability and accuracy of our findings, we employed appropriate statistical analyses that enable a robust comparison and evaluation of the reindeer samples collected from different Paleolithic sites in Moldova. In this study, we grouped together the remains of geologically older reindeer from the Paleolithic "cave assemblages" (Duruitoarea Veche and Brînzeni 1) for comparison with a "glacial maximum" reindeer sample (Cosăuți). Our research focuses on the analysis of new biometric data obtained from isolated lower fourth premolar (P_4) specimens of reindeer originating from several Paleolithic sites, namely

Brînzeni-1, Rașcov 7, and Cosăuți. These data are used to estimate and compare the size of reindeer dentition.

In addition to the lower fourth premolars (P_4), the third lower premolars (P_3) of reindeer exhibit a similar level of molarization. To distinguish between P_3 and P_4, we utilized the K-means clustering technique, which enables differentiation based on distinct crown measurements such as mesodistal length and labiolingual breadth. Crown measurements were recorded as maximum values; however, it is important to acknowledge that dental wear, particularly in heavily worn teeth, may introduce bias to the mesodistal length measurement. This consideration is taken into account during the biometric analysis and interpretation of results.

The biometric data concerning postcranial bones of fossil reindeer from Moldova, as well as data from modern reindeer used for independent t-tests, were adapted from a previous study by Croitor (2010). It is worth noting that the fossil reindeer samples exhibit varying male-to-female ratios. Therefore, we conducted independent t-tests separately for male and female samples to account for this variability. The data analysis was performed using the Python programming language in Jupyter Notebook.

Abbreviations used in the article: L, length; D, breadth; S, surface; DAP, antero-posterior measurement; DLM mediolateral measurement; dist, distal; br., brow tine; tr., trez tine; st., second tine; pt., posterior tine; bm., beam; cr., crown tine; pcr., posterior crown tine; plm., antler palmation.

3. Description

The taxonomic position and evolutionary affinity of Rangifer tarandus remains from the older cave faunas (Duruitoarea Veche and Brînzeni 1) require a reassessment of shed antler Nr. 290 from Duruitoarea Veche (Fig. 1). This particular specimen was initially identified as reindeer by David [3, Fig. 30, p. 147]. However, the morphological characteristics of this antler suggest that it actually belongs to the red deer species *Cervus elaphus*, which is also present in the fauna of Duruitoarea Veche. Unlike reindeer antlers, the surface of antler Nr. 290 exhibits a pearled texture rather than a smooth one. The antler is further characterized by consistently circular transverse sections of its beam (with an anteroposterior diameter of 43.0 mm above the brow tine and a lateromedial diameter of 41.8 mm) and tines. The brow tine displays a cylindrical shape and an additional spontaneous prong on its middle part, an uncommon feature. Notably, the bez tine is missing, and the trez tine differs from the flattened middle tine observed in forest caribou from North America. The main measurements of antler Nr. 290 are as follows: the anteroposterior diameter of the burr is 72.2 mm, the beam circumference above the brow

tine is 140 mm, the length of the basal tine is approximately 300 mm, the anteroposterior diameter of the antler above the burr is 67.7 mm, the lateromedial diameter of the antler above the burr is 48.0 mm, and the distance between the brow and trez tines is 260 mm.

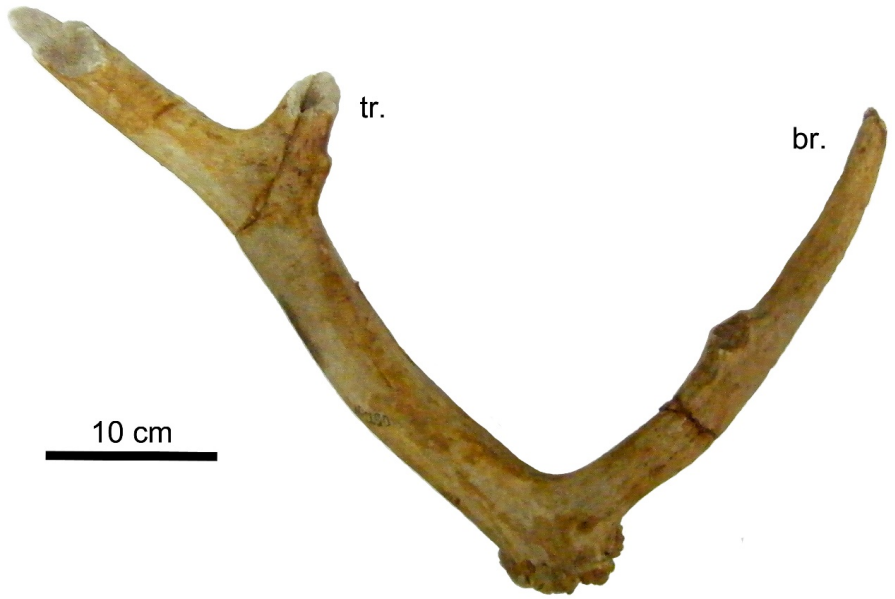

Figure 1. The left shed antler Nr. 290 of *Cervus elaphus* from Duruitoarea Veche originally described as reindeer antler [3].

Other fragments of antlers from Duruitoarea Veche and Brînzeni 1 exhibit less complete preservation but demonstrate typical morphology observed in reindeer: a remarkably smooth antler surface and a beam that is compressed more strongly from the sides, with the second tine located in close proximity to the basal tine, resembling the modern American barren ground caribou and Eurasian reindeer. The fragment of reindeer antler 30/1586 from Brînzeni 1 displays the longest distance between the basal and second tines, although this distance remains relatively small, measuring only 8 cm.

Unfortunately, the nearly complete antler of reindeer from Cosăuți has not been preserved. However, the provided photograph by Dr. Serghei Covalenco includes all the important features of the specimen (Fig. 2). The first basal tine, which exhibits strong asymmetry in reindeer and is often absent in one of the antlers, is not visible in the Cosăuți specimen and likely did not develop. The second tine terminates with a moderately developed palmation. The antler beam deviates caudally from the second tine ramification and displays a bowed shape. The posterior tine is well-developed. The distal portion of

the antler is characterized by a large hook-shaped posterior crown tine that points downward. The distal palmation of the antler is well-developed and bears four digitations. The antler from Cosăuți belongs to a large mature male; however, its distal palmation and crown tines are less developed when compared to the reindeer from Villestofte depicted by Aaris-Sørensen et al. [4].

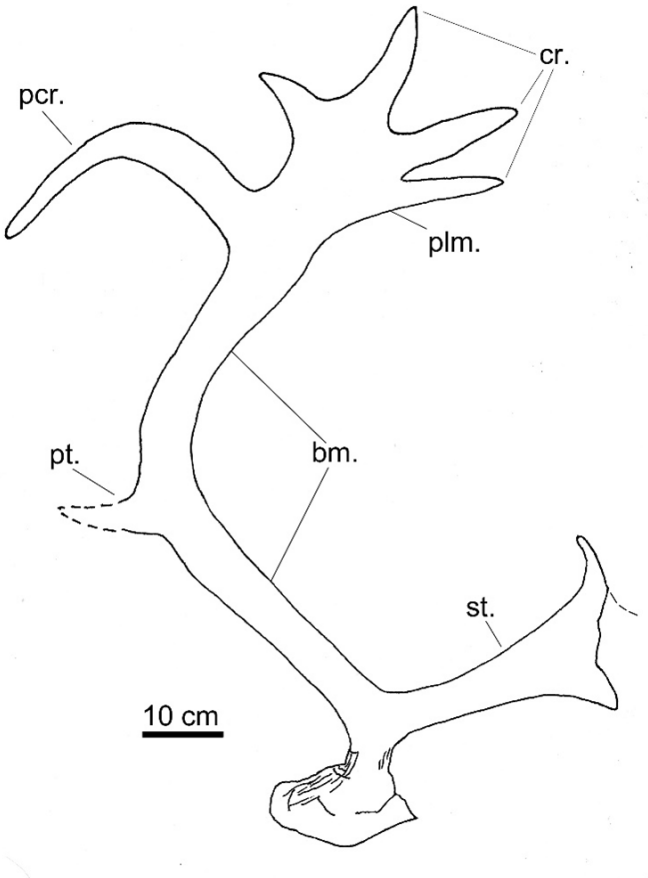

Figure 2. The almost complete antler of male reindeer *Rangifer tarandus constantini* from Cosăuți. The figure is based on photograph kindly provided by Dr. Serghei Covalenco.

During the statistical analysis of postcranial measurements in two forms of reindeer, we employed the Independent t-test since the studied reindeer populations are statistically unrelated. The postcranial measurements of the older form of reindeer from Duruitoarea Veche and Brînzeni 1 were found to be slightly larger than those of reindeer from Cosăuți [1]. However, there is a broad overlap in the data (Fig. 3, Tab. 1). The t-test comparing the

lumped sample of male reindeer talus from Duruitoarea and Brînzeni with the sample from Cosăuți indicated that the observed difference in body size is not statistically significant. The obtained T-statistic was 1.198, with a P-value of 0.248. Based on these results, we failed to reject the null hypothesis, which assumes no statistical difference between the compared samples. In other words, there is insufficient evidence to suggest a true difference in the distal breadth of the talus between the fossil reindeer males. The effect size, as indicated by Cohen's d value of 0.7, suggests a medium-to-large difference between the means of the two reindeer samples. The effect size provides information about the magnitude of the difference, regardless of statistical significance. Notably, the statistical difference in talus length was even less significant (T-statistic: 0.037, P-value: 0.971). Overall, the data does not provide strong evidence to support a true difference in the distal breadth of the talus between the two reindeer samples. While there may be a medium-to-large effect size, the statistical analysis does not support the presence of a significant difference between the means of the two samples.

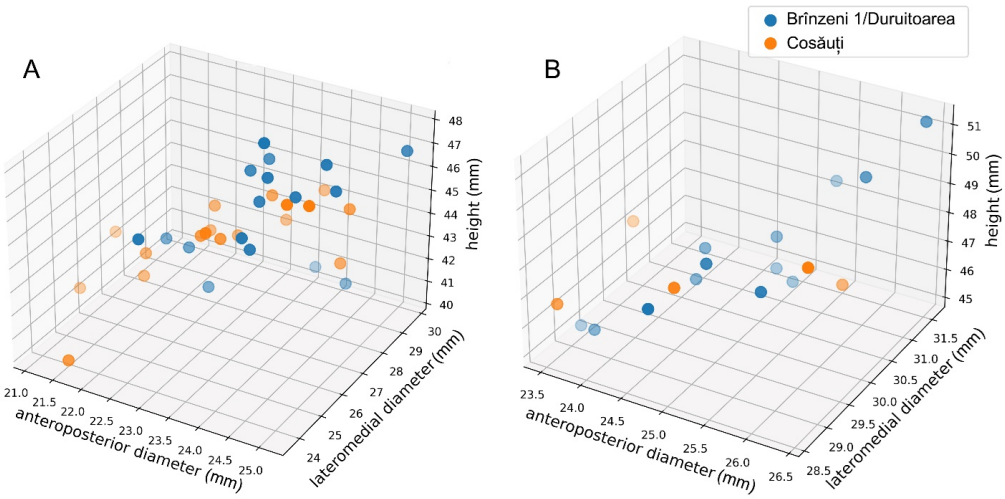

Figure 3. Measurements of tali of reindeer from the Paleolithic sites of Moldova: A, females; B, males.

Similar results were obtained in the statistical comparison of female distal breadth of talus: T-statistic: 1.668, P-value: 0.105, Cohen's d: 0.582.

To sum up, while there may be a moderate effect size and a moderate difference between the means of the two samples, the statistical analysis does not provide strong evidence to support a significant difference. To gain further insights into the obtained results of the statistical comparison of Paleolithic reindeer samples, we conducted a comparison

of small but informative samples of modern *R. tarandus tarandus* (n=4) and *R. tarandus terranovae* (n=4) from the Natural History Museum of London. The Student's t-test for metacarpal length yielded a T-statistic of 7.17 and a P-value of 0.00037, indicating a statistically significant difference between the metacarpal lengths of the modern reindeer samples. The comparison of distal talus breadths also revealed a statistically significant difference, with a T-statistic of 5.0457 and a P-value of 0.00234.

However, the statistical comparison of tooth size in Paleolithic reindeer from Moldova (Fig. 4, Tab. 2) yielded interesting results. The independent t-test for the length of the lower fourth premolar (P_4) between the samples from Cosăuți and Brînzeni 1 yielded a T-statistic of 3.379 and a P-value of 0.00222, suggesting a statistically significant difference between the samples (Fig. 4 A).

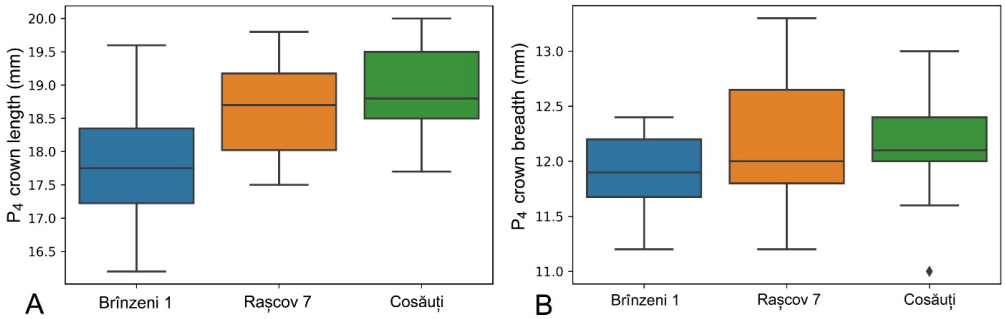

Figure 4. Boxplot showing range of measurements of lower fourth premolar (P_4) of Paleolithic reindeer from Moldova: A, crown length; B, crown breadth.

The independent t-test for P_4 between Cosăuți and Rașcov resulted in a T-statistic of 1.0635 and a P-value of 0.2932, indicating that the difference between the samples from Cosăuți and Rașcov is not statistically significant.

On the other hand, the independent t-test for P_4 between Rașcov and Brînzeni yielded a T-statistic of 2.7572 and a P-value of 0.0096, suggesting a statistically significant difference between the samples. However, the significant statistical difference only applies to tooth crown length. The independent t-test for P_4 breadth (Fig. 4, B) showed that the measurement differences among samples are not statistically significant: for the samples from Cosăuți and Brînzeni 1, the T-statistic is 1.4307 and the P-value is 0.1639; for the samples from Cosăuți and Rașcov, the T-statistic is -0.0342 and the P-value is 0.9729; and for the samples from Rașcov and Brînzeni 1, the T-statistic is 1.2322 and the P-value is 0.2268.

4. Discussion

The new data on antler material of reindeer from the Paleolithic sites of Moldova, combined with the t-test analysis of postcranial and dental remains, have provided valuable insights into the morphological characteristics of Late Pleistocene reindeer in southeastern Europe. Through the revision of shed antler Nr. 290 from Duruitoarea Veche, it has been determined that this antler actually belongs to red deer. Consequently, the assumption of the presence of a reindeer form in the Paleolithic of Moldova that evolved antler shapes similar to those of forest caribou from North America can be rejected. Instead, all known antlers of fossil and modern reindeer from Eurasia exhibit the characteristic antler morphology of barren ground American caribou, thereby confirming Geist's hypothesis [5] that the origin of Eurasian reindeer is related to barren ground caribou.

This type of antler morphology is also observed in the reindeer from Cosăuți. Although the complete antler of a mature male reindeer from Cosăuți is not preserved due to the extreme fragility of the specimen, it stands as the sole complete Late Pleistocene reindeer antler from Eastern Europe that reveals specific morphological characteristics. The most prominent features of the reindeer antler from Cosăuți are the large hook-like posterior crown tine and the development of palmation in the distal part of the antler. This particular shape of the crown part of the antler differs from the distal part of the antler in reindeer from Villestofte, which is characterized by elongated palmation and the development of several posterior crown tines of more or less equal size. It is worth noting that the Cosăuți variant of antler morphology is not documented in the extensive Paleolithic material (including archeozoological remains and Paleolithic art) from Western Europe, as summarized by Bouchud [6].

The reindeer specimens from Duruitoarea Veche and Brînzeni 1 were previously reported [1] as belonging to a large forest form. However, the t-test conducted in this study demonstrates that the postcranial measurement differences between these reindeer and those from Cosăuți are not statistically significant. It is important to note that many significant ecomorphological features of Paleolithic reindeer from Moldova remain unavailable. For instance, the length of metacarpals in reindeer from Duruitoarea Veche and Brînzeni 1 (represented by three specimens) closely resemble those of modern caribou from North America (Fig. 5 A). Unfortunately, a direct comparison of limb bone length between reindeer from Duruitoarea Veche, Brînzeni 1, and the reindeer form from Cosăuți is not possible. The only complete long bone available from the reindeer at Cosăuți is a tibia belonging to a female (Croitor, 2010). This tibia falls within the variation range of

modern *R. tarandus tarandus* (Fig. 5 B), suggesting that the reindeer from Cosăuți can be characterized as a short-limbed form.

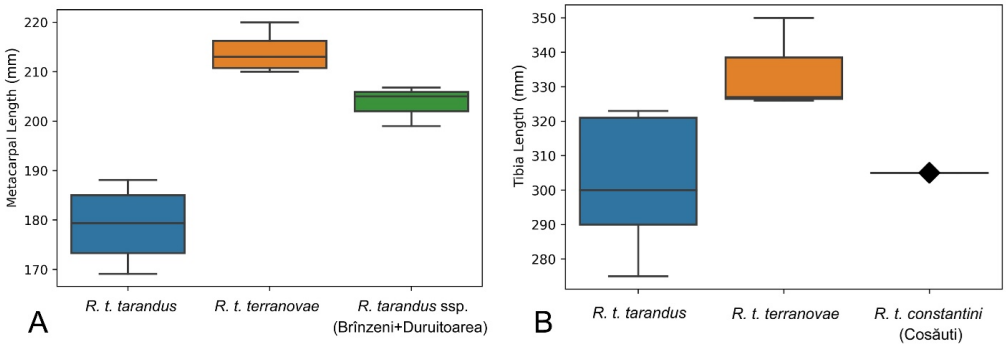

Figure 5. Lengths of Paleolithic reindeer long bones compared to modern reindeer subspecies: A, metacarpals; B, tibia. The data are adapted from Croitor [1].

Examining the dentition measurements provides interesting insights, as it reveals a statistically significant difference in tooth size, with larger teeth observed in the reindeer from Cosăuți compared to those from Brînzeni 1. The difference in P_4 crown breadth, however, is not statistically significant, although the mean value of the sample from Cosăuți is slightly higher than that of the sample from Brînzeni 1. Nevertheless, the difference in P_4 crown length is statistically significant, with the reindeer from Cosăuți exhibiting larger crown length. Therefore, the statistical analysis confirms the taxonomical significance of cheek tooth size, which is larger in the reindeer from Cosăuți identified as *Rangifer tarandus constantini* Flerov [1]. The increased size of cheek teeth is an adaptation to abrasive herbaceous forage in the Late Pleistocene tundra-steppe conditions. While there is no existing literature on tooth size dimorphism in ruminants, including reindeer, it was not possible to address the issue of tooth size dimorphism in this research due to the study's focus on isolated teeth without available sex identification. Nonetheless, the results indicating statistically significant larger cheek teeth in the reindeer from Cosăuți are consistent, as the total sample of reindeer comprises approximately 75% female remains. Therefore, the obtained results are not biased by potential sexual dimorphism in tooth size.

The conducted t-tests have enhanced our understanding of the systematic position of the reindeer from Rașcov 7, characterized by relatively large cheek teeth, which can be classified as *R. tarandus constantini*. Considering the broad range of variability observed

in the Rașcov 7 sample, I suggest referring to this reindeer form as *R. tarandus* cf. *constantini*.

5. Conclusions

The reevaluation of antler Nr. 290 from Duruitoarea Veche, originally attributed to reindeer, has revealed that it actually belongs to red deer (*Cervus elaphus*). Therefore, the assumption that reindeer from Duruitoarea Veche possessed antlers with a middle tine, similar to modern forest-dwelling American caribou, should be rejected.

All reindeer forms from the Paleolithic sites in Moldova are characterized by a distinct antler structure, with the second tine positioned close to the basal one. This antler bauplan represents the only recorded in Eurasia type of reindeer antlers that shows similarity to antlers of North American barren ground caribou.

The t-test conducted on talus samples of reindeer from the Paleolithic sites of Moldova did not reveal statistically significant differences in body size between the so-called "cave faunas" of Brînzeni 1 and Duruitoarea Veche and the Last Glacial Maximum reindeer from Cosăuți. However, the available material does not provide sufficient data on limb bone lengths, which are important ecomorphological characteristics of reindeer subspecies. Generally, reindeer from the "cave faunas" exhibit relatively long metapodials, similar to the metapodial length of modern forest-dwelling caribou, *R. tarandus terranovae*, from North America. Data on metapodial length in the reindeer from Cosăuți is lacking, but the single complete female tibia from Cosăuți falls within the variation range of modern tundra reindeer, *R. tarandus tarandus*.

The t-test conducted on P_4 crown length revealed the statistical significance of this characteristic, distinguishing the reindeer from the "cave faunas" from the reindeer form from Cosăuți, which is characterized by relatively larger cheek teeth. Therefore, large cheek tooth size serves as a reliable taxonomic character distinguishing *R. tarandus constantini* from Cosăuți from older reindeer forms, which are considered here as R. tarandus ssp. The reindeer from Rașcov 7 also exhibits relatively larger cheek teeth and is considered here as *R. tarandus* cf. *constantini*, given the broad range of individual variation observed in the Rașcov 7 sample.

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(Roman Croitor) Université Aix-Marseille, Aix-en-Provence, France; Institute of Zoology, State University of Moldova

E-mail address: romancroitor@europe.com, roman.croitor@zoology.md

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The assessment of the nutrient content in the plants of the hybrid *Sorghum bicolor* x *Sorghum sudanense* harvested in different development stages

Victor Țîței, Sergiu Coșman, Natalia Cîrlig, Valentina Coșman, Ana Guțu, Natalia Mocanu, Serghei Cozari, Alexandru Teleuță, and Nina Garștea

Abstract. The article presents the results of the research aimed at evaluating the nutrient content in the leaves, stems and panicles of the hybrid sorghum x Sudan grass "SAȘM-4" plants harvested in different development stages. It has been determined that the leaves have a higher content of crude protein, crude fat, carotene, ash, calcium and phosphorus; the stems have a high content of crude cellulose, nitrogen-free extract and sugar; while the panicles are rich in starch, with high energy value. During the growing season, in all component parts, the content of dry matter increases, the concentration of proteins decreases and the content of cellulose and nitrogen-free extract essentially increases.
Keywords: sorghum x Sudan grass hybrid "SAȘM-4", nutrients, leaf, stem, panicle.

Evaluarea conținutului de nutrienți în plantele hibridului *Sorghum bicolor* x *Sorghum sudanense* recoltat în diferite perioade de vegetație

Rezumat. În articol sunt prezentate rezultatele cercetărilor care au avut ca scop evaluarea conținutului de nutrienți în frunze, tulpini și panicule a plantelor hibridului sorg x iarba de sudan „ȘAȘM-4" recoltat în diferite faze de dezvoltare. S-a stabilit că frunzele au un conținut mai înalt de proteină brută, grăsimi brute, carotenă, cenușă și calciu și fosfor, tulpinile au un conținut ridicat de celuloză brută, substanțe extractive neazotate și zaharuri, paniculul este bogat în amidon cu o valoare energetică înaltă. Pe parcursul vegetației în toate părțile componente sporește conținutul de substanțe uscate, se diminuează concentrația de substanțe proteice și crește esențial conținutul de celuloză și substanțe extractive neazotate.
Cuvinte-cheie: hibridul sorg x iarba de Sudan SAȘM-4, nutrienți, frunză, tulpină, panicul.

1. Introduction

Climate change, which affects our region and the extent of desertification and salinization of some areas, implies reconsidering the structure of agricultural crops, because maize and other traditional leguminous crops do not withstand long-term drought and heat, and if such crops as millet and sorghum will be cultivated again on a large scale, it will be easier to ensure a fodder base. Genus *Sorghum* Moench, tribe *Andropogoneae*, sub-family *Panicoideae*, family *Poaceae*, includes 31 species, native to Europe, Asia, America and Australia. In our region, in the 17th century, broomcorn *Sorghum technicum* (Körn.) Trab. was introduced, and during the last century, other species were also introduced: grain sorghum *Sorghum bicolor*, Sudan grass *Sorghum sudanense* as a fodder source, *Sorghum bicolor var. saccharatum* and *Sorghum bicolor var. oryzoidum* for the food industry, as well as the Columbus grass *Sorghum* × *almum* for forage. Research on *Sorghum* species, the identification of valuable forms and the creation of cultivars with high productivity and tolerant to environmental conditions, has been carried out over the years in several scientific centres in our country [1-8].

The hybrids of sorghum x Sudan grass (*Sorghum bicolor* (L.) Moench × *Sorghum sudanense* (Piper) Stapf.) are valuable because they inherited the advantages of both species: thinner and taller stems, high content of leaves in the harvested mass, rapid regeneration after harvest, reaching up to 3 cuts annually, forage rich in protein and carbohydrates. As compared with corn, it develops a stronger root system, and the leaves are narrower, that is why it uses water more efficiently and posesses high tolerance to drought and heat.

It is known that the quality of fodder depends on the chemical composition and biomorphological structure of the plants, whose content and structure change as they grow. Therefore, a feed poorer in nutritional elements is less consumable, less digestible and, finally, its relative feed value is lower, and it cannot ensure the well-being of animals (health, reproduction functions) and planned productivity indices (meat, milk), the low content of proteins leads to an overconsumption of feed per production unit. The research aimed at evaluating the nutrient content in leaves, stems and panicles of the sorghum x Sudan grass hybrid, harvested at different stages of development.

2. Materials and Methods

The plants of the sorghum x Sudan grass hybrid 'SAȘM-4' created at the Institute of Genetics, Physiology and Plant Protection, by Gheorge Moraru, Ph.D., and grown on the experimental sector of the "Alexandru Ciubotaru" National Botanical Garden (Institute)

from Chisinau, served as subjects of study. For research, sorghum x Sudan grass plants were harvested by hand, form the stage of stem elongation until panicle formation – tassel, milk-wax kernels and wax kernels. Samples of 10 plants were taken from the harvested mass to determine the ratio per organs (component parts of the plant) by separating the leaves and inflorescences from the stem, weighing them separately and establishing the ratios of these quantities and being fixed by forced dehydration to evaluate the dry matter content and the nutrient one. According to the traditional methodical indications, in the Laboratory of Nutrition and Forage Technology of the Scientific-Practical Institute of Biotechnology in Animal Husbandry and Veterinary Medicine, the following indices were determined: moisture, hygroscopic moisture, dry matter content, nitrogen, crude protein, crude fat, crude cellulose, crude ash, nitrogen-free extract, starch, sugar, calcium, phosphorus, carotene; the nutritional and energy value of the feed were evaluated.

3. Results and Discussions

When harvesting sorghum x Sudan grass plants in mid-June, the content of leaves in the harvested mass was very high – about 55%, and in the following stages of development the structure of the crop changed essentially, the content of leaves decreased to 20.1% -23.9%, and the stem content increased to 56.4%-66.2%.

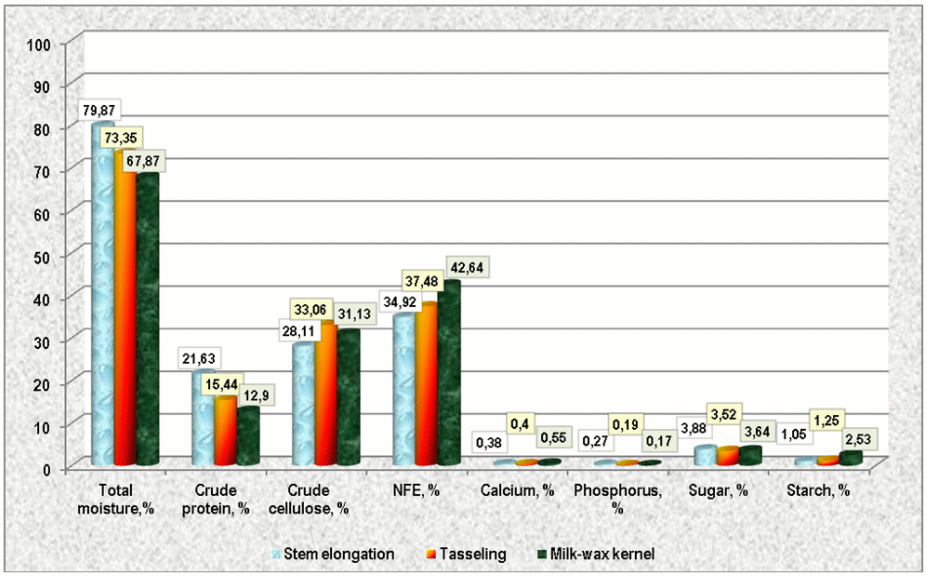

Figure 1. The nutrient content of leaves of the sorghum x sudan grass hybrid 'SAȘM-4' depending on the development stage.

