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FRACTIONAL COMPOSITION OF DUST EMITTED FROM BOILERS WITH CIRCULATING FLUIDIZED BEDS

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Keywords: Dust, fraction, emission, hard coal, combustion, CFB.

Abstract: The paper presents investigations of dust fractional composition concerning combustion of hard coal in circulating fluidized bed boilers (CFBs). Experimentally determined emission factors for such boilers, and also typical pulverized-fuel furnaces, furnaces with mechanical stoker and domestic furnaces are presented. They all have been obtained for Upper Silesian type of coal. Total dust and characteristic fractions: PM2.5 and PM10 are covered.

INTRODUCTION

Combustion of hard and brown coals for energy production is in Poland, aside of making use of liquid fuels in car engines, the greatest source of gases and dusts emission to the atmosphere. Polish statistics of fuels utilization explains this phenomenon quite well: hard and brown coals make up 62.3%, oil and natural gas 32.8% of the power resources. The renewable energy share in the total energy produced is only 0.2% [3]. Production of energy by combusting coal in various furnaces yields annual emission of about 270 thousands Mg of dust, what makes 61% of 443 thousands Mg of total dust emitted per year [3].

Especially harmful are small, local heating systems and also, in great cities districts, domestic furnaces combusting hard coal, built-up with old houses at the beginning of the 20th century. They cause considerable concentrations of PM10, i.e. dust particulates having equivalent diameters up to 10 µm. Due to the PM10 adverse effects, relatively low permissible annual value (40 µg/m³) was established for its concentration in atmospheric air. The actual annual concentrations of PM10 in some cities are higher, for instance in the Upper Silesian agglomeration 51.3 µg/m³ and in the Krakow agglomeration 67.5 µg/m³ [3].
Ecological effects of atmospheric dust significantly depend on its granular composition – share of grains with various diameters. Most hazardous to humans are fractions (grains of equivalent diameter belonging to a defined range) less than 5 µm with special importance of those not greater than 2 µm, due to its ability to deposit in alveoli and infiltrate into the vascular system (in practical use, as a specimen of all these fractions, PM2.5 is considered). For this reason the granular composition is commonly taken into account while assessing air quality and environmental effects of emission sources. In the USA, for instance, data on concentrations of PM2.5 from a monitoring network are used to determine the Air Quality Index (AQI). The knowledge of dust composition is needed in designing de-dusting and gas emission reducing installations, modernizations of power and heat producing utilities, defining the Best Available Techniques for large combustion plants and assessing emissions of compounds located on dust surface [9]. Also air pollutant propagation models demand data on the granular composition and physical properties of dust to assess its time of floating in the air and range of transport of particular fractions.

Concentrations of PM2.5 are measured in some Polish agglomerations along with overall dust concentration. In circumstances of lacking standard for this fraction, the value of 25 µg/m³ is assumed as a desirable limit for PM2.5 concentration in ambient air. This value is not ever fulfilled. In Zabrze (population of 200 000) in the Silesian Agglomeration – the area environmentally degraded by intensive, lasting over 150 years mining, coking, steel and energy production – the annual PM2.5 concentration in 2005 was about 41 µg/m³ and PM2.5 mean winter value was about 57 µg/m³. Also high daily PM2.5 concentrations, reaching 188 µg/m³, were observed. The American AQI computed for Zabrze falls between 151 and 200, meaning conditions harmful to human health (unhealthy) [5].

In practice, while assessing the hazard to the biosphere from dust by investigating emissions, standard PM10 and PM2.5 dust emission factors determined for unclean flue gas, are commonly used. Then the total de-dusting efficiency is considered to calculate final dust emission. Modern de-dusting devices working in big power plants usually maintain concentration of dust in flue gases below relatively low level of 50 mg/m³. However, due to their greater efficiency in removing coarse fractions, share of fine dust in cleaned gas is much greater than in gas before de-dusting. Consequently, actual concentration of PM10 and PM2.5 dusts in emitted gases remains unknown, what makes applicability of unabated emission factors useless for such calculations [2, 4, 10, 13].

The authors have assessed emissions from the combustion of coal in energy production for years. Among others, their works resulted with experimentally determined emission factors for estimating amount of dust introduced into atmosphere during combustion of Upper Silesian hard coal in typical pulverized-fuel boilers used in power industry without wet desulphurization but equipped with high-efficient electrofilters, furnaces with mechanical stoker and typical cyclones used in municipal heating systems and individual domestic furnaces. Brand and quality parameters of the coal were properly selected for each kind of furnace to go along with local statistics of fuel consumption. They are applicable in determinations of emission of total dust, PM10 and PM2.5 [6, 8, 14].
Emission from the fluidized furnaces is less known than emission from conventional ones. The fluidized furnaces are promising as they may be applied in energy production on municipal, industrial or commercial scale and fuelled with various kinds of coal, slurry, banded coal and recycled wastes [12]. The fluidized bed combustion technique has been developed from combustion under atmospheric pressure (Atmospheric Fluidized Bed Combustion, AFBC); through pressurized circulating fluidized bed (Pressurized Fluidized Bed Combustion, PFBC) till combustion in binary circulating bed (Multi-Solid Fluidized Bed, MSFB). Also, technologies of coal and wastes combustion in fluidized bed with internal circulation of granular matter (Twin Interchanging Fluidized Bed, TIFB) or with heat exchangers immersed in a fluidized bed (Internally Circulating Fluidized Bed, ICFB) were developed. Combustion in a circulating bed, in presence of alkaline additive, at relatively low temperature and with highly-efficient de-dusting devices allows for meeting very high ecological demands [6].

The paper presents results of fractional composition determination of dust emitted from circulating fluidized bed boilers (CFB). Measurements were performed in 4 power stations; results from only 2, fuelled with Upper Silesian hard coal for energetic purposes, are presented here (the other 2 plants are fuelled with coal and slurry mixed in rather random proportion).

In the paper, the word “dust” stands for solid residuals of fuel combustion, products of reactions between additive and sulphur dioxide, chlorides and fluorides, particles of unutilized additive and components of the bed itself mixed all together in the flue gas leaving de-dusting system.

INVESTIGATED OBJECTS

The fractions of emitted dust were measured in power plants equipped with circulating fluidized bed (CFB) boilers where, to lower SO$_2$ emission, the lime-stone additive was applied.

TYCHY Power Plant (Elektrociepłownia Tychy S.A., PP. Tychy):
- Boiler type – CFB (Aker Kvaerner ASA),
- Boiler capacity – 70 MW$_t$, 40 MW$_e$,
- Steam rated output – 135 Mg/h,
- Boiler brand – CFB Cymic,
- Started working – 2000,
- Average flue gas flow during measurements – 157730 m$^3$/h,
- Average concentration of dust in emitted flue gas – 26.5 mg/m$^3$,
- Density of dust (captured by the electrofilter) – 2.5551 kg/dm$^3$.
Coal parameters are presented in Table 1.
Table 1. Parameters of coal used in PP. Tychy

<table>
<thead>
<tr>
<th>Coal parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorific value</td>
<td>[KJ/kg]</td>
<td>19915</td>
</tr>
<tr>
<td>Moisture content</td>
<td>[%]</td>
<td>10.51</td>
</tr>
<tr>
<td>Sulphur content</td>
<td>[%]</td>
<td>0.92</td>
</tr>
<tr>
<td>Ash content</td>
<td>[%]</td>
<td>24.78</td>
</tr>
<tr>
<td>Volatile matter</td>
<td>[%]</td>
<td>28.91</td>
</tr>
</tbody>
</table>

ELCHO Chorzów Power Plant Ltd. (Elektrociepłownia Chorzów ELCHO Sp. z o.o., PP. Elcho):
- Boiler type – 2 × CFB (Foster-Wheeler),
- Boiler capacity – 274 MWt,
- Steam output – 404 Mg/h,
- Live steam pressure – 13.5 MPa,
- Live steam temperature – 811 K,
- Boiler brand – CFB Compact,
- Started working – 2003,
- Average flue gas flow during measurements – 420340 m$^3$/h,
- Average concentration of dust in emitted flue gas – 21.9 mg/m$^3$,
- Density of dust (captured by the electrofilter) – 2.6456 kg/dm$^3$.

Coal parameters are presented in Table 2.

Table 2. Parameters of coal used in PP. Elcho

<table>
<thead>
<tr>
<th>Coal parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorific value</td>
<td>[KJ/kg]</td>
<td>19775</td>
</tr>
<tr>
<td>Moisture content</td>
<td>[%]</td>
<td>14.90</td>
</tr>
<tr>
<td>Sulphur content</td>
<td>[%]</td>
<td>1.22</td>
</tr>
<tr>
<td>Ash content</td>
<td>[%]</td>
<td>14.90</td>
</tr>
<tr>
<td>Volatile matter</td>
<td>[%]</td>
<td>25.08</td>
</tr>
</tbody>
</table>

METHOD OF MEASUREMENT

Dust total concentration in flue gas and its total emission were determined according to the Polish Norm [11]. A gravimetric semiautomatic meter P-10 ZA with a pressure balance-type probe and exchangeable nozzles was used. The apparatus configuration allows for multipoint isokinetic sampling, independent of actual variations of flue gas velocity at a sampling point.

The fractional composition of dust was measured with the P-10 ZA meter with the seven-stage cascade impactor MARK III mounted in a place of the probe, according to the impactor operating manual [1]. Each sampling was preceded by measurements of velocity, temperature and static pressure of the flue gas stream. The velocity and pressure were measured according to [11] by using a Prandtl probe. Humidity of the flue gas was also determined.

During fractional composition measurements, a single, representative of the whole investigated cross-section sampling point was selected and proper gas stream gauge in the meter, ensuring isokinecity of sampling, was computed and maintained. The volume of flue gas drawn through the sampler was calculated from the time of sampling and flow.
The time of sampling, determined experimentally before aspiration for each object, varied from 180 to 220 minutes depending on flow velocity and dust content in flue gas.

DEVELOPMENT OF RESULTS

From the size of the nozzle used, temperature and volume of flue gas passed through the device, the cutoff diameters were determined for the impactor stages. The characteristics provided by the device producer were applied [1]. The cutoff diameter for an impactor stage is defined as the equivalent diameter of particles, of which 50% are arrested at this stage along with all greater ones; all smaller grains pass further to the next impactor stage. Dust particles of equivalent diameter falling in the range between cutoff diameter of current and preceding impactor stage create, for particular measurement, one fraction.

After sampling the fractional dust composition was determined, as the fractional proportion (FP), expressing mass contributions of particular fractions in total collected dust, and also cumulated mass (CM), as a sum of FPs of this fraction and all finer ones.

The cutoff diameters for impactor stages depend on gas flow velocity at a place and in time of sampling. Due to differences in gas velocity assuring isokinetic sampling, these diameters differ for various objects of investigations and often even for consecutive measurements at the same object. Comparison of fractional dust composition between various objects demands defining uniform diameter intervals for particular fractions, to refer results of all measurements to. Four such intervals were defined for the purposes of the presented work: diameters less than 1.0 µm, diameters between 1.0 and 2.5 µm, diameters between 2.5 and 10.0 µm, diameters greater than 10.0 µm. Values of FPs and CMs obtained in particular measurements were interpolated for these intervals. The sequence of computations for the first considered object, PP. Tychy, is presented as an example in Tables 3 and 4.

### Table 3. Measured fractional composition of dust, PP. Tychy

<table>
<thead>
<tr>
<th>Impactor stage</th>
<th>Diameter interval [µm]</th>
<th>Weight of fraction [mg]</th>
<th>FP&lt;sup&gt;a)&lt;/sup&gt;</th>
<th>CM&lt;sup&gt;b)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.52–1.26</td>
<td>2.18</td>
<td>0.0805</td>
<td>0.0805</td>
</tr>
<tr>
<td>6</td>
<td>1.26–2.53</td>
<td>9.90</td>
<td>0.3654</td>
<td>0.6452</td>
</tr>
<tr>
<td>5</td>
<td>2.53–4.00</td>
<td>5.90</td>
<td>0.2178</td>
<td>0.8630</td>
</tr>
<tr>
<td>4</td>
<td>4.00–8.50</td>
<td>2.57</td>
<td>0.0949</td>
<td>0.9579</td>
</tr>
<tr>
<td>3</td>
<td>8.50–13.70</td>
<td>0.66</td>
<td>0.0244</td>
<td>0.9823</td>
</tr>
<tr>
<td>1</td>
<td>&gt; 13.70</td>
<td>0.48</td>
<td>0.0177</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

<sup>a)</sup> – fractional proportion, <sup>b)</sup> – cumulated mass

### Table 4. Uniform fractional composition of dust, PP. Tychy

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Diameter interval [µm]</th>
<th>FP&lt;sup&gt;a)&lt;/sup&gt;</th>
<th>CM&lt;sup&gt;b)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>&lt; 1.0</td>
<td>0.2098</td>
<td>0.2098</td>
</tr>
<tr>
<td>II</td>
<td>1.0–2.5</td>
<td>0.4269</td>
<td>0.6366</td>
</tr>
<tr>
<td>III</td>
<td>2.5–10.0</td>
<td>0.3283</td>
<td>0.9649</td>
</tr>
<tr>
<td>IV</td>
<td>&gt; 10.0</td>
<td>0.0352</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

<sup>a)</sup> – fractional proportion, <sup>b)</sup> – cumulated mass
Ultimate results of measurements of fractional dust composition for the two investigated plants, related to the uniform fractions, are presented in Table 5.

Table 5. Fractional uniform dust composition for investigated plants

<table>
<thead>
<tr>
<th>Power station</th>
<th>Diameter interval [μm]</th>
<th>FP (^a)</th>
<th>CM (^b)</th>
<th>FP (^a)</th>
<th>CM (^b)</th>
<th>FP (^a)</th>
<th>CM (^b)</th>
<th>FP (^a)</th>
<th>CM (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP. Tychy</td>
<td>&lt; 1.0</td>
<td>0.2098</td>
<td>0.2098</td>
<td>0.4269</td>
<td>0.6366</td>
<td>0.3283</td>
<td>0.9649</td>
<td>0.0351</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>1.0–2.5</td>
<td></td>
<td></td>
<td>0.4103</td>
<td>0.5805</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5–10.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3693</td>
<td>0.9498</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 10.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0502</td>
<td>1.0000</td>
</tr>
<tr>
<td>PP. Elcho</td>
<td>≤ 1,0</td>
<td>0.1702</td>
<td>0.1702</td>
<td>0.4103</td>
<td>0.5805</td>
<td>0.3693</td>
<td>0.9498</td>
<td>0.0502</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>1.0–2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5–10.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 10.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(a\) – fractional proportion, \(b\) – cumulated mass

Measured, uniform fractional compositions of dust are presented in the chart shown in Figure 1.

Fig. 1. Fractional uniform composition of dust for investigated plants

Along with measurements of the fractional composition of dust, the total dust emission from both plants was measured in a standard way. The proper CMs were superimposed on dust emissions, and the cumulated fractional emissions received were related to the flux of burned fuel. This yielded the cumulated emission factor for each dust fraction, expressed as a mass of this fraction and all finer ones emitted during combusting of a unitary mass of fuel, kg/Mg. They have been presented in Table 6.

Table 6. Emission factors for investigated CFBs

<table>
<thead>
<tr>
<th>Power station</th>
<th>Diameter interval [μm]</th>
<th>Total dust</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 1,0</td>
<td>≤ 2,5</td>
</tr>
<tr>
<td></td>
<td>Cumulated emission factors for dust fraction, kg/Mg</td>
<td></td>
</tr>
<tr>
<td>PP. Tychy</td>
<td>0.035</td>
<td>0.107</td>
</tr>
<tr>
<td>PP. Elcho</td>
<td>0.028</td>
<td>0.095</td>
</tr>
</tbody>
</table>
The cumulated emission of particular dust fractions, related to the unit of burned fuel, is presented in a form of chart – Figure 2.

![Graph showing the cumulated emission of particular dust fractions](image)

**Fig. 2.** The cumulated emission of particular dust fractions, related to the unit of burned fuel

### CONCLUSION

The results for the two boilers, concerning the fractional composition of the emitted dust and cumulated factors of its emission were similar. The factors for total dust emission are 0.164 kg/Mg for PP. Tychy and 0.167 kg/Mg for PP. Elcho (0.166 kg/Mg in average). The PM2.5 factors are 0.095 and 0.107 kg/Mg, respectively (0.101 kg/Mg in average) and the PM10 factors – 0.156 and 0.161 kg/Mg (0.159 kg/Mg).

The mass share of PM10 in total emission of dust is 95.0% in PP. Tychy and 96.5% in PP. Elcho, the share of PM2.5 is 58.0 and 63.7%, respectively. The PM1 mass shares differ relatively more and are 17.0 and 21.0% for PP. Tychy and PP. Elcho, respectively.

The efficiency of de-dusting systems is high in both plants; concentration of dust in flue gases after electrofilters does not exceed 26.5 mg/m$^3$. Share of coarse fractions, with particle diameters greater than 10 μm, is small due to their almost total elimination by the electrofilters. Relatively small amounts of respirable dust are also emitted: considerably smaller than from boilers with pulverized fuel furnaces applied in typical power plants and tens times smaller than from boilers with mechanic grate furnaces.

The PM2.5 emission factor for the fluidized bed boilers is 0.101 kg/Mg (on average), for pulverized fuel boilers – 0.34 kg/Mg and 2.97 kg/Mg for mechanic grate boilers (Tab. 7). For power plants applying wet methods of de-sulphurization of flue gas this factor is even lower. The factors determined in the presented work contribute to earlier knowledge on dust emission factors [7, 8].
Table 7. Emission factors for various boilers types

<table>
<thead>
<tr>
<th>Furnace</th>
<th>Emission factors [kg/Mg]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total dust</td>
</tr>
<tr>
<td>Pulverized fuel fed</td>
<td>0.82</td>
</tr>
<tr>
<td>Mechanical stocker</td>
<td>9.0</td>
</tr>
<tr>
<td>Domestic stove</td>
<td>5.4</td>
</tr>
<tr>
<td>CFB</td>
<td>0.166</td>
</tr>
</tbody>
</table>

In this work the Authors have used the results of investigations conducted within the scope of research project number 4 T10b O6525 Characteristics of Emissions of Dust and Gaseous Substances Causing Contamination in the Process of Coal Burning in Fluidized Beds, financed by the Ministry of Science and Higher Education (Poland)

REFERENCES


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W artykule przedstawiono wyniki badań nad składem frakcyjnym pyłu pochodzącego ze spalania węgla kamiennego w kotłach z fluidalnymi złożami cyrkulacyjnymi (CFB). Dla kotłów tego typu, a także dla typowych kotłów pyłowych, kotłów z rusztem mechanicznym i palenisk domowych określono w drodze pomiarowej wskaźniki emisji pyłu całkowitego oraz charakterystycznych frakcji PM2,5 i PM10. Wskaźniki dotyczą spalania górnoshląskiego węgla kamiennego.
AIR QUALITY INDEX AND ITS SIGNIFICANCE IN ENVIRONMENTAL HEALTH RISK COMMUNICATION

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Keywords: Air quality index, daily mortality, environmental epidemiology.

Abstract: Air Quality Index (AQI) is a standardized summary measure of ambient air quality used to express the level of health risk related to particulate and gaseous air pollution. The index, first introduced by US EPA in 1998 classified ambient air quality according to concentrations of such principal air pollutants as PM10, PM2.5, ozone, SO2, NO2 and CO. Subsequently similar, index-based approach to express health risk was developed in France, Great Britain and Germany. No such environmental warning system exists in Poland, although some test-trials took place in Katowice area and the city of Gdańsk. However, the operational value of AQI under environmental circumstances in Poland remains unknown. The aim of the study was to examine current air pollution levels in Katowice area and to confront AQI categories with local air quality, also in terms of health impact on the population as expressed by daily total and specific mortality. The data on daily average PM10 and sulphur dioxide concentrations available in regional network (PIOŚ in Katowice) and data on daily number of total deaths and deaths due to cardiorespiratory diseases from the Central Statistical Office in Warsaw were collected. The data covered the period 2001–2002. The percentage of days with individual Air Quality Index, created by American, French, British and German method of indexation was calculated. Then, the relationship between values of air quality indexes and daily total and specific mortality according to Spearman correlation coefficients was assessed. Finally, the obtained results were verified according to ANOVA Kruskal-Wallis test. The obtained results suggest significant discrepancy in the range of air quality categories depending on applied system of classification. Percentage of days with “unhealthy” air quality (in the period 2001–2002) was running from 0.1% (American method of indexation) to 11.2% (British method) and usually referred to winter season. Statistically significant Spearman correlation coefficients were obtained for the relationship between air quality and total number of deaths, as well as the number of deaths due to cardiovascular and respiratory diseases in elderly population (aged 65 and more). The observed values of correlation coefficients are very low and do not exceed value 0.2 for each chosen method of indexation.

BACKGROUND

The impact of ambient air pollution on human health has been subject to many epidemiological investigations performed worldwide and targeting morbidity and mortality,
both total and specific mostly due to respiratory and circulatory diseases. The accumulated epidemiologic evidence provided scientific background for regular assessment of air pollution-related health risk, summarized in the reports published under the auspices of World Health Organization [15]. A wide spectrum of health risk estimates and their presentation are commonly used by public health professionals; however their meaning is less clear to the public, more and more interested in environmental health hazards. A need to communicate the results and inform the public about potential health impacts of the measured and/or projected ambient air pollution levels prompted an effort to develop easily understood information, of every-day use by the public, including administrative authorities [6, 13]. The concept was addressed as early as in 1970 at the European level and was followed by initiatives taken by the Environmental Protection Agency in the USA in 1998 [2, 5]. As a result summary index, known as the Air Quality Index (AQI) was introduced in order to express an increasing health risk to the public in response to increasing ambient air pollution, on a daily basis. The construction of AQI allows for distinction between “good” and “bad” air quality.

The Air Quality Index developed in the USA is based on the combined effects of five criteria pollutants: suspended particulate matter – aerodynamic diameter below 10 and 2.5 μm (PM_{10}, PM_{2.5}), sulphur dioxide (SO_2), carbon monoxide (CO), ozone (O_3) and nitrogen dioxide (NO_2) [1]. Concentrations of the pollutants are recorded by automatic air monitoring stations permitting prompt data analysis and transformation of the readings into the AQI scale. The range of AQI values includes seven categories grouped into “good”, “moderate” and “dangerous” air quality zones [1]. The “good” air quality zone is defined if the AQI is in the range of 0 to 50. The “moderate” air quality zone is defined if the AQI is between 51 and 100. The “dangerous” air quality zone is defined if the AQI exceeds 100. A similar three-level approach has been adopted in the European countries. Table 1 shows the cut-off values for the specific air quality zones used in France, Great Britain and Germany, compared to the US standards [12, 18–19]. In addition to the between-country differences in the decisive cut-off values of air pollution the country-specific AQIs differ in the internal composition, as shown in Table 2 [12, 18–19]. Moreover, in each country the AQI is calculated on the daily basis.

The presentation of AQIs implies their practical application, both in environmental health risk communication to the public (via media) and as an evidence-based support for preventive measures. Because of its simplicity the AQI serves as a convenient early warning tool. No such environmental warning system exists in Poland, although some test-trials took place in Katowice area and the city of Gdańsk [4, 16]. However, the operational value of AQI under environmental circumstances in Poland remains unknown. Because of poor ambient air quality in our country it is essential to examine the potential for AQI application and to explore the system’s functioning given ambient air quality in Poland. For a number of reasons relevant findings could be provided by a pilot study implemented under the “worst case scenario”, in terms of ambient air pollution. Hence, the aim of the study was to examine current air pollution levels in Katowice area and to confront local air quality with AQI categories, also in terms of health impact on the population as expressed by daily total and specific (cardiovascular and respiratory) mortality.
MATERIAL AND METHODS

Data concerning ambient air pollution, such as particulate matter PM10 and sulphur dioxide, and meteorological conditions were obtained from regional network providing on-line measurements by the State Environmental Agency in Silesia voivodeship. There were calculated as 24-hour area averages and applied to measurements in 14 regional stations. Mortality data (total and specific mortality) were obtained from the register at the Central Statistical Office in Warsaw. The records were analyzed according to the classification scheme of the International Classification of Diseases – 10th Edition (ICD-10) [11] and included the number of daily deaths in population living in the urban area of Katowice from January 01 to December 31 in the period 2001–2002. Daily mortality was arranged in three categories: all deaths, deaths due to cardiovascular causes (ICD-10 codes: I00-I99) and deaths due to respiratory causes (ICD-10 codes: J00-J99). Moreover, analysis was taken for the two aged groups: inhabitants aged 0–64 years and aged 65 and more. The percentage of days with selected AQI categories was calculated according to available method (American, French, British and German), but established way of indexation refers to only 24-hourly PM10 concentrations. Next, mean value of daily number of deaths characteristic for days with particular categories quality of air, expressed by 33th and 66th percentile of PM10 concentration or specific AQI value was calculated. The association between air quality and daily number of deaths was calculated by ANOVA Kruskal-Wallis procedure. Moreover, the relationship between daily number of deaths and value of AQI was estimated by means of Spearman correlation analysis. Finally, the obtained results were verified by ANOVA Kruskal-Wallis procedure. Interpretation of statistical significance of the results was based on the criterion p < 0.05. Statistica 7.1 statistical software was used for all calculations.

RESULTS

Values of daily particulate matter (PM10) and sulphur dioxide (SO2) concentrations measured in the urban area of Katowice, in the study period (year 2001–2002), are presented in Table 3. A mean value was below the acceptable limit value for both sorts of pollution, but maximum concentrations observed exceeded the established norms only in winter time.

Percentage of days with selected value of AQI calculated for PM10 concentrations, defined such as “dangerous air quality”, depends on chosen indexation method and amounted from 0.1% (American method – AQI in the range of 4–7) and 6.1% (German – AQI 6, and French method – AQI in the range of 8–10) to over 11% (British method – AQI in the range of 7–10) and usually concerned winter season.

