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# The ontogenetic development of *Rana dalmatina* Bonaparte, 1840 species (Amphibia: Ranidae) in the context of climate change

TUDOR COZARI  AND ELENA GHERASIM 

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**Abstract.** Globally, amphibians represent one of the main objects of study in the context of climate change, because the temperature variations recorded and in the Republic of Moldova also represent a major threat to their development and sustainability.

For this purpose, a complex ecological study was carried out regarding the embryonic and larval development strategies of the *Rana dalmatina* Bonaparte, 1840, species and the main particularities related to its population structure and dynamics were described. This research allowed us to evaluate the degree of ecological plasticity of natural populations of *Rana dalmatina* in relation to the plurality of environmental factors, which allows us to develop suitable and safe measures for monitoring and conservation of the species.

**Keywords:** *Rana dalmatina*, ecology, climate change, Moldova.

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## Dezvoltarea ontogenetică a speciei *Rana dalmatina* Bonaparte, 1840 (Amphibia: Ranidae) în contextul schimbărilor climatice

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**Rezumat.** Pe plan mondial, amfibienii reprezintă unul din principalele obiecte de studiu în contextul schimbărilor climatice, deoarece, variațiile temperaturilor înregistrate la ora actuală și în Republica Moldova reprezintă o amenințare majoră asupra dezvoltării și sustenabilității acestora.

În acest scop, a fost efectuat un studiu ecologic complex referitor la strategiile de dezvoltare embrionară și larvară a speciei *Rana dalmatina* Bonaparte, 1840 și s-au descris principalele particularități referitoare la structura și dinamica populațională a acesteia. Aceste cercetări ne-au permis de a evalua gradul de plasticitate ecologică a populațiilor naturale de *Rana dalmatina* în raport cu influența complexă a factorilor de mediu, ce ne-a permis de a elabora unele măsuri efective de monitorizare și conservare a speciei.

**Cuvinte-cheie:** *Rana dalmatina*, ecologie, schimbări climatice, Moldova.

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### 1. INTRODUCTION

In recent years, amphibians have emerged as a reliable model of biological indicators under the continuous pressure of environmental climate change, their effects on

ecosystems, and the degree of evolutionary adaptability resulting from specific ecological relationships.

The sustainability of the species is determined by the population's capacity to produce new generations capable of surviving under conditions of instability and high fluctuations in non-periodic ecological factors. However, this particularity, in turn, distinguishes itself throughout specio-specific evolution, thus representing one of the distinctive criteria of the species.

Reproductive behavior, morpho-physiological adaptive characteristics during the growth and development period in fluctuating environmental conditions, constitute one of the indispensable and extremely important components of the ontogeny of all amphibians for their perpetuation, including the species *Rana dalmatina* Bonaparte, 1840. The specificity of reproduction and development in amphibians is determined by some characteristic adaptations to the environment and their living conditions [4, 6, 7].

## 2. MATERIALS AND METHODS

The study area encompasses both natural and anthropized aquatic ecosystems specific to the *Rana dalmatina* species within the Codrii Centrali region. Species identification, including both adult forms and embryonic and larval stages, was conducted using classical deductive methods, focusing on morphometric parameters and body coloration [1, 2, 3].

To obtain scientific results regarding the embryonic and larval developmental characteristics of the *Rana dalmatina* species, its phenology was evaluated in relation to environmental fluctuations across various types of aquatic ecosystems, both natural and anthropized. Additionally, the population structure of the species (age structure, size structure, spatial structure) and 142 egg masses were monitored to assess the numerical abundance of eggs, embryonic and larval developmental stages, as well as to obtain a detailed and comprehensive characterization of these aspects.

Given that the investigations were focused on studying the entire annual life cycle with reference to deciphering the embryonic and larval developmental characteristics (which occur exclusively in aquatic environments), we specifically examined all aquatic basins within the study area. This enabled us to obtain valuable data regarding the specific developmental strategies (which constituted the main objective of our research) of the tailed amphibian species *Rana dalmatina* Bonaparte, 1840.

Climate change represents a global phenomenon that endangers natural, social, and economic systems due to their sensitivity and vulnerability to climatic factors. In the current pedoclimatic conditions of the Republic of Moldova, the animal world is influenced



by the degradation of plant associations, food and water deficits, fluctuations in temperature regimes and breeding grounds caused by climate change, as well as anthropogenic impacts. The increased vulnerability of the animal world in the Republic of Moldova is the result of the low functionality of natural ecosystems. Most natural ecosystems are fragmented, degraded, and polluted. Recently, there has been an intensification of water eutrophication processes in river basins, which has a direct negative impact on the development of amphibian embryonic and larval stages, their growth, reproduction, as well as the ontogeny of other groups of invertebrate and vertebrate animals. Deforestation along riverbanks directly leads to increased water evaporation processes and reduces the ecological capacity of aquatic basins, ponds, or streams to maintain a certain degree of ecological balance - favorable for amphibian development, as well as for maintaining rich aquatic fauna diversity.

To assess the state of amphibian adaptation as a biological resource to climate change, a study was conducted on the relationship between the environment with all its characteristics and the growth and development process of the species *Rana dalmatina* Bonaparte, 1840 (Agile frog), encompassing all stages of ontogenetic development in various natural and anthropized, aquatic and terrestrial ecosystems of the Codrii Centrali region in the Republic of Moldova.

Agile frog is a woodland species, inhabiting marshy areas or habitats with increased humidity, forming isolated and sparse populations in the Central and Northern regions of the country.

As an early breeding species, it becomes active when the environmental temperature reaches +5.8°C, migrating from hibernation sites to summer habitats once specific favorable climatic conditions of temperature and humidity are established. Together, these conditions play a crucial role in the reproductive process: spatial distribution within breeding basins, mate attraction and pair formation, egg deposition, and fertilization.

Dependent on the composition of suitable substrates for egg attachment, sunlit areas, zones occupied by dense aquatic-air vegetation, shrubbery, and arboreal vegetation, they exhibit distinct spatial distributions within breeding aquatic basins. Correspondingly, according to our research, freshly deposited egg masses are found along the bottoms of breeding aquatic basins, at depths ranging between 15 and 45 cm. After hydration, the majority of egg masses (65%) rise to the water's surface. Some of these masses are within the water column (27%), while only a relatively small number (8%) remain free on the basin floor.

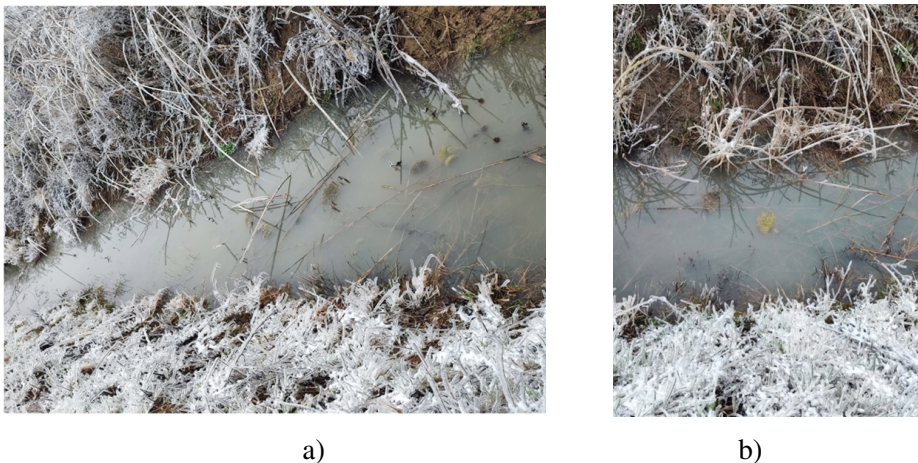
Following deposition, the egg masses resemble spheres, with diameters ranging between 6-12 cm, depending on the age of the breeding pair. After hydration, most egg

## THE ONTOGENETIC DEVELOPMENT OF *RANA DALMATINA* BONAPARTE IN THE CONTEXT OF CLIMATE CHANGE

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masses appear at the water's surface, with their volume increasing by approximately 3-5 times. While at the water's surface, these masses are subject to direct temperature influence, which accelerates their embryonic development.

In recent years, temporary fluctuations in temperature regime have been increasingly observed in the Central region of the country, particularly in the Codrii Centrali, with values falling below the permissible limit for the development of *Rana dalmatina* egg masses, where the surface layer of water or vegetation partially freezes (Figure 1). Nevertheless, due to the transient nature of spring frosts as limiting factors, the egg masses withstand these unfavorable temperature conditions, and once optimal ecological conditions are established, they resume their embryonic development.

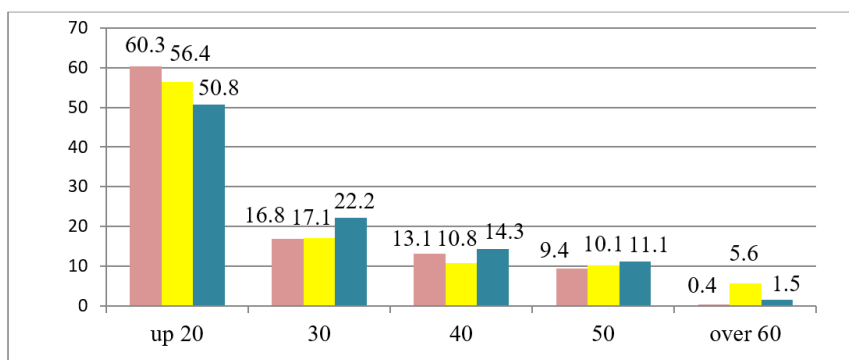


**Figure 1.** The appearance of the temporary aquatic pools for the reproduction of the *Rana dalmatina* species and the phenomenon of short-term freezing in the conditions of the ecosystems of the Forest Codrii.

The spatial distribution pattern of egg masses depends on several natural ecological factors, such as lake size and depth, the presence and distribution of submerged vegetation, solar radiation, water temperature, and the degree of protection of oviposition sites from wind action, among others. These specified ecological factors, through their complex interactions, influence the spatial distribution of egg masses, with their abundance varying from one aquatic basin to another. Following a comprehensive assessment, it was determined that in the ecosystems of the “Codrii” Reserve, the most favorable lakes for species reproduction are lakes No. 4, 5, and 6, where the egg mass density at oviposition stations ranges from 0.7 to 1.4 egg masses/m<sup>2</sup>.

According to scientific data from certain authors (Sherbac, Sherbani), in colder zones (mountainous areas) within the species' range, egg masses are deposited in clusters, which, upon full hydration, form true "islands" on the water surface. This grouping strategy of egg masses represents an efficient adaptation to protect the eggs from negative diurnal temperature fluctuations, as it has been established that the temperature within these egg mass clusters is 2-4 °C higher than that of the water in the breeding basins.

As a result of evaluating the placement of egg masses based on water depth in the breeding stations, it was determined that the maximum depth at which egg masses can be found is 0.7 m, with the majority of them (up to 73%) being located at depths of 20-30 cm (Figure 2).



**The depth of the ponds**

**Figure 2.** Distribution of ponds of the *Rana dalmatina* depending on the water pool depth.

Among the 142 egg masses of *Rana dalmatina* examined, the number of eggs ranged from 404 to 1560, with the average fecundity of females in the populations from the Codrii Centrali being  $784 \pm 89$ .

Given that the population structure of *Rana dalmatina* varies from one aquatic basin to another, as well as the size and age structure of females depositing egg masses in the same breeding aquatic basin, it was observed that the fecundity of females is closely correlated with individual age. Similarly, the numerical quota of egg masses based on the number of eggs contained in them also varies [2].

Therefore, based on the obtained data, it can be noted that based on fecundity, *Rana dalmatina* females are categorized as follows:

- (1) females with low fecundity (300-500 eggs) – 29.2%;
- (2) females with medium fecundity (501-700) – 37.5%;

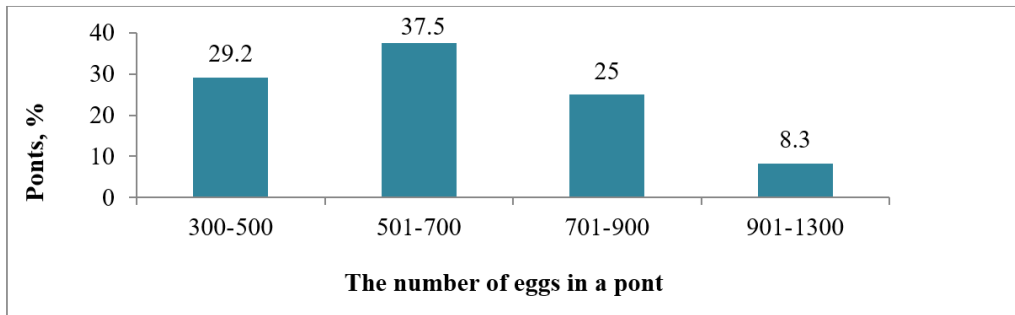
THE ONTOGENETIC DEVELOPMENT OF *RANA DALMATINA* BONAPARTE IN  
THE CONTEXT OF CLIMATE CHANGE

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(3) females with relatively high fecundity (701-900) – 25.0%;

(4) females with high fecundity (901-1300) – 8.3% (Figure 3).

Subsequently, with the establishment of favorable environmental conditions, when the average water temperature in the aquatic basins ranges between 8.1-9.7 °C, the embryonic development of the *Rana dalmatina* species occurs, but as temperature values increase, larval development also takes place.

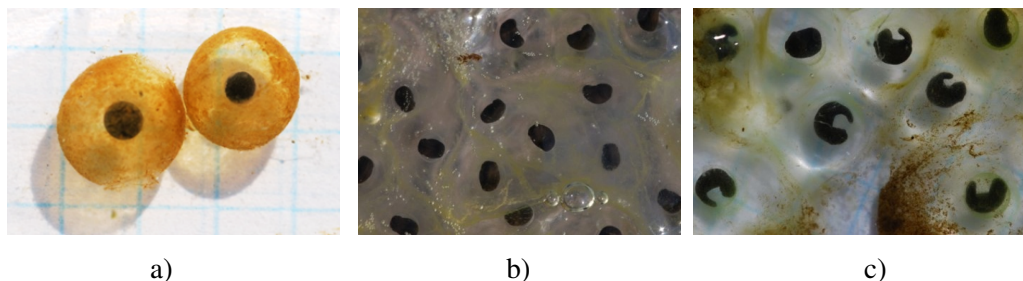


**Figure 3.** Numerical share (%) of *Rana dalmatina* ponds depending on the number of eggs contained in them (Forest Codrii lake no. 7).

The embryonic development of the *Rana dalmatina* species progresses through the following 3 stages:

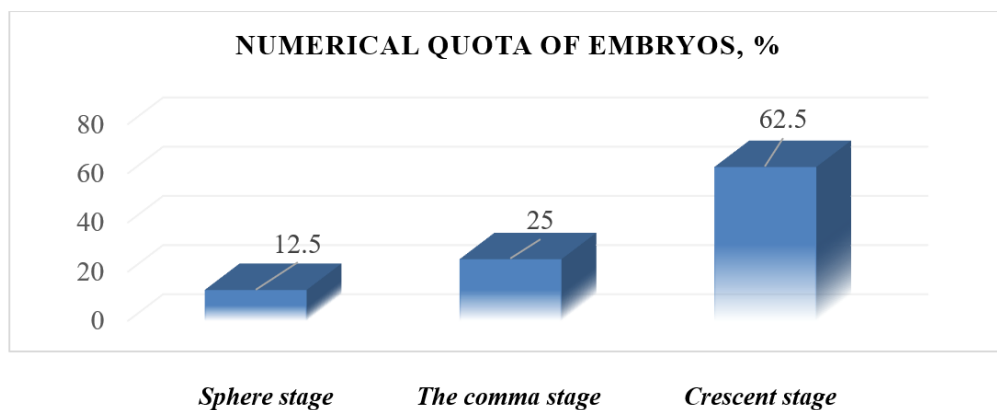
- (1) **Initial embryonic stage.** At this stage, the eggs are freshly deposited, aged 5-7 hours, and fully hydrated. Morphologically, they have a spherical shape, both the ova themselves and their gelatinous proteinaceous envelope. The diameter of the eggs is 8.3-8.5 mm, including the ova - 2.4 mm, and the thickness of the proteinaceous envelope is 3.0 mm. The eggs are black in color, and their proteinaceous envelope initially appears colorless. However, due to biocenotic relationships within the respective aquatic ecosystem, the proteinaceous envelope of the eggs later becomes covered with microscopic algae, imparting a brownish-green color to them (Figure 4, a).
- (2) **Intermediate stage of embryonic development (the “crescent” stage).** Embryos within the eggs are 7-9 days old. As a result of consecutive divisions, the ova elongate slightly and curve, acquiring the appearance of a “crescent.” Embryos at this stage have two distinct regions: the head - with small primordia of the gills, and the trunk - with the yolk sac (Figure 4, b).
- (3) **3. Final stage of embryonic development (the “pre-hatching” stage).** Embryos at this stage are greatly elongated, curving into a ring shape, so that their tail

tips almost reach their heads. The embryos already exhibit the three distinct characteristic body regions - head, trunk, tail, and are prepared for hatching, which is why this stage is referred to as “pre-hatching.” At this ontogenetic stage, the embryos already perform their first movements within the egg (Figure 4, c).



**Figure 4.** The embryonic development stages of the *Rana dalmatina* species in the ecosystems of the Forest Codrii: a – the initial embryonic stage, b – the “crescent” stage, c – the pre-hatching stage.

Depending on the specific microclimatic conditions in the aquatic basins where the egg masses of the *Rana dalmatina* species were deposited, the frequency of embryonic developmental stages was evaluated, and this is depicted in Figure 5.



**Figure 5.** The frequency of embryonic stages of development of the *Rana dalmatina* species in a populations of Codrii Forest.

After the hatching of embryos in the aquatic environment, the larval development process of the species begins, which, under natural conditions, progresses through the following developmental stages:

- (1) **Initial larval stage:** Larvae are one day old, with a length ranging from 8.4 to 10 mm ( $M \pm m = 9.2 \pm 0.3$ ,  $n = 12$ ). The larval body is distinctly differentiated into head, trunk, and tail, with mean dimensions of  $1.1 \pm 0.1$  ( $n = 12$ ),  $3 \pm 0.1$  ( $n = 12$ ), and  $5.1 \pm 0.3$  ( $n = 12$ ), respectively (Figure 5, a). The percentage ratio of these three body regions is 12%:32.6%:55.4%, indicating that the tail fin is approximately 1.2 times larger than the combined length of the head and trunk. The larvae lack a mouth opening at this stage and are attached to the proteinaceous envelope of the eggs using the prebuccal sucker for nourishment from the yolk sac. Larvae are black in color, while the tail fins appear brown.

The two transparent lobes of the caudal fin are not equal in size: the lower lobe begins near the anal opening, while the upper lobe begins at the posterior end of the head, and is therefore much longer than the lower lobe. This morpho-functional peculiarity gives the larvae a high degree of stability during swimming. The larvae at this stage of development do not yet have a mouth opening, and due to this fact, in the first hours of their existence, the larvae are fixed with the help of the preoral suction cup to the protein coating of the eggs. They feed on the nutrients of the yolk sac. The color of the larvae is black, and that of the caudal lobes – brown.

- (2) **Stage of mouth opening and initiation of active life.** The larvae are 2-3 days old post-hatching. Their body length ranges from 11.4 to 12.4 mm ( $M \pm m = 12 \pm 0.3$ ,  $n=12$ ), and the dimensional ratio of “head + trunk : tail” is 29.3%:70.7% or 1:2.4. According to the obtained data, it can be concluded that the length of the tail, crucial for swimming, has significantly increased compared to its length in the initial developmental stage when it was still inactive (Figure 5, b).
- (3) **Stage of hind limb appearance:** In this developmental stage, larvae reach their 40th day of existence. Throughout their larval development, their appearance undergoes considerable changes. Their bodies become robust, with large heads and trunks, while the tail fin becomes tall and the caudal trunk very strong, ensuring rapid and maneuverable swimming. Posterior limbs emerge at the anal opening line between the trunk and tail. These limbs are incompletely developed, short, with a length of up to 4 mm, and undifferentiated into thigh, shank, and foot, but with the initial formation of the first three toes. The hind limbs lie flat, oriented along the tail.

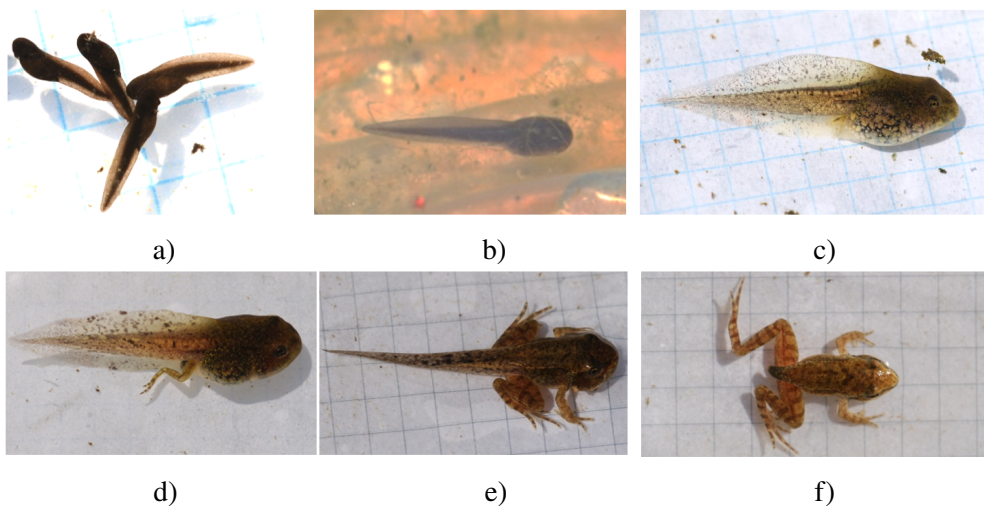
The larvae’s body length ranges from 40.8 to 41.9 mm ( $M \pm m = 41.6 \pm 0.4$ ), with the ratio of the three body regions (**head-trunk-tail**) being 12.6%:24.7%:62.1% (or 1:2:5). In comparison, it is noted that in the initial larval developmental stage,

this dimensional ratio was 1:2.7:4.6, indicating an increase in the trunk and tail proportions at the current developmental stage. The color of larvae at this ontogenetic stage becomes gray-silver, and numerous amoeboid-shaped melanin spots appear on the tail fin (Figure 5, c).

- (4) **Stage of complete hind limb development:** Larvae at this ontogenetic stage are 62 days old. The hind limbSs have reached full development, possessing all three characteristic regions – **thigh, shank, and foot**. The shape and orientation of the larvae’s limbs are identical to those of mature individuals, differing only in size. The hind limbs are already used for both locomotion (larvae pushing against submerged vegetation, the bottom of the aquatic basin, or other objects on the bottom) and for support on substrates during stationary periods. The larvae’s dimensions are 43 mm, with the ratio of the three body regions (head - trunk - tail) being 1:1.4:3.7. On the lateral sides of the larvae’s trunk, at the posterior end of the gill chamber, a characteristic concavity appears. In this location, the wall of each gill chamber will subsequently rupture, allowing the release of the fully developed forelimbs, which have completed their development (Figure 5, d).
- (5) **Stage of forelimb appearance:** Larvae of the species enter this stage of development on the 63rd day of existence. The length of larvae at this stage coincides with the length of larvae from the previous stage of development ( $M \pm m = 43 \pm 0.2$ ,  $n = 13$ ). However, in addition to the fully developed hind limbs (thigh length = 5 mm, shank length = 6.4 mm, foot length = 9.2 mm), fully developed forelimbs are also present (arm length = 3.1 mm, forearm length = 4 mm, hand length = 2.1 mm). Larvae at this stage of development closely resemble future juveniles: they represent a transitional stage from a true larva (which has a tail) to a true juvenile (which has both pairs of limbs) because, at the same time, they have both a tail and limbs. The dimensional ratio between the three body regions at the stage of forelimb appearance – **head - trunk - tail**, is 5 mm:10 mm:28 mm or 1:2:5.6. At this ontogenetic stage, individuals already exhibit morphological features specific to adult forms of the species: their color is brownish, the hind limbs are long and graceful with dark oblique stripes on the thigh and shank, on the dorsal side of the trunk, they exhibit the two characteristic dermal folds extending from behind the head to the hind limbs, and on the back, at the level of the forelimbs, there is a characteristic pattern resembling the letter “V” (Figure 5, e).
- (6) **The stage of metamorphosis and emergence of juveniles onto land** (Figure 5, f). At this stage, the larvae are 63-65 days old (between June 9-11) and are characterized by radical morpho-physiological transformations associated with

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the reorganization of certain larval organs and organ systems into adult ones. Externally, only the reduction of the caudal fin is noted, replaced by the presence of a “caudal bud” measuring 1.5-2.5 mm. Additionally, it is observed that the juveniles already emerge to the water surface and swim freely on its mirror, or they stay on emergent vegetation, indicating their transition from branchial to cutaneous and pulmonary respiration. Gradually, the juveniles concentrate in the shoreline area, where they position themselves at the “water-land” demarcation line or stay on aquatic-aerial vegetation in well-insolated aquatic basin sectors. Juveniles exhibit species-specific coloration, with dimensions ranging from 13.2 to 16.1 mm ( $M \pm m 15.1^* \pm 0.2$ ,  $N = 17$ ), depending on the functional state of the given ecosystem.

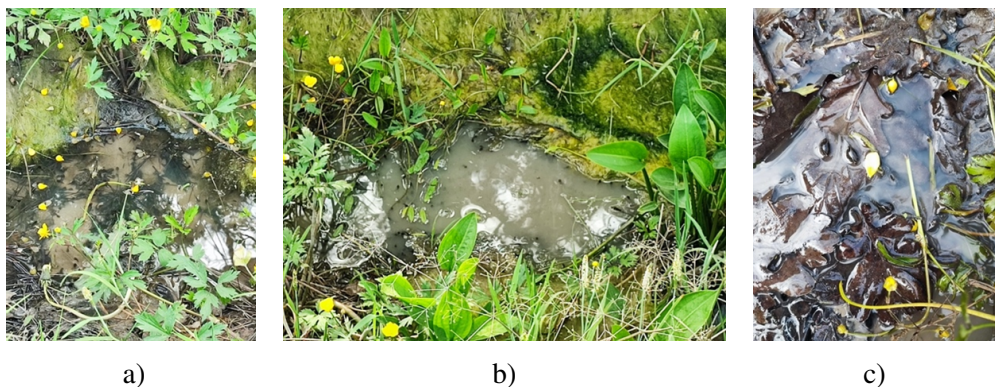


**Figure 6.** The larval development of the *Rana dalmatina* species in the Forest Codrii area.

Due to the species *Rana dalmatina*'s secure adaptation to environmental conditions, presently within our country, however, its population size is not reduced solely because of temperatures falling below the permissible threshold during oviposition. Additionally, abrupt increases in temperature during larval development directly contribute to the evaporation of water from small habitats crucial for the reproduction and development of the species, or even complete desiccation (Figure 7).

Therefore, the embryonic and larval development of the species *Rana dalmatina* in the conditions of the Republic of Moldova is determined by the direct and continuous influence of inappropriate climatic factors, which represents an increasingly significant





**Figure 7.** Temporary breeding pools, the phenomenon of heating and evaporation of water.

risk factor for the population structure of the species and, overall, a threat to amphibian diversity.

### 3. CONCLUSIONS

The swiftness of the direct influence of climatic factors on biodiversity varies greatly in different regions, with amphibians, in particular, demonstrating a high degree of adaptability. Moreover, the impact of these factors is challenging to assess by scholars, although science in recent times is characterized by the most advanced research methodologies and equipment.

The fire-bellied toad exhibits a certain degree of morpho-physiological and eco-ethological specialization, ensuring its existence in various environmental conditions with the most unfavorable fluctuations in temperature and water regime during embryonic and larval development.

In the context of the aforementioned, conducting detailed and comprehensive research on the embryonic and larval development characteristics in various aquatic ecosystems, both natural and anthropogenic, of the species *Rana dalmatina* is crucial for the sustainable management and protection of populations of this amphibian species in relation to the succession of natural ecological factors.

### Acknowledgements

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## The biochemical composition of the biomass of the oat cultivar 'Sorin' grown under the conditions of the Republic of Moldova and its possible uses

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**Abstract.** We investigated the quality indices of the biomass from common oat *Avena sativa*, romanian cultivar 'Sorin' which was grown in the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chisinau, Republic of Moldova. The results revealed that the dry matter of whole oat plants contained 9.5% CP with forage value 598 g/kg DDM, RFV= 89, 11.84 MJ/kg DE, 9.72 MJ/kg ME and 5.37 MJ/kg NEL; prepared oat hay contained 10.5% CP, 574 g/kg DDM, 11.40 MJ/kg DE, 9.36 MJ/kg ME and 5.39 MJ/kg NEL. The oat haylage is characterized by pH = 3.77, 38.1 g/kg lactic acid, 5.9 g/kg acetic acid, 10.2% CP, 567-619 g/kg DDM, 11.28 MJ/kg DE, 9.26 MJ/kg ME and 5.29 MJ/kg NEL. The biochemical methane potential of the studied substrates from *Avena sativa* cv. 'Sorin' reaches 329-355 l/kg VS.

**Keywords:** *Avena sativa*, biochemical composition, biomethane potential, cultivar 'Sorin', fodder value, green mass, hay, haylage.

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## Compoziția biochimică a biomasei de ovăz a soiului 'Sorin' cultivat în condițiile Republicii Moldova și posibilități de valorificare

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**Rezumat.** Au fost investigați indicii de calitate ai biomasei de ovăz *Avena sativa*, soiul românesc „Sorin”, cultivat pe lotul experimental al Grădinii Naționale Botanice (Institutului) „Alexandru Ciubotaru”, Chișinău, Republica Moldova. Rezultatele au evidențiat că masa uscată a plantelor întregi de ovăz conținea 9.5% PB cu o valoare furajeră de 598-603 g/kg MUD, VFR= 89, 11.84 MJ/kg ED, 9.72 MJ/kg EM și 5.37 MJ/kg ENI; fânul preparat -10.5% PB, 574 g/kg MUD, 11.40 MJ/kg ED, 9.36 MJ/kg EM și 5.39 MJ/kg ENI. Furajul însilozat (semifân) este caracterizat de pH = 3.77, 38.1 g/kg acid lactic, 5.9 g/kg acid acetic, 10.2% PB, 567-619 g/kg MUD, 11.28 MJ/kg ED, 9.26 MJ/kg EM și 5.29 MJ/kg ENI. Potențialul biochimic de producție a biometanului al substraturilor studiate de *Avena sativa* ajunge la 329-355 l/kg VS.

**Cuvinte-cheie:** *Avena sativa*, compoziția biochimică, fân, fânaj, masa verde, potențial de biometan, soiul 'Sorin', valoare furajeră.

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# THE BIOCHEMICAL COMPOSITION OF THE BIOMASS OF THE OAT CULTIVAR 'SORIN' AND ITS POSSIBLE USES

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## 1. INTRODUCTION

Because of the thermal regime with high temperatures and the lack of precipitation, droughts during the growing season occurred 2-3 times more often over the last 40 years, as compared to the entire period of observations made by the national meteorological services [3]. The cumulative action of high temperatures and the low amount of precipitation and its uneven distribution, soil erosion and salinization have a negative impact on the harvest and quality of traditional agricultural crops, and therefore, on the feed supply for livestock. The growth prospects of the agricultural-food market depend on solving the problem of providing a balanced diet for farm animals in accordance with their physiological and production requirements. In order to respond to these challenges, it is necessary to capitalize on the productive potential of traditional crops by identifying valuable forms, creating and implementing new cultivars and technologies, for a more efficient use of solar energy, soil and water resources, providing food for people and animals, and on the other hand, using these plants as a source of raw material for various industries (textiles, pharmaceuticals, cosmetics etc.) and last but not least, biomass for the production of renewable energy.

The genus *Avena* L. fam. Poaceae includes 29 species distributed in Europe, the Mediterranean Basin, North Africa, West Asia and East Asia, North and South America [17]. Most species and subspecies with a huge diversity of forms are found in Asia Minor, being considered the gene centre of the *Avena* genus [19]. The following species are widely cultivated: *Avena abyssinica* Hochst. (Ethiopian oat), *Avena byzantina* K.Koch (red oat), *Avena nuda* L. (hulless oat), *Avena sativa* L. (common oat), *Avena strigosa* Schreb. (black oat).

