CONTENTS

Emission of Airborne Fibers from Mechanically Impacted Non-Asbestos Fiber-Containing Materials: Preliminary Results – Józef S. Pastuszka........................................ 3
Influence of Type of Substrate and Water Chemistry on the Structure and Succession of Periphytic Ciliate Communities in Hypertrophic Lake – Tomasz Mieczan, Agnieszka Puk.............................................................. 13
The Bayesian Model of the Interdependencies between Soil Sorption Features – Stanisław Gruszczyński ................................................................. 25
Molasses as a Carbon Source for Denitrification – Dorota Kulikowska, Karolina Dudek .......................................................... 35
Effect of Organic Fertilization on Development of Proteolytic Bacteria and Activity of Proteases in the Soil for Cultivation of Maize (Zea Mays L.) – Alicja Niewiadomska, Hanna Sulewska, Agnieszka Wolna-Maruwka, Justyna Klama ................................................................. 47
Inactivation of Escherichia Coli during Composting Process of Organic Wastes with Sewage Sludge – Beata Szala, Zbigniew Paluszak .............. 57
Polycyclic Aromatic Hydrocarbons in PM1, PM2.5, PM10 and TSP in the Upper Silesian Agglomeration, Poland – Krzysztof Klejnowski, Barbara Kozielska, Andrzej Krasa, Wioletta Rogula-Kozłowska ......................... 65
Phenol Biodegradation by Pseudomonas Putida PCM2153 – Grzegorz Przybyłek, Sławomir Ciesielski ................................................................. 73
Consequences of Water Engineering Projects in the Mokašnica River Basin (Bosnia and Herzegovina) – Božo Soldo, Josip Mesec, Mladen Zelenika ........................................................................ 79
Variability of Nitrogen and Phosphorus Concentration and the Net Primary Production of Vaccinium Vitis-Idaea L. and Vaccinium Myrtillus L. in Chosen Woodland Ecosystems of the Słowiński National Park – Agnieszka Parzych, Zbigniew Sobisz, Jan Trojanowski .......... 91
EMISSION OF AIRBORNE FIBERS FROM MECHANICALLY IMPACTED NON-ASBESTOS FIBER-CONTAINING MATERIALS: PRELIMINARY RESULTS

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Keywords: Airborne fibers, fiber-containing materials, emission factor.

Abstract: The emission rate of fibers released from the new/fresh and used/worn ceramic fiber material, glass wool and man-made mineral fiber material due to mechanical impact was determined experimentally. The emission rate has been defined as a number of fibers emitted per unit mass and unit impaction energy. The averaged emission rate of short fibers (L \leq 5 \mu m) for all studied fresh non-asbestos fiber materials ranged from 2.2 to 20 fibers/(g·J), while the emission of long fibers (L > 5 \mu m) was between 2.2 and 100 fibers/(g·J). The susceptibility of worn fiber-containing materials to emitting fibrous particles due to mechanical impaction was significantly diverse. Emission from glass wool unchanged with the exploitation, while the emission rate of the mineral fiber material increased by a factor of \( 10^4 \) compared to new material. The dominating population of emitted fibers from studied materials ranged from 2 to around 8 \mu m in length.

INTRODUCTION

People in homes, as well as in the work environment, may be exposed to various fibrous aerosols. There are various diseases, including lung cancer, which are caused by inhalation of airborne fibrous dust clouds (e.g.: [7, 14, 15, 20]). Over the past two decades a great deal has been learned about how and why fibers cause pulmonary diseases (e.g.: [1]). There are three essential factors that are required to develop such disease [6]: adequate dose, dimensions of the fibers in the alveolar region, and fiber biopersistence [8]. Other fiber properties, such as presence of iron or other transition metals on fibers, ability of fibers to generate free radicals [5], and the ability of fibers to interact with and alter biologically relevant molecules, as well as the ability of fibers to produce reactive oxygen/nitrogen species (ROS) may also be determinants of fiber toxicity [6], especially among biopersistent fibers. The fundamental property of the fiber toxicity is, that in contrast to chemicals, fibers are believed to cause disease trough a physical/chemical interaction, what means that the health effects depend not only on the type of fiber but also upon...
its diameter and length. Typically, it is assumed that the most hazardous fibers are those longer than 5 μm and of a diameter up to 3–4 μm [22]. It is possible that the physical form of a fiber is even more important than its chemical composition [6, 21].

Since the aerodynamic behavior of airborne fibers depends on the orientation of these fibers in the flow, theories and data valid for spherical particles are not applicable for fibers [16]. Estimation of the exposure to fibrous aerosol requires knowledge about the emission of fibers, their atmospheric and indoor transport, and penetration from outdoor into indoor environment, as well as deposition and resuspension. This knowledge is still very poor. One of the reasons is the lack of a method to inexpensively produce length and diameter mono-dispersive fibers. Only recently Gilbertson et al. [3] have developed such a method, which produces straight fibers with controllable lengths, using thin film grown by physical vapor deposition. On the other hand, the health effects of inhalation of fibrous aerosol can concern the huge groups of people becoming very important for some local populations. For example, many buildings in Central Europe are covered with thermal insulation containing asbestos-cement sheets, which weather and corrode. Cement particles, asbestos fibers and agglomerates of both particles and fibers are released from the plate surface and become dispersed in the air [18]. It has been also documented that fibers emitted from the asbestos-cement facades of the buildings migrate into indoor air, significantly elevating the concentration of fibrous aerosol in the flats [10–13]. These airborne asbestos fibers can create a health risk, including a lung cancer risk. However, because the carcinogenic properties of asbestos are most probably due to its fiber geometry, also other fibers with the same characteristics may be carcinogenic [21]. This hypothesis can have very important implications in the future, because in the last five years in a number of buildings in many countries, including Poland, the asbestos-cement sheets have been replaced by different isolating materials, mainly glass wool and other synthetic fibers. In fact, synthetic vitreous fibers (SVS), also called man-made mineral fibers [9], have been used extensively in residential and industrial settings for more than one century. They are used primarily for thermal and acoustical isolation, liquid and gas filtration, industrial textiles, and for reinforcing other materials. SVS include a very broad variety of inorganic fibrous substances with an amorphous (vitreous, i.e., non-crystalline) molecular structure. Traditionally, they have been arbitrarily divided into three general categories based on composition and application [4]: fiberglass (including glass wool and the thicker glass filament), mineral wool (rock, stone and slag wool), and refractory ceramic fibers. However, in the last few years, these three categories have become antiquated; the categories are useless for a number of new “hybrid” SVS formulations and are irrelevant for hazard classification [4]. The commercial production of these materials became especially important when the adverse health effects associated with asbestos prompted the search for a substitute material.

It should be noted that fiber-containing materials become dangerous only when the microscopic fibers are emitted from these materials into air. Such phenomenon appears if fiber-containing materials become friable (crumbling), for example due to the atmospheric weather, or if building maintenance, repair, renovation or other activities (vibration
and vandalism) disturb these materials. Therefore, it can be concluded that the important factor that generates, or considerably increases, the emission of fibers from these fiber-containing materials used in buildings is the mechanical impact. Also in the industry the health risk of workers exposed to fiber-containing materials is directly related to the condition of these materials and their property to emit airborne fibers due to a mechanical contact (impact or/and vibration).

In the last decade some papers describing in qualitative way the process of fibers emission have been published. For example, the release of asbestos fibers from the brake pads of overhead industrial cranes has been described by Spencer et al. [17]. USEPA prepared the memorandum on the testing carpet, being the asbestos reservoir [19]. Crossman et al. [2] reported the quantification of fiber releases for various floor tile removal methods. They documented that fibers are released when floor tile is broken and/or abraded during removal procedures. They established that fiber levels vary with the aggressiveness of the procedures but they did not study the emission rate. After the pioneering work of Spurný [18] on the release of asbestos fibers from weathered and corroded asbestos-cement products, recently the emission of fibers from asbestos-cement plates due to the mechanical impact has been determined experimentally [11]. However, till now the phenomenon of the mechanically generated emission of fibers from fiber-containing non-asbestos materials has not been satisfactorily recognized.

The aim of this work was to determine the emission rate of fibers released from the new/fresh and used/worn ceramic fiber material, glass wool and man-made mineral fiber material due to the mechanical impaction.

**EXPERIMENTAL**

A simple experimental set-up has been developed to measure the emission of fibers from the selected materials. The samples of studied materials having a surface area between 0.02 and 0.03 m² were placed inside the AURA 2000 M.A.C. Cabinet adapted for the fiber emission experiments. Masses of the samples ranged from about 7 g to 58 g. During the experiments the ventilation system as well as UV lamps was switched off. The basic principle of the experiments was as follows: the falling weight (10 iron balls of 19 g each, or one iron weight of 450 g) generated the emission of fibers from the samples of fiber-containing materials. Since the falling height was 23 cm, the impaction energy was 0.4 J and 1.0 J respectively. Such low impact energy values were selected to simulate both: vibrations of the fiber-containing building facades caused by the turbulence of wind, and weak mechanical impacts made by workers during the renovation of the thermal isolation of buildings and industrial devices. Additional reason was to keep the same conditions like in the previous study of the emission of fibers from the asbestos materials [11]. The increasing concentration of fibers in the cabinet volume was measured by the Laser Fiber Monitor (MIE, Inc., Billerica, MA). The method used in this monitor is based on the electric field-induced alignment and oscillation of particles, combined with light scattering, resulting in the highly selective detection of individual fibers, even in the presence
of a population of predominantly non-fibrous particles. After each experiment the cabinet was cleaned using the ventilation system with HEPA filter. During all experiments the air temperature and humidity inside the cabinet were almost constant, maintained at the level 24–25°C and 30–31%, respectively.

This set-up had been previously successfully used for the study of fibers emission from mechanically impacted asbestos-cement sheets [11]. Therefore, its detailed description, including the photograph of the Laser Fiber Monitor connected with the cabinet, can be found in this paper.

Figure 1 illustrates the main part of the experimental set-up, located inside the cabinet. It can be seen the photographed sample of mineral fiber material after the mechanical impaction by the iron balls (which can be also observed near the sample) and on the sample surface as well. Over the sample is seen the box for the balls. These balls could drop down through the hole, located in the bottom of the box, and opened from the outside of the cabinet.

![Figure 1](image-url)

Fig. 1. Photograph of the main part of the experimental set-up inside the cabinet  
a – the sample of a mineral fiber material, b – the iron balls, c – the box with iron balls d – the tube connected with the Laser Fiber Monitor (located outside the cabinet)
The emission rate is defined in this study as a number of fibers emitted from the unit mass of investigated material due to the impaction of unit impaction energy. This factor was calculated using the following equations:

\[ C_m = \Delta C_{\text{max}} \cdot V/(m \cdot E) \]  

(1)

where:
- \( C_m \) \([1/(g \cdot J)]\) – is the mass emission factor (using the traditional units \([\text{fibers}/(g \cdot J)]\)),
- \( \Delta C_{\text{max}} \) \([\text{fibers/m}^3]\) – is the highest increase in the measured concentration of fibers inside the cabinet after impaction of 10 balls or one iron weight,
- \( V \) \([m^3]\) – is the volume of the cabinet,
- \( m \) \([g]\) – is the mass of the sample,
- \( E \) \([J]\) – is the impaction energy.

The emission rate has been determined for the following, new/fresh and used man-made fiber-containing materials: glass wool, ceramic fibers and man-made mineral fibers. The length distributions of the emitted fibers have been also investigated.

The new/fresh samples were the production residuals while all samples of the used/worn fiber-containing materials were prepared from the materials previously used as a thermal isolating medium. The glass wool has been used for about 20 years as a thermal wrapper of the hot water pipe-line crossing over the ground. Other materials have been used in the industrial plants.

RESULTS AND DISCUSSION

Table 1 shows the averaged values of the mass emission factor \( C_m \) for 3 types of fiber-containing materials, fresh and used/worn: glass wool, ceramic fibers and man-made mineral fibers. It can be seen that the mechanically induced emission of long (\( L > 5 \mu m \)) and short (\( L \leq 5 \mu m \)) fibers from the new/fresh materials is the lowest for the man-made mineral fibers. It should be noted that the emission factor of short fibers for all fresh materials is between 2 and 20 fibers/(g\cdot J) while the emission rate of long fibers shows more significant differences and ranged from 2 fibers/(g\cdot J) for material containing man-made mineral fibers through 69 fibers/(g\cdot J) for glass wool, up to 100 fibers/(g\cdot J) for ceramic fiber material.

<table>
<thead>
<tr>
<th>Numbers of samples studied</th>
<th>Material</th>
<th>( C_m ) [1/(g\cdot J)]*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(&lt; 5 \mu m)</td>
<td>( \geq 5 \mu m)</td>
</tr>
<tr>
<td>Glass wool</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>new (fresh)</td>
<td>6.9\cdot10</td>
</tr>
<tr>
<td>4</td>
<td>old (used)</td>
<td>4.5\cdot10</td>
</tr>
<tr>
<td>Ceramic fiber</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>new (fresh)</td>
<td>1.0\cdot10^2</td>
</tr>
<tr>
<td>7</td>
<td>old (used)</td>
<td>3.0\cdot10^3</td>
</tr>
<tr>
<td>Man-made mineral fibers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>new (fresh)</td>
<td>2.2</td>
</tr>
<tr>
<td>4</td>
<td>old (used)</td>
<td>7.0\cdot10^4</td>
</tr>
</tbody>
</table>

*in traditional units \([f/(g \cdot J)]\)
It is interesting that the increase of the emission factor of used fiber-containing material highly depends on the type of material. The analysis of the Table 1 indicates that the mass-oriented emission factor of the used mineral fiber material is ten thousand times higher comparing with fresh material while the emission rate of glass wool does not change with long term exploitation. The used ceramic fiber material has the emission factor ten times higher than the emission rate of the fresh material.

Figures 2–4 show the length distributions of airborne fibers emitted during the mechanical impaction. These length distributions have been averaged for the data obtained from both the new and old (used) materials. Although these distributions differ from each other, it can be found that every distribution seems to be two-modal with a first peak appearing for short fibers \((L \leq 5 \, \mu m)\) and the second one observed in the 5–8 \(\mu m\) length range. In this context it is important to note that the length distribution of airborne fibers emitted from asbestos-cement sheets is one-modal, having a peak concentration level in the 4–5 \(\mu m\) length range [11].

**Fig. 2.** Length distribution of the fibrous aerosol generated from the glass wool by the mechanical impaction

**Fig. 3.** Length distribution of the fibrous aerosol generated from the ceramic fiber-containing material by the mechanical impaction
Finally, it was measured the factor $\varepsilon'_S$, defined as a number of emitted fibers per unit area of the impacted surface of the sample, for some impaction energies: from 0.04 J to 1.44 J. The example result, obtained for the sample of the man-made mineral fiber material, is presented in Figure 5 indicating that in the studied range of impaction energy the relationship between $\varepsilon'_S$ and impaction energy $E$ can be described by a linear function.

![Man-made mineral fibers](image)

**Fig. 4.** Length distribution of the fibrous aerosol generated from the man-made mineral fiber-containing material by the mechanical impaction

![Figure 5](image)

**Fig. 5.** Fibers emission from the area unit of the man-made mineral fiber-containing material as a function of the impaction energy
Because this paper discusses only the preliminary results of the emission of fibers from some kinds of fiber-containing mechanically impacted materials it was not possible to described how the exploitation conditions and exploitation time influence the emission factor. To find these relationships further studies are needed.

CONCLUSIONS

Various insulating materials made from natural or artificial fibers are susceptible to emitting fibrous particles generated by impact.

The mass emission rate of short fibers for all new/fresh non-asbestos fiber materials ranges from about 1 to 10 fibers/(g·J) while the emission of long fibers was more diverse; it was about 1 fiber/(g·J) for man-made mineral fibers, 10 fibers/(g·J) for glass wool and about 100 fibers/(g·J) for ceramic fibers.

The susceptibility of worn/used fiber-containing materials to emitting fibrous particles due to a mechanical impaction is significantly diverse:

a) Emission from glass wool did not change after the exploitation.

b) The emission rate of the worn/used ceramic fiber material increased nearly ten times compared to the emission from the new material.

c) The emission rate of the mineral fiber material increased by $10^4$ times above the emission level from the new material.

The dominating population of fibers emitted during a mechanical impaction from the examined materials contains fibers from 2 to around 8 µm of length.

The length-distribution of fibers emitted from materials containing man-made fibers is two-modal, with the first maximum appearing for short fibers ($L \leq 5 \mu m$) and with the second maximum in the range of fibers of 5–8 µm long.

The method of the estimation of the emission rate, described in this work, could be useful in the assessment of the health risk related to human contact with the fiber-containing materials, as well as in the control measurements of the safety level of these materials.

Acknowledgements

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Author is grateful for the assistance of Dr. Beata Kita-Witkowska and Mr. Jan Mastalski of the Institute of Wastes Management, Katowice, Poland for their efforts in sampling non-asbestos fiber-containing materials.

Author also wishes to thank Mrs. Beata Łudzeń-Izbińska, IOMEH, Sosnowiec, for helping with the experiments.

REFERENCES


W pracy wyznaczono doświadczalnie wskaźnik emisji włókien wydzielanych z nowych oraz zużytych materiałów włóknistych (włókna ceramiczne, wełna szklana, sztuczne włókna mineralne) na skutek mechanicznego impakcji (uderzenia). Wskaźnik emisji zdefiniowano jako ilość włókien emitowanych z jednostki masy na skutek jednostkowej energii uderzenia. Średni wskaźnik emisji krótkich włókien (L ≤ 5 µm) dla wszystkich badanych nowych/świeżych materiałów nieazbestowych zawierał się w przedziale od 2,2 do 20 włókien/
(g·J), natomiast emisja długich włókien (L > 5 µm) wynosiła od 2,2 do 100 włókien/(g·J). Podatność zużytych materiałów włóknistych do emitowania cząstek włóknistych na skutek uderzenia była znacząco zróżnicowana. Emisja z wełny szklanej nie zmieniała się na skutek eksploatacji materiału, podczas gdy wskaźnik emisji z zużytego materiału zawierającego sztuczne włókna mineralne był większy w stosunku do wskaźnika emisji z nowego materiału aż 10⁴ razy. Dominującą populację włókien emitowanych z badanych materiałów stanowiły cząstki włókniste o długości od 2 do około 8 µm.
INFLUENCE OF TYPE OF SUBSTRATE AND WATER CHEMISTRY ON THE STRUCTURE AND SUCCESSION OF PERIPHYTIC CILIATE COMMUNITIES IN HYPERTROPHIC LAKE

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Keywords: Hypertrophic lake, natural and artificial substrata, colonization, periphytic ciliates.

Abstract: The aims of the study was to establish whether differences exist between periphytic ciliate communities on different substrates; to determine whether colonization time would yield an abundance and taxonomic composition of ciliates; to assess the effect of physical and chemical factors on the distribution of ciliates in a shallow hypertrophic lake. Generally the species richness as well as the abundance of periphytic ciliates are determined mostly by the habitats and chemical properties of the waters (especially the content of total organic carbon and nitrate nitrogen), and, to a lesser extent, by the type of the colonized substrate. Moreover, exposition time of the substrates affected both an increase in the richness of periphytic ciliates and the changes in their trophic structure. At the beginning of the experiment the substrates were intensively colonized by typically bacterivorous species, yet prolonged exposition time resulted in an increasing proportion of omnivorous species.

INTRODUCTION

In recent years, there has been renewed interest in studies of colonization and successional patterns of periphytic communities on artificial and natural substrates [13, 16]. A few investigators have noted that artificial substrates are biased with respect to characterizing natural communities, while others argue that natural substrates pose problems with sampling design and quantification [13]. The periphyton forms on the surface of artificial and natural substrata of freshwater lakes and rivers and it consists of mucilage of slime, bacteria, algae, fungi, protozoa and small metazoans [10]. Periphyton plays a very important role in carbon fixation and nutrient cycling in aquatic ecosystems. Natural substrata that maintain periphyton are different in origin and size; this variability in the nature of substrata, and the corresponding variation of microbial communities, has always made quantitative studies difficult. To facilitate such studies, artificial substrata have been used for several years. Their usage simplifies the natural complexity and reduces the disruption of the habitat because there is no need to remove large amounts of natural substrata. Furthermore, since the total surface area is known, problems with the measurement of irregular natural substrata are eliminated. Because of their uniform size and inert surface, glass slides are among the most frequently used artificial substrata [13,
16]. Many recent studies have shown that ciliates play a very important trophic role in periphytic communities [19, 20, 24]. Protozoa are known to be an important food source for metazoa and effectively transfer picoplanktonic production to higher trophic levels. They can feed on bacteria, auto- and heterotrophic pico- and nanoplankton and provide dissolved organic matter as nutrients to bacteria. Rapid growth, high turnover rates and short generation times allow protozoan communities to respond immediately to changing environmental conditions. Hence many species can be highly valuable bioindicators in water quality analyses [22, 24]. However, still more attention is paid to planktonic ciliates of marine and river ecosystems [1, 2, 22]. In Polish limnological studies only a few publications have reported abundance and taxonomic composition of periphytic ciliata on natural substrates in eutrophic lakes [19, 20, 21]. Another publication dealing with periphytic ciliates presented mainly the data on ciliate numbers belonging to the order Peritrichida on Phragmites australis in oligo-, mezo- and eutrophic Hungarian lakes [17]. Spatial distribution of periphytic ciliates on natural substrates in a eutrophic lake has been also studied by Primc-Habdija et al. [24]. Boothroyd and Dickie [5] have reported the presence of similar epiphytic communities on macrophytes as well as on natural and artificial substrates. To date, no research has been carried out on periphytic ciliates on natural and artificial substrates in the hypertrophic lakes.

The aim of the present study, therefore, was to establish differences between periphytic ciliate communities on different substrates; to determine whether colonization time would yield an abundance and taxonomic composition of ciliates; to assess the effect of physical and chemical factors on the distribution of ciliates in shallow hypertrophic lake.

STUDY AREA, MATERIALS AND METHODS

Samples were collected in a hypertrophic Lake Syczyńskie (surface area 6.0 ha, max depth 4.0 m). Lake Syczyńskie is situated on Pagóry Chełmskie. Its southern shore borders with carbonate peat-bogs, while the rest of the lake is surrounded by village Syczyn [14, 15]. Lake Syczyńskie is characterized by intensive development of emergent vegetation, dominated by reed (Phragmites australis (Cav.) Trin. ex Steud.) and temporal blooms of Planktotrix agardhii (Gomont).

The periphyton was collected from the reed stems and glass slides in littoral zone at a depth of 0.1–0.5 m. Three perspex frames with 6 microscopic glass-slides (2 x 5 cm each) were placed near the Phragmites bed. The frames were placed horizontally and the slides vertically. Sampling was done monthly from April to November 2007. On each sampling occasion 6 periphyton samples were collected from each type of substrate. New substrata were then placed for colonization in the next month. One sample consisted of 10 cm$^2$ of periphyton taken from the macrophyte stems and glass-slides by means of a scalpel. In order to determine the density of ciliates, 4 samples were fixed with Lugol’s solution (1% v/v) and settled for at least 24 h in plankton chambers. The laboratory experiment was performed in order to trace the colonization and succession patterns of periphytic ciliate communities on particular types of substrates. In laboratory conditions natural (stems of Phragmites australis) and artificial substrates (glass slides) were placed in three aquariums (depth of 0.1–0.5 m) filled with water collected from the studied lake. Three aquariums, contained sediment of about 4–5 cm depth to serve as a benthic substrate. The
microcosms were maintained at 20 ± 2°C. The course of substrates colonization processes was observed during the period of one month, in 4-day intervals. The ciliates were counted and identified with an inverted microscope at magnification x 400–1000. Taxonomic determination was based primarily on Foissner and Berger [8], Foissner et al. [9].

The water samples for chemical analyses were taken simultaneously with the periphyton samples. Direct water measurements comprised transparency, by means of a Secchi disc, pH and conductivity using an electrode. Concentrations of N-NO₃ and Pₜₒₜ were done according to Hermanowicz et al. [11]. Total organic carbon (TOC) was determined by using the PASTEL UV.

One-way ANOVAs were run on the number of species per square centimeter and total number of cells on the reed stems and glass slides. Correlation between physical and chemical parameters and ciliate density were analyzed by calculating Pearson’s correlation.