The results presented in Figure 1 indicate significant changes in the chemical composition of the green mass of leaves depending on the plant development stage and harvest time. Thus, the moisture content of the leaves decreased from 79.87% in the pre-tasselling stage to 73.35% in the tasselling one and then decreased to 67.87% in the milk-wax stage of kernels. A similar trend was noticed in the crude protein content, from 21.63% in the pre-tasselling stage to 15.44% in the tasselling period and only 12.90% in the grain ripening stage. However, the amount of crude cellulose, as expected, gradually increased from 28.11% in the pre-tasseling stage to 33.06% in the tasseling stage and decreased insignificantly to 31.13% in the milk-wax stage of the grains. The crude ash content in leaves during the growing season was more constant, with small variations from 8.09 to 8.38%. Of the mineral substances, there was a substantial increase in the content of calcium – from 0.38% to 0.55% in the milk-wax stage of the grains, accompanied by a decrease in the content of phosphorus from 0.27% to 0.17%. The level of nitrogen-free extract (NFE) in the leaves increased with the age of the plant from 34.92% to 42.64%. The content of sugars in the leaves was quite constant 3.52-3.88%, and that of starch increased essentially, by more than 2.4 times (from 1.05 to 2.53%). During the growing season, a significant increase in the carotene content was observed: 94.83 mg/kg in the pre-tasselling stage, 122.5 mg/kg in the tasselling stage and 136.5 mg/kg during the grain ripening one. The leaves of the hybrid sorghum x Sudan grass are rich in fats, and their content decreased from 6.53% in the pre-tasselling stage to 4.95% in the milk-wax stage of the grains. Nutritive and energy values of the leaves harvested in the pre-tasselling stage were 0.19 nutritive units /kg and 2.08 MJ/kg metabolizable energy, in the tasselling stage being the highest – 0.26 nutritive units /kg and 2.82 MJ/kg metabolizable energy, while in the milk-wax stage of the grains, they decreased to 0.23 nutritive units /kg and 2.77MJ/kg metabolizable energy, respectively.

The results of the evaluation of the chemical composition of the sorghum x Sudan grass SAȘM-4 plant stems are presented in Figure 2. We would like to mention that the stems had a lower content of dry matter as compared with the leaves in the pre-tasselling and tasselling stages. The stems of this plant in the pre-tasselling stage had very high moisture content – 90.68%. Along with the development of the plant, this index gradually decreased to 79.14% during the tasselling stage and to 69.05% in the milk-wax stage of the grains. These indices are important in order to determine the optimal time for harvesting the given plants for ensiling. The amount of crude protein in the absolutely dry matter dropped very quickly and quite sharply from 13.45% in the pre-tasselling stage to only 4.03% in the tasselling stage and to only 2.42% in the wax-milk stage of the grains. The crude cellulose content increased with age from 29.29% in the first stage to

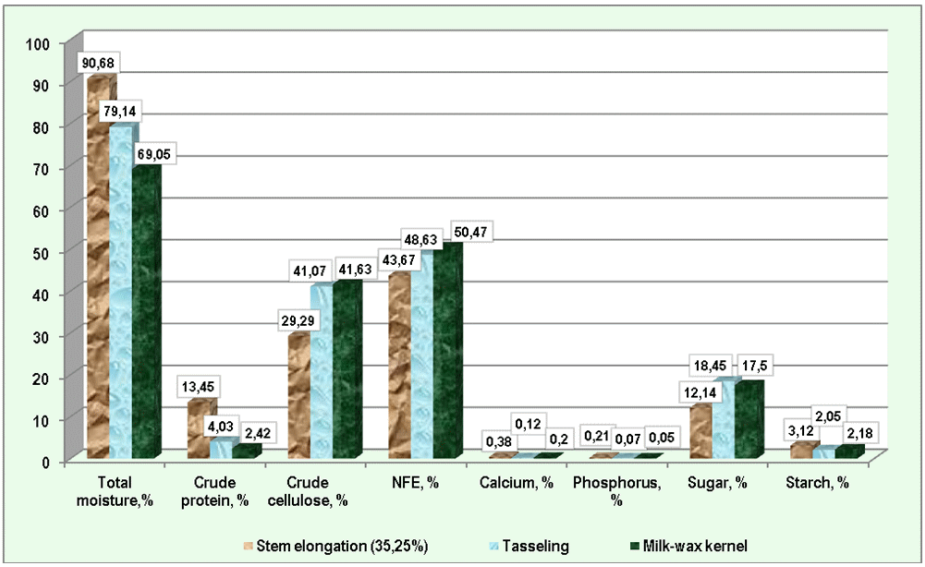

Figure 2. The nutrient content of stems of the sorghum x sudan grass hybrid 'SAȘM-4' depending on the development stage.

41.07% in the tasselling stage and to 41.63% in the milk-wax stage of the grains, so in the last two stages this index stabilized and was practically at same level, i.e. a very high level for green mass. A very high amount of nitrogen free extract is characteristic of the stems of this plant throughout their entire development 43.67-50.47%. A feature, which is characteristic only for the chemical composition of the stems of this hybrid, is the high content of sugars from 12.14% in the pre-tasselling stage, 17.50% in the milk-wax stage of the grains and up to 18.45% in the tasselling stage. The starch content was much lower and varied between 2.05% and 3.12%. The sugar content of the leaves was quite constant at 3.52-3.88%, and the amount of starch increased essentially by more than 2.4 times, i.e. from 1.05% to 2.53%. The amount of carotene and fats in the green mass of stems was low. During the growing season, there was a decrease in the ash content from 11.84% to 4.48%, in the calcium content from 0.38% to 0.12% and in phosphorus – from 0.21% to 0.05%. The nutritive and energy values of the stems harvested in the pre-tasselling were 0.08 nutritive units /kg and 0.88 MJ/kg metabolizable energy, during the tasselling stage there were 0.20 nutritional units /kg and 2.10 MJ/kg metabolizable energy, and the milk-wax stage of the grains had 0.21 nutritional units /kg and 2.64 MJ/kg metabolizable energy, respectively.

The results regarding the content of nutrients in the panicles of the hybrid sorghum x Sudan grass SAȘM-4 depending on the development stage are shown in Figure 3.

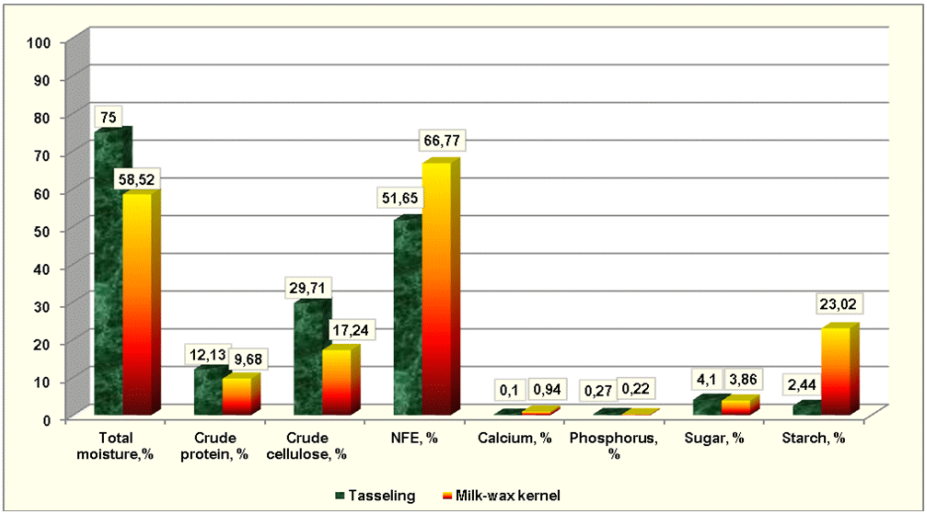

Figure 3. The nutrient content of panicles of the sorghum x sudan grass hybrid 'SAȘM-4' depending on the development stage.

An increase in the content of dry matter was observed along with the changes in its composition, thus, there was a decrease in the crude protein amount from 12.13% to 9.68% and in the crude cellulose from 29.71% to 17.24%, an essential increase in the crude fat content from 2.17 % to 4.14% and in the nitrogen-free extract from 51.65 to 66.77%. The most significant increase was observed in the content of starch – from 2.44 to 23.02% and in calcium – from 0.1% to 0.94%, which is more than nine times. The carotene content in panicles was low – 10.33-12.33 mg/kg. The nutritive and energy value of the fresh mass of panicles was of 0.08 nutritive units/kg and 0.88 MJ/kg metabolizable energy, during the tasselling period being 0.25 nutritive units/kg and 2.69 MJ/kg metabolizable energy, while in the milk-wax stage of the grains – 0.35 nutritive units/kg and 3.87 MJ/kg metabolizable energy, respectively.

4. Conclusions

During the growing season, in the plants of the hybrid sorghum x Sudan grass 'SAȘM-4' the content of dry matter increases, the concentration of proteins decreases and the amount of cellulose and nitrogen-free extract essentially increases. Leaves have higher content of crude protein, crude fat, carotene, ash, calcium and phosphorus, and the stems have high content of crude cellulose, nitrogen-free extract and sugars, and the panicle is rich in starch and provides the feed with high energy value.

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(Victor Țîței, Natalia Cîrlig, Ana Guțu, Natalia Mocanu, Serghei Cozari, Alexandru Teleuță, Nina Garștea)
"Alexandru Ciubotaru" National Botanical Garden (Institute) of Moldova State University

(Sergiu Coșman, Valentina Coșman) Scientific-Practical Institute of Biotechnologies in Animal Husbandry and Veterinary Medicine, Republic of Moldova

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Aspects of bird diversity and ecology in ”Riscani” forest park, Chisinau municipality

Tatiana Cîrlig

Abstract. Our study has been focused on the diversity and ecology of birds in the “Rîșcani” forest park, Chisinau municipality. The scientific investigations were conducted between April and August 2022. We inventoried 52 species of birds with uneven distribution in the territory included in this study. The greatest diversity was found in forest areas subjected less to the anthropogenic impact. Vegetation management practices applied in the park and the expansion of recreational areas are disruptive factors for bird species, especially for their breeding population. Of the avifauna of the area, 50 species, or 96,2% of identified birds are included in the lists of the International Union for Conservation of Nature (IUCN), and 44 species, or 84,6%, are also included in the lists of the Bern Convention.
Keywords: avifauna, biodiversity, ecology, anthroposystem.

Aspecte privind diversitatea și ecologia păsărilor din parcul-pădure „Rîșcani”, mun. Chișinău

Rezumat. Studiul nostru se referă la diversitatea și ecologia păsărilor din cadrul parcului-pădure „Rîșcani”, mun. Chișinău. Investigațiile au fost realizate în perioda aprilie-august 2022. Am inventariat 52 de specii de păsări cu distribuție inegală în teritoriul inclus în acest studiu. Cea mai mare diversitate a fost întâlnită în spațiile forestiere cu presiune antropică mai redusă. Practicile de întreținere a vegetației în cadrul parcului și extinderea zonelor de agrement prezintă un factor perturbator pentru speciile de păsări, în special, asupra populației lor de reproducție. Din ornitofauna zonei 50 de specii sau 96,2% de păsări identificate sunt incluse în listele Iniunii Internaționale pentru Conservarea Naturii (IUCN), 44 specii sau 84,6% din zonă se găsesc și în listele Convenției Berna.
Cuvinte-cheie: ornitofauna, biodiversity, ecology, anthroposystem.

1. Introduction

Biodiversity in the Republic of Moldova is permanently threatened by human activities. Environmental pollution and climate change, fragmentation of habitats, illegal logging and the increase in the number of rare species are some of the factors that negatively

influence biodiversity in the territory. The biodiversity of anthropogenic landscapes in the republic is often richer in terms of number of species than in wild areas.

The presence and diversity of birds in anthropogenic ecosystems plays an important role, because having a very high plasticity to environmental changes, they provide us with information about environmental quality. The previous research conducted within the anthropogenic ecosystems of Chisinau municipality has revealed a tendency towards certain changes in the composition of the urban avifauna. In the last decades, new species of birds have been detected that have visited and are visiting or even managed to become common for the parks in the municipality of Chisinau. Thus, such new species as: *Tadorna tadorna, Gavia stelata, Tacybaptus ruficolis, Dryocopus martius* were found in the "Valea Trandafirilor" Park, *Aythya marila* was found in the "La Izvor" Park, the species *Turdus merula* has become a common species in several green spaces of the city; the populations of some species of birds found, such as: *Garrulus glandarius, Columba palumbus* [5], sporadically in urban parks, in recent years had considerably increased.

There are several studies on the bird fauna of Chisinau, carried out within the Institute of Zoology [3; 6; 7; 8]. Most of the studies on the urban avifauna of the municipality refer to the forest spaces in the parks "La Izvor", "Valea Trandafirilor", Botanical Garden and Dendrological Park. The avifauna of the "Rîșcani" forest park has been less studied. To study the diversity of birds in the research area, two itineraries were identified. The first includes the southern part of the park, which is about 2,5 km long Within the given itinerary, the phytocenosis is represented by a natural, spontaneous forest, which is then gradually replaced by ornamental forms characteristic of parks. The relief is heavily uneven; there are many ravines, but also clearings with spontaneous forest vegetation. The second itinerary includes the meadow on the bank of the stream near the park, which, being dammed, forms two lakes.

The "Rîscani" forest park was founded in 1970 on the basis of a forest massif, being located between two sectors of Chişinău, Ciocana and Rîscani. The total area of the park is 32 hectares. The park is divided into two parts by Aleco Russo str., which crosses it. There are water bodies in both parts of the park. The vegetation of the park, under the influence of anthropogenic factors, has become poor in herbaceous forest plants, and the maple (*Acer platanoides*) predominates in the stands on the hills, the elm (Ulmus glabra, U. pumila) and the poplar (*Populus canescens, P. nigra*) is in the valley [4]. The vertebrate fauna within the park is represented by about 72 species. Among them, there are 7 species of amphibians, 3 species of reptiles, 52 species of birds and 10 species of mammals.

2. Materials and Methods

The field survey was carried out between April and August 2022. The established transects were monitored twice a month using binoculars (Carl Zeiss 8x30) and guidelines to identify birds [1]. The list of avifauna observed in the “Rîșcani” forest park includes 52 species, most of which are typical forest species and are characterized by uneven distribution in the territory.

3. Results and Discussions

The birds found in the researched area belong to 9 orders, the most numerous being the representatives of *Passeriformes* – 67,3% (35 species) (Fig. 1). The passerines detected in the area belong to 14 families: *Hirundinidae, Motacillidae, Laniidae, Oriolidae, Sturdidae, Corvidae, Sylvidae, Turdidae, Paridae, Sittidae, Certhidae, Passeridae, Fringillidae* and *Emberizidae*. The most numerous are: *Turdidae* – 7 species, *Corvidae, Sylvidae* and *Fringillidae*, the other families are represented by 1 to 4 species. It is followed by

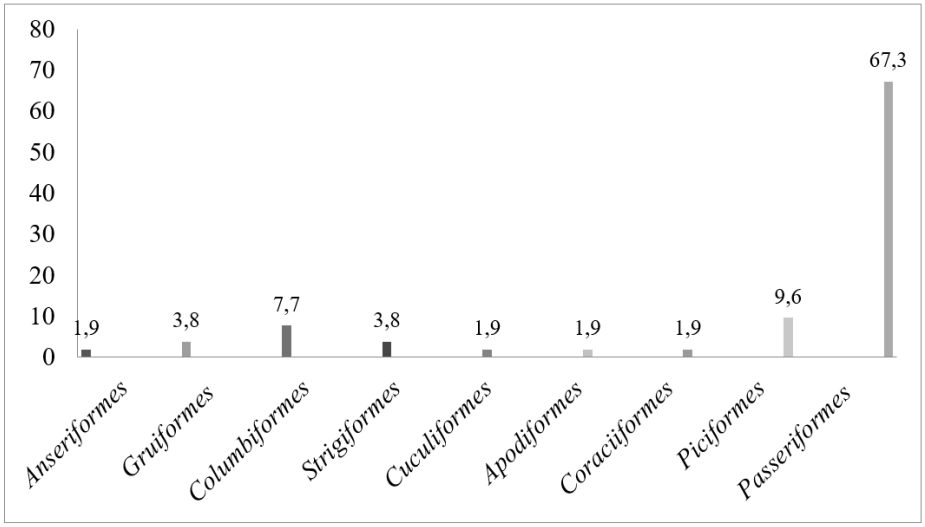

Figure 1. The taxonomic structure of the avifauna in the “Rîșcani” park.

the order *Piciformes* – 9,6% (5 species): *Dendrocopos syriacus, D. major, D. minor, Picus canus, Jynx torquilla*. Of the order *Columbiformes*, the following species were detected: *Columba livia domestica, C. palumbus, Streptopelia decaocto, S. turtur*. The representatives of *Gruiformes* and *Strigiformes* were *Gallinula chloropus, Fulica atra* and, respectively, *Asio otus* and *Otus scops*. The other orders are represented by the species *Anas platyrhynchos, Cuculus canorus* and *Apus apus*.

The habitat preferences of the avifauna of the park are quite varied: forest spaces and open spaces, areas with bushes and wetlands near water bodies, as well as spaces where various human-made constructions are present. Forest areas are home to 55.8% (29 species) of birds (Fig. 2). Next, the spaces with buildings follow. Here 10 species were

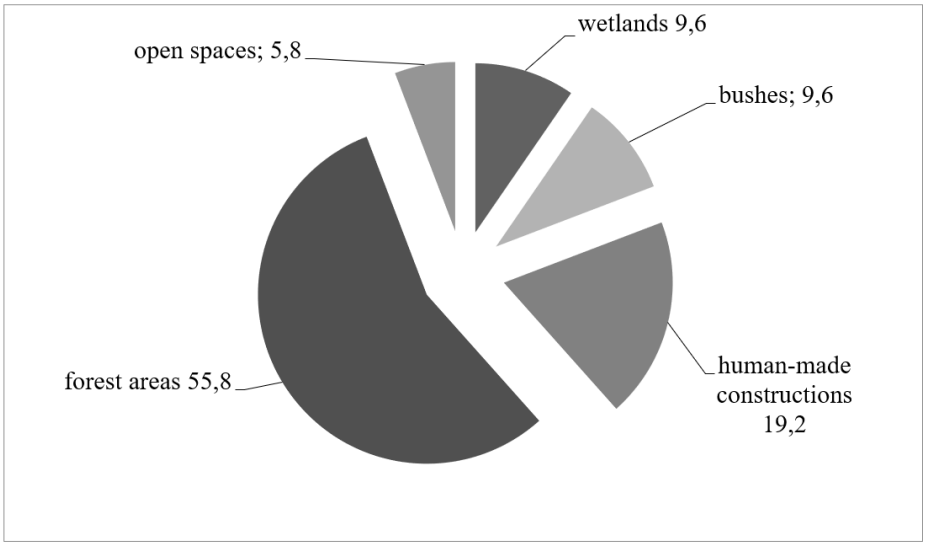

Figure 2. The habitat preferences of the avifauna in the "Rîșcani" park, %.

observed, or 19,2% of the total number of birds recorded within the park. The spaces of the park occupied by scrub, the wetlands – the water bodies and adjacent territories, as well as the open spaces within the park are less preferred by birds. Thus, by 5 species of birds were recorded in the scrub areas as well as in the water bodies and their adjacent territories, which represent by 9,6% each. In the open areas of the park, with stony soil and with sparse shrubby vegetation, only 3 bird species, or 5,8%, were recorded.

The preferences of the avifauna of the "Rîșcani" park over forest spaces and spaces with buildings can be explained by the fact that in the given areas birds have more opportunities to obtain food, build nests and ensure their safety, defence from enemies etc. Likewise, in forest areas, birds are less disturbed by people, who use the given park area for walks, rest, recreation etc.

According to nutritional preferences, most of the birds in the park are insectivores and omnivores (Fig. 3). Thus, there are 21 species of insectivores, or 40,4%, and 20 species of omnivores, or 38,5%. The nutritional spectrum of omnivorous birds represents a wide variety of food types: seeds, fruits, plant parts, insects, their larvae, gastropod molluscs, worms, earthworms and other invertebrates, and sometimes small vertebrates. Insectivorous birds usually eat only various insects and their larvae.

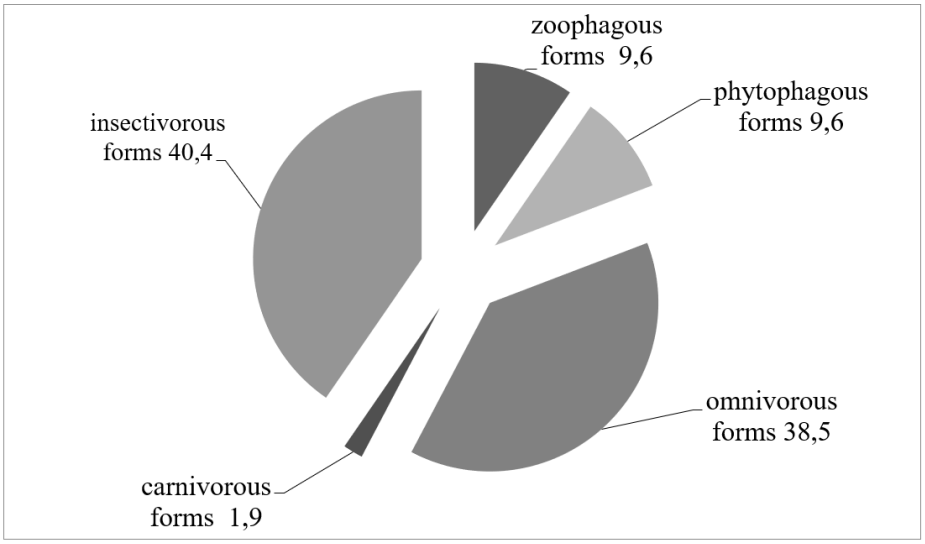

Figure 3. The nutritional spectrum of the avifauna in the “Rîșcani” park, %.

The zoophagous and phytophagous forms in the area are less common, represented by 5 species each, or by 9,6% each. Zoophagous birds feed on various invertebrates and small vertebrates: insects, their larvae, molluscs, worms, earthworms, lizards, frogs, tadpoles, fish etc. The phytophages have a diet that consists of food of plant origin, such as plant parts, seeds, fruits etc.

The carnivorous birds in the studied territory are represented by a single species – the long-eared owl (*Asio otus*). The long-eared owl feeds mainly on field mice, wood mice, rats, less often shrews, small birds, squirrels, moles and bats.

During the period of observations in the study area, only the representatives of the following phenological groups were observed: migratory birds present during summer and sedentary birds. The species that appear only during the warm period of the year for reproduction make up 29 bird species, which is 55,8%, the sedentary ones are also numerous – 23 species, or 44,2%. Of the migratory birds present during summer, we observed the representatives of the following genera: *Luscinia, Sylvia, Cuculus, Oriolus, Saxicola, Phoenicurus, Phyloscopus* etc. The sedentary birds found in the park belong to the families: *Picidae, Corvidae, Paridae, Fringillidae* etc.

Analysing the frequency of bird species in the research area, we concluded that rare species are very few – only 2 species, which represent 3,8%. The rare bird species detected in the research area are *Corvus monedula* and *Streptopelia turtur*.

Common bird species in the research area are the most numerous – 53,8% (28 species). There are such species as: *Anas plathyrhynchos, Fulica atra, Columba livia, Columba*

palumbus, Apus apus, Dendrocopos syriacus, Dendrocopos major, Picus canus, Hirundo rustica, Delichon urbica, Motacilla alba, Oriolus oriolus, Sturnus vulgaris, Garrulus glandarius, Pica pica, Corvus frugilegus, Corvus corone cornix, Phyloscopus colibita, Phoenicurus ochrurus, Turdus merula, Parus major, Parus caeruleus, Passer domesticus, Passer montanus, Fringilla coelebs, Coccothraustes coccothraustes, Carduelis chloris, Carduelis carduelis.

The frequent species of the park birdlife, likewise, are quite numerous – 42,4% (22 species): *Gallinula chloropus, Streptopelia decaocto, Cuculus canorus, Alcedo athis, Otus scops, Jynx torquilla, Acrocephalus arundinacesus, Sylvia communis, Sitta europea, Luscinia luscinia* etc.

Analysing the state and the conservation status of the avifauna in the "Rîșcani" park, we would like to mention that, of 52 species of birds present in the territory, not a single species is found in the Red Book of the Republic of Moldova. In contrast, 50 species or 96,2% of identified birds are included in the lists of the International Union for Conservation of Nature (IUCN), 44 species or 84,6% are mentioned in the lists of the Bern Convention and 3 species or 5,8% are present in the lists of the Bonn Convention (Fig. 4).

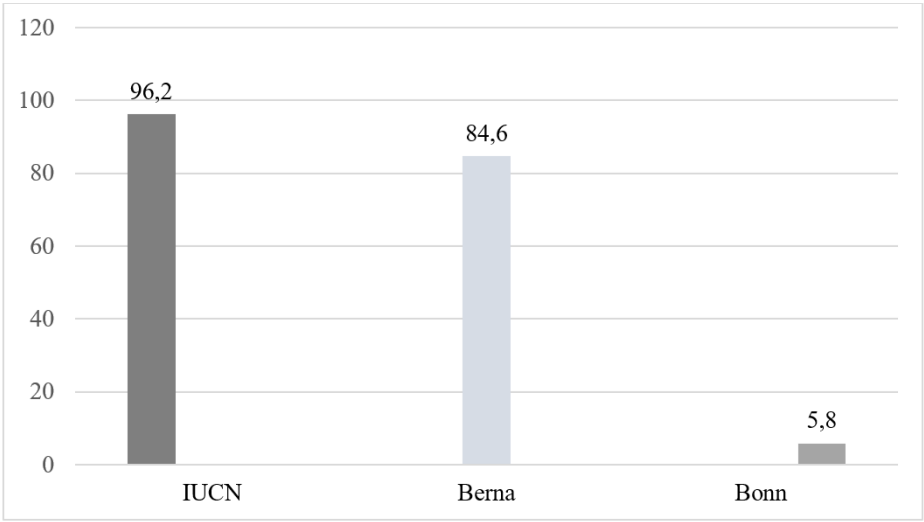

Figure 4. The conservation status of the avifauna of the "Rîșcani" park, %.

Bird species in the park on the Bern Convention lists are included in Appendix II - *Strictly protected fauna species* and Appendix III - *Protected fauna species*. For the protection of the species listed in Appendix II, the following is prohibited: all forms of deliberate capture and keeping, deliberate killing, the deliberate damage to or destruction

of breeding or resting sites, the deliberate disturbance of wild fauna, particularly during vulnerable periods of life (breeding, hibernation etc.), the deliberate destruction or taking of eggs from the wild or keeping these eggs even if empty, the possession of and internal trade in these animals, alive or dead, including stuffed animals and any readily recognisable part or derivative thereof. Appendix III of the Convention covers protected species whose exploitation must be regulated in order to protect them [2]. In the lists of Appendix II of the Bern Convention, 33 species of birds found in the “Rîșcani” forest park are included. In the lists of Appendix III – 11 species.

4. Conclusions

(1) In the territory of the “Rîscani” forest park, 52 bird species were identified.
(2) We recorded the greatest diversity of birds in the areas of the park with various bushes and trees of very different ages, but also with a low level of human presence.
(3) Vegetation maintenance practices applied in the park and the expansion of recreational areas present a disturbing factor for bird species, in particular, for their reproduction process.
(4) Most of the bird species are included in the lists of the International Union for Conservation of Nature and the Bern Convention.

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(Tatiana Cîrlig) "Ion Creangă" State Pedagogical University of Chisinau
E-mail address: carlig.tatiana@upsc.md

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The importance of the EMERALD sites for the conservation of biodiversity

Elena Moşanu, Anatol Tariţă, Raisa Lozan, and Maria Sandu

Abstract. Biodiversity illustrates the extraordinary variety of life on Earth, but technological progress and the intensive use of natural resources have considerably increased the anthropogenic impact on biological diversity. Therefore, to stop the decline of biodiversity through the long-term conservation of the most valuable and endangered species and habitats of European interest to identify, maintain, and restore areas of special interest for the conservation of wild fauna and flora species, the EMERALD Network was established. To integrate the objectives of conservation and protection of species and habitats of national and local interest, education, information, and public involvement in the efficient management of the heritage of the Emerald Network site and Natura 2000, it is necessary to develop management plans for EMERALD sites.
Keywords: biodiversity, EMERALD site, management plan, conservation.

Importanța siturilor EMERALD pentru conservarea biodiversității

Rezumat. Biodiversitatea ilustrează varietatea extraordinară a vieții de pe Pământ, dar progresul tehnologic și utilizarea intensivă a resurselor naturale au sporit considerabil impactul antropic asupra diversității biologice. Prin urmare, pentru a stopa declinul biodiversitatii prin conservarea pe termen lung a celor mai valoroase și pe cale de dispariție specii și habitate de interes european cu scopul de a identifica, menține și reface zonele de interes deosebit pentru conservarea speciilor de faună și floră salbatică, a fost înființată Rețeaua Emerald. Pentru integrarea obiectivelor de conservare și protecție a speciilor și habitatelor de interes național și local, educație, informare și implicare publică în gestionarea eficientă a patrimoniului sitului Rețelei Emerald și Natura 2000, este necesar de elaborat planuri de management a siturilor EMERALD.
Cuvinte-cheie: biodiversitate, sit EMERALD, plan de management, conservation, conservare.

1. Introduction

One of the main concerns of humanity in Mineniul III is the conservation of biological diversity at the level of ecosystems, species, populations, and genes. But with the advancement of technological progress and the intensive use of natural resources,

the anthropogenic impact on biological diversity has considerably increased, essentially reducing the number of species and varieties of living organisms that populate the Earth.

In Europe, globally, the loss of biodiversity is accelerating. The current rate of species extinction is estimated to be 100 to 1000 times higher than the natural rate, which is much higher than the rate that allows the emergence of new species. According to the International Union for Conservation of Nature, 15% of mammals, 13% of birds, 37% of freshwater fish, and 23% of amphibians in Europe are threatened with extinction [8].