Oat *Avena sativa* was firstly cultivated approximately 3000 years ago, under the more favourable climatic conditions of Europe. It is an annual herbaceous plant with a stem (straw, culm) of 5-7 internodes, smooth, glabrous or hairy in the area of the nodes, hollow inside, 100-150 cm tall, forms a bush of 3-4 shoots and the main stem has one node more than the secondary stems. The leaves are flat, with 11-13 veins, up to 15 mm wide, glabrous or ciliate on margins, with glabrous sheaths, rarely hairy, leaf lobes absent, ligule white, limbed, medium or short, toothed at apex. The leaf blade is twisted from right to left, opposite to the other cereals. The inflorescence is a pyramidal panicle, 15-30 cm long, with ramifications arranged in 5-6 tiers on the main axis. Each ramification ends in a spikelet attached to a short peduncle. The spikelets have 2-3 flowers. The glumes are veined, 18-25 mm long, completely covering the flowers. The lower palea is elongated, convex, straight or geniculate on the dorsal side, yellowish-white or brown, obviously

veined, glabrous or rarely with short hairs only at the base, with 2 teeth without awns. There are forms with compact panicle (standard) and with loose panicle. It blooms in June; pollination is autogamous, but not excluding allogamy. Fruit is a hairy caryopsis, with whitish-yellowish, sometimes grey-brown, adherent petals. The shape of the grain is fusiform, with a groove on the ventral side. The weight of 1000 seeds is 20-35 g. At germination, oats develop three embryonic roots, form a fibrous root system, which is stronger and deeper as compared with other grassy cereals, possesses a great capacity to absorb the nutritional elements from the poorly soluble compounds of the soil. Oat forms are identified as withstanding temperatures below -10 °C at the root collar. Winter hardiness also depends on the protective snow cover and temperature fluctuations. Oat plants are quite demanding in terms of moisture, thus, they need more water as compared to other cereals, especially in the stages of straw formation and budding-flowering. It does not have high requirements towards soil and can be grown on loamy and loamy-sandy soils, chernozem, forest red-brown soil, podzols, with pH between 5.5 and 7.0, while sandy soils and compacted loamy soils are less suitable. Oats make the best use of soils with nutrients left from heavily fertilized predecessor plants. Oats should never be planted on an area after sugar beet or another oat crop [27].

In the Catalog of Plant Varieties of the Republic of Moldova [29], 3 cultivars of common oat have been registered, while in the Official Catalog of the Varieties of Crop Plants in Romania [30] - 6 varieties of oat have been registered, of which 4 varieties were created at the Agricultural Research and Development Station Lovrin.

In recent years, special attention, both globally and locally, has been paid to the use of renewable energy sources. In our region, the focus is on the production and use of biomass to obtain second-generation fuels.

The goal of our research was to evaluate the quality of the harvested biomass of the romanian cultivar 'Sorin' of common oat *Avena sativa*, as natural fodder and haylage for feeding the livestock, as well as as a substrate for the production of biomethane by anaerobic digestion, as a renewable energy resource.

## 2. MATERIALS AND METHODS

The plants of common oat *Avena sativa* cv. 'Sorin' created at the Agricultural Research and Development Station Lovrin, Romania, and cultivated in the experimental sector of the "Alexandru Ciubotaru" National Botanical Garden (Institute) Chisinau, Republic of Moldova, served as research subjects. Alfalfa (*Medicago sativa*) and maize (*Zea mays*) plants served as control samples. The experiments started in autumn, at the end of September, were made in 4 repetitions, on non-irrigated land. Common oat plants

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were harvested during the panicle formation - flowering period (June). The haylage was prepared from pre-wilted in the field plant, shredded and then packed in airtight containers. The hay was dried directly in the field. The fresh and conserved mass samples were dehydrated in an oven with forced ventilation at a temperature of 60 °C; at the end of the fixation, the biological material was finely ground in a laboratory ball mill. The assessment of the content of crude protein (CP), crude ash (CA), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS) was done by near infrared spectroscopy (NIRS) technique, using the PERTEN DA 7200 at the Research and Development Institute for Grasslands, Braşov, Romania, according to standard methods. The content of cellulose (Cel) and hemicellulose (HC), the digestible dry matter (DDM), the relative feed value (RFV), the digestible energy (DE), the metabolizable energy (ME) and the net energy for lactation (NEI) were calculated according to accepted equations.

The carbon content in the organic matter was calculated using the equation reported by Badger et al. [5]. The biochemical methane potential was calculated based on the basis the crude protein content and chemical compounds of the cell wall, acid detergent lignin (ADL) and hemicellulose (HC), according to Dandikas et al. [7].

### 3. RESULTS AND DISCUSSIONS

The results regarding the biochemical composition of the dry matter and the nutritional value of the fresh mass and hay of the oat cultivar 'Sorin' are presented in Table 1. Oat plants were found to have higher crude protein content as compared with maize, but lower as compared with alfalfa. The content of structural carbohydrates was much higher, while that of soluble carbohydrates was lower, a fact that had a negative effect on the digestibility and energy supply of natural oat fodder as compared with that of maize. Oat plants have a lower content of ash and lignin, and contain a quite high amount of soluble sugars and hemicellulose as compared with alfalfa fodder. There are no essential differences in the digestibility of the dry matter and the energy supply of the oat and alfalfa fodder. In the process of making hay, the content of structural carbohydrates and acid detergent lignin increases and the digestibility of nutrients becomes lower. Oat hay has an optimal concentration of crude protein and hemicellulose.

In the specialized literature, there are different data regarding the quality of fresh mass and hay from *Avena* species. According to the data presented by Burlacu et al. [6] oat plants contained 170 g/kg dry matter, 90.1% organic matter, 10.3% crude protein, 3.1% fat, 26.5% crude cellulose, 50.2% nitrogen free extract, 9.9% ash, 14.2% sugars, 0.8% starch, 30.0% ADF, 6.6 % lignin and 17.8 MJ/kg gross energy, while hay was made up

Indices	<i>Avena sativa</i>		<i>Medicago sativa</i> fresh mass	<i>Zea mays</i> fresh mass
	Fresh mass	Hay		
Crude protein (CP), g/kg	95	105	170	84
Crude fibre (CF), g/kg	356	381	341	248
Crude ash (CA), g/kg	65	74	90	52
Acid detergent fibre (ADF), g/kg	374	404	365	271
Neutral detergent fibre (NDF), g/kg	627	660	558	474
Acid detergent lignin (ADL), g/kg	46	50	63	48
Total soluble sugars (TSS), g/kg	167	111	63	336
Cellulose (Cel), g/kg	328	354	302	223
Hemicellulose (HC), g/kg	258	256	193	203
Digestible dry matter (DDM), %	59.8	57.4	60.5	67.8
Digestible energy (DE), MJ/kg	11.84	11.4	11.96	13.28
Metabolizable energy (ME), MJ/kg	9.72	9.36	9.82	10.9
Net energy for lactation (NEL), MJ/kg	5.73	5.39	5.83	6.91
Relative feed value (RFV)	89	81	101	133

**Table 1.** The biochemical composition and nutritional value of oat fresh mass and hay.

of – 8.0 % crude protein, 3.1% fat, 36.4% crude cellulose, 44.6% nitrogen free extract, 39.7% ADF, 3.9 % lignin, 7.9% ash and 19.3 MJ/ kg gross energy. Kamble et al. [13] reported that oat green mass had a concentration of 121 g/kg dry matter, 13.5% protein, 3.1% fat, 50.8% NDF, 1.7% ADL, 32.9% ADF, 17.9% HC and 22.5% Cel. Kocer & Albayrak [14] determined that the concentration of nutrients in *Avena sativa* plants was 10.87% protein, 59.12% NDF, 34.4% ADF, 566.7 g/kg total digestible nutrients, RFV=97.45. Heuze et al. [11] reported that the nutritional value of *Avena sativa* plants was 263 g/kg dry matter, 10.5% protein, 4.9% fat, 30.2% crude cellulose, 54.2% NDF, 31.0% ADF, 4.5% lignin, 7.1% soluble sugars, 10.1% ash, 3.8 g/kg Ca and 2.2 g/kg P, 67.0% digestible matter, 18.0 MJ/kg gross energy, 11.5 MJ/kg digestible energy and 9.3 MJ/kg metabolizable energy, while that of hay was, respectively, 9.1% protein, 2.2% fat, 34.0% crude cellulose, 61.7% NDF, 38.1% ADF, 8.3% ash, 17.7% starch, 4.7 g/kg Ca and 2.0 g/kg P, 60.1% digestible matter, 18.0 MJ/kg gross energy, 10.2 MJ/kg digestible energy and 8.3 MJ/kg metabolizable energy. Kumar et al. [15] mentioned that the biochemical composition and nutritive value of oat fresh mass was 8.80% protein, 2.74%

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fat, 53.25% NDF, 27.41% ADF, 3.73% ADL, 25.84% HC, 20.22% Cel, 11.97% ash, 8.34 MJ/kg metabolizable energy and 567.2 g/kg total digestible nutrients. Tambara et al. [25] determined that the biochemical composition of the dry matter of *Avena sativa* plants was 24.11% protein, 40.50% NDF and 18.86% ADF, while that from *Avena strigosa* plants was – 24.38% protein, 43.53% NDF and 19.83% ADF. Horst et al. [12] mention that the oat green mass harvested until flowering period contained 185.1-243.4 g/kg dry matter, 8.85-9.96% protein, 68.85-70.97% NDF, 38.69-44.89% ADF, 26.08-28.16% HC, 5.57-5.62% ash, 564.4-607.6 g/kg total digestible nutrients, RFV=74.09-84.59 and 0.664-1.029 Mcal/kg net energy for lactation. Abdelraheem et al. [1] mentioned that oat hay was characterized by 7.50-7.86 % protein, 1.80-2.12 % fat, 54.13-54.20 % NDF, 28.72-31.17% ADF, 4.60-5.50 % ash, 61.96-63.82% digestible organic matter and 17.71-17.92 MJ/kg gross energy. Bacchi et al. [4], evaluating forage quality indices of annual forage crops, determined that when it is harvested in the inflorescence emergence period, *Avena sativa* plants contain 201.4 g/kg dry matter with 10.56 % protein, and *Lolium multiflorum* plants contain 217.2 g/kg dry matter and 8.67% protein, while, when it is harvested during the ripening period of the grains, *Avena sativa* plants contain 358.4 dry matter with 6.63 % protein and *Lolium multiflorum* plants contain 322.9 g/kg dry matter with 6.41 % protein. Ma et al. [18] reported that the nutrient content of *Avena nuda* forage was 5.94% protein, 63.83% NDF, 38.28% ADF, 7.99% ash, 3.81% starch, 53.61% total digestible nutrients, 59.08% digestible dry matter, RFV=86.11 and RFQ=81.95. Patidar et al. [21] indicated that the nutritional value of oat plants was 87.68% organic matter, 10.80% protein, 2.10% fat, 10.84% starch, 53.50% NDF, 47.86% ADF, 10.80% lignin, 55.06% total digestible nutrients, 16.62 MJ /kg gross energy, 10.23 MJ/kg digestible energy and 8.44 MJ/kg metabolizable energy; while that of hay was 88.91% organic matter, 9.48% protein, 1.80% fat, 7.86% starch, 73.24% NDF, 48.24% ADF, 11.48% lignin, 55.40% total digestible nutrients, 16.64 MJ/kg gross energy, 8.81 MJ/kg digestible energy and 7.31 MJ/kg metabolizable energy. Rady et al. [22] mentioned that the nutritional value of oat plants was 901.9 g/kg organic matter, 8.30% protein, 2.58% fat, 169.16% NDF, 64.67% ADF, 4.48% HC, 55.39% Cel, 11.29% lignin, 533.1 g/kg total digestible nutrients. Shekara et al. [24] reported that quality parameters of fodder oats genotypes were 170-212 g/kg dry matter, 9.0-11.1% crude protein and 25.99-27.36% crude cellulose.

Fermented forage is the key element for productive and efficient livestock farms, providing an even amount of high-quality nutrients, especially during the autumn – mid-spring period, but also, for some farms, throughout the year. When opening the glass containers with oat haylage, there was no gas or juice leakage, the preserved mass retained its consistency in comparison with the initial green mass, without mould or fungi. The



oat haylage had dark green-olive colour, with a pleasant, specific smell of pickled apples, and the maize silage was homogeneous yellow, with a pleasant smell like pickled fruits. Following the analyses carried out (Table 2), it was determined that the fermentation profile of the fermented fodders was pH=3.77-4.10, 38.1-38.8 g/kg lactic acid, 5.9-10.3 g/kg acetic acid, which corresponded to the standard in force SM 108, quality 1. In oat haylage, the concentration of acetic acid was lower than in maize silage, and there was no butyric acid. The dry matter of the researched pickled fodder contained 8.0-10.2% protein, 5.9-7.8% ash, 24.5-39.3% crude fiber, 25.8-41.3% ADF, 46.9-69.9% NDF, 3.7-4.0 % ADL, 2.6-32.6% soluble sugars, 56.7-68.8% g/kg digestible dry matter, RFV=76-136, 9.26-11.04 MJ/kg metabolizable energy and 5.29-7.06 MJ/kg net energy for lactation. Oat haylage had a higher content of crude protein and structural carbohydrates as compared with maize silage. The dry matter digestibility and energy supply of oat haylage was than that of maize silage.

The quality of fermented forage from oat plant has been presented in several sources. Burlacu et al. [6] reported that the silage from oat plants contained 185 g/kg dry matter, 91.2% organic matter, 11.1% protein, 3.7% fat, 31.5% crude cellulose, 44.9% nitrogen-free extract, 5.7% sugars, 4.1% starch, 33.1% ADF, 4.7% lignin and 18.3 MJ/kg gross energy. Geren et al. [9] mentioned that *Avena sativa* silage was characterized by pH 3.91-4.64, 9.2-12.2% protein, 51.3-54.9% NDF and 34.2-39.7% ADF. Herrmann et al. [10] stated that *Avena sativa* silage contained 379 g/kg dry matter, 92.4% organic matter, pH 4.3, 5.6% lactic acid, 1.1% acetic acid, 9.2% protein, 3.1% fat, 51.5% NDF, 34.3% ADF and 5.5 % ADL, while that – of *Zea mays* contained – 302 g/kg dry matter, 95.8% organic matter, pH 3.7, 5.1% lactic acid, 1.6% acetic acid, 7.8% protein, 2.6% fat, 41.2% NDF, 24.0% ADF and 2.9% ADL. Heuze et al. [11] reported that the nutritional value of *Avena sativa* silage was 305 g/kg dry matter, 9.5% protein, 5.8% fat, 37.4% crude cellulose, 53.4% NDF, 35.9% ADF, 4.1% lignin, 9.7% starch, 10.1% ash, 4.6 g/kg Ca and 3.1 g/kg P, 17.6 MJ/kg gross energy, 10.7 MJ/kg digestible energy and 8.7 MJ/kg metabolizable energy. Leão et al. [16] indicated that *Avena sativa* silage contained 54-78 g/kg protein, 50-54 g/kg ash, 660-711 g/kg NDF, 417-459 g/kg ADF, 243-253 g/kg HC, 485-509 g/kg total digestible nutrients, while *Avena strigosa* silage contained 43-60 g/kg protein, 55-58 g/kg ash, 709-799 g/kg NDF, 443-520 g/kg ADF, 265-279 g/kg HC, 451-494 g/kg total digestible nutrients. Teixeira & Fontaneli [26] determined that the nutrient concentration in oat silage was 6.57-10.72% protein, 37.23-39.02% ADF, 59.45-62.46% NDF and 58.53-60.66 DMD. Horst et al. [12] mentioned that fermented oat feed contained 538.1-613.4 g/kg dry matter, 8.29-10.1% protein, 69.62-72.03% NDF, 42.05-47.22% ADF, 24.81-27.57% hemicellulose, 4.91-5.41% ash, 54.79- 58.41% total

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Indices	Avena sativa haylage	Zea mays silage
pH	4.10	3.77
Organic acids, g/kg DM	44.7	48.6
Free acetic acid, g/kg DM	2.5	5.1
Free butyric acid, g/kg DM	0	0
Free lactic acid, g/kg DM	10.7	17
Fixed acetic acid, g/kg DM	3.4	5.2
Fixed butyric acid, g/kg DM	0	0.2
Fixed lactic acid, g/kg DM	28.1	21.1
Total acetic acid, g/kg DM	5.9	10.3
Total butyric acid, g/kg DM	0	0.2
Total lactic acid, g/kg DM	38.8	38.1
Lactic acid, % organic acids	86.8	78.4
Crude protein (CP), g/kg DM	102	80
Crude fiber (CF), g/kg DM	393	245
Crude ash (CA), g/kg DM	78	59
Acid detergent fiber (ADF), g/kg DM	413	258
Neutral detergent fiber (NDF), g/kg DM	699	469
Acid detergent lignin (ADL), g/kg DM	40	37
Total soluble sugars (TSS), g/kg DM	26	326
Cellulose (Cel), g/kg DM	373	221
Hemicellulose (HC), g/kg DM	286	211
Digestible dry matter (DDM), %	56.7	68.8
Digestible energy (DE), MJ/kg	11.28	13.45
Metabolizable energy (ME), MJ/kg	9.26	11.04
Net energy for lactation (NEL), MJ/kg	5.29	7.06
Relative feed value (RFV)	76	136

**Table 2.** Fermentation profile, biochemical composition and nutritional value of ensiled fodder.

digestible nutrients, RFV=70.78-78.10 and 0.527-0.832 Mcal/kg net energy for lactation. Ruckaya et al. [23] determined that the oat haylages contained 474-526 g/kg dry matter, 6.9% protein, 4.9-5.8 % ash and 9.0-9.1 MJ/kg metabolizable energy. Özyazıcı et al. [20]

reported that the nutritional value of *Avena sativa* oat silage is 170 g/kg organic matter, pH = 5.50, 6.01% protein, 64.83% NDF, 40.27% ADF and RFV = 82.55.

Energy production from biomass is considered an important component in the transformation of the current energy system in order to reduce greenhouse gas emissions and decrease the dependency on fossil energy sources. Biogas has become important as a renewable source of energy due to its decentralized approach, and it can be used to obtain heat and electrical power in special installations, as well also as fuel in internal combustion engines. The anaerobic digestion process of biomass consists in the conversion of organic matter, by anaerobic bacteria, into biogas, mixture of methane, carbon dioxide and other substances, and also the fermentation residue – digestate rich in nutrients that may be used as fertilizer in organic farming systems. The results regarding the quality indices of the substrates for anaerobic digestion and the biomethane production potential are presented in Table 3. Oat substrate is characterized by a higher content of hemicellulose and optimal content of proteins, a fact that had a positive effect on the reduction of the carbon/nitrogen ratio as compared with maize substrates. The biochemical methane potential reached 355 l/kg in the oat haylage substrate, as compared with 338 l/kg in the maize silage substrate.

Dubrovskis et al. [8] mentioned that the methane yields of oat-barley silage were 288.3-299.2 l/kg those of maize silage were 293.4-296.8 l/kg, respectively. Heiermann et al. [10] noted that the carbon/nitrogen ratio and the specific methane yield of oat silage substrate were C/N = 33 and 277 l/kg, while those of maize silage substrate were C/N = 37 and 329 l/kg organic matter. Allen et al. [2] established that oat silage substrates were characterized by a content of 43.39% C, 6.38% H, 0.55% N, C/N = 80.70 with a biogas potential of 450 l/kg and methane of 281.26 l/kg. Zhang et al. [28] reported that the biomethane potential of oat substrates ranged from 203 to 402 l/kg organic matter.

#### 4. CONCLUSIONS

Oat plants of the cultivar 'Sorin' harvested before flowering have a dry matter nutrient content of 9.5% crude protein, 35.6% crude fiber, 6.5% crude ash, 4.6% acid detergent lignin, 16.7% soluble carbohydrates, 32.8% cellulose and 25.8% hemicellulose, with a feed value of 59.8% dry matter digestibility, 11.84 MJ/kg digestible energy, 9.72 MJ/kg metabolizable energy, and 5.73 MJ/kg net energy for lactation. The natural oat fodder is richer in protein as compared with maize, and as compared with alfalfa, the fresh mass oat fodder has a lower content of lignin.

The hay from oat plants has a nutrient content in dry matter of 10.5% crude protein, 38.1% crude fiber, 7.4% crude ash, 5.0% acid detergent lignin, 11.1% soluble sugars, 35.4% cellulose and 25.6% hemicellulose with a forage value of 57.4% digestible dry

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Indices	<i>Avena sativa</i>			<i>Zea mays</i>	
	fresh mass	hay	haylage	fresh mass	silage
Crude protein (CP), g/kg	95	105	102	84	80
Crud ash (CA), g/kg	65	74	78	52	59
Nitrogen (N), g/kg DM	15.2	16.8	16.3	13.44	12.8
Carbon (C), g/kg DM	519	514.44	512.2	527	511.1
Carbon/nitrogen ratio (C/N)	34	33.5	31.38	39	39.93
Hemicellulose (HC), g/kg	258	256	286	203	211
Acid detergent lignin (ADL), g/kg	46	50	40	48	37
Biomethane potential, L/kg O.M.	329	324	355	321	338

**Table 3.** The biochemical methane production potential of the investigated substrates.

matter, 11.4 MJ/kg digestible energy, 9.36 MJ/kg metabolizable energy, and 5.39 MJ/kg net energy for lactation.

Oat haylage is characterized by pH = 3.77, 38.1 g/kg lactic acid, 5.9 g/kg acetic acid, no butyric acid, 10.2% crude protein, 567 g/kg dry matter digestibility, 9.26 MJ/kg metabolizable energy and 5.29 MJ/kg net energy for lactation.

The biochemical methane production potential in the studied substrates of *Avena sativa* reaches values of 329-355 l/kg organic matter, while in the substrates of *Zea mays* the values are of 321-338 l/kg organic matter.

In the Republic of Moldova the Romanian cultivar 'Sorin' of *Avena sativa* can be cultivated in pure culture or mixed with leguminous plants, and used as fresh mass fodder, hay and haylage for feeding farm animals, as well as a substrate at biogas stations for the production of biomethane.

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## New (*o*-phenylenediaminglyoximato)-(*o*-phenylenediaminglyoxime)zinc(II) chloride. Synthesis and crystal structure

DUMITRU URECHE , ION BULHAC , AND PAVLINA BOUROSH 

**Abstract.** The interaction of dichloroglyoxime and *o*-phenylenediamine with zinc(II) acetate dihydrate leads to the formation of a new compound of Zn(II) with *o*-phenylenediaminglyoxime (*o*-FDH<sub>2</sub>), very little studied ligand. The X-ray study, on the basis of which the crystal structure of this compound was determined, established that this oxime ligand was obtained from the condensation of organic components and coordinates to the metal atom like most vic-dioximes – bidentate-chelate through two nitrogen atoms, forming metallic rings of five atoms. The presented mononuclear complex compound is of a molecular type, the coordination polyhedron of Zn(II) being a pyramidal-tetragonal one is formed by a neutral *o*-FDH<sub>2</sub> ligand, a monodeprotonated *o*-FDH<sup>-</sup> one and a chloride anion.

**Keywords:** mononuclear complex, Zn(II), dioxime ligand, chloride anion.

## O nouă clorură de (*o*-fenilendiaminglioximato)-(*o*-fenilendiaminoximă)zinc(II). Sinteză și structură cristalină

**Rezumat.** La interacțiunea dicloroglioximei și *o*-fenilendiaminei cu acetatul de zinc(II) dihidrat a fost obținut un compus nou al Zn(II) cu *o*-fenilendiaminglioxima (*o*-FDH<sub>2</sub>), un ligand foarte puțin cercetat. Studiul cu raze X, în baza căruia a fost determinată structura cristalină a acestui compus, a stabilit că acest ligand oximic s-a obținut la condensarea componentelor organice și coordonează la atomul de metal ca și majoritatea vic-dioximelor – bidentat-chelat prin doi atomi de azot, formând cicluri metalice din cinci atomi. Compusul complex mononuclear prezentat este unul de tip molecular, poliedrul de coordinare al Zn(II), fiind piramidal-tetragonal, este format de un ligand neutru *o*-FDH<sub>2</sub>, unul monodeprotonat *o*-FDH<sup>-</sup> și de un anion clorură.

**Cuvinte-cheie:** complex mononuclear, Zn(II), ligand dioximă, anion de clorură.

### 1. INTRODUCTION

*vic*-Dioximes and their complexes represent an important class of compounds with versatile reactivity [1-3]. With reporting dimethylglyoxime as a gravimetric reagent for the quantitative determination of nickel(II) by Tschugaeff [4], vicinal dioximes with chelating



ability and diprotic nature have become an indispensable part in various research fields, including analytical chemistry, supramolecular chemistry, biochemistry and biomedical applications [5]. Currently, the Cambridge Structural Database includes more than 2000 entries on the structure of transition metal dioximates, the formation of which is determined by several factors: nature of the complexing agent, the properties of the dioxime itself, pH of the solution, etc.

*vic*-Dioximes can form coordination compounds in which the dioximes are entrained in molecular [6, 7], monodeprotonated [8-11] and bis-deprotonated [12] forms. Nickel(II), palladium(II) and platinum(II) dioximates with the planar-square coordination polyhedron [13], binuclear copper(II) dioximates with tetragonal structure [14], as well as octahedral-shaped iron(II) [15], iron(III) [16], cobalt(II) [2] and cobalt(III) [17, 18] dioximates are well-known in scholarly sources. In most of the complex compounds known, the central atom coordinates two dioxime ligands as monoanions located approximately in a same plane and being linked together by strong intramolecular O–H...O hydrogen bonds.

Recent studies report Zn(II), Mn(II) and Ni(II) complexes with polyhedron structure in which one dioxime ligand coordinates to the metal atom in a neutral form [6, 19], while in the octahedral Co(III) complexes to which already coordinate two oxime ligands, both are mostly monodeprotonated [20], although there are cases when one of them exists in a neutral, while the other in a bideprotonated form [21].

The aim of this paper was considering a Zn(II) compound with *o*-phenylenediamine glyoxime obtained by condensing of dichloroglyoxime with *o*-phenylenediamine and complexing it with zinc(II) ion, together with its crystal structure. *o*-phenylenediamine glyoxime ligand is not sufficiently studied as the Cambridge Structural Database contains only data on its structure and a single Fe(II) compound, which coordinates a macrocyclic ligand consisting of two fragments of diphenylglyoxime and one of *o*-phenylenediamine glyoxime, these being joined by BF<sub>2</sub> [22, 23].

## 2. MATERIALS AND METHODS

*Synthesis of [Zn(*o*-FDH<sub>2</sub>)(*o*-FDH)Cl]·6,5H<sub>2</sub>O.* A mix of *o*-phenylenediamine (C<sub>6</sub>H<sub>4</sub>(NH<sub>2</sub>)<sub>2</sub>) (0.022 g, 0.2 mmol), dichloroglyoxime (0.031 g, 0.2 mmol) and Zn(CH<sub>3</sub>COO)<sub>2</sub>·2H<sub>2</sub>O (0.022 g, 0.1 mmol) was dissolved in DMF – methanol (8 mL) in 1:4 ratio (vol./vol.). A yellowish sediment was formed. After adding 3 drops of concentrated HCl, the sediment disappeared, but a yellowish solution was formed. The solution was filtered and left at room temperature for crystallization. In 11 days, colourless cubic crystals were formed. The product is soluble in DMSO, insoluble in methanol, water, ethanol, DMF and diethyl ether. 0.037 g were formed. Yield – 31%.

NEW (*O*-PHENYLENEDIAMINGLYOXIMATO)-(*O*-  
PHENYLENEDIAMINGLYOXIME)ZINC(II) CHLORIDE

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Found, %: C, 32,01; H, 4,73; N, 18,67; Zn, 10,89;

For C<sub>16</sub>H<sub>28</sub>ClN<sub>8</sub>ZnO<sub>10,5</sub>

Calculated, %: C, 31,96; H, 4,69; N, 18,63; Zn, 10,88.

IR spectrum ( $\nu$ , cm<sup>-1</sup>): 3112 s, 2873 w, 2790 w, 2738 w, 1692 w, 1631 v.s., 1607 v.s., 1492 s, 1448 s, 1366 a, 1318 a, 1264 a, 1240 sh, 1157 w, 1123 w, 1007 v.s., 950 a, 930 a, 853 w, 749 s, 712 w, 655 a, 618 a, 593 a, 524 a, 503 a, 453 a. (relative intensity of the absorption bands: v.s – very strong, s – strong, a – average, w – weak, sh – shoulder).

C, H and N content in the synthesized compounds was determined using the elemental analyser Elementar Analysensysteme GmbH Vario El III.

Quantitative determination of metal in the coordination compound was performed on the Shimadzu AA-7000 atomic absorption spectrophotometer.

FTIR (ATR) spectra were recorded on Spectrum-100 Perkin-Elmer FTIR spectrometer with ATR accessory (attenuated total reflection) in the 4000-650 cm<sup>-1</sup> range and in Vaseline oil in the 4000-400 cm<sup>-1</sup> range in the Department of Physical and Inorganic Chemistry of the Institute of Chemistry, MSU.

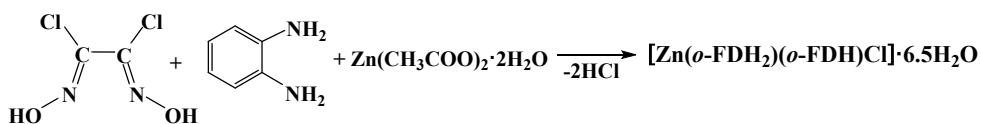
*X-ray study.* Crystal structure was determined using single-crystal X-ray diffraction on Xcalibur E diffractometer with a CCD detector and a graphite monochromator with MoK $\alpha$  radiation source with  $\lambda = 0.71073 \text{ \AA}$ . Determination of unit cell parameters and analysis of experimental data were performed using CrysAlis Oxford Diffraction Ltd [27]. The SHELXS97 and SHELXL2014 program packages were used to solve and refine the structure in the anisotropic full-matrix variant for non-hydrogen atoms and the hydrogen atoms in an isotropic environment [28, 29]. Bonded H to C atoms were located in the positions calculated using a rigid model with Uiso(H)=1.2Ueq(C) and Uiso(H)=1.5Ueq(C), while H atoms of water molecules and amino groups were found from Fourier syntheses in intermediate refinement steps, using geometric restraints. Occupancy was determined for 10 molecules of water of crystallization and ranged between 1 and 0.5. The crystallographic data obtained for the compound in the crystalline phase are shown in Table 1. The crystal data for compound was deposited with the Cambridge Crystallographic Data Centre (CIF files CCDC nr. 2345901, respectively; deposit@ccdc.cam.ac.uk, <http://www.ccdc.cam.ac.uk>).

### 3. RESULTS AND DISCUSSIONS

As a result of reaction between dichloroglyoxime, *o*-phenylenediamine and zinc(II) acetate dihydrate in a 1:2:1 molar ratio a mononuclear Zn(II) complex was obtained, in which the ligand obtained from condensation of the two organic proligands coordinates to the metal atom. The reaction proceeded according to the scheme from Figure 1.

Parameters	Compound
Formula	$C_{16}H_{28}ClN_8ZnO_{10.5}$
$M_r$	601.28
Crystal system	monoclinic
Space group	$Cc$
$a/\text{\AA}$	28.3571(19)
$b/\text{\AA}$	13.1707(9)
$c/\text{\AA}$	7.1199(3)
$\beta/^\circ$	96.140(5)
$V/\text{\AA}^3$	2643.9
$Z$	4
$\rho_{\text{calc.}}/\text{g/cm}^3$	1.511
$\mu/\text{mm}^{-1}$	1.095
$F(000)$	1244
Crystal sizes, mm	0.3x0.3x0.05
Collected reflexions / Independent reflexions	4540/3155
Completeness to theta/ % ( $\theta = 25.05$ )	99.7
Parameters	337
$GOOF$	1.004
$R_1, wR_2$ final	$R_1 = 0.0628,$ $wR_2 = 0.1762$
$R$ index (overall data)	$R_1 = 0.0836,$ $wR_2 = 0.1952$

**Table 1.** Crystallographic data, experimental characteristics, and structure refinement parameters for Zn(II) compound.



