MODERN PROBLEMS AND SOLUTIONS IN ENVIRONMENTAL PROTECTION – 2021

Post-conference monograph

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Contents

Dear Readers

We present you with the next edition of the monograph Modern Problems and Solutions in Environmental Protection. The book you have received was created as a result of the 16th international interdisciplinary conference "Current Environmental Issues – 2021" organized in cooperation with the Faculty of Biology and Ecology of the Yanki Kupala State University of Grodno during the period 24-26th September 2019 at the Faculty of Biology and Chemistry of the University of Bialystok.

The current year – 2021 – is a specific pandemic year. The ongoing covid-19 pandemic has forced the organization of the CEI-2021 conference online. We did not have the opportunity to meet face-to-face, exchange thoughts, ideas and achievements. But the prevailing state of a pandemic does not release the academic community from dealing with, caring for, and researching the state of the natural environment, on the contrary, it creates new problems related to the new pollutants e.g. massive amounts of used masks, the use of new disinfectants, virus biology research, their mutations, etc. Problems that have arisen in the last 12 months, you can count indefinitely.

The main goal of the monograph was to present the most recent results of the participants' scientific activity during the last 12 months. As the broadly understood protection of the environment and natural resources is multidimensional, covering issues from chemical, biological, and econometric analysis, or environmental management, to education, the presented monograph also reflects this complexity. Therefore, the content discussed in the monograph is very diverse. The monograph is divided into three parts. The first part deals with chemical analysis, the second with issues related to microbiology, ecology, botany, and the third with environmental education.

The monograph begins with a chapter devoted to issues related to the problem of reducing nitrogen oxides. For this purpose, a catalyst based on natural zeolite such as clinoptilolite was proposed. The stability of the catalyst was checked by XRD methods. The efficiency of the proposed catalyst was checked. It was stated that raw, as well as modified by iron species, catalyst exhibited a high rate of NOx conversion.

The next chapter presents the current state of achievements in the field of application of ion-selective electrodes for nitrates determination. The importance of the development of simple, fast, and reliable methods for their content determination in environmental samples was presented.

The third discusses commercial methods of production of polycarbonates. The advantages and disadvantages of applied reactions are presented and their environmental and human health impact is assessed.

The fourth chapter deals with the electrochemical determination of indium(III) ions in environmental aqueous samples. The influence of the type of electrodes used at the limit of detection is discussed. The procedures applied different working electrodes: a mercury film electrode with a silver substrate (Hg(Ag)FE), a lead film electrode (PbFE), and a bismuth film electrode (BiFE) were compared. The influence of substances naturally present in aqueous

samples was checked. All presented procedures were validated and applied to the analysis of certified reference material and surface waters taken from the surroundings of Lublin.

The next chapter deals with the problem of recovering rare earth elements from waste materials. For this purpose, an alginate-based biosorbent (named alginate-biochar composite/ ALG-BC) is proposed. The efficiency of lanthanum (III) preconcentration and recovery were checked. The parameters that influenced the sorption process of La(III) were evaluated.

The second part of the monograph begins with work discussing the phenomenon of bacterial opportunism. As opportunistic human pathogens can contribute to a significant reduction in the effectiveness of treatment with antibiotics and antimycotics, their influence on human health and the spread of bacterial and fungal infections cannot be ignored. This subject is continued in the next chapter where the environmental problems caused by cyanobacteria are discussed. Their negative impact on surface water quality as well the conditions of aqueous organisms is presented.

The eighth chapter is devoted to dogs' and cats' health issues. The Authors looked at some factors that allowed to identify patterns of occurrence and development of various diseases and to predict their dynamics in connection with changes in the complex conditions of their habitat.

Chapter nine presents the results of research on the number and activity of bacteria in Lake Hańcza in northern Poland. The analysis of these parameters was carried out both in the vertical section of the water body and horizontally in individual water zones. The study was carried out in early September 2018. Neither horizontal nor vertical changes in bacterial number were observed. There were recorded only some differences in bacterial abundance in samples taken from two coastal sampling points located in the southern part of the lake at the direct catchment area with commercial development.

Chapter ten focuses on differentiation of bacterioplankton in 23 lowland springs at protective area of Gryżyński Landscape Park (western Poland). Authors present relationships between hydrogeological, hydrochemical features and development of water bacteria in spring niches. This study shows that microbiological parameters can be used as important indicators of ecological status of groundwater condition.

The next chapter deals with studies on long-term changes of crustacean zooplankton communities in Lake Wigry. There are summarized 100 years of observation of zooplankton structures in relation to the changing trophic conditions. As a result, the qualitative and quantitative changes during the last century can be summarized and some conclusions regarding the development prospects of some species can be made.

The twelfth chapter presents the results of studies of some ground beetles of the genus Coleoptera and Carabidae in 6 provinces of Russia and Belarus. The provided data was based on morphimetric traits. The presented results concern nine species for which the left and right sides were measured and the variable asymmetry (FA) parameter was estimated. It was found that the size of FA changes depended on the biotope, species, and interactions between them.

In eudominant of arable lands biotopes – Poecilus cupreus – the highest values of FA were recorded in the meadows, being about equal in all types of crops.

The next chapter presents results of observation of the influence of the secondary succession of cleared bilberry pine forest on changes in bird population. The fieldwork was performed in the years 1996–2018 applying conventional bird counting methods. It was found at the beginning the bird population comprised European, European-Turkestan, and Palearctic types of fauna. The diversity of species has changed over the years and is now dominated by the European types of fauna.

Chapter 14 discusses the problems associated with the invasive species, the American mink. The action of catching these animals was carried out in 13 fish pond complexes in February-April 2021. The caught specimens were tested in order to determine the genetic diversity and origin of the wild American mink population in the south-eastern part of Poland. The action of trapping mink will continue this fall.

This part ends with Chapter 15 on conditions for rational use of medicinal plants. For this purpose, it is necessary to define the phytocoenotic limitations of a species and information about its productivity in various communities. Such an analysis is presented based on the example of yarrow (Achillea millefolium L.). its occurrence, the association of communities with phytocoenoses and the impact of the most important environmental factors on them, as well as the frequency of occurrence, phytocenotic activity, the average predicted coverage and productivity in various types of communities have been determined.

The last part of the monograph concerns problems related to modern methods of teaching about the environment and for the environment. The Authors of chapter 16 present their experiences with the participation of students of secondary school no 12 in Grodno in the local environmental ecological initiative "URA Grodno!". As part of this initiative, the project "Public involvement in environmental monitoring and improvement of environmental management at the local level", financed by the EU and implemented by UNDP in cooperation with the Ministry of Natural Resources and Environmental Protection of the Republic of Belarus, was implemented.

The last chapter discusses the competences in biology that a graduate of Belarusian secondary schools should demonstrate when applying for medical, biological, environmental or agricultural studies. The subject teachers are responsible for the preparation for the central test examination in Belarus and the candidate's success depends on their work. Each teacher has a task: to organize the work in such a way that the graduate can get the maximum grade from the test. The teacher, guided by the students' skills, selects forms, methods and techniques of work to achieve the highest effect. Preparation for the centralized test begins in 7th grade, when students start learning botany. Using the example of the subject block "Zoology", the article presents methods of using the competency approach in the lessons.

We hope that the monograph we have prepared, due to the variety of topics, will interest many specialists dealing with various aspects of both ecology and environmental protection.

Editorial team

CATALYTIC ACTIVITY OF NATURAL CLINOPTILOLITE MODIFIED WITH IRON BY VARIOUS METHODS IN SELECTIVE CATALYTIC REDUCTION OF NITROGEN OXIDES WITH AMMONIA (NH_3-SCR)

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Abstract

Nitrogen oxides (NO_x) are one of the most harmful air pollutants emitted by stationary sources. The mixture of NO_x has a great contribution to acid rain and photochemical smog formation and ozone layer depletion. In order to abate the amount of nitrogen oxides, governments around the world implemented a number of emission control legislations. Meeting more severe restrictions can be achieved only by using effective methods of NO_x reduction. One of the widespread and efficient technologies used on the industrial scale is selective catalytic reduction with ammonia (NH_3-SCR). The reaction between nitrogen and the reducing agent (NH_3) takes place on the surface of the catalyst, consequently, nitrogen and water vapor are yielded. The commercial catalyst of the process is V_2O_5-WO_3(MoO_3)- TiO_2. Despite satisfactory activity above 300 °C, it causes some operational problems. The most important are its narrow temperature window and secondary contamination by toxic vanadia and oxidation of SO_2 to SO_3. The mentioned problems can be solved by the replacement of the commercial system by substitutive catalyst, free of these drawbacks. One of the most promising precursors of new NH_3-SCR catalysts are natural zeolites, such as clinoptilolite. It is mainly due to its acidic character, micro- and mesopore texture and high thermal stability. Vanadium active phase can be replaced by iron species that have already found wide application in NH_3-SCR. Therefore, in the presented work, natural clinoptilolite was modified with iron by co-precipitation and adsorption from solution and tested as a catalyst of NH_3-SCR. It was found that even raw zeolite exhibits 56% of NO conversion at 450 °C. Catalytic activity of the material was much higher when co-precipitation was applied as the modification procedure. Furthermore, when the active phase was introduced by adsorption from solution, the amount of emitted N_2O was higher in the entire temperature range of the reaction. XRD analysis performed in the temperature range of 100-500 °C indicated that the structure of both the raw zeolite and the catalysts remains stable within the temperature range of the reaction.

Keywords: selective catalytic reduction, nitrogen oxides, natural zeolite catalyst

Introduction

The emission of nitrogen oxides (NO_x) caused by the progressive development of industry and increasing demand for energy is one of the most significant environmental problems. Above all, contamination with NO_x contributes to the formation of photochemical smog, acid rain, inorganic aerosols and ozone layer depletion. Despite the fact that the gases were of greatest concern and under strict regulation in many countries, they still have a big share in air pollution (P. et al., 2016; Huang et al. 2017; Gelves et al. 2019). One of the most important industrial catalytic technologies for the abatement of NO_x emission is selective catalytic reduction with ammonia (NH_3-SCR) (Kim et al. 2020; Szymaszek et al. 2020; Vignesh and Ashok 2020). The most important reactions of the process are depicted by equations (1-2):

$$4NH_3 + 4NO + O_2 \rightarrow 4N_2 + 6H_2O \quad (1)$$
$$4NH_3 + 2NO + 2NO_2 \rightarrow 4N_2 + 6H_2O \quad (2)$$

The reactions (1-2) stand for so-called "standard SCR" and "fast SCR", respectively. The main difference between the mechanisms is that in "standard SCR" equimolar amounts of NH_3 and NO form N_2 and H_2O, while in the presence of NO_2 the reaction rate is higher and the process is much faster (Arnarson et al. 2017). The commercial catalyst of NH_3-SCR installations in power plants is V_2O_5-TiO_2 promoted with MoO_3 or WO_3 ('Research Status and Prospect on Vanadium-Based Catalysts for NH3-SCR denitration' 2018; Chen et al. 2020). The material provides more than 90% of NO conversion and high selectivity to N_2, especially above 300 ºC (Kompio et al. 2017; Gan et al. 2018). Nonetheless, the material has some significant operating limitations. One of the most important is its narrow temperature window that requires the placement of the catalyst in "high dust" position. In such a placement the system undergoes contamination by fly ash and sulphur compounds which leads to its poisoning and lower activity. The alternative option is to install the catalyst in "tail end position" and heat the flue gas. However, this solution generates higher energy demand and, in consequence, additional costs of the whole SCR installation (Grzybek 2007; Szymaszek et al. 2020). What is more, vanadium-based catalyst is sensitive to poisoning with SO_2 and catalyzes its oxidation to SO_3 (Ma et al. 2015; Xu et al. 2017). Additionally, the emission of vanadium during exploitation of the catalyst can cause secondary contamination. The element accumulates in soil for many years and negatively affects the quality of environment due to its carcinogenic character (Yannopoulos 1967; Zwolak 2014; Liu et al. 2015). As a consequence of all of the raised problems, replacement of the vanadium-based SCR catalyst with alternative material has gained a lot of attention among scientists. During the last decades, a number of substitutional catalytic systems for NO_x abatement has been developed, including mixed metal oxides (Liu et al. 2021; Tan et al. 2021), modified activated carbon (Saad et al. 2020a, 2020b), supported metal oxides (Saad et al. 2020b; Si et al. 2020; Zhou et al. 2021), and modified zeolites (Ghasemian et al. 2014; Bergman et al. 2020; Chen et al. 2020; López-Hernández

et al. 2020). Among them, the most promising are zeolites doped with iron or/and copper (Chen et al. 2020; Lei et al. 2021; Tang et al. 2021; Xiao et al. 2021). A representative of the group of natural zeolites is one of the most abundant on Earth – clinoptilolite that belongs to the group of heulandite (HEU) (Valdiviés-Cruz, Lam and Zicovich-Wilson, 2017; Ramishvili et al., 2019). Crystalline structure of the material was refined as a monoclinic cell of C2/m space group (Alberti 1975). Si/Al molar ratio of clinoptilolite oscillates around 4.5 – 5.5, which means that the material is highly acidic (Valdiviés-Cruz et al. 2017). The conventional cell parameters of the zeolite are a = 17.64 Å, b = 17.89 Å, c = 7.39 Å, and β = 116.22 º. The values correspond to a primitive cell possessing a = 12.41, b = 12.57, c = 7.28, and α = 69.04 º, β = 105.58 º, and γ = 88.38 º parameters that have half of the volume of the conventional one. Clinoptilolite is a 2D zeolite that contains three channels along the dimensions. Two channels A and B that exhibit 8- and 10-member rings, respectively, run along the conventional axis c. The third channel C with 8-member rings goes parallel to the conventional axis a. Apart from that, clinoptilolite can confine about twenty molecules of water in one unit cell and the negative charge caused by the partial replacement of Si^{4+} cations by Al^{3+} cations is compensated usually by Na^+, K^+, and Ca^{2+} (Alberti 1975; Ramishvili et al. 2019). The advantage of the material is that its structural and chemical properties can be easily modified to obtain the features required for the specific application. For example, Valdiviés-Cruz et al. (Valdiviés-Cruz et al. 2017) described the influence of strong acid on the dealumination process over protonated form of clinoptilolite. It was found that treatment of the material with concentrated HCl results in the formation of new Brönsted acid sites and weakening of Al – O bonds. Additionally, in other works it was confirmed that elimination of framework aluminum increases the amount of empty spaces in porous materials (Yi et al. 2018; Zhu et al. 2021). Consequently, it allows to obtain molecular sieves with pores that facilitate the diffusion of molecules of specific size to the active centers of zeolite. Clinoptilolite can be successively utilized as an adsorbent (Abukhadra et al. 2020; Nasiri-Ardali and Nezamzadeh-Ejhieh 2020) or as a component of composites used for photodegradation in waste water purification processes (Derikvandi and Nezamzadeh-Ejhieh 2020; Tan et al. 2020). According to a number of studies, the zeolite exhibits promising catalytic properties in selective catalytic reduction of nitrogen oxides, with hydrocarbons (Ghasemian et al. 2014; Favvas et al. 2016) as well as with ammonia (Moreno-Tost et al. 2004; Szymaszek et al. 2019). Ghasemian et al. (Ghasemian et al. 2014) tested clinoptilolite protonated with NH_4^+ cations in propane-SCR. The authors found that even without the addition of metallic active phase, the zeolite exhibits catalytic activity of around 75% at 425 ºC. Moreover, after consideration of the nitrate species formed during the HC-SCR process, it was suggested that the reaction mechanism was of Langmuir-Hinshelwood type. Gelves et al. (Gelves et al. 2019) studied the catalytic performance of iron-containing Colombian clinoptilolite before and after protonation modification. It was found that the maximum conversion over the tested zeolites was reached at around 400 ºC, but above that temperature it dropped down due to ammonia oxidation reaction. Additionally, catalytic tests performed in the presence of 5 mol% of water

in the inlet stream narrowed the temperature window of the catalyst from 350-500 ºC to 400-500 ºC. Nevertheless, despite the fact that H_2O decreased the NO adsorption capacity of the catalyst, it improved that of NO_2. As a consequence, the "fast-SCR" process was facilitated.

Another factor that has a big impact on the catalytic activity of SCR catalysts is the procedure of active phase deposition (Boroń et al. 2014; Chen et al. 2020; Jiang et al. 2020; Zhang et al. 2020). Chen et al. (Chen et al. 2020) compared catalytic activity of MWW-type structured MCM-22 modified with iron by various methods in NH_3-SCR. Fe was introduced into the zeolite structure using one-pot synthesis or post-synthesis methods, such as incipient wetness impregnation, mechanical mixing, and solid-state ion exchange. It was found that the modification route determined the form of the active phase of the catalyst. One-pot synthesis resulted in the formation of well-dispersed isolated Fe^{3+} moieties that give rise to low-temperature activity in NH_3-SCR, while post-synthesis modifications yielded higher concentration of oligomeric iron oxides and aggregated Fe_2O_3.

According to the above, the aim of this study was to prepare clinoptilolite-supported NH_3-SCR catalyst modified with iron by co-precipitation and adsorption from solution. Since the zeolite exhibited satisfactory performance in propane-SCR without any modifications, it is expected that it will be as active in NH_3-SCR after deposition of iron species. What is more, high acidity of clinoptilolite can facilitate ammonia adsorption of the active centers and speed-up the reaction rate of NO_x reduction.

Materials and Methods

Preparation of the catalysts

In order to prepare SCR catalysts supported on natural clinoptilolite, the zeolite was ion-exchanged with 15 wt.% solution of hydrochloric acid three times with mass ratio zeolite/acid = 1/3. The modification was performed at room temperature and each ion-exchange step lasted 2 hours. Subsequently, the protonated zeolite was dried overnight at 120 ºC. Afterwards, it was modified with iron using two different procedures:

(a) Co-precipitation: 10 g of H-zeolite was dispersed in aqueous $FeSO_4$ solution of 0.05 mol · dm^{-3}, heated to 50 ºC and vigorously stirred for 4 hours maintaining pH ~ 3.0. After that time, 25 wt.% of NH_3 solution was added dropwise to the mixture until pH ~ 9.0 was reached. While the suspension became alkaline, it was stirred for another 1 hour. Afterwards, it was filtered and washed several times with distilled water in order to remove the residual solution of ammonia. The zeolite with precipitated iron was dried at 120 ºC overnight and calcined at 450 ºC for 2 hours in air. The material was labelled as Fe-Clin (co-p).

(b) Adsorption from solution: 10 g of H-zeolite was dispersed in aqueous solution of $Fe(NO_3)_3$ of 1 mol · dm^{-3} and vigorously stirred for 24 hours at 50 ºC. After that time,

iron-modified zeolite was dried overnight at 120 °C and calcined at 450 °C for 2 hours in air. The material was labelled as Fe-Clin (ads).

The codes of the analyzed samples with the description are listed in Table 1.

Table 1. List and codes of the analyzed materials.

No.	Sample code	Modification procedure
1	Clin non-mod	Non-modified clinoptilolite
2	H-Clin	Clinoptilolite ion-exchanged with HCl
3	Fe-Clin (co-p)	H-Clin modified with iron by co-precipitation procedure
4	Fe-Clin (ads)	H-Clin modified with iron by adsorption from solution procedure

Characterization of the catalysts

The structure and the presence of iron species on the parent, protonated and iron-modified clinoptilolite, was examined using X-ray diffraction analysis (XRD). XRD patterns were obtained using Empyrean (Panalytical) diffractometer equipped with copper-based anode (Cu-K_α LFF HR, λ = 0.154059 nm). The diffractograms were collected within 2θ range of 5.00-55.00 ° (2θ step scans of 0.02 °, counting tine of 1 s per step). Additionally, high-temperature XRD analysis was performed over the zeolitic support and the catalysts in the temperature range 100-500 °C.

Coordination and agglomeration of iron species deposited on the zeolite was analyzed using UV-vis spectroscopy. The spectra were recorded using Lambda 35 UV-Vis Spectrometer in the wavelength range of 200-900 nm with 1 nm resolution.

The presence of chemical groups present in the structure of the parent material and the catalysts was studied using Fourier-transform-infrared spectroscopy (FT-IR). The spectra were collected using Perkin Elmer Frontier spectrometer in wavelength region of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}. Before each measurement, the sample was mixed with potassium bromide (KBr) with the sample/KBr ratio of 1:100 and pressed into a disk.

NH_3-SCR catalytic tests

NH_3-SCR catalytic tests over non-modified and iron-doped clinoptilolite were carried out in a fixed-bed flow microreactor under atmospheric pressure. The reaction set-up is presented in Figure 1.

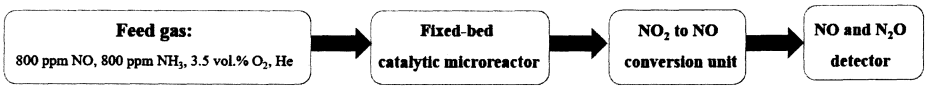


Figure 1. NH_3-SCR catalytic setup.

The reaction mixture consisting of 800 ppm of NO, 800 ppm of NH_3, 3.5 vol.% O_2 was introduced into the catalytic microreactor through mass flow controllers and helium was added as an inert gas in order to maintain the total flow rate of 100 $cm^3 \cdot min^{-1}$. NO_2 formed during the reaction was converted to NO by the catalytic unit placed downstream of the reactor. The concentrations of the residual NO and N_2O in the flue gas after the catalytic reaction were analyzed by FT-IR detector (ABB 200 AO series). The catalytic reaction was performed in the temperature range of 150-450 ºC, each temperature step lasted 30 minutes. NO conversion was calculated using the following formula (3):

$$NO_{conversion} = \frac{NO_{in} - NO_{out}}{NO_{in}} \cdot 100\%, \qquad (3)$$

where NO_{in} – inlet concentration of NO, NO_{out} – outlet concentration of NO.

Results and discussion

XRD results

The results of XRD analysis performed over natural clinoptilolite, protonated form of clinoptilolite and clinoptilolite modified with iron by co-precipitation and adsorption from solution are presented in Figure 2. The characteristic diffraction maxima of clinoptilolite structure at 2θ values of 9.8, 11.2, 13.1, 19.1, 22.4, 30.1, 32.0, 32.7, 33.6 º can be observed in the pattern of non-modified zeolite. The outcomes confirmed that the main crystalline phase of the samples is clinoptilolite (Favvas et al. 2016; Szymaszek et al.) Clinoptilolite was ion-exchanged with NH4NO3 in order to increase the content of Brönsted acid sites, indispensable for NH3 adsorption during the reaction. Subsequently, iron as an active phase was deposited on the modified supports by various methods, including incipient wetness impregnation, ion-exchange and co-precipitation. The efficiency of these methods was compared as NOx conversion obtained for each material. XRD analysis indicated that the initial modifications affected the structure of the raw aluminosilicates. FT-IR measurement confirmed the presence of characteristic Si-O and Al-O bonds and H2O molecules that occur naturally in the materials. UV-Vis spectroscopy results indicated that different types of Fe species were deposited on the catalysts' surface and their form strongly depends on the type of support. NH3-SCR catalytic tests showed that all of the analyzed materials exhibit satisfactory levels of NO conversion and negligible concentration of byproduct (N2O and all of the characteristic reflections are ascribed in the pattern by the specific Miller's indices. Additionally, the observed reflection peaks are in accordance with the clinoptilolite crystalline structure data in the library of the instrument [JCPDS No. 39-1383] and also in the literature of the subject (Nezamzadeh-Ejhieh and Kabiri-Samani 2013). The appearance of the maxima at 2θ values of 17.3 and 26.5 º suggests the presence of stilbite [00-011-0695] and SiO_2 [00-046-1045] phases, respectively. Additionally, the material before modifications contained a low amount of rhombohedral α-Fe_2O_3, which

can be confirmed by the reflection at $2\theta \sim 35.5$ º (Lassoued et al. 2017). However, the peak is rather broadened, which suggests that iron oxide is present in the parent material in the form of small particles. After protonation, intensity of the reflection at $2\theta \sim 9.8$ º slightly decreased. Nevertheless, the majority of the diffraction maxima remained unchanged upon pretreatment with HCl. The lack of any significant structural changes in clinoptilolite after protonation is in agreement with the previous research over dealumination of natural zeolites (Müller et al. 2015; Burris and Juenger 2016; Wang et al. 2018). Additionally, it was not evident from the obtained XRD pattern that hydrochloric acid reduced the amount of impurities, such as stilbite or SiO_2 in the sample. Modification of H-Clin with iron by co-precipitation and adsorption from solution resulted in decreased intensity of the maxima characteristic for clinoptilolite. What is more, as a consequence of the application of the adsorption form solution procedure, the majority of the reflections characteristic for zeolite crystalline structure were broadened or removed. Therefore, the methods used for clinoptilolite modification had an impact on the crystallinity of the material. However, it can be concluded that the introduced iron is well-dispersed, since there are no diffraction maxima ascribed to aggregated iron oxide phase, except from that detected for the parent material.

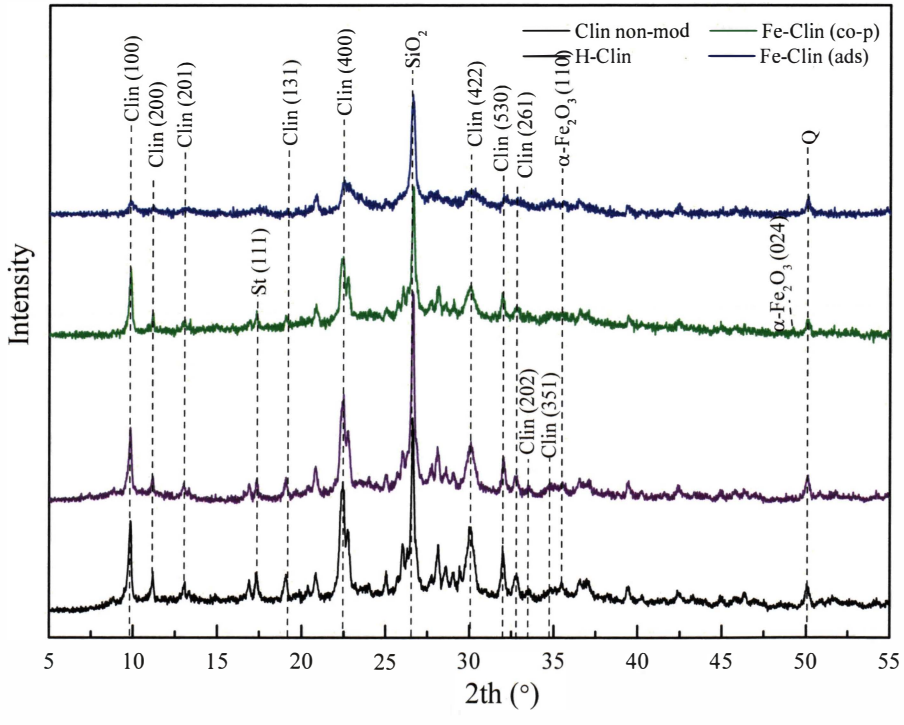


Figure 2. XRD patterns of non-modified clinoptilolite, protonated form of clinoptilolite, clinoptilolite modified with iron by co-precipitation and clinoptilolite modified with iron by adsorption from solution.

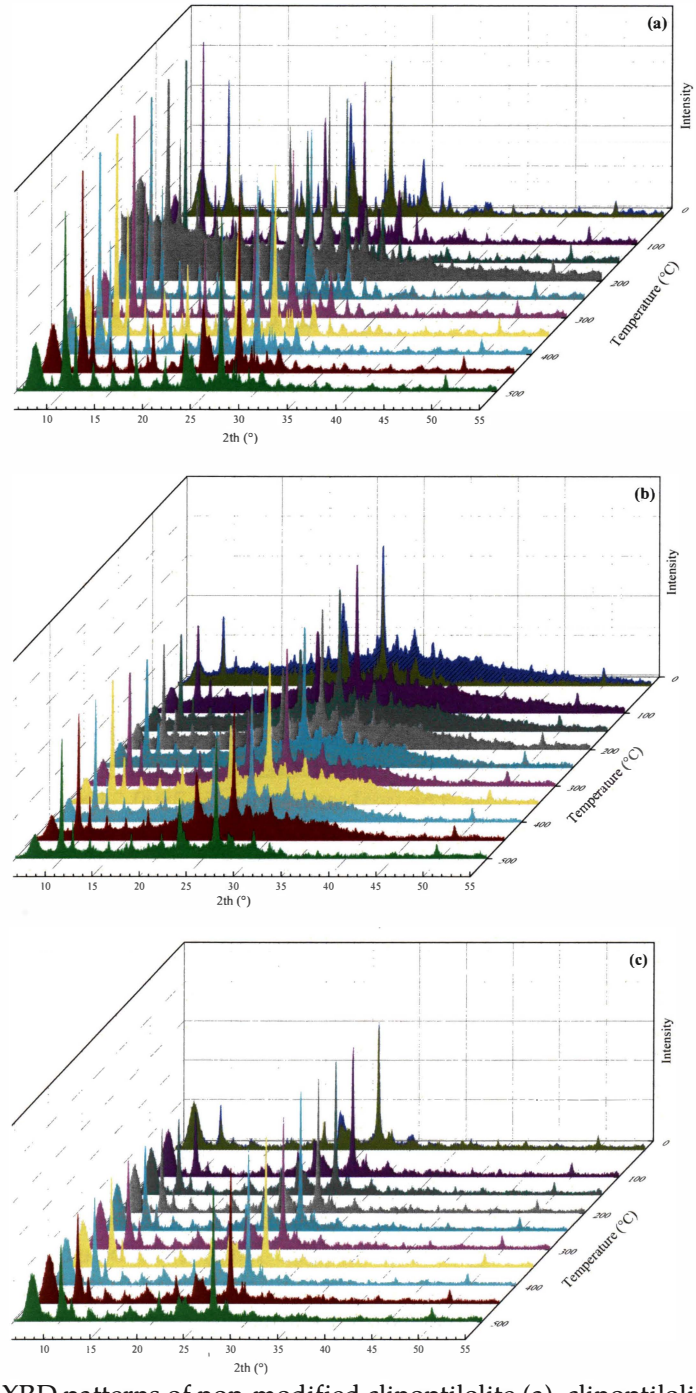
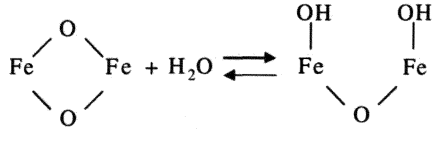


Figure 3. XRD patterns of non-modified clinoptilolite (a), clinoptilolite modified with iron by co-precipitation (b), clinoptilolite modified with iron by adsorption from solution (c) obtained in the temperature range of 100-500 ºC

In order to analyze the influence of temperature on the crystal structure of clinoptilolite and the catalysts, high-temperature XRD analysis in the temperature range of 100-500 °C was performed. The obtained patterns are depicted in Figure 3 (a-c). It can be observed that no significant changes appear in the intensity or the shape of characteristic zeolitic diffraction maxima in the case of raw clinoptilolite. Hence, the material is stable in the applied temperature range, which is in agreement with data from the literature, confirming that heulandite-type framework is highly resistant to thermal decomposition (Alberti 1975). What is more, regardless of the applied method of modification with iron, crystallinity of the catalysts remained stable in the whole temperature range. In fact, the analysis was performed in air, nevertheless the results suggest that clinoptilolite-based catalysts have a potential to preserve their structural features in the temperature range of the NH_3-SCR process.

FT-IR results

The results of FT-IR spectroscopy carried out on the raw zeolite, protonated zeolite, and the catalysts are presented in Figure 4. The spectra can be divided into three wavelength ranges: O-H stretching vibrations (3800-3400 cm^{-1}) (1), Si-O stretching vibrations, Al-Me-OH (where Me = metal cation, for example Ca, Mg) bending vibrations, and O-H bending vibrations from H_2O molecules (1700-700 cm^{-1}) (2), and pseudo lattice vibrations (700-450 cm^{-1}) (3). It can be observed that regardless of the modifications, the framework of clinoptilolite maintained its characteristic structural groups. The peak appearing at 3800-3400 cm^{-1} (shoulders at 3635, 3445 and 3400 cm^{-1}) corresponds to the bridging OH groups of ≡Al-OH-Si≡ and its form depends on the location of hydrogen atoms bonded to various oxygen atoms in the framework. It can be observed that the bands at 3635, 3445 and 3400 cm^{-1} disappeared in the case of Fe-Clin (ads). The lack of vibrations in this region of the sample is related to the structural changes that took place during ion-exchange during the adsorption from the solution modification. According to the literature (Oelkers and Schott 1995; Filippidis et al. 1996), ≡Al-OH-Si≡ surface groups, ≡Al-OH, and ≡Si-OH play a role of the active, ion-exchange sites of the zeolites. Therefore, it can be predicted that during the modification procedure the negatively charged sites ≡Si-O^--Al and ≡Al-O^- were neutralized by Fe^{3+} cations. Additionally, the band at 3445 cm^{-1} present in non-modified zeolite, protonated zeolite and zeolite with co-precipitated iron arises from monomeric hydrogen bonds. For Fe-Clin (ads) the peak almost disappears, which suggests significant decrease of hydrogen bonding for that sample. Thus, it can be expected that the co-precipitation method rather than adsorption from solution resulted in the formation of structurally hydroxylated surface iron species, according to the reaction depicted by equation (4) (Doula 2007):



(4)

According to the literature, these species can play a crucial role in NH_3-SCR, since they act as Brönsted active sites (Chen et al. 2020). The intense peak at 1635 cm^{-1}, appearing for all of the materials, corresponds to the bending vibration mode of hydroxyl groups in the zeolite (Nezamzadeh-Ejhieh and Kabiri-Samani 2013). The bands at 1202 and 1048 cm^{-1} are attributed to the Me-O asymmetric stretching vibrations of free tetrahedral groups MeO_4 and O-Me-O stretching vibration that is sensitive to the framework Si and Al, respectively (Yang and Xu 1997; Doula 2007). The peaks at 794, 788, and 602 cm^{-1} can be observed in the spectra of all of the analyzed samples. The first two arise as a consequence of the presence of symmetric O-Me-O stretching vibrations (Doula 2007). The sharp peak at 602 cm^{-1} in all of the spectra corresponds to O-Me-O bending vibrations in the free tetrahedral group TO_4 (Doula and Ioannou 2003). The bands at 679 and 524 cm^{-1} are attributed to the presence of so-called "pore opening" vibration modes (Doula and Ioannou 2003). The absence of the peak at 524 cm^{-1} in the spectra obtained for Fe-Clin (ads) suggests that iron could somehow interact with the pore openings of the zeolite and change the structural features of the material. Interestingly, for Fe-Clin (co-p) the band is also much less intense, although it is not completely washed from the spectrum. Thus, the modification procedure influenced the form of the clinoptilolite framework. Finally, the peak at 470 cm^{-1} in the pseudo lattice vibration region appears for all of the materials and is assigned to internal vibrations in MeO_4 tetrahedra (Doula 2007).

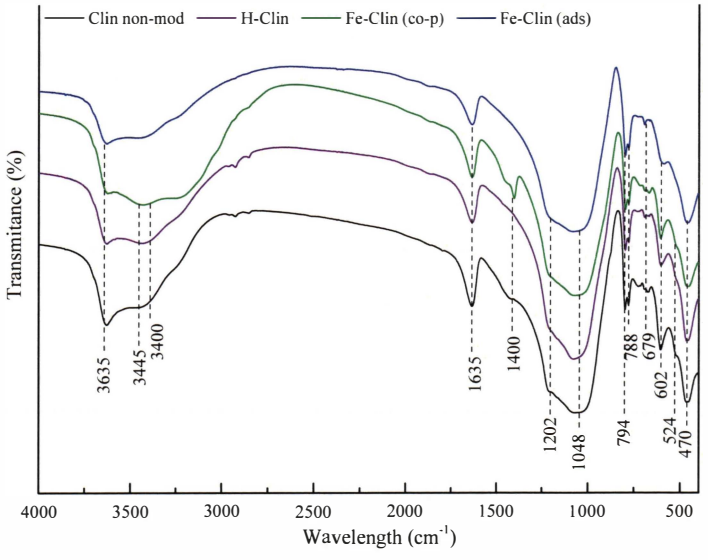


Figure 4. FT-IR spectra of non-modified clinoptilolite, protonated form of clinoptilolite, clinoptilolite modified with iron by co-precipitation, and clinoptilolite modified with iron by adsorption from solution.

UV-vis results

The UV-vis spectra obtained for non-modified clinoptilolite, protonated clinoptilolite and clinoptilolite modified with iron by co-precipitation and adsorption from solution are presented in Figure 5. The band at 220, 248, and 260 nm, appearing for all of the materials, can be assigned to isolated Fe^{3+} ions in tetrahedral coordination (Pérez-Ramírez et al. 2005; Nedyalkova et al. 2013). Interestingly, the band at 260 nm is almost absent for Fe-Clin (co-p), which suggests a lower amount of these species in the sample, in comparison to the parent material. Furthermore, due to the presence of bands in the range of 220-300 nm in the spectra of raw and protonated material, it is predicted that non-modified clinoptilolite can exhibit high activity in the NH_3-SCR reaction. The bands in the 310-500 nm range indicate that the parent zeolite and the modified materials contain some amount of Fe^{3+} ions in the form of small oligonuclear clusters (Fe_xO_y) (J. Chen et al. 2020). In the case of H-Clin only one band at 360 nm can be observed in this region, confirming that acid treatment results in the exfoliation of aggregated metal oxides from natural aluminosilicates (Ghasemian et al. 2014; Valdiviés-Cruz et al. 2017). Moreover, it can be predicted that pretreatment with HCl resulted in the removal of larger particles of Fe_2O_3, since the band at 500 nm ascribed to these iron species (Janas et al. 2009) is absent only for H-Clin. It is worth to emphasize that despite the usage of $FeSO_4$ for co-precipitation of iron on Fe-Clin (co-p), the contribution of Fe^{2+} species was not taken into consideration, due to the fact that divalent iron species are visible only in near-infrared range (around 1000 nm), which is invisible in UV-vis spectra (Pérez-Ramírez et al. 2004; Gao et al. 2016).

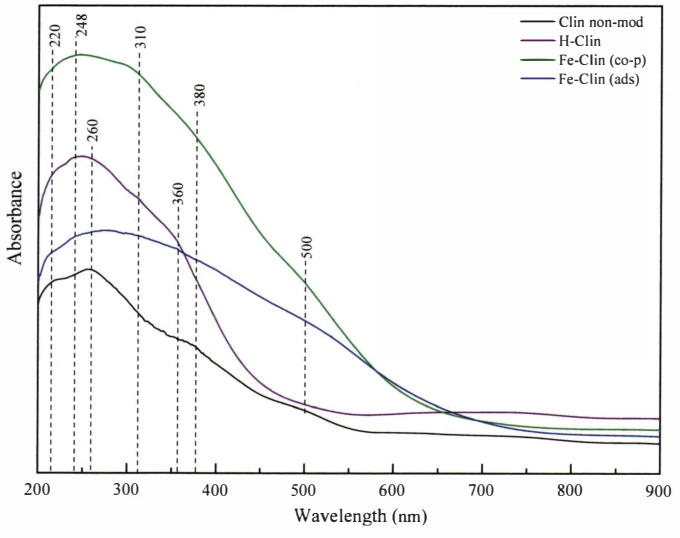


Figure 5. UV-vis spectra of non-modified clinoptilolite, protonated form of clinoptilolite, clinoptilolite modified with iron by co-precipitation, and clinoptilolite modified with iron by adsorption from solution

Results of NH_3-SCR catalytic tests

NO conversion obtained during NH_3-SCR catalytic tests performed for the parent zeolite and iron-functionalized samples is depicted in Figure 6., while N_2O concentration in the flue gas after reaction is presented in Figure 7. It can be observed that non-modified clinoptilolite exhibited relatively high NO conversion of 58% at 450 °C. The result suggests that the raw material contains some amount of catalytically active species. As it was proved by UV-vis and XRD outcomes, natural clinoptilolite contain some amount of oligomeric Fe_xO_y clusters. According to the literature (Brandenberger et al. 2008, 2010; Pariente and Sánchez-Sánchez 2018), these species are dominant active sites in NH_3-SCR at high temperature (>300 °C). Thus, activity of the zeolite can be correlated with its natural chemical composition. Furthermore, as presented in Figure 6., NO conversion curves obtained for Fe-Clin (ads) and Fe-Clin (co-p) are overlapping in the temperature range of 150-250 °C. With the increase of temperature of the reaction, the difference between activity of the catalysts was much more clear, since at 300 °C Fe-Clin (co-p) reached 94% of NO conversion, while for Fe-Clin (ads) it was only 62%. The effect can be explained by the postulated strong dependency between NH_3-SCR activity and speciation of Fe active sites on zeolites. As confirmed by UV-vis results, in case of Fe-Clin (co-p) Fe is present mainly in the form of isolated Fe^{3+} species in tetrahedral coordination that give rise to NO conversion below 300 °C. Nevertheless, at 350 °C there is only a small difference between activity of the catalysts. Hence, it can be suggested that both materials contain similar amounts of oligomeric Fe^{3+} active sites. The presence of highly aggregated Fe_2O_3 in Fe-Clin (ads) confirmed by UV-vis results in significant drop of NO conversion above 400 °C, caused by undesired side reaction of ammonia oxidation ($2NH_3 + 2O_2 \rightarrow N_2O + 3H_2O$) (J. Chen et al. 2020). The effect is confirmed by the concentration of N_2O which is one of the products of NH_3 oxidation. As depicted in Figure 7., the amount of nitrous oxide increases with increasing temperature, which confirms the oxidating character of the catalyst caused by the presence of bulk Fe_2O_3 particles (Brandenberger et al. 2010). Nonetheless, it was observed that in case of Fe-Clin (ads) N_2O concentration started to gradually decrease at 450 °C. According to the previous findings, the diminished yield of nitrous oxide at this temperature can be ascribed to its decomposition over iron-active sites present in the catalyst ($2N_2O \rightarrow 2N_2 + O_2$) (Wang et al. 2018). In addition, considering the results of N_2O concentration, it can be assumed that the analyzed catalysts exhibited satisfactory selectivity. The generation of by-product did not exceed 50 ppm of concentration in the flue gas, therefore the dominant reaction over the catalysts in the entire temperature range was NH_3-SCR.

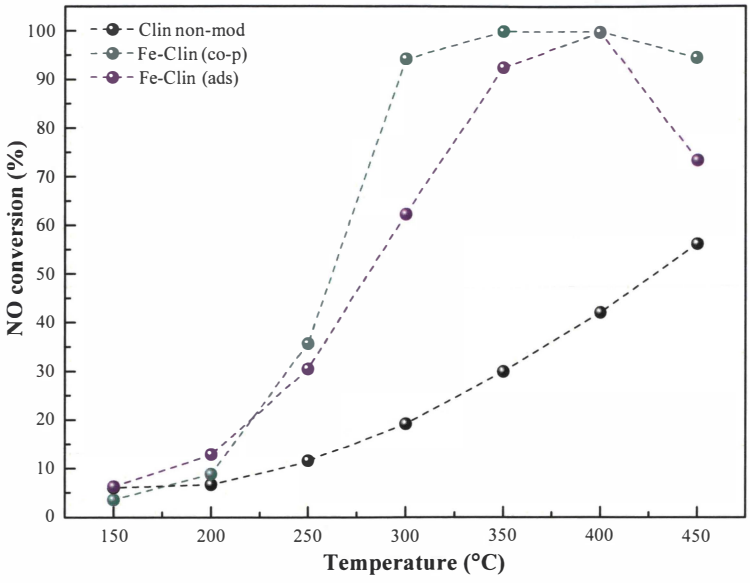


Figure 6. NO conversion obtained during NH_3-SCR catalytic tests carried out over non-modified clinoptilolite and clinoptilolite functionalized with iron by co-precipitation and adsorption from solution.

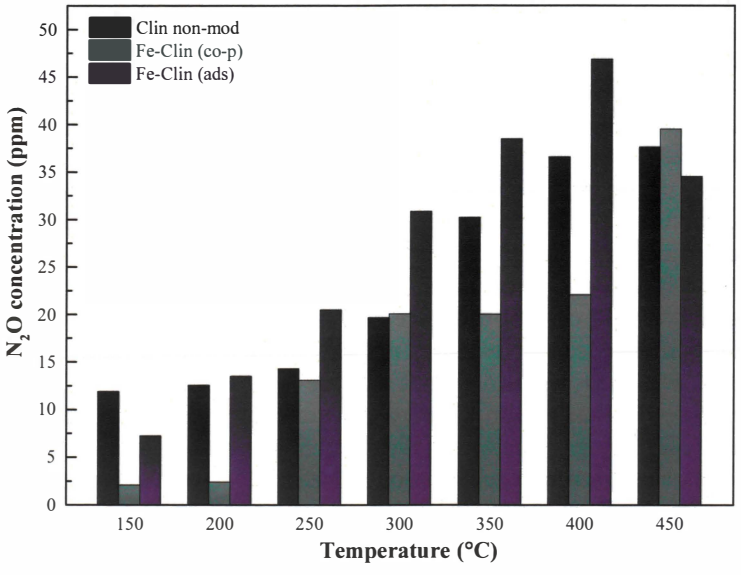


Figure 7. N_2O concentration in the flue gas after NH_3-SCR catalytic tests carried out over non-modified clinoptilolite and clinoptilolite functionalized with iron by co-precipitation and adsorption from solution

Conclusions

The catalytic activity of natural clinoptilolite modified with iron by co-precipitation and adsorption from solution were analyzed and compared. It was found that even before modifications, raw zeolite exhibits catalytic activity in NO conversion at high temperature (450 °C). It was showed that modification procedures had an impact on the type of iron active species introduced into the zeolite structure. Co-precipitation yielded significant amounts of isolated Fe^{3+} tetrahedral sites, while adsorption from solution resulted in the formation of more aggregated Fe_xO_y species. Despite the fact that the catalytic activity of the former sample decreased above 400 °C, due to ammonia oxidation side reaction, the emission of N_2O for that sample gradually decreased. The effect was ascribed to the decomposition of nitrous oxide catalyzed by iron oxide in this temperature range. Therefore, the procedure of active phase introduction was a crucial factor influencing the catalytic performance of clinoptilolite in NH_3-SCR.

Acknowledgements

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NITRATE ION-SELECTVE ELECTRODES – NEW CONSTRUCTIONS AND APPLICATIONS IN THE MONITORING OF NITRATE IONS IN ENVIRONMENTAL SAMPLES

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Abstract

Ion-selective electrodes, due to many advantages, such as simplicity of use, low cost and high speed of measurements, as well as very good analytical parameters (low detection limits and high selectivity), have been widely used in recent years to determine various types of ions in environmental samples. Nitrates are ubiquitous in the environment, both in air, soil and water, and are essential for the proper growth of plants and animals. Research is still ongoing to unambiguously determine the influence of consumption of increased doses of nitrates on human health. Therefore, it is important to develop appropriate methods for determining their content in natural samples and in food products. The paper reviews articles on the latest knowledge on the influence of nitrates on human health as well as on the development and application of potentiometric sensors for determining the content of nitrates in environmental samples, including food products and various types of natural waters.

Keywords: nitrates, potentiometry, ion selective electrodes, solid contact

Nitrates are abundant in the natural environment, both in soil and water, as well as in all living organisms. They are also widely used by humans, inter alia, as artificial fertilizers to increase the yield of agricultural crops (Iammarino et al. 2013) and as preserving additives for meats and cold cuts, to improve the taste and color of products, inhibit the growth of bacteria and delay food spoilage as a result of rancidity (Bryan and Ivy 2015; Sindelar and Milkowski 2012).

Nitrates supplied to the body with food and drinking water are certainly very important biological compounds and have a good effect on human health. They have a protective effect on the cardiovascular system, lowering blood pressure and improving its flow in blood vessels (Sindelar and Milkowski 2012). Nitrates and nitrites are also nutrients necessary to maintain the homeostasis of nitric oxide (II) in the body, which, being a product of enzymatic synthesis, is involved in the process of wound healing and in the immune and neurological response (Bryan et Ivy 2015; Sindelar and Milkowski 2012). The fact that nitrates and nitrites are necessary in the human diet is also evidenced by, inter alia, the

fact that in the early postpartum period in breast milk there is a relatively high concentration of these compounds, with the nitrite concentration outweighing the nitrate concentration, due to the absence of appropriate bacteria in the gastrointestinal tract of the newborn, which ensure the reduction of nitrates to nitrites. Later, however, this ratio is gradually reversed to the benefit of nitrates with obtaining appropriate commensal bacteria (Bryan et Ivy 2015).

In the right concentration, nitrates provide health benefits, but as with other chemicals, taking them in more than recommended amounts can have negative health effects, in this case mainly related to problems with the digestive system. Although nitrates have been used by humans for many different applications for centuries, the alarming results concerning the ill effects of increased doses of nitrates on human health resulting from numerous scientific studies conducted in the last century caused the use of nitrates to almost be banned in the 1970s, due to fears of carcinogenic nitrosamine formed during the thermal processing of meat products (Bedale et al. 2016). It was believed that their excessive consumption may also be associated with the occurrence of cancer and Parkinson's disease. At the beginning of the 20th century, research was also carried out on the so-called "blue baby syndrome", otherwise known as methaemoglobinaemia. The disease was expected to affect mainly infants and cause a significant reduction in the oxygen content in the blood of children (Alahi and Mukhopadhyay 2018; McKnight et al. 2009).

The main source of nitrates and nitrites in the human diet are vegetables and fruits, as well as, to a lesser extent, drinking water, animal products and grains. Nitrates are essential for the proper growth of plants, but the excess of nitrates absorbed by the plant, which the plant is no longer able to absorb, can accumulate in its tissues, especially in the leaves. Therefore, it is the leafy vegetables (lettuce, arugula, spinach, parsley) that contain the highest nitrate concentrations among various other vegetable species (Colla et al. 2018; McKnight and al. 2009). The content of nitrates in plant tissue may also be influenced by plant growth conditions (e.g. CO_2 content in the air, temperature and sunlight), as well as the harvest time and the conditions for their subsequent storage (Colla et al. 2018). The content of nitrates and nitrites in the body is due to both their consumption and their endogenous production due to the presence of denitrifying bacteria and oxidation of nitric oxide (II). The bioavailability of food nitrates is 100% (Bryan and Ivy 2015).

All nutrients should be supplied to the body in appropriate amounts so as not to cause negative health effects related to both their deficiency and excess. Also, nitrates and nitrites should be present in appropriate doses that enable proper growth and functioning. The increased content of nitrate ions has a negative effect on the balance in the environment and the proper functioning of living organisms. Therefore, in order to ensure the safety of humans and animals, it is necessary to monitor the state of the natural environment. For many essential vitamins as well as macro- and microelements, the optimal intake and the recommended diet for maintaining health and good condition of the body have been determined based on research. Therefore, governmental and international organizations have developed certain regulations to define and control

the maximum concentration of nitrates in food and the environment (Alahi and Mukhopadhyay 2018). Recommendations established by the World Health Organization (WHO) allow a maximum concentration of nitrate in drinking water of 50 mg L^{-1} in Europe and 44 mg L^{-1} in the United States (Bryan and Ivy 2015), while the average daily nitrate intake was estimated at 43-141 mg. For nitrates and nitrites, optimal doses are much lower than harmful and lethal doses (Sindelar and Milkowski 2012).