As a result, biodiversity constitutes a natural heritage that must be preserved and passed on to future generations, especially because of its essential value and the services it provides to humans (food production, climate regulation, water purification, pollination, soil fertility, etc.). Some of the major causes of biodiversity loss are the fragmentation of habitats, pollution, over-exploitation of natural areas, and artificialization of landscapes. The solution for neutralizing these causes and, consequently, for the protection of wild flora and fauna is the preservation of the natural environment.

2. Methods and materials used

The detailed analysis of the current situation of the environmental components (water, air, soil, flora, fauna) in the sites of the Emerald Network formed the basis of the research methodology used. The methods of analysis (in the field) of animal and plant species were used according to [14]. The inventory of rare species was carried out using the transect method [10]. The protection status of rare species was determined according to national and international documents [1-2, 4-7]. Water samples were collected and analyzed in accordance with SM SR ISO 5667-6:2011 [3].The soil samples were collected according to the ”satellite” method [9]. As well as the national legislative-normative framework in force [11-12, 15-16].

3. Obtained results and discussion

Nature does not take political and administrative borders into account and the best method to protect the natural environment is to coordinate the efforts of countries and jointly strengthen the means. Thus, the survival over time of the numerous endangered species was ensured by a coordinated joint effort of the European community, and consequently, each country was obliged to assume individual responsibilities.

Thus, in 1993, the Republic of Moldova, together with the member countries of the Council of Europe, undertook to actively participate in ensuring the conservation of natural habitats, spontaneous flora and fauna, including migratory bird species, on the verge of extinction by ratifying the Convention on the Conservation of Wild Life and of

Natural Habitats (Bern, 1979) [5], which is an indispensable international legal instrument for strengthening the conservation of wild flora and fauna and their natural habitats and for promoting interstate cooperation. For the same purposes, in 1995, the Republic of Moldova also ratified the Convention on Biological Diversity [5], becoming a party to ten international and regional conventions and agreements in the field of biodiversity conservation.

For the more effective protection of biodiversity, in 1989, the contracting parties of the Berne Convention initiated the creation of a special instrument for the protection of Europe's natural environment: the EMERALD Network [8]. As a continuation of the European biodiversity conservation process, in the Republic of Moldova by Law no. 94/2007 regarding the ecological network, the National Ecological Network was established as a component of the Pan-European Ecological Network (Fig. 1).

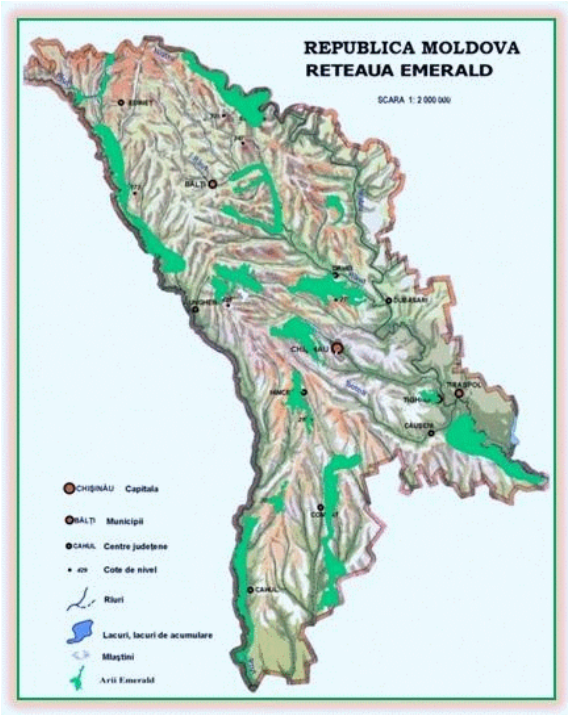

Figure 1. The Emerald network in the Republic of Moldova [9].

And to stop the decline of biodiversity through long-term conservation, with the aim of identification, of the most valuable and endangered species of flora and fauna and habitats of European interest, maintaining and restoring areas of special interest, the Emerald Network was established. The given network is composed of areas of special interest for conservation (ASIC) [15]. These are areas of essential value, with the potential to

contribute to the maintenance or restoration of species and habitats in a favorable state of conservation, especially in terms of:

- endangered, endemic, migratory, and rigorously protected species under the Berne Convention;
- endangered and exemplar habitat types, as well as mosaics of various habitat types;
- migratory species that constitute a general heritage for European countries.

ASIC are areas assessed from a scientific point of view as suitable to achieve the objective of the conservation of species and habitats. Especially when the sites represent fairly well, the species and habitats in their distribution area, their diversity, and their specific conservation needs as well as the area of habitat and the percentage of populations of the species included in the sites are considerable concerning the overall national resource.

The Emerald network includes around 3,500 candidates or certified EMERALD Sites in 16 countries, approximately 600,000 km^2, and an average of 11%-12% of the national surface of the countries involved [8].

To integrate the objectives of conservation and protection of species and habitats of national and local interest, education, information, and public involvement in the management of the heritage of the Emerald Network site and Natura 2000, it is necessary to develop management plans for EMERALD sites.

The Management Plan is to promote a management model that allows the sustainable development of human communities and the conservation of species and habitats, biological diversity, and other values of the natural environment in the protected area.

In the Republic of Moldova, EMERALD NETWORK Sites are devoid of Management Plans. Their lack forced us to elaborate up the first management plan for the ”Cărbuna” Landscape Reserve - Emerald site in 2020, after which, within the framework of the institutional project (with institutional funding), ”Completion of the data bank of the register of the automated information system of the fund of natural areas protected by the state” natural areas protected by the state”, for the stage of 2022, the Management Plan of the EMERALD Site ”Pădurea Hâncești” was developed [19]. Thus, the developed plans serve as a model of sustainable management of human communities and the conservation of species and habitats, biological diversity, and other values of the natural environment in protected areas.

In the Lower Dniester area, the ”Cărbuna” Landscape Reserve is the second most important protected area. Founded back in 1933 on an area of 35 ha, by 1975 it had expanded its area to 356 ha, today exceeding 600 ha.

The natural reserve ”Cărbuna” - EMERALD Site with the code MD0000022 with an area of 678 ha, includes 2 habitats regarding the conservation of wildlife and natural habitats in Europe (Berna, 1979): 1. Oak-hornbeam forests - G1A1; 2. Ponto-Sarmatic deciduous thickets - Ponto-Sarmatic deciduous - F3.247; 3 bird species, and 4 other species according to the updated list of officially adopted EMERALD Sites in December 2019 [8] (Tab. 1). Some species of birds, reptiles, and insects from the given list are given below:

Table 1. Updated list of Emerald Officially Adopted Sites (2019)

Republic of Moldova							
Site Code	Site Name	Site Area (ha)	Birds species number	Other species number	Habitat number	Total features	Biogeo region(s)
MD0000022	”Carbuna” Natural Reserve	678	3	4	2	9	CON
MD00000019	Pădura Hâncești	11290	18	8	4	30	CON, STE

Updated list of officially adopted Emerald sites (December 2019), pag. 9.

A 1188 Bombina bombina, B A429 Dendrocopos syriacus, I 1083 Lucanus cervus, B A073 Milvus migrans, M 1323 Myotis bechsteinii, A 1166 Triturus cristatus (A = Amphibians, B = Birds, I = Invertebrates, M = Mammals, P = Plants, R = Reptiles) [8].

The EMERALD Site ”Cărbuna” has a varied biodiversity, due to the divergent climatic and topographical conditions found in this area. Natural forces, combined with human interaction over time, have created a complex and confusing patchwork of natural and semi-natural habitats, each with a different content of plants and animals.

Biodiversity in the EMERALD Site ”Cărbuna” is, however, damaged. The main pressures and factors behind this decline are habitat destruction, degradation, and fragmentation due to land use change. Key pressures include over-exploitation of natural resources and environmental pollution. The effects of climate change on biodiversity are also visible, with changes in species distribution, migration, and reproduction patterns. Through the lens of the management plan of the EMERALD Site ”Cărbuna” , there are

proposed the following general actions to improve the situation: Stopping the decline of biological diversity represented by genetic resources, species, ecosystems, and landscape and restoring the degraded systems, to stop the reduction of the areas with fundamental natural stands proposes that the fundamental natural oak stands (*Quercus pubescens, Quercus petraea* and *Quercus robur*) be managed only by the method of installation and development of the seed, the regeneration of the oak will be carried out only from the account of natural regeneration. Areas with *Carpinus orientalis* require particular attention. It is also necessary to regulate the rest and recreation of the population in the territory of the protected area according to the regulations in force, it is recommended to integrate the policies regarding the conservation of biodiversity in the researched area and the promotion of knowledge, practices and traditional innovative methods and clean technologies as support measures for the conservation of biodiversity as support of sustainable development [18].

Recommended: activities to maintain or improve the conservation status of rare species: species of flora and fauna: A 1188 *Bombina bombina*, B A429 *Dendrocopos syriacus*, I 1083 *Lucanus cervus*, B A073 *Milvus migrans*, M 1323 *Myotis bechsteinii*, A 1166 *Triturus cristatus* as well as the two habitats: - G1A1 Oak and hornbeam forests - Oak-hornbeam forests, F3.247 Ponto-Sarmatic deciduous thickets - Ponto-Sarmatic deciduous; monitoring the quality of water bodies, to identify those that are at the limit of achieving the objectives of the Water Framework Directive, and using the appropriate measures to restore their condition, as well as evaluating the extent of the eutrophication process by determining the trophic index, monitoring avifauna from the EMERALD Site ”Cărbuna” [18].

It is necessary to monitor the avifauna from the EMERALD Sites ”Cărbuna”, and the results obtained will be recorded regarding the sex of the birds, the rearing behavior of the chicks, and the age class. Habitat data will be recorded for each segment. Floating nests (platforms) will be installed to improve the breeding conditions of water-nesting bird species, creating favorable nesting conditions for birds and prohibiting the presence of humans in the nesting areas during April-June; Realization of a continuous regime of protection of bird colonies during the breeding period; Improving the trophic base of birds through actions to maintain the water level in aquatic ecosystems (performing some dyke activities, cleaning and preventing the phenomenon of clogging, planting new protective strips, etc.), monitoring reptiles, etc.

In the same way, the permanent assessment of air quality and atmospheric precipitation at the EMERALD Sites ”Cărbuna” (local and transfrontier pollution) is required, because

the accumulation of large amounts of atmospheric pollutants have an impact on the environment, such as the greenhouse effect, global warming, air pollution, ozone depletion, and acid rain. And the inclusion of landscape elements and biodiversity conservation principles are indicated to be included as major conditions for the development of tourism infrastructure.

The Emerald site - MD code 00000019 "Pădura Hâncești" is located between the villages of Lăpușna and Merești in the Hâncești district, Republic of Moldova. The specific objective of the Site is the protection of the fundamental natural groves pedunculate oak, rare plant species, and landscape landscapes. This site includes the Natural Reserve of Medicinal Plants "Logănești" with an area of 710 ha and the Landscape Reserve "Pădurea Hâncești" with an area of 4499.0 ha [7].

The protected area includes three habitats of European importance, they are 9170 (A): Oak forests of *Quercus robur* and *Quercus petraea* on rocky slopes and gravel alluvium; 9170 (B), rocky oak forests with the predominance of *Quercus pubescens* on developed carbonate soils; 91 HO and Balkan forests with *Quercus pubescens*.

The ecological state of the mentioned habitats is satisfactory, the dominant stand, according to the state of health, is assigned to the category of healthy trees and trees with the crown partially affected by the accompanying species. Biological diversity includes 29 rare plant species and 19 animals with national and international protection status, including 8 species of plants and 12 animals found in the Red Book of the Republic of Moldova. Here you can find 2 plant species (*Fritillaria Montana* and *Pulsatilla grandis*) and 6 animal species (*Bombina bombina, Triturus cristatus, Lucanus cervus, Morimus asper funereus, Zerynthia polyxena*, and *Euplagia quadripunctaria*), species from the Reference List of species of Union interest.

There have been identified 27 species of birds, of national and international importance that require priority protection, of which 6 species of woodpeckers (including 2 species from the RBM – the oak woodpecker and the black woodpecker). Birds Directive 2000 – 10 species; - Bern Convention- 8; GEO Romania – 17. It was established that the average density of the oak woodpecker in the forest ecosystem of the Emerald site "Pădurea Hâncești" is approximately 0.9 individuals per km^2, and the black woodpecker - 0.3 individuals per km^2. Common bird species identified are 50 species, most of them being from the Passeriformes Order [19].

The elaborated management plan of the site has actions that would allow the improvement of the quality of the environmental components in the area, such as: management, protection and conservation of forest sectors of European importance, where the ecological management prevails over the economic one; restricting any intervention in the

state-protected areas (LR Pădurea Hânceşti and NRMP Logănesti) in the territory of the Site; sustainable collection of natural resources without affecting the trophic base of habitats; performing sustainable Biomonitoring; the installation of artificial nests in poorly productive sectors, with low densities of trees older than or equal to 70 years; in the execution, modification or expansion of the activities in the field (use of water resources for different purposes, constructions, installations on water or related to water, use of minor riverbeds) the Regulation on the procedure for establishing the protected natural area regime [12]; continuous assessment of air quality and atmospheric precipitation at the EMERALD Site "Pădurea Hânceşti" (local and transfrontier pollution), etc.[19].

Prohibition of hunting activities in protected areas, they represent the only place of refuge for fauna. It is also recommended to carry out afforestation in deforested areas, for faster regeneration of affected forest habitats.

The systematic assessment of the ecological state of the surface and underground waters as well as the continuous assessment of air quality and atmospheric precipitation at the level of the Emerald site "Pădurea Hânceşti" (local and transfrontier pollution), etc.[19]

The Management Plans include actions to improve the administrative and scientific staff of the EMERALD Sites "Cărbuna" and "Pădurea Hânceşti" of the ecological education of the young generation from the localities in the area through the prism of the development of some thematic stands on the conservation of biodiversity, of the scientific evaluation courses of different groups of animals in nature and of acquiring contemporary methods of fauna assessment in field conditions, etc. as well as consultation, awareness, and information activities through the organization of lessons in educational institutions, conferences, round tables, seminars, distribution of leaflets, participation in profile television shows, etc.

4. Conclusion

These studies address and integrate the recommended actions, respecting national and European legislation on environmental components and especially biodiversity, and the results obtained contribute to the scientific substantiation of the development of recommendations to improve the sustainable management of the EMERALD Sites.

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(Elena Moşanu, Anatol Tariţă, Raisa Lozan, Maria Sandu) Institute of Ecology and Geography of the Moldova State University

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Enterobacteria from the genera *Salmonella* and *Shigella* with an etiological role in acute diarrheal diseases

Nadejda Guțu, Olga Postolachi, and Elena Zariciuc

Abstract. The research was carried out during the years 2011-2022 in PHMI TMA Buiucani, 12496 samples were processed and 129 strains (1.03%) were isolated. The major causative agents of acute diarrheal disease were pathogenic enterobacteria *Salmonella* spp. (90.7%) and *Shigella* spp. (9.3%). The dominant serovars were *Salmonella* Enteritidis (53.49%) and S. Typhimurium (37.21%), and *Shigella* serogroups constituted 4.65% each. The isolation rate of *Salmonella* and *Shigella* strains decreased ranging from 1.67% (year 2011) to 0.12% (year 2022), except for 2014 (2.01%) and 2015 (1.78%). To mitigate acute bacterial diarrhea, it is necessary to apply infection prevention strategies.
Keywords: pathogens, Enterobacteriaceae, *Salmonella*, *Shigella*, acute diarrheal diseases.

Enterobacteriile din genurile *Salmonella* și *Shigella* cu rol etiologic în bolile diareice acute

Rezumat. Cercetările au fost efectuate în perioada anilor 2011-2022 în IMSP AMT Buiucani, au fost prelucrate 12496 de probe și izolate 129 tulpini (1,03%). Agenții cauzali majoritari ai bolii diareice acute au fost enterobacteriile patogene *Salmonella* spp. (90,7%) și *Shigella* spp. (9,3%). Dominante au fost serovarurile *Salmonella* Enteritidis (53,49%) și S. Typhimurium (37,21%), iar serogrupurile de *Shigella* au constituit 4,65% fiecare. Rata izolării tulpinilor de *Salmonella* și *Shigella* a scăzut variind de la 1,67% (anul 2011) până la 0,12% (anul 2022), cu excepția anilor 2014 (2,01%) și 2015 (1,78%). Pentru atenuarea diareii bacteriene acute este necesar de a aplica strategii de prevenire a infecțiilor.
Cuvinte-cheie: agenți patogeni, Enterobacteriaceae, *Salmonella*, *Shigella*, boli diareice acute .

1. Introduction

Among the enteric pathogens of bacterial origin, which endanger human life worldwide, there are included *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Vibrio cholerae*, *Escherichia coli, Yersinia enterocolitica*. Among these, the genera *Salmonella* and *Shigella* continue to be the major cause of acute diarrheal disease (ADD) in many

countries, thus presenting a serious challenge to health authorities. The aforementioned pathogens cause a well-characterized spectrum of disease in humans, ranging from asymptomatic states to hemorrhagic colitis and fatal typhoid fever [2]. Bacteria of the genus *Salmonella* are actively fermentative and cause the disease called salmonellosis in humans. Salmonellosis remains a serious socio-economic problem for the most countries of the world. Actually, even in all economically developed countries of the world, salmonellosis is widespread. Currently, more than 2500 serotypes of *Salmonella* are described, some of them having host specificity, found only in humans or only in certain species of animals and birds [10]. In the United States, 4 serotypes represent nearly half of all reported human isolates, namely *Salmonella* Typhimurium (19%), *Salmonella* Enteritidis (14%), *Salmonella* Newport (9%), and *Salmonella* Javiana (5%) [1].

All representatives of the genus *Shigella* are capable of causing a widespread disease - dysentery. Dysentery has been reported since ancient times, today recognized as "dysenteric syndrome" (muco-sanguineous stools, tenesmus and abdominal cramps, frequent stools) characteristic for several diarrheal diseases. Since the last century, it has also traditionally been called "bacillary dysentery", to differentiate it from amoebic dysentery. *Shigella* are gram-negative, aerobic, non-sporulating, immobile microorganisms. They differ in antigenic structure, biochemical activity, pathogenicity and virulence. A major virulence is possessed by *Shigella dysenteriae*, especially the S. *shigae* serotype, which secretes exotoxin with hemolytic properties. Fairly high virulence possesses S. *flexneri* and less pronounced – S. *sonnei*. Shigelloses are typical anthroponoses. Both the reservoir and the sources of the pathogen are hosted by the sick person, with an acute or chronic form of infection. A major risk represent the patients with mild or cleared forms of infection, especially people who are part of the socially vulnerable contingents [8].

In the Republic of Moldova, in 2021 the dysentery morbidity increased insignificantly to 0.06 cases per 100 thousand, compared to 2020 (0.03). In the Eastern districts, morbidity remains at the same level, constituting 1.71 cases per 100 thousand. During the years 2021 and 2020, no outbreak of dysentery was reported, the last outbreak being recorded in 2017. After the worsening of the epidemiological situation due to salmonellosis in 2014 (52.9 per 100 thousand), when the maximum indicators were reached after zero in 1992, in 2021 the morbidity increased insignificantly to 7.31 (by 12.4% compared to 2020), including by increasing the number of epidemic outbreaks – 8 (2020 – 7, 2019 – 34). As in previous years, in 2021 there were no significant changes in the etiological structure of salmonella in the population and in the environment, predominant are S. Enteritidis – 84.5% (2020 – 84.6%, 2019 – 76.9%) and S. Typhimurium – 11.6% (2020 – 14.1%, 2019 – 17.2%). According to National Agency for Food Safety (ANSA) data, 36 strains of

salmonella were detected in animal products (raw material and finished products) in 2021, including: S. Infantis – 25.0%, S. Kentucky – 13.9%, S. Fillmore – 5.6%, S. Enteridis – 5.6%, S. Montevideo – 11.7%, *Salmonella* spp. – 33.4%, etc. [6].

Morbidity through salmonellosis is mainly recorded among the urban population. The multi-year dynamics of morbidity due to salmonellosis in the country has a cyclical character. In the last 70 years two periods are highlighted: the first one – 1961-1989, with a tendency of increasing morbidity, period associated with the intensification of animal breeding in zootechnical complexes, and the second – period marked by a tendency of decreasing morbidity, associated with the lowering of animal number maintained in complexes and their increasing in the private sector. The periodicity of the epidemic process is largely conditioned by the changes in the etiological structure. The morbidity increase in the 70s of the last century was caused by S. Typhimurium. The share of this *Salmonella* serovariant in the etiological structure in the given period was 60.8%. The increase in morbidity in the 80s was mostly (≈70%) caused by S. Enteritidis. In the years 1999-2005, S. Typhimurium serovar prevailed in the etiological structure (60%), and in the last period (2006-2012) dominated S. Enteritidis (59.13%). Adequate sanitary-hygienic rules must be strictly followed when transporting, storing, preparing and marketing food products. An important measure concerns the deratisation and disinsection of storage and food processing objects [8].

Morbidity through shigellosis is also more frequently recorded in the urban population in comparison with the rural population. Most illnesses occur in the warm season of the year. In the summer-autumn months, 70-80% of the annual morbidity is registered. During the warm time of the year, the need for drinking water essentially increases and favorable conditions are created for the multiplication of shigella in food products, especially in dairy products, but also in water, conditions that can lead to more frequent occurrence of both sporadic cases and of outbreaks or epidemics [8].

The purpose of this work is to identify the pathogens from genera *Salmonella* and *Shigella* from the Enterobacteriaceae family, their share in the etiology of acute diarrheal disease and the dynamics of the spread in the Public Health Medical Institution TMA Buiucani, Chisinau.

2. Materials and Methods

The research was carried out during the years 2011-2022. In total, 12496 clinical samples were investigated in the microbiological laboratory of the Public Health Medical Institution (PHMI) TMA Buiucani. The etiological confirmation of the disease was carried out on the basis of coproculture, according to the methodological indication

"Microbiological diagnosis of intestinal infections" recommended by MH RM [4]. Before being inoculated, each fecal matter sample was homogenized with sterile saline solution NaCl 0.85% - 2 ml. The loop was loaded with fecal matter and then discharged on the surface of the agar plate in the form of perpendicular streaks. The primary seeding of the biomaterial was done in Petri dishes on culture media (Endo agar, UTI chromogenic agar, Bismuth sulfite agar, Columbia agar + 5% blood) and thermostated at 37°C for 24 hours. After the incubation period, the Petri dishes and the tubes containing the sample are removed from the thermostat and the lactose-negative colonies are examined. For the identification of microorganisms from the Enterobacteriaceae family, the Kligler Iron Agar medium was used, based on the double fermentation of sugar and the production of H2S, and biochemical tests on differential media Urea agar, Citrate agar, Simmons agar, Acetate agar. The confirmation of pathogenic bacteria (*Shigella* spp., *Salmonella* spp.) was performed by the serological method of slide agglutination with specific polyvalent and monovalent immune sera [4, 5].

3. Results and Discussion

From the total number of investigated clinical samples (12496) there were identified 129 strains (1.03%) in which the etiological agent was represented by pathogenic microorganisms from 2 genera of the Enterobacteriaceae family: *Salmonella* (S. Enteritidis, S. Typhymurium) and *Shigella* (*S. sonnei, S. flexneri*). Among the representatives of these two genera the *Salmonella* genus had the highest share with 90.7% (117 strains), and the *Shigella* genus – 9.3% (12 strains). The analysis of the share of pathogenic strains identified during the study period showed that in most cases there were isolated the serotypes S. Enteritidis - 53.49% (69 strains) and S. Typhimurium - 37.21% (48 strains) (Fig.1) . The most frequently isolated *Shigella* serogroups were *S. flexneri* and *S. sonnei*, which recorded a share of 4.65% each (6 strains each).

Analyzing the etiological structure of the pathogenic strains depending on the frequency of isolation, during the study period the dominant serovars of the *Salmonella* enterica species were S. Enteritidis (83.33%) and S. Typhimurium (75%), and the species of the *Shigella* genus varied between 16.67% (*S. flexneri*) and 33.33% (*S. sonnei*) (Fig. 2).

One of the sources of salmonellosis infection are the products of sick poultry (meat, eggs) or clinically healthy poultry - carriers of different *Salmonella* serotypes pathogenic for humans [3]. Identifying the food source that caused the illness is sometimes difficult, but it is the most important measure to prevent the spread of salmonellosis infection. The European Center for Disease Prevention and Control (ECDC) reports that "compared to 2015 there was a 23.6% increase in the number of outbreaks caused by S. Enteritidis

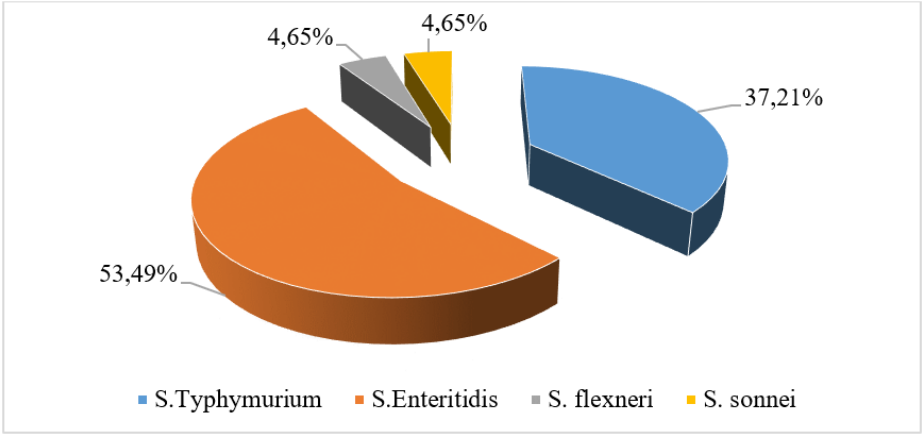

Figure 1. Etiological structure of pathogenic strains from the Enterobacteriaceae family isolated during the study period.

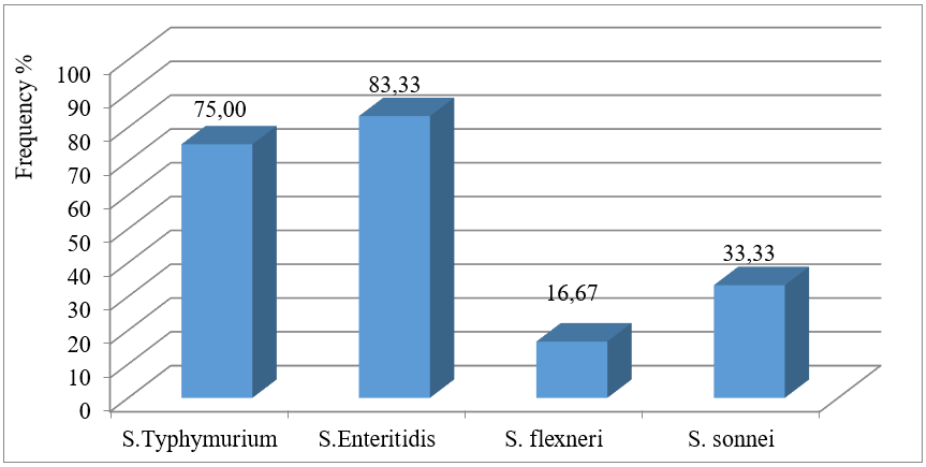

Figure 2. The etiological structure of pathogenic strains of the Enterobacteriaceae family depending on the frequency of isolation during the study period.

reported at EU level in 2016, with 13 Member States". The most important *Salmonella* serovars involved in the outbreaks were S. Enteritidis followed by S. Typhimurium [7]. *Shigella* spp. species are mainly transmitted by the fecal-oral route. The house fly (*Musca domestica*) acts as a vector of transmission of the pathogen. They are easily transmitted through personal contact with the infected person or by consuming contaminated food or water. Even a minimal bacterial load of 10-100 cells, can lead to the development of the infection. The pathogen transmission is influenced by the variations of environmental

factors such as temperature and rainfall. The increase of the infection rate was observed during the summer months. They are highly contagious and occur especially in areas with unsatisfactory sanitary conditions [9].

Enterobacteriaceae pathogens *Shigella* spp. and *Salmonella* spp. were annually isolated. The highest circulation of pathogens *Shigella* spp. and *Salmonella* spp. was highlighted in the years 2014 (2.01%), 2015 (1.78%) and 2011 (1.67%) (fig. 3). Between 2013 and 2017, the share of positive results was practically similar, constituted about 1%, and in the years 2012, 2016, 2018-2022 – it varied from 0.89% to 0.12%.

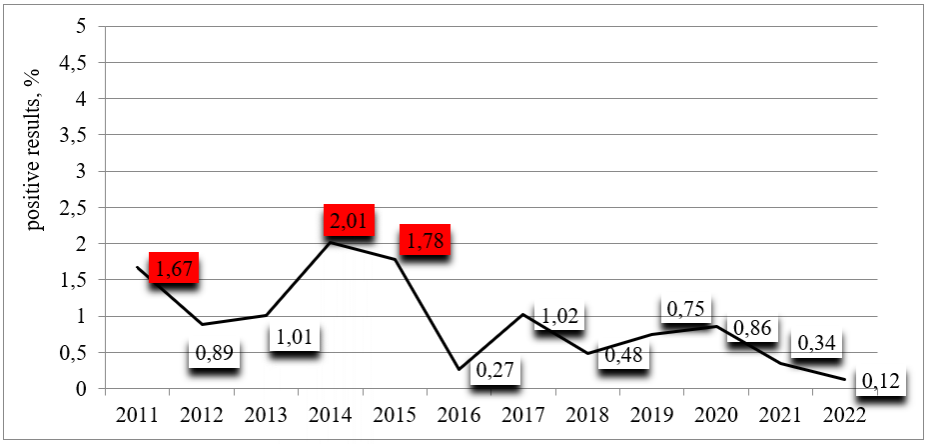

Figure 3. Dynamics of the pathogenic strains circulation of the Enterobacteriaceae family during the study period.