RESULTS

Physicochemical properties of water
Physicochemical properties of water were different in particular months. The transparency of water (SD) was the lowest in summer (August – 0.23 m) and the highest in early spring (0.54 m). Water pH did not reveal any significant differences and in all months it amounted to the values adequate for neutral or alkaline water (pH = 7.3–8.42). Conductivity was the highest in October, reaching 496 µS·cm⁻¹. In the remaining months its values were from 339 µS·cm⁻¹ to 495 µS·cm⁻¹. The mean concentration of oxygen varied from 10.3 mg O₂·dm⁻³ in April to 12.4 mg O₂·dm⁻³ in August. Total organic carbon content fluctuated between 6.4 mg C·dm⁻³ in summer and > 7.7 mg C·dm⁻³ in spring and autumn. The mean content of nitrate nitrogen was 0.07 mg N·dm⁻³, reaching its highest values in autumn. Total phosphorus concentrations were highest in spring and autumn (0.303 mg·dm⁻³ and 0.283 mg·dm⁻³, respectively), and lowest in summer, when they did not exceed 0.194 mg·dm⁻³ (Tab. 1). Physicochemical properties of water used in the experiment were very different on particular days of exposition. The differences were highest in the case of conductivity, total organic carbon (TOC) and nitrate nitrogen (N-NO₃), and lowest as regards temperature, pH, dissolved oxygen and concentration of total phosphorus (Tab. 1).

Species richness and abundance of ciliates in the field
The number of species of periphytic ciliates was not significantly different in the studied types of substrates. Thirty nine ciliate taxa were identified on natural substrates (reed steams), and slightly less (37) on artificial substrates (glass slides). Thirty-seven taxa (94.8%) were common for the studied substrates. Mean abundance of periphytic ciliates on the studied substrates revealed slight differences (P > 0.05). Their higher density was observed on reed stems and it amounted to 77 ind·cm⁻². A slightly lower abundance was noted on glass slides – 71 ind·cm⁻². Irrespective of the type of the substrate, ciliate abundance was the highest in autumn (November) and the lowest in summer (June or July) (Fig. 1). The domination structure of periphytic ciliates was similar on natural and artificial substrates. Both types were dominated by Hymenostomatida, Pleurostomatida and Oligotrichida (Fig. 2 A, B). Also, the percentage share of individual trophic groups
was similar on both types of the examined substrates. Both substrates were dominated by omnivorous species which constituted 28% and 29% of ciliate density, respectively. Bacterivorous ciliates occurred in high numbers (25%) as well (Fig. 3 A, B).

<table>
<thead>
<tr>
<th>Months</th>
<th>SD [m]</th>
<th>pH</th>
<th>Conductivity [µS·cm(^{-1})]</th>
<th>Dissolved oxygen [mg·dm(^{-3})]</th>
<th>(P_{\text{tot}}) [mg·dm(^{-3})]</th>
<th>N-NO(_3) [mg·dm(^{-3})]</th>
<th>TOC [mg C·dm(^{-3})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>0.54</td>
<td>7.6</td>
<td>495</td>
<td>10.3</td>
<td>0.203</td>
<td>0.04</td>
<td>7.7</td>
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<tr>
<td>VI</td>
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<td>8.42</td>
<td>451</td>
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<td>0.303</td>
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<td>X</td>
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Table 1. Physical and chemical characteristics of the water of investigated lake and in laboratory experiment.

Fig. 1. Seasonal patterns of the density of periphytic ciliates community on natural and artificial substrata in littoral of investigated lake.
Species richness and abundance of ciliates in laboratory experiment

Nineteen taxa of periphytic ciliates were observed in the course of experiment. Species diversity of periphytic ciliates revealed some slight differences between the studied substrates (P > 0.05). Fourteen taxa (74%) were common for the substrates examined. The most significant differences were observed at the end of the experiment (day 31, 35 and 39), as well as on the 15th, 19th and 23rd day of substrates’ exposition (ANOVA F(4.2) = 21, P = 0.018). The mean density of ciliates on glass slides reached 38 ind. cm⁻². A lower abundance was noted on reed stems, amounting to 30 ind. cm⁻². The lowest number of ciliates was observed on the third day of exposition, whereas the highest increase in the density of these microorganisms occurred on the 31st day of the substrates’ exposition (Fig. 4). On the 3rd day ciliates representing merely two orders: Cyrtophorida and Hypotrichida. Reed stems were definitely dominated by Hypotrichida, constituting 75% of the total

Fig. 2 A, B. Seasonal patterns of abundance of the common Ciliata on natural and artificial substrata in littoral of investigated lake (% of total numbers)
number. Glass slides, on the other hand, were dominated by Cyrtophorida which made 54% of the total density. On the 7th day of exposition both natural and artificial substrates were dominated by the species representing Hypotrichida, constituting, respectively, 57% and 47% of the total numbers. During the successive days of the exposition the proportion of Pleurostomatida and Peritrichida clearly increased (Fig. 5 A, B). The percentage share of particular trophic groups revealed only insignificant differences between the studied substrates. Bacterivorous ciliates were prevalent on both reed stems and glass slides. Their proportion on the natural substrates reached 53%, whereas it was as much as 63% on the artificial ones. Omnivorous ciliates constituted 20% of the total number on reed stems and 16% on glass slides. Predatory and mixotrophic species constituted 13% each of all trophic groups on natural substrates. In the course of exposition, bacterivorous and omnivorous ciliates were more abundant (Fig. 6 A, B).

Fig. 3 A, B. Seasonal patterns of trophic groups of periphytic ciliates found on natural and artificial substrata in littoral of investigated lake.
Fig. 4. The relative density of ciliates on artificial and natural substrata in laboratory experiment from day 3 to 39 day period.

Fig. 5 A, B. Domination structure of Ciliata on artificial and natural substrata in laboratory experiment from day 3 to 39 day period (% of total numbers).
The effect of chemical properties of water on the abundance of periphytic ciliates

Both in the lake and in the laboratory environment, irrespective of the type of the substrate, the abundance of ciliates correlated positively with conductivity. Correlation coefficient on reed stems reached the value of 0.53, \( p \leq 0.05 \), whereas its value on glass slides was \( r = 0.43–0.54 \), \( p \leq 0.05 \). On natural substrates the abundance of periphytic ciliates correlated positively with the content of TOC in water (\( r = 0.53, p \leq 0.05 \)). On artificial substrates the correlations between ciliate numbers and concentrations of total organic carbon were slightly stronger (\( r = 0.64–0.66, p \leq 0.01 \)). Irrespective of the type of substrate, the abundance of ciliates correlated positively with total phosphorus and concentrations of nitrate nitrogen (\( r = 0.43–0.45, p \leq 0.05 \) and \( r = 0.40 \ p \leq 0.05 \), \( r = 0.63, p \leq 0.01 \), respectively) (Tab. 2).

Fig. 6 A, B. Trophic groups of periphytic ciliates found on natural and artificial substrata in laboratory experiment from day 3 to 39 day period
DISCUSSION

On the basis of the conducted studies it was concluded that the number of species of periphytic ciliates on natural and artificial substrates is almost identical. Such a situation occurred both in the case of samples collected from the lake and in laboratory conditions. An extremely high similarity in the taxonomic composition of ciliates inhabiting natural and artificial substrates was also observed by Chadwick and Canton [7], Boothroyd and Dickie [5], and Mieczan [21]. In late summer and in autumn Cladophora sp. was developing intensively on the stems of Phragmites australis in Lake Syczyńskie, forming a periphyton of “thread-like” character. This type of periphyton seems to create larger numbers of micro-niches and, consequently, it is willingly inhabited by periphytic ciliates. On the other hand, glass slides were dominated by the periphyton of “shell-like” character with a clear prevalence of diatoms. Characteristic (exclusive) species constituted a small group on both the stems and on glass slides. In Lake Syczyńskie reed stems were inhabited by only two species of the group, Litonotus varsaviensis and Holophrya sp. Litonotus varsaviensis was also found as a characteristic species in a eutrophic lake situated in Łęczna-Włodawa Lakeland [19]. Ciliates representing Holophrya on the stems of Phragmites australis were also noted in eutrophic and dystrophic lakes [21]. In laboratory conditions two characteristic taxa, Chlamydonella sp. and Litonotus cygnus, occurred. Chlamydonella sp. genus comprises species of wide ecological tolerance and it was observed in different types of trophic lakes, as well as in peat ecosystems [21]. Litonotus cygnus occurred quite abundantly on the stems of Phragmites australis in the eutrophic lake [21]. Glass slides revealed 3 characteristic taxa, namely Vorticella microstoma – complex, Carchesium sp. and Litonotus sp.

The density of periphytic ciliates in Lake Syczyńskie showed slight differences between natural and artificial substrates. Their higher numbers (77 ind·cm⁻²) were found on reed stems and slightly lower ones on glass slides (71 ind·cm⁻²). The abundance of periphytic ciliates revealed similar tendencies in eutrophic lakes [20]. On the other hand, studies focused on the density of periphytic ciliates on glass substrates in a eutrophic lake in Croatia showed that the abundance of the communities fluctuated between 40 and up to 2,400 ind·cm⁻² [23], whereas in dystrophic lakes in Germany their numbers on glass slides did not exceed 30 ind·cm⁻² [27]. In laboratory conditions a higher density was noted on artificial substrates. A lower abundance of ciliates on reed stems may result from the fact that throughout the whole period of the studies in the experimental laboratory...
conditions the substrate was intensively colonized by rotifers and nematodes which can control to a high extent the abundance of protozoan. In laboratory conditions the abundance of ciliates did not increase after a long period of exposition (day 35 and 39). The occurrence of protozoa species on new substrate is in accordance with the Mac Arthur and Wilson [18] model for the colonization of islands. At the early stage of colonization, the immigration rate of species to the substrate is high. As the colonization proceeds, the immigration rate declines.

In field conditions the densities of periphytic ciliates remained on a similar level in particular months. The highest richness was noted on both reed stems and glass slides in autumn (November). The lowest density for both types of substrates was observed in summer (June – July). The increase in the number of ciliates in autumn could have resulted from feeding conditions profitable for that group of micro organisms. In autumn the littoral zone was characterized by a significant content of organic matter. High densities of such organisms at that period may also have been a consequence of a distinct increase in their abundance in the pelagial zone. The studies performed by Sanders and Wickham [25] point at a periodical occurrence of planktonic species in the periphyton complex. On the other hand, low abundance of periphytic ciliates in summer may have resulted from their being consumed by the organisms representing higher trophic levels. In the studied lake, especially in June, extremely high numbers of planktonic crustacean fauna were observed, particularly the ones representing Daphnia genus. Indeed, as proved by the studies performed by Carrias et al. [6], these organisms do control the abundance of protozoans. The natural substrates of Lake Syczyńskie were dominated by ciliates representing the genera of Hymenostomatida and Pleurostomatida. Another numerous group was Prostomatida. The prevalence of this order was also noted in eutrophic lakes [19]. The most numerous groups on artificial substrates were Oligotrichida and Hymenostomatida. In the laboratory reed stems were dominated by ciliates belonging to the orders of Hypotrichida, Peritrichida and Cytophorida. The species representing these orders include mainly typically periphytic forms and are especially abundant in eutrophic and hypertrophic waters [8]. In Lake Syczyńskie, irrespective of the type of the substrate, omnivorous and bacterivorous species prevailed. High numbers of omnivorous ciliates in freshwater reservoirs were also observed by other authors [2]. The examinations of periphytic ciliates carried out in eutrophic lakes revealed that at the early stages of colonization natural and artificial substrates were dominated by bacterivorous species, whereas after approximately two weeks of exposition the proportion of algivorous and mixotrophic species increased as well. The studies performed by Beaker [1] prove that bacteria appear already 4 hours after the exposition of the substrates. Most probably, such an early and numerous occurrences of bacterivorous organisms is the effect of beneficial feeding conditions. Natural substrates also revealed large numbers of mixotrophic ciliates. Mixotrophs occur copiously in reservoirs of different trophic status [4]. Physiological flexibility of mixotrophs provides them with an advantage in changeable and unpredictable conditions which characterize shallow hypertrophic lakes [12]. Observed in this study low numbers of algivorous ciliates in the periphyton may have resulted from difficulty in finding appropriate food since periphyton was dominated by large-cell algae, filamentous and colony-forming organisms which were inaccessible or hardly accessible for the ciliates. The results of the laboratory studies were similar. The trophic structure of periphytic ciliates in field conditions showed relatively low seasonal variability. In spring
natural substrates were dominated by omnivorous species, whereas artificial substrates were abundantly inhabited, apart from omnivorous, by predatory species. In summer the prevalence of mixotrophic species was noted on both types of substrates. An increase in the number of mixotrophic species was also recorded in a eutrophic lake, and their occurrence may be related to an increase in the density of fine phytoplankton [21]. In autumn the proportion of bacterivorous ciliates increased on both reed stems and glass slides. Natural substrates revealed also very numerous omnivorous species in that period, whereas artificial substrates were abundant in mixotrophic species. The increase in the share of bacterivorous species was probably related to advantageous feeding conditions resulting from a high content of total organic carbon in the water. Both in the lake and in the laboratory conditions the densities of ciliates on the two studied substrates were closely correlated with conductivity and the content of TOC in the water. An increase in the numbers of ciliates on natural and artificial substrates accompanying an increase of the two agents was observed by other authors [20]. Clearly positive correlations between the content of TOC in the water and the abundance of ciliates on the stems of *Phragmites australis* and on glass slides were also noted in the eutrophic reservoir [21]. The abundance of ciliates on the studied substrates was also strongly related with the content of nitrate nitrogen and total phosphorus in the water. Similar correlations were observed in shallow eutrophic lakes [3, 20, 21].

**CONCLUSIONS**

Species richness as well as the abundance of periphytic ciliates are determined to the highest degree by the habitats and chemical properties of the waters (especially the content of TOC and nitrate nitrogen), and, to a lower degree, by the type of the colonized substrate. Moreover, exposition time of the substrates affected both an increase in the richness of periphytic ciliates and the changes in their trophic structure. At the beginning of the experiment the substrates were intensively colonized by typically bacterivorous species, yet prolonged exposition time resulted in an increasing proportion of omnivorous species.

**REFERENCES**

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Wpływ rodzaju podłoża oraz właściwości chemicznych wody na strukturę i sukcesję zespołu orzęsków peryfitonowych w jeziorze hypertroficznym

Celem pracy było porównanie składu taksonomicznego i liczebności zespołu orzęsków peryfitonowych zasiedlających różne podłoż; określenie, w jaki sposób czas ekspozycji podłoży wpływa na obfitość tych mikroorganizmów oraz analiza zależności pomiędzy wybranymi właściwościami fizyczno-chemicznymi wód a orzęskami w jeziorze hypertroficznym. Wykazano, że zarówno bogactwo gatunkowe jak i obfitość orzęsków peryfitonowych w największym stopniu determinują zasoby pokarmowe siedliska oraz właściwości chemiczne wód (głównie zawartość całkowitego węgla organicznego oraz azotu azotanowego), w mniejszym zaś stopniu rodzaj kolonizowanego podłoż. Czas ekspozycji podłoży wpływał zarówno na wzrost obfitości peryfitonowych orzęsków jak i zmianę ich struktury troficznej. Na początku eksperymentu podłoża były intensywnie kolonizowane przez gatunki typowo bakteriożerne, wraz ze wzrostem czasu ekspozycji podłoży wzrastał udział gatunków wszystkożernych.
THE BAYESIAN MODEL OF THE INTERDEPENDENCIES BETWEEN SOIL SORPTION FEATURES

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Keywords: Belief network, soil sorption feature, interdependencies.

Abstract: The paper presents a qualitative, Bayesian model used to determine some interdependencies between sorption features for mineral soils in southern Poland. Sorption properties are very important, crucial for measure of fertility, nutrient retention capacity, and the capacity to protect groundwater from contamination. Cation exchange capacity (CEC) is a commonly applied indicator of the soils conditions or vulnerability. Base saturation (BS) is an important element of hazard degree assessment in soils lying within reach of impact of acidifying agents. The considered soils represented different valuation classes and differed in their typology. The Bayesian model is used for interdependences assessment.

INTRODUCTION

The soil sorption properties (Cation Exchange Capacity – CEC and Base Saturation – BS) are regarded as important soil valuation criteria. It is assumed that soils characterized by higher cation exchange capacity (the CEC value) retain applied nutrients better than those with smaller values. Similarly, base saturation indicating percent saturation of cation exchange capacity by base cations is an important soil condition indicator. Complete vulnerability estimation except for CEC and BS, needs to introduce the intensity parameter. It informs us about the sorption aspect resulting, for instance, from soil density, soil moisture and structure variation.

Traditionally, the characteristic of soil sorption properties (CEC and BS) is most often determined using the Kappen method. It involves determination of ions with base reaction and ions with acid reaction in a soil sample water extract. The sum of their capacity gives the computed cation exchange capacity, while the sum of ions with base reaction, given in percent, constitutes base saturation. The determination is relatively complex and time-consuming, therefore in some cases its substitute is being used (e.g. the extent of methylene blue sorption), or empirical equations [4, 7]. This is so because their parameters transform other soil properties, which are easier for laboratory testing. In soil science, these equations are called PedoTransfer Functions – PTF [6].

However, in some circumstances, it is enough to have only qualitative information, referring to the scale and state of soil sorption. This approach was successfully applied,
for example, in making the database of the soils of the European Union, where so-called PedoTransfer Rules (PTR) were adopted. The idea of constructing such a qualitative model results from the general knowledge of the relationship between sorption properties and the content of clay fraction and organic carbon, on one side, and the soil reaction on the other. Such a model can successfully be formalized. An efficient tool for the formalization can be a properly designed Bayesian belief network.

In Bayesian belief network, a prior probability distribution is assigned to each variable and then the strength of the dependence between each pair of variables is defined. A Bayesian network [1–3] is a probabilistic model that represents a set of random variables and their conditional independencies via a directed acyclic graph. Formally, Bayesian networks are directed acyclic graphs whose nodes represent variables, and whose edges encode conditional interdependencies between the variables. Generalizations of Bayesian networks that can represent and solve decision problems under uncertainty are called influence diagrams. A Bayesian network could represent the probabilistic relationships between reasons and effects.

MATERIAL AND METHODS

The soil material originated from studies carried out in the scope of implementation of a project concerning determination of soil quality in reclaimed areas [5]. In the scope of these studies, samples were taken from 67 soil pits in order to determine some physical and chemical properties. This was aimed to develop a database containing characteristics of soils in various (almost all) valuation classes. Soil pits were made in soils belonging to various valuation classes, from class II to VI of arable land. As regards typology, the soils represented Luvisols, Cambisols and Gleyic Phaeozems. Open pits were made in Southern Poland. Within the carried out investigations on 381 soil samples numerous physical and chemical properties were determined. The following analytical methods were employed for the purposes of determining the properties used in this work:

- grain-size distribution – Casagrande areometric method,
- MH, maximum hygroscopicity – in vacuum desiccator over 10% sulphuric acid,
- CEC and BS, cation exchange capacity and base saturation – the Kappen method,
- pH(1) soil reaction in water suspension, pH(2) soil reaction in KCL suspension – electrometrically,
- OC, organic carbon – in the CS-500 Eltra elementary analyzer, after having deducted inorganic carbon determined in the Scheibler apparatus,
- SBM, methylene blue sorption – the Peter-Markert method according to Myślińska [8].

The results of determinations were used to develop the Bayesian, qualitative, empirically

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cal model interrelations between properties of soils. Table 1 specifies elementary measures of central tendency and dispersion of data contained in the database.

**BAYESIAN MODEL OF SORPTIVE RELATIONS**

Two circumstances connected with the issue of CEC and BS estimation are worth consideration: correlations between these factors and thus variables forming them, and considerable fuzzy relations between valuation of both components and qualitative classification of soils. In other words, oftentimes linguistic, imprecise and fuzzy evaluations are sufficient, apart from the cases of absolute need to use numerical values of estimations of both quantities. In these conditions, the Bayesian model may constitute a good tool to perform an analysis of correlations determining sorption effects in soil.

The Bayesian model, which belongs to the group of graphical models, is known in reference literature as the Bayesian network (Bn – Bayesian network, or belief network), and by assumption it reflects statistical relations between the system elements. This concluding model assumes [1, 2] the existence of domain \( X \), for which the following are specified: probability distribution \( \Omega \) and attributes \( a_1, a_2, \ldots, a_n \) for \( a_i : X \rightarrow A_i \) for \( i = 1, 2, \ldots, n \). Conventionally, it is assumed for simplicity that the attributes are solely nominal, and we are interested only in the relationship between them. In practical applications of this model, it is common to use different terminology: attributes are referred to as variables, and the domain is constituted by the set of all possible attributions of variable values [2].

In recent years, the Bn networks have become popular as the concluding systems, mainly due to the fact that computers considerably facilitate rather complex calculations required in order to estimate probability of a certain event. At the same time, packages make the Bn design easier and their utilization have been popularized as well [3].

The key problem involved in generating the Bn cause-effect networks is the algorithm applied to reconstruct the form of relationships between the system variables. In general, two approaches are possible here, depending on certain circumstances:

1. Construction of a Bn only with participation of experts, based on their belief, while an expert may design graph of the network and parameters for its processing; or else a database will be used to determine these parameters after having designed the graph.

2. Construction of the network as a whole – as a reflection of knowledge contained in the database only, in practice without direct participation of an expert. There are many algorithms allowing to perform this operation [1–3], from extremely simplified to complex ones.

In the Bn networks, individual variables are bound up with conditional distribution tables determined by an expert or as a result of applying the learning procedure. This allows to update individual distributions after having observed a specific value of any variable, that is to establish distributions a posteriori. Each Bn network node has an attributed table of variable state probability on condition of the state of its predecessors (so-called „parents”) in the network. Concluding in the Bayesian networks is executed by propagation of successive conditional distributions at observed value of a specific variable, according to the formula [2]:

\[
P(x_1, \ldots, x_n) = \prod_{i=1}^{n} P(x_i \mid \text{Predecessor}(x_i))
\]  

(1)
The GeNiE computational system has been used in the paper, developed at Pittsburgh University for research purposes. The obtained model was evaluated using the Netica application from NorSys.

**Discretization**
Most of packages used to generate the Bn network require discrete variables. Certainly, this involves the need to convert continuous variables into nominal variables.

One may assume – while analyzing regression examination results – that the network of connections determining sorption properties of soils includes the following variables: colloidal clay content, organic carbon content (OC), maximum hygroscopicity (MH), methylene blue sorption (SBM), reaction (pH), and of course cation exchange capacity (CEC) and base saturation (BS). In view of model construction requirements, these variables had to be discretized by rating their individual values in proper classes. Discretization resulted in the conversion of raw variables into modified variables: clay, carbon, higr, mblue, react, cec and bs. Table 2 shows the digitizing principles. Note that some variables are classified (named) with certain exaggeration (for example those related to soil reaction), but this results solely from the need to ensure the model communicativeness.

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<tr>
<td>MH</td>
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<td>CEC</td>
<td>cmol/kg cec</td>
<td>VLOW(&lt; 5), LOW(5.1–10), HIGH(10.1–20), VHIGH(&gt; 20)</td>
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<td>Reaction</td>
<td>pH react</td>
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<tr>
<td>BS</td>
<td>% bs</td>
<td>LOW(&lt; 50), MED(50–75), HIGH(&gt; 75)</td>
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**The model graph**
Construction of a Bayesian model requires many tests to be performed. Employment of an algorithm producing network graph in an automated procedure has the value of objectivism, but it also carries with it the risk of occurrence of incidental connections between variables. The graph of the model shown in Figure 1 is one of possible versions of the concluding system. From this point of view, the ‘mblue’ and ‘react’ variables function as diagnostic variables, connected with the variables we are interested in, which are difficult to observe.

Figure 2 illustrates the distribution of probabilities for variables at determined (by assumption observed) values of the ‘react = NEUTR’, ‘mblue = VHIGH’ variables. This configuration results in the \( p(HIGH) = 0.99 \) value being indicated for the ‘bs’ variable, and \( p(HIGH) = 0.59 \) and \( p(VHIGH) = 0.4 \), respectively, for the ‘cec’ variable. In Figure 3, the configuration has changed, since it has been observed that ‘react = VACID’. In this situation, the following estimate has been obtained for the ‘bs’: \( p(LOW) = 0.12, p(MED) = 0.7, \) and \( p(HIGH) = 0.18 \). Also, the estimates of the ‘cec’ variable probability value have changed: \( p(HIGH) = 0.27, p(VHIGH) = 0.7 \), respectively, with further consequences.
Fig. 1. The structure of a Bn network for analyzing sorption properties of soils

Fig. 2. Probability distributions for variables of the Bn model variables at determined 'react = NEUTR', 'mblue = VHIGH' variables
Fig. 3. Probability distributions for variables of the $Bn$ model variables at determined 'react = VACID', 'mblue = VHIGH' variables

Fig. 4. Probability distributions for variables of the $Bn$ model variables at determined 'react = VACID', 'mblue = VLOW' variables
Figure 4 illustrates the situation in which ‘react = NEUTR’ and the observed value ‘mblue = VLOW’. As a consequence of these changes, we obtain the following configuration for the ‘bs’: $p(LOW) = 0.13$, $p(MED) = 0.36$, and $p(HIGH) = 0.51$. Significant changes have occurred in the distribution of probabilities for the ‘cec’ variable, which assume the following values: $p(VLOW) = 0.67$ and $p(LOW) = 0.32$.