The observed number of total deaths, deaths due to cardiovascular and respiratory diseases, their average values and number of deaths during days with low, medium or high concentration of particulate matter (PM10) are presented in the Table 4. Most of deaths (about 47% out of 39 222 cases) concerned deaths due to cardiovascular diseases, and most of them appeared in the older population (people aged 65 year or more). Deaths due to respiratory diseases represent nearly 4.1% of the total mortality in the study period, but it is significant that most of them (78.2%) concerned the elderly. Moreover, it was observed that the number of total deaths and deaths due to cardiorespiratory diseases in total and old population depends on a chosen level of PM concentration, defined by the values
Table 1. Comparison of the definition of three categories of ambient air quality (“good”, “moderate”, “dangerous”) used in Air Quality Indices in the USA, France, Great Britain and Germany – example for 24 hour PM10 concentration

<table>
<thead>
<tr>
<th>Category of air quality</th>
<th>Cut-off values for daily PM10 concentration [μg/m³]</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>France</td>
</tr>
<tr>
<td>Good</td>
<td>0–54</td>
</tr>
<tr>
<td>Moderate</td>
<td>55–154</td>
</tr>
<tr>
<td>Dangerous</td>
<td>155 and more</td>
</tr>
</tbody>
</table>

Table 2. Composition of Air Quality Index in selected countries

<table>
<thead>
<tr>
<th>AQI</th>
<th>USA</th>
<th>France</th>
<th>Great Britain</th>
<th>Germany</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>good air quality</td>
<td>good air quality</td>
<td>good air quality</td>
<td>good air quality</td>
</tr>
<tr>
<td>2</td>
<td>moderate air quality</td>
<td>good air quality</td>
<td>good air quality</td>
<td>good air quality</td>
</tr>
<tr>
<td>3</td>
<td>moderate air quality</td>
<td>good air quality</td>
<td>good air quality</td>
<td>good air quality</td>
</tr>
<tr>
<td>4</td>
<td>dangerous air quality</td>
<td>good air quality</td>
<td>moderate air quality</td>
<td>moderate air quality</td>
</tr>
<tr>
<td>5</td>
<td>dangerous air quality</td>
<td>moderate air quality</td>
<td>moderate air quality</td>
<td>moderate air quality</td>
</tr>
<tr>
<td>6</td>
<td>dangerous air quality</td>
<td>moderate air quality</td>
<td>good air quality</td>
<td>dangerous air quality</td>
</tr>
<tr>
<td>7</td>
<td>dangerous air quality</td>
<td>moderate air quality</td>
<td>dangerous air quality</td>
<td>dangerous air quality</td>
</tr>
<tr>
<td>8</td>
<td>dangerous air quality</td>
<td>dangerous air quality</td>
<td>dangerous air quality</td>
<td>dangerous air quality</td>
</tr>
<tr>
<td>9</td>
<td>dangerous air quality</td>
<td>dangerous air quality</td>
<td>dangerous air quality</td>
<td>dangerous air quality</td>
</tr>
<tr>
<td>10</td>
<td>dangerous air quality</td>
<td>dangerous air quality</td>
<td>dangerous air quality</td>
<td>dangerous air quality</td>
</tr>
</tbody>
</table>

Table 3. Daily means concentrations of PM10 and SO$_2$ in ambient air in urban area of Katowice, in the period 2001–2002

<table>
<thead>
<tr>
<th>Sulphur dioxide SO$_2$ [μg/m$^3$]</th>
<th>Particulate matter PM10 [μg/m$^3$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value ± SD</td>
<td>35.21 ± 24.18</td>
</tr>
<tr>
<td>Median</td>
<td>27.10</td>
</tr>
<tr>
<td>Minimum</td>
<td>10.50</td>
</tr>
<tr>
<td>Maximum</td>
<td>239.80</td>
</tr>
<tr>
<td>33.3 percentile</td>
<td>21.90</td>
</tr>
<tr>
<td>66.6 percentile</td>
<td>36.50</td>
</tr>
</tbody>
</table>

Table 4. Total and daily number of deaths in the Urban Area of Katowice, in the study period 2001–2002

<table>
<thead>
<tr>
<th>Deaths due to</th>
<th>Age</th>
<th>Total number of deaths</th>
<th>Average ± SD</th>
<th>Number of deaths during days with selected concentrations of PM10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>low (≤ 31,8 μg/m$^3$)</td>
</tr>
<tr>
<td>Cardiovascular diseases</td>
<td>0–64 years</td>
<td>4391</td>
<td>6.0 ± 2.4</td>
<td>6.2 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>65 and more</td>
<td>14065</td>
<td>19.2 ± 4.8</td>
<td>18.7 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>18456</td>
<td>25.2 ± 5.5</td>
<td>24.9 ± 5.3</td>
</tr>
<tr>
<td>Respiratory diseases</td>
<td>0–64 years</td>
<td>348</td>
<td>0.4 ± 0.7</td>
<td>0.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>65 and more</td>
<td>1246</td>
<td>1.7 ± 1.3</td>
<td>1.5 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>1594</td>
<td>2.1 ± 1.6</td>
<td>1.9 ± 1.4</td>
</tr>
<tr>
<td>Total</td>
<td>0–64 years</td>
<td>14220</td>
<td>19.4 ± 4.7</td>
<td>19.4 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>65 and more</td>
<td>25002</td>
<td>34.2 ± 6.3</td>
<td>33.1 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>39222</td>
<td>53.7 ± 8.2</td>
<td>52.6 ± 8.3</td>
</tr>
</tbody>
</table>
Table 5. Total and daily number of deaths in the Urban Area of Katowice, in the study period 2001–2002

<table>
<thead>
<tr>
<th>Deaths due to</th>
<th>Age</th>
<th>Great Britain</th>
<th>France</th>
<th>USA</th>
<th>Germany</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g</td>
<td>m</td>
<td>d</td>
<td>g</td>
</tr>
<tr>
<td>Cardio-vascular diseases</td>
<td>&lt; 65</td>
<td>6.0 ± 2.4</td>
<td>5.7 ± 2.3</td>
<td>6.3 ± 2.8</td>
<td>6.0 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>18.8 ± 4.6</td>
<td>19.9 ± 4.7</td>
<td>20.6 ± 5.3</td>
<td>18.8 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>24.8 ± 5.4</td>
<td>25.6 ± 5.3</td>
<td>26.9 ± 6.1</td>
<td>24.8 ± 5.4</td>
</tr>
<tr>
<td>Respiratory diseases</td>
<td>&lt; 65</td>
<td>0.4 ± 0.7</td>
<td>0.5 ± 0.8</td>
<td>0.6 ± 0.8</td>
<td>0.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>1.6 ± 1.3</td>
<td>1.7 ± 1.4</td>
<td>2.0 ± 1.5</td>
<td>1.6 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>2.1 ± 1.6</td>
<td>2.3 ± 1.5</td>
<td>2.6 ± 1.7</td>
<td>2.0 ± 1.6</td>
</tr>
<tr>
<td>Total</td>
<td>&lt; 65</td>
<td>19.4 ± 4.7</td>
<td>19.5 ± 4.8</td>
<td>19.7 ± 4.5</td>
<td>19.3 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>33.6 ± 6.2</td>
<td>34.9 ± 6.3</td>
<td>36.0 ± 6.4</td>
<td>33.4 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>53.0 ± 8.3</td>
<td>54.5 ± 8.0</td>
<td>55.9 ± 7.9</td>
<td>52.7 ± 8.5</td>
</tr>
</tbody>
</table>

*Table 5. Total and daily number of deaths in the Urban Area of Katowice, in the study period 2001–2002*

_The table shows the number of deaths due to cardiovascular and respiratory diseases, categorized by age group, in Great Britain, France, USA, and Germany, during days with selected AQI values (mean value ± SD). The AQI is categorized into three levels: g – good, m – moderate, d – dangerous._
Table 6. Spearman correlation coefficients for relationship between daily count of deaths and air quality index by different methods of indexation, p value in brackets

<table>
<thead>
<tr>
<th>Mortality</th>
<th>Population aged</th>
<th>AQI Great Britain</th>
<th>AQI France</th>
<th>AQI USA</th>
<th>AQI Germany</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiorespiratory diseases</td>
<td>0–64</td>
<td>0.00 (NS)</td>
<td>0.01 (NS)</td>
<td>0.04 (NS)</td>
<td>0.00 (NS)</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>0.15 (p &lt; 0.05)</td>
<td>0.15 (p &lt; 0.05)</td>
<td>0.16 (p &lt; 0.05)</td>
<td>0.14 (p &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>0.13 (p &lt; 0.05)</td>
<td>0.13 (p &lt; 0.05)</td>
<td>0.16 (p &lt; 0.05)</td>
<td>0.12 (p &lt; 0.05)</td>
</tr>
<tr>
<td>Cardiovascular diseases</td>
<td>0–64</td>
<td>-0.02 (NS)</td>
<td>-0.01 (NS)</td>
<td>0.03 (NS)</td>
<td>-0.01 (NS)</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>0.13 (p &lt; 0.05)</td>
<td>0.14 (p &lt; 0.05)</td>
<td>0.14 (p &lt; 0.05)</td>
<td>0.13 (p &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>0.11 (p &lt; 0.05)</td>
<td>0.11 (p &lt; 0.05)</td>
<td>0.14 (p &lt; 0.05)</td>
<td>0.10 (p &lt; 0.05)</td>
</tr>
<tr>
<td>Respiratory diseases</td>
<td>0–64</td>
<td>0.07 (NS)</td>
<td>0.08 (NS)</td>
<td>0.08 (NS)</td>
<td>0.07 (NS)</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>0.10 (p &lt; 0.05)</td>
<td>0.11 (p &lt; 0.05)</td>
<td>0.10 (p &lt; 0.05)</td>
<td>0.10 (p &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>0.12 (p &lt; 0.05)</td>
<td>0.13 (p &lt; 0.05)</td>
<td>0.13 (p &lt; 0.05)</td>
<td>0.12 (p &lt; 0.05)</td>
</tr>
<tr>
<td>Total number of deaths</td>
<td>0–64</td>
<td>0.03 (NS)</td>
<td>0.03 (NS)</td>
<td>0.04 (NS)</td>
<td>0.04 (NS)</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>0.17 (p &lt; 0.05)</td>
<td>0.17 (p &lt; 0.05)</td>
<td>0.13 (p &lt; 0.05)</td>
<td>0.16 (p &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>0.15 (p &lt; 0.05)</td>
<td>0.15 (p &lt; 0.05)</td>
<td>0.11 (p &lt; 0.05)</td>
<td>0.14 (p &lt; 0.05)</td>
</tr>
</tbody>
</table>

NS – not statistically significant

Table 7. Results of ANOVA Kruskal-Wallis and median procedure (p values) in relationship between AQI and daily mortality, urban area of Katowice in the period 2001–2002

<table>
<thead>
<tr>
<th>Mortality</th>
<th>Population aged</th>
<th>AQI Great Britain</th>
<th>AQI France</th>
<th>AQI USA</th>
<th>AQI Germany</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiorespiratory diseases</td>
<td>0–64</td>
<td>0.0009</td>
<td>0.0009</td>
<td>0.0005</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>0.01</td>
<td>0.007</td>
<td>0.0007</td>
<td>0.001</td>
</tr>
<tr>
<td>Total number of deaths</td>
<td>total</td>
<td>0.004</td>
<td>0.02</td>
<td>0.004</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>0.001</td>
<td>0.0009</td>
<td>0.0005</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

K-W – Kruskal-Wallis procedure, M – median test

of 33.33 and 66.66 percentile. The highest mortality concerned days with high level of air pollution, exceeding the concentration of 49.3 μg/m³.

Moreover, mean value was calculated of daily total and specific mortality characteristic for days with particular AQI defined as: good, moderate and dangerous category of air quality, determined by particular methods of indexation. It was observed that the highest mortality concerned the days with dangerous quality of air. Detailed results are presented in Table 5. The association between mortality and quality of air was similar for German, British and French method of indexation, but finally the obtained results confirm that the highest mortality concerned the days with dangerous quality of air, and the lowest concerned the days with good quality of air. The observed variability was statistically significant in each of AQI categories.

The relationship between daily number of deaths, separately for total and specific mortality, and value of particular AQI was estimated by means of Spearman correlation analysis. The obtained results are presented in Table 6 and confirm existence of statistic-
cally significant correlation for each chosen air quality index and daily count of deaths in total and elderly population, although values of correlation coefficients were below 0.20. It was observed that particular values of correlation coefficient were similar in each applied method of AQI indexation. It was documented that the higher level of AQI is associated with the increase of daily mortality.

Finally, the obtained results were verified by ANOVA Kruskal-Wallis procedure and additionally by median test. Table 7 presents particularly data (statistically significant expressed by “p” value) in both tests for separate air quality indexes. The results suggest that the difference between medians of compared groups is not statistically significant only in population aged 0–64 years.

DISCUSSION

The obtained results suggest disagreement on the range of air quality according to selected classification method. Percentage of days with “unhealthy” air quality (in the period 2001–2002) was between 0.1% (American method of indexation) and 11.2% (British method). The frequencies of days with air quality dangerous for health (PM10 concentrations) calculated by French and German AQI were similar and amounted nearly 6%. Moreover, it was observed that the highest number of daily mortality was characteristic for days with the highest level of PM10 concentration expressed by percentile value of PM10 concentration or by AQI value. The association between mortality and quality of air was similar for German, British and French method of indexation, the course of relationship for American AQI was quite different, but finally the highest mortality was assigned to dangerous air quality. We noted statistically significant relationship between daily number of total deaths and deaths due to cardiovascular/respiratory diseases and air quality index in the elderly (population aged 65+ years) and in total population. These results confirm the fact that older people are the most sensitive group of population in environmental health and our data are comparable with well-known published data [3, 8–10, 14].

According to poor ambient air quality in Silesia region, especially during winter time, it is essential to inform inhabitants about environmental health hazard. Confrontation data of local air quality with AQI categories and with daily total and specific (cardiovascular and respiratory) mortality confirm the fact that British and French methods of AQI indexation are the best way to inform about risk in Poland. Probably, similar climate conditions and specific of air pollution are comparable in all described countries, so the association between air quality index and health effect is similar too.

Despite existence of very interesting handbooks prepared for older people [7, 17] the information is not clear enough and available to protect a person with cardiovascular or respiratory problems from undesirable health effects. It is necessary to disclose the knowledge about air quality index and its association with health effect. Very important source of this information are medical doctors, especially general practitioners. Moreover, well known websites or regional TV channels are very useful sources to transmit important information about environmental health risk.
REFERENCES


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INDEKS JAKOŚCI POWIETRZA I JEGO ZNACZENIE DLA KOMUNIKOWANIA ŚRODOWISKOWEGO RYZYKA ZDROWOTNEGO

Indeks jakości powietrza (AQI) jest wskaźnikiem określającym jakość powietrza atmosferycznego i jednocześnie wskazującym potencjalne ryzyko zdrowotne ponoszone przez populację wskutek narażenia na standardowo mierzone stężenia zanieczyszczeń pyłowych i gazowych w danym regionie. Po raz pierwszy został użyty przez US EPA w 1998 r. i klasyfikował jakość powietrza atmosferycznego w oparciu o stężenia podstawowych zanieczyszczeń: PM_{10}, PM_{2,5}, ozonu, SO_{2}, NO_{x} oraz CO. Podobne wskaźniki, oparte na danych regionalnych opracowano również we Francji, Wielkiej Brytanii i Niemczech. Właściwie w naszym kraju nie funkcjonuje spójny system komunikowania ryzyka zdrowotnego, który byłby oparty na własnym indeksie jakości powietrza, chociaż pewne próby podejmowane są w Katowicach i Gdańsku. Celem prezentowanej pracy była ocena jakości powietrza atmosferycznego w Katowicach na podstawie przyjętych kategorii AQI oraz porównanie uzyskanych danych z danymi opisującymi potencjalne ryzyko zdrowotne wyrażone w postaci dobowej umieralności całkowitej lub specyficznej. Zebrano dane dotyczące średnich dobowych stężeń pyłu PM10 oraz dwutlenku siarki dostępne w ramach regionalnego monitoringu środowiska (PIOŚ w Kato-

Uzyskane wyniki sugerują występowanie istotnego zróżnicowania w zakresie kategorii jakości powietrza atmosferycznego, zależnie od przyjętego sposobu klasyfikacji. Procent dni z tzw. „niezdrową” jakością powietrza kształtował się w badanym okresie (2001–2002) w zakresie od 0,1% (amerykański sposób indeksowania) do 11,2% (brytyjski sposób indeksowania) i zazwyczaj kategoria dotyczyła okresu zimy. Statystycznie znamienne wartości współczynników korelacji Spearmana uzyskano jedynie dla zależności pomiędzy jakością powietrza a dobową liczbą zgonów ogółem oraz zgonów z powodu chorób układu oddechowego i krążenia w grupie osób po 65 roku życia. Jednakże zaobserwowane wartości współczynników były niewielkie i nie przekraczały wartości 0,2 dla każdej z przyjętych metod klasyfikacji.
DISTRIBUTION OF POLYPORUS FUNGI IN CZAPLINEK
(DRAWSKO LAKE DISTRICT, NORTH-WESTERN POLAND)

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Keywords: Polyporus fungus, Czaplinek.

Abstract: Research on the occurrence of polyporus fungi in Czaplinek were conducted in 2004 and 2005 in 6 designated regions, using the route method (area search). 363 sites containing polyporus fungi belonging to 24 species and 4 families – Coriolaceae, Ganodermataceae, Hymenochaeteceae and Polyporaceae – have been found. The most numerous among the species were Bjerkandera adusta (Wills. Fr.) P. Karst. (87 sites) and Ganoderma applanatum (Pers.) Pat. (66 sites). Three species listed on the Red List of makrofungi in Poland (Datronia mollis (Sommer. Fr.) Donk, Inonotus hispidus (Bull. Fr.) P. Karst. and Ganoderma lucidum (W. Curt. Fr.) P. Karst.) have been identified. It should be noted that G. lucidum is one of the fungi under strict legal protection.

INTRODUCTION

Polyporus fungi are one of well known types of fungi decomposing wood. They belong to Basidiomycetes class. They often produce imposing fruit bodies and play an important role in forests as pathogens of living trees and decomposers of dead wood [22]. Nowadays 222 species of these fungi are known in Poland [32]. Many of them, for example: Meripilus giganteus (Pers. Fr.) P. Karst., or Grifola frondosa (Dicks. ex Fr.) S.F. Gray are under protection by forest regulation [32]. Some of them, for example: Datronia mollis (Sommer. Fr.) Donk, Inonotus hispidus (Bull. Fr.) P. Karst. are on the Red List of makrofungi endangered in Poland [33].

Urban environment is essentially different from the forest phytocoenoses. Average air temperature in cities is a few degrees higher than outside, which is connected with accumulation of heat by walls of buildings, and higher air pollution caused by gases and dust [31]. There are significant fluctuations of temperature and air humidity is lower than in forests, which is not profitable for spore sprouting and fungi growing. Windiness, similarly to forests, is not strong because buildings slow down wind velocity [6]. However, in cities there are phytocoenoses (parks, gardens, cemeteries, recreational areas) with different species of trees and bushes that can be hosts of polyporus fungi.
Polyporus fungi can accumulate great concentrations of mercury and other toxic metallic elements and metalloids, such as cadmium, lead or arsenic and radionuclides [4, 5]. Compared to green plants, fungi can build up large concentration [2]. This would suggest that fungi have effective mechanism which allows them to take out toxic substances from substrate. This mechanism may be more effective in parasitic and saprotrophic fungi than in another group of fungi [24, 30].

Fungi are used to estimate heavy metals accumulation. They can be bioindicator of heavy metals accumulation in environment [26].

The research presented in this study was conducted in Czaplinek where there are two complexes of allotments, a cemetery, and two parks with different species of trees which can be hosts to fungi. The aim of this study was to analyze the occurrence of polyporus fungi in the Czaplinek area, especially, to analyze species composition and the frequency their occurrence.

CHARACTERISTICS OF THE RESEARCH AREA

Czaplinek is situated in the Drawskie Lake District (Pojezierze Drawskie), north-western Poland (symbol 314.45) which is a part of the Zachodniopomorskie Lake District (Zachodniopomorskie Pojezierze) macro region (symbol 314.4) [11].

The Czaplinek area is situated in the southern part of the West-Pomeranian Voivodship, in the area of Powiat Drawski. The terrain relief was shaped during Baltic glaciations [11, 29]. When it comes to climate the Drawskie Lake District belongs to Pomeranian district and is under the influence of the Atlantic Ocean and the Baltic Sea [16]. Diversified relief comes from various degrees of intensity of postglacial relief and genetic layout of groups of forms, as well as from terrain fragmentation by valleys into separated elevations, which cause diversification and differences of mesoclimate [7].
It is worth mentioning that the whole northern part of the Czaplinek district is a part of Drawsko and Insk Landscape Park [23].

**METHODOLOGY**

The research was carried out from July 2004 to October 2005 in Czaplinek in the 6 following regions:
- Region A – allotment garden area of Adam Mickiewicz;
- Region B – cemetery;
- Region C – shore of Czaplineo lake;
- Region D – shore of Drawsko lake within the town;
- Region E – allotment garden area within the town;
- Region F – streets of the town including the area of two parks.

Our observation was carried out by route method of walking around and taking notes of trees, stumps and branches on the ground. After a fruit body was found the site of its occurrence was marked as a test stand. The test stand was described by an identification of the host plant inhabited by the fungus and by a description of macroscopic features that helped to identify the fungi species. To make a herbarium and some microscopic tests fruit bodies or their fragments were collected according to Domański’s method [3]. The collected material was dried at room temperature and kept in paper bags. A photographic documentation of the test stands and fruitbodies’ natural environment was also made.

To determine fungi species the keys of Domański [3], Ryverden and Gilberstone [22], Ratusznia [19] were used. Species nomenclature was taken according to Ryverden and Gilberstone [22]. The results have been presented in tables and on charts below.

**RESULTS**

Within two years of observation 363 stands of polyporus fungi were found. Most of the stands were found in region F – 163 stands; in region D – 90, in region C – 56. The number of stands in the remaining regions (A, B and E) was similar: 17, 19 and 18 respectively.

On the registered stands 24 species of polyporus fungi belonging to 4 families were identified: *Coriolaceae*, *Ganodermataceae*, *Hymenochaetaceae* and *Polyporaceae*. The alphabetic lists of species and stands number are shown in Table 1. The greatest species variety was found in region F. Among 24 species generally identified region F contained as many as 18. Half of the identified species occurred in region D (11), and in the remaining regions – A, B, C and E: 7, 6, 9 and 7 respectively.

The most frequent species were: *Bjerkandera adusta* (Wills. Fr.) P. Karst. found on 87 stands, *Ganoderma applanatum* (Pers.) Pat. – on 66 and *Phellinus igniarius* (L. Fr.) Quel – on 51. The rarest species that occurred only on one of the stands were: *Daedaleopsis confragosa* (Bolt. Fr.) Schroet., *Piptoporus betulinus* (Bull. Fr.) P. Karst., *Inonotus hispidus* (Bull. Fr.) P. Karst. and *Daedalea quercina* (L. Fr.).

Three of the identified species occurred very rarely and can be found on the Red List of makrofungi endangered in Poland [33, 35]. These were: *Datronia mollis*, *Inonotus hispidus* (Bull. Fr.) P. Karst. and *Ganoderma lucidum* (W. Curt. Fr.) P. Karst. The last one also belongs to funguses under strict legal protection [21].
Table 1. Taxonomic specification of denoted species of polypore fungi with regard to their occurrence and number of sites

<table>
<thead>
<tr>
<th>FAMILY / Sort</th>
<th>Test stands number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Region A</td>
</tr>
<tr>
<td>Corylaceae</td>
<td></td>
</tr>
<tr>
<td>Bjerkandera adusta (Wills. Fr.) P. Karst.</td>
<td>1</td>
</tr>
<tr>
<td>Bjerkandera fumosa (L. Fr.)</td>
<td>–</td>
</tr>
<tr>
<td>Daedalea quercina (L. Fr.)</td>
<td>1</td>
</tr>
<tr>
<td>Daedaleopsis confragosa (Bolt. Fr.) Schroet.</td>
<td>–</td>
</tr>
<tr>
<td>Datronia mollis (Sommer. Fr.) Donk</td>
<td>–</td>
</tr>
<tr>
<td>Fomes fomentarius (L. Fr.) Krickx</td>
<td>–</td>
</tr>
<tr>
<td>Fomitopsis pinicola (Schwartz Fr.) P. Karst.</td>
<td>4</td>
</tr>
<tr>
<td>Gledophyllum sepiarium (Wulf. Fr.) P. Karst.</td>
<td>–</td>
</tr>
<tr>
<td>Heterobasidion annosum (Fr.) Bref.</td>
<td>–</td>
</tr>
<tr>
<td>Laetiporus sulphureus (Bull. Fr.) Murr.</td>
<td>1</td>
</tr>
<tr>
<td>Piptoporus betulinus (Bull. Fr.) P. Karst.</td>
<td>1</td>
</tr>
<tr>
<td>Trametes gibbosa (Pers.) Fr.</td>
<td>–</td>
</tr>
<tr>
<td>Trametes hirsuta (Fr.) Pilat</td>
<td>2</td>
</tr>
<tr>
<td>Trametes ochracea (Pers.) Gilb &amp; Ryvarden</td>
<td>–</td>
</tr>
<tr>
<td>Trametes pubescens (Schumach. Fr.) Pilat</td>
<td>–</td>
</tr>
<tr>
<td>Trametes versicolor (L. Fr.) Pilat</td>
<td>–</td>
</tr>
<tr>
<td>Gomonephelaceae</td>
<td></td>
</tr>
<tr>
<td>Ganoderma applanatum (Pers.) Pat.</td>
<td>–</td>
</tr>
<tr>
<td>Ganoderma lucidum (W. Curt. Fr.) P. Karst.</td>
<td>–</td>
</tr>
<tr>
<td>Polyporaceae</td>
<td></td>
</tr>
<tr>
<td>Polyporus varius (Fr.)</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
</tr>
</tbody>
</table>
DISCUSSION

Flora of urban environments is under strong anthropopressure, and the species composition of trees and bushes living there differs significantly from forest phytocenoses. Trees distribution and their density are also diversified, which is closely connected with a possibility of spreading infections. Infection efficiency by pathogenic fungi depends, to a large extent, on a plant’s surface because this is the first contact and further reaction place between a pathogen and a plant [8]. In the case of polyporus fungi, the age of the host is one great significance. Old trees are more susceptible to infection than younger ones [13].

Region B is an area of the communal cemetery. Polyporus fungi occur here only on stumps of cut down trees that are a perfect place of infection thanks to big surface of a cut. Living trees are tended by city service and places where branches were cut off are protected against fungi infections.