**Figure 1.** Reaction scheme for the synthesis of compound  $[\text{Zn}(\text{o-FDH}_2)(\text{o-FDH})\text{Cl}] \cdot 6.5\text{H}_2\text{O}$

NEW (*O*-PHENYLENEDIAMINGLYOXIMATO)-(*O*-  
PHENYLENEDIAMINGLYOXIME)ZINC(II) CHLORIDE

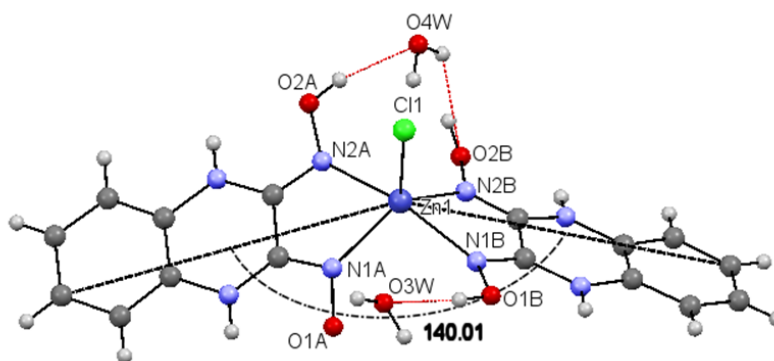
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A broad band of high intensity is observed at  $3112\text{ cm}^{-1}$  in the IR spectrum of the compound, which includes the oscillations:  $\nu(\text{OH})_{\text{water}}$  of water of crystallization molecules,  $\nu(\text{OH})_{\text{oxime}}$  and  $\nu(\text{NH})$ . The bands at  $2873$ ,  $2790$  and  $2738\text{ cm}^{-1}$  are attributed to  $\nu(\text{CH})$  oscillations. The intense absorption bands at  $1631$  and  $1007\text{ cm}^{-1}$  might be attributed to the  $\nu(\text{C}=\text{N})$  and  $\nu(\text{N}-\text{O})$  vibrations of the oxime groups, respectively [24]. Planar vibrations of the  $\text{C}=\text{C}$  skeleton appear as absorption bands at  $1607$ ,  $1492$ ,  $1448\text{ cm}^{-1}$  [25].

The  $\delta(\text{CH})_{\text{nonpl.}}$  absorption band at  $749\text{ cm}^{-1}$  corresponds to 1,2-substituted aromatic ring (presence of 4 adjacent hydrogen atoms) [26].

Structure of the  $\text{Zn}(\text{II})$  compound represents a molecular complex compound, in which oxime ligands coordinate at the central atom, both being formed as a result of condensation of dichlorglyoxime with *o*-phenylenediamine. The formula of this compound is  $[\text{Zn}(\text{o-FDH}_2)(\text{o-FDH})\text{Cl}]\cdot 6.5\text{H}_2\text{O}$ , in which one of the organic ligands coordinates in neutral form (*o*- $\text{FDH}_2$ ), and the other – in mono-deprotonate form (*o*- $\text{FDH}$ ), zinc charge being compensated by that of the chloride ion (Figure 2).

$\text{Zn}(\text{II})$  compound crystallizes in *Cc* monoclinic space group (Table 1). In the asymmetric part of the unit cell, the metal atom and the two oxime ligands being dentate-chelate coordinated via nitrogen atom while a chlorine atom and 10 water molecules are located in general positions.



**Figure 2.** Structure of the mononuclear complex of the  $\text{Zn}(\text{II})$  compound highlighting the positioning of the oxime ligands coordinated to the metal ion and the intermolecular hydrogen bonds with the involvement of water of crystallization molecules.

The coordination polyhedron of the  $\text{Zn1}$  atom in the complex takes shape of a tetragonal pyramid and consists of four nitrogen atoms of the two oxime ligands, located at the base, with the  $\text{C11}$  atom located at the apex. As a result, the metal atom is pentacoordinated,

Bonds	(Å)
Zn(1)–N(1A)	2.043(10)
Zn(1)–N(2A)	2.184(10)
Zn(1)–N(1B)	2.171(9)
Zn(1)–N(2B)	2.070(11)
Zn(1)–Cl(1)	2.274(3)
Angles	(°)
N(1A)– Zn(1)–N(2A)	75.9(3)
N(1A)– Zn(1)–N(1B)	96.1(3)
N(1A)– Zn(1)–N(2B)	126.0(4)
N(1A)– Zn(1)–Cl(1)	117.2(3)
N(2A)– Zn(1)–N(1B)	162.2(3)
N(2A)– Zn(1)–N(2B)	95.9(4)
N(2A)– Zn(1)–Cl(1)	98.8(2)
N(1B)– Zn(1)–N(2B)	75.7(4)
N(1B)– Zn(1)–Cl(1)	99.0(2)
N(2B)– Zn(1)–Cl(1)	116.8(3)

**Table 2.** Bond Lengths (Å) and Angles (°) in Coordination Metal Environment of Zn(II)

ZnN<sub>4</sub>Cl being the coordinating node (Figure 2). The Zn–N interatomic distances in the coordination polyhedron of the complex vary in the range of 2.049–2.184 Å, and the Zn–Cl distance is 2.274 Å (Table 2).

The oxime ligands are positioned at an angle of 140.01° in the complex and the deviation of the four coordinated nitrogen atoms from their mean plane is ±0.29 Å, and the Zn1 metal atom is displaced from this plane towards the Cl1 atom by 0.635 Å. The same data are also obtained for the pentacoordinated compounds of Zn(II), with the same donor set of N<sub>4</sub>Cl atoms, the four nitrogen atoms belonging to macrocyclic or open-loop tetracoordinated ligands [30, 31].

As a result, no intramolecular hydrogen bonds are formed between the two oxime ligands in the zinc(II) compound, but in the crystal a complicated system of O<sub>oxime</sub>–H...O intermolecular hydrogen bonds is formed via water molecules located in the outer sphere; the donor-acceptor distance varies in the range of 2.67(1)–2.94(2) Å (Table 3).

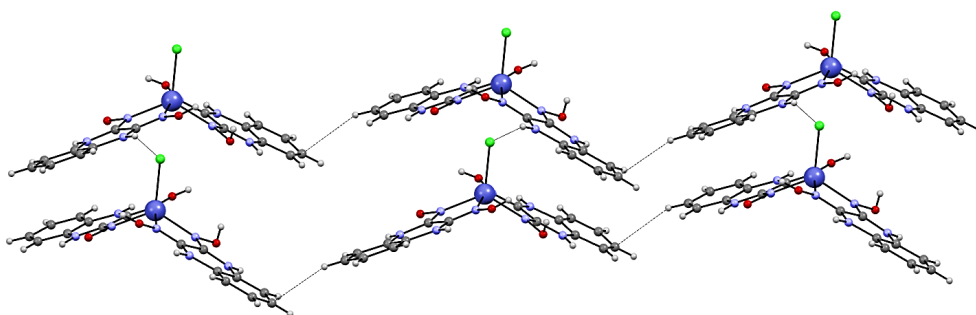
The mononuclear complexes in the crystal are connected in chains by N–H...Cl intermolecular hydrogen bonds and in layers by fine intermolecular C–H...π interactions

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PHENYLENEDIAMINGLYOXIME)ZINC(II) CHLORIDE

**Table 3.** Hydrogen Bond Distances (Å) and Angles (°) in crystal

D–H···A	d(H···A)	d(D···A)	∠(DHA)	Symmetry transformation for acceptor
N(3A)–H(1)···Cl(1)	2.56	3.348(10)	153	$x, -y+1, z+1/2$
N(4A)–H(2)···O(2W)	2.30	3.12(2)	161	$x, y, z$
N(3B)–H(3)···O(1W)	2.47	3.28(2)	158	$x, y, z$
N(4B)–H(4)···Cl(1)	2.57	3.377(10)	157	$x, -y, z+1/2$
O(2A)–H(2)···O(4W)	1.90	2.70(2)	165	$x, y, z-1$
O(1B)–H(1)···O(3W)	1.86	2.67(1)	173	$x, y, z-1$
O(2B)–H(2)···O(4W)	2.16	2.94(2)	157	$x, y, z-1$
O(2W)–H(2)···O(7W)	2.38	2.94(3)	124	$x, -y+1, z-1/2$
O(3W)–H(1)···O(7W)	1.88	2.70(3)	162	$x, y-1, z$
O(3W)–H(1)···O(9W)	2.25	2.90(3)	136	$x, y-1, z$
O(3W)–H(2)···O(7W)	2.09	2.76(3)	136	$x, -y+1, z-1/2$
O(3W)–H(1)···O(9W)	2.03	2.83(3)	157	$x, -y+1, z-1/2$
O(4W)–H(1)···Cl(1)	2.68	3.48(3)	147	$x, y, z+1$
O(4W)–H(2)···O(2B)	2.34	2.94(2)	124	$x, y, z+1$
O(4W)–H(2)···O(5W)	2.43	3.19(3)	142	$x, y, z$
O(5W)–H(1)···O(4W)	2.51	3.10(3)	128	$x, -y+1, z+1/2$

(Figure 3). The complexes are additionally interconnected with water molecules of co-crystallization.



**Figure 3.** Formation of layers in crystal with entrapment of mononuclear complexes.

#### 4. CONCLUSIONS

Upon dichloroglyoxime and *o*-phenylenediamine interaction with zinc(II) acetate dehydrate, a complex molecular compound with the formula  $[Zn(o\text{-FDH}_2)(o\text{-FDH})Cl]\cdot 6.5H_2O$  was obtained, in which the organic *o*-phenylenediamineglyoxime (*o*-FDH<sub>2</sub>) ligand is very little studied. The crystal structure of this compound revealed that this new oxime ligand was obtained by condensation of two organic components and coordinates to the metal atom like most *vic*-dioximes - bidentate-chelate through two nitrogen atoms, forming metallic rings of five atoms. The complex compound has a molecular type, as it is formed by one neutral *o*-FDH<sub>2</sub> ligand, one mono-deprotonated *o*-FDH<sup>-</sup> ligand and a chloride anion.

#### Acknowledgements

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PHENYLENEDIAMINGLYOXIME)ZINC(II) CHLORIDE

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## Influence of fertilization and row spacing on seed yield and quality of *Lathyrus sativus* L.

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**Abstract.** Pulses provide, along with other species, a rich source of nutrients needed both for human food and animal feed. These include *Lathyrus sativus* L., a grain legume that is less cultivated worldwide, especially in Europe, but of interest because of its ecological plasticity such as tolerance to drought, waterlogging, salinity, etc.

The present research attempts to bring a novelty to the technology of growing grasspea, a less known and studied grain legume crop in Romania. The bifactorial experiment was set up at the Didactic Station, Ezăreni Farm, University of Life Sciences “Ion Ionescu de la Brad”, Iași, arranged in randomized blocks design where several parameters (MMB, MH, quantity and quality of production, etc.) were followed.

According to the following results obtained from the research: the highest mass of 1000 grains was 165.1 g at 25 cm row spacing fertilized with  $N_{92}P_{92}K_{32}$ . The highest average value of hectolitre mass was 79.99 kg/hl at 37.5 cm row spacing, unfertilised, and the highest average yield obtained was 2755 kg/ha at 37.5 cm row spacing, fertilised with  $N_{36}P_{36}K_{16}$ .

Fertilization with  $N_{60}P_{60}K_0$  at 12.5 cm row spacing yielded highest amount of crude protein content in grass pea seeds (34,8%), which was about 11% greater than control.

**Keywords:** fertilization, grass pea, *Lathyrus sativus* L., production, protein, row spacing.

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## Influența fertilizării și distanței dintre rânduri asupra producției de boabe și calității lor la *Lathyrus sativus* L.

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**Rezumat.** Leguminoasele oferă, alături de alte specii, o sursă bogată de nutrienți necesari atât pentru hrana umană, cât și pentru hrana animalelor. Printre acestea se numără *Lathyrus sativus* L., o cultura de leguminoase pentru boabe care este mai puțin cultivată la nivel mondial, în special în Europa, dar prezintă un deosebit interes datorită plasticității sale ecologice, cum ar fi toleranța la secetă, inundație temporară, salinitatea etc.

Prezenta cercetare încearcă să aducă o noutate în tehnologia de cultivare a latirului, o cultura de leguminoase pentru boabe mai puțin cunoscută și studiată în România. Experimentul bifactorial a fost înființat la Stațiunea Didactică, Ferma Ezăreni a Universității de Științe ale Vieții „Ion Ionescu de la Brad” Iași, amplasată în blocuri randomizate, în care mai mulți parametri (MMB, MH, cantitatea și calitatea producției de boabe etc.) au fost urmărite.

Rezultate obținute în urma cercetării: cea mai mare masă a 1000 de boabe a fost de 165,1 g la distanța dintre rânduri de 25 cm și fertilizare cu  $N_{92}P_{92}K_{32}$ . Cea mai mare valoare a masei hectolitrică a fost de 79,99 kg/hl la distanța dintre rânduri de 37,5 cm, nefertilizat, iar cea mai mare recoltă fiind de 2755 kg/ha la distanță de rânduri de 37,5 cm și fertilizarea  $N_{36}P_{36}K_{16}$ .

Fertilizarea cu  $N_{60}P_{60}K_0$  și distanța dintre rânduri de 12,5 cm a produs cea mai înaltă productivitate și conținut de proteină brută în semințele de latir (34,8%), care a fost cu aproximativ 11% mai mare decât la lotul de control.

**Cuvinte-cheie:** distanța dintre rânduri, fertilizare, latir, *Lathyrus sativus* L., producție de boabe, proteine.

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## 1. INTRODUCTION

A complementary perspective for the continued improvement of key crop species to ensure the maintenance of sustainable food and feed production in the context of climate change, as well as the current energy crisis, involves targeting agricultural species that are currently less exploited globally but which have traits of interest such as tolerance to drought [15], salinity [14], short-term flooding [11]. These include *Lathyrus sativus* L, commonly called grass pea, an annual legume crop and the most important species of *Lathyrus* genus [6], which is a large genus with 160 species. Grass pea is shown both for seed production as a source for human food, as a forage crop for animal feeds, and also it is cultivated for use as a green manure or cover crops [27]. Additionally, grass pea is one of the crops with great agricultural potential, due to its very high protein concentration of up to 34% [16], starch content [4] and high lysine content up to 20.4 mg/kg [22]. Typically, as with other grain legumes, protein quality is limited only by its low concentrations of methionine, cysteine and tryptophan [20].

*Lathyrus sativus* L. covers small areas globally, with an estimated cultivated area of about 1.50 million hectares [24], but it is a species that integrates perfectly in a sustainable agricultural system, being a natural source of nitrogen, which allows reducing the cost of cultivation and the environmental impact of nitrogen-based fertilizers.

Grass pea grows and develops very well under adverse agricultural conditions, performs well in a broad spectrum of soil types and its cultivation requires low or zero agricultural inputs [22] thereby, it can be successfully incorporated in crop rotation systems [4]. Grass pea is able to fix nitrogen very efficiently through symbiosis with *Rhizobium leguminosarum* bv. *viciae* [9] and in the presence of a specific protein called leghemoglobin, add about 124 kg/ha N in a single growing season [26].

## INFLUENCE OF FERTILIZATION AND ROW SPACING ON SEED YIELD AND QUALITY OF *LATHYRUS SATIVUS* L.

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It is also necessary for the rehabilitation of physical structure of the soil [30], and offers an efficient alternative in areas with land overexploited by cereal cultivation, which is why interest in this species has been renewed in Europe, and its cultivation has now extended to include marginal lands of the Mediterranean basin (France, Spain, Lebanon, Egypt, Syria, Libya, Morocco, Algeria), [8].

Since world food production is largely sustained by major crop plants (wheat, maize, etc.), as well as major nutrients and micronutrients that impact crop nutrition and productivity [29], [28], less emphasis has been accorded to minor crops [5], such as grass pea.

Sowing seeds at optimal row spacing allows efficient use of soil resources and fertilizer management is a key aspect which affects crop growth, development and productivity. Very limited studies regarding the judicious use of fertilizer on grass pea has been laid out worldwide, especially in Europe and Romania.

Therefore, the present study was undertaken to study the effect of row spacing and different levels of NPK fertilizer on seed yield and quality, and to identify the optimum row spacing and the optimum level of NPK fertilizers for obtaining maximum yield of *Lathyrus sativus* L.

## 2. MATERIALS AND METHODS

### **Experimental design**

The bifactorial experiment was set up in the research field of Phytotechny at the Didactic Station, Ezăreni Farm, of the University of Life Sciences “Ion Ionescu de la Brad”, Iași, to study the effect of row spacing and fertilization levels on yield of grass pea (*Lathyrus sativus* L.).

The study consisted of four row spacing (12,5 cm, 25 cm, 37,5 cm, 50 cm) with four different levels of NPK ( $N_0P_0K_0$ ,  $N_{36}P_{36}K_{16}$ ,  $N_{60}P_{60}K_0$ ,  $N_{92}P_{92}K_{32}$ ) using a randomized block design with three replications. The required quantities of fertilizer in the form of NPK (20% N, 20%  $P_2O_5$  and 16%  $K_2O$ ) as per treatment were applied one day before sowing the crop in each plot independently and was mixed properly with the soil. The plot size kept for each treatment measured  $3 \times 3 \text{ m}^2$ . Sowing of crop was done on 26th April, 2021 and harvested at its full maturity on 24th August, 2021.

The crop of grass pea was grown in rainfed conditions without using irrigation and the other management practices were in accordance with the recommended practices for the crop.

The 1000-seed weight (g), hectolitre mass (kg), seed yield (kg ha) were calculated and additionally the seeds were analyzed for their protein composition. The crude protein in

seeds was determined by the Kjeldahl method described by Nelson and Sommers (1980). The principle of method consists in the determination of total nitrogen content and its conversion into crude protein by multiplying by the factor 6.25. The crude protein content is given by the relation:

$$PB = Nt \times 6,25$$

The meteorological data (average air temperature and rainfall) of the five months in which the research was carried out are given in table (1) taken from Ezăreni meteorological station.

### Statistical analyses

For all mentioned descriptors the experimental results were calculated using the analysis of variation method [10] to determine the significance of the difference between variants (fertilization, row spacing), calculating the limiting differences for the 5%, 1% and 0.1% probability of transgression.

Year/Month	April	May	June	July	August	Average
Temperature (°C)						
Average°C	7.8	14.9	19.8	22.2	20.9	17.1
2021	10.1	16.1	19.4	21.3	20.6	17.5
Deviation	-2.3	-1.2	0.4	0.9	0.3	0.38
Rainfall (mm)						
Σmm	56.4	86	107.4	80.3	155.6	97.14
2021	40.3	52.5	75.1	69.2	57.6	58.94
Deviation	16.1	33.5	32.3	11.1	98	38.2

**Table 1.** Temperature and precipitation during the growing season.

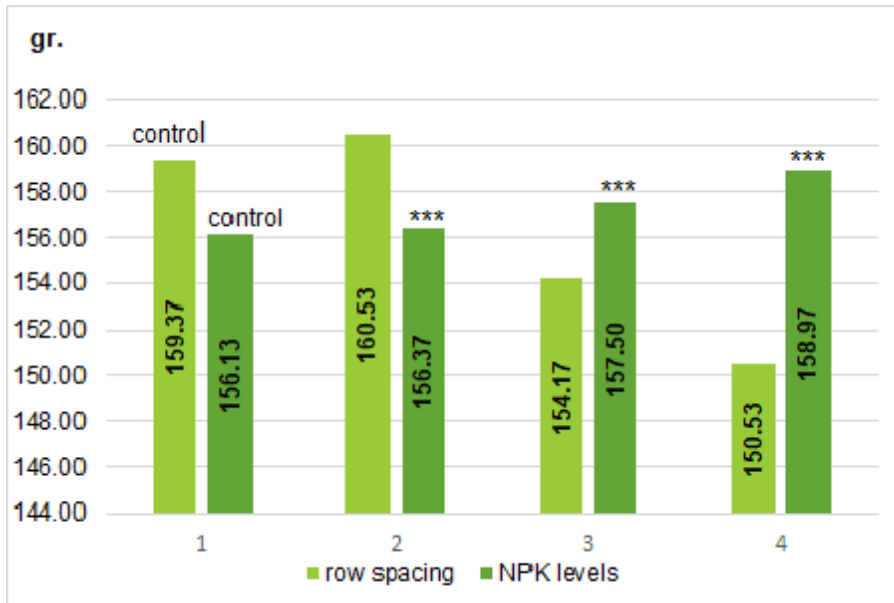
### 3. RESULTS AND DISCUSSIONS

**1000-seeds weight (g).** In this study, the highest value of 1000-seed weight was 160.53 g, obtained at 25 cm row spacing with a difference from the control of 1.17 g, and the lowest value was 150.53 g at 50 cm row spacing. This is explained by better optimization of the nutrition space at row spacings of 25 cm and 37.5 cm, respectively.

Grass pea crop showed a consecutive improvement in 1000-seed weight with each increase in NPK doses. Thus, in the control without fertilization, the weight of 1000 seeds

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was 156.13 g. The applying of N<sub>93</sub>P<sub>92</sub>K<sub>32</sub>, with a very significant positive difference from the control, resulted in the highest value of 1000 seeds, which was 158.97 g. Also, the increasing level from N<sub>36</sub>P<sub>36</sub>K<sub>16</sub> to N<sub>60</sub>P<sub>60</sub>K<sub>0</sub> determined an increase in the weight of 1000 seeds, the differences from the control find very significant, positive (Figure 1).

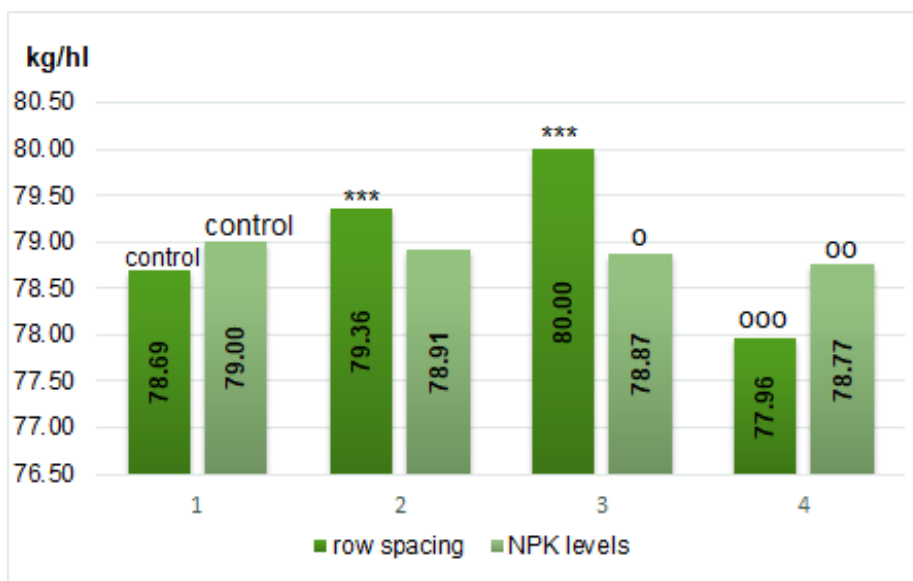


**Figure 1.** Effects of row spacing and levels of NPK on 1000 seeds weight.

The results from this study on the weight of 1000 seeds were lower than those of Karadag Y. [12], and higher than those of Seydosoglu Y. [25] and Kaminkyi V. [11].

**Hectolitre weight.** In *Figure 2*, it can be observed that the row spacing of 37.5 cm produced the highest value of hectolitre weight, which was 80.0 kg/hl, with a difference compared to the control of 1.31 kg/hl. The applied NPK fertilizer did not have a positive influence on the hectolitre weight, the highest value obtained being 79.0 kg/hl for the control. Although the hectolitre weight has not been evaluated in many studies, the results are almost in agreement line with the findings of Campbell C. [7], which reported a hectolitre weight between 61.22 kg/hl and 82.88 kg/hl.

**Yield.** The highest seeds yield was obtained at 25 cm row spacing (2444 kg/ha), which was significantly higher than the yield obtained at the others row spacings applied. In figure 3 it can be observed that at 25 cm row spacing the difference is distinctly significant, with a difference of 514 kg compared to the control, and at 37.5 cm row spacing the difference is significant, with 340 kg higher than the control. In the case of row spacing of 50 cm, the difference to the control is significant, but negative. It



**Figure 2.** Effects of row spacing and different levels of NPK on hectolitre weight.

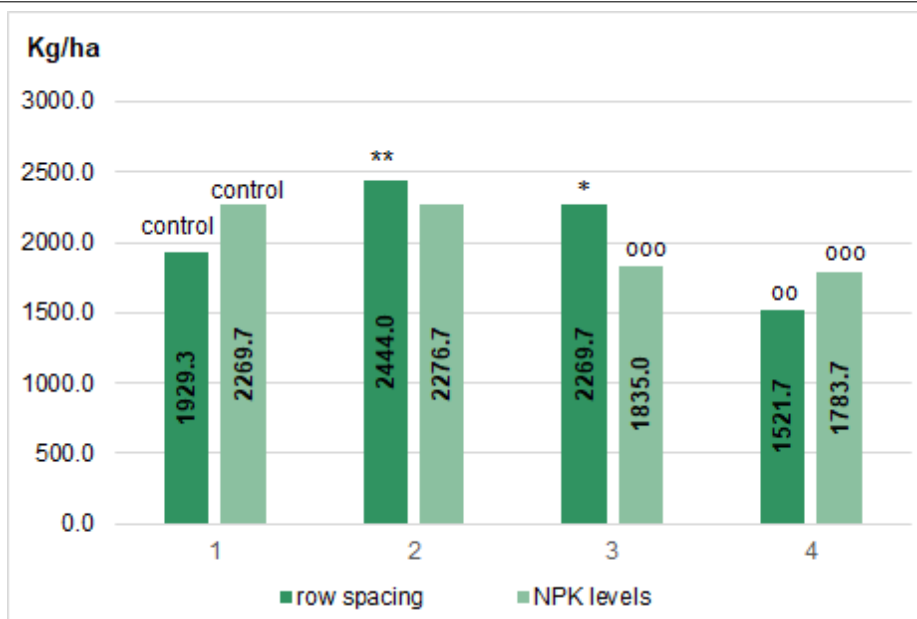
might due, to the lower number of plants per unit area as compared to the other spacings. Nandini Devi [18] and Luikham E. [13] reports similar results for *Lathyrus sativus* L.

In the case of fertilization, the application of complex fertilizers at the rate of  $N_{36}P_{36}K_{16}$ , recorded the highest yield value of 2276 kg/ha, but it should be noted that the application of  $N_{60}P_{60}K_0$  and  $N_{92}P_{92}K_{32}$  resulted in a decrease in yield, 1835 kg/ha and 1783 kg/ha respectively, the differences being very significant but negative (Figure 3). Kaminkyi V. [11] reported similar findings.

The effectiveness of lower fertilizer rates has been confirmed by several scientific studies. Thus, the application of potassium fertilizer had a significant effect on production parameters (pod/plant, seed/pod, MMB, biomass yield and seed yield), with the highest values obtained when applying a 20 kg/ha  $K_2O$  dose [1]. Mesfin S. [17] obtained the highest seed yields when  $N_{20}P_{20}$  was applied, thus the use of nitrogen-phosphorus fertilizers had a positive effect on root system formation at early stages of plant development and on legumes productivity. Sahu B. [23] showed that the application of mineral fertilizers with nitrogen, phosphorus and potassium at the rate of 20.40.20 significantly increased crop yield.

**Protein content (%) in *Lathyrus sativus* L.** Analysing the influence of row spacing on the crude protein content of the grass pea seeds, it can be seen that the row spacing of 37.5 cm resulted in the highest protein content of 33.99%, the difference from the

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**Figure 3.** Effects of row spacing and levels of NPK on seeds yield.

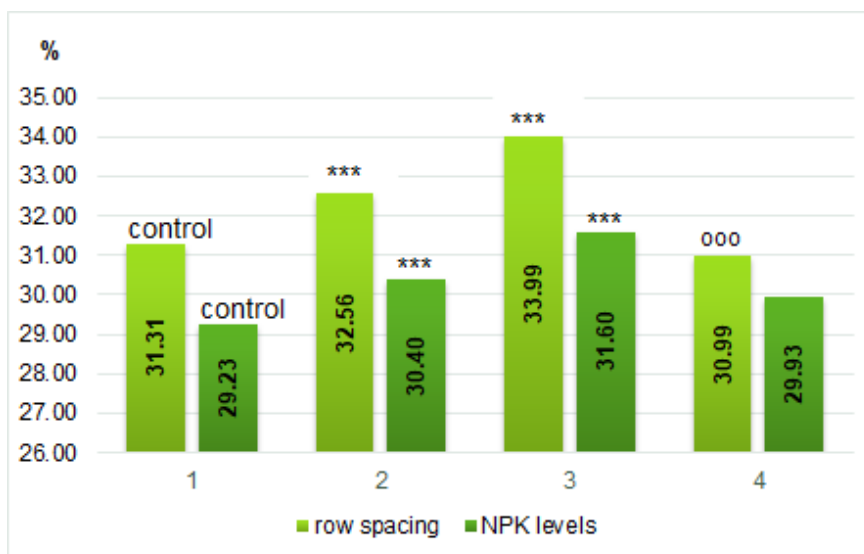
control being very significant, positive. The lowest value was recorded at 50 cm row spacing, with a difference to the control very significant but negative. According to the results obtained in this study, the highest crude protein value of 31.6% was recorded when  $N_{60}P_{60}K_0$  fertilizer was applied, followed by 30.4% when  $N_{36}P_{36}K_{16}$  treatment was applied. The differences between the doses of NPK and control are highly significantly, positive (Figure 4). In this study, these findings regarding the protein content of grass pea seeds are similar with the results obtained by Banerjee P. *et al* [3].

There is a positive correlation between row spacing and production (kg/ha), with the quadratic regression having a value of 0.988%. Between 1000-seeds weight and production the regression factor is 0.933%. Between hectolitre weight and production the regression factor is 0.915%. A low interaction was found between protein and row spacing.

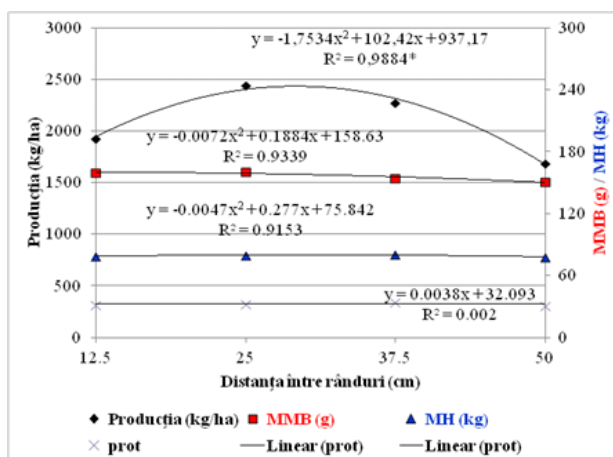
In the case of fertilization, a strong correlation was found between this factor and 1000-seeds weight, with the quadratic regression having a significant value of 0.989%.

A low interaction was found between fertilizer doses (NPK) and yield, hectolitre mass and protein content, the regression factors having the following values: 0.644%, 0.512%, 0.181%.





**Figure 4.** Effects of row spacing and levels of NPK on the protein content in grass pea.

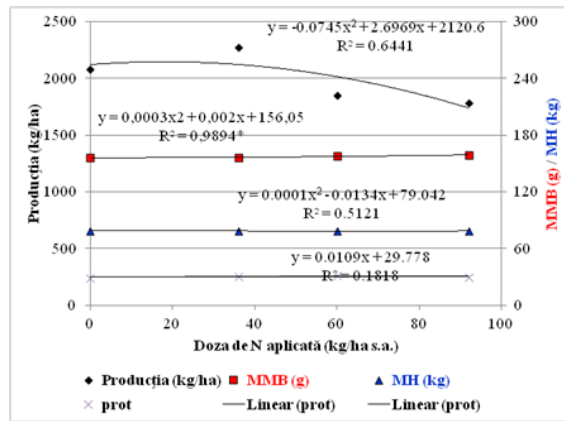


**Figure 5.** Correlation between row spacing and production elements.

#### 4. CONCLUSIONS

The seeds of *Lathyrus sativus* L. have potential as an alternative source of protein, according to the experimental results, the protein content is rich, ranging from 29.23% to 33.99%.

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**Figure 6.** Correlation between levels of NPK and production elements.

It was found that one dose of N<sub>60</sub>P<sub>60</sub> is sufficient for better grass pea production. Also, sowing grass pea at 50 cm row spacing for forage, green manure or cover crop production, and sowing at 25 cm row spacing is recommended when seed consumption is desired.

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## Diversity of avifauna in the orchards of the Republic of Moldova

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**Abstract.** The study was conducted in the period 1991-2018 in 86 different types of orchards from the central part of the Republic of Moldova, in all phenological periods. The bird fauna in the orchards is well represented, with a rich diversity of 95 species from 13 orders, which represents 35% of the avifauna of the republic. The dominant species were the passerine birds that are widespread and have high density in various arboreal biotopes. There were recorded 5 protected species: *Ciconia ciconia*, *Circus pygargus*, *Milvus migrans*, *Falco vespertinus* and *Dendrocopos medius*. In terms of phenology, the predominance of migratory species was found, followed by sedentary ones, winter guests, occasional and passage species. Further research is needed on orchard avifauna, which provide a range of economically valuable ecosystem services.