According to scientists, the main risk of consuming increased levels of nitrates is their derivatives, such as nitrites, oxides and N-nitroso compounds (Colla et al. 2018). The nitrosation process is thought to be very important. There is S-nitrosation and N-nitrosation, the latter of which is harmful to the body and may result in the formation of carcinogenic compounds. According to scientists, however, this process does not occur under conditions of normal metabolism (Bryan et al. 2012). Although scientists currently claim that many civilizational diseases are mainly caused by poor nutrition, physical inactivity and stimulants, and there are no clear results of studies on the carcinogenic effect of nitrates in humans, not only in laboratory animals, there is still a need for further research and control of consumed nitrates in the diet. There are many review articles that collect research on the effects of nitrates and nitrites on animal organisms depending on the doses taken (Bedale et al. 2016; Bryan et al. 2012; Bryan and Ivy 2015; McKnight et al. 2009; Sindelar and Milkowski 2012).

Deviating from the subject of human health, poor management of available natural resources by people, excessive use of nitrate fertilizers in agriculture and the production of pollutants and waste also disturb the balance of the natural environment and the nitrogen cycle in nature by significantly increasing the concentration of nitrates and nitrites. Water is particularly susceptible to pollution, the purity of which should be particularly important to us nowadays. Too high concentration of nitrates in surface water bodies causes excessive growth of algae and phytoplankton, which contribute to the death of aquatic organisms that do not have access to dissolved oxygen in the water and to overgrowth of water bodies (eutrophication phenomenon) (Alahi and Mukhopadhyay 2018).

Concerns about the impact of increased consumption of nitrates and nitrites both on human health and the state of the natural environment made it very important to monitor their content in both the environment and in food products and drinking water. Therefore, it is important to develop effective, quick and accurate methods for determining the content of these ions in natural samples.

Scientists developed and implemented many analytical methods to determine nitrates in various types of natural samples, including spectrophotometry (Yue et al., 2004), gas chromatography (Akyüz and Ata 2009; Campanella et al. 2017), liquid chromatography (Akyüz and Ata 2009; Croitoru 2012), colorimetry (Woollard and Indyk 2014), spectrophotometry (García-Robledo et al. 2014), spectrofluorimetry (Biswas et al. 2004), amperometry (Can et al. 2012), voltammetry (Guadagnini and Tonelli 2013).

Potentiometric methods are often used to determine the concentration of nitrate ions in water samples because they allow for direct, quick and cheap measurements and do not require a complicated sample preparation process. In

addition, potentiometric sensors with special parameters can be used for real-time and in situ research, which is particularly useful in monitoring the state of the natural environment. Ion-selective electrodes often show very good selectivity for selected main ions and they also reach lower and lower detection limits each year. In addition, the use of solids contact in ion-selective sensors, enabling the removal of the internal solution, allowed to increase the mechanical resistance of the electrodes, facilitate their storage and transport, and allowed for their miniaturization, which is particularly useful in the construction of multi-sensor platforms for applications in environmental measurements (Alahi and Mukhopadhyay 2018).

The first use of potentiometry for the determination of nitrate ions in water took place in the 1980s (Alahi and Mukhopadhyay 2018). Since then, there are still new research articles describing the innovative use of active substances and solid contacts, making it possible to obtain sensors with better and better analytical parameters.

In 2007, the Álvarez-Romero team constructed a potentiometric sensor using a composite material, polypyrrole doped with nitrate as recognition agent, to ensure selectivity for nitrate ions. The sensors obtained in this way were characterized by extended life time (~ 6 months) compared to unmodified electrodes and the possibility of regeneration of the sensor's active surface. Efficacy was tested in a real sample – in a drug (determination of isosorbide mononitrate, the active ingredient of Elantan) (Álvarez-Romero et al. 2007).

All-solid-state potentiometric sensors for nitrate determination using graphene as an ion-electron transducer were also constructed. The water layer test and impedance measurements were performed, confirming a significant reduction of the charge transfer resistance for the tested electrodes. In this way, sensors with a very good slope of the electrode characteristic curve (-57.9 mV / dec) and a fast response time (~ 10 s) were obtained, which were then successfully used to determine the nitrate content in drinking water samples (Tang et al. 2012).

Lipophilic multiwalled carbon nanotubes (f-MWCNT) were also used as the ion-electron transducer placed between the solid electrode material and the ion-selective membrane layer. According to the authors, the obtained sensors showed many advantages, such as insensitivity to pressure and light, the possibility of vertical or horizontal orientation, and high mechanical and chemical strength, which may be particularly useful in the construction of multi-sensor research platforms for determining the content of selected ions in the environment in situ (Yuan et al. 2015).

An interesting solution was proposed by scientists from the Garland team, who used laser induced graphene (LIG) as an innovative way to produce ion-selective electrodes. According to the authors, it is to be a one-step, easy and cheap process of producing laser recording on polyamide substrates, which can then be used for scalable roll production and disposable sensors in technologies. The publication focuses on the determination of nitrogen available to plants in soil in the form of both nitrate (NO_3^-) and ammonium (NH_4^+) (Garland et al. 2018).

Again, in order to test soil samples, sensors were constructed using a novel nanocomposite of poly(3-octyl-thiophene) and molybdenum disulfide (POT-MoS_2) as a solid contact layer. The nanocomposite is characterized by high hydrophobicity and redox properties. Based on the research, it was found that the modification of the POT chain with MoS_2 increased both the conductivity and the anion exchange, and minimized the formation of a water layer at the interface between the ion-selective membrane and the substrate (in this case, the Au electrode). The purpose of the sensor is long-term use and continuous monitoring of nitrate content in the soil (Ali et al. 2019).

Sensors with very good stability, repeatability and potential reproducibility were produced by using platinum nanoparticles on a carbon black support (Pt-NPs-CB) as solid contact. The research used chronopotentiometry with current reversal and the potentiometric test of the water layer. Additionally, the use of this type of transducer made it possible to significantly reduce the resistance of the ion-selective membrane (Paczosa-Bator et al. 2013).

The use of the association complex of nitron-nitrate ions (Nit^+/NO_3^-) and multi-wall carbon nanotubes (MWCNT) as an intermediate layer between the ion-selective membrane (ISM) and the glassy carbon substrate made it possible to significantly reduce the potential drift after modification and over 10-fold increase in double layer capacitance compared to a conventional ion selective electrode. The generated sensors were successfully used to test real samples for the presence of nitrate ions – wastewater, fertilizers and gun powder samples (Hassan et al. 2019).

Another method of obtaining ion-selective electrodes sensitive to nitrate ions was electropolymerization of N-methylpyrrole with the use of potassium nitrate as an auxiliary electrolyte by the Bomar research team. It has been found that N-methylpyrrole is better than pyrrole for this purpose, and electrodes with an ionic imprinted polymer exhibit very good selectivity and stability of potential and a long lifetime (Bomar et al. 2017).

The electrodes were also constructed with a graphene-tetrathiafulvalene interlayer acting as a solid contact. The use of graphene allowed to obtain a much lower resistance of the ion-selective membrane and a potential drift compared to the unmodified electrode. In this case, the best parameters were obtained for the electrode modified with GR-TTF(NO_3) nanocomposite (Pi k et al. 2016).

A completely new active substance used in the ion-selective electrodes was a cobalt(II) complex with 4,7-diphenyl-1,10-phenanthroline (Bphen) with the formula $Co(Bphen)_2(NO_3)_2(H_2O)_2$. The obtained sensor with simple operation and construction, without an additional intermediate layer, which was characterized by very good analytical parameters, a wide measuring range and pH range in which the sensors could work (5.4 – 10.6), fast response time and very good potential stability. The electrodes worked properly for many months. Their practical application has been checked in the determination of nitrate ions in natural samples: water (mineral, tap and river water) and in vegetables (Pietrzak et al. 2020).

In order to improve the analytical parameters of the electrodes, an addition of ionic liquid (IL) – trihexyltetradecylphosphonium chloride (THTDPCl) was

also used as a component of the ion-selective membrane consisting of polyvinyl chloride (PVC) and a plasticizer (NPOE). The ionic liquid played a very important role in this case, both the ionophores and the ionic lipophilic component reducing the resistance of the ion-selective membrane, as well as the converter aimed at stabilizing the potential of the internal electrode. It is an inexpensive and easy to manufacture sensor with good potential stability and selectivity. It was used in samples of water and vegetables, in which the content of nitrate ions was successfully determined (Wardak 2014).

Table 1. presents a summary of analytical parameters for selected sensors sensitive to nitrate ions, which have been described in the literature over the last 15 years.

Table 1. Comparison of basic analytical parameters of nitrate ion-selective electrodes with solid contact.

Active substance	Contact	Slope [mV/ dec]	LOD [mol L^{-1}]	Linear range [mol L^{-1}]	Response time [s]	Life time [months]	Refence
Ppy(NO_3^-) composite	graphite powder	-57.1	5.4×10^{-5}	1.5×10^{-4} – 1.0×10^{-1}	20	~6	(Álvarez-Romero et al., 2007)
MTDDA-NO_3	graphene	-57.9	3.0×10^{-5}	5.0×10^{-5} – 1.0×10^{-1}	~10	-	(Tang et al., 2012)
TDMAN	f-MWCNTs	-57.7	2.5×10^{-6}	3.2×10^{-6} – 1.0×10^{-1}	-	-	(Yuan et al., 2015)
TDMAN	laser induced graphene	-54.8	2.1×10^{-5}	5.0×10^{-5} – 1.0×10^{-1}	-	-	(Garland et al., 2018)
TDMAN	POT-MoS_2 nanocomposite	-64.0	9.2×10^{-5}	7.1×10^{-4} – 1.0×10^{-1}	-	-	(Ali et al., 2019)
TDMAN	PtNPs-CB	-58.6	5.0×10^{-7}	1.0×10^{-6} – 1.0×10^{-1}	5	~5	(Paczosa-Bator et al., 2013)
(Nit+/ NO_3–) complex	MWCNTs	-55.1	2.8×10^{-8}	8.0×10^{-8} – 1.0×10^{-2}	<10	2	(Hassan et al., 2019)
poly(N-methylpyrrole)	-	-56.3	2.8×10^{-8}	5.0×10^{-6} – 1.0×10^{-1}	-	6	(Bomar et al., 2017)
nitrate ionophore V,	TTF(NO_3)	-59,4	6.3×10^{-7}	1.0×10^{-6} – 1.0×10^{-1}	-	-	(Pięk et al., 2015)

Active substance	Contact	Slope [mV/ dec]	LOD [mol L^{-1}]	Linear range [mol L^{-1}]	Response time [s]	Life time [months]	Refence
nitrate ionophore V,	GR-TTF	-59.1	6.3×10^{-7}	1.0×10^{-6} – 1.0×10^{-1}	-	-	(Pięk et al., 2016)
$Co(Bphen)_2(NO_3)_2$	Ag/ AgCl/Cl^-	-56.3	4.0×10^{-6}	1.0×10^{-5} – 1.0×10^{-1}	-	> 3	(Pietrzak et al., 2020)
THTDPCl	Ag/ AgCl/Cl^-	-60.1	2.8×10^{-6}	1.0×10^{-5} – 1.0×10^{-1}	5-10	> 4	(Wardak, 2014)

LOD – limit of detection;
MTDDA-NO_3- methyltridodecylammonium nitrate,
TTF-TCNQ – tetrathiafulvalene-tetracyanoquinodimethane;
f-MWCNTs – lipophilic multiwalled carbon nanotubes;
TDMAN – tridodecylmethylaminium nitrate;
THTDPCl – trihexyl- tetradecylphosphonium chloride;
Ppy(NO_3^-) composite – composite comprising graphite powder, polypyrrole doped with nitrate and epoxy resin;
CRGNO – chemically reduced graphene oxide;
PtNPs-CB – carbon black supporting platinum nanoparticles;
(Nit^+/NO_3^-) – nitron-nitrate ion association complex;
GR-TTF – graphene–tetrathiafulvalene nanocomposite;
TDMACl – tridodecylmethylammonium chloride;

Table 2 shows examples of the application of solid contact ISEs for the determination of nitrates in natural samples, e.g. in various water samples and vegetables using ion-selective electrodes.

Table 2. Examples of application of solid contact ISEs for the determination of nitrates in real samples.

Type of ISE (solid contact/inner electrode)	Sample	Method	Ref
Graphene/GCE	Mineral water Tap water	Calibration curve	(Tang et al., 2012)
TTF/NO_3^-/GCE	Ground water Well water Tap water River water	Calibration curve	(Piek et al. 2015)

Type of ISE (solid contact/inner electrode)	Sample	Method	Ref
Ionic liquid(TDMACl)/ Ag/AgCl	Mineral water Tap water	Multiple standard addition	(Wardak et Grabarczyk, 2016)
	Vegetables (Iceberg lettuce Butterhead lettuce Fresh spinach)	Multiple standard addition	
MWCNTs/GCE	Waste waters (Aerated lagoon effluent, Raw sewage plant inflo, Nitrate fertilizer factory, outfalls)	Calibration curve	(Hassan et al., 2019)
Ionic liquid(TDMACl)/ Ag/AgCl	Tap water Mineral water River water	Calibration curve	(Pietrzak et al., 2020)

Recently our team developed all solid state nitrate ion-selective electrodes in which ionic liquid and multiwalled carbon nanotubes were used as additional membrane components (Pietrzak and Wardak 2020). Such modification of membrane composition caused noticeable improvement in the electrode performance, especially in potential stability and reversibility. The electrode with the best analytical parameters was successfully used for nitrate determination in natural waters samples (water from Bystrzyca River and Zemorzyce Lake) and vegetables. The water samples were analyzed without pretreatment, whereas vegetables were analyzed after minimum sample preparation without mineralization. They were collected from local markets during the period of March –April 2019. A 5g portion of previously homogenized vegetable samples was mixed with deionized water and the mixtures were stirred and heated (80∘C) for 30 min. After cooling the solutions were transferred to a 500 mL volumetric flask and diluted to volume with deionized water.

Potentiometric measurements of electromotive force (EMF) were performed in a two-electrode system with an ion-selective electrode sensitive to nitrate ions (NO_3-SCISE) as an electrode working against a silver-chloride electrode as a reference electrode. The determination of nitrate was performed by the standard addition method. Recovery was also examined. Obtained results are presented in Table 3 where it can be seen that our electrode is suitable for nitrate monitoring in environmental samples.

Table 3. Result of nitrates determination in various samples (unspiked and spiked) using nitrate ion-selective electrode.

Sample	Nitrate found by NO_3^-SCISE, mg L^{-1} or mg kg^{-1}	Recovery, %
River water	14.6±0.4	-
River water +50 mg L^{-1} NO_3^-	65.2±0.6	100.9
Lake water	17.2±0.7	-
Lake water +50 mg L^{-1} NO_3^-	66.8±1.1	99.4
butterhead lettuce	916 ± 18	-
butterhead lettuce + 300 mg kg^{-1} NO_3^-	1226 ± 23	100.8
Radish	396 ± 16	-
Radish +300 mg kg^{-1} NO_3^-	732 ± 19	105
Fresh spinach	1322 ± 11	-
Fresh spinach + 300 mg kg^{-1} NO_3^-	1546 ± 13	95.3
Cucumber	532 ± 23	-
Cucumber + 300 mg kg^{-1} NO_3^-	815 ± 27	97.9
Cabbage	312 ± 23	-
Cabbage + 300 mg kg^{-1} NO_3^-	612 ± 22	100
Tomato	414 ± 16	-
Tomato + 300 mg kg^{-1} NO_3^-	688 ± 27	96.4

Conclusions

Ion-selective electrodes are a cheap and simple analytical tool for determination of many ions in environmental samples, including nitrates. In recent years scientists constructed many types of ion-selective electrodes with lower and

lower detection limits and better potential stability, at the same time with very good selectivity, which is a special feature of ion-selective electrodes. This creates more and more possibilities to monitor nitrate in the environment in different samples, also online.

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POLYCARBONATES – SYNTHESIS, PROPERTIES AND ENVIRONMENTAL IMPACT

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Abstract

In this work a synthesis of commercially available polycarbonates as well as selected applications of these unique polymers are discussed. A traditional method of synthesis using phosgene and Bisphenol A, as well as methods based on the transesterification of methyl carbonate and diphenyl carbonate are presented. The advantages and disadvantages of the above procedures are shown. The possibilities of replacing the phosgene technology, used by most polycarbonate manufacturers, with more environmentally friendly methods were assessed. In addition, the work includes general characteristics of polycarbonates and their wide application.

Keywords: polycarbonate, Bisphenol A, phosgene

Introduction

Polycarbonates are materials we encounter every day. It is a class of thermoplastic polymers that are formally esters of carbonic acid. They have a lot of advantages such as hardness, ductility, rigidity, transparency, toughness and excellent mechanical properties. These materials are amorphous and polar polymers (Hammani et al. 2012, Shu et al. 2019). Polycarbonate, as a commercial thermoplastic engineering plastic, has been widely applied in electronics, medical equipment, food packaging, automotive industries, aerospace because of its unique thermal, mechanical, electrical and optical properties (Hauenstein et al. 2016, Zhencai et. al 2020). Due to this set of favorable properties, polycarbonates have many applications. The most advantageous properties, compared to other thermoplastics, are as follows: high impact strength, good dielectric properties, good dimensional stability, wide operating temperature range, creep strength, low water absorption, and self-extinguishing tendency. In addition, most polycarbonates, are non-toxic, very hard, abrasion and chemical resistant materials (Schnell 1964, Tabell et al. 1959, Hubacher 1957).

A number of properties of this polymer depends on the chemical structure of a polycarbonate particle. Polycarbonate macromolecules are characterized by high stiffness, limited rotation of aromatic rings and relatively long segments without polar groups (Serini 2000, Distaso et al. 2006, Darensbourg et al. 2011). The general characteristics of polycarbonates have been well described in literature. The thermal properties of unmodified polycarbonate, which can operate

in the temperature range from minus 100°C to plus 130°C, are well understood. The melting point is high and exceeds 220°C. The decomposition temperature is above 320°C but the polymer may start to decompose earlier due to additives (e.g. presence of water). Polycarbonate is considered to be a self-extinguishing material, i.e. one that ignites in a flame and tends to go out when the flame is removed (Schnell 1964, Christopher 1962).

To study the behavior of polycarbonates, thermogravimetric analysis (TG) and differential scanning calorimetry (DSC) are used. Polycarbonate distinguishes itself from the thermoplastic polymers with exceptionally favorable mechanical properties. They depend on the average molecular weight. When it comes to the construction of polycarbonates, we include them among polyesters. So they are generally not resistant to alkaline substances that will cause the hydrolysis of ester bonds. These materials are resistant to acid substances (including organic acids) and neutral salt solutions (Wnuczek et al. 2021, Lee 1964).

Bisphenol A polycarbonate is the carbonic acid polyester derived from 2,2-bis(4-hydroxyphenyl)propane. It is the best known resin polycarbonic material of this type because of its good mechanical, thermal and electrical properties, as well as being made from easily available raw materials. Most of the commercially available polycarbonates have been synthesized using Bisphenol A. This compound is a precursor of important plastics, primarily certain polycarbonates and epoxy resins. Plastic materials based on Bisphenol A are clear and tough and are used to produce a variety of common consumer goods, such as plastic bottles, food storage containers, sports equipment. Epoxy resins derived from Bisphenol A are used to manufacture water pipes, serve as coatings on the inside of many food and beverage cans and thermal paper such as the one used in sales receipts. The use of Bisphenol A for products that will come into contact with food is still controversial and raises many concerns (Pivnenko et al. 2015). Bisphenol A is a minor skin irritant but not as much as phenol (Fiege et al. 2000). Bisphenol A is a xenoestrogen with hormone-like properties. Although the effect is very weak, the pervasiveness of Bisphenol A – based materials raises concerns. However, the multitude of advantages of these plastic materials is so great that in industrial applications, Bisphenol A is still irreplaceable. For this reason the search for new components for the synthesis of polycarbonates is very important (Thoene et al. 2020, Egan 2014)

Overall, polycarbonates are a very promising group of polymers. Seeking new polycarbonates with special properties, combining mechanical and thermal resistance, as well as light construction, is still a challenge for scientists. In addition, ecological methods with the use of safe technologies are being searched for. That is why the production of polycarbonates would increasingly fit the term of „green chemistry".

Production

The main polycarbonate material is produced by the phosgene method by the reaction of bisphenol A and phosgene $COCl_2$ (carbon dichloride oxide,

dichloromethanal). Phosgene is an essential raw material for the synthesis of polycarbonate by the interfacial polycondensation method and for the synthesis of phenyl carbonate, which in turn is a raw material for the preparation of polycarbonate by the ester exchange method.

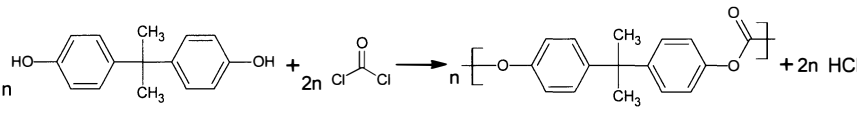

Figure 1. Scheme of reaction of bisphenol A and phosgene.

The first stage of the synthesis involves the treatment of Bisphenol A with sodium hydroxide which deprotonates the hydroxyl groups of Bisphenol A. The diphenoxide reacts with phosgene to form the chloroformate that is then attacked by another phenoxide (Serini 2000)

$$(C_6H_4(OH))CMe_2 + 2NaOH \rightarrow [Na_2(C_6H_4O_2)CMe_2] + 2\,H_2O$$
$$[Na_2(C_6H_4O_2)CMe_2 + COCl_2 \rightarrow [OC(C_6H_4O)_2CMe_2] + 2NaCl$$

Synthesis by direct phosgenation is performed either in a system of two solvents that are immiscible with each other (a kind of interfacial polycondensation) or in a single-phase system using pyridines. Pyridine acts as a solvent and hydrogen chloride acceptor. However, the traditional synthetic process of polycarbonates from Bisphenol A with phosgene or diphenyl carbonate face problems because phosgene is a highly toxic compound and Bisphenol A is also a toxic substance that affects the health of human beings, especially children (Zhencai 2020, Darensbourg et al. 2011, Christopher et al. 1962, Lee 1964, Ozyildiz et al. 2019, Dong et al. 2019, Samikannu et al. 2019). The traditional synthetic process of polycarbonates from Bisphenol A and phosgene contributes to environmental pollution due to toxic raw materials and large-scale use of dichloromethanal. The hydrochloric acid is scavenged with aqueous base. Therefore, the development of green non-phosgene routes for the synthesis of polycarbonates is urgently needed (Erythropel et al. 2018, Lounsbury et al. 2018, Clarke et al. 2018, Rogers et al. 2019, Anastas et al. 2010, He et al. 2013).

Transesterification of diphenyl carbonate with Bisphenol A is an ester exchange method, which is discussed for industrial application in the production of polycarbonate (next to the method of direct phosgenation in solvents). This method has a number of advantages. The polymer obtained by ester exchange is suitable for granulation because it is in molten state after the end of the process. In addition, this method does not use solvents, which allows for high yield. The waste product in transesterification is phenol, which can be used for other purposes (Schnel 1964, Tarbell 1959, Serini 2000). Unfortunately, phenol, a by-product of the reaction, can only be removed under severe conditions (Clarke et al. 2018).

$$(C_6H_4(OH))CMe_2 + (C_6H_5O)_2CO \rightarrow [OC((C_6H_4)_2O)CMe_2] + 2C_6H_5OH$$

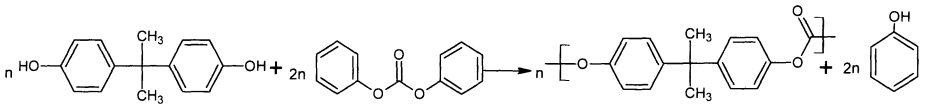

Figure 2. Scheme of transesterification reaction of bisphenol A and diphenyl carbonate.

Dimethyl carbonate can react with Bisphenol A to prepare a precursor of polycarbonate. Transesterification of dimethyl carbonate with Bisphenol is ecological because dimethyl carbonate is qualified as a green reagent. Moreover, the byproduct methanol can be easily removed in low temperature. Dimethyl carbonate is not only a less toxic organic synthesis ingredient but also a raw material for the synthesis of diphenyl carbonate (Liang et al. 2019, Tong et al. 2007, Niu et al. 2007).

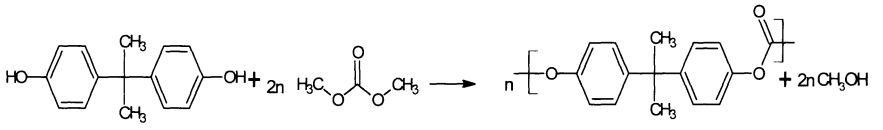

Figure 3. Scheme of transesterification reaction of bisphenol A and dimethyl carbonate.

Although well-developed and commercial, phosgene-free methods are complicated ones involving multiple reaction steps. Some of them are under severe equilibrium constraints (Kim et al. 2002, Tundo et al. 1988). The direct oxidative carbonylation of Bisphenol A into polycarbonate oligomers could be a promising method of non-phosgene polycarbonate synthesis because it converts CO and Bisphenol A directly to polycarbonate (Kim et al. 1999, 58-59, King 1999, Lago et al. 2016). The direct oxidative carbonylation of Bisphenol A has been reported with the use of homogeneous Pd catalysts (58).

Properties

Polycarbonates attract much attention because of their several interesting properties. There are some features that all polycarbonates have in common. They are unique in the polymer market owing to their excellent mechanical stiffness (2.0–2.4 GPa), electrical and optical properties, as well as self-extinguishing characteristics. This group is characterized by the unique high impact resistance and strength in a wide range of operating temperatures. Most polycarbonates are transparent (over 80% in visible spectrum, with a refractive index of 1.59) (58, King 1999, Lago et al. 2016). Polycarbonate, which has the advantages of ductility, rigidity, hardness, transparency, toughness and excellent mechanical properties, is an amorphous and polar thermoplastic polymer (Laurenti et al. 2016, Levchik et al. 2005, Eshaghi et al. 2014).

On account of the aromatic structure in macromolecular chains, polycarbonates show the capability of self-carbonation to some degree (Naik et al. 2012). Aliphatic polycarbonates, compared with traditional aromatic polycarbonates, have received little attention because of their worse thermal stability and high susceptibility to hydrolysis (Kuran et al. 2000). On the other hand, the aliphatic polycarbonates are getting more attention because of their biomedical application, e.g. the composition of biomedical implants and acting as drug delivery devices, due to their biodegradability, biocompatibility and low toxicity (Huang et al. 2015, Sun et al. 2016, Wnuczek et al. 2021).

Table 1. Properties of polycarbonates (Karazil et al. 2017). Table lists the most commonly quoted properties of PC. Table was collated from the most representative values found in different literature sources.

Property	Value
Refractive index	1.583-1.586
Transmittance [%]	89
Density [g/cm^3]	1.17-1.45
Thermal conductivity [W/m*°C]	0.19-0.21
Specific heat (J/g*°C]	1-1.2
Melting temperature [°C]	150
Glass transition temperature [°C]	147

Environmental impact

The use of Bisphenol A as a diol for the synthesis of polycarbonates is controversial. The detection of Bisphenol A in the environment and food products has been the subject of much recent research. Some studies prove that thermal treatment of any product containing Bisphenol A causes it to be released into food. Further, Bisphenol A is also found in other everyday objects, such as thermally printed paper receipts, medical devices, phones and sunglasses. It is estimated that over 100 tons of Bisphenol A are released into the environment every year (Vandenberg et al. 2009, Geens et al. 2012, Geens et al. 2011, Sun et al. 2016). Studies have shown that at temperatures above 70°C and at high humidity, polycarbonate is hydrolyzed to Bisphenol A (Bair et al. 1981).

Table 2. Occurrence data for Bisphenol A in food and beverages. Table section from WHO table (Raport of Joint FAO/WHO Expert Meeting 2010).

Matrix/Reference	Concentration (µg/l or µg/kg)	
	Number of samples	**Maximum**
Baby bottles	6	15
Human breast milk	20	7.3
Tableware	3	2
Tap water and bottle water	>100	1
Canned food	**Number of samples**	**Average**
Fruit	70	9.8
Vegetables	305	32.4
Meat	70	69.8

Bisphenol A is the subject of well-developed medical research. It is a known endocrine-disrupting compound. Its structural form is similar to natural hormones such as estradiol and diethylstilbestrol. It can affect the functions of the hormone estrogen by binding to estrogen receptors (Sun et al. 2016). It has been demonstrated that even in very low doses (even in concentration on picogram levels) Bisphenol A could have an impact on physiological functions of the brain, endocrine pancreas, ovaries, and reproductive organs, as well as the immune system (Cabaton et al. 2011). Recently, some new studies have suggested that Bisphenol A is associated with global diseases such as obesity, diabetes and also cardiovascular diseases, neurotoxicity and behavioral problems (Michałowicz et al. 2014). The large production of plastics, including polycarbonates, creates a worldwide problem with waste disposal. Research on the degradation of polycarbonates by bacteria has been carried out since the 1990s. Soil bacteria are capable of degrading aliphatic polycarbonates such as polyhexamethylene carbonate and polyethylene carbonate (Suyama et al. 1998). When it comes to thermal degradation, polycarbonate decomposes in high temperatures. The polycarbonate waste decomposes to form solid, liquid and gaseous pollutants (Collin et al. 2012). In the presence of UV light, oxidation of polycarbonates can occur, leading to the formation of compounds such as phenols, ketones and other unsaturated compounds.

Application

The use of polycarbonates is very wide. We can really meet them anywhere. They are present in every aspect of our lives. Their unique properties mean that they dominate many industries. Generally, polycarbonates are used wherever

transparency, thermal resistance and high impact strength is required (Wnuczek et al. 2021).

Polycarbonates are widespread in the household both in construction and in everyday use items. Currently, polycarbonate is increasingly replacing glass and is successful in various areas of construction. Polycarbonate is used to make the layers of panes resistant not only to breaking, but also to fire. In the construction industry these compounds are applied to manufacture glazing, roofing sheets and sound walls. In addition polycarbonate can also be found in relays, contacts, housings and elements of household appliances (60, Andrzejewski et al. 2018).

Electrical devices also contain polycarbonate in mounting frames. Polycarbonate is mainly used for electronic appliances to ensure their safety. Being a good electrical insulator and having heat-resistant and flame-retardant properties, it is used in various products associated with electrical and telecommunications hardware. Some major smartphone manufacturers use polycarbonate for finishing their products (Serini 2000, King 1999).

A major application of polycarbonate is the production of Compact Discs (CD), Digital Video Discs (DVD) and Blu-ray Discs. These discs are produced by injection molding polycarbonate into a mold cavity with two sides. One has a metal stamper containing a negative image of the disc data, while the other side is a mirrored surface (Suna et al. 2016).

Pure polycarbonate is used in such places as airplanes, astronaut helmets, or even in advertising panels, oil pumps, filters, lamp shades, control devices and security components (Feng et al 2012). In the streets there are large products such as: street letter boxes, switch cabinets, mounting plates, lamp posts, which are made from polycarbonates (Andrzejewski et al. 2018). Research is ongoing on the use of polycarbonates for medical applications (Feng et al 2012) and a variety of healthcare products such as dental sealants and tooth coatings (Li et al. 2015). The second largest consumers of polycarbonates are the manufacturers of food packing, drink bottles, water supply pipes (Zhu et al. 2018).

Conclusions

In summary, due to the prevalence of polycarbonates, research on synthesis as well as preparation of new materials based on polycarbonates and their use is very promising. Thanks to their excellent properties, this polymer group has many applications, both in daily life and in many sectors of industry. To conclude, these plastics have excellent mechanical and thermal properties (in particular impact resistance) which makes them very versatile. However, their most important feature is that they are transparent and durable. Polycarbonate is used in every place where a transparent material with extremely high mechanical parameters is needed. All in all, polycarbonates are a promising group of polymers that should be further developed with a special focus on improving its environmental performance.

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INFLUENCE OF THE TYPE OF WORKING ELECTRODE ON THE LIMIT OF DETECTION IN VOLTAMMETRIC PROCEDURES FOR THE DETERMINATION OF IN(III) IN ENVIRONMENTAL WATER SAMPLES

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Abstract

Three simple and fast adsorptive stripping voltammetric procedures for trace determination of indium in environmental water samples were described. For all indium ion determination procedures, cupferron was used for the first time as a complexing agent. However, these procedures differed in the key element, which is the working electrode, which largely determines the detection limit and range of quantification. The following working electrodes were used in the research: a mercury film electrode with a silver substrate (Hg(Ag)FE), a lead film electrode (PbFE) and a bismuth film electrode (BiFE). The influence of humic substances and foreign ions on the voltammetric indium signal was examined in all procedures, which allowed for the evaluation of their practical application for direct analysis of environmental water samples. Finally, the developed procedures were used for analysis of certified reference materials: waste water and surface water, as well as natural waters taken from the Lublin region: from the Bystrzyca river and the Zemborzycki Lake.

Key words: mercury film electrode, lead film electrode, bismuth film electrode, humic substances

Introduction

Indium is not a widespread element, but very valuable, due to its unique properties and wide application, which has grown significantly in recent years. This, in turn, led to a sharp increase in indium production; it is estimated that today about 2,000 tons of indium are obtained annually. The vast majority of this is used for the production of liquid crystal LCD displays, both television screens, computer monitors, telephones, and solar panels. Indium is mainly used in the form of indium tin oxide (ITO), which is also electrically conductive and transparent (Alfantazi and Moskalyk 2003, Craig et al. 2001, Merian et al. 2004). Indium is considered to be of low toxicity and there is little information regarding the adverse health effects in workers exposed to indium compounds. Indium toxicity is related to its chemical form and route of exposure; insoluble

indium oxides are more toxic than indium ions. In 2003, the first case of ITO-induced pneumonia, resulting from its passage through the respiratory tract, was reported (National Toxicology Program 2009, Tanaka et al. 2010).

Along with the increase in demand for and use of indium in various areas, the amount of indium that ends up in the environment along with waste grows. This raises the need to develop analytical procedures allowing for the determination of this element in various environmental matrices. One of the methods with low limit of determination is adsorptive stripping voltammetry (AdSV). The development of the AdSV procedure requires, first of all, the selection of a complexing agent specific for the metal to be determined and the selection of a working electrode ensuring effective accumulation of the complexes of analyzed element on it. Analyzing the literature data, it could be noticed that in the case of In(III) determination, only three procedures based on adsorptive stripping voltammetry were described. They used the following complexones as complexing agents: morin, xylene orange, APDC (Paolicchi et al. 2004, Benvidi and Ardakani 2009, Farias et al. 1994]. The use of cupferron, which is known from the literature as a complexone forming stable and electrochemically active complexes with various metals (Grabarczyk and Koper 2011, Grabarczyk and Wardak 2014, Adamczyk and Grabarczyk 2020), has been proposed as a complexing agent in our studies. Regarding the choice of the working electrode, special attention is currently paid to the fact that the working electrode should be the least toxic while ensuring at the same time a low detection limit, a wide range of linearity and good selectivity. Unfortunately, all AdSV procedures of In(III) determination described before used a hanging mercury drop electrode (HMDE), which is considered harmful to the laboratory environment due to the toxicity of mercury. Therefore, the main goal of our work was to develop indium determination procedures using the AdSV technique with the use of more environmentally friendly electrodes. These electrodes were: a mercury film electrode with a silver substrate (Hg(Ag)FE), a lead film electrode (PbFE) and a bismuth film electrode (BiFE). The choice of these electrodes was dictated by the possibility of obtaining low detection limits of indium in aqueous environmental samples. In this way, three procedures for the determination of In(III) with the AdSV technique with the use of cupferron as a complexing agent and three different working electrodes were developed: Hg(Ag)FE (Grabarczyk and Wasąg 2016a), PbFE (Grabarczyk and Wasąg 2016b) and BiFE (Wasąg and Grabarczyk 2016). These procedures will be presented below and compared in terms of the optimization of key parameters and the obtained parameters, such as linear range and, first of all, the limit of detection.

Results

Procedure using cupferron and Hg(Ag)FE

The voltammetric measurement consisted of the following stages:

- simultaneous deoxidation of the solution with nitrogen gas and formation of a complex In(III)-cupferron for a period of 4 min.;
- adsorption of In(III)-cupferron complexes on Hg(Ag)FE at 0 V potential within 30 s;
- recording of the voltammogram using the differential pulse technique as a result of changing the electrode potential in the range from -0.4 to -0.8 V.

In order to optimize the parameters of the determinations, the influence of the concentration and pH of the base electrolyte on the indium signal, the concentration of cupferron, the potential and the time of accumulation were examined. Acetate buffer was used as the primary electrolyte, the pH of which was selected on the basis of research on the effect of its value on the indium peak size. Measurements were carried out in the pH range from 3.5 to 6.2, the highest peak current values were obtained at pH = 5.5 and this value was chosen as the most optimal. The influence of the buffer concentration in the range from 0.01 to 0.2 mol L^{-1} was also checked and it turned out that it did not affect the indium signal; in standard measurements a buffer with a concentration of 0.1 mol L^{-1} was used. Another parameter, the influence of which on the indium peak current value was investigated, was the concentration of the complexing agent, which was cupferron. It was found that the indium peak appears only at a cupferron concentration of 5×10^{-6} mol L^{-1} and increases with the increase of its concentration to 5×10^{-5} mol L^{-1} and then slightly decreases. Therefore, a cupferron concentration of 5×10^{-5} mol L^{-1} was used. The influence of the accumulation potential was tested in the range of its value from 0.2 V to -0.5 V. As the potential value increased in the positive direction, the indium signal clearly increased, the highest value was obtained at the 0 V potential and this value was chosen as the most optimal. When analyzing the effect of the accumulation time, it was found that the highest peak for a given concentration was obtained at a 60 s accumulation time.

With such parameters, a series of measurements was carried out to determine the performance of this procedure. The calibration curve was determined using the following measurement conditions: 5×10^{-5} mol L^{-1} cupferron and 0.1 mol L^{-1} CH_3COOH/CH_3COONa (pH = 5.5), 0 V accumulation potential and 30 s accumulation time. Linearity in the concentration range was obtained from 5×10^{-10} mol L^{-1} to 5×10^{-8} mol L^{-1}. Under the same conditions, the limit of detection was determined based on three times the standard deviation of 5×10^{-10} mol L^{-1} In(III) and was $1.5 \times \times 10^{-10}$ mol L^{-1}. The relative standard deviation of the peak current values was determined for two different concentrations and it was 3.7% and 3.3% for the indium concentration of 1×10^{-9} mol L^{-1} and 5×10^{-9} mol L^{-1}, respectively (Grabarczyk and Wasąg 2016a). For comparison, measurements were also carried out using a classic hanging mercury drop electrode (HMDE) as a working electrode. The obtained results confirmed that with the use of

Hg(Ag)FE a better sensitivity was obtained than for HMDE and additionally, the Hg(Ag)FE electrode, due to the use of amalgam instead of mercury drop, is much less toxic to the environment.

Procedure using cupferron and PbFE

The voltammetric measurement consisted of the following stages:

- -1.6 V for 10 s (PbFE formation);
- -0.7 V for 60 s (adsorption of In(III)-cupferron complexes on PbFE);
- potential scan from -0.7 V to -1.1 V (recording of voltammogram).

Before each measurement, the electrode was electrochemically cleaned by applying first a potential of -1.6 V for 10 s and then 0.2 V also for 10 s. All measurements were carried out without deoxidizing the solution.

In this procedure, apart from the standard parameters of voltammetric measurement, the conditions for the formation of the lead film had to be optimized. The basic electrolyte was the acetate buffer, whose pH value = 4.5 in this case turned out to be the most optimal. The selection of its concentration was also important, because it turned out that with its increase above 0.3 mol L^{-1}, the indium peak decreased. Therefore, its concentration equal to 0.2 mol L^{-1} was selected as the most optimal. A very important parameter in the case of using a lead film electrode created in situ is the concentration of lead ions introduced into the analyzed sample. On the basis of the research on the dependence of the current value of the indium peak on the concentration of lead ions, it was found that their optimal value is 5×10^{-5} mol L^{-1}; a further increase in the concentration did not cause an increase in the peak current, but caused its shape to deteriorate. A number of measurements were carried out to select the accumulation potential, which affects both the formation of the lead film and the adsorption of In(III)-cupferron complexes on it. The potential was varied over a wide range from -0.7 V to -1.8 V by applying it to the glassy carbon electrode at different time combinations. It was found that the highest indium signal was recorded using a two-step potential change, first -1.6 V for 10 s and then -0.7 V for 60 s. When examining the effect of cupferron concentration, it was observed that a high concentration of 5×10^{-4} mol L^{-1} is necessary in order to obtain the highest indium peak value.

A calibration curve was established using the following measurement conditions: 5×10^{-5} mol L^{-1} Pb (II), 5×10^{-4} mol L^{-1} cupferon and 0.2 mol L^{-1} CH_3COOH/CH_3COONa (pH = 4.5). Linearity was obtained in the concentration range from 5×10^{-10} to 2×10^{-7} mol L^{-1}. Under the same conditions, the limit of detection was determined on the basis of three times the standard deviation of 5×10^{-10} mol L^{-1} In(III) and it was 1.65×10^{-10} mol L^{-1}. The relative standard deviation of the peak current values was determined for the indium concentration of 1×10^{-9} mol L^{-1} and it was 4.7% (Grabarczyk and Wasąg 2016b).

Procedure using cupferron and BiFE

The voltammetric measurement consisted of the following stages:

- -0.65 V for 60 s (BiFE formation and adsorption of In(III)-cupferron complexes on it);
- potential scan from -0.5 V to -1.0V (recording of voltammogram).

During the first stages, simultaneous formation of the bismuth film and the accumulation of In(III)-cupferron complexes takes place. During the second stage, the voltammogram is recorded using the differential pulse technique. Before each measurement, the electrode was electrochemically cleaned by applying first a potential of -1.4 V for 15 s and then 0.3 V also for 15 s. All measurements were carried out without deoxidizing the solution.

In this case, as in the PbFE procedure, it was necessary to optimize, in addition to standard parameters, the conditions of bismuth film formation. The basic electrolyte was traditionally an acetate buffer, the pH value of which = 4.5, as in the case of the PbFE electrode, turned out to be the most optimal. As for its concentration, in the case of its value below 0.05 mol L^{-1} the indium peak did not appear at all, therefore a 0.2 mol L^{-1} acetate buffer was used for the measurements to ensure a stable indium signal. As mentioned before, an important parameter in the case of using an in situ film electrode is the concentration of metal ions in the solution from which the film is formed. On the basis of the research on the dependence of the size of the indium peak on the concentration of bismuth ions, it was found that their optimal value is 4×10^{-4} mol L^{-1}. At higher concentrations of bismuth, the indium peak continued to increase but its shape deteriorated. The measurements carried out to select the accumulation potential proved that in this case the best parameters are obtained using one potential equal to -0.65 V, at which the formation of the bismuth film and the accumulation of In(III)-cupferron complexes took place simultaneously. In the tests, the potential was changed in the range from -0.5 V to -1.0 V by applying it to the glassy carbon electrode in various time combinations. It was found that the indium signal increases with increasing accumulation time to 60 s. Analyzing the influence of the cupferron concentration on the indium signal, it was observed that its increase to the value of 1×10^{-4} mol L^{-1} causes a gradual increase in the peak current and after exceeding this concentration the peak current slowly decreases.

The calibration curve was established under the following measurement conditions: 4×10^{-4} mol L^{-1} Bi(III), 1×10^{-4} mol L^{-1} cupferron and 0.2 mol L^{-1} CH_3COOH/CH_3COONa (pH = 4.5). Linearity was obtained in the concentration range from 5×10^{-9} to 5×10^{-7} mol L^{-1}. Under the same conditions, the limit of detection was determined on the basis of three times the standard deviation of 5×10^{-9} mol L^{-1} In(III) and it was 1.6×10^{-9} mol L^{-1}. Relative standard deviation of the peak current value was determined for the concentration of indium 1×10^{-8} mol L^{-1} and it amounted to 3.7% (Wasąg and Grabarczyk 2016).

Influence of the matrix of environmental water samples on the In(III) signal

In adsorption striping voltammetry, the main interferences associated with the analysis of environmental samples result from the presence of humus substances, which are commonly present in greater or lesser concentrations. The humic substances include: humic acids, fulvic acids and humins. These substances are produced in the microbiological and chemical processes of decomposing plant residues (humification). Humic acids are a group of humic compounds which contain about 58% of carbon. These compounds are not soluble in an acidic environment. They are dark brown to black in color. Fulvic acids contain about 55% of carbon, are yellow to yellow-brown in color and easily dissolve in the entire pH range. Humins are dark humus compounds, insoluble in water in the entire pH range. These compounds play an extremely important role in the biosphere. They create the appropriate soil structure, accumulate nutrients for plants, transform water-insoluble microelements and macronutrients into a form available for crops, and regulate the geochemical flow of metals in aquatic and terrestrial ecosystems. However, in the case of voltammetric methods when determining metal ions in environmental samples, they can be a serious interferent. Therefore, in all three procedures for the determination of In(III) ions, their influence on its voltammetric signal was examined.

In this work, the researchers used humic substances such as humic acids (HA), fulvic acids (FA) and natural organic matter (NOM). The conducted studies confirmed that in all three above described In(III) determination procedures, these compounds at a concentration of several mg × L^{-1} suppress the voltamperometric indium signal (Grabarczyk and Wasąg 2016a, Grabarczyk and Wasąg 2016b, Wasąg and Grabarczyk 2016). Therefore, in order to reduce these interferences, it was proposed to premix the analyzed sample with Amberlite XAD-7 resin, on which they are adsorbed, thereby reducing their concentration in the sample. Figure 1 shows the scheme of removing humic substances from the analyzed sample. As demonstrated by the procedure using the BiFE electrode as the working electrode premixing with the resin allows for the determination of In(III) ions in a solution in which the initial concentration of HA and NOM substances was even 15 mg × L^{-1} and FA 10 mg × L^{-1} (Wasąg and Grabarczyk 2016).

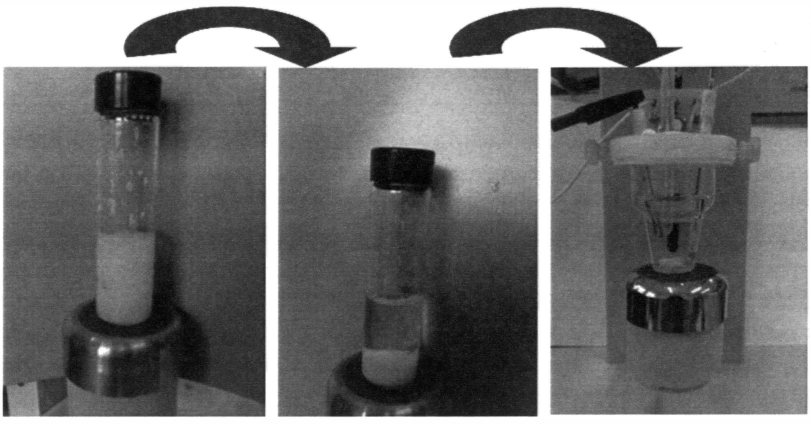

Figure 1. The scheme of removing humic substances from the analyzed sample: mixing the sample with the resin, sedimentation of the resin, transfer of the solution from above the resin to the electrochemical cell.

Other interferences accompanying adsorptive voltammetric procedures for determining a given ion are related to the presence of associated ions. In order to determine the selectivity of the developed In(III) determination procedures, the influence of a number of foreign ions on its voltammetric signal was investigated. Table 1 shows the foreign ions together with their concentrations at which they do not affect the voltammetric In(III) signal for the presented procedures.

Table 1. List of foreign ions with their concentrations at which they do not affect the voltammetric indium signal depending on the working electrode used.

Procedure using cupferron and Hg(Ag)FE	**Procedure using cupferron and PbFE**	**Procedure using cupferron and BiFE**
1×10^{-5} mol L^{-1} Al(III), Ca(II), Cd(II), Co(II), Cr(III), Cr(VI), Cu(II), Mg(II), Mn(II), Ni(II), Pb(II), Zn(II) 1×10^{-6} mol L^{-1} Fe(III), Sb(III) 5×10^{-7} mol L^{-1} W(VI) 1×10^{-7} mol L^{-1} Bi(III), Ga(III), Ge(IV), Mo(VI)	5×10^{-6} mol L^{-1} Al(III), As(III), As(V), Bi(III), Ca(II), Cd(II), Cr(III), Cr(VI), Co(II), Fe(III), Ga(III), Ge(IV), Hg(II), Mg(II), Mn(II), Mo(VI), Ti(IV), V(V), Zn(II) 1×10^{-6} mol L^{-1} Fe(III), Sb(III) 2×10^{-7} mol L^{-1} Cu(II), Ni(II), Sb(III)	1×10^{-5} mol L^{-1} Cu(II), As(III), Fe(III), Cr(III), Cr(VI), Co(II), Zn(II), Ca(II), Mn(II), Mg(II), Ni(II), Al(III), Na(II), K(II), Pb(II), Hg(II), Ge(IV), Cd(II), Tl(I), Fe(III), Sb(III) 5×10^{-6} mol L^{-1} Sb(III) 2×10^{-6} mol L^{-1} As(V), W(VI) 1×10^{-6} mol L^{-1} Pd(II), Mo(VI), Se(IV)

The most interfering ion turned out to be Sn(II), which at a concentration above 5×10^{-7} mol L^{-1} suppressed the In(III) signal by 70% and at higher concentrations even made this signal completely disappear.

Analytical application

The developed procedures were used for the analysis of certified reference materials: waste water (SPS-WW1) and surface water (SPS-SW1), as well as natural waters taken from the Lublin region: from the Bystrzyca river and the Zemborzycki Lake. Satisfactory results were obtained in all cases and the recoveries ranged from 93 – 106%.

Conclusions

Three voltammetric procedures for the determination of trace amounts of indium were developed using the accumulation of In(III)-cupferron complexes on different working electrodes. As can be seen, the best results were obtained using Hg(Ag)FE and PbFE as the working electrodes but PbFE had a wider linear range. The procedure using BiFE as the working electrode has a limit of detection one order of magnitude higher than that of the other two electrodes. All three procedures were used for the analysis of environmental waters, which confirms the possibility of their practical use.

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APPLICATION OF LOW-COST ALGINATE-BASED BIOSORBENTS FOR EFFECTIVE RECOVERY OF RARE EARTH ELEMENTS

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Abstract

Rare earth elements are used in many high-tech applications but their rarity and availability require the development of methods for their recovery from low-quality sources and recycling from waste materials. Sorption processes, including biosorbents, are an interesting method for their recovery from dilute solutions. This work investigates the sorption of lanthanum(III) ions using cheap, renewable biosorbents: calcium alginate and biochar (considered as reference materials) and an alginate-based biosorbent named alginate-biochar composite (ALG-BC). Sorption properties of these materials were compared using batch-adsorption techniques, under various sorption conditions. The obtained sorption results show the applicability of the biosorbents for rare earth elements recovery.