Region F is the one where the highest number of fungi stands was recorded. This region comprises mainly city parks with a great amount of old trees. Due to the closeness of lakes, great humidity as well as trees density it favors the spread of spores and infections.

The regions A and E are areas of allotments gardens and the density of fungi stands occurring there was similar. It was probably connected with the similar age of trees. Trees are tended by owners which mean that if pathological symptoms appeared, trees like these or branches were removed. That is why the number of stands was not great.

The regions C and D include lake shores and they are very similar as regards humidity, trees species and also inhabiting fungi. Trees growing in that vicinity are old and often damaged by wind and grow close to one another. The region D had more species of fungi and more numerous probably because it is a bigger area in comparison to the region C. The neighborhood of forests could also have had some influence as fungi spores could easily find the way to the urban area.

Research on the influence of urban environments and anthropopression on the occurrence of polyporus fungi were carried out sporadically. Adamczyk and Ławrynowicz [1] claim that first data on fungi appearing in urban areas go back to the second half of the 20\textsuperscript{th} century. The oldest information concerns cities such as Gdańsk, Jelenia Góra and Wrocław [1]. It only briefly mentions the occurrence in one of those cities of one or just a few fungi species.

Research on the occurrence of polyporus fungi in urban areas of Pomerania: in Gdynia, Starogard Gdański and Sztum were carried out by Milewska and Ratuszniak [14]. The number of registered stands in individual cities was 169, 195 and 107 respectively and the total number of 25 species of polyporus fungi was recorded.

In our own research the number of stands in Czaplinek (363) outnumbered the number of stands in each of the above mentioned cities but the number of the determined species was similar. The species composition of the fungi determined by the above authors and the species obtained in our own research are shown in Table 2. Among the species found in Czaplinek as many as 6 did not occur in any of the cities researched by Milewska and Ratuszniak; however, 6 species, from the ones determined by the above researchers, did not occur in Czaplinek.
It is worth pointing out that among species determined in Czaplinek, *Ganoderma lucidum* is the one which belongs to the list of fungi legally protected, and *Datronia mollis* is on the Red List of Makrofungi endangered in Poland. When it comes to the species not found in Czaplinek, all of them except *Trametes suaveolens*, were saprophytes or weak parasites of coniferous trees. In the research region of Czaplinek coniferous trees occur sporadically and probably that is the reason why fungi preferring such types of hosts were not found.

In years 2002 and 2003 in Słupsk Ratusznia found number of 160 stands, which belong to 20 species of polyporus fungi. Among fungi which didn’t appear in Czaplinek there were noted such species as: *Meripilus giganteus* (Pers. Fr.) P. Karst. – 5 stands, *Oxyporus populinus* (Schumach. Fr.) Donk, *Inonotus cuticularis* (Bull. Fr.) Karst. [20]. It is worth pointing out that the surface area of Słupsk is four times bigger than the surface area of Czaplinek and quantity of observed stands were almost three times less than in Czaplinek.

The influence of industrial pollution on the growth of fungi diseases spreading among trees and forest stands began to be researched in detail from the beginning of the 1970’s. The research involved trees diseases occurring in the closest neighborhood of industries emitting different gas and dust pollutants [10]. According to Norden [15] we know very little about how saprophyte fungi respond to changes in urban environment. Sokół and Piątek indicate synantrophication of fungi in cities [18, 27]. In urban environment many fungi change their ecological preference, e.g. they use another substance [28]. Sokół after Wojewoda presented the phenomenon of fruit bodies nanizm in cities and another urban area [28].

The town of Czaplinek is situated in the Drawski district in which, according to Voivodeship Inspectorate for Environmental Protection in Szczecin [12], pollutants and environmental dangers do not appear or they occur on a minimal scale. Probably for this reason fungi occurred in Czaplinek in years 2004 and 2005 were in typical size and appeared on their typical hosts. In 2002–2004 the Inspectorate carried out air quality research in which they studied the concentration of sulphur dioxide, nitrogen oxide and ozone. The research showed that all these pollutants did not exceed acceptable standards introduced by the Ministry of Environment in the decree from the 5th of December, 2002 on the reference values of substances in the air. Some increase of pollutants can be observed during heating season, which is caused by energy sector. In the town there are seven small factories and three boiler houses. They emit insignificant amounts of pollution. Some, however insignificant, concentration of nitrogen oxides can be observed in summer, which is generally connected with transport and heavier traffic in the tourist season. Transport causes emission of about 69% of lead, 66% of carbon oxides, 31% of ozone, 43% of carbon oxide and 20% of insoluble pollutants [19].

<table>
<thead>
<tr>
<th>Species found only in Czaplinek</th>
<th>Species not found in Czaplinek</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bjerkandera fumosa</em></td>
<td><em>Antrodia serialis</em> (Fr.) Donk</td>
</tr>
<tr>
<td><em>Daedalea quercina</em></td>
<td><em>Gleophyllum odoratum</em> (Fr.) Sing</td>
</tr>
<tr>
<td><em>Datronia mollis</em></td>
<td><em>Oligoporus stipticus</em> (Pers.) Gilb. &amp; Ryvarden</td>
</tr>
<tr>
<td><em>Ganoderma lucidum</em></td>
<td><em>Trametes suaveolens</em> (L. ex Fr.) Fr.</td>
</tr>
<tr>
<td><em>Polyporus varius</em></td>
<td><em>Trichaptum abietinum</em> (Pers. Fr.) Ryv.</td>
</tr>
</tbody>
</table>
CONCLUSIONS

1. Research carried out in 2004 and 2005 in 6 regions of the town of Czaplinek helped to locate 363 stands of polyporus fungi belonging to 24 species within the following families Coriolaceae, Ganodermataceae, Hymenochaetaceae and Polyporaceae.

2. The region F was the richest in species variety and the number of fruit bodies. 163 stands of funguses belonging to 18 species were located. The fewest fruit bodies were found in region A (17 stands with 7 species).

3. The most numerous species of polyporus fungi were: Bjerkandera adusta, found on 87 stands and Ganoderma applanatum – 66 stands. The least frequent were Daedalea quercina, Inonotus hispidus, Daedaleopsis confragosa and Piptoporus betulinus – each only on 1 stand.

4. Three species of protected fungi Datronia mollis, Inonotus hispidus and Ganoderma lucidum that are on the Red List of Makrofungi endangered in Poland were found. The latter is also on the list of fungi under strict legal protection.

5. Significant numbers of stands in the region E (near Drawsko Lake) is connected with high humidity, which favors fungi infection.

6. There data can suggest that polyporus fungi may be used as bioindicator.

![Czaplinek street map devised on research regions](image-url)

Fig. 2. Czaplinek street map devised on research regions
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ROLE OF RECYCLED SLUDGE AGE IN COAGULATION OF COLORED WATER

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Keywords: Optimum coagulant dose, recirculation, post-coagulation sludge.

Abstract: All multifunctional units combine flocculation and sedimentation to improve efficiency of treatment process. The characteristic feature of the unit is a contact of raw water with previously generated flocs which enhances flocculation by increasing interparticle collisions and sorption ability of flocs. On the basis of the authors’ experience it was stated that in spite of significant differences between the procedure of conventional treatment and treatment in multifunctional units, an optimum coagulant dose is determined in jar testing which is commonly used for conventional treatment. The influence of sludge recirculation is not taken into consideration resulting in reagent overdosing. In this paper the results of the research on coagulation with post-coagulation sludge recirculation in aspect of sludge age are presented. It was stated that for the tested water, sludge recirculation may result in significant lowering of optimum alum doses provided that a proper degree of fresh sludge recirculation is applied.

INTRODUCTION

In water treatment the process of coagulation is applied to remove colloidal dispersed minerals and natural organic substances in a dissolved or colloidal form. Organic impurities found in natural waters are mainly humic substances which cause yellow or brown color of the waters. These organics are undesirable by consumers not only because of aesthetic reasons. The most important is the fact that they negatively influence water quality in a distribution system. This influence may be direct, when organics while chlorinated may be the source of carcinogenic trihalomethans or indirect, when they decrease biological stability of water, being nutrients for microorganisms.

However, in waterworks practice most of water treatment plants are optimized mainly for the removal of impurities which cause turbidity, not for minimization of a required disinfectant dose [5, 13]. It is caused by the fact that in the majority of cases it is more difficult to decrease turbidity of water after rapid filters to the desired level (0.1–0.2 NTU), taking into account Cryptosporidium oocyst presence, than to lower concentration of organic matter measured as TOC (Total Organic Carbon) to a value 5 mg C/dm³. Usually technological parameters for required turbidity removal stated by the operators of a treatment system meet quality requirements in the aspect of organics content. However,
if organics’ contamination of water is considered as a source of DBP (Disinfection By-Products) on the one hand and a source of assimilable organic carbon (AOC) on the other, the concentration of organic matter in water after treatment may be still too high.

According to many authors variable effectiveness of turbidity and NOM (Natural Organic Matter) removal at a given configuration of flocculation parameters results from the fact that optimum technological conditions for the removal of turbidity are different from these for NOM [1–4, 6, 10, 15]. The basic parameters influencing the coagulation of humic substances are a coagulant dose and the pH at which treatment is carried out. Narkis and Rebhun [14] found that the presence of organic matter in solution or as a complex with the mineral clay particle inhibits the process of flocculation. So those to make flocculation possible, large doses of flocculant are required. According to a number of studies [7, 8, 12] the optimum pH for color removal with alum is noted to be in the range of 5–6.5. According to Kim et al. [13] turbidity and TOC removal regions mostly overlap. The difference is only in the restabilization region which is wider for turbidity removal than for TOC removal. Hence, it may be concluded that most TOC is removed together with turbidity.

The processes of coagulation and separation of post-coagulation solids may be run in various technological systems. If the system consists of a rapid mix tank, followed by a flocculator and then a sedimentation basin, a treatment process is called conventional coagulation. Conventional treatment plant requires a large area to construct tanks for mixing, flocculation and sedimentation resulting in high investment and exploitation costs of the system. So that, to make the process more effective, flocculation and sedimentation may be run in one device [9]. All multifunctional units combine flocculation and sedimentation to enhance efficiency of treatment process by using the recirculation of sludge. The role of sludge recirculation is to accelerate agglomeration of freshly produced flocs and to increase sorption capacity of flocculated sludge solids. One of crucial factors which decide about a proper coagulation course in this type of unit is a coagulant dose. However, no research on the influence of suspended sludge on treatment effectiveness has been done. A coagulant dose for coagulation in a multifunctional unit is determined in jar testing which is commonly used for conventional treatment. It means that a dose determined in this way is not optimal, because when using this method the influence of sludge is not taken into consideration.

This paper focuses on the possibility to enhance coagulation by post-coagulation sludge recirculation and to decrease an optimum coagulant dose in aspect of sludge age. The influence of recirculation degree on treatment effectiveness was also presented.

MATERIALS AND METHODS

Water sample
Water sample was prepared by mixing peat extract with tap water to simulate colored water sample. The peat was separated by utilizing differences in solubility at different pH values. A stock solution of humic substances was obtained by extraction with 0.1 N sodium hydroxide. After 1 week ageing the clarified solution of humic acids was collected. Test samples were prepared by diluting the stated volume of aquatic humic extract in 0.7 dm³ of tap water with the corresponding apparent color of 80–90 mg Pt/dm³ (true color of 70–80 mg Pt/dm³). Color was determined spectrophotometrically according to the pro-
ducer method. Because the results of color measurements after treatment in filtered and unfiltered samples were very close and the difference was not higher than 5%, it was decided to make measurements only in unfiltered samples, i.e. apparent color. Absorbance UV in unfiltered samples at the wavelength 254 nm (it is commonly used as an indicator of organic contaminants in water) was in the range of 21.76–24.86 m^{-1}. Absorbance UV_{254} measurements of samples filtered through 0.45 μm membrane filter were in the range of 84–90% of the values noted in unfiltered samples. It means that most of organic substances were in a dissolved form. Aluminium concentration in raw water did not exceed 0.2 mg Al/dm³ and was in the range from 0.15–0.17 mg Al/dm³, which is an acceptable value in drinking water. Turbidity didn’t exceed 1 NTU. So that, to prepare homogeneous sample while extract diluting it was mixed at 200 rpm for 120 s.

**Preparation of coagulant solution**

The stock solution of alum was prepared by dissolving Al₂(SO₄)₃·18H₂O in D.I. water to obtain 2% concentration. Coagulant doses reported in this study are presented in mg Al/dm³.

**Apparatus**

A Carl Zeiss UV/VIS spectrophotometer was used for all absorbance measurements. Measurements were carried out in a 20 mm quartz cuvette. Color was measured with a photometer Nanocolor (MECHERY NAGEL). The mixing device was a six-beaker Floculator SW1 (Stuart Scientific).

**JAR TESTS**

**Fresh sludge recirculation**

The research consisted of four stages. In the first stage jar tests were performed to evaluate an optimum coagulant dose. Rapid-mixing for 1 min at rotational speed of 200 rpm was followed by 30-min slow-mixing at 30 rpm. After settling for 30 min residual color, absorbance UV_{254}, turbidity and dissolved aluminium (residual coagulant) of the supernatant were measured. All experiments were carried out at pH = 6.9–7.2. The optimum coagulant dose was the lowest one which allowed to gain high treatment effectiveness and higher doses did not allow to achieve significant improvement of water quality.

In the second stage the optimum coagulant dose was injected into six beakers to produce sludge. After coagulation and settling at the operational parameters stated in the first stage of the study the supernatant was removed and the stable volume of sludge from each beaker was collected into one beaker. The collected sludge was immediately used in the third stage of the experiment.

In the third stage the treatment process was conducted at six coagulant doses as follows: 20%, 30%, 50%, 70%, 90% and 100% of the optimum dose stated in the first stage. After 1-min rapid-mixing the rotational speed was decreased from 200 to 60 rpm. At the lower speed, which was held for 2 min, the stated volume of fresh sludge (collected in the second stage) was injected into the first five beakers, where coagulant doses were lower than the optimum one. The purpose of speed lowering, while sludge mixing with water, was to prevent sludge flocs from breaking-up. The sixth beaker (with the optimum dose of coagulant) was a comparative one and no sludge was injected into it. After sludge
injection the speed was decreased again to 30 rpm and 30-min flocculation started. The sludge was added in the amount 20 cm$^3$ and 40 cm$^3$ in the first and second series, respectively. Taking into consideration the fact that water samples were 0.7 dm$^3$ the volume degree of recirculation was 2.9% and 5.7%. Similarly to the first stage of the study, after flocculation and settling for 30 min residual color, absorbance UV$^{254}$ and aluminium of supernatant were measured.

The fourth stage was a comparative one. At that stage conventional coagulation process and settling (rapid-mixing for 1 min at 200 rpm, 30-min slow-mixing at 30 rpm, settling for 30 min) was run with coagulant doses applied in the previous stage (20%, 30%, 50%, 70%, 90% and 100% of the optimum dose) but without sludge recirculation.

**Aged sludge recirculation**

The aim of the research was to study the influence of the age of post-coagulation sludge on treatment effectiveness. The studies were conducted according to the same method as applied in the series where fresh sludge was recycled. However, instead of fresh sludge, the sludge after 1-day or 6-day ageing was used.

**RESULTS AND DISCUSSION**

**Influence of recirculation degree on treatment effectiveness**

According to the stated method the testing was conducted for two different recirculation degrees: 2.9% and 5.7%. The optimum alum dose stated at the first stage was 3 mg Al/dm$^3$. The residual color at that dose was 20 mg Pt/dm$^3$ (75% of color removal). Residual UV absorbance was 9.21 m$^{-1}$ (57.7% removal). The results of treatment effectiveness in these series are presented in Figures 1–4. In the analyses residual color and absorbance changes were taken into consideration. The results show that sludge recirculation causes distinct decrease in the optimum alum dose. When 20 cm$^3$ of sludge (2.9% recirculation degree) was added with the coagulant dose of 1.5 mg Al/dm$^3$ absorbance removal was 57.2% with corresponding residual color 25 mg Pt/dm$^3$ (Figs. 1 and 2.). This effectiveness was similar to the effectiveness of treatment achieved in the stage without sludge recirculation but at the dose twice higher, i.e. 3 mg Al/dm$^3$. The results of treatment without sludge recirculation at the dose of 1.5 mg Al/dm$^3$ were much worse in comparison to the process with sludge addition. Absorbance was decreased only by 22%; residual color was noted at 60 mg Pt/dm$^3$. With increase of alum dose the better effects of coagulation with sludge recirculation were observed. At 70% of the optimum coagulant dose the quality of treated water with sludge addition was even better (67.3% of absorbance removal, 15 mg Pt/dm$^3$ of residual color) than the quality achieved in conventional treatment at the optimum dose.

The increase in the amount of recycled sludge to 40 cm$^3$ (5.7% recirculation degree) caused more significant effects on the contaminants removal (Figs. 3 and 4). The results indicate that sludge recirculation enhances coagulation and treatment is more effective than in conventional coagulation, even if the alum dose is much lower than the optimum one. Figures 3 and 4 show that at 30% of the optimum dose i.e. 0.9 mg Al/ dm$^3$ and sludge injection the effectiveness of organic matter removal (59.8% of absorbance removal, 20 mg Pt/dm$^3$ of residual color) was similar to the results of conventional coagulation (stage 1) but at a much higher dose (3 mg Al/dm$^3$). When a dose of 0.9 mg Al/dm$^3$ was applied
conventional coagulation was totally ineffective. The residual color was at the same value as in raw water. The increase of a coagulant dose to 1.5 mg Al/dm$^3$ during coagulation with sludge injection resulted in 65.9% of absorbance removal and 15 mg Pt/dm$^3$ of residual color. At the same alum dose coagulation without sludge recirculation resulted only in 22% of absorbance removal and residual color 60 mg Pt/dm$^3$. Taking into consid-
etration the conventional coagulation effectiveness at the dose of 3 mg Al/dm$^3$ it may be concluded that the sludge addition may not only allow to decrease an alum dose but may also improve water quality after treatment in comparison to the effects of conventional coagulation at the optimum process parameters. In the tested range of coagulant doses, besides the lowest one, absorbance UV$_{254}$ values in filtered samples were 47.3–49.5% and 53.2–66.7% of values noted for unfiltered samples for conventional coagulation and with

![Fig. 3. Effectiveness of coagulation with recirculation of 40 cm$^3$ of fresh sludge (absorbance removal)](image1)

![Fig. 4. Effectiveness of coagulation with recirculation of 40 cm$^3$ of fresh sludge (residual color)](image2)
sludge recirculation, respectively. At 30% of the optimum alum dose without sludge injection real values were 21.15 m$^{-1}$ and 10 m$^{-1}$ in unfiltered and filtered water. When sludge was added 9.28 m$^{-1}$ and 4.44 m$^{-1}$ values respectively, were measured. In this case sludge addition improved sorption ability and enhanced agglomeration of generated flocs. At higher alum doses both in samples collected after coagulation with and without sludge recirculation values of absorbance in filtered samples were close. It means that at this range of doses for the tested water sludge recirculation mainly improves flocs agglomeration and this way accelerates solids separation in sedimentation process. In all samples where alum doses were not below 30% of the optimum one the concentration of residual coagulant was very low and did not exceed the value of 0.2 mg Al/dm$^3$. Thus the aluminium concentration resulted from added coagulant was only 0.03–0.05 mg Al/dm$^3$.

Fresh sludge injection during flocculation may improve the process effectiveness and decrease coagulant dose, even three times. However, the condition of such effects is that treatment is run with proper recirculation degree. For the tested water such effects were achieved at 5.7% recycle ratio. On the other hand, the effectiveness is strictly connected with raw water quality and the treatment results are not always so significant [11]. The purposefulness of recirculation should always be proceeded by research.

**Influence of sludge age on treatment effectiveness**

The studies of the influence of post-coagulation sludge age on treatment effectiveness were made for 5.7% recirculation degree. At this degree, in the series when fresh sludge was applied, the best effects were achieved. In Figures 5 and 6 the results for 1-day-old sludge are presented. The optimum coagulant dose was stated at the same value as in the previous series, i.e. 3 mg Al/dm$^3$, but the quality of water after treatment was better (63% of absorbance removal and 15 mg Pt/dm$^3$ of residual color). The probable reason of different results achieved in both series at the same coagulant dose may be explained by changes of tap water parameters not being under control. The analysis of the results for 1-day sludge indicates that there is significant deterioration in treatment effectiveness when aged sludge is injected. It was impossible to produce water of better quality than in conventional coagulation at the optimum coagulant dose. Though, it was noticeable that at coagulant doses, lower than optimal one, sludge addition enhanced treatment in comparison to conventional coagulation run at the same reagent doses. For example, at the dose of 1.5 mg Pt/dm$^3$ (50% of the optimum dose) in conventional coagulation no changes of absorbance after treatment were noted, while at the same dose with 5.7% sludge recirculation 19.3% decrease of absorbance value was measured. Residual color of samples obtained after treatment both with and without sludge recirculation was 60 mg Pt/dm$^3$. The effectiveness close to that noted during conventional coagulation at the optimum coagulant dose (3 mg Al/dm$^3$) was observed at 90% of the optimum dose with sludge recirculation resulting in 60.3% organic matter as absorbance removal and color at 20 mg Pt/dm$^3$ were noted. Such results suggest that treatment with 1-day post-coagulation sludge recirculation is ineffective and economically unexplainable for the water tested in these investigations.

Figures 7 and 8 present results for 6-day sludge. The analysis of samples after coagulation, with and without sludge recirculation, at the same coagulant doses, indicates that sludge addition may cause deterioration in water quality after treatment, e.g. at 30% of the optimum alum dose.
In samples where fresh sludge was added absorbance values decreased with the decrease of color. Comparing the results of treatment for 1-day and 6-day-old sludge besides differences in effectiveness, different behavior of absorbance and color was observed. When 1-day sludge was injected at 20% and 30% of the optimum alum dose any removal of color and noticeable changes of absorbance were noted. A little increase of absorbance values resulted from coagulant addition, which in this case, caused only the increase

![Fig. 5. Effectiveness of coagulation with recirculation of 40 cm³ 1-day-old sludge (absorbance removal)](image)

![Fig. 6. Effectiveness of coagulation with recirculation of 40 cm³ 1-day-old sludge (residual color)](image)
of turbidity and hence absorbance higher values. For 6-day sludge at the lowest alum doses residual color after treatment with sludge recirculation was higher in comparison to conventional treatment. So that, to make treatment with sludge addition possible, higher coagulant doses than in series with fresh sludge had to be applied, basing the process on sweep coagulation rather than electrostatic coagulation and charge neutralization. It was necessary to increase a dose to 50% of the optimum one. Higher doses (70%, 90%) had
no significant influence on treatment operation with sludge recirculation and differences of absorbance values result from the fact that absorbance UV measurement depends not only on DOC concentration in water, but suspended solids, as well. For example, when coagulation without sludge was carried out at 70% of the optimum alum dose, 66.2% absorbance removal and residual color of 15 mg Pt/dm$^3$ were observed. For samples with sludge addition 61.2 % and 20 mg Pt/dm$^3$ values respectively were noted. Absorbance measurements in filtered samples, besides the lowest alum doses, indicate that floc ageing caused a little deterioration of sorption abilities, so the problem was agglomeration. The difficulty may be explained by the changes in sludge structure (lower dimension and higher density), which makes agglomeration more difficult.

On the basis of the comparative analysis of coagulation with sludge recirculation for 1-day and 6-day-old sludge the most distinct were differences of treatment effectiveness at 50% of the optimum alum dose. It may be concluded that this dose is a limiting value above which the sweep coagulation is dominating mechanism. This type of coagulation is needed to gain the proper agglomeration and clarification.

CONCLUSIONS

1. Sludge recirculation significantly enhances coagulation. When fresh post-coagulation sludge is recycled treatment effectiveness is much better in comparison to conventional coagulation.

2. When coagulation is conducted with fresh sludge recirculation much lower alum doses may be applied. The alum dose depends on the degree of sludge recirculation.

3. The influence of sludge recirculation on coagulation effectiveness depends on sludge age. Yet, 1-day sludge makes it impossible to produce water of better quality than at the optimum coagulant dose in conventional coagulation. Extension of retention time of sludge in a recirculation system up to 6 days results not only in lack of any positive effects on treatment process; it may cause its significant deterioration.

REFERENCES


ROLA WIEKU OSADU RECYRKULOWANEGO W PROCESIE KOAGULACJI WÓD BARWNYCH

Celem stosowania urządzeń wielofunkcyjnych jest zwiększenie efektywności uzdatniania wody poprzez jednoczesne prowadzenie procesu flokulacji i sedymentacji. Cechą charakterystyczną tego typu urządzeń jest kontakt dopływającej wody z uprzednio wytworzonymi podczas flokulacji kłaczkami, umożliwiający pełne wykorzystanie ich właściwości sorpcyjnych. Z doświadczeń autorów wynika, iż dotychczas pomimo zasadniczych różnic w sposobie realizacji procesu uzdatniania pomiędzy układem konwencjonalnym, a układem, w którym zastosowano urządzenie wielofunkcyjne, dawka optymalna koagulantu dla obu układów jest ustalana według tej samej metodyki tzn. w oparciu o tzw. testy zlewkowe. W przypadku urządzeń wielofunkcyjnych metoda ta jest wysoce zawodna i często prowadzi do znacznego przedawkowania reagenta. W prezentowanej pracy przedstawiono wyniki badań nad rolem wieku osadu recyrkulowanego podczas koagulacji wód, których podstawowym zanieczyszczeniem są barwne związki organiczne. Na podstawie przeprowadzonych badań stwierdzono, iż możliwe jest obniżenie dawki optymalnej koagulantu w stosunku do dawki stosowanej w układzie koagulacji konwencjonalnej, jednak o skuteczności takiego rozwiązania decyduje zarówno stopień recyrkulacji osadu, jak również wiek osadu recyrkulowanego.
THE EFFECT OF SOME TECHNOLOGICAL PARAMETERS ON THE DIVERSITY OF IMMOBILIZED MICROORGANISMS

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Keywords: Microbial diversity, immobilized biomass, RISA.