There is a problem of qualitative valuation of the obtained models. The following indicators are used conventionally in models valuation: error frequency coefficients, error matrixes, and indicators taking into consideration probability distribution for the states of the variable we are interested in. The Netica Package offers the following three indicators which analyze the distribution of probability indications: $L_l$ (logarithmic loss), computed using formula:

$$L_l = \frac{1}{n} \sum_{i=1}^{n} [-\log(P_i)]$$

$L_B$ (Brier, quadratic loss), computed using formula:

$$L_B = \frac{1}{n} \sum_{i=1}^{n} [1 - 2 \cdot P_i + \sum_{j=1}^{m} (P_j)^2]$$

and $C_S$ (spherical payoff), computed using formula:

$$C_S = \frac{1}{n} \sum_{i=1}^{n} \frac{P_i}{\sqrt{\sum_{j=1}^{m} (P_j)^2}}$$

Lower limit of indicator $L_l = 0$; this value shows the best efficiency. The $L_B$ indicator value ranges within $(0; 2)$, where $0$ indicates the best efficiency. The $C_S$ indicator value ranges within $(0; 1)$, where $C_S = 1$ shows best efficiency.

Tables 3, 4 and 5 contain indicators for correct estimates made using the $Bn$. They show serious scattering of results, and difficulties with building of a reliable model, since the indicators have been obtained for the training set, assuming complete knowledge of the explanatory variables distribution (apart from predicted variables).

<table>
<thead>
<tr>
<th>Pr(VLOW)</th>
<th>Pr(LOW)</th>
<th>Pr(HIGH)</th>
<th>Pr(VHIGH)</th>
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<td>4</td>
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<tr>
<td>Obs(LOW)</td>
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<tr>
<td>Obs(HIGH)</td>
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<td>169</td>
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<tr>
<td>Obs(VHIGH)</td>
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<table>
<thead>
<tr>
<th>Pr(LOW)</th>
<th>Pr(MED)</th>
<th>Pr(HIGH)</th>
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<tbody>
<tr>
<td>Obs(LOW)</td>
<td>15</td>
<td>28</td>
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<tr>
<td>Obs(MED)</td>
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<td>81</td>
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<tr>
<td>Obs(HIGH)</td>
<td>0</td>
<td>27</td>
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</table>
The Bayesian network indicates probability distribution for states, related to certain configuration of variables. Besides certain cases of close function-type relations it does not allow to determine what exactly will be the value of the variable. However, a diagnostic model of this type may be a useful tool helping to make correct technical decisions, for example during reclaiming works, in particular considering suitably extensive database used to build it. Making of decisions concerning neutralizer doses, aimed to reach certain saturation state may be based on a similar model.

Quite a serious problem in making adaptation models is defining their generalization properties. Adaptation models often contain many free parameters, being justed in the training procedure. With a relatively small amount of data, there is a risk of adjusting a model to a concrete set, which then manifests in a disproportional growth of the error in the classification of the data outside the training set. A commonly applied way of the

<table>
<thead>
<tr>
<th>Indicator</th>
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<th>BS</th>
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<tr>
<td>Error rate [%]</td>
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<td>17.6</td>
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<tr>
<td>Logarithmic loss</td>
<td>0.32</td>
<td>0.36</td>
</tr>
<tr>
<td>Quadratic loss</td>
<td>0.21</td>
<td>0.23</td>
</tr>
<tr>
<td>Spherical loss</td>
<td>0.88</td>
<td>0.86</td>
</tr>
</tbody>
</table>

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Table 5. Indicators of the Bayesian network indications quality for the CEC and BS valuation

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<th>L₂</th>
<th>Cₛ</th>
<th>ER</th>
<th>L₁</th>
<th>L₂</th>
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</table>

Table 6. Results of the Bayesian network cross-validation (10-Folds method) for the CEC and BS valuation

(ER – error rate in %)
assessment of this risk is cross validation [1]. The results of this procedure for the discussed model are presented in Table 6. It is natural that the validation usually gives worse results of the correctness of classification than it takes place with the application of data used in the training. This is also true in this case, although the results of the validation are relatively little significant, they are, generally, different than the assessment of the model, based on the training data. Looking through the indicators of the classification correctness, the model can be regarded as reflecting real relationships between soil features.

SUMMARY AND CONCLUSIONS

Problems on land use, soil conservation and environmental management require increasingly accurate information on soil properties and their geographical location. Qualitative models, particularly in GIS/LIS application as a soils inference system, are developed for estimation of attributes related to management, planning practices, environmental impact assessment, environmental risk assessment and other areas.

All the soil properties are linked with one another in numerous relationships. The activities, undertaken, e.g. to improve a certain soil property, can lead to the modification of many features. A comprehensive model of the functioning of the soil system would probably be useful, but nowadays we have to do with extremely simplified models, neglecting some conditions. The Bayesian belief network is helpful in the construction of such models. They were constructed with the objective to assess the impact on the environment of different soil management scenarios. The attempt presented in the paper shows the advantages and disadvantages of such modeling: despite significant simplifications and obvious removals of some aspects, the obtained result can be regarded satisfactory. A model of this type enables to bind comprehensively the observed parameters of the ecosystem and analyzing its behavior in case of the intervention in its structure. Indirectly, the model of this type shows the need of analyzing, if possible, many soil properties, because rudimentary data do not allow the estimation of the directions of potential threats connected with the intended activities, e.g. reclamation measures. An obvious disadvantage of this is the need of basing on a rather large set of empirical data, because, otherwise, there is a high risk to obtain a model insufficiently reflecting general relationships between soil properties.

REFERENCES

MODEL BAYESOWSKI WSPÓŁZALEŻNOŚCI MIĘDZY CECHAMI SORPCYJNYMI GLEB

Praca przedstawia jakościowy, bayesowski model niektórych współzależności między cechami sorpcyjnymi mineralnych gleb opróbowanych w południowej Polsce. Właściwości sorpcyjne są ważnymi cechami, współdecydującymi o poziomie nawożenia, zasobności w składniki pokarmowe oraz zdolności do ochrony wód gruntowych przed zanieczyszczeniem. Pojemność wymienna kationów jest powszechnie używanym wskaźnikiem stanu gleb i ich odporności na różnorodne wpływy. Stopień wysycenia kationami zasadowymi jest ważnym czynnikiem kształtowania ryzyka środowiskowego związanego z procesami zakwaszania gleb. Badane gleby reprezentują różne klasy bonitacyjne i typologię. Do oceny współzależności zastosowano model bayesowski.
MOLASSES AS A CARBON SOURCE FOR DENITRIFICATION

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ul. Słoneczna 45G, 10-907 Olsztyn-Kortowo, Poland
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Keywords: SBR, denitrification, molasses, hydrolyzed molasses, observed biomass yield coefficient.

Abstract: The paper presents the results of an experiment with sugar-industry waste (molasses) as an organic carbon source for denitrification. The investigations concern the influence of untreated molasses and molasses after pretreatment (hydrolyzed molasses) and variable COD/N ratio (6.0; 5.0; 4.0) on denitrification efficiency and kinetics. Moreover, sludge production, in dependence on tested carbon source, was estimated. At COD/N ratio 6 and 5, regardless of applied organic carbon source (untreated molasses, hydrolyzed molasses), the denitrification efficiency was over 98%. However, from kinetic analysis it results that a kind of carbon source and COD/N ratio have an effect on denitrification rate. The highest nitrate removal rate – 9.5 mg NO₃⁻/(dm³·h) was obtained at COD/N = 6 in the reactor with hydrolyzed molasses as a carbon source and the lowest – 5.14 mg NO₃⁻/(dm³·h) in reactor with untreated molasses at COD/N = 5.0. The lowering of COD/N ratio to 4 caused decrease of the process efficiency to 27.6% (untreated molasses) and 44.3% (hydrolyzed molasses). Hydrolyzed molasses as a carbon source caused higher production of activated sludge. In reactors with untreated molasses Yobs equals 0.40 mg VSS/mg COD at COD/N ratio 6 and 0.31 mg VSS/mg COD at COD/N ratio 5. In reactors with molasses after hydrolysis Yobs were 1.35-fold and 1.5-fold higher, respectively. Since, the molasses hydrolysis results in rising costs of wastewater treatment and cause higher sludge production, untreated molasses seems to be a more suitable carbon source for denitrification.

INTRODUCTION

The biological oxidation of nitrogen compounds occurring in wastewater leads to nitrates as the final products. The biological removal of nitrate can be achieved via heterotrophic denitrification, which requires a carbon source as an electron donor. In the case of wastewater, especially with a low COD/N ratio, the readily biodegradable organics can be a limiting factor for complete nitrate removal.

From the review of the literature, it can be concluded that short-chain fatty acids and low molecular weight alcohols are the most often applied as an external carbon source in denitrification [5, 7, 9, 10, 11, 15, 20]. In the case of wastewater containing high concentrations of nitrate, the use of commercially available chemicals represents a critical cost in the process. Therefore, researchers use waste materials as an alternative carbon source, e.g. industrial effluents, primary sludge, supernatant of thermally treated wastewater sludge or organic fraction of municipal solid waste [2, 3, 18, 21, 27].

In spite of a multiplicity of investigations, the dose of an external organic carbon source required for denitrification process, has not been established. The main reason is that the process efficiency depends on many factors, such as the kind of external carbon...
source, the type of reactor used and the microorganisms used in the process (pure cultures, mixed populations). So, it can be concluded that the optimal C/N ratio for the denitrification system for treating by different wastewater must be experimentally determined.

Nowadays, with the rising costs of sludge disposal, the minimization of sludge production during wastewater treatment has become of an increasing importance. However, studies concerning denitrification with an external carbon source have dealt with process efficiency without the examination of activated sludge production. Therefore, the aim of this study was to examine sugar-industry waste (molasses) as an organic carbon source for denitrification, to determine process efficiency and kinetics in dependence on the pretreatment of molasses and COD/N ratio and to determine the defined sludge production in term of $Y_{obs}$.

**METHODS**

**Process configuration**
Experiments were carried out in sequencing batch reactors SBRs with the working volume of 2.5 dm$^3$. The reactors were operated in a 24-h cycle mode (filling 0.25 h, anoxic 23 h, settling 0.5 h and decantation 0.25 h).

Three series were carried out (series 1–3) differed in COD/N ratio in the influent, i.e. 6.0 (series 1), 5.0 (series 2) and 4.0 (series 3). At each series the investigations were conducted in parallel research stations (SBR 1 and SBR 2) with untreated molasses and molasses after pretreatment (hydrolyzed molasses) as an organic carbon source (Tab. 1).

<table>
<thead>
<tr>
<th>Technological parameters</th>
<th>SBR 1</th>
<th>SBR 2</th>
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<tbody>
<tr>
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<td>hydrolyzed molasses</td>
</tr>
<tr>
<td>Hydraulic retention time (HRT) [d]</td>
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<td>1.25</td>
</tr>
<tr>
<td>Volumetric exchange rate [-]</td>
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<td>0.8</td>
</tr>
<tr>
<td>$C_{0,N-NO_3}$ [mg N$_{NO_3}$/dm$^3$]</td>
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<td>100</td>
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</table>

All reactors were operated at a room temperature (19 ± 3°C) for 2 months at each COD/N ratio.

**Wastewater and carbon source characterization**
SBRs were supplied with the activated sludge from anoxic chamber of municipal wastewater treatment plant. The initial MLVSS concentration in SBRs was 3.5 g/dm$^3$. The reactors were fed with synthetic wastewater – composition and concentration of micronutrients were taken from Coelho et al. [4] (modification, without NH$_4$Cl). As a consequence, the treatment process was not limited by the lack of these components.

As a nitrate source KNO$_3$ was used in specific amount to maintain its concentration on the level of 100 mg N$_{NO_3}$/dm$^3$ at the beginning of SBR cycle. As an external carbon source in SBR reactors sugar beet molasses (untreated and hydrolyzed) were used.
Hydrolysis of molasses
To increase susceptibility of molasses to biodegradation, it was hydrolyzed. A portion of 240 g molasses was dissolved in 760 g of deionised water and hydrolyzed for 30 min at 100°C and at pH 1.5 (adjusted with 98% H$_2$SO$_4$). Next, the solution was cooled to about 60°C and neutralized to pH 7.5 with Ca(OH)$_2$. After 12 hours, it was centrifuged at 4500 rpm for 10 min. to remove precipitated CaSO$_4$.

Preparation of stock solutions
Depending on COD/N ratio, an established amount of molasses stock solution was put into SBR 1 or hydrolyzed molasses was put to SBR 2, prepared as follows: 260 g of molasses was filled up with deionised water to 1 dm$^3$ and diluted 200-times. Stock solution of hydrolyzed molasses was prepared in similar way. The characteristic of stock solutions is given in Table 2 (chapter Carbon source characterization).

Analytical methods
The influents and effluents from the SBRs were subjected to measurements with the following parameters:
- organic compounds expressed as COD (dichromate reflux method) – according to Standard Methods (1997) [25] and BOD$_5$ – according to DIN EN 1899-1/EN 1899-2 official EPA method using OxiTop® made by WTW company,
- nitrogen compounds (nitrite nitrogen (colorimetric method with sulfanilic acid and 1-naphthylamine) and nitrate nitrogen (colorimetric method with phenolsulfonic acid) – according to the Standard Methods (1997) [25],
- volatile suspended solids (VSS) and total suspended solids (TSS) in the settled effluent (according to the Standard Methods, 1997) [25].

The mixed reactor content was measured for the mixed liquor total suspended solids (MLTSS) and volatile suspended solids (MLVSS) (according to the Standard Methods, 1997) [25].

RESULTS AND DISCUSSION

Carbon source characterization
Molasses hydrolysis caused increase of biodegradability in comparison with untreated molasses. It was confirmed by higher BOD$_5$/COD ratio and value of constant rate of oxygen uptake $k$, which in the case of molasses were 0.58 and 0.203 1/d, respectively. However, for hydrolyzed molasses they were 1.3- and 1.5-fold higher, respectively (Tab. 2).

Table 2. Characteristics of the carbon sources

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Molasses</th>
<th>Hydrolyzed molasses</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>[mg/dm$^3$]</td>
<td>918 ± 9.07</td>
<td>917 ± 11.76</td>
</tr>
<tr>
<td>BOD$_5$</td>
<td>[mg/dm$^3$]</td>
<td>533 ± 7.9</td>
<td>721 ± 6.98</td>
</tr>
<tr>
<td>BOD$_5$/COD</td>
<td>[-]</td>
<td>0.580 ± 0.012</td>
<td>0.79 ± 0.014</td>
</tr>
<tr>
<td>$k$</td>
<td>[1/d]</td>
<td>0.203 ± 0.006</td>
<td>0.303 ± 0.010</td>
</tr>
</tbody>
</table>

In the literature, it is usually stated that the value of $k$ depends primarily on the rate at which the organic substances can be biologically oxidized. Thus, for instance, in raw
municipal wastewater, values of $k$ are much higher (0.3–0.5 1/d) than in some industrial wastewater containing primarily slowly degradable compounds (0.2 1/d) [23].

**The efficiency and rate of denitrification**

The efficiency of denitrification was investigated in 3 series differed in COD/N ratio in raw wastewater, which in individual series was 6.0 (series 1), 5.0 (series 2) and 4.0 (series 3). In each series, the research was conducted in 2 SBR reactors, with carbon source in a form of untreated molasses (SBR 1) and hydrolyzed molasses (SBR 2).

Nitrate and nitrite concentrations in treated wastewater are given in Table 3. From the data obtained, it is clear that in the effluent from SBR reactors both at COD/N = 6.0, and COD/N = 5.0 nitrite and nitrate concentrations did not exceed 1 mg N_{NOx}/dm$^3$.

<table>
<thead>
<tr>
<th>Carbon sources</th>
<th>COD/N = 6 (series 1)</th>
<th>COD/N = 5 (series 2)</th>
<th>COD/N = 4 (series 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N_{NO_2}$ [mg/dm$^3$]</td>
<td>$N_{NO_3}$ [mg/dm$^3$]</td>
<td>$N_{NO_2}$ [mg/dm$^3$]</td>
</tr>
<tr>
<td>Molasses (SBR 1)</td>
<td>0.03 ± 0.001</td>
<td>0.28 ± 0.03</td>
<td>0.9 ± 0.024</td>
</tr>
<tr>
<td>Hydrolyzed molasses (SBR 2)</td>
<td>0.05 ± 0.002</td>
<td>0.26 ± 0.03</td>
<td>0.61 ± 0.02</td>
</tr>
</tbody>
</table>

Lowering of COD/N ratio to 4.0 caused increase of the sum of nitrite and nitrate concentration in the effluent to 72.5 mg N_{NOx}/dm$^3$ (molasses) and 55.5 mg N_{NOx}/dm$^3$ (hydrolyzed molasses), what responded to the denitrification efficiency of 27.6% and 44.3%, respectively. Åsøy et al. [1] stated that obtaining complete denitrification of nitrate (25 mg N_{NO3}/dm$^3$) in biological bed was possible at COD/N ratio of 8–10 mg COD/mg N_{NO3} and when organic fraction of municipal waste was used as carbon source. The investigations of Tsonis [26] with use of wastewater from olives processing in modified Bardenpho system revealed that demand for that substrate was from 4.6 to 5.4 mg COD/mg N_{NO3}. Quan et al. [24] examined removal of nitrate with use of hydrolyzed molasses as a carbon source. The authors stated that the content of readily accessible organics in molasses after acid hydrolysis was 47.5%, what allowed to achieve 91.6% efficiency of denitrification at COD/N_{NO3} 4.8–5.3. Thus, the authors demonstrated that hydrolyzed molasses can be an effective carbon source in denitrification process. From our investigations it is clear that at similar COD/N ratio complete denitrification can be achieved using both hydrolyzed molasses and molasses without pretreatment.

**Denitrification rate**

In series 1 (COD/N = 6.0) and 2 (COD/N = 5.0), for which complete denitrification was achieved, after attainment of steady-state conditions in both reactors, kinetic analysis was performed. On the basis of changes in nitrite and nitrate concentrations in the SBR operating cycle, a reaction rate was estimated.

The analysis of experimental results showed that denitrification was a zero-order kinetics defined by the following differential equation:

$$r_{deni,NO_3} = \frac{dC_{NO_3}}{dt} = -k_{deni,NO_3}$$  (1)
The solution for this could be fitted to the experimental data according to (2):

$$C_{NO_x} = -k_{deni,NO_x} \times t + C_{0,NO_x}$$  \hspace{1cm} (2)

where:

- $r_{deni,NO_x}$ – denitrification rate [mg N$_{NOx}$/dm$^3$·h],
- $k_{deni,NO_x}$ – constant of denitrification rate [mg N$_{NOx}$/dm$^3$·h],
- $C_{NOx}$ – sum of nitrite and nitrate concentration after time $t$ [mg N$_{NOx}$/dm$^3$],
- $t$ – time [h],
- $C_{0,NOx}$ – sum of nitrite and nitrate concentration at the beginning of the SBR operating cycle [mg N$_{NOx}$/dm$^3$].

The changes of nitrite and nitrate concentration in SBR operating cycle, depending on applied carbon source at COD/N equal 6.0 is presented in Figure 1. Denitrification rate was 8.4 mg N$_{NOx}$/dm$^3$·h (molasses) and 9.5 mg N$_{NOx}$/dm$^3$·h (hydrolyzed molasses). By the first hours of the cycle an increase of nitrite concentration was observed in both reactors (Fig. 1), and their highest concentrations 39.7 mg N$_{NO2}$/dm$^3$ (SBR 1) and 34.5 mg N$_{NO2}$/dm$^3$ (SBR 2) were noted at 7th and 3rd h of the cycle, respectively. Complete reduction of nitrate and nitrite was stated during 12 h of the cycle.

![Fig. 1. Concentrations profile of nitrite and sum of nitrite and nitrate and reaction rates described by zero-order kinetics at COD/N = 6.0 a) – molasses, b) – hydrolyzed molasses](image)

MOLASSES AS A CARBON SOURCE FOR DENITRIFICATION
In Figure 2, changes of nitrite and nitrate concentration in SBR operating cycle, depending on type of carbon source at COD/N = 5.0 are shown.

Fig. 2. Concentrations profile of nitrite and sum of nitrite and nitrate and reaction rates described by zero-order kinetics at COD/N = 5.0 a) – molasses, b) – hydrolyzed molasses

Higher denitrification rate at this COD/N ratio was obtained in SBR 2 (hydrolyzed molasses) (Fig. 2b). It was 8.21 mg N_NOx/(dm^3·h) and 1.6-fold higher in comparison with SBR 1 (Fig. 2a), where untreated molasses was a carbon source.

In reactor with hydrolyzed molasses (SBR 2), after 10 h of the cycle, the concentration of the sum of nitrite and nitrate concentration was on the level of about 30 mg N_NOx/dm^3, and after next 2 h decreased to about 1 mg N_NOx/dm^3 (Fig. 2b). It means that already after 12 h of the cycle complete denitrification was obtained. However, in the case of molasses without hydrolysis the decrease of the sum of nitrite and nitrate concentration reached 58%.

A significant lowering of the denitrification rate with untreated molasses as a carbon source after decreasing of COD/N_NO3 from 6 to 5, is very significant because of practical use. At the rate of 5.14 mg N_NOx/(dm^3·h), the time necessary to complete reduction of nitrite and nitrate is about 20 h, which poses over 80% of the cycle length. It means that
<table>
<thead>
<tr>
<th>Carbon source</th>
<th>C/N ratio</th>
<th>Kind of wastewater</th>
<th>Reactor system</th>
<th>Denitrification rate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>CH$<em>3$OH/N$</em>{N\text{O}_3}$ &gt; 2.5</td>
<td>synthetic wastewater</td>
<td>continuous-flow stirred reactor</td>
<td>4.35 mg/(g VSS·h)</td>
<td>Foglar, Briski [9]</td>
</tr>
<tr>
<td>Methanol</td>
<td>3.9 g COD/g N</td>
<td>wastewater</td>
<td>single activated sludge</td>
<td>3 mg/(g VSS·h)</td>
<td>Nyberg et al. [20]</td>
</tr>
<tr>
<td>Methanol</td>
<td>8 mg CH$_3$OH/dm$^3$</td>
<td>wastewater</td>
<td>SBR</td>
<td>0.8 mg/(g VSS·h)</td>
<td>Louzeiro et al. [15]</td>
</tr>
<tr>
<td>Methanol</td>
<td>–</td>
<td>wastewater</td>
<td>pre-denitrification system</td>
<td>3.2 mg/(g VSS·h)</td>
<td>Peng et al. [22]</td>
</tr>
<tr>
<td>Methanol</td>
<td>1.8–7.3 mg COD/mg N$_{N\text{O}_3}$</td>
<td>landfill leachate</td>
<td>SBR</td>
<td>39.9–48.4 mg/(dm$^3$·h)</td>
<td>Kulikowska, Klimiuk [12]</td>
</tr>
<tr>
<td>Ethanol</td>
<td>C/N = 6.0</td>
<td>synthetic wastewater</td>
<td>columns with beads of immobilized <em>Pseudomonas butanovora</em></td>
<td>67.9 mg/(dm$^3$·h)</td>
<td>Kesserű et al. [11]</td>
</tr>
<tr>
<td>Ethanol</td>
<td>4.4 g COD/g N</td>
<td>wastewater</td>
<td>single activated sludge</td>
<td>10 mg/(g VSS·h)</td>
<td>Nyberg et al. [20]</td>
</tr>
<tr>
<td>Ethanol</td>
<td>–</td>
<td>wastewater</td>
<td>pre-denitrification system</td>
<td>9.6 mg/(g VSS·h)</td>
<td>Peng et al. [22]</td>
</tr>
<tr>
<td>Ethanol</td>
<td>COD/N$_{\text{utilization}}$ = 7.4</td>
<td>landfill leachate</td>
<td>SBR</td>
<td>6.67 mg/(g VSS·h)</td>
<td>Doyle et al. [6]</td>
</tr>
<tr>
<td>Acetate</td>
<td>COD/N$_{\text{utilization}}$ = 7.4</td>
<td>landfill leachate</td>
<td>SBR</td>
<td>6.67 mg/(g VSS·h)</td>
<td>Doyle et al. [6]</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>C/N = 6.0</td>
<td>synthetic wastewater</td>
<td>columns with beads of immobilized <em>P. butanovora</em></td>
<td>63.75 mg/(dm$^3$·h)</td>
<td>Kesserű et al. [11]</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>2.7 mg COD/mg N$_{N\text{O}_3}$</td>
<td>landfill leachate</td>
<td>SBR</td>
<td>7.14 mg/(g VSS·h)</td>
<td>Kulikowska, Racka [13]</td>
</tr>
<tr>
<td>Acetic acid + propionic acid 1:1</td>
<td>2 mg C/mg N$_{N\text{O}_3}$</td>
<td>synthetic wastewater</td>
<td>batch experiment</td>
<td>0.35–1.75 mg/(g·h)</td>
<td>Elefsiniotis, Li [7]</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>2 mg C/mg N$_{N\text{O}_3}$</td>
<td>synthetic wastewater</td>
<td>batch experiment</td>
<td>0.058–1.2 mg/(g·h)</td>
<td>Elefsiniotis, Li [7]</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>5.3 mg COD/mg N$_{N\text{O}_3}$</td>
<td>landfill leachate</td>
<td>SBR</td>
<td>5.08 mg/(g VSS·h)</td>
<td>Kulikowska, Racka [13]</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>5.1 mg COD/mg N$_{N\text{O}_3}$</td>
<td>landfill leachate</td>
<td>SBR</td>
<td>12.2 mg/(g VSS·h)</td>
<td>Kulikowska, Racka [13]</td>
</tr>
<tr>
<td>Maltose</td>
<td>COD/N$_{\text{utilization}}$ = 7.4</td>
<td>landfill leachate</td>
<td>SBR</td>
<td>7.08 mg/(g VSS·h)</td>
<td>Doyle et al. [6]</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>C/N = 6.0</td>
<td>synthetic wastewater</td>
<td>columns with beads of immobilized <em>P. butanovora</em></td>
<td>48 mg/(dm$^3$·h)</td>
<td>Kesserű et al. [11]</td>
</tr>
</tbody>
</table>
with a slight increase of nitrate concentration in the influent, the length of the cycle may turn out to be insufficient to complete nitrate reduction and may lead to loss of process stability.