**Keywords:** avifauna, orchard, diversity, dominant species, rare species, phenology, importance.

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## Diversitatea avifaunei în livezile din Republica Moldova

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**Rezumat.** Studiul a fost realizat în perioada 1991-2018 în 86 de livezi de diferite tipuri din zona centrală a Republicii Moldova, în toate perioadele fenologice. Fauna de păsări din livezi este bine reprezentată, cu o bogată diversitate de 95 de specii din 13 ordine, ceea ce reprezintă 35% din avifauna republicii. Speciile dominante au fost paseriformele care sunt larg răspândite și au densități mari în diverse biotopuri arboricole. Au fost înregistrate 5 specii protejate: *Ciconia ciconia*, *Circus pygargus*, *Milvus migrans*, *Falco vespertinus* și *Dendrocopos medius*. Din punct de vedere fenologic, s-a constatat predominanța speciilor migratoare, urmate de cele sedentare, oaspeți de iarnă, specii ocazionale și de pasaj. Sunt necesare cercetări suplimentare asupra avifaunei din livezi, care oferă o serie de servicii ecosistemice valoroase din punct de vedere economic.

**Cuvinte-cheie:** avifauna, livadă, diversitate, specii dominante, specii rare, fenologie, importantă.

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### 1. INTRODUCTION

Due to the favorable pedological and climatic conditions, the Republic of Moldova represents a viticulture and fruit growing region par excellence. That is why ornithological studies in the area often have connections with the anthropic landscape and fruit growing

in particular. Among the first studies, which address aspects of avifauna in the Republic of Moldova, there can be mentioned several books dedicated to the biocenotic distribution of bird communities, the influence of anthropogenic factors on avifauna, as well as their role in orchard pest control [1, 2, 13]. Further research addresses the distribution and other ecological aspects of birds in natural and anthropogenic ecosystems, as well as the specific composition and density of bird populations in intensive orchards [3, 4, 5]. Similar studies also extend to the avifauna of protective forest belts, both from agricultural fields and from orchards [8, 12, 19]. The studies on the avifauna of orchards have been focused on the adaptation of birds to the conditions of anthropogenic ecosystems [14], the particularities of the ecology of bird nesting in intensive orchards [6, 7], the importance of birds in the fight against agricultural pests and the importance of biocenotic oases in enriching the avifauna of agrocenoses [10, 12, 14] and spatial distribution of birds in orchards [11, 20]. In the last 20 years, the studies on the avifauna of orchards are very few and sporadic [18].

After the year 2000, new forms of private property began to appear – farmers and orchard collectives. The planting of new orchards, which are almost exclusively of intensive and super-intensive form, has started and the structure of their tree species composition has also changed. Thus, the share of seed orchards, such as apple and pear, decreased in favor of stone fruit ones, such as plum, peach, apricot, cherry, sour cherry, as well as nut trees, such as walnut, almond, rarely hazelnut. In this context, there have been changes in the quantitative and qualitative structure of bird communities in various types of orchards. Hence the need for an updated study on orchard avifauna has arisen.

The aim of the paper is to analyze the diversity of the avifauna in the orchards of the republic in the last decades, highlighting the dominant species and the importance of birds in the functioning of anthropogenic ecosystems.

## 2. MATERIALS AND METHODS

The study was conducted in the period 1991-2018. There were selected 86 different orchards, in terms of tree essence, age, structure and location, with an area between 0.02 and 60 ha each, in total – 615 ha. The studied orchards were mixed, of different species and varieties, from apple and pear to cherry, peach and apricot, located in the Central Codri forest area, in the suburbs of Chisinau and in the Criuleni district.

In order to highlight the seasonal dynamics of the avifauna distribution, the annual life cycle of the birds was divided, according to established principles, into 5 phenological aspects. They are the following: hiemal, prevernal, vernal, serotinal and autumnal. The estimation of bird density was carried out using the method of plots and the method of

transects [15, 16, 17]. The evaluation of the diversity and bird number was carried out by the mapping method, recording on the sketch of the territory all the individuals identified visually and after sound. The research was systematically carried out twice for each phenological aspect in the period 1991-2001 and in the vernal, serotinal and autumnal aspects in the period 2002-2018.

### 3. RESULTS AND DISCUSSIONS

Obtained results and discussions As result of the studies carried out in different types of orchards, a diversity of 95 bird species from 13 orders was established (Tab. 1). The classification was made according to the World Bird Database - Avibase; the Bird World and Bird Life International databases.

**Table 1.** Avifauna diversity in the orchards from the central part of the Republic of Moldova

No.	Order, Species	H	P	V	S	A
Order Galliformes						
1.	Phasianus colchicus	-	+	+	+	-
2.	Perdix perdix	+	+	+	+	+
3.	Coturnix coturnix	-	-	+	-	-
Order Ciconiiformes						
4.	Ciconia ciconia	-	-	+	+	+
Order Accipitriformes						
5.	Circus aeruginosus	-	+	-	-	-
6.	Circus pygargus	-	+	-	-	-
7.	Buteo buteo	-	+	+	+	+
8.	Buteo lagopus	+	-	-	-	-
9.	Milvus migrans					
10.	Accipiter nisus	+	+	-	+	+
11.	Accipiter gentilis	-	+	-	+	-
Order Falconiformes						
12.	Falco tinnunculus	-	+	+	+	+

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13.	<i>Falco vespertinus</i>	-	-	-	+	+
Order Columbiformes						
14.	<i>Columba livia</i>	+	+	+	+	+
15.	<i>Columba palumbus</i>	-	-	-	+	-
16.	<i>Streptopelia turtur</i>	-	+	+	+	-
17.	<i>Streptopelia decaocto</i>	-	-	-	+	-
Order Cuculiformes						
18.	<i>Cuculus canorus</i>	-	+	+	+	-
Order Apodiformes						
19.	<i>Apus apus</i>	-	-	+	+	-
Order Caprimulgiformes						
20.	<i>Caprimulgus europaeus</i>	-	-	-	+	-
Order Strigiformes						
21.	<i>Asio otus</i>	+	+	+	-	-
22.	<i>Athene noctua</i>	+	+	-	-	-
Order Coraciiformes						
23.	<i>Merops apiaster</i>	-	-	+	+	-
Order Bucerotiformes						
24.	<i>Upupa epops</i>	-	-	+	-	-
Order Piciformes						
25.	<i>Dendrocopos syriacus</i>	+	+	+	+	+
26.	<i>Dendrocopos medius</i>	+	-	-	-	-
27.	<i>Dendrocopos major</i>	+	+	+	+	+
28.	<i>Dendrocopos minor</i>	+	-	-	-	-
29.	<i>Picus canus</i>	-	+	-	-	-
Order Passeriformes						
30.	<i>Regulus regulus</i>	+	+	-	-	-
31.	<i>Coccothraustes coccothraustes</i>	+	+	+	+	-



32.	<i>Carduelis cannabina</i>	-	+	+	-	-
33.	<i>Jynx torquilla</i>	-	-	+	+	-
34.	<i>Serinus serinus</i>	-	-	+	-	-
35.	<i>Fringilla coelebs</i>	+	+	+	+	+
36.	<i>Fringilla montifringilla</i>	+	-	-	-	-
37.	<i>Corvus frugilegus</i>	+	+	+	-	+
38.	<i>Corvus corone</i>	+	+	+	-	+
39.	<i>Galerida cristata</i>					
40.	<i>Melanocorypha calandra</i>	-	+	-	-	-
41.	<i>Alauda arvensis</i>	-	+	+	+	-
42.	<i>Lullula arborea</i>	-	+	+	+	+
43.	<i>Turdus pilaris</i>	+	+	-	-	-
44.	<i>Motacilla alba</i>	-	+	+	+	+
45.	<i>Phoenicurus phoenicurus</i>	-	-	-	+	-
46.	<i>Phoenicurus ochruros</i>	-	-	+	+	+
47.	<i>Certhia familiaris</i>	+	+	+	+	+
48.	<i>Corvus corax</i>	+	+	+	+	+
49.	<i>Pica pica</i>	+	+	+	+	+
50.	<i>Anthus campestris</i>	-	-	-	+	+
51.	<i>Anthus trivialis</i>	-	+	+	+	+
52.	<i>Carduelis chloris</i>	+	+	+	+	-
53.	<i>Hippolais icterina</i>	-	-	+	-	-
54.	<i>Garrulus glandarius</i>	+	+	+	+	+
55.	<i>Oriolus oriolus</i>	-	+	+	+	-
56.	<i>Sturnus vulgaris</i>	-	+	+	-	-
57.	<i>Delichon urbica</i>	-	-	-	+	-
58.	<i>Erithacus rubecula</i>	-	+	-	+	+
59.	<i>Saxicola rubetra</i>	-	-	+	+	-

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60.	<i>Saxicola torquata</i>	-	+	+	+	+
61.	<i>Bombycilla garrulus</i>	+	-	-	-	-
62.	<i>Turdus merula</i>	+	+	+	+	+
63.	<i>Pyrrhula pyrrhula</i>	+	+	-	-	+
64.	<i>Ficedula albicollis</i>	-	+	-	-	-
65.	<i>Ficedula hypoleuca</i>	-	+	-	-	-
66.	<i>Muscicapa striata</i>	-	+	+	+	-
67.	<i>Oenanthe oenanthe</i>	-	+	-	-	-
68.	<i>Phylloscopus trochilus</i>	-	-	-	+	-
69.	<i>Phylloscopus collybita</i>	-	+	+	+	+
70.	<i>Parus caeruleus</i>	+	+	-	+	+
71.	<i>Aegithalos caudatus</i>	-	+	-	-	+
72.	<i>Parus major</i>	+	+	+	+	+
73.	<i>Parus palustris</i>	+	+	-	-	+
74.	<i>Emberiza hortulana</i>	-	-	+	+	-
75.	<i>Emberiza schoeniclus</i>	+	+	-	-	-
76.	<i>Emberiza citrinella</i>	+	+	+	+	+
77.	<i>Emberiza calandra</i>	-	-	+	-	-
78.	<i>Luscinia luscinia</i>	-	-	+	+	-
79.	<i>Hirundo rustica</i>	-	+	+	+	-
80.	<i>Carduelis spinus</i>	+	-	-	-	+
81.	<i>Lanius minor</i>	-	-	+	+	-
82.	<i>Lanius excubitor</i>					
83.	<i>Lanius collurio</i>	-	-	+	+	-
84.	<i>Sylvia atricapilla</i>	-	-	+	+	-
85.	<i>Sylvia communis</i>	-	-	+	+	-
86.	<i>Sylvia borin</i>	-	-	+	-	-
87.	<i>Sylvia curruca</i>	-	-	+	-	-

88.	<i>Sylvia nisoria</i>	-	-	+	+	-
89.	<i>Corvus monedula</i>	-	+	+	-	+
90.	<i>Carduelis carduelis</i>	+	+	+	+	+
91.	<i>Turdus philomelos</i>	-	+	+	+	+
92.	<i>Turdus viscivorus</i>	-	+	-	-	-
93.	<i>Sitta europaea</i>	-	-	-	+	+
94.	<i>Passer montanus</i>	+	+	+	+	+
95.	<i>Passer domesticus</i>	-	-	+	+	+
Total species	95	32	56	58	58	38

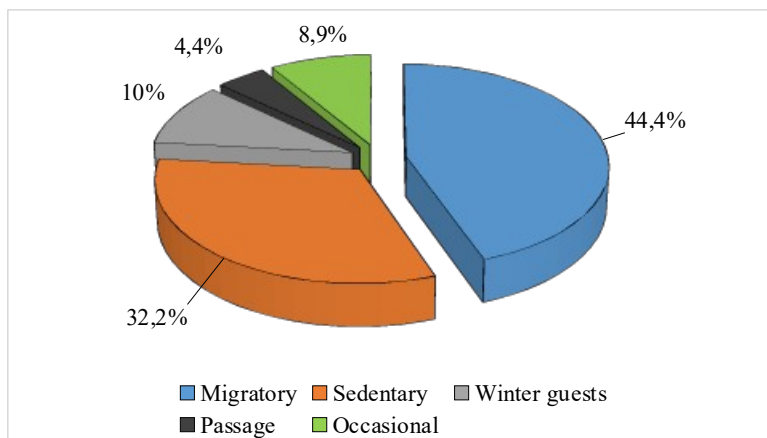
Note: phenological period: H – hiemal, P – prevernal, V – vernal, S – serotinal, A – autumnal.

The avifauna of any habitat is formed on the basis of biotic and abiotic factors, specific to a certain area, settling as a component of the regional avifauna. Thus, the regional avifauna of the Republic of Moldova includes about 280 species of birds [3], 95 species of birds were identified in orchards, which constitutes approximately 34% of the avifauna of the republic. It is quite a large diversity, taking into account the fact that orchards are anthropogenic ecosystems and not natural ones. The dominant species in orchards were *Fringilla coelebs*, *Passer montanus*, *Emberiza citrinella*, *Cardueliscarduelis*, *Parus major*. Also, 5 rare species listed in the Red Book of the Republic of Moldova [9, p. 266-230], were registered, namely *Ciconia ciconia*, *Circuspygargus*, *Milvus migrans*, *Falco vespertinus* and *Dendrocopos medius*.

Following the analysis of the avifauna in terms of phenological aspect, it was found that migratory species predominate with over 44% of the total number of species, followed by sedentary ones, winter guests, occasional and species of passage (fig. 1). The increased share of nesting species (migratory and sedentary), found in orchards, does not necessarily mean breeding, but only their presence in this habitat. The phenological category of each species was assessed in relation to the regional avifauna in general [3] and not in relation to the specific orchard habitat. If we take it strictly, there are almost no sedentary birds in the orchards. Even tits and woodpeckers retire for the night in certain periods of the year, especially in winter, to the neighbouring arboreal biotopes. Synanthropic species, such as corvids, retreat to the localities during the cold period of the year. According to the given study, the nesting of 48 species (about 50% of the total number) was established in the orchards, both by finding the nests and by their nuptial song or dance. However,

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even in cases of direct registration of birds with nuptial behaviour, difficulties may arise. For example, the common buzzard and the raven often include orchards in their breeding territories, but their nests are located in adjacent biotopes.



**Figure 1.** Phenological groups of birds in orchards.

As species of passage were considered the birds, which are assigned to this category in relation to the regional avifauna. But many migratory birds, as well as the winter guests observed on the territory of the republic, can be met in orchards only during migrations. Thus, a part of chaffinches, like other migratory birds are birds of passage in orchards. That is why their real share, being difficult to assess accurately, is much higher. The share of occasional species is relatively small, they meet sporadically in certain periods of the year in search of food, and some species, such as *Ciconia ciconia*, *Milvus migrans*, *Falco vespertinus*, were reported only a few times.

The study of the avifauna of orchards is of particular importance, because in such biotopes stable communities of birds are formed, which contribute to the biological regulation of pests in all phenological periods. Also, the orchards, especially the old ones, provide favourable conditions for rare species as well, being biocenotic oases for the protection and preservation of the avifauna diversity in the agricultural landscape.

### 4. CONCLUSIONS

The bird fauna in the orchards from the central part of the Republic of Moldova is well represented, constituting 95 species from 13 orders, which represents 35% of the avifauna of the republic. The dominant species are the widespread Passeriformes, which have large numbers in various arboreal biotopes. 5 protected species *Ciconia ciconia*, *Circus pygargus*, *Milvus migrans*, *Falco vespertinus* and *Dendrocopos medius* were reported.

In terms of phenology, the predominance of migratory species was found, followed by sedentary ones, winter guests, occasional and species of passage. Further research is needed on orchard avifauna, which contribute to the stable functioning of ecosystems and provide a range of economically valuable ecosystem services.

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## ISSR fingerprinting of genetic diversity within and among *Orobanche cumana* Wallr. populations from different countries

INA BIVOL 

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**Abstract.** Molecular genetic diversity was analysed for 23 *Orobanche cumana* populations from four countries using 13 ISSR markers. Descriptive population genetic statistics and AMOVA analysis revealed a marked degree of intrapopulation differentiation. PCA and UPGMA clustering showed a clear division into two main regional groups, i.e. the first group was more closely related to the Middle East and the second group belonged to Eastern Europe. The results of this research would suggest that the Moldavian, Bulgarian, and Romanian populations, which have many molecular markers in common, may share many genetic traits that indicate their monophyletic origin.

**Keywords:** *O. cumana*, markers, genetic diversity, populations, differentiation.

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## Amprentarea ISSR a diversității genetice în cadrul și între populațiile de *Orobanche cumana* Wallr. din diferite țări

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**Rezumat.** Diversitatea genetică moleculară a fost analizată la 23 de populații de *Orobanche cumana* din patru țări folosind 13 markeri ISSR. Statistica genetică descriptivă și analiza AMOVA a datelor obținute au relevat un grad ridicat de diferențiere intrapopulațională. Gruparea PCA și UPGMA a evidențiat o divizare clară în două grupuri regionale principale, respectiv primul grup a fost mai strâns legat genetic de Orientul Mijlociu, iar al doilea grup a aparținut Europei de Est. Rezultatele acestor cercetări ar sugera că populațiile din Moldova, Bulgaria și România cu un număr mare de markeri moleculari în comun, pot împărtăși multe trăsături genetice care ar indica originea lor monofiletică.

**Cuvinte-cheie:** *O. cumana*, markeri, diversitate genetică, populații, diferențierea.

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### 1. INTRODUCTION

Broomrape (*Orobanche cumana* Wallr.) sunflower infestation is a significant reason for its yield decrease and gross seed harvests in many regions worldwide [1]. On average, sunflower seed losses due to broomrape can be more than 50% when susceptible hybrids are grown and as high as 100% in heavily infested fields [2]. The degree of negative effects of broomrape parasitism depends critically on the host resistance, the parasite's aggressiveness, the sunflower's developmental stage at the time of infection, the level of



contamination, and environmental factors [3]. Intensive sunflower cultivation in violation of crop rotation, import of foreign breeding seeds with susceptibility to the local broomrape, rapid parasite evolution, climate changes favorable for broomrape expansion, and other causes have led to the emergence of sudden genetic changes in broomrape populations and the active spread of new highly virulent races of the parasite in almost all sunflower producing countries [4]. A species' genetic relatedness and population structure are key determinants of its natural distribution, environmental adaptability, survival, and evolutionary potential under changing conditions [5]. The genetic structure of a species is formed by the influences of both internal (size, density, distribution of the species' population, gene flow, genetic drift, natural selection, and plant mating system) and external factors (changes in the boundaries of the species' distribution) determined by past and present evolutionary processes. Changes in climate and habitat often accelerate the expansion or contraction of a species' range. Studies of the genetic structure and diversity of the parasitic species *O. cumana* are important for understanding the underlying genomic, evolutionary, and demographic processes occurring in populations, which is of great importance for the development of effective control strategies of the pathogen and breeding programs for resistance to broomrape in sunflower.

This study aimed to investigate the genetic diversity and differentiation of 23 Black Sea broomrape populations of different origins using ISSR molecular markers.

## 2. MATERIALS AND METHODS

269 plants of 23 sunflower broomrape populations from different regions of Bulgaria (notation keys B1-B4), Turkey (T1-T5), Republic of Moldova (RM1-RM13) and Romania (R1), belonging to 3 virulent races (race E- populations RM12, RM13; race G -B1, B2, T5, RM10, RM11; and race H- B3, B4, T1-T4, RM1-RM9, R1), were used in this study.

Total genomic DNA was isolated from frozen stem material, using the Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit #K0791 (Thermo Fisher Scientific, USA). The quantitative and qualitative content of isolated DNA was assessed using a spectrophotometer (T60 UV-VIS, PG Instruments Limited, England), and verified by 1% agarose gel electrophoresis [6]. Thirteen ISSR primers, representing di-, tri-, and tetra- repeats that produced clear and reproducible bands, previously reported by Benharrat [7], were selected to investigate genetic diversity. DNA amplification was carried out on a Genset 9700 thermocycler (Applied Biosystems, USA), using the following parameters: initial denaturation - 5 min at 95°C (1 cycle); denaturation - 30 s at 95°C, annealing - 45 s at 45°C, extension - 2 min at 72°C (35 cycles); and final extension - 5 min at 72°C (1 cycle). PCR products were analysed by electrophoresis on a 2% agarose

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gel containing 0.5  $\mu\text{g/ml}$  ethidium bromide in Tris-acetate-EDTA (TAE) buffer (40 mM Tris-acetate, pH 8.0; 1 mM EDTA) and visualised under UV light (wavelength  $\lambda=305$  nm). The ready-to-use GeneRuler Express DNA Ladder, SM1553, (Thermo Fisher Scientific, USA) was used as the standard molecular weight marker. Photographs were taken, using the Doc-Print VX2 gel documentation system, model SXT-F20.M (Vilber Lourmt, France).

The binary data matrix of amplification profiles for all samples, obtained by the Photo-Capt V.15.02 program, was used to calculate descriptive population genetic statistics parameters, total genetic diversity, genetic structure, and genetic relationships of the broomrape populations, using the POPGENE V.1.32, GenAlex 6.503, and XLSTAT V.2016.02.28451 software packages.

### 3. RESULTS AND DISCUSSIONS

13 ISSR primers were used to assess the genetic diversity of a group of *O. cumana* populations from several countries in the Black Sea basin. Of the 23 populations sampled, population RM11 had the highest observed number of alleles ( $N_a = 1.53$ ), but population B2 had the lowest ( $N_a = 1.17$ ), the maximum value of the effective number of alleles was recorded in population T2 ( $N_e = 1.30$ ) and the minimum in population B2 ( $N_e = 1.08$ ) (Fig. 1). The Nei's gene diversity index ( $H$ ) for all populations was estimated to be between 0.17 (RM11 and T2) and 0.05 (B2). Shannon's information index ( $I$ ) has varied between 0.26 (RM11) and 0.08 (B2). The number of polymorphic loci (NPL) and the percentage of polymorphic loci (PPL) ranged from 175 (RM11) to 55 (B2) and 52.87 (RM11) to 16.62 (B2), respectively.

The results, obtained on the average value of the indices of genetic diversity among 23 studied broomrape populations, showed that the highest observed number of alleles ( $N_a = 1.37$ ) and the effective number of alleles ( $N_e = 1.24$ ), the highest value of Nei's gene diversity ( $H = 0.14$ ) and Shannon's Information index ( $I = 0.21$ ), as well as the largest number of polymorphic loci ( $NPL = 123$ ) and the highest percentage of polymorphic loci ( $PPL = 37.16$ ) were found for populations from Turkey, followed by Moldova and Romania (Fig. 1). The lowest mean values were observed for populations from Bulgaria (1.19, 1.09, 0.06, 0.09, 62.75 and 18.96, respectively). Moreover, it was revealed that the range of variation of  $N_a$ ,  $N_e$ ,  $H$ ,  $I$ , NPL, and PPL values was much wider for Moldavian populations (1.21-1.53, 1.11-1.28, 0.07-0.17, 0.10-0.26, 69-175, 20.85-52.87, respectively) than for the Turkish ones (1.29-1.43, 1.15-1.29, 0.09-0.17, 0.14-0.25, 95-143, 28.70-43.20, respectively) (Fig. 1). The Bulgarian population, on the other hand, had a relatively



**Figure 1.** Genetic diversity indices for each separate population *O. cumana* revealed by ISSR markers: *Na* – observed number of alleles, *Ne* – effective number of alleles, *H* – Nei's gene diversity, *I* – Shannon's Information index, *NPL* – number of polymorphic loci, *PPL* – percentage of polymorphic loci.

small range of variation (1.17-1.23, 1.08-1.12, 0.05-0.07, 0.08-0.11, 55-77, 16.62-23.26, respectively) compared to all the populations studied.

At each polymorphic locus, the total allelic diversity is known to be represented by the expression  $H_t = H_s + D_{st}$ , where  $H_s$  is the mean within-population allelic diversity and  $D_{st}$  is the among-population allelic diversity. These quantities are, in turn, related to the fraction of total allelic diversity found between populations ( $G_{st}$ ) by the expression  $D_{st}/H_t$  [8]. According to the analysis of the population genetic structure for all loci of *O. cumana* species, the genetic diversity at the species level ( $N_a = 2.00$ ,  $N_e = 1.38$ ,  $H = 0.23$ ,  $I = 0.37$ ,  $H_t = 0.24$ ,  $H_s = 0.18$ ,  $NPL = 331$ ,  $PPL = 100\%$ ) was higher compared to the genetic diversity at the population level (Fig. 1, Table 1). However, when the expected proportion of heterozygous genotypes per total sample ( $H_t = 0.24$ ) was compared with the expected proportion of heterozygous genotypes within populations for all loci ( $H_s = 0.18$ ), the low population heterozygosity among populations ( $D_{st} = 0.07$ ) was noted, suggesting that the genetic variation of the broomrape studied is mainly within populations (Table 1). Based on the analysis of genetic diversity parameters, it can be concluded that Turkish broomrape exhibits the highest genetic diversity among the broomrape populations compared. It is

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also clear that Turkish broomrape is more distantly related to broomrape from European countries. However, Moldavian populations showed a wider range of variation in genetic diversity indices. Thus, the frequency and abundance of common alleles in the broomrape gene pool in the Black Sea basin have changed, and there is a high probability that new virulent genes will emerge over time.

**Table 1.** Genetic diversity parameters and differentiation of *O. cumana* for all loci.

	Sample size	Na	Ne	H	I	Ht	Hs	Dst	Gst	Nm (Gst)	NPL	PPL
Mean	269	2.00	1.38	0.23	0.37	0.24	0.18	0.07	0.28	1.30	331	100
SD	-	0.00	0.32	0.17	0.22	0.03	0.02	-	-	-	-	-

*Na* – observed number of alleles, *Ne* – effective number of alleles, *H* – Nei’s gene diversity, *I* – Shannon’s Information index, *Ht* – total gene diversity, *Hs* – gene diversity within populations, *Dst* – gene diversity among populations,  $Gst = (Ht-Hs)/Ht$ , coefficient of gene differentiation among populations, *Nm* – gene flow among populations from *Gst*, *NPL* – number of polymorphic loci, *PPL* – percentage of polymorphic loci, *SD* – standard deviation

**Table 2.** Distribution of genetic diversity in *O. cumana* populations by AMOVA.

Variation source	DF	SS	MS	Est. Var.	Phi-statistics	Variance percentage, %
AC	3	2461.45	820.48	12.69	PhiRT=0.28	28
AP	19	3760.41	197.92	15.44	PhiPR=0.47	34
WP	246	4209.91	17.11	17.11	PhiPT=0.62	38
TOTAL	268	10431.8	-	45.25	-	100

*AC* – among countries, *AP* – among populations, *WP* – within populations, *DF* – degree of freedom, *SS* – sum of squares, *MS* – mean squares, *Est. Var.* – estimate of variance component, *PhiRT* – among region variation, *PhiPR* – among population variation within region, *PhiPT* – total variation within all populations.

The *Gst* is a measure of population differentiation and its values range from zero to one. Genetic differentiation among populations of *O. cumana* species was high (*Gst* = 0.28) (Table 1), meaning that approximately 72% of the total genetic variation occurred

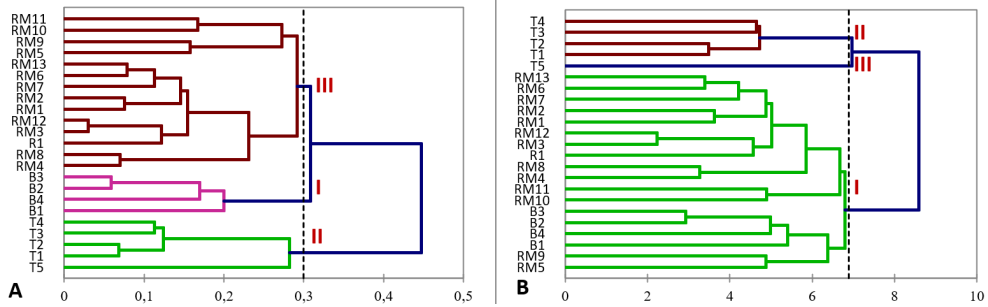
within populations, while only 28% of the genetic variation was among populations, following the classification of  $G_{st}$  degree established by Buso [8]. The transfer of genetic material from one population to another can significantly influence the dynamics of gene frequencies within an entire species. This process can also promote local adaptation and co-evolution between parasites and their hosts by introducing new alleles or beneficial mutations to populations with limited genetic diversity. Populations will differentiate locally when  $N_m$  is less than 1, and there will be little differentiation among populations when  $N_m$  is greater than 1 [9]. At the species level, the gene flow value among the broomrape populations exceeded 1 ( $N_m=1.30$ ) (Table 1). This indicates that there has been moderate gene flow among populations with little differentiation [10].

A dendrogram of the 23 abovementioned populations was constructed, based on Euclidean genetic distances and Pearson's dissimilarity, using UPGMA cluster analysis. The populations studied were partitioned into three main groups in both methods (Fig. 2). However, the clustering patterns generated, using Euclidean genetic distances and Pearson's dissimilarity, were unidentical and contained a slightly different composition of the groups. Based on Euclidean clustering, group I comprised the populations of Bulgaria, Moldova, and Romania. Meanwhile, the Turkish populations T1-T4 formed group II, while the T5 population fell into a separate third group. According to the Pearson clustering approach, the genetic variation among the Bulgarian (B1- B4) and Turkish (T1-T5) broomrape populations placed these populations in the first and second separate groups, respectively. The third group contained thirteen populations from Moldova (RM1-RM13) and one Romanian population (R1). These results suggest that the Turkish populations are genetically distant from the European broomrape populations according to these clustering patterns. The same cannot be said for the Bulgarian populations, which both resembled and differed from the Moldavian and Romanian broomrape populations, forming a separate group in one case and a combined group in the other. The Romanian and Moldavian populations were the most diverse of all the populations, showing many similarities and invariably forming a separate group.

The PCA analysis supported population clustering of different origin broomrape, derived from the dendrograms (Fig. 2, 3). The proportions of variation of the first and second components were 32.97 and 15.53%, respectively, of the total variability of the molecular data in 48.50%.

The results of PCA showed that the Middle East broomrape collection from Turkey was far from the other 18 Eastern European populations from Bulgaria, Moldova, and Romania and formed a separate group with two subgroups (T5 and T1-T4). Moreover, the PCA presented that both groups were distributed along two opposite coordinate

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**Figure 2.** The relationships between 23 sunflower broomrape populations based on ISSR data using UPGMA methods with Pearson's dissimilarity (A) and Euclidean genetic distances (B).

axes. Such a distribution mainly reflects the geographical location of populations. The genetically closest populations (Moldova, Romania, and Bulgaria) were combined, while those farthest apart resulted in separate groups (the first group – Turkey, the second group – Moldova, Bulgaria, and Romania).

In the present study, the different origin broomrape populations from the Black Sea basin were selected to acquire a deeper insight into their population structure and genetic variability at the species level. As the emergence of more aggressive broomrape populations (races), which can rapidly spread to new areas, has been observed in the last 20 years in the sunflower-producing countries covered in this article (Moldova, Turkey, Romania, and Bulgaria), many breeders and geneticists are interested in the operational detection of the different interactive genetic processes (mutation, genetic drift, gene flow, etc.) within species for constant monitoring of broomrape resistance [11, 12].

Genetic analysis, using ISSR markers in different broomrape populations, showed the presence of very high genetic diversity, especially within populations, whereas little differentiation was observed among populations. The level of ISSR diversity was quite high among 269 accessions studied according to the genetic diversity indices ( $N_a = 2.00$ ,  $N_e = 1.38$ ,  $H = 0.23$ ,  $I = 0.37$ ) (Table 1). The gene diversity values among and within broomrape populations were the indicators of total genetic polymorphism in the species. It was found that the gene diversity within all populations ( $H_s = 0.18$ ) was significantly higher than the gene diversity among populations ( $D_{st} = 0.07$ ) to the total gene diversity ( $H_t = 0.24$ ), signifying the low interpopulation heterozygosity and suggesting that the genetic variation of the studied broomrape was mainly within populations. The  $G_{st}$  value of 0.28 confirmed that 72% of the genetic variation was within populations. Moreover, the moderate gene flow value ( $N_m = 1.30$ ) also confirmed that a significant degree of gene

exchange between different populations was one of the reasons for little differentiation among populations [10]. The AMOVA test revealed the same pattern, showing high genetic differences within populations (38%), a low level among the populations (34%), and a lower level among countries (28%) (Table 2). Based on these results, it is possible to conclude that a main genetic pool exists in the Black Sea Basin, comprising populations originating from Bulgaria, Turkey, Moldova, and Romania. This study demonstrated the existence of regular gene flow among populations of the *O. cumana* gene pool distributed in the Black Sea basin, which could be attributed to the frequency of cross-pollination and self-pollination within a species. The recent study confirms the occurrence of a relatively high rate of cross-fertilization in *O. cumana* plants, ranging from 14.8% to 40.0% [13], which may be a major creative force of the race evolution in *O. cumana*, with reassortment of avirulence genes conferring specificity against resistance genes, as also proposed by Joel [14]. The opposite results of low intrapopulation and high interpopulation genetic variation in *O. cumana* from several countries (Bulgaria, Turkey, Romania, and Spain) using RAPD markers were reported by Gagne [15]. Pineda-Martos [16] also demonstrated extremely low intra- and interpopulation genetic variation in two main gene pools of *O. cumana* in Spain, using SSR markers, probably due to a founder effect. However, in another study, RAPD analysis revealed high intrapopulation diversity in Serbian *O. cumana*, which can be explained by the fact that broomrape plants collected from different agricultural regions in Serbia, belong to the same population with high genetic heterogeneity [17]. Similar results about a rather high proportion of the intrapopulation genetic diversity were obtained from the genetic variability studies in populations from Russia, Kazakhstan, Romania, Tunisia, and Turkey, using SSR markers [18, 19, 20]. Two different methods of multivariate analysis, PCA and cluster analysis were used to group the broomrape accessions in this study. The resulting UPGMA-based dendrograms divided 23 populations by region into two main groups, with some subgroups depending on which distance methods were used (Fig. 2, 3). Group I was closely related to the Middle East, while Group II, consisting of Moldavian, Bulgarian, and Romanian population collections, belonged to Eastern Europe. The second group of populations shared some of the same genetic characteristics, which could indicate their monophyletic origin. The clustering results of both UPGMA and PCA analyses support this conclusion (Fig. 2, 3). The same results were obtained in another of our studies on the genetic relationships between broomrape of different origins, based on the frequency distribution of alleles at the country level using ISSR markers. The genome of Turkish broomrape was shown to have a specific microsatellite allele distribution that differed from that of Bulgarian, Moldavian, and Romanian broomrape [21].