Keywords: alginate, biochar, biosorbents, rare earth elements, adsorption abilities

Introduction

In recent years rare earth elements (REEs) have gained increasing importance due to their special functionality in many fields, including electronics, glass, ceramics, catalysis, optics, magnets and superconductors. REEs are important strategic elements. Their largest deposits are located in China, which is the main exporter of these raw materials. However, China has recently changed its policy significantly and limited the export of these raw materials, due to the depletion of their natural resources (Anastopoulos et al. 2016, Maksymowicz 2019). The constantly growing demand for these elements all over the world necessitates finding alternative sources. The commonly used methods for preconcentration and recovery of metal ions from different solutions include adsorption, ion exchange, solvent extraction, precipitation, electrochemical or membrane processes (Gupta et al. 2019). Among these methods a very promising one is biosorption using low-cost natural materials, such as alginate, chitosan, zeolite, cellulose, lignin, and solid wastes, such as biochar, fly ash or activated carbon (Galhoum et al. 2017, Gładysz-Płaska et al. 2003, Kołodyńska et al. 2018, Xiaoqi et al. 2016, Zhang et al. 2011). For example Galhoum et al. (2017) studied the sorption of La(III), Nd(III) and Er(III)) using cellulose and the maximum

sorption capacities for rare earth metal ions were in the range of 31-53 mg/g. Unfortunately, very often adsorption capacities of natural adsorbents are not as efficient as expected. Therefore, the aim is to improve their adsorption properties through the synthesis of hybrid biosorbents by doping various organic or inorganic materials.

Alginate salts, for example calcium alginate, are some of the carriers for the immobilization of microorganisms or biocatalysts (Pankhurst et al. 2003). They can be also used as a matrix for immobilization of some sorbent materials with very small particle size. This fact is influenced by factors such as the presence of carboxyl groups (Figure 1), natural origin, hydrophilicity, relatively good mechanical strength, and a very cheap and easy procedure to obtain a gel that is non-toxic and benign to the immobilized material.

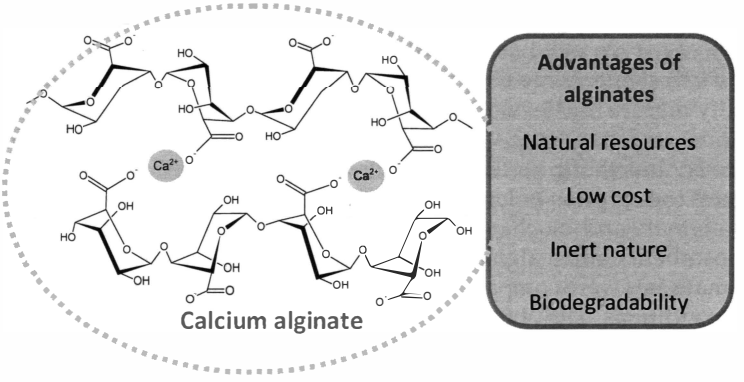

Figure 1. Egg-box structure of calcium alginate and alginate advantages.

Immobilization of biomass on a granular or polymeric matrix can increase the efficiency and adsorption capacity of the biosorbent towards metal ions and facilitates separation of biomass from solution. Wu et al. (2011) prepared magnetic alginate-chitosan gel beads by incorporating iron oxide nanoparticles onto a mixed alginate and chitosan adsorbent. Glutaraldehyde was used as a crosslinking agent. The obtained composite was used for La(III) ions sorption from aqueous solutions. Sorption equilibrium was achieved after 10 hours and the obtained maximum capacity was 97.1 mg/g.

The overall aim of the paper was to study the sorption properties of calcium alginate and biochar as reference materials and to explore the potential of alginate-based biosorbents prepared using sodium alginate as a matrix, different amounts of biochar as an additive, and calcium chloride as a crosslinking agent for the removal and recovery of REEs.

Materials and Methods

To synthetize alginate based composites, sodium alginate purchased from Carl Roth (Germany), calcium chloride purchased from Chempur (Poland), and

biochar derived from animal manure combustion were used. The procedure for the synthesis was as follows: a 1% solution of sodium alginate (ALG) was prepared to which various amounts of biochar (BC) were added to obtain ALG:BC ratios of 5:1, 2:1, 1:1, 1:2, 1:5. The whole mixture was stirred using a magnetic stirrer (ES 21, Wigo, Poland). Then this mixture was dripped with 1% calcium chloride solution using a peristaltic pump (BT100S-1, Lead Fluid Technology Co., Ltd. China). After 24 h, the obtained beads were washed several times with distilled water and were allowed to dry. Additionally, calcium alginate beads without additives were prepared and their sorption properties towards rare earth ions were compared.

Sorption studies were performed using the batch sorption method. Nitrate salts of rare earth elements purchased from Sigma Aldrich (Germany) were used for sorption studies. The influence of several operating parameters i.e. solution pH (1-7), contact time (1-1440 minutes), and initial REE concentration (25-500 mg/dm^3) was tested and presented. The metal ions concentration after sorption experiments was analyzed using an ICP-OES spectrometer (720-ES Varian Inc., USA).

Results and Discussion

The ALG-BC composite with different amounts of ALG and BC was prepared and used for adsorption tests. The prepared composite beads were named: ALG (1:0, ALG:BC), BC (0:1 ALG:BC), ALG_5BC_1 (5:1, ALG:BC), ALG_2BC_1 (2:1, ALG:BC), ALG_1BC_1 (1:1, ALG:BC), ALG_1BC_2 (1:2, ALG:BC), and ALG_1BC_5 (1:5, ALG:BC), respectively.

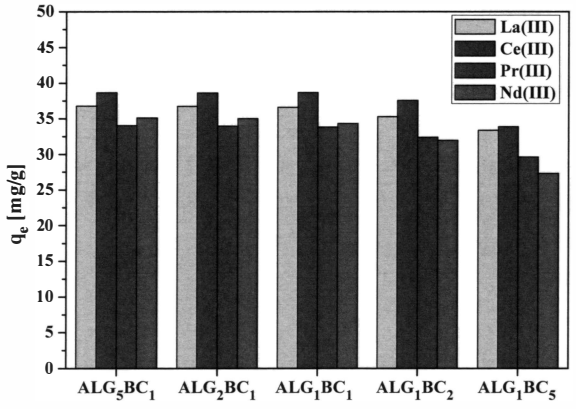

Figure 2. Effect of different amount of ALG and BC on REE sorption process (m= 0.05 g, V= 20 cm^3, C_0= 100 mg/dm^3, t= 480 min, T= 293 K).

ALG_5BC_1, ALG_2BC_1, and ALG_1BC_1 composites had the highest values of q_e (Figure 2) and %S equal to about 100% towards La(III), Ce(III), Pr(III), and

Nd(III) ions sorption, hence one of them, i.e. ALG_5BC_1 composite was chosen in subsequent adsorption experiments (i.e. for effect of solution pH, effect of phase contact time, and effect of initial metal concentration).

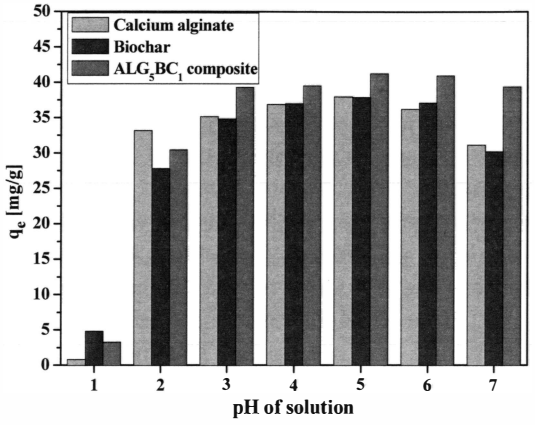

Figure 3. Effect of La(III) solution pH on sorption (m= 0.05 g, V= 20 cm^3, C_0= 100 mg/dm^3, t= 480 min, T= 293 K).

The influence of solution pH value on REE sorption efficiencies was noted. Figure 3 shows the obtained equilibrium adsorption capacities for different solution pH values on the example of La(III) ions for calcium alginate, biochar and their composite. When pH increased from 1 to 5, the equilibrium adsorption of lanthanum(III) ions enhanced. The optimum pH value for REE ions sorption on the biosorbents was 5.

In the next step, the effect of phase contact time on the amount of adsorbed La(III) ions on calcium alginate, biochar, and ALG_5BC_1 composite was investigated.

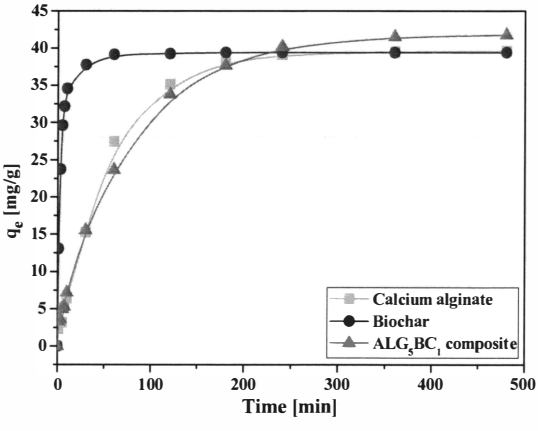

Figure 4. Effect of phase contact time on La(III) sorption effectiveness (m= 0.05 g, V= 20 cm^3, C_0= 100mg/dm^3, pH= 5, t= 480 min, T= 293 K).

As shown in Figure 4, with time increasing, the amount of La(III) ions adsorbed increased until sorption equilibrium was established. For biochar the sorption equilibrium state was achieved after 60 minutes, but for calcium alginate and ALG_5BC_1 composite after 240 minutes, so the biosorbents are characterized by good kinetic properties.

Effect of initial concentration of La(III) ions on the sorption process was investigated (Figure 5). The results showed that the equilibrium sorption capacity of La(III) ions increased with the increase of initial metal concentration. The maximum q_e values were obtained for 500 mg/dm^3 and were: 100.46 mg/g for calcium alginate, 68.42 mg/g for biochar, and 110.28 mg/g for ALG_5BC_1 composite. The synthesized ALG_5BC_1 composite showed better removal efficiency of La(III) ions than the reference materials i.e. calcium alginate and biochar.

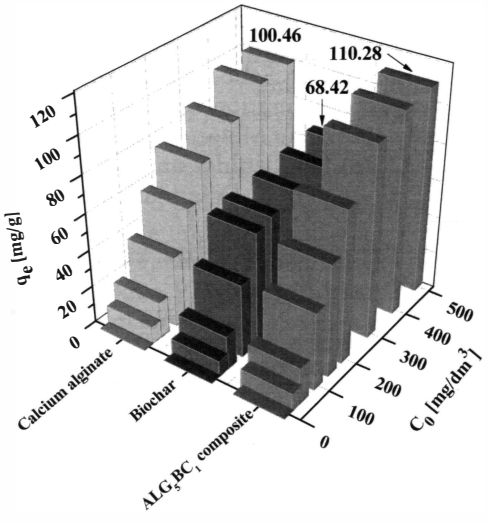

Figure 5. Effect of initial concentration on La(III) ions sorption (m= 0.05 g, V= 20 cm^3, C_0= 100 mg/dm^3, t= 480 min, T= 293 K).

The effectiveness of the La(IIII) ions sorption process on the used biosorbents followed the order: ALG_5BC_1 composite > calcium alginate > biochar.

Conclusions

In this study, it was confirmed that alginate can be used to synthesize alginate-biochar composite. The obtained ALG_5BC_1 composite demonstrated effective sorption of La(III) ions from aqueous solutions and proved to be a very promising biosorbent for REE ions sorption and recovery.

Acknowledgements

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REMOVAL OF VANADIUM(V) BY ADSORPTION ONTO ION EXCHANGERS FROM AQUEOUS SOLUTIONS

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Abstract

Vanadium is considered a strategic metal, and its limited resources and high consumption make its production and recovery of significant importance. The Dowex PSR2's and Dowex PSR3's applicability for vanadium removal from aqueous solutions was examined. The adsorption process optimization was performed for the adsorbent dose (0.01 – 0.1 g) and pH (2 – 10) effects. It was found that the optimum adsorbent dose is equal to 0.1 g and optimum pH = 6. V(V) can be removed with high efficiency (the amount of vanadium adsorbed at time t, q_t = 9.75 mg/g; percentage removal of vanadium, %R = 97.6% - gel Dowex resin or q_t = 9.86 mg/g; %R = 98.6% macroporous Dowex resin).

Key words: adsorption, removal, anion exchanger, vanadium

Introduction

Vanadium was first discovered in 1801 by Andreés Manuel del Rio in lead and vanadium ores, but it was mistaken for a form of chromium (Habashi, 2002). The element was named after the goddess of beauty and fertility Vanadis. It was rediscovered in the 1830s in converter slag by the Swedish chemist Nils Gabriel Seftström and isolated in 1867 by Sir Henry Roscoe, whereas production of pure vanadium (99.7% purity) began after 1925 when two American chemists, J.W. Marden and M.N. Rich reduced vanadium pentoxide (V_2O_5) by calcium (Moskalyka et al. 2003; Imtiaz et al. 2015). Vanadium belongs to the fifth group of the periodic table. Vanadium has a high melting point and good corrosion resistance at low temperature. Vanadium usually possesses a steel grey colour with a bluish tinge. It exists in a variety of oxidation states: –1, 0, +2, +3, +4, and +5 (Imtiaz et al. 2015). Vanadium is found in different minerals, as well as phosphate rock, iron ores, crude oils, or meteorites. Among different vanadium minerals carnotite, vanadinite, roscoelite, and patronite play a significant role. Vanadium is soluble in HNO_3, H_2SO_4 acids but insoluble in HCl, dilute NaOH, and dilute alcohol (Moskalyka et al. 2003). Vanadium forms different types of compounds, e.g. ammonium metavanadate (NH_4VO_3), sodium metavanadate ($NaVO_3$), sodium orthovanadate (Na_3VO_4), but the most commonly used form

of vanadium is vanadium pentaoxide (V_2O_5) (Imtiaz et al. 2015). Vanadium applications are found in three main areas. Due to its high resistance to corrosion, vanadium is commonly applied as an additive to stainless steel alloys (Nazarov et al. 2007) and aerospace alloys. In the aerospace industry vanadium is applied with aluminum as well as titanium (this area covers 4.5% of the vanadium market). Another application area is the chemical industry. Vanadium in the form of vanadium pentaoxide is a very important component of catalyst matrices used in the refining and particular sulfuric acid production industries. The third area of vanadium application is the redox battery industry (Baranova et al. 2012; Barceloux et al. 1999). As it was found, the vanadium supply as well as demand increase year by year (Fig. 1). The vanadium demand increase is a results of steel industry development as well as its new applications. Taking into account that the demand is greater than the supply and the very low inventory levels for vanadium, its recovery is of particular importance. Therefore, the purpose of the study was to check Dowex ion exchange resins' applicability for vanadium removal from the model and real solutions. Optimization of the adsorption process was performed and the effect of pH was evaluated.

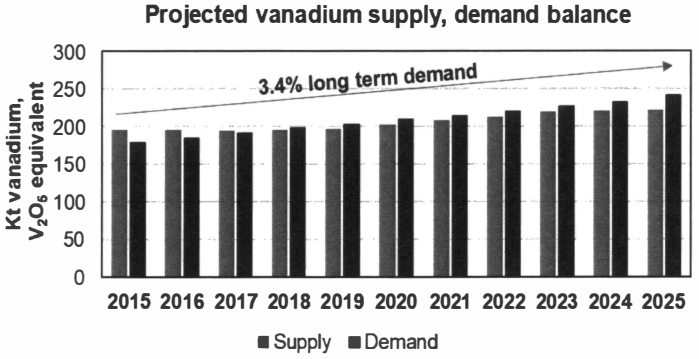

Figure 1. Vanadium supply and demand (expectation).

Materials and Methods

The vanadium(V) stock solution of 1000 mg/L concentration was prepared from sodium metavanadate which was dissolved in distilled water with 5 mL of concentrated ammonia (25%) and 10 mL of concentrated nitric acid(V) (65%). The working V(V) solutions were prepared by dilution and their pH values were adjusted using the HNO_3 and NaOH solutions of 1 mol/L concentrations. The properties of ion exchangers applied for V(V) removal are presented in Table 1 and Fig 2.

The studies of vanadium(V) ions removal by the static method were carried out in 100 mL conical flasks tightly sealed with silicone stoppers. Various weights of Dowex ion exchange resin were placed in the flasks: for the effect

of pH - 0.1 g, for the effect of weights - 0.01 g, 0.025 g, 0.05 g, 0.075 g, 0.1 g. All samples were weighed with an accuracy of ± 0.0005 g. Then 20 mL of the stock solution, which contained V(V) ions at a concentrations of 50 mg V(V)/L was added to the flasks. The samples were set up in an Elpin+ mechanical shaker, type 357, and its operating parameters were as follows: shaking speed=170 rpm, vibration amplitude A=8, room temperature T=295 K. The flasks were shaken for 4 hours. Then the aqueous phase was separated from the ion exchanger by filtration and the V(V) concentration was determined using Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) (Varian AA240Z spectrometer with a GTA120 graphite cell and a PSD120 automatic dispenser). After that the percentage removal of vanadium was calculated according to the formula (Manohar et al. 2005):

$$\%R = \frac{C_0 - C_t}{C_0} * 100\% \qquad (1)$$

where: C_0 is the initial concentration of V(V) ions in the aqueous phase (mg/L), C_t is the concentration of V(V) ions in the aqueous phase after time t (mg/L).

Table 1. Physicochemical properties of Dowex ion exchangers (Wołowicz et al. 2020).

Functional resin	Dowex PSR2	Dowex PSR3
Type	Strongly basic anion exchanger	Strongly basic anion exchanger
Matrix	Cross-linked polystyrene	Cross-linked polystyrene
Structure	Microporous	Macroporous
Functional groups	Quaternary ammonium, type, tri-n-butyl amine	
Mean bead size (mm)	0.3 – 1.2	0.3 – 1.2
Total capacity (val/L)	0.65	0.6
Water retention (%)	40 – 48	50 – 65

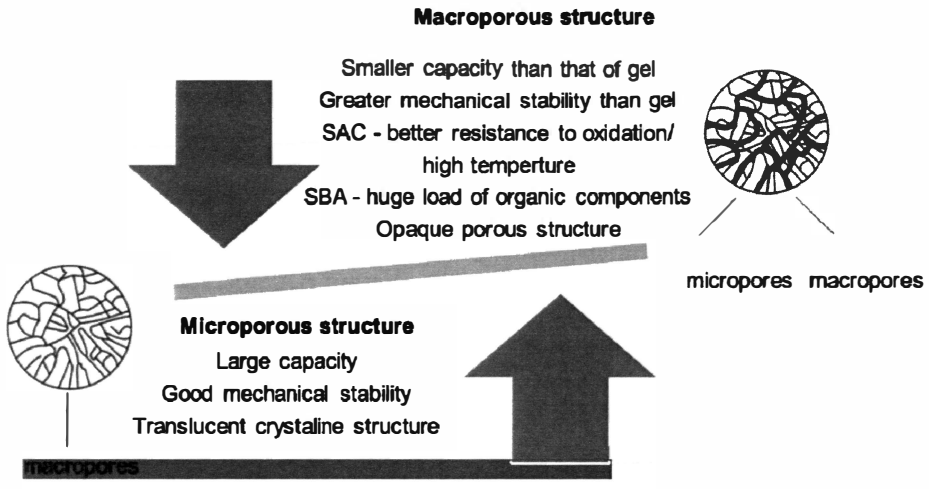

Figure 2. Difference between the micro- and macro-porous ion exchange resins (SBA – the strongly basic anion exchange resin, SAC – the strongly acidic cation exchange resin).

Results

The effect of pH on adsorption of V(V) on Dowex PSR2 and Dowex PSR3 was studied in the pH range from 2 to 10. The results are presented in Figure. 3.

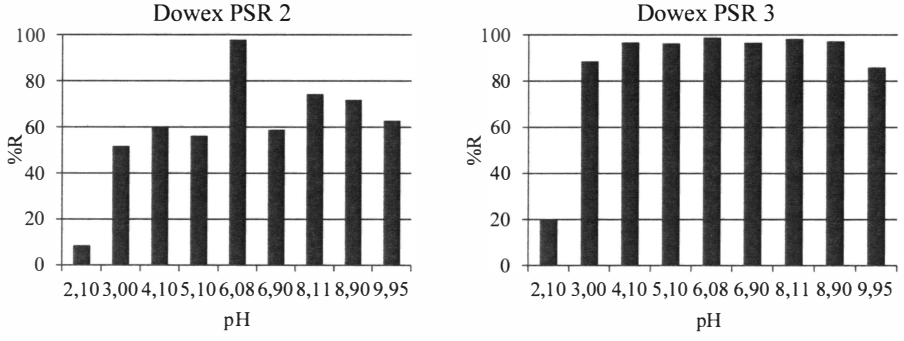

Figure 3. Effect of pH on the percentage removal on Dowex PSR2 and Dowex PSR3.

Vanadium can exist in different forms, depending on the solution pH and solution concentration. At least 11 species can be found. At pH 12-14 the tetrahedral orthovanadate ion is the principal species, whereas at lower pH values the monomer $[HVO_4]^{2-}$ and dimer $[V_2O_7]^{4-}$ are formed. As the pH value is reduced, further protonation and condensation to polyvanadates occur. At pH 4-6 $[H_2VO_4]^-$ is predominant, while at higher concentrations trimers and tetramers

are formed. At pH 2-4 decavanadate predominates (Baes et al. 1976; Bartecki 1996, Peacock et al., 2004, Takaya et al. 1994). The form of vanadium can affect its percentage removal. As it was found, pH = 6 was the optimum for the adsorption of V(V) on Dowex PSR2 as well as PSR3. In the case of microporous Dowex PSR2 the effect of pH is more evident than in the case of macroporous Dowex PSR3. At pH = 6 (initial V(V) concentration 50 mg/L) vanadium exists in anionic forms (Fig. 4) and the ion exchangers belong to the anion exchange resin type therefore, the uptake is large and %R is equal to 97.6% for the Dowex PSR2 and %R = 98.6% for Dowex PSR3 resins. A slightly greater or much greater adsorption was observed for macroporous Dowex PSR3.

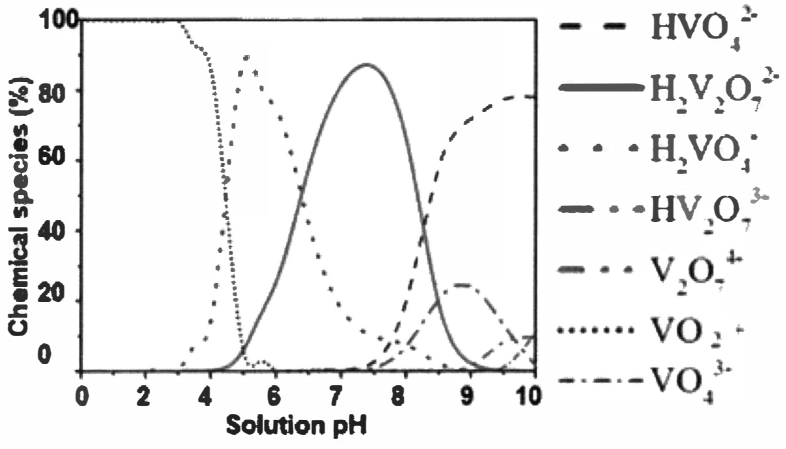

Figure 4. Forms of vanadium in aqueous solution calculated using Visual MINEQL ver. 3.0 at the initial concentration 50 mg/L.

As is reported in literature, pH is an important parameter affecting the adsorption efficiency of V(V) ions. The elimination efficiency of vanadium ions at the initial concentration C_0 = 40 mg/L on the composite adsorbent ranged from 46.1% (pH=7) to 72.1% (pH=3.5). The removal of vanadium ions decreased with the pH increasing above 3.5. The optimum pH was 3-3.5 (Mojiri, 2017), while when using the porous ceramic containing amino groups (C_0 = 50 mg V(V)/L) the optimum pH was 4-4.5 (99.8% of ions was removed). Additionally, a decrease in %R was observed with the increasing pH (He et al. 2018). The optimum pH for vanadium adsorption on Fe(III)/Cr(III) hydroxide treated with hydrochloric acid was 4.0 (Prathap et al. 2010).

The adsorption optimization was also achieved by changing the adsorbent dose from 0.01 g to 0.1 g. The experimental conditions were as follows: adsorbent mass = 0.1±0.0005 g, initial concentration C_0 = 50 mg V(V)/L, volume of added solution, V = 20 mL, pH = 6, agitation speed = 170 rpm, amplitude, A = 8, temperature, T = 295 K, t = 4 h. It was found that the increase in the adsorbent dose increased the removal of V(V) (Fig. 5). With the increase in the ion exchanger resin dose, larger numbers of active sites in the adsorbent surface are

accessible for V(V) adsorption, which results in greater adsorption (Jana et al. 2015).

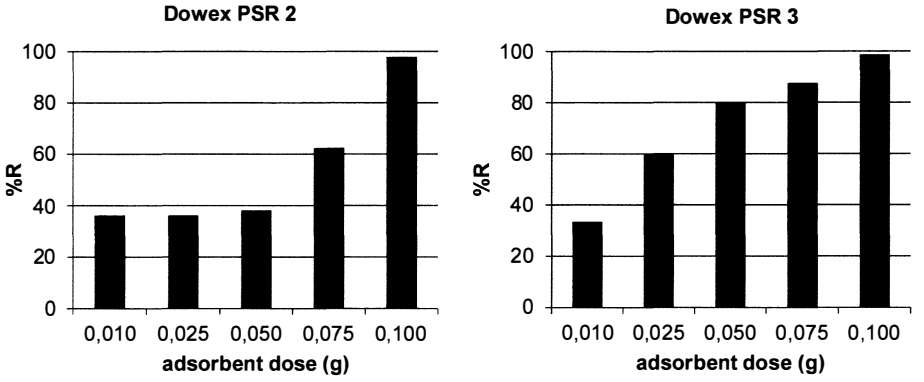

Figure 5. Effect of adsorbent dose on the V(V) adsorption on Dowex PSR2 and Dowex PSR3.

Conclusions

Studies of optimization of adsorption of V(V) on the Dowex ion exchange resins prove that the best adsorption uptake was obtained at pH=6 and adsorbent dose 0.1 g. Moreover, the studies show a high percentage removal of V(V) from aqueous solutions. Further studies to prove the ion exchange resins' applicability for V(V) removal from real wastewaters are in progress.

Acknowledgments

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IT'S GETTING WORSE: ANTIBIOTIC RESISTANCE AMONG OPPORTUNISTIC PATHOGENS

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Abstract

The phenomenon of bacterial opportunism has been known for two centuries. It means the occurrence of species that generally remain completely harmless, but in conditions unfavorable for the host may lead to the development of disease symptoms. Until now, opportunistic pathogens have often been marginalized and the number of infections caused by them has been underestimated. In recent years, however, the importance of opportunistic human pathogens in the general pool of bacterial and fungal infections has been increasingly emphasized. Unfortunately, as the frequency of infections increases, so does the percentage of resistant strains among these bacteria and fungi. This is a serious therapeutic challenge, but also clearly demonstrates the need to take measures to prevent the emergence of antibiotic resistance among opportunists. These species, apart from causing infection, may constitute a natural reservoir of resistance for other, often more virulent, microorganisms and contribute to a marked reduction in the effectiveness of treatment with antibiotics and antimycotics.

Keywords: facultative bacteria, horizontal gene transfer, side effects, Bacillus cereus sensu lato

Introduction

Since the discovery of the first antibiotics in the first half of the 20th century, the era of antibiotics has begun. Thanks to the possibility of using effective bactericidal drugs in treatment, it became possible to control the most dangerous infectious diseases, which were the cause of numerous deaths, both among people susceptible to infections (patients with immunodeficiency, children, the elderly, people with comorbidities) as well as immunocompetent individuals.

Unfortunately, with the spread of antibiotic therapy, more and more cases of resistance to selected groups of drugs began to be noted. Already in the first years after the use of penicillin (β-lactam antibiotics class) for treatment, an increasing incidence of infections caused by gram-positive cocci resistant to these antibiotics was noted. Increasing insensitivity to selected aminoglycosides (e.g. streptomycin) and tetracycline was also noted fairly quickly. This resulted in the

need to search for new drugs and revise the treatment procedures used so far (Davies and Davies 2010).

The points where the phenomenon of antibiotic resistance seems to develop most rapidly proved to be an additional threat. These are mainly hospitals, where people carrying different drug-resistant strains contact each other, and the phenomenon of horizontal gene transfer and selection pressure, associated with the widespread use of antibiotics that favors strains showing a high degree of insensitivity to various medicinal preparations, manifest.

Even more worrying is the phenomenon associated with numerous infections caused by species of bacteria that are facultative pathogens. For many years, the greatest risk from staphylococci was associated with *Staphylococcus aureus* infections, while nowadays, serious illnesses caused by coagulase-negative staphylococci (CNS) are more common. Similar observations were made for bacteria from the *Enterobacteriaceae* family, but also for *Bacillaceae*, especially *Bacillus cereus*, and related species (Iredell et al. 2016, Forrester et al. 2018, Bartoszewicz et al. 2019, Bartoszewicz and Czyżewska 2021).

The aim of the present work is to discuss the occurrence of antibiotic resistance among selected species of opportunistic pathogens and point out important sources of these kind of resistant bacteria.

Opportunistic pathogens in humans

Commensal microorganisms have aroused scientific interest since the end of the 19th century. At that time, people wondered about the influence of these microorganisms on human health. Such considerations were taken up, among others, by microbiologist Louis Pasteur after he had observed non-pathogenic bacteria present both in the environment and on the body surfaces of humans and animals. Consequently, the belief that colonization by bacteria is always synonymous with disease has gradually been abandoned. Commensalism refers to microorganisms that do not cause disease symptoms (or cause them only sporadically and only under strictly defined conditions) and thus do not meet Koch's third postulate, which says that a microorganism isolated from an infected person, introduced into another organism, must cause the same disease. The first concept of commensalism appeared in the 1860s. It was formulated by Pierre-Joseph van Beneden, presenting almost 270 examples showing a relationship between two species, in which one of them (commensal) gets benefits (e.g. access to nutrients, shelter), while the other (host) gets no profit or loss (Poreau 2014). Currently, this term is also used in relation to the commensal bacterial biota of humans. However, it is worth mentioning that it is not limited to bacteria only. A number of yeast-like fungi act the same way (mainly *Candida* and *Malassezia* spp). Species naturally related to the human microbiota, as a result of a significant reduction in the level of immunity of the body, cause candidiasis of the mouth, throat or mucous membranes of the gastrointestinal tract (caused mainly by *Candida albicans*) or dermatoses, e.g. atopic dermatitis associated with *Malassezia furfur* (Czyżewska et al. 2018).

All microorganisms considered as facultative pathogens have certain features that determine the predisposition to colonize individual parts of the organism, thanks to which they can be part of the natural microbiota. Commensal microorganisms usually do not show the ability to produce toxins typical for obligatory pathogens, or they are less effective due to the low level of gene expression that determines their synthesis. Facultative pathogens possess other factors of pathogenesis like adhesins of CNS used in order to adhere to the host's extracellular matrix proteins. Among them, the key role is played by the SdrG protein, which determines contact with the layers coated with fibrinogen, or the SdrF protein, which is responsible for binding collagen, which is the main component of the skin (Nowicka et al. 2012, Milles et al. 2018). *Clostridioides difficile* (formerly *Clostridium difficile*) can attach to components such as collagen, fibrinogen or fibronectin, by the fibronectin-binding protein (Fbp68), which facilitates colonization of colon enterocytes (Mehlich et al. 2015). In addition, adhesion is provided by extracellular structures, characteristic of gram-negative bacteria, i.e. fimbriae, thanks to which *Klebsiella pneumoniae* can adhere to tracheal or lung cells. *E. coli* binds to the surface of intestinal epithelial cells in the same way. In turn, secretion of mucous enables the formation of aggregates and biofilm which make it easier for bacteria to oppose the host's immune mechanisms. Most facultative microbial pathogens, including microscopic fungi, exhibit the ability to produce various enzymes that act as virulence factors. Studies indicate that 50% of CNS isolated from patients with infectious endocarditis showed the ability to produce proteases and 30% of these strains produced fibrinolysin (Nowicka et al. 2012). Thanks to the ability to secrete urease, *K. pneumoniae* can successfully oppose the antibacterial effect of urea.

In the case of opportunistic fungi of the genus *Malassesia*, the virulent factor is a lipophilic cell wall, consisting of approximately 20% fatty acids, which facilitates the binding of these yeasts to the host cells and protects against phagocytosis (Jagielski et al. 2013, Czyżewska et al. 2018). There are also proteases or phospholipases, synthesized by both *C. albicans* and *M. furfur*. Human skin is a very complex and diverse habitat for microorganisms. It is the first line of defense (included in the physiological and anatomical immune mechanisms. However, in every human being the composition of microorganisms depends on the local microenvironment of an individual body and its parts. Podgórska and Kędzia (2018a, 2018b) indicated that poly-γ-DL-glutamic acid produced by CNS plays a major protective role. In addition, the hyperosmotic nature of the environment hinders the proliferation of other bacteria, thus reducing the pressure from a potential competitive biota. The dermal environment is also favorable for lipophilic fungi of the genus *Malassezia*, which prefer areas with abundant and active sebaceous glands, such as facial skin (Malinowska et al. 2018). Another potential limitation for the growth of microorganisms could be skin pH (4.2-5.9). The microbes also easily adapted to this, both through the ability to produce urease and through the formation of a biofilm, effectively protecting against acidic skin reactions. Interestingly, *S. epidermidis*, as a facultative anaerobic, is able to colonize areas with reduced oxygen content, e.g. the mucous membranes of the pharynx (Podgórska and Kędzia 2018a). It should be

noted that cutaneous staphylococcus also supports the host's defense mechanisms. Literature overview shows that about 96% of strains have the ability to produce proteins – bacteriocins, which effectively inhibit the multiplication of bacteria with a much higher pathogenic potential, such as *S. aureus* (Podgórska and Kędzia 2018a, 2018b).

The digestive tract is colonized by microorganisms in almost every part, although significant differences in the quantitative and species composition of individual parts of the intestine are observed. The reasons for this are different conditions prevailing there. The first limitation is the lack of oxygen availability, as a result of which this environment will be preferred by obligatory anaerobes represented mainly by *C. difficile* or facultative anaerobes such as *E. coli* and *E. faecalis*. The concentration of bacteria in the colon is in the range of 10^{11}-10^{12} cells/ml. An alkaline environment with a pH of ~ 8.4 and a temperature of about 37°C are favorable conditions for these species. The mucus secreted by specialized epithelial cells also plays a protective role, but *C. difficile* is able to penetrate the thick layer of mucus with the help of flagella to reach the target site, i.e. the cells of the colon. In addition, the main component of mucus, a protein named mucin 2 (MUC2), influences the biofilm formation capacity of non-pathogenic *E. coli*, increasing its colonization capacity. The microbes of the colon microbiota confer many benefits to the host (Malinowska et al. 2018). By competing effectively with pathogenic microorganisms for the same ecological niche, they eliminate them from the environment. In addition, they take part in the decomposition of food residues and also support the synthesis of folic acid or vitamins from groups B and K.

Facultative pathogens, although in most cases associated with the normal microbiota of the organism, appear more and more frequently in reports of endogenous infections. Most of them are related to the hospital environment. For example, data from the National Institute of Hygiene (PZH) indicate that in 2019 the total number of cases caused by *C. difficile* was 11,310, of which almost 86% required hospitalization. Moreover in 2014, we noted less than 17 cases per 100 thousand people, but now this rate is almost two times higher. As a result of the disturbed balance of microorganisms in the colon, this bacterium can multiply uncontrollably, synthesizing toxins that damage the intestinal epithelial cells leading to fatal cases (Mehlich et al. 2015). Another serious risk is posed by the extra-intestinal pathogenic *E. coli* (ExPEC) strains. They are responsible for approximately 75-85% of primary urinary tract infections. Within this group 80-90% of cases is attributed to uropathogenic coliform strains (UPEC). The frequency of infections is also related to the progress of medicine and the increasingly common use of invasive medical procedures. Despite the low incidence level (1.5-4.95 per 100,000 people), mortality among the elderly is significantly increased, ranging 14-46% (Podgórska and Kedzia 2018a, 2018b). *K. pneumoniae* infections also occur in hospitalized patients with additional severe diseases. In Poland, 2016 saw a sharp increase in the number of cases compared to previous years. The situation is getting worse every year. According to the report of the National Reference Center for Antimicrobial Susceptibility (KORLD), about 2,355 infections (mainly pneumonia and urinary tract infections) were registered

in 2018, which is five times more than in 2015. They were also caused by the strain *K. pneumoniae* NDM-1, which is difficult to treat due to multi-resistance.

Another organism that poses a serious challenge is the bacteria of the *Bacillus cereus* group. Although they generally do not cause health issues, they may lead to mild food poisoning causing diarrhea (due to the production of a number of enterotoxins by bacteria) and vomiting (due to cereulide intoxication), they can also cause serious infections, which can be life threatening. The literature describes cases of inflammation of the eye, periodontitis, but also inflammation of the joints caused by *B. cereus* and *B. cytotoxicus*. Sepsis caused by these microorganisms has also been reported. In their case, however, the environment plays an extremely important role. On the one hand, these bacteria are commonly found in soil, water, air and food, and on the other hand, they are easily persistent in various products as they form spores (Bartoszewicz and Czyżewska, 2017). Interesting observations were made by Bartoszewicz and Czyżewska (2021), who showed significantly higher minimum inhibitory concentrations for selected antibiotics and more frequent occurrence of multiresistant strains among isolates derived from food of animal origin, primarily milk. In addition, recent data also indicates that these bacteria may be a source of resistance to other bacteria (unpublished data). On the other hand, transfer of *B. cereus* and related species can take place both through water, soil, as well as through food and synanthropic species of urban birds (unpublished results). Finally, recent data suggests, that among bacteria we often deal with evolution leading to the formation of distinct ecotypes and one such ecological form could be a multiresistant variant (Bartoszewicz et al. 2019).

Summing up, we must be aware that in recent years numerous infections are caused by distinct opportunistic bacteria, not only by obligatory pathogens. These microbes are not the primary goal for antibiotic therapy and sometimes are omitted by reports, which causes their role to be underestimated. Consequently, we are still convinced that they pose little risk. Nevertheless, the deteriorating immunity among people, the aging of the population, and unhealthy lifestyle increase our susceptibility to infections with opportunistic bacteria. And in a situation where our immunity is not optimal, even the less effective mechanisms of virulence of such bacteria can lead to health disorders that require antibiotic therapy, which is increasingly limited by the common resistance of these bacteria.

Antibiotics against bacteria

Antibiotics constitute a group of therapeutics with antibacterial activity, diverse in terms of origin, structure and mechanisms of action. In lower concentrations, they are often bacteriostatic by blocking metabolic pathways in susceptible cells. Higher concentrations may be bactericidal by disturbing processes of cell wall synthesis, replication and protein biosynthesis. Most of the antibiotics act as inhibitors of cell synthesis (mainly β-lactams), substances that disrupt the activity of the cell membrane (gramicidin), blockers of nucleic acid

synthesis (rifampin) or protein synthesis inhibitors (tetracyclines, macrolides, aminoglycosides).

Discovered as the first, but still frequently applied: β-lactams

The most common and preferred antibiotics for treating numerous infections are β-lactams. In the natural state, they are most often produced by fungi of the genus *Aspergillus* and *Penicillum*, but also by some bacteria belonging to the genus *Streptomyces* and *Nocardia*. Their name is related to their structure, namely the presence of the β-lactam ring in the antibiotic molecule, as showed in Figure 1. This class includes the first isolated antibiotic, penicillin, and related substances – carbapenems, cephalosporins and monobactams with higher effectiveness, lower side effects and more resistant to β-lactamases (Bush and Bradford 2016, Tooke et al. 2018).

The mechanism of their action is based on the inhibition of the last stage of peptidoglycan biosynthesis, which is the main component of the cell wall. This is because the antibiotic blocks the enzyme transpeptidase (penicillin-binding protein, PBP), which catalyzes the proper cross-linking of murein (main component of prokaryotic cell wall). Ultimately, the cell lyses due to osmotic stress.

In response to the action of β-lactams, many bacteria, including commensal and pathogenic isolates, have developed a number of resistance mechanisms that protect them from the adverse effects of these substances. One of the best known mechanisms, both among gram-negative bacteria (represented by *E. coli* and other enterobacteria) and gram-positive bacteria (staphylococci), is the production of β-lactamases, which are designed to hydrolyze the amide bond in the β-lactam ring. What is worse, as a result of mutations in the genes responsible for coding β-lactamases, a significant number of microorganisms began to produce enzymes with a much broader substrate spectrum. Metallo-β-lactamases (MBL) produced by *Enterobacteriaceae*, especially by *K. pneumoniae*, pose a particular risk (Khan et al. 2017). They are not only capable of hydrolyzing all β-lactams (except of monobactams), but also the genes encoding the enzyme are located on mobile genetic elements, which facilitates their transfer between bacteria (HGT, horizontal gene transfer). Moreover, hospital-origin strains of *S. epidermidis* resistant to methicillin (MRSE) possess the *mecA* gene on mobile SCCmec genetic cassettes (Podgórska and Kędzia 2018a, 2018b). It conditions the production of additional or modified PBP proteins (PBP2a), which is more insensitive, enabling bacteria to synthesize the cell wall even in the presence of high drug concentrations. On the other hand, in gram-negative bacteria, e.g. *E.* coli, resistance may be the result of limited outer membrane penetration due to loss or alteration of the porin channels conformation. Interestingly, the β-lactam antibiotics also include atypical therapeutics, e.g. clavulanic acid, sulbactam or tazobactam, which, unlike the rest of the class, do not show a therapeutic effect by inhibiting murein synthesis, but act as β-lactamase inhibitors. Therefore, the combined use of an inhibitor (clavulanic acid) with an antibiotic (e.g. amoxicillin)

enhances the bactericidal effectiveness of the described preparations (Bush and Bradford 2016). Nevertheless, we must still bear in mind that resistance to this class of antibiotics can spread rapidly by horizontal gene transfer. In the case of infections caused by opportunistic bacteria, therapy should be continued until the infection is completely eliminated, to prevent the survival of strains with increased resistance, which may later become a source of re-infection.

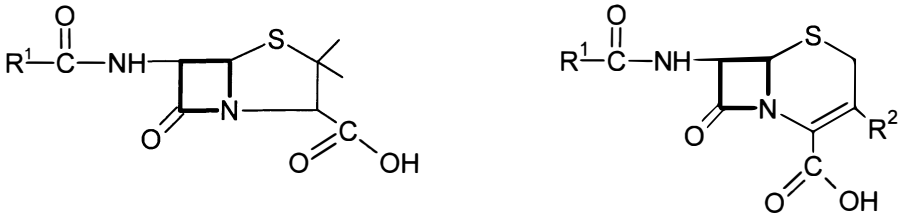

Figure 1. Chemical structure of penicillin (a) and cephalosporin (b), including the β-lactam ring (bold). Figure made in ChemSketch 14.0.1.

Still relevant, but less frequently used: aminoglycosides

Aminoglycosides have a broad spectrum of activity, including both gram-negative and gram-positive bacteria. In addition, streptomycin is an effective weapon against mycobacteria, including *Mycobacterium tuberculosis*, the tuberculosis causative factor. Most natural aminoglycosides are produced by actinomycetes belonging to the genus *Streptomyces*. A characteristic feature of all of them is the presence in the antibiotic molecule of amino sugars linked by a glycosidic bond with a ring – streptamine, as is the case in neomycin and kanamycin molecules or streptidin in streptomycin (Zaffiri et al. 2012, Becker and Cooper 2013), as shown in Figure 2. The mechanism of action of aminoglycosides is related to the inhibition of translation by binding to the minor subunit of the bacterial ribosome. This leads to a breakdown in interaction of the mRNA codon with the tRNA anticodon and the incorporation of incorrect amino acids into the forming polypeptide (Hobson et al. 2021). Due to the diverse chemical structure of this class of antibiotics, bacteria have developed a variety of resistance mechanisms against them.

Resistant bacteria most often produce enzymes that modify the hydroxyl or amino groups of the antibiotic molecule by acetylation, phosphorylation or adenylation. Some strains of the genus *Enterococcus* (mainly *E. faecalis*) and *Staphylococcus* spp. (especially *S. epidermidis*) have several genes, the products of which display N-acetyltransferase and O-phosphotransferase activity, responsible for inactivating gentamicin or kanamycin (Hobson et al. 2021). From the medical point of view, this property is problematic mainly due to its location on the

transposon (mobile genetic element), the presence of which has been demonstrated in numerous opportunistic and obligatory pathogenic bacterial species. On the other hand, other facultative pathogens can methylate the target site, namely the 16S rRNA, which is part of the 30S subunit. Such a mechanism, conditioned by the *rmtA* and *rmtB* plasmid genes, has been reported in *K. pneumoniae* NDM-1 and *E. coli* (Hobson et al. 2021).

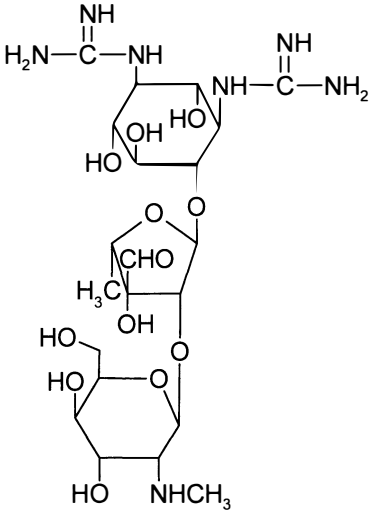

Figure 2. Chemical structure of streptomycin. Figure made in ChemSketch 14.0.1.

Enterobacteria with the aforementioned resistance mechanisms, due to their commonness, are becoming a more and more serious problem. *K. pneumoniae* in particular is indicated as an extremely important etiological factor of difficult-to-treat infections and the spread of resistance genes among other, also pathogenic, strains increase the threat. Hence, an emphasis on a high level of hygiene is important, also in health care facilities, in order to limit the transmission of these resistant bacteria between patients.

Antibiotics with wide spectrum of activity: tetracyclines

Tetracyclines are a relatively early described class of natural and semi-synthetic antibiotics. Its first representative, discovered at the turn of the 1940s and 1950s, was chlortetracycline produced by *Streptomyces aureofaciens* (Zaffiri et al. 2012, Hobson et al. 2021). Unfortunately, quite soon after the introduction of chlortetracycline to therapy, numerous resistant strains appeared and this phenomenon was probably exacerbated by HTG with the participation of commensal strains and opportunistic pathogens. Currently, therapy is dominated by semi-synthetic drugs, including doxycycline, metacycline and minocycline. Their spectrum of activity includes a number of gram-negative and

gram-positive bacteria, as well as some protozoa. The compounds have four characteristic six-membered rings joined together (Figure 3). In addition, they are also characterized by a relatively high molecular weight (>400 Da).

The target structure, exposed to tetracyclines, is mainly the 30S ribosome subunit. Within it, the antibiotic strongly binds to proteins' S4, S18 and 16S rRNA. As a result, it is impossible to bind the tRNA carrying the amino acid at the acceptor site A of the mRNA-ribosome complex and elongate the polypeptide. It should be emphasized that this process is reversible, therefore regular use of the drug is so important in tetracycline therapy.

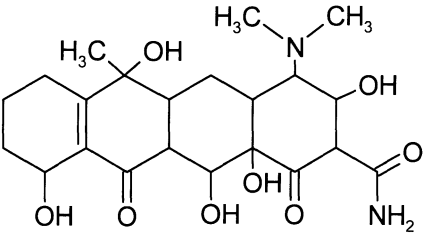

Figure 3. Chemical structure of tetracycline. Figure made in ChemSketch.

Unfortunately, the issue of tetracycline resistance is also a serious problem. This resistance can be of two types: non-specific (limited drug influx through porin channels) or specific for a given bacterial species. In clinical terms, specific resistance is assigned much more importance. Its most important mechanism is related to the active pumping by pumps belonging to the MSF group (major facilitator subfamily), anchored in the cytoplasmic membrane. In gram-negative bacteria of the genus *Escherichia*, the presence of *tetA* genes was confirmed on plasmids (including the RP1 plasmid) and *tetB* genes were noted on the Tn10 transposon (Coleman et al. 1982). Another well-known mechanism of resistance, confirmed in *C. difficile*, is the production of ribosome protective proteins (RPP) responsible for introducing conformational changes to the ribosome, which in turn leads to displacement of the antibiotic (Fyfe et al. 2016). As in the case of the previous mechanism, this type of resistance is also determined by both plasmid genes (the *tetO* gene) and those located on transposons (the *tetM* gene). In *Bacteroides* spp. in turn, the ability to synthesize flavin-dependent monooxygenase, encoded by the *tetX* gene also, located on the Tn4351 transposon, inactivates the drug. However, due to the location of this gene and the ease of its spread, its presence was noted among the *Enterobacteriaceae* (Fyfe et al. 2016).

Distinct antibiotics with similar mode of action: MLS_b antibiotics

Medicinal substances included in this group constitute a group that is extremely diverse in terms of their chemical structure, although they all have

a common mechanism of action, which also translates into a similar mechanism of resistance. These antibiotics are produced by actinomycetes of the genus *Streptomyces*. However, their spectrum of activity is different. In the case of macrolides, it includes a number of gram-positive bacteria and gram-negative cocci. As for lincosamides, they are also gram-positive bacteria (staphylococci, streptococci), as well as anaerobic non-sporulating bacteria and some protozoa. On the other hand, streptogramins are particularly effective against multi-drug-resistant strains representing mainly gram-positive bacteria. As for their chemical structure, substances classified as macrolides usually consist of 14-, 15- or 16-element lactone rings (without a nitrogen atom), conjugated with sugar residues. One of the primary and most famous representatives of this class is erythromycin, which was isolated in 1952 (Zaffiri et al. 2012). Clarithromycin and azithromycin are among the present representatives of the new generation of macrolides. Lincosamides antibiotics have a simpler chemical structure. They consist of an amino sugar (lincosamine) linked by an amide bond with L-proline. Representatives of this class are lincomycin and its derivative, clindamycin. Another group that is structurally different are streptogramins. Quinupristin (streptogramin B) is a cyclic hexadepsipeptide, and dalfopristin (streptogramin A) is a macrolactone with multiple bonds (Figure 4) (Wolstenholme and Kaplan 2012, Marosevic et al. 2017).