From the review of the literature, it is clear that values of denitrification rate using fatty acids and alcohols as an external carbon source are numerous. However, the data obtained by individual authors differed by order of magnitude even for the same carbon source (Tab. 4). The differences could result from the fact that individual experiments varied from each other by operational conditions, dosage of organic carbon, reactor type, and kind of treated wastewater (industrial, municipal or synthetic). On the other hand, the literature data concerning denitrification rate with the use of alternative carbon sources are sparse. Barlindhaug, Ødegaard [3] noticed denitrification rate of 1.4 kg N\(_{NO_3}\)/m\(^3\)-d at COD/N\(_{NO_3}\) = 8.0 in a biological bed with hydrolyzed excessive sludge as a carbon source. Usage of a similar carbon source (a mixture of hydrolyzed sludge and organic fraction of municipal waste) and COD/N\(_{NO_3}\) Åsøy et al. [1] enabled to achieve a denitrification rate nearly 2-fold higher. Elefsiniotis et al. [7] investigated the ability of VFA generated from anaerobic digester (treating a mixture of starch rich industrial and municipal wastewater) to act as a carbon source for denitrification. According to the authors the mean denitrification rate was 0.0111 g N\(_{NO_3}\)/g VSS d.

**Observed biomass yield coefficient**

In present study an observed biomass yield coefficient \(Y_{obs}\) was determined, depending on the type of organic carbon source and COD/N ratio in raw wastewater. The value of the \(Y_{obs}\) corresponds to net biomass yield coefficient and can be calculated from the following equation (4):

\[
Y_{obs} = \frac{X_{org} \cdot (V_{w} / t_{c}) + X_{e} \cdot (V_{eff} / t_{c})}{(C_{s} - C_{e}) \cdot (V_{in} / t_{c})}
\]

where:

\(Y_{obs}\) – observed biomass yield coefficient [mg VSS/mg COD],

\(X_{org}\) – volatile suspended solids in SBR [mg VSS/dm\(^3\)],

\(V_{w}\) – volume of suspended solids disposed in SBR operating cycle [dm\(^3\)],

\(t_{c}\) – time of SBR operating cycle [d],

\(X_{e}\) – effluent volatile suspended solids concentration [mg VSS/dm\(^3\)],

\(V_{eff}\) – volume of wastewater effluent in SBR operating cycle [dm\(^3\)],

\(V_{in}\) – volume of wastewater influent in SBR operating cycle [dm\(^3\)] \((V_{in} = V_{eff} + V_{w})\),

\(C_{s}\) – concentration of COD in raw wastewater [mg COD/dm\(^3\)],

\(C_{e}\) – concentration of COD in the effluent [mg COD/dm\(^3\)].

The values of observed biomass yield coefficient \(Y_{obs}\) depending on carbon source and COD/N ratio in raw wastewater calculated from equation 4 are given in Table 5.

<table>
<thead>
<tr>
<th>(Y_{obs}) [mg VSS/mg COD]</th>
<th>COD/N = 6.0</th>
<th>COD/N = 5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>molasses</td>
<td>0.40</td>
<td>0.31</td>
</tr>
<tr>
<td>hydrolyzed molasses</td>
<td>0.54</td>
<td>0.47</td>
</tr>
</tbody>
</table>
Higher values of $Y_{obs}$ were stated in reactors, where hydrolyzed molasses was applied as a carbon source – 0.54 mg VSS/mg COD (COD/N = 6.0) and 0.47 mg VSS/mg COD (COD/N = 5.0). It means that in the case of hydrolyzed molasses 47–54% of COD was converted to biomass during the denitrification process. In reactors with untreated molasses $Y_{obs}$ was 1.35-fold and 1.52-fold lower, respectively.

It is known that pretreatment of molasses enables better assimilation of substrate by activated sludge microorganisms. On the other hand, readily biodegradable substances give more biomass growth [23].

In the effluent from SBRs with molasses and hydrolyzed molasses concentrations of organics expressed as COD were similar and equalled 112 mg COD/dm$^3$ (molasses) and 108 mg COD/dm$^3$ (hydrolyzed molasses) at COD/N = 6.0 and 98 mg COD/dm$^3$ and 89 mg COD/dm$^3$, respectively, at COD/N = 5.0. Therefore, it may be supposed that polysaccharides (the major components in molasses) have carbon chains that are too long to be used by microorganisms. On the other hand, the main product of hydrolyzed molasses are reduced sugars – glucose or sugars similar to glucose [19], which are considered as readily biodegradable organics and it is known that this type of substances gives higher biomass growth. Therefore, in the case of hydrolyzed molasses $Y_{obs}$ was higher than for untreated molasses.

Majone et al. [16] studied the removal mechanisms and sludge production during the pre-denitrification in anoxic/aerobic sequencing process with different substrates (commercially available acetate, ethanol, glucose, glutamic acid as carbon source). According to the authors, the observed biomass yield coefficient for glucose was the highest among applied substrates – 0.74 g COD/g COD (0.5 g VSS/g COD) and was comparable with the results obtained from our own research for hydrolyzed molasses.

In the literature, there are conflicting reports on biosolids production. It is known that the value of $Y_{obs}$ depends on a type of substrate [23]. According to the authors, values of $Y_{obs}$ for aerobic heterotrophs in a system with plug flow pattern and in completely mixed system changed in the range of 0.53–0.62 g COD/g COD for glucose, 0.35–0.49 g COD/g COD for acetic acid, 0.25–0.48 g COD/g COD for methanol and 0.35–0.37 g COD/g COD for ethanol. McClintock et al. [17] have found that biosolids production is lower when anoxic conditions are incorporated in the process. However, from other author’s researches it results that sludge production in anoxic system was the same or higher than in aerobic system (Lishman et al. [14] (after Smyth 1994).

**CONCLUSIONS**

On the basis of the obtained results the conclusions are as follows:

1. Molasses hydrolysis caused increase in organics biodegradability. The values of BOD$_5$/COD ratio and rate constants of oxygen consumption $k$ for hydrolyzed molasses were 1.3-fold and 1.5-fold higher, respectively, in comparison with non-hydrolyzed molasses.
2. Complete denitrification (the efficiency above 98%), irrespective of organic carbon source (untreated molasses, hydrolyzed molasses) was obtained both at COD/N ratio of 6.0 and 5.0. Decreasing of COD/N ratio to 4.0 caused decline of denitrification efficiency to 27.6% (untreated molasses) and to 44.3% (hydrolyzed molasses).
3. Denitrification rate at COD/N ratio of 6.0 was 9.5 mg $N_{NOx}$/dm$^3$·h in the reactor
with hydrolyzed molasses and 8.4 mg N$_{NOx}$/ (dm$^3$·h) in reactor with non-hydrolyzed molasses. It means that in both cases complete denitrification proceeded within 12 h of the cycle. After decreasing of COD/N ratio to 5.0, the time necessary to reduction of nitrate for hydrolyzed molasses remained unchanged. However, in case of non-hydrolyzed molasses it lengthened to 20 h.

4. Sludge production was dependent on the substrate used. In reactors with untreated molasses, the value of observed yield coefficient was 1.35-fold (COD/N = 6.0) and 1.52-fold (COD/N = 5.0) lower in comparison with hydrolyzed molasses.

5. Taking into account the economical aspects (molasses hydrolysis increases the cost of wastewater treatment) and the fact that molasses after hydrolysis causes higher sludge production, untreated molasses seems a more suitable carbon source for denitrification.

REFERENCES


MOLASSES AS A CARBON SOURCE FOR DENITRIFICATION

Praca zawiera wyniki badań dotyczące możliwości wykorzystania melasy – produktu odpadowego powstającego w przemyśle cukrowniczym – jako źródła węgla organicznego w procesie denitryfikacji. Badano wpływ hydrolizy melasy oraz stosunku ChZT/N (6,0; 5,0; 4,0) na efektywność i kinetykę procesu. Określono również przyrost osadu czynnego w zależności od rodzaju źródła węgla (melasa, melasa zhydrolizowana) oraz stosunku ChZT/N. Przy stosunku ChZT/N wynoszącym 6,0 i 5,0, niezależnie od formy występowania źródła węgla organicznego (melasa, melasa zhydrolizowana), efektywność denitryfikacji przekraczała 98%. Badania kinetyki procesu wykazały natomiast, że rodzaj źródła węgla oraz stosunek ChZT/N wpływały na szybkość denitryfikacji. Najwyższą szybkość procesu – 9,5 mg NNOx/(dm$^3$·h) odnotowano przy ChZT/N wynoszącym 6,0 w reaktorze z melasą zhydrolizowaną a najniższą – 5,14 mg NNOx/(dm$^3$·h) w reaktorze z melasą niezhydrolizowaną przy ChZT/N wynoszącym 5,0. Obniżenie stosunku ChZT/N do 4,0 spowodowało spadek efektywności procesu do 27,6% (melasa niezhydrolizowana) oraz 44,3% (melasa zhydrolizowana). Zastosowanie melasy zhydrolizowanej powodowało wyższy przyrost osadu czynnego. W reaktorach z melasą zhydrolizowaną $Y_{obs}$ wynosił 0,4 mg smo/mg ChZT przy stosunku ChZT/N wynoszącym 6,0 i 0,31 mg smo/mg ChZT przy stosunku 5,0. W reaktorach z melasą zhydrolizowaną $Y_{obs}$ był odpowiednio 1,35-krotnie i 1,5-krotnie wyższy. Biorąc pod uwagę, że hydroliza melasy podnosi koszty oczyszczania ścieków oraz przy- czynia się do wyższej produkcji osadu nadmiernego, melasa niezhydrolizowana wydaje się być lepszym źródłem węgla organicznego w procesie denitryfikacji.
EFFECT OF ORGANIC FERTILIZATION ON DEVELOPMENT OF PROTEOLYTIC BACTERIA AND ACTIVITY OF PROTEASES IN THE SOIL FOR CULTIVATION OF MAIZE (ZEA MAYS L.)

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Keywords: Proteolytic bacteria, proteases, organic fertilization, sewage sludge, slurry, manure.

Abstract: The objective of this work was investigation of the growth dynamics of proteolytic bacteria and the enzymatic activity in soil for the cultivation of maize (Zea mays L.), as well as the maize yield under application of some selected organic fertilizers. Intensity and the direction of the developed changes in the soil depended on the type of applied organic fertilizer, the size of its dose introduced into the soil and on the developmental phase of the grown plant (maize). On the basis of obtained results it was found that all tested organic substances stimulated the activity of proteases. Yields of maize as raw material for silages obtained from soil fertilized with sewage sludge were similar to those obtained after the application of pig slurry and they exceeded maize yields harvested from other fertilization objects.

INTRODUCTION

Polish soils are deficient in humic compounds and, therefore, it seems very important to select an adequate organic fertilizer and its proper application. These practices appeared to be important in the protection of the natural environment and the preservation of the biodiversity of agroecosystems as well as soil quality (fertility). The selection of an adequate fertilizer plays a significant role in the increase of soil fertility and primarily in the intensification of the humification process. The introduced organic fertilizers can disturb the biological balance of soil and the congeneric vegetation composition [23].

Actually, beside the traditional organic fertilizers (manure, cattle slurry), attempts are made to fertilize soil with sewage sludge [11, 15]. Because of a high fertilizing value, sewage sludge plays an essential role primarily in the intensification of the humification process [4]. The introduction into the soil of natural fertilizers including sewage sludge satisfying the standard recommendations may have a significant effect on the growth and development of microorganisms as well as on their enzymatic activity of soil. According to Koper et al. [7], excessive and particularly unilateral fertilization, frequently used in agricultural practice, can disturb the balance of nutritive components in water solution.
Next to the proper bacteria, actinomycetes and fungi responsible for important transformations of carbon compounds, there are also many species of microorganisms able to utilize proteins and aminoacids as nutritive and energetic substrates. Organic nitrogen compounds which get into the soil in the form of plant or animal remains are subject to biochemical transformations. In all particular stages of the transformations, the participating microorganisms’ specific intercellular or extracellular enzymes are secreted into the environment (soil). Different ways of decomposition by proteolytic microorganisms are known. However, this process is always carried out in such a way that from proteins develop peptones which are hydrolyzed into polypeptides and peptides and from them, due to further decomposition, aminoacids develop [24]. The measure of soil biological activity, which includes the total transformations of compounds and the energy taking place in it, can be an enzymatic activity [1]. According to many authors [2, 9, 19] the cognition of the enzymatic activity of soil gives an objective picture of the processes taking place in it. Actually, it is recognized that the enzymatic activity of soil is the measure of soil fertility and productivity in a much higher degree than other biological indicators such as e.g. the number of microelements in their biomass [6, 17].

The objective of the present work was to investigate the growth dynamics of proteolytic bacteria and the enzymatic activity in the soil for the cultivation of maize (Zea mays L.), as well as the maize yield under the application of some selected organic fertilizers.

MATERIALS AND METHOD

The studies were carried out in 2006 on plots of the Experimental and Didactic Farm of the Department of Soil and Plant Cultivation in Swadzim belonging to the University of Life Sciences in Poznań. Studies were carried out in a field after a 5-year maize monoculture. The experiment was established in randomized block method in four replications.

This interval matched the successive developmental phases of maize:
- term 1 – before sowing (BBCH 0),
- term 2 – phase of germination (BBCH 09),
- term 3 – phase of 2–3 leaves (BBCH 12–13),
- term 4 – phase of 6–7 leaves (BBCH 16–17),
- term 5 – tasseling phase (flowering) (BBCH 65),
- term 6 – phase of cob setting (BBCH 70).

Soil samples for analyses were collected in ten replications according to Polish standard [5].

The soil of experimental plots according to PTG classification [14] belongs to typical grey-brown podsolic soils created of light loamy sands shallowly lying on light loam. According to soil classification, it is counted to the IVa class and according to agricultural suitability; it belongs to the 5th complex (good rye complex). All cultivation treatments were carried out according to the principles of correct agrotechniques of maize cultivation designed for silage.

In the experiment, the following fertilization combinations were applied: control (mineral NPK), manure 15 Mg·ha⁻¹ of fresh matter,
5 Mg d.m. of wheat straw + pig slurry 40 m\(^3\)-ha\(^{-1}\),
5 Mg d.m. wheat straw + 15 kg nitrogen·Mg wheat straw·ha\(^{-1}\).

Sewage sludge was used according to the regulation of the Minister of the Environment permitting the application of it in a dose of 10 Mg·ha\(^{-1}\) d.m., once in 5 years. In the used fertilizers, the NPK content was determined and the dose of these components was balanced by mineral fertilization to the levels of: nitrogen (N) – 130 kg·ha\(^{-1}\); phosphorus (P) – 15.274 kg·ha\(^{-1}\); potassium (K) – 96.28 kg·ha\(^{-1}\). Nitrogen fertilization was applied in the form of ammonium saltpeter, phosphorus in the form of triple superphosphate and potassium in the form of 60% potassium chloride salt. Organic (natural) fertilizers were covered by autumn ploughing, while sewage sludge was covered by spring ploughing.

Sewage sludges used in our own studies were examined in respect of their microbiological condition and heavy metals content in the sewage treatment plant in Szamotuły. Results of these analyses indicated that they were safe in reference to their sanitary condition. Also the content of heavy metals was significantly lower than the standards admitted for agricultural utilization. Consequently, in the doses of sewage sludge used in the experiments, the amounts of lead and copper introduced into the soil were respectively 0.5% and 53% of the norm, according to Directive of the Minister of Agriculture and Rural Development. Additionally, the concentration of heavy metals in the gathered plant material (seeds, leaves and stem) was determined and compared against the control value. Manure came from a farm in Swadzim and pig slurry from a farm in Złotniki. Both farms belong to the University of Life Sciences in Poznań.

Experimental plots were situated on a typical grey-brown podsolic soil created of postglacial formations of light loamy sand, shallowly lying on light loam, classified to the IVa class and to the 4\(^{th}\) complex of agricultural suitability (very good rye complex). The soil reaction was neutral and it was characterized by a good content of potassium, phosphorus and magnesium (Tab. 1). During the experimental period, the suitability of soil – climate conditions was estimated as moderate. The meteorological conditions during the vegetation season are shown in Table 2. The additional indicator of the impact of organic fertilization applied was the yield of the cultivated plant determined by the weighing method.

<table>
<thead>
<tr>
<th>Soil levels [cm]</th>
<th>pH</th>
<th>C [g·kg(^{-1})]</th>
<th>N [g·kg(^{-1})]</th>
<th>C:N</th>
<th>Mg [mg MgO·100 g(^{-1})]</th>
<th>P [mg P(_2)O(_5)·100 g(^{-1})]</th>
<th>K [mg K(_2)O·100 g(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–30</td>
<td>6.5</td>
<td>8.7</td>
<td>0.811</td>
<td>10.7</td>
<td>8.8</td>
<td>16.2</td>
<td>16.9</td>
</tr>
</tbody>
</table>

Table 1. Some soil physical – chemical properties at site of study

<table>
<thead>
<tr>
<th>Months</th>
<th>Mean day temperature [°C]</th>
<th>Mean of precipitation [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Decade I</td>
<td>Decade II</td>
</tr>
<tr>
<td>April</td>
<td>8</td>
<td>9.5</td>
</tr>
<tr>
<td>May</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>June</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>July</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>August</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>September</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>October</td>
<td>16.5</td>
<td>9.5</td>
</tr>
</tbody>
</table>

Table 2. Decade distribution of temperature and precipitation in the Experimental and Didactic Farm in Swadzim in 2006
**Enzymatic studies**

Studies of soil enzymatic activity were based on the determination of the activity of proteases by spectrophotometric method [10] in which sodium caseinate was used as substrate after 1-hour incubation at 50°C with 578 nm wave length. Enzyme activity was expressed in l mg tyrosine·kg⁻¹·ha⁻¹.

**Microbiological analyses**

In soil samples taken from underneath the plants from interrows at the depth of 15–20 cm, the number of microorganisms was determined by plate solutions according to Koch’s method on adequate agar mediums (in five replications). The mean number of colonies was converted into soil dry matter [18] [CFU·g⁻¹ d.m. soil] number of proteolytic bacteria [CFU·g⁻¹ d.m. soil] was determined in a selective medium according to Rodina [16]. The bacteria were incubated at 22°C for 48 h. Additionally, in the determination of the number of the grown colonies, Frazier’s reagent was applied in order to increase the content of the substrate in relation to the proteolytic bacteria (white colonies creating bright sites).

**Statistical analysis**

The accumulated results were statistically analyzed with the use of analysis of variance for factorial experiments established in random block design. The significance of the effect of the studied factors was defined by Fisher-Snedecor test at the confidence levels \( P = 0.95 \) and \( P = 0.99 \). Differences between the objects were tested according to the t-Student’s procedure using the same confidence levels. In the case when the statistical effect of the factor on the value of the analyzed feature was proven, the calculus of correlation and regression was carried out.

**RESULTS AND DISCUSSION**

The results of this study have shown that the fertilization of grey-brown podsolic soil with natural fertilizers and sewage sludge evoked changes in soil enzymatic activity. Also the cultivation of maize benefited from the changes in soil enzymatic activity. Intensity and the direction of the developed changes depended on the type of the applied organic matter, the size of its dose introduced into the soil and on the developmental phase of maize. On the basis of data presented in a diagram (Fig. 1), it was found that all tested organic substances stimulated the activity of proteases. This phenomenon, however, was most visible in conditions of the use of 5 Mg d.m. wheat straw·ha⁻¹·year⁻¹ + 15 kg nitrogen per 1 Mg of straw. Martens et al. [12], who studied the effect of organic matter introduced into the soil in the form of bird dung, barley straw, sewage sludge and green fertilizer, obtained results similar to our studies indicating that the activity of the evaluated enzymes increased in the highest degree after soil fertilization with straw.

The analysis of data presented in Figure 1 indicated that the highest activity of the studied enzymes, in the majority of combinations occurred in term 1 – before sowing. The above phenomenon could have been connected with the fact that biochemical determination was carried out directly after the introduction of organic matter into the soil in the form of natural fertilizers and sewage sludge which could have contributed to the increase of the biological activity of soil. Additionally, one should keep in mind the fact that soil after the application of organic fertilizers is enriched with autochthonous microflora.
which can reveal strong metabolic activities. However, some controversies are evoked by the recorded increase of the activity of proteases also in the control combinations (1\textsuperscript{st} term of analyses). One can suppose that the reason of such phenomenon was the absence of plants on the plots. This is because starting with the 2\textsuperscript{nd} phase (plant germination), the activity of the studied enzymes showed sharp decline in the majority of combinations with the exception of plots fertilized with 5 Mg d.m.·ha\textsuperscript{-1} wheat straw + 15 kg of nitrogen per 1 Mg of straw. According to Wielgosz [20], some plants can secrete to the soil substances which impede the development of definite microorganisms causing in effect a decrease in the enzymatic activity of soil.

In the successive terms of analyses, the activity of proteases was subject to significant oscillations depending on the type of fertilizer combination; however, it was maintained on a low level until the end of the experiment. The studies of Koper and Piotrowska [8] indicate that the level of soil enzymatic activity depends, in a high degree, on the season of the year. According to these authors, enzymes are particularly active at the end of spring, while in summer, there follows a decrease of their activity.

Results presented in Figure 2 indicate that the number of proteolytic bacteria was determined by the fertilization method and by the developmental phase of maize. The greatest number of proteolytic bacteria was recorded in two developmental phases (terms 2 and 4), in soils where sewage sludge was used. It is reported that the number of proteolytic bacteria depends on the quality of organic fertilizer (Reference). In the experiment, it was recorded that the number of microorganisms decomposing protein, in soils fertilized with sewage sludge, significantly increased. Similar dependences were also recorded by Emmerling \textit{et al.} [3] and Wolna-Maruwka \textit{et al.} [21]. Sewage sludges are a very rich source of nutritive components for microorganisms, plants and soil microfauna. They
play an important role in the intensification process of the humification of organic substance [4, 22].

![Graph showing the number of proteolytic bacteria in terms of analyses (developmental phases of plants) depending on organic fertilizations.](image)

On the basis of statistical analysis, it was found that in the control sample and in soils fertilized with manure at 5 Mg d.m·ha⁻¹ wheat straw + 15 kg nitrogen per 1 Mg of straw, the dynamics of proteolytic bacteria development had a similar course as the changes of proteases activity, since the Pearson’s correlation coefficient was positive and it oscillated within 0.21–0.51 (Figs 3, 4 and 5). In the remaining fertilization combinations, no linear correlation was found between the number of bacteria and proteases activity in the soil (Figs 6 and 7).