#### 4. CONCLUSIONS

In conclusion, our results demonstrated the effectiveness of ISSR marker systems in elucidating species population structure and genetic variability. The investigation revealed that the different broomrape populations have a significant level of genetic diversity, especially within populations, whereas little differentiation was observed among populations. We suggest that there is a main gene pool of *O. cumana* in the Black Sea basin, comprising populations from Bulgaria, Turkey, Moldova, and Romania, supported by regular gene flow among populations. Based on the results of UPGMA and PCA analyses, Moldavian, Bulgarian, and Romanian broomrape populations were found to have more genetic similarities with each other than with Turkish populations within the main gene pool of *O. cumana* in the Black Sea basin. The groupings of broomrape were mainly influenced by its geographical origin, as well as genetic differences and similarities that have accumulated over time. Clustering and PCA analyses confirmed that these patterns were not associated with virulence.

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## [CaL<sub>3</sub>][Co(NCS)<sub>4</sub>] - potential agent for enhancing the productivity of *Chlorella Vulgaris* Beijer. microalga

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EDUARD COROPCEANU , AND LILIA BRÎNZĂ 

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**Abstract.** The advancing of modern biotechnologies requires the development of new procedures for stimulating the biological activity of some cultures that produce substances of vital importance. Coordination compounds present an effective solution for achieving this objective. The inclusion of some biometals in the composition of the coordination compounds, as well as some organic ligands with electron-donating atoms, creates premises for the assembly of molecules that can influence biological systems. The productivity of the microalgae *Chlorella vulgaris* Beijer., cultivated on modified nutrient media and supplemented with a coordination compound containing calcium, was evaluated. It was found that increased concentrations of this compound have an inhibitory action on *Chlorella* strain. A stimulatory effect was registered when the concentrations of 10 mg/L, 5 mg/L and 1 mg/L of the tested coordination compound were administered, the biomass obtained was higher by 10.8%, 7.6% and 5.4% in relation to the control group.

**Keywords:** *Chlorella vulgaris*, Ca(II), biomass, biostimulator, cultivation.

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## [CaL<sub>3</sub>][Co(NCS)<sub>4</sub>] - potențial agent pentru sporirea productivității microalgei *Chlorella Vulgaris* Beijer

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**Rezumat.** Dezvoltarea biotehnologiilor moderne solicită elaborarea noilor procedee de stimulare a activității biologice a unor culturi producătoare de substanțe cu importanță vitală. Compușii coordinațivi prezintă o soluție eficientă pentru realizarea acestui obiectiv. Includerea în componența compușilor coordinațivi a unor biometale, precum și a unor liganzi organici cu atomi donori de electroni crează premise pentru asamblarea unor molecule ce pot influența sistemele biologice. A fost evaluată productivitatea microalgei *Chlorella vulgaris* Beijer., cultivată pe medii nutritive modificate și suplimentate cu un compus coordinațiv cu conținut de calciu. S-a constatat că concentrațiile sporite ale acestui compus au acțiune inhibitoare asupra tulpinii *Chlorella*. Efect stimulator s-a înregistrat la administrarea concentrațiilor de 10 mg/L, 5 mg/L și 1 mg/L a compusului coordinațiv testat, unde biomasa obținută a fost mai mare cu 10,8%, 7,6% și 5,4% în raport cu martorul.

**Cuvinte-cheie:** *Chlorella vulgaris*, Ca(II), biomasă, biostimulator, cultivare.

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## 1. INTRODUCTION

The growing needs of society in substances of natural origin that possess properties to influence biological systems require new solutions for increasing the productivity of certain cultures. Algae are a rich natural source of bioactive compounds, which have different applications in agriculture, pharmaceutical, food, cosmetic and perfumery industries, etc. Microalgae and cyanobacteria biomass extracts, as well as the filtrates resulting after their cultivation, can be used as biostimulators and antimicrobial substances in cultivation of some crops. These substances can have a positive impact on some organisms with an important role in substituting synthetic products with toxic action on the environment and human health [6; 8]. A number of factors, including the chemical one, can influence the synthesis of biologically active substances. A class of chemical compounds with a special potential are the coordination complexes which, due to the diversity of their composition and structural architecture, show various useful properties, including stimulation of biochemical processes in some species of algae [2; 3]. Combining in one compound one or more bioactive metal ions, as well as organic molecules containing various functional groups, creates favorable prospects for expressing the synergistic effect of metal complexes on the physiological systems of microorganisms.

*Chlorella vulgaris* Beijer. is a single-celled autotrophic protist with a coccoidal structure. The cells are spherical or ellipsoidal, solitary of 2.2-7.5  $\mu$  in diameter (or aggregated), with thin and smooth cellulosic walls, which do not gel. In some senescent cells (larger in size – 10.0-13.3  $\mu$ ) the vacuoles can be observed [5]. They have a parietal cupular chromatophore, with a single pyrenoid (sometimes missing) surrounded by 2-4 starch granules. *Chlorella* reproduces mainly asexually via 2-8 or, rarely, 16 spores, which are formed in autosporangia (mother cells). Autosporangia have a spherical-ellipsoidal shape with a diameter of 7-8  $\mu$ . It is a  $\alpha$ -mesosaprobe species commonly distributed in all types of freshwater pools. It can be found on all types of soil. *Chlorella vulgaris* is an autotroph, but under conditions of excess organic substances dissolved in the water tank, it switches to heterotrophic mode of nutrition. This species has a high tolerance to environmental conditions, increased adaptability; therefore, it can easily be cultivated in controlled laboratory conditions, serving as test object in various physiological, biochemical, biophysical, genetic researches, etc. [1].

The purpose of this work is evaluating the action of the coordination complex containing calcium and cobalt metal cations  $[\text{CaL}_3][\text{Co}(\text{NCS})_4]$  ( $\text{L}_3$  – dimethylpyridine-2,6-dicarboxylate) on the accumulation of the microalga *Chlorella vulgaris* Beijer. biomass.

## 2. MATERIALS AND METHODS

Heterobimetallic complex [CaL<sub>3</sub>][Co(NCS)<sub>4</sub>] was obtained in the reaction of 2,6-pyridinedicarbonyl dichloride with calcium(II) thiocyanate tetrahydrate and cobalt(II) thiocyanate trihydrate in methanol, according to the method described in the literature [10, 11]. The synthesis of the coordination complex was carried out in the Laboratory of Coordination Chemistry of the Institute of Chemistry of the MSU.

The biological investigations were carried out in the “Ecological Biotechnologies” scientific laboratory of “Ion Creanga” State Pedagogical University. The *Chlorella vulgaris* Beijer. microalga strain supplied by the Institute of Microbiology and Biotechnology (deposited in the National Collection of Nonpathogenic Microorganisms) served as the object of study. Cultivation was carried out in 100 mL Erlenmeyer flasks closed with cotton plugs with periodic slow stirring. Borsch liquid medium with the following chemical composition was used as a nutrient substrate (g/L): NH<sub>4</sub>NO<sub>3</sub> - 0.1; KH<sub>2</sub>PO<sub>4</sub> - 0.04; FeSO<sub>4</sub>·7H<sub>2</sub>O - 0.00001; MgSO<sub>4</sub>·7H<sub>2</sub>O - 0.04; CaCl<sub>2</sub> - 0.02; solution of microelements - 1.8 mL (g/L: H<sub>3</sub>BO<sub>3</sub> - 2.86; MnCl<sub>2</sub>·4H<sub>2</sub>O - 1.82; ZnSO<sub>4</sub>·7H<sub>2</sub>O - 0.222; MoO<sub>3</sub> - 0.01764; NH<sub>4</sub>VO<sub>3</sub> - 0.02296) [9].

In the case of the experimental series, the liquid culture medium was supplemented with [CaL<sub>3</sub>][Co(NCS)<sub>4</sub>] coordination complex in concentrations of 50 mg/L, 10 mg/L, 5 mg/L and 1 mg/L.

The amount of inoculum was 0.625 g/L of fresh biomass [4]. The Erlenmeyer flasks inoculated with *Chlorella* chlorophyte were placed on special racks under artificial light of about 4000 lx and a temperature of 27°C.

After 8 days of action of the coordinating compound, the *Chlorella vulgaris* strain was subjected to study. The statistical processing of the data obtained was carried out using the “STATISTICA 7” software, and the standard error of the mean was also determined. The productivity of *Chlorella vulgaris* chlorophyte was determined according to the current methodology [6; 7].

## 3. RESULTS AND DISCUSSIONS

For the successful cultivation of microalgae under controlled conditions, providing them with the chemical elements necessary for mineral nutrition is of great importance. For the synthesis of proteins, carbohydrates, lipids and other cellular components, most of them require such macroelements as N, P, K, Mg, S, Ca, etc., as well as the microelements Fe, Mn, B, Sr, Cu, Zn, Ba, Ti, Mo et al. Under favourable nutritional conditions microalgae cultures accumulate impressive amounts of biomass in a short time [6].

The investigations carried out demonstrated that the calcium coordination compound content added to the Borsch liquid nutrient medium has a different stimulatory/inhibitory action on *Chlorella vulgaris* chlorophyte. The result depends primarily on the concentration of the chemical complex used, as well as on the light intensity, temperature and other abiotic environmental factors that accompany the cultivation process. Thus, after 8 days of *Chlorella vulgaris* Beijer strain cultivation on the Borsch culture medium supplemented with the calcium coordination compound, a greater amount of biomass was detected in the experimental series with the calcium compound concentration ranging from 1 mg/L to 10 mg/L. The optimal concentration of the calcium coordination complex with a stimulating effect on the productivity of *Chlorella vulgaris* strain proved to be 10 mg/L, where the fresh biomass obtained was of about 7.38 mg/L, being 10.8% higher than the accumulated biomass in the control series (Tab. 1).

The accumulated biomass of the microalgae tested in this experimental series after 8 days of cultivation was about 12-fold higher compared to the one initially administered in Erlenmeyer flasks. The microalgae productivity decreased together with concentration of the complex. In the case of series supplemented with the calcium compound in a concentration of 5 mg/L, the productivity of the chlorophyte was about 7.17 g/L, while at the concentration of 1 mg/L it accumulated about 7.02 mg/L, exceeding the control values by 7.6% and 5.4%, respectively. The complex administered in concentrations lower than 1 mg/L had an insignificant stimulatory effect and the fresh biomass accumulated by the *Chlorella* strain was approximately at the respective level in the control series.

**Table 1.** Fresh biomass accumulated by *Chlorella vulgaris* when treated with the  $[\text{CaL}_3][\text{Co}(\text{NCS})_4]$  compound

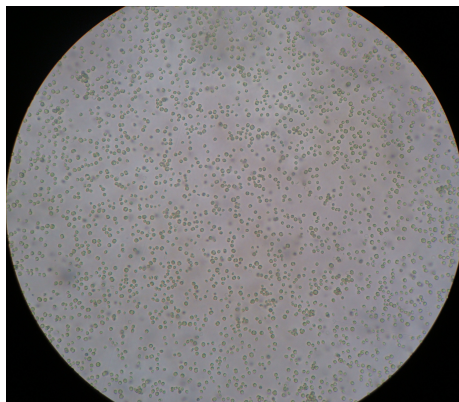
Nr.	Series	Starting fresh biomass, g/1000 mL	Fresh biomass after 8 days of cultivation, g/1000 mL		$\Delta$	
			$\bar{x} \pm m_x$	$\sigma$		
1.	Control	0.625	6.66 $\pm$ 0.18	0.36	-	
2.	$[\text{CaL}_3][\text{Co}(\text{NCS})_4]$	50 mg/L	0.625	6.04 $\pm$ 0.41	0.72	-9.3
3.		10 mg/L	0.625	7.38 $\pm$ 0.08	0.17	10.8
4.		5 mg/L	0.625	7.17 $\pm$ 0.18	0.36	7.6
5.		1 mg/L	0.625	7.02 $\pm$ 0.10	0.20	5.4

In the experimental series with the calcium complex supplemented to the Borsch culture medium in a concentration of 50 mg/L, a lower productivity of the *Chlorella* culture was

[CAL<sub>3</sub>][CO(NCS)<sub>4</sub>] - POTENTIAL AGENT FOR ENHANCING THE  
PRODUCTIVITY OF *CHLORELLA VULGARIS* BEIJER. MICROALGA

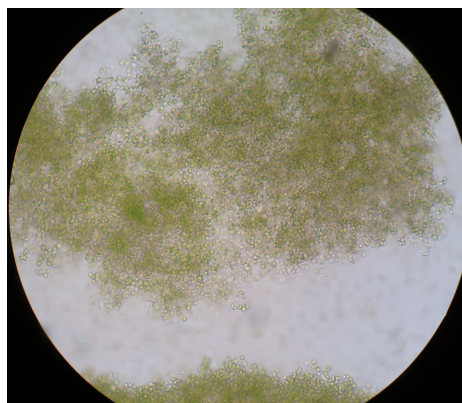
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recorded, the biomass accumulated being by about 6.04 g/L, or 9.3% lower than the values in the control group. Thus, increased concentrations of the complex inhibit the growth and development of the *Chlorella vulgaris* strain.



**Figure 1.** *C. vulgaris* strain in series with complex 1 in concentration of 10 mg/L (400 x).

In the first days of cultivation, the *Chlorella vulgaris* strain was in the phase of latency and growth acceleration. Under the microscope, numerous small cells with dimensions of 2.5-3.0  $\mu$  (autospores), distributed among larger and senescent cells of 5.5-7.5  $\mu$ , were observed. On the 8th day of cultivation, the biomass accumulated in the series administrated with 50 mg/L of the Ca(II) had a different colour compared to the control – a green-yellowish hue, while the series treated with coordination compound concentrations of 10 mg/L, 5 mg/L and 1 mg/L biomass had a green colour (Fig. 1; 2).



**Figure 2.** *C. vulgaris* strain in series with complex 1 in concentration of 50 mg/L (400 x).

In case of the samples treated with the Ca(II) complex in concentrations of 50 mg/L, the cells viewed in the microscope had a more rounded shape, were deformed, had a diameter of 4.0-8.0  $\mu$  and were included in large agglomerations (aggregates).

The results obtained confirm the importance of the calcium coordination complex content added to the Borsch liquid nutrient medium for the growth of the *Chlorella vulgaris* Beijer. chlorophyte, as well as the concentration of the administered active substance, which stimulates its development.

#### 4. CONCLUSIONS

- (1) The results obtained demonstrated that the calcium coordination compound supplemented to the Borsch liquid nutrient medium has a different stimulating action on the *Chlorella vulgaris* culture. Its effect depends primarily on the concentration of the administered compound, as well as on the abiotic factors (temperature, lighting, etc.) accompanying the cultivation.
- (2) Increased concentrations of 50 mg/L proved to be inhibitory on the *Chlorella* culture. The most significant data were obtained in the series with the Ca(II) complex concentration of 10 mg/L, where the investigated strain accumulated up to 7.38 g/L of the biomass, as well as the concentration of 5 mg/L, and where a higher productivity of 10.8% and 7.6% was recorded compared to the control series.

#### Acknowledgements

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[CAL<sub>3</sub>][CO(NCS)<sub>4</sub>] - POTENTIAL AGENT FOR ENHANCING THE  
PRODUCTIVITY OF *CHLORELLA VULGARIS* BEIJER. MICROALGA

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# The dependence of the adaptation of the cardiovascular system in adolescents on the state of some environmental factors

DIANA KOSHKODAN  AND LORA MOSHANU-SHUPAC 

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**Abstract.** This article presents the experimental data related to the ecological factors that have an impact on the adaptation of children and adolescents. The selected ecological factors are the air and water quality in the areas surrounding the investigated educational institutions. It was established that there is a correlation between the degree of pollution of the external environment and the cardiovascular indices of adolescents. Thus, for the students from the academic institutions located in the more polluted areas, the indices of heart economy and the restoration of the frequency of heart contractions after physical effort are lower compared to those of students located in the educational institutions from the less polluted areas.

**Keywords:** adolescents, ecological factors, health, adaptation, cardiovascular system.

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## Dependența adaptării sistemului cardiovascular la adolescenți de starea unor factori de mediu

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**Rezumat.** Sunt prezentate datele experimentale legate de factorii ecologici care au impact asupra adaptării copiilor și adolescenților. Ca factori ecologici au fost selectați starea aerului și a apei din zonele instituțiilor de învățământ. S-a stabilit prezența corelației dintre gradul de poluare a mediului extern și indicii cardiovasculari ai adolescenților. Astfel, pentru elevii din instituțiile situate în zonele mai poluate, indicii de economie a inimii și de restabilire a frecvenței contracțiilor cardiace după efort fizic sunt mai mici față de valorile elevilor din liceele din zonele mai puțin poluate.

**Cuvinte-cheie:** adolescenți, factori ecologici, sănătate, adaptare, sistem cardiovascular.

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### 1. INTRODUCTION

The incidence of neurosis of children increases as the learning period extends [4]. Neurotic disorders are more common in boys, most of who are in their crisis age. School inadequacy also contributes to neurosis [1,5]. The incidence of neurosis of adolescents is higher, especially with a predominance of anxiety and depression, which are more common in girls.

## THE DEPENDENCE OF THE ADAPTATION OF THE CARDIOVASCULAR SYSTEM IN ADOLESCENTS

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One of the strategies employed by psychologists is monitoring and intervening during the pupils' adaptation to school. According to research by Furdui T. et al. [6], the process of adapting by education includes several stages:

- First stage - characteristic to the first stage are stormy reactions and significant tension of all organ systems. This lasts 2-3 weeks.
- Second stage – adaptation is unstable when the organism searches the optimal body side of impact factors. Energy costs are decreased.
- Third stage - the adaptation is unstable when the body finds optimal variants of reactions, with minimal costs, but the possibilities of a child's body are limited, and long-term effects can influence their health.

Children are classified as children with easy, moderate and difficult adaptation. In easy adaptation, the organism's tension is compensated during the first half of the year. In moderate adaptation, functional disorders may be present also in the first half of the year. In difficult adaptation, health disorders increase towards the end of the school year, which shows that the educational system and information put pressure on children. The proposed criterion of easy adaptation is the level of work capacity during the first half of the year, the absence of adverse changes in health and better assimilation of the curriculum. The indicator of difficult adaptation are the changes in the child's behavior [2].

The factors that that cause inadequacy are:

- School factor - lack of individual approach towards the child, inadequate educational measures, signs of disrespect.
- Family factor - unfavorable emotional and material situation in the family, parents' alcoholism, lack of attention or the contrary, hyperattention.
- Microsocial factor - negative influence, access to money and alcohol.
- Macrosocial factor - deformation of social and moral ideals, propaganda of violence.
- Somatic factor - severe chronic somatic diseases, disorders of locomotion, hearing, vision, speech.

## 2. PURPOSE OF THE WORK

Adaptation is an important factor in the educational process, which itself is influenced by many factors. This article analyzes the influence of ecological factors on the physiological parameters of children in order to determine their level of adaptation.

### 3. MATERIALS AND METHODS

The study was conducted in two high schools, located in different zones of the anthropic factor. All children (90 people, aged 16-17) participating in the studies were practically healthy. The degree of environmental pollution was proved by the number of factories in the zone, as well as by objective indicators of the air and water quality. To assess the functional state of the cardiovascular system, the heart rate (HR), systolic and diastolic blood pressure (PS and PD) were measured. In order to study the degree of functional recovery of the cardiovascular system, measurements were taken before the load, immediately after it, after 2 minutes and after 10 minutes. Calculation of the coefficient of efficiency of blood circulation (KEK) was carried out according to the formula:  $KEK = (PS - PD) * Heart\ rate\ (HR)$

The assessment of the vegetative status of the child included the calculation of the Kerdo index:  $IK = PS / HR$  [4].

### 4. RESULTS AND DISCUSSIONS

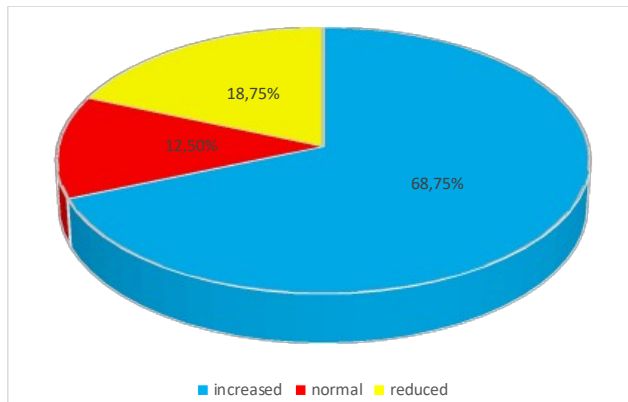
The 21st century is characterized not only by the rapid development of the scientific and technological revolution, but also by the emergence of universally recognized dangers for the continued existence of civilization – ecology, nutrition, energy, and demography. Most countries of the European continent are facing demographic problems. Therefore, it is all the more crucial to ensure the younger generation's health. At present, heart diseases occupy the leading place in the structure of the causes of death in the economically developed countries. In different countries, the share of these diseases in total mortality ranges from 30% to 60%. Among other causes of cardiovascular diseases, the effect of air pollution has been noted [3].

The studies of the economy of blood circulation showed that in the studied schools, the KEK increased in most student, which indicates the stress of the state of the cardiovascular system. KEK was increased in 68.75% of students living in a less polluted zone (Fig. 1), and 50% of teenagers from a more polluted area.

The increase in the coefficient indicated a reorganization of the central circulation, which is associated with an increased costs of body reserves. At the same time, there was no student found in the polluted zone who has a normal coefficient of efficiency of blood circulation (KEK) [5]. On the other hand, in an ecologically more favorable zone, a normal KEK was observed in 12.5% of the children.

Thus, a greater number of children in the ecologically favorable area (87.5%) have the HR index within normal range over one minute after exercise. However, this index is

## THE DEPENDENCE OF THE ADAPTATION OF THE CARDIOVASCULAR SYSTEM IN ADOLESCENTS

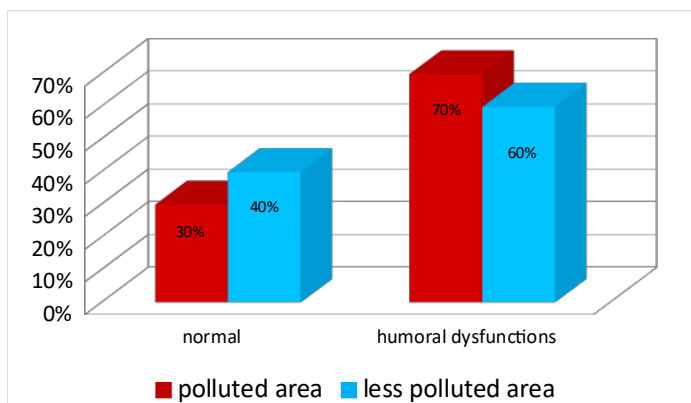


**Figure 1.** Coefficient of efficiency of blood circulation in the less polluted area.

smaller in value for the children in the polluted area (only 70%). HR over five minutes after exercise is not restored to normal in 60% of children in the polluted area and 37.5% of children in the ecologically favorable area (the difference between the results is statistically significant) [7]. Thus, cardiovascular parameters are influenced by ecological factors – particularly by the composition of the air.

The assessment of the vegetative status of the child are demonstrate in Fig. 1. Research on the functions of the endocrine system (Kerdo Index) has shown that most students have hormonal disorders - 60% in less polluted areas, and 70% in polluted areas.

It is concerning that endocrine disorders are present in most children, regardless of the degree of pollution of the area (Fig. 2).



**Figure 2.** Kerdo index for students in areas with different levels of pollution.

## 5. CONCLUSIONS

The cardiovascular parameters of children are influenced by ecological factors, particularly by the composition of air.

Students from both ecological areas showed disturbances in the activity of the cardiovascular and endocrine systems. For the students from the academic institutions located in the more polluted areas, the indices of heart economy and the restoration of the frequency of heart contractions after physical effort are lower compared to the values displayed by the students in the academic institutions from the less polluted areas

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## Floristic notes from Bessarabia No. 241-276

PAVEL PÎNZARU , VICTOR SFECLĂ , AND VALENTINA CANTEMIR 

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**Abstract.** The paper presents new growing stations for 36 species of rare vascular plants from the spontaneous flora of the Republic of Moldova, highlighted as the result of the new floristic investigations carried out from the spring to the summer of the current year. The species *Valerianella eriocarpa* Desv. was recorded for the first time in the local flora, the presence of 7 rare species, little known previously, was confirmed and they were proposed to be included in *the List of protected species* and in *The Red Book of the Republic of Moldova*, 4th edition: *Carex pseudocyperus* L., *C. strigosa* Huds., *C. vesicaria* L., *Epipactis leptochila* (Godfery) Godfery, *Fraxinus coriariifolia* Scheele, *Linum catharticum* L. *Senecio macrophyllus* M.Bieb., and for the species *Klasea bulgarica* (Acht. & Stoj.) Holub, indicated only for one station in Batîr commune, Cimişlia district, from where it disappeared, another station was registered in the same locality.

**Keywords:** spontaneous vascular flora, rare species, chorology, Republic of Moldova.

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## Note floristice din Basarabia Nr. 241-276

**Rezumat.** În lucrare sunt prezentate noi stațiuni de creștere pentru 36 specii de plante vasculare rare din flora spontană a Republicii Moldova, evidențiate ca rezultat al noilor investigații floristice din primăvara – vara anului curent. Specia *Valerianella eriocarpa* Desv. este înregistrată prima dată în flora locală, a fost confirmată prezența a 7 specii rare, puțin cunoscute anterior și se propun a fi incluse în *Lista speciilor ocrotite* și în *Cartea Roșie a Republicii Moldova*, ediția a 4-a: *Carex pseudocyperus* L., *C. strigosa* Huds., *C. vesicaria* L., *Epipactis leptochila* (Godfery) Godfery, *Fraxinus coriariifolia* Scheele, *Linum catharticum* L. *Senecio macrophyllus* M.Bieb., iar pentru specia *Klasea bulgarica* (Acht. & Stoj.) Holub, indicată numai pentru o singură stațiune din comuna Batîr, raionul Cimişlia, de unde a dispărut, a fost înregistrată o altă stațiune în aceeași localitate.

**Cuvinte-cheie:** flora vasculară spontană, specii rare, corologie, Republica Moldova.

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### 1. INTRODUCTION

In accordance with the release of the new scientific research project for the assessment of the plant species state and the elaboration of the rare species list, and with its algorithm for presenting them in the 4th edition of *The Red Book of the Republic of Moldova*,

new expeditions were organized on the territory of the Republic. The new floristic data highlighted in the current vegetation year is also the subject of continuity for the work “*Floristic Notes from Bessarabia*” No. 217-240 [14].

## 2. MATERIALS AND METHODS

The floristic and phytocenological investigations were carried out on the field in the districts from the north to the south of the Republic of Moldova such as: Ocnîța, Briceni, Edineț, Dondușeni, Rîșcani, Glodeni, Florești, Șoldănești, Fălești, Ungheni, Călărași, Orhei, Strășeni, Hîncești, Criuleni, Cimișlia, Taraclia and Cahul. Determining species was carried out according to the classical comparative-morphological method. Species nomenclature corresponds to the monograph “*Vascular flora of the Republic of Moldova (species list and ecology)*” [12]. In order to identify new growth characteristics of the plant species, there were consulted such sources as the bibliographic information and the dried collections from the Herbariums of the National Botanical Garden “Al. Ciubotaru” (CHGB) and of “Ion Creangă” State Pedagogical University of Chisinau (CH-HBPP), overlapping them with the field study results. The rarity category was established according to the criteria of the International Union for the Conservation of Nature (IUCN) [32]. The collected and herborized plants are kept in the CH-HBPP and CHIS herbarium collections in Chisinau. Phytocenological research was carried out by applying the methods of the Central European School [1].

The presence of the species is indicated by +RAR – rare species, /A -adventive species, +RM – new species for the flora of the Republic of Moldova, +TN – new species for a geographical sector.

Citation method: PÎNZARU, P. Note no. 218. *Carex curvata* Knaf (Cyperaceae) in PÎNZARU, P., CANTEMIR, V. (eds.) Floristic notes in Bessarabia. In: *Journal of Botany*, 2023, vol. XV, Nr. 1(26), pp. 56. ISSN 1857-2367 E-ISSN 2587-3814.

## 3. RESULTS AND DISCUSSIONS

As a result of the new floristic and phytocenological investigations in the field, of the analysis of dried specimens from the Herbarium collections (CHGB and CH-HBPP) and of the study of bibliographic references, new places of growth were found for 36 species of spontaneous vascular plants, out of which 1 new species for the flora of the Republic of Moldova (*Valerianella eriocarpa* Desv.) and 7 rare species, little known previously, were confirmed, which are proposed to be included in the *List of protected species* and in *The Red Book of the Republic of Moldova*, 4th edition: *Carex pseudocyperus* L., *C. strigosa* Huds., *C. vesicaria* L., *Epipactis leptochila* (Godfery) Godfery, *Fraxinus coriariifolia*

Scheele, *Linum catharticum* L. *Senecio macrophyllus* M.Bieb. For the first time in the flora of the “Pădurea Domnească” Scientific Reserve there are recorded 8 new species (*Androsace elongata* L., *Bromus ramosus* Huds., *Draba nemorosa* L., *Carex vesicaria* L., *Carex strigosa* Huds., *Ornithogalum umbellatum* L., *Scleranthus uncinatus* Schur, *Serratula coronata* L.) and 2 new species in the flora of the “Plaiul Fagului” Scientific Reserve (*Carex strigosa* Huds. and *Bromus ramosus* Huds.). Following, new chorological data for the researched species are presented.

PÎNZARU P.

**241. *Androsace elongata* L.** 1763, Sp. Pl., ed. 2: 1663 (Primulaceae).

+ **TN:** the species is recorded for the first time in the flora of the Scientific Reserve “Pădurea Domnească”, Vasileuți forest body, outskirts of the forest, on sandy soil, collected by P. Pînzaru, 15.06.2024 (CH-HBPP 9125). Eurasian species, annual, therophytic, xeromesophilic, steppe, sporadic throughout the territory of the republic [23].

PÎNZARU P.

**242. *Draba nemorosa* L.** 1753, Sp. Pl.: 643 (Brassicaceae).

+ **TN:** the species is recorded for the first time in the flora of the “Pădurea Domnească” Scientific Reserve, the “Vasileuți forest stand, outskirts of the forest, on sandy soil, collected by P. Pînzaru, 15.06.2024 (CH-HBPP 9126). Circumpolar species, annual, therophytic, mesophyllous. rare in the Republic of Moldova [10].

PÎNZARU P.

**243. *Euphrasia pectinata* Ten.** 1811, Prodr. Fl. Napol: 36 (Orobanchaceae).

+ **RAR:** Bumbota commune, Ungheni district, grows sporadically on a sandy hill with northern exposure, in phytocenoses dominated by *Anthericum ramosum* L., collected by P. Pînzaru, 29.07.2024 (CH-HBPP 9121; CHGB). Eurasian species, annual, therophytic, mesophilic, rare on the territory of the Republic of Moldova.

PÎNZARU P.