Abstract: The effect of hydraulic retention time (HRT) and internal circulation capacity (q_c) on microbial diversity of immobilized biomass in the porous ceramic carrier was determined. The bioreactor, operating at HRT of 70 and 60 min, and with q_c in the range of 20–70 dm³.h⁻¹, was employed for the removal of organic compounds from municipal wastewater. Microbial diversity was estimated on the basis of RISA patterns using the Shannon-Wiener index (H'). At HRT of 70 min, H' lowered from 2.48 ± 0.14 to 2.13 ± 0.23 as q_c was increased from 20 to 60 dm³.h⁻¹. At HRT of 60 min, an increase in q_c from 40 to 70 dm³.h⁻¹ resulted in H' drop from 2.41 ± 0.13 to 2.08 ± 0.19. At every HRT the highest efficiency of removal of organic compounds was obtained at the lowest value of q_c and the highest biomass diversity.

INTRODUCTION

In wastewater treatment technology systems with high concentrations of biomass, various reactor types are used including column or membrane reactors, fluidized beds and air-lift reactors, that are characterized by high volumetric reaction rate and long solids retention time. These reactors are considered as ecosystems of high process stability [8] and provide a larger biodiversity in comparison to reactors with suspended biomass [4].

Ecosystem stability is the outcome of functional redundancy, which is ensured by the presence of many species able to perform the same ecological function [3]. The presence of many microorganisms that are able to conduct a specific process heightens the probability that a sudden change of environmental conditions will not worsen the effectiveness of wastewater treatment, because one or more of the species will manage to adapt and assure maintenance of the specific metabolic pathway [13].

Genetic techniques offer promising opportunities for quickly analyzing the community diversity, because many samples can be analyzed relatively rapidly [12]. Recently, several molecular techniques have been developed in order to study a number and di-
versity of natural samples [6]. One of the methods is based on Ribosomal Intergenic Spacer (RIS) region analysis. RIS is located between the 16S and 23S rDNA genes in the ribosomal operon. Research showed that this region is extremely variable both in size and sequence even within closely related taxonomic groups [9]. The patterns obtained can be used to characterize different communities with respect of their species diversity [10, 17].

Microbial diversity in a bioreactor depends on the scale of process being conducted and on its operating parameters. In our column reactor with biomass immobilized in a porous carrier, internal circulation is of high importance, preventing the carrier fouling. Zielińska [23] claimed that lowering the internal circulation capacity from 60 to 20 dm$^3\cdot$h$^{-1}$ resulted in an increase in sludge yield by 20% on average.

From a technological point of view, it is important to evaluate the impact of operating parameters on microbial diversity in a bioreactor. The aim of the experiment was to estimate the effect of hydraulic retention time and internal circulation capacity on microbial diversity of biomass immobilized in the ceramic carrier. The results obtained may help in selecting optimal values of the parameters at which the microbial diversity is maximized, and thus positively influence the wastewater treatment efficiency.

MATERIALS AND METHODS

Characteristics of the bioreactor
Activated sludge was immobilized inside a porous carrier formed as an 8-channeled cylinder. The carrier external diameter was 25 mm, length 1178 mm, total volume 0.6 dm$^3$. The carrier volume was the total volume with the volume of eight channels. The carrier producer (TAMI Industries, Germany) provided the following carrier characteristics: hydraulic diameter 6 mm, internal surface 0.2 m$^2$, pore diameters from 4 to 6 μm, and the material porosity of 35–40%. The carrier was made from the mixture of aluminum oxide (Al$_2$O$_3$), titanium oxide (TiO$_2$) and zirconium oxide (ZrO$_2$) and was called a ceramic carrier. From this powdered mixture a paste was made and formed in the shape of multichanneled tube. The tube was then sintered at $> 1000^\circ$C.

Activated sludge, derived from a full scale wastewater treatment plant with nitrification, was the source of inoculum. The value of the Shannon-Wiener index for activated sludge used as inoculum was 2.31 ± 0.28. First, activated sludge was thickened to the concentration of about 23 g TSS$\cdot$dm$^{-3}$. Then, the immobilization was made by circulating the activated sludge in the reactor for 24 h. The circulation was conducted in such a way that allowed for biomass flow through internal channels of the carrier and not through the space outside the carrier. As a result, biomass was immobilized both inside the pores and on the internal surfaces of the carrier. The initial carrier loading reached 18.2 g TSS$\cdot$dm$^{-3}$. The carrier loading was calculated from the total volume of a carrier.

The carrier with immobilized biomass comprised the stationary filling of the reactor with internal circulation. The reactor scheme and its dimensions in mm (in italics) are shown in Figure 1. The carrier was fixed into the bioreactor using O-rings. Two spaces were created: the internal channels and the external space. Raw wastewater flux and the circulating stream were mixed before they flowed into the reactor. The influent was divided into two streams flowing parallel through the external space and internal channels. This allowed the pressure on the internal and external surfaces to be kept equal.
Operational conditions

The reactor worked under aerobic conditions. In order to maintain the oxygen concentration in the reactor at the level of 2 mg O\textsubscript{2} ⋅ dm\textsuperscript{-3}, it was necessary to supply about 120 dm\textsuperscript{3} ⋅ h\textsuperscript{-1} of air to the reactor. DO was measured in the upper part of the reactor in the effluent. The experiment was carried out at the temperature of 20°C.

During the experimental period, municipal wastewater was taken each day directly from the inspection chamber of a sewer pipe. Typical contents of organic compounds, nitrogen compounds and total suspended solids were as follows: 274 ± 49.7 mg COD ⋅ dm\textsuperscript{-3}, 47 ± 7.6 mg TKN ⋅ dm\textsuperscript{-3}, 25 ± 4.0 mg N\textsubscript{NH4} ⋅ dm\textsuperscript{-3}, 150 ± 45.9 mg TSS ⋅ dm\textsuperscript{-3}. Chemical analyses of wastewater were performed according to Polish Standards [16]. We analyzed 130 samples of wastewater.

The experiment was organized as presented in Table 1. Firstly, the study was carried out for a hydraulic retention time (HRT) of 70 min, and volumetric organic loading rate (VLR) of 5.4 kg COD ⋅ m\textsuperscript{-3} ⋅ d\textsuperscript{-1}. The experiment was repeated for a HRT of 60 min, and a VLR of 5.8 kg COD ⋅ m\textsuperscript{-3} ⋅ d\textsuperscript{-1}. HRT was altered by changing the wastewater feed rate. The volumetric loading rate was calculated per total volume of the carrier according to German ATV directions concerning dimensioning of biological beds. For both HRTs the internal circulation (q\textsubscript{C}) was changed as shown in Table 1.

### Table 1. Experimental set-up

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<tr>
<td>VLR [kg COD ⋅ m\textsuperscript{-3} ⋅ d\textsuperscript{-1}]</td>
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<td>q\textsubscript{C} [dm\textsuperscript{3} ⋅ h\textsuperscript{-1}]</td>
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All experimental series were carried out consecutively in the same reactor in the following order: 1, 2, 3, 5, 6, 4. Before the start of the first experimental series, inoculum was immobilized in the carrier and used throughout the whole experiment. The adaptation period before every series lasted about 30 days and was considered complete when the range of changes of particular parameters of the effluent (COD, TKN, N\textsubscript{NH4}) within 7 days did not exceed 5–10\%. At each hydraulic retention time, after biomass adaptation for the experimental conditions, the research was carried out for about 2 weeks. During the experiment, samples were collected twice a day.

**DNA isolation and RISA**

The samples of biomass were taken from the effluent at the end of each experimental series and stored at -20°C prior to molecular analysis. DNA was extracted from approximately 0.5 g of immobilized biomass using a commercial DNA isolation kit (Fast DNA\textsuperscript{®} SPIN\textsuperscript{®} Kit for Soil, Q-BIOgene, USA) according to manufacturer’s instructions. The quality and quantity of isolated DNA were measured spectrophotometrically using a Biotech Photometer (WPA, UK).

The bacterial RIS was amplified in duplicate with primers 1 and 2 described previously by Dolzani *et al.* [7]. The amplified fragment contained RIS plus approximately 380 bp, corresponding to flanking regions of genes coding for 16S and 23S rRNA. PCR was performed in an Eppendorf\textsuperscript{®} Mastercycler Gradient (Eppendorf, Germany). The PCR mixture contained 50 ng of extracted total DNA, 0.5 μM of each primer, 100 μM of deoxynucleoside triphosphate (Promega, USA), 1 U of Taq DNA polymerase (Sigma, USA), 3⋅10\textsuperscript{-6} dm\textsuperscript{3} of reaction buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM MgCl\textsubscript{2}, 0.01% gelatin, pH 8.3 at 25°C), and sterile water to a final volume of 30⋅10\textsuperscript{-6} dm\textsuperscript{3}. The PCR amplification was carried out using the following program: 95°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 43°C for 30 s, extension at 72°C for 1 min, and a final elongation at 72°C for 5 min. The presence of PCR products was confirmed by analyzing 5⋅10\textsuperscript{-6} dm\textsuperscript{3} of the product on a 1.2% agarose gel stained with ethidium bromide.

After the successful DNA amplification, 10⋅10\textsuperscript{-6} dm\textsuperscript{3} of PCR products were applied directly to 6% polyacrylamide gel (29 : 1 acrylamide : bisacrylamide). Electrophoresis was carried out at 60 V for 110 min in 1x TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA; pH 8.0). After electrophoresis, the gel was stained with SYBRgold (Molecular Probes, USA) at 10 000x dilution in 1x TAE buffer for 30 min.

Stained gel was viewed with an ultraviolet transilluminator and recorded with a CCD camera (Gel Logic 200, Eastman Kodak Company, USA). Bands were detected automatically from digital images of the gel using KODAK 1D 3.6 Image Analysis Software (Eastman Kodak Company, USA). The size of PCR products was estimated using 1 kb DNA Ladder (Promega, USA). Both polyacrylamide gels were used for the determination of the Shannon-Wiener index.

**Calculation methods**

The structural diversity of the microbial community was examined by the Shannon-Wiener index of general diversity H’ [18, 20]. H’ was calculated on the basis of the bands on the gel tracks, using the densitometric curves. The intensity of the bands was reflected as peak heights in the densitometric curve. The Shannon-Wiener index was calculated according to the equation:
\[ H = - \sum \left( \frac{n_i}{N} \right) \ln\left( \frac{n_i}{N} \right) \]

where: \( n_i \) was the height of the peak,

\( N \) was the sum of all peak heights in the densitometric curve.

The relationships between microbial diversity and technological parameters of the process were determined by correlation analyses. The dependence between the number of bands in RISA patterns and internal circulation was assessed with Pearson’s correlation \( (R_p) \). Since a normal distribution could not be assumed, the correlation between \( H’ \) values and internal circulation intensity was performed as Spearman’s rank correlations \( (R_s) \). A statistical significance of the differences between the TSS values in a particular series was determined using the Kruskall-Wallis test. All statistical analyses were performed at significance level of 0.05 using the program STATISTICA 6.0 (StatSoft, USA).

On the basis of RISA patterns, distance matrix analyses were performed according to the method of Nei Li [14] using the program DGGEstat 1.0 (van Hannen, the Netherlands Institute for Ecological Research, NIOO-KNAW, the Netherlands). The samples were clustered using the unweighted pair group method of arithmetic averages (UPGMA), bootstrapping was conducted with 1000 replicates.

The liquid superficial velocity was determined according to Zaiat et al. [22].

The calculated mean values are followed by standard deviations.

RESULTS AND DISCUSSION

In the experiment, the impact of hydraulic retention time and internal circulation capacity on the microbial diversity changes in biomass immobilized in the porous ceramic carrier was examined. Both at HRT 70 and 60 min, at variable \( q_C \), the efficiency of organic compounds (COD) removal remained at the level of about 80% (Tab. 2). However, it could be noticed that, at every HRT, the highest efficiency of organic compounds removal \( (E_C) \) was obtained at the lowest value of internal circulation capacity.

Table 2. Mean values of parameters in the effluent of the bioreactor with immobilized biomass

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<td>( q_C ) [dm(^3)⋅h(^{-1})]</td>
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<td>40</td>
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<tr>
<td>TSS in the effluent [mg⋅dm(^{-3})]</td>
<td>64 ± 58.6</td>
<td>65 ± 39.7</td>
<td>30 ± 24.8</td>
<td>113 ± 109.0</td>
<td>54 ± 62.0</td>
<td>57 ± 35.0</td>
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<tr>
<td>( E_C ) [%]</td>
<td>76.8 ± 15.0</td>
<td>81.8 ± 15.2</td>
<td>84.6 ± 12.7</td>
<td>82.6 ± 16.3</td>
<td>80.6 ± 15.7</td>
<td>87.1 ± 12.0</td>
</tr>
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</table>

In order to investigate the microbial diversity of biomass immobilized in the reactor, molecular analyses were carried out. Electrophoretic separation of PCR-amplified RISA region from immobilized biomass, sampled at the end of each experimental series, resulted in distinct banding patterns (Fig. 2).

The Shannon-Wiener index \( (H’) \) is more and more widely used for evaluation of microbial diversity both in natural and technical biocoenoses [2, 5, 19]. This index can be successfully used for estimating the diversity of communities, however it is difficult to interpret the results obtained, and so further investigations into the dependence between technological parameters and Shannon-Wiener index values are needed [11]. In our research no statistically significant dependence between HRT and Shannon-Wiener
index values was proved. The obtained results are in agreement with the research carried out by Nogueira et al. [15]. The authors did not show any effect of HRT ranging from 0.7 h to 5.0 h on the composition of nitrifiers in biofilm. In the presented experiment, however, a statistically significant correlation between internal circulation capacity ($q_c$) and $H'$ values, both at HRT 70 min and 60 min was noted ($R_s = -0.85$, $p < 0.05$) (Fig. 3A). These data indicated that an increase in the internal circulation capacity resulted in a decrease in microbial diversity of biomass immobilized in the porous carrier. At a HRT of 70 min an increase of $q_c$ from 20 to 60 $\text{dm}^3\cdot\text{h}^{-1}$ caused a decrease of $H'$ from 2.48 $\pm$ 0.14 to 2.13 $\pm$ 0.23, whereas at a HRT of 60 min an increase of $q_c$ from 40 to 70 $\text{dm}^3\cdot\text{h}^{-1}$ reduced $H'$ from 2.41 $\pm$ 0.13 to 2.08 $\pm$ 0.19. Parallelly, a statistically significant negative correlation between the number of bands, representing different bacterial species, and internal circulation capacity ($q_c$) was observed ($R_p = -0.92$, $p < 0.05$) (Fig. 3B). A decline of immobilized biomass diversity did not result from the biomass washout, because no statistically significant differences between TSS values in the effluent during experimental series were observed (Tab. 2). The calculated values of the liquid superficial velocity in the reactor (from 1.55 to 5.3 $\text{cm} \cdot \text{s}^{-1}$) indicated that the flow speed of the stream through the bioreactors could have not caused the biomass washout. In general, the increase of the internal circulation capacity results in the larger total contaminants load in the wastewater stream introduced to the bioreactor. According to Atlas [1], at high nutrient loading a less diverse community develops. Our research results are in agreement with this opinion. In the reactors with bacterial communities immobilized in the carriers, part of the biomass is attached to the carrier; the other part is suspended [21]. It seems that intensive wastewater flow due to increased internal circulation capacity mainly favors the growth of bacterial species with greater ability to attach, whereas the contribution of suspended biomass is minor.

![Fig. 2. Ribosomal Intergenic Spacer (RIS) region analysis of bacterial community immobilized in the porous carrier; lane labels along the top show the number of experimental series; lane M: 1 kb DNA Ladder (Promega, USA); the gel was stained with SYBRgold (Molecular Probes, USA)](image-url)
A comparison of RISA patterns obtained in different experimental series was conducted in order to determine the similarities between microbial communities in the reac-

Fig. 3. Correlations between: (A) internal circulation intensity and Shannon-Wiener index values (Spearman’s rank correlation), (B) internal circulation intensity and the number of bands in the RISA patterns (Pearson’s correlation); black circles represent values obtained for HRT 60 min, whereas grey squares represent values obtained for HRT 70 min.

Fig. 4. Dendrogram of RISA patterns similarities among different experimental series calculated on the basis of distance matrix according to the method of Nei Li with the clustering algorithm of UPGMA; numbers adjacent to branch points are bootstrap percentages (n = 1000 replicates); in the description of branches the first number refers to HRT, the second number to the \( q_c \) value.
tor in relation to technological parameters. The cluster analysis divided the RISA patterns of the microbial community immobilized in the porous carrier into two clusters (Fig. 4). A clear distinction between microorganisms communities at HRT of 70 and 60 min was found. The first cluster embraced RISA patterns obtained in the series 4–6 at HRT of 60 min, while the second cluster included patterns from the series 1–3 at HRT of 70 min. It is worth mentioning, however, that RISA similarity patterns indicated in the dendrogram corresponded to the order of series, in which the experiment was conducted. As the reactor was inoculated once only, at the beginning of the experiment, it can be assumed that biocoenoses shaped in every series fluently passed one into another. For this reason, the effect of HRT on similarities between bacterial communities immobilized in the carrier cannot be stated unequivocally.

**CONCLUSIONS**

1. At a given HRT, the microbial diversity depends on the internal circulation capacity. Along with an increase of wastewater flow, the Shannon-Wiener index values and the number of different bacterial species decrease.

2. At any given HRT, the highest efficiency of organic compounds removal ($E_C$) can be obtained at the lowest value of internal circulation capacity and the highest biomass diversity.

**SYMBOLS USED**

- DO – dissolved oxygen,
- COD – chemical oxygen demand,
- $E_C$ – effectiveness of organic compounds removal,
- HRT – hydraulic retention time,
- $H'$ – Shannon-Wiener index,
- $p$ – significance level,
- $q_C$ – internal circulation capacity,
- RISA – Ribosomal Intergenic Spacer Analysis,
- $R_p$ – Pearson’s correlation,
- $R_s$ – Spearman’s rank correlation,
- TKN – total Kjeldahl nitrogen,
- TSS – total suspended solids,
- VLR – volumetric loading rate.

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Celem pracy było określenie wpływu hydraulicznego czasu zatrzymania (HRT) i wydajności cyrkulacji wewnętrznej ($q_C$) na różnorodność mikroorganizmów w biomasie unieruchomionej w porowatym nośniku ceramicznym. Bioreaktor, wykorzystywany do usuwania związków organicznych ze ścieków komunalnych, był eksploatowany przy HRT 70 i 60 min oraz $q_C$ w zakresie 20–70 dm$^3$·h$^{-1}$. Różnorodność mikroorganizmów była określana na podstawie wzorów RISA przy użyciu indeksu Shannona-Wienera ($H'$). Przy HRT równym 70 min, $H'$ obniżył się z 2,48 ± 0,14 do 2,13 ± 0,23 ze wzrostem $q_C$ z 20 do 60 dm$^3$·h$^{-1}$. Przy HRT 60 min, zwiększenie $q_C$ z 40 do 70 dm$^3$·h$^{-1}$ spowodowało spadek $H'$ z 2,41 ± 0,13 do 2,08 ± 0,19. Przy każdej wartości HRT, najwyższą efektywność usuwania związków organicznych uzyskano przy najniższej wartości $q_C$ i najwyższej bioróżnorodności.
SEASONAL CHANGES IN THE NUMBER OF SULPHATE-REDUCING BACTERIA IN THE WATER, SOIL AND PLANT OF THE WETLANDS NEAR OLSZTYN

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Keywords: Bacteria, sulphate-reduction, water, soil, plants, wetland.

Abstract: Seasonal changes in the numbers of sulphate-reducing bacteria in water, soil, the surface of sedge (Carex acutiformis Ehrb.) immersed in water and roots (dead and live) were studied. The study on one of larger wetland near Olsztyn (Masurian Lake District) was carried out in two annual cycles. Sulphate-reducing bacteria in the studied ecosystems occurred sporadically and generally in inappreciable count. Their count did not exceed 20 cells in 1 cm³ of water; in the soil and in different parts of sedge the number of bacteria ranged from several to over dozen thousands cells in 1 g of dry weight. In the first year of studies these bacteria were the most numerous in June, July and during first days of December, but in the second – in April (in soil and sedge immersed in water), in August (in soil and dead and live roots), in November (in water, soil, sedge immersed in water and dead roots) and exceptionally in other months.

INTRODUCTION

The sulphate-reducing bacteria (SRB) play an important role in many anaerobic processes, especially mineralization of organic substance, biodegradation of xenobiotics, mercury methylation, heavy metals removal from contaminated environments or metal reduction by insoluble sulphite precipitation [2, 5, 40, 42, 44]. SRB activity is conditioned by the presence of sulphate (electron acceptor) and easily soluble organic substance (electron donor). In anaerobic ecosystems, equally important are reaction (pH) and temperature. In natural ecosystems, the process of sulphate reduction has been observed in the range of pH 3.0–9.2 [11]. Low-molecule organic compounds utilized by SRB can be generated by the heterotrophic bacteria decomposing organic substance during fermentation of cellulose, pectin and other organic carbon compounds. The quantity and quality of organic substrate, as well as the vulnerability to degradation, are important factors regulating sulphate reduction also in wetlands. Some SRB oxidize these intermediary products of organic substance degradation/fermentation only partially, i.e., to acetate, and prefer simple products of fermentation, such as hydrogen, lactates or ethanol produced at the oxic/
anoxic interface in sediments (*Desulfovibrio*, *Desulfomicrobium*, *Desulfovibulus*), while other – oxidize them completely, i.e., into CO$_2$ (*Desulfonema*). SRB are very common in most of the anaerobic environments [1, 13–15, 17, 19, 20, 26, 29, 35, 36, 40, 46]. In surface waters (rivers, lakes, ponds), where aerobic conditions prevail, SRB quantities do not exceed several dozen, or less frequently – several hundred cells in 1 cm$^3$ [25, 31]. They are more abundant in lake bottom deposits (up to several thousand cells in 1 g of fresh mass) [12–14, 30, 31]. The highest SRB numbers in the bottom sediments of some eutrophic and mesotrophic lakes have reached $2.0 \times 10^3 – 4.7 \times 10^5$ cells in 1 g of fresh mass [25]. SRB occurrence and their role in mineralization of organic substance produced in wetlands have been scarcely studied [1, 9, 11]. Among the reasons for such little interest in this group of bacteria is the low sulphate content in the water (50–450 μM) needed as the acceptor of electrons in the process of organic substrate mineralization in the anaerobic conditions [11]. In such ecosystems nearly 11–14% of organic carbon is oxidized with participation of SRB whereas in salty marshes, where sulphate content is higher, as much as 50% of organic substance produced in situ can be degraded through dissimilative sulphate reduction. In such environments, the number of SRB in the bottom deposits can amount to $1.0 \times 10^6$ cells in 1 g of fresh mass [25]. Occurrence and seasonal variability of the number of these microorganisms in water, soil and rhizosphere of the plants growing in the wetlands of Mazurskie Lakeland is a missing subject in the reference literature. Therefore the aim of this study was determining the number of sulphate-reducing bacteria in different kinds of biotopes in one of the largest wetlands in the Olsztyn city area.

**MATERIALS AND METHODS**

**Study area**

The study covered the Lakeland area in the vicinity of the Stary Dwór forest’s lodge. It is located among slopes afforested with pine and spruce in the upper parts and birch in the lower parts and with willow in coastal zone. The surface area of the Lakeland covers approximately 0.2 km$^2$, with a channel (6 m in width and 4–5 m in depth) running through in the center (a remnant of an old lake). The entire area is periodically flooded in spring and autumn, whereas in the summer the water count outside the channel zone falls to a dozen or so cm. The flora is dominated by sedges (Carex) and rushes (Juncus) that form a permanent cover or isolated tufts surrounded by water [32].

**Materials**

The number of sulphate-reducing bacteria was determined in water of the littoral zone overgrown with dense phytomass and between tufts of plants emerging from water; on plants emerging from water – separately on submerged stem and aerial leaves; in the root system (rhizosphere) – on old (the previous year’s) and new (the current year’s) roots, as well as in soil.

**Collection of samples**

Samples of water and tufts of sedge (Carex acutiformis Ehrb.) including the soil and root system were taken in 1 month intervals, in the vegetative seasons from March to December of the two following years. Water samples were collected from the depth of 0.3 m directly into sterile glass bottles. Site 1 was located 30 m from a forest tract leading along
the edge of wetland on its eastern side. Sites 2 and 3 were located on the edge of wetland, on the northeastern side, 80 m from a path leading to the Stary Dwór forest’s lodge [32]. Under laboratory conditions, the material collected was separated into parts of plant immersed in water (steams), part protruding from water (aerial leaf), previous-year’s roots (dead) and current year’s roots (live), and soil from the root system. Aseptically weighed 10 g of soil samples and particular fragments of plants were transferred into flask with a 90 cm$^3$ of sterile physiological NaCl solution and shaken in a shaker for 30 min. The obtained suspension of soil and plants and roots was diluted (1:10 ÷ 1:1000) and transferred in the volume of 1 cm$^3$ on respective media appropriate for individual physiological group of bacteria. Simultaneously weighed 10 g samples of soil and particular fragments of plants were dried at 105°C to measure the dry matter.

Microbiological analyses
The number of sulphate-reducing bacteria was determined on Tauson’ medium modified by Szturm [37]. The medium contained: 3.5 g calcium lactate, 4.0 g $(\text{NH}_4)_2 \text{SO}_4$, 0.5 g $\text{K}_2\text{HPO}_4$, 1.0 g $\text{MgSO}_4\cdot7\text{H}_2\text{O}$, 0.5 g $\text{CaSO}_4\cdot2\text{H}_2\text{O}$, 0.5 g $(\text{NH}_4)_2\text{Fe(SO}_4)_2\cdot6\text{H}_2\text{O}$ (Mohr’ salt), 1 dm$^3$ distilled water, after neutralization by KOH to pH 7.0 and solidified by Difco agar (18 g agar per 1 dm$^3$ medium). The medium (without Mohr’ salt) was autoclaved in 121°C per 10 minutes. Mohr’ salt was sterilized with using sterile membrane filters with pores of 0.22 μm in diameter and added to dissolved and cooled to 42°C agars medium before inoculation of samples. BRS inoculated in test tubes with rubber stoppers on Tauson’s medium, were incubated in 25°C for 7, 14 and 21 days. The presence of BRS was identified by precipitation of black iron sulphide around colony of bacteria and hydrogen sulphide smell (after stopper removing).

Each measurement was done in three simultaneous repetitions of the same samples. MPN of BRS in 1 cm$^3$ of water or 1 g of plant parts were measured according to the guidelines and read out from McCrady’s charts [28].