![Graph showing the relationship between a number of proteolytic bacteria and proteases activity in control soil.](image)
Fig. 4. Relationship between a number of proteolitic bacteria and proteases activity with manure fertilized

![Graph showing the relationship between proteolitic bacteria and proteases activity with manure fertilization.](image)

\[ y = 0.7763x - 3.3637 \]

Fig. 5. Relationship between a number of proteolitic bacteria and proteases activity in straw and nitrogen fertilized

![Graph showing the relationship between proteolitic bacteria and proteases activity with straw and nitrogen fertilization.](image)

\[ y = 0.5769x + 7.3328 \]

Fig. 6. Relationship between a number of proteolitic bacteria and proteases activity with straw and slurry fertilized

![Graph showing the relationship between proteolitic bacteria and proteases activity with straw and slurry fertilization.](image)

\[ y = 0.0061x + 8.979 \]
In the growing of maize for silage, the application of each of the natural fertilizers and sewage sludge, in comparison with mineral fertilizers, caused an increase of fresh and dry matter of the whole plants, and the obtained differences were not statistically proven only for objects fertilized with straw + mineral nitrogen (Tab. 3). The highest yield of fresh matter of the whole plants was obtained using sewage sludge at 43.7 Mg·ha\(^{-1}\).

Table 3. Yield of whole plants fresh matter [Mg·ha\(^{-1}\)] and cobs share in fresh matter of whole plants [%]

<table>
<thead>
<tr>
<th>Specification</th>
<th>Fresh matter yield [Mg·ha(^{-1})]</th>
<th>Cobs share in fresh matter of whole plants [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>35.4</td>
<td>33.4</td>
</tr>
<tr>
<td>Manure 15 Mg</td>
<td>40.2</td>
<td>33.8</td>
</tr>
<tr>
<td>Straw + N</td>
<td>38.0</td>
<td>31.8</td>
</tr>
<tr>
<td>Straw + slurry</td>
<td>40.9</td>
<td>35.3</td>
</tr>
<tr>
<td>Sludge 10 Mg d.m</td>
<td>43.7</td>
<td>35.8</td>
</tr>
<tr>
<td>LSD = 0,05</td>
<td>3.93</td>
<td>1.98</td>
</tr>
</tbody>
</table>

* fertilized NPK

In the studies of Mazur and Sądej [13], a comparison of the yields of the whole plants obtained with interchangeable use of traditional natural fertilizers in the form of manure or pig slurry have shown that they were similar to those obtained with mineral fertilization while after the application of a double dose of cattle or pig slurry, they were higher. The doses of natural fertilizers in our own studies and in the quoted studies are in agreement with the Nitrogen Directive of the European Union which admits this form of fertilization up to 170 kg N·ha\(^{-1}\) annually.

Furthermore, it was found that similar results were obtained in soils fertilized with sewage sludge and pig slurry, these treatments were characterized by the highest share of cobs indicating that it was of a high quality.

Sewage sludges are characterized by a high hygroscopicity and thanks to this feature; they preserve and accumulate water improving thereby its retention in soil. Information about such properties of sewage sludge was given by Żurawski et al. [25] on the basis of their lysimetric studies. This may explain the good effect of sewage sludge in conditions of drought.
The above cited reports from other studies fully support the fact that agricultural utilization of communal sewage sludge is justified. Intensification of such studies is particularly recommended in regions where there is significant level of sewage sludge production for agricultural use.

CONCLUSIONS

1. The direction of changes in the soil depended on the type of the applied organic fertilizer, its dose introduced into the soil and the developmental phase of the maize. The greatest number of proteolytic bacteria was recorded in soils where sewage sludge was used.
2. All tested organic substances stimulated the activity of proteases.
3. Yields of maize as raw material for silages obtained from soil fertilized with sewage sludge were similar to use of traditional natural fertilizers in the form of manure or pig slurry.

REFERENCES

WPŁYW NAWOŻENIA ORGANICZNEGO NA DYNAMIKĘ ROZWOJU BAKTERII PROTEOLITYCZNYCH, AKTYWNOŚĆ PROTEAZ W GLEBIE POD UPRAWĄ KUKURYDZY (ZEAMAYS L.)

INACTIVATION OF *ESCHERICHIA COLI* DURING COMPOSTING PROCESS OF ORGANIC WASTES WITH SEWAGE SLUDGE

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Keywords: *Escherichia coli*, composting, sewage sludge.

Abstract: The aim of the study was to estimate the effect of the composting process in the container technology Kneer on *E. coli* inactivation. The bacteria placed in the special carriers were introduced into the composted material. The elimination rate of *E. coli* differed depending on both the carriers' location in the biomass and the thermal conditions. The most effective hygienization of the material was noticed in summer – after 48 h in the middle layer, 6 days in the top layer and 10 days in the bottom layer. In spring and autumn, the bacteria survived the longest in the bottom layer – 83 and 45 days, respectively. Apart from the high temperature, the research points out the action of other factors such as competition, antagonism and antibiosis.

INTRODUCTION

The management of organic municipal waste and sewage sludge for fertilization and land reclamation is an essential aspect of shaping the environment. Sewage sludge is a rich source of organic compounds, biogenic elements and microelements [2, 11, 21]. Apart from chemical compounds, sludge can contain a considerable amount of microorganisms which pose a hazard for human health and environmental [7]. Its application as a fertilizer to arable land, for forest cultivation and for land reclamation depends on its previous treatment. One of the methods for obtaining a high-quality and environmentally safe product is the composting process. It allows for obtaining the stable material, being a good soil conditioner and a source of nutrients for plants and microorganisms [10, 15]. Moreover, during the disinfection stage of this process pathogenic bacteria are reduced to low levels. On account of environmental safety, it is crucial that its quality is assessed microbiologically in order to prevent soil and organisms from contamination with pathogenic microorganisms [19].

The aim of the study was to evaluate the hygienic effectiveness of the composting process conducted in closed containers, based on the inactivation of the indicator bacteria *E. coli*. The bacteria are commonly used for assessing the effectiveness of composting organic wastes, as they occur in composted biomass considerably more often than other pathogens and methods of their determination are simpler and safer [3, 8, 12].
MATERIAL AND METHODS

The research was carried out in spring, summer and autumn in a composting plant working in the container technology system Kneer. The material composted consisted of wastes from municipal greens, scraps and sewage sludge mixed in a proportion of 2/3:1/3:1. It was placed in containers, where the intensive composting stage proceeded, lasting about 14 days. Then the material was removed from the containers and formed in a windrow. To provide a suitable biomass aeration, the windrow was turned mechanically every 2 weeks. The compost maturation process lasted for about 4–6 weeks.

In order to perform the microbiological evaluation of the composting process effectiveness, spherical carriers of about 5 cm in diameter were made of pasteurized compost and inoculated with 1 cm$^3$ of solution of E. coli. The concentration of the bacteria in the solution was 10$^8$–10$^9$ MPN/cm$^3$ of a suspension. The carriers were additionally surrounded with compost and placed in special nylon sacks. The carriers with microorganisms were placed in the container in the top, middle and bottom layers of the biomass. Throughout intensive composting the level of E. coli inactivation was determined. After 2 weeks that is during the time of windrow forming part of the carriers from the container were transferred to the windrow. In order to estimate the effectiveness of hygienization of the second stage of composting proceeding in the windrow, additional carriers were introduced to it, which were also subjected to microbiological analyses.

The material from each carrier was accurately fragmented and mixed. 1 g weighed portions were taken from each compost carrier and a series of 10-fold dilutions in 0.9% NaCl was prepared. The number of E. coli was determined in triplicate using the selective MacConkey medium (MERC105396). The samples were incubated at a temperature of 43°C for 24 h. Next, the material was sieved into the solid selective medium Lactose TTC agar with Tergitol® (MERC107680). The samples were incubated at 37°C for 24 h. In dubious cases the material was transferred on the Standard I nutrient agar (MERC105450). After 24 h of incubation at 37°C the pure colonies obtained were subjected to the IMViC tests. The determination of bacteria number was made based on the method of the most probable number (MPN). The results were analyzed statistically and regression lines were drawn to determine the theoretical survival time of E. coli in the tested material.

RESULTS

The results of the research are presented in Tables 1–3. The elimination rate of E. coli differed significantly and was dependent both on the location of the carriers and thermal conditions.

In the spring cycle E. coli died the most slowly in the bottom part of the biomass. During 14 days of intensive composting their number decreased only by 2 log$_{10}$. At the same time the full inactivation of E. coli occurred in the top parts (Tab. 1).

After forming a windrow, a biomass hygienization did not proceed evenly. After 9 days the number of the tested bacteria in the top part decreased from 1.48·10$^9$ MPN/g to 4.67·10$^2$ MPN/g and the total elimination occurred in the middle part. In the bottom part the bacteria number was still high and amounted to 2.65·10$^8$ MPN/g (Tab. 2).

In the summer cycle the reduction of E. coli proceeded very quickly. After 48 h their presence was not detected in the middle part of the composted material (Tab. 1). The
Table 1. The average number of *E. coli* in the carriers placed in the containers and the windrow [MPN/g]

<table>
<thead>
<tr>
<th>Cycles</th>
<th>Layers of biomass</th>
<th>Time of sampling [days]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in the container</td>
<td>in the windrow (after transfer the carriers from the container)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Spring</td>
<td>top</td>
<td>1.30·10⁹</td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bottom</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>top</td>
<td>1.48·10⁹</td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bottom</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>top</td>
<td>3.83·10⁹</td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bottom</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td></td>
</tr>
</tbody>
</table>

nd – tested bacteria not detected

* – experiment was not continued due to lack of bacteria presence in the carriers

Table 2. The average number of *E. coli* in additional carriers with a high concentration of bacteria placed in the windrow [MPN/g]

<table>
<thead>
<tr>
<th>Cycles</th>
<th>Layers of biomass</th>
<th>Time of sampling [days]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Spring</td>
<td>top</td>
<td>1.48·10⁹</td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td>1.80·10²</td>
</tr>
<tr>
<td></td>
<td>bottom</td>
<td>2.83·10⁸</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>2.83·10⁹</td>
</tr>
<tr>
<td>Summer</td>
<td>top</td>
<td>3.17·10⁸</td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>bottom</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>2.83·10⁸</td>
</tr>
<tr>
<td>Autumn</td>
<td>top</td>
<td>2.65·10⁸</td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td>3.17·10⁹</td>
</tr>
<tr>
<td></td>
<td>bottom</td>
<td>3.83·10⁶</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>3.83·10⁸</td>
</tr>
</tbody>
</table>

nd – tested bacteria not detected

* – experiment was not continued due to lack of bacteria presence in the carriers
survival calculated on the basis of regression lines in the top part was 6 days, and it was only 4 days longer in the bottom part (Tab. 3). In spite of the high concentration of *E. coli* 
\(3.17 \times 10^8\) MPN/g) in additional carriers introduced into the composting windrow the first analysis carried out two days after its forming did not show the bacteria presence at any level (Tab. 2).

In the autumn cycle, as well as in spring one, *E. coli* died the most quickly in the top parts of the composted material. In the middle part of the biomass, they were not detected after 13 days. At the same time the concentration of the bacteria in the top part was \(2.34 \times 10^2\) MPN/g, and in the bottom part – \(3.83 \times 10^6\) MPN/g (Tab. 1). *E. coli* were not found in the windrow after 9 days in the middle part and after two weeks in the top part. In the bottom part of the composted biomass the theoretical time of their survival amounted to 53 days (Tab. 3). This reflects a considerable difference between a daily reduction rate of the tested bacteria in the top part – 0.59 log\(_{10}\) and in the bottom part – 0.16 log\(_{10}\) (Tab. 3).

### Table 3. Regression line equations showing the dynamics of *E. coli* inactivation in the composted material

<table>
<thead>
<tr>
<th>Cycles</th>
<th>Location of the carriers</th>
<th>Layers of biomass</th>
<th>Regression equations</th>
<th>(r^2) [%]</th>
<th>Survival of bacteria [days]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>container top</td>
<td>y = -0.60x + 7.36</td>
<td>62.41</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>top middle</td>
<td>y = -0.67x + 9.69</td>
<td>77.44</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>top bottom</td>
<td>y = -0.11x + 9.14</td>
<td>92.16</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>top control</td>
<td>y = -0.06x + 9.19</td>
<td>53.29</td>
<td>153</td>
<td></td>
</tr>
<tr>
<td>windrow</td>
<td>top</td>
<td>y = -0.54x + 9.82</td>
<td>73.96</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td>y = -0.06x + 9.35</td>
<td>53.29</td>
<td>156</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bottom</td>
<td>y = -0.05x + 9.83</td>
<td>70.56</td>
<td>197</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>container top</td>
<td>y = -0.95x + 5.67</td>
<td>51.84</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>top middle</td>
<td>y = -0.90x + 9.37</td>
<td>79.21</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>top bottom</td>
<td>y = -0.27x + 8.80</td>
<td>72.25</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>top control</td>
<td>y = -0.06x + 9.35</td>
<td>53.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>windrow</td>
<td>top</td>
<td>y = -0.59x + 8.03</td>
<td>82.81</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td>y = -0.16x + 8.43</td>
<td>57.76</td>
<td>5*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bottom</td>
<td>y = -0.16x + 8.35</td>
<td>47.61</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>container top</td>
<td>y = -0.52x + 8.60</td>
<td>72.25</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>top middle</td>
<td>y = -0.77x + 8.79</td>
<td>70.56</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>top bottom</td>
<td>y = -0.21x + 9.45</td>
<td>79.21</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>top control</td>
<td>y = -0.07x + 9.28</td>
<td>77.44</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

nd – tested bacteria not detected

* – the day of the process when the bacteria were last isolated
DISCUSSION

The properly conducted composting process is an effective method of pathogen elimination in the biomass [4, 13]. In the experiment by Savage [14] the author reported a reduction in coliform concentration from $10^7$ to $10^3$ cfu/g during 35 days of the composting process. Similar results were obtained by Hassen [9]. After the stage of hygienization he observed a decrease in \textit{E. coli} concentration from $2 \cdot 10^7$ to $3.1 \cdot 10^3$ cfu/g d.m.

The present studies, conducted during the spring and summer, also confirmed a high efficiency of the composting process in indicator bacteria inactivation, but only in the top and middle layers of the biomass. In both layers the survival rates of \textit{E. coli} ranged from 12 days in spring to 17 days in autumn (Tab. 3). The highest effectiveness of the composted biomass hygienization was noticed in summer. After 24 h a reduction in indicator bacteria concentration in the top layers of the biomass amounted to $7 \log_{10}$. The elimination rate of \textit{E. coli} was particularly rapid in additional carriers introduced into the compost windrow. The total inactivation of the microorganisms occurred within 48 h (Tab. 2). This was mainly due to the rise in temperature from $39^\circ C$ to $66^\circ C$ in the composted material (Fig. 1). Numerous authors point out the decisive effect of high temperature on pathogen elimination [6, 17, 18]. A high temperature should be obtained in all parts of the biomass [4].

![Fig. 1. Temperature distribution in composted biomass in the container and in the windrow in the top (T) and bottom (B) layers – summer cycle](image)

Tateda [20] reported that during the composting process in a closed reactor the highest temperature occurred in the top part, and the lowest in the bottom part of the material. Similar observations were made in the studies conducted in spring and autumn after building a compost windrow. In the top layer of material the temperature was remarkably higher than in the bottom (Figs 2 and 3). It might have resulted from improper material aeration. Such temperature distribution caused a notably slower course of \textit{E. coli} elimination in the bottom part of the biomass. Epstein [5] also found out that a number of fecal coliforms was high in the lower parts of the compost pile, which he ascribed to the excessively low temperature generated in the biomass.
In the spring and autumn cycles, a decrease in the bacteria number was observed in the top and middle parts of the biomass in the container, though the temperature obtained was lower than required. This indicates the presence of other important factors such as competition, antagonism or antibiosis. Competition among microorganisms for available carbon and nitrogen compounds can be an essential mechanism destroying pathogens in the compost [1, 9]. Being in competition for food with organisms which naturally occur in the compost, pathogens are definitely weaker rivals. The antagonistic effect of autochthonic microorganisms is strongest during initial composting stages and decreases slowly during compost maturation [16]. Such interactions might have caused \textit{E. coli} inactivation in the biomass intensive composting in the container in the present study.

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INAKTYWACJA PAŁECZEK ESCHERICHIA COLI PODCZAS KOMPOSTOWANIA ODPADÓW ORGANICZNYCH WRAZ Z OSADEM ŚCIEKOWYM

Badano wpływ procesu kompostowania w kontenerowej technologii Kneer na inaktywacje pałeczek E. coli. Bakterie, w specjalnych nośnikach, wprowadzano do warstwy górnej, środkowej i dolnej kompostowanego materiału. Doświadczenie prowadzono w cyklu wiosennym, letnim i jesieniowym. Tempo eliminacji pałeczek coli było zróżnicowane i zależało zarówno od usytuowania nośników w biomasie, jak i warunków cieplnych. Higienizacja materiału nastąpiła najszybciej w cyklu letnim – po 48 godz. w warstwie środkowej, 6 dniach w górnej i 10 dniach w dolnej. Wiosną i jesienią, bakterie najdłużej przeżywały w warstwie dolnej – odpowiednio 83 i 45 dni. Badania wskazują również na działanie, oprócz wysokiej temperatury, innych czynników, takich jak konkurencja, antagonizm czy antybioza.
Keywords: TSP, PM10, PM2.5, PM1, impactor, dust fraction, suspended dust, PAHs, benzo(a)pyrene, organic pollutants, air pollution.

Abstract: Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants, ubiquitous in the whole environment. They are relatively well known and are still of interest due to their well documented carcinogenic and mutagenic properties. In ambient air of urban regions they mostly occur as adsorbed to particles of suspended dust. The richest in these compounds and therefore most hazardous to humans, fraction of dust is the fraction of the finest particles. The paper presents results of investigations of dust sampled with use of an impactor Dekati PM10 in Zabrze, a site typical of Upper Silesian conditions. While sampling, the impactor segregates sampled particles into four fractions by their aerodynamic diameters. Sixteen PAHs were determined chromatographically. PAH content in the fraction of the finest particles, i.e. in PM1, was of particular interest.

INTRODUCTION

Properties of ambient dust (PM, Particulate Matter), its environmental and health effects as well as its fate in the environment depend on size of its particles [9]. Elevated health hazard in urban areas highly polluted by the finest ambient particles has been pointed out by many papers [3, 4, 27]. The finest particles easily penetrate into alveoli – the particles with the diameter of 1–2 μm are arrested there with the highest efficiency. Present in the body, such particles can cause intoxication, upper respiratory tract inflammation, bronchitis, lung cancer, allergies and asthma [27, 30]. Chemical and mineralogical composition of the dust, also that depending on the particle size fraction, affects the human health itself. Many volatile organic compounds, metal oxides and other, often carcinogenic, substances may be found on the particle surface [4, 11, 27]. Polycyclic aromatic hydrocarbons (PAHs) 48 of which are carcinogens [10, 33, 35], due to their specific strong
effects on living beings are of special concern \[34, 35\]. Urban ambient PAHs come from combustion processes, such as production of coke, combustion of organic fuels for energy production \[38\], road traffic \[1, 7, 13, 18\] and domestic furnaces \[17, 20, 21\]. They occur in gas phase and are adsorbed to dust particles. In areas dominated by vehicular air pollution, up to 95% of total ambient PAHs may be in PM (in PM3 rich in elemental carbon) \[28\]. PM1.3 may contain 60–90% of total PAHs from PM \[25, 26, 31\].

The paper presents results of investigations of 16 ambient PAHs (Tab. 1) in the great Upper Silesian agglomeration, Poland, in winter. Ambient concentrations of the 16 PAHs and distribution of their mass between four PM fractions: PM1 (particles with the aerodynamic diameter less than 1.0 μm), PM1–2.5 (particles with the diameter between 1.0 and 2.5 μm), PM2.5–10 (particles with the diameter between 2.5 and 10.0 μm) and particles with the aerodynamic diameter greater than 10 μm were determined. Till now, in Poland, PAH content in ambient PM fractions has not been investigated.

<table>
<thead>
<tr>
<th>Compound</th>
<th>&lt; 1 μm</th>
<th>1–2.5 μm</th>
<th>2.5–10 μm</th>
<th>&gt; 10 μm</th>
<th>PM1</th>
<th>PM2.5</th>
<th>PM10</th>
<th>TSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM [μg/m³]</td>
<td>38.84</td>
<td>8.86</td>
<td>3.40</td>
<td>1.32</td>
<td>38.84</td>
<td>47.70</td>
<td>51.10</td>
<td>52.42</td>
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<td>Naphthalene</td>
<td>0.00</td>
<td>0.26</td>
<td>0.06</td>
<td>0.00</td>
<td>0.00</td>
<td>0.26</td>
<td>0.32</td>
<td>0.32</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>0.07</td>
<td>0.12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.07</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
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<tr>
<td>Acenaphthene</td>
<td>0.19</td>
<td>0.09</td>
<td>0.15</td>
<td>0.00</td>
<td>0.19</td>
<td>0.28</td>
<td>0.43</td>
<td>0.43</td>
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<tr>
<td>Fluorene</td>
<td>0.22</td>
<td>0.29</td>
<td>0.23</td>
<td>0.00</td>
<td>0.22</td>
<td>0.51</td>
<td>0.74</td>
<td>0.74</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>2.69</td>
<td>0.68</td>
<td>1.20</td>
<td>0.00</td>
<td>2.69</td>
<td>3.37</td>
<td>4.57</td>
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<td>Anthracene</td>
<td>0.56</td>
<td>0.21</td>
<td>0.46</td>
<td>0.00</td>
<td>0.56</td>
<td>0.77</td>
<td>1.23</td>
<td>1.23</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>3.33</td>
<td>0.63</td>
<td>0.36</td>
<td>0.00</td>
<td>3.33</td>
<td>3.96</td>
<td>4.32</td>
<td>4.32</td>
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<tr>
<td>Pyrene</td>
<td>3.78</td>
<td>0.43</td>
<td>1.11</td>
<td>0.00</td>
<td>3.78</td>
<td>4.21</td>
<td>5.32</td>
<td>5.32</td>
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<tr>
<td>Benzo(a)anthracene</td>
<td>7.83</td>
<td>0.98</td>
<td>1.10</td>
<td>0.00</td>
<td>7.83</td>
<td>8.81</td>
<td>9.91</td>
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<td>Chrysene</td>
<td>8.56</td>
<td>1.31</td>
<td>2.45</td>
<td>0.00</td>
<td>8.56</td>
<td>9.87</td>
<td>12.32</td>
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<tr>
<td>Benzo(b)fluoranthene</td>
<td>4.30</td>
<td>0.23</td>
<td>1.08</td>
<td>0.00</td>
<td>4.30</td>
<td>4.53</td>
<td>5.61</td>
<td>5.61</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>4.36</td>
<td>0.25</td>
<td>0.20</td>
<td>0.00</td>
<td>4.36</td>
<td>4.61</td>
<td>4.81</td>
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</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>5.82</td>
<td>0.45</td>
<td>0.89</td>
<td>0.00</td>
<td>5.82</td>
<td>6.27</td>
<td>7.16</td>
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<tr>
<td>Indeno(1,2,3.cd)pyrene</td>
<td>2.09</td>
<td>1.67</td>
<td>0.50</td>
<td>0.00</td>
<td>2.09</td>
<td>3.76</td>
<td>4.26</td>
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<tr>
<td>Dibenzo(ah)anthracene</td>
<td>0.28</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.28</td>
<td>0.28</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>Benzo(ghi)perylene</td>
<td>2.27</td>
<td>0.00</td>
<td>0.29</td>
<td>0.00</td>
<td>2.27</td>
<td>2.27</td>
<td>2.56</td>
<td>2.56</td>
</tr>
<tr>
<td>Σ PAHs</td>
<td>46.35</td>
<td>7.60</td>
<td>10.08</td>
<td>0.00</td>
<td>46.35</td>
<td>53.95</td>
<td>64.03</td>
<td>64.03</td>
</tr>
</tbody>
</table>

**METHOD**

Dust was sampled from December 2006 to February 2007 in the central part of Zabrze (Fig. 1) by taking 48 hour samples with use of a Dekati PM10 four stage impactor. The sampling site is representative of the Upper Silesian ambient air quality conditions and it was characterized in \[12\]. The impactor collects dust from ambient air and segregates particles into four fractions: PM1, PM1–2.5, PM2.5–10 and particles greater than 10 μm. The finest dust was collected on a glass fiber after filter, the other fractions – on aluminium plates. The inlet of the impactor was 7 m above the ground level – the altitude
precluding effects of the secondary emission. The detailed description of the impactor is in [12]. In total, there were 30 samples taken.

![Location of the sampling point](image)

Fig. 1. Location of the sampling point

Concentrations of the dust fractions were determined gravimetrically. Prior to each weighing, before and after exposing, all substrates were conditioned in the weighing room for 48 hours.