**244. *Fraxinus coriariifolia* Scheele,** 1843, Linnaea 17: 350 (Oleaceae).

+ **RAR:** Baurci village from Chircăiestii Noi commune, Căușeni district, collected by P. Pînzaru, 23.05.2024, grows sporadically in phytocenoses from the association *Iridio variegatae-Quercetum pubescentis* Pînzaru et al. 2022 (CH-HBPP 8750, 8751; CHGB), the trees are 8-10 m tall, fruiting. A species insufficiently known for the local flora it was cited on the basis of a single dried specimen (CHGB), collected by T. Gheideman, in the forest near Cioburciu commune, Slobozia district. [6]. Ponto-Caucasian species, micromagnerophyte-megaphanerophyte (can reach a height of up to 20 m), xeromesophilous, characteristic for the *Quercetalia pubescenti-petraeae* Klika 1933 order vegetation [19]. Very rare species it is proposed to be included in the *List*



of protected species of the Republic of Moldova and in the Red Book of the Republic of Moldova, 4th edition, Endangered category (EN).

PÎNZARU P.

**245. *Linum catharticum* L. 1753, Sp. Pl.: 281 (Linaceae).**

+ **RAR:** Bumbota commune, Ungheni district, grows sporadically on a sandy hill with northern exposure, in phytocenoses dominated by *Anthericum ramosum* L., collected by P. Pînzaru, 29.07.2024 (CH-HBPP 9096-9099; CHGB). This species was recorded in the glades of the northern districts of the Republic of Moldova: Ocnîța (Ocnîța and Mihălășeni communes), Dondușeni (Dondușeni town [18, 27] and Briceni (Cotiujeni) [7]. European/West-Asian species, therophyte, mesophyll, characteristic for the *Molinetalia caeruleae* Koch 1926 order vegetation [19]. Very rare species it is proposed to be included in the *List of protected species of the Republic of Moldova* and in the *Red Book of the Republic of Moldova*, 4th edition, status Critically Endangered (CR).

PÎNZARU P.

**246. *Linum nervosum* Waldst. & Kit. 1803, Descr. Icon. Pl. Hung. 2: 100 (Linaceae).**

+ **RAR:** Baurci village in Chircăiestii Noi commune, Căușeni district, growing in small groups, in a glade of downy oak (*Quercus pubescens* Willd.) with variegated stilts (*Iris variegata* L.), collected by P. Pînzaru, 23.05.2024 (CH-HBPP 8759, 8760). It is rarely found on the steppe hills of central and southern Bessarabia. Widespread species in South-Eastern and Eastern Europe, Caucasus, Turkey, Iran, Iraq, perennial, hemicryptophyte, xeromesophilous, characteristic for *Festucetalia valesiacae* Soó 1947 order vegetation [19].

PÎNZARU P.

**247. *Ornithogalum umbellatum* L. 1753, Sp. Pl.: 307 (Asparagaceae).**

+ **TN:** Cobani commune, Glodeni district, “Pădurea Domnească” Scientific Reserve, forest borders, 15.06.2024, was recorded by P. Pînzaru. Western and Central European species, perennial plant, geophyte, mesophilous. Species recorded for the first time in the flora of the Scientific Reserve “Pădurea Domnească”.

PÎNZARU P.

**248. *Valerianella eriocarpa* Desv. 1809, in J.Bot. (Desvaux) 2: 314 (Caprifoliaceae).**

+ **RM:** Baurci village in Chircăiestii Noi commune, Căușeni district, collected by P. Pînzaru, 23.05.2024 (CH-HBPP 8790, 8792; CHGB), grows in a glade of downy oak (*Quercus pubescens* Willd.) forest with variegated irises (*Iris variegata* L.), on an area of about 100 m<sup>2</sup>, with abundance + dominance 1-2. Species recorded in the local flora for the first time. Beyond the country's borders, it is spread in Western, Central, South-Eastern

and Northern Europe (Great Britain), Crimea, Caucasus, Turkey, North Africa. Annual, therophytic, xermesophilic species [34].

PÎNZARU P.

**249. *Valerianella rimosa*** Bastard 1814, in J.Bot. Agric. 3: 20 (Caprifoliaceae).

Syn.: = *Valerianella auriculata* DC. 1815, Fl. Franç., ed.3, 5: 492. – *V. bessarabica* Lipsky, 1894, Tr. Sanct.-Pet. Bot. Sada 13: 305. – *Valeriana auriculata* (DC.) Tardent, 1841, Essai Hist. Nat. Bessarabie: 65.

+ **RAR:** Baurci village in Chircăieștii Noi commune, Căușeni district, collected by P. Pînzaru, on 23.05.2024, 05.06.2024 (CH-HBPP 8791, 8797; 8918; CHGB), grows together with *Valerianella eriocarpa* Desv. in a glade within a downy oak (*Quercus pubescens* Willd.) forest with variegated iris (*Iris variegata* L.), covering an area of about 100 m<sup>2</sup>, with an abundance + dominance of 1-2. This species is also known from two other locations: Cornești commune, Ungheni district [27] and near Tătărăuca Veche commune, Soroca district [11, 29]. A species distributed in Central and Southern Europe, therophyte, xeromesophilic, characteristic of the *Caucalidion* Tx. ex von Rochow 1951 alliance vegetation [18].

PÎNZARU P., CANTEMIR V.

**250. *Hypericum tetrapterum*** Fr. 1823, in Novit. Fl. Svec.: 94 (Hypericaceae).

+ **RAR:** Căpriana commune, Strășeni district, was collected by P. Pînzaru, V. Cantemir, 01.08.2024 (CH-HBPP 9127; CHGB) grows in the meadow, about 80 specimens. In the Republic of Moldova it was known only from the Scientific Reserve “Codru” near Lozova commune, Strășeni district, it is included in *The Red Book of Moldova* (2015, status Critically Endangered (CR). A perennial plant, hemicryptophyte, mesohygrophyte. Outside the country it can be met in Western and South-Eastern Europe, Crimea, Caucasus, Asia Minor and North-Western Africa [36].

PÎNZARU P., CANTEMIR V.

**251. *Klasea bulgarica*** (Acht. & Stoj.) Holub, 1977, Folia Geobot. Phytotax. 12: 305 (Asteraceae).

Syn.: = *Serratula bulgarica* Acht. & Stoj. 1932, in Izv. Bulg. Bot. Družh. 5: 111. – *S. caput-najae* Zahar. 1946, in Bill. Sect. Sci. Acad. Roumanie, 28: 318.

+ **RAR:** Batîr commune, Cimișlia district, grows on the side of a forest road, in a phytocenosis of the *Iridio variegatae-Quercetum pubescentis* Pînzaru et al. 2022 association, collected by P. Pînzaru and V. Cantemir, 30.06.2021 (CH-HBPP 9122). 2 small groups of 7 and 21 specimens were recorded. Plants usually reproduce vegetatively, forming underground horizontal rhizomes that produce new plants. In ex situ conditions, they bloom in the 3rd year, reach a height of about 80 cm, form up to 5 anthodia, the

duration of flowering of flowers from an anthodia is 10 days, purple-pink flowers, form few glabrous, blackish achenes. This species was first indicated in Batîr, Cimişlia district by D. Gociu in 1979 (CHGB), where it vegetated in a meadow near a well [26], from where it later disappeared.

Rare species, a Balkan subendemyte, spread only in Bulgaria (north-eastern), Romania (south-eastern) and the Republic of Moldova (Batîr commune, Cimişlia district). A perennial plant, hemicryptophyte, mesophyllous (xeromesophylos), included in: *The Red Book of Moldova* (2015, Critically Endangered (CR)), *The Red Book of vascular plants Romania* (2009, Vulnerable (VU) [2]), *The Red Book of Bulgaria* (2011, Critically Endangered (CR) [25]). It is proposed to avoid collections for the herbarium in the known resort.

PÎNZARU P., CANTEMIR V., SLIVCA V.

**252. *Carex alba*** Scop. 1772, Fl. Carn. Ed. 2, 2: 216 (Cyperaceae).

+ **RAR:** Naslavcea commune, Ocnîţa district, “La 33 de vaduri” Landscape Reserve, acorn on the rock, collected by P. Pînzaru, V. Cantemir, V. Slivca, determined by P. Pînzaru, 15.06.2024, (CH-HBPP 8953); Arioneşti commune, Donduşeni district, sessile oak forest with white sedge, collected by P. Pînzaru, V. Cantemir, V. Slivca, determined by P. Pînzaru, 17.06.2024 (CH-HBPP 8954). Eurasian species, perennial, hemicryptophyte, chalcephy, xerophilous. In the Republic of Moldova, it is also nfound in the rocky forests surrounding the village of Verejeni, Ocnîţa district, Saharna commune, Rezina district, Trebujeni commune, Orhei district, Beloci and Molochişul Mare communes in Rîbniţa district [5].

PÎNZARU P., CANTEMIR V., SLIVCA V.

**253. *Carex vesicaria*** L. 1753, Sp. Pl.: 979 (Cyperaceae).

+ **RAR:** Cobani commune, Glodeni district, Scientific Reserve “Pădurea Domnească”, in “La Fontal” meadow, collected by P. Pînzaru, 19.06.2023 (CH-HBPP 6917); Balatina commune, Glodeni district, Scientific Reserve “Pădurea Domnească”, on the edge of “Potcoava” lake, collected by P. Pînzaru, V. Cantemir, V. Slivca, determined by P. Pînzaru, 11.06.2024, (CH-HBPP 8898, 8932; CHGB), other collections are not from the territory of the Republic of Moldova, although it is cited in 1841 by Ch. Tardent [23] from Chisinau, Orhei, Bălţi and in 1924 by T. Săvulescu and T. Rayss [16] from Corneşti, Ungheni district, from the last station disappeared, following the drying of the land. Circumpolar species, perennial, hemicryptophyte, mesohydrophilic, rare on the territory of the Republic of Moldova, it is proposed to be included in the *List of protected species* and *The Red Book of the Republic of Moldova*, 4th edition, status Endangered (EN).

PÎNZARU P., CANTEMIR V., SLIVCA, V.

**254. *Crambe tataria*** Sebeök, 1779, Diss. Tatar.: 7 (Brassicaceae).

+ **RAR:** Pietrosu commune, Făleşti district, grows on a steppe-like hill, collected by P. Pînzaru, V. Cantemir, V. Slivca on 10.06.2024 (CH-HBPP 8856). A Pannonian-Pontic-Sarmatian species, perennial, hemicryptophyte, xeromesophilic, included in *The Red Book of the Republic of Moldova* (2015), Endangered category (EN).

PÎNZARU P., CANTEMIR V., SLIVCA V.

**255. *Spergula arvensis*** L. 1753, Sp. Pl.: 440 (Caryophyllaceae).

+ **RAR:** Pietrosu commune, Făleşti district, grows sporadically on the edge of a field, collected by P. Pînzaru, V. Cantemir, V. Slivca, determined by P. Pînzaru, on 10.06.2024 (CH-HBPP 9011). A circumpolar species, annual, therophyte, mesophilic, known only from the central districts of the Republic.

PÎNZARU P., CANTEMIR V., SLIVCA V.

**256. *Stipa tirsia*** Steven, 1857, Bull. Soc. Imp. Naturalistes Moscou 30(II): 115 (Poaceae).

+ **RAR:** Pietrosu commune, Făleşti district, steppe-like hill, collected by P. Pînzaru, V. Cantemir, V. Slivca, determined by P. Pînzaru, on 14.06.2024 (CH-HBPP 9104). A eurasian species, perennial, hemicryptophyte, xerophilic, included in *The Red Book of the Republic of Moldova* (2015), Endangered category (EN), forms phytocenoses with an abundance + dominance of 2-5.

PÎNZARU P., CANTEMIR V., MANIC [218?]t.

**257. *Carpinus orientalis*** Mill. 1768, Gard. Dict., ed. 8: no. 3 (Betulaceae).

+ **RAR:** Suruceni commune, Ialoveni district, grows rarely in a sessile oak forest (*Quercus petraea* (Matt.) Liebl.), collected by P. Pînzaru, V. Cantemir, [218?]. Manic, on 20.05.2024 (CH-HBPP 8721). In the local flora, it is known from the districts of Strășeni (Scoreni), Ialoveni (Cărbuna, Rezeni), and Cimișlia (Zloți), included in *The Red Book of the Republic of Moldova* (2015), Endangered category (EN). A species distributed in Southeast Europe, Hungary, Crimea, the Caucasus, and Turkey, microphanerophyte, xeromesophilic-mesophilic, characteristic of the *Quercetalia pubescenti-petraeae* Br.-Bl. 1932 order vegetation [19].

PÎNZARU P., CANTEMIR V., SFECLĂ V.

**258. *Carex strigosa*** Huds. 1778, Fl. Angl., ed. 2: 411 (Cyperaceae).

+ **RAR:** Căpriană commune, Strășeni district, Căpriană forest district, in a riparian forest, collected by P. Pînzaru, V. Cantemir, determined by P. Pînzaru, on 01.08.2024 (CH-HBPP 9105, 9106; CHGB); Rădenii Vechi commune, Ungheni district, “Plaiul Fagului” Scientific Reserve, plot no. 33, riparian forest, collected by P. Pînzaru, V. Sfeclă, determined by P. Pînzaru (CH-HBPP 9108). This species was also collected by

L. Toderaş from the forest near Scoreni commune, Străşeni district, on 05.05.1976, and Cioreşti, Nisporeni district, on 06.05.1976 (LE) [30]. It is proposed to be included in the *List of protected species* and *The Red Book of the Republic of Moldova*, 4th edition, status Endangered (EN). A West/Central European species, perennial, hemicryptophyte, mesohydrophilic.

PÎNZARU P., CANTEMIR V., TOFAN-DOROFEEV E.

**259. *Serratula coronata*** L. 1763, Sp. Pl., ed. 2: 1144 (Asteraceae.)

**+RAR:** Horodca village from Draguşenii Noi commune, Hînceşti district, within the “Codru” Scientific Reserve, grows in a floodplain with tall herbaceous vegetation surrounded by forest, collected by P. Pînzaru on 11.09.2020 (CH-HBPP 5154); Moara Domnească commune, Glodeni district, “Pădurea Domnească” Scientific Reserve, on a forest path, a small group of plants, collected by P. Pînzaru, V. Cantemir, E. Tofan-Dorofeev, determined by P. Pînzaru, on 06.08.2024 (CH-HBPP 9109). In the Republic of Moldova, it is known from Caracuşenii Vechi commune, Briceni district, from Stejăreni village, Lozova commune, Străşeni district (in the “Codru” Scientific Reserve, Păunului glades), and from the vicinity of Codreni and Lipoveni communes, Cimişlia district. A Pontic species, perennial, hemicryptophyte, mesophilic, included in *The Red Book of the Republic of Moldova* (2015), Endangered category (EN).

PÎNZARU P., CANTEMIR V., TOFAN-DOROFEEV E., DOROFEEV A.

**260. *Carex pseudocyperus*** L. 1753, Sp. Pl.: 978 (Cyperaceae).

**+RAR:** Crihana Veche commune, Cahul district, Lake “Manta”, grows in a phytocenosis of the association *Telypterido palustris-Phragmitetum australis* Kuper 1957 em. Segal ex Westh. & den Held 1968, collected by P. Pînzaru, V. Cantemir, E. Tofan-Dorofeev and A. Dorofeev, determined by P. Pînzaru, 17.07.2024 (CH-HBPP 9110, 9111; CHGB). In the Herbarium of the Chisinau Botanical Garden there is only one dried specimen collected by V. Andreev (14.06.1946, along a stream near Peresecina commune, Orhei district) [CHGB]. Very rare species, it is proposed to be included in *The List of protected species of the Republic of Moldova* and in *The Red Book of the Republic of Moldova*, 4th edition, status Critically Endangered (CR). Circumpolar species (subcosmopolitan), helohydatorphyte (hemicryptophyte), hygrophilous (mesohygrophilous) [3].

PÎNZARU P., CANTEMIR V., TOFAN-DOROFEEV E., DOROFEEV A.

**261. *Dryopteris filix-mas*** (L.) Schott, 1834, Gen. Fil.: 18 (Dryopteridaceae).

+ **RAR:** Holercani commune, Criuleni district, rarely grows on the steep slopes of a deep ravine, under the crown of trees, collected by P. Pînzaru, P. Cantemir, E. Tofan-Dorofeev, A. Dorofeev, 12.08.2024 (CH-UBPP 343; CHGB). Circumpolar species, perennial, geophytic, (xeromesophilic) mesophilic (mesohygrophilous), is included in *The Red Book of the Republic of Moldova* (2015, status Vulnerable (VU)).

PÎNZARU P., CANTEMIR V., TOFAN-DOROFEEV E., STELEA V.

**262. *Elodea nuttallii*** (Planch.) H.St. John, 1920, *Rhodora*, 22: 29 (Hydrocharitaceae).

+ **RAR/A:** Moara Domnească commune, Glodeni district, in the Prut riverbed, forms small clumps near the shore, collected by P. Pînzaru, V. Cantemir, E. Tofan-Dorofeev, V. Stelea, 06.08.2024 (CH-HBPP 9123; CHGB) This species was only recorded on the territory of the “Pădurea Domnească” Scientific Reserve, in the Prut riverbed, near Cuhnești commune, Glodeni district [13]. Adventitious species, native to North America, perennial, helohydatophyte, hydrophilic.

PÎNZARU P., SFECLĂ V.

**263. *Euphorbia maculata*** L. 1753, Sp. Pl.: 455 (Euphorbiaceae).

+**RMC/A:** Cornești village from Cornești commune, Ungheni district, ruderal, near the railway station, abundance + dominance of 1-2, collected by P. Pînzaru and V. Sfeclă, determined by P. Pînzaru, 21.07.2024 (CH-CHPP 9112; CHGB). This species was collected by C. Zahariadi (21.09.1935, from Etulia commune, Vulcănești district)[CHGB] and by P. Pînzaru 13.07.2015, near Zloți Station, Cimișlia district, along the railway, 13.07 .2015 (CH-HBPP 7648) [8]. Adventitious species (native to North America), annual, therophytic, xeromesophilic.

PÎNZARU P., SFECLĂ V.

**264. *Scleranthus uncinatus*** Schur, 1851, Verh. Siebend.Ver. Naturw, 2: 10 (Caryophyllaceae).

+ **RAR:** Cobani commune, Glodeni district, Scientific Reserve “Pădurea Domnească” Lake “La Fontal”, on sandy soil, collected by P. Pînzaru, 17.05.2024 (CH-HBPP 9093); Pietrosu commune, Fălești district, segetal, collected by P. Pînzaru, 10.06.2024 (CH-HBPP 9013); Albota de Sus commune, Taraclia district, on the edge of a field, collected by P. Pînzaru, V. Sfeclă, determined by P. Pînzaru, 28.06.2024, (CH-HBPP 8975). This species was indicated by Tr. Săvulescu and T. Rayss (1926), from the vicinity of Corpaci commune, Edineț district [17], considered extinct by T. Izverscia [4]. In the summer of 2023 it was registered in the “Dobrușa” landscape reserve, it grows in the outskirts of the forest, on the edge of fields with alfalfa or corn, near the communes of Chipeșca (CH-HBPP 7265, 7237, 7238; CHGB) and Olișcani, Șoldănești district (CH -HBPP 7239) [20,

21]. *Carpatho-Balkan-Anatolian-Caucasian* species, annual, therophytic, mesophyllous, segetal, on sandy soils.

PÎNZARU P., SFECLĂ V.

**265. *Senecio macrophyllus*** M.Bieb. 1808, Fl. Taur.-Cauc. 2: 308 (Asteraceae).

Syn.: = *Senecio schvetzovi* Korsh. 1898, Mém. Acad. Sci. Pétersb. 7, 1: 519.

**+RAR:** Bumbăta commune, Ungheni district, rarely grows on sandy hills with northern, northeastern exposure, in phytocenoses dominated by *Anthericum ramosum* L. or *Stipa ukrainica* P. Smirn., collected by P. Pînzaru and V. Sfeclă, determined by P. Pînzaru, 02.08.2024 (CH-HBPP 9114). Eurasian species, perennial, hemicryptophyte, mesophilic, rare in the flora of the Republic of Moldova, it is proposed to be included in the *List of protected species* and in the *Red Book of the Republic of Moldova*, 4th edition, status Critically Endangered (CR).

PÎNZARU P., SFECLĂ V., MANOLE S.

**266. *Beta trigyna*** Waldst. & Kit. 1800, Pl. Rar. Hung. 1: 34. (Amaranthaceae).

**+ RAR:** Vadul lui Isac commune, Cahul district, in *Quercus robur* subsp. *pedunculiflora* (K.Koch) Menitsky, collected by P. Pînzaru, 09 VI 2022, (CH-HBPP 5147); Pelinei commune, Cahul district, registered by P. Pînzaru, V. Sfeclă and S. Manole 27.06.2028 (CH-HBPP 9115) in the phytocoenoses of the *Iridio variegatae-Quercetum pubescentis* association Pînzaru et al. 2022. Pontic-Mediterranean, perennial, hemicryptophyte, xeromesophilic, rare species, included in the *List of protected species of the Republic of Moldova* [33].

PÎNZARU P., SFECLĂ V., MANOLE S.

**267. *Chaerophyllum nodosum*** (L.) Crantz, 1767, Class. Umbell. Emend.: 76 (Apiaceae).

**+ RAR:** Frumușica village from Chioselia Mare commune, Cahul district, grows in small groups under the canopy of *Quercus robur* subsp. *pedunculiflora* (K.Koch) Menitsky, recorded by P. Pînzaru, V. Sfeclă, and S. Manole, 28.06.2024, dry plants, in the dissemination phase. Mediterranean, annual, therophyte, xerophilous species, included in *The Red Book of the Republic of Moldova* (2015), Critically Endangered category (CR).

**Note.** This species is characteristic for the forests of the *Quercetalia pubescenti-petraeae* Klika 1933 order, in recent years several places of growth have been detected and it is proposed to be excluded from *The Red Book of the Republic of Moldova*, 4th edition, the presence of the given species is not in danger, it forms many seeds.

PÎNZARU P., SFECLĂ V., MANOLE S.

**268. *Dictamnus gymnostylis*** Steven, 1856, Bull. Soc. Nat. Moscou, 29, 1: 333 (Rutaceae).

+ **RAR:** Frumușica village from Chioselia Mare commune, Cahul district, grows sporadically in glades and under the canopy of downy oak trees from the association *Iridio variegatae-Quercetum pubescentis* Pînzaru et al. 2022, collected by P. Pînzaru, V. Sfeclă and S. Manole, determined by P. Pînzaru, 28.07.2024 (CH-HBPP 8979; CHGB). Eurasian species, perennial, hemicryptophyte, xerophilous, included in *The Red Book of the Republic of Moldova*, Endangered category (EN).

PÎNZARU P., SFECLĂ V., MANOLE S.

**269.** *Gypsophyla glomerata* Pall. ex Adams, 1805, Beitr. Naturk. 1: 54 (Caryophyllaceae).

Syn.: = *Gypsophyla pallasii* Ikonn. 1976, Novosti Sist. Vyssh. Rast. 13: 118, nom. nud.

+ **RAR:** Dermengi village from Budăi commune, Taraclia district, grows sporadically on a clay, steppe hill, collected by P. Pînzaru, V. Sfeclă and S. Manole, determined by P. Pînzaru, 27.06.2024 (CH-HBPP 8821; CHGB); Văleni commune, Cahul district, develops on a sandy slope in the Prut river valley, abundance + dominance is 2, on relatively large areas, south-west slope exposure, slope inclination of about 60°, collected by P. Pînzaru, 17.07.2024 (CH -HBPP 9115). Balkan-Pontic species, perennial, (hemicryptophyte) camephyte, xerophilous, included in *The Red Book of the Republic of Moldova* (2015), Vulnerable category (VU).

PÎNZARU P., SFECLĂ V., MANOLE S.

**270.** *Jurinea multiflora* (L.) B.Fedtsch. 1911, in Consp. Fl. Turkest. 4: 285 (Asteraceae).

+ **RAR:** Dermengi village from Budăi commune, Taraclia district, rare, grows on steppe clay hill, collected by P. Pînzaru, V. Sfeclă and S. Manole, determined by Pînzaru, 28.06.2024, (CH-HBPP, 8822). Pontic, perennial, hemicryptophyte, xerophilous, steppe species, rare on the territory of the Republic of Moldova, it is proposed to be included in the *List of protected species*.

PÎNZARU P., SFECLĂ V., MANOLE S.

**271.** *Pulsatilla grandis* Wend. 1831, Schr. Ges. Beföod. Gessamt. Naturwiss. Marburg, 2: 257 (Ranunculaceae).

+ **RAR:** Dermengi village from Budăi commune, Taraclia district, very rarely, vegetates on a clay, steppe hill, recorded by P. Pînzaru, V. Sfeclă and S. Manole, determined by P. Pînzaru, 27.06.2024. European, perennial, hemicryptophyte, xeromesophilic species, included in *The Red Book of the Republic of Moldova* (2015), Endangered category (EN).

PÎNZARU P., SFECLĂ V., MANOLE S.



**272. *Quercus × rosacea*** Bechst. 1810, Forstbot.: 333 (*Q. petraea* × *Q. robur*) (Fagaceae).

+ **RAR:** Dermengi village in Budăi commune, Taraclia district, in a forestry plantation, collected by P. Pînzaru, V. Sfeclă and S. Manole, determined by P. Pînzaru, 27.06.2024, (CH-HBPP 8985, 8986, 8987; CHGB). European hybrid species, megaphanerophytic, xeromesophilic. In the Republic of Moldova, it was first collected by P. Pînzaru on 14.06.2009, in a forestry plantation near the village of [21A?]ipova in Lalova commune, Rezina district, determined by P. Pînzaru on 27.11.2022, (CH-HBPP 4299, 4300) [14]

PÎNZARU P., SFECLĂ V.

**273. *Bromus ramosus*** Huds. 1762, Fl. Angl.: 40 (Poaceae).

+ **RAR:** this species was indicated only by И. Пачоский (1912) from the forests of Cornești, Otaci [28] and by Tr. Săvulescu, T. Rayss (1924) from the forests near Cornești, Bahmut and Rădeni [15], in subsequent publications it was no longer cited. In the current year, this species was recorded in the sessile oak forest, near Chipeșca commune, [218?]oldănești district, collected by V. Sfeclă, 06.08.2024 (CH-HBPP 8944). New collecting activities were carried out in the forests of the northern districts of the republic: Ocnîța (Ocnîța village, Bîrnova, Mihălășeni, Calarașovca), Briceni (Cotiu-jeni), Dondușeni (Arionești), Rîșcani (Șaptebani), Fălești (Pietrosu), Glodeni (Moara Domnească) and from the center of the country: Strășeni (Lozova, Căpriana), Ungheni (Rădenii Vechi, Cornești), Hîncești (Lăpușna), Orhei (Lopatna), Călărași (Sipoteni & Bahmut). It grows rarely or sporadically in various types of forests from mesohygrophilous to xeromesophilic ones. Widespread species in Europe, Iran, Tibet and India, perennial, hemicryptophyte, xeromesophilic-mesophilic (mesohydrophilic) [35].

SFECLĂ V.

**274. *Iris aphylla*** L. 1753, Sp. Pl.: 38 (Iridaceae).

+ **RAR:** Dobrușa commune, Șoldănești district, growing together with *Hupocaeris maculata* L. in the clearings of the “Dobrușa Mică” forest, plot 14 “N”, altitude 265 m, south-west exposure, inclination 20°, recorded by Sfeclă V., 15.04.2024. Central-European/Eastern-European species, perennial, geophyte, xeromesophilic, in the Republic of Moldova it is found more frequently in the downy oak forests on the cliffs, in the phytocoenoses of the *Iridio aphyllae-Quercetum pubescentis* Pînzaru 2019 association [9].

SFECLĂ V.

**275. *Symphytum tuberosum* subsp. *angustifolium*** (A.Kern.) Nyman, 1881, Consp. Fl. Eur.: 510 (Boraginaceae).

Syn.: = *Symphytum popovii* Dobrocz. 1968, Ukrayins'k. Bot. Zhurn. 25(5): 60. – *S. tuberosum* subsp. *nodosum* (Schur) Soó, 1941, in Acta Geobot. Hung. 4: 192.

+ **RAR**: Dobruşa commune, Şoldăneşti district, “Dobruşa Mare” forest, plot no. 32, collected by V. Sfeclă V., 09.05.2024, determined by P. Pînzaru P. (CH-HBPP 6765). Widespread species in the Central and South-Eastern Europe, perennial, hemicryptophyte, mesophyllous, little known on the territory of the Republic of Moldova.

SFECLĂ V., PÎNZARU P.

**276. *Epipactis leptochila* (Godfery) Godfery** 1921, J. Bot. 59: 146 (Orchidaceae).

+ **RAR**: Rădenii Vechi Commune, Ungheni District, “Plaiul Fagului” Scientific Reserve, plot 26, identified by Sfeclă V., 06.07.2023; Căpriana Commune, Străşeni District, Căpriana Forestry, plot 53, in a meadow forest, identified by P. Pînzaru & V. Sfeclă, 25.07.2024. Given that there were 1-3 specimens in these populations, no herbarium was collected to avoid reducing the population. These locations are new growth points, following the first identification on the territory of the Republic of Moldova, in the “Dobruşa” landscape reserve [22]. Endemic to the European continent, ranging from the west to the North Caucasus, it grows on limestone soils, prefers shaded and moist places, is perennial, geophyte, and mesophytic. Rare in the flora of the Republic of Moldova, it is proposed to be included in the *List of protected species* and in *The Red Book of the Republic of Moldova*, 4th edition, status Critically Endangered (CR).

#### 4. CONCLUSIONS

As a result of the floristic research carried out during the vernal-summer period of the current year, new places of growth for 36 species of spontaneous vascular plants were registered, including 12 species included in *The Red Book of the Republic of Moldova* (2015).

7 species of rare plants have been confirmed for the flora of the Republic, which are proposed to be included in the *List of protected species of Republic of Moldova* and in *The Red Book of the Republic of Moldova*, 4th edition: *Carex pseudocyperus* L., *C. strigosa* Huds., *C. vesicaria* L., *Epipactis leptochila* (Godfery) Godfery., *Fraxinus coriariifolia* Scheele, *Linum catharticum* L. and *Senecio macrophyllus* M. Bieb.

The species *Bromus ramosus* Huds., which was no longer cited for the local flora after T. Săvulescu and T. Rayss (1924) was confirmed, grows sporadically or rarely in the forests of the northern and central districts of the Republic, from mesohydrophilous to xermesophilic ones, it is proposed to be considered as distinctive for the *Quercus-Fagetum* Br.-Bl forest class. et Vliieger in Vliieger 1937.

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- (2) “Development and implementation of good practices of sustainable agriculture and climate resilience (GREEN)” – 020407.

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## DFT analysis of intermolecular and intramolecular double proton transfer during the condensation process of 4-pyridinecarboxaldehyde with isomers of aminobenzoic acid

ION ARSENE  AND VIORICA PURCEL 

**Abstract.** To understand in detail the double proton transfer in the condensation reaction of 4-pyridinecarboxaldehyde with isomers of aminobenzoic acid (in the gas phase and in solvent), the dynamic mechanism was studied using the DFT method implemented in the GAUSSIAN 09 program and the 6-31G basis set. All condensation processes described in this study proceed in two stages: the first stage involves the interaction of the reactants and the methanol-mediated proton transfer from the amine group to the aldehyde group, forming an intermediate compound; the second stage is identified by the intramolecular donation of the second proton from the (-NH) group to the hydroxyl oxygen, forming highly stable Schiff base products with the elimination of water and methanol molecules. From the perspective of activation energy, it is observed that the solvent has a positive influence on intermolecular transfer, which is not the case for intramolecular transfer. Additionally, the solvent positively contributes to the energetic stability of the final reaction products.

**Keywords:** DFT study, double proton transfer, activation energy, energetic stability.

## Analiza DFT a transferului dublu de protoni inter și intramolecular în timpul procesului de condensare a 4-piridincarboxaldehydei cu izomerii acidului aminobenzoic

**Rezumat.** Pentru a înțelege în detaliu transferul dublu de protoni în reacția de condensare a 4-piridincarboxaldehydei cu izomerii acidului aminobenzoic (în fază gazoasă și în solvent), a fost studiat mecanismul dinamic, utilizând metoda DFT implementată în programul GAUSSIAN 09 și setul de bază 6-31G. Toate procesele de condensare descrise în acest studiu decurg în două etape: prima etapă decurge cu interacțiunea reactanților și transferul protonului mediat de metanol de la gruparea amină la gruparea aldehydică formându-se un compus intermediar; a doua etapă se indentifică prin donarea intramoleculară a celui de-al doilea proton din gruparea (-NH) la oxigenul hidroxilic, formându-se produși Baze Schiff cu stabilitatea mare, cu eliminarea moleculei de apă și matanol. Din punct de vedere a energiei de activare se constată că solventul influențează pozitiv în cazul transferului intermolecular, ceea ce nu putem spune în cazul intramolecular. De asemenea solventul are un aport pozitiv în cazul stabilității energetice a produșilor finali de reacție.