All these therapeutics bind to the 50S subunit of the prokaryotic ribosome, preventing further elongation of the peptide. It happens as a result of blocking the proper operation of peptidyl transferase responsible for the formation of peptide bonds between adjacent amino acids. In addition, macrolides prevent the attachment of peptidyl-tRNA at the P site and lincosamides inhibit the binding of aminoacyl-tRNA at the A site (Wolstenholme and Kaplan 2012, Kozińska and Sitkiewicz 2017). The synergistic effect of streptogramin antibiotics should also be mentioned. On the one hand, this consists in modifying the active site of the enzyme by streptogramin A and on the other hand, in hindering the translocation of tRNA with an attached polypeptide chain by streptogramin B. Ultimately, the mechanisms of both of these antibiotics result in a much stronger antibacterial effect.

Unfortunately, the problem of resistance to these therapeutics is particularly serious. One of the main mechanisms by which bacteria are insensitive to macrolides is the modification of the ribosome by adenine-N6-methyltransferase. This enzyme catalyzes the methylation of adenine, a component of 23S rRNA. As it turns out, in the case of the other two classes of antibiotics, we are dealing with a similar mechanism. This is due to the fact that the binding site of streptogramin B partially coincides with the binding site of other antibiotics. This phenomenon presents a therapeutic challenge as resistance to at least one antibiotic will often equate to resistance to other drugs, which is termed cross-resistance (MLS_b cross-resistance). The *ermB* genes responsible for this type of resistance, included in genomes of *E. coli* or *S. epidermidis*, can be located both on the chromosome and on plasmids, which is why it is transferred horizontally to other bacterial species, contributing to the increase in drug resistance. In addition to modifying the ribosome target site, many facultative pathogens

can also produce enzymes that inactivate antibiotic molecules. The literature review shows that most of the bacteria belonging to the *Enterobacteriaceae* family (*E. coli, K. pneumoniae*) have the *ereA* and *ereB* genes encoding the erythromycin lactone ring-cleaving esterases (Fyfe et al. 2016). The same gene is also found in coagulase-negative staphylococci, e.g. *S. epidermidis*, and also the *msrA* gene encoding the ATP-dependent ABC transporter, responsible for active pumping of the drug out of the bacterial cell (Fyfe et al. 2016, Szemraj et al. 2019).

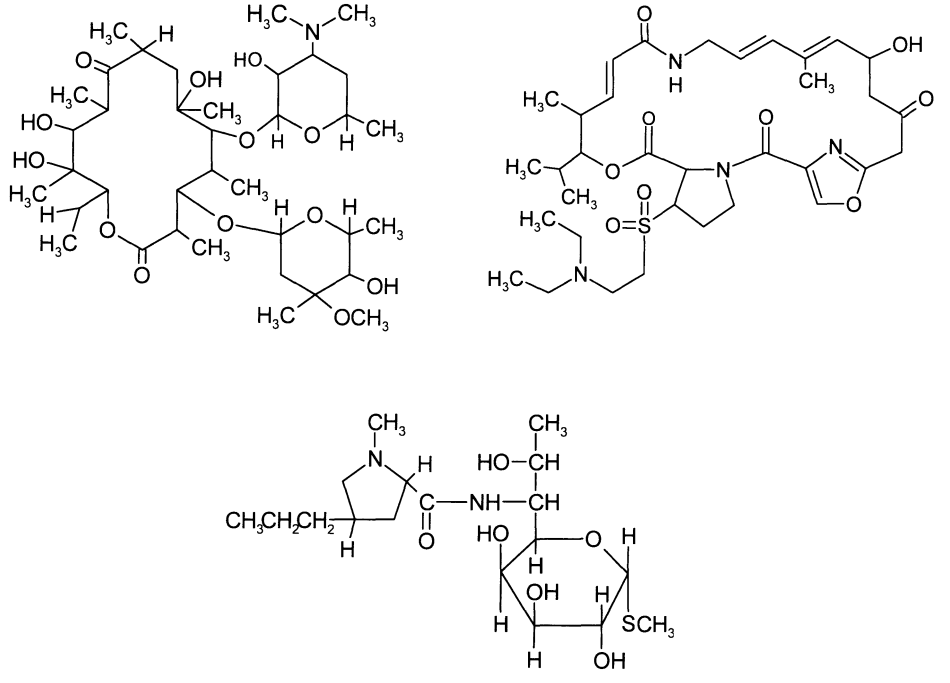

Figure 4. Chemical structure of erythromycin (a), dalfopristin (b) and lincomycin (c).

The MLS_b group of antibiotics is very commonly used in therapy. However, due to the variety of resistance mechanisms present among pathogens, all these limitations of effective therapy of infections should be considered. Moreover, even in the case of the effectiveness of treatment with MLS_b antibiotics, one should be aware of the risks associated with the selection of opportunistic bacteria resistant to these drugs in a particular patient. Such a phenomenon may cause therapeutic difficulties in subsequent infections, especially endogenous ones.

Powerful, but with important side effects: chloramphenicol

Therapeutics classified into this class are characterized by a fairly strong antibacterial effect. The beginnings of therapy with chloramphenicol date back to 1949, when chloramphenicol (also known as chloromycetin or detreomycetin), produced naturally by *Streptomyces venezueale* (Yunis 1988), was introduced into treatment. Currently, due to their ease of production, both chloramphenicol and its derivative thiamphenicol are obtained synthetically. Both of them have a wide range of action, especially against the common anaerobic bacteria, including of the genus *Bacteroides*, but also pathogenic and relatively pathogenic gram-negative and gram-positive bacteria. Recently it has been reported that chloramphenicol could induce invasion of solid tumor cells and cause mitochondrial stress (Li et al. 2010). In the chemical structure, we note two chlorine atoms in its molecule, with chloramphenicol having a nitro group on the benzene ring, and thiamphenicol having a sulfomethyl group (Figure 5).

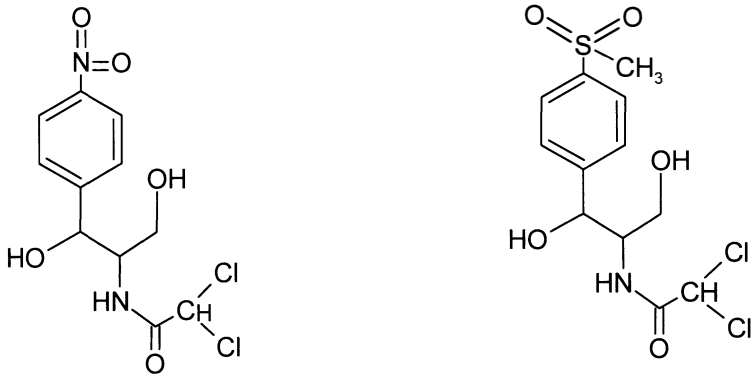

Figure 5. Chemical structure of chloramphenicol (a) and thiamphenicol (b). Figure created in ChemSketch.

The effect of both of these substances is due to their ability to attach to the 50S ribosome subunit, close to the active site of the peptidyltransferase. As a result, there is no binding of the aminoacyl-tRNA at the aminoacyl site of the ribosome. In addition, the formation of a peptide bond between amino acids is disturbed, and the resulting peptide is unable to dissociate from the translation complex, which blocks protein biosynthesis and stops bacterial cell division.

Resistance to this class of antibiotics is most often determined by the presence of chloramphenicol acetyltransferase (CAT), an enzyme responsible for translocation of acetyl residues from acetyl-CoA to the chloramphenicol hydroxyl group. The CAT enzyme is most often encoded in plasmids by the *catA* gene, although its presence was also found on transposons in *E. coli* (Potrykus and Węgrzyn, 2001). Moreover, many other gram-negative bacteria have other types of acetyltransferases encoded by plasmid genes with relatively high homology indicating their common genesis. The second mechanism of resistance

occurs in some gram-negative bacteria, including a fairly common component of the warm-blooded microbiota of the species *Pseudomonas aeruginosa* and is associated with reduced outer membrane penetration due to loss of integral protein. Unfortunately, in this case, the genes are located on transposons, which makes them very easily spread between bacteria, both typically pathogenic and opportunistic pathogens of humans and animals.

Chloramphenicol is a highly effective antibiotic, but its use is severely limited due to its serious side effects. Nowadays, apart from exceptional cases, it is used in the treatment of skin infections as an ingredient of external ointments. Unfortunately, skin infections are often caused by opportunistic species, which show a fairly high level of resistance to this drug and are a reservoir of resistance genes for other, potentially more dangerous species.

With regard to opportunistic pathogens, the phenomenon of resistance to numerous other antibiotics could be discussed, but this is beyond the scope of the present study. Therefore, our goal was to demonstrate examples of the mechanisms of resistance to selected antibiotics and to show their importance not only in terms of the most common medical terms related to obligatory pathogens, but also in relation to species whose pathogenicity is generally negligible.

Conclusions

In the era of constant stress, intense work, accompanied by an unhealthy lifestyle and the related decrease in immunity affecting a significant part of society, the phenomenon of increasing antibiotic resistance among seemingly non-pathogenic bacteria must raise concerns and increased vigilance. These microorganisms are an important element of the microbiota of every human being, and therefore they are often underestimated by us. As a rule, we do not realize that in the presence of immunodeficiency we are exposed to opportunistic infections and their treatment becomes more and more difficult, because facultative pathogens are, like obligatory pathogens, commonly resistant to antibiotics. The presence of various resistance mechanisms and the ability to transfer them horizontally due to genes placed on mobile genetic elements only increase this threat. As opportunistic bacteria are often not the target of antimicrobial therapy, their presence is not monitored during treatment, but the effect of a strong selective pressure leads to the accumulation of various resistance genes, so that in the future they may not only be a factor of etiologically difficult to treat infections, but also act as a reservoir of resistance genes from which obligatory pathogens can also draw. The more frequently described hospital and multidrug resistant opportunistic bacteria pose a huge challenge for modern medicine and epidemiology. They also require an enormous investment of time and resources to develop new, alternative treatments for bacterial infections and to search for other substances with antimicrobial effect. In addition, great care is needed for the effective treatment of diseases caused by strains resistant to certain antibiotics, to eliminate them from the human microbiota, limiting the risk of the formation of bacterial isolates that are even more difficult to eradicate,

as well as the implementation, especially in medical facilities, of the principles of proper hygiene and isolation to reduce potential contact of patients infected with different bacteria with different immune mechanisms. It is also necessary to implement new and continue existing educational and preventive programs, such as the National Antibiotic Protection Program.

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CYANOBACTERIA – THE PROBLEM OF THE MODERN WORLD

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Abstract

Cyanobacteria are a very expansive group of microorganisms, showing a number of adaptations, allowing them to live in various environmental conditions. Cyanobacteria are characterized by massive growth which manifests itself as water blooms. Blooms are stimulated by environmental factors, including high concentration of biogenic compounds, high water temperature. Blooming is a major threat because it has many negative consequences, including disturbance of the entire ecosystem and the production of toxins by cyanobacteria. Contact with cyanotoxins is extremely dangerous, both for humans and animals. Cyanotoxins can be divided according to the nature of their interaction. There are: neurotoxins, dermatotoxins, hepatotoxins and cytotoxins. Each of these groups has a different mechanism of action and causes different effects in the body. There are many possible routes of exposure to toxins. Toxins can also bioaccumulate leading to risk of consuming fish caught in bloomed water bodies. At the moment, there are no effective ways to combat water blooms or clean them of toxins. The aim of this study is to draw attention to the problem posed by mass blooms of cyanobacteria in water reservoirs.

Key words: cyanobacterial blooms, bioaccumulation, toxins.

General characteristics of cyanobacteria

Cyanobacteria are gram-negative prokaryotes with the ability to carry out the photosynthetic process. They form a very expansive group of microorganisms, occurring mainly in aquatic environments. The feature that offers a significant advantage to the growth of cyanobacterial cells, compared to other phytoplankton organisms, is the ability to perform photosynthesis at low concentrations of carbon dioxide and pH in the range of 7-9 (Błaszczyk et al. 2010). They also have a mechanism for fixing atmospheric nitrogen. Nitrogen fixation takes place in special cells called heterocytes; inside them molecular nitrogen is reduced to ammonia (Błaszczyk et al. 2010). This process takes place under anaerobic conditions with the participation of the enzyme nitrogenase. In addition, some species of cyanobacteria produce aerotopes, structures consisting of numerous cylindrical gas bubbles that allow vertical movement in the water column, thereby optimizing access to light and biogenic compounds (Błaszczyk et al. 2010). Cyanobacteria reproduce in a vegetative way, through cell division or disruption of a thread formed by a group of cells. Bloom-forming cyanobacteria

are remarkably resistant to environmental extremes. This group of organisms manages to tolerate huge changes in salinity and temperature and their ability to survive low light intensity gives them an extremely large competitive advantage in numerous environments. Due to the formation of large aggregates, ranging from 0.2 mm to 5.0 mm, cyanobacteria cannot be eaten by zooplankton, which results in a lack of natural enemies (Błaszczyk et al. 2010).

Cyanobacterial blooms

Definition of cyanobacterial blooms

Cyanobacteria are characterized by rapid and massive growth, which is called bloom. Water blooms consist of massive phytoplankton growth, accompanied by a characteristic smell, strong turbidity and intense color of the water. Blooms are commonly observed worldwide in seas and oceans, lakes and other bodies of water. In temperate climates, they occur most frequently in the summer. Furthermore, recent studies have reported that global climate change can also favor hazardous cyanobacterial species, increasing their growth rate, dominance, persistence, geographical distribution, and activity. Among the species that create blooms, we can distinguish unicellular and colony forms of picoplankton, nanoplankton and microplankton. On the basis of such features as: size, shape, and method of cell division, we distinguish five orders: *Chroococcales, Pleurocapsales, Oscillatoriales ,Nostocales, Stigonematales* with main representatives listed in Table 1.

Table 1. Examples of bloom-forming cyanobacteria.

Order	Representative of the species
Chroococcales	*Microcystis aeruginosa,* *Microcystis flos-aquae,*
Oscillatoriales	*Planktothrix agardhii,* *Planktothrix rubenscens*
Nostocales	*Nodularia spumigena,* *Dolichospermum lemmermannii*

Causes of cyanobacterial blooms

The massive growth of cyanobacteria is influenced by environmental factors, such as high nutrient content, low nitrogen to phosphorus ratio, water temperature exceeding 20 °C, intense sunlight, no wind (Rzymski 2009, Sierosławska et al. 2012). The rate of multiplication is most strongly related to the temperature and length of the day. Increasing the content of biogenic compounds in the water with a longer period of warm, sunny weather contributes to the appearance of blooms (Błaszczyk et al. 2010). High concentrations of biogenic compounds

in water have an anthropogenic source. Cultural eutrophication from domestic, industrial, and agricultural wastes as well as global climate change can play a major role in the global expansion of harmful algal blooms. Most often we are dealing here with irrational agriculture, too intensive fertilization of farmland (Rastogi et al. 2015). The water eutrophication process is also influenced by soil water erosion and the increasing emission of nitrogen oxides to the atmosphere and their increased content in rainfall. The growth and composition of blooms is also influenced by xenobiotics entering the environment, e.g. heavy metals, antibiotics and other synthetic growth regulators (Rzymski 2009).

Blooms can be divided into two types: appearing on the surface and those that occur in the deeper layers of the water column (Błaszczyk et al. 2010). Surface blooms are most often formed by the species *Microcystis spp., Dolichospermum spp., Nodularia spumigena,* while the deep-sea blooms are *Planktothrix rubenscens* and *Limnothrix redeckei* (Błaszczyk et al. 2010). Cyanobacterial blooms cause many adverse effects, including changes in the abiotic conditions in reservoirs, e.g. a decrease in the concentration of oxygen dissolved in water, a change in pH, water turbidity. This causes changes in the structure of aquatic ecosystems, the structure of the food chain, and loss of biodiversity (Sierosławska et al. 2012). There is a change in the biomass and species composition of zooplankton, which is food for pelagic fish, including herring and sprat (Pastuszak et al. 2015). Areas inhabited by zoobenthos, which are a very important part of the diet of cod, are being reduced. This leads to a reduction in the cod breeding areas. Massive development of cyanobacteria reduces the transmittance of sunlight, which deteriorates the light conditions of deeper water layers and leads to the disappearance of vegetation from the coastal zone. As a result, the plant composition of the reservoir changes (Pastuszak et al. 2015).

Cyanobacteria vs human and animal health

Cyanobacteria can interact directly with the organisms found in blooming water bodies by producing and releasing toxins (Sierosławska et al.2012). Due to the nature of the impact, toxins can be divided into four groups: neurotoxins, hepatotoxins, dermatotoxins and cytotoxins. Examples of illnesses caused by cyanobacterial toxins are presented in Table 2. Neurotoxins damage the central and peripheral nervous systems and they are the most toxic compounds produced by cyanobacteria. They interfere with the neuromuscular system, causing paralysis of respiratory muscles, and death by respiratory failure in rats after only a few minutes. Hepatotoxins impair activity of hepatocytes, leading to extensive liver injury. Dermatotoxins cause irritation and damage to the skin and mucous membranes. Cytotoxins disrupt the course of metabolic processes in cells, which leads to disturbances in a whole range of biochemical pathways (Adamski et al. 2016). Toxins most often find their way into the water during the lysis of cyanobacterial cells, which in the case of intensive blooms can cause high concentrations of them when mass cell death occurs (Adamski et al. 2016). It can happen that we do not see the characteristic features of the bloom in the

water, such as changed color, smell, but toxins are present in it anyway. Due to the fact that the toxins are odorless and colorless, it is not possible to observe them directly (Zanchett and Oliviera-Filho 2013).

Table 2. Reports of animal illnesses and deaths that serve as sentinel events for cyanobacteria-associated human health risk between 1989 and 2010. Based on Hilborn and Beasley 2015.

Location	Year	Cyanobacteria	Toxin	Animal illness	Human illness
Lake Rutland Water in Leicestershire, United Kingdom	1989	*Microcystis aeruginosa*	Microcystin	Dog and sheep deaths	Gastroenteritis, dermatitis among those who recreated in water
Zeekoevlei Lake, South Africa	1994	*Nodularia spumigena* and *Microcystis aeruginosa*	Microcystin Nodularin	Dog and livestock deaths	None reported
Pond in Mymensingh, Bangladesh	2002	*Anabaena flos-aquae* and *Microcystis aeruginosa*	Unknown	Fish and goat deaths	Rash, eye and ear irritation
River Meuse, Venlo Municipality, Netherlands	2003	Unspecified cyanobacteria	Unknown	Fish and bird deaths	Rash
Buccaneer Bay Lake, Eastern Nebraska, United States	2004	*Anabaena, Microcystis, Oscillatoria*	Microcystin	Dog, livestock, wildlife deaths	More than 50 reports of rash, skin lesions, headache and/or gastroenteritis
Lakes, Ohio, United States	2010	*Anabaena, Aphanizomeno, Planktolyngbya limnetica*	Microcystin Anatoxin-a	Dog, fish deaths, bird illness	Multiple effects including dermatologic, respiratory, neurologic illness and/or gastroenteritis

Toxins of cyanobacteria

In the Baltic Sea, the toxin producing species are *Nodularia spumigena*, *Dolichospermum lemmermannii*, and *Dolichospermum flos-aquae*. Species of the genus *Dolichospermum* are massively present in the Gulf of Gdańsk, they produce

microcystins. *Nodularia spumigena* is responsible for the production of nodularins. Both nodularins and microcystins are hepatotoxins (Trevino-Garrison et al. 2015). Microcystins and nodularins are very similar in structure and biological activity and are among the most commonly synthesized cyanobacterial toxins (Błaszczyk et al. 2010). Their toxic effects result from the strong binding of serine-threonine phosphotases in the cytosol of liver cells. This reaction inhibits the activity of phosphatases, leading to hyperphosphorylation of proteins, including intermediate filaments and microfilaments, and damage to the cytoskeleton of liver cells (Błaszczyk et al. 2010, Rzymski 2009). Moreover, microcystins and nodularins induce apoptosis and necrosis of hepatocytes, they can also be promoters of cancerous tumors (Błaszczyk et al. 2010). The characteristic symptoms of poisoning are: fever, vomiting, diarrhea, while skin exposure causes symptoms of irritation, rash or dermatitis (Rzymski 2009). Both microcystins and nodularins bioaccumulate in the food chain. Microcystins have been found in the tissues of zooplankton crustaceans, snails, crab larvae, clams and fish. Nodularins are detected in the tissues of animals intended for consumption, e.g. clams, shrimps, flounder, cod. It has also been found that cooking does not break down these toxins (Rzymski 2009). Another toxin produced by species of the genus *Dolichospermum* is anatoxin-a. In Poland, the presence of this toxin during blooms was detected in the water of the Gulf of Gdańsk (Sierosławska et al.2012). It is a neurotoxin. The main toxic effect is disturbance of the transmission of nerve impulses (Sierosławska et al. 2012). This is caused by the binding of the anatoxin-a to the nicotinic receptor, to which it has a greater affinity than acetylcholine. This leads to the opening of ion channels and depolarization of the muscle cell membrane. Acetylcholinesterase does not break down toxins, so there is a constant depolarization of the neuromuscular synapse, causing contractions (Rzymski 2009). A characteristic symptom of anatoxin-a poisoning is salivation (Błaszczyk et al. 2010). The toxin was originally called "the factor causing rapid death", because of the very vigorous effects after intraperitoneal administration of toxic cyanobacterial cells or filtrates from their cultures to mice (Sierosławska et al. 2012). Contact with the toxin caused seizures and paralysis, followed by death within minutes (Sierosławska et al. 2012).

Cyanobacteria of the genus *Dolichospermum* also produce lipopolysaccharides (LPS) classified as endotoxins, which are a component of the cell wall and occur in all cyanobacteria (Błaszczyk et al. 2010). Lipopolysaccharides are dermatotoxins and irritants (Rzymski 2009). Contact with LPS in humans and animals may cause septic shock. Other symptoms are: fever, chills, cough, sore throat (Błaszczyk et al. 2010). Moreover, LPS may cause skin and eye irritation (Rzymski 2009).

Routes of exposure to toxins

The most important route of exposure of humans and animals to cyanobacterial toxins is through water intake (Sierosławska et al. 2012). Domestic and wild animals are endangered due to drinking water directly from blooming

reservoirs and numerous cases of fatal poisoning of cattle, dogs and horses have been reported (Błaszczyk et al. 2010). In recreational reservoirs, where massive water blooms occur, there is a risk of accidental ingestion of cyanotoxin water, as well as of the cells themselves, which may contain much higher concentrations of toxins than water (Zanchett and Oliviera-Filho 2013, Sierosławska et al. 2012). There is also the possibility of intoxication by inhalation with aerosols containing cyanotoxins while swimming or practicing other water sports (Sierosławska et al. 2012, Błaszczyk et al. 2006). Another route of exposure is skin contact with contaminated water, causing skin irritation and allergies. Consuming fish and aquatic invertebrates such as clams, snails and shrimp, caught in ecosystems susceptible to cyanobacterial blooms, can also be hazardous to health (Rzymski 2009).

Bioaccumulation of toxins

Invertebrates are less susceptible to the effects of cyanotoxins, but have the ability to accumulate them in their tissues (Rzymski 2009). The literature shows the phenomenon of accumulation of microcystins and nodularins in zooplankton and benthic organisms, as well as in muscles and organs of fish (Błaszczyk et al. 2010). The accumulation of hepatotoxins in zooplankton organisms indicates that they constitute an important link in the transport of these compounds to higher levels of the food chain (Błaszczyk et al. 2010). Studies confirm the increased rate of toxin accumulation during the increase in cyanobacteria. In the case of fish, the source of cyanotoxin contamination may be their food, i.e. mollusks, smaller fish. The highest concentrations of microcystins and nodularins were detected in the liver, the organ which is the main site of hepatotoxin action. Nodularin has been identified in samples of many different Baltic fish, including: salmon, herring, stickleback, cod. The presence of hepatotoxins in the muscles of bream, zander and roach caught from the Gulf of Gdańsk has been demonstrated (Błaszczyk et al. 2010). Microcystins can also accumulate in other parts of the fish's body: gills, kidneys, gonads and the digestive tract. Some of the toxins have even been detected in the silver crucian brain, suggesting the ability to cross the blood-brain barrier. Drinking water is considered to be the main source of danger cyanobacterial toxin poisoning for humans. However, bearing in mind the increasing number of studies proving the high levels of cyanotoxins in fish, shrimp and other edible aquatic organisms, we can conclude that this is another, no less important source of risk (Błaszczyk et al. 2010).

Environmental factors of toxin synthesis

An important aspect to pay attention to is what the synthesis of toxins is dependent on. The production of cyanobacterial toxins is regulated by environmental factors. Studies have shown that the cultivation of cyanobacteria under conditions of elevated temperature promoted an increase in the amount of

produced toxins, even under conditions of nutrient limitation. Cyanobacteria that produce toxins, not only withstand various stressors in the environment, but also increase the level of toxin production in response to them. It has also been found that toxins play a role in the growth rate of cyanobacterial cells (Boopathi and Ki 2014).

Conclusions

Cyanobacterial toxins pose a real threat to human health, moreover, this threat manifests itself on many levels. For this reason, it is necessary to constantly control aquatic ecosystems for the occurrence of blooms. The blooms should be investigated, the species of cyanobacteria that compose them should be observed and the level of cyanotoxins monitored. Strategies based on physical, chemical and biological manipulation can reduce the amount of harmful cyanobacteria. However, these strategies are largely confined to relatively small ecosystems and some of them can cause ecological and environmental damage, including disturbances of plankton and benthic populations and fish habitats. Due to the lack of an effective and side-effect free method enabling the removal of cyanobacteria from water reservoirs, research should be conducted towards the development of such a method. Endangered ecosystems should be strictly excluded from recreational use and fishing for aquatic organisms. Bathing in reservoirs exposed to cyanobacterial blooms should be strictly avoided. Reclamation of degraded water reservoirs, where conditions may favor the formation of cyanobacterial blooms, is also very important. Efforts should be made to eliminate the causes of this degradation by introducing changes to the irrational agricultural economy, which is mainly responsible for the excessive introduction of nitrogen and phosphorus compounds into the waters. The ecological state of the Baltic Sea also depends on the ecological state of the natural environment in the river basins that constitute the sea basin. For this reason, the protection of the Baltic Sea must be closely related to the protection of inland waters, which in turn requires the reduction of nitrogen and phosphorus compounds pollution in the river basins. Research into cyanobacterial toxins and the role of environmental factors in their production must be continued to fully understand their environmental impact. Much field research is still needed to tackle the problem of cyanobacteria in the Baltic Sea and other bodies of water.

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CYTOGENETIC AND MEDICO-ECOLOGICAL ASSESSMENT OF INDICATORS OF THE SKIN OF DOMESTIC ANIMALS OF THE *CANIDAE* AND *FELIDAE* FAMILIES OF CENTRAL BELARUS

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Abstract

The study of the genesis of domestic animals in zoogeographic, phylogenetic and cytogenetic aspects allowed us to identify patterns of occurrence and development of various diseases and to predict their dynamics in connection with changes in the complex conditions of their habitat. It was found that among the examined group of dogs in 80,3% of cases there were benign neoplasms; in 19,7% of cases the possibility of the occurrence and development of malignant neoplasms was diagnosed. Malignant neoplasms of the skin among the examined cats were detected more often and made up 63,0% of cases (benign – 37,0%).

Key words: benign neoplasms, malignant neoplasms, habitat impact.

Introduction

The skin is the most important organ of humans and animals, serving as a protective barrier against exogenous factors: physical, chemical, and microbial pathogens (Candi et al.2005, Elias et al. 2008). The genetic basis of skin diseases is widely postulated as a risk factor for the occurrence and development of specific skin diseases in both humans and animals (Sugarman et al. 2008). Certain breeds of dogs and cats are prone to skin diseases. In this regard, for the final diagnosis, additional laboratory tests are required, including thorough pathocytohistological studies.

Materials and methods

The objects of research are biopsies from the affected areas of the skin and its derivatives, mucous membranes, lymph nodes, and neoplasms obtained from the veterinary clinic "WellVet". The subject of the research is the cytogenetic study of the analyzed cytopathological preparations.

Sampling techniques for cytological examination of skin pathologies differ depending on the site and the type of lesion analyzed. The main sampling techniques are scraping, smear imprint, fine needle biopsy with or without aspiration. A total of 515 cytological samples were obtained.

Results

1. Results of the differential diagnosis of inflammation patterns and identification of their frequency of occurrence among dogs

In the presented study, the number of cytopathological preparations of the inflammatory type was 260 samples. Septic neutrophilic inflammation is predominant and is found in 95% of pathologies. Within the framework of this inflammatory process, the main population of cells is represented by neutrophils, accounting for up to 80-95% of the total number of cells (Figure 1). As a result of the studies, it was found that the neutrophilic inflammatory type of cytological preparation is one of the most common in dogs (Table 1).

Table 1. Differential diagnosis of inflammation patterns among dogs.

Inflammation pattern	Pathology (n=)	Extensive coefficients,%
Septic neutrophilic inflammation	247	95,0
Sterile neutrophilic inflammation	1	0,4
Neutrophilic and macrophage inflammation	8	3,1
Eosinophilic inflammation	4	1,5

Staphylococcal pyoderma commonly occurs in dogs with atopic dermatitis. The adhesion of *staphylococci* to the corneocytes of these dogs is higher than to the corneocytes of healthy dogs (Olivry et.al.2015).

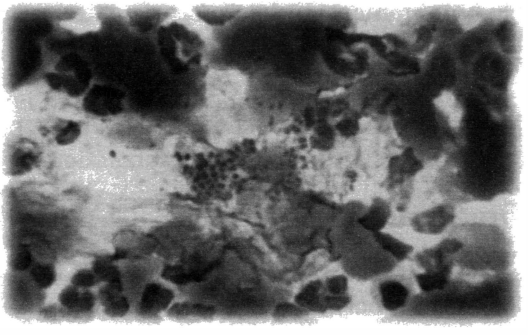

Figure 1. Septic neutrophilic inflammation. Bacterial overgrowth (cocci), Magnification X 1000

Adhesive bacteria *(Staphylococcus intermedius)* were identified using a cytological scotch test and a fingerprint smear in case of exudation on the surface of the skin lesion.

2. Results of the differential diagnosis of inflammation patterns and identification of their frequency of occurrence among cats

In the study, a total of 152 samples of inflammatory cytology of the skin and mucous membranes among cats were identified. The most common type of inflammation in cats is eosinophilic inflammation (65,1%). Table 2 presents the results of differentiation and frequency of occurrence of patterns of inflammation among cats.

Table 2. Differential diagnosis of inflammation patterns among cats.

Inflammation pattern	Pathology (n=)	Extensive coefficients,%
Septic neutrophilic inflammation	48	31,6
Sterile neutrophilic inflammation	3	2,0
Neutrophilic and macrophage inflammation	99	65,1
Eosinophilic inflammation	2	1,3

Flea allergic dermatitis and eosinophilic-granuloma complex are the most common pathologies in this cytological picture.

The eosinophilic-granuloma complex is a common diagnostic finding in veterinary dermatology.

3. Results of differential diagnosis of skin neoplasms and the revealed frequency of skin neoplasms among dogs

Due to the fact that the skin has a complex structure, a large number of different neoplasms can develop in it, including their metastases, localized in other organs and tissues. About 2/3 of all canine neoplasms are single benign neoplasms formed by epithelial cells or adnexal structures [1-3] (Table 3).

Table 3. Differential diagnosis of dog skin neoplasms.

Type of neoplasm	Pathology (n=)	Extensive coefficients,%, %
Melanocytic tumors (melanoma / melanocytoma))	3	4,0
Lymphoma	2	2,6
Histiocytoma	9	11,8
Mastocytoma	12	15,8
Lipoma	22	29,0
Cerumenous gland adenoma	2	2,6
Sebaceous adenoma	4	5,3
Keratin follicular cyst	3	4,0
Fibrosarcoma	3	4,0
Sebaceous gland hyperplasia	13	17,1
Fibroma	2	2,6
Epulis	1	1,3

According to the results of these studies, it was found that 80,3% were benign neoplasms, while in 19,7% of cases, the possibility of the onset and development of malignant neoplasms was diagnosed.

All neoplasms were surgically removed.

A case of melanoma has been reported in a Black Russian Terrier (Figure 2).

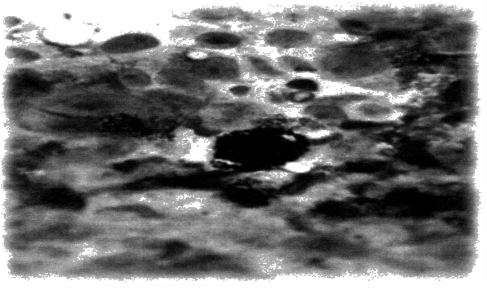

Figure 2. Melanoma. The preparation is formed of melanocytes and epithelial-like pseudogroups. Melanin is visualized in the cytoplasm and in the background of the micropreparation. Magnification X 1000.

Dogs of this breed have a genetic predisposition to this oncopathology. In dogs, cutaneous melanoma is recorded predominantly in aging animals and most often it affects breeds with intensely pigmented skin.

Canine mastocytoma is a malignant tumor that arises from the mast cells of the skin (Figure 3). The neoplasm is the most common skin tumor in dogs. Among dogs, predisposed breeds include Boxers, Boston Terriers, Labradors, Weimaraners, Beagles, Sharpei and Golden Retrievers.

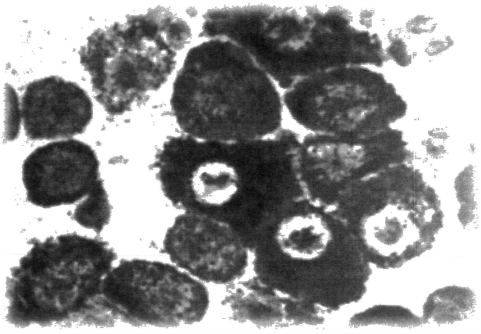

Figure 3. Highly differentiated mastocytoma. The preparation is formed of mast cells with well-visualized round-oval nuclei and purple granules, which are present in the background of the preparation. Magnification X 1000.

Lipoma in dogs is a benign neoplasia of subcutaneous adipocytes. It is most common in middle-aged or older dogs, especially Dobermans, Labradors, and Miniature Schnauzers (Figure 4).

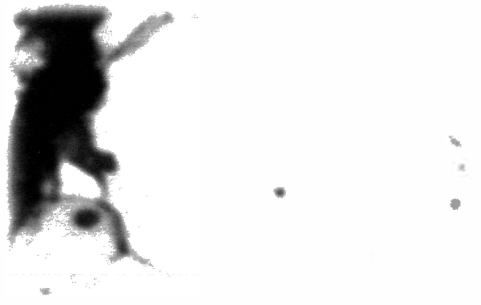

Figure 4. Group of mature adipocytes. Lipoma. Magnification X 1000.

Lipoma is common in dogs and much less common in cats.

4. Results of differential diagnosis of skin neoplasms and the revealed incidence of malignant skin neoplasms among cats

It was revealed that among cats, malignant neoplasms are recorded more often than benign ones. The etiology of neoplasms in small domestic animals remains largely unexplored. Among the known etiological factors, it is necessary to note the effect of teratogens, hormones, as well as viral infections and genetic factors.

Table 4. Differential diagnosis of feline skin neoplasms.

Type of neoplasm	Pathology (n=)	Extensive coefficients %
Lymphoma	1	3,7
Mastocytoma	12	44,4
liposarcoma	2	7,4
Cerumenous gland adenoma	1	3,7
Keratin follicular cyst	1	3,7
Fibrosarcoma	3	11,1
Sebaceous gland hyperplasia	7	25,9

As a result of the conducted cytological studies, it was revealed that malignant neoplasms of the skin among the examined cats are detected more often and account for 63.0% of all studied cases, while benign ones – 37,0%. Mastocytoma accounted for 44,4% of detected pathologies, fibrosarcoma 11,11%, liposarcoma – 7,4%, lymphoma – 3,70%, cerumenous gland adenoma – 3,7%, hyperplasia of the sebaceous glands – 25,9% of cases.

Epidermal and follicular inclusion cysts in cats are cystic structures that have epithelial walls. These lesions are uncommon in cats, with the highest incidence in middle-aged animals.

Fibrosarcoma is a malignant neoplasm that develops in the skin as well as in soft tissues (Figure 5).

There are cases when fibrosarcoma in cats can grow in the fascia, bones, ligaments, great vessels.

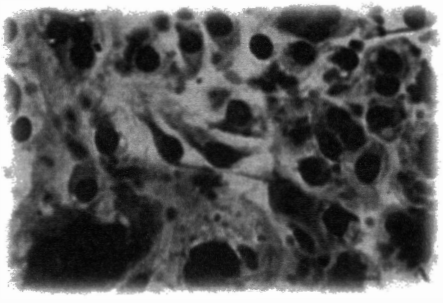

Figure 5. The preparation is formed of multinucleated cells with pronounced dysmetry of nuclei and large cells in a state of mitosis. Expressed anisocariosis. Fibrosarcoma. Magnification X 1000.

Discussion

One important aspect is the differentiation of inflammation patterns. The following inflammations are distinguished: neutrophilic inflammation, neutrophilic sterile inflammation, neutrophilic and macrophage inflammation, eosinophilic inflammation, lymphocytic and plasmacytic cell inflammation. In addition, the neutrophilic inflammatory process by its nature can be septic (purulent inflammation) or sterile. Diseases causing neutrophilic and macrophage inflammation have different etiologies, including bacterial, fungal, p rasitic, protozoal diseases, as well as storage diseases (skin calcification, xanthomatosis) and diseases caused by foreign bodies. Among the diseases causing eosinophilic inflammation, eosinophilic granuloma, eosinophilic furunculosis, sterile pustular dermatitis the most common pathology is hypersensitivity reaction to insect bites. Carrying out such differential diagnostics is extremely important in modern veterinary medicine not only in the Republic of Belarus but also in world practice.

A large number of different tumors can develop in the skin; The prognosis for these cancers varies from fav rable to unfavorable, depending on the type of neoplasm. A curate identification of tumors is necessary for the choice of optimal treatment.

In dogs, cutaneous melanoma occurs predominantly in aging animals, and most often affects breeds with intensely pigmented skin, such as Scottish Terriers. This tumor also occurs in aging cats, but they do not have a sexual or breed predisposition to this cancer. Outwardly, melanocytic tumors look like flat, pla ue-like or button-like neoplasms with a diameter of up to 2 cm, immersed in the dermis. They are usually black-colored and fairly well distinguishable. Malignant tumors can become quite large, less intensely pigmented, and often ulcerate. In cats, melanoma should be differentiated from a basal cell tumor, which is more often highly pigmented.

A number of reactive and malignant histiocytic tumors have been described in small domestic animals. In dogs, the most common diagnosis (10% of all reported cases of skin cancer) is cutaneous histiocytoma, a benign skin tumor not found in other animal species. Most often, it affects y ung animals. In typical cases, the neoplasm is localized on the head, limbs and body. It is a rapidly growing tumor immersed in the dermis. Small animal histiocytic skin tumors are actually benign and it is not uncommon for them to spontaneously disappear without any treatment. The regression of these tumors is associated with their infiltration with cytotoxic *T*-lymphocytes. In most publications devoted to the histological study of these tumors, experts note the presence of lymphocytic infiltrates in them. Epithelial lymphoma is more common in dogs. During histological examination in thin sections of this tumor, diffuse infiltration of the epidermis by neoplastic lymphocytes and other inflammatory cells, microabscesses and tropism to the accessory structures of the skin are noted. As the tumor develops, the transformed cells penetrate into the deep layers of the skin (Fontaine et al.2009).

Tumors arise when exogenous factors interact with the genetic apparatus of the cell. Expressed geographical differences in morbidity, as well as a direct relationship with individual risk factors, indicate the possibility of avoiding the occurrence of a tumor in some cases.

From the standpoint of cell biology, tumor transformation is the result of a gradual accumulation of genetic disorders in cells, which can also be genetically determined, affecting various regulatory mechanisms.

Conclusions

1. Through applied cytodiagnostics, the frequency of occurrence of inflammatory patterns was revealed and their etiology was determined among dogs. The most common pattern among dogs – septic neutrophilic inflammation – was 95%, sterile neutrophilic inflammation-0,38%, neutrophilic and macrophage inflammation-3.08%, eosinophilic inflammation-1,54%. Dermatitis associated with overgrowth of yeast fungi of the genus *Malassezia*, resulting in septic neutrophilic inflammation, was diagnosed in two dachshunds. Dachshunds have a genetic predisposition to this pathology. In 5 dogs of the Jack Russell Terrier breed, a clinically verified diagnosis of atopic dermatitis was made, which is genetically determined in this breed.

2. By means of diagnostics using methods of cytological studies, the frequency of occurrence of patterns of inflammation was revealed and the etiology among cats was determined. The most common pattern among cats – eosinophilic inflammation – accounted for 65,1%, lymphocytic-plasmacytic inflammation – 1,3%, neutrophilic and macrophage inflammation – 2,0%, septic neutrophilic inflammation – 31,6%. No connection was found between the occurrence of patterns of inflammation and genetic predisposition.

3. Through cytological studies, it was found that among malignant neoplasms in dogs, the most common skin neoplasm is a mastocytoma -15,79%, among benign ones, the most common is a lipoma – 28,95%. Histiocytoma was 11,84%, lymphoma-2,63%, fibrosarcoma-3,95%, fibroma-2,63%, keratin follicular cyst-3,95%, sebaceous gland hyperplasia-17,10%, sebaceous gland adenoma-5,26%, cerumen gland adenoma-2,63%, epulis-1,32%, melanoma-3,95%. A case of melanoma was reported in a Russian black terrier. Dogs of this breed have a genetic predisposition to this oncopathology. Differentiation of malignant neoplasms from benign ones made it possible to choose the necessary protocol for subsequent therapy in a timely and accurate manner.

4. Through cytological studies, it was revealed that malignant skin neoplasms among the examined cats are detected more often and make up 62,97% (benign 37,03%). Mastocytoma was 44,44%, fibrosarcoma 11,11%, liposarcoma-7,42%, lymphoma-3,70%, adenoma of the cerumen gland-3,70%, hyperplasia of the sebaceous glands-25,93%.

5. The impact of environmental factors on the body is cumulative and subsequently causes the development of structural abnormalities in somatic cells.

The role of environmental factors in the development of oncopathologies was considered as a result of the analysis of epidemiological studies.

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VERTICAL AND HORIZONTAL CHANGES IN THE ABUNDANCE AND ACTIVITY OF BACTERIA IN LAKE HAŃCZA, NE POLAND

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Abstract

A bacteriological study was conducted in late summer (early September 2018) in the deepest postglacial reservoir in the central lowland depression – Lake Hańcza (northeastern Poland). The aim of the study was to determine the spatial variation in the density of planktonic bacteria, the level of their hydrolytic activity and the ratio of live to dead bacterial cells. During the fieldwork 92 water samples were collected from thirteen stratified sites located in different parts of the lake. The bacterial number ranged from 0.57 to 3.97 (10^6 cells ml^{-1}). No horizontal or vertical changes in bacterial number were observed in a uniform pattern. The highest bacterial abundance was recorded at two coastal sampling points, both located in the southern part of the lake at the direct catchment area with commercial development ("agroturism" guesthouses). There was little difference in bacterial number across the horizontal gradient ($F = 1.865$; $p = 0.170$). Many water quality parameters appeared to be significant factors correlating with bacterial abundance in Lake Hańcza. There were no statistically significant differences in hydrolitical bacterial activity and live/dead ratios between investigated sites and across horizontal gradient. Our results indicate that bacterial activity in deep lakes is affected by many water quality parameters, such as temperature (r=0.667), Ca^{2+} (r=-0.4510), Mg^{2+}(r=0.511), NO_3^- (r=-0.587) and oxygen saturation (r=0.582).). Taking into account previous hydrobiological studies, Lake Hańcza was found to be very patchy in terms of bacterioplankton abundance.

Key words: bacterioplankton, hydrolytic activity, spatial variation

Introduction

Lake Hańcza (LH) is considered to be a homogeneous natural water reservoir due to its structure – the shape of the lake basin and shoreline of little variety. Despite the increasing anthropopressure in the catchment area, the lake still maintains its water quality level. It retains a fairly natural character and low trophy. Differences in environmental conditions in the presence of human activity in various parts of the lake can lead to significant changes of water quality across the reservoir. It is usually assumed that those differences are negligible for small, shallow lakes with a more compact shape and greater stability of water masses (Łopata et al. 2014). In the case of large lakes with an extensive

shoreline, the differences in water quality in individual parts of the reservoir are very well documented, e.g. as recorded for Lake Wigry (Górniak and Zieliński 2006). However, even lakes such as LH, with poorly differentiated lake basin morphology, may also have significant spatial variation in water quality. This may be influenced by the hydrological character of the lake, through which the Czarna Hańcza River flows. The distribution and activity of bacteria may be a good indicator of spatial variation in water microbiological quality. According to Bergström and Jansson (2000) the contribution of allochthonous bacteria can account for up to 70% of the internal bacterioplankton production in the lake's epilimnion. Since there is such a high proportion of these allochthonous bacteria, there is a differential distribution of bacterioplankton in the epilimnion related to organic matter availability (Lindström and Bergström 2004).

Bacteria play a substantial role in shaping water quality through cycling of organic and inorganic matter of freshwater ecosystems (Chróst and Siuda 2006). The rate of this circulation depends on chemical, physical and biological factors. Recent studies show that bacterioplankton can also be an indicator of the trophic state of a lake in relation to anthropogenic factors and their influence on water quality (Zieliński et al. 2006). LH is well investigated in terms of zooplankton (Jekatierynczuk-Rudczyk et. al 2014; Karpowicz and Ejsmont-Karabin 2017; Ejsmont-Karabin et al. 2020), phytoplankton (Napiórkowska-Krzebietke and Hutorowicz 2013; Karpowicz et al. 2016) and bacterioplankton (Niewolak and Gotkowska-Płachta 1999; Gotkowska-Płachta et al. 2003; Zieliński et al. 2006; Gotkowska-Płachta 2008), hydrochemistry (Hillbricht-Ilkowska and Wiśniewski 1993; Pyka et al. 2007), macrophytes (RIEP 2017) and fish (Brylińska et al. 2002, Kotusz et al. 2004; Dziekońska-Rynko et al. 2018).

The aim of the study was to determine the spatial variation in the occurrence of planktonic bacteria density, the level of their hydrolytic activity and the ratio of live to dead bacterial cells, which was crucial for establishing whether LH is homogeneous in terms of microbiological and hydrochemical water quality on the vertical and horizontal scale.

Materials and methods

LH, commonly regarded as a model mesotrophic lake, is the deepest water reservoir not only in Poland, but in the European Lowlands, with depth reaching 105.6 m (Borowiak et al. 2020). The surface area of the lake is 303.6 ha and the volume of the lake is 0.1186 km^3 (Popielarczyk and Templin 2014). LH is a part of the East Suwalskie Lake District and it is a flow-through lake, with the Czarna Hańcza River (CHR) flowing through it. CHR is a tributary of the Nemen River and is characterised in its upper section by a very natural, meandering riverbed (Kołodziejczyk 1999). The main factor in the exchange of lake water, which takes about 144 years, is rainwater and water from surface runoff (Mitręga et al. 1993). The lake lies within the Suwałki Landscape Park. It has been protected in its entirety, along with its shores, as the Lake Hańcza Natural Reserve, since 1963. It is a unique body of water, located in a post-glacial trough,

the catchment area of which is mostly occupied by agricultural and agrotourism areas. The rest of the catchment area consists mainly of forests (Figure 1).

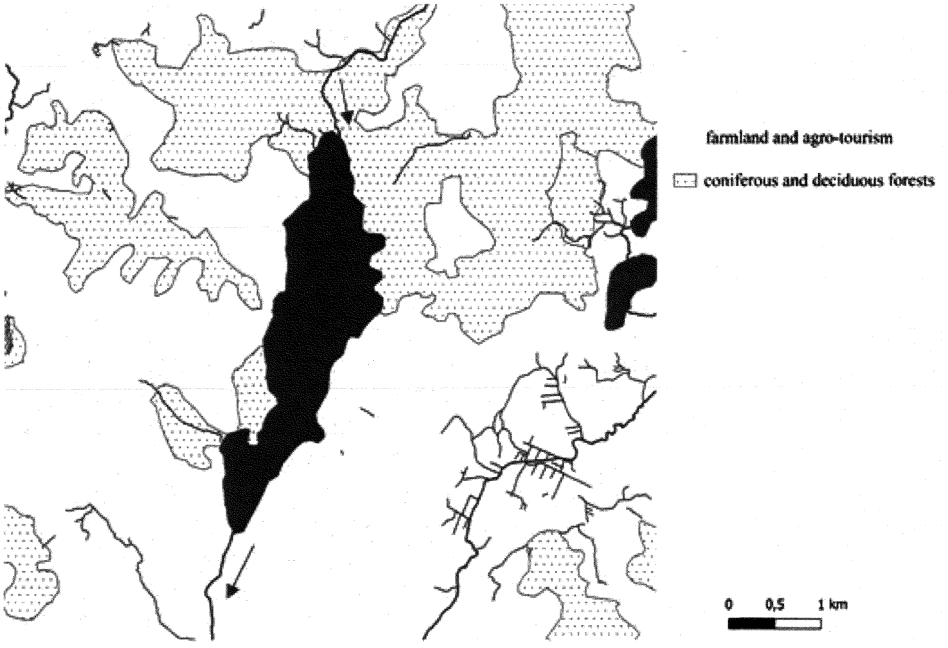

Figure 1. Management map of the Lake Hańcza and its direct catchment.

Considered one of the cleanest lakes in Poland (Gotkowska-Płachta et al. 2003), due to its unique structure and depth, it has been listed as a UNESCO Aqua Project since 1974. It is estimated that LH, with a maximum length of 4.5 km, a width of 1.2 km and a total shoreline length of 12.6 km, was created during the Late Weichselianglacier outburst floods in North-Eastern Poland around 15-17 thousands years ago (Weckwerth et al. 2019).

It is assumed that the oldest rocks forming the deep bedrock surrounding LH are the highly fractured and fault-cut Precambrian anorthosite and norite rocks of the so-called Suwałki Anorthosite Massif. The sediments shaping the surface of LH's surroundings are Quaternary formations reaching the thickness of up to 281 m and differentiated into glacial, fluvioglacial and limnoglacial sediments and form an organic sediment of the Eemian interglacial. The surface formations are represented mainly by peats, silts and sands of river terraces and bottoms, lacustrine organic and mineral sediments (gyttjas), as well as sands and deluvial clays. Along the lake shores there are accumulations of boulders constituting a layer often exceeding 2 meters (Pochocka-Szwarc et al. 2013).

In our study, all water samples were collected on one day during the summer stagnation period (September 2018), using a Ruttner apparatus, from 13 sites designated for the deep lake sites (no 1-3) and the coastal sites (no 4-13), according to the bathymetric map (Figure 2). In this study, sampling points were

delineated taking into account the lake's direct catchment area, including the community beach (station 4).

A total of 92 samples was obtained, 62 of which were collected at deep lake sites, every meter from a depth of one meter to 15 meters and then at 10 meter intervals to the bottom. Water transparency level was measured at these sites using a Secchi disk. The remaining 30 samples were collected at coastal sites from the three stratification layers of the studied lake. Water for microbiological analysis from each site was collected into sterile containers.