The collected dust was extracted from the after-filter and plates in a dichloromethane (CH\textsubscript{2}Cl\textsubscript{2}) ultrasonic bath. The extract was percolated, washed and dried by evaporating in the helium atmosphere. The dry residue was diluted in propanol-2 (CH\textsubscript{3}CH(OH)CH\textsubscript{3}) and distilled water was added (proportion of propanol-2 to water – 15/85, v/v). For selective purification, the samples were extracted to solid phase (SPE) in columns filled with octadecylsilane (C\textsubscript{18}, Supelco). The columns were pre-conditioned in the extraction setting by washing, first with methanol, next with the 15/85 propanol-2/water mixture. They were kept wet during the whole conditioning. Directly before the extraction, 5 cm\textsuperscript{3} of the propanol-2/water mixture was percolated through each column in normal conditions (no vacuum). The samples were percolated under vacuum, rinsed with the propanol-2/water mixture and dried under vacuum. PAHs were eluted with use of dichloromethane. The extract of PAHs was condensed in the helium atmosphere to the volume of 0.5 cm\textsuperscript{3}.
A Perkin Elmer Clarus 500 gas chromatograph, with a flame ionization detector (FID), was used. An RTX-5 (Restek) 30 m x 0.32 mm x 0.25 μm capillary column with nonpolar stationary phase was used to separate the sample components. The flow of carrier gas, helium, was 1.5 cm$^3$/min.

Calibration curves for the 16 PAH standards were used in quantitative determinations. Linear correlation of peak surfaces with PAH concentrations was checked in concentration range 10−40 ng/mm$^3$. The samples were introduced into a split/splitless injector. Temperatures of the vaporizer and detector were 240 and 280°C, respectively. The temperature of the column was 60°C during the first 4 min of the analysis and increased at 10°C/min up to 280°C, at which the column was maintained for 14 min. The whole analysis lasted 40 min. The rates of providing the detector with hydrogen, air and helium were 45 cm$^3$/min, 450 cm$^3$/min and 30 cm$^3$/min, respectively.

Recoveries of the 16 PAHs were calculated by using the internal standard method. They ranged from 85% to 93%. The detection limit was 0.1 ng/mm$^3$.

RESULTS AND DISCUSSION

The measurement results are presented in Table 1. Concentrations of dust fractions are averages of 30 measurements (10 measurements per one month from December 2006 to February 2007). Each ambient PAH concentration is an average of nine measurements, three from each month. In this period, the average TSP concentration was almost equal to average PM10 concentration. PM1 was more than 74% of TSP; average content of PM1 in PM2.5 was 81.4%. It proves combustion and transformation of gaseous precursors contributing greatly to ambient PM. The average concentration of PM10 (Tab. 1) exceeds 40 μg/m$^3$, the limit value defined in [24]; the PM2.5 concentration exceeds 25 μg/m$^3$, the European Commission’s proposal for the limit value for yearly ambient PM2.5 concentrations [4]. However, the summer concentrations of PM in this area are much lower (averages of 48 hour PM10 and PM2.5 concentrations in summer 2006 were 38 and 32 μg/m$^3$, respectively [12]) and yearly concentrations of PM also may be lower than these presented in Table 1.

Concentrations of particular PAHs belong to a wide interval. They are high – concentration of B(a)P, the compound expressing carcinogenicity of PM, differs from 1 ng/m$^3$, the standard for yearly average from the Regulation [24], by one order (in fact, the concentrations should not be compared but the comparison is very figurative). B(a)P and other US EPA carcinogens – benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz(a,h)anthracene and indeno[1,2,3-cd]pyrene – are 72, 64 and 62% of the total mass of the 16 PAHs in PM1, PM1−2.5 and PM2.5−10, respectively. High contribution of these seven compounds (over 33 ng/m$^3$) to PM1 is of special significance. These PAHs adsorbed on such fine particles may penetrate human body very deeply.

Pyrene, fluoranthene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene – 4- and 5-ring hydrocarbons prevailed in PM1 and, in PM1, their concentration was about 60% of total concentration of the 16 PAHs (Tab. 1).

Considerable amounts of total and particular PAHs were found in PM1 (Tab. 1). There were no PAHs in the fraction of particles with diameters greater than 10 μm. It is probably due to low mass of the analyzed sample (in average 30 times lower than the mass of PM1, Table 1) and mechanical origin of these particles.
Total PAHs from PM1 had the greatest ambient concentration, and consequently the greatest mass (all the fractions were in the same volume of air), next were PAHs from PM2.5–10 and PAHs from PM1–2.5 had the lowest ambient concentration. In some conditions there are more 4-, 5-, 6- and 7-ring ambient PAHs in particles with aerodynamic diameter 7 µm than in particles with the diameter 3 µm [28]. It corresponds very well with the obtained results.

Most of the 16 PAHs (acenaphtene, phenanthrene, anthracene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, Tab. 1 and Fig. 2) behaved like the sum of PAHs. Some of the rest (fluoranthene, benzo(k)fluoranthene, indeno(1,2,3.cd)pyrene), like PM (Fig. 3), had concentrations increasing along with decreasing diameters defining the fractions. Naphthalene was entirely comprised in PM1–2.5 and PM2.5–10 (probably majority of this compound in the air, especially its gas phase, comes from its vapor condensed on surfaces of greater particles) and dibenzo(ah)anthracene did not occur in particles greater than 1 µm at all – Figure 3.

![Fig. 2. Mass distribution of nine PAHs among dust fractions](image)

Some combinations of PAHs may be characteristic of their sources [2, 6, 8, 15, 16, 19, 32, 37]. Chrysene, and benzo(k)fluoranthene are markers of coal combustion, benzo(ghi)perylene, coronene and phenanthrene – of car engine emission, phenanthrene, fluoranthene, pyrene are connected with absorbing vehicular gaseous PAHs particles from salted (in winter) roads, pyrene, fluoranthene, phenanthrene – with incinerators, volatile PAHs (fluorene, fluoranthene, pyrene, benzo(b)fluoranthene and indeno(1,2,3-cd)pyrene) are tied with oil combustion.
The dominance of chrysene and benzo(k)fluoranthene suggests coal combustion being the main source of the dust [5, 14, 22, 23, 29]. Their distribution among the PM fractions (almost all ambient PAHs are in PM1) and high content of PM1 in PM reflect the structures of air pollution in Zabrze – majority of houses in the centre of Zabrze are heated by furnaces burning hard coal very inefficiently. The sum of concentrations of acenaphthene, fluoranthene and pyrene is in average 21, 23 and 28% of the total PAH concentrations in PM1, PM1–2.5 and PM2.5–10, respectively. It means that in Zabrze PM, these compounds may come from adsorption of their gas phase by particles of dust [8]. Their content in – having greater mass – coarse fractions is relatively higher. In PM1, oil combustion markers – benzo(b)fluoranthene and indeno(1,2,3-cd)pyrene – occur in high concentrations. Only several of the 16 PAHs came from other than hard coal burning sources – among them very clearly naphthalene.

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WIELOPIERŚCIENIOWE WĘGLOWODORY AROMATYCZNE ZWIĄZANE Z PM1, PM2.5, PM10 I CAŁKOWITYM PYLEM ZAWIESZYM ZAGŁÓW ZAWIESZONYCH W AGLOMERACJI GÓRNOŚLĄSKIEJ

Wielopierścieniowe węglowodory aromatyczne (WWA) należą do grupy trwałych zanieczyszczeń organicznych obecnych we wszystkich elementach środowiska. Są licznie reprezentowane i stosunkowo dobrze poznane, a ich udokumentowane właściwości mutagene i kancerogene sprawiają, że nadal są w centrum zainteresowań. W powietrzu atmosferycznym obszarów zurbanizowanych najczęściej występują w postaci zaadsorbowanej na cząsteczkach pyłu zawieszonego. Najbogatszą w te związki, i stąd najniebezpieczniejszą, jest frakcja cząstek najdrobniejszych. Przedmiotem badań był pył zawieszony pobrany impaktem Dekati PM10 w punkcie charakterystycznym dla Aglomeracji Górnosłaskiej w Zabrzu. Impaktor umożliwia rozdział pyłu zawieszonego na cztery frakcje, każda o cząstkach mających średnice aerodynamiczne w innym z czterech przyległych rozłącznych przedziałów. W każdej z frakcji oznaczono zawartość WWA, a za szczególnie interesujące uznano wyniki zawartości WWA w pyle PM1.

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PHENOL BIODEGRADATION BY PSEUDOMONAS PUTIDA PCM2153

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COMMUNICATION

Keywords: 16S rRNA, aromatic hydrocarbons, degradation, phenolic compounds.

Abstract: Phenol degradation efficiency of Pseudomonas putida PCM2153 free cells was experimentally studied. Bacterial cells were acclimatized to phenol what relied on gradually increasing the phenol concentration in the medium. The highest phenol degradation rate was calculated as approximately 15.2 mg dm$^{-3} \cdot$ h$^{-1}$. Investigated strain degraded the phenol at the concentration of 400 mg dm$^{-3}$ in 24 h. The result of toxicity analysis showed that acclimatized cells of P. putida PCM2153 are able to survive even at as high concentration of phenol as 3000 mg dm$^{-3}$. The obtained result suggests that the analyzed strain can be used for effective treating of high strength phenolic wastewater. Due to resistance of the strain to high phenol concentration it may be applied in bioremediation of exceedingly contaminated sites, especially where dilution of pollutants cannot be implemented.

INTRODUCTION

One of the main human impacts on the natural environment is pollution of soil and ground water by inorganic and organic chemicals. Among these chemicals, low molecular weight aromatic hydrocarbons such as phenols, the simplest structurally aromatic compounds, can be found. Phenolic compounds are extensively used in polymer resin production, oil refinery, dye industry, pharmaceutical and pesticide industry, semiconductor industry etc [15, 16]. Phenolic compounds are very stable in the environment; therefore various chemical and biological methods for their removal have been extensively investigated. The concentration of phenols in effluents has been observed to vary from 10 to 104 mg dm$^{-3}$ [18], but microorganisms can survive even at higher concentrations and efficiently biodegrade this hazardous compound [1]. Traditionally, activated sludge process has been used to remove that pollutant from industrial wastewater but this method does not appear to be absolutely satisfactory, because this system is known to be very sensitive to high phenol loading rates [10]. An alternative approach applies pure cultures that are physiologically adapted to metabolize such as contaminant even in very high concentrations. This approach is based on degradation using specially selected microorganisms, which utilize xenobiotics as their energy, carbon, nitrogen and phosphorus or sulfur sources. For this purpose, numerous bacteria mainly from the genera Pseudomonas, Acinetobacter, Kleb-
siella and Bacillus are used for phenols degradation. Application of pure cultures is a very promising strategy; therefore finding new microorganisms capable of fast decomposition of phenols is still a hot research topic [3–8, 14].

Among others, the pseudomonads are renowned for their abilities to degrade compounds which are highly refractory to other microorganisms, including aliphatic and aromatic hydrocarbons. Pseudomonas metabolism has been the subject of intensive biochemical research, and many of the unique catabolic pathways found in Pseudomonas have been described. Studies on the microbial degradation of naturally and artificially synthesized pollutants have been exploited in order to solve the problems of environmental pollution. The ability of Pseudomonas strains to degrade various substrates results mainly from their genetic and physiological diversity. The high degree of their evolvability makes a chance for finding new strains that could be applied for pollutants decay during bioremediation processes.

The main goal of this work was to estimate the rate of phenol decomposition by pure culture of Pseudomonas putida PCM2153 and to determine the inhibitory effect of high phenol concentration on this species strain.

MATERIALS AND METHODS

Culture media and microorganism
Pseudomonas putida PCM2153 was obtained from the Polish Collection of Microorganisms (Polish Academy of Science, Wroclaw, Poland). The stock culture was stored at 4°C. The cells were cultivated in mineral salt medium (MSM) supplemented with glucose (1 g⋅dm⁻³). The composition of modified MSM was: (NH₄)₂SO₄ (0.5 g⋅dm⁻³), MgSO₄·7H₂O (0.4 g⋅dm⁻³), KH₂PO₄ (2.65 g⋅dm⁻³) and Na₂HPO₄·12H₂O (9.65 g⋅dm⁻³). In addition, 1 cm³ of a trace element solution was added to the medium. This solution contained (g): 20 FeCl₃·6H₂O, 10 CaCl₂·H₂O, 0.03 CuSO₄·5H₂O, 0.05 MnCl₂·4H₂O, and 0.1 ZnSO₄·7H₂O in 1 dm³ 0.5 N HCl.

25 cm³ of inoculum from the late exponential growth phase was transferred aseptically to flask with 475 cm³ of MSM supplemented with phenol up to concentration of 100 mg⋅dm⁻³ at the beginning of the culture. After 24 and 48 hours the concentration of phenol was increased up to 200 and 400 mg⋅dm⁻³, respectively. Cells were grown in 1000 cm³ flask temperature of 30°C with shaking at 200 rpm. A flask with the same amount of bacterial inoculum and without phenol was used as control. Samples were withdrawn periodically for cell density and phenol concentration analyses. From 48 until 72 hour the culture was monitored more frequently in order to determine the rate of phenol decay. Cell growth was monitored spectrophotometrically by measuring absorbance at 600 nm after 24, 48, and 72 h of cultivation.

Analytical methods
For the phenol concentration determination samples were initially diluted with distilled water to obtain OD value at the level of 0.6. An amount of 1.0 cm³ of diluted culture medium was collected in Eppendorf tube and centrifuged at 13 000 rpm for 5 min, and supernatants were used for further analysis. The residual phenol concentration was determined in triplicate using standard colorimetric assay, where phenolic compounds react with 4-aminoantipyrine dye. The absorbance of color solution was measured at 550 nm [11].
The toxic effect of phenol on *P. putida* PCM2153 cells was studied by cells cultivation at various phenol concentrations. After 72 hours of acclimatization, the culture was divided into six 50 ml samples and transferred to 250 cm$^3$ flasks. The cultivation in medium supplemented with phenol up to 500, 1000, 1500, 2000, 2500, and 3000 mg·dm$^{-3}$ were conducted for 24 hours. After that, the cells growth was estimated spectrophotometrically.

**RESULTS AND DISCUSSION**

Accordingly to our knowledge, even bacteria possessing necessary genetic ability to phenol degradation must be initially adapted to the environment containing such compound. During acclimatization process certain enzymes are induced, what is needed for their usefulness in the metabolism reaction. At the beginning of acclimatization process, the stock culture was transferred into flasks with phenol concentration at the level of 100 mg·dm$^{-3}$. After 24 and 48 hours the concentration of phenol was increased up to 200 and 400 mg·dm$^{-3}$, respectively. This procedure was necessary to prepare the cells for effective phenol biodegradation. The low concentration of pollutant in the first stage of culturing helped to perform this process easily without the risk of inhibition of the bacterial growth [9].

In this study, after acclimatization period, the culture of *P. putida* PCM2153 was supplemented with phenol at concentration of 400 mg·dm$^{-3}$, as it turned out such concentration was best for microorganism’s growth. What is worth of indicating, very short lag phase was observed (Fig. 1). Margesin *et al.* [13] studied the efficiency of phenol degradation by cold-tolerant *Arthrobacter* sp. AG31 and mesophilic *Pseudomonas putida* DSM6414. Both strains degraded 200 and 400 mg phenol·dm$^{-3}$ within 48–72 h. Kumar *et al.* [12] studied phenol and catechol degradation by *P. putida* MTCC1194. The strain utilized 1000 mg phenol·dm$^{-3}$ within 162 hours and 500 mg catechol·dm$^{-3}$ within 94 hours. Annadurai *et al.* [2] tested the ability of phenol degradation by *Pseudomonas putida* ATCC31800 as well as by mixture of this strain cells with activated sludge. The best results were achieved using the mixed liquor (activated sludge and *P. putida* cells). When the initial phenol concentration in medium was 0.2 g·dm$^{-3}$, the maximum phenol degradation, at the level of 80% was reached within 48 h. Comparing the phenol degradation ability of strains mentioned above and strain used in our studies, *P. putida* PCM2153 exhibits better phenol degradation ability because it could degrade ca. 400 mg phenol·dm$^{-3}$ within 24 h with the efficiency of phenol decay at the level of 96% (Fig. 1).

In this study phenol degradation rate reached 15.2 mg·dm$^{-3}$·h$^{-1}$. Comparing this value with other strains of *Pseudomonas* e.g. *P. pseudomallei* and the *P. putida* strains or mixed liquor of activated sludge and *P. putida* ATCC31800 it places *P. putida* PCM2153 slightly above them [2, 12, 17]. This value shows that this strain has great potential in terms of biodegradation of phenol.

During the toxicity test, any of applied concentrations did not influence the survival of bacterial cells. Growth of bacteria and phenol degradation was observed in a medium containing 1000 mg·dm$^{-3}$ (data not shown). At higher phenol concentrations (up to 3000 mg·dm$^{-3}$) the decrease of cells number was not observed after 24 hours of culturing. This observation could suggest that the analyzed strain is extremely resistant to high phenol concentration and could be used for bioremediation of exceedingly contaminated sites, where dilution of pollutants cannot be implemented.
Fig. 1. Efficiency of phenol biodegradation by *Pseudomonas putida* PCM2153 (OD – optical density measured at $\lambda = 600$ nm)

- Phenol concentration [mg dm$^{-3}$]
- Time (h)
The results of this study suggest that investigated strain can be applied for effective removing of phenolic compounds from wastewater. The results presented here, indicate that acclimatization is required for successful decay of phenol by studied \textit{P. putida} strain. Due to the high phenol utilization rate it is worth to consider the designing of degradation system with periodical loading of phenol. We propose adding previously diluted phenol-containing wastewater periodically after every 24 hours, what guarantees efficient phenol removal as well as the proper bacterial growth. The phenomenon of the cell survival at concentrations as high as 3 g phenol⋅dm\(^{-3}\) shows a great potential of this strain in bioremediation.

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**BIODEGRADACJA FENOLU PRZEZ PSEUDOMONAS PUTIDA PCM2135**

W doświadczeniu analizowano efektywność rozkładu fenolu przez szczep Pseudomonas putida PCM2135. Komórki bakteryjne zostały poddane adaptacji do wysokich stężeń fenolu, która polegała na stopniowym zwiększaniu jego stężenia w pożywce. Maksymalna szybkość rozkładu fenolu wyniosła 15,2 mg·dm$^{-3}$·h$^{-1}$. Badany szczep usunął 400 mg fenolu w ciągu 24 godzin. Przeprowadzone testy toksyczności wykazały, że zaadaptowane komórki P. putida PCM2153 są zdolne do przeżycia w roztworze fenolu nawet o stężeniu 3000 mg·dm$^{-3}$. Uzyskane wyniki sugerują, że badany szczep może być wykorzystany do efektywnego oczyszczania ścieków charakteryzujących się wysokim stężeniem fenolu. W zawiązku z odpornością szczepu na wysokie stężenia fenolu, może on być wykorzystywany do bioremediacji terenów silnie zanieczyszczonych fenolem, gdzie rozcieńczanie zanieczyszczeń jest niemożliwe.
CONSEQUENCES OF WATER ENGINEERING PROJECTS IN THE MOKAŠNICA RIVER BASIN (BOSNIA AND HERZEGOVINA)

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COMMUNICATION

Keywords: Mokašnica River basin, karst, aquifer, water engineering project, water, environment.

Abstract: The article deals with the state of utilization and protection of the water resources in the Mokašnica river basin (MRB) within the larger Mostarsko Blato catchment area (MBB). The authors present data on the Mostarsko Blato basin, provided through the analyses of the results of earlier research projects dealing with surface and groundwater flows, particularly related to the research carried out in the MRB. The previous water engineering works in the MRB have been analyzed for effects on the natural environment and the influence of such works on the regime of surface flow and groundwater aquifer geometry. In order to protect the area of the MRB from further devastation, the authors suggest some protective measures and due improvements. A special environmental protection study is necessary for better management of the MRB waters and the natural environment, including the analysis of the possibility of losing said water resources due to the implemented engineering interventions.

INTRODUCTION

The Mokašnica river basin (hereinafter: the MRB), covering an approximate area of 120 km², is the second largest and the second most important river basin of Mostarsko Blato (hereinafter: MB). The characteristics of the MB basin were examined before and during the 20th century in order to facilitate better protection from floods, to intensify agricultural production using irrigation, and lately in order to use the water for production of electricity. The Mokašnica River, its branches, wellsprings, sinking streams, estavelles, underground aquifers and other MRB features are seldom mentioned in reports on MB examination. Most research, according to reported data, pertained to the Lištice river basin, whose mainstay spring is the largest in the MB basin (hereinafter: the MBB).

The springs of Mokašnica and Lištice rivers are located northwest from MB, and their sinkholes (Orline for Mokašnica; Renkovača, Košina, Kabanica and Plitonje for Lištice) at the eastern edge of the MB plain. The Mokašnica and Lištice rivers flow almost parallel through their individual water beds from their springs to sinkholes. Dr. Polić used
(color) tracers to show that the waters from the Lištica follow mainly flow toward the Jasenice spring, and those from the Orline toward the Arape and Miljačić Oko springs [12]. Every summer the Mokašnica dried up in its upper part of stream, along with its right incoming branches. Its two tributaries entering the MB field with their entire lengths, as well as the Mokašnica itself in its lower part of stream (from Biograci to the Orline sinkhole), used to have enough water to support life in them all year long [10].

Mokašnica was always functioning as an open drainage „sewer system” to a congruence of over 2000 homes with septic tanks, dunghills, farming land with crops being fertilized and protected. A lot of solid waste is being thrown away within the MRB without any care or control [3]. Earlier engineering research and interventions did not observe any environmental analyses related to the ecological aspect of the MRB or the MBB, nor the consequences on the quality and quantity of underground and surface waters in the MRB.

Natural sinkholes at the eastern edge of MB (Fig. 1) are insufficiently large in order to take in the large waters of the Mokašnica, the Lištica, the Crnašnica, the Orovnik, the Žvatić and numerous occasional streams appearing during the rainy season. As a result, a larger portion of MB gets flooded, and the waters from the above sub-inlets, get mixed forming an intermittent lake which was known to reach 40 km$^2$ in size [4]. In order to have shorter floods periods and smaller flooded area of the MB field, a tunnel was constructed for evacuation of large water masses. The tunnel is only partially used, because

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Fig. 1. The situation map of Mostarsko Blato plain with water flow streams and directions
1 – spring, 2 – intermittent spring, 3 – estavelle, 4 – perennial open flow, 5 – intermittent open flow, 6 – sinkhole zones, 7 – the field border between the alluvial aquifer and the karst zone, 8 – dug well, 9 – bore-hole, 10 – spring, 11 – sinkhole, 12 – canal, 13 – alluvial aquifer MB
of the limitation of the Jasenica River not being able to transport all water from the tunnel to the Neretva River. Conflicting opinions exist regarding the tunnel’s effect on the environment in the MRB.

Inhabitants of the MRB are peasant who and get their water from some 2500 rain water tanks and numerous draw-wells found along the edges of the MB field. The largest number of water supply wells is constructed in the alluvial aquifer of the MRB, which earlier MBB projects chose to ignore. Rural households in the MRB, with their 1000-odd shallow wells, drain and consume from the alluvial aquifer more than 5000 m³ water per day. In addition to the rain water tanks and shallow wells, the locals used to purchase water from private providers who transported it from other river basins – approximately 50000 m³/year at 2–6 EUR/m³.

Due to insufficient earlier research of the intermittent water springs in the MRB, the hydrogeological characteristic of the karst aquifers of the area is unknown. The demand for water and the prices are high in the MRB, and they continue to rise. There are no programs or concepts for investigation of groundwater aquifers near the intermittent springs, which are generally neglected. Therefore, the authors of this paper examined the largest portion of available data from earlier research in the MBB and gave their initial estimations on consequences of individual water engineering projects, i.e. activities pertaining to the change in quantity or quality of surface and underground waters. Results acquired through this research are presented here, along with the suggestions of improving environmental conditions and for revitalization of former water sources and perennial tributaries in the MRB.

WATER ENGINEERING RESEARCH IN THE MOKAŠNICA RIVER BASIN

Mokašnica is an intermittent river comprising many intermittent springs, carrying rain water and waste water to the river basin through the surface stream and a very complex system of underground canals. Only two water-measuring stations are placed in the MRB in order to control the surface dissipation. The older of them, and more regularly recording, is stationed in Jare (232.5 m a.s.l.), while the other is in Mokro (approximately 259 m a.s.l.). The purpose of the Mokro hydrological station is to control the surface water flow from the upper part (approximately 16.8 km²), the highest part of the MRB. The average water flow is estimated at 0.425 m³/s. The Jare hydrological station controls a larger portion of the MRB, an estimated 28 km². The average water flow in Jare station is 0.748 m³/s. Regular measuring of precipitation is continually being performed only by the weather service station in Mostar. According to the water-measuring station in Mokro, the average value of water outflow (with precipitation of 1770 mm/year) would amount to 25 dm³/(s·km²), and to 27 dm³/(s·km²) according to the water-measuring station in Jare (Fig. 1). If we presume the average value of outflow for the entire Mokašnica river basin of 120 km² up the stream from the Orline sinkhole (223 m a.s.l.) to be 26 dm³/(s·km²), then the total average Mokašnica flow can be estimated at 3.12 m³/s for the same annual precipitation value.