RESULTS
In the water of the examined wetland SRB have been isolated rarely; the highest number observed in November, the second year of the study, at the sampling site 2 did not exceed 20 cells in 1 cm$^3$. Periodically, they were present in larger numbers on the submerged in water parts of the sedge (to $7.5 \times 10^3$ cells in 1 GWD at site 1 and to $9.0 \times 10^3$ cells in 1 GWD at site 2), in the soil surrounding the root system of the plant (to $2.4 \times 10^3$ cells in 1 GWD at site 1 and to $2.2 \times 10^3$ cells in 1 GWD at site 2), and on the surface of dead roots (to $1.86 \times 10^3$ cells in 1 GWD at site 1 and $11.6 \times 10^3$ cells in 1 GWD at site 2) and live roots (to 645 cells in 1 GWD at site 1 and to $2.1 \times 10^3$ cells in 1 GWD at site 2). On the surface of the emerged (aerial/aerated) parts of the sedge SRB were examined only at site 1. Throughout the study they were observed only twice and their quantity was lower than 15 cells in 1 GWD (Tab. 1).

In the water sampled from sites 1, 2 and 3, in soil and in individual parts of the sedge from sites 1 and 2 the differences in SRB numbers over the same period were less than 1 order of magnitude. In the first year of the study, in the soil surrounding the root system of the sedge, on the submerged in water parts of the plant and on the dead roots, more SRB were found in June, July and December, on the live roots – in July and December. In the
Table 1. The number (MPN·cm$^{-3}$/MPN·GDW$^{-1}$) of sulfate-reducing bacteria in water, soil and the surface of sedge (*Carex acutiformis* Ehrb.) of natural wetland near Olsztyn in two following years of studies

<table>
<thead>
<tr>
<th>Date</th>
<th>Water (in 1 cm$^3$)</th>
<th>Rhizosphere soil (in 1 GDW)</th>
<th>Sedge (in 1 GDW)</th>
<th>Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Submersed stem</td>
<td>Aerial leaf</td>
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<td></td>
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<td></td>
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<td>Site</td>
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<tr>
<td>April</td>
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<tr>
<td>May</td>
<td>0</td>
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<td>1.5</td>
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<tr>
<td>July</td>
<td>0</td>
<td>0.4</td>
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<tr>
<td>August</td>
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<td>September</td>
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<td>0.3</td>
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<tr>
<td>October</td>
<td>0</td>
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<tr>
<td>December</td>
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<tr>
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<tr>
<td>Mean</td>
<td>0.4</td>
<td>0.2</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Range</td>
<td>0–4.5</td>
<td>0–20</td>
<td>0–4.5</td>
<td>0–4.5</td>
</tr>
</tbody>
</table>

– not tested
second year of the study, in the soil surrounding the root system more SRB were counted on the dead and live roots in August whereas on the submerged in the water parts of the plant – in April. In other months of the study, they either did not occur in the examined soil mass and on the individual parts of the sedge, or their number was negligible. The exemption comprised the soil samples taken in April, the second year of the study, containing up to 250 cells in 1 GWD (Tab. 1).

DISCUSSION

Sporadic occurrence and very low numbers of SBR in the water of the examined wetland can be explained by the very good oxygenation during the vegetative period. Low depth of the water on the sampling sites has allowed for free oxygen diffusion from the atmosphere and its release through photosynthesis by the phytoplankton and hydromacrophytes. In the investigated wetland little sulphate content (36–37 mg SO$_4$·dm$^{-3}$ – 380–390 μM) was probably the key factor in organic substance mineralization by SRB under anaerobic conditions. The data quoted by Feng and Hsieh [11] reveal that sulphate content in the fresh-water wetlands is generally low (50–450 μM), unlike the salty marshes and marine sediments, and it also limits the proliferation of SRB. The water reaction (pH 6.0) of the examined wetland may have had no effect on the number of SRB. Although the optimum pH for these microorganisms is 7.0–7.2 [35], the majority tolerates a broader range: 3.0–9.2 [11]. Seemingly, temperature did not limit the SRB growth either, as they occurred even in November, the second year of the study, reaching a number higher than in the summer. As SRB occurred in the water of the wetland, their number did not vary from that given in the literature for the inland-lake water [12, 25, 31]. SRB occurrence in the water of the examined wetland should be associated with the higher number of these bacteria in the soil surrounding the root system of the sedge, on the submerged in the water parts of the plant and in the rhizosphere.

The higher numbers of SRB observed periodically on the surface of submerged in water parts of the sedge, in the rhizosphere and in the soil surrounding the root system of that plant may have been stimulated by the release to the environment of the soluble organic compounds (ethanol, lactates, presumably also acetates) by roots and root hairs, or the fatty acids and amino acids from the necrosis zone of the dead roots and/or through shedding of the tips of live roots and root hairs [39]. In the ecosystems, like soil and rhizosphere, simple organic carbon compounds (formates, lactates, ethanol, fatty acids, secondary alcohols, isobutyrates, propionates, hydrogen and some other) can be made available to SRB by fermenting bacteria or acetogenic bacteria [43]. Mineralization processes of organic substance by heterotrophic bacteria use up oxygen, therefore, conditions are created for the SRB occurrence. The maximum numbers of SRB determined in the soil and rhizosphere (on the surface of dead and live roots) and on the submerged in water parts of the sedge occurred in parallel with the maximum numbers of heterotrophic bacteria in July, the first year of the study, amounting to a few million up to a billion and more cells in 1 GDW [23]. Assuming that live roots of sedge release much oxygen to the environment which allows for existence of nitrifying bacteria [34] in the rhizosphere and in the soil surrounding the plant’s root system (literature data deal with the amounts of oxygen released only by *Phragmites* – the values range from 0.02 g·m$^{-2}$·24 h$^{-1}$ to 5–12 g·m$^{-2}$·24 h$^{-1}$ depending on the applied measurement technique [3]), niches must be created
for the suitably low redox potential (E_h = -150 to -200 mV) needed for SRB survival [6–8, 10, 41], although they do not reduce sulphate [16, 21, 22]. SRB are strict anaerobes vulnerable to oxygen. The critical concentration of oxygen which still allows for sulphate reduction is 0.1–1.0 mg∙dm^{-3} [18]. Provided the level is higher than 1 mg O_2∙dm^{-3}, the reduction will not occur due to increased redox potential and inhibition of SRB. However, if water, sediment or soil contain larger amounts of soluble organic substance SRB become active in the anaerobic micro-niches, although these comprise the elements of an aerobic environment [45]. It is believed that oxygen inactivates or inhibits enzymes or proteins active in the sulphate reduction process [4]. Literature data of the past few decades revealed that most of the known SRB may periodically survive in contact with oxygen; some cause oxygen reduction, however the growth in such conditions is rather slow [42]. SRB can also migrate from the aerobic zone or make aggregates of cells providing for their survival [24].

The individual cases of small SRB amounts detection on the surface of the emerged from water (aerated) parts of the sedge may have been the result of accidental contamination caused by moose, roe deer, or elk using the wetland as drinking reservoir in the near shore area [33]. Some SRB strains living in the rumen of ruminants together with autogenic bacteria can interact with fermentative bacteria decomposing carbohydrates to organic acids (e.g., acetic acid) and molecular hydrogen, oxidized then by SRB [29].

Seasonal changes in SRB numbers in the rhizosphere and in the soil surrounding the root system of the sedge and on the surface of the submerged in water parts of the plant may have been stimulated by the release of photosynthesis products (soluble organic C compounds). The example of card grass Spartina alterniflora Loisel, inhabiting salty marshes (Chapman Marsh) in the south-eastern area of New Hampshire, USA, has shown that larger numbers of SRB may occur in the rhizosphere in the first months of growth (May and June) and then drop when the plant starts blooming [19, 27, 38, 39]. These authors share the opinion that as soon as the first phase of the vegetative growth plant “re-mobilizes” the non-structural carbohydrate accumulated in root hairs and transfers the new products of photosynthesis to the fast-growing roots and root hairs. An increase of the store of soluble carbohydrates together with the products of the root tips lysis is the reason for larger leakage of soluble organic carbon compounds from the roots and root hairs. Once the plant enters the phase of the generative (reproductive) growth, organic carbon is transferred to the blooming structures, carbohydrates are immobilized in root hairs, and in consequence, the amount of the released organic carbon compounds in the rhizosphere rapidly decreases. Simultaneously, the number of SRB drops. It is possible that similar phenomena have occurred in the case of the sedge inhabiting the examined wetland in the Olsztyn city area. As in the case of Spartina alterniflora, the maximum numbers of SRB occurred in the rhizosphere of the plant during the vegetative growth (in June and July of the first year of the study, and in August of the second year). Next, the SRB number decreased or the bacteria were absent from the examined root mass. The repeated increase of the SRB number in the rhizosphere of the sedge observed in early December of the first year of the study or in late November of the second year may have been caused by the mineralization of the dead parts of the plant occurring with the participation of the heterotrophic bacteria and saprophytic fungi [23, 32] and by the environment’s enrichment in the intermediate products of the decomposition, utilized by SRB.
CONCLUSIONS

1. Sporadic occurrence of small amounts of SRB in the water of the examined wetland can be caused by the low depth (between ten and several dozen cm) on the individual sampling sites which enables constant oxygen diffusion to the atmosphere.

2. The higher SRB numbers observed periodically on the submerged in water parts of the sedge, in the rhizosphere and in the soil surrounding the root system of the plant can be related to the larger amount of the intermediary products of the organic substance decomposition of the dead plant parts or secreted by the plant.

3. The increased number of SRB on the surface of the submerged parts of the sedge, in the rhizosphere and in the soil surrounding the root system of the plant in the summer (in June and July of the first year of the study and in August of the second year) can be related to the release of organic carbon substance by the roots and root hairs, while in the autumn (December of the first year of the study and end of November of the second year) to decomposition of organic remnants by the heterotrophic bacteria and fungi.

4. The differences in the number of SRB in the water samples, in the samples of various parts of the sedge and in the samples of soil surrounding the root system, collected from different sampling sites in the wetland were usually negligible.

5. The single cases of SRB detection on the surface of the emerged from water (aerated) parts of the sedge may be due to accidental contamination caused by ruminants (moose, roe deer, elk) living in the wetland area.

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SEASONAL CHANGES IN THE NUMBER OF SULPHATE-REDUCING BACTERIA IN...

Badano sezonowe zmiany liczebności bakterii redukujących siarczany w wodzie, glebie, zanurzonych w wodzie i wynurzonych z wody (napowietrznych) częściach turzycy błotnej (*Carex acutiformis* Ehrb.) oraz jej korzeniach (obumarłych i żywych). Badania przeprowadzono na jednym z większych mokradel śródleśnych w okolicy Olsztyna (Pojezierze Mazurskie) w dwóch kolejnych latach badawczych. Bakterie redukujące siarczany występowały sporadycznie w badanych ekosystemach i z reguły w niewielkich ilościach. W wodzie ich liczba nie przekraczała 20 komórek w 1 cm³, w glebie i różnych częściach turzycy błotnej – kilku (wyjątkowo kilkunastu) tysięcy komórek w 1 g suchej masy. W pierwszym roku badań występowały one głównie w czerwcu i lipcu oraz w pierwszych dniach grudnia, w drugim zaś – w kwietniu (w glebie i zanurzonych w wodzie częściach turzycy błotnej), w sierpniu (w glebie i na korzeniach martwych i żywych) oraz w listopadzie (w wodzie, glebie, zanurzonych w wodzie częściach turzycy błotnej oraz korzeniach martwych), wyjątkowo w innych miesiącach.
CHANGES OF PHOTOSYNTHETIC PIGMENTS CONCENTRATION IN THE SYNCHRONOUS CULTURE OF CHLORELLA VULGARIS AS AN INDICATOR OF WATER QUALITY IN GOCZAŁKOWICE RESERVOIR

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Keywords: Chlorella vulgaris, synchronous culture, photosynthetic pigments, water quality control, Goczałkowice Reservoir.

Abstract: The aim of this study was to examine the possibility of use of synchronous culture of Chlorella vulgaris algae as a biotest in water quality control. In the experiment the samples of water collected from seven sampling points in Goczałkowice Reservoir were used. The criterion of changes was the concentration of photosynthetic pigments, from 24th hour of the cell life cycle, separated by HPLC technique. On the basis of changes taking place in the cells of the algae cultures it was possible to establish the timing of the flood period and autumnal changes in a water quality. It was also proved that the water quality in the main water current that fall into the western part of reservoir carried by the Wisła River after the flood period significantly differed from the water quality in the other parts of the reservoir, particularly in its eastern section.

INTRODUCTION

It is estimated that water is contaminated by about one million pollutants. In Poland the water quality is assessed by the physical, chemical and bacteriological analyses carried out according to the Polish standards, which do not include testing for all pollutants. The precise analysis of toxic substances in the water environment requires chemical analyses of a large number of samples. This is complicated and costly. Therefore, the use of biotest is a useful method of supporting the physicochemical analyses. Biotests are recommended in the USA and UE countries in the procedures of aquatic monitoring [17, 27]. In such biotests the general influence of contaminated environment on the growth of chosen living organisms is investigated. Since on the basis of biotests alone it is impossible to determine which components of complex mixture are responsible for the biological activity detected, it is necessary to combine these methods with the proper physicochemical analyses [20].
Asynchronous cultures of *Chlorella vulgaris* algae are often used and recommended by Polish [13] and European [27] directives. Published results of studies show that synchronous cultures of *Chlorella vulgaris* algae, in which the cells are at the same stage of life, give more uniform answers to the changes of toxicity than asynchronous cultures. Synchronous cultures allow investigating of processes at the all life stages of cells affected by the tested substances [8–10, 12, 16, 18–19, 22–23].

The first step of the investigations with synchronous cultures of *Chlorella vulgaris* algae in the environmental studies was undertaken in the Department of Molecular Biology, Biochemistry and Biopharmacy at Medical University of Silesia. The studies were focused on changes of culture absorbance and the rate of cellular division in synchronous culture of *Chlorella vulgaris* algae (cultivated in industrial wastewater from the Odra river watershed diluted with the distilled water) was determined [21]. Lodowska *et al.* [7] cultivated those algae in synchronous culture in tannery wastewater from Skoczów diluted with the distilled water. Czaplicka-Kotas [2], who investigated the water from the Goczałkowice Reservoir, showed that the changes of the concentration of photosynthetic pigments are the best criterion of water quality changes.

In the investigations mentioned above the following criteria were used:

- changes in the cell culture absorbance (per million cells) at 680 nm, spectrophotometric measurements were performed every hour during the first ten hours and at the 24th hour of synchronous growth;
- changes in the number of algae cells in the given culture, the number of cells was counted in the Bürker’s chamber, the results were used to establish the rate of cellular division;
- changes of the concentration of photosynthetic pigments (using HPLC).

The aim of this study was to examine the possibility of the use of synchronous culture of *Chlorella vulgaris* algae as a biotest in water quality control in the Goczałkowice water reservoir.

**MATERIALS AND METHODS**

Investigated *Chlorella vulgaris* (Beijerinck 1890; strain A-8) were synchronously cultured (Fig. 1) on the sterile mineral Kühl and Lorenzen medium [6] modified by Borns [1] (5·10⁶ ± 0.5·10⁶ cells/cm³). Every experiment was conducted in two measurement series. In the first, algae were cultivated in a medium prepared with clean water (control). In the second algae were cultivated in media prepared with water from sampling points from the Goczałkowice Reservoir. The algae cultures were carried under the stable conditions: temperature 30°C, illumination 15000 lux on the reactor surface during 10 hours of the light period followed by 14 hours of the dark period. During the light period, the cultures were aerated by air (30 dm³/hour) supplemented with 2% CO₂, and during the dark period by air without CO₂. According to scheme of the cell life cycle of *Chlorella vulgaris* (Fig. 1) the development and growth of all stadium from G1 to cytokinesis is strongly dependent on illumination and culture conditions. From the very beginning of the dark phase, cytokinesis and sporulation take place [11].
Water samples, used in the experiment, were taken in the seven sampling points from the Goczałkowice Reservoir (Southern Poland, Upper Silesia region) (Fig. 2). Water samples were taken into acid clean polyethylene containers. Before the experiment water samples were filtered (0.45 m Millipore filters).

Fig. 2. Goczałkowice Reservoir with the localization of the water sampling points
Samples (20 cm$^3$) of *Chlorella vulgaris* suspension from the 24th hour of the life cycle were centrifuged (2400 $\cdot g$, 15 minutes), dried and resuspended in the pure methanol. Pigments were extracted from this mixture by boiling at 65 ± 2°C for 10 minutes. Extracts were brought up to 3 cm$^3$ with methanol and then centrifuged (2500 $\cdot g$, 3 minutes) [12].

Suspensions were filtered (0.22 m Millipore filters) and analyzed using 1050 Hewlett Packard liquid chromatograph equipped with an on-line degasser and variable wavelength detector UV/VIS (set at 440 nm). Aliquots (20·10$^{-3}$ cm$^3$) were injected on C$_{18}$ column Eurospher 100, Knauer (250 x 4 mm) using an autosampler.

Photosynthetic pigments were eluted by the gradient mixture of methanol – water (85–100% methanol in 15 minutes, followed by 100% methanol for 35 minutes). The flow rate was 1 cm$^3$/minute, the temperature 30°C and the total time of analysis 50 minutes. The chromatographic procedure was controlled by the HPLC ChemStation A.06.03 (Hewlett Packard). Photosynthetic pigments were identified by comparison with the commercially available standards and the literature references [14–15, 24–25].

To assess the impact of the Goczałkowice Reservoir water quality changes on the photosynthetic pigments synthesis in *Chlorella vulgaris* algae, the relative concentration factor ($W_m$) was used according to the equation:

$$W_m = \sqrt{\sum_{i=1}^{L_B} \sum_{j=1}^{L_P} (a_{ij}^m - 1)^2}$$

where: $a_{ij}^m$ – relative value (to the control sample) of $i$ photosynthetic pigment and $j$ sampling point in $m$ month,

$m$ – month (VI – X),

$L_B$ – number of photosynthetic pigments (nine),

$L_P$ – number of sampling points (1–7).

This factor integrates the effect for the nine photosynthetic pigments. Each photosynthetic pigment can be described separately as was shown in the monograph of Czaplicka-Kotas [2].

The study of similarity profile of concentration changes of the nine identified photosynthetic pigments was performed using the cluster analysis. The applied similarity measure was the Euclidean distance. The distance among clusters was calculated by average between groups linkage method [4]. Statistic analysis (p = 0.05) was performed with the SPSS software.

RESULTS

On the basis of performed investigations it was concluded that the analyzed algae cells contain the following photosynthetic pigments: neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, chlorophyll a, chlorophyll b, α-carotene, β-carotene.

To compare synthesis changes of all photosynthetic pigments in *Chlorella vulgaris* algae from the seven sampling points localized in the Goczałkowice Reservoir the relative concentration factor of photosynthetic pigments was calculated. The relative concentrations of photosynthetic pigments were calculated on the basis of quotient for the concentration of photosynthetic pigment in the investigated and control cultures. The
relative concentration in the control culture was equal 1. The Figure 3 shows that the relative concentration factor of photosynthetic pigments ($W_m$) was close to the control culture (factor equal 1) in the July and August. In the September and October the difference was the biggest.

To group profiles of the concentration changes of all photosynthetic pigments the clusters analysis with Euclidean distances as a criterion of grouping was used. In each profile the sum of the relative concentrations of all identified pigments in each sampling site in a particular month was taken into consideration. Results are presented as the dendrogram (Fig. 4).

![Fig. 3. Relative concentration factor of the photosynthetic pigments ($W_m$)](image)

DISCUSSION AND CONCLUSIONS

In the investigated period (from June to October 1997) the water quality changes were affected by July flood and additional autumnal loading of contaminated waters from farm-fishing ponds.

The relative concentration factor of the photosynthetic pigments extracted from the algae cultivated on the water from the Goczałkowice Reservoir (Fig. 3) had the closest values to the factor for the control culture in the flood period and the following month (July and August). It is obvious that contaminants in the reservoir are diluted with water inflow from the watershed. The flood inflow is enriched with the rock material during the fall period. This material comes from the watershed and forms a new lake bed. As the result of natural processes in the lake the quantity of biogenic substances is increased [3]. Because of the decreased temperature and decreased algae activity these substances are not used by algae. Increased amount of biogenic substances is caused by the phosphorous and ammonia nitrogen from farm-fishing ponds in the Goczałkowice Reservoir water-
Fig. 4. Similarity dendrogram of the relative Euclidean distances calculated for profile of photosynthetic pigments changes (neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, chlorophyll b, chlorophyll a, α-carotene and β-carotene) in Chlorella vulgaris cells cultivated in the water from Goczałkowice Reservoir in June – October (VI–X) 1997.
shed [5]. The farm-fishing ponds complex in this region is the biggest in Central Europe with the area of 900 ha [26]. *Chlorella vulgaris* algae cultivated on the Goczałkowice Reservoir water sampled in September and October synthesized the highest amounts of photosynthetic pigments. The relative factor values in this period were the most different from the factor for the control culture.

The dendrogram showing the similarity profiles of relative concentrations of all photosynthetic pigments changes in *Chlorella vulgaris* cells cultivated in the water from the Goczałkowice Reservoir is presented in the Figure 4. This dendrogram reflects the similarity and differences of water quality from June to October 1997. The Euclidean distances showed similarity for the pigments from algae cultivated on the water from July and August. Profiles of photosynthetic pigments from algae cultivated on water sampled in June and in sampling sites 1 and 2 (located in the western part of the reservoir close to the Wisła River inflow) in September and October created a separate cluster (similarity 63%). The significant difference (similarity 0%) was found among described clusters and the cluster of photosynthetic pigments profiles in algae cultivated on waters from sampling points 4, 5, 6, 7 (localized in the eastern part of the reservoir) from September and October. These profiles show that the water quality in the main water flow from the Wisła River was significantly different after the flood period, especially in the eastern part of the reservoir, what has not been documented by the results of the standard chemical and biological investigations [2].

The analysis of the results presented in the study allows for drawing the final conclusions:

1. On the ground of the photosynthetic pigments synthesis, it was shown that the *Chlorella vulgaris* algae Beijerinck 1890 of the A-8 strain are sensitive to water quality changes of the Goczałkowice Reservoir.
2. The analyses of concentration changes of photosynthetic pigments distinguish the July flood period and autumnal water quality changes.
3. Basing on the relative concentration factor of photosynthetic pigments in algae cultivated in water from July and August, it could be stated that concentrations of pigments are the closest to control, thus the water was the cleanest.
4. Concentration variations of photosynthetic pigments in *Chlorella vulgaris* cultivated synchronously in water collected from different points of the Goczałkowice Reservoir demonstrate that the water quality in the Wisła mainstream (western part of the reservoir) after the flood period differed greatly from the water quality in other parts of reservoir, particularly in the eastern section.

**Acknowledgements**

This work was a part of PhD thesis of Anna Czaplicka-Kotas entitled “Synchronic culture of Chlorella vulgaris algae in the water quality control” supervised by Professor Beata Cwalina. The authors would like to express cordial thanks to Professor Beata Cwalina for the revision of the manuscript.

**REFERENCES**


ZMIANY KONCENTRACJI BARWNIKÓW FOTOSYNTETYCZNYCH W SYNCHRONICZNEJ Hodowli Chlorella vulgaris jako wskaźnik jakości wód zbiornika Goczałkowice

Celem niniejszej pracy było wykazanie możliwości wykorzystania synchronicznej hodowli Chlorella vulgaris jako biotestu w kontroli jakości wód. Próbki wody wykorzystane w eksperymencie pochodziły z siedmiu punktów pomiarowych zlokalizowanych w obrębie zbiornika Goczałkowice. Kryterium zmian była koncentracja barwników fotosyntetycznych pochodzących z dwudziestoczterogodzinnego cyklu życiowego komórek analizowanych techniką HPLC. Interpretując zmiany zachodzące w komórkach glonów hodowanych w wodach ze zbiornika Goczałkowice wyodrębniono okres lipcowej powodzi oraz jesiennych zmian jakości wód związanych z naturalnymi przemianami zachodzącymi w jeziorach. Wykazano również, że jakość wody w głównym nurcie strumienia wód wnoszonych do zbiornika przez Wisłę po okresie powodzi różniła się istotnie od jakości wód w innych miejscach zbiornika, zwłaszcza w jego wschodniej części.
CHANGES OF CHOSEN CHEMICAL ELEMENTS CONCENTRATION IN ALLUVIAL SEDIMENTS: AN EXAMPLE OF THE LOWER COURSE OF THE OBSA RIVER (WESTERN POLAND)

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Keywords: Chemical elements, alluvial deposits, the Obra River.

Abstract: Research concerning chemical constitution of alluvial sediments was done in the lower course of the Obra River (Western Poland). The fragment of vertical profile, which consisted of various alluvial sediments (fine sands, peats and sandy silts) was chosen for detailed analysis. Main research problem was to determine if lithology and chemical constitution of alluvial deposits are interconnected in a distinct way within studied section of the Obra river valley, and if changes of chemical elements concentration could be used to illustrate depositional processes which take place in river bed and floodplain. Concentrations of Fe, Mn, Cu, Zn, Ca, Mg and K were determined in collected sediment samples. Investigated changes of chemical elements concentration show that there is a distinct border between the organic sediments, which mark the place of former functioning of the Obra river bed, and sandy silts, which were deposited within floodplain during floods. Besides, dependence between lithologic variability of alluvial sediments and their chemical constitution was observed. However, this dependence is not clear in some cases. The authors concluded that it is necessary to use statistic analyses to define connection between lithology and chemical constitution of alluvial deposits (distinguishing geochemical groups of alluvial sediments).

INTRODUCTION

Studies regarding Late-glacial and Holocene sediments concern mainly the deposits, which were accumulated in lakes [11, 24, 29, 30]. The research works, which referred to chemical constitution of sediments, were done in the areas polluted with heavy metals originating from mine industry [10, 15, 17, 19] which were entrained to transport in a river bed during extreme events (floods, landslides) [27, 28]. The authors of the mentioned above research works tried to distinguish sedimentation periods, which took place before, during and after intensive mining exploitation of researched areas. They found out that it is difficult to achieve this goal because of secondary migration of investigated metals to the sediments, which had no such contaminants before. They also noted that the minerals, which contained these metals, were subjected to disintegration or metamorphosis [13]. Other research [28] pointed to very important role of floodplain in periodic accumulation of chemical elements together with sediment during flood and repeated entraining to
transport during next such event. The authors demonstrated that even 50% of chemical compounds transported annually in river bed cross-section can be deposited within flood-plains as in the case of the Aire and the Swale rivers (Great Britain) [28]. Other researchers [26] paid attention to various sources of origin of increased concentrations of zinc, lead, copper and cadmium in the Subernarekha River (India). In the upper course of the river, the investigated metals originated from natural sources (valley bedrock) and in the lower course, from industrial pollution [26].