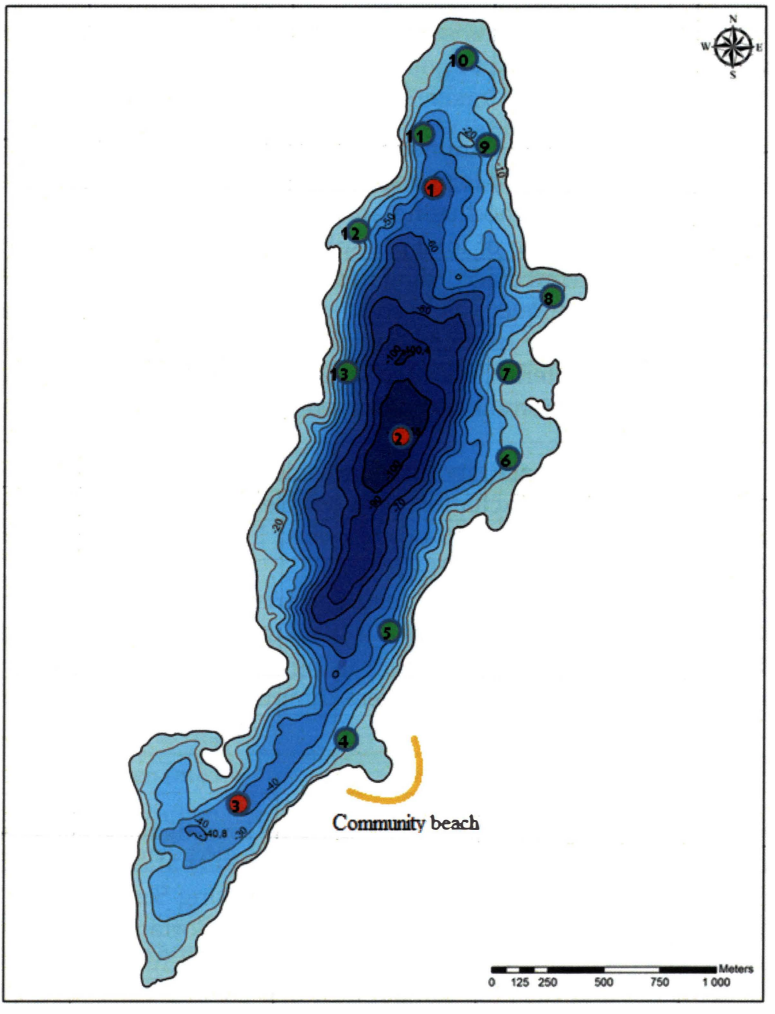

Figure 2. Map of the location of research sites (red circles – *deep lake sites*; green circles – *coastal sites*) on the background of the bathymetric map of Lake Hańcza (*based on:* Popielarczyk and Templin 2014).

Directly in the field, in-situ measurements were performed with the Hach-Lange multi-parameter probe (HQ-40) taking into account: water temperature,

electrolytic conductivity, pH and dissolved oxygen concentration and oxygen saturation. The main hydrochemical parameters were quantified using standard procedures as described by WEF and APHA (2005) approximately 24 hours after collection to ensure that the sample did not deteriorate significantly during storage. Dissolved organic carbon (DOC) and total nitrogen (TN) were determined in the laboratory using a high temperature combustion method with the Shimadzu TOC-L analyser. Specific UV absorbance (SUVA) was used to evaluate the aromaticity of dissolved organic matter. UV absorbance normalized to the DOC concentration of the sample was computed using the formula:

$$SUVA = \frac{Abs_{254} \times 1000}{DOC}$$

(Chin et al., 1994, modified). Parameters such as different nitrogen (NH_4^+ and NO_3^-) and phosphorus (PO_4^{3-}) ions and content of Ca^{2+} and Mg^{2+} were determined using the HPLC method using an ion chromatograph Thermo Dionex ICS-1100. The determination of total phosphorus has been made with the molybdenum method measured according to standard methods (APHA 1998).

Epifluorescence technique was used to determine the total bacterial number (BN) after staining the bacterial cells with 4′,6-diamidino-2-phenylindole (DAPI). To count the bacteria deposited on 0.2 μm filters, they were placed on basal slides with immersion oil and observed using an epifluorescence microscope (Olympus BX43).

The bacteria hydrolytic activity (BHA) was determined using fluorescein diacetate and analysed at an excitation light wavelength of 480 nm and emission of 505 nm with the modified method described by Adam and Duncan (2001) following Burkowska-But et al. (2014).

To determine the ratio of live to dead bacteria (LD), samples were stained with LIVE/DEAD® Bacterial Viability Kit (BacLight™) according to the manufacturer's instructions and then counted using an epifluorescence microscope (Olympus BX43). The final LD values were computed using the formula:

$$Ratio_{G/R} = \frac{F\ cell,em1}{F\ cell,em2}$$

Various statistical models have been used to investigate relationships between BN, BHA, LD and the hydrochemical parameters at each site and at different depths. Statistical analyses were performed using Statistica ver. 13.3 for analysing the level of statistical significance with importance level $p < 0.005$. The standardised coefficients of variation (CV = [SD / m] 100%, where SD is the standard deviation and m is the sample mean), were also calculated and used for comparison of bacterial and hydrochemical parameters from all collected samples. To determine if there was a significant difference between studied parameters, the Kruskal-Wallis test was used. Spearman's rank correlation analysis, with the level of statistical significance of $p < 0.05$ and $p < 0.01$, was used to show the relationship between hydrochemical and bacteriological parameters.

Results

The highest visibility level measured with a Secchi disk for LH during the sampling period was 4.2 m. The average temperature of the lake was 12 °C, the average temperature of surface water was 17.7 °C, electrical conductivity 266 μS and pH 7.5. The nitrate extreme values ranged from 0.001 mgN l^{-1} to 0.27 mgN l^{-1} and both of them were detected at sampling site No 3. The ammonium value was the lowest at 6 meters of the first sampling site – 0.0002 mgN l^{-1}, the highest in the thermocline of site No 9 – 0.31 mgN l^{-1}. Soluble reactive phosphorus (PO_4^{3-}) ranged from 0.0002 mgP l^{-1} in metalimnion at No 7 sampling site to 0.02 mg l^{-1} in surface water from the first sampling site – where the dissolved organic carbon was also the highest – 7.14 mg l^{-1}. Average total phosphorus was 0.05 mg l^{-1} and the average total nitrogen – 0.88 mg l^{-1} (Table 1).

Table 1. The hydrochemical characteristic of Lake Hańcza – mean values for all samples (September 2018).

Parameter	Mean value	Standard deviation	Min -Max
	n=92		
Temperature (°C)	12	5.9	4 – 20
Electrical conductivity (μS cm^{-1})	266	5.4	254 – 279
pH	7.5	0.42	6.45 – 8.37
Oxygen saturation	102.8	13.8	67.7 – 119.8
Ca^{2+} (mg l^{-1})	42.49	3.38	28.17 – 47.55
Mg^{2+} (mg l^{-1})	8.55	0.35	8.54 – 8.66
NO_3^- (mgN l^{-1})	0.1	0.11	0.001 – 0.27
NH_4^+ (mgN l^{-1})	0.02	0.05	0.00002 – 0.31
PO_4^{3-} (mgP l^{-1})	0.003	0.003	0.0002 – 0.02
Total phosphorus (mg l^{-1})	0.05	0.04	0.0001 – 0.17
Total nitrogen (mg l^{-1})	0.88	0.15	0.64 – 1.24
Dissolved organic carbon (mg l^{-1})	5.69	0.42	4.98 – 7.14
Specific UV absorbance (Abs_{254} gC^{-1})	20.4	3.02	14.8 – 28.8

On average, the highest BN was recorded in the epilimnion layer – 2.38 10^6 cells ml^{-1} in coastal sampling sites and 2.34 10^6 cells ml^{-1} in deep lake ones. The lowest average BN value was recorded at site 1 where it was 1.33 10^6 cells ml^{-1}, and the highest at site 4 with the count of 3.08 10^6 cells ml^{-1} (Figure 3).

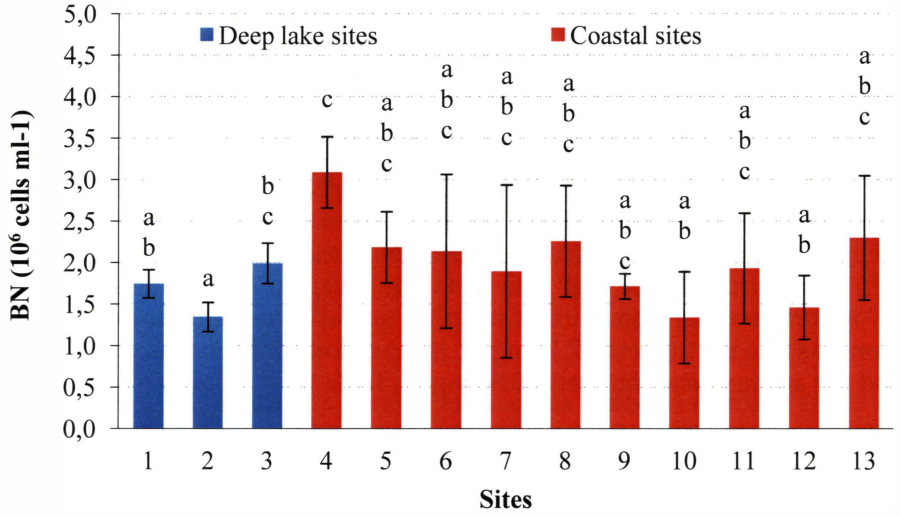

Figure 3. The average bacterial number (BN) (±SD) of 13 sampling sites in Lake Hańcza. Letters indicate statistically significant differences.

The vertical and horizontal changes of BN in LH ranged from 0.57 10^6 cells ml^{-1} at a depth of 14 meters at one of the central sites to 3.97 10^6 cells ml^{-1} at depths of 1 and 15 meters of the nearshore site designated as site 6 (Figure 4).

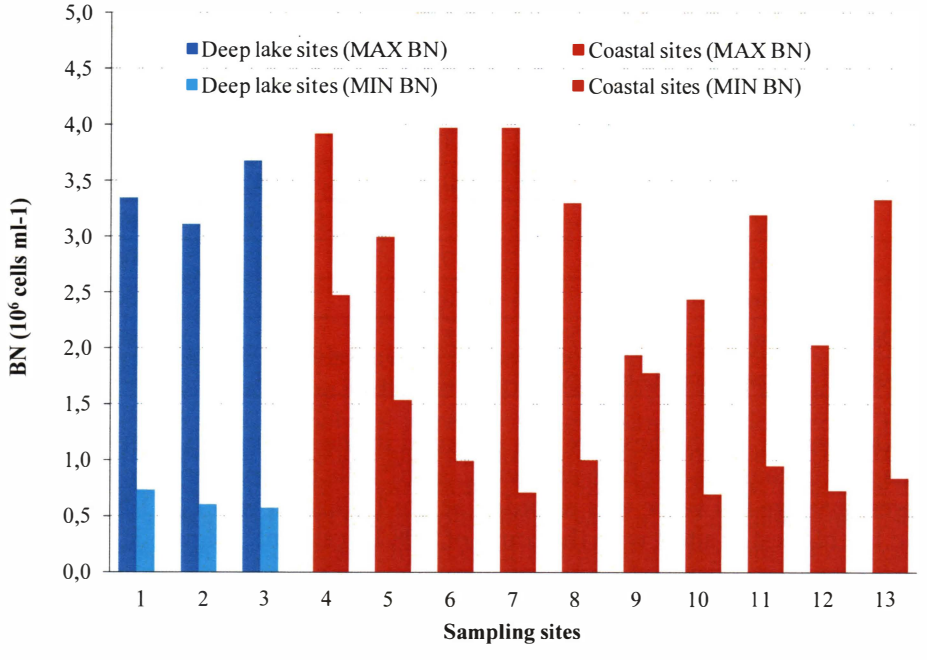

Figure 4. The extreme values (Min, Max) of bacterial number (BN) of all investigated sampling sites in Lake Hańcza.

The average abundance of bacteria in LH was 1.66 10^6 cells ml^{-1} for deep lake sites and 2.02 10^6 cells ml^{-1} for the coastal sites at the end of the growing season (September 2018) (Figure 5).

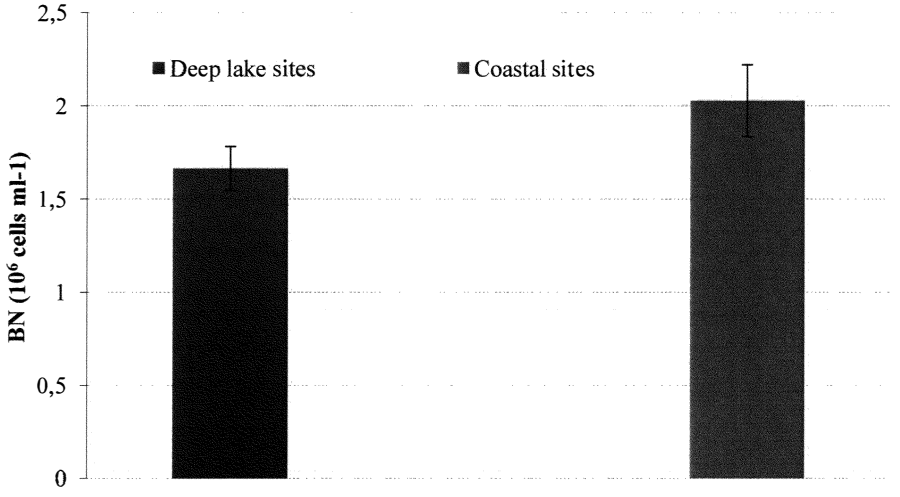

Figure 5. Average bacterial number (± SD) on deep lake sites and coastal sites in Lake Hańcza.

The most statistically significant differences in BN were observed between shore sampling point No 4 and deep lake sites: 1 and 2 as well as north eastern sites, near the forested shore: 10 and 12 ($p<0.005$). Mean bacterial abundance varied considerably among the strata of the deep lake stations. The coastal sites were less diverse in mean BN values. In both cases, the highest BN was recorded in the epilimnion layer, but the lowest number of bacteria of deep lake stations was in the hypolimnion layer, in contrast to coastal stations – metalimnion. The greatest difference in mean BN was noted in the hypolimnion at 1.05 10^6 cells ml^{-1} for deep lake stations and 1.9 10^6 cells ml^{-1} for coastal stations ($t = 3.8036$ $p=0.0005$) (Figure 6).

The highest bacterial number at the deep lake sites was recorded at site No 3 in the epilimnion – 3.7 10^6 cells ml^{-1}. Each deep site was characterized by large BN fluctuations both in the epilimnion and in the metalimnion. Only after the thermocline passes, in the hypolimnion, the bacterial abundance decreased significantly, fluctuating between 0.7 and 1.8 10^6 cells ml^{-1} (Figure 7).

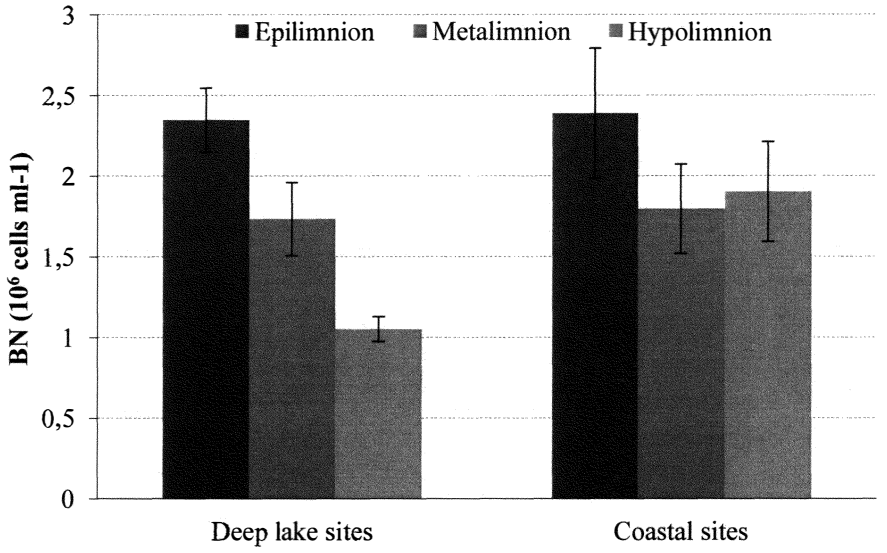

Figure 6. Average bacterial number (BN; ± SD) in different stratification layers on deep lake and coastal sites of Lake Hańcza.

The BN reduces in hypolimnion by up to three times, compared to epi- and metalimnion. The biggest differences are between site 1, closest to the inflow, and site 3, closest to the outflow. The greatest rise of BN at each of the deep lake sites was recorded in the metalimnion (Figure 7).

One of the most varied factors examined in this study was BHA, averaging 813 µgFl. $ml^{-1} h^{-1}$ for the entire lake (CV=31.2%). The lowest average BHA in LH was found at site No 2 – the deepest sampling point – 648 µgFl. $ml^{-1}h^{-1}$ and that was also the station with the lowest hydrolytic activity in all samples tested, with a value of 239 µgFl. $ml^{-1} h^{-1}$. The highest average value of all the coastal sampling sites was in the north-eastern part of the lake (No 9) with a rate of 1004 µgFl. $ml^{-1} h^{-1}$. The most significant differences were found between the deepest sampling site and coastal sites numbered 8, 9, 11 and 12 (Figure 8), located on both sides of the northern part of the lake.

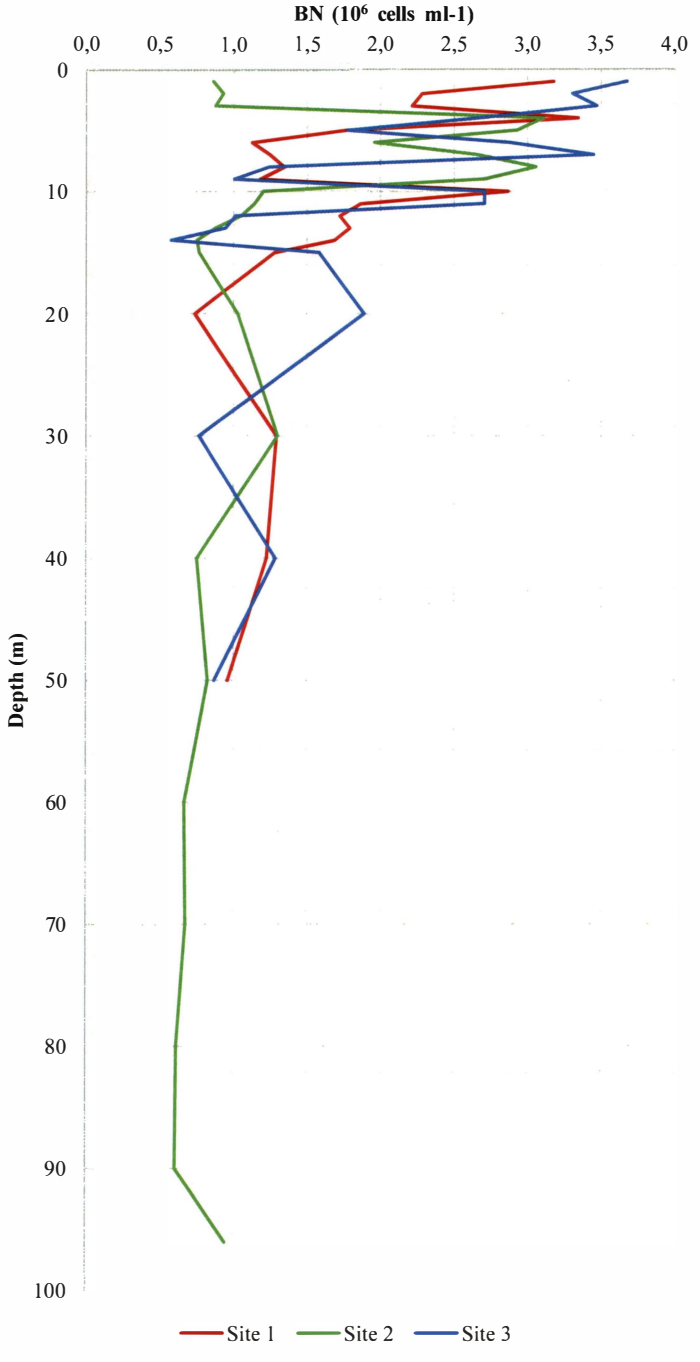

Figure 7. Vertical changes of bacterial number in three deep lake sites of Lake Hańcza.

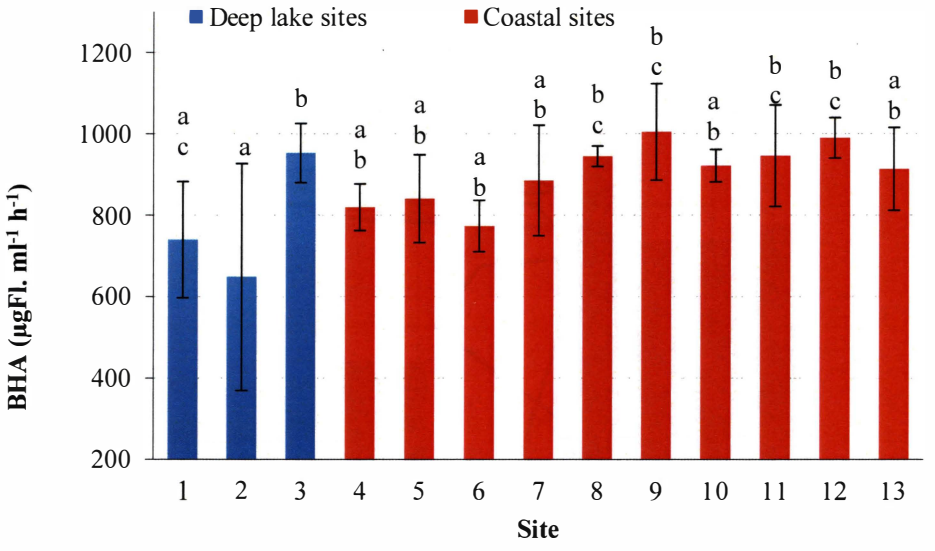

Figure 8. The average bacterial hydrolytic activity (± SD) in Lake Hańcza at each of 13 investigated sites.

There was also a significant difference in BHA between the most southern deep lake site located nearest to the lake outflow and both other deep lake sampling sites. The two deep lake sites furthest from the lake centre (No 1, 3) also displayed the most significant differences in BHA between stratification layers of the lake (Figure 9).

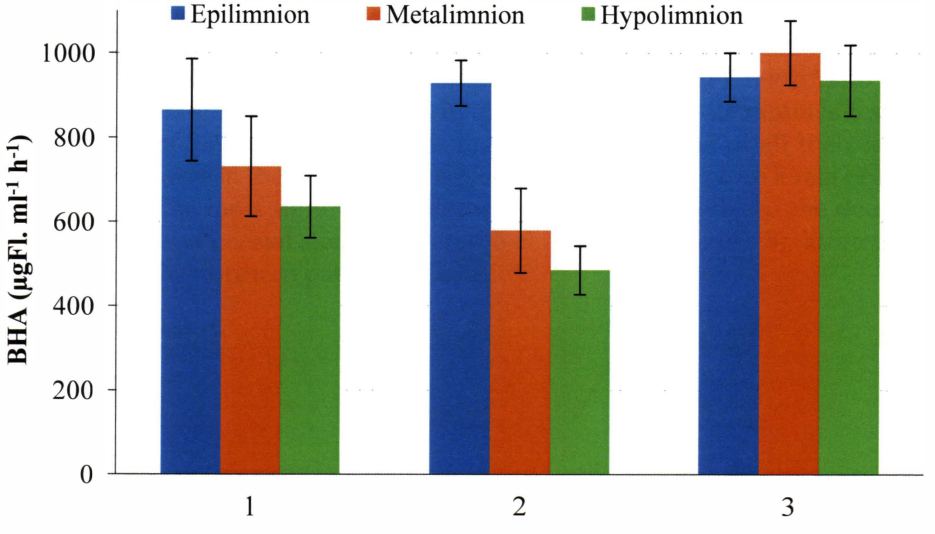

Figure 9. The mean bacterial hydrolytic activity (± SD) in the different thermal layers of Lake Hańcza measured in deep lake sites 1, 2 and 3.

The highest average live and dead bacterial cells ratio was noticed at north-eastern forested coastal sites (No 6) with a value of 0.081, while the lowest at station No 1, which is the deep lake sampling site closest to the inflow of river Czarna Hańcza, with a value of 0.064. There was also a substantial difference be een the two furthest deep lake si es numbered 1 and 3. The study showed no statistically significant differences in the ratio of L/D within the studied lake.

Discussion

The hydrochemical studies carried out in this work (Tab. 1) confirm the low trophic state of the lake. LH, being a flow-through lake of the greatest depth in Europe (105.6 m) (Borowiak et al. 2020), should be characterised by a low trophic level – currently mesotrophic (Karpowicz and Kalinowska 2018). In comparison with the results conducted by Jekatierynczuk-Rudczyk et al. (2014), the TSI_{SD} value was very similar at 39.2, while the TSI_{TP} value was more than slightly lower and reached a value of 60.6. LH is often considered to be very homogeneous due to its depth, the shape of the lake and the little diversified shoreline, which is confirmed by earlier studies on zooplankton (Jekatierynczuk-Rudczyk et. al 2014; Ejsmont-Karabin et al. 2020), phytoplankton (Napiórkowska-Krzebietke et al. 2012; Karpowicz and Ejsmont-Karabin 2017) and bacteria (Niewolak and Gotkowska-Płachta 1999; Zieliński et al. 2006; Gotkowska-Płachta 2008;).

However even lake Hańcza, protected as a reserve and part of the Suwałki Landscape Park, has noticeable human influence. Studies conducted by Gotkowska-Płachta et al. (2005) show that the amount of bacteria in LH is increasing, which may indicate intensification of allochtonic factors, eg. enrichment with organic matter or biogenic substances. Even though our studies clearly classify LH as mesotrophic, even if phosphorus level is relatively high, which is common in lakes of NE Poland (Hillbricht-Ilkowska 1993; Hillbricht-Ilkowska and Kostrzewska-Szlakowska 1996; Zdanowski 2003).

Bacterioplankton can be considered a useful factor showing the horizontal and vertical variation of microbiological water quality. The discussed studies indicate that LH has a diverse distribution of bacteria both vertically and horizontally, ranging from 0.57 10^6 cells ml^{-1} to 3.97 10^6 cells ml^{-1} for all the lake samples with coefficient of variation (CV = 54.67%). The average BN in LH was 1,66 10^6 cells ml^{-1} for deep lake sites and 2.02 10^6 cells ml^{-1} for coastal ones, which is respectively 53.4% and 43.2% less than in studies conducted ea lier (Zieliński et al. 2006). This may possibly be due to seasonality – sampling took place in a significantly colder period of the summer, but still shows the same pattern of decreasing the amount of bacterioplankton with depth. Compared to other oligo-mesotrophic lakes like Mamry or Przystań of the Masurian Lake District, Lake Hańcza has noticeably lower average BN (Górniak and Świątecki 2007).

The most of the statistically significant differences in horizontal distribution of BN were observed between deep lake sites (1, 2, 3) and shore sites located in the north eastern part of the lake ($p<0.005$) (Figure 3). While the maximum BN values do not differ much, the values in deep lake sites were less variable.

The minimum values are significantly lower on deep lake sites and much less variable than coastal sites, as confirmed by the CVs, for which the values are 12.2% and 49.8% respectively. It is possible that smaller values of BN in the northern part of LH are due to the inflow of the Czarna Hańcza River. This may be due to the character of the direct catchment area of the investigated lake, which is covered with wetlands. The statistically significant differences in average BN occur at the forested shoreline and the station located near the community beach (Figure 2). Statistically significant differences in BN were found between deep and coastal sites (Figure 4), which confirms the spatial patchiness of bacterioplankton.

The average abundance of bacteria in LH was lower at deep lake sites with an abundance of 1.66 10^6 cells ml^{-1} and 2.02 10^6 cells ml^{-1} for the coastal sites (Figure 5). In comparison of stratification layers of both deep and coastal lake sites, the highest value was recorded in the epilimnion layer. The difference of BN between types of sites was visible in comparison of metalimnion – lowest for coastal sites – and hypolimnion – lowest for deep lake ones (Figure 6). Differences in bacterial abundance in stratification layers between different types of sites show that Lake Hańcza is not homogenous in terms of spatial distribution of bacterioplankton.

The thermocline depth is known to be an important factor for bacterioplankton structuring in aquatic ecosystems and it is natural for the BN to be highest there (Mieczan 2008, Gotkowska – Płachta et al. 2003). The overall distribution of bacterioplankton differs a lot between station 1 and 3 (Figure 7), the epilimnion layer has a higher BN in epilimnion, probably due to the nearer outflow. Vertical changes in BN at the deep sites of LH are comparable to those in other lakes of similar trophy (Mieczan 2008). It is likely that the hypolimnion BN difference effect is due to the influence of bottom sediments in shore sites with relatively higher temperature. Research by Bajkiewicz-Grabowska (2004) shows that LH is heavily fed by groundwater and it is possible that the movement of groundwater may influence the bacterial number derived from the external environment, or from bottom sediments located near the water supplying sites.

Differences were also found in the hydrolytic activity of bacteria in LH. The most significant differences in BHA were found between the deepest sampling site No 2 and coastal sites located on both sides of the north part of the lake (nr 8, 9, 11 and 12), where the BHA was the highest (Figure 9). All mentioned sites are located in the part of the lake with river inflow (Figure 1). It can be assumed that wetlands are an important source of metabolically active bacteria from the direct Czarna Hańcza River catchment during the full growing season, although the study was conducted at the end of the growing season. Unfortunately, no correlations were found between the type of management of the direct catchment area and the BHA. Perhaps this type of analysis requires further study in full summer and with denser shoreline sampling sites. We assume that the forested catchment may be the main source of active bacteria for the northern part of the lake. Statistically significant differences in BHA indicate vertical differentiation of the BHA even between deep lake sites (Figure 8) and may be caused by the close outflow location of the third sampling station, which

showed very little BHA differences between the stratification layers, as opposed to the other two deep lake sites. The BHA is a very important indicator and is used to assess the condition of bacterioplankton and distinguish water types (Zieliński et al. 2020).

The dependence of investigated bacterial parameters on water quality was confirmed in the study. The development of bacteria in LH is influenced by different hydrochemical factors at deep and coastal lake sites. For the first type, the most important parameters influencing BN were temperature (r=0.623), oxygen saturation (r=0.588) and concentration of Ca^{2+} (r=-0.607), NO_3^- (r=-0.539) as well as dissolved organic carbon (r=0.534). There were no significant correlations at the coastal sites between BN and hydrochemical parameters, as confirmed by the highly variable habitat of bacteria assemblages in coastal zones, while the deep lake sites are more stable. Some other correlations were also noted for BHA at deep lake stations: temperature (r=0.667), oxygen saturation (r=0.582) Ca^{2+} (r=-0.451), Mg^{2+}(r=0.511), NO_3^- (r=-0.587). BHA at coastal sites showed correlations with temperature (r=0.545), oxygen saturation (r=0.520) and specific UV absorbance (r=-0.556). The negative correlation found between BHA and SUVA confirms the great importance of organic matter quality in the rapid growth of bacteria in the aquatic environment. Both BN and BHA at deep lake sites were correlating negatively with Ca^{2+}, which indicates that the hardness of the water is not conducive to bacterial growth. Maybe it is the precipitate – bacteria may sink to the bottom with it. We assume that the alkalising effect may be caused by the groundwater phase of water circulation.

Most of the bacteria were metabolically inactive and the proportion of live bacteria did not exceed 8%, averaging 6.79%. That might be caused by nitrogen and phosphorus deficits, which may result in a low proportion of viable bacteria (Chróst et al. 2009). There were no significant differences in spatial L/D ratio distribution in LH.

Although the lake is considered to be very homogeneous, the study does not confirm this and indicates that the water quality, including the microbiological one, varies considerably – especially between the central part of the lake and its shores, but also between the northern and southern parts. The conducted study shows many differences in both vertical and horizontal changes of BN as well as BHA. As a result of the study it was established that LH is heterogeneous in terms of hydrochemical water quality and microbiological quality.

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IMPACT OF WATER QUALITY ON SUMMER BACTERIOPLANKTON IN SPRINGS OF GRYŻYŃSKI LANDSCAPE PARK

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Abstract

The study aimed to analyze hydrochemical and bacteriological features in selected springs of the Gryżyński Landscape Park (GLP) in western Poland. The basic hydrochemical parameters and the abundance and biomass of bacteria were analyzed in water samples collected from 22 springs in GLP in July 2018. The hydrochemical composition of the GLP spring waters depends mainly on the specific geological structure and character of catchment land use. The research showed significant differences in the water quality of GLP springs in the case of many hydrochemical parameters, e.g., dissolved organic carbon concentration, biogenic substances which influence the bacterial abundance and biomass. The average bacterial number (BN) in the waters of the Gryżyński Landscape Park springs was 0.656 10^6 cells/ml, with a range from 0.181 10^6 cells/ml to 1.656 10^6 cells/ml. The coefficient of variation of this parameter was 75.67%. In 12 investigated springs of GLP, BN was below 0.5 10^6 cells/ml, in 6 springs, the average BN was between 0.5 and 1 10^6 cells/ml, and in 4 springs BN, it was in the range from 1 to 2 10^6 cells/ml. The mean bacterioplankton biomass was 22.98 µgC/l and from 6.35 to 61.54 µgC/l. Based on the collected material, many correlations between BN and selected water quality parameters were found (temperature, phenolics, phosphates, nitrites, and dissolved organic carbon). The microbiological condition of investigated springs depends mainly on the geological structure of the spring niches and water quality. Moreover, the research shows that GLP springs are susceptible to human activity in the direct catchment area. Therefore, this research indicates the potential possibility of using hydrochemical and microbiological parameters together to assess the ecological condition of lowland springs or in planning protective measures.

Key words: bacterioplankton, hydrochemistry, spring

Introduction

Microbiological tests of waters focus mainly on their sanitary and epidemiological condition. Less attention is paid to the role of aquatic microorganisms

in shaping the ecological state and the condition of the ecosystem. Among inland waters, most studies concern bacterioplankton in lakes, rivers, and dam reservoirs. Scientists consider bacteria in natural groundwater outflows much less frequently. They can be divided, among others, by the way water flows out (springs, effluents), the type of aquifer (fissures, karst, pore springs – fed with water from loose formations), or the direction of the hydrodynamic force (ascension, descent) (Moniewski 2007). All these features determine the quality of water, and thus also the groups of organisms inhabiting them. It is worth noting, especially, that the springs are characterized by very high natural value and usually occur in areas with a low degree of anthropogenic transformation. Although springs have been recognized as necessary, rare, and globally threatened ecosystems, there is no consistent and comprehensive classification or common lexicon for springs, especially in lowland areas (Springer et al. 2008). Springs play a critical role in periods without rainfall, because by supplying rivers they determine, among others, the amount of water flowing in the river beds. Hence, it is crucial to protect the springs to maintain the proper level of surface waters. In Poland, especially in lowland areas, the degradation and disappearance of wetlands and springs are observed (Siwek 2004); therefore, research on the springs is becoming more and more critical (Michalczyk 2001, Michalczyk et al. 2004, Michalczyk et al. 2015, Puczko, Jekatierynczuk-Rudczyk 2020). The waters of Quaternary springs are exposed to area pollution of agricultural origin or resulting from urbanization in the direct catchment area (Jekatierynczuk-Rudczyk 2008). This affects the changes in the chemical composition of groundwater manifested by a disturbance in the proportion of micronutrients (Górniak, Jekatierynczuk-Rudczyk 1997, Małecki 1998, Jekatierynczuk-Rudczyk 1999, Szczucińska 2017) and dissolved organic matter (Zieliński et al. 2020). The organisms that respond to the changes in water quality in the first place are water microorganisms. The number of bacterioplankton is a good illustration of the intensity of biogeochemical changes in these naturally poor habitats. Moreover, the abundance of bacterioplankton in the water of the springs is determined by different geological, hydrological, and catchment conditions. To better understand their ecology in the area with little anthropogenic transformation, hydrochemical and microbiological studies of selected springs of the Gryżyński Landscape Park were carried out. The study considers the nature of the outflows and the type of land development as additional factors determining bacterioplankton in springs.

Materials and methods

Study area

Gryżyński Landscape Park (GLP) is located in the west of Poland, in Lubuska Upland. It was established on April 15, 1996. The area of the park is 3064.80 ha, and the buffer zone is 7911.20 ha. The park was established to protect postglacial relief, hydrography, various habitats, and rare flora and fauna. The

landscape is dominated by a post-glacial gutter drained by the river Gryżynka, 17 km long. The gutter is cut into the sander surface to a depth of 30 m. A deep erosive incision is conducive to the functioning of groundwater outflows, of which more than 350 have been inventoried in the gutter. These outflows are located mainly under the slopes and drain Quaternary waters from alternating sand and gravel pores. They are characterized by high-performance stability throughout the year and a stable temperature. The spring zone with the highest efficiency of approximately 50 dm^3/s is located in Gryżyna. The average temperature of spring waters is around 9°C. More than 70% of these waters belong to the hydrochemical type HCO_3-SO_4-Ca. This chemical composition is characteristic of shallow groundwater in the hypergeneic zone.

However, for some indicators, e.g., nitrates, sulfates, iron, and manganese, different concentrations were found even in the adjacent outflows. This diversity may be influenced by how the catchment area and the immediate vicinity of the outflow are managed. Forests dominate the direct drainage catchment. They cover 76% of the GLP area and are dominated by pine forests. Less numerous are deciduous forests, preserved mainly in the Gryżyński Potok valley. Some spring niches are located near farmland and built-up areas. Agricultural land and built-up land, which can be a source of anthropogenic pollution, account for 16% and 4%, respectively. Spring zones are a place where groundwater flows to the surface of the area, and hence they are a convenient place for sampling and obtaining information about the quality of the water drained by them. The GLP area is slightly anthropogenically transformed, and therefore it was selected to recognize both the hydrochemical and microbiological status of groundwater occurring in the semi-natural area. The study covered 22 springs located in the GLP area (Figure 1).

Research methods

Twenty-two springs in the Gryżyński Landscape Park were selected for detailed hydrochemical and microbiological analyses (Table 1, Figure 1). The field studies were conducted in the summer (July 9, 2018). Temperature, pH, electrolytic conductivity (EC), and oxygen concentration in water were measured directly in the field using the Hach-Lange HQ-40d multi-parameter probe. Water samples for hydrochemical analyses were collected following the recommendations for the Geochemical Map of Europe (Salminen et al. 1998) and samples for microbiological determinations were collected in sterile 50 ml containers. Hydrochemical analyzes were performed at the Department of Water Ecology at the University of Białystok. Concentrations of anions: NO_3, NO_2, PO_4, and cations: NH_4 were determined by high-performance liquid chromatography (HPLC). The content of total phosphorus (TP) was determined by the molybdate method with ascorbic acid and the total iron content (TFe) was determined using the phenanthroline method. SpecraMaxM2 spectrofluorimeter was used in spectrophotometric determinations. The analyses of dissolved organic carbon (DOC) and total nitrogen (TN) concentrations were carried out using the

high-temperature catalytic combustion method with Shimadzu carbon and nitrogen analyzer (TOC 5050A, Japan). Water samples for DOC determinations were filtered through a 0.45 μm filter, acidified with 2 M HCl to a pH of about 2, and rinsed with pure nitrogen to remove traces of inorganic carbon.

The total number of bacteria (BN) was determined in formalin preserved samples (2% final conc.) using epifluorescence microscopy. DAPI-stained bacterial cells were counted on 0.2 μm pore-size black polycarbonate membrane filters (Nuclepore, Whatman) with Olympus epifluorescence microscopy (BX61, Japan) using the Porter and Feig (1980) method. BN was recalculated for bacterial biomass (BB) using the Theil-Nielsen, Sondergaard (1998) method.

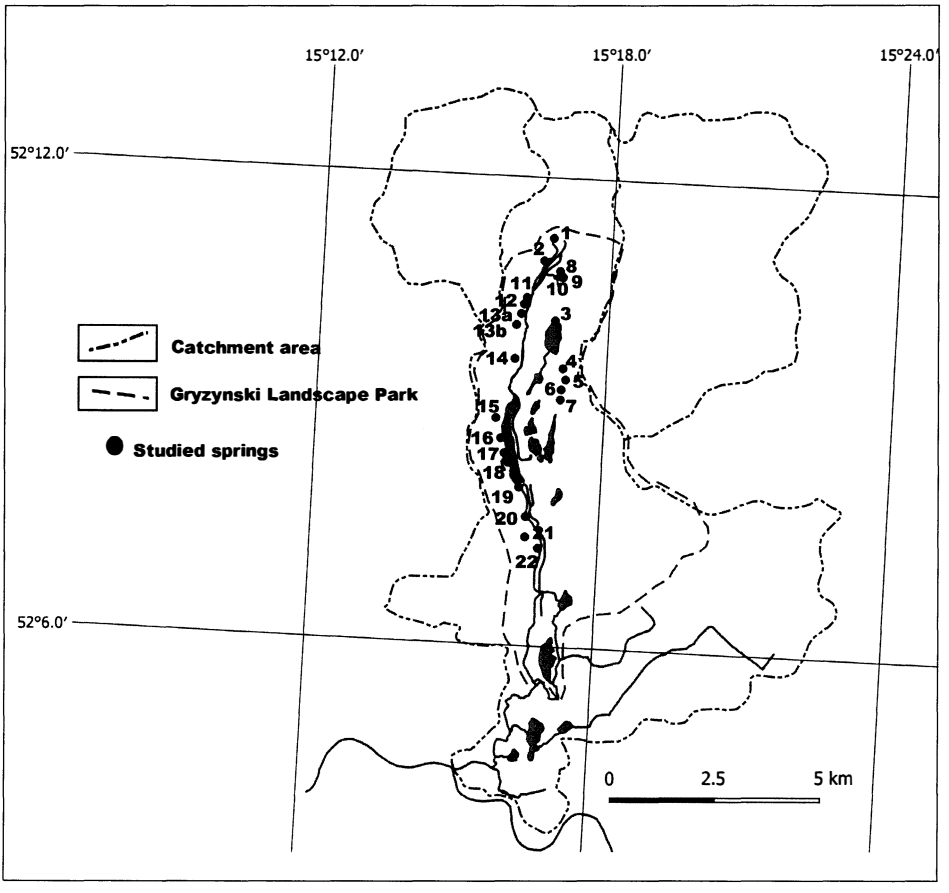

Figure 1. Distribution of the studied springs in the Gryżyński Landscape Park, considering the Gryżynka River catchment area.

Table 1. Hydrological characteristics of the studied springs in GLP.

Spring No.	Morphological type	Q (dm^3/s)	Outflow type	Development
1	bottom-slope	0.5	descending	rural buildings
2	bottom-slope	0.5	descending	forest
3	bottom-slope	2.5	ascending	forest
4	bottom-slope	0.1	descending	forest
5	bottom-slope	0.1	descending	forest
6	bottom-slope	0.1	descending	forest
7	bottom-slope	0.1	descending	forest
8	bottom-slope	0.2	descending	forest
9	bottom-slope	0.3	descending	forest
10	bottom-slope	0.4	descending	forest
11	bottom-slope	0.1	descending	field, forest
12	bottom-slope	0.15	descending	field, forest
13	bottom-slope	2.5	descending	field, forest
14	bottom-slope	0.8	descending	forest
15	bottom-slope	0.8	descending	forest
16	bottom-slope	6	descending	forest
17	bottom-slope	0.3	descending	forest
18	bottom-slope	0.15	descending	forest
19	bottom-slope	0.15	descending	forest
20	slope	0.1	descending	forest
21	bottom-slope	0.6	descending	forest
22	valley	0.1	ascending	forest

Results

Hydrochemical water quality

The average air temperature during the sampling period (July 2018) in GLP was 20.8°C, while the average water temperature of the studied springs was 12.2°C. The water temperature in the samples from GLP ranged from 10.1°C in spring 13 to 16.1°C in spring No. 1. A low coefficient of temperature variation was noted for the waters of GLP springs (16.5%). The average water pH in the GLP springs was 7.45. The average electrolytic conductivity in the GLP springs was 410.78 µS/cm. The EC values ranged from 159.9 to 538 µS/cm. The dissolved oxygen content in the waters of GLP springs was on average 4.29 mg/l.

The amount of oxygen in the water ranged from 0.11 in spring No. 22 to 9.04 mg/l in spring No. 11 (Table 2).

Table 2. Statistical characteristics of physical and chemical parameters in the springs of the Gryżyński Landscape Park, average results for the summer period of 2018.

Parameter	Number of samples	Mean	Standard deviation	Min-max value	Variation coefficient
Temperature (°C)	22	12.24	2.02	10.1 – 16.1	16.48
pH	22	7.45	0.098	7.28 – 7.61	13.4
Specific electrolytic conductivity (µS/cm)	22	410.27	92.27	159.9 – 538	23.01
Oxidation-reduction potential (mV)	22	175.04	6.580	155.9 – 185.6	3.84
Oxygen (mg/l)	22	4.35	2.917	0.11 – 9.04	68.32
Oxygen saturation (%)	22	42.38	29.69	1.1 – 92.3	71.21
Ammonium ions (µgN/l)	22	62.81	35.52	1.0 – 138	56.55
Nitrites (µgN/l)	22	2.65	2.27	0.4 – 13.2	100.94
Nitrates (µgN/l)	22	411.95	849.98	2.4 – 3360	206.33
Total nitrogen (µgN/l)	22	876.81	1239	197 – 5012	141.32
Phosphates (µgP/l)	22	10.97	5.56	2.4 – 22.7	50.75
Total phosphorus (µg/l)	22	64.8	27.1	13.5 – 145.3	41.72
Total iron (mg/l)	22	0.329	0.138	0.028 – 0.560	87.93
DOC (mg/l)	22	2.75	1.20	1.58 – 6.48	43.46
Phenolics (mg/l)	22	0.39	0.12	0.19 – 0.61	31.08

Nitrogen compounds

The average concentration of ammonium ions in the GLP springs was 62.8 µgN/l (Table 1). The minimum values of NH_4 were recorded in springs No. 13 and 18 (26 and 32 µgN/l, respectively). A significantly higher NH_4 concentration, exceeding 120 µgN/l, was found in springs No. 1 and 2, located close to human settlements. The average concentration of nitrates in the GLP springs was 0.412 mg/l. The values of this parameter ranged from 24 (spring No. 6) to 3368 µgN/l (spring No. 9). Due to the high variability of this parameter, a

high coefficient of variation was noted (206.33%). The less variable parameter in terms of concentration in water were nitrites (CV = 100.94%). The average concentration of nitrites in the GLP springs was 2.65 μgN/l. The concentration of nitrites in the GLP springs ranged from 0.4 μgN/l to 13.2 μgN/l (in springs No. 13 and 3, respectively). The mean value of total nitrogen in the GLP springs was small and amounted to 0.88 mg/l. In GLP springs, the value of this parameter ranged from 0.197 mg/l to 5.12 mg/l. In GLP, a high coefficient of variation was noted: 149.49% (Table 2).

Phosphorus compounds

The average concentration of phosphates (SRP) in the waters of GLP springs was 10.97 μgP/l. The concentration of SRP ranged from 2 μgP/l to 23 μgP/l. The average concentration of total phosphorus in the GLP springs was 65 μgP/l. This concentration in the GLP springs ranged from 14 μgP/l to 145 μgP/l (Table 2).

Organic compounds are essential for the functioning of microorganisms in spring niches. The average content of dissolved organic carbon in GLP was 2.754 mg/l. In the waters of the GLP springs DOC range was between 1.577 and 6.478 mg/l DOC. The mean concentration of phenolics in the GLP springs was 0.391 mg/l. On the other hand, the content of phenolics ranged from 0.197 mg/l to 0.612 mg/l (Table 2).

Total number and biomass of bacteria

The average number of bacteria in the waters of the GLP springs was 0.611 10^6 cells/ml, and the coefficient of variation was 75.67%. The number of bacteria in the samples from GLP ranged from 0.182 10^6 cells/ml (spring No. 10) to 1.656 10^6 cells/ml (spring No. 13). In 12 of the examined GLP springs, the number of bacteria did not exceed 0.5 10^6 cells/ml, in 6 springs, the average BN ranged from 0.5 to 1 10^6 cells/ml, and in 4 springs, BN ranged from 1 to 2 10^6 cells/ml (Figure 2). Thus, bacterial biomass in water samples from GLP springs was small and amounted to 22.975 μgC/l. Bacterial biomass ranged from 6.352 (spring No. 10) to 61.537 μgC/l (spring No. 13).

Based on the performed statistical analyses, numerous dependencies were found between water quality parameters and the BN. For example, positive correlations were found between BN and water temperature (Figure 3), the content of dissolved organic matter (Figure 4), and the concentration of phenolics (Figure 5). On the other hand, negative correlation coefficients were noted between BN and the concentration of phosphate ions (Figure 6) or the concentration of NO_2 ions (-0.377; $p<0.005$).

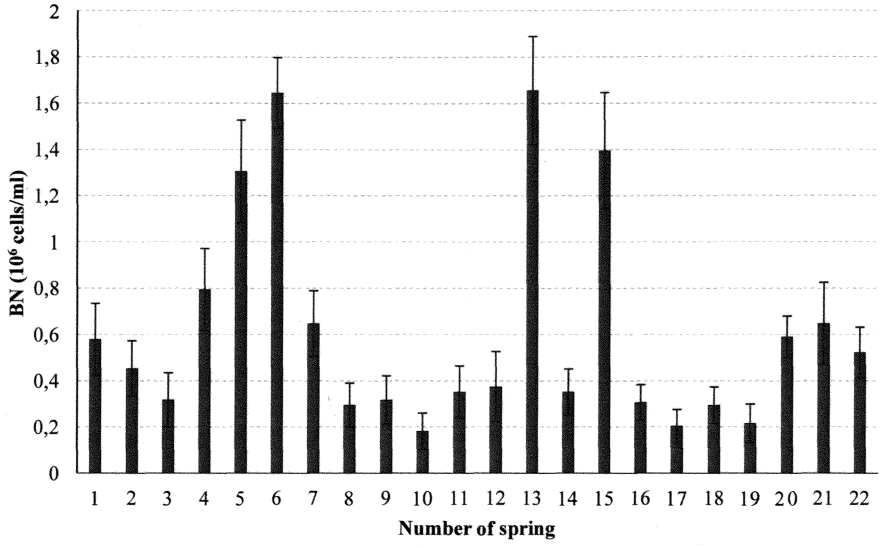

Figure 2. The average number of bacteria (BN) in the studied springs of the Gryżyński Landscape Park, average results from the summer period of 2018.