**Cuvinte-cheie:** studiul DFT, transfer dublu de protoni, energie de activare, stabilitate energetică.

## 1. INTRODUCTION

Aliphatic and aromatic aldehydes constitute a significant category of organic compounds involved in numerous essential chemical reactions. One of the most remarkable reactions is their condensation with primary amines, resulting in the formation of N-substituted imines, known as Schiff bases. These imines are crucial in chemical synthesis and biological processes, being widely used in organic chemistry to generate complex compounds. Moreover, Schiff bases are of major importance in the pharmaceutical industry for drug synthesis, in molecular recognition studies, and as intermediates in various catalytic processes. Theoretical analysis of the properties and reactivity of Schiff bases provides valuable information for understanding the mechanisms of chemical reactions and for developing new materials and functional compounds [1].

The condensation reactions leading to the formation of Schiff bases involve essential proton transfers, both within individual molecules and between different molecules. In the process of forming a Schiff base, a proton is transferred from the amino group to the carbonyl group, thereby facilitating the formation of the characteristic carbon-nitrogen double bond of N-substituted imines. This proton transfer can occur through intramolecular mechanisms, where the proton moves between functional groups within the same molecule, or through intermolecular mechanisms, via hydrogen bonds formed with water molecules or other molecules present in the environment. In these proton transfer processes, quantum tunneling plays an important role, accelerating the reactions and influencing the dynamics of Schiff base formation. Thus, understanding these proton transfer mechanisms, both inter- and intramolecular, is essential to fully elucidate the formation and reactivity of Schiff bases under various chemical and biological conditions [2].

The theoretical mechanism of double proton transfer in aldehyde-amine condensation reactions is described in the paper [3], where the authors have developed and described the reaction mechanism, comparing the energetic data of direct proton transfer and double proton transfer. Additionally, in the works [4, 5] the theoretical mechanisms of the condensation reaction between 4-pyridinecarboxaldehyde and o-, m-, and p-aminobenzoic acids are also studied and described. The authors mention the reduction of activation energy when the reaction occurs in a solvent.

The aim of this research is to develop the mechanism of double proton transfer in the condensation process of 4-pyridinecarboxaldehyde with isomers of aminobenzoic acid, calculating the activation energy for each system.

## 2. COMPUTATIONAL METHODS

The optimization of geometric structures for condensation reactions was performed using Density Functional Theory (DFT), employing the hybrid exchange-correlation functional B3LYP (Becke's three-parameter exchange functional with Lee, Yang, and Parr's correlation functional) and the standard basis set 6-31G [6]. This combination provides a balance between accuracy and computational cost, and is widely used in theoretical studies to predict the electronic structure and properties of molecules. To characterize the optimized stationary points as minima and to evaluate the vibrational zero-point energies, there were calculated harmonic vibrational frequencies for all species involved in the reaction using the B3LYP/6-31G level. All calculations were performed using the GAUSSIAN 09 software package [7].

## 3. RESULTS AND DISCUSSIONS

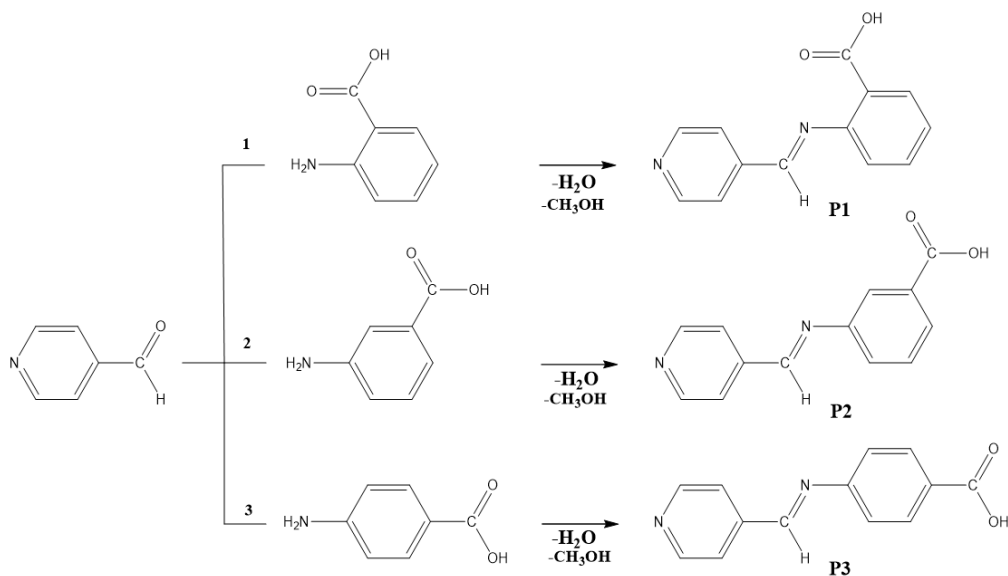
Proton transfer is one of the most common processes in chemical and biochemical reactions. A rigorous analysis of this phenomenon requires, first, a consistent explanation of the quantum-chemical behaviour of the proton. Secondly, proton transfer is often associated with charge transfer, which involves a change in the interaction between charge and the polarization of the environment, behaving in a classical manner. Based on the aim of this study, there have been developed general schemes for condensation reactions (Figure 1), which will be theoretically studied using advanced computational methods. The essence of these reactions involves the interaction of an aromatic aldehyde (4-pyridinecarboxaldehyde) with three isomeric amino acids: 2-aminobenzoic acid (1), 3-aminobenzoic acid (2), and 4-aminobenzoic acid (3). The resulting reaction products are N-substituted imines, known as Schiff bases: P1, P2, and P3.

### **Condensation of 4-Pyridinecarboxaldehyde with o-Aminobenzoic Acid**

Theoretical investigation of the condensation mechanism between 4-pyridinecarboxaldehyde and o-aminobenzoic acid was conducted according to the scheme shown in Figure 2. The reaction yields 4-(pyridin-2-ylmethyleneamino)benzoic acid as the reaction product.

The condensation reaction proceeds in two stages. In the first stage, as illustrated in Figure 2, methanol, forming hydrogen bonds between the involved functional groups, facilitates the intermolecular transfer of a hydrogen atom from the amino group to the aldehyde oxygen. This reduces the energy barrier for the formation of the intermediate compound (**Inter1**), contributing to the stabilization of the intermediate structure and enhancing the efficiency of the reaction.





**Figure 1.** General scheme for the condensation of 4-pyridinecarboxaldehyde with aminobenzoic acid isomers.

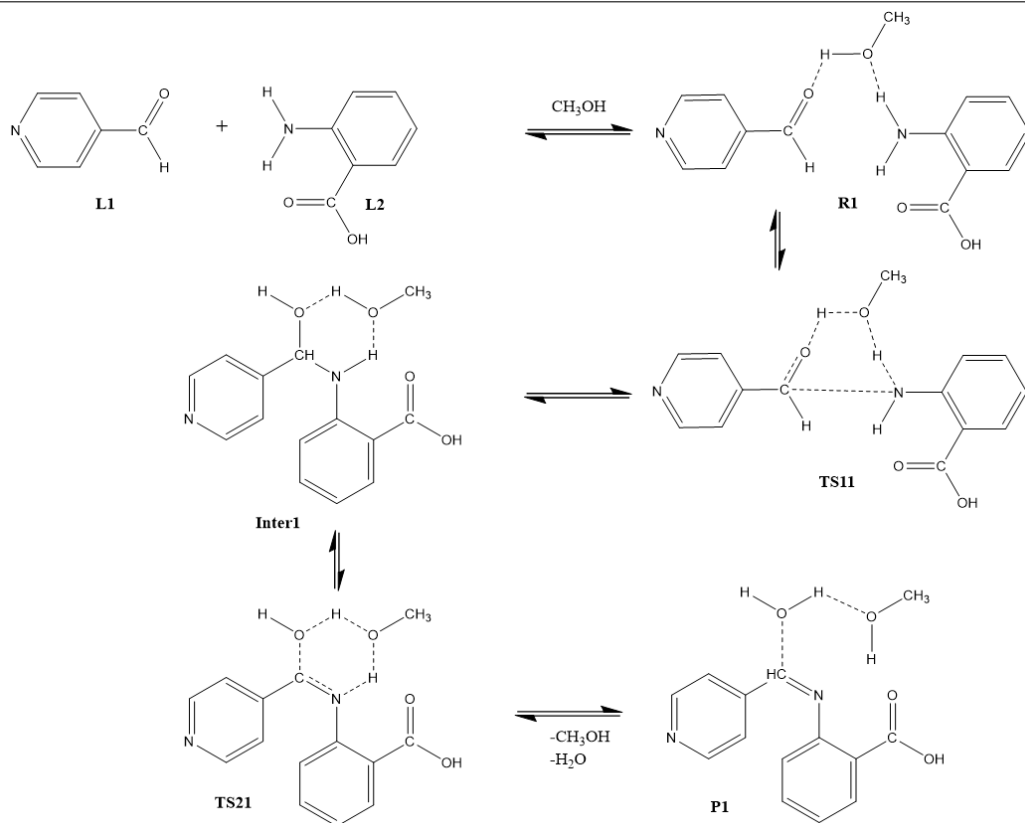
**Table 1.** Activation energy and imaginary frequency values for the transition state in stage I and II.

Reaction stage	Reaction environment	Activation energy, kcal/mol	The imaginary frequency, $\text{cm}^{-1}$
TS11	vacuum	25.98	-1016.87
	methanol	21.52	-833.45
TS21	vacuum	30.94	-1257.07
	methanol	31.62	-800.32

In the second stage, methanol continues to play an important role by facilitating the intramolecular donation of the second hydrogen atom from the (-NH) group to the hydroxyl oxygen, resulting in the formation of 4-pyridin-2-ylmethyleaminobenzoic acid (P1) with the elimination of a water molecule.

Through these mechanisms, methanol not only supports the reaction but also optimizes the conditions for obtaining the final product in an energy-efficient manner. Table 1 presents the numerical values of the activation energies and vibrational frequencies for the studied reaction.

DFT ANALYSIS OF 4-PYRIDINECARBOXALDEHYDE WITH ISOMERS OF  
AMINOBENZOIC ACID



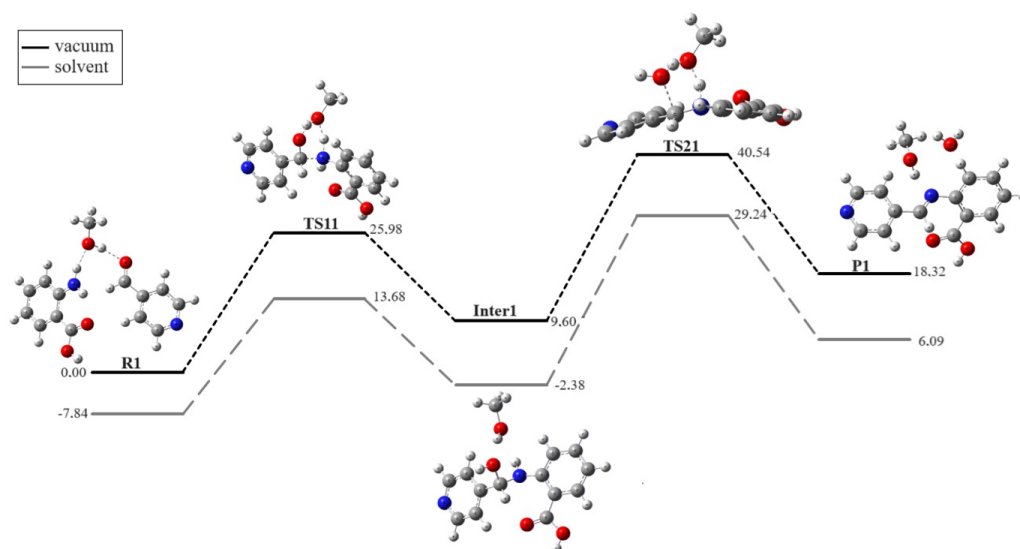
**Figure 2.** Mechanism of the double proton transfer condensation reaction of 4-pyridinecarboxaldehyde with o-aminobenzoic acid.

According to the data presented in Table 1, the solvent, in this case, methanol, plays a significant role in reducing the activation energy in the first stage of the condensation reaction. This reduction is from 25.98 to 21.52 kcal/mol and is due to the solvent's solvation capacity and its ability to form hydrogen bonds, which facilitates the formation of the final product in an energy-efficient and kinetically efficient manner. In the second stage of the reaction, where intramolecular transfer occurs, the activation energies do not show a similar decrease, increasing from 30.94 to 31.62 kcal/mol. This phenomenon suggests a different influence of the solvent depending on the specific stage of the condensation reaction.

Analyzing the transition states in this condensation reaction, in the first stage, the activated complex corresponds to the moment when the hydrogen atom moves from the amino group to the oxygen of the aldehyde group. At this point, the value of the imaginary frequency is negative (Table 1) and reflects a critical point in the double proton transfer,

indicating how the hydrogen atom oscillates between the donor and acceptor. In the second stage, the transition state occurs when the hydrogen atom moves from the amino group to the hydroxyl oxygen, forming a water molecule and eliminating methanol. The imaginary frequencies associated with this transition state reflect the oscillations of the hydrogen atom before the formation of a stable covalent bond.

Based on the energies obtained from the optimization of the species involved in the condensation reaction, an energy profile was constructed, both in the vacuum and in methanol (Figure 3).



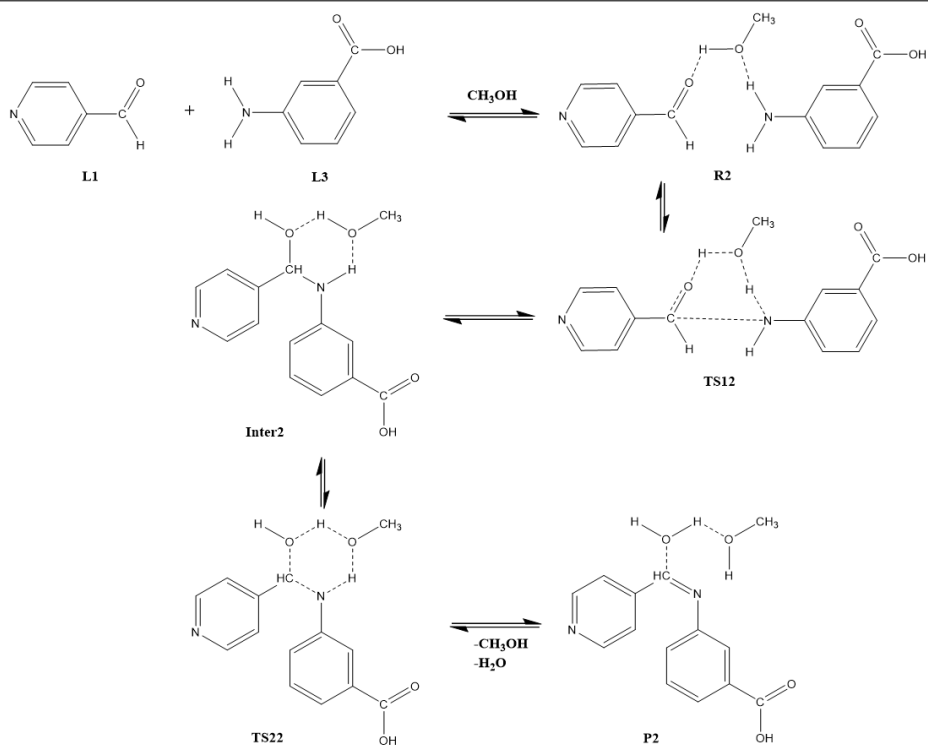
**Figure 3.** Energetic profile of the reaction between 4-pyridinecarboxaldehyde and o-aminobenzoic acid expressed in kcal/mol.

Analyzing this reaction, it is an endothermic one, meaning it absorbs heat, with the reaction energy values in the vacuum and methanol being 18.32 kcal/mol and 13.93 kcal/mol, respectively. This aspect is consistent with experimental data, indicating that the synthesis or reaction was carried out under severe conditions, at high temperatures.

**Condensation of 4-pyridinecarboxaldehyde with m-aminobenzoic acid.** Theoretical investigation of the condensation mechanism between 4-pyridinecarboxaldehyde and m-aminobenzoic acid was conducted according to the scheme shown in Figure 4. The reaction yields 4-(pyridin-3-yl methylene amino)-benzoic acid as the reaction product.

To form the final product, the condensation reaction undergoes two consecutive stages. In the first stage, as shown in Figure 4, the intermolecular transfer of a hydrogen atom from the amino group ( $-\text{NH}_2$ ) to the carbonyl oxygen ( $\text{HC}=\text{O}$ ) occurs via hydrogen bonds

DFT ANALYSIS OF 4-PYRIDINECARBOXALDEHYDE WITH ISOMERS OF  
AMINO BENZOIC ACID



**Figure 4.** Mechanism of the double proton transfer condensation reaction of 4-pyridinecarboxaldehyde with m-aminobenzoic acid.

created by methanol, forming an intermediate compound (**Inter2**). Methanol continues to play an important role in enhancing the reaction in the second stage by facilitating the intramolecular donation of the second hydrogen atom from the (-NH) group to the hydroxyl oxygen, resulting in the formation of 4-(pyridin-3-yl methylene amino)-benzoic acid (**P2**), with the elimination of a water molecule and the release of methanol. Table 2 presents the numerical values of the activation energies for the given reaction.

According to the data presented in Table 2, it is obvious that methanol plays an important role in reducing the activation energy required in the initial phase of the condensation reaction. The energy variation in this case is 2.01 kcal/mol, with the decrease attributed to the solvent's solvation capacity and its ability to form hydrogen bonds, thus accelerating the formation of the final product in an energy-efficient and kinetically favourable manner. Moving to the next stage of the reaction, characterized by intramolecular transfer, the activation energy increases from 24.16 to 25.67 kcal/mol.

Transition states for this reaction were studied, characterizing the moment when the hydrogen atom undergoes a transfer from the amino group to the oxygen atom in the

**Table 2.** Activation energy and imaginary frequency values for the transition state in stage I and II of the condensation reaction with direct proton transfer.

Reaction stage	Reaction environment	Activation energy, kcal/mol	The imaginary frequency, $\text{cm}^{-1}$
TS21	vacuum	20.96	-995.69
	methanol	18.95	-881.73
TS22	vacuum	24.16	-891.65
	methanol	25.67	-839.76

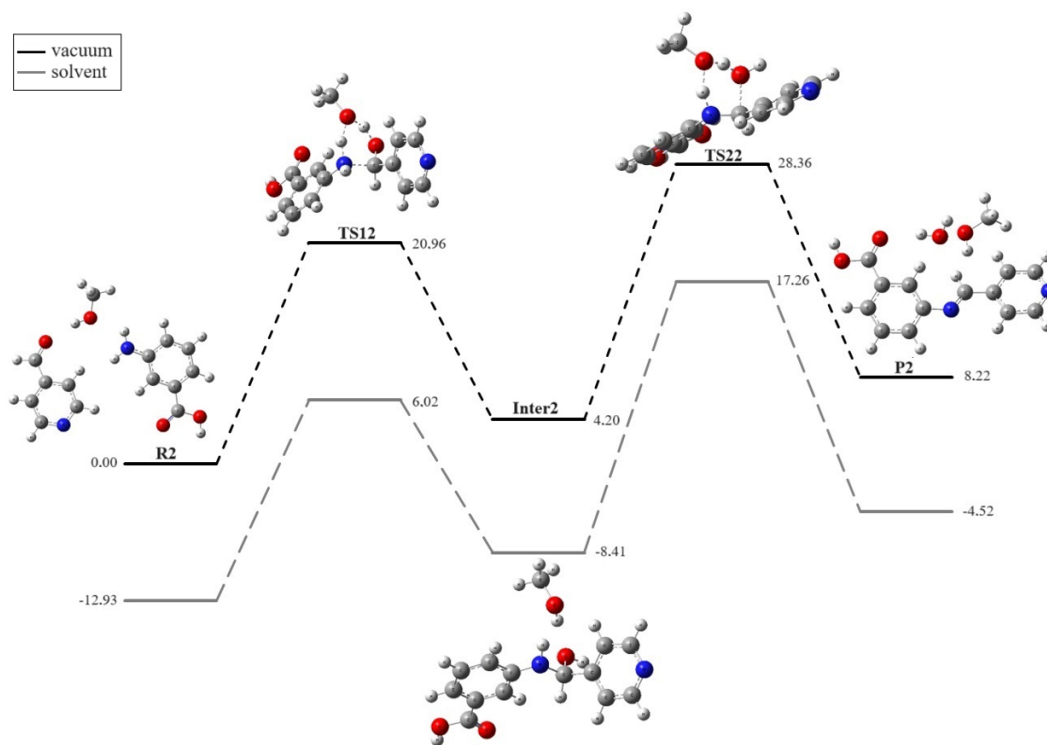
aldehyde group. This critical juncture is characterized by a negative imaginary frequency, as shown in Table 2, symbolizing a stage in the double proton transfer mechanism. In the subsequent stage, the transition state occurs when the hydrogen atom moves from the amino group to the hydroxyl oxygen, leading to the formation of a water molecule and the simultaneous expulsion of methanol from the system.

By analyzing the energies derived from the optimization process of the various species involved in the condensation reaction, there was constructed an energy profile, including calculations performed both in the vacuum and in the presence of methanol, as illustrated in Figure 3.

The thermodynamic study of the chemical reaction denotes that it is an endothermic process, when it takes place in vacuum and in the presence of methanol, the energy of the reaction being equal to 8.22 kcal/mol and 8.41 kcal/mol, respectively. These numerical values align with experimental observations, suggesting that the synthesis took place under demanding conditions, likely involving high temperatures to facilitate the desired chemical transformation.

**Condensation of 4-pyridinecarboxaldehyde with p-aminobenzoic acid.** The theoretical investigation of the process of condensation of 4-pyridinecarboxaldehyde with p-aminobenzoic acid has been studied in detail according to the mechanism shown in Figure 6. The reaction results in the formation of 4-(pyridin-4-yl methylen amino)-benzoic acid, commonly known as Schiff Base.

The condensation reaction involves two sequential steps, as illustrated in Figure 6, the process begins with the intermolecular transfer of a hydrogen atom from the amino group ( $-\text{NH}_2$ ) to the oxygen of the aldehyde group ( $\text{HC}=\text{O}$ ). This transfer is facilitated by methanol-mediated hydrogen bridging, resulting in the creation of an intermediate compound (**Inter2**). Subsequently, in the second step, methanol continues to be an

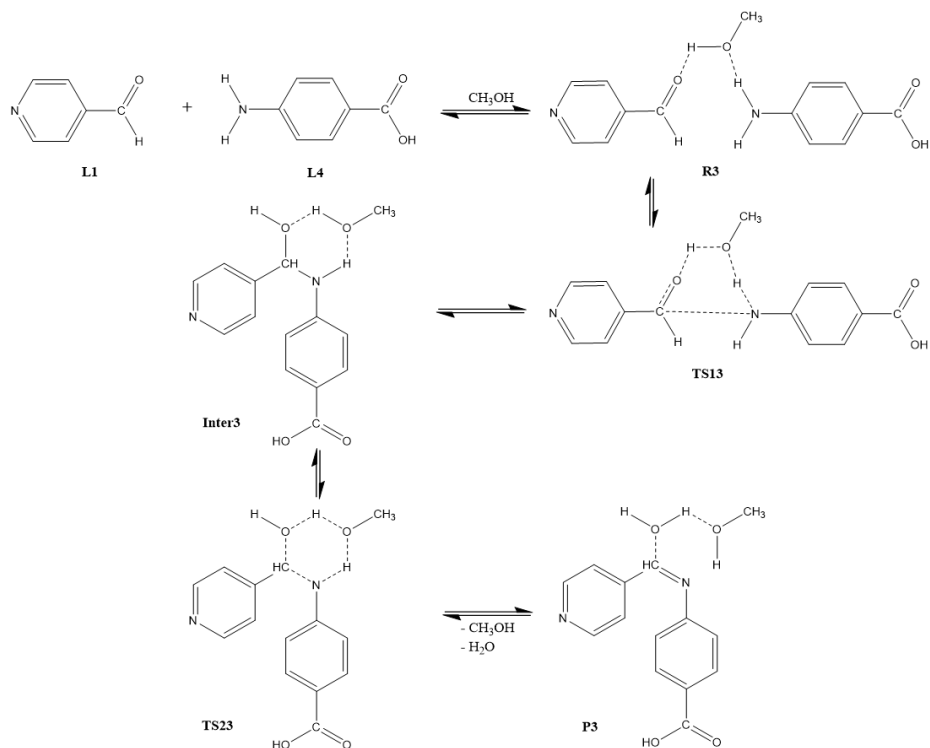


**Figure 5.** Energetic profile of the reaction between 4-pyridinecarboxaldehyde and m-aminobenzoic acid expressed in kcal/mol.

**Table 3.** Values of activation energies and imaginary frequencies for the transition state in stage I and II of the double proton transfer condensation reaction.

Reaction stage	Reaction environment	Activation energy, kcal/mol	The imaginary frequency, cm <sup>-1</sup>
TS31	vacuum	19.95	-1029.07
	methanol	19.33	-949.15
TS32	vacuum	25.58	-1054.70
	methanol	27.30	-824.29

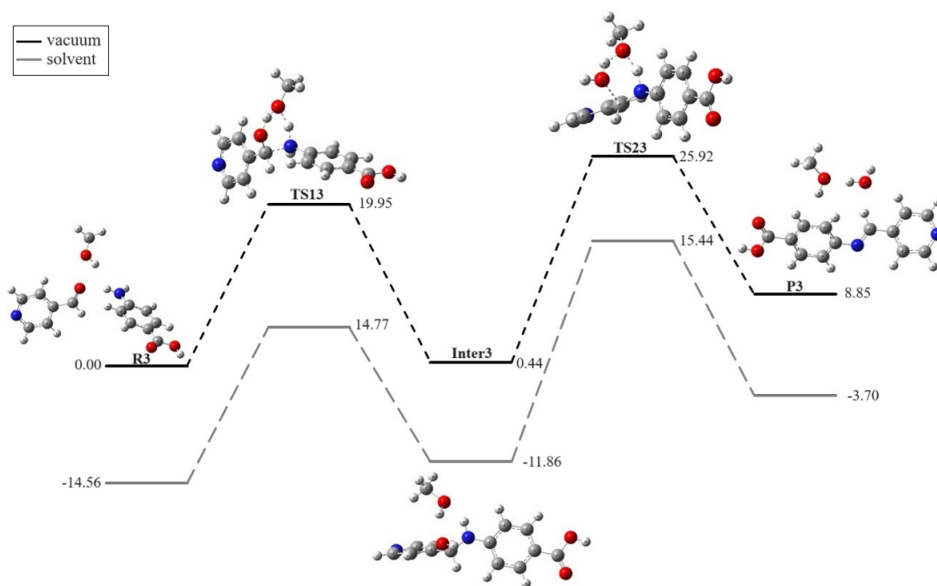
indispensable component, aiding the intramolecular transfer of the second hydrogen atom from the (-NH) group to the oxygen of the hydroxyl group. This transfer results in the formation of 4-pyridin-(4-yl-methylene)-aminobenzoic acid (**P3**), simultaneously eliminating a water molecule and methanol. Detailed information on the activation energies of the reaction can be found in the data presented in Table 3.



**Figure 6.** Mechanism of the double proton transfer condensation reaction of 4-pyridinecarboxaldehyde with p-aminobenzoic acid.

According to the information in Table 3, it is obvious that methanol as a solvent plays an essential role in reducing the activation energy required in the initial stage of the condensation process, with values of 19.95 and 19.33 kcal/mol in vacuum and methanol, respectively. The ability of the solvent to form hydrogen bonds thus accelerates the production of the final product in an energy-efficient and kinetically advantageous manner. Progressing to the later phase of the reaction, which is characterized by intramolecular proton transfer, there is a noticeable increase in the activation energy values from 25.58 to 27.30 kcal/mol.

In examining the transition states for this condensation reaction, the initial phase aims to identify the activated complex, which indicates the specific case when the hydrogen atom passes from the amino group to the oxygen atom in the aldehyde group. This transition point is distinguished by a negative value of the imaginary frequency, (Table 2), representing an essential step in the double proton transfer mechanism. Moving to step II, the transition state materializes as the hydrogen atom moves from the amino group to the hydroxyl oxygen, leading to the formation of a water molecule and the expulsion



**Figure 7.** Energetic profile of the reaction between 4-pyridinecarboxaldehyde and p-aminobenzoic acid expressed in kcal/mol.

of methanol from the system. Theoretically investigating the energies obtained from the optimization process of the different entities participating in the condensation reaction and the energy profile was developed, including energy data both in vacuum and in methanol (Figure 3).

The examination of the chemical reaction involves the investigation of a phenomenon characterized by the absorption of thermal energy. The quantification of the energy associated with this particular reaction, which occurs under vacuum conditions and in the presence of methanol, amounts to 8.22 kcal/mol and 8.41 kcal/mol, respectively. These numerical values are in agreement with the experimental data, indicating that the synthesis or reaction took place under specific conditions requiring high temperatures to facilitate the desired chemical synthesis.

#### 4. CONCLUSIONS

Double proton transfer is a scientific approach that can provide important insights into the mechanism of condensation reactions and can contribute to the understanding of key aspects of reactions in biological and chemical systems. The use of Density Functional Theory (DFT) has proven to be effective in optimizing geometries and determining the transition states involved in condensation reactions.



Harmonic vibrational frequency harmonic calculations confirmed that all transition states are characterized by single negative imaginary frequencies, indicating that they are veridical saddle points on the potential energy surface. These data confirm the relative energy stability of intermediate and transition species.

The study revealed the complex mechanism of double proton transfer at both intermolecular and intramolecular levels. In the first step, intermolecular proton transfer is facilitated by the formation of an unstable intermediate compound, which initiates intramolecular proton transfer in the second step. The study has shown that methanol, as a solvent, reduces the activation energy at the intermolecular proton transfer step due to its solvation and hydrogen bond formation ability  $\Delta E_a$ (1st step)=4.46; 2.01; 0.62 kcal/mol. However, at the intramolecular transfer step, the activation energy increases, indicating a complex influence of the solvent on the different reaction steps  $\Delta E_a$ (II stage)= 0.68; 1.51; 1.72 kcal/mol.

The study confirmed the endothermic nature of the condensation reaction, requiring harsh conditions and high temperatures to be realized. This observation is consistent with the experimental data, emphasizing the need for strict control of the reaction conditions in practical synthesis, thus validating the accuracy of the DFT method for this type of study.

## Acknowledgements

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## Physiological particularities of calves' organisms in early postnatal ontogenesis

SERGIU BALACCI , ION BALAN , NICOLAE ROȘCA , AND VLADIMIR BUZAN 

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**Abstract.** This article presents a synthesis of the specialized literature regarding some aspects of the physiological state, growth, and development of calves in early postnatal ontogenesis. Calves have a distinct physiology, by comparison to adult cattle, adapted to their period of rapid development. Studying the physiology of calves during the early postnatal period is essential for ensuring their healthy growth and development, directly influencing long-term health and productivity. This critical period is marked by numerous physiological changes and adaptations, and understanding them is vital for implementing effective management and care strategies.

**Keywords:** calves, physiological peculiarities, postnatal ontogenesis.

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## Particularități fiziologice ale organismului vițelilor în ontogeneza postnatală timpurie

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**Rezumat.** Acest articol prezintă o sinteză a literaturii de specialitate privind unele aspecte ale stării fiziologice, creșterii și dezvoltării vițelilor în ontogeneza postnatală timpurie. Vițelii au o fiziologie distinctă, comparativ cu bovinele adulte, adaptată la perioada lor de dezvoltare rapidă. Studiul fiziologiei vițelilor în perioada postnatală timpurie este esențial pentru asigurarea unei creșteri și dezvoltări sănătoase a acestora, influențând în mod direct sănătatea și productivitatea pe termen lung. Această perioadă critică este marcată de numeroase schimbări fiziologice și adaptări, iar înțelegerea lor este vitală pentru implementarea unor strategii eficiente de management și îngrijire.

**Cuvinte-cheie:** viței, particularități fiziologice, ontogeneza postnatală.

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### 1. INTRODUCTION

Numerous experiments and world practice have shown that many problems that arise in the process of animal breeding cannot be solved without an in-depth study of the development of the organism at various developmental stages of postnatal ontogenesis. From the very beginning, from the amphimixis of the sperm with the egg, when the zygote is formed and until senescence, living organisms go through various stages and complex processes of ontogenesis [1, 2].