This paper presents the first part of the study regarding the determination of connections between lithology and chemical constitution of alluvial sediments. In the second part of the study [23] an attempt was made to define these connections by distinguishing geochemical groups of alluvial sediments using cluster analysis.

Main research problem was to determine whether lithology and chemical constitution of alluvial sediments are connected within studied section of the Obra river valley and, whether changes of chemical elements concentration could be used to illustrate depositional processes, which take place in the river bed and floodplain. The following research tasks were done:

1. Lithology of alluvial deposits was recognized to choose vertical profiles, which geologic structure of which is particularly variable.
2. Analyses of chosen chemical elements concentration were done to determine connections between lithology and chemical constitution of alluvial sediments.
3. Analysis of Fe/Mn, Fe/Ca, Ca/Mg and Cu/Zn ratios was done to determine changes of oxidation/reduction conditions in alluvial deposits. On the basis of the analysis, an attempt was made to identify the dominant type of denudation within studied area.

The problem of concentration changes of chemical elements in alluvial deposits was a part of research concerning the lower course of the Obra river valley (Western Poland). The reasons of choosing this area were the following:

− the Obra River is the biggest water course situated between the Warta and the Odra rivers (Fig. 1), which flows through the middle and western part of Wielkopolska lowland;
− specific hydrological regime, which is influenced by the presence of lakes in the course of the Obra River. The lakes smooth the amplitude of water stages, discharges and floods [1, 4, 6]. The Obra River has a regular rhythm of water stages fluctuations. According to Dynowska [8] this water regime is counted among temperate regimes with spring flood and groundwater-rainfall water supply.

RESEARCH AREA

The study concerning changes of chemical elements concentration in alluvial deposits was done in the lower course of the Obra River, which flows through a geographic region named the Depression of Obra (Fig. 1). This is a concave form of landscape spreading from north-west to south-east. Detailed characteristics of this area relating to geologic structure, geomorphology and waters are presented in other publications [2, 7, 25].
Detailed research was done near St Wojciech village, 3.5 km north-west of the town of Międzyrzecz (Fig. 1). The Obra is a meandering river within the chosen section of the valley. Hydro-technical works, which took place in the past, were limited in this area and did not change neither the river bed geometry nor the structure of alluvial sediments. The Obra River formed its valley in glacial sediments represented by glacial till and fluvioglacial fine sands, which can be seen in exposures occurring in concave riverbanks.

**RESEARCH METHODS**

Tube sample borings were done in the Obra valley floor in five geological cross-sections (Fig. 2A) to choose vertical profiles, the geologic structure of which is particularly variable. The fragment of vertical profile from geologic cross-section no 1 (Fig. 2A) was chosen for detailed analysis. There are fine sands in the base of the profile (Fig. 2B) followed by peat sediments with numerous, 1–2 cm thick inserts of fine sands in its middle part. At the top of the profile, there are sandy silts (Fig. 2B).
A variable geological structure makes it possible to analyze changes of particular chemical elements concentration depending on lithologic variability of alluvial deposits. There are sediments within the fragment of investigated vertical profile, which have relatively high filtration coefficient (fine sands), and organic deposits, where migration of most macro- and microelements can be limited [20]. These sediments contain colloidal, mineral and humus substances, which form stable bonds with migrating substances and chemical elements. Peat sediments are the example of geochemical barrier of adsorptive type [20].

To determine changes of chosen chemical elements concentration in alluvial sediments, 71 samples were taken from the fragment of vertical profile. The samples were collected in 1 cm intervals. Next, the analyses of Fe, Mn, Zn, Cu, Ca, Mg and K were done. Changes of these elements concentration are interpreted in literature as indicators of particular type of environment (for example: reductive environment) or dominant type of denudation [3, 29]. The concentrations of particular chemical elements were defined using atomic absorption spectrometry method. The measurements were done using AAnalyst 300 Perkin Elmer spectrometer (atomization in air-acetylene flame after previous wet mineralization and dilution of the sample in concentrated nitric acid and hydrogen peroxide).

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and periods joined with more stable discharges (an increase of reductive conditions and concentrations of Fe).

Ca/Mg – refers to determining the type of denudation. Ca/Mg proportion can be used as indicator of domination of mechanic denudation (Ca < Mg) or chemical denudation (Ca > Mg) [3].

Fe/Mn and Zn/Cu – indicators of changes of oxidation/reduction conditions.

The fragment of investigated vertical profile was divided into the following three parts to analyze changes of chosen chemical elements concentrations:

- **bottom part**: fine sands, which are the result of point bar accumulation;
- **middle part**: peat sediments with inserts of fine sands. In the studied section of the Obra river valley, agglomerations of organic deposits occur in the places of former functioning of the river bed. The inserts of fine sands illustrate intensive flood events, which took place in the past [22];
- **top part**: sandy silts, which are the result of vertical accretion in the area of floodplain during floods.

The division presented above shows that the bottom part of the profile represents river bed sediments and the remaining two parts – the deposits, which were accumulated in the nearest vicinity of the river bed or in the area of floodplain.

It should be noted that the presented analysis refers only to the fragment of single vertical profile within studied section of the Obra river valley. Only preliminary conclusions regarding the research problems presented above can be suggested on the basis of this study.

**CHANGES OF THE CHEMICAL ELEMENTS CONCENTRATIONS IN ALLUVIAL SEDIMENTS: ...**

Sediments of the bottom part of the profile, represented by fine sands, are the result of point bar accumulation process, which was especially intensive during period of low water stages and relatively small intensity of lateral erosion [22]. Ca and Mg, the elements joined with mineral deposits, have the biggest concentrations here. High content of Ca (maximum 22.4 mg·g⁻¹, Fig. 3) is the result of its concentrations in glacial till, which underlies alluvial sediments. Ca is the chemical element which easily migrates in water environment. Mg (maximum 8.45 mg·g⁻¹, Fig. 3) is much less susceptible to migration in water and biological cycle. This element is much more related to sorption complex of soils and wastes. Besides, Mg is joined with clay grain-size fraction and increased content of silica [3]. The concentrations of K were also slightly increased (above 300 μg·g⁻¹, Fig. 3). This chemical element, as well as Mg, in the case of lacustrine sediments is connected to clay grain-size fraction [21] and silica content. Besides, it is inversely related to organic matter content [3, 29].
Fig. 3. Changes of concentration of chosen chemical elements in alluvial sediments of the Obra river valley (geologic section no 1, fragment of vertical profile no 18); 1 – fine sands, 2 – peats, 3 – sandy silts, A – bottom part of investigated fragment of vertical profile, B – middle part, C – top part; concentrations of Zn, Cu and K were presented in a scale 1000 times smaller than other chemical elements.
The sediments, which are situated in the middle part of the profile, were accumulated during the period of frequent occurrence of flood events and significant influence of lateral erosion process. These deposits mark the place where the Obra river bed was functioning in the past [22]. Organic sediments were deposited in a lateral, stagnant water channel, which was formed in the vicinity of laterally migrating river bed during the period of high water stages [22]. Accumulation of the sediments in stagnant water within such channel or within inundated fragment of floodplain was accompanied with reductive conditions. The presented results (Fig. 3) show that there are two zones in the profile with strongly reductive environment. The first one is situated at the depth of 1.67–1.77 m below land surface and is marked with high concentrations of Fe and Cu, which indicate reductive environment with sulphuretted hydrogen. Fe/Mn proportion is also high here. Research done by McArthur (in: [12]) show that the environment of peat sediments is the place of iron hydroxides reduction. According to Borówka [3] and Salminen et al. [21], Fe has low ability to migrate in such environment because of precipitation of iron sulphur, which is difficult to dissolve.

The second zone, at the depth of 1.95–2.03 m below land surface, is characterized by high concentrations of Cu, Mn and Zn. Increased concentrations of Mn are situated at the depths of 2.18 m below land surface (0.7 mg·g⁻¹) and 1.98 m below land surface (0.3 mg·g⁻¹, Fig. 3). Mn usually occurs in lower concentrations than Fe, which is the result of low solubility of its salts [9]. Increased Mn concentrations are accompanied by high Fe contents (more than 5 mg·g⁻¹). Presumably, there are reductive conditions with the presence of H₂S at the depths mentioned above. Cu and Zn are also indicators of such environment [3, 14, 20]. Increased Cu concentrations were detected in peat deposits at the depth of 2.03 m b.l.s. (9.5 μg·g⁻¹), 1.72 m b.l.s. (8.1 μg·g⁻¹) and 1.77 m b.l.s. (7 μg·g⁻¹) (Fig. 3). Zn content in peat sediments amounts 15–25 μg·g⁻¹ at the depth of 1.95–2.00 m b.l.s. (Fig. 3). The proportions of Cu/Zn and Fe/Mn are also high here (Fig. 4). It should be noted that low values of Cu/Zn ratio do not always mean the lack of reductive conditions and important role of oxidative environment. This could happen in the case when Zn concentrations exceed Cu content and indicate the domination of reductive conditions (Zn creates hardly soluble bonds in such environment [14, 20]).

The inserts of fine sands within peat deposits are the traces of intensive flood events, which caused inundation of floodplain. When water level was falling down, the transported material was accumulated as 1–2 cm thick layers of sand deposits. Increased contents of Ca, Mg and K were observed in some of such mineral inserts (Fig. 3). In fine sand layers with high concentrations of Ca, increased concentrations of K were detected (maximum 2.5 mg·g⁻¹, Fig. 3). However, increased contents of Mg and K also occur in peat deposits. This could be explained by increased content of mineral fraction in organic sediments. Such admixtures could be added to peats during floods of lesser magnitude. Besides, Mg and K could migrate vertically from the inserts of fine sands. When comparing concentration of Ca and Mg, it can be observed that the first of these two elements is characterized by much higher concentrations. According to Borówka [3] greater concentrations of Ca would suggest that chemical denudation is dominant within the studied area. Lower values of Ca/Mg ratio in most cases refer to fine sands in the profile (Fig. 4), which is the
result of an increase of Ca and Mg content within inserts of sand deposits (Fig. 3). However, such raise concerns not only fine sands but it is also observed within peat sediments. This may be caused by greater content of mineral material in some peat layers. Besides, glacial till, which is situated beneath alluvial sediments, can also influence the greater concentrations of Ca because it contains CaCO$_3$ [22]. The area of the Obra river valley is supplied with ground waters originating from Lubuska moraine plateau [5]. It is possible that the compounds of Ca were being washed out from glacial till and later redeposited in alluvial sediments. Such processes are still active, especially during flood events when Ca concentrations increase together with water level [22].

CHANGES OF THE CHEMICAL ELEMENTS CONCENTRATIONS IN THE TOP PART OF THE PROFILE

The top part of investigated profile consists of sandy silts, which are floodplain sediments. These deposits contain great amount of colloidal substances accumulated through decantation during flood events in stagnant waters within floodplain area. Distinct increase of Fe (maximum 61.5 mg·g$^{-1}$), Mn (3.35 mg·g$^{-1}$), Mg (3.4 mg·g$^{-1}$) and Zn (21 μg·g$^{-1}$) concentrations was observed here (Fig. 3). Such increase is illustrated by the highest in studied profile values of Fe/Mn and Fe/Ca ratios (Fig. 4). The biggest contents of Fe, Mn and Zn are usually present in mineral deposits enriched with colloidal substances [3, 21]. Research done by Liu et al. [16] has shown that heavy metals concentrations are 2–4 times greater in fine deposits than in the sediments enriched with coarser grain-size fractions. Similar results were achieved in the case of Pb, Zn, Cu, Ni and Cr concentrations in the deposits of the Mekhna River (Bangladesh) [12] where contents of these elements were much higher in silts than in sands.

Fe/Ca ratio also has maximum value (> 6) in the top part of the profile (Fig. 4). In the case of the deposits of the Obra river valley this proportion does not reflect water level changes in the past. Alluvial sediments are formed in the environment of much greater dynamics and variability than lacustrine sediments where Fe/Ca ratio can be used to reconstruct changes of water level [29].

Silts are the finest deposits present within studied section of the Obra river valley (mean diameter: 3 phi) [22]. In the top part of studied profile, rapid increase of concentration of chemical elements mentioned above (Fig. 3) marks the border between the environment of organic sediments, which were accumulated in the place where the Obra river bed was functioning in the past, and the environment of floodplain sediments.

CONCLUSIONS

On the basis of the analysis of chosen chemical elements concentrations within investigated fragment of vertical profile, the following conclusions can be drawn:

1. In the studied fragment of the profile, there are two zones representing strongly reductive conditions with the content of H$_2$S within peat deposits. They are situated at the depths of 1.67–1.77 below land surface and 1.95–2.03 m below land surface, and are characterized by increased contents of Fe, Mn, Cu and Zn. Reductive conditions in these two zones are strongly connected with the way of forming peat sediments. Peats were accumulated on wet area of floodplain or in a lateral, stagnant water...
Fig. 4. Changes of Fe/Mn, Fe/Ca, Ca/Mg and Cu/Zn ratio regarding to alluvial sediments of the Obra river valley (geological section no 1, investigated fragment of vertical profile no 18); explanations are the same as at Fig. 3
channel, which was shaped by migrating Obra river bed during period of frequent flood events. It can be suggested that organic sediments mark the place where the Obra river bed was functioning in the past [22].

2. There is a distinct border between the environment of organic sediments, which fill the place of former river bed and the environment of floodplain represented by silt deposits (accumulated through vertical accretion during flood events within inundated fragment of floodplain). The border is marked with rapid increase of Fe, Mn, Mg and Zn concentrations (Fig. 3).

3. High values of Ca/Mg ratio show much greater contents of Ca in the profile. According to Borówka [3] this would suggest that chemical denudation is dominant within investigated section of the valley.

4. The dependence between lithologic variability of alluvial deposits and its chemical constitution is observable. There is increase of Ca, Mg and K content in the inserts of fine sands within peat deposits. These 1–2 cm thick sandy layers illustrate especially intensive flood events in the past [22]. However, it should be noted that increased contents of these chemical elements also occur in peat deposits. This may be caused by admixtures of mineral material in organic sediments.

5. On the basis of achieved results it is difficult to distinguish particular geochemical groups of alluvial deposits. Floodplain sediments (sandy silts) in the top part of the profile are the only exception (conclusion no. 2). Determining the dependence between lithology and chemical constitution of alluvial sediments can be done in more detailed way using statistical analyses.

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kach osadów określono koncentrację żelaza, manganu, miedzi, cynku, wapnia, magnezu i potasu. Wykazano, że zmiany koncentracji pierwiastków chemicznych wskazują na wyraźną granicę pomiędzy środowiskiem osadów organicznych wypełniających miejsce dawnego funkcjonowania koryta, a środowiskiem pozakorytowym reprezentowanym przez osady mułkowe. Ponadto w badanym fragmencie profilu zauważalny jest związek między zmiennością litologiczną osadów aluwialnych dna doliny Obry a ich składem chemicznym. Zależność ta nie jest jednak ścisła. Wskazano na konieczność wykorzystania analiz statystycznych w celu bardziej szczegółowego określenia związku między litologią i składem chemicznym osadów rzecznych (wydzielenie grup geochemicznych osadów).
GEOCHEMICAL GROUPS OF ALLUVIAL SEDIMENTS OF THE LOWER COURSE OF THE OBRA RIVER: AN EXAMPLE OF USING CLUSTER ANALYSIS

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Keywords: Cluster analysis, Ward’s method, alluvial deposits, chemical elements, the Obra River.

Abstract: Research concerning changes of chosen chemical elements concentration in alluvial sediments was conducted in the lower course of the Obra river valley. The analyses of Fe, Mn, Cu, Zn, Ca, Mg and K concentration were done in a fragment of vertical profile, which was characterized by variable lithology. On the basis of statistical analysis (cluster analysis) an attempt was made to distinguish geochemical groups of alluvial sediments of the Obra river valley. Six geochemical groups, which represent reductive conditions within peat deposits, the environment of flood sediments (inserts of fine sands within peats and sandy silts in the top of the profile) and the environment of river bed sediments (fine sands in the bottom part of the profile), were singled out. Results of the study show that it is possible to distinguish the above mentioned depositional environments on the basis of variations of sediments’ chemical constitution.

INTRODUCTION

The first part of the study [12] has shown that it was difficult to distinguish geochemical groups of alluvial sediments, which would represent particular types of depositional environment. In this paper, which presents results of the second part of the study, cluster analysis was used to single out geochemical groups of alluvial sediments. This analysis allows to combine together particular objects or features on the basis of similarity function [4]. The aim of grouping is to obtain n groups of objects in such a way that the loss of information during grouping process in minimized. The first step is joining two objects (features), which results in the smallest square distances increase between the two grouped objects (features) [1]. Another stage is checking if the third object can be included in a 2-element cluster formed in the first stage of the analysis or can form another cluster. The procedure is repeated until the moment when all objects and clusters of objects are connected together [13]. Connection of objects or features is done using one of agglomeration methods [13] and matrix of square distances. Each cluster refers to definite level described by Euclidean distance [4].
Cluster analysis is widely applied in various studies concerning medical problems [9, 10, 15] or regarding the choice of variables to neural network model [14]. In the case of natural studies, this method was used to distinguish types of meadow plants [7]. Cluster analysis was also applied to sort out climate types in the area of Saudi Arabia [1] and Europe [3] using the function which helped to establish optimal number of achieved clusters [3]. Another study [2] concerned intensity of erosion and mass movements. Using cluster analysis, interactions between river network density, type of vegetation and land use were shown. Besides, the units of land use type, which are endangered with erosion of various intensity, were sorted out [2]. In the case of research regarding intensity of soils erosion using Cs\(^{137}\) radionuclide method, cluster analysis was used to distinguish among soil erosion classes depending on lithology, location of studied profiles and slope shape [8]. The analysis was also applied to determine the dependence between soil type and shape of slopes [16].

In the second part of the research, which is presented in this paper, the main study problem was to define more closely the connections between type of sediment, its chemical constitution and properties of depositional environment. The following research tasks were done:

1. Determination of variability of alluvial deposits lithology.
2. Analyses of chosen chemical elements concentration in alluvial sediments of investigated fragment of vertical profile.
3. Distinguishing geochemical groups of the Obra river valley alluvial deposits using cluster analysis.

Description of research area and figures illustrating its localization were presented in the first part of presented study [12].

**RESEARCH METHODS**

A fragment of the vertical profile from the middle part of geologic cross-section no. 1 was chosen for detailed analysis (Fig. 2A in [12]). The deposits of the profile, the way of samples collection and laboratory analyses were described in the first part of the study [12]. Variable geologic structure makes it possible to analyze changes of particular chemical elements concentration depending on lithologic variability of alluvial deposits. Within the fragment of investigated vertical profile, there are sediments, which have relatively high filtration coefficient (fine sands), and organic deposits, where migration of most macro- and microelements can be limited [6]. Concentrations of the following chemical elements were subjected to detailed analysis: Fe, Mn, Cu, Zn, Mg, Ca and K.

Geochemical groups of the Obra river valley alluvial sediments were distinguished on the basis of concentration changes of particular chemical elements in the studied fragment of vertical profile (Fig. 2). The groups were singled out using cluster analysis (Ward’s method, 1-r Pearson’s distances) [4, 13] (Fig. 1). Ward’s method is different from other agglomeration methods. It uses variance analysis to determine distances between clusters. It also assumes minimal square distance between each two clusters, which are formed during particular stages of analysis. It is thought to be one of the best agglomeration techniques [5] and is frequently used. In the simulation, which was done by Mangiamelli et al. [5], 252 datasets were tested using all agglomeration techniques. Ward’s method gave one of the best results in joining objects into logically ordered clusters.
Before the analysis, the input data were standardized to eliminate considerable difference between values of particular variables (chemical elements concentrations). The values were standardized using formula:

\[
\frac{x_i - \bar{x}}{\delta}
\]

where:
- \(x_i\) – value of variable \(x_i\),
- \(\bar{x}\) – mean value of variable,
- \(\delta\) – standard deviation.

The following variations of chemical elements concentrations were taken into account when forming geochemical groups:

1. Distinct increase or decrease of chemical element concentration.
2. Referring chemical constitution of alluvial sediments to its lithology within each distinguished group of clusters. Recognition of geologic structure of analyzed profile makes it possible to describe processes, which formed particular types of sediments. Knowledge concerning properties of depositional environment may be useful to analyze the way of accumulation of particular chemical elements in various types of deposits.

Six geochemical groups were distinguished. Mean value of each chemical element was calculated within each group. The obtained results are presented in Table 1.

RESULTS OF THE STUDY

The following classification geochemical group of alluvial sediments of the Obra river valley was done:

\textit{I – geochemical group of fine sands and peats containing inserts of fine sands, characterized by increased Ca, Mg and K concentrations (Fig. 1)}

The sediments, which belong to this group, are situated in the bottom part of the studied fragment of vertical profile at depth 2.24–2.27 m below land surface (fine sands) and 2.04–2.12 m b.l.s. (peats with inserts of fine sands) (Fig. 2). This geochemical group is featured with high concentrations of Ca (mean concentration: 10.88 mg⋅g⁻¹, maximum: 22.4 mg⋅g⁻¹) and distinct variations of Mg (maximum concentration: 8.45 mg⋅g⁻¹) and K content (mean concentration 131.6 μg⋅g⁻¹, Tab. 1). Mg and K are accumulated within colloidal elements of sand deposits. Ca is one of the main chemical components of the Obra river valley, which is the result of high Ca concentrations in glacial till beneath alluvial

<table>
<thead>
<tr>
<th>Group</th>
<th>Fe [mg⋅g⁻¹]</th>
<th>Mn [mg⋅g⁻¹]</th>
<th>Ca [mg⋅g⁻¹]</th>
<th>Mg [mg⋅g⁻¹]</th>
<th>Zn [μg⋅g⁻¹]</th>
<th>K [μg⋅g⁻¹]</th>
<th>Cu [μg⋅g⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3.25</td>
<td>0.217</td>
<td>10.88</td>
<td>1.59</td>
<td>11.44</td>
<td>131.6</td>
<td>1.78</td>
</tr>
<tr>
<td>II</td>
<td>3.08</td>
<td>0.16</td>
<td>9.6</td>
<td>1.37</td>
<td>18.69</td>
<td>222.71</td>
<td>2.5</td>
</tr>
<tr>
<td>III</td>
<td>1.72</td>
<td>0.061</td>
<td>4.74</td>
<td>0.476</td>
<td>10.06</td>
<td>180.8</td>
<td>2.14</td>
</tr>
<tr>
<td>IV</td>
<td>4.48</td>
<td>0.107</td>
<td>3.38</td>
<td>0.631</td>
<td>9.51</td>
<td>101.64</td>
<td>3.88</td>
</tr>
<tr>
<td>V</td>
<td>5.31</td>
<td>0.234</td>
<td>5.05</td>
<td>0.937</td>
<td>11.29</td>
<td>69.74</td>
<td>2.16</td>
</tr>
<tr>
<td>VI</td>
<td>22.55</td>
<td>0.695</td>
<td>4.49</td>
<td>0.889</td>
<td>13.25</td>
<td>92.27</td>
<td>1.9</td>
</tr>
</tbody>
</table>
sediments [11]. Deposits belonging to this group were accumulated in most part during formation of meander point bar in period of low water levels [11] and represent river bed depositional environment.

**II – geochemical group of peat deposits with inserts of fine sands, characterized by increased concentrations of Ca, Mg and K, and high contents of Zn and Cu (Fig. 1)**

Distinguished geochemical group is situated at the depth of 1.96–2.00 m below land
surface, 1.89 m b.l.s. and 1.92 m b.l.s. (Fig. 2). It is featured with increased concentrations of Ca (mean concentration: 9.6 mg·g⁻¹, Tab. 1) and chemical elements joined with clay grain-size fraction and sorption complex of wastes (Mg with mean concentration 1.37 mg·g⁻¹ and K with mean concentration 222.71 μg·g⁻¹, Tab. 1). Comparatively high contents of these elements are probably the result of the presence of fine sand inserts within...
peat deposits. Similar situation can be observed in the case of group I. However, group II is characterized by high contents of Zn (maximum concentration: 24.3 μg⋅g⁻¹) and Cu (maximum concentration: 3.77 μg⋅g⁻¹), which are the result of strongly reductive environment within peat deposits, with presence of sulphuretted hydrogen. Such conditions accompanied the accumulation process of organic deposits within inundated fragment of floodplain or in a lateral channel, which was formed during the period of high water levels [11]. It can be seen that mean concentrations of Zn and Cu in group II (Tab. 1) are distinctly higher than in group I.

III – geochemical group of peat deposits with inserts of fine sands, featured with increased concentrations of K and low contents of Fe, Mn, Ca and Mg (Fig. 1)

Group III is represented by the sediments at the depths of 1.77–1.87 m below land surface, 1.90–1.91, 1.93 and 2.01–2.02 m b.l.s. (Fig. 2). It is characterized by high concentrations of K, which reach maximally 200 μg⋅g⁻¹. K indicates close connection with clay grain-size fraction content. Increased concentrations of K are probably the result of increased amount of mineral admixtures in peats. Fe and Mn have low concentrations within this group (Tab. 1). These chemical elements are subjected to active migration in reductive conditions without the presence of sulphuretted hydrogen. Zn content is also low, because this element migrates easily in acid environment (also without the presence of sulphuretted hydrogen).

IV – geochemical group of peat deposits with high concentrations of Cu and Mn (Fig. 1)

The discussed group of alluvial sediments is situated at the depths of 2.17–2.23 m below land surface and 1.73, 1.76, 1.88, 2.03 m b.l.s. (Fig. 2). The group is joined only with peats and with strongly reductive environment (high Cu concentrations – maximum 9.19 μg⋅g⁻¹ and Mn concentrations – maximum 0.717 mg⋅g⁻¹). Other chemical elements are featured with relatively low contents in this group (Fig. 1, Tab. 1). Sediments of group IV were formed in the way similar to group II. Two separate groups were distinguished because of rapid increase of Mn (2.17 m b.l.s.) and Cu concentrations (1.73 and 1.76 m b.l.s.) in group IV.