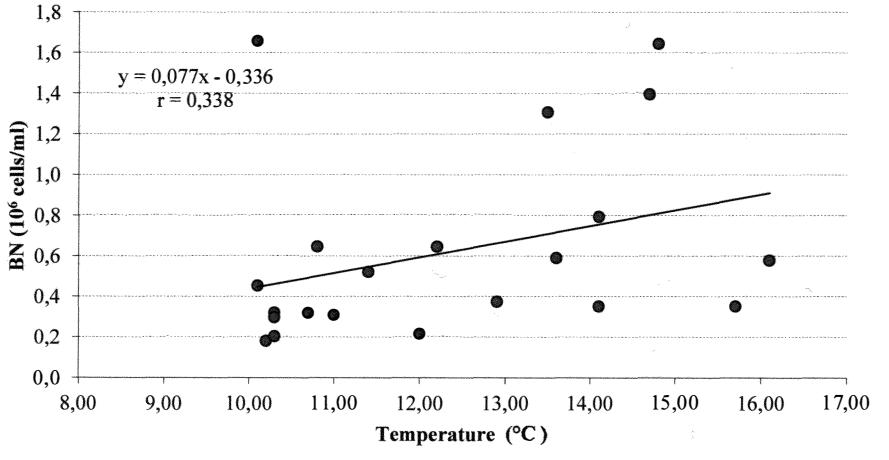

Figure 3. Correlation between the number of bacteria (BN) and the temperature in the waters of the Gryżyński Landscape Park springs.

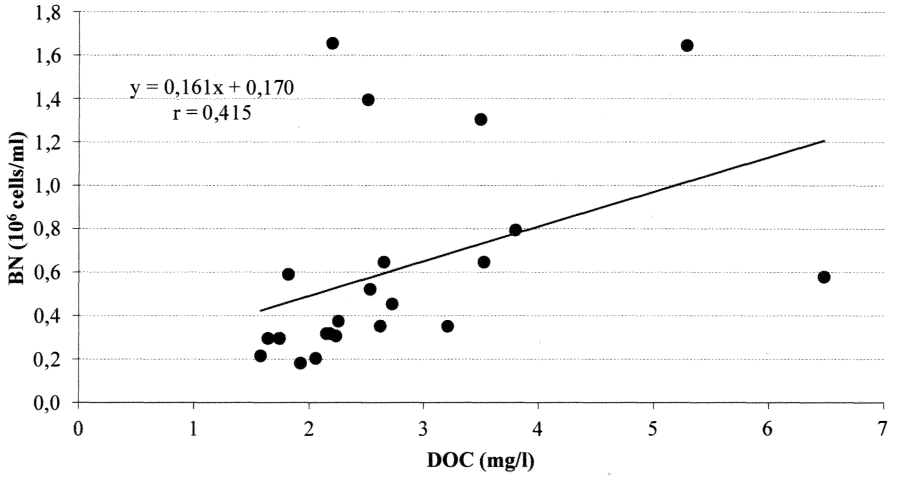

Figure 4. Correlation between the number of bacteria (BN) and the concentration of dissolved organic carbon (DOC) in the waters of the Gryżyński Landscape Park springs.

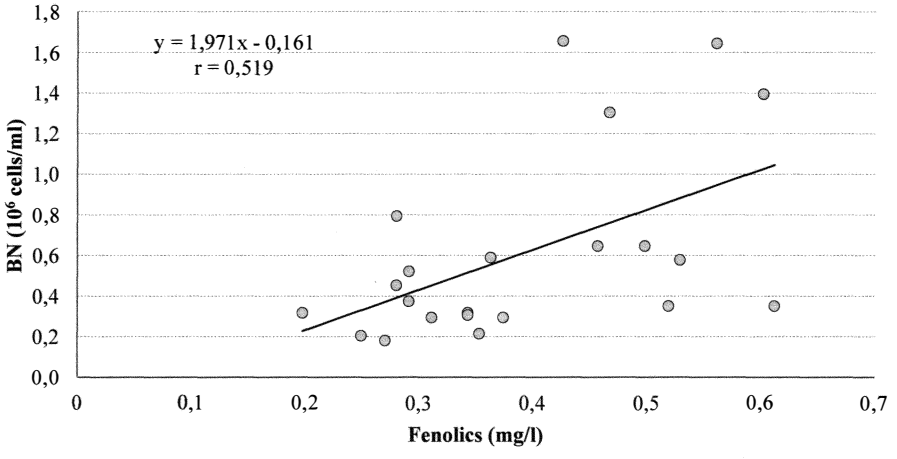

Figure 5. Correlation between the number of bacteria (BN) and the concentration of phenols in the waters of the Gryżyński Landscape Park springs.

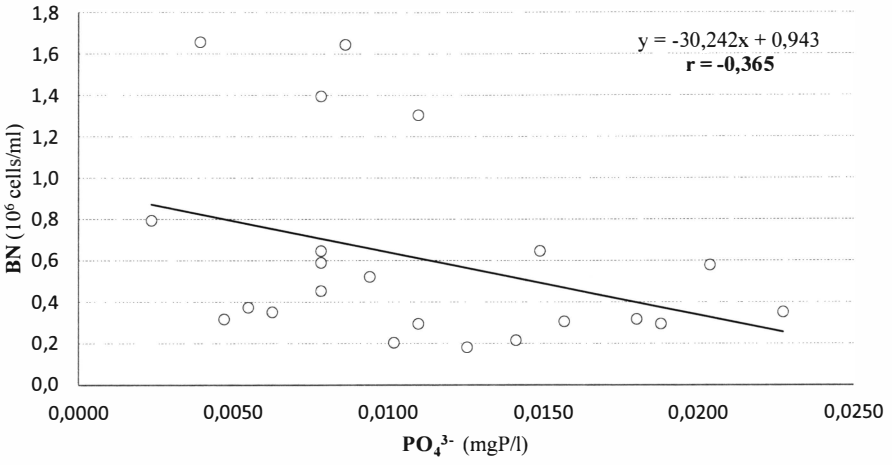

Figure 6. Correlation between the number of bacteria (BN) and the concentration of soluble reactive phosphates (PO_4^{3-}) in the waters of the Gryżyński Landscape Park springs.

Discussion

Microorganisms, including bacteria, constitute the most numerous group of organisms living in all aquatic ecosystems. The specific aquatic environments include springs, where the degree of recognition of the diversity of organisms is still insufficient (Puczko et al. 2018, Zieliński et al. 2020). The high hydrochemical stability of natural groundwater outflows allows for identifying those factors that determine the development of bacterioplankton in lowland spring niches. The number of bacteria depends on many hydrological, physical, and chemical parameters of water. For spring waters, BN is usually from several dozen to several hundred thousand cells per milliliter. The total number of bacterioplankton in the GLP springs exceeded several hundred thousand cells per milliliter (Figure 2). It may be related to the research period — the high growing season. According to the literature, usually, the highest BN values are recorded in warmer periods (Sinreich et al. 2014). Probably the increased BN value in GLP springs is primarily the result of favorable thermal and oxygen conditions. Psychrophilic bacteria predominate in the examined GLP springs because the water temperature of these springs did not exceed 20 °C. The groundwater of lowland areas is characterized by relatively stable temperature (Jekatierynczuk-Rudczyk 2007, Szczucińska, Wasilewski 2013). It depends mainly on the climatic conditions in a given area and the geological structure and management of the catchment area (Michalik, Szczucińska 2011). The slight variation in water temperature in the springs is one of the main factors affecting the occurrence and diversity of aquatic microorganisms (Rychła et al. 2015, Sinreich et al. 2014), which is

confirmed by this study (Figure 3). The development of aquatic bacteria is favored by a pH that is close to neutral. The optimal pH for bacterial growth ranges from 5.5 to 8.5 (Chomutowska 2009). This feature of water mainly depends on the electrolytic dissociation of water and the dissociation and hydrolysis of the compounds dissolved in it. Most shallow groundwater in post-glacial areas is naturally neutral (Bodora 2016, Jekatierynczuk-Rudczyk et al. 2017). The pH range noted in GLP springs favors the development of bacterioplankton (Table 2). The periodic changes in the water reaction in the springs are also influenced by the quantity and quality of precipitation, the content of carbonates, the nature of the substrate, and the degree of anthropogenic pollution (Jekatierynczuk-Rudczyk 1999, Zieliński et al. 2020). In the case of GLP springs, there were no differences in water pH resulting from changes in the catchment area management. Another factor influencing the growth of bacteria is the availability of oxygen. The oxygen in the water of the springs comes from the atmosphere and, to a lesser extent, from photosynthesis. The content of dissolved oxygen in groundwater is usually lower than in surface waters, hence more challenging conditions for the development of bacteria. The average oxygen concentration in the waters of GLP springs was similar to that in the lowland NE springs of Poland, among others, Knyszyn Forest Landscape Park and Suwałki Landscape Park (Jekatierynczuk-Rudczyk 2008, Jekatierynczuk-Rudczyk et al. 2017, Zieliński et al. 2020). As the springs show a relatively constant water temperature daily and annually, they rarely experience extreme oxygen conditions. No oxygen deficits were found in the examined GLP springs, which can also be considered a factor favoring the growth of bacteria.

Phenolics are organic compounds that occur naturally in water (leached from the soil) or constitute industrial, less so communal, pollution. The amount of phenolics in groundwater depends mainly on the immediate catchment area and soil erosion. Favorable conditions for accumulating phenols in the soil are created by low soil pH, increased humidity, and easy oxygen access. In the waters of GLP springs, relatively low concentrations of phenols were found (Table 2). Still, it turns out that due to the limited access to organic matter (Table 2), phenolics can be an attractive source of substrates for bacteria (Figure 5), similarly to other organic compounds dissolved in water (Figure 4). Other studies confirm a substantial contribution of dissolved organic carbon to groundwater microbial biomass build (Schw b et al. 2017).

Nitrogen compounds of both inorganic and organic origin play an essential role in the proper functioning of aquatic ecosystems. These compounds can get into the water from both natural and anthropogenic sources. Some bacteria can fix nitrogen from various compounds (Adamczy , Jachimowski 2013), while others release this element during the reduction of nitrates. The average concentration of total nitrogen and nitrates in the GLP springs was lo and did not differ from other glacial areas (Table 2). However, in 2006-2007 Michalik and Szczucińska (2011) recorded much higher values of nitrate concentrations in GLP waters. Thus, the condition of the springs' waters in terms of these parameters has improved within 14 years.

On the other hand, increased mean concentrations of nitrites and ammonium ions in GLP springs were noted (Table 2). In the summer period, intensive denitrification processes probably occur in the studied niches, which are much more efficient at higher BN values. Recent studies demonstrated a high potential for denitrification linked to the capacity for anaerobic ammonium oxidation in springs (Savio et al. 2019).

The source of various phosphorus compounds (including orthophosphates) is the natural process of weathering of phosphate minerals and human economic activity (Igras, Jadczyszyn 2008). However, the average concentration of orthophosphates is so high that it is not a factor limiting the growth of bacteria in the springs of GLP. Therefore, it can be assumed that phosphorus from anthropogenic sources is a good indicator of the microbiological condition of the springs. Presumably, bacterioplankton of the springs develop better under conditions of lower SRP values, hence the observed negative relationship between the concentration of SRP and BN (Figure 6).

The increased value of the summer BN in the waters of GLP springs is probably the result of the specific geological structure, water quality of the springs, and the fact that they are not mountain springs (springs efficiency). The GLP area is characterized by high forest cover, and forest areas constantly supply organic matter to the water and thus increase the number of bacteria. The relatively small range of the total number of bacteria in GLP results from the fact that the springs are located close to each other, are supplied from practically the same aquifer, and lie within a protected area. In GLP, the highest abundance of bacterioplankton was recorded at spring No. 13 (Figure 2), where a field and a forest surround a descending spring. The impact of land use on shallow groundwater quality is often observed in shallow groundwater environment (Eftimi, Zojer 2015, Ramos et al. 2018). Low concentrations of most chemical parameters of water were found in forest springs. More considerable changes in the chemical composition of water occurred in the outflows located in arable lands, rural areas, and the greatest in the rural development zone; similar observations were recorded by Michalczyk et al. (2015), Jekatierynczuk-Rudczyk et al. (2017). However, this did not lead to significant differences in BN between springs with different catchment area management. It is also worth noting that the assessment of the microbiological condition of springs may play an essential role in determining their ecological status. In the future, using this parameter should be considered an obligatory factor in assessing the condition of natural groundwater outflows.

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LONG TERM CHANGES OF CRUSTACEAN ZOOPLANKTON COMMUNITIES IN LAKE WIGRY AS A RESPONSE TO CHANGING TROPHIC CONDITIONS – 100 YEARS OF OBSERVATIONS

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Abstract

Wigry Lake has one of the longest zooplankton data sets in Poland and has been investigated for over 100 years. Therefore, our goal was to present long-term changes in zooplankton structures against the background of changing trophic conditions. The species composition of crustacean zooplankton in the first half of the 20th century was typical for a large mesotrophic lake, with a high share of *Daphnia cucullata* and stenotherm species: *Eurytemora lacustris*, *Heterocope appendiculata, Cyclops lacustris*, *Bythotrephes brevimanus*, *Daphnia longiremis*. The results of the research from the years 1965-1985 were very pessimistic, because the lake was exposed to intense eutrophication and there were important changes in zooplankton communities. The glacial relicts and species characteristic for low trophic condtions declined, while the share of species typical for high trophic conditions (*Mesocyclops leuckarti*, *Thermocyclops oithonoides*, *Diaphanosoma brachyurum*) increased. As a result, species characteristic for eutrophic conditions made up to 70 % of crustacean zooplankton, when in 1921 their share did not exceed 25%. The results of hydrochemical and biological studies of the last 30 years indicate a gradual tendency to improve the trophic state of Lake Wigry, due to biomanipulation activities carried out in the lake and its catchment area. We also have observed a marked increase in the occurrence of glacial relicts and the dominance of large *Daphnia* individuals, which indicates an improvement in water quality.

Keywords: crustacean zooplankton, long-term changes, water quality, glacial relicts, reoligotrophication

Introduction

Wigry is a large lake with an area of 21.7 km^2 and a maximum depth of 73 m, which makes it one of the largest lakes in Poland. The lake is located in northeast Poland, and the Czarna Hańcza River flows through its North Basin. Lake Wigry is unusual due to its diversified morphometry, shape, and

bathymetry, which create diverse habitat conditions, although most parts of the lake have mesotrophic conditions (Karpowicz et al. 2019). However, in the 70's and 80's the lake underwent strong eutrophication and its ecological status significantly decreased. Nowadays we are observing a reoligotrophication process (Kamiński 1999) due to the construction of sewage water treatment plants in the catchment area and active protection through biomanipulation (Karpowicz et al. 2019). The composition of zooplankton has also changed with the trophic status. Research on Lake Wigry began in 1920 by setting up the Hydrobiological Station, managed by Dr. Alfred Lityński, which contributed greatly to the European limnology until 1939. The scientists of the Station and its visitors published about 100 research papers on hydrography, hydrochemistry, phytoplankton, zooplankton, bottom fauna, vegetation, fish, and other groups of water organisms of Lake Wigry. The outbreak of World War II halted the activity at the Hydrobiological Station and its work has never been resumed. After the war, fragmentary information about zooplankton came only from studies of lake fisheries. Comprehensive hydrochemical and biological studies of Lake Wigry have been conducted in 1981-1995 by the Polish Academy of Sciences, and then by the Department of Hydrobiology of the University of Bialystok. Thus, with 100 years of observations, Lake Wigry is probably the best understood Polish lake in terms of planktonic fauna (Karpowicz et al. 2013).

Long-term data is integral to understanding and quantifying complex processes in ecosystems and for predicting their response and future development under changing ambient conditions (Straskrabova et al. 1999). Therefore, our goal was to describe the long-term changes in the zooplankton composition of Lake Wigry in the context of changing trophic conditions. The zooplankton can very quickly respond to changes in the environment and is widely used as an indicator of trophic status (Andronikova 1996; Jeppesen et al. 2011; Ejsmont-Karabin 2012; Ejsmont-Karabin et al. 2013; Karpowicz et al. 2020). Generally, zooplankton of eutrophic lakes is characterized by high abundance, low diversity, and small body size (Hillbricht-Ilkowska et al. 1979; Jekaterynczuk-Rudczyk et al. 2012). While in oligotrophic lakes zooplankton is characterized by low abundance, high diversity, large body size, and the presence of stenotherm species with high environmental requirements (Andronikova 1996; Karpowicz et al. 2020)

Materials and methods

Lake Wigry and 41 other lakes in the Wigry National Park are located in NE Poland close to the Lithuanian border. The landscape was shaped during the last glaciations (Marks 2005). We analyzed long-term changes based on the North Basin of Lake Wigry (Figure 1). The samples were collected in the midsummer in the years 2007, 2009, 2015, and 2016. The samples were taken using a 5l Limnos sampler from the epilimnion, matalimnion, and hypolimnion. For zooplankton analysis, 10-20 liters of water were filtered through a 40 µm plankton net and were preserved in a 4% formalin solution. The animals were

identified to species level and 10 individuals of each species were measured for estimated biomass, using length-weight regression relationships (Błedzki et al. 2016). Our results were presented against the background of long-term changes in the structure of crustacean zooplankton (Lityński 1922; Adlerówna 1929; Karabin et al. 1992). The zooplankton structure was also used to calculate trophic status based on the equation proposed by Ejsmont-Karabin and Karabin (2013). Comparative limnology studies were conducted to present the effect of environmental conditions on crustacean communities. The trophy of the lake was calculated using the Carlson trophic status index (TSI) as an average of three parameters: Secchi disc visibility (SDV), chlorophyll *a*, and total phosphorus (Carlson 1977). Chlorophyll *a* concentration in water was determined by spectrophotometry, after homogenization filtered on GF/C and extraction with boiling 90% ethanol (Lorenzen 1965; Nush 1980). For the determination of phosphorus forms, we used the standard molybdate colorimetric method (Murphy and Riley 1962). Assay of total phosphorus accomplished after prior digestion with UV radiation, with concentrated H_2SO_4 and 30% H_2O_2. We measured *in situ* the Secchi disc visibility (SDV), and dissolved oxygen concentrations in the hypolimnion using an HQ40D Multi Meter (Hach-Lange GmbH, Germany).

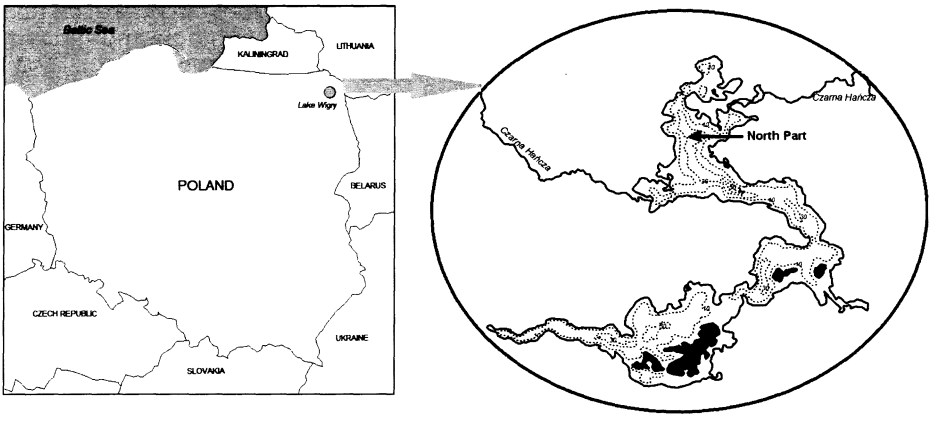

Figure 1. Lake Wigry bathymetry with the location of the sampling site.

Results and discussion

In the early twentieth century, Lake Wigry was characterized by high water transparency, which ranged from four to six meters in July (Figure 2A), while in winter it was more than 11 meters (Lityński 1926). In the 80s, when the lake underwent strong eutrophication, water transparency decreased significantly to a maximum of 1.8 m. Low water transparency persisted until 1998 and then slightly increased in the following years, reaching 3.5 meters (Figure 2A). There were also changes in oxygen concentrations in the hypolimnion, which were caused by intense eutrophication. High oxygen concentrations in the hypolimnion were

observed in the 70s (Figure 2B). Then, the concentration of dissolved oxygen in the lower layers decreased to only 1.6 mg/L, indicating a hypoxic state disturbing biotic interactions and causing significant changes in food webs (Kolar et al. 1993). From 2002 we have observed improving oxygen conditions in the hypolimnion (Figure 2B) and nowadays oxygen concentration in hypolimnion ranges from 8.2 to 9.6 mg/L.

In the 20s, high water transparency and good oxygen conditions indicated low trophic conditions, which were also emphasized by Dr. Lityński (1922). However, there is a lack of data on hydrochemistry from that period, causing a gap in the calculations of the Carlson Trophic State Index (TSI). TSI values in 1986-2007 ranged from 50.5 to 54 (Figure 3A), which indicates eutrophic conditions. Since 2009 we observed improving trophic conditions and TSI values ranging from 34 to 48.5 indicate mesotrophic conditions.

We also analyzed the concentrations of orthophosphates in the surface and lower layers of the lake. From 1986 to 2002, the concentrations of orthophosphates in the surface layer oscillated between 15 and 22 µg/L and in the lower water layers these values were particularly high, ranging from 95 to 106 µg/L (Figure 3B). This was caused by the uncontrolled inflow of nutrients to the northern part of the lake with the Czarna Hańcza river, which provided 80% of pollutants as a recipient of sewage from nearby Suwałki (70 thousand inhabitants) and it also collects pollution from its catchment area of 170 km^2 (Kamiński 1999). Since 2007, the values both in the upper and lower water layers were at the same level, ranging from 6 to 20 µg/L (Figure 3B). We can observe an improvement in orthophosphates levels, thanks to the launch of the sewage treatment plant in Suwałki in 1986 and its modernization in the years 1993-1995, introducing the dephosphatation process (Ćwikła et al. 2011).

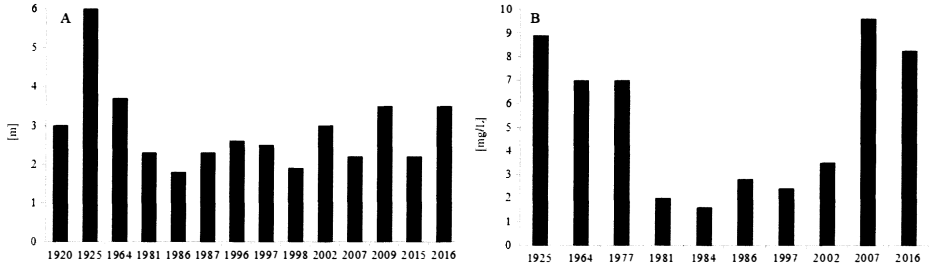

Figure 2. Long-term changes of the Secchi disk visibility (A) and hypolimnetic oxygen concentrations (B) in Lake Wigry during 1920-2016.

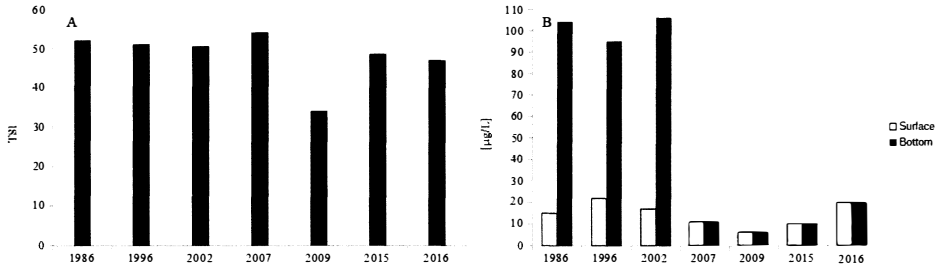

Figure 3. Changes in the value of Carlson's Trophic State Index (A) and concentrations phosphorus forms (B) in surface and bottom water layers on Lake Wigry.

During the activity of the Hydrobiological Station, many detailed studies were carried out on the structure and ecology of crustacean zooplankton in various parts of Lake Wigry. Lityński (1922) noticed qualitative and quantitative differences in the composition of zooplankton in the northern part of the lake, where biomass was much higher than in other parts of the lake. In the 1920s and now, sampling methods are slightly different, which makes a comparison of abundance very difficult. Therefore long-term succession was analyzed based on the percentage of species in groups. The share of the main zooplankton groups was similar over the years, but nowadays the share of Cladocera is increasing with a decrease in copepods (Figure 4A), which indicates an improvement in water quality. The Cladocera at the beginning of the 1920s was dominated by *Daphnia cucullata* and *Eubosmina coregoni*, while *Diaphanosoma brachyurum* and *Daphnia cristata* had a smaller share (Figure 4B). The share of *D. cucullata* in 1986-2009 increased (Figure 4B) and larger individuals of this species dominating in recent years (Karpowicz et al. 2019). The body size of *D. cucullata* is a good indicator of the trophic state (Karpowicz et al. 2020), and increasing body size also indicating improvement of environmental conditions of Lake Wigry. In the eighties decreased share of *E. coregoni* and increased share of species characteristic for eutrophic conditions like *Chydorus sphaericus*, *Ceriodaphnia quadrangula*, *Bosmina berolinensis* (Figure 4B). The greatest changes took place in Calanoida. At the beginning of the twentieth century, *Eurytemora lacustris* and *Heterocope appendiculata* dominated the Calanoida (Figure 4C), both of these species are stenotherm and are considered as glacial relicts (Karpowicz et al. 2021). In the second half of the twentieth century, the share of this species significantly decreased (Figure 4C). Recent studies from 2007 and 2009 show increased importance of Calanoida in the overall zooplankton biomass and also more common were *E. lacustris* and *H. appendiculata*. In the Cyclopoidae was observed slow change in the dominance from *Thermocyclops oithonoides* to *Mesocyclops leuckarti* (Figure 4D). Last research from 2007 and 2009 also showed the decline importance of this group, which is one of the indicators for the trophic state proposed by Karabin (1985).

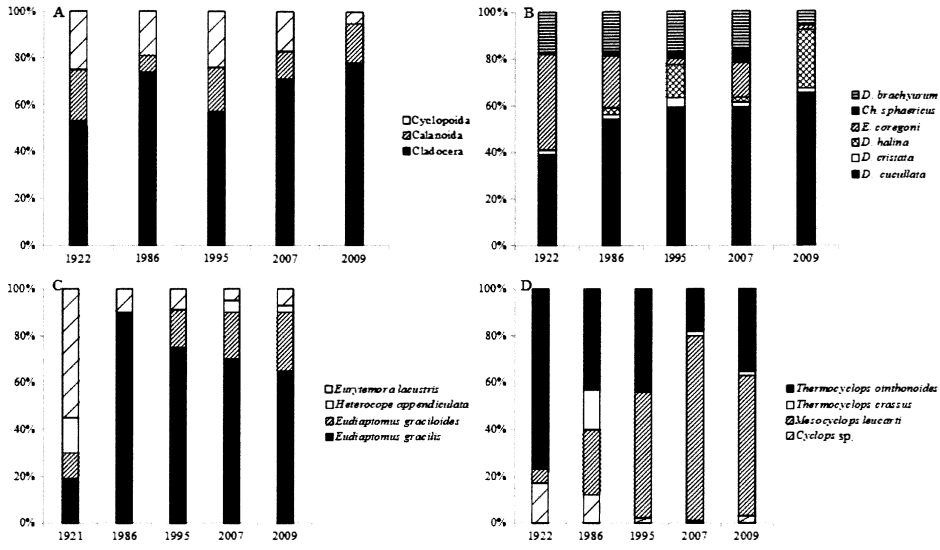

Figure 4. Long-term changes of crustacean zooplankton structure. A – main crustacean groups; B – Cladocera; C – Calanoida; D – Cyclopoida.

During progressive eutrophication in the second half of the twentieth century, changes in the structure of zooplankton took place. However, thanks to protection activities carried out in the lake and its direct catchment area, nowadays the structure of the zooplankton indicates low trophy, which is a good sign for the future. In the summer, at the majority of the stations, the *Daphnia cucullata* dominates in zooplankton biomass. High species diversity of the pelagic zooplankton gives a very specific value to the lake, particularly due to the occurrence of relic and rare crustaceans species, like *Eurytemora lacustris*, *Heterocope appendiculata*, *Cyclops lacustris*, *Bythotrephes brevimanus*, and *Daphnia longiremis*. These rare species in recent years were more frequent at most sampling sites, which indicates improving environmental conditions in the lake.

Conclusions

The species composition of crustacean zooplankton in the first half of the 20th century was typical for a large mesotrophic lake. In the second half of the twentieth century, increased anthropopressure resulted in: decreased water transparency, increase of phosphorus contents, and lack of oxygen in the hypolimnion. Studies conducted in the eighties have shown changes in zooplankton structure, with increased the importance of species typical for eutrophic conditions (*Mesocyclops leuckarti*, *Thermocyclops oithonoides*, *Diaphanosoma brachyurum*), while stenotherm species, which prefer cool oxygenated waters (*Eurytemora lacustris*, *Heterocope appendiculata*, *Cyclops lacustris*, *Bythotrephes brevimanus*,

Daphnia longiremis) were found occasionally. However, thanks to protection activities carried out in the lake and its direct catchment area, in the early twenty-first century we have observed improvement of trophic conditions and increasing water transparency. Nowadays relict species, which are especially sensitive to environmental deterioration are more frequently found.

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BIOTOPE IMPACT ON FLUCTUATING ASYMMETRY MANIFESTATION IN GROUND BEETLES (*COLEOPTERA, CARABIDAE*)

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Abstract

In our study we used the data set on morphimetric traits in ground beetles species. It has been constantly replenished for 20 years with samples received from different regions of Russia and abroad. In this case we have selected data on nine species for which the left and right sides had been measured and fluctuating asymmetry (FA) could be estimated. The samples were from 6 provinces of Russia and Belarus, which ranged along 57 degrees in longitude and included more than 150 plots in different types of biotopes. FA was established in 5265 specimens in one dimensional and one meristic trait. Biotope, species, and their interaction affected FA in both traits, that is: different species reacted differently to biotope type. In uncommon biotopes FA was increased. In forest species a negative relationship between FA in dimensional and meristic traits was revealed. In those species only were FA values sex-biased. In generalist species FA varied similarly in both sexes and in both traits, being the highest in open biotopes. In the eudominant of arable land biotopes – *Poecilus cupreus* – the highest values of FA were recorded in the meadows, being about equal in all types of crops.

Keywords: fluctuating asymmetry; ground beetles; morphometric variation; biotope impact; species*biotope interaction; negative relationship between dimensional and meristic traits.

Introduction

The body size of an animal is one of the most widely studied traits in biology because it has a potent impact on various aspects of the life-history of a given organism, from physiology to ecology and evolution (Peters 1983). In a long-term macroevolutionary context, body size may have serious effects on speciation and extinction processes mediated by its effects on population density, resource exploitation, generation time, etc. (Martin and Palumbi 1993; Silva and Downing 1995). On the short-term ecological and microevolutionary timescale, body size is a crucial feature affecting individual fitness.

That is why body size variation is frequently used in studies concerning the level of adaptation of certain species. Apparently it is reflected in research when fluctuating asymmetry (FA) is taken into account. The latter is an indicator of development stress in certain environmental conditions. In this aspect the most valuable studies were done on insects with holometabolism, because their imagoes do not change body size and its proportions during the remaining ontogenesis (Schmeller et al. 2011; Trotta et al. 2005). Ground beetles are no exception. Being recognized bioindicators (Speight 1986; Labrie et al. 2003; Rainio and Niemelea 2003; Elek et al. 2014), they are widely used in bioindicative practice. Effects of urbanization and sampling region on FA manifestation were revealed, while FA in carabids turned out to be species and sex dependent (Sukhodolskaya et al.2019).

The aim of this study was to research the impact of biotope peculiarities on FA in ground beetles, emphasizing biotope vegetation. Ground beetles are characterized by biotope confinement and if unaccustomed to one, they decrease in size (Sukhodolskaya 2014; Sukhodolskaya et al. 2018) and change their population characteristics (Luff 1965; Luff 1966; Wallin et al. 1992; Dennis et al. 1994; Bommarco 1999; Kromp 1999; Cole et.al 2002; Thorbek and Bilde 2004; Pywell et al. 2005; Hatten et al. 2007; Korenko and Pekar 2010; Haschek et al. 2012; Knapp and Saska 2012).

We hypothesized that: (i) the factors "biotope and species" interact when affecting FA; (ii) FA level is biotope dependent; (iii) in uncommon biotopes FA level is increased; (iv) FA in males is higher than in females.

Materials and Methods

Studied organisms. We analyzed FA in nine carabid species: Carabus aeruginosus (Fischer von Waldheim, 1823), Carabus *(Carabus) granulatus* Linnaeus 1758, *Carabus (Tachypus) cancellatus* Illiger 1798, *Carabus arvensis* Hbst. 1784, *Pterostichus melanarius* Illiger 1798, *Pterostichus niger* Schaller 1783, *Pterostichus oblongopunctatus* Fabricius, 1787, *Poecilus cupreus* Linnaeus 1758, *Pseudoophonus rufipes* Dejean, 1828. All of them (except *C. aeruginosus*) are widespread in the Paleartic, generalists, zoophagous and mesophilous. *C. aeruginosus* is a Siberian species.

Collection sites and sampling methods. In our investigations we used our data set of morphometric measurements in ground beetles. It was funded in 1996

and has been complemented to date with new information: within the framework of scientific cooperation our colleagues – carabidologists have been sending the beetles to our laboratory, where we have been measuring them for several morphological traits. At present this data set includes more than 60000 rows and this data is used for different purposes. For the purposes of this study we have extracted the data, including the left and right sides measurements of beetles. So we formed the data set for our further analysis including data on seven species of ground beetles (Table 1). They were sampled in different years and in different provinces of Russia and Belarus (Figure 1). But we compiled all data taking into account only two variables – species and biotope. The others were ignored, because the impact of the factor "Province" had been studied earlier (Sukhodolskaya et al. 2019). Sampling year can be also ignored, because sampling period in each province is no less than three years and the possible fluctuations in FA year by year neutralize each other.

The beetles were sampled with a unified method (pitfall traps); all photos were taken by one person using the same method. Morphometric data was collected from images taken with a Nikon D5100 camera with custom opaque light disperser and a box with opaque reflective surface.

Table 1. Sample size when estimating FA in studied species of carabids.

		C. granulatus	*C. cancellatus*	*C. aeruginosus*	*C. arvensis*	*P. melanarius*	*P. niger*	*P. oblongopunctatus*	*P. rufipes*	*P. cupreus*
Linden	f	30	57	-	-	67	-	-	114	-
	m	25	73	-	-	192	-	-	167	-
Pine	f	8	7	49	9	279	44	157	22	-
	m	0	7	34	14	128	17	17	33	-
Meadow	f	26	0	218	-	94	-	31	-	41
	m	122	40	159	-	111	-	7	-	51
Birch	f	4	16	-	-	0	-	-	-	-
	m	4	35	-	-	3	-	-	-	-
Lawn	f	56	11	33	-	-	-	32	-	-
	m	8	21	30	-	-	-	90	-	-
Spring wheat	f	-	22	-	-	-	-	-	-	124
	m	-	25	-	-	-	-	-	-	74
Winter wheat	f	-	-	-	-	-	-	-	-	188
	m	-	-	-	-	-	-	-	-	207

		C. granulatus	*C. cancellatus*	*C. aeruginosus*	*C. arvensis*	*P. melanarius*	*P. niger*	*P. oblongopunctatus*	*P. rufipes*	*P. cupreus*
Raspberry	f	-	41	-	-	-	-	-	89	-
	m	-	23	-	-	-	-	-	59	-
Schrubs	f	5	-	-	-	-	-	-	-	-
	m	9	-	-	-	-	-	-	-	-
Swamp	f	0	-	-	-	-	-	-	-	-
	m	16	-	-	-	-	-	-	-	-
Barley	f	-	-	-	-	-	-	-	-	166
	m	-	-	-	-	-	-	-	-	124
Maize	f	-	-	-	-	-	-	-	-	41
	m	-	-	-	-	-	-	-	-	30
Lucerne	f	-	-	-	-	-	-	-	-	36
	m	-	-	-	-	-	-	-	-	64
Oats	f	-	-	-	-	-	-	-	-	229
	m	-	-	-	-	-	-	-	-	117
Pea	f	-	-	-	-	-	-	-	-	105
	m	-	-	-	-	-	-	-	-	69
Carrot	f	-	-	-	-	-	-	-	-	48
	m	-	-	-	-	-	-	-	-	29

Measurements were made using a program designated specifically for the given method of measurement and utilized distances between manually pointed out elements of photos' arrays as terminal points of measurement and fiducial scale, using the latter to bind real scale to array output data (Figure 2). For each specimen we measured the width of the right and left elytra (further – dimensional trait). Besides the dimensional trait, we analyzed meristic traits and counted: the number of tubercles in the first line near the medial ridge of the scutellum (in *C. granulatus, C. cancellatus*), the number of spots on the left and right elytra (in *P. oblongopunctatus*), the number of furrows on the left and right elytra (in *P. melanarius, Poec. cupreus*). *C. aeruginosus* and *C. arvensis* have no such meristic traits, so data on it is absent in certain figures in Results and in the tables.

Statistical analysis. For each specimen we calculated a fluctuating asymmetry (FA) index $FA = |R - L| / (R + L)/2$, where R is the value of a trait at the right elytra, while L denotes the value of a trait at the left elytra.

Figure 1. Provinces of Russia and Belarus, where the beetles were received from: 1 – Belarus Republic, 2 –Tatarstan Republic, 3 – Bashkortostan Republic, 4 – Sverdlovsk Province, 5 – Kostroma Province, 6 – Mariy El Republic, 7 – Kemerovo province.

We analyzed measurements from *Poec. cupreus* separately, because it is an arable lands species and its biotopes differed drastically from all the others, where the other species were sampled.

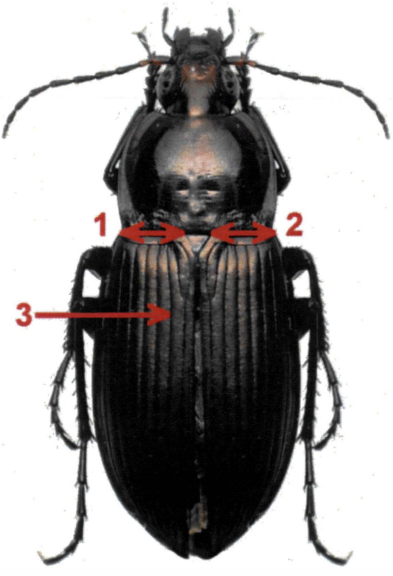

Figure 2. The scheme of dimensional and meristic traits estimation: 1 – the width of the left elytra, 2 – the width of the right elytra, 3 – furrows on *Poec. cupreus* elytra (as illustration).

We used LM to recognize what kind of biotope affected FA in ground beetles and how it is connected with species specificity and sex:

lm(formula = FA_.dimensional ~ fBiotope * fSpecies + fBiotope * FA_.dimensional ~ fBiotope * fSpecies + fBiotope * fSex

lm(formula = FA_.dimensional ~ fBiotope + fSpecies + fSex, data = p) FA_.dimensional ~ fBiotope + fSpecies + fSex

lm(formula = FA_meristic ~ fBiotope * fSpecies + fBiotope * fSex, data = p) FA_meristic ~ fBiotope * fSpecies + fBiotope * fSex

lm(formula = FA_meristic ~ fBiotope + fSpecies + fSex, data = p) FA_meristic ~ fBiotope + fSpecies + fSex

Results

FA in the dimensional trait appeared to be affected by species*biotope, but not biotope*sex interactions (Table 2). In other words, FA level in the beetles from the same type of biotope was depended on species, but it was not dependent on those beetles' sex. This is especially clear for *C. granulatus, C. cancellatus* and *P. melanarius.*

Table 2. ANOVA results when studying species*biotope and biotope*sex interactions in FA in the dimensional trait in studied species of carabids.
FA_.dimensional ~ fBiotope * fSpecies + fBiotope * fSex

Source	Df	Sum of Sq	RSS	AIC	Fvalue	Pr(>F)	
<none>			6.8809	-15950			
fBiotope:fSpecies	10	0.38842	7.2694	-15823	14.9590	< 2e-16	***
fBiotope:fSex	7	0.03617	6.9171	-15950	1.9899	0.05288	.

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

This conclusion became more pronounced when modeling different variables' impact against the background of others (Table 3): biotope type and species affected FA significantly in studied samples. This was again especially clear for *C. granulatus, C. cancellatus* and all types of biotopes except shrubs and swamp.

Table 3. ANOVA results when studying the influence of biotope, species and sex on FA in the dimensional trait in studied species of carabids.
FA_.dimensional ~ fBiotope + fSpecies + fSex

Source	Df	Sum of Sq	RSS	AIC	Fvalue	Pr(>F)	
<none>			7.3023	-15825			
fBiotope	8	1.24616	8.5485	-15418	56.8914	<2e-16	***
fSpecies	8	0.80809	8.1104	-15559	36.8917	<2e-16	***
fSex	1	0.00645	7.3088	-15825	2.3572	0.1248	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

FA in meristic traits was affected by species*biotope, but not biotope*sex interactions, similar to the dimensional trait, though with lower significance. Intercepts in all models were significant, as well as biotope and species impact on FA. But we did not find interactions biotope*species and biotope*sex affecting the FA value.. So FA in meristic traits was practically independent of biotope type where the carabid species dwelled.

Table 4. ANOVA results when studying species*biotope and biotope*sex interactions in FA for meristic traits in studied species of carabids.
FA_meristic ~ fBiotope * fSpecies + fBiotope * fSex

Source	Df	Sum of Sq	RSS	AIC	Fvalue	Pr(>F)	
<none>			5.8319	-16380			
fBiotope:fSpecies	10	0.045564	5.8775	-16380	2.0688	0.02376	*
fBiotope:fSex	7	0.010954	5.8428	-16389	0.7106	0.66314	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

That conclusion was confirmed by the next analysis (Table 5): FA in meristic traits was species-specific in all studied species.

Table 5. ANOVA results when studying the influence of biotope, species and sex on FA in meristic traits in studied species of carabids.
FA_meristic ~ fBiotope + fSpecies + fSex

Source	Df	Sum of Sq	RSS	AIC	Fvalue	Pr(>F)	
<none>			5.8941	-16386			
fBiotope	8	0.02657	5.9207	-16390	1.5014	0.1513	
fSpecies	8	0.83126	6.7254	-16048	46.9817	<2e-16	***
fSex	1	0.00082	5.8949	-16388	0.3707	0.5427	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Similarly, we analyzed the data set for *Poec. cupreus*. FA in the dimensional trait was significantly affected by biotope, but not biotope*sex interactions, sex by itself did not affect FA (Tables 6,7, S5,6). In meristic traits, on the contrary, FA was significantly affected by biotope, biotope*sex interaction, but not by sex by itself (Tables 8, 9).

Table 6. ANOVA results when studying biotope*sex interactions in FA in the dimensional trait in *Poec. cupreus*. FA_dimensional ~ fBiotope * fSex

Source	Df	Sum of Sq	RSS	AIC	Fvalue	Pr(>F)
<none>			0.81375	-13332		
fBiotope:fSex	8	0.0038391	0.81759	-13340	1.0173	0.4206

Table 7. ANOVA results when studying the influence of biotope and sex on FA in the dimensional trait in *Poec. cupreus*.
FA_dimensional ~ fBiotope + fSex

Source	Df	Sum of Sq	RSS	AIC	Fvalue	Pr(>F)	
<none>			0.81759	-13340			
fBiotope	8	0.089004	0.90659	-13176	23.582	<2e-16	***
fSex	1	0.000327	0.81792	-13341	0.694	0.4049	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 8. ANOVA results when studying biotope*sex interactions in FA in the dimensional trait in *Poec. cupreus*. FA_meristic ~ fBiotope * fSex

Source	Df	Sum of Sq	RSS	AIC	Fvalue	Pr(>F)	
<none>			0.69716	-13601			
fBiotope:fSex	8	0.010471	0.70763	-13591	3.2387	0.001154	**

Signif.codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 9. ANOVA results when studying the influence of biotope and sex on FA in the dimensional trait in *Poec. cupreus*. FA_meristic ~ fBiotope + fSex

Source	Df	Sum of Sq	RSS	AIC	Fvalue	Pr(>F)	
<none>			0.70763	-13591			
fBiotope	8	0.095860	0.80349	-13386	29.3455	<2e-16	***
fSex	1	0.000472	0.70810	-13592	1.1563	0.2824	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

The actual values of FA in different species and in different biotopes are presented in the following series of figures.

In *C. granulatus* FA in the dimensional trait in females followed the pattern: linden=shrubs=birch<meadow<lawn, that is: the highest FA level was in the lawn. In males FA was roughly speaking similar in all biotopes (Figure 3). If we exclude some biotopes from the analysis, due small sample size (i.e. pine, birch, shrubs, swamp), then we can obtain the following picture of dimensional trait FA variation in different biotopes: linden<meadow<lawn. It is noteworthy that for meristic traits in the females relation was the opposite: lawn=meadow<linden. For *C. granulatus* the opposite trend occurred: FA in the dimensional trait was the highest in the forest biotope and in meristic traits – in the open one.

In *C. cancellatus* FA in the dimensional trait in females followed the pattern: spring wheat<linden<birch<lawn<raspberry (biotope "pine" was ignored because of the small sample). In males the tendencies of FA variation were similar (Figure 4).

In meristic traits (similar to the previous species – *C. granulatus*) there was the opposite trend of FA in dimensional and meristic traits: the highest FA in *C. cancellatus* dimensional trait was observed in open habitats (lawn), and in meristic traits – in forest (linden).

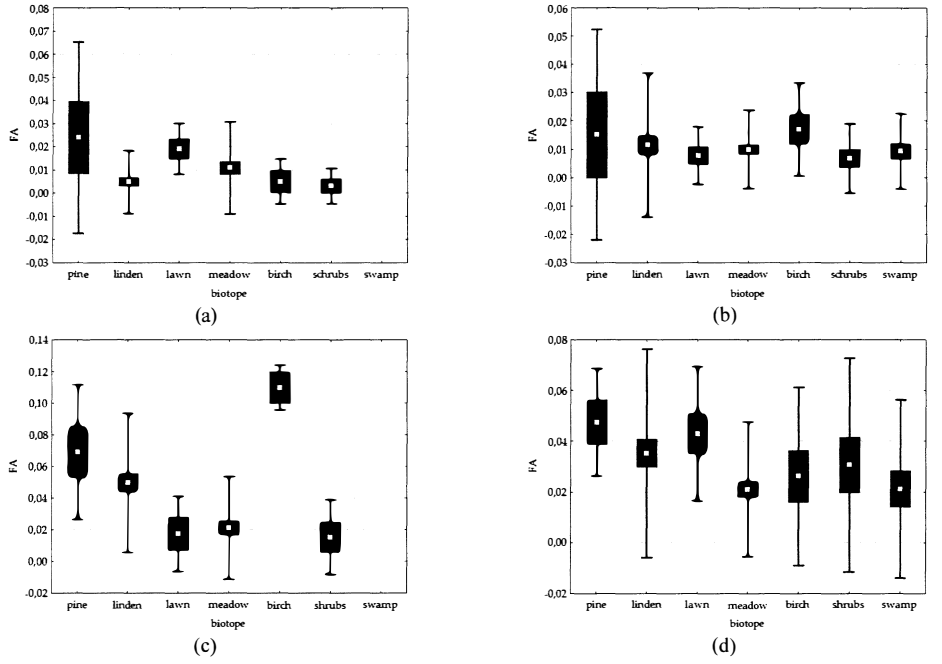

Figure 3. FA in *C. granulatus* in different biotopes. Mean value (square), mean±SE (boxes), mean±SD (whiskers); a, c – females; b, d – males; a, b, – dimensional trait, c, d – meristic traits.

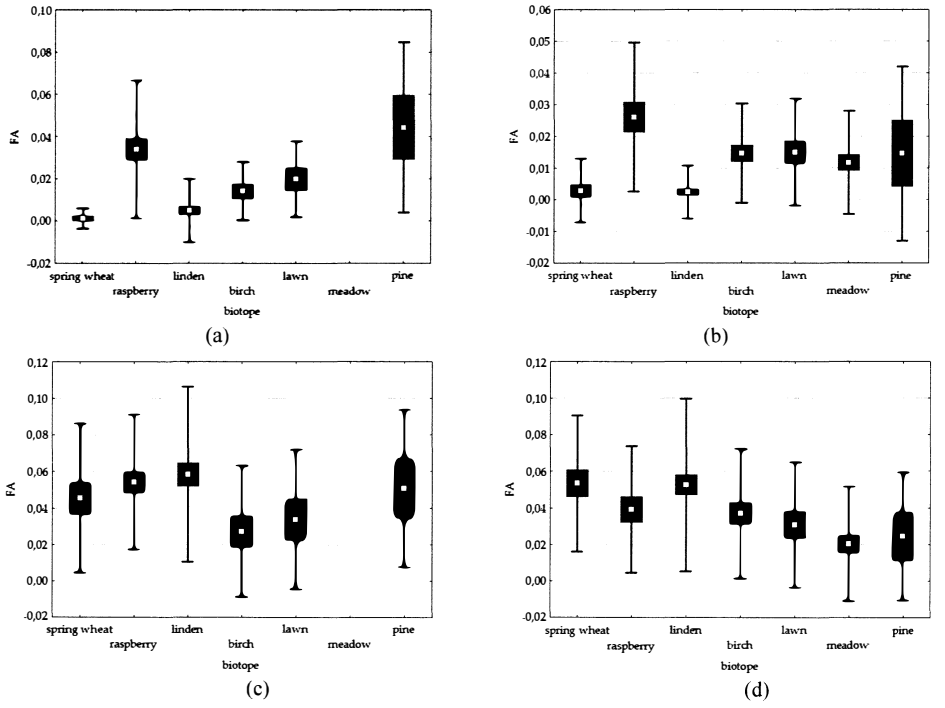

Figure 4. FA in *C. cancellatus* in different biotopes. Mean value (square), mean±SE (boxes), mean±SD (whiskers); a, c – females; b, d – males; a, b, – dimensional trait, c, d – meristic traits.

C. aeruginosus did not show any differences in FA values in different biotopes neither in females nor in males (Figure 5).

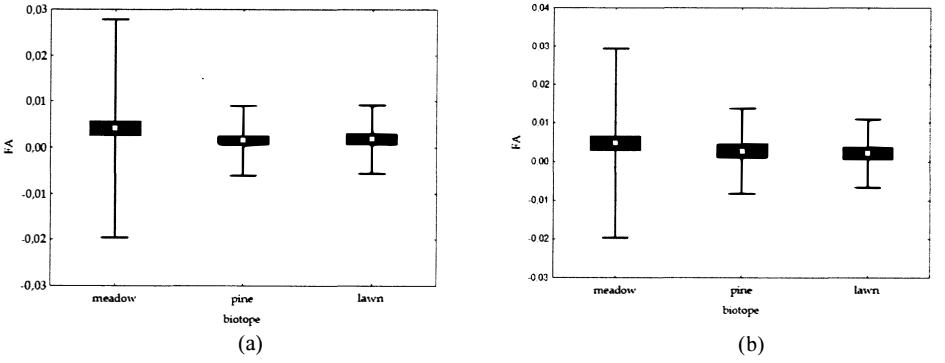

Figure 5. FA of dimensional trait in *C. aeruginosus* in different biotopes. Mean value (square), mean±SE (boxes), mean±SD (whiskers); a – females; b – males.