## PHYSIOLOGICAL PARTICULARITIES OF CALVES' ORGANISMS IN EARLY POSTNATAL ONTOGENESIS

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In each period of development, the organism is different from a physiological point of view. The early postnatal period is of great importance because, as the calves are born, they lose contact with the mother's organism, which leads to a complex restructuring of the physiological systems. The physiological systems of the calves' organisms during this period are characterized by their immaturity, thus, their organisms are sensitive to all the external conditions to which they are exposed [1, 3, 4].

The growth and development of the organism's functional systems in the first stages of postnatal ontogenesis occurs unevenly and is characterized by a pronounced heterochronism. In the process of evolutionary development, the calves' organisms have developed a series of protective and adaptive mechanisms to maintain homeostasis.

### 2. NERVOUS SYSTEM

If we refer to the nervous system, in newborn calves it is an immaturity of the cerebral cortex, which performs a constant regulation of all physiological processes, ensuring the unity of the organism with the external environment. Therefore, many physiological processes during the neonatal period are carried out on the basis of unconditioned reflexes, for example, the sucking reflex, motor or protective reflexes. Conditioned reflexes are gradually formed throughout life and make it possible for the organism to adapt to changing environmental conditions.

### 3. THERMOREGULATION

Thermoregulation is a complex neuro-humoral process of maintaining a constant body temperature using physical and chemical processes. Newborn calves are characterized by imperfect thermoregulation due to poor development of the internal thermoregulatory system. This is due to the peculiarity of the central nervous system, as its regulatory effect on thermoregulation gradually increases. There is every reason to believe that the formation of thermoregulation in ontogeny consists in the maturation of the temperature information integration apparatus and the development of control signals to peripheral thermoregulation effectors.

### 4. RESPIRATORY SYSTEM

The respiratory system begins to function from the moment of birth. At birth, the first extrauterine respiratory movements occur, which are facilitated by the resulting significant negative pressure in the pleural cavity that favours the expansion of the lungs. Its function is regulated in the neonatal phase by the reticular formation of the brainstem, which has a significant regulatory effect on the functional state of the respiratory centre and other

autonomic functions. Newborn calves have rapid and shallow breathing in the first hours after birth. Typically, the respiratory rate of a newborn calf is about 30-60 breaths per minute. This gradually normalizes as the calf adapts to the outside environment. During the first few days, the calves' lungs develop and expand to replace the amniotic fluid with air. During this period, it is important to ensure that the calf breathes properly and does not have respiratory problems.

## 5. DIGESTIVE SYSTEM

Newborn calves do not have a fully developed digestive system to digest solid food. Their digestive system is adapted to process milk, which is their main source of nutrition. The stomach of calves is divided into four compartments: rumen, reticulum, omasum and abomasum. At birth, the rumen and reticulum are underdeveloped, while the abomasum is functional and essential for milk digestion [5].

Calves have well-developed swallowing reflexes that allow them to consume milk without inhaling. However, at first their reflexes can sometimes be immature and calves may require close supervision to prevent milk aspirating into the airways.

The digestive system of calves undergoes significant changes in the first months of life. In the early days, calves are dependent on milk for their nutrition. The stomach of calves is predominantly of "proventricular" type with a relatively well-developed abomasum. During this period, the stomach lining is adapted for the digestion of lactose and milk protein. As calves grow, the digestive system goes through a transition to a "ruminant" digestive system. The intestinal microflora begins to develop and the rumen begins to form, adapting to the diet based on solid fodder. This transition is essential for developing the ability to digest fibrous forages and maximizing nutritional efficiency.

As one of the characteristics of the gastrointestinal tract of newborn calves, it should be noted that it is devoid of microflora. During birth, passing through the narrow birth canals, the calf swallows different microflora, thus, the microflora of the mucous membrane of the genital tract enters its gastrointestinal tract. From the very first day the gastrointestinal tract of the calf is populated with lacto-bacteria, bifidu-bacteria, enterococci, E. coli, staphylococci and a small number of other bacteria. Being located in the intestines, they constantly compete with each other. This temporary instability of microflora composition is called the period of transient dysbiosis. During the colostrum period, the intestinal microbial landscape stabilizes, both quantitatively and qualitatively. The composition of the normal microflora of the intestines of healthy calves consists of an equal amount of lacto-bacteria, bifidu-bacteria and E. coli, which then gradually becomes unbalanced and the number of potentially pathogenic bacteria and the concentration of E. coli decreases

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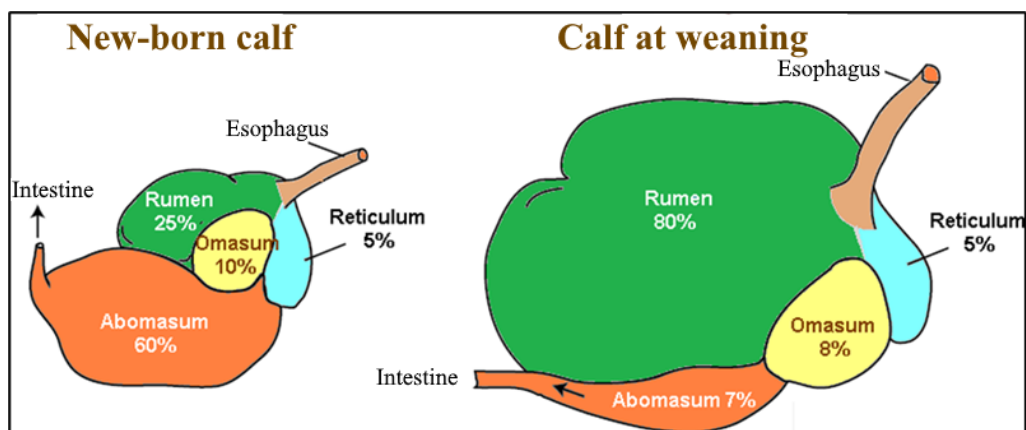
and populates the posterior part of the the intestine. One of the most important functions of the normal microflora is that it, together with the host organism, provides resistance to colonization by foreign microorganisms [6].

Liquid calf feed constitutes the majority of the diet until the calf begins to consume sufficient solid feed. In order to assimilate the milk, the calf only uses the abomasum. This is made possible by means of the esophageal gullet, which is formed reflexively and through which the milk reaches directly from the esophagus into the abomasum. In the acidic environment, the milk coagulates, forming a lump of milk, which gradually breaks down in the intestines, so the calf can assimilate all the nutrients. As the calf begins to grow and receive solid food, the function of the stomach compartments also develops. The newborn calf, therefore, finds it difficult to break down solid food. He can chew food even after a few weeks of life, but only around the age of 6-8 months he does become a real ruminant and can "procure" his nutrients from roughage as well [7].

After birth, physiologically mature calves assume a confident standing posture. During this period, a high excitability of the alimentary centre is registered. This is expressed through exploratory food reactions and the manifestation of the sucking reflex. Endogenous stimulation of the alimentary centre in calves lasts about 3 hours and manifests itself in the form of movements in searching for mothers [8, 9, 10]. The calf begins to suckle within the first three hours after birth. In the case of a delay in feeding a newborn, the food centre loses its excitability. The amount of milk sucked is regulated by the capacity of the calf's stomach cavity. As soon as the milk fills the stomach cavity and expands it, the receptors are excited. The related impulses that appear in this case trigger the inhibition reflex of the alimentary centre, which is expressed by the cessation of sucking movements. Long and irregular intervals between feedings cause a strong excitation of the alimentary center. When the alimentary centre is strongly excited, the time of inhibition of the alimentary centre during feeding is prolonged, and overfeeding of newborn calves is recorded, which can lead to diarrhea [11].

The gastrointestinal tract of newborn calves has the following relative parameters: the capacity of the rumen – 730 ml, the capacity of the abomasum – 1250 ml, the length of the small intestine is 14.5-16 m, the length of the large one is 2.3-3 m. In the neonatal phase, in calves the capacity of the rumen and especially of the abomasum increases rapidly (Figure 1) [12].

Prestomach tissues in newborn calves are poorly differentiated. In the first weeks of postnatal life, there is an intensive growth and differentiation of the cellular elements of the mucous membrane. The functional system of the abomasum at birth reaches such a degree of maturity that it fully ensures the adaptation of calves to a new way of feeding. A



**Figure 1.** Stages in the development of prestomachs in calves.

high activity of oxidative enzymes is recorded in the parietal cells of the abomasum. The principal cells in the abomasum glands in newborn calves are still few and they secrete pepsin, renin and lipase. The predominant enzyme of the gastric juice in the neonatal phase is renin, which coagulates the milk. In general, clot secretion in newborn calves is poor. In the newborn period there is little pepsin and chymosin in the abomasum. In the first 2-4 days, free hydrochloric acid is formed in minimal amounts and this provides the necessary conditions for the assimilation of some components of maternal colostrum, which are extremely important for the organism of newborns in the first days of life. The increase in the amount of HCl in the content of the abomasum occurs from the end of the first month of life, after the gradual development of nervous tissue and the function of the glandular epithelium. During the absence of hydrochloric acid in the clot, the function of activating the conversion of pepsinogen into pepsin is performed by lactic acid, which is formed as a result of the breakdown of glycogen [13]. According to Coleen M. Jones [7], the acidity of the clot during this period depends on the acidity of the colostrum and the time elapsed after its administration.

Due to the fact that the development of digestive organs has not been completed yet, the digestive enzymes are initially adapted only for digesting the nutrients of colostrum and milk. The major importance at this time belongs to the parietal digestion. The reflex mechanism for regulating the functions of the abomasum has limited capabilities and, due to this, spontaneous secretion occurs. By the end of the neonatal phase, the secretory activity of the abomasum increases, the reflex and hormonal phases of the regulation of secretion from the abomasum begin to be clearly manifested.

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In the intestine, due to the presence of lactic acid, secretin is formed by the mucous membrane of the anterior part, which in turn stimulates the secretion of pancreatic juice. Pancreatic juice contains trypsinogen, which is transformed into trypsin under the action of enteropeptidase. The trypsin of the intestine in newborn calves, as well as the pepsin of the stomach, predominantly performs the proteolysis of casein. The bile of newborns is poor in bile acids and does not significantly activate pancreatic juice enzymes. Intestinal glands secrete phosphatase, galactosidase and other enzymes of intestinal juice in small amounts. In newborn calves, the villi are well developed, while the glandular and muscular structures are less developed, with parietal digestion predominating. Albumin and globulins from colostrum, without being subjected to hydrolysis, reach the intestine and, unchanged, are absorbed through the intestinal wall into the blood [14,15]. This ensures the creation of a new internal environment in the newborn, thus, creating its own natural physiological immunity. Lysozyme also enters the calf's blood out of the colostrum. Gamma globulins and lysozyme are not formed in the neonatal phase. The timing of the first feeding and the observance of appropriate intervals between feedings are of great immunobiological importance for the newborn.

### 6. IMMUNE SYSTEM

The body protection function against various pathogens is implemented by special organs, tissues and cells, which are part of a system called immune system. Calves are born with an immature immune system that develops gradually in the first months of life. In the first period, the body immunity is supported by colostrum, which provides essential antibodies for the protection against diseases. The transfer of immunoglobulins through colostrum is crucial for the protection of calves until their own immune systems become functional.

The level of immunoglobulins in colostrum on the first day after birth is several times higher than in the mother's blood. In the first hours in newborns, the absorption of immunoglobulins is conditioned by a high permeability of the intestinal mucosa. The main types of immunoglobulins in colostrum are Ig A, G and M [14, 15]. The high immunoglobulin content in colostrum does not last long. In 6 hours, the average capacity of the intestinal walls to absorb immunoglobulins decreases by a third. By 24 hours, the walls absorb less than 10% of what could have been absorbed initially [7]. Thus, in one day, the number of immunoglobulins is reduced by 2 times and in two days by 4 times. The duration of colostrum immunity is short, the half-life of IgM is 3-5 days, IgG – 10-25 days, IgA – 4-6 days [8, 16]. The immunity of a newborn calf is sufficient if



the immunoglobulin content in the colostrum is at least 50 g/l and this corresponds to a density of 1.048 g/cm<sup>3</sup> [8, 17, 18, ].

The immunoglobulins that are absorbed by the intestinal mucosa enter the lymphatic tract and from there into the blood, and serve to protect the organism from infections in the form of humoral antibodies. Since these antibodies were formed as a result of the mother's immune response, they are directed primarily against those microbes which the mother's organism came into contact with. Antibodies also have some opsonizing activity against other pathogens with a similar antigenic structure. Colostral antibodies are also involved in the local protection mechanism [11].

The supplying of colostral antibodies to newborns is determined by the immunoglobulin content in the colostrum, the amount of colostrum drunk and the permeability of the intestinal walls. Leukocytes are transmitted to newborns along with colostrum and increase in the blood of calves after the first feeding by 1.5-2 times. They pass into the mammary gland shortly before birth and can reach a concentration that is tens times higher than their level in blood. Colostral lymphocytes produce antibodies, mainly IgA, and perform an adaptive transfer of cellular immunity, especially delayed-type hypersensitivity [11].

The synthesis of immunoglobulins in calves begins with the formation of IgM, then IgA and IgG. M Class immunoglobulins can block the spread of the pathogen agent in the organism, but are ineffective against toxins. Therefore, young animals are very sensitive to poisoning and toxicoinfections [19].

In the neonatal period, it is necessary to emphasize that the barrier function of the liver in newborn calves is insufficient, the neutralization of toxic substances is weak, therefore, calves often have cases of intestinal intoxication and inflammation in the gastrointestinal tract. The excretory function of the liver in calves is at a low level. In the first days of life, the activity of the calf's liver depends on the amount of proteins in the blood, hematopoietin. During this period there is a lower capacity to bind and release bilirubin than in older calves. All this indicates that the liver during this period is functionally immature [20].

There are noted some features in the functioning of the immune system and non-specific resistance of calves in the neonatal and early postnatal period. Before the intake of colostrum, in the blood of calves it is a low content of total proteins, immunoglobulins, leukocytes, including lymphocytes, amino acids and other defense factors. After the first administration of colostrum, these indicators increase significantly during the first week. Then, a slight decrease in these parameters is recorded, particularly, in the period from the 14th day to the 21st one. During this period, the activity of the passive immunity,

## PHYSIOLOGICAL PARTICULARITIES OF CALVES' ORGANISMS IN EARLY POSTNATAL ONTOGENESIS

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received from the mother-cows altogether with the colostrum, decreases, and the calves' own immunity is just forming. This period of life is characterized by a low content of own immunoglobulins, low phagocytic activity; mucous membranes and skin are easily accessible to pathogenic microflora. Thus, timely feeding with high-quality colostrum plays a crucial role in raising healthy offspring [21].

In the early postnatal period of ontogenesis, cellular factors of immunity predominate and they compensate for the deficiency of humoral factors, which are formed during the growth and development of calves in different periods. Thus, 80% of the lymphocytes in the lymph nodes, spleen and blood are T cells. At the same time, there is a deficiency of helper T cells and suppressor T cells, which affects the development of humoral immunity. The immune system in calves begins to stabilize at 1.5-2 months, and the final formation of cellular defense factors is completed by 6 months of age. A weak humoral response during this period is associated, on the one hand, with the presence of maternal antibodies which block incoming antigens, and, on the other hand, with an underdevelopment of B immune system, which is responsible for the synthesis of various classes of immunoglobulins. Later on, with age, the phagocytic activity of calves decreases slightly, while the activity of humoral factors increases significantly.

The implementation of the immune response is achieved through the interaction of cellular and humoral immunity connection laws. The cellular connection is represented by the functioning of T cells, while the humoral one is represented by B cells and the formation of antibodies. The place to form T and B lymphocytes is the bone marrow, from which these types of cells migrate to the thymus, lymph nodes and spleen [19].

Serum bactericidal activity is a complex indicator due to the combined action of immunoglobulins, complement, muramidase, beta-lysinase, that is, the sum of the action of all antimicrobial factors. Lysozyme acts on gram-positive bacteria, while complement lyses gram-negative bacteria and many protozoa. An important role in implementing the bactericidal activity of blood is attributed to the immune cells, such as T and B lymphocytes, macrophages and neutrophils. T cells, through secreted factors, regulate the activity of macrophages that synthesize lysozyme [22].

It is believed that the reduced resistance in calves in early ontogeny is associated with a special biochemical state of body cells. The presence of age-related characteristics of non-specific resistance was confirmed as early as 1979 by Plyashchenko and Sidorov [23], who observed an increase in the level of phagocytosis in calves up to five days old, then, starting at ten days old, a sharp decrease was observed. At the same time, the formation of bactericidal activity of blood serum continued gradually. Therefore, by ten days of age in calves, the high level of phagocytic activity compensates for the low level of bactericidal

activity. In the next 2-3 weeks of life, there is a rapid increase in humoral factors, which reach a relative stability at the age of 6 months, and the final formation at the age of 11-12 months [10, 11].

## 7. CONCLUSIONS

Calves go through a series of significant physiological changes in the postnatal period, starting with the adaptation of the digestive and respiratory systems up to the development of the immune system and the ability to regulate their body temperature. Understanding these physiological aspects in the breeding, maintenance and operation of animals is essential for optimizing the calf's growth and welfare. Careful monitoring of the physiological characteristics of the organism during the critical periods of ontogenesis contributes to ensuring a healthy transition from the early postnatal period – a period in which important physiological processes change rapidly and in which the foundations are laid for the future productivity of the organism in adulthood.

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## Diversity of small rodents (Mammalia: Rodentia) in various sectors of the municipality of Chisinau

NATALIA CARAMAN 

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**Abstract.** The small rodents in various sectors of the municipality of Chisinau are represented by 10 species. In the recreational sectors of the municipality of Chisinau, a rather rich fauna of small rodents was recorded due to the existence of various types of biotopes, including natural and wetland, which denotes the presence of stable communities of rodents, favoring in turn the existence of a series of predatory vertebrates.

**Keywords:** rodents, trappability index, abundance, municipality of Chisinau.

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## Diversitatea rozătoarelor mici (Mammalia: Rodentia) în unele sectoare ale municipiului Chișinău

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**Rezumat.** Rozătoarele mici din diverse sectoare ale municipiului Chișinău sunt reprezentate de 10 specii. În sectoarele de agrement ale municipiului Chișinău a fost înregistrată o faună destul de bogată de rozătoare mici datorită existenței diverselor tipuri de biotopuri, inclusiv cele naturale și zonele umede, ceea ce denotă prezența unor comunități stabile de rozătoare, favorizând la rândul său existența unei serii de vertebrate prădătoare.

**Cuvinte-cheie:** rozătoare, indice de capturare, abundență, municipiul Chișinău.

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### 1. INTRODUCTION

In recent decades the world has undergone rapid changes, including demographic explosions and massive urbanization, as result of human activities natural ecosystems have been affected, either positively or negatively. As an example of the negative effects of urbanization is the loss of many plant and animal species.

The degradation of natural habitats and their replacement by anthropogenic habitats have caused, over time, the decrease in the number of ecological niches and an intensification of interspecific competition, lax food requirements as well as the high degree of adaptability, representing an advantage of synanthropic species over other representatives of fauna from natural areas.

Synanthropic species are often generalist species, and in non-urban areas they commonly develop [5]. Mammals that are able to adapt to even the most developed portion of the urban environment are called urban explorers or synanthropes. They represent a

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group of mammal species that are highly adapted to urban environments [6]. They are usually omnivores and their populations depend on man human resource.

The first rodents identified on the territory of the municipality of Chisinau from the Late Miocene fossil sites were discovered in the Otovasca site, which is part of the southeastern sector of Chisinau city. This archaic rodent belongs to the species *Chalicomys jaegeri* (Kaup, 1832) of the Castoridae family [4].

Small mammals are an indispensable component of urban ecosystem fauna and can serve as ecological indicators of urban ecosystem stability. In the last century, there are only a few studies on the urban fauna of small mammals, where 8 species of rodents are mentioned [9]. After the year 2000, the faunal studies of small mammals in the municipality of Chisinau were more intense. Thus, research was carried out in various types of more or less anthropized biotopes of the municipality of Chisinau [1-3, 8]. In these papers it is mentioned that urban ecosystems are populated by 11 species of rodents and three species of insectivores from three ecological groups: synanthropes, hemisynanthropes and exoanthropes, some ecological peculiarities of synanthropes species were elucidated.

As a result of these studies, the specific composition and diversity of the fauna of small mammals in the selected territories were elucidated.

The aim of the work was the ecological analysis indices of small rodent have been calculated: abundance, trappability and diversity in different types of biotopes of the small rodents in the sectors of the municipality of Chisinau, which are considered to be areas with intense recreational activity. As result of these studies, it was elucidated diversity of the small rodent fauna in the various sectors of the Chisinau city.

## 2. MATERIALS AND METHODS

The research was carried out between the years 2008-2009 in the municipal parks of the municipality of Chisinau. Valea Trandafirilor Park is located in the Botanica sector. It covers an area of 145 hectares, of which 9 hectares are water surface. It was set up in 1968 on the site of a hill cultivated with roses, paths were built and the banks of the waters were dammed. Valea Morilor Park is part of the Buiucani sector of the municipality of Chisinau and is located on the shore of Lake Valea Morilor on a plot of land with a varied relief, having 4 entrances [13]. The park area was initially about 114 hectares, the lake having an area of 34 hectares. A 2.5-kilometer-long ring road was arranged around the lake. The Botanical Garden is located in the South-East part of Chisinau city, Botanica sector. The territory of the Botanical Garden is crossed by four constructed water reservoirs, which, being at different levels, form a cascade of lakes. Groundwater is at different depths between 1.5 - 7.0 m [12]. The Zoological Garden is located near the

Botanical Garden and is located in the southern part of the city, stretching over an area of 24,306 hectares [12]. The Arboretum Park is located in the Buiucani sector of Chisinau city. The park is located in the central-western part of the municipality, in the valley of Durlești river, the surface of the park has been extended to 77.8 hectares. “La Izvor” park is part of the Buiucani sector of Chisinau. Being the second largest in the municipality, it covers an area of 150 hectares and includes 3 lakes with several islets.

Data on the structure and density of the populations of small rodents were obtained according to the standard method of collecting the material in the field during 4-5 days with the help of traps arranged in lines of 25 pieces with the interval between them of 5 m and between the lines of 20 m. Black bread soaked in unrefined sunflower oil was used as bait. All captured animals were identified.

The ecological indices of small rodent have been calculated: abundance, trappability and diversity in different types of biotopes.

### 3. RESULTS AND DISCUSSIONS

Research in different sectors of the city proved to be the most difficult to carry out due to the increased crowding of residents and the presence of stray animals. Thus, the species diversity of small rodents varies from one species in the Botanica sector to 10 (*C. glareolus*, *Arvicola terrestris*, *Microtus arvalis*, *M. rossiaemeridionalis*, *Apodemus uralensis*, *A. sylvaticus*, *A. flavicollis*, *A. agrarius*, *Mus musculus* and *Rattus norvegicus*) in the Sculeni sector.

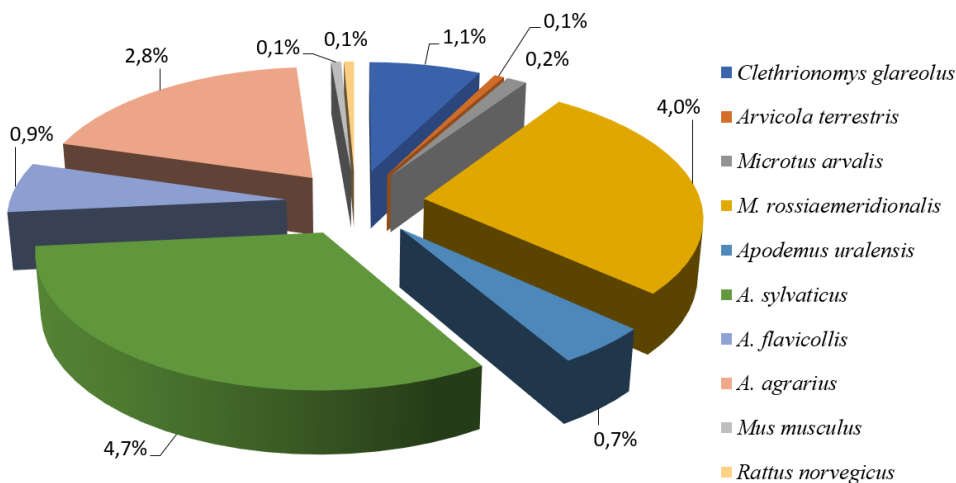
The diversity of small rodent species collected in various types of biotopes in the Sculeni sector of the municipality of Chisinau was the richest, compared to other types of the biotopes located on the territory of Chisinau city, possibly due to the fact that the works were mostly carried out in biotopes further away from inhabited houses, on the banks of the Bîc river and its surroundings.

The most numerous species was *A. sylvaticus* with trappability index of 4.7%, followed by *M. rossiaemeridionalis* with 4.0%, *A. agrarius* was reported with a trappability index of 2.8%. The species *Arvicola terrestris*, *Mus musculus* and *Rattus norvegicus* had the lowest trappability index with 0.1% each (Fig. 1).

The relative abundance in the Sculeni sector biotopes is the highest in the species *A. sylvaticus*, which constitutes 32.8%, followed by *M. rossiaemeridionalis* with 17.2%, *A. agrarius* – 14.9%. The lowest relative abundance was reported in the species *Arvicola terrestris* with 0.6%. Regarding the synanthropic species, we mention that *M. musculus* had a relative abundance of 4.6%, being captured in the warehouse, and *Rattus norvegicus* - 2.9% was captured not far from the warehouse, which is located on the banks of the

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Bîc river. On the territory of the deposit, the most abundant species was *A. sylvaticus* with 83.3%, due to the fact that the deposit was bordered by a forest strip, while the synanthropic species *M. musculus* was reported with a much lower relative abundance only by 16.7%.



**Figure 1.** Trappability index of small rodent species in the Sculeni sector.

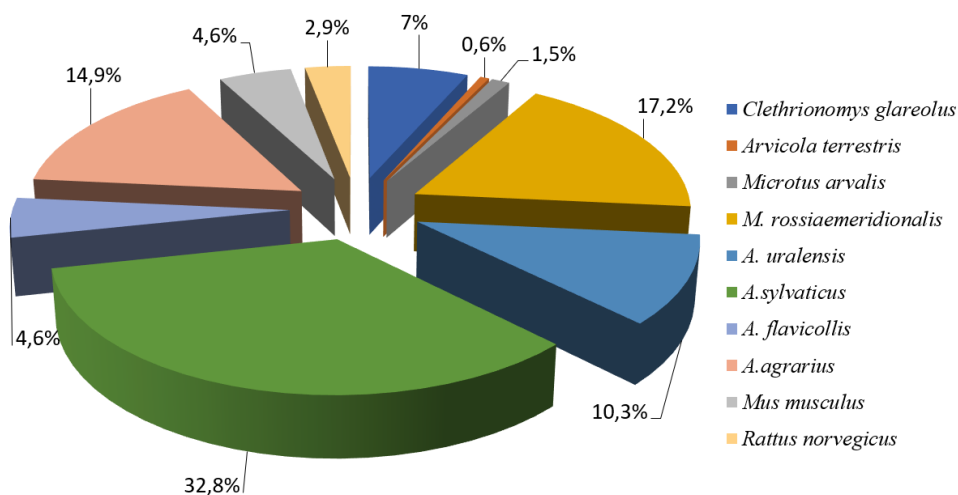
The deposit, which is located near to the Bîc river bank, the most abundant species was *A. sylvaticus* with 62.5%, followed by *M. rossiaemeridionalis* - 12.5%. The species *C. glareolus*, *A. terrestris*, *A. agrarius* and *R. norvegicus*, the last species being strongly synanthropic species, they had a relative abundance of 6.25% each at the time of the research (Fig. 2.).

After the research carried out in the anthropized biotopes of Valea Morilor Park of Chisinau city, 3 species of small rodents were identified: *A. sylvaticus*, *A. uralensis* and *Mus musculus*. The highest trappability index in these biotopes was reported for the strongly anthropized species *M. musculus* with 4.4% and an abundance of 58.3%, followed by *A. sylvaticus* with – 1.9% and a relative abundance of 25%. While the species *A. uralensis* had a trappability index of 1.3% and a relative abundance of 16.7%.

In the Botanica sector of Chisinau city, research was carried out in Valea Trandafirilor Park, where 2 species were identified, both belonging to the genus *Apodemus*: *A. sylvaticus* and *A. uralensis*. Both species had close values of trappability index *A. sylvaticus* 5% and *A. uralensis* 4%, the first species having an abundance of 55.6% and the second 44.4%.

The diversity of rodent species is very poor in the city parks, it was represented by only 3 species, of which the species *A. sylvaticus* had a major abundance of 46.7%, and





**Figure 2.** The abundance of small rodent species in the Sculeni sector.

the smallest one was reported in the synanthropic species *M. musculus* – 20%, because it inhabits the biotopes near houses.

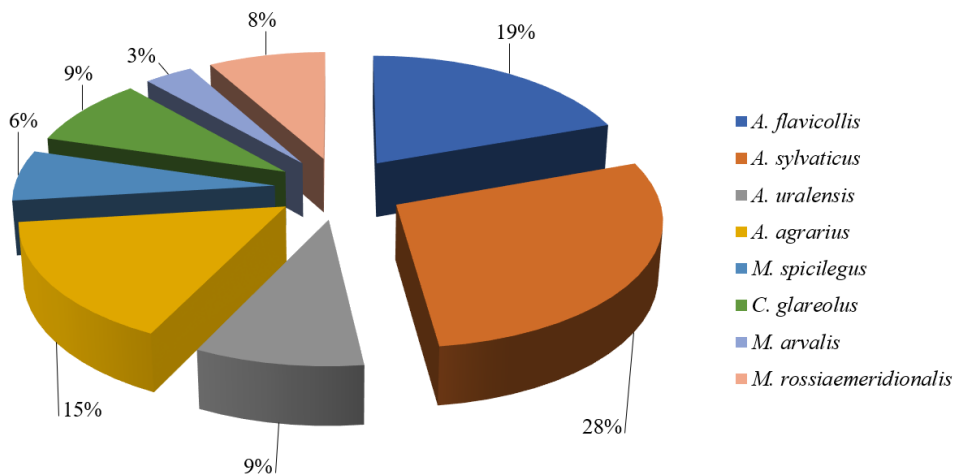
The landscape parks (Botanical Garden and Zoological Garden) from the faunal point of view were much richer than the aforementioned parks, being identified 8 species of rodents. The maximum abundance was held by the species *A. sylvaticus* with 27.5% followed by *A. flavicollis* with 18.9%, *A. agrarius* with 15.3%, and the species with the lowest abundance value was *M. arvalis* with 3% (Fig. 3.).

As result of the processes of development and urbanization (the growth of cities and suburban areas), the forest cover of the region decreased significantly, the native forest coenoses were gradually replaced by parks, protective forest belts, agrocoenoses, etc. [11]. The emergence and subsequent development of settlements, especially those as large as cities, could only affect the state of the surrounding natural landscapes. Urban biota (including small rodents) is exposed to sound and electromagnetic pollution, which affects the functioning of cellular and molecular structures.

Undoubtedly, green spaces, which are of particular importance for improving the ecological condition of the urban environment, should be considered the most important components of urban landscapes. Plants retain dust and absorb 50-60% of toxic gases [10]. They essentially reduce noise pollution and wind intensity. Tree crowns, especially poplars, absorb up to 20–70% of sound energy [10].

As result of the research carried out, some practical recommendations can be proposed such as:

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**Figure 3.** The abundance of small rodent species in the landscape parks of Chisinau city.

1. Prohibiting the storage of household waste in unauthorized places, in order to avoid the accumulation of a large number of synanthropic and hemi-synanthropic rodents – harmful to the health of the population.
2. Monitoring of drains, sewers and basements of blocks of flats, houses, abandoned buildings, as these are breeding sites for synanthropic species of small rodents (*R. norvegicus* and *M. musculus*).
3. Awareness of the general public through lectures, projects, articles in newspapers and popularizing magazines, participation in various events regarding the maintenance of faunal diversity in urban ecosystems, rodents being an indispensable component of them.

#### 4. CONCLUSIONS

In the recreational sectors of the municipality of Chisinau a rather rich fauna of small rodents was recorded due to the existence of various types of biotopes, including natural, which denotes the presence of stable communities of rodents, which in turn favor the existence of a series of ownerless animals.

In Chisinau city, lawns and boulevards were the most unfavorable for the existence of small rodents, i.e. the biotopes most intensely subject to urbanization factors. The most attractive for rodents in the municipality of Chisinau are the landscaped parks. The dominant group was that of the hemisynanthropic species.

A considerable negative influence on rodent species is the high disturbance factor from the local population and recreational activity in populated areas.

Thus, the fauna of small rodents is well represented in the recreational and urban ecosystems of the municipality of Chisinau. The obtained results are close to the existing data for other European cities [11].

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