Analyzing the etiological structure of the pathogenic strains for each year, it was found that ADD were most frequently caused by 2 strains - 58.3%, less often 3 strains - 25% and very rarely 1 strain - 16.7% (fig. 4).

Throughout the study period ADDs were caused by both salmonella and shigella, but the analysis of the etiological structure demonstrated that salmonellosis was predominant, namely the strains *S. enterica* with the serovars S. Enteritidis and S. Typhymurium. In the last 2 years of study, all ADD cases investigated in the microbiological laboratory of PHMI TMA Buiucani were identified as salmonellosis - S. Enteritidis (2021) and S. Typhimurium (2022) with 100% each.

ADD caused by *Shigella* spp. strains were reported as sporadic cases in 2011 (*S. flexneri* 9.09%) and 2015 (*S. sonnei* 4.55%). The incidence of ADD caused by *Shigella* spp. serogroups has significantly increased in the following years. Thus, in 2017, 33.3% of disease cases were caused by *S. flexneri*, and during 2018-2020, the *S. sonnei* strain was identified as the causative agent of ADD in 33.3% - 50% of cases.

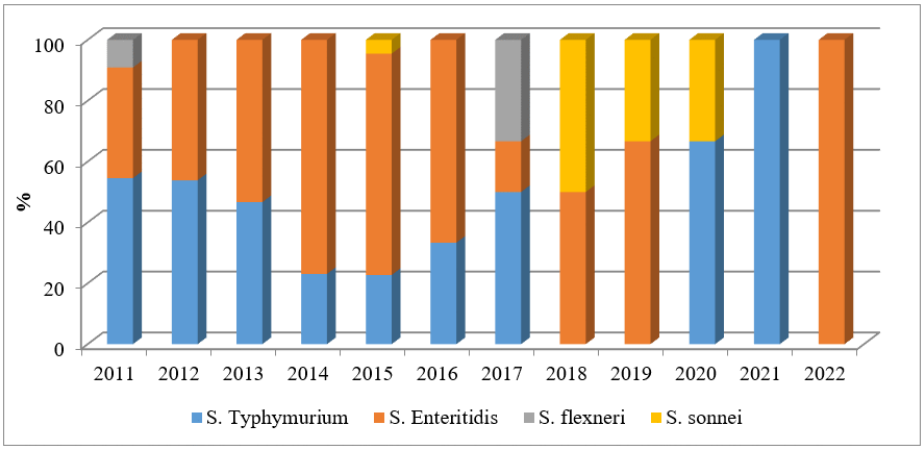

Figure 4. Etiological structure of pathogenic strains of the Enterobacteriaceae family determined during the study period.

The isolation rate of *Salmonella* and *Shigella* strains from ADD patients has decreased since 2019. During this period, the sanitary-epidemiological rules in institutions and public transport were strengthened, there were used various disinfectant solutions and compliance with personal hygiene rules is the safe condition of disease avoidance.

4. Conclusions

During the research period (2011-2022) it was established that in most cases the acute diarrheal disease has a bacterial etiology, the pathogens belonging to the Enterobacteriaceae family, the *Salmonella* and *Shigella* genera. The morbidity through salmonellosis constituted 90.7%, and through shigellosis 9.3%.

The isolation of pathogens in salmonellosis revealed the presence of the *Salmonella enterica* species, S. Enteritidis (53.49%) and S. Typhimurium (37.21%) serovars, and in shigellosis there were determined the *S. flexneri* and *S. sonnei* serogroups with a share of 4.65% each.

The bacteriological confirmations of ADD caused by enterobacteria had the highest incidence in 2011 (1.67%), 2014 (2.01%) and 2015 (1.78%), after which the dynamics of the circulation of these pathogens was decreasing continuously. In the etiological structure of ADD, 2 strains predominated more frequently - 58.3%, less often 3 strains - 25% and very rarely 1 strain - 16.7%.

The study was carried out as part of the doctoral project with the theme "Pathogenic agents of acute diarrheal diseases - morpho-cultural peculiarities, identification methods, antibiotic resistance and the spread dynamics in Chisinau".

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(Nadejda Guțu) Public Health Medical Institution TMA Buiucani, Chisinau

(Olga Postolachi) Institute of Microbiology and Biotechnology, Technical University of Moldova

(Elena Zariciuc) Department of Preventive Medicine, Discipline of Microbiology and Immunology of the State University of Medicine and Pharmacy "Nicolae Testemițanu"

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Water quality of the Dniester river right tributaries depending on the pollution sources

Maria Sandu, Anatol Tariţă, Elena Moşanu, and Raisa Lozan

Abstract. According to the water quality Index calculated in different sections of the Dniester River tributaries the water was from medium pollution to polluted state, in Botna river (Căuşeni town) in 1995 and 2004 being very polluted. The water in Răut river in 2015 was of average pollution, at Balti town being polluted. A detailed study was carried out for the water from Bâc river, Chisinau municipality, in 2009, through the calculated ICAcc, it was demonstrated that the water entering the territory of Chisinau municipality was already polluted, reaching very polluted downstream of the sewage treatment plant from the municipality.

Keywords: water, quality Index, tributaries, Dniester river, sources of pollution.

Calitatea apei din afluenții din dreapta râului Nistru în funcție de sursele de poluare

Rezumat. Conform indicelui de calitate a apei calculate în diferite secțiuni ale afluenților râului Nistru, apa era de la o poluare medie la o stare poluată, în râul Botna (orașul Căușeni) în anii 1995 și 2004 apa era foarte poluată. A fost efectuat un studiu detaliat pentru apa din râul Bâc, municipiul Chișinău. În anul 2009, prin calculul ICAcc, s-a demonstrat că apa care intră pe teritoriul municipiului Chișinău era deja poluată, ajungând foarte poluată în avalul de epurare din municipiu.

Cuvinte-cheie: apă, Indexul calității, afluenți, râul Nistru, surse de poluare.

1. Introduction

The hydrographic network of the Dniester river basin is represented by 1591 rivers, which also have territories adjacent to natural areas protected by the state, represented by all the variety of categories of protected areas. Among the most significant protected areas in the basin are the natural reserves Codru (5177 ha), Iagorlîc (836 ha), Plaiul Fagului (5642 ha) and Orhei National Park (33 792.09 ha) [1]. The longest tributaries in the Dniester river basin are the Răut, Bâc and Botna rivers.

The influence of the anthropogenic factor and the caused pollution change the composition of the water, affect the fauna and flora in the aquatic environment. The sources of

surface water pollution in the Republic are insufficiently purified or untreated wastewater discharges from the domestic and industrial sectors, meteoric water discharges, from various waste deposits, from agricultural fields, domestic livestock, etc.

The existence of pollution sources from recent years is mentioned in the Yearbook of the Inspectorate for Environmental Protection - 2020 [2]. The main activities and urban agglomerations that cause water pollution are: industry (activities in the energy field, sugar/alcohol/bakery industry, etc.), urban agglomerations with the most numerous household waste deposits, agriculture and animal husbandry.

In 2020, in the Dniester river basin there were 154 waste water discharge and treatment complexes, only 20 units (13%) were with standard treatment, and 74 (48%) systems were with insufficient treatment, 19 (13%) with partial treatment and 41 (26%) treatment stations were not working. Most systems (14 units) did not work in Donduşeni district, 5 stations in Teleneşti district, 4 units each in Rezina and Străşeni districts, and in other districts 1-2 wastewater treatment systems did not work. Annually in the hydrographic network of the Dniester basin, on average, are discharged 1212.6 thousand m3 of waste water, of this volume only 13% is sufficiently purified [1].

Another point source of pollution is waste dumps. In the Republic of Moldova the disposal of municipal waste at the moment is mainly carried out by storing it on the ground. According to the data of the Environmental Protection Inspectorate [2] in 2020 in the districts of the Dniester river basin there were 619 waste depots in operation, organized practically in every locality by the local public authorities. In the protection zone of water bodies, 66 municipal waste deposits were located, which represent a source of water pollution. At the same time, 150 waste deposits were located at a distance of less than 500 m from the housing sector, especially in the rural sector, which endanger the health of the population and the quality of local surface and underground waters.

Some of the diffuse pollution sources are agricultural land, which in the Dniester river basin occupies 76,53% of the total area, and atmospheric deposition, involving in the process of discharge wastewater, wastes, chemical fertilizers, pesticides, etc. [1]. Thus, studies on the quality of surface waters have an important role because in the Republic they are exposed to pollution.

In order to reflect the influence of different parameters on water quality, transforming large amounts of data into a single number, have been developed different water quality indices, which have a value of the water quality level. A synthesis of surface water quality indices was made by Couillard D., et al (1985) [3] to transform water quality data into a unique format.

The purpose of the present study is to evaluate the ecological state of the water in the right tributaries Răut, Bâc, Botna and Ichel of the Dniester River using the calculated Water Quality Index.

2. Materials and methods

A water quality index is a tool for transmitting information to the general public, water users, scientific researchers, managers, parliamentarians, engineers, etc.

In the assessment of the surface water quality index (ICAcc,%), which is applied to assess water quality according to the requirements specified in Government Decision no. 890 of 12.11.2013 [4], was used the national standard SM 354: 2021 [5].

The surface water quality index includes a scale of 100% based on quality classes (I-V) according to the concentration of substances specified in the Regulation on environmental quality requirements for surface water [4].

The general classification of surface water quality was proposed according to the US National Sanitation Foundation's scoreboard, mentioned in table 1 [6, 7].

Table 1. Legend of the Surface Water Quality Index

IPAcc, %	State	Quality class
90-100	Excellent	I
70-90	Good	II
50-70	Medium pollution	III
25-50	Polluted	IV
0-25	Very polluted	V

In the present study the ICAcc is evaluated in the water of the right tributaries of the Dniester river in different years (1995-2015), using in the calculation the information from scientific publications [8-13], resulting from the hardness and mineralization of the water, the concentration of NH_4^+, NO_2^-, NO_3^-, Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, SO_4^{2-} ions, of the pH, CBO_5 and $CCO - Cr$ values.

3. Results and Discussion

The study shows that the water quality of the Dniester River tributaries in the years 1995-2015 was from medium pollution to the polluted state (Fig. 1):

- ICAcc in the Bâc river water varied from 42% (polluted) to 63
- The water in the Botna river had ICAcc from 31%-36% at Căuşeni city (polluted), to 66% (medium pollution).

- In the Răut river in 2015 the water was as medium polluted (Floresti - Orhei town, ICAcc 62-65%) and in the years 1995-2009 it was polluted in all sections (ICAcc 42%-58%).
- In Ichel river (Paşcani village) ICAcc was within the limits of 46%-58% (polluted).

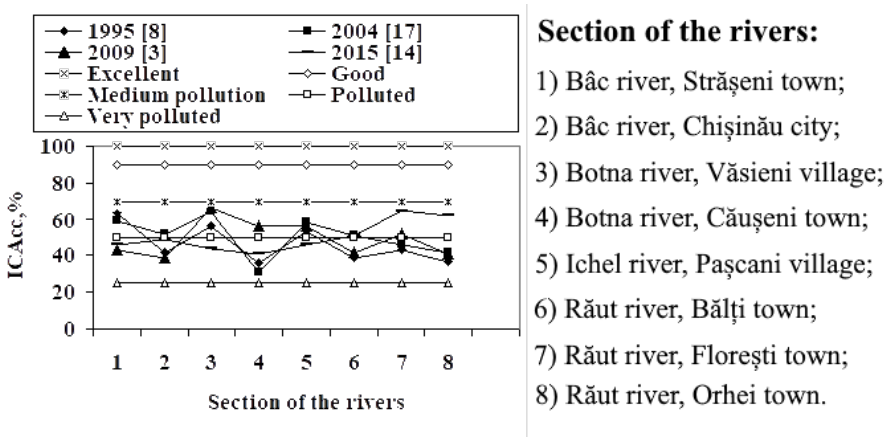

Figure 1. Dynamics of the water quality index in the rivers Răut, Bâc, Botna and Ichel.

A detailed study was carried out for the water in the Bâc river, Chişinău municipality, in the local environmental action plan of Chişinău municipality, year 2009 [11]. Through the water quality index, calculated on the basis of the physical-chemical composition of the water in the text of the Plan, it is specified that the water from the Bâc river at the entrance to the territory of the municipality was already polluted (ICAcc, 53%), reaching a low index of 31% quality (highly polluted) downstream of the municipal wastewater treatment plant (SEB) (Fig. 2).

ICAcc (%) of the tributaries water when it flows into the Dniester river, calculated based on the information from the publication of Viorica Gladchi, etc (2013) [12], based on the annual average from the years 2009-2010, is higher for the water from the Răut river (65%), which corresponds to the medium pollution category. In the Ichel (58%) and Botna (55,5%) rivers, the water at the discharge into the Dniester river was polluted, being very polluted in the Bâc river (46%) (Fig. 3).

Based on the fact that insufficiently treated waste water is discharged into the hydrographic network of the Dniester river basin or, in general, some wastewater treatment systems do not work, in order to improve the quality of natural waters, it is necessary to

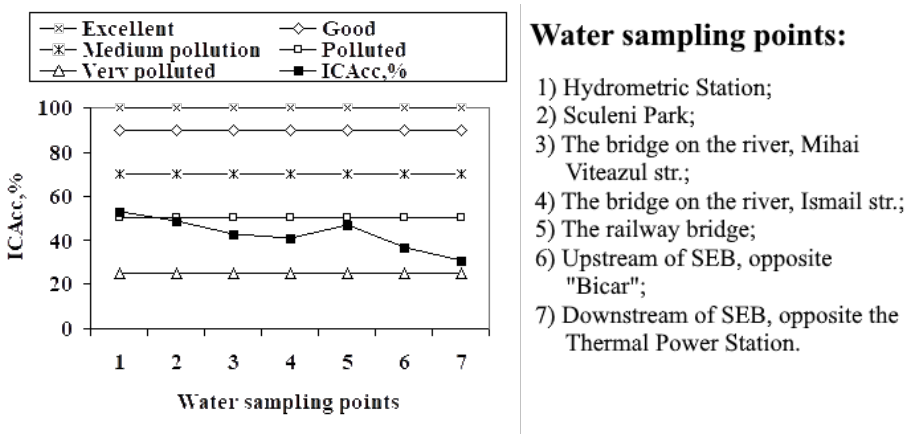

Figure 2. Dynamics of the water quality index in the Bâc river, Chişinău city, year 2009.

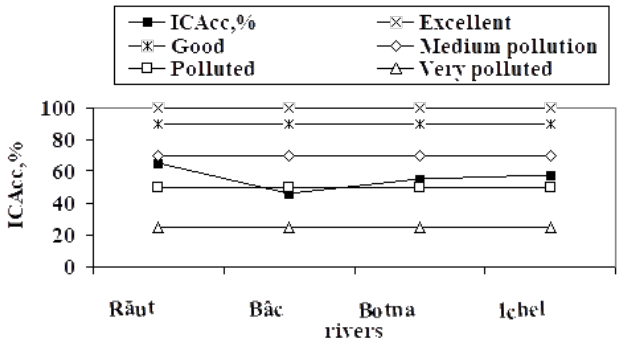

Figure 3. The water quality index of the Răut, Bâc, Botna and Ichel rivers on them flow into the Dniester river (years 2009-2010).

implement efficient domestic and industrial from all fields of activity wastewater treatment stations, through which the normative requirements of Government Decision no. 950 of 25.11.2013 [14] will be respected.

In order to intensify measures to protect and reduce pollution of aquatic resources, the Republic of Moldova has signed various conventions, including the Convention on the Use and Protection of Transboundary Watercourses and Lakes (Helsinki, 1992), the Danube Convention (Sofia, 1994) and the Convention on the wetlands important for wading birds (Ramsar, 1971). In order to comply with the Convention on the use and protection of watercourses, the forested areas and riparian strips of water protection have an important role for the ecological state of the environmental components in the Dniester river basin [14].

The total national forest fund is of 419,1 thousand ha with an area covered by forests of 374,5 thousand ha. In the river basin, forested areas constitute 11,07% of the territory, the average for the country being 10,4% [15]. The largest forest bodies are in the districts of Străşeni – 35,8%, Călăraşi – 30,1% and Orhei – 19,4% and Şoldăneşti – 18,8%, many of them being included in the natural areas protected by the state [16].

The area and forested share of the sub-basins, which are part of the Dniester river basin, are: the Bâc river basin is covered with 45,797 ha of forests (20,69%), followed by the Ichel river basin with 17,782 ha (20,26 %), of the Botna river with 26155 ha (15,39%) and of the Răut river with 57597 ha (7,4%) [1]. The need to ensure river water protection through protection zones and strips is specified in Law no. 440 of 27.04.1995 [17]. In article 4 of the law it is mentioned that in the water protection zone the riparian strip is separated within the boundaries of which the economic activity is strictly limited. The dimensions of the water protection zones of rivers and water basins (article 6) are established with a width of at least 500 meters from the edge of the river bed slope on the banks, but not further from the water table, and those of the river protection strips of waters (article 7) depend on the length of the rivers: streams and small rivers of at least 20 meters, medium rivers of at least 50 meters and for large rivers of at least 100 meters.

In order to minimize or prevent the pollution of the aquatic environment in the Republic by pollutants from waste dumps, it is necessary to use efficient waste management in compliance with the requirements of Law no. 1347 of 09.10.1997 [18], where are the requirements regarding the storage (burial) and preservation of waste (article 18), the manner of storage (burial) and preservation of waste (article 19) and restrictions (article 20). In article 20, point a, of the law it is prohibited to throw waste into drainage systems and aquatic objects, their storage and processing on the territory of the protection zones of basins and watercourses, in the sanitary protection zones of water supply sources drinking water and aqueducts, in recreation areas, nature reserves and parks, as well as in the protection strips of railways and roads. Only by complying with the conditions of the national legislation will the prevention or minimization of the level of pollution of the waters of the tributaries of the Dniester river be ensured, being proven to be a pollution over a long period.

4. Conclusions

- The water quality of the right tributaries of the Dniester river in all the years of study (1995-2015) was from medium pollution to the highly polluted state.
- ICAcc (%) of the water of the tributaries when it flows into the Dniester river, based on the annual average from 2009-2010, is higher for the water from the

Răut river (65%), being of medium pollution, and in the Ichel rivers (58%) and Botna (55.5%) the water was polluted, being the most polluted in the Bâc river (46%).

- In order to improve the water quality of small rivers, it is necessary to use efficient wastewater treatment plants for domestic and economic activities in order to comply with the requirements of the legislation on discharge into the environment.
- In the minimization and prevention of the pollution of the aquatic environment in the Republic by pollutants from household waste deposits and from all areas of economic activity, it is necessary to use an efficient waste management according to the requirements of the national legislation in force.

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(Maria Sandu, Anatol Tariţă, Elena Moşanu, Raisa Lozan) Institute of Ecology and Geography of the Moldova State University

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Regression analysis in the process of studying the correlation between climate factors of the Chisinau weather station

Anatolie Puțuntică and Vitalie Puțuntică

Abstract. The study presents linear and nonlinear mathematical models that analyze the regression in the process of correlation between climatic factors. The elaborated research is carried out for the period 1960-2019, analyzing the experimental data of the average annual temperature and the amount of annual precipitation. Various forms of regressions (linear, parabolic, cubic, etc.) and predictions for the correlation between the year and the average annual temperature, the year and the amount of annual precipitation, the average annual temperature and the amount of annual precipitation were obtained.
Keywords: regression, covariance, correlation coefficient.

Analiza de regresie în procesul de studiere a corelației dintre factorii climatici ai stației meteo Chișinău

Rezumat. Studiul prezintă modele matematice liniare și neliniare care analizează regresia în procesul de corelație între factorii climatici. Cercetarea elaborată este realizată pentru perioada 1960-2019, analizând datele experimentale privind temperatura medie anuală și cantitatea de precipitații anuale. Au fost obținute diverse forme de regresii (liniare, parabolice, cubice etc.) și prognoze pentru corelația dintre an și temperatura medie anuală, an și cantitatea de precipitații anuale, temperatura medie anuală și cantitatea de precipitații anuale.
Cuvinte-cheie: regresie, covarianță, coeficient de corelație.

1. Introduction

The forms of manifestation of the interdependence relations between the processes and the natural phenomena are extremely varied and most often difficult to notice. An essential problem to be solved in the analysis of the link between a dependency variable (denoted by y) and one or more independent variables (denoted by x_i) is the existence of the link between them. In practice, the following situations are encountered [1]:

a) the independent variable determines the modification of the dependent variable in case there is an univocal;
b) the two variables influence each other;

c) the variables evolve similarly independently, but influenced by another variable simultaneously;

d) the variables have a similar evolution without any connection between them.

Thus, for the systematic study of the relations between the two types of variables it is necessary to classify them according to certain criteria:

- by the nature of the interdependence relationship (functional and statistical links);
- by the number of factorial variables (single and multiple links);
- by the nature of the characteristics (association and correlation links);
- by the direction of the connection (direct and inverse connections);
- according to the shape of the function by which the connection is described (linear and nonlinear connections);
- after the connection time (synchronous and asynchronous connections).

2. Analytical method of measuring links

Analytical methods are those that allow the precise determination of both the relationship between two or more variables and its intensity. The analytical methods are:

- regression method;
- correlation method.

2.1. Regression method

This method is based on the use of mathematical functions to describe the shape of the connection between variables. The regression function has the following form:

$$y = f(x_1,\ x_2,\ \ldots,\ x_n) + \varepsilon,$$

where y - the dependent variable; $x_1,\ x_2,\ \ldots,\ x_n$ - independent variables; n - the number of influencing factors; ε - random variable or error that synthesizes the influence of unspecified factors.

In relation to the number of registered influencing factors are [2]:

- simple regression (unifactorial);
- multiple regression (multifactorial).

Only simple regression will be used in this research. It is based on function $y = f(x)+\varepsilon$ and studies the variation of a dependent variable y in relation to a single independent variable x, the other factors being considered neglected and with constant action.

The choice of the function is made with the help of the correlation graph. The most common simple correlation functions used are:

(1) $y = ax + b$ (linear);
(2) $y = ax^2 + bx + c$ (parabolic);
(3) $y = ax^3 + bx^2 + cx + d$(cubic);
(4) $y = ax^4 + bx^3 + cx^2 + dx + e$ (polynomial of degree IV);
(5) $y = ax^b$ (power);
(6) $y = ab^x$ (exponential);
(7) $y = a + b\ln x$ (logarithmic);
(8) $y = \frac{1}{a+bx}$ (hyperbole);
(9) $y = \frac{ax}{x+b}$ (Törniquist),

where a, b, c, d, e, f are parameters to be determined.

To determine the parameters a, b, c, d, e, f, the least squares method is usually used, according to which in order for the chosen regression function to be really significant we must have:

$$S = \sum_{i=1}^{n} \left(y_i - y_{x_i}\right)^2$$

to be minimal, where $i = \overline{1, n}$ - number of statistical units observed, y_i – the observed (empirical) values of the dependent variable, y_{x_i} – the theoretical values expressed by the regression equation. Determination of the values of each parameter (a, b, c etc.) is done by applying the conditions for obtaining the minimum value in the partial derivatives of the function S considered in the variables a, b, c etc.:

$$\frac{\partial S(a,\ b,\ c\ ...)}{\partial a} = 0,\quad \frac{\partial S(a,\ b,\ c,\ ...)}{\partial b} = 0,\quad \frac{\partial S(a,\ b,\ c,\ ...)}{\partial c} = 0, \ldots\ .$$

The formulas deduced for the mentioned correlation functions are brought in Tab. 1

Table 1. Functions formula correlation

Linear	$y = ax + b$	$\begin{cases} a\sum x^2 + b\sum x = \sum xy, \\ a\sum x + bn = \sum y; \end{cases}$
Parabolic	$y = ax^2 + bx + c$	$\begin{cases} a\sum x^4 + b\sum x^3 + c\sum x^2 = \sum x^2 y, \\ a\sum x^3 + b\sum x^2 + c\sum x = \sum xy, \\ a\sum x^2 + b\sum x + cn = \sum y; \end{cases}$
Cubic	$y = ax^3 + bx^2 + cx + d$	$\begin{cases} a\sum x^6 + b\sum x^5 + c\sum x^4 + d\sum x^3 = \sum x^3 y, \\ a\sum x^5 + b\sum x^4 + c\sum x^3 + d\sum x^2 = \sum x^2 y, \\ a\sum x^4 + b\sum x^3 + c\sum x^2 + d\sum x = \sum xy, \\ a\sum x^3 + b\sum x^2 + c\sum x + dn = \sum y; \end{cases}$

Polynomial of degree IV	$y = ax^4 + bx^3 + cx^2 + dx + e$	$\begin{cases} a\sum x^8 + b\sum x^7 + c\sum x^6 + d\sum x^5 + e\sum x^4 = \sum x^4 y, \\ a\sum x^7 + b\sum x^6 + c\sum x^5 + d\sum x^4 + e\sum x^3 = \sum x^3 y, \\ a\sum x^6 + b\sum x^5 + c\sum x^4 + d\sum x^3 + e\sum x^2 = \sum x^2 y, \\ a\sum x^5 + b\sum x^4 + c\sum x^3 + d\sum x^2 + e\sum x = \sum xy, \\ a\sum x^4 + b\sum x^3 + c\sum x^2 + d\sum x + en = \sum y; \end{cases}$
Power	$y = ax^b$	$\begin{cases} n\lg a + b\sum \lg x = \sum \lg y, \\ \lg a\sum \lg x + b\sum (\lg x)^2 = \sum \lg x \cdot \lg y; \end{cases}$
Exponential	$y = ab^x$	$\begin{cases} n\lg a + \lg b\sum x = \sum \lg y, \\ \lg a\sum x + \lg b\sum x^2 = \sum x \lg y; \end{cases}$
Logarithm	$y = a + b\ln x$	$\begin{cases} an + b\sum \ln x = \sum y, \\ a\sum \ln x + b\sum \ln^2 x = \sum y \ln x; \end{cases}$
Hyperbole	$y = \frac{1}{a+bx}$	$\begin{cases} an + b\sum x = \sum 1/y, \\ a\sum x + b\sum x^2 = \sum x/y; \end{cases}$
Törniquist	$y = \frac{ax}{x+b}$	$\begin{cases} \frac{n}{a} + \frac{b}{a}\sum \frac{1}{x} = \sum \frac{1}{y}, \\ \frac{1}{a}\sum \frac{1}{x} + \frac{b}{a}\sum \frac{1}{x^2} = \sum \frac{1}{xy}. \end{cases}$

2.2. Correlation method

The correlation method is used to measure the intensity of the link between the dependent variable y and the independent variable x. Depending on the nature of the link between the dependent variable y and the independent variable x, the correlation can be positive (in the case of the direct link) or negative (in the case of the reverse link). In this method the following indicators are used: covariance, correlation coefficient and correlation ratio [1,2].

Covariance captures the existence and direction of the link between the dependent variable y and an independent variable x. It is calculated as the simple arithmetic mean of the products of the deviations of the two correlated variables y and x from their arithmetic average $\bar{y}$ and $\bar{x}$ using the relation:

$$\operatorname{cov}(x, y) = \frac{\sum (x_i - \bar{x})\,(y_i - \bar{y})}{n}.$$

The positive values of this indicator reflect a direct link, and the negative ones reflect an inverse link.

High values of the indicator show a strong link, while values close to zero signify the lack of links between the variables y and x. The correlation coefficient is determined according to one of the relations:

$$r = \frac{n\sum x_i y_i - \sum x_i \cdot \sum y_i}{\sqrt{\left(n\sum x_i^2 - (\sum x_i)^2\right) \cdot \left(n\sum y_i^2 - (\sum y_i)^2\right)}} \Leftrightarrow r = \frac{\sum (x_i - \bar{x})\,(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \cdot \sum (y_i - \bar{y})^2}}.$$

The correlation coefficient can take values between -1 and 1. If $r \in [-1, 0)$ – reverse link, $r \in [0,\ 1)$ – direct link, $r = 0$ – the two variables do not correlate linearly.

The correlation ratio measures the intensity of the connection between the dependent variable y and the independent variable x in the case of nonlinear regression functions. The correlation ratio is determined by the relation:

$$F = \sqrt{1 - \frac{\sum \left(y_i - y_{i,\ curba}\right)^2}{\sum (y_i - \bar{y})^2}}.$$

Thus, the value of the correlation ratio is always positive and between 0 and 1. In the case of linear type connections the correlation ratio must be equal to the correlation coefficient.

Calculating this coefficient F for each of the variants of the functions considered we obtain a value close to 1. The most appropriate function is the one with the value F closest to 1.

3. Study of the correlation between some climatic elements at the Chisinau Meteorological Station

In this study, we will study the correlation between: the calendar year and the annual temperature, the calendar year and the annual amount of precipitation, the annual temperature and the annual amount of precipitation. According to the State Hydro meteorological Service of Chisinau station [3,4], in Tab. 2 we indicated the average annual temperature ($^{\circ}C$) and the amount of annual precipitation (mm) in the period 1960-2019, here by MT we denoted the medium temperature, and by AP - the amount of precipitation.