In *C. arvensis* in a single type of biotope FA was significantly higher in males than in females in studied traits (Figure 6), but in *P. niger* in the same biotope FA was similar in males and females (Figure 7).

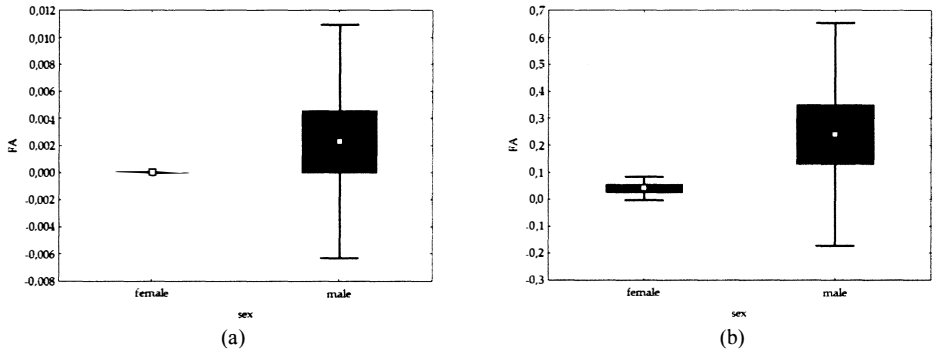

Figure 6. FA of dimensional trait in *C.* arvensis in different biotopes. Mean value (square), mean±SE (boxes), mean±SD (whiskers); a – females; b – males.

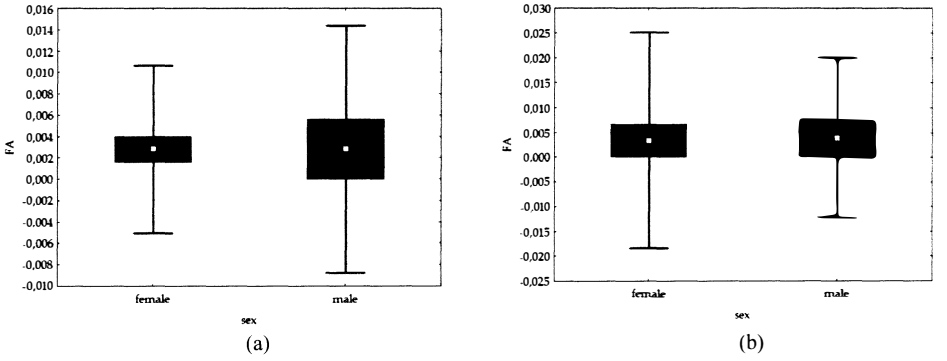

Figure 7. FA in *P. niger* in different biotopes. Mean value (square), mean±SE (boxes), mean±SD (whiskers); a – dimensional trait; b – meristic trait.

In *P. melanarius* FA in both traits and in females and males also followed the pattern: birch<pine<linden<meadow, thus, FA being the highest in the open biotope (Figure 8).

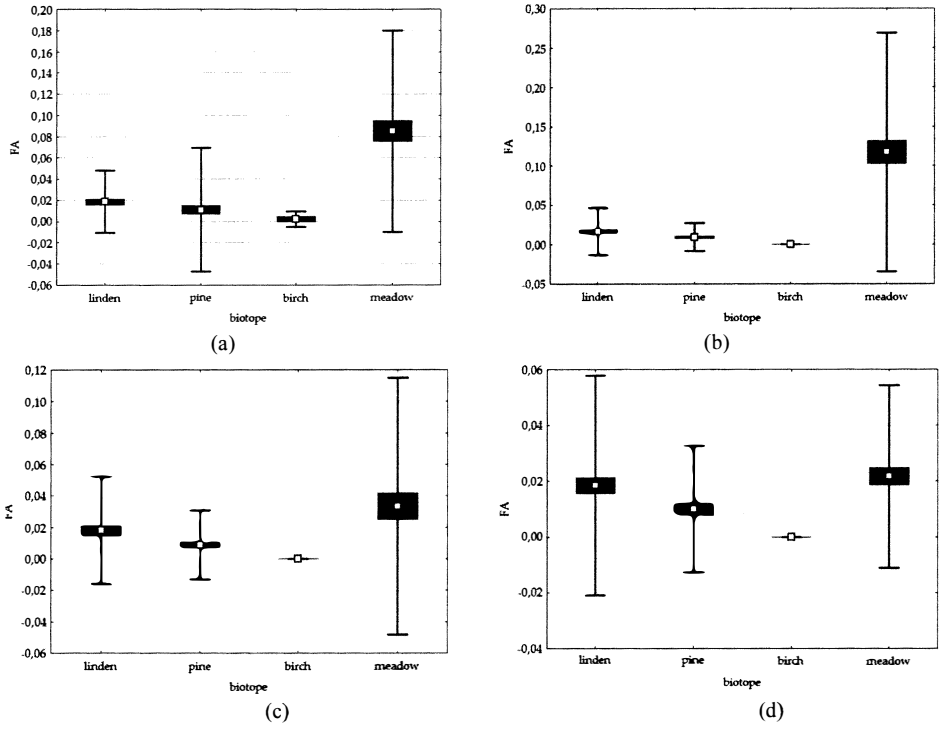

Figure 8. FA in *P. melanarius* in different biotopes. Mean value (square), mean±SE (boxes), mean±SD (whiskers); a, c – females; b, d – males; a, b, – dimensional trait, c, d – meristic traits.

The opposite trends in the dimensional trait were observed in *P. oblongopunctatus*, the highest value of FA being in open biotope (lawn) and the lowest – in the forest (pine) (Figure 9). But in meristic traits FA values were the highest in the forest biotopes (pine, linden), that is: the opposite trends in relation to two studied traits were revealed. Those trends were similar to those observed in *C. granulatus* and *C. cancellatus.*

In *Ps. rufipes* FA in both traits and in females and males also followed the pattern: raspberry<linden<pine (Figure 10). In the similarity of the variability of asymmetry for both traits it is similar to *P. melanarius*.

As for the last species studied – *Poec. cupreus* – the highest value of FA in it was in the meadow. It was true both for females and males in the dimensional trait and meristic traits (Figure 11–14).

Discussion

In a previous paper (Sukhodolskaya et al. 2019) significant influence of species, anthropogene, sex and locality on FA manifestation was shown. In this article a similar biotope effect is revealed. Especially important is that that effect

interacted with species. In other words, FA manifested differently in different species in the same biotope. This conclusion has the direct relation to bioindication where FA is used. That is to say, for instance, if we estimate FA in the *C. cancellatus* in the city biotopes (these are lawns and meadows as a rule) and in natural ones (linden, for instance), the high level of FA in the city populations will be determined by biotope peculiarities, but not by factor of "being the city". And the conclusions about the negative environment in the city will be wrong.

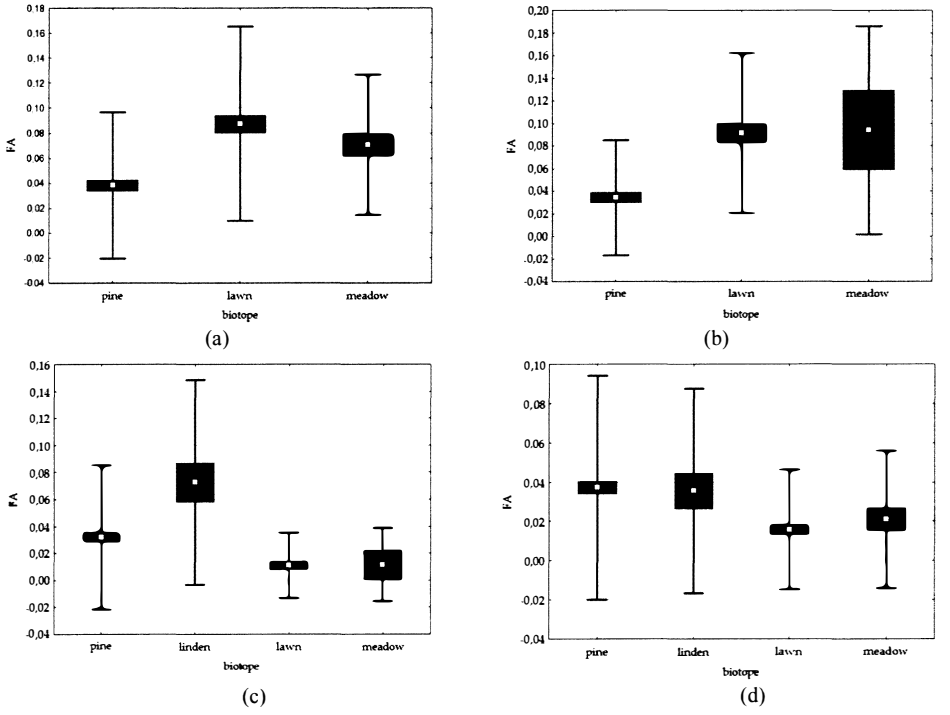

Figure 9. FA in *P. oblongopunctatus* in different biotopes. Mean value (square), mean±SE (boxes), mean±SD (whiskers); a, c – females; b, d – males; a, b, – dimensional trait, c, d – meristic traits.

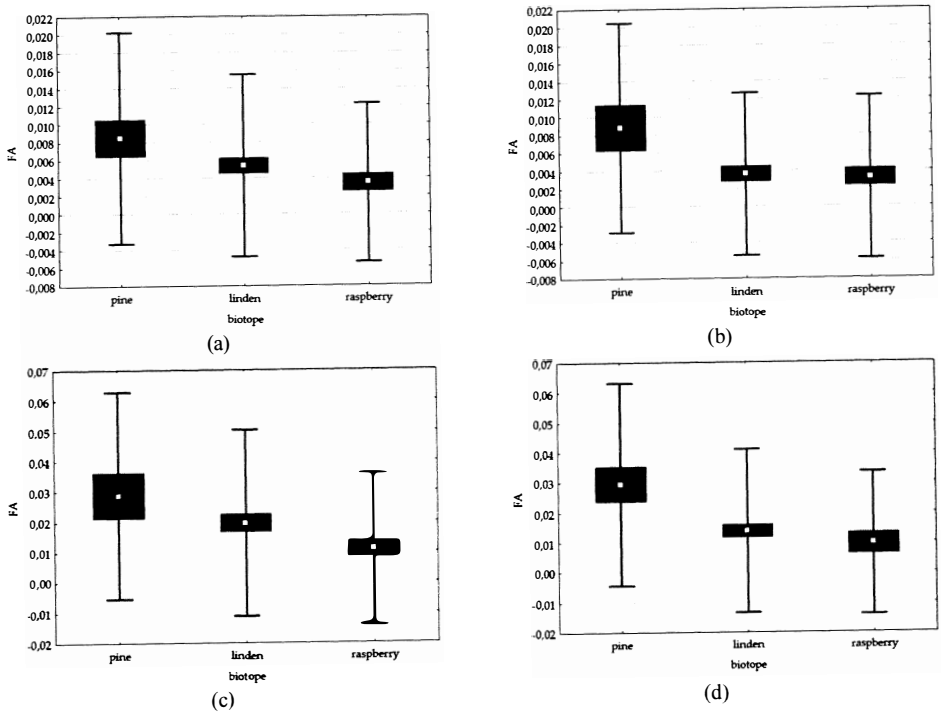

Figure 10. FA in *Ps. rifipes* in different biotopes. Mean value (square), mean±SE (boxes), mean±SD (whiskers); a, c – females; b, d – males; a, b, – dimensional trait, c, d – meristic traits.

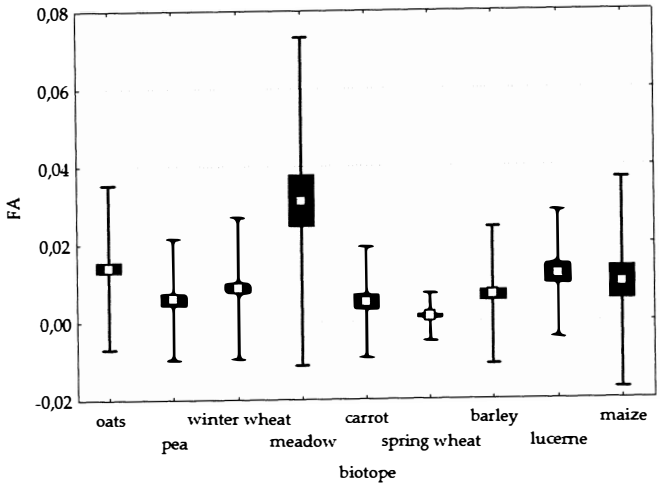

Figure 11. FA in females' dimensional trait in *Poec. cupreus* in different biotopes. Mean value (square), mean±SE (boxes), mean±SD (whiskers).

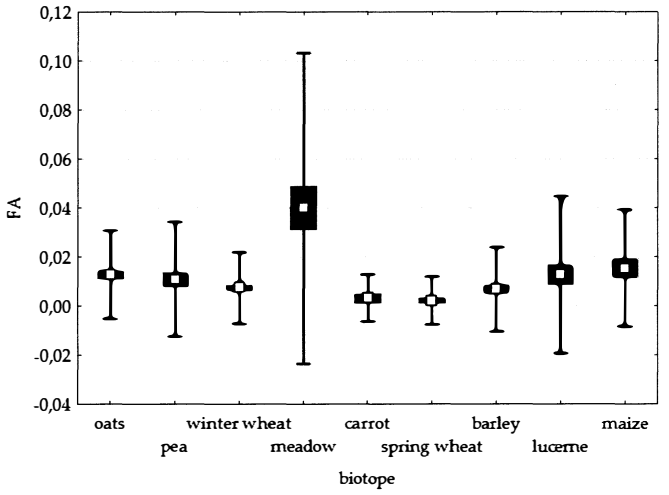

Figure 12. FA in males' dimensional trait in *Poec. cupreus* in different biotopes. Mean value (square), mean±SE (boxes), mean±SD (whiskers).

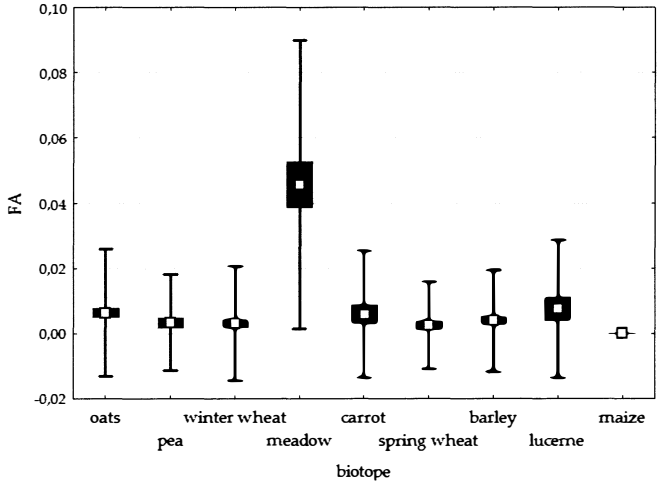

Figure 13. FA in females' meristic traits in *Poec. cupreus* in different biotopes. Mean value (square), mean±SE (boxes), mean±SD (whiskers).

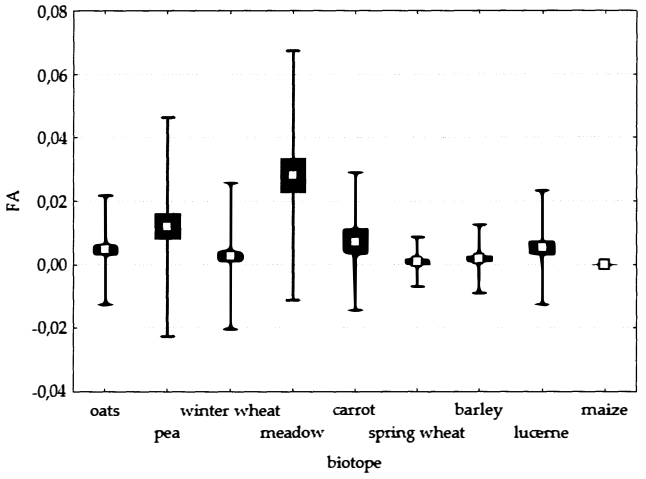

Figure 14. FA in males' meristic traita in *Poec. cupreus* in different biotopes. Mean value (square), mean±SE (boxes), mean±SD (whiskers).

In our work we used modeling not by chance. Environmental factors are unpredictable and occur at random in time and space. The course of the weather or disruptive changes in the environment are environmental factors that can affect the FA in insects. In our data set we compiled samples taken in different provinces, in gradient of anthropogenic pressure and in different years, so that possible random deviations of FA could neutralize each other and only the biotope's impact on FA variation could be estimated.

We confirmed the hypothesized biotope*species interaction affecting FA. Every species has its own preferences for humidity, light, canopy cover etc.

The negative relation of FA variation in dimensional and meristic traits in *C. granulatus* and *C. cancellatus* remains unexplained: in both species FA levels in dimensional and meristic traits changed in opposite in relation to biotope. And exactly in those species FA in males was higher than in females. In other studied species males responded to biotope characters about the same as females. So unlike the previous studies (Sukhodolskaya et al.2019) we did not find sex*biotope interaction in affecting FA in most cases. Perhaps it can be explained by species-level peculiarities of *C. granulatus* and *C. cancellatus.* Both are large forest zoophagous species and are very rarely met in open habitats and arable landscapes. These uncommon habitats affect those species negatively, including developmental stability, and lead to high FA values in lawns and raspberry (Figure 3, 4). *P. oblongopunctatus* is a forest species too. And the discussed negative effect on FA variation in dimensional and meristic traits was revealed in it too, though it is a relatively small species.

Unlike the forest species discussed above, *P. melanarius* and *Ps. rufipes* are ubiquists. They feel equally good both in forest and open biotopes. Perhaps due to their "generalist" characters they did not show differences in male/female

response to habitats and multidirectional variation of FA in dimensional and meristic traits (Figure 8, 10).

Separate part of the discussion should be devoted to *Poec. cupreus* because its data set was based on arable land habitats, which are characterized by soil and pesticide treatments. Crops provide different environmental conditions and food availability, which are two of the most important factors influencing carabid beetle distribution (Luff 1987; Holland 2002; Thomas et al. 2002). In addition, crops also differ in the intensity and timing of management practices, such as soil tillage and harvesting, which are determined by crop successions (Joannon et al. 2008). Agricultural practices have been shown to influence carabid beetle abundance either directly, through mortality and emigration, or indirectly, by affecting local microhabitat conditions (Kromp 1999; Cole et.al 2002; Thorbek and Bilde 2004; Hatten et al. 2007). Accordingly, studies which investigated the effect of crop management practices on the species composition of carabid communities concluded that the crop type was one of the most influential factors (Booij and Noorlander 1992; Weibull and Östman 2003; Eyre et al. 2013). Taking into account all these factors it was not surprising that FA in *P. cupreus* dimensional and meristic traits varied greatly in different crops. But some conclusions can be drawn: the lowest FA was observed in spring wheat (as in *C. cancellatus*, by the way). The low soil disturbance in that crop can lead to the more stable development of beetles, especially their larva. The second feature was the highest FA in both traits and in both sexes in the meadows. That fact can be related to the natural conditions in meadows, where *P. cupreus* has its natural enemies and competitors, unlike arable lands where it is the eudominant. And the last feature of FA variation in this species: in tall crops (lucerne, maize) FA standard error was significantly higher, suggesting that those kinds of crops affected beetles selectively.

So we confirmed three out of four hypothesized suggestions: factor "biotope" and its interaction with factor "species" affected FA in ground beetles, the latter being higher in uncommon biotopes. FA was higher in males than in females in a small number of cases.

Conclusions

The use of FA as a bioindicator of environmental stress is complicated by the fact that information on the basic ecology of individual species is relatively scant and contradictory for even the most common species. Revealed habitat impact on FA values in ground beetles demands a detailed understanding of species distribution among habitats at different life stages. The latter can provide insights into their ecological requirements, possibly allowing to design ecological strategies of management through environmental engineering.

Author Contributions

Conceptualization, R.A.S.,N.I.E. and N.L.U.; methodology, R.A.S. and A.A.S.; software, A.A.S.; validation, R.R.Shagidullin; formal analysis, T.A.G. and N.L.U.; investigation, I.A.S., A.L.A. and I.G.V.; resources, I.A.S., A.L.A., N.I.E. and I.G.V; data curation, R.R.S.; writing—original draft preparation, R.A.S. and A.L.A.; writing—review and editing, R.A.S. N.I.E. and T.A.G.; visualization, T.A.G.; supervision, R.A.S. N.I.E. and A.A.S.; project administration, R.R.S.; funding acquisition, N.L.U.

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CHANGES IN BIRD COMMUNITIES THROUGHOUT SECONDARY BILBERRY PINE FOREST SUCCESSION IN SOUTH-WESTERN BELARUS

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Abstract

The article tracks the changes in the bird population during secondary succession of cleared bilberry pine forest. The field work was performed in the years 1996–2018 applying the conventional bird count methods. The study revealed that the bird species diversity in the course of succession (6 stages, 1–90 years old) increased from 10 to 45 species, total abundance – from 159.2±1.25 to 687.0±3.80 birds/km², total biomass – from 5.71±0.25 to 31.34±1.10 kg/km². The ornithological diversity included six faunal types. At the initial stages of succession the bird population comprised the European, European-Turkestan and Palearctic types of fauna. At the stage of 80–90 years old the species structure (55.6%) and the total biomass (47.7%) were dominated by the Palearctic types of fauna, and the bird population (48.6%) was dominated by the European types of fauna.

Key words: succession; bilberry pine forest; ornithological diversity; dominant; rare bird species; Belarus

Introduction

Secondary successions of vegetation and avifauna of forest ecosystems have been studied most fully. With the appearance of fresh cutting or after a fire, there is a spatial redistribution of dendrophilous species that inhabited the forests prior to the deforestation, birds of open landscapes (meadows, fields, shrubs) and forest edges. The change in the bird population in the forests is caused by a consistent succession of phytocenoses, accompanied by a change in the life forms of the dominant plants. At present the main reason for the occurrence of successions in Belarus and other regions are anthropogenic factors (forestry activities, reclamation of agricultural lands adjacent to forests) (Głowaciński 1975, 1979, 1981; Abramova 2007). Cutting down trees on large land areas changes the habitat for birds radically.

The papers devoted to secondary successions of avifauna show that the diversity of birds increases in parallel with the succession of plant communities (Novikov et al. 1956; Danilov 1958; Johnson and Odum 1975; Bednorz and

Bogucki 1982; Helle and Mőnkkőnen 1986). The successions of various bird communities in forest ecosystems in Belarus and other regions have not been sufficiently studied. This paper aims to study the successions of bird populations in bilberry pine forests in the south-western part of the Belarusian Polesie, as well as to analyze the taxonomic and faunal structure of bird communities at different stages of succession.

Material and Methods

The material for this work was collected in 1996–2018 in south-western Belarus in the Brest timber enterprise (Tomashovka, Medno and Domachevo forestries), in the Malorita timber enterprise (Pozhezhin and Malorita forestries), in the Ivatsevichi timber enterprise (Ivatsevichi and Bronnaya Gora forestries), 52º–52º30′N 23°40′–25º30′E. This territory is located in the subzones of broad-leaved pine and hornbeam-oak dark coniferous forests.

The research was conducted in bilberry pine forests *Pinetum myrtillosum*, which occupy 9.5% of the pine forests of Belarus. Pine *Pinus sylvestris* predominates in the forest stand there; in admixture to it – Silver Birch *Betula pendula* (up to 30%), Common Aspen *Populus tremula* (up to 5%) and single trees of Common Oak *Quercus robur*, Common Hornbeam *Carpinus betulus*, Common Alder *Alnus glutinosa* (Lovchiy 2012). The undergrowth contains Bird Cherry *Prunus padus*, Common Rowan *Sorbus aucuparia*, Common Hazel *Corylus avellana*, Alder Buckthorn *Frangula alnus*, and others. In the shrub-grass cover, the background consists of European Blueberry *Vaccinium myrtillus*, Lingonberry *Vaccinium vitis-idaea*, Wood Small-reed *Calamagrostis epigeios*, False Lily of the Valley *Maianthemum bifolium*, Common Bracken *Pteridium aquilinum* and others. The moss layer is dominated by *Pleurozium schreberi*, *Dicranum polysetum*, *Polytrichum commune*, *Hylocomium splendens*, and others.

During the period of secondary succession in pine forests, we have identified six stages of vegetation development: from fresh cutting to mature forest of 90 years old:

1–3 years – grassy vegetation of meadow type (recently clear-cut area);

4–9 years – clear-cut area with plantation of birch, aspen and pine trees;

10–20 years – continuous thickets of bushes, shrubs and undergrowth;

30–40 years – young pine forest;

60–70 years – mature pine forest;

80–90 years – a high preclimax forest of pine with an admixture of birch, oak and other species.

Bird counts were conducted on routes with no capacity limit with subsequent calculation of the average population density according to the average detection range of the birds.

Transect lines (200 m wide, 1–2 km long) were laid in communities at various stages of succession. The first three stages (up to 20 years) of succession were traced on the same sites, and later – on sites occupied by pine phytocenoses of different ages with the same type of conditions. The total length of the routes

covered was 450 km. Generally accepted counting techniques were used for bird inventory (Ravkin 1967; Järvinen and Väisänen, 1976; Bibby et al. 2000). Counting was performed in clear weather in the morning (1 hour after sunrise) and in the evening (stopped 1–2 hours before sunset), when birds are most active, by mapping meetings at trial sites and transects. The calculation of the abundance of birds per area unit was carried out separately by average detection ranges (voice, visual). The dominant species were those whose share in the bird community is 10% or more. Indices of species similarity, species diversity and evenness were determined by well-known methods. Stability is here understood as in Jarvinen's (1979) study: the more stable a community is, the less its properties (e.g. density, diversity, densities of individual species) vary from year to year. To measure stability, we selected the following 3 criteria

1) Coefficient of variation (*CV*%) in bird density;
2) Species diversity (Shannon's index) (*H'*);
3) Evenness of species-abundance distribution (Pielou's evenness index) (*J'*).

Latin names of birds are given according to the Clements checklist of birds of the world: v2019. Faunal types are given according to K. Voous (1962).

Results and their discussion

In the first year, in the clear-cut areas the microclimate, species composition of herbaceous vegetation and invertebrates change dramatically. At the site of fresh cutting, 10 species were counted. Here there are birds of forest edges and open spaces: Tree Pipit *Anthus trivialis*, Meadow Pipit *Anthus pratensis*, Eurasian Skylark *Alauda arvensis*, Woodlark *Lullula arborea*, Whinchat *Saxicola rubetra* and White Wagtail *Motacilla alba* (Table 1).

Some birds (Tree Pipit, Common Redstart *Phoenicurus phoenicurus*, etc.) use cuttings as feeding stations. The total abundance on average is 159.2±1.25 birds/km^2, with the dominant species (Tree Pipit, Woodlark and Yellowhammer *Emberiza citrinella*) accounting for 67.4% of the bird population and 30.0% of the species composition (Table 2). The abundance of this group of birds species varies from 18.78 to 25.24 %. Background bird species have a higher level of abundance variation (CV up to 45.53 %). The total biomass is 5.71±0.25 kg/km^2, the biomass is dominated by Grey Partridge *Perdix perdix*, Tree Pipit and Yellowhammer; these three species account for 68.6% of the total indicator.

At the stage of clear-cut area with plantation of birch, aspen and pine trees (4–9 years), birds of shrubby thickets appear: Common Whitethroat *Sylvia communis*, Eurasian Blackcap *Sylvia atricapilla*, Common Linnet *Carduelis cannabina*, Red-backed Shrike *Lanius collurio* and others. The number of species increases to 18, the total abundance is 218.8±1.28 birds/km^2. The composition of the dominant species does not change in comparison with the previous stage, they make up 56.8% of the bird population and 16.7% of the species composition. The abundance of species varies significantly (from 14.67 to 135.00 %). The abundance is more stable in Woodlark (CV = 14.67 %), Tree Pipit (CV = 17.70%) and Meadow Pipit (CV = 17.95%). An extremely high level of variation in this

parameter (more than 90%) was observed in three species: European Goldfinch *Carduelis carduelis*, Spotted Flycatcher *Muscicapa striata* and Corn Crake *Crex crex*, with their abundance less than 1.5 birds/km^2. The biomass is dominated by Grey Partridge, Tree Pipit, Woodlark and Yellowhammer, which make up 66.6% of the total biomass (6.46±0.28 kg/km^2).

20 species of birds were recorded in dense thickets of bushes (10–20 years old). Birds of the edges and open spaces (Meadow Pipit, Whinchat, Eurasian Skylark, Grey Partridge) begin to fall out from the community. They are replaced by birds of tree and shrub tiers. The total abundance is significantly reduced – 179.1±1.40 birds/km^2. The share of dominant species (Tree Pipit, Yellowhammer, Common Chaffinch *Fringilla coelebs*) in the total abundance is 38.1% (Table 2). The variability of abundance of this group of bird species ranges from 20.45 to 25.60 %. High variability of abundance is typical for species with low population, the abundance of which does not exceed 5 birds/km^2. The biomass is dominated by Common Blackbird *Turdus merula* and Song Thrush *Turdus philomelos*, Common Chaffinch, Yellowhammer and Tree Pipit; their share in the total biomass (5.08±0.26 kg/km^2) is 69.7%.

By 30–40 years, the bird community acquires a characteristic appearance for this type of forest, the number of species increases to 27. The total abundance is 1.3 times higher than at the previous stage. As the age of the main forest-forming species (common pine) increases, typical forest bird species begin to dominate the bird community. At this stage, Common Chaffinch dominates by abundance (50,0±3,14 birds/km^2, CV = 21,76 %). The lower the abundance, the greater the role of stochastic variations (random fluctuations), so in species with an abundance of less than 2 birds/km^2, the CV ranges from 63.30% to 119.5%. The dominant biomass species are Common Blackbird, Common Chaffinch, Fieldfare *Turdus pilaris* and Carrion Crow *Corvus corone*, which together account for 59.5% of the total biomass of the bird community.

Table 1. Population (a, birds/km²) of bird communities at different stages of secondary succession of bilberry pine forests.

Species	Age of biocenoses (years)											
	1–3		4–9		10–20		30–40		60–70		80–90	
	± x	CV	± x	CV	± x	CV	± x	CV	± x	CV	± x	CV
Motacilla alba	9.4±0.69	25.53	10.4±0.80	27.10	3.5±0.45	44.00						
Anthus trivialis	61.7±3.35	18.78	65.3±3.34	17.70	25.2±1.52	20.45	12.5±0.99	27.52	71.4±3.40	16.48	72.0±3.05	14.65
Anthus pratensis	8.4±0.68	28.09	6.5±0.65	34.30								
Saxicola rubetra	11.2±0.75	23.25	15.1±0.78	17.95								
Perdix perdix	4.7±0.62	45.53	3.2±0.56	60.93								
Alaunda arvensis	15.0±0.98	25.53	11.3±0.77	23.71								
Lullula arborea	25.0±1.40	19.56	30.6±1.38	14.67	15.4±1.15	25.84	8.2±0.82	34.33	3.0±0.59	67.66	3.2±0.58	62.50
Lanius collurio			2.6±0.45	60.00	1.8±0.39	75.50						
Emberiza citrinella	20.6±1.50	25.24	28.4±1.51	18.38	18.5±1.35	25.30						
Phoenicurus phoenicurus	2.4±0.47	67.50	5.8±0.57	34.00	3.2±0.59	63.75			4.3±0.58	47.21	10.6±1.15	37.64
Carduelis cannabina			9.7±0.83	29.70	1.0±0.30	50.54						
Carduelis carduelis			0.6±0.15	135.00	1.8±0.39	76.11						
Erithacus rubecula			7.0±0.64	31.70	10.2±0.70	23.70	5.8±0.69	41.30	10.3±0.57	19.20	14.1±0.88	21.56
Turdus philomelos			2.0±0.36	63.00	7.0±0.52	25.60	8.1±0.51	21.60	36.9±1.59	14.93	38.6±1.41	12.59
Regulus regulus									4.0±0.62	53.75	6.0±0.53	30.05
Turdus merula					13.8±1.09	27.53	16.7±1.19	24.67	18.5±1.17	21.84	20.4±1.16	19.60
Turdus pilaris							13.1±1.33	35.10	15.8±1.38	80.32	16.2±1.26	27.08
Sylvia communis			9.8±0.95	33.57	15.6±1.12	24.80	4.2±0.51	42.38				
Sylvia atricapilla			7.7±0.83	37.27	12.4±1.05	29.35	8.5±0.68	27.50	14.6±1.02	24.22	15.2±0.96	21.84
Certhia familiaris							3.6±0.59	56.34	8.2±0.64	26.42	13.8±0.0.30	23.55
Sitta europaea							2.0±0.58	101.00	5.4±0.74	29.62	2.3±0.37	56.10

Species	Age of biocenoses (years)											
	1–3		4–9		10–20		30–40		60–70		80–90	
	± x	CV	± x	CV	± x	CV	± x	CV	± x	CV	± x	CV
Troglodytes troglodytes							1.5±0.27	63.30	4.2±0.38	33.09	5.7±0.43	25.96
Fringilla coelebs					24.5±1.81	25.60	50.0±3.14	21.76	160.2±6.75	14.59	166.6±6.96	11.17
Parus major					6.3±0.53	28.86	16.8±0.96	19.88	30.6±1.49	16.83	38.7±1.58	14.30
Lophophanes cristatus							2.2±0.39	61.80	6.7±0.71	36.57	9.4±0.82	30.10
Poecile montanus					3.7±0.58	54.05	6.2±0.59	32.90	18.8±1.21	22.40	20.2±1.22	20.89
Poecile palustris									1.0±0.25	86.00	1.4±0.28	69.28
Cyanistes cyanus							1.0±0.25	86.00	2.2±0.39	61.80	2.0±0.36	63.00
Aegithalos caudatus							2.7±0.41	52.22	4.6±0.61	45.87	8.5±0.64	27.05
Phylloscopus trochilus					3.8±0.45	40.79	13.0±1.25	38.40	24.3±1.35	19.30	32.7±1.33	14.09
Phylloscopus sibilatrix					4.5±0.62	39.63	12.6±1.04	28.49	60.5±2.53	14.50	64.4±0.2.89	15.43
Phylloscopus collybita					3.7±0.58	54.05	18.2±1.06	20.10	40.6±1.48	12.58	42.5±1.57	12.80
Muscicapa striata			1.4±0.38	94.28	3.2±0.77	78.43	6.5±0.87	46.15	14.3±1.46	35.45	22.4±1.59	24.64
Ficedula parva									0.6±0.20	115.00	0.8±0.24	102.80
Ficedula hypoleuca							3.4±0.76	77.64	5.9±0.77	44.91	8.0±1.01	43.75
Oriolus oriolus							3.2±0.77	78.43	6.0±0.91	52.67	6.5±1.08	57.54
Garrulus glandarius							1.5±0.41	94.00	4.2±0.61	52.85	8.5±1.29	52.47
Corvus corone							1.8±0.45	85.50	3.4±0.79	80.59	6.2±0.81	45.16
Pica pica									1.0±0.28	96.00	1.2±0.28	12.50
Corvus corax									1.0±0.29	100.00	1.4±0.51	95.00
Columba oenas									0.5±0.19	135.00	1.0±0.25	86.00
Caprimulgus europaeus									0.3±0.11	130.00	0.4±0.14	120.00
Picus canus							1.1±0.35	119.50	1.1±0.36	111.80	0.8±0.26	130.00
Dendrocopos major							4.5±0.59	45.60	8.0±0.97	42.08	8.5±0.99	49.26

Species	Age of biocenoses (years)											
	1–3		4–9		10–20		30–40		60–70		80–90	
	± x	CV	± x	CV	± x	CV	± x	CV	± x	CV	± x	CV
Dryocopus martius									1.8±0.76	90.00	2.0±0.58	101.80
*Dendrocopos medius**									2.0±0.61	105.50	2.4±0.69	100.00
Bonasa bonasia									1.6±0.55	118.75	3.7±0.58	54.60
Scolopax rusticola									1.9±0.56	102.63	2.0±0.65	112.00
*Ciconia nigra**									0.5±0.18	124.00	0.7±0.22	108.60
Cuculus canorus									1.0±0.30	102.00	1.2±0.32	91.70
*Crex crex**	0.8±0.26	112.10	1.4±0.40	99.70								
Accipiter gentilis									1.0±0.33	112.90	1.1±0.35	110.90
Accipiter nisus									1.4±0.41	102.10	1.6±0.46	99.37
Buteo buteo									1.0±0.38	132.00	0.8±0.32	140.00
Falco subbuteo *									0.6±0.21	123.30	0.5±0.20	142.00
Pernis apivorus									0.8±0.27	116.20	0.9±0.31	118.90
Number of species	10		18		20		27		45		45	
Total abundance, birds/km^2	159.2±1.25		218.8±1.28		179.1±1.40		228.9±1.94		606.0±3.56		687.1±3.80	
Total biomass, kg/km^2	5.71±0.25		6.46±0.28		5.08±0.26		8.39±0.30		25.71±0.93		31.32±1.10	
Species diversity (H')	2.67		3.36		3.86		4.10		3.99		4.14	
Evenness of species-abundance distribution (J')	0.80		0.81		0.89		0.86		0.73		0.75	

Note: * – species listed in the Red Book of the Republic of Belarus (2015)

Table 2. Dynamics of bird species dominance in bilberry pine forests in summer during succession (% of total abundance and total biomass).

Species	Indicator	Age of biocenoses (years)					
		1–3	4–9	10–20	30–40	60–70	80–90
Anthus trivialis	abundance	38.8	29.8	14.1	-	11.8	10.5
	biomass	24.9	23.2	11.4	-	-	-
Emberiza citrinella	abundance	12.9	13.0	10.3	-	-	-
	biomass	10.8	13.2	10.9	-	-	-
Lullula arborea	abundance	15.7	14.0	-	-	-	-
	biomass	-	10.4	-	-	-	-
Fringilla coelebs	abundance	-	-	13.7	21.8	26.4	24.2
	biomass	-	-	10.6	13.1	13.7	11.7
Phylloscopus sibilatrix	abundance	-	-	-	-	10.0	-
	biomass	-	-	-	-	-	-
Perdix perdix	abundance	-	-	-	-	-	-
	biomass	32.9	19.8	-	-	-	-
Turdus merula	abundance	-	-	-	-	-	-
	biomass	-	-	26.6	19.5	-	-
Turdus philomelos	abundance	-	-	-	-	-	-
	biomass	-	-	10.2	-	10.6	-
Turdus pilaris	abundance	-	-	-	-	-	-
	biomass	-	-	-	15.6	-	-
Corvus corone	abundance	-	-	-	-	-	-
	biomass	-	-	-	11.3	-	10.4

A further increase in the main total indicators (number of species, abundance, biomass) was observed in the middle-aged forest (60–70 years). At this stage, the bird community is enriched with new species, and the abundance of birds in the bilberry pine forest increases. The total abundance reaches 606.0±3.56 birds/km^2, the total biomass is 25.71±0.93 kg/km^2. The bird population is dominated by Common Chaffinch (160,2±6,75 birds/km^2, CV = 14,59 %), Tree Pipit (71,4±3,40 birds/km^2, CV = 16,48 %) and Wood Warbler *Phylloscopus sibilatrix* (60,5±2,53 birds/km^2, CV = 14,50 %). By total abundance, they account for 48.2%. A group of species with low abundance (1–10 birds/km^2) is formed by 26 species, most of them characterized by high rates of abundance variability (CV up to 118.75 %). This indicator is especially high (up to 135.00 %) for rare species with an abundance of less than 1 bird/km^2. By biomass, Common Chaffinch (13.7%) and Song Thrush (10.6%) dominate.

At the stage of the preclimax forest (80–90 years), these indicators are slightly higher (Tables 1 and 2). The total abundance is 687.1±3.80 birds/km^2 and the biomass is 31.32±1.10 kg/km^2. The abundance is dominated by Common Chaffinch

(166,6±6,96 birds/km^2, CV = 11,17 %) and Tree Pipit (72,0±3,05 birds/km^2, CV = 14,65 %), while the biomass is dominated by Common Chaffinch and Carrion Crow (Table 2).

Thus, each of the 6 selected stages of succession is characterized by certain dominant species (Table 2). As the cuttings are overgrown with young saplings, birds of open ecosystems disappear or become scarce, and they are not recorded at later stages of succession. On the contrary, tree and shrub birds (typical warblers, warblers, Common Chaffinch, etc.) become numerous. In mature pine forest and high preclimax forest Common Chaffinch dominates.

At various stages of succession, there are bird species belonging to 11 orders (Table 3). At all stages of succession, the Passeriform birds predominate, accounting for 66.8–100.0% of the species diversity. At the fifth and sixth stages of succession, the Accipitriformes and the Piciformes are in the second place in terms of the number of species (8.9% each). The Passeriform birds also dominate the bird population (in terms of abundance and biomass).

Table 3. Participation of bird species of different orders in species diversity, total abundance and total biomass (%) at different stages of succession of bilberry pine forests.

Orders	Indicator	Age of biocenoses (years)					
		1–3	4–9	10–20	30–40	60–70	80–90
Galliformes	number of species	10.0	5.6	-	-	2.2	2.2
	abundance	3.0	1.5	-	-	0.3	0.5
	biomass	32.9	19.8	-	-	2.4	4.5
Ciconiiformes	number of species	-	-	-	-	2.2	2.2
	abundance	-	-	-	-	0.1	0.1
	biomass	-	-	-	-	5.8	6.7
Accipitriformes	number of species	-	-	-	-	8.9	8.9
	abundance	-	-	-	-	0.7	0.6
	biomass	-	-	-	-	9.9	8.4
Falconiformes	number of species	-	-	-	-	2.2	2.2
	abundance	-	-	-	-	0.1	0.1
	biomass	-	-	-	-	0.5	0.4
Columbiformes	number of species	-	-	-	-	2.2	2.2
	abundance	-	-	-	-	0.1	0.1
	biomass	-	-	-	-	0.5	0.9

Caprimulgiformes	number of species	-	-	-	-	2.2	2.2
	abundance	-	-	-	-	0.1	0.1
	biomass	-	-	-	-	0.1	0.1
Gruiformes	number of species	10.0	5.6	-	-	-	-
	abundance	0.5	0.6	-	-	-	-
	biomass	2.2	3.4	-	-	-	-
Charadriiformes	number of species	-	-	-	-	2.2	2.2
	abundance	-	-	-	-	0.3	0.3
	biomass	-	-	-	-	2.1	1.8
Cuculiformes	number of species	-	-	-	-	2.2	2.2
	abundance	-	-	-	-	0.2	0.2
	biomass	-	-	-	-	0.4	0.4
Piciformes	number of species	-	-	-	7.4	8.9	8.9
	abundance	-	-	-	2.5	2.1	2.0
	biomass	-	-	-	5.6	5.8	5.0
Passeriformes	number of species	80.0	88.8	100.0	92.6	66.8	66.8
	abundance	96.5	97.9	100.0	97.5	96.0	96.0
	biomass	64.9	76.8	100.0	94.4	72.5	71.8

At different stages of successions, 3 species were found included in the Red Book of the Republic of Belarus (2015): Eurasian Hobby *Falco subbuteo* (category IV), Corn Crake (category III), Black Stork *Ciconia nigra* (category III). 25 bird species registered during the study are of international conservation significance (SPEC), including Corn Crake (category I), Woodlark and Common Redstart (category II), Eurasian Skylark, Red-backed Shrike *Lanius collurio*, Spotted Flycatcher and Grey-headed Woodpecker *Picus canus* (category III) (European birds of conservation concern... 2017).

The ecological structure of bird communities changes parallel to the succession of vegetation cover (Figure 1). In the first stage (recently clear-cut area), ground-nesting birds predominate, with the exception of the Common Redstart, which collects food on the ground. Later the number of birds building nests on the ground or immediately above it gradually decreases, at the fifth and sixth stages of succession, the share of this group is 22.2%. This trend is not typical for birds that nest in bushes.

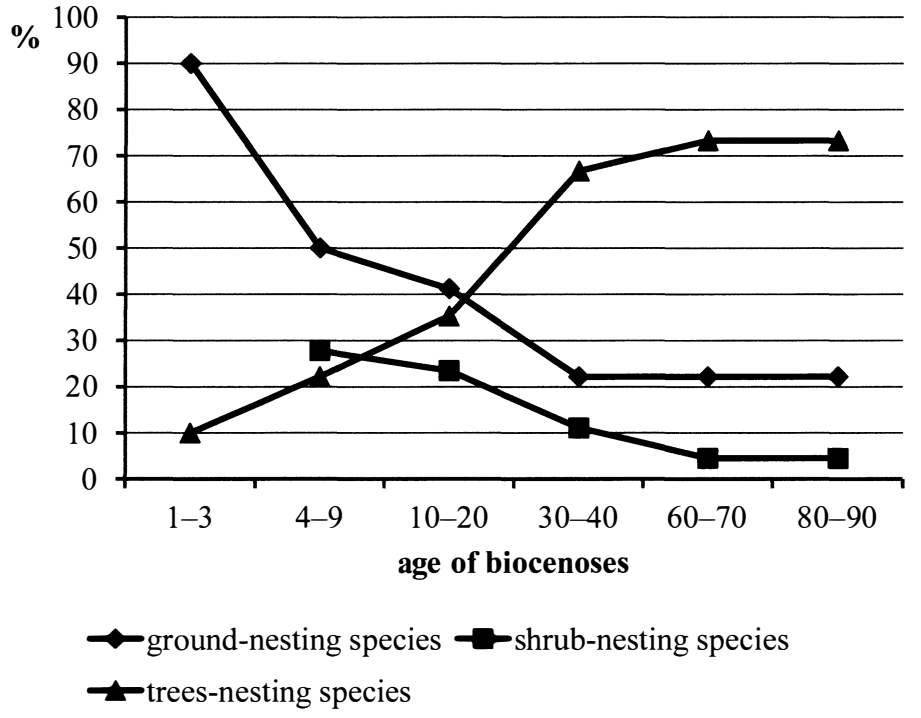

Figure 1. The distribution of bird species according to the levels of nesting in bilberry pine forests of different age.

On the contrary, the number of bird species building nests in the shrub and tree layer increases with the age of the main forest-forming species and reaches the maximum at the last two stages – 33 species (73.3%). In the high preclimax pine forest, birds populate all levels: there are birds that nest and forage on the ground, on fallen trees or dead wood, on shrubs and undergrowth, many species nest in hollows and crowns of trees, where they forage.

At the stages of recently clear-cut area (1–3 years) and clear-cut area with plantation of birch, aspen and pine trees (4–9 years), 3 types of fauna are represented in bird communities: European, European-Turkestan and Palearctic (Figure 2). The population is dominated by species of the European-Turkestan type of fauna (41.1–41.7% of the total abundance, 48.8–57.8% of the total biomass), in terms of the number of species, European-type species lead the way (38.9–50.0). Starting from the 10–20-year-old succession stage, the bird community is dominated by a complex of Palearctic faunal types (45.0–60.1% of the number of species, 35.9–48.6% of the total abundance, 49.7–65.7% of the total biomass). The participation of European faunal types in the species diversity and abundance of bird communities decreases with the development of succession (50.0–24.4%) and the share of birds in the total abundance increases (30.0–48.3% respectively). At the last two stages of succession, there are 6 types of fauna. The main contribution to the structure of bird communities is made by the Palearctic and

European types of fauna, the Holarctic type is represented by 2 species (Northern Goshawk *Accipiter gentilis* and Common Buzzard *Buteo buteo*), the Siberian and Afro-Eurasian types – by 1 species each (respectively, Hazel Grouse *Tetrastes bonasia* and Eurasian Golden Oriole *Oriolus oriolus*). The participation of birds of these three types of fauna in the species structure is no more than 4.4%, in the population of birds it does not exceed 1.5%, in the total biomass it is no more than 6.5%. Bird complexes of the Holarctic, Siberian and Afro-Eurasian faunal types make up no more than 4.4% of species, no more than 5.7% of the bird population, and no more than 15.6% of biomass.

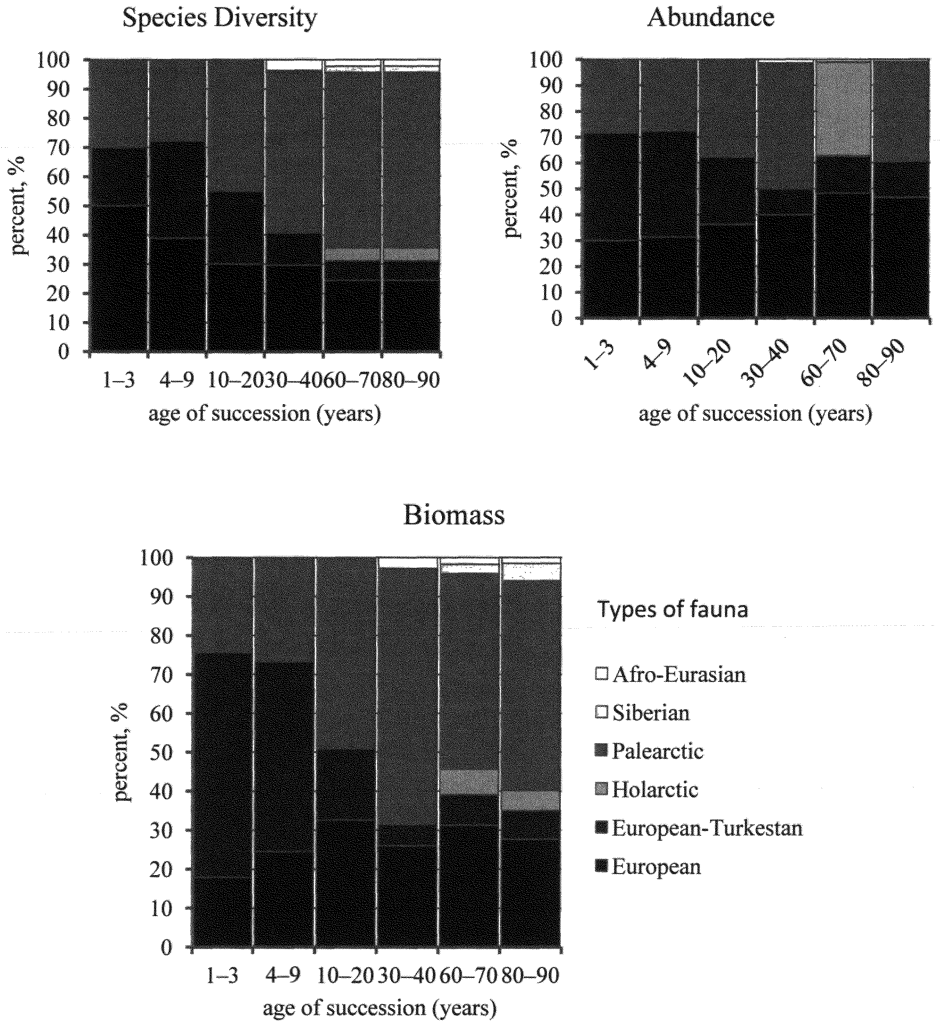

Figure 2. Faunal structure of the bird communities at different stages of succession of the bilberry pine forest.