V – geochemical group of peats with inserts of fine sands, characterized by high concentrations of Fe, Mn, Ca and Mg (Fig. 1)

The deposits, which belong to group V, are situated at the depth of 2.13–2.16 m below land surface (Fig. 2). Increased concentrations of Mn (mean concentration: 0.234 mg⋅g⁻¹, Tab. 1) and Ca (mean concentration: 5.05 mg⋅g⁻¹, Tab. 1) were noted in this group. Increased content of Mn could be the effect of reductive conditions in peat sediments. It should be noted that mean Mn concentration (0.234 mg⋅g⁻¹, Tab. 1) is higher than in group IV. Besides, rapid increase of concentrations of Mn is not observed in this group. High contents of Ca are joined with inserts of fine sands in peats. Similar situation occurred in group II. However, mean Ca concentration is almost two times higher in group II than in group V (Tab. 1).

VI – geochemical group of silt and peat deposits with high concentrations of Fe, Mn, Ca, Mg and Zn (Fig. 1)

The discussed group is situated in the top part of the profile consisting of peats and sandy silts, which were deposited through vertical accretion in the area of floodplain during floods. The maximum concentrations of Fe (61.5 mg⋅g⁻¹), Mn (3.356 mg⋅g⁻¹) and Ca
(10.88 mg·g⁻¹) occur in silt deposits and are the result of precipitation of these metals in the form of colloidal suspensions. Increased amounts of Fe (12.56 mg·g⁻¹) are also present within peat deposits in this group (Fig. 2). Here, they are caused by reductive conditions with the presence of sulphuretted hydrogen. It can be seen that rapid increase of Fe, Mn, Mg and Zn concentrations shows a distinct border between flood sediments and organic deposits, which mark the place, where the Obra river bed was active in the past.

CONCLUSIONS

Distinguished geochemical groups of alluvial sediments show the following environment types:

- reductive environment within peat deposits (high concentrations of Fe, Mn, Cu and Zn) observed in the deposits, which belong to geochemical groups II, IV and V. Organic deposits within these groups were accumulated on inundated fragment of floodplain or in a lateral, stagnant water channel, which was formed during period of high water stages;
- flood deposits environment with high concentrations of Ca, Mg and K modified by migration of these elements in groundwater. The environment is represented by thin layers of fine sands within peat deposits (geochemical group I, II and V). Sandy sediments were accumulated during intensive floods. However, it should be noted that the connection between thin inserts of fine sands and changes of studied elements concentration is not precise enough to distinguish geochemical group, which would contain only thin layers of fine sands in the profile;
- flood deposits environment with high contents of Fe, Mn, Ca, Mg and Zn (top part of group VI). Silt deposits, which belong to this group, were accumulated through vertical accretion. The process of chemical elements precipitation in the form of colloidal suspensions played very important role here;
- river bed environment (fine sands of meander point bar in the bottom part of the profile) with increased concentrations of Ca, Mg and K (geochemical group I). The sediments, which belong to this group, were deposited during accumulation process on the convex bank of the Obra river bed (point bar deposition) during period of low water levels.

The conclusions presented above should be treated initially and not fully solving the problem of changes of chemical elements concentrations in alluvial sediments as indicators of particular type of depositional environment. In this study, only a fragment of vertical profile was investigated. It should be noted that the obtained results include some disturbances caused by changes of chemical constitution, which take place within alluvial deposits. The reason could be migration of chemical elements in groundwater environment, especially in mineral deposits characterized by relatively high filtration coefficient.

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GRUPY GEOCHEMICZNE OSADÓW ALUWIALNYCH DOLNEGO ODCINKA DOLINY OBRY:
PRZYKŁAD ZASTOSOWANIA ANALIZY SKUPIEŃ

W dolnym odcinku rzeki Obry przeprowadzono badania nad zmianami koncentracji pierwiastków chemicznych w osadach aluwialnych. Analizy zawartości żelaza, manganu, miedzi, cynku, wapnia, magnezu i potasu przeprowadzono we fragmencie profilu pionowego cechującego się zróżnicowaną budową litologiczną. Na podstawie analiz statystycznych (analiza skupień) podjęto próbę wydzielenia grup geochemicznych osadów aluwialnego wypełnienia dna doliny Obry. Wydzielinio sześć grup geochemicznych osadów reprezentujących środowisko redukcyjne w obrębie osadów torfowych, środowisko pozakorytowe (warstwy piasków drobnoziarnistych w torfach oraz mułki piaszczyste w stropie profilu) oraz środowisko korytowe (piaski drobnoziarniste w spągu profilu). Z przeprowadzonych badań wynika, że rozróżnienie wyżej wymienionych środowisk sedymenta-

MARCIN SŁOWIK, TADEUSZ SOBCZYŃSKI, ZYGMUNT MŁYNARCZYK
INFLUENCE OF SOIL CONTAMINATION WITH NICKEL AND LIMING ON LEAD AND MANGANESE CONTENTS IN RED CLOVER BIOMASS

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Keywords: Soil contamination with nickel, liming, red clover, lead, manganese.

Abstract: In general, industrial pollution contributes to deeper degradation of agricultural production space, which leads to accumulation of heavy metals in soils. Nickel is a heavy metal. At small amounts, it is necessary for the growth and development of living organisms, while it is toxic in excess. The influence of soil contamination with nickel (50, 100, and 150 mg Ni/kg of soil – NiSO₄⋅7H₂O) on Pb and Mn at red clover was studied in four-year pot experiments on a background of varied liming levels (according to 0.5, 1, and 1.5 H₂ of soil – CaCO₃). Metal contents were determined by means of ICP-AES technique after sample dry digestion in muffle furnace at 450°C and dissolution of ash in 10% HCl. The results were statistically processed using variance analysis and F-Fisher-Snedecor’s distribution according to F.R. Anal. ver. 4.1. software, while LSD₀.₀⁵ values were calculated according to Tukey’s test. To find interactions between studied traits, a linear correlation analysis was performed. Both metals concentrations at plants grown on polluted soils were higher as compared to those cultivated on non-contaminated ones, which may indicate the synergism between nickel and both discussed heavy metals. Applied liming (regardless the amount of CaCO₃) caused significant decrease of both metals contents at a test plant. The studies revealed synergistic dependencies between nickel and lead and manganese.

INTRODUCTION

Contamination of agricultural production area due to heavy metals has been the subject of many studies for over twenty years. The elements contents at vegetables, namely in their parts for direct consumption, are of special interests. However, there are few works upon the heavy metals content in general crop species cultivated under production conditions [2, 8, 12, 14, 15].

Soil is a principle source of heavy metals for plants. Due to the soil, majority of metals are incorporated into the trophic chain [7, 11]. The process of their uptake by plants depends on a variety of factors: their total contents in the soil, sorption capacity, acidity, organic matter content, humidity [6, 9, 12], and other heavy metals the up taken element can enter the antagonistic or synergistic reactions with [1, 3].

The aim of the present study was to evaluate the influence of soil contamination with nickel on lead and manganese contents at red clover (Trifolium pratense L.) on a background of varied liming.
MATERIAL AND METHODS

The 4-year pot experiments were carried out at a greenhouse of University of Podlasie in Siedlce in completely randomized pattern in three replications. The following factors were examined:

I. soil contamination with nickel (50, 100, 150 mg Ni/kg soil);
II. liming (without liming or with liming in doses calculated for 0.5, 1.0, and 1.5 $H_h$ of soil).

Liming was applied at the beginning of May in a form of CaCO$_3$, while nickel was introduced into the soil at the beginning of June in a form of NiSO$_4$\cdot7H$_2$O solution. The 15 dm$^3$ pots were filled with 10 kg of soil material collected from the humus layer of dusty sandy loam podsollic soil with the following physicochemical features: pH in 1 M KCl – 5.49, $C_{org}$ – 6.5 g/kg soil, $N_{tot}$ – 0.61 g/kg soil, available P and K [mg/kg soil] – 71 and 110, respectively; total contents of Ni, Pb, and Mn [mg/kg] – 10.1, 5.9, and 76.0, respectively. Pots were left with no tillage and cultivation for one vegetation period, maintaining the moisture contents at 60% of the field water capacity, and then red clover (Trifolium pretense L. of Jubilatka cv.) was cultivated for four seasons. Every year, before sowing, the following mineral fertilization was applied: N – 0, P – 0.053 g/kg of soil in a form of granulated triple superphosphate (19% P), K – 0.17 g/kg of soil in a form potassium salt (41% K).

For four years and for every red clover cut, concentrations of Pb and Mn in each cut of test plant were determined by means of ICP-AES technique after dry digestion in muffle furnace at 450°C and subsequent ash grinding and dissolution in 10% HCl [13]. Results were statistically processed using variance analysis and F-Fisher-Snedecor’s distribution according to F.R. Anal. ver. 4.1. software, while LSD$_{0.05}$ values were calculated according to Tukey’s test. To find interactions between studied traits, a linear correlation analysis was also performed.

RESULTS AND DISCUSSION

Lead contents at particular cuts of plants (Figs 1–4) were significantly differentiated by both examined factors: soil contamination with nickel and liming. Less lead was found at plants of the 2$^{nd}$ and 3$^{rd}$ cuts of plants cultivated with uncontaminated soil for all study years than those grown on contaminated soils regardless the nickel amount introduced into the soil. In majority, there were statistically significant differences. At the same time, for all years, in 1$^{st}$ cut plants as well as in the first and fourth years in 3$^{rd}$ cut plants cultivated on soils limed with the highest calcium rate calculated according to 1.5 $H_h$, lower lead contents in relation to red clover grown on non limed and limed using lower calcium doses were recorded, and the differences were in most cases significant. Both studied factors differentiated mean values (for four years) of lead level in particular red clover cuts (Tab. 1). Considerably lower mean concentration of lead was found at plants of the first three cuts and grown on soils that were not nickel-contaminated as compared to those cultivated on contaminated soils, regardless the pollution extent. Different dependencies were observed in 4$^{th}$ cut plants. Considerably the lowest mean lead concentration was determined at plants cultivated on soils polluted with nickel to the highest level (150 mg Ni/kg of soil). In the case of all red clover cuts in plants grown on limed soils according to 1.5 $H_h$, lower mean value of discussed element was recorded as compared to plants
grown on other fertilization objects, and in majority there were significant differences. For all experimental years, the lowest mean lead content was found in plants grown on soils that were not polluted with nickel (Fig. 5) and those limed with the highest calcium dose calculated according to 1.5 $H_h$ of soil (Fig. 6), which was consistent with studies by Lipiński and Lipińska [7]. Those authors examined the influence of organic matter and acidity on heavy metals contents in soils and plants of green lands finding that the soil acidity exerted the strongest effects on heavy metals concentrations. The increase of pH contributed to the decrease of lead content at these plants. For all experimental years, lead contents at particular cuts of red clover did not exceed 10 mg/kg D.M., which allows for using it for fodder purposes [2, 5].

Fig. 1. Lead content in red clover [mg/kg D.M.] (first experimental year)

<table>
<thead>
<tr>
<th>LSD_{0.05}</th>
<th>I cut</th>
<th>II cut</th>
<th>III cut</th>
</tr>
</thead>
<tbody>
<tr>
<td>doses of nickel</td>
<td>n.i.</td>
<td>0.534</td>
<td>0.454</td>
</tr>
<tr>
<td>liming</td>
<td>0.653</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>interaction: doses of nickel x liming</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
</tbody>
</table>

Fig. 2. Lead content in red clover [mg/kg D.M.] (second experimental year)

<table>
<thead>
<tr>
<th>LSD_{0.05}</th>
<th>I cut</th>
<th>II cut</th>
<th>III cut</th>
<th>IV cut</th>
</tr>
</thead>
<tbody>
<tr>
<td>doses of nickel</td>
<td>n.i.</td>
<td>0.648</td>
<td>0.438</td>
<td>n.i.</td>
</tr>
<tr>
<td>liming</td>
<td>0.582</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>interaction: doses of nickel x liming</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
</tbody>
</table>
Fig. 3. Lead content in red clover [mg/kg D.M.] (third experimental year)

LSD_{0.05} for:
doses of nickel | n.i. | 0.287 | 0.584 | n.i. 
liming | 0.621 | n.i. | n.i. | n.i. 
interaction: doses of nickel x liming | n.i. | n.i. | n.i. | n.i. 

Fig. 4. Lead content [mg/kg D.M.] in red clover (fourth experimental year)

LSD_{0.05} for:
doses of nickel | n.i. | 0.310 | 0.365 | n.i. 
liming | 0.472 | n.i. | 0.365 | n.i. 
interaction: doses of nickel x liming | n.i. | n.i. | n.i. | n.i. 

Fig. 5. Lead content (mean for cuts) in red clover depending on soil contamination with nickel [mg/kg D.M.]
Fig. 6. Lead content (mean for cuts) in red clover depending on different liming [mg/kg D.M.]

Table 1. Four-year mean lead content in red clover [mg/kg D.M.]

<table>
<thead>
<tr>
<th>Cut</th>
<th>Doses of nickel [mg/kg soil]</th>
<th>Liming according to $H_h$</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 $H_h$</td>
<td>0.5 $H_h$</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>1.50</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2.33</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.42</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>1.93</td>
<td>1.88</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>2.05</td>
<td>1.95</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>1.18</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>1.51</td>
<td>1.35</td>
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<tr>
<td></td>
<td>100</td>
<td>1.64</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>2.05</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1.59</td>
<td>1.48</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>1.19</td>
<td>1.29</td>
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<tr>
<td></td>
<td>50</td>
<td>2.07</td>
<td>1.86</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.32</td>
<td>2.04</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>1.98</td>
<td>1.75</td>
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<tr>
<td></td>
<td>Mean</td>
<td>1.89</td>
<td>1.73</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>1.65</td>
<td>2.09</td>
</tr>
<tr>
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<td>2.53</td>
<td>2.30</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.32</td>
<td>2.26</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>2.03</td>
<td>2.09</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>2.13</td>
<td>2.18</td>
</tr>
</tbody>
</table>

LSD$_{0.05}$ for:
doses of nickel 0.210 0.178 0.183 0.107
liming 0.210 0.178 0.183 0.107
interaction: doses of nickel x liming 0.419 n.i. 0.365 0.214
The statistical analysis revealed significant dependencies between soil contamination with nickel and mean value (for four years) of lead content at particular cuts of red clover. The correlation coefficients were: for the 2\textsuperscript{nd} cut $r = 0.83^{**}$ ("**" – significant at 0.01), for the 3\textsuperscript{rd} cut $r = 0.70^{**}$, for the 4\textsuperscript{th} cut $r = 0.89^{**}$. Positive values of these coefficients may prove the synergism of nickel and lead. The analysis also revealed significant interactions between soil liming and content (mean for experimental years) of lead at particular cuts of red clover. The correlation coefficients amounted to: for the 1\textsuperscript{st} cut $r = -0.78^{**}$, for the 2\textsuperscript{nd} cut $r = -0.72^{**}$, for the 3\textsuperscript{rd} cut $r = -0.67^{**}$, and for the 4\textsuperscript{th} cut $r = -0.51^{*}$ ("*" – significant at 0.05). Negative values of these coefficients confirm well-known fact that the solubility of heavy metals as well as mobility decreases with the increase of pH value [4, 7, 9, 10].

Contents of the second studied heavy metal – manganese – at red clover of particular cuts are presented in Figures 7–10. In the first experimental year (Fig. 7), in 2\textsuperscript{nd} cut plants grown on soils not contaminated with nickel, lower level of the metal was found than at those cultivated on polluted soils; the differences were statistically significant in relation to those grown on soils where 150 mg Ni/kg of soil was introduced. Similar dependence was observed for the 3\textsuperscript{rd} cut, for which in plants cultivated on soil contaminated with nickel to the highest extent, significantly higher content of examined metal was recorded as compared to red clover grown on less polluted soils (50 and 100 mg Ni/kg of soil). At the same time, considerably lower manganese concentration was recorded in the 2\textsuperscript{nd} cut plants cultivated on non-limed vs. limed soils, regardless the amount of the calcium fertilizer introduced into the soil, which was not consistent with other authors’ findings [2, 3, 5], and which can be attributed to slow transformations of calcium fertilizers in soil. In plants of 3\textsuperscript{rd} and 4\textsuperscript{th} cuts in the second year (Fig. 8), 1\textsuperscript{st}, 2\textsuperscript{nd}, and 3\textsuperscript{rd} cuts in the third year (Fig. 9), as well as 2\textsuperscript{nd}, 3\textsuperscript{rd}, and 4\textsuperscript{th} cuts in the last experimental year (Fig. 10) cultivated on non-nickel-contaminated soils, lower levels of manganese in relation to those grown on polluted soils were found, and in most cases, the contents of the metal in plants increased along with the increase of nickel amounts introduced into the soil. At the same time, at plants of the 1\textsuperscript{st} and 4\textsuperscript{th} cuts in the second year (Fig. 8), 1\textsuperscript{st}, 2\textsuperscript{nd}, and 4\textsuperscript{th} cuts in the third year (Fig. 9), and 3\textsuperscript{rd} and 4\textsuperscript{th} cuts in the fourth year (Fig. 10) cultivated on non-limed soils, higher contents of manganese were recorded, and in majority, its concentration at plants decreased along with the increase of calcium rate introduced as a fertilizer.

![Fig. 7. Manganese content in red clover [mg/kg D.M.] (first experimental year)](image-url)

<table>
<thead>
<tr>
<th>LSD (_{0.05}) for:</th>
<th>I cut</th>
<th>II cut</th>
<th>III cut</th>
</tr>
</thead>
<tbody>
<tr>
<td>doses of nickel n.i.</td>
<td>3.759</td>
<td>4.635</td>
<td></td>
</tr>
<tr>
<td>liming n.i.</td>
<td>3.759</td>
<td>n.i.</td>
<td></td>
</tr>
<tr>
<td>interaction: doses of nickel x liming n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 8. Manganese content in red clover [mg/kg D.M.] (second experimental year)

\[
\begin{align*}
\text{LSD}_{0.05} & \text{ for:} \\
\text{doses of nickel} & \quad \text{n.i.} \quad \text{n.i.} \quad 4.396 \quad 5.018 \\
\text{liming} & \quad 5.333 \quad \text{n.i.} \quad \text{n.i.} \quad 5.018 \\
\text{interaction: doses of nickel x liming} & \quad \text{n.i.} \quad \text{n.i.} \quad \text{n.i.} \quad \text{n.i.}
\end{align*}
\]

Fig. 9. Manganese content in red clover [mg/kg D.M.] (third experimental year)

\[
\begin{align*}
\text{LSD}_{0.05} & \text{ for:} \\
\text{doses of nickel} & \quad 3.783 \quad 5.055 \quad 4.415 \quad \text{n.i.} \\
\text{liming} & \quad 3.783 \quad \text{n.i.} \quad \text{n.i.} \quad 3.793 \\
\text{interaction: doses of nickel x liming} & \quad \text{n.i.} \quad \text{n.i.} \quad \text{n.i.} \quad \text{n.i.}
\end{align*}
\]

Fig. 10. Manganese content in red clover [mg/kg D.M.] (fourth experimental year)

\[
\begin{align*}
\text{LSD}_{0.05} & \text{ for:} \\
\text{doses of nickel} & \quad \text{n.i.} \quad 3.781 \quad 3.033 \quad 5.860 \\
\text{liming} & \quad \text{n.i.} \quad \text{n.i.} \quad \text{n.i.} \quad 5.860 \\
\text{interaction: doses of nickel x liming} & \quad \text{n.i.} \quad \text{n.i.} \quad \text{n.i.} \quad \text{n.i.}
\end{align*}
\]
Mean value of manganese content for all experimental years at particular cuts of red clover significantly differentiated (Tab. 2), both due to soil nickel contamination, and liming. In all cuts plants grown on soils that were not polluted with nickel, significantly lower manganese contents were recorded as compared to its concentration in plants cultivated on contaminated soils. Plants of all cuts cultivated on soils polluted with nickel to the highest extent (150 mg Ni/kg of soil) were characterized by considerably the highest manganese content, which seems to confirm a thesis on the synergism of nickel and manganese. In plants of the 1\textsuperscript{st}, 2\textsuperscript{nd}, and 4\textsuperscript{th} cuts grown on non-limed soils, significantly higher manganese contents were found in relation to red clover cultivated on limed soils; in most cases, its content in plants considerably decreased along with the increase of calcium fertilizer rate introduced into the soil. In the first experimental year, the lowest mean value (for cuts) of discussed element was found in plants cultivated on soils, to which 50 mg Ni/kg of soil was introduced (Fig. 11) as well as those grown on non-limed soils (Fig. 12). In subsequent years, the lowest mean manganese levels for cuts were recorded in plants cultivated on soils that were not polluted with nickel (Fig. 11), and those grown on soil limed with the highest calcium fertilizer rate calculated according to 1.5 H\textsubscript{h} of soil (Fig. 12), which was confirmed by experiments of Rogoż [9].

Values of correlation

<table>
<thead>
<tr>
<th>Cut</th>
<th>Doses of nickel [mg/kg soil]</th>
<th>Liming according to H\textsubscript{h}</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 H\textsubscript{h}</td>
<td>0.5 H\textsubscript{h}</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>25.5</td>
<td>26.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>27.2</td>
<td>25.7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>30.5</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>32.5</td>
<td>29.1</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>28.9</td>
<td>27.2</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>24.7</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>23.6</td>
<td>23.5</td>
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<td>Mean</td>
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<td>24.0</td>
</tr>
<tr>
<td>III</td>
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<td>11.7</td>
<td>13.2</td>
</tr>
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<td>50</td>
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<td>17.1</td>
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</tr>
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<td>150</td>
<td>20.1</td>
<td>18.7</td>
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<tr>
<td>Mean</td>
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<td>16.1</td>
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<tr>
<td>IV</td>
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<td>50</td>
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<td>27.2</td>
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<td>30.5</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>29.9</td>
<td>27.3</td>
</tr>
</tbody>
</table>

LSD\textsubscript{0.05} for:
doses of nickel 2.488  3.058  3.209  1.570
liming 2.488  3.058  n.i.  1.570
interaction: doses of nickel x liming n.i.  n.i.  n.i.  n.i.
coefficients achieved from the statistical computations revealed significant dependence between nickel content in the soil and mean manganese concentration (for experimental years) in plants of the 1\textsuperscript{st} cut ($r = 0.74^{**}$), 2\textsuperscript{nd} cut ($r = 0.61^*$), and 3\textsuperscript{rd} cut ($r = 0.50^*$), as well as between soil liming and mean value of manganese content for years in red clover of the 1\textsuperscript{st} cut ($r = -0.71^{**}$).

![Fig. 11. Manganese content (mean for cuts) in red clover depending on soil contamination with nickel [mg/kg D.M.]](image1)

It may be suggested that both studied experimental factors (soil contamination with nickel and liming) significantly differentiated lead and manganese contents at red clover. Both metals concentrations in plants grown on polluted soils were higher as compared to those cultivated on non-contaminated ones, which may indicate the synergism between nickel and both discussed heavy metals. Applied liming (regardless the amount of CaCO$_3$) caused a significant decrease of both metals contents in test plant, whereas the

![Fig. 12. Manganese content (mean for cuts) in red clover depending on different liming [mg/kg D.M.]](image2)
lowest mean values of them were found in plants grown on soils limed according to 1.5 Hₜ of soil, which was consistent with other authors studies [7, 9, 10].

CONCLUSIONS

1. Soil contamination with nickel caused the increase of lead and manganese contents in red clover.
2. Liming caused significant decrease of lead and manganese concentrations in test plant.
3. Studies revealed synergistic interactions between nickel and lead and manganese.

REFERENCES


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Zaliczany jest nikiel, który w małych ilościach jest niezbędny dla wzrostu i rozwoju organizmów żywych, natomiast występujący w nadmiarze jest toksyczny. W czteroletnim doświadczeniu wazonowym badano wpływ zanieczyszczenia gleby nikiel (50, 100 i 150 mg Ni/kg gleby zastosowanego w formie NiSO₄⋅7H₂O) na tle zróżnicowanego wapnienia (wg 0,5; 1 i 1,5 H₂O gleby zastosowanego w formie CaCO₃) na zawartość Pb i Mn w koniczynie czerwonej. Zawartość metali oznaczono metodą ICP-AES po wcześniejszej mineralizacji materiału roślinnego „na sucho” w piecu muflowym w temperaturze 450°C i rozpuszczeniu popiołu w 10% roztworze HCL. Wyniki badań opracowano statystycznie analizą wariancji z wykorzystaniem rozkładu F-Fishera-Snedecora wg programu F.R. Anal.var 4.1., a wartość NIR₀,₀₅ wyliczono wg testu Tukeya. W celu znalezienia związków między badanymi cechami w pracy przeprowadzono również analizę korelacji liniowej. Zawartość obu metali w roślinach uprawianych na glebach zanieczyszczonych nikiel była większa w odniesieniu do roślin uprawianych na glebach niezanieczyszczenych, co może świadczyć o synergizmie niklu i omawianych metali. Zastosowane wapnienie (niezależnie od ilości CaCO₃ wprowadzonego do gleby) powodowało istotne zmniejszenie zawartości obu metali w roślinie testowej. Przeprowadzone badania wykazały synergistyczne zależności pomiędzy nikiel a ołowiem i manganem.
DISINFECTION OF MEAT INDUSTRY EQUIPMENT
AND PRODUCTION ROOMS WITH THE USE OF LIQUIDS
CONTAINING SILVER NANO-PARTICLES

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Keywords: Meat industry, disinfection, nanosilver.

Abstract: Essential sorts of disinfecting agents and principles of their action have been considered in the paper. Results of research on application of washing-disinfecting liquids, containing silver nano-particles, in meat industry have been presented. It has been established that liquids characterized by very efficient bactericidal and fungicidal properties can be prepared by composition of toxically performing silver nano-particles, some degreasing agents, reducing surface tension, and frothing agents. The listed components eliminate “insulating effect” of fat particles in which bacteria are suspended and facilitate silver nano-particle contact with bacteria and fungi, increasing this way biochemical effect of silver nano-particles.

INTRODUCTION

Biocides are applied in various industries in order to reduce population of hazardous microorganisms harmful to health of human beings or animals as well as those affecting quality of food products in meat industry [3, 13].

According to UE directive [2], regulations concerning biocidal products [15] and law regulating categories towards their objectives [12], the biocidal products are defined as “active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means”. According to the above regulations [12] biocidal substances can be divided into 23 groups in 4 categories. The products of the 1st category, including disinfecting and bactericidal products of general application, are presented in Table 1.