We will describe the process, for example, for the function of the parabolic form between the average annual temperature and the amount of annual precipitation. For this model, we complete the following table:

According to Tab. 1 (parabolic model), we obtain:

Table 2. Average annual temperature and amount of annual rainfall in the period 1960-2019, Chisinau

Year	MT	AP	Year	MT	AP	Year	MT	AP	Year	MT	AP
1960	10,575	537	1975	10,808	484	1990	11,342	361	2005	10,492	638
1961	10,408	450	1976	8,342	600	1991	9,433	673	2006	10,208	564
1962	10,092	559	1977	9,525	464	1992	10,117	417	2007	12,042	480
1963	9,192	532	1978	8,725	563	1993	9,408	532	2008	11,308	466
1964	9,442	511	1979	9,783	684	1994	11,342	415	2009	11,408	446
1965	9,033	537	1980	8,300	712	1995	10,017	702	2010	10,558	734
1966	10,858	774	1981	9,667	536	1996	9,050	711	2011	10,450	428
1967	10,042	481	1982	9,783	384	1997	9,400	607	2012	11,217	522
1968	9,992	532	1983	10,458	549	1998	10,250	668	2013	11,083	531
1969	8,692	525	1984	9,167	669	1999	11,025	485	2014	10,917	604
1970	10,067	672	1985	8,000	591	2000	11,150	437	2015	11,983	431
1971	9,967	590	1986	9,625	402	2001	10,308	618	2016	11,225	644
1972	9,758	621	1987	8,075	592	2002	10,842	604	2017	11,208	635
1973	9,500	396	1988	9,025	652	2003	9,800	459	2018	11,200	609
1974	9,958	562	1989	10,933	460	2004	10,300	591	2019	12,225	403

Table 3. Parabolic function model

n	x	y	x^2	x^3	x^4	xy	x^2y
1	10,575	537	111,830625	1182,608859	12506,08869	5678,775	60053,04563
2	10,408	450	108,333333	1127,569084	11736,11112	4683,7485	48750,00003
...	...	...	...	...	...	...	...
59	11,2	609	125,44	1404,928	15735,1936	6820,8	76392,96
60	12,225	403	149,450625	1827,033891	22335,48931	4926,675	60228,60188
Σ	609,1	33036	6240,452323	64502,06588	672353,4868	333539,6911	3398332,086

$$\begin{cases} 672353,486759a + 64502,065881b + 6240,452323c = 3398332,085749; \\ 64502,065881a + 6240,452323b + 609,1c = 333539,69105; \\ 6240,452323a + 609,1b + 60c = 6240,452323; \end{cases} \Leftrightarrow$$

$$\Leftrightarrow \begin{cases} a = & -8,8035497; \\ b = & 145,4784458; \\ c = & -10,6131547. \end{cases}$$

Therefore, the parabolic regression will be:

$$y = -8,8035497x^2 + 145,4784458x - 10,6131547.$$

If the connection between the average annual temperature and the amount of average annual precipitation is assumed to be linear, then the coefficients of the equation $y = ax+b$ based on the system of equations must be determined using the least squares method (see Tab.1):

$$\begin{cases} 6240,4523236a + 609,1b = 333539,69105; \\ 609,1a + 60b = 33036; \end{cases} \Longleftrightarrow \begin{cases} a = -32,0781457; \\ b = 876,2466428. \end{cases}$$

So the regression line will be $y = 876,2466428 - 32,0781457x$. For the covariance sizes and the correlation coefficient of the regression line we will have to complete the table:

Table 4. Calculating covariance and regression.

n	x	y	$x-\overline{x}$	$(x-\overline{x})^2$	$y-\overline{y}$	$(y-\overline{y})^2$	$(x-\overline{x})(y-\overline{y})$
1	10,575	537	0,423333	0,179211	-13,6	184,96	-5,757333
2	10,408	450	0,256663	0,065876	-100,6	10120,36	-25,820331
...	...	...	...	...	...	...	...
59	11,2	609	1,048333	1,099003	58,4	3410,56	61,222667
60	12,225	403	2,073333	4,298711	-147,6	21785,76	-306,024
Σ	609,1	33036	0	57,07216	0	584508,4	-1830,76895

As a result we get:

$$r = \frac{\Sigma(x_i - \overline{x})(y_i - \overline{y})}{\sqrt{\Sigma(x_i - \overline{x})^2 \cdot \Sigma(y_i - \overline{y})^2}} = \frac{-1830,76895}{\sqrt{57,072156 \cdot 584508,4}} = -0,3169757358483542.$$

Because $\mathrm{cov}(x, y) < 0$, respectively $r < 0$, then we have a strong inverse link between the average annual temperature and the amount of average annual rainfall, well increasing the average annual temperature leads to a decrease in the amount of average annual rainfall.

To determine the correlation ratio for parabolic regression:

$$y_{parabola} = -8,8035497x^2 + 145,4784458x - 10,6131547,$$

we complete Table 5.

Table 5. Correlation for the parabolic regression.

x_i	y_i	$y_{i,parabola}$	$y_i - y_{i,parabola}$	$(y_i - y_{i,parabola})^2$	$y_i - \overline{y}$	$(y_i - \overline{y})^2$
10,575	537	543,3149	-6,31495	39,87855	-13,6	184,96
10,408	450	549,8566	-99,8566	9971,347	-100,6	10120,36
...	...	...	...	...	...	...
11,2	609	514,4282	94,57183	8943,832	58,4	3410,56
12,225	403	452,1648	-49,1648	2417,182	-147,6	21785,76
Σ	33036	33036	-25,6656	519295,7	0	584508,4

So,

$$F = \sqrt{1 - \frac{519295,65987597423}{584508,4}} = 0,3340187496937681.$$

The graphical representation of the initial data, the parabolic regression and the linear regression is represented in Fig. 1.

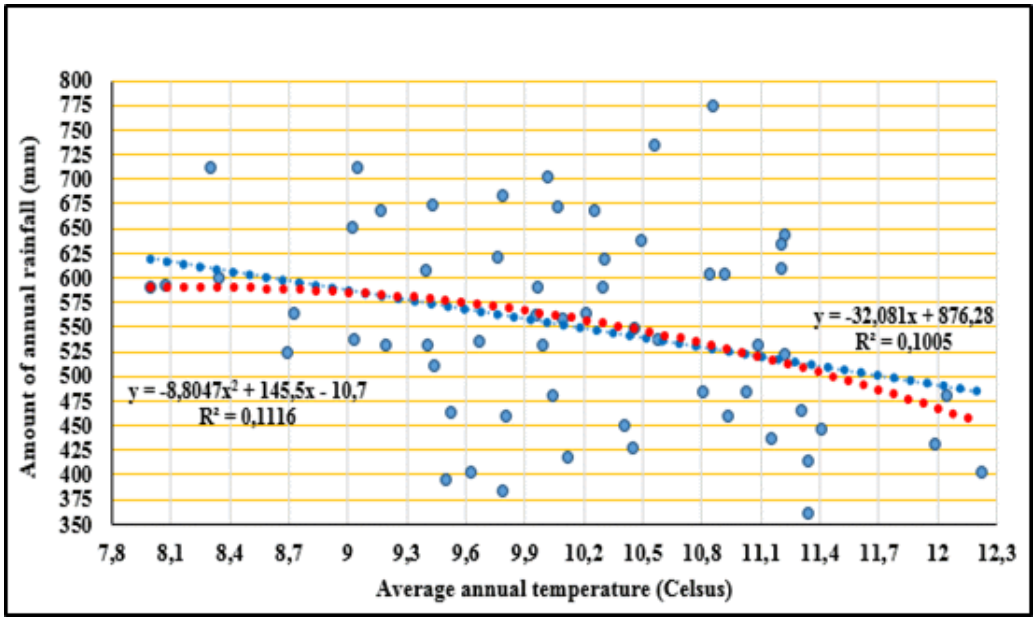

Figure 1. Linear regression (blue) and parabolic regression (red).

Correlation	Regression curve	Regression of curve coefficients	Correlation ratio	Forecast for 2020
Year and average annual temperature (°C)	linear	0,031261439	0,555102	11,1051
		-52,04296569		
	parabolic	0,001411821	0,677351	11,9951
		-5,586376217		
		5535,678664		
	cubic	$-2{,}8101 \cdot 10^{-5}$	0,687447	11,6603
		0,169132863		
		-339,2522238		
		226791,6331		
	quartic	$-2{,}56495 \cdot 10^{-7}$	0,687637	11,6044
		0,00201309		
		-5,922092462		
		7739,21872		
		-3790846,75		
	exponential	0,018015157	0,564372	11,1691
		1,003188088		
	logarithmic	-460,9379324	0,553585	11,0976
		62,02137463		
	hyperbole	0,741389957	0,573484	11,2382
		-0,000322974		
	Törniquist	$6{,}45085 \cdot 10^{14}$	0,303971	10,3113
		$1{,}26373 \cdot 10^{17}$		
Year and amount of annual rainfall (mm)	linear	-0,122422895	0,02148	546,866
		794,1603501		
	parabolic	-0,01772693	0,052732	535,692
		70,41303189		
		-69365,6667		
	cubic	0,000725385	0,06064	544,334
		-4,347184658		
		8683,477728		
		-5780743,873		
	quartic	-0,000120363	0,096406	518,114
		0,958571053		

		-2862,705381		
		3799573,226		
		-1891091005		
	exponential	854,0270487	0,021398	546,903
		0,999779385		
	logarithmic	2384,28375	0,021292	546,918
		-241,4139201		
	hyperbole	0,001025088	0,021316	546,94
		$3{,}97657 \cdot 10^{-7}$		
	Törniquist	385,6266587	0,02094	547,042
		-596,0389292		
Average annual temperature and amount of annual rainfall (mm)	linear	-32,07814573	0,316976	520,016
		876,2466428		
	parabolic	-8,803549677	0,334019	467,739
		145,4784458		
		-10,61315474		
	cubic	-11,75781748	0,374376	486,022
		346,625388		
		-3407,48275		
		11730,63422		
	quartic	-0,907613644	0,374624	494,343
		25,02808731		
		-208,9526932		
		297,6295967		
		2526,383239		
	exponential	977,2843906	0,313286	518,977
		-0,056666872		
	logarithmic	1276,94661	0,310794	521,142
		-314,038121		
	hyperbole	0,000806208	0,309868	518,131
		0,0000999988		
	Törniquist	365,1741364	0,297274	542,413
		-3,369321499		

Table 6. Correlations results for other types of regression.

From Fig.1 we notice that the increase of temperature leads to a decrease in the amount of precipitation. Therefore, we can make the following predictions: for an average annual air temperature of $11,015^\circ$ C, we will have according to the parabolic regression the amount of annual precipitation $519,257$ *mm* and according to the linear regression $520,014$ *mm*.

Proceeding according to the above model, the other types of regressions are also studied: cubic, degree IV curve, logarithmic, exponential, etc. The results are presented in Tab. 6.

Also, the correlation between the year and the average annual temperature, the year and the amount of annual precipitation are analyzed (see Tab. 6).

To estimate the amount of average annual rainfall for 2020, the average annual temperatures were obtained by correlating the year with the average annual temperatures.

4. Conclusions

As a result of analyzing all forms of correlation (calendar year and annual temperature, calendar year and annual rainfall, annual temperature and annual rainfall) it can be concluded that the best function is grade IV. Cubic function as well provides good results. In addition to the correlations of mathematical functions, obviously, we will take into account the geographical conditions of the weather station location (altitude, latitude, exposure, vegetation, transparency of the atmosphere, pollution, fragmentation of the relief, etc.). Similar works in the future could take into account other logical correlations, passed through the mathematical apparatus (temperature and humidity, cloudiness and precipitation, cloudiness and visibility, visibility and humidity - very important correlation for road, air, sea, river, rail transport traffic).

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(Anatolie Puțuntică, Vitalie Puțuntică) "Ion Creangă" State Pedagogical University of Chisinau
E-mail address: aputuntica@gmail.com, putunticavitalie@gmail.com

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Anthropogenic influence on the water resource quality in the village of Bălăneşti, Nisporeni district

Eugenia Melentiev and Mirela Rotaru

Abstract. Water is one of the fundamental elements of life, and, at the same time, the factor that defines social-economic development. That is why the current state of water resources is a pressing and current problem of contemporary society. The evaluation of the anthropogenic impact on some water sources in the village of Bălănești, Nisporeni district, highlighted the real state of the surface and underground waters in this area by determining some physico-chemical quality indices; establishing the correlation of the content of some main ions (Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, HCO_3^-) with the content of nitrates as well as determining the degree of pollution of the Nârnova River.
Keywords: surface water, groundwater, quality index, pollution index.

Impactul antropic asupra calității unor resurse de apă în satul Bălănești, raionul Nisporeni

Rezumat. Apa este unul dintre elementele fundamentale ale vieţii, şi, în acelaş timp, factorul care definește dezvoltarea social - economică. De aceea starea actuală a resurselor de apă este o problemă stringentă și actuală a societății contemporane. Evaluarea impactului antropic asupra unor surse de apă din satul Bălănești, raionul Nisporeni, au scos în evidență starea reală a apelor de suprafață și subterane din această zonă prin determinarea unor indici fizico-chimici de calitate; stabilirea corelației conținutului unor ioni principali (Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, HCO_3^-) cu conținutul nitraților precum și determinarea gradului de poluare a râului Nârnova.
Cuvinte-cheie: ape de suprafață, ape subterane, indice de calitate, indice de poluare.

1. Introduction

Water represents the most important element in the natural environment, without which life on earth is inadmissible, and at the same time, a natural resource that defines the socio-economic development factor. The demand for water has grown enormously, with the rapid increase of the population on Earth, because of the progress in social-economic activities, with the acceleration in the urbanization process and with the increase in comfort of the modern life.

Republic of Moldova depends a lot on surface water, especially on water captured from the Nistru River. This makes Moldova's water resource balance highly dependent on the external water flow. Annually, Moldova uses approximately 800 million m^3 of water, of which 85% is obtained from surface water resources. The Republic is sourced by two main sources of water: the Nistru River, which provides almost 90% of water consumption from surface waters, and also the Prut River [1].

The drinking water supply of localities in rural areas is ensured from three important sources: underground water, which accounts for 65% of the total volume of consumed water, surface water - 34.8% of the volume of consumed water, reservoirs and springs - 0.2% [2].

The quality of drinking water is a current topic of major importance, in the assessment of the anthropogenic impact on the health of the population [3]. The attention given to this theme is directly proportional to the degree of influence of the various factors, that define drinking water and the assessment of the health status of the population and the environment in general. Ensuring the population with drinking water in quantity and quality, is one of the priority tasks of the state, in order to improve the health indicators and the well-being of the population of the Republic of Moldova.

The aim of this study is to evaluate the ecological state of some underground and surface water sources in Bălănești, by determining the quality and physico-chemical parameters, the correlation between the content of the cations and nitrate anions, the determination of the surface water pollution index and the coefficient of irrigation. It is the first important study carried out in this area.

2. Materials and Methods

The water quality index is a tool for transmitting information to the public, water users, scientific researchers, managers, engineers, etc. about water quality. In the assessment of the surface water quality index (ICAcc, %), which is applied to assess water quality according to the requirements specified in Government Decision no. 890 of 12.11.2013 [4], the national standard SM 354: 2021 [5] was used.

The surface water quality index includes a 100% scale based on quality classes (I-V) according to the concentration of substances specified in the Regulation on environmental quality requirements for surface waters [6].

Different physico-chemical methods were used to estimate the degree of water pollution and perform the analysis of the collected samples:

- The quantitative titrimetric method is used to determine carbonic and total alkalinity (neutralization); carbonic and total (complexonometric) hardness; calcium

(complexonometric); chemical oxygen demand (redox), content of Cl- ions (exchange).

- The gravimetric method is applied to determine the fixed residue (mineralization). The spectrophotometric method is used to determine nitrites NO_2^-, nitrates NO_3^-, sulfates SO_4^{2-}, Na^+, K^+ ions. The instrumental method applied to the determination of the pH value.

Table 1. List of collected samples

1.- Well nr.I, the right bank of river Nârnova	6.- Well III,the left bank of river Nârnova
2. – Well II, the right bank of river Nârnova	7. - Rriver Nârnova; upstream
3.- Well III, the right bank of river Nârnova	8. - River Nârnova; centre of village
4.- Well I, the left bank of river Nârnova	9. - River Nârnova; downstream
5.- Well II, the left bank of river Nârnova	

3. Results and Discussion

The analysis of the water collected from different sources, to determine the quality indices (hardness, mineralization, pH) and the physico-chemical indices of the main ions in the composition of the waters, was carried out in two stages, between the years 2020-2021 and the years 2022-2023. The list of collected samples are indicated in Table 1.

Characteristic of the physico-chemical quality parameters of natural waters.

Table 2. Average values of physico-chemical parameters of groundwater and surface water in the study area during 2023

Sample nr.	$Na^+ + K^+$ mg/L	Ca^{2+} mg/L	Mg^{2+} mg/L	NH_4^+ mg/L	NO_2^- mg/L	Cl^- mg/L	SO_4^{2-} mg/L	NO_3^- mg/L	HCO_3^- mg/L
1.	41,3	97,5	74,2	0	0	50,9	143,32	25,5	477,1
2.	326,5	281,9	132,5	0	0	199,8	1101	338,4	521,3
3.	176	241,8	92,5	0	0	150,2	598,2	99,1	538,7
4.	42,5	125,3	85,3	0	0	50,9	85	28,4	501,7
5.	79	107,6	56,9	0	0	72,3	163	121,3	571
6.	37	119,5	57,6	0	0	50,9	87	23,4	526
7.	90,5	109,64	65,5	0	0	86,4	139	146,1	521
8.	171	125,54	70,9	0	1,37	128,92	405	73,3	525
9.	377	200,5	139,5	2,87	1,4	178,6	841	2,5	661

The results obtained are indicated in Tables 2 and 3.

Table 3. Average values of groundwater and surface water quality indices in the studied area during 2023

Sample nr.	pH	General hardness		Mineralization
		me/dm^3	°dH	mg/dm^3
1.	8,06	10,8	30,28	664
2.	7,13	25,03	70,1	2672
3.	7,01	19,64	56,08	1642
4.	7,27	13,21	37,01	623
5.	7,29	10	28	939
6 .	7,58	10,61	29,72	617

The comparative study of groundwater.

Nitrate ion concentration values during the years 2021-2023 did not vary significantly, which indicates that the pollution is of an anthropogenic nature (Figure 1).

According to the data of the analyzes carried out, it was found that in three wells the concentration of nitrates exceeds the maximum permissible concentration by 2-9 times (CMA of NO_3^- ions 50 mg/dm^3).

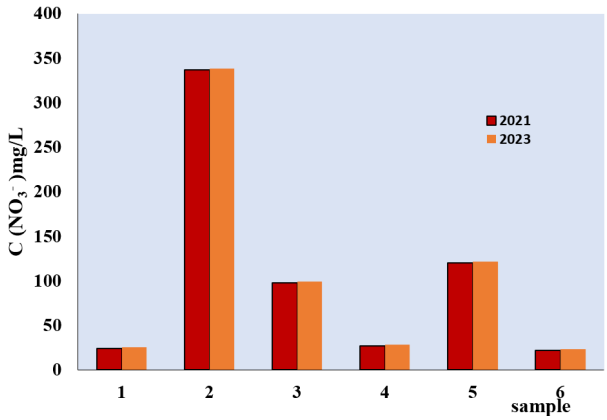

Figure 1. Variation of the concentration of nitrate ions in the analyzed water samples, collected from 6 wells in the years 2021-2023.

During the year 2021, the values of mineralization in the waters of the wells varied within the limits of 626-2680 mg/dm^3. Water from wells number 2 and 3 has increased mineralization, exceeding the CMA for drinking water. It is obvious that the dynamics of

mineralization and the main ions depend on natural factors, but the variation of the ratio between ions can also be influenced by the human factor. The value of mineralization is determined by the content of the main cations (Ca^{2+} with a range of 94-280 mg/dm^3; Mg^{2+}46 -170 mg/dm^3) and anions (Cl^- 49-200 mg/dm^3, SO_4^{2-} 86-556 mg/dm^3, HCO_3^- 475-573 mg/dm^3). The water of the Nârnova river is of hydrogen carbonate type, Ca group.

There is a balanced dependence between mineralization values and main ions (Figures 2 and 3).

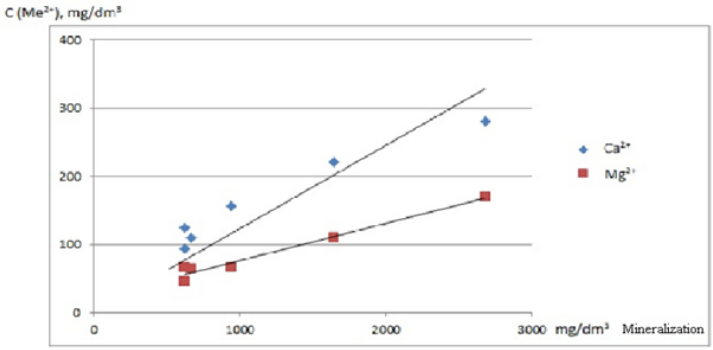

Figure 2. Dependence of the content of Ca^{2+} and Mg^{2+} cations on the mineralization values of the investigated waters.

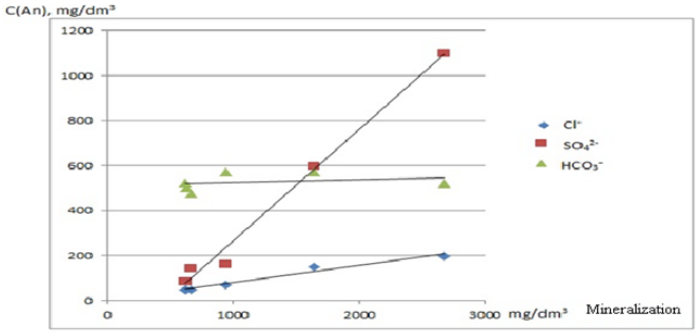

Figure 3. Dependence of the content of HCO_3^-, SO_4^{2-}, Cl^- anions on the mineralization values of the analyzed waters.

The waters from wells 1,4,5,6 under study from the territory of Bălănești, according to the regulations in force, correspond to class II which is of good quality (Tables 2 and 3).

Chemical oxygen demand COD in the waters of the Nârnova river

The chemical oxygen demand (COD) value is widely used in aquatic ecosystem investigations. The given quantity indirectly characterizes the content of organic and mineral

substances in water, oxidized by one of the strong oxidants such as $KMnO_4$ or $K_2Cr_2O_7$, under certain conditions. COD can be expressed by the amount of oxygen required for the oxidation of organic substances in a certain volume of water. Average values of COD-Mn during the study period (May and July 2021-2023) ranged from 8,65 mg O_2/dm^3 to 24,4 mg O_2/dm^3 (Figure 4).

Depending on the season and the water sampling points, the COD-Mn values vary significantly, indicating that the most polluted waters with organic substances are those discharged from the territory of the town (downstream) and reach the maximum value of 24 mgO_2/dm^3 and less polluted are the waters upstream of the locality (8,65-15,7 mgO_2/dm^3). The direct influence of the human factor on water pollution is obvious. Taking into account the COD-Mn value and according to the Regulation on the quality requirements for surface waters [7], the upstream water quality corresponds to the III^a quality class (moderately polluted); and the downstream water - quality class IV (polluted).

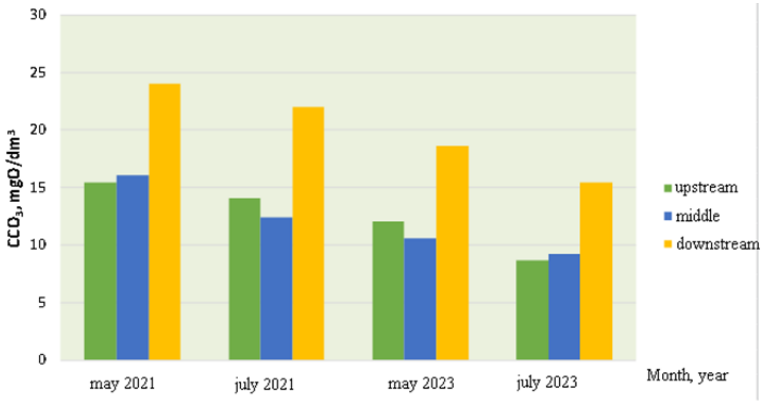

Figure 4. COD-Mn values in the waters of the Nârnova river during the years 2021 and 2023.

Water pollution index in the Nârnova river.

The Pollution Index was used to assess the ecological state of the water in Nârnova, which was determined using the 5 classes of surface water quality (IPAcc) in accordance with the National Normative. The IPAcc assessment frames the selection of the set of water quality parameters. The calculation was made based on the following parameters: pH, mineralization, hardness, COD-Mn, co-concentration of Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, HCO_3^-, NO_3^- ions. The intermediate values of the quality level (VNC, %) between quality classes of the water for a specific amount of physico-chemical parameters were calculated according to [8].

The evaluation of water quality is recommended after the stoppage (IPAcc, %) according to [8]. The values of the pollution index (IPAcc), ecological status and quality level of the investigated waters are presented in Table 4.

Table 4. The water pollution index of Nârnova river, year 2021.

Methods of analysing the samples	IPAcc, %	Quality level	Environmental status
In upstream	60,5	III	Moderately polluted
Middle	40,2	IV	Polluted
In downstream	37,9	IV	Polluted

Recent data indicate that the flowing waters of this stream, which traverses the village downstream, are polluted by the unauthorized waste from the population in this area.

Determination of water irrigation coefficients in Bălănești village, Nisporeni district.

A series of irrigation indices, calculated based on the chemical parameters of the water, were used to evaluate the quality of the irrigation water.

SAR index – Sodium absorption capacity, [SAR].

The SAR index is used to estimate the water quality for irrigation purposes. The determination of the coefficient is carried out according to the ratios between the milliequivalents of Na^+, Ca^{2+} and Mg^{2+}.

$$SAR = \frac{rNa^+}{\sqrt{\frac{rCa^{2+}\, rMg^{2+}}{2}}}$$

In general, the higher the SAR, the less suitable the water is for irrigation. The water with a high SAR value, before use, will require the use of specialized soil additives in order to avoid the long-term effect of sodium on soil quality.

If irrigation water has a high SAR and is used over many years, sodium can replace calcium and magnesium in the soil. Sodium is an element that disperses the soil and leads to the loss of its structure. It will also increase soil compaction, a fact that will have a negative effect on plant growth and expenses for mechanized works.

According to the value of the SAR coefficient, the waters used in irrigation can be classified as:

- if SAR<10 – excellent;
- if SAR 10-18 – good;

- if SAR 18-26 – satisfactory;
- if SAR >26 – unsatisfactory [9].

The calculated data regarding the SAR index values is indicated in Table 5.

Stebler coefficient or Irrigation coefficient [Ka] [10].

This coefficient is used to evaluate the probability of a secondary salinization of the soil, triggered by the poor quality of the water used for irrigation. The Stebler method involves the calculation of alkaline characteristics in the form of irrigation coefficients, K (in me/L):

$$K1 = \frac{288}{5\,\mathrm{rCl}^-}, \quad \text{at} \ \ \mathrm{rNa}^+ < \mathrm{rCl}^-$$

$$K2 = \frac{288}{\mathrm{rNa}^+ + 4\,\mathrm{rCl}^-}, \quad \text{at} \ \ \mathrm{rCl}^- + \mathrm{SO}_4^{2+} > \mathrm{rNa}^+ > \mathrm{rCl}^-$$

$$K3 = \frac{288}{\mathrm{rNa}^+ + 5\,\mathrm{rCl}^- + 9\,\mathrm{rSO}_4^{2-}}, \quad \text{at} \ \ \mathrm{rNa}^+ > \mathrm{rCl}^- + \mathrm{SO}_4^{2-}$$

Stebler:

- K>18 – good;
- K 18-6 – satisfactory;
- K 6-1,2 – unsatisfactory;
- K <1.2 – bad [10].

The calculated data regarding the values of the STEBLER index are indicated in Table 5.

Magnesium Adsorption Ratio (MAR).

The magnesium content of water is considered one of the most important qualitative criteria in determining the quality of irrigated water. The magnesium content is calculated by the following formula: MAR = [Mg^{2+} / (Mg^{2+}+ Ca^{2+})] /100 (Concentrations are in meq/l). Water is suitable for irrigation at MAR <50% and unsuitable at MAR>50%, as it will damage the soil [11].

The irrigation coefficients calculated for the taken water samples are presented in Table 5.

According to the SAR coefficient, all the waters analyzed are good for irrigation, and according to the Stebler coefficient, 56% correspond to the "good" qualification, and 44% - to the "satisfactory" qualification, according to the MAR qualification, 33% of the waters sampled cannot be used for irrigation, because they can damage the quality of the soil.

Table 5. Quality of surface water according to irrigation coefficients.

Nr	SAR	Rating	Stebler	Rating	MAR	Rating
1	0,76	Excellent	39,2	Good	56	Not satisfactory
2	3,75	Excellent	7,9	Satisf.	50	Good
3	2,4	Excellent	11,8	Satisf.	40	Good
4	0,9	Excellent	38,97	Good	38	Good
5	1,32	Excellent	25,3	Good	41	Good
6	0,7	Excellent	40	Good	51	Good
7	1,6	Excellent	21,4	Good	48	Good
8	2,76	Excellent	13,2	Satisfactory	57	Not satisfactory
9	5,6	Excellent	7,9	Satisfactory	72	Not satisfactory

4. Conclusions

The water from 50% of the wells under study cannot be used for human consumption, they are classified as polluted water, because the concentration of nitrates exceeds the permissible norm of 2-9 CMA.

Consequently, according to the chemical parameters (hardness, sulfates, nitrates, pollution index), the waters of the Nârnova river correspond to quality class III - IV.

As a result of the research conducted according to the SAR coefficient, all analyzed waters are good for irrigation, and according to the Stebler coefficient, 56% correspond to the ”good” qualification, and 44% - to the ”satisfactory” qualification, and only according to the MAR qualification, 33% of the sampled waters cannot be used for irrigation, because they can damage the quality of the soil.