An assessment of the similarity of species composition by the Jaccard index of bird community at different stages of secondary succession showed that the greatest similarity was observed between communities formed at the first two stages of succession (55.6), and at the age of 30–40 years or more (56.5–100.0%) (Table 4).

Table 4. The similarity of the bird communities at different stages of secondary succession in bilberry pine forests by the Jaccard index (%).

Age of succession, years	4–9	10–20	30–40	60–70	80–90
1–3	55.6	20.0	5.7	5.8	5.8
4–9		52.0	18.4	12.5	12.5
10–20			42.4	27.5	27.5
30–40				56.5	56.5
60–70					100.0

The Shannon diversity index increases with the growth of the stand and reaches the highest indicators at the last stages of succession (Table 1). Pielou's evenness index increases at the fi st four stages, and decreases slightly at the last two stages due to an increase in the share of the three dominant species, primarily Common Chaffinch, in the bird population (Tables 1 and 2).

Information about the summer avifauna of pine forests in south-western Belarus is available in monographs (Abramova 2007; Abramova and Haiduk 2009, 2013). It is shown (Abramova 2007) that in summer 38 species of birds live in bilberry-green moss pine forests in combination with shrub-longmoss pine forests (the Brest timber enterprise, Tomashovka forestry), the total abundance of which was 643.5 birds/km^2, the total biomass – 24.88 kg/km^2.

According to M. S. Dolbik (1974), 11 species of songbirds with a total number of 2.21 p/1 ha were recorded in bilberry pine forests in the territory of the Belarusian Polesie (Lelchitsy and Luninets districts), including Willow Warbler *Phylloscopus trochilus* – 0.80, Common Chaffinch – 0.37, Spotted Flycatcher – 0.22, Eurasian Golden Oriole – 0.14, European Pied Flycatcher – 0.14, other species – 0.07 p/1 ha. These numbers are equal to or significantly higher than our data and the data indicated by Ravkin et al. (2001), according to which the average total density of the bird population for the subclass of small-leaved forests is 669 birds/km^2.

The succession of avifauna of bilberry pine forests is similar to broad-leaved pine forests in terms of species structure and bird population (Abramova 2007). In broad-leaved pine forests, 6 stages were identified from recently clear-cut area to climax forest (120–150 years). The number of species ranged from 12 in recently clear-cut area to 60 in climax forests. The total abundance gradually increased during the succession from 67.4 to 934.1 birds/km^2, with the exception of the age stage of 10–14 years, when it was the lowest (107.4 birds/km^2). The total biomass changed in parallel with abundance, increasing from 3.4 to 54.2 kg/km^2.

The studied indicators are somewhat lower, especially at the last stages of succession (90–100 years) in spruce forests, where 59 bird species were identified, the total abundance of which is 689.5 birds/km^2, and the biomass is 47.7 kg/km^2 (Abramova 2017, 2018). The main ecological parameters of the bird population (species diversity, abundance, biomass) are progressively increasing from recently clear-cut areas to high preclimax forest. The exception is the stage of dense thickets of bushes, shrubs and undergrowth (9–14 years), where the total abundance and biomass of birds is lower than at previous and next stages.

For comparison, we present data on the state of bird communities in coniferous forests in other regions. In the Western Moscow region, according to systematic observations in 1956–1968, from 24 to 37 species of birds nest in pine forests, the population density of birds varies from 150 to 300 p/km^2 (Inozemtsev 1987). In the mossy pine forests of the Berezinsky biosphere reserve (Belarus) in the first half of the summer in 1986–1987, 31 species were registered, the total abundance was 470 birds/km^2, the dominant species by abundance were Common Chaffinch, Tree Pipit and European Crested Tit *Parus cristatus* (Byshnev 1989).

In the pine forests of Poland, at various stages of renewal after a recent clear-cut, it was found that the number of breeding bird species increases from 2 to 30, the number of pairs per 10 ha increases from 2.3 to 62.9 (Głowaciński 1979) and the community of breeding birds with a large species diversity is more stable (Głowaciński 1981).

In the coniferous forests of the southern boreal forest (Kostroma region), the change of bird population during the overgrowth of various types of cuttings is similar. At recently clear-cut areas there are 5–8 species with an abundance of more than 0.1 pairs per 10 ha. These indicators increase 2–4 times during the subsequent stages of coniferous forest succession (Preobrazhenskaya and Borisov 1987).

Conclusions

Studies of the succession of bird communities in pine forests in southwestern Belarus and analysis of literature data have shown that the change of bird populations in forests is caused by a consistent succession of forest phytocenoses. Over the past 50 years, the succession of biocenoses has been significantly influenced by anthropogenic factors: forestry activities, reclamation of agricultural land adjacent to forests, and recreation.

In the process of secondary succession of bilberry pine forests, 6 stages of vegetation development were identified from a fresh clearcut to a 90-year-old forest. In parallel with the regular change of vegetation, the succession of bird communities also occurs. In the course of the study, 56 bird species belonging to 11 orders were registered. Passerine birds predominate at all stages of succession (66.8–100.0% of the total number of species). The main total indicators of the population in pine forests initially increase with the development of succession, but at the stage of young brush (10–20 years), the total number decreases. At the stage of plantings aged 30–40 years, species diversity and other indicators

increase and this trend continues as the age of the main forest-forming species increases. In the succession process of this ecosystem from the stage of fresh clear cut to the age of 80–90 years old, the number of species in the bird community increases 4.5 times, total abundance – 4.3 times and biomass – 5.5 times. As the age of the main tree species increases, the number of birds nesting in the trees rises and reaches the maximum at the last two stages – 33 species (73.3%), while the share of ground-nesting species falls. The highest variability of abundance (CV from 50.00 to 135.00 %) is typical for species whose abundance does not exceed 10.0 birds/km^2. In dendrophilous species with an average abundance, the values of the coefficient of variation range from 14.34 to 29.12 % at the last three stages of succession. The highest stability of abundance was found in Common Chaffinch (CV at the fifth stage was 14.59 %, at the sixth stage – 11.17 %) and Common Chiffchaff (12.58 and 12.80% respectively).

At the stages of grassland vegetation of meadow type (1–3 years) and young pine saplings (4–9 years), 3 types of fauna are represented in bird communities. The population is dominated by species of European-Turkestan type of fauna (41.1–41.7% of the total abundance, 48.8–57.8% of the total biomass). Starting from the 10-20-year-old succession stage, the bird community is dominated by a complex of Palearctic fauna types (45.0–60.1% of the number of species, 35.9–48.6% of the total abundance, 49.7–65.7% of the total biomass). The participation of European fauna types in the species diversity of bird communities and populations decreases with the development of succession (50.0–24.4%), and the share of birds in the population increases (30.0–48.3% respectively). In the last two stages of succession, there are 6 faunal types. The main contribution to the structure of bird communities is made by the Palearctic European fauna type, Holarctic type is represented by 2 species, Siberian and Afro-Eurasian type – by 1 species each.

At various stages of succession, 3 species included in the Red Book of the Republic of Belarus (2015) were identified, while 25 species were of international conservation significance.

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PRELIMINARY STUDIES ON ORIGIN AND DISTRIBUTION OF AMERICAN MINK *NEOVISION VISION* WILD POPULATION AT THE EDGE OF ITS INVASION RANGE

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Abstract

The paper presents the preliminary results of wild American mink trapping in 13 fishpond complexes. A total of 13 American minks have been captured in 7 fishpond complexes from February to April 2021. The index of mink trapping success was from 1.7 to 11.5 individuals per 100 trap-nights. Capturing of the American mink will be continued in autumn in order to determine genetic differentiation and origin of the wild American mink population in the south-eastern part of Poland.

Key words: alien species, American mink, distribution and abundance

Introduction

The American mink *Neovison vison* is an alien species in Polish fauna and it has been noted in Poland for about 40 years. Its successful invasion caused it to now inhabit most of Poland. The colonization of Poland by this species began in the north-eastern part of the country, then it settled in western Poland and migrated southwards, where it is still scarce or absent. Genetic studies of wild and farm-bred American minks indicate their two origins which include migration of wild individuals and running away from fur farms (Zalewski et al. 2010).

The process of colonization of new areas by American mink is still not fully recognized. It is not entirely clear whether the area is colonized by a small group of animals that increase in numbers in subsequent breeding seasons, or if there is a continuous influx of minks from neighbouring areas. In the first case, the genetic differentiation of the newly created population will be low and there will be little genetic similarity between the newly created population and the neighbouring populations. In the second case, the newly formed and adjacent populations will not show a significant difference in genetic variation (Dlugosch, Parker 2008, Zalewski et al. 2010). Previous studies of the genetic differentiation of wild American minks showed the presence of at least four subpopulations occurring in western and north-western, central and north-eastern Poland (Zalewski et al. 2010).

The main aim of the study is the initial verification of the hypothesis that the genetic differentiation of the wild American mink population in the south-eastern part of Poland has decreased. Genetic similarity of the population from this part of Poland with the minks from north-eastern Poland will be examined. Furthermore, the morphological variability of the American mink will be determined on the basis of measurements of body length and weight. Minks from fur farms are larger than wild animals. As a result of natural selection, feral individuals become similar in size and body structure to wild minks in successive generations (Zalewski, Bartoszewicz 2012). The paper presents the preliminary results concerning the distribution and abundance of wild American mink in the fishponds of the Lublin region, i.e. the results of American mink capturing.

Materials and Methods

The American minks were caught in 13 fishpond complexes in the southern part of the South Podlasie Lowland, the Lublin Upland and the Western Polesie in south-eastern Poland, in cooperation with pond managers and hunters. The ponds are situated in river valleys, the largest of which is the Wieprz River valley, and they are surrounded by a diverse landscape (Figure 1, Table 1).

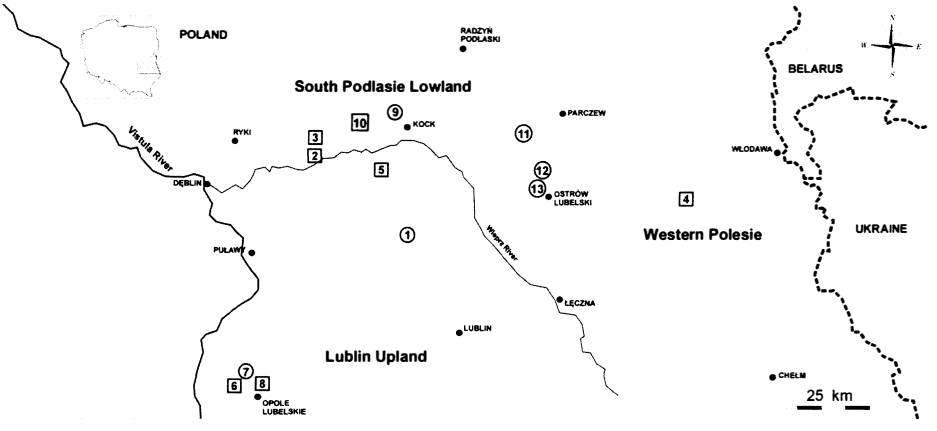

Figure 1. Location of the fish pond complexes. Square – fishpond complex where at least on mink was trapped; circle – fishpond complex where no mink was caught. The fishpond complexes are numbered according to the order in which they were visited in the period from February to April 2021.

Table 1. Selected details of the studied areas, methods and obtained results. The numbering of fishpond complexes corresponds to the numbering on the map (Figure 1).

No.	Fishpond complex	River	Surrounding landscape	Number of captured individuals	Number of traps	Period (day and month)
1.	Samoklęski	Minina	fields	0	12	1-6 Feb.
2.	Sobieszyn	Wieprz, Świnka	meadows, wetlands	1	11	18-22 Feb.
3.	Wólka Sobieszyńska-Podlodów	Świnka	forest	1	13	25 Feb.-1 Mar.
4.	Stary Brus	Mietułka	forest, meadows, fields, wetlands	6	13	3-7 Mar.
5.	Rawa	Minina	forest, meadows	2	11	9-13 Mar.
6.	Jankowa	Leonka	forest, meadows	1	10	18-22 Mar.
7.	Pomorze	Chodelka	forest, meadows	0	5	18-22 Mar.
8.	Wola Rudzka (Pustelnia, Kulig, Grabówka)	Chodelka, Poniatówka	forest, fields	1	15	24-28 Mar.
9.	Kock	Czarna	fields, forest	0	8	29 Mar.-2 Apr.
10.	Kawęczyn	Struga	fields	1	6	29 Mar.-2 Apr.
11.	Siemień	Tyśmienica, Piskornica	forest, fields	0	13	8-12 Apr.
12.	Tyśmienica	Tyśmienica	forest, meadows	0	8	15-19 Apr.
13.	Babianka	Tyśmienica	forest, meadows	0	5	15-19 Apr.

The minks were caught in the period from February to April 2021 (Table 1) using a live-trapping method (Brzeziński et al. 2019). From 5 to 15 traps were placed at each fishpond complex and left for the next 4 nights (except from Samoklęski fishponds where the traps were left for 5 nights) (Table 1). The number and location of the traps within the fishpond complex depended on the environmental conditions, the size of the complex, the length of the watercourses and the presence of sites where minks were observed in the past. The traps were usually placed at the base of a dike along the rivers and channels surrounding or running inside the complexes. The traps were checked every morning. Parts of fish, mainly Prussian carp *Carassius gibelio* obtained from a fish farm, were left as bait in the traps. The bait was replaced or replenished each day if it was necessary. The density of captured minks was defined as the number of

individuals trapped per 100 trap-nights (the index of mink trapping success), 1 trap-night means 100 traps set during 1 night or 1 trap left for 100 nights (Zalewski, Brzeziński 2014, Brzeziński et al. 2019).

Genetic differentiation of captured animals will be determined based on the analysis of 21 microsatellite markers. The obtained results will be compared with genetic data for wild minks from north-eastern and western Poland (Zalewski et al. 2016).

Results

Thus far, 13 American minks have been captured. These individuals were caught in 7 fishpond complexes located near Lower Wieprz and Chodelka River and Western Polesie wetlands. No minks were captured in the remaining studied fishpond complexes (Table 1). The mean number of trapped minks was 1 individual per fishpond complex. The index of mink trapping success for 6 fishpond complexes was from 1.7 to 4.5 individuals per 100 trap-nights. The fishponds and surrounding wetlands in Stary Brus (Western Polesie) stood out because the trapping success in this area was equal to 11.5 individuals per 100 trap-nights. In April, no mink was trapped although they had been previously observed at these fishponds (Figure 2).

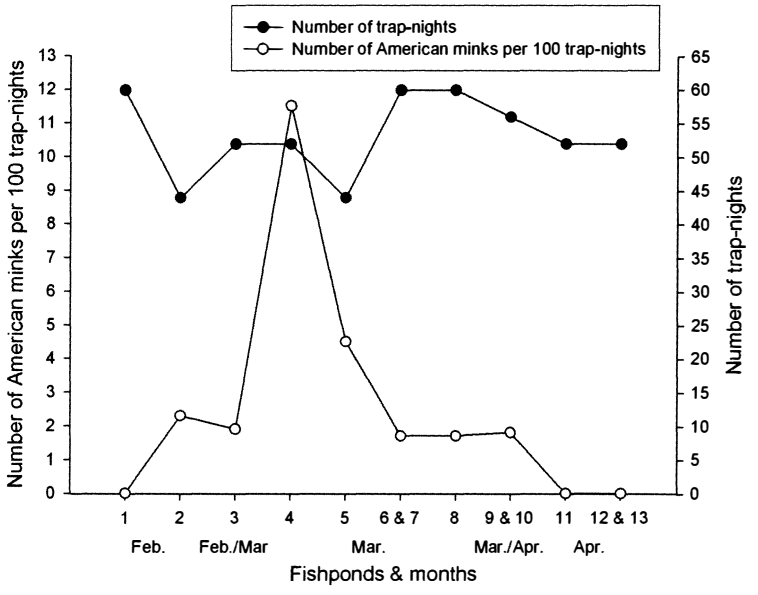

Figure 2. The number of American minks per 100 trap-nights (the mink trapping success index) and the number of trap-nights. Data are presented according to the order of visiting fishpond complexes. Numbering of fishpond complexes corresponds to the numbering in Table 1 and Figure 1. Data that was collected at the same time, i.e., 6 and 7, 9 and 10, as well as 12 and 13, are presented in total.

Discussion

Previous research revealed the presence of at least four genetic groups of wild American mink in Poland. Three groups from north-eastern and central Poland include the populations inhabiting the areas from the Masurian Lake District through the Biebrza basin and the lower Narew river, to the middle Vistula river. The fourth group consists of minks from northern and north-western Poland (Zalewski, Brzeziński 2014). The planned research aims to determine whether the minks captured in south-eastern Poland are related to the populations from north-eastern Poland, especially to the population of the Vistula River. Genetic comparison with domestic minks from fur farms will be examined too. The influx of individuals that escaped from farms might increase genetic diversity. However, it might be supposed that this impact in south-eastern Poland will be much smaller than in western Poland due to the small number of farms (Zalewski et al. 2010). Capturing of the American mink will be continued in autumn and harvesting a total of ca. 20-30 minks from various parts of the Lublin region is planned. Minks will be also monitored at the studied fishponds using rafts during the summer (Reynolds et al. 2004).

Considering that the average index of mink trapping success in Poland is between 7 and 16 individuals per 100 trap-nights, American mink densities obtained in the present study are quite low (Brzeziński et al. 2019). However, Stary Brus (Western Polesie) was a distinct area among other studied fishponds. According to Zalewski and Brzeziński (2014) a density index equal to 10 individuals per 100 trap-nights is high. It suggests that the American mink population in this area is quite large. Since this area borders the Polesie National Park, implementation of an active protection program consisting of catching invasive predators should be considered. Similar programs have been implemented in other national parks, which protect wetlands in western and northern Poland (ptaki-life.pl). Moreover, the whole UNESCO (MAB) biosphere reserve "Polesie Zachodnie", together with the Lower Tyśmienica and Wieprz rivers, should be covered by this protection program. Since the American mink is an alien and invasive mammal, the captured individuals were not released into the environment. These semiaquatic predators harm native animals species, mainly birds and fish in fishpond habitats and other wetlands (Zschille et al. 2014, Niemczynowicz et al. 2017, Brzeziński et al. 2020).

Previous studies concerning the American mink were focused on populations from north-eastern and western Poland. It was due to the high densities of wild minks and a large number of fur farms, as well as threats to local fauna in national parks protecting the wetlands in these parts of Poland. Therefore, it is justified to extend the studies on the American mink population in southern and eastern Poland. Since the south-eastern population of these animals can be increased not only by migrating individuals from the north but also the east, genetic analyses of animals from Belarus and Ukraine are also planned (Zalewski, Brzeziński 2014). The research will be connected with monitoring of the American mink in poorly known areas in south-eastern Poland. It will allow for the determination of the mink's range and interactions between this

species and local animal populations. Studies of the impact of the colonization model and American mink density changes on other vertebrates distribution will be considered. Effect of the American mink population from north-eastern and western Poland on the breeding success of birds will be analysed and then compared to similar data collected in south-eastern Poland. The negative impact of the American mink on the breeding success of waterbirds from inland water bodies is still not well understood.

Conclusions

Results of the present study enable further wide-ranging research aimed at determination of the occurrence and distribution of the wild American mink population in south-eastern Poland, as well as continuation of genetic and morphological studies to assess its descent. Explanation of the process of colonization of new areas by mink will allow for the preparation of guidelines for limiting its expansion rate.

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RESOURCE CHARACTERISTICS AND DETERMINATION OF THE VALUE OF THE RAW PHYTOMASS OF COMMON YARROW (*ACHILLEA MILLEFOLIUM L.*)

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Abstract

As a result of the analysis of the obtained data, the average projective cover of common yarrow in various types of meadow communities was determined – 4.85±0.57%. The highest average projective coverage was recorded in plant communities of the unions Festucion pratensis – 6.01±1.12% and Dauco-Melilotion (albi) – 6.40±2.16%. The value of the projective cover of common yarrow in these unions of meadow communities varied from 0.5 to 40% and from 2 to 13%. The maximum values of the projective cover (15% or more) of Achillea millefolium were recorded only for 14 populations found in plant communities of the following unions: Arrhenatherion elatioris, Festucion pratensis, Deshampsion caespitosae, and Filipendulion ulmariae. In them, the average projective coverage of yarrow was 23.43± 2.21%. The highest frequency of occurrence (more than 40%) of this species was noted for the meadow communities of the unions Festucion pratensis and Dauco-Melilotion (albi), in which its high phytocenotic activity is observed. The dependence of the distribution of this species in meadow ecosystems on the ground water level, which was determined on the basis of the availability of these types of plant communities in certain environmental conditions, was revealed.

Key words: projective cover, frequency of occurrence, meadow ecosystems

Introduction

Rational use of medicinal plants is impossible without identifying the phytocenotic confinement of the species and information about its productivity in various communities. In this regard, we analyzed the distribution of common yarrow (*Achillea millefolium L.*) by communities, their association with phytocenoses and environmental factors most significant for them, and also determined the frequency of occurrence, phytocenotic activity, average projective cover and productivity in various types of communities. The data obtained is given for the objects of study, which are placed in groups depending on the raw material used.

Materials and Methods

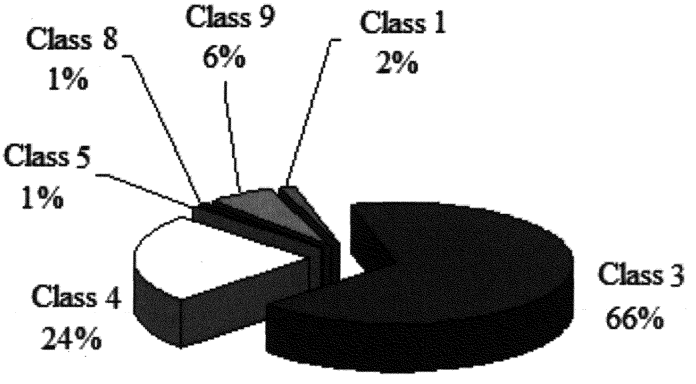
The object of the study is the population of common yarrow (*Achillea millefolium L.*) (medicinal plant), which are of particular economic importance, representing a perennial herb, the intensity and direction of use, the nature of the harvested raw material (shoots, leaves, rhizomes), and its occurrence. The subject of research is productivity, dynamics, state and stability of populations, stocks and rates of withdrawal of raw materials of the model species.

Results and Discussions

An analysis of the phytocenotic confinement of common yarrow (*Achillea millefolium L.*), according to the descriptions of 218 populations, showed that their largest number is concentrated in meadows – 197 (90% of the total), of which 132 are registered in mesophilic herbal communities that belong to Class 3 – *Arrhenatheretea (elatioris)* Tx 1937 em. Br.-Bl. 1947 (Avohora et al. 2004; Figure 1).

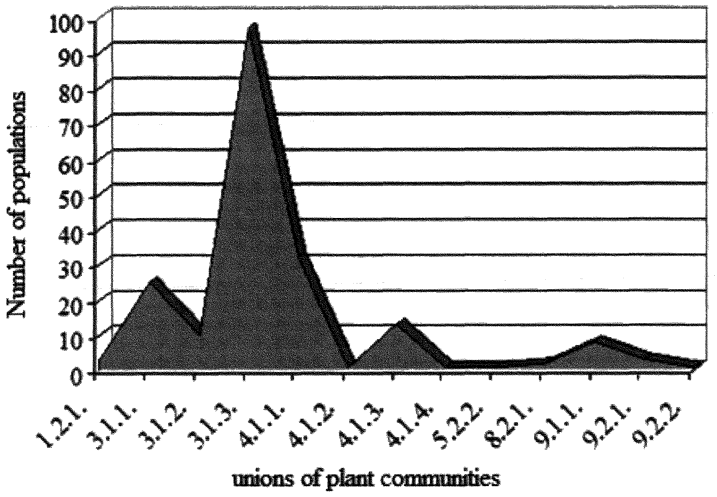
The distribution of this species over 13 unions of plant communities of meadows was analyzed (Figure 2).

It was revealed that common yarrow (*Achillea millefolium L.*) is widespread in mesophilic communities of the *Festucion pratensis* union (Pogosic et al. 2015). They form on fresh and moderately moist, sufficiently rich, mainly slightly acidic, neutral and alkaline soils (Liddle et al. 1975).



Class 1 – *Sedo-Scleranthetea (biennis) Br.-Bl.* 1955 *em. Moravec* 1967; Class 3 – *Arrhenatheretea (elatioris) Tx.* 1937 *em. Br.-Bl.* 1947; Class 4 – *Molinio-Juncetea (effusi)* Br.-Bl. (1947) 1949; Class 5 – *Phragmitetea (communis) Tx. et Prsg.* 1942; Class 8 – *Plantaginetea majoris Tx. et Prsg.* 1947 *in Tx.* 1950; Class 9 – *Artemisietea vulgaris Lohm., Prsg. et Tx. in Tx.* 1950 *em. Kopecký in Hejný et al.* 1979

Figure 1. Distribution of *Achillea millefolium* by plant community class.



1.2.1. *Sedo-Scleranthion (biennis) Br.-Bl.* 1955; 3.1.1. *Arrhenatherion elatioris (Br.-Bl. 1925) Koch* 1926; 3.1.2. *Cynosurion R. Tx.* 1947; 3.1.3. *Festucion pratensis Sipajlova et al.* 1985; 4.1.1. *Deshampsion caespitosae Horvatić* 1930; 4.1.2. *Molinion (caeruleae) Koch* 1926; 4.1.3. *Filipendulion ulmariae (Br.-Bl.* 1947) *Lohm. ap. Oberd. et al.* 1967 *em. Balátová-Tuláčková* 1978; 4.1.4. *Alopecurion pratensis Passarge* 1964; 5.2.2. *Magnocaricion elatae W. Koch* 1926; 8.2.1. *Agrostion stoloniferae (Soó* 1957) *Oberd. in Oberd. et al.* 1967; 9.1.1. *Dauco-Melilotion (albi) Görs* 1960 *em. Eliáš* 1980; 9.2.1. *Arction (lappae) (Tx.* 1937) *Siss* 1946 *em. Gutte* 1972; 9.2.2. *Carduo (crispi) – Urticion dioicae Hadač* 1962

Figure 2. Distribution of common yarrow (*Achillea millefolium L.*) by alliances of plant communities.

As a result of the analysis of the data obtained, the average projective cover of common yarrow in various types of meadow communities was determined – 4.85 ± 0.57%. The largest average projective cover was recorded in plant communities of *Festucion pratensis* – 6.01 ± 1.12% and *Dauco-Melilotion (albi)* – 6.40 ± 2.16% (Table 1). The projective cover of common yarrow in these unions of meadow communities varied from 0.5 to 40% and from 2 to 13%.

Table 1. Average projective cover of common yarrow (*Achillea millefolium L.*) in the most common unions of plant communities.

Union of plant communities	Average projective cover,%
Arrhenatherion elatioris (Br.-Bl. 1925) Koch 1926	4,76 ± 1,04
Cynosurion R. Tx. 1947	2,78 ± 0,81
Festucion pratensis Sipajlova et al. 1985	6,01 ± 1,12
Deshampsion caespitosae Horvatić 1930	4,23 ± 1,16

Union of plant communities	Average projective cover,%
Filipendulion ulmariae (Br.-Bl. 1947) Lohm. ap. Oberd. et al. 1967 em. Balátová-Tuláčková 1978	4,85 ± 1,57
Dauco-Melilotion (albi) Görs 1960 em. Eliáš 1980	6,40 ± 2,16
Arction (lappae) (Tx. 1937 Siss 1946 em. Gutte 1972	3,00 ± 1,15

The maximum values of the projective cover (15% or more) of common yarrow (*Achillea millefolium L.*) were recorded only for 14 populations found in plant communities of the following unions: *Arrhenatherion elatioris, Festucion pratensis, Deshampsion caespitosae, Filipendulion ulmariae*. In them, the average projective cover of yarrow was 23.43 ± 2.21%.

The highest frequency of occurrence (more than 40%) of this species was noted for meadow communities of the *Festucion pratensis* and *Dauco-Melilotion (albi)* unions, in which its high phytocenotic activity is observed (Minarchenko et al. 2013).

The dependence of the distribution of this species over meadow ecosystems on the groundwater level, which was determined on the basis of the confinement of these types of plant communities to certain environmental conditions, was revealed (Nath et al. 2016). It has been established that the largest number of common yarrow (*Achillea millefolium L.*) populations is observed in plant communities, which are formed under conditions of groundwater occurrence from 0.55 to 1.55 m (Smolarz et al 1999).

As a result of the analysis of the phytocenotic features of common yarrow, it was noted that 10% of the total number of its populations is concentrated in forests. The largest share of common yarrow (*Achillea millefolium L.*) growth sites was recorded in pine forests (68% of the total number of forest plant communities), and most often the species was found in mossy pine forests – 71% of all pine forests.

The average projective cover of this species in all studied types of communities was calculated – 4.66 ± 0.54%.

Analysis of the data obtained made it possible to distinguish plant communities, in which it is promising to harvest medicinal raw materials, by the largest average projective cover, frequency of occurrence and phytocenotic activity of yarrow. These are plant communities of the *Festucion pratensis* and *Dauco-Melilotion (albi)* unions.

In order to assess the stocks of raw materials of common yarrow (*Achillea millefolium L.*) in these communities at the UP, its projective cover, number and height of flowering shoots were assessed, and all raw phytomass was collected.

In the plant communities of the union *Festucion pratensis*, the average projective cover of yarrow was 19.52 ± 2.44% (values ranged from 0.5 to 70%), the average height of flowering shoots was 53.11 ± 2.03 cm (from 33.8 to 98 cm), their average number was 58.185 ± 7.63 pieces / m^2 (from 1 to 236 pieces / m^2), the average value of USF was 52.47 ± 4.99 g / m^2 (from 1.73 to 159.14 g / m^2) when wet and 15.44 ± 1.70 g / m^2 (from 0.42 to 51.66 g / m^2) in the air dried state, the

mass of 1 leafy shoot was 1.30 ± 0.12 g (from 0.44 to 4.89 g) in the raw state and 0.33 ± 0.02 g (from 0.16 to 1.05 g) in the air dried state.

For the plant communities of the *Dauco-Melilotion (albi)* union, the projective cover of common yarrow (*Achillea millefolium L.*) varied from 2.73 ± 0.47% to 26.40 ± 5.05% (mean 7.46 ± 2.17%). The average height of flowering shoots in such communities was 42.68 ± 2.97 cm (with values ranging from 0 to 68 cm). Their number ranged from 7.74 ± 1.55 to 56.00 ± 11.66 pieces / m^2, the average value was 19.32 ± 5.09 pieces / m^2. The value of the raw phytomass in the raw state varied from 8.35 ± 1.66 g / m^2 to 50.44 ± 10.38 g / m^2, the average values were 20.13 ± 4.88 g / m^2, and in the air dried state – from 3.39 ± 0.68 g / m^2 to 20.96 ± 4.92 g / m^2, with an average value of 6.91 ± 1.78 g / m^2. The average weight of 1 leafy shoot of yarrow was 1.08 ± 0.14 g (from 0 to 3.68 g) in a raw state and 0.36 ± 0.04 g (from 0 to 1.11 g) in an air dried state.

When processing the materials, the average values of the main population indicators were obtained for all PPs. Thus, the average projective cover of common yarrow (*Achillea millefolium L.*) was 15.70 ± 1.90% (with values ranging from 0 to 70%), the average height of flowering shoots was 49.81 ± 1.75 cm (from 0 to 98 cm), their average number was 45.89 ± 5.81 pcs / m^2 (from 0 to 236 pcs / m^2), the average value of the raw phytomass was 42.24 ± 4.10 g / m^2 (from 0 to 159.14 g / m^2) when wet and 12.74 ± 1.36 g / m^2 (from 0 to 51.66 g / m^2) in the air dried state, the mass of 1 leafy shoot in the wet state was 1.23 ± 0.09 g (from 0 up to 4.89 g), and in an air dried state – 0.34 ± 0.02 g (from 0 to 1.11 g). The yield of freshly harvested air dried raw materials was 29.35 ± 0.62%.

The results obtained agree with the data from other authors in other countries. So, in the territory of the north-western regions of the Kemerovo region of the Russian Federation, the productivity of air dried raw material of yarrow ranged from 9.90 ± 0.45 to 37.00 ± 4.90 g / m^2 and in the southern regions – from 4.36 ± 0, 20 to 27.90 ± 0.53 g / m^2; the average productivity was 17.90 ± 0.53 g / m^2 (Trampling et al. 2010). On the forb-cereal floodplain meadows of the Parabel district of the Tomsk region, the value of its raw phytomass reached 11 g / m^2, and on upland forb-cereal meadows in the eastern and southeastern regions of the Perm region the density of the stock of raw materials ranged from 11.9 ± 1, 6 to 134.2 ± 19.2 kg / ha. In the Republic of Azerbaijan, this number was 186.2 ± 19.0 kg / ha (18.62 g / m^2).

Correlation analysis of the research results revealed a very strong relationship between the mass of raw and air dried medicinal raw materials of common yarrow (*Achillea millefolium L.*) and the projective cover (r = 0.93 and r = 0.95, respectively), as well as the number of flower-bearing shoots (r = 0, 92 and r = 0.95, respectively). A very weak correlation was established between the raw phytomass of this species and the height of flowering shoots (Rajput et al. 2014)

Based on regression analysis, models of the dependence of the value of the raw phytomass of common yarrow (*Achillea millefolium L.*) on the projective cover of the species and the number of flowering shoots were constructed:

– MSStys = 1.9906 PP + 10.9781 and MSStys = 0.6750 PP + 2.1435 (Figure 3);

– MSStys = 0.6494 KP + 12.4376 and MSSthys = 0.2224 KP + 2.5385 (Figure 2);

– MSS thousand = 1.0962 PP + 0.3158 KP + 10.5340;

– MVSStys = 0.3270 PP + 0.1228 KP + 1.9707;
where MSStys is the value of raw phytomass in a raw state, g / m^2;
PP – projective cover, %;
MVSStys – the value of the raw phytomass in the air-dry state, g / m^2;
KP – the number of flowering shoots, pcs / m^2.

Linear models of the dependence of the raw material phytomass of yarrow in the raw and air dried state on the projective cover of the species and the number of flowering shoots fit the initial data, since the calculated significance level (4.24E-34, 3.59E-39 and 3.53E-33, 1.17E-40, respectively) is much less than the specified one (0.05). Multiple regression models are valid because P (3.5699E-36 and 6.2638E-45) is much less than the target (0.05). Model coefficients are statistically significant (P <0.05).

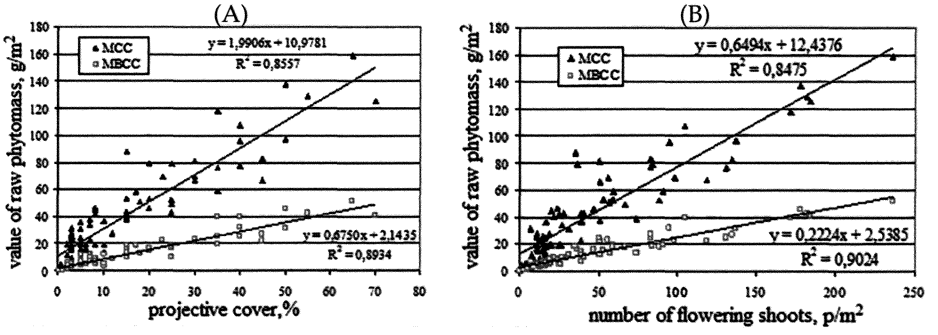


Figure 3. Dependence of the value of raw phytomass of common yarrow (*Achillea millefolium L.*) in the raw (MSS) and air dried state (MSS) on the projective cover (A) and the number of flowering shoots (B).

The constructed models were checked. The productivity of yarrow was determined in the field and according to the regression equations, using the required measured parameters.

Analysis of the obtained data showed that the values of the mass of air dried raw material of common yarrow (*Achillea millefolium L.*) depending on the projective cover calculated using the equation MVSStys = 0.6750 PP + 2.1435 differed by 1% from the values determined in the field. Using a multiple regression model of the dependence of the value of raw phytomass of yarrow in an air dried state on the projective cover of the species and the number of flowering shoots (MVSStys = 0.3270 PP + 0.1228 CP + 1.9707), its productivity was calculated, the values of which were 13% differed from the actual determined values.

Therefore, the constructed regression equations can be used to determine the value of the raw phytomass of a given species without cutting off medicinal raw materials, but only by determining the projective cover of the species and the number of flowering shoots.

For the northern part of the territory of Belarus, the dependence of the dynamics of quantitative indicators of the population of common yarrow (*Achillea millefolium L.*), the degree of its stability, recovery time from various modes of raw material procurement and recreational load have been established.

Conclusions

1. A comprehensive analysis of common yarrow (*Achillea millefolium L.*), a perennial herbaceous plant of the natural flora of Belarus, has been carried out, regarding its life form, occurrence, intensity, and use;

2. The distribution of the isolated common yarrow (*Achillea millefolium L.*) on the territory of the northern part of the country has been studied, its association with various plant communities and dependence on the main ecological factors of the environment have been analyzed;

3. The productivity of common yarrow (*Achillea millefolium L.*) in different phytocenoses was determined and the dependence of the value of its raw phytomass on the main population parameters was revealed;

4. On the basis of a comprehensive analysis of the population of common yarrow (*Achillea millefolium L.*) of the natural flora of the northern part of Belarus, the state, resources, rates of removal, stability and the possibility of recovery were assessed in connection with various degrees of anthropogenic impact.

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URA GRODNO: TOGETHER WE CAN DO MORE!

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Key words: environmental monitoring, improvement of environmental management, innovations

The experience of participation of the secondary school № 12 in Grodno in the implementation of the local environmental initiative "URA Grodno!", which is a part of the project "Public involvement in environmental monitoring and improvement of environmental management at the local level", funded by the EU and implemented by UNDP in partnership with the Ministry of Natural Resources and Environmental Protection of the Republic of Belarus, is presented. A description of innovations and effective tools and brief characteristics of the most significant events of the initiative are given.

Continuity and succession, as well as the inclusive participation of each participant in the interaction, are important provisions in Education for Sustainable Development (hereinafter – ESD). Environmental education enables them under certain conditions.

In secondary school №12 in Grodno the organization of ESD practices is based on an open educational environment and support for children's and youth initiatives. The first allows for attracting out-of-school specialists and preserving the continuity and succession of environmental education in the age range 3 - 80 years old. The second not only motivates participants to care for the result, but also promotes participation in the development of useful cases and achievement of meaningful results.

One of the prime examples of this approach is the development and implementation of the local environmental initiative "URA Grodno!"

The idea of creating a multicenter for environmental education and monitoring, which arose in the intellectual association "Prudent Masters' School" of secondary school № 12 in Grodno, was reborn into a joint environmental initiative of Yanka Kupala State University of Grodno (applicant of the initiative), Secondary School No. 12 in Grodno (partner of initiative 1), Kindergarten No. 45 in Grodno (partner of initiative 2), Association "Education for Sustainable Development" (partner of the initiative 3) under the title: "Inclusive environmental

management by motivated inclusion of the residents of the city of Grodno in the development and implementation of measures aimed at improving the quality of the environment and monitoring environmental risks, in the context of the educational multicenter for comprehensive support of the environmentally friendly lifestyle of local communities "URA Grodno!" ("Sustainable Development – Grodno Activity" or "URA Grodno!" for short).

The initiative was recognized as the winner of the competition for environmental initiatives in the project "Public involvement in environmental monitoring and improvement of environmental management at the local level", funded by the EU and implemented by UNDP in partnership with the Ministry of Natural Resources and Environmental Protection of the Republic of Belarus (hereinafter referred to as the Project).

"URA Grodno!" has been successfully implemented with the support of this project in 2019 – 2021.

The participation of all three institutions in the work of the Education for Sustainable Development Association allowed them to become partners in a large-scale environmental start-up, agree on interaction, expand and complement each other's capabilities and get the expected result.

The goal of the initiative is inclusive environmental management by motivated inclusion of Grodno residents in the development and implementation of measures aimed at improving the quality of the environment and monitoring environmental risks, in the context of the educational multicenter for comprehensive support of the environmentally friendly lifestyle of local communities "URA Grodno!", creation of an educational multicenter that ensures the continuity of environmental education at the stages of "kindergarten – school – university" and the involvement of a large number of Grodno residents in the implementation of environmentally significant events and actions.

The initiative is based on the idea of a "green office" created on the basis of an open demonstration platform by three institutions. The well-organized educational environment of the "green office" allows you to efficiently use your own resources and thereby save the resources of the planet.

The main innovative instrument of the initiative is the open educational multicenter "URA Grodno", which includes eight mini-centers located on the basis of partner institutions:

Yanka Kupala State University of Grodno:

- Laboratory of environmental monitoring.

State Educational Institution "Kindergarten No. 45 of Grodno":

- Mini-center "Little Meteorologist";
- Mini-center "Ecology and Health".

State Educational Institution "Secondary School No. 12 of Grodno":

- Mini-center "Green Workshops";
- Mini-center "Green Foyer";
- Mini-center "Green class";
- Mini-center "Energy";
- Mini-center "Water".

Mini-center "Green Workshops" – equipment for recycling materials, tables for drawing with sand, ornamental plants were purchased and installed in the center, funded by the Project. Master classes, exhibitions and training sessions are held here. More than 800 people have been involved in environmental practices in two years.

A decorative waterfall, rattan furniture, and ornamental plants have been purchased for the Green Foyer mini-center. The center organizes exhibitions, master classes, eco-theater performances, training sessions, thematic changes. The coverage is over 1000 people annually.

A gazebo, ornamental plants, and wooden decor were purchased for the Green Class mini-center. This made it possible to conduct educational and recreational activities with children and adults in the fresh air.

A unique interactive stand "Is it easy to be a power plant?" Has been ordered for the mini-center "Energy" (exercise bike for generating energy and turning on electrical appliances) along with other equipment, as well as games for workshops, research projects, experiments. More than 1,500 residents of Grodno have already taken part in them.

The mini-center "Water" is equipped not only with materials for laboratory workshops, but also with an interactive stand "Precious Water", which makes it possible to determine the loss of water during various human activities in everyday life. More than 1200 people have already taken part in trainings, laboratory workshops, seminars, research on the issues of careful use of water.

At present, all centers are actively interacting, functioning, purchasing and installing new equipment.

On the basis of mini-centers, large-scale and chamber events are organized with the active participation of representatives of local communities: preschoolers, adolescents, youth in Grodno and the region, teachers, residents of educational institution microdistricts, elderly people, etc.

Favorable conditions have been created for the birth and support of socially significant children's and youth environmental initiatives.

The partners of the initiative have successfully implemented most of the planned activities. On the basis of secondary school No. 12 in Grodno, active participation in all environmental practices of the initiative was organized, 6 trainings and 10 ESD workshops were conducted using the capabilities of the created educational mini-centers.

The most striking, meaningful, large-scale of them are:

- inclusive bike ecoquest "Give the Planet an Hour!" (September 2019, 2020, March 2021, more than 800 participants; popularization of cycling as an environmentally friendly way of life, ecological cycling events);
- information and educational campaign "Energy efficient planet: define your position" (November 2019, 2020, more than 1800 participants; including a series of trainings, workshops, master classes on energy efficiency, an information campaign in the microdistrict, LED show);
- a long-term environmental and educational campaign "Clean Business" (December 2019 – November 2020, more than 2600 participants; including a series of information and game programs, ecological events, trainings,

workshops, master classes on the recycling of raw materials and the separate collection of waste, the show "Second-Art", etc.);
- action "Earth Hour: arithmetic and geography of our participation" (March 2020, 2021, more than 600 participants, 44 Grodno streets, 16 settlements in the region and the city of Shakhtinsk, Kazakhstan);
- open city forum of environmental initiatives (March – April 2020, more than 300 participants);
- ecological shift during the school health camp (June-July 2020, more than 200 people involved; a series of educational workshops and environmental campaigns);
- ecological marathon "2020 useful summer activities" (June – August 2020, more than 700 participants) and a summer methodological school (July 2020, 30 teachers from the educational institution of the republic).

In total, the partner institutions ensured the participation of more than 10,000 residents of Grodno and the region in the activities of the initiative. More than two dozen scenarios have been developed, a program for the ecological shift during the school health camp; more than 2,000 leaflets were created and distributed; the creative album "The ABC of Saving the Planet" was created; a collection of creative works of the forum participants was created.

The innovativeness of the approaches consisted in continuity, openness and succession in the field of additional environmental education in the context of the "kindergarten – school – university" triad, the wide use of the possibilities of remote interaction and information and computer technologies, the implementation of the objectives of the initiative through the organization of ESD (education for sustainable development) practices, as well as in close cooperation with local (city) government.

Preschoolers, schoolchildren, students, teachers, local residents acted as agents of positive change in the interest of the environmentally friendly lifestyle of local communities. The importance of the initiative has been repeatedly noted by representatives of the city government, the education system, and the media.

The useful experience of implementing a local environmental initiative was presented 13 times in mass media publications, more than 260 times in Internet publications in regional and national sources, at five national forums.

The obtained results allow us to predict high activity and demand for the multicenter "URA Grodno!" for several years ahead:
- close cooperation of the partners of the initiative has been established;
- a functional educational multicenter was created, on the basis of which the school alone conducted more than 15 laboratory workshops and 5 field laboratory workshops, 6 trainings; more than 600 people (including teachers, students and schoolchildren) were trained in monitoring techniques within the framework of the events;
- a program for holding workshops in the State Educational Institution "Secondary School No. 12 in Grodno" was developed, using the equipment of educational mini-centers as resource support;

- as a result of the measures taken, about 6,000 residents of Grodno are involved in an environmentally friendly lifestyle;
- More than 2,500 Grodno residents were able to familiarize themselves with the methods of environmental monitoring and resource conservation in everyday life, to determine their contribution to solving energy problems.

Thus, the local environmental initiative "URA Grodno!" is a worthy example of engaging the local community in meaningful environmental practices, ensuring openness, continuity and succession of additional environmental education for all generations. The organizers of the initiative are ready for cooperation and invite all people who are not indifferent to the fate of the planet to join its activities.

The materials of the initiative can be found in open access in the network community "URA Grodno!": https://www.facebook.com/groups/760173954420940/.

APPLICATION OF THE COMPETENCE APPROACH IN BIOLOGY LESSONS IN THE STUDY OF THE "ZOOLOGY" BLOCK TO PREPARE FOR CENTRALIZED TESTING

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Key words: educational competencies, cognitive competencies, competence approach

Every year, to gain admission to higher education institutions of medical, biological, environmental, and agricultural profiles, students take a centralized test in biology. Successful completion guarantees admission to university. However, only a well-trained student can obtain a high result. Therefore, each teacher has a task: to organize the work in such a way that the graduate can get the maximum score on the test. The teacher, based on the abilities of the students, chooses the forms, methods, and techniques of work to achieve the result.

To prepare and achieve high results students must possess educational and cognitive competencies, i.e. be ready for independent cognitive activity. This activity consists in the ability to plan and analyze your activities, evaluate the level of educational achievement, analyze and compare, work with different sources of information, select the necessary material for use and memorization. Preparation for the centralized testing begins with the 7th grade, when students begin to study botany. Let us consider in more detail the methodology of using the competence approach in the classroom when studying the block "Zoology" (Bogacheva 2008).

Students work with a large amount of information when repeating or summarizing the material. Therefore, we suggest that you make a plan for repeating a specific topic, planning the time for work. Systematically, in the classroom, we apply the task of compiling summary tables. To compile a table, students need to have a good command of the material, choose the right features to compare. When compiling tables, students usually have difficulties choosing the main information. Most often, the entire text of the textbook is copied. In this case, we teach students to divide the text into separate semantic parts. In the tasks of centralized testing, the names of representatives and taxa are suggested to compare different systematic groups. The oral survey shows that students remember and can reproduce the general features of the organization of different groups, classes, and types. However, if it is necessary to name a representative, then students find it much more difficult to cope with such a task. Therefore, we

use the "Find a Pair" technique when students need to correlate the taxonomic rank and representatives of animals.

In the CT, there are tasks that involve comparing classes of animals, establishing similarities and differences. Tasks of this type teach you to analyze, compare, and find differences. We use the "50×50" technique, which involves dividing the proposed features of the structure of organisms into two groups and choosing those that correctly characterize the required class.

When preparing for testing, it is important to work systematically with biological terms. The student needs to memorize and master a large number of biological concepts and definitions. In order to facilitate the process of memorization, you should create your own dictionary. The student writes down on the first page of the notebook all the terms with their interpretation on the letter "A", on the second-on the letter "B", etc. However, it is necessary to provide space in the notebook in order to make adjustments and additions from other sources. It is important to teach students to relate the term to a certain group of animals, to know not only the meaning of the concept, but also examples of it. After all, in the CT, there are often tasks that require explaining the relationship of the concept with certain animals.

The ability to determine the systematic affiliation of biological objects can be affected by several factors. Few students demonstrate knowledge of the main systematic categories used in the classification of animals, as well as the principle of hierarchy (subordination), on the test. Therefore, we use the game technique "Do you know the taxonomy of animals?" Students need to remember the main taxonomic units of animals. Then the students place the taxa on the board, starting with the smallest, and then correlate with a certain representative.

It is interesting to use the "Logical Domino" technique for the correlation of concepts with their explanations. The task activates mental activity, develops memory. When using the "Domino" technique, we distribute sheets with white spots. One student fills in the information on the right, the other on the left. After that, they put the sheets together, correct the errors. Organized in this way, the work allows students to develop the ability to highlight the main thing, compare, analyze, remember, work with information (Ponomareva 2007).

To familiarize students with the features of the external structure, the principles of inheritance of traits in animals, we turn to the developed electronic simulator: https://testygenetika.blogspot.com.by/?zx=172f84092510db25. Students have the opportunity to get acquainted with theoretical and practical materials. For students interested in biology, we suggest solving problems for determining the inheritance of traits based on an algorithm for solving problems of a certain type. For example, the inheritance of the color of feathers in birds, coat in rats, rabbits, dogs, pigs, and the body of flies. After all, tasks of different types are found in CT (Yarygin 2003).

Thus, the teacher's use of a variety of methodological techniques for developing the ability to schematize information, establish correspondences, compare, highlight similarities and differences, work with drawings, mark the correct features of these biological objects, analyze facts and solve biological problems will

allow students to navigate well in a large amount of information, thoroughly prepare for the CT exam.

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