Microorganisms, after entering the animal- or human-organism can cause numerous infectious diseases. They can be transferred from the environment by atmospheric air, water or food products, contaminated carrier or by contact with contaminated surface [1, 14]. In food industry a very good medium for microorganism growth is created by residual pieces of food left in technological machines. As a result, fresh food subjected to technological process may undergo microbiological recontamination [10]. Probability of food contamination and human or animal organism infection is low when the environment of technological line is kept properly clean. Cleanliness, adequate to the require-
ments of technological process can be ensured by application of washing and disinfection processes. The aim of these processes is removing of the mechanical, chemical and microbiological impurities from the surface of machines, appliances, packing materials and also from production compartments [7, 10]. Washing and disinfecting process has been presented in Figure 1.

In the washing process mechanical and chemical impurities are removed, a however,

<table>
<thead>
<tr>
<th>Category</th>
<th>Group of biocidal products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disinfecting and biocidal products of general application</td>
<td>1. Biocidal products used for personal hygiene</td>
</tr>
<tr>
<td></td>
<td>2. Disinfecting products for personal and public use and other biocidal products</td>
</tr>
<tr>
<td></td>
<td>3. Biocidal products used for veterinarian hygiene</td>
</tr>
<tr>
<td></td>
<td>4. Products used for disinfection of surfaces that come into contact with food and animal</td>
</tr>
<tr>
<td></td>
<td>5. Products applied for water, used by people and animals, disinfection</td>
</tr>
</tbody>
</table>

Table 1. Classification of bactericidal products of the 1st category [3, 12]

Fig. 1. Stages of washing and disinfection
A – detergent dissolves organic and inorganic components of the dirt, B – washing, separation of dirt layers, uncover the microorganisms in biofilm, C – disinfection (penetration into biofilm and inactivation of microorganisms [19]
number of microorganisms is reduced only from the level of $10^6$ to the level of $10^3$. A disinfection process can reduce the microorganism’s population down to the level of $10^1$ [19]. Disinfection kills vegetative forms of microorganism by using chemicals or applying physical methods [7]. The most frequently used methods, are based on the application of chemical agents.

In the present work results on application of disinfecting, containing silver nanoparticles alkali liquids for meat production compartments, smoke chambers, technological machines and appliances in meat industry disinfection have been presented.

### DISINFECTING METHODS AND DISINFECTING AGENTS

Distribution of a disinfecting agent on the disinfected surface can be achieved by using “cold steam”, foaming or spraying method. [4]. Fog, made by “cold steam” method, covers all surfaces uniformly making a film of active substance. Foaming method enables precise observation of the disinfecting sites and longer exposure time to disinfectant. Spraying method is the most widespread method consisting in preparation of working solution for the disinfection. Disinfecting agents are selected according to the aim, range, and application place [7, 19]. Chemical disinfectants should be characterized by efficient bactericidal action towards hazardous microorganisms; however, they should not induce immunity mechanisms in bacteria, viruses or fungi. Disinfecting agents, by wetting the surface perfectly, should act fast, efficiently even at low concentrations used, and be not toxic for the people and animals. They should also be water soluble, biodegradable and not inducing corrosion [19]. Bactericidal effect of chemical agent depends mainly on the sort and number of undesirable microorganisms, concentration and time of exposure to the chemical, and also on the environmental factors such as temperature, pH, humidity and organic substance [7, 18].

Disinfecting agents affect microorganisms (viruses, bacteria, fungi) by damaging their cytoplasm and causing protein denaturation and degradation of nucleic acids. Hydro-sulphur (-SH) groups- elements of protein structure- undergo oxidation that results in malfunctioning of their system [7, 11, 19]. Concentration of the active substance determines the effectiveness of the disinfecting agent, that can be bactericidal (irreversible structural and metabolic changes in cells) or biostatic (inhibited growth of microorganisms).

Viruses are microorganisms of a transitory form between simple protein molecules and bacteria. Such primitive structures consist of nucleic acid sheathed with proteins [1, 8, 16]. They contain genetic material (DNA, RNA), however, they cannot metabolize independently, so, they use for this purpose cells of a host organism causing its disease.

Bacterium cell is built of nucleus, where genetic material is located and protoplasm closed within cell membrane, adjacent to the rigid wall, impervious for high-molecular compounds. Cell membrane, built of lipids and proteins is permeable for metabolic compounds [1, 8, 16]. A large number of bacteria form endospore enclosed in thick membrane. Under favorable conditions spores transform into vegetative cells [8]. Fungus cell, containing glycogen, fats and dye is enclosed in chitin membrane. Fungi cells feed on products resulted from organic matter decomposition [8, 16].
The most important disinfecting compounds are quaternary ammonium compounds, peroxides, Cl- and J- containing compounds, alcohols, aldehydes, bactericides, phenols, cresols and heavy metal salts [6, 7, 10, 18, 19].

Quaternary ammonium compounds perform disinfecting, wetting and emulsifying functions. Preparations containing ammonium salts affect Gram-positive, Gram-negative, yeast, fungi, and viruses. Their action consists in attraction of salt cation to negatively charged surface of the microorganism cell. That decreases wall permeability of the cell and hinders transport of feeding components to cytoplasm. Cation can also penetrate to cytoplasm, where, through enzyme inactivation inhibits metabolism of the cell. The disadvantage of the agents containing quaternary ammonium compounds is formation of immune to their action microorganisms. To counteract, an alternate disinfecting agents are to be applied. Some limitations in the quaternary ammonium compounds application can also result from their inactivation by such substances as protein, fat or blood.

Compounds, such as peracetic acid, hydrogen peroxide, potassium persulphate are able to damage vegetative forms of bacteria as well as bacterial spore. The most widely used preparations, containing the above compounds, are mixtures of peracetic acid, hydrogen peroxide and water. The compounds used, oxidize the SH-groups in proteins into disulphide forms, causing malfunctioning of membrane and wall of the microorganism cell. Particular advantage of peroxides is their biodegradability.

Cl-containing compounds are strong oxidants and effectively destroy microorganisms. This group includes chloramine T, and sodium hypochlorite. Effectiveness of the sodium hypochlorite is based on its hydrolysis resulting in hypochlorous acid formation. The latter, after penetration into microorganisms, destroys bacteria, fungi und viruses. Ionized oxygen, released in the process, denaturizes proteins, inactivates enzymes and oxidize -SH groups. Chlorine concentration in solutions, used for disinfection purpose, should be on the level of 100 to 200 ppm. Bactericidal effect of chlorine can be reduced by the presence of organic compounds in the environment.

Iodophors are complex compounds of iodine with polymers, biopolymers or surface active compounds. Iodine, released from iodophors, inhibits activity of respiration enzymes of microorganisms through oxidation of hydro-sulphur groups and through complexing of proteins of cell membrane. Iodophors affect all microorganisms at concentrations of active form as low as 12–25 ppm.

Alcohol containing disinfecting agents cause denaturation of protein cells of microorganisms. The highest effects are achieved when alcohol concentration is ~ 65%. The most important for disinfection are ethyl- and propyl alcohol. Alcohols, however, should be applied to the surfaces previously washed.

Aldehyde containing agents, because of their toxicity, can be applied only to walls and floors disinfection. An example is preparation of the mixture of formalin of the concentration of 3–20%. It is a strong bactericidal agent, destroying also bacterial spore.

Bactericides are amphoteric organic compounds, for example, aminoacids with substituted groups. They display acidic or alkaline properties, depending on pH of the environment.

Phenol and its derivatives destroy vegetative forms of bacteria and fungi; however, they do not affect much bacterial spore. At present, the phenol is not applied in disinfecting preparations. Instead, biphenols, cresols, xylen phenols are used. They react with
structural proteins of microorganism cells consequently inactivate cell wall and membrane. Phenols, due to their toxic properties, are not in application.

Among heavy metal salts, applied for disinfection, are silver and mercury salts. They react with -SH structural groups of proteins and enzymes negatively affecting metabolism of microorganisms. Particular recognition was received by nitrate, citrate and lactate of silver. However, solutions containing colloidal metallic silver are applied the most. Such solution, containing nano-particles of metallic character, displays stronger biochemical activity.

Disinfecting chemicals can destroy microbiological source of impurities or make it harmless. Nevertheless, the microbiological organisms can recuperate when the environmental conditions change [9]. Favorable conditions for microorganism growth can be reduced by regular washing and disinfection. The very important factor is, however, application of disinfectants, containing different sorts of active substances.

The basic problem in food industry is disinfection of production rooms and production lines in order to prevent the growth of different kind of bacteria, and mould fungi. For this purpose, different kinds of agents have been applied for a very long time. Historically, the first conserving and disinfecting agents applied were vegetable extracts from horseradish, garlic, onion, nettle, or acetic acid solution. Peracetic acid solution has been one of the most frequently used bactericidal agent until now. Application of calcium hydroxide in the form of lime is also frequently used as a bactericidal and fungicidal agent for household room disinfection and for tree lime whiting.

Modern disinfecting and bactericidal agents usually contain several, typical for washing, components such as surface actives, emulsifiers, complexones, anti-flocculants, foaming and other components with different proportions, depending on the application purpose. The main purpose of these mixtures is to create physico-chemical conditions enabling effective bactericidal action of alkali, particularly sodium hydroxide – the main component of all disinfecting solutions.

Among the newest bactericidal substances nano-silver in water solution can be distinguished. Bactericidal properties of nano-silver have been known since ancient times, when silver cups for drinking water were used to protect from infection [17]. The ability to kill bacteria, moulds, fungi and bacterial spores by silver compounds were already known at the beginning of the 19th century, however the mechanisms of silver action had not been described. Nowadays, it is known that toxic for the microorganisms silver ion can affect directly an individual cell. Owing to catalytic properties silver oxidizes genetic material of the cell.

Water solution, containing nano-particles of metallic silver of $10^{-12}$ m in large deposited on different carriers, for example, silica or polymer, is one of the modern disinfecting and bactericidal agents. Tests on its application for technological line and industrial compartments disinfection gave positive results. However, there was fear that concentration of silver used (300 ppm) might be toxic to the environment and that its application could be expensive.

The presence of fats hinders bacteria and fungi contact with silver particles and restrain the oxygen access to the silver surface resulting in a decrease of disinfecting effects.
EXPERIMENTAL PART

The aim of the work was preparation of solutions, enabling to create the most effective bactericidal and fungicidal properties of silver and its compounds. Simultaneously, the silver concentrations should not be at a toxic level and technological procedure should be economically profitable. That is particularly important for meat, food and related industries, where the problem comes from fatty surfaces, creating perfect conditions for bacteria and fungi of the mould group growth.

The liquids manufactured for this purpose should be characterized by the properties listed below.

- Ability to reduce surface tension at the interface between the washing liquid and dirty (fatty) surface. This requires an introduction of a surface-tension agent into the disinfecting liquid.
- Ability to form complex compounds with metal ions present as impurities. This requires application of adequate complexing agents.
- Ability to prevent secondary precipitation of dirt. This requires application of emulsifiers.
- Ability to ensure permanent foaming by addition of foam stabilizer in order to increase components interaction. This is particularly important when the washed surface has places not easily accessible.
- Ability to ensure alkali medium helping in dissolving fats. This requires sodium hydroxide addition.
- The content of nano-silver should be adequate to ensure its efficient action without threat of toxic effect of the metal.
- Disinfected elements of technological appliances and rooms should meet the European requirements, according to permitted number of microorganisms TVC, (0–10/cm²), and Enterobacteriae (0–1/cm²).

In the present work formula for several disinfecting liquids has been worked out. 100 kg of concentrate K1 characterized by bactericidal, washing-disinfecting, non-foaming properties of alkaline liquid was prepared using 0.5 kg of anionic surface active agent in the form of 86.5% solution of alkyl ether of carboxylic acid (trade name Akyl LF4); 15 kg of sodium hydroxide solution (50% NaOH); 1 kg of complexing agent in the form of solution, containing 24% of hexasodium salt of diethyl triamine-penta (trade name Dequest 2066); 1 kg of the agent that prevents secondary precipitation of dirt, in the form of solution containing 45% of sodium acrylate and aliphatic aminoacid (trade name Sokalan PA 30); 0.5 kg of nano-composite, containing 2000 ppm of Ag in the form of particles ~ 10^{-12} m deposited on silica carrier (trade name Nano Silver) and 82 kg of water.

Concentrate K2 of bactericidal, washing-disinfecting, and foaming characteristics in the amount of 100 kg of alkaline liquid was prepared using 5 kg of anionic surface agent of emulsifying and dissolving properties in the form of alkyl ether of carboxylic acid (trade name AKYPO RLM 45CA); 5 kg of sodium hydroxide (50% NaOH), 3 kg of the agent that prevents secondary precipitation of dirt, in the form of solution containing 45% of sodium acrylate and aliphatic aminoacid (trade name Sokalan PA 30); 10 kg of foam stabilizer in the form of oxide of amine alkyl dimethyl coconut oil (trade name Oxidet DMC LD); 16 kg of nano-composite, containing 2000 ppm of Ag in the form of particles ~ 10^{-12} m deposited on silica carrier (trade name Nano Silver) and 59 kg of water.
Working solutions of different concentrations were prepared by diluting the K1 and K2 concentrates as below:

- 100 kg of concentrate K1 was diluted, under continuous stirring, by addition of 400, 900 and 1900 kg of water.
- 100 kg of concentrate K2 was diluted, under continuous stirring, by addition of 900, 2900 and 4900 kg of water.

In the next stage of work three new agents, containing silver nano-composite were investigated:

- Alkaline washing-disinfecting agent (CLIX M.01) appropriated for washing machines, walls and floors with use of foam. It is a highly alkaline agent efficiently removing organic impurities, such as fat and proteins. Its composition: dispersants, alkali, detergents, silver nano-composite. Application parameters: concentration 3–4.0%, temperature: 40–50°C, time of exposure 10–20 minutes.
- Alkaline washing-disinfecting agent (CIP-CLIX P.01) appropriated for washing plastic containers. It is a not-foaming alkaline agent. It removes heavy dirt of organic nature. Its composition: alkali, detergents, sequestration agent, dispersants, silver nano-composite. Application parameters: concentration 0.5–1.0%, temperature: 30–55°C, time of exposure 5–10 minutes.

The solutions obtained by diluting the concentrate K1 and K2 were applied for washing of four technological systems in meat industry. After washing, means of two measurements of total number of microorganisms TVC (standard is 0–10/cm²) and number of bacteria of Enterobacteriaceae group (standard 0–1/cm²) were obtained. The objects were analyzed according to PN-ISO 18593:2005 and the results obtained (Tab. 2) were interpreted according to [5].

From Table 2, it can be concluded that working solutions prepared by dilution of the concentrates K1 and K2 meet the standards concerning the total number (0–10/cm²) of microorganism TVC and number of Enterobacteriaceae (0–1/cm²).

Tests on application of alkaline foaming liquid CLIX K.01 for washing smoke chamber gave satisfactory washing and foaming results. Stable, slowly sliding down the

<table>
<thead>
<tr>
<th>Liquid applied</th>
<th>Number of TVC microorganisms/cm²</th>
<th>Number of microorganisms from Enterobacteriaceae group/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1 (1:4)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>K1 (1:9)</td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>K1 (1:19)</td>
<td>2.0</td>
<td>0.25</td>
</tr>
<tr>
<td>K2 (1:9)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>K2 (1:29)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>K2 (1:49)</td>
<td>0.5</td>
<td>0.25</td>
</tr>
</tbody>
</table>
wall foam accumulated dirt, fat and tar from the surface. The liquid applied contained 4% of concentrate in water of the temperature ~ 20°C. The temperature inside the smoke chamber before the washing started was 60°C; contacting time of foam with washing surface was ~ 15 minutes.

Tests on application of alkaline foaming liquid CLIX M.01 for washing and disinfecting surfaces of the machines and installations gave satisfactory results. Stable, slowly sliding down the surface foam accumulated dirt and fat. The liquid applied contained 3% of concentrate in water of the temperature ~ 45°C. Contacting time of foam with washing surface was ~ 15 minutes.

Satisfactory results were also obtained using alkaline liquid CLIX P.01 for washing containers. Microbiological smear tests provided results within the standard requirements for plastic materials. The concentrate was diluted with water of 55°C to yield concentration of 0.5%. Contacting time of washing solution with washed surface was ~ 15 minutes.

CONCLUSIONS

The formula of two concentrates K1 and K2 and several disinfecting liquids was been worked out experimentally.

It has been established that liquids obtained with use of concentrates K1, K2 and those with symbols CLIX K.01, CLIX M.01, CLIX P.01 are characterized by very efficient bactericidal and fungicidal properties. These properties are enhanced by addition of silver nano-particles (toxically affecting bacteria), some degreasing agents, reducing surface tension and foaming agents. The listed components eliminate “insulating effect” of fat particles in which bacteria are suspended, and facilitate access of nano-silver particles to bacteria and fungi. This results in an increase of biochemical effect of nano-silver. Additional effects consist in removing particles of dust and other impurities from washed surfaces.

REFERENCES

ZASTOSOWANIE PŁYNÓW ZAWIERAJĄCYCH NANO-CZĄSTECZKI SREBRA DO DEZYNFEKCJI URZĄDZEŃ I POMIESZCZEŃ PRODUKCYJNYCH W PRZEMYŚLE MIĘSNYM

W pracy przeanalizowano podstawowe rodzaje środków dezynfekcyjnych i zasady ich działania. Przedstawiono wyniki badań zastosowania płynów myjąco-dezynfekujących zawierających nano-cząsteczki srebra w przemyśle mięsnym. Stwierdzono, że można otrzymać płyny o doskonałych własnościach bakteriobójczych i grzybobójczych w kompozycjach z dodatkiem nano-cząsteczek srebra działających toksycznie na bakterie oraz odpowiednich środków odtłuszczających, zmniejszających napięcie powierzchniowe i spieniających. Składniki te eliminują „izolacyjne” działanie tłuszczu, w którym zawieszone są bakterie i rozwijając powierzchnię kontaktu cząsteczek srebra z bakteriami i grzybami zwiększają oddziaływanie biochemiczne nano-cząsteczek srebra.

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STABILIZATION OF POST-GALVANIC SLUDGE USING PORTLAND CEMENT AND SILICA FUME

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Keywords: Post-galvanic waste material, cement, silica fume, physical and mechanical properties, leaching.

Abstract: Solidification in mineral binders is one of the useful methods of neutralizing hazardous materials. In this work some waste created after neutralization of used etching baths, obtained in the process of wire galvanizing and copper plating were solidified by cement mortar with and without silica fume. The researches showed that the addition of waste to cement mortar in the amount of up to 5% is safe in respect of ecology. At such an amount of waste addition to cement, metals are permanently immobilized in a matrix. While the addition of 10% of silica fume to cement improved the strength properties of products, it had no influence on the enhancement of immobilization of metals in cement mortars.

INTRODUCTION

Solid waste and by-products obtained from industrial plants are one of the basic causes of environmental degradation. Bigger and bigger amounts of waste lead to the need of expansion of existing waste dumps and limitation of soil area. Therefore, it is essential to eliminate the results of dump storing and to find a proper way of coping with unavoidable.

Post-galvanic waste materials, containing heavy metals, are classified as hazardous substances, so they must be neutralized. The solid part of such waste consists mainly of insoluble metal hydroxides, carbonates or sulphides, which, with lowered pH, can undergo leaching and endanger the environment.

One of the popular methods of waste neutralization used on a large scale in the USA is solidification in mineral binders, especially by means of cement [2]. This way of isolating hazardous materials is interesting because of beneficial for the environment immobilization of ingredients, lowered percolation of materials, and reduced leaching of the stabilized substances [1, 10, 11]. This method does not require huge expenses and can be introduced in any plant which produces construction components, such as setts, pavement plates, openwork pavements and kerbs. To improve features of products applied in construction or highway engineering, various types of additives, such as pozzolans enhancing physicomechanical parameters of cement materials, can be used. Pozzolan additions, when they take part in the process of hydration of cement, are conductive to the microstructure forming and beneficially influence keeping metals in a matrix [3, 12, 14].
The waste that was put to the processes of stabilization and solidification needs some assessment with respect to its influence on environment, among others, by means of the evaluation of toxic substances leaching and physicomechanical properties of admixed cement materials. The way of immobilization of particular components can influence leaching in utilization conditions, which was confirmed by the trials of introducing mixed sludge after fluoroborate leading [17], copper hydroxide [15], nickel [9, 18], or lead chromate [16] to ceramics.

In this work the influence of the addition of post-galvanic waste to cement mortar, with and without silica fume, on physicomechanical properties of received mortars was studied. Furthermore, the researchers tested, by means of leaching tests according to TVA. AS. 1991 [8] and TCLP [6] methods, whether the stabilized waste was bound firmly enough with a cement matrix so that it did not endanger the environment.

**METHODS**

*Waste*

Post-galvanic sludge from “Galmar” galvanizing plant in Poznan was used. This sludge is created in the neutralization processes of used etching baths diluted with washings, obtained from the process of zinc and copper plating of wire. The sludge was in the form of brown powder – humidity 10% and bulk density 1.24 kg/dm$^3$. A sample of waste was taken once in the quantity of about 10 kg, dried and homogenized by means of sieving (with sieve meshes d = 1 mm).

The waste was poured with concentrated hydrochloric acid solution as well as *aqua regia* and boiled for a few hours in order to analyze the post-galvanic sludge composition. After cooling, the solutions with the rest of suspended matter were diluted and filtered. Some analysis aimed at checking if any heavy metals and sulphates were present in the solution. Metals, the presence of which was most important and most probable because of the conducted technological processes, were determined taking into consideration their toxicity.

The main component of sludge was iron which constituted about 15% of dry weight (DW). The composition of sludge is presented in Table 1.

<table>
<thead>
<tr>
<th>Components</th>
<th>Content of component in waste (in DW) [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>14.672</td>
</tr>
<tr>
<td>Zn</td>
<td>2.915</td>
</tr>
<tr>
<td>Cu</td>
<td>1.874</td>
</tr>
<tr>
<td>Ni</td>
<td>1.263</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>1.152</td>
</tr>
<tr>
<td>Ca</td>
<td>1.000</td>
</tr>
<tr>
<td>Pb</td>
<td>0.253</td>
</tr>
<tr>
<td>Cr</td>
<td>0.199</td>
</tr>
<tr>
<td>Mn</td>
<td>0.094</td>
</tr>
<tr>
<td>Co</td>
<td>0.002</td>
</tr>
<tr>
<td>Cd</td>
<td>0.002</td>
</tr>
<tr>
<td>Insoluble substances</td>
<td>67.79</td>
</tr>
</tbody>
</table>

Table 1. Approximate percentage constitution of post-galvanic sludge
Portland cement
In order to prepare cement mortar with waste addition, ordinary Portland cement (CEM I 42.5 R, Górażdże EN 197-1) and norm sand for cement strength tests (PN EN 196-1, Certificate IMMD o. Krakow KWARCMIX Tomaszów Mazowiecki) were used. Concrete additive containing micro-silica SikaFume®HR (Poland Sika Company) with the bulk density of 0.65 ± 0.1 kg/dm³ was introduced to some part of prisms.

Preparation of specimens
The cement mortar was made of 450 g of cement, 1350 g of sand, 225 cm³ of water and post-galvanic sludge added in the amounts of 2.5, 5 and 10%. A batch of prisms with 10% additive of silica fume as well as 2.5, 5 and 10% additive of waste was also prepared. Reference prisms, i.e. without sludge addition, were also made. All amounts of additives are given in mass fractions in relation to cement. The determination of particular cement samples with additives used in this work are presented in Table 2.

<table>
<thead>
<tr>
<th>Kind of additives</th>
<th>Amount of additives [%] and the samples’ symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pozzolans</td>
<td>C      C2.5  C5  C10  CP  CP2.5  CP5  CP10</td>
</tr>
<tr>
<td>Waste</td>
<td>0      2.5  5  10  0   2.5  5  10</td>
</tr>
</tbody>
</table>

40 x 40 x 160 mm prisms were prepared in order to determine the compression and flexural strength of cement mortar and for freeze-thaw resistance tests. Roller-shaped specimens with diameter of 30 mm and height 50 mm were made for leaching tests by means of TVA. AS. method. 24 hours after the preparation of the mortar, the prisms were demoulded and placed in water bath for 4 weeks. After this period they were ready for tests of physicomechanical properties and leaching tests.

Physicomechanical properties

Testing the setting time was conducted by means of the Vicata apparatus. The Vicata ring was filled with cement paste of norm consistence and placed under the needle of the apparatus. The movable parts were quickly released in order to make the needle sink vertically into cement paste. The period of time between the moment when cement was poured into mixer and the moment when the needle was 4 ± 1 mm distant from glass plate was treated as the beginning of cement setting.

Six cement prisms with all kinds of additives after 28 days of hardening and all prisms after freeze-thaw resistance tests underwent the compressive strength tests. Louis Schopper Staafl – a press for testing strength – was used, with the maximum acceptable load 100 kN. A prism was put into the apparatus with its lateral surface on support rollers so that its oblong axis would be perpendicular to the rollers. The load was vertically transferred to the opposite lateral surface of the prism by means of the loading roller, while pressure was gradually increased until the prism was broken. The halves of prisms broken during the flexural strength tests were analyzed in compression strength tests (on lateral surfaces 40 x 40 mm). The halves were placed with their lateral surfaces in the centre of
the plate while the load was gradually increased (with pressure increase $2400 \pm 200$ N/s), until the prisms were destroyed.

Determining the absorbability consists in finding the mass of water that can be absorbed by a cement specimen dipped in water under standard atmospheric pressure. For the test 6 cement prisms with all kinds of additive were used. All samples were dried at $105^\circ$C, and then, after precise weighing and measuring, they were placed in a bath and poured with water at $20^\circ$C to $\frac{1}{4}$ of height. After 3 hours the next portion of water was added – to $\frac{1}{2}$ of height, and after next 3 hours – to $\frac{3}{4}$ of height. After 24 hours the samples were completely poured with water in order to have the upper surfaces of the prisms 2 cm under the water level. When 24 hours passed the samples were taken out of the water, dried, and weighed. Then, the prisms were again immersed in water and weighed after 24 hours. Weighing was repeated till the mass was steady.

After soaking 4 prisms their freeze–thaw resistance was tested (while the tests were conducted, the two remaining prisms were conditioned in water as reference prisms). After taking out of water and drying the prisms were put in to a freezer at minus