Initial water engineering research was performed by the Hydrotechnical Institute of the Technical Faculty in Zagreb during the early 1960’s [4]. This and subsequent research was performed mainly in order to ensure the basis for designing hydro-melioration structures and other projects in the MBB. An earlier MB hydrological research program
related to the hydro-power station on the Neretva (the Institute for Study and Development in Mostar) consisted in performing 18 hydrogeological boreholes in MB. The report of the results of these operations contains mostly geological and technical borehole profiles, without any data on hydro-geological parameters of the alluvial aquifer, changes in groundwater levels, etc. [7]. The basic 1:100 000 geological maps, Mostar and Metković sheets, and the hydro-geological map of the Neretva river basin at 1:200 000, gave valuable orientation data on the MBB. The MBB hydro-geological map at 1:25 000 [12] shows the geological data, sites of faults, the slip and folds, and presents much significant hydrogeological information, enabling us to determine the conditions and causes for majority of intermittent and permanent water-springs in the MRB.

By examining unpublished technical documentation, including the reports related to the melioration in the MRB available in Vodoprivreda (Water Management Co.), Mostar, the research of bauxite in Western Hercegovina, the water supply and, lately, the energy needs, the authors familiarized themselves with numerous hydrogeological characteristics of the MRB.

SURFACE WATERS

The Mokašnica begins in Mokro polje, between Mokro and Turčinovići villages, and disappears into the Orline sinkhole at the south-eastern end of Mostarsko Blato (Fig. 1). During the rainy season, numerous springs of water appear in Mokro polje, some representing estavelles. The upper route of Mokašnica is considered to be its largely hilly portion, approximately 8 km long, reaching from the spring at 260 m a.s.l. to the Jare water measuring station at 232.5 m a.s.l. This portion of the river bed has several occasional wellsprings, the largest of which are Lasića vrelo (Lučica) and Anića vrelo, offering water for longer periods than the upper springs in Mokro polje and Luke. Surface water in the occasional upper flow of Mokašnica is mainly used as water supply for cattle and for running one paddle-wheel mills. Some intermittent water springs water in the upper part of the MRB and on northern slopes of Trtla occasionally permit surface water to percolate into the muddy alluvial sand deposits, or pržina, and recharge the alluvial aquifer in the MRB. It is a natural way of drainage of underground karst aquifer into the MRB. It is assumed that a certain portion of water from the orographic MRB flows into lower permanent wellsprings in Studenci, Vitina and some springs in the Neretva Valley, which should be investigated. Permanent springs and water sources of two Mokašnica’s (left) tributaries, Rika and Govnuša, and springs of water along the lower permanent Mokašnica flow (Bilila, Otok, etc.) are natural points of drainage of the alluvial aquifer at the Mostarsko Blato plain (AAMB). Quantities of water inflow along the permanent part of the Mokašnica flow were not measured, nor were the controlled quality of water present at that location – something that should be done as soon as possible.

Both left tributaries of Mokašnica, as well as Mokašnica itself, had enough water for cattle, fishing and irrigation of a limited number of lots even during the driest seasons, ranging from the Rika confluence up to the Orline sinkhole. Mokašnica in Biograci had several fresh water sources (Bilila, Otok) and several larger sources of water along the river (Buć, Modraš, Grumeničovac, Prokopica, kaluža), where high willows and other useful plants grew. These spots were important and popular locations (Bilila, Otok, Dubalj, Prokopica and Kaluža) for the people from more than 10 villages, who came to
these locations to rest, relax, enjoy fresh drinking water, play sports, dance, participate in religious ceremonies, etc.

The water flow of the Mokašnica’s lower end has made numerous meanders in the MB plain, causing a gentle slope without erosion. The relations between the lengths of the longer intervals along the meanders of the river flow and the shorter (straight) intervals, measured between characteristic points on the 1:10 000 scale maps are shown in Table 1. Mokašnica flow segments are 1.50 to 1.97 times longer along the meanders than the straight length measurements (Table 1). The average decrease of slopes at certain segments of the upper MRB ranges from 0.3 to 10.8, but in the lower portion segments are only 0.2–1.2 m/km (Table 1).

<table>
<thead>
<tr>
<th>Intervals</th>
<th>Distance [km]</th>
<th>Meters above sea level [m a.s.l.]</th>
<th>Dip [m/km]</th>
<th>Meandering coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>longer shorter</td>
<td>start. ending ending diff.</td>
<td></td>
<td></td>
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<tr>
<td>Mokro polje</td>
<td>2.00</td>
<td>260.0 259.4 0.6 0.3</td>
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<td></td>
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<tr>
<td>Ćužići</td>
<td>1.23</td>
<td>259.4 257.5 1.9 1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luke</td>
<td>0.48</td>
<td>257.5 256.0 1.5 3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uzarići Gornji</td>
<td>2.00</td>
<td>256.0 252.0 4.0 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uzarići Donji</td>
<td>1.80</td>
<td>252.0 232.5 19.5 10.8 (water mill)</td>
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<td></td>
</tr>
<tr>
<td>Total upper flow</td>
<td>7.51</td>
<td>260.0 232.5 27.5 3.7</td>
<td></td>
<td></td>
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<tr>
<td>Jare/Biogradci</td>
<td>2.85</td>
<td>232.5 229.0 3.5 1.2 1.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biogradci</td>
<td>3.55</td>
<td>229.0 225.0 4.0 1.1 1.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blato Donje</td>
<td>11.25</td>
<td>225.0 223.0 2.0 0.2 1.73</td>
<td></td>
<td></td>
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<tr>
<td>Total lower flow</td>
<td>17.65</td>
<td>232.5 223.0 9.5 0.5 1.73</td>
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<td></td>
</tr>
<tr>
<td>Deep drainage canal (DDČ)</td>
<td>8.8</td>
<td>231 222 9.0 1.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rika (tributary)</td>
<td>2.30</td>
<td>231.5 229.0 2.5 1.1 1.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Govnuša (tributary)</td>
<td>4.20</td>
<td>229.5 225.0 4.0 0.95 1.68</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Lengths and slopes of certain segments of the Mokašnica, its left tributaries and the DDC

In addition to the above mentioned water springs and two tributaries from the left side, the Mokašnica also has numerous water springs coming from the right: Anića spring, Blaž, Gromolj, Kaćun, Zelenikove Babe and Dedo, Zveč, and numerous springs in the alluvial sand deposits (Blatska pržina) along the southern margins of the MB plain, consumed by forest cutting, active lime production and goat herding.

It is well-known that the cutting of forests and reforestation of areas may cause changes in precipitation recharge of surface streams and the groundwater aquifer. Especially noted is the reforestation of areas in the United Kingdom, which caused an almost 20% decrease in the surface runoff [2]. Similar changes in surface runoff values were also determined through latest research results in Ireland [1]. Many changes occurred in the forestlands of the MRB during the last 40 years, but the adequate hydrological analysis of surface water quantities in the MRB was not possible because of the lack of necessary data related to forestland changes and hydrological data for the recent period.
GROUNDWATER

Groundwater has some important advantages over surface water. Most underground waters demand little or no cleaning before being used in households or by industry. That fact gives groundwater a large economic advantage with leading persons in various public institutions and industries. However, contrary to popular belief, groundwater is not infinite: once tapped out from the aquifer or contaminated, it requires decades or hundreds of years to replenish or cleanse itself [5].

Two significant groundwater aquifers are available in the MRB. The larger one – karstic aquifer exists in the carbonate rock, while the smaller one is found in the alluvial deposits of the MB plain and along the river and streams. The karstic aquifer was built by water-saturated cracked and cavernous limestone and (less permeable) dolomites, which are present in the entire river basin, at the surface and deeper, under the neogene and quaternary deposits. The alluvial aquifer contains water-saturated alluvial sand, gravel and crushed limestone (Fig. 2). The alluvial aquifer sits in the lower Mokašnica area, between the hydro-station in Jare and Kaluža, in the western part of Mostarsko Blato plain and along the southern edge of the low-lying portion of the MRB from Jare to Čule (Fig. 1).

Divides within the groundwater aquifers in the MRB carbonate rock and the groundwater in aquifers of the neighboring river’s basins, the Lištica to the north, the Lukoć to the south, the Neretva to the east and the Topola to the west, are yet to be investigated. Appearances of the intermittent karst water springs at various elevations in the MRB are proof of changes in groundwater levels, as is probably the position of the groundwater divide in the carbonate rock. The groundwater level in the alluvial aquifer features lesser changes during the rainy and dry seasons in relation to groundwater level changes in the MRM karst aquifer.

Rain water infiltrates and percolates into the karst aquifer and flows through complex, difficult to follow paths; therefore, it is very difficult to determine the exact process of groundwater recharge, its flows, accumulations and springs. The karst groundwater aquifer in the MRB has not been closely investigated. Only 3 groundwater investigation boreholes were made in Mokro polje, but the groundwater level (GWL) was not systematically monitored, nor were water samples regularly taken in order to control water quality. Intermittent water springs on the northern slopes of Trtla in the MRB are natural phenomena, with no record of appearances and durations, quantity and quality controls, or any other physical and chemical characteristics of their water samples.
The most significant alluvial groundwater aquifer is the one found in the western part of the MB plain (the AAMB). It used to be recharged by precipitation through direct soil infiltration and percolation into alluvial sandy gravels, and indirectly through the contact of the AAMB with the karstic aquifers. During rainy seasons, the alluvial aquifer is also recharged by the neighboring Lištica water stream. The alluvial aquifer is naturally drained through springs in the MRB along the river banks during its surface flow over the alluvial sandy gravel. Traditional water springs along the Mokašnica River (Bilila and Otok) and the numerous dug shallow wells in the MRB were an excellent supplementary rural water supply when their traditional rain water tanks dry out during dry season.

WATER ENGINEERING PROJECTS

Until the mid-20th century, water belonging to the permanent part of the Mokašnica water stream was used mainly as a water supply for cattle, smaller agricultural lots, fishing, washing clothes and, to a lesser extent, for domestic use. There was no conflict among the users because there were unwritten rules in place where the fishermen muddled the water only downstream from the locations where the water was used for cattle and washing clothes. Smaller water engineering projects were designed, realized and used in the MRB before the 20th century as well. Local population used to dig shallow wells a few meters deep and drew up water for vegetables and other domestic use (in Odanci), or divert water from the Mokašnica stream toward their land. During the 20th century, the local government and the authority in charge of water sources began to design more expensive and more complex water engineering projects, which were not maintained at all. Individual MBB projects have significant negative effects on the MRB, as witnessed by the following examples:

1. A small group of households in Biograci constructed a weir (small earth dam) at Bilila, on the permanent flow portion of Mokašnica in order to divert water for irrigation. They also constructed a water course to transport diverted water toward the south-east in order to flood their agricultural land. This project had several positive effects, but the dug water course was a hindrance for cattle carriages, horses and people passing through the cut-off area. This first soil dam was active for a very long time, since its users reconstructed it every year and took good care of it. Later, the local government constructed a concrete dam on the same location at Bilile, but it did not last long because no-one knew how to maintain it.

2. A larger group of households from Ljuti Dolac village built two earth dams in Biograci, the main one at Kaluža (on the Mokašnica river, downstream from Bilila), and the second one in the middle of the permanent stream of the left Mokašnica’s tributary, plus the construction of new canals across the field towards the south-east edges of the MB plain. These structures drew their water from the original permanent stream of Mokašnica leading toward the agricultural plots in Ljuti Dolac, without consent from the Biograci villages, i.e. the owners of the land. It caused numerous negative consequences.

3. It is well-known that the natural sinkholes cannot evacuate larger inflow of water during heavier rains in the MBB, where the hundred-year waters calculation is $Q_{100} = 360 \text{ m}^3/\text{s}$ and that of the fifty-year waters $Q_{50} = 137 \text{ m}^3/\text{s}$ [4]. Therefore, a tunnel was constructed to evacuate the flood waters from the lowest point of the MBB (222 m.a.s.l.) into the Jasenica river. The tunnel can receive only 40% of larger inflows,
since it is limited by the flow-through capacities of the Jasenica River [10]. The environmental consequences of this tunnel in the MRB should be studied and estimated as soon as possible.

4. The Water Sources Management Office in Mostar designed and constructed a deep, steep drainage canal (DDC) in the lower Mokašnica area during the early 1970’s. The excavated material during the construction of the DDC was transported and buried the original streams of the permanent river Mokašnica and its left tributaries. This material covered up the permanent drinking water springs along the Mokašnica (Bilila, Otok and others), where masses were held even during the driest weeks of the year. The village people from Ljuti Dolac, Biograci and Jare used to drink water there use it for their cattle and gardens, learned to swim, had fun, transported water from the springs for the domestic use, and also fished conger eels, crabs, frogs, etc. The construction of the DDC began at the centre of the spring zone (230 m above sea level) of the left Mokašnica’s tributaries, and ended in the lowest area of the MB plain, in the zone of sinkholes (223 m a.s.l.). The construction of the DDC caused harmful reduction of the GWL in the AAMB of more than 1 m.

5. As designed and approved by the Water Sources Management Office in Mostar, a massive gravel excavation commenced during the 1970’s, starting at the Lištica river bed – very near to the Mokašnica river and the springs of its 2 left tributaries (AAMB). Although this massive excavation of gravel from the AAMB was ordered by ‘responsible’ designers, it caused additional degradation of the river bed and a very damaging decrease in GWL of AAMB in the area of permanent water springs.

6. Higher water consumption by the local population in the MRB demanded deeper excavation of their wells, the installation of more powerful pumps, and intensification of capturing more water from the relatively thin AAMB, in order to supply the households and gardens during the entire year. Majority of private wells are in the MRB and a smaller number of wells in the Lištica river basin.

7. The inhabitants of Biograci and Ljuti Dolac villages initiated and performed the excavating of the buried river Mokašnica from Bilila to Kaluža in the late 1990’s. After this excavation, the permanent water flow was established again, but the surface water levels were significantly lower than before; no well-known water springs (Bilila and Otok) were recorded since the GWL was lowered due to the steep DDC, excavation of gravel and intensified AAMB drainage because of deeper wells.

8. A civil work contractor from Uzarići village obtained a permit to excavate the gravel and sand from the precious AAMB near the zone of previously active springs of the Mokašnica’s permanent tributaries. These excavations lowered the bed of the Lištica River and the GWL in the precious AAMB.

9. Another private (amateur) group from Uzarići village constructed a low dam in the Lištica river bed, near the previously mentioned spring zone of the Mokašnica’s tributaries. This dam was made with stone blocks driven in from elsewhere, and filled up with local soil using a bulldozer. The works were performed without an approved design and without expert supervision, but the GWL in the AAMB was raised a few decimeters at least.

10. Special threat to the natural environment in the MRB are numerous illegal dumps, the growing number of large warehouses for fuel, lubricants and other industrial and agricultural materials found in the MRB. An estimated 2000 liquid-manure pits,
1500 septic fills and numerous underground fuel and lubricant tanks can be found in the area [3]. The largest, already filled public dump was located on the largest underground aquifer, on water-permeable terrain of the karst aquifer called „Krtine”, also containing several illegal landfills. Illegal landfills are numerous, the largest of which is Čajsula, found in the area of the most used underground aquifer in the MRB, is situated in the most delicate of locations, because it stands on the open AAMB in the MRB, where illegal excavation occurs for the construction needs in Ljuti Dolac and Biograci villages.

The quality of surface water and groundwater depends on various factors [6]: quantity of precipitation, evaporation intensity, chemical make-up of precipitation, the soil and the rocks through which the water flows, soil additives for purposes of fertilization and protection from weed and other damaging effects, materials let out from the industrial plants and various other facilities, and from the community. Peterson and Wollheim reported [8] that even the smallest surface water flows may remove 50% of nonorganic nitrogen taken in by the stream. Therefore, the disappearance of the permanent surface water flow in the MRB presents an immeasurably high damage to the environment.

CONCLUSIONS

Obligatory protocols have been proclaimed, and numerous institutions established within the United Nations system and the European Union, for the implementation of adequate measures of protecting the natural environment and revitalization of endemic and endangered species. Unfortunately, the reliable necessary data regarding the changes in the natural environment, quantities and qualities of water and soil in the MRB is not available. Therefore, it is difficult to plan effective protection activities aimed at revitalizing the natural environment, and adequate measures against further damages of the environment. To estimate realistic data related to changes in the natural environment, a necessary program of investigation should be implemented.

The permanent Mokašnica’s flow and its two permanent tributaries with numerous intermittent streams drained the MRB, which was the home for numerous animal species, abundant vegetation and featured large annual fish growth before the arrival of water engineering activities. Eight out of nine hydrotechnical interventions in the MRB reduced the value of its natural environment. The increasing number and types of pollutants also increased the risk of polluting the soil and water in the MRB. The AAMB, intensively used for the rural water supply, was badly damaged, and if this is to continue, this precious aquifer may be depleted and even dry up. The burying and the drying-out of the permanent water streams with meanders shortens the surface water flow in the MB plain, and lowers the possibility of natural cleansing of water before sinking in the Orline sinkhole. This, in turn, can present a significant risk to the quality of groundwater and surface water in the lower Neretva river basin, and this situation should be systematically monitored and appropriate safety and protection measures applied. Therefore, an adequate investigation program is necessary in order to design and implement the needed revitalization activities.

The consequences of earlier noted water engineering projects in the MBB are as follows:

It has been estimated that the construction of the soil dam on Bilile and the Biograci
canal had no negative effects on the lower water stream. The water quantity downstream from the dam was replenished due to underground waters below the dam and strong springs of water downstream from the dam; therefore, the biological need for the river bed downstream from Bilile was fulfilled [11]. The Bilile dam and the canal protected the water levels in a greater portion of the alluvial aquifer, so less energy was being spent to lift water from shallow wells.

However, negative environmental effects occurred due to the diverting of entire flow of the Mokašnica using dams at the Mokašnica in Kaluža and the Govnuša in the middle of the stream by villagers from Ljuti Dolac. Water was taken using man-made canals to transport this water in Ljuti Dolac in order to irrigate the agricultural land. All the water downstream from these dams in Kaluža to the Orline sinkhole dried up, doubtlessly destroying numerous plant and animal life. The scope of these damages needs to be re-examined using adequate rational projects [11].

The construction of the canal definitely decreased the flooded areas, the duration of floods in the MRB, the duration of the geese hunting season, as well as the duration of the use of boats for transport of goods and people over flooded areas. The construction of the canal also encouraged irresponsible issuing of water-supply office permits for the construction of various objects at lower elevations in danger of possible floods. The consequences the canal had on flora and fauna in the MBB, and the general environmental effects on the quality of life in the MBB are yet to be investigated.

The construction of the steep DDC lowered the groundwater level in the valuable alluvial aquifer for more than 1 m. This decrease of the groundwater level and burying the original permanent streams caused the drying out of all water springs and natural river flows, as well as earlier canals in the MRB. The disappearance of permanent water springs and water streams in the MRB also meant the disappearance of fish species such as prikanac, conger eels, frogs and numerous other species; water could no longer be used for supply, recreation, fishing, there were no new willow branches for basket weaving (krtote), etc., and no one ‘competent’ offered a reason or an explanation to the local populace for this direct and all-too-real loss.

The excavation of the shortest course of the buried Mokašnica River from Bilile to Kaluža, caused some erosion by surface waters during rainy seasons. If additional deeper wells were to be dug and more powerful pumps used, the excavated portion of the Mokašnica River would dry up during dry seasons. Further deepening of the river bed may cause additional lowering of groundwater levels in the AAMB and in all private wells in the MRB, presenting danger for total characteristics of the alluvial aquifer in MB.

Uncontrolled excavation of gravel and sand in the area formerly occupied by springs of permanent tributaries of the Mokašnica caused a quicker degradation of the river bed and the lowering of the groundwater level in the alluvial aquifer for another meter, maybe more during certain periods of the year. The consequence of this is drying-out of the more shallow private wells, their deepening and a greater expansion of energy used for raising the groundwater.

A low dam on the Lištica River in the area near the former permanent springs of the two Mokašnica’s tributaries was constructed by locals from the Uzarići village. The dam was not professionally designed and did not have an experienced expert to implement and supervise the works. It caused the GWL to rise 0.50 m in the MRB. This rise of GWL is a positive effect of this first low dam, but it was not enough to facilitate the return of the
important water springs. When these works were performed, the owner of the dam was advised to engage local experts to monitor the hydrogeological changes in the area, and perform the necessary evaluations in order to recognize the total impact of this first low dam in the MBB. The results of such evaluation may help to prepare a relevant guide to designing, locating and constructing any additional necessary structure(s) which may spearhead the rise of the GWL, initiate the return of the lost water springs and generally offer other positive benefits to the location.

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COMMUNICATION

Keywords: Nitrogen, phosphorus, N:P, C:N, C:P, net primary production, bilberry, crowberry.

Abstract: Vaccinium uliginosi-Betuletum pubescentis and Empetro nigri-Pinetum are characteristic of their diverse frequency and density Vaccinium vitis-idaea L. and Vaccinium myrtillus L. on the forest floor. The examined berry under-shrubs show differences in content of nitrogen and phosphorous compounds and the volume of the over-ground net primary production. An average content of the examined biogenes in leaves of the bilberry (Vaccinium myrtillus) within the area of the Słowiński National Park was 1.311% N, 0.102% P and 40.8% C in Vaccinium uliginosi-Betuletum pubescentis and 1.159% N, 0.095% P and 38.7% C in Empetro nigri-Pinetum. Red bilberry leaves are thought to contain nitrogen, phosphorus and carbon in such proportions: 1.083% N, 0.097% P and 44.70% C in Vaccinium uliginosi-Betuletum pubescentis and 0.868% N, 0.085% P and 44.70% C in Empetro nigri-Pinetum. The variable concentration of nitrogen and phosphorus in sprouts of the examined species of berries shows a positive correlation of those elements. The over-ground net primary production of Vaccinium myrtillus is 534.905 kg/ha in Vaccinium uliginosi-Betuletum pubescentis and 216.594 kg/ha within the coastal crowberry coniferous forest. Vaccinium vitis-idaea in Empetro nigri-Pinetum reaches 155.283 kg/ha of over-ground net primary production and only 113.220 kg/ha in Vaccinium uliginosi-Betuletum pubescentis.

INTRODUCTION

Nitrogen and phosphorus belong to more important elements among nutrients on which the growth of plants depends in the forest ecosystems [6, 9, 13, 20, 52]. Seasonal variability of the environment and variability of plants requirements during their life span decide on dynamic character of the plant – environment relations. Intake of most elements by plants is a metabolically regulated process [8, 42] and their concentration is connected with the processes of growth and aging occurring in them [28, 34]. The humus of forest soils constitutes a source of nutritional (biogenic) elements for plants [12, 28, 39].
Many factors, such as air and soil humidity, availability of nutrients [17] rainfall as well as character of vegetation have impact on concentration of mineral components in plants. Scarcity of soil humidity strongly enhances the nitrogen content in leaves, and with increasing soil humidity, the content of nitrogen and phosphorus usually decreases [54]. Intake of ammonium, nitrate and phosphorous ions by plants is differentiated during a vegetation season, and their rise strictly dependent on biomass, is a result of a photosynthesis and assimilation of NH$_4^+$ and NO$_3^-$ ions from the subsoil [45]. The volume of chemical components in leaves of bilberry (Vaccinium myrtillus L.), as in the red bilberry (Vaccinium vitis-idaea L.), is connected with phenological stages of plants development [20]. The status of mineral nutrition of plants depends not only on concentration of particular mineral components, but also on mutual balance between them [5]. Analysis of quantitative proportions between individual elements both in the soil and in the plants, can be the evidence, among others, of the status of their nutrition [7], regularity of the course of physiological processes in a plant [34] and indirectly, of its health status [47]. Changeable environmental conditions disable maintenance of optimum C:N proportion in plants. Plants use a big volume of nutritional ingredients included in the soil. Availability of these components is a main factor which controls biomass production and stability of ecosystems [48]. Increase of biomass production depends especially on the fecundity of forest community [49], the structure of the undercover, the role of a given species in the ecosystem [30], and rapidity of biogenes in soil and plant tissues [8]. In the undercover of pinewoods and mixed woods, the bilberry is a dominant species. In Vaccinio myrtilli-Pinetum, it reaches ecological optimum, which is the biggest frequency, density, biomass, production and intake surface of the leaves [30]. Nearly in all forest communities Vaccinium myrtillus returns large volumes of potassium, calcium, magnesium, manganese and iron to soil surface along with fallen leaves, which enriches the habitat with elements vital for adequate functioning of forest ecosystems. Vaccinium vitis-idaea populations, in comparison to other species of the kind, are characteristic of the highest phenological diversity [15] and only in some complexes they undergo annual, full developmental cycle. Vaccinium vitis-idaea most often appears in fresh coniferous forest (Vaccinio myrtilli-Pinetum), and the highest coverage ratio is reached in the coastal crowberry coniferous forest (Empetro nigri-Pinetum) [23].