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(Eugenia Melentiev, Mirela Rotaru) „Ion Creangă" State Pedagogical University of Chisinau

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Coordination compounds and chemical compositions with antioxidant properties

Ion Bulhac, Anastasia Ștefîrță, Lilia Brînză, Eduard Coropceanu, Leonid Voloșciuc, and Maria Cocu

Abstract. In the paper there are presented the main results that argue the antioxidant effect of BACC and some chemical compositions on plants. The anti-stress action of the new preparations - Conimid, Difecoden, Fludisec, Polyel, Galmet and Thiogalmet, is provided by increasing the antioxidant protection capacity due to the decrease of the content of malondialdehyde, intensification of the activity of antioxidant enzymes, increase in the content of non-enzymatic antioxidants with low molecular mass. The possibilities of optimizing the resistance of plants to oxidative stress, caused by drought, and stabilization of plant productivity are promising. The biological performance of plants pre-treated with BACC and antioxidant preparations are more fully realized under both optimal moisture and moderate drought conditions.

Keywords: biologically active coordination compounds, antioxidant chemical compositions, enzymatic and non-enzymatic system of antioxidant protection, resistance, productivity.

Compuși coordinativi și compoziții chimice cu proprietăți antioxidante

Rezumat. În lucrare sunt prezentate principalele rezultate care argumentează efectul antioxidant al compușilor coordinativi biologic activi (CCBA) și unele compoziții chimice asupra plantelor. Acțiunea anti-stres a noilor preparate - Conimid, Difecoden, Fludisec, Poliel, Galmet și Tiogalmet, este asigurată prin creșterea capacității de protecție antioxidantă datorită scăderii conținutului di-aldehidei malonice, intensificării activității enzimelor antioxidante, creșterii conținutului de antioxidanți non-enzimatici cu masă moleculară mică. Posibilitățile de optimizare a rezistenței plantelor la stresul oxidativ, cauzate de secetă și stabilizarea productivității plantelor sunt promițătoare. Performanțele biologice ale plantelor pre-tratate cu CCBA și preparate antioxidante sunt realizate mai complet, atât în condiții optime de umiditate, cât și în condiții de secetă moderată.

Cuvinte-cheie: compuși coordinativi biologic activi, compoziții chimice antioxidante, sistem enzimatic și neenzimatic de protecție antioxidantă, rezistență, productivitate.

1. Introduction

It has become an axiom the affirmation that one of the reactions of plants to the action of stress factors, including drought, is the intensification of reactive oxygen species (ROS) formation, which, depending on their concentration, have the role of signalling and activating protective mechanisms or induction of oxidative stress and destruction of cellular structures [1, 2]. Under normal conditions, ROS in cells are involved in metabolism, participate in the synthesis of some substances (phytohormones, physiologically active substances), in immunity reactions, in the inactivation of toxic substances, in the processes of cell division, in the annihilation of affected structures etc. Active oxygen compounds are formed continuously throughout the entire ontogenesis of plants, but in adverse environmental conditions, their formation intensifies and oxidative stress becomes a priority. Under optimal conditions, antioxidant enzymes and metabolites neutralize ROS, minimizing oxidative destruction. ROS, generated during drought, induce chlorophyll degradation in chloroplasts, causes stomatal closure, stop photosynthesis and reduce plant productivity [3, 4]. The key mechanisms, related to the adequate response to the unfavourable fluctuation of humidity and the establishment of drought conditions, are mechanisms coupled with the self-regulation of the formation and neutralization of ROS by activating the antioxidant potential, with the intensification of the synthesis of compounds with regulatory and protective functions. Avoidance of over-accumulation of ROS in cells is ensured by enzymatic and non-enzymatic antioxidant protection systems, which include antioxidant enzymes and low molecular weight compounds with antioxidant properties. A possibility to increase the productivity and quality of agricultural crops is the use of physiologically active substances (PhAS) with antioxidant properties [5, 6, 7]. In this sense, the coordination compounds which possess antioxidant properties deserve attention [8].

In recent years it has been shown that exogenous antioxidants can be used to increase the tolerance of plants to stress factors. The existence of a positive correlation between the exogenous administration of many PhAS, antioxidant protection and the formation of plant drought tolerance is known [3,6,9]. It is considered that the intensification of the activity of antioxidant enzymes increases the tolerance of plants to oxidative stress, conditioned by different unfavourable factors, through a more efficient elimination of ROS. Enzymatic decomposition and with the participation of low molecular mass compounds (ascorbic acid, glutathione, α-tocopherol) protect the plant from excessive ROS production.

Based on the above, one of the objectives of the investigations for several years consisted in the elucidation of the properties of new coordination compounds to increase the activity

of antioxidant protection enzymes and to reduce the impact of oxidative stress, caused by unfavourable conditions in the external environment.

2. Materials and Methods

Zea mays L., *Phaseolus vulgaris* L., *Glycine max* Merr. (L.), as well as *garlic Allium sativum* L. plants, pre-treated with solutions of BACC - Difecoden, Conimid, Fludisec, and chemical compositions Polyel, Galmet and Thiogalmet, synthesized in the Laboratory of Coordination Chemistry of the Institute of Chemistry within the Moldova State University, served as objects of study. The testing of the effect of the new preparations on the antioxidant protection systems of plants was carried out in the laboratories, the Vegetation Complex and in the fields of the Institute of Genetics, Physiology and Plant Protection of the MSU on small plots according to the requirements of the State Centre for the testing and approval of fertilizer products and plant improvers. In the experiments, the seeds were embedded with the respective solutions before sowing, and foliar treated during the vegetative growth of the plants.

The molecular and crystal structure of the coordination compounds was carried out in the Institute of Applied Physics of MSU at the Nonius Kappa CCD diffractometer (MoKα radiation, graphite monochromator, ω-2θ scan) [12]. The IR spectra were recorded on the FT-IR spectrometer Perkin-Elmer Spectrum instrument in Nujol in a range of 4000-400 cm^{-1} and in the attenuated total reflectance (ATP) mode in a range 4000-650 cm^{-1}, and their interpretation was carried out using the sources: [15,16, 17].

The estimation of the adaptive changes of the enzymatic system of antioxidant protection was carried out by comparing the degree of intensification of the activity of antioxidant enzymes in the leaves of control plants and those pre-treated with BACC and exposed to the action of oxidative stress, caused by the action of moderate drought [6]. The effect of plant pre-treatment on the indices that characterize the intensity of oxidative destruction (content of malondialdehyde - MDA) and the activity of antioxidant defence enzymes (superoxide dismutase - SOD, catalase - CAT, ascorbate peroxidase - APX, glutathione peroxidase - GLPX, glutathione reductase - GLR) were studied. SOD activity was determined by the inhibition of nitroblue tetrazolium photochemical reduction assay, described in detail by Becana M., Aparicio-Tejo P.M., Irigoyen J.J., Sanchez-Diaz M. [18]. The incubation medium contains K-Na-phosphate buffer (60 mmol, pH 7.8), methionine (13 mmol), riboflavin (2 μmol), nitroblue tetrazolium (63 μmol), EDTA (0.1 mmol) and 100 μL of extract. The reaction runs for 10 minutes at light intensity of 15W fluorescent lamps. Samples incubated in the dark served as controls. Enzyme activity that inhibits 50% of the nitroblue tetrazolium photo-reduction is considered as a conventional unit of

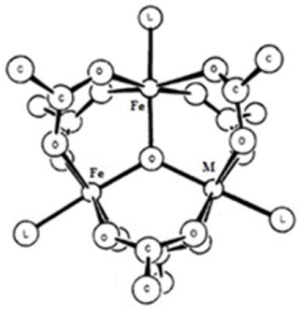
SOD activity. CAT activity was estimated by the method of Chance B. and Machly A. [19] by spectrophotometric determination at λ 240 nm of H_2O_2 decomposition; APX – by monitoring the oxidation rate of ascorbate at λ 290 nm [20]; GLR - by reducing oxidized glutathione in the presence of $NADP^+H$ [21]. Homogenization of plant material and extraction – as described in [17]. The activity of the antioxidant enzymes was expressed in mmol of oxidized substrate and was estimated as a percentage of the enzymes' activity in leaves of the control plants.

The effect of PhAS on plants was concluded by identifying differences in physiological and biochemical parameters. The results were statistically analyzed using the "Statistic 7" software package for computers.

3. Results and Discussion

a) *Characteristics of BACC*, chemical compositions *and obtaining methods*

Syntheses of coordination compounds and chemical compositions or carried out in the Laboratory of Coordination Chemistry of the Institute of Chemistry of the MSU. Two types of coordination compounds (BACC) were obtained and investigated: clusters of the "μ3-oxo" class (Figure 1) [10, 11] and complexes from the "dioximate" class of metals (Figures 2 and 3) [12].



$[Fe^{III}_2M^{II}O(CH_3COO)_6L_3]$ (L=H_2O, 3-$CONH_2$-Py, 3-$CON(C_2H_5)_2$-Py, 3-, 4-$COOC_2H_5$-Py

M=Fe^{III}, L=nicotinamide (**Trifenamid**);
M=Fe^{III}, L=N,N-dietilnicotinamide (**Trifeden**);
M=Co^{II}, L= N,N-dietilnicotinamide (**Difecoden**);
M=Mn^{II}, L=N,N-dietilnicotinamide (**Difemanden**)

Figure 1. General structure of "μ3-oxo" class clusters.

The chemical composition, spectral and structural properties of the chemical products were determined using the following methods: elemental analysis, IR spectroscopy and single crystal X-ray diffraction. It should be noted that the Fludisec preparation consists of 3 complexes in different molecular ratios, which differ from each other by the axial component of the complexes: a) Seu-Seu, b) Seu-SeSeu and c) Seu-SeSe (Figure 4).

The structure of the coordination compound Fludisec was established by the single crystal X-ray diffraction method [12].

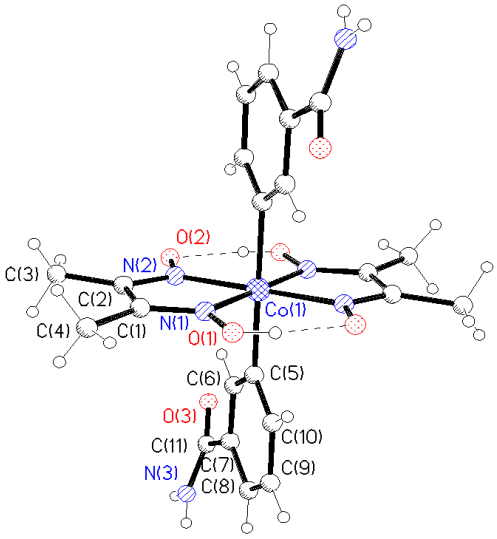


Figure 2. Complex cation structure in $[Co(DH)_2(Nia)_2][BF_4]\cdot 2H_2O$ compound

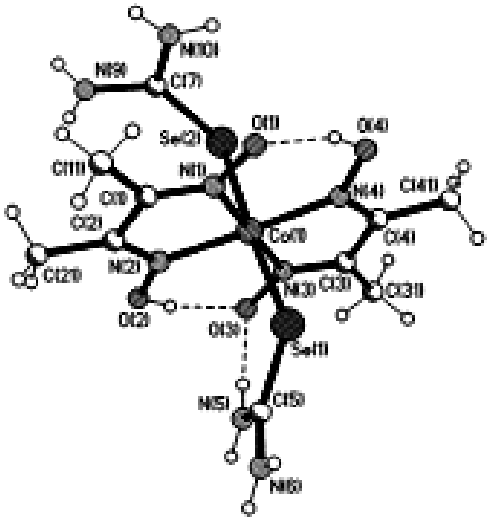
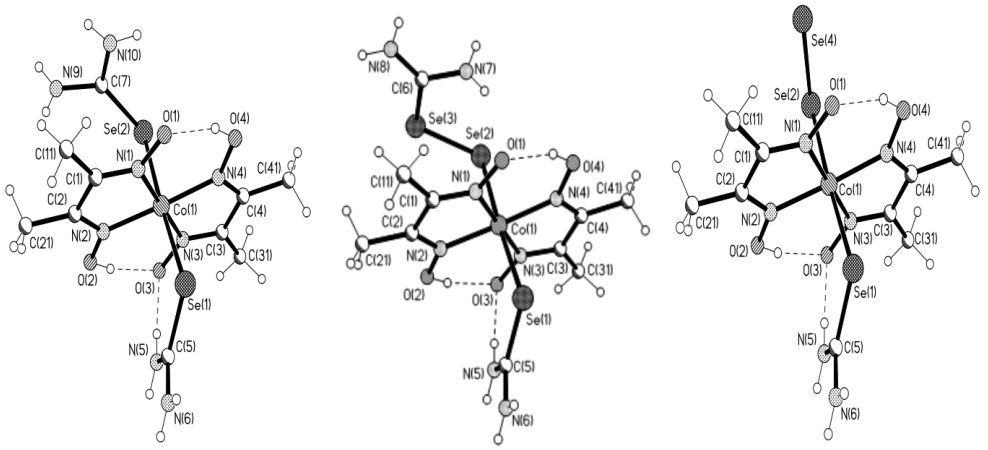


Figure 3. Cation structure $[Co(DH)_2(Seu)_2]^+$ in $[Co(DmgH)_2(Seu)_{1.4}(Se\text{-}Seu)_{0.5}(Se_2)_{0.1}][BF_4]$ (“**Fludisec**”) compound

Difecoden complex – with the chemical formula $[Fe_2^{III}Co^{II}O(CH_3COO)_6(DENA)_3]\cdot H_2O$ is obtained by substituting coordinated water molecules in the initial complex with the ligand having the nitrogen atom as donor (DENA). The complex $[Fe_2^{III}Co^{II}O(CH_3COO)_6(H_2O)_3]\cdot 2H_2O$ with a mass of 2 g was suspended in 40 mL of acetone. 2 mL of diethylnicotinamide (DENA) was added to the obtained suspension. The reaction mixture is heated to 50°C until the initial complex is completely dissolved. The solution is filtered and left at room temperature for the acetone to evaporate. As a result, a light brown colored substance was formed, which is separated by filtration, washed 3-4 times with diethyl ether in a volume of 30 mL and air-dried. 2.2 g of final product is obtained.

The coordination compound, conventionally called Conimide, represents the nitrate of trans-bis(dimethylglyoxymato)bis(nicotinamide)cobalt(III) dihydrate, with the chemical formula $[Co(DmgH)_2(PP)_2]NO_3\cdot 2H_2O$, in which: DmgH – monoanion of dimethylglyoxime (CH_3-C(=NOH)-C(=NO-)-CH_3); PP – vitamin PP (nicotinic acid amide, 3-$CONH_2$-C_5H_4N), having the crude formula $C_{20}H_{30}CoN_9O_{11}$ and a molecular mass equal to 631,445. The compound was obtained from the mixture of 0.29g (0.001 mol)



a) b) c)

Figure 4. Structure of $[Co(DmgH)_2(Seu)_{1.4}(Se\text{-}Seu)_{0.5}(Se_2)_{0.1}][BF_4]$ ("Fludisec") compound: a) $[Co(DmgH)_2(Seu)_2]^+$; b) $[Co(DmgH)_2(Seu)(Se\text{-}Seu)]^+$; c) $[Co(DmgH)_2(Seu)(Se_2)]^+$.

$Co(NO_3)_2{\cdot}6H_2O$, 0.23g (0.002 mol) dimethylglyoxime in 100 mL of water: methanol mixture in a 1:1 volume ratio. The reaction mixture was heated in a water bath at a temperature of 50-60°C until the complete dissolution of the dimethylglyoxime. 0.24g (0.002 mol) of nicotinamide dissolved in 50 mL of methanol is added to the obtained solution, after oxidizing with oxygen in the air for 30 minutes. As a result, a light tan crystalline product is formed with a yield of 70%. The substance is soluble in water, methanol, ethanol and insoluble in diethyl ether. The IR spectrum shows the absorption bands that characterize trans-dimethylglyoximates of cobalt(III) with nicotinamide: $\nu_{as}(NH_2)$ 3379 cm^{-1}, $\nu_s(NH_2)$ 3203 cm^{-1}, ν(C=O) 1694 and 1674 cm^{-1}, $\delta(NH_2)$ 1604 cm^{-1}, $\delta_{as}(CH_3)$ 1446 cm^{-1}, $\delta_s(CH_3)$ 1384 cm^{-1}, ν_{as}(NO) 1234 cm^{-1}, ν_s(NO) 1093 cm^{-1}, δ ($CH_{arom.}$) in plane 1135 and 1067 cm^{-1}, $\delta(CH_{arom.})$ out of plane 755 cm^{-1}.

Fludisec is an ionic coordination compound of selenium tetrafluoroborate-[bis(dimethylglyoxymato)-(selenocarbamide)$_{1.4}$-(selenium-selenocarbamide)$_{0.45}$-(selenium-selenium)$_{0.15}$cobalt(III)] with the chemical formula $[Co(DH)_2Seu_{1.4}Se\text{-}Seu_{0.45}Se\text{-}Seu_{0.15}]BF_4$, in which two monodeprotonated DH^-/$DmgH^-$ ligands were coordinated to the complex generator atom, where DH_2/$DmgH_2$ is the dimethylglyoxime molecule, Seu – selenocarbamide molecule, and Se-Seu – a neutral carbamide molecule containing two Se atoms. To obtain 0.34 g (0.001 mol) of $Co(BF_4)_2{\cdot}6H_2O$ in 30 mL of water, 0.23 g

(0.002 mol) of dimethylglyoxime in 40 mL of methanol and 0.25 g (0.002 mol) of selenourea in 30 mL of methanol were added. The obtained solution was heated for 10 minutes in the water bath in a graphite crucible at ~70°C. Crystals of the same color precipitated from the dark-brown solution upon slow evaporation (yield ~32%). The compound is soluble in alcohols, partially in water. In the IR spectrum, the characteristic absorption bands of the $Co(DmgH)_2$ fragment are present, cm^{-1}: $\nu_{as}(CH_3)$=2928, $\nu_s(CH_3)$=2871,ν_{as}(C=N)=1546, δ_{as}as(CH_3)=1461, $\delta_s(CH_3)$=1376,ν_{as}(N=O)=1237, ν_s(C=N)=1285, ν_s(N=O)=1083, γ(OH)=972, δ(CNO)=730, ν_{as}(Co-N)=507 and ν_s(Co-N)=428. For the $[BF_4]^-$ ions in the outer sphere, the bands $\nu_{as}(BF_4)$=1084, $\nu_s(BF_4)$=761, δ(F–B–F)=524 cm^{-1} can be assigned.

The crystal structure of $[Co(DmgH)_2Seu_{(1+0.4)}Se\text{-}Seu_{0.45}Se\text{-}Se_{0.15}]BF_4$ was determined by the X-ray analysis method in the X-calibre diffractometer equipped with a CCD detector at room temperature. Crystallographic data indicate triclinic syngony, symmetry space group P-1, unit cell parameters: a=7.9163(9), b=11.679(2), c=13.433(3) Å, α=64.50(2), β=75.31 (1), γ=82.05(1)°, V=1083.8(3) $Å^3$, the number of independent structural units Z=2, ρ(calc.)=1.906 g/cm^3 for the composition $C_{10}H_{22}BCoF_4N_8O_4Se_2$. The structure was determined by direct methods, and the coordinates of the basic (non-hydrogen) atoms were specified by the least squares method in the anisotropic variant within the SHELX-97 program complexes.

The preparation, called **Polyel**, is a light-brown chemical composition, hygroscopic, well soluble in water, soluble in alcohols, stable over time and in light. The composition contains thiourea, coordination compounds with biological activity, macro- and microelements in the following composition of active ingredients, %: thiourea - 50.00; $Mg(NO_3)_2{\cdot}6H_2O$ - 20.12; $Ca(NO_3){\cdot}24H_2O$ - 14.51; potassium salicylate -11.43; $Mn(CH_3COO)_2{\cdot}4H_2O$ - 0.55; $[Co(DmgH)_2(SeUrea)_2]BF_4{\cdot}2H_2O$ - 1.73; $[Fe_3O(CH_3COO)_6(H_2O)_3]NO_3{\cdot}3H_2O$ - 0.69; $[Co(DmgH)_2(Nia)_2]BF_4{\cdot}2H_2O$ - 0.36; $Zn(NO_3)_2{\cdot}6H_2O$ - 0.26; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ - 0.19; $(HOC_6H_4COO)_2Cu{\cdot}4H_2O$ - 0.16.

The coordination compounds of iron(III) and cobalt(III) were obtained according to the following protocols: $[Fe_3O(CH_3COO)_6(H_2O)_3]NO_3{\cdot}3H_2O$, $[Co(DmgH)_2(Nia)_2]BF_4{\cdot}2H_2O$ - according [8], $[Co(DmgH)_2(Se\text{-}Urea)_2]BF_4{\cdot}2H_2O$ [12], where DmgH – dimethylglyoximate monoanion, Nia – nicotinamide (vitamin PP). The starting substances for the synthesis of coordination compounds and other components of the Polyel preparation were purchased from SIGMA-ALDRICH and used without further purification.

Thiogalmet is a solid, powdery substance of light-brown colour, stable at room temperature, well soluble in water. It was obtained by mixing two parts (m/m) of thiourea with one

part of the known biologically active preparation Galmet. The calculated content of bioactive components in Thiogalmet, %: thiourea – 66.66; gallate anion ($(OH)_3C_6H_2COO^-$) – 21.52; K^+ - 1.63; Mg^{2+} - 0.97; Mo(VI) – 0.99; NH_4^+ - 1.07; total nitrogen – 25.36.

The IR spectrum of the Thiogalmet composition is characterized by the presence of several absorption bands of different intensity, the knowledge of which will allow the use of the spectrum as a benchmark when obtaining the respective composition repeatedly. IR (ν, cm^{-1}): 3623w, 3361m, 3251s, 3163s, 3083m, 2989s, 2686m, 2647w, 1680w, 1607m, 1539v.s, 1456s, 1399v.s, 1366v.s, 1345v.s, 1317sh, 1276s , 1262s, 1220w, 1201s, 1096m, 1082m, 1055s, 1044s, 965w, 890sh, 876m, 836w, 800m, 792m, 773m, 748m, 729s, 672m, 631s, 488v.s, 467s, 412w (v.s – very strong, s – strong, m – medium, w – weak, v.w – very weak, sh – shoulder).

b) *Arguing the antioxidant properties of the newly synthesized BACC and chemical compositions*

It is known that the resistance of plants to unfavourable conditions consists of a complex of protective mechanisms - physiological, biochemical, anatomical-morphological, oriented towards maintaining the internal environment of the organism at a relatively constant level, a necessary condition for the normal realization of the growth and development program each species and variety of plants encoded in the genetic apparatus. Those physiologically active substances, including BACC, which under suboptimal environmental conditions have a positive impact on these mechanisms, can be considered tolerance inducers. Much of the damage caused by drought is associated with oxidative damage at the cellular level as a result of excessive ROS formation caused by cell dehydration. Logically, it is correct to assume that the property of plants to control and maintain at a certain level the ROS content is directly correlated with their resistance to the action of unfavourable factors.

It has been demonstrated that some coordination compounds and some chemical compositions possess the property of protecting plant cells from the destruction of cellular structures by reactive oxygen species. It was established (Table 1), that BACC **Conimid** $[Co(DmgH)_2(PP)_2]NO_3 \cdot 2H_2O$ and **Difecoden** $[Fe_2CoO(CH_3COO)_6(DENA)_3] \cdot H_2O$, differ in antioxidant protection capacity.

Treatment of seeds and foliage of *Phaseolus vulgaris*, L plants with Conimid and Difecoden significantly reduces the formation of malondialdehyde - the end product of the oxidative destruction of phospholipids, and intensifies the activity of antioxidant protection enzymes. The obtained data demonstrate that the new preparation, - Conimid, has a significantly stronger antioxidant effect compared to Difecoden. The treatment of seeds and foliage with Conimid reduces the formation of malondialdehyde by 32.87%

Table 1. The content of malondialdehyde and the activity of antioxidant enzymes in the leaves of plants pre-treated with Difecoden and Conimid

Variant	**MDA**, μmol · g-1 fr. m. *		**SOD**, conv. un. · g-1 fr. m.		**APX**, mmol · g-1 fr. m.		**CAT**, mmol · g-1 fr. m.	
	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
Control	15.9 ± 0.8		145.7 ± 2.2		3.1 ± 0.1		2.10 ± 0.05	
Difecoden	12.4 ± 0.6	-21.8	154.5 ± 1.9	6.0	4.7 ± 0.2	52.1	3.06 ± 0.06	45.7
Conimid	10.7 ± 0.4	-32.8	165.6 ± 0.9	13.6	5.3 ± 0.3	61.7	3.12 ± 0.09	48.6

fr. m. * - fresh mass

compared to the content of malondialdehyde in the leaves of control plants and by 14.15% compared to plants treated with Difecoden. The activity of the antioxidant enzymes SOD, APX and CAT under the influence of this BACC increases compared to the control by 13.6; 61.7 and 48.6 % respectively. Enhancing the antioxidant protection capacity of plants ensures the stabilization of the complex of assimilatory pigments and reduces the oxidative destruction of chlorophyll (Table 2). Pre-treating the plants with the respective compounds protect the assimilatory pigments from oxidative destruction. The plants pre-treated with Difecoden and, especially, with Conimid, are characterized by an assimilative apparatus with a significantly higher content of assimilative pigments compared to the control plants.

Table 2. Effect of Difecoden and Conimid on content of assimilatory pigments (mg·dm^{-2}) in leaves

Variant	**Chlorophyll *a***		**Chlorophyll *b***		**Carotenoids**		**Cl *a* + Cl *b***	
	M ± m	Δ, % C	M ± m	Δ, % C	M ± m	Δ, % C	M ± m	Δ, % C
Control C	1.441 ± 0.002		0.601 ± 0.003		0.424 ± 0.001		2.091 ± 0.002	
Difecoden	1.910 ± 0.002	32.6	0.902 ± 0.003	38.5	0.621 ± 0.001	47.6	2.802 ± 0.001	34.0
Conimid	2.320 ± 0.001	61.8	1.043 ± 0.005	59.7	0.715 ± 0.001	70.1	3.373 ± 0.001	61.2

Carbon assimilation processes in *Phaseolus vulgaris* plants pre-treated with Conimid prevailed over the control by 40.4 and 8.3 percent. Plant productivity in moderate drought conditions in pre-treated plants exceeded the productivity value of control plants in the same humidity conditions by 30.2%.

Therefore, BACC Difecoden and Conimid, show antioxidant properties, and the pre-treated plants possess a significantly higher antioxidant protection capacity compared to control plants, with a positive impact on plant growth and productivity.

Currently, one of the important objectives of modern agriculture is to obtain production with an increased content of microelements and vitamins with a protective function from reactive oxygen species, which cause oxidative stress both in the body of plants and animals, including humans. Special attention is paid to products with a high selenium content due to its importance in the food chain. The organic form of selenium, obtained by the human body from vegetable products, is considered more active and useful. It has been demonstrated that selenium in small concentrations conditions the amplification of the adaptive potential of plants, diminishes the negative action of drought, stabilizes the surface of the assimilation apparatus, reduces the fall of flower buds and contributes to the activation of growth processes during the repair period after the improvement of humidity conditions [13].

The ionic coordinating compound Fludisec, which has selenium in its composition $[Co(DH)_2(Seu)_{1.4}(Se\text{-}Seu)_{0.5}(Se_2)_{0.1}][BF_4]$, has the property of stimulating the growth processes of garlic seedlings already at the initial stages of ontogenesis. It has antioxidant properties, significantly increases the activity of antioxidant protection enzymes, the content of photosynthetic pigments and the productivity of garlic plants (Table 3). The character of the changes in the content of malondialdehyde and the activity of antioxidant protection enzymes in the treated plants confirms the effect of reducing the oxidative stress induced by selenium.

Antioxidant action of selenium is manifested by the tendency to normalize these parameters; malondialdehyde values decreased significantly not only compared to those of untreated plants. The optimization of the antioxidant protection capacity provided by BACC Fludisec has a positive impact on the functional state of the plant's consequence of drought-caused oxidative stress is abundant generation of singlet oxygen, destruction of chloroplasts and decrease in the content of assimilatory pigments in leaves. According to our data [12], the supplementation of garlic plants with Se by treating the foliar apparatus with Fludisec caused an increase in content of assimilatory pigments, rate of photosynthesis and plant productivity. Selenium content in leaves and bulbs of the plants

Table 3. Malondialdehyde content and activity of antioxidant defence enzymes in garlic (*Allium sativum* L.) leaves and bulbs

Parameter	Control	Fludisec 0,00001%	
	M ± m	M ± m	Δ, %
In leaves			
MDA, μmol · g^{-1} fr. m.	32.35 ± 0.38	25.52 ± 0.36	-21.11
SOD, conv. un. · g^{-1} fr. m.	62.81 ± 0.73	78.39 ± 0.47	24.80
CAT, mmol · g^{-1} fr. m.	1.30 ± 0.01	1.94 ± 0.04	4.23
APX, mmol · g^{-1} fr. m.	8.43 ± 0.13	12.44 ± 0.35	47.57
GlR, mmol · g^{-1} fr. m.	172.81 ± 2.08	118.30 ± 2.12	38.46
GlPX, mmol · g^{-1} fr. m.	85.44 ± 1.94	217.01 ± 3.64	25.56
In bulbs			
MDA, μmol · g^{-1} fr. m.	16.18 ± 0.20	12.11 ± 0.19	-25.15
SOD, conv. un. · g^{-1} fr. m.	52.81 ± 0.77	70.14 ± 0.52	32.82
CAT, mmol · g^{-1} fr. m.	2.14 ± 0.01	2.98 ± 0.05	39.25
APX, mmol · g^{-1} fr. m.	7.16 ± 0.10	11.31 ± 0.02	57.96
GlR, mmol · g^{-1} fr. m.	182.84 ± 2.95	227.01 ± 3.89	24.16
GlPX, mmol · g^{-1} fr. m.	95.15 ± 2.31	120.21 ± 2.05	26.34

pre-treated with Fludisec exceeds the respective control values by about 19 and 49% (Table 4).

Maximum effect of increasing selenium content in both leaves and bulbs were recorded in plants treated with Fludisec. Biomass accumulation of the plants pre-treated with Fludisec was significantly higher compared to the control plants. The effect of plant mass increasing is respectively 6.87 and 21.20%. Average bulb mass exceeded control values by 7.47 and 22.20 percent respectively. Plant treating with the new preparation provided a 22.18% increase in production compared to the control. Supplementation of garlic plants

Table 4. Effect of garlic treatment on selenium content in leaves and bulbs ($\mu g \cdot kg^{-1}$ fr. m.)

Organ	Control	Fludisec 0,00001%	
	M ± m	M ± m	Δ, % Control
In leaves	74.0 ± 1.8	88.0 ± 1.9	18.92
In bulbs	47.0 ± 0.7	70.0 ± 1.1	48.94

(*Allium sativum* L.) with selenium by treating them with Fludisec increases tolerance to oxidative stress caused by drought.

A number of new chemical compositions also have been found to possess significant antioxidant properties. Plants treated with **Polyel** possess a significantly higher capacity of antioxidant protection compared to the plants treated with thiourea and, in particular, with those from the control group (Table 5).

Seed and leaf treatment of corn and soybean plants during vegetative growth with complex preparation **Polyel** ensures the increase of plants' resistance to drought, stabilizes productivity under fluctuating environmental conditions by 24.1% higher than in the control plants and by 13.8% higher than the plants treated with thiourea.

So, new chemical composition Polyel has a genuine influence on the antioxidant protection capacity of plants both under favourable conditions and under drought conditions.

It has been proven with certainty that the compositions **Galmet** and **Thiogalmet** possesses antioxidant properties and its use for the pre-treatment of plants in optimal humidity conditions ensures an improvement of the red-ox status by activating antioxidant enzymes and reducing the content of MDA (Table 6).

MDA content in plants pre-treated with Thiourea, Galmet and Thiogalmet under moderate drought conditions was maintained at a lower level by 7.79; 18.63 and 15.31% in corn and with 14.40; 13.08 and 22.02% in soybean, compared to the index values of untreated and drought-exposed control plants. MDA content in plants pre-treated with thiourea and Thiogalmet under moisture deficiency exceeded the MDA levels of control plants under optimal moisture by 28.93 and 18.42% in corn and by 29.93 and 15.36% - in soybean plants, which represents a significantly lower degree of MDA content modification than in untreated plants but exposed to drought-caused oxidative stress. Activity of antioxidant enzymes under optimal humidity increases in corn and soybean plants by 9.31 and 8.89 percent under the influence of thiourea and by 24.1 and 14.4 respectively when pre-treated with Galmet. Under these conditions, overall enzyme activity in corn and soybean plants

Table 5. Influence of plant treatment with PhAS on antioxidant defence capacity of *Glycine max (L.) Merr plants. 'Deia'* variety under drought conditions

Parameter	Control	Thiourea		Polyel	
	M ± m	M ± m	Δ, % M	M ± m	Δ, % M
MDA, μmol · g^{-1} fr. m.	17.4 ± 0.3* 28.5 ± 0.2**	16.0 ± 0.2 18.9 ± 0.4		14.8 ± 0.3 17.6 ± 0.5	
SOD, conv. un. · g^{-1} fr. m.	68.2 ± 0.6 81.7 ± 1.4	74.5 ± 0.1 96.5 ± 1.6	18.1	82.3 ± 2.1 109.6 ± 1.2	34.3
CAT, mmol · g^{-1} fr. m.	6.5 ± 0.1 8.1 ± 0.2	7.3 ± 0.2 9.3 ± 0.1	14.4	7.5 ± 0,2 13.9 ± 0.4	70.4
APX, mmol · g^{-1} fr. m.	8.3 ± 0.1 13.1 ± 0.3	10.9 ± 0.3 17.8 ± 0.4	35.7	13.7 ± 0.2 20.3 ± 0.5	55.1
GPX, mmol · g^{-1} fr. m.	51.8 ± 0.9 68.8 ± 0.5	60.6 ±1.3 76.0 ± 2.1	10.5	62.8 ± 1.1 78.7 ±1.4	14.4
GwPX, mmol · g^{-1} fr. m.	39.3 ± 0.6 66.4 ± 0.9	58.1 ± 0.8 93.8 ± 2.1	41.3	59.8 ± 1.3 120.5 ± 2.6	81.6
GR, mmol · g^{-1} fr. m.	121.4 ± 1.8 152.4 ± 2.7	132.3 ± 3.8 173.1 ± 5.2	13.6	148.8 ± 4.3 176.7 ± 3.5	15.9

*- control optimum 70% TWC; **- control drought 30% TWC

increased by 27.5 and 16.9% respectively compared to the index values of control plants. The beneficial physiological effect of Thiogalmet is also confirmed by the higher levels of assimilation and growth process in plants (Table 6). The intensity of photosynthesis in soybean and corn plants pre-treated with Thiogalmet constitutes an increase of 54.5 and 62.0%, respectively, compared to the value of carbon dioxide assimilation recorded in the control plants. Under insufficient moisture, the intensity of photosynthesis of the plants pre-treated with Thiogalmet was 2-fold higher compared to the control plants, exposed to drought. As a result of these changes, the efficiency of water use in assimilation process

Table 6. Antioxidant protection capacity and CO_2 assimilation process of plants pre-treated with Thiogalmet and exposed to moderate drought

Variants		**Control**	**Thiourea**	**Galmet**	**Thiogalmet**
Indices		M ± m	M ± m	M ± m	M ± m
***Zea mays*, L. cv. P458**					
MDA,	*optimum*	33.7 ± 0.2	31.4 ± 0.1	26.8 ± 0.1	24.9±0.1
µmol · g^{-1} fr. m.	*drought*	47.1 ± 0.9	43.4 ± 0,2	39.9 ± 0.2	39.8±0.1
Total enzymatic activity	*optimum*	145.4 ± 8.4	158.9 ± 6.8	180.5 ± 6.7	185.4±9.9
	drought	210.2±6.6	249.0 ± 6.7	280.5 ± 6.7	300.3±5.0
Assimilation,	*optimum*	5.0 ± 0.5	6.5 ± 0.2	7.6 ± 0.2	8.1±0.3
mmol CO_2· m^{-2}·h^{-1}	*drought*	1.9 ± 0.4	2.90 ± 0.09	2.8 ± 0.2	3.7±0.3
***Glycine max (Merr)*, L., 'Nadejda' variety**					
MDA,	*optimum*	32.9 ± 0.1	25.4 ± 0.06	26.2 ± 0.1	24.7±0.7
µmol ·g^{-1} fr. m.	*drought*	49.9 ± 0.4	42.70 ± 0.04	43.4 ± 0.1	37.9±0.1
Total enzymatic activity	*optimum*	203.7 ± 6.5	221.9 ± 6.5	232.5 ± 6.6	238.1±6.8
	drought	291.2 ± 5.3	326.4 ± 6.9	339.4 ±6.9	356.2±9.6
Assimilation,	*optimum*	5.2 ± 0.2	7.0 ± 0.2	7.2 ± 0.5	7.9±0.3
mmol CO_2· m^{-2}·h^{-1}	*drought*	1.2 ± 0.2	2.1 ± 0.1	2.5 ± 0.1	2.80±0.16

in plants pre-treated with Thiogalmet was higher by 72.8% compared to control plants under same humidity conditions [14]. The intensification of carbon dioxide assimilation processes conditions a significant increase in plant growth and productivity. Plant pre-treatment with Thiogalmet caused an increase in growth of soybean and corn plants under optimal humidity conditions by 5.6 and 14.6% compared to the control plants. Under conditions of insufficient moisture, soybean plants pre-treated with Thiogalmet were 17.8% taller than control plants exposed to drought. The productivity of soybean and corn plants pre-treated with the new composition was higher by 21.9 and 25.6% under optimal moisture and by 35.6 and 48.3% - under moderate moisture deficiency. The biological performance and productivity potential of soybean and corn plants pre-treated

with Thiogalmet are more thoroughly fulfilled under both optimal moisture and moderate drought conditions.

4. Conclusions

(1) Changing the chemical composition by substituting coordinated water molecules in "μ_3-oxo" type complexes $[M^{III}_3O(CH_3COO)_6\,(H_2O)_3]NO_3$, $[M^{III}_2M^{II}O(CH_3COO)_6\,(H_2O)_3]$, as well as by the variation of ligands on axial coordinates in "dioximate" type compounds with biologically active ligands such as nicotinamide (vitamin PP), N,N-diethylnicotinamide (cordiamine), selenourea leads to obtaining coordination compounds with pronounced antioxidant properties.

(2) The coordination compounds Conimid, Difecoden, Fludisec and the chemical compositions Polyel, Galmet and Thiogalmet possess properties of antioxidant substances, and can be used to protect plants from oxidative stress, caused by unfavourable environment, in particular, by drought.

(3) Plant tolerance to adverse conditions of external environment can be enhanced by pre-treating seed prior to sowing and plants during vegetative growth with BACC solutions and chemical compositions with antioxidant properties.

(4) The anti-stress action of the new preparations - Conimid, Difecoden, Fludisec, Polyel, Galmet and Thiogalmet, is provided by increasing the antioxidant protection capacity due to the decrease of the content of malondialdehyde, intensification of the activity of antioxidant enzymes.

(5) Plants pre-treated with BACC have a higher antioxidant protection capacity compared to untreated plants.

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(Ion Bulhac, Lilia Brînză, Maria Cocu) Institute of Chemistry of Moldova State University

(Anastasia Ștefîrță, Leonid Volоșciuc) Institute of Genetics, Physiology and Plant Protection of Moldova State University

(Lilia Brînză, Eduard Coropceanu) Institute for Research, Innovation and Technology Transfer of "Ion Creangă" State Pedagogical University in Chișinău

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The role of bioindicators and the modern instrumental methodology of ^{1}H NMR applied in ecological forensic expertise

Viorica Trifăuțan, Elena Gorincioi, and Boris Nedbaliuc

Abstract. Bioindicators and NMR performance offer new possibilities for forensic examination, directly in nature, at the site of the incident/ecological impact aimed at quickly finding out the truth about the crime and the criminal. The advantage of using biotic parameters (bioindication) lies in their greater objectivity and validity. The state of the boita is determined by the general state of the environment and promptly reacts to negative actions from various sources.

Keywords: pollution, bioindicators, Nuclear Magnetic Resonance (NMR), ecological forensic expertise.

Rolul bioindicatorilor și metodei instrumentale moderne ^{1}H RMN aplicate în cadrul expertizelor judiciare ecologice

Rezumat. Bioindicatorii și performanța RMN oferă noi posibilități pentru examinarea criminalistică, direct în natură, la locul incidentului/impactului ecologic menite să asigure aflarea rapidă a adevărului cu privire la infracțiune şi infractor. Avantajul utilizării parametrilor biotici (bioindicaţia) constă în obiectivitatea și temeinicia lor mai mare. Starea boitei este determinată de starea generală a mediului și reacționează prompt la acțiunile negative de diverse proveniențe.

Cuvinte-cheie: poluare, bioindicatori, Rezonanță Magnetică Nucleară (RMN), expertiza judiciară ecologică.

1. Introduction

The disturbance of environmental factors has a direct effect on the evolution of living beings, initially on their ability to adapt and later on their ability to survive, constituting, in extreme cases, factors for the elimination of certain species from food webs with drastic consequences on the evolution of biodiversity at the local level and with impact at a general level. This desideratum determined the need to evaluate the water quality of an aquatic basin and anthropogenic changes in aquatic ecosystems by means of bioindicators and modern technologies [1].

The large number of chemical compounds found in environmental factors requires the use of specific methods of analysis with high selectivity, specificity and sensitivity. Due to the continuous increase in the number of chemical compounds released into environmental factors that can contribute to the alteration of its quality, the development of new analysis protocols for their identification and quantification, even at the ultra trace level, has become a priority. Thus, in this article we highlight the role of NMR analysis in the assessment of environmental quality and the results of research carried out in this regard [2].

Numerous species of organisms manifest themselves as natural bioindicators of ecosystems, responding to changes in the environment to the presence of pollutants through changes in vital functions, or which accumulate pollutants in their bodies. Lake pollution indicators can be differentiated into sensitive species, which indicate the presence of a pollutant by the appearance of lesions or malformations, and accumulating species, which concentrate the pollutant in their body [1].

Diatoms are of particular importance in environmental monitoring, as they are good indicators of organic pollution in the aquatic environment. The dynamic nature of an environment leads to the incorporation of natural and anthropogenic materials of diverse provenance, characterizing a site to such an extent that it becomes highly distinctive and useful for forensic comparisons [1,3].

Diatoms have been used in forensic pathology for the diagnosis of death by drowning and to reconstruct or estimate the submergence of the post mortem interval (PMSI) of a body recovered from water. The use of diatoms as a diagnostic test for vital drowning is based on the assumption that, as long as the blood circulation is functioning (suggesting that the person was alive), these microorganisms will be transported and deposited in organs some distance from the lungs. In the absence of blood circulation (the individual was dead when placed in the water) they will stop at the walls of the lung alveoli. The blood circulation generated post-mortem by the autolysis process does not have the power to transport the diatoms and fix them at the level of the organs [4].

The consensus opinion currently accepted by specialists is that, provided adequate precautions are taken to prevent contamination, the demonstration of the presence of diatoms in organs such as bone marrow, brain, kidney, liver, spleen is strong evidence confirming death by drowning. If the degradative processes in the corpse are very advanced, the error in the assessment of the diagnosis of drowning is very possible, since the contamination - so not the destruction of the diatoms already fixed by inhaling the water – is obvious. In order to establish the place of immersion (where the vital drowning practically occurred), it must be confirmed, by histological examination, if the species

of diatoms found in the organs of the deceased are of the same kind as those present in the water from which the body was removed. Demonstrating the presence of diatoms and/or other physical particles or chemical pollutants in certain organs of the human corpse removed from the water is of real use both in the investigation of homicide and the investigative version, by establishing the exact cause of death and by locating the submersion area [1,5].

NMR is a versatile technique that is capable of analyzing any analyte that has magnetically active nuclei such as ^{1}H or ^{13}C, as well as many heteronuclei (e.g. ^{19}F, ^{15}N, ^{31}P), a requirement that is met by almost all relevant compounds from an ecological point of view. NMR is not only useful for structure determination and compound identification, it can also be used to examine non-covalent interactions between wastewater components, such as pollutant-protein or pollutant-humic binding – which is very difficult to achieve using other techniques. The authors aimed at examining the use and potential of NMR application as a tool for understanding polluted water and wastewater treatment processes and effects. They mention that a deeper exploration of the various NMR techniques that have been and can be applied to the study of polluted waters is warranted. The mentioned authors aim at showing, that even a short case study can be convincing, that when different NMR characterization approaches are combined with NMR-based toxicity assessment, the result is a comprehensive understanding of both wastewater and its impacts, which cannot be matched by any other modern analytical approach [2,9].

NMR spectroscopy is undoubtedly the most powerful tool for the study of molecular structures and interactions and is increasingly applied to environmental research such as the study of wastewater. With over 97% of the planet's water being saltwater and two-thirds of fresh water frozen in ice sheets and glaciers, there is a significant need to maintain and reuse the remaining 1%, which is a precious resource critical to the sustainability of the most life on Earth. The sanitation and reuse of wastewater is an important method of water conservation, especially in arid regions, making the understanding of wastewater itself and its treatment processes a highly relevant area of environmental research. Here, the benefits, challenges, and subtleties of using NMR spectroscopy for wastewater analysis are considerable [5, 7].

The study demonstrates that, when applied comprehensively, NMR can provide unique insights into not only the structure, but also the potential impacts of wastewater and wastewater treatment processes. Finally, low-field NMR, which holds considerable future potential for on-site wastewater monitoring, is briefly discussed [7].

NMR spectroscopy is one of the most versatile tools in modern science, with abilities to study all phases (gases, liquids, gels, and solids), chemical structures, interactions,

interfaces, toxicity, and more. The authors hope that this analysis will inspire more scientists to adopt NMR, given its huge potential for both wastewater analysis in particular and environmental research in general [2].

Experimental research studies are capitalized with the results which determine the presence and abundance of a range of environmental indicators and guide subsequent strategy for the collection and analysis of a more in-depth forensic sample. Evidence from the aquatic environment is recognized as having the potential to contribute valuable circumstantial information regarding a particular crime: ecological (anthropogenic impact), death by drowning (accidental, suicidal, criminal) or a murder by other means, later concealed by drowning. This paper presents current applications of limnology, particularly algae and diatom analysis to introduce new and ongoing research into ecological forensics and forensics itself [3].

2. Metods and Materials

The water samples were collected from Valea Morilor Lake in Chisinau municipality seasonally during the years 2021-2023 according to the unified methods of collection and processing of field and experimental hydrobiological samples. Algae species were identified with the Optica B-510 POL microscope equipped with a digital camera, species determination according to the determinants in force and the literature in the field. The saprobiological analysis was based on the list of water quality indicator algae species [4], consulting specialists from the academic environment. In forensic expertise, chemists apply a set of approved methodologies, procedures and instructions, using calibrated measuring equipment and analytical techniques subject to strict quality control [4,8,9].

During the research, various methods were applied to highlight the degree of pollution of Valea Morilor lake in Chisinau municipality: chemical, biological, ecological and forensic. The versatility of NMR spectroscopy has led to the development and implementation of various types of NMR techniques, intended for the examination of the structure of various types of environmental samples, living and non-living, as well as the study of critical processes in the aquatic environment. Fixing, lifting, transporting and preserving traces of a biological/chemical nature according to methodical and forensic recommendations meet the conditions to be admitted as evidence in solving environmental crimes. From an applied point of view, forensics is inextricably linked to different fields, such as chemistry, biology, physics, anatomy, etc. Due to crimes, forensics requires a series of modern technical-scientific means and methods specially adapted and perfected to meet the needs of the criminal investigation process. Chemistry makes forensics available, some of which allow specialists and experts in this field to go beyond the classical sphere

by continuously processing new discoveries and adapting them to the methods and means specific to forensic chemistry.

The water samples from the Valea Morilor lake in Chisinau municipality were taken at the end of October 2021 from three distinct sectors of the lake, with different accumulation of green algae, as the given research is part of a larger prospective study, also aiming involvement of algae in the ecobiological indication of water quality [3]. Valuing evidence by applying logic, scientific and forensic methods is the basic requirement in conducting ecological forensic expertise. Since the evidence collected in the case of a crime has an inhomogeneous character or a different chemical composition, they require various high-performance technical means and new examination methods. The veracity of the conclusions depends on the appropriate research methods and techniques, and modern high-performance technologies ensure the examination of the small volume of the presented material with high certainty. The amount of evidence in forensic chemical analysis is often at the level of microtraces/microparticles. In some perishable cases, for this reason, it requires fast (express) chemical methods of investigation and automated processing of the obtained data.

To perform the ^{1}H NMR experiments, the water samples were diluted with 10% D2O (v/v) (99.9%, Sigma-Aldrich), being subsequently transferred directly into the NMR vial (5 mm). The measurements were made at 25°C on Bruker AVANCE III 400 NMR spectrometer, 400 MHz operating frequency, equipped with 5 mm PABBO BB-1H/D Z-GRD Z108618/0071 sample head.

To suppress the water resonance, the pulse sequence "Water suppression using 3-9-19 pulse sequence with gradients" (eng.) was used. Direct ^{1}H NMR analysis of DOM took one hour per sample. This acquisition time of the ^{1}H spectrum, which involved the recording of 1024 scans, allowed the qualitative functional analysis study of DOM to be performed. After recording, the NMR spectra were subjected to Fourier transformation, using LB 0.3 Hz (LB- line broadening, eng) and the usual procedures of automatic spectrum post-processing (including baseline and phase adjustment). The ^{1}H NMR spectra of DOM in all analyzed samples demonstrated the most numerous resonances in the typical region for unsubstituted aliphatic hydrocarbons (0.5-2.05 ppm). Signals characteristic of protons adjacent to functional groups, such as carbonyl, ester or amide in the 2.1-3.10 ppm region of the ^{1}H spectra are missing, but instead, resonances are present in the 3.1-3.90 ppm region, which identify C-H bonded to the oxygen atom, i.e. hydrogen from carbohydrates [2,8,9].

3. Obtained Results and Discussion

Currently, a large amount of waste, plastic, vegetable remnants has accumulated in Valea Morilor lake, among which fish die daily. Dozens of dead fish can be seen floating on the surface of the lake. Given the high temperature that is favourable for the development of bacteria and algae, this fact has led to the so-called "blooming" of the water [5,6]. Phosphorus and nitrogen concentrations also contributed to this. Phosphorus and nitrogen reach the lake water through the small streams, including the Durlești stream. The big danger now appears as a consequence of last year blue-green algae death because when they decompose, they absorb the oxygen that is in the water and release toxins that are harmful to other aquatic life, also causing fish to die [6].

According to the specialists from the Environment Agency, the exact cause of the death of the fish is not known yet. Over the course of several years at the same time, in their opinion, this phenomenon occurs because the water is not sufficiently oxygenated. The O_2 share in the water varies from 85% to 140%, compared to the saturation norm, being maximum in the summer period, together with the massive development of algal vegetation.

Water mineralization oscillates between 700 and 1070 mg/l. The water of the lake belongs to the hydrocarbonate-sulfate-chlorine and hydrocarbonate-chlorine-sulfate category from the Mg+K and Mg+Na groups; the pH is slightly alkaline. To solve the problem, the municipal authorities proposed to procure 25 additional aerators, to activate in addition to the 13 already existing. Later, the specialists took samples that proved that there is insufficient oxygen in the water, which leads to the death of the fish.

Eutrophication contributes to the overgrowth of various species of algae in water bodies. The presence of an increased amount of nutrients in the water contributes to the intensification of the process of photosynthesis and cell division, which ensures the exaggerated reproduction of algae. Water pollution occurs due to a large concentration of chemical or biological waste that reaches the lake. They are dangerous, posing a great threat to human health. Some of the most common problems faced by fish species are: insufficient oxygen and organic pollution, thermal pollution, pollution with persistent synthetic compounds, etc.

In order to obtain a more complete picture regarding the state of water quality, the assessment was also extended to the biological components that can store information at a structural and functional level, in time and space, etc. The ecobiological indication of water quality allows for the express assessment of the level of pollution of the aquatic environment, presenting an advantage for forensics in the framework of ecological forensic

expertise that requires a quick assessment. The rapid assessment of the quality of the aquatic environment is becoming more and more effective and current.

The dominant composition of algae species differs in different research periods. The development of the phytoplankton of the Valea Morilor lake reaches maximum levels in spring and summer. In the winter period, the phytoplankton is very poor and contains mostly diatom algae. The diversity of phytoplankton species varies under the influence of the hydrological regime, climatic conditions and the degree of significant water pollution.

Based on these data we tended to highlight the role of modern technologies (NMR) and bioindicators in the assessment of anthropogenic impact in highly polluted areas with application of data in forensic expertise. As a result of the examination of the samples, the strongest signals in the obtained spectra are those of the solvent (waste water) in the region 4.40-5.40 ppm. In the spectrum, you'll notice that there are no signals in the range of 5.60-6.10 ppm, which is characteristic of derivatives containing alkenyl groups. However, in the region corresponding to aromatic protons at 8.30 ppm, you will observe the respective signals.

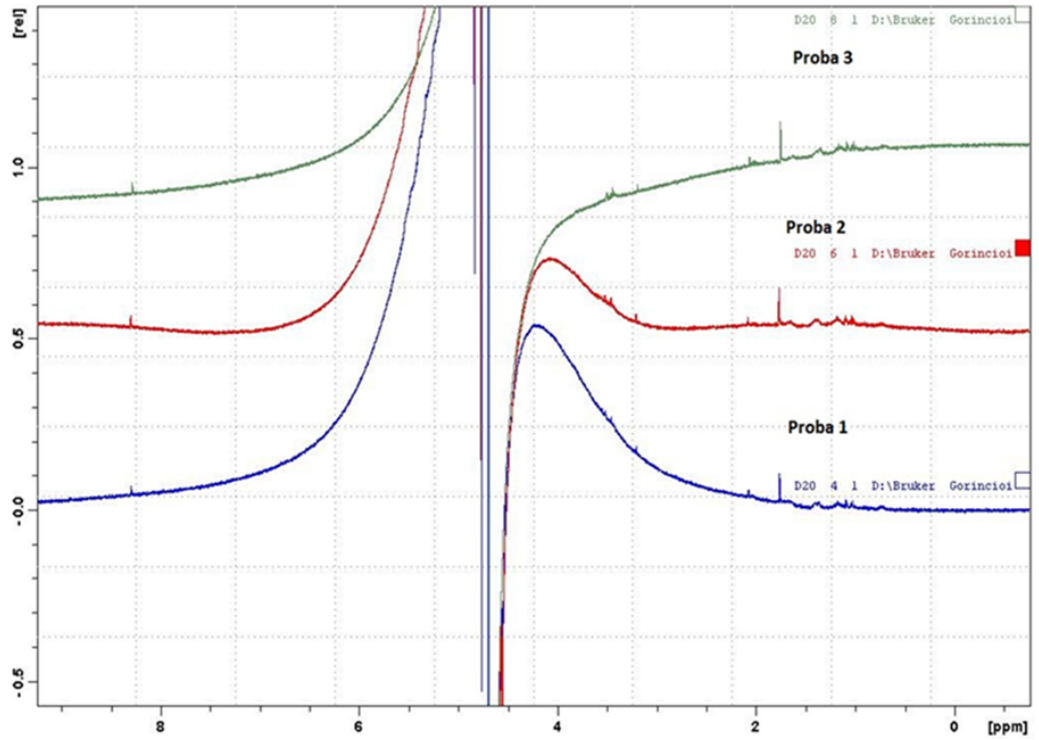

Figure 1. ^{1}H NMR spectra of DOM in water samples taken at the end of October, 2022 from Valea Morilor lake in Chisinau municipality.

Analyzing the water sample from Valea Morilor lake in Chisinau municipality, which was taken in June 2022 from the area with many rotting organisms (dead fish), in the ^{1}H NMR spectrum, the same groups of signals were found at a qualitative level, as it was mentioned above for the samples taken in autumn Fig. 1. The signals characterizing the spectrum area typical for unsubstituted aliphatic hydrocarbons (0.5-2.05 ppm) include the signal at 1.76 ppm apparently more intense, which would be favorable to a higher concentration of these compounds in this sample. Based on these data, it can be concluded about the composition of DOM in the warm period of the year, as the result of both water eutrophication due to algae (unsubstituted aliphatic hydrocarbons, carbohydrates) and, probably, of anthropogenic pollution (aromatic compounds). In the discussed sample, the signal was attested at 0.012 ppm, which can be attributed to organometallic compounds, probably water pollutants. Also, the typical area for aromatic compounds is richer in signals: signals at 6.45, 6.96, 7.10, 7.23 and 8.29 ppm are attested, which probably speaks of a more extensive pollution (although the signals are of low intensity).

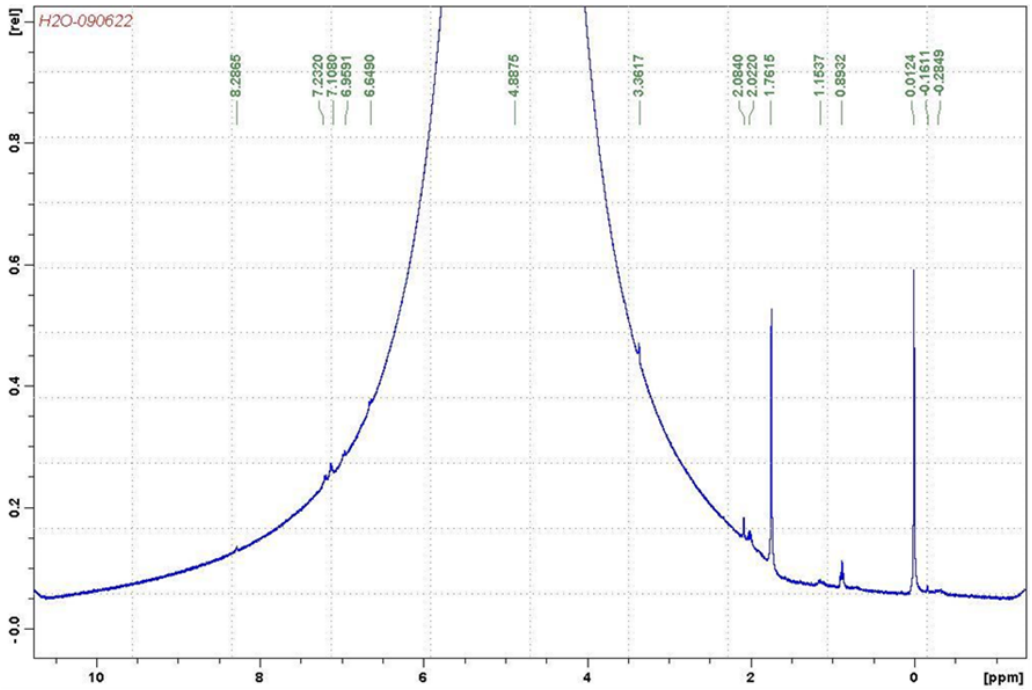

Figure 2. The ^{1}H NMR spectrum of DOM in the water sample taken on June 9, 2022, from the Valea Morilor lake in Chisinau municipality.