The objectives of this paper are:
1. systematic measurement of concentration of nitrogen and phosphorous compounds (inorganic and total form) in leaves, stems and fruit of Vaccinium myrtillus and Vaccinium vitis-idaea in two different forest ecosystems of the Słowiński National Park in the period of 2002–2005,
2. comparison of variability concentration of N and P in the shoots of bilberry and cowberry in researched forest ecosystems,
3. comparison of their frequency, density and the volume of over-ground net primary production.

MATERIAL AND METHODS

Sampling sites
The research was held at the Słowiński National Park situated in northern Poland, in a region affected by the Baltic Sea. The research areas are situated 1.5 km from the coastal...
line of the Baltic Sea, along the road running from the Smołdziński Forest to Czołpino, at a distance of 600 m one from the other. Location of the sampling sites and their surroundings with more detailed characteristics in presented in Figure 1.

Fig. 1. Situation plan of the Słowiński National Park; locations of the study sites: I – research plot I (Vu-Bp), II – research plot II (En-P)

The research was held in two separate forest complexes of SNP: Vaccinio uliginosi-Betuletum pubescentis, Libbert 1933 and in Empetro nigri-Pinetum, (Libb. et Siss. 1939 n.n.) Wojt. 1964. Vaccinio uliginosi-Betuletum pubescentis (Vu-Bp) covers the Podzol Soil developed on the fossil peat soil, and Empetro nigri-Pinetum (En-P) covers poor barren Podzol Soil. The forest stand of the examined forest areas is diverse as to species and age. Vu-Bp is a loose pine and birch forest stand of 18–19 m in height. Contribution of the 60 year old common pine (Pinus sylvestris L.) to the forest stand is 25% (128 trees), and the 47 year old downy birch (Betula pubescens) 75% (392 trees). En-P with 715 trees is covered by a 140 the year old pine forest stand (Pinus sylvestris L.) with low (7 m) distorted canopies and inclined trunks [38, 40]. The Vu-Bp of forest stands is of natural origin, the En-P formation is the result of artificial afforestation. Choosing the researched plots the first were taken into consideration the natural resources among them the situation the level of ground water.

Sampling and analytical procedure
Measurement of frequency, density, biomass and net primary production (NPP) of the over-ground part of the Vaccinium vitis-idaea and Vaccinium myrtillus under-shrubs was performed in July and August 2005 applying the Traczyk’s method [51]. In the years 2002–2005, systematic measurement of concentration of nitrogen and phosphorous compounds in leaves, stems and berry fruit was performed. The plant samples were taken
from several sites of each research area; then, they were combined, taking into consideration the species diversity. Plant samples were taken several times during four vegetation seasons (2002–2005). After having transported them to the laboratory, the plant material was cleaned of the mineral particles of the soil, separated into individual species, leaves were separated from stems and fruit. After initial preparation, the plants were dried to a solid mass at the temperature of 65°C, which was then homogenized in a mill. T-N was determined by Kjedhal’s method after mineralization in the mixture of \( H_2SO_4 \) and \( H_2O_2 \) [11]. Organic carbon was determined by Alten’s method. N-NH\(_4^+\) and N-NO\(_3^-\) were determined spectrophotometrically (UV-VIS 1202, Shimadzu, Japan) with Nessler’s reagent and sodium salicylate, respectively. Both T-P and P-PO\(_4^{3-}\) were determined spectrophotometrically according to molybdate method with ascorbic acid as reducing agent (T-P after mineralization in the mixture of \( H_2SO_4 \) and \( H_2O_2 \)). Both inorganic nitrogen and phosphorus were determined after extraction with 1% \( K_2SO_4 \) [33]. The limit of detection and quantification of the method depend on the purity of the reagents used. In the case of spectrophotometric determinations the calibration solutions were prepared basing on Merck standards with the nominal PO\(_{4}^{3-}\), NO\(_3^-\) and NH\(_4^+\) concentrations as follows: 1002 ± 5 mg/dm\(^3\), 1004 ± 2 mg/dm\(^3\) and 1001 ± 2 mg/dm\(^3\). The limit of quantification for PO\(_{4}^{3-}\), NO\(_3^-\) and NH\(_4^+\) was 0.01 mg/dm\(^3\), 0.02 mg/dm\(^3\) and 0.005 mg/dm\(^3\), respectively. The QA/QC ratio was carried out by analyzing field samples fortified with the analytes of interest. The repeatability of methodology calculated according to the formulas presented by Konieczka et al. [26] reached RSD(PO\(_{4}^{3-}\)) = 1.4%, RSD(NO\(_3^-\)) = 1.6%, RSD(NH\(_4^+\)) = 1.4%, RSD(T-N) = 2.1% and RSD(T-P) = 1.8%.

**Other variables (soil-related)**

In order to assess possible relation between trophic state of investigated habitats and soil properties focused on total nitrogen and phosphorus abundance in the Vaccinio uliginosi-Betuletum pubescentis and Empetro nigri-Pinetum were investigated as well (Tab. 1). Among possible soil layers only rooting zones rich in nutrients (AEes in Vu-Bp and En-P) were evaluated in this study.

<table>
<thead>
<tr>
<th>Soil horizon</th>
<th>Vaccinio uliginosi-Betuletum pubescentis</th>
<th>Empetro nigri-Pinetum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O (Ol + Ofh)</td>
<td>AEes</td>
</tr>
<tr>
<td>Depth [cm]</td>
<td>8–0</td>
<td>0–13</td>
</tr>
<tr>
<td>N [%]</td>
<td>1.06</td>
<td>0.06</td>
</tr>
<tr>
<td>P [%]</td>
<td>0.111</td>
<td>0.007</td>
</tr>
<tr>
<td>C [%]</td>
<td>27.1</td>
<td>0.95</td>
</tr>
</tbody>
</table>


**RESULTS AND DISCUSSION**

The examined forest ecosystems of SNP are characteristic of uneven distribution of examined species of berries on the forest floor. The under-shrubs of bilberry (Vaccinium
*Vaccinium myrtillus* have the highest frequency (43%) in the marshy birch wood (*Vu-Bp*), reaching a density of 52.7 sprouts per 1 m² and the highest individual increase of 1.015 g (Tab. 2). The net primary production (NPP) of *Vaccinium myrtillus* under-shrubs in *Vu-Bp* constituted as much as 534.905 kg/ha (48.68% of the whole production of the ground cover). *Vaccinium myrtillus* is characteristic of a slightly lower participation in the crowberry coniferous forest (*En-P*), in which it reaches the frequency of 23%, which constitutes 37.8 sprouts per 1 m². In this complex, it reaches significantly lower individual increase (0.573 g), net primary production (216.594 kg/ha), having only 19.21% participation in the over-ground production of the ground cover. The under-shrubs of the red bilberry (*Vaccinium vitis-idaea*) have slightly different distribution of frequency in the coastal crowberry coniferous forest (*En-P*), reaching the frequency of 54% and density of 57.3 sprouts per 1 m². The high frequency does not reflect the size of individual increase. *Vaccinium vitis-idaea* in the pine coniferous forest complex (*En-P*) reaches only 0.271 g of average increase and 155.283 kg/ha of net primary production, which constitutes 19.21% of the over-ground production of the ground cover. The research results confirm a thesis that *Vaccinium vitis-idaea* has the highest cover ratio in the coastal crowberry coniferous forest (*Empetro nigri-Pinetum*), [23]. The under-shrubs of the red bilberry are characteristic of a visibly lower frequency in the complex of marshy birch coniferous forest (*Vu-Bp*). Their frequency is maintained at the level of 32%, which constitutes 30.6 sprouts per 1 m². Much better development conditions in *Vaccinio uliginosi-Betuletum pubescentis* make that species reach much higher individual increase (0.370 g) than in *Empetro nigri-Pinetum*. The total net primary production (113.220 kg/ha) is, however, lower than in a pine coniferous forest (*En-P*) due to the lower frequency and density of that species (Tab. 2). The plants of the layer of the ground cover of the pine coniferous forest (*En-P*) reached density of 232.2 sprouts per 1 m² of the surface area [35], which is characteristic of dry pine forests [3].

<table>
<thead>
<tr>
<th>Forest association</th>
<th>Species</th>
<th>Frequency [%]</th>
<th>Density of the plants, D [m²] [%]</th>
<th>Number of shoot</th>
<th>Average individual growth [Gi], [g]</th>
<th>Net productivity [kg/ha] [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vu-Bp</em></td>
<td><em>Vaccinium myrtillus</em></td>
<td>43</td>
<td>52.7</td>
<td>527</td>
<td>1.015</td>
<td>534.905 [48.68]</td>
</tr>
<tr>
<td></td>
<td><em>Vaccinium vitis-idaea</em></td>
<td>32</td>
<td>30.6</td>
<td>306</td>
<td>0.370</td>
<td>113.220 [10.39]</td>
</tr>
<tr>
<td><em>En-P</em></td>
<td><em>Vaccinium myrtillus</em></td>
<td>23</td>
<td>37.8</td>
<td>378</td>
<td>0.573</td>
<td>216.594 [26.70]</td>
</tr>
<tr>
<td></td>
<td><em>Vaccinium vitis-idaea</em></td>
<td>54</td>
<td>57.3</td>
<td>573</td>
<td>0.271</td>
<td>155.283 [19.21]</td>
</tr>
</tbody>
</table>

The trophic status [16], weather conditions and density as well as frequency of the berry species have big impact on the production yield of the forest ground cover. Relatively low rainfall (579 mm) was a negative factor, which had substantial impact on the limitation of production of ground cover of the examined ecosystems. Substantial lowering of the level of underground water during the vegetation season of 2005 had negative impact on the development and growth of plant cover at SNP, and in consequence on...
productivity of berries [37]. The level of underground waters during intensive period of
growth of the plants (from June to September) remained on average at the level of -91.3

cm in Vaccinio uliginosi-Betuletum pubescentis and -112.7 cm in Empetro nigri-Pinetum,
therefore being inaccessible for the examined under-shrubs under consideration [35].

According to the research done by Kimsa [23], Vaccinium vitis-idaea reaches the
highest production of biomass in a mixed forest Pino Quercetum (2151.6 kg/ha), and the
lowest in TC (0.2 kg/ha). The research done by Gerdol [18] indicates that NPP Vaccinium
myrtillus in Hyperico richeri-Vaccinietum remains at the level of 2670 kg/ha and 70 kg/ha
in Empetrum Vaccinietum. The volume of over-ground net primary production of Vac-
cinium vitis-idaea and Vaccinium myrtillus under-shrubs reflects, among others, average
volume of ions absorbable by the plants [1].

An average content of examined biogenes in the leaves of bilberry (Vaccinium myr-
tillus) at the area of the SNP was 1.311% N, 0.102% P and 40.8% C in Vaccinio uligi-
nosi-Betuletum pubescentis (Tab. 3) and 1.159% N, 0.095% P and 38.7% C in Empetro
nigri-Pinetum. Higher concentration of N was found in the leaves, and that of P in stems
of Vaccinium myrtillus in both examined forest complexes. Bilberry stored in its stem
on average 1.023% N and 0.112% P in Vu-Bp and 0.914% N and 0.114% P in En-P. An
average nitrogen content in both species of the examined berries was substantially higher
in Vu-Bp than in En-P (Tab. 3), while average content of phosphorus and carbon showed
much smaller difference. Higher concentration of the above mentioned biogenes in the
berries of the complex of Vaccinio uliginosi-Betuletum pubescentis is the result of much
higher content of the above mentioned elements at top genetic levels of such soils (Tab. 1)
[35]. It confirms that the regularity of biogenes layout in plant tissues is closely depend-
ent on the soil richness. [32, 41]. In both forest complexes, the content of nitrogen was
the highest in the leaves of both species of berries (Tab. 3), and the lowest in stems in
the case of red bilberry, and in the fruits in the case of bilberry. Such distinctive relations
were not found as to the content of phosphorus. Only in the case of bilberry in both forest
complexes, its highest content was found in the stems, and the lowest in the fruits thereof.

The stems of the red bilberry contained the lowest volume of phosphorous compounds. A
slightly higher concentration of nitrogen (1.75–2.01% N) and phosphorus (0.096–0.153%

<table>
<thead>
<tr>
<th>Forest association</th>
<th>Species</th>
<th>N [%]</th>
<th>P [%]</th>
<th>C [%]</th>
<th>C:N</th>
<th>C:P</th>
<th>N:P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vu-Bp</td>
<td>Vaccinium vitis-idaea</td>
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P) in leaves of *Vaccinium myrtillus* was found by Gerdol [18] during research held in Italy than in the forest complexes of SNP. The plant growth is limited especially by concentration of nitrogen and phosphorus in the soil [9, 21, 35].

Concentration of nitrogen and phosphorus in leaves of the red bilberry (*Vaccinium vitis-idaea*) remained on average at the level of 1.083% N and 0.097% P in *Vaccinio uliginosi-Betuletum pubescentis* and 0.868% N and 0.085% P in *Empetro nigri-Pinetum*. An average concentration of examined biogenes in stems of the red bilberry was lower and remained at the level of 0.870% N and 0.083% P in *Vu-Bp* and 0.734% N and 0.077% P in *En-P* (Tab. 3). Concentration of nitrogen and phosphorus in fruits of the examined berries was higher in *Vu-Bp* complex than in *En-P*. In *Vaccinio uliginosi-Betuletum pubescentis* higher volumes of biogenes were found in *Vaccinium vitis-idaea*, and in *Empetro nigri-Pinetum* in *Vaccinium myrtillus*. The *Vaccinium vitis-idaea* under-shrubs showed slightly higher concentration of carbon in leaves (44.7% C) than in stem (40.2–42.4% C) in both examined forest complexes. The reverse situation was observed in the case of bilberry. Higher concentration of C as well as P in stems than in leaves both in *Vaccinio uliginosi-Betuletum pubescentis* and in *Empetro nigri-Pinetum* (Tab. 3) can be the result of the economic management of such biogenes. It protects the shrubs of the bilberry against the loss of such elements during the period of autumn leaves shedding. Such situation most frequently takes place in poor habitats such as pine coniferous forests.

The dynamics of concentration of N and P in sprouts of the examined berries shows positive correlation of those elements (Figs 2 and 3). The graphs below indicate, that variations of nitrogen and phosphorus during the four examined vegetation seasons are vitally interrelated, which can be confirmed by the values of the Pearsons correlation coefficient. Slightly lower values of R coefficient were reached in the shoots of the berries in *Vaccinio uliginosi-Betuletum pubescentis*. Literature confirms existence of positive correlation between N and P in plants. Positive, important correlation of N and P in the leaves of the trees was evidenced by Pugnaire [41], in shoots of moor grass (*Molinia caerulea*), by Güsewell [21] and Garten [17] in various fragments of plants.

An analysis of quantitative relations between particular elements in the under-shrubs of berries provides valuable information on their nutritional status. The N:P relation remained within the limits of 8.02–12.85 in *Vaccinium myrtillus* and 8.82–11.37 in *Vaccinium vitis-idaea*. In each of the analyzed cases, the N:P ratio was each time higher in leaves than in stems of the berries in examined forest complexes (Tab. 3). According to Zhiguo

![Fig. 2. Relationships between concentration of nitrogen and phosphorus in shoots *Vaccinium vitis-idaea* in *Vu-Bp* and *En-P*, R – Pearson’s correlation coefficients, p < 0.05, n = 25, R_{crit} = 0.323](image-url)
et al., [56] the maximum growth of plants and maximum supply of nutrients occurs at the N:P relation approximating 9.5. Consequently, the value of the N:P ratio is characteristic for each species [50]. According to Güsewell [21] during the vegetation period it can have the values of 10–20. According to Koerselman and Meuleman [24] the N:P correlation > 16 in Vaccinium myrtillus leaves indicates substantial deficit of phosphorus.

The C:N correlation in leaves and stems of berries was between 31.12 and 54.77. However, higher values were reached in Empetro nigri-Pinetum than in Vaccinio uliginosi-Betuletum pubescentis. According to the data found in literature, the most frequent C:N correlation in plants is between about 5 in algae and over 100 in trees [43]. The values of the C:N correlation in the plant cover of the examined forest complexes at SNP are within the limits of the values provided in literature. As far as the C:P correlation is concerned, the scope of the values was much wider and was between 377.19–525.88 (Tab. 3). Enwezor [14] during the examination of various plants found the values of the C:P correlation within the limit from 112 to 501. This value in plants depends on concentration of the above mentioned elements in soil.

The plants during vegetation season show variable demand for mineral compound of nitrogen and phosphorus. The same species in different vegetation conditions absorbs nutritional ingredients in variable proportions [22]. This diversification is one of the reasons of variable concentration of ammonium nitrate and phosphorous ions in plants. Nitrogen and phosphorus occur in plant tissues mainly in the form of organic complexes, therefore concentration of mineral forms of nitrogen and phosphorus constitutes only minor part of general forms. Mineral forms of nitrogen and phosphorus absorbed by plants are transformed into complex organic compounds.

The concentration of N-NH$_4$ in Vaccinium vitis-idaea has average values from 35.33 mg/100 g dry mass in leaves (CV = 26.35%) to 21.02 mg/100 g d.m. in fruits (CV = 5.42%) (Tab. 4). Slightly higher concentration of those ions was found in Vaccinium myrtillus leaves and stems. Those values were between 56.74 mg/100 d.m. in En-P stems to 15.88 mg/100 g d.m. in fruit. The highest dynamics of concentration of ammonium ions was in Vaccinium myrtillus leaves (CV = 52.26% in Vu-Bp) and (CV = 47.18% in En-P). The concentration of nitrate ions in berry tissues was lower than the content of ammonium ions. The lowest concentration of N-NO$_3$ was found in Vaccinium myrtillus fruits in En-P (8.21 mg/100 g d.m., CV = 14.22%). The obtained values of N-NO$_3$ in examined plant cover are slightly lower from those found in tissues of Phalaris arundinacea (26–
The highest dynamics of N-NO$_3$ was also characteristic of bilberry leaves in both examined forest complexes. The dynamics of nitrate ions (Tab. 4) is closely connected with rainfalls which could have significantly washed out N-NO$_3$ from plant tissues [36]. The concentration of phosphorus ions was also maintained at average level with the values from 23.77 mg/100 g d.m. in leaves to 17.28 mg/100 g d.m. in stems of Vaccinium vitis-idaea and from 32.6 mg/100 g d.m. in leaves from Vaccinium myrtillus fruit.

The concentration of nitrogen and phosphorus in examined species of berries indicated variation during examined vegetation seasons. The largest concentration of nitrogen in leaves and stems of Vaccinium vitis-idaea was in summer during their maximum growth (Fig. 4). From August, a gradual decrease of concentration of that biogene both in leaves and stems of red bilberry in both forest complexes was observed.

The lowest volume of nitrogen in plant tissues was found in the autumn months, which is a consequence of lower demand of plants for nitrogen in that period. The largest concentration of phosphorus was found in sprouts of bilberry in June, both in Vu-Bp and in En-P, and then it gradually decreased by the end of vegetation season (Fig. 5). Similar concentration of nutrients in leaves during vegetation season was described by other researchers [10, 42, 44, 54]. Over both examined forest

<table>
<thead>
<tr>
<th>Forest association</th>
<th>Species</th>
<th>N-NO$_3$ (mg/100 g d.m.)</th>
<th>N-H$_4$ (mg/100 g d.m.)</th>
<th>P-PO$_4$ (mg/100 g d.m.)</th>
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</thead>
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<td>43.70</td>
<td>15.15</td>
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<td>22.50</td>
<td>22.20</td>
<td>1.76</td>
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areas, much lower concentration of P was found in the autumn season in leaves than in stems. Such status can be a consequence of retranslocation of this element from leaves to stems connected with storage of valuable biogenes in thicker parts of berries [30]. It is the evidence that the bilberry withdrawing biogenes before autumn dropping of leaves behaves as tall trees [31]. Among ions containing nitrogen N-NH\textsubscript{4} dominated in examined species of berries. Ammonium ions exceeded concentration of nitrates by many times (Tab. 5). Similar relations among mineral forms of nitrogen in plant cover were discovered by Andrews \textit{et al.} [2]. In berries of examined forest complexes, mineral forms of nitrogen exceeded phosphates.

CONCLUSIONS

Forest ecosystems at the Słowiński National Park are characteristic of uneven species of berries on the forest floor. Bilberry under-shrubs (\textit{Vaccinium myrtillus}) have the largest frequency (43\%), individual growth 1.015 g and NPP (534,91 kg/ha) in marshy coniferous birch forest (\textit{Vu-Bp}). Under-shrubs of the red bilberry (\textit{Vaccinium vitis-idaea}) had the highest frequency (54\%) at the coastal crowberry coniferous forest (\textit{En-P}), reaching at the same time with modest individual growth (0.271 g) much larger net primary pro-
duction (155.28 kg/ha) than in the marshy coniferous birch forest. Frequency, density, average individual growth and low atmospheric rainfall in 2005 (579 mm) had an impact on reduction of net primary production of the examined berries. The NPP volume of *Vaccinium vitis-idaea* and *Vaccinium myrtillus* is also a reflection of average concentration of compounds of nitrogen and phosphorus absorbable by plants. The concentration of nitrogen and phosphorus in leaves of red bilberry (*Vaccinium vitis-idaea*) was on average at the level of 1.083% N and 0.097% P in *Vaccinium uliginosus-Betuletum pubescentis* and 0.868% N and 0.085% P in *Empetrum nigri-Pinetum*. An average concentration of biog enes in stems of the red bilberry was slightly lower and remained at the level of 0.870% N and 0.083% P in *Vu-Bp* and 0.734% N and 0.077% P in *En-P*. The dynamics of nitrogen and phosphorus in sprouts of the examined species of berries showed positive correlation which can be evidenced by the levels of the correlation of Pearson’s coefficients. The concentration of nitrogen and phosphorus in the fruits of examined berries was larger in *Vu-Bp* complex than in *En-P*. The N:P relation remained between 8.02–12.85 in *Vaccinium myrtillus* and 8.82–11.37 in *Vaccinium vitis-idaea*. In examined forest complexes, each analyzed N:P correlation was each time higher in leaves than in stems of berries.

**REFERENCES**


ZMIENNOŚĆ KONCENTRACJI AZOTU I FOSFORU ORAZ PRODUKCJA PIERWOTNA NETTO VACCINIUM VITIS-IDAEA L. AND VACCINIUM MYRTILLUS L. W WYBRANYCH EKOSYSTEMACH LEŚNYCH SŁOWIŃSKIEGO PARKU NARODOWEGO

Vaccinium uliginosi-Betuletum pubescentis i Empetro nigri-Pinetum charakteryzują się zróżnicowaną frekwencją i zagęszczeniem Vaccinium vitis-idaea L. i Vaccinium myrtillus L. na dnie lasu. Badane krzewinki borówek wykazują różnice w zawartości związków azotowych i fosforowych oraz wielkości nadziemnej produkcji pierwotnej netto. Średnia zawartość badanych biogenów w liściach borówki czarnej (Vaccinium myrtillus) na...
terenie Słowińskiego Parku Narodowego wynosiła 1,311% N, 0,102% P i 40,8% C w *Vaccinio uliginosi-Betuletum pubescentis* oraz 1,159% N, 0,095% P i 38,7% C w *Empetro nigri-Pinetum*. Zawartość azotu, fosforu i węgla w liściach borówki brusznicy (*Vaccinium vitis-idaea*) wynosiła: 1,083% N, 0,097% P i 44,70% C w *Vaccinio uliginosi-Betuletum pubescentis* oraz 0,868% N, 0,085% P i 44,70% C w *Empetro nigri-Pinetum*. Zmienna koncentracja azotu i fosforu w pędach badanych gatunków borówek wykazuje dodatnią korelację tych pierwiastków. Nadziemna produkcja pierwotna netto *Vaccinium myrtillus* wynosi 534,905 kg/ha w *Vaccinio uliginosi-Betuletum pubescentis* oraz 216,594 kg/ha w nadmorskim borze bażynowym. *Vaccinium vitis-idaea* w *Empetro nigri-Pinetum* osiąga 155,283 kg/ha produkcji pierwotnej netto oraz jedynie 113,220 kg/ha w *Vaccinio uliginosi-Betuletum pubescentis*. 

AGNIESZKA PARZYCH, ZBIGNIEW SOBISZ, JAN TROJANOWSKI