It is interesting to compare the ^{1}H NMR spectrum of DOM in the water sample from the Valea Morilor lake in Chisinau municipality, taken on June 9, 2022, with that of DOM

spectra in the water samples taken on March 27, 2023, i.e. during the cold period (Fig. 2). The spectra were recorded with the same NMR experimental parameters set, practically demonstrating the absence of DOM in the analyzed water, i.e. confirming the absence of eutrophication in the cold period of the year and the absence of pollution.

Ensuring the validation of the obtained results, we resorted to the use of scientific materials from the country and abroad, the creation and interpretation of diagrams according to data from the literature in the field [9].

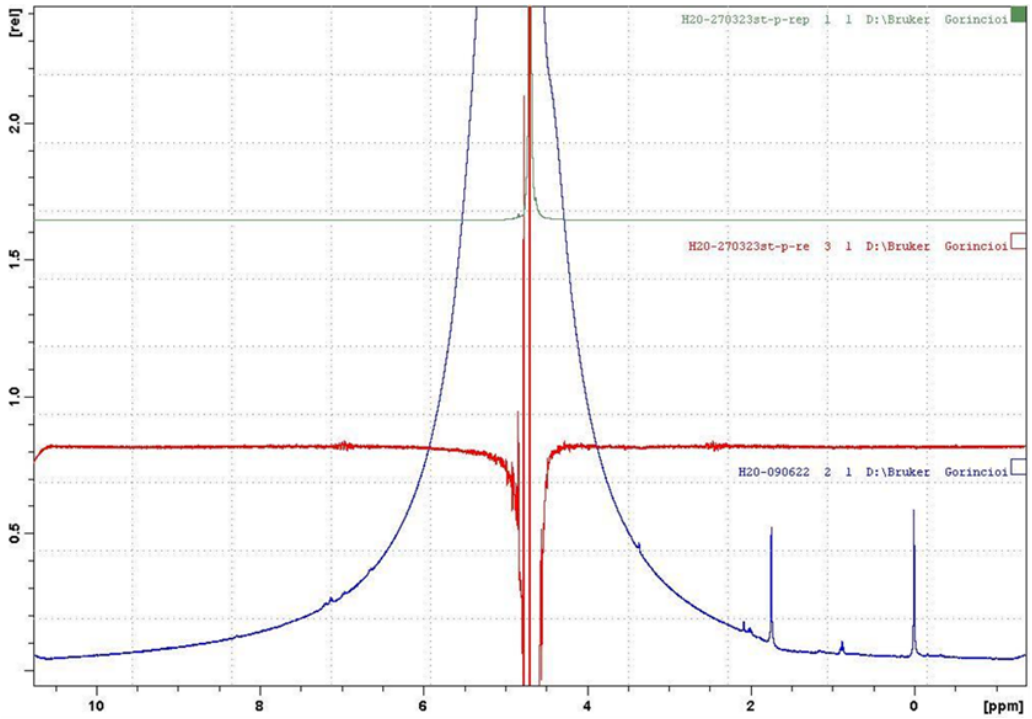

Figure 3. ^{1}H NMR spectra of DOM in water samples taken at the end of March, 2023 (red, green), June 9, 2022 (blue), from Valea Morilor lake in Chisinau municipality.

The samples were examined during the sampling period, using the NMR method for the detection and measurement of substances. In addition to these, the research activity was guided by the recommendations offered by international research with reference to modern technologies and chemical forensic expertise. The research results demonstrate that separation techniques and advanced NMR techniques are indispensable in environmental studies. According to the results obtained in the research carried out, the environmental factors are polluted/contaminated with a varied range of chemical compounds that create a permanent chemical stress on the environment.

The samples taken in different periods of the years of studies capable of determining the chemical compounds were examined, thus creating the premises for a more detailed assessment of the chemical stress to which the Valea Morilor lake hydroecosystem is subjected. The use of biomonitors and the application of specific methods to identify pollution sources together with NMR analysis made it possible to determine the sources of pollution of environmental factors and obtain useful information for the authorities responsible for water quality management.

The development of this method allows the introduction of a new direction that we want to apply in forensics in the case of pollution of watercourses based on persistent organic pollutants in water samples or sediments. We believe that this direction of research is welcome, considering the fact that, at the international level, it is widely applied in the ecological assessment of the environment.

Databases can be built with the NMR spectra of DOM, which can later be used in the authentication of unknown samples. NMR spectroscopy plays an essential role in understanding the nature of different types of environmental components and associated processes, including the different forms of organic matter found in soil, water and air, and is used to elucidate the state of water, organics, pollutants and metals in the environment.

4. Conclusion

Experimental research studies by evaluating algae as biological monitors capitalize through this work on the application of limnology, especially diatoms, to introduce new and ongoing research in ecological and forensic forensics.

DOM research in natural waters with the application of ^{1}H NMR spectroscopy is pioneering in the Republic of Moldova. The use of the ^{1}H NMR technique allowed the determination of the organic matter dissolved in the waters of Valea Morilor lake in the Chisinau municipality, which consists of a complex mixture of aliphatic and carbohydrate structures, with a minor contribution of aromatic compounds. NMR provides a very large amount of information on the chemical composition of water in a relatively short time and with minimal sample preparation.

The results of this initial qualitative functional analysis study are to be completed by quantitative analysis studies, based on the same method and by comparison with the currently applied and validated methods.

The methodological assurance of the actions of the ecological judicial expert in the examination of aquatic ecosystems is a good ground of conduct in the case of ecological damage, and the compensation of the damage from the polluter is the right of everyone not to be deprived of the normal state of the environment in which they live.

The theoretical-practical significance of the paper consists in the proposal and application of the modern instrumental method ^{1}H NMR in forensics. Nuclear Magnetic Resonance Spectroscopy (NMR) and the opportunities offered by this analytical method to apply measurement data at both molecular and macroscopic scales facilitate its rapid advancement in environmental science studies. The obtained results provide evidence of how the bioindicators respond to changes and react to pollutants in the aquatic environment. The ecological damage that affects the ecosystem, man and his goods, constitutes the first and most important stage of the process of perfecting the normative framework in the field.

The complex of analysis methods applied in carrying out chemical-forensic expertise by licensed forensic experts, with the use of advanced technologies and equipment, with the application of approved procedures and instructions, as well as with ensuring the quality control of the results, make this type of expertise a source of valid results, with high precision, scientifically substantiated and correlated with the factual data of the criminal prosecution.

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(Viorica Trifăuțan) National Center for Judicial Expertise Ministry of Justice (NCJE MJ), Chisinau municipality, Republic of Moldova
E-mail address: viorica.trifautan@mail.ru

(Elena Gorincioi) Institute of Chemistry, Moldova State University, Chisinau, Republic of Moldova
E-mail address: elena.gorincioi@gmail.com

(Boris Nedbaliuc) "Ion Creangă" State Pedagogical University,
Institute for Research, Innovation and Technology Transfer of CSPU, Chisinau, Republic of Moldova
E-mail address: boris.nedbaliuc@gmail.com

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The analysis of the chemical composition of the grape seed oil and peel

Liliana Sergheev

Abstract. The increased interest in grapevines and their products, which are universal in terms of taste and healing properties, results from the diverse content of beneficial compounds that have a positive effect on the human body. The valuable chemical composition of grape seed oil, as well as the peel of both white and red grape varieties, supports the importance of using this product not only in food but also in other areas.
Keywords: lipids, oil, fatty acids, aromatic acids, flavonoids, physico-chemical indices, phenolic compounds.

Analiza compoziției chimice a pieliței și a uleiului din sâmburii de vița-de-vie

Rezumat. Interesul sporit pentru vița-de-vie și produsele ei, care sunt universale după însușirile gustative și curative, rezultă din conținutul variat al compușilor utili cu acțiune benefică asupra organismului uman. Compoziția chimică valoroasă a uleiului din sâmburii de struguri, dar și a pieliței soiurilor albe și roșii, argumentează importanța utilizării acestui produs atât în alimentație, cât și în alte domenii.
Cuvinte-cheie: lipide, ulei, acizi grași, acizi aromatici, flavonoide, indici fizico-chimici, compuși fenolici.

1. Introduction

The grapevine has been known in the territories occupied by the Romans since prehistoric times, from the Neolithic period, over 3000 years BC. The cultivation of this vine in our country is confirmed by numerous sources, including archaeologists, paleontologists, as well as in the folklore and ethnography of the native region as part of our people's culture.

The highly favorable natural conditions, as well as people's attachment to this plant, explain the increased interest in cultivating grapevines, which first developed among the Thracians, then among the Greeks and Romans. Evidence of the knowledge of the Getae-Dacians and the development of this culture is seen in the preservation of Dacian-origin

words in the Romanian language, such as “butuc” (grapewine), “strugure” (grape), or “răvac” (fermented grape juice drained prior crushing and pressing).

Grapevines are cultivated for their delicious and aromatic grapes, which contain 10-30% sugars (glucose, fructose) with high nutritional, dietary, and healing qualities. Grapes are used in the treatment of liver, kidney, and stomach diseases, as well as tuberculosis. They are used to make juice, compote, jam, fruit paste and others. Grape seeds contain up to 20% of fatty oil, which is used in food and for certain technical purposes. The extract from grape seeds, which is rich in antioxidants, accelerates wound healing and benefits the cardiovascular, skeletal, and immune systems, as well as the cognitive function.

The period of grape development and maturation is characterized by several biochemical processes that produce qualitative and quantitative changes in the composition of the berries, determining the optimal time for harvesting. Both the raw material and the by-products obtained from grape processing must meet the applicable standards.

The grape seed oil is a valuable product with multiple properties and uses. It is one of the most potent oils with antioxidant effects, due to the presence of flavonoids, polyphenols, tannins, anthocyanins, and proanthocyanins.

The most significant stages of analyzing these substances include their extraction from plant sources, separation, purification, and isolation of individual components from crude extracts. Typically, polyphenols are extracted from plant sources using methanol [1], ethanol [2], acetone [3], or mixtures of these solvents with water in various proportions. Good results are also obtained with extractions using mixtures of methanol-water or acetone-water as extractants of proanthocyanidins from plant sources, as these mixtures have enhanced extraction capacity [3, 4]. In the extraction process with solvents such as methanol, ethanol, or acetone, in addition to proanthocyanidins, other substances are also extracted, making the purification procedure of the extract more challenging and reducing the extraction rate of proanthocyanidins [5]. If the extract is accompanied by lipophilic compounds, then non-polar solvents such as petroleum ether and other non-polar solvents are typically used for extraction [6].

It is known that besides water, methanol, or acetone, proanthocyanidins are highly soluble in ethyl acetate, and this solvent exhibits significant selectivity concerning natural products, making it a good alternative in the extraction process of proanthocyanidins from grape seeds [7].

2. Methods and materials used

For the study of the chemical composition of lipids and phenolic compounds from grape seeds and peel there were used the following methods: Soxhlet extractions, gas

chromatography (GC), and high-performance liquid chromatography (HPLC). The practical part of the work was carried out in the laboratories of the *Institute of Chemistry* in Chișinău.

3. Obtained results and discussion

The main substances in the composition of grape peel are:

- Phenolic compounds: anthocyanins, flavonoids, tannins, phenolic acids.
- Aromatic substances with a heterogeneous composition: alcohols, acids, esters, aldehydes, ketones, terpenes, etc.
- Oxidase enzymes (tyrosinases in healthy grapes and laccases in grapes affected by mold), which oxidize anthocyanins and tannins.
- Pectolytic and proteolytic enzymes.

Grape seeds constitute 2-6% of the grape, mature along with their ripening, and have the following chemical composition: 28-40% water, 28% cellulose, 0.8-1.2% nitrogenous substances, 4-6% tannins, 10-25% fatty substances, 2-4% minerals, fatty acids, and other substances. In order to determine the quality indicators of the oils, *Moldova* and *Izabella* grape varieties were analyzed. The fatty substances from grape seeds were determined by extraction with petroleum ether using the Soxhlet apparatus.

Table 1. Physicochemical indicators of grape seed oils.

Name of the variety	Acidity index	Saponification index	Esterification index	Iodine index	Peroxide index
Izabella	1.009	190.47	204.05	121.06	0.185
Moldova	0.505	188.47	195.85	119.73	0.175

The results obtained from the research were compared with the indicators provided in the Technical Regulation for *Edible Vegetable Oils* [9], which demonstrated that all indices meet the quality standards.

The acidity index determines the amount of free fatty acids in the oils, indicates the degree of rancidity and the duration and storage conditions of the oil as well. Fresh lipids usually have a minimal acid content. When pomace and seeds are stored in a moist state or when oil is obtained at high temperatures, the acidity index increases.

The iodine value characterizes the quantity of unsaturated fatty acids in the oil.

The peroxide value indicates the degree of oxidative rancidity of the fat. During the oxidation of fats, various products (peroxides, aldehydes, ketones) are formed, which alter the taste of the oil and give it an unpleasant odor.

The quality of grape seed oil is influenced by the grape variety, the drying and storage conditions of the seeds, as well as their processing technology.

The oil obtained from different varieties differs in physicochemical properties: it was determined that red varieties contain 13.69 - 16.35%, while white varieties contain 12.88 - 20.58% oil. The quantity and quality of oil depend on the soil composition, the ripeness of the grapes, seed quality, and the duration of seed storage. If the seeds are stored for a year, the oil content decreases by 1.45% - 13.55%, so processing should be done within 3 - 4 months after harvest [8].

To identify the chemical composition of seeds from different varieties, the samples were dried and ground, and then the extracts were obtained. Upon analyzing the data by varieties, it was found that the *Aligote* variety has the highest content of (+)-catechin, with a value of 2.640 mg, followed by (-)-*epi*-catechin with 2.574 mg, and chlorogenic acid with 0.247 mg. In descending order, other compounds present in the seeds of this variety include rutin, gallic acid, 3,4-dihydroxybenzoic acid, syringic acid, ellagic acid, quercetin, ferulic acid, resveratrol, 4-hydroxybenzoic acid, p-coumaric acid, while caffeic acid was not detected in the seeds of this variety.

The chemical composition of the seeds of the *Lidia* variety is characterized by an increased amount of (+)-catechin, specifically 0.771 mg, (-)-*epi*-catechin - 0.574 mg, and chlorogenic acid - 0.139 mg. In decreasing order, the sequence continues with syringic acid, rutin, gallic acid, 3,4-dihydroxybenzoic acid, ellagic acid, ferulic acid, quercetin, and 4-hydroxybenzoic acid, resveratrol, p-coumaric acid, and in a quantity of 0.001 mg, caffeic acid was also detected.

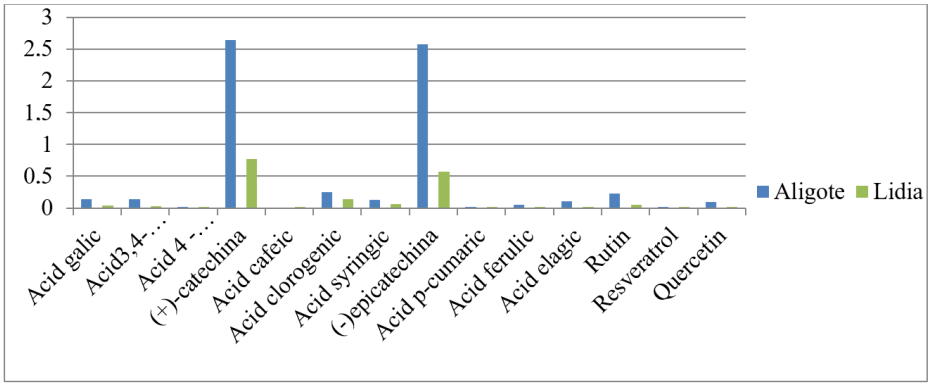

Figure 1. Chemical composition of seeds by grape varieties (mg).

Conducting a comparative study, we reached the conclusion that the grape seeds of the two analyzed varieties contain a significant amount of flavonoids: (+)-catechin and (-)-*epi*-catechin. In the *Aligote* variety, (+)-catechin predominates, followed by (-)-*epi*-catechin and chlorogenic acid. As for the other flavonoids, rutin and quercetin, the difference between the varieties is not as significant, but the *Aligote* variety has the highest content of these two compounds.

The group of hydroxycinnamic acids includes: caffeic acid, chlorogenic acid, *p*-coumaric acid, and ferulic acid. The values for these compounds are also higher in the white variety compared to the red grape variety. An exception is caffeic acid, which is present only in the *Lidia* variety (0.001 mg).

Regarding the derivatives of benzoic acid, the following were analyzed: syringic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, and gallic acid. For these compounds, no significant differences were observed, except for syringic and gallic acid, which are present in smaller amounts in the red variety.

In comparison to other classes of compounds, grape seeds contain a lower amount of polyphenols, such as resveratrol and ellagic acid, with the red variety having less content of these compounds compared to the white variety.

The seeds of the respective varieties were analyzed in comparison to observe the changes that occur in the chemical composition of the berries over 11 days. In the seeds of the *Aligote* variety there was observed a decrease in the values of all studied flavonoids over time, except for quercetin, which increased by 0.001 mg.

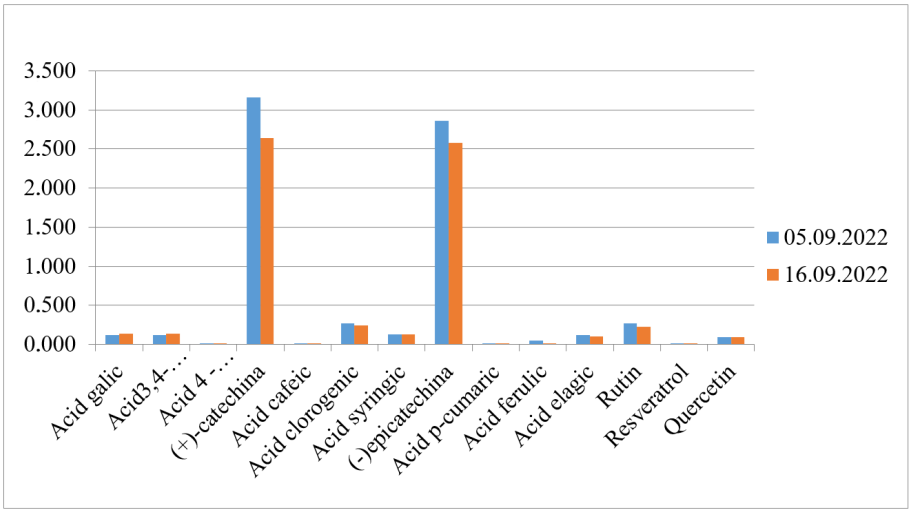

Figure 2. Chemical composition of *Aligote* grape seeds at different time periods (mg).

In the seeds of *Lidia* grape variety, the content of flavonoids and hydroxycinnamic compounds also undergoes changes over time. The derivatives of benzoic acid also decrease in quantity over the 11-day period, except for 3,4-dihydroxybenzoic acid, which increases from 0.152 mg to 0.156 mg. An increase is also observed in the polyphenolic compounds: ellagic acid and resveratrol.

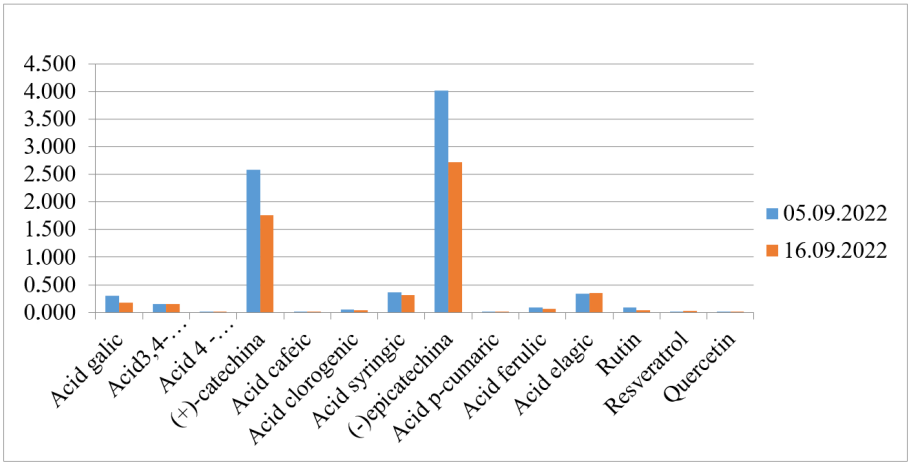

Figure 3. Chemical composition of *Lidia* grape seeds at different time periods (mg).

If the quantity of chemical compounds in the seeds decreases over time, the situation is reversed in the composition of the peel. Observing the evolution of chemical compounds in the peel of *Aligote* grape variety over time, it was noticed that flavonoids: (+)-catechin, (-)-*epi*-catechin, and rutin significantly increase their values, while quercetin, like in the seeds, decreases in quantity. Two representatives of hydroxycinnamic acids, chlorogenic and p-coumaric acids, show decreased values over 11 days, while the other two representatives, caffeic and ferulic acids, exhibit an opposite situation. The derivatives of benzoic acid of this white variety, as the harvest period approaches, concentrate more in the peel, while the polyphenols show the opposite trend.

Performing a comparative analysis of the chemical compounds in the peel of *Lidia* grape variety, it was observed that over 11 days, the peel of red varieties become richer in flavonoids, except for rutin. The content of hydroxycinnamic acids in the peel also increases. The derivatives of benzoic acid decrease quantitatively in the peel, except for syringic acid, which shows an increase. As for the polyphenols in this variety, the values of resveratrol decrease, while the value of ellagic acid remains constant. The study conducted on the peel and seeds of grapes, which includes the extraction and

identification of compounds present in these sources rich in antioxidants and essential fatty acids necessary for the body, justifies their separation and use in various fields.

4. Conclusions

(1) The chemical composition of Aligote, Izabela, Moldova, and Lidia grapes was investigated.
(2) The total content of fatty oil in the grape seeds of Moldova and Izabela varieties was determined through extractions with petroleum ether, using a Soxhlet apparatus.
(3) To confirm the quality of the oils, their physicochemical indices were determined, which were similar, since both varieties are red.
(4) Fourteen phenolic compounds were determined in the seeds and peels using the HPLC method.
(5) A decrease in the content of polyphenols was observed in the seeds during the pre-harvest period, while an increase in polyphenol content was observed in the peel during the same period.
(6) Additionally, a difference in the chemical composition between the white and red varieties was observed. The white variety is characterized by a high content of flavonoids, while the red variety has a higher content of polyphenols.

Recomandations: The wine industry represents one of the main branches of our country's economy, but the by-products accumulated after grape processing are not fully utilized. The bioactive components in grapes with antioxidant, antibacterial, anti-inflammatory, cardiovascular, and hepatic protective properties can be used in various fields: food industry, pharmaceuticals, cosmetics, animal husbandry and agriculture.

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(Liliana Sergheev) „Ion Creangă" State Pedagogical University of Chisinau
E-mail address: sergheevliliana@gmail.com

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Book Review

TO THE MONOGRAPH STUDY OF USEFUL PROPERTIES OF SOME COORDINATION COMPOUNDS CONTAINING OXIMIC LIGANDS, AUTHORS: E. COROPCEANU, A. CILOCI, A. ŞTEFÎRŢĂ, I. BULHAC, PUBLISHING HOUSE ACADEMICA GREIFSWALD, 2020

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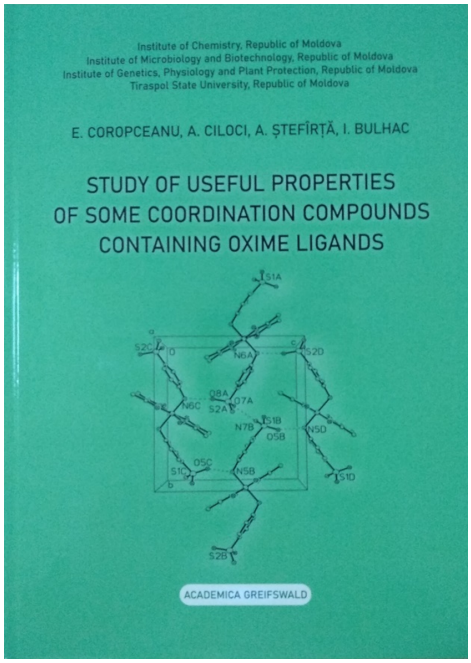
One of the important goals of contemporary chemical synthesis is to obtain substances with useful properties for various economic fields. Coordination compounds present complex substances, which include metal ions, as well as the organic part, very diverse, possessing different functional groups with electron-donating atoms, which confer a wide diversity of composition, structure, and properties. In most metal complexes, the atoms generating the complex are the biometals – the action of which is linked to specific proteins and enzymes. Metal ions play an important role in enzyme catalysis processes. Ligands in the composition of coordination compounds can present molecules with biological properties, etc.

The monograph STUDY OF USEFUL PROPERTIES OF SOME COORDINATION COMPOUNDS CONTAINING OXIMIC LIGANDS is a synthesis of the works of the Coordination Chemistry, Enzymology and Plant nutrition and water regime laboratories during the last two decades. The monograph consists of four chapters, accompanied by bibliographic sources. In chapter I. AN OVERVIEW OF THE PERSPECTIVES OF USING COORDINATION COMPOUNDS BASED ON DIOXIME LIGANDS, the specifics of the compounds of this class and the properties they exhibit for different fields are analyzed: biotechnologies, dyes, materials useful in water decomposition processes, etc. Cobalt dioximates are compared to vitamin B12, a fact that allows the initiation of syntheses for obtaining artificial molecules based on natural models with an impact on biological systems. In addition, a series of vitally important compounds are metal complexes: hemoglobin (central atom – Fe^{2+}), chlorophyll (central atom – Mg^{2+}), etc. The high activity of metal complexes can be explained by the energetic and conformational state of the molecular tension, conditioned by the spatial geometry, the specific system of bond lengths, the coordination number, etc.

In chapter II. THE INFLUENCE OF DIOXIME LIGANDS BASED COMPLEXES ON THE BIOLOGICAL ACTIVITY OF SOME ENZYME-PRODUCING FUNGI STRAINS, the influence of coordination compounds on different species of industrially important fungi is analyzed. The high technological properties of fungi that ensure their priority over other microorganisms are: short development cycle (2-10 days), adaptive metabolism, unusual intensity of cell synthesis, assimilation of a wide range of different substrates presented in the most frequent cases of by-products and waste of different productions and agriculture, the high yield and purity of the final product. The ability of micromycetes to secrete enzymes in the culture medium gives them additional biotechnological importance. The following strains were selected for research: *Aspergillus niger* 33 (V.Tiegh, Rape, Fenell), CNMN FD 06 and *Aspergillus niger* 33-19 (V.Tiegh, Rape, Fenell) CNMN FD 02 – amylase-producing *Rhizopus arrhizus* (File 67) and *Penicillium viride* (Fresenius Beitz) CNMN FD 04 – producing pectinases, *Aspergillus niger* 412 CNMN FD 01 and *Rhizopus arrhizus* Fișer CNMN FD 03 – producing lipases, *Trichoderma koningii* Oudemans CNMN FD 15 and *Fusarium gibbosum* CNMN FD 12 – producing proteases, *Aspergillus niger* (V.Tiegh) CNMN FD 10, *Aspergillus terreus*, *Aspergillus flavus* (Link), Reaper, Fenell BKM F3292D and *Penicillium expansum* (Link) Thom CNMN FD 05 – cellulase and xylanase complex producers.



In Chapter III. THE EFFECT OF SOME COORDINATION COMPOUNDS ON PLANT PHYSIOLOGICAL PROCESSES UNDER THE IMPACT OF ECOLOGICAL STRESS it is analyzed the influence of some coordination compounds and some compositions on some higher crop plants: *Zea mays* L., hybrids M 291 and P 459; *Phaseolus vulgaris*, L., Porumbita variety; *Glycine max*, Merr., (L.), Bucuria variety; *Cucumis sativus* L., Concurent and Mirabella varieties and Rodnicioc F1 and Icar F1 hybrids; *Lycopersicon esculentum* L., cv. Leader; *Arachis hypogeae* (L.), variety Fazenda 2., beetroot plants (*Béta vulgáris* L. var. conditiva Alef.); sugar beet (Béta vulgáris L., Baracuda soil), garlic (*Allium sativum*, L., Izumrud variety) and al.

The results of the study demonstrate that the treatment of the seeds for sowing and the leaf apparatus during the vegetation, with aqueous solutions of Difecoden, Difemanden, Coditiaz, Conimid, Cobamid conditions the optimization of the functional state, growth and development of corn, soybean, sugar beet, cucumber, tomato plants, red beets, peanuts, both in favorable moisture conditions and in a moderate water deficit. The coordination compounds Difecoden, Conimid and Fludisec possess antioxidant properties that are manifested in increasing the antioxidant protection capacity of the organs of the treated plants as a consequence of the intensification of antioxidant enzyme activity in them with a positive impact on plant productivity.

In chapter IV. PERSPECTIVES OF USING COORDINATION COMPOUNDS BASED ON DIOXIME LIGANDS IN INDUSTRIAL PROCESSES there are presented potential fields of use of compounds of this class: the textile and chemical industry, in the urban household to protect and extend the term of exploitation of closed networks, where it is used as water agent, in the electronics industry and others.

The monograph was awarded at the EuroInvent International Invention Fair, Iași, Romania, with the gold medal. The monograph is valuable and useful for familiarizing students of the first cycle with modern achievements in the field of coordination chemistry, serving as course support for the Basics of Coordination Chemistry subjects, for the second cycle – for the course Oximates of Transition Metals, as well as for doctoral students in the field of coordination chemistry and biotechnologies.

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(Boris Nedbaliuc) Ion Creangă State Pedagogical University, 1 Ion Creangă St., Chişinău, Republic of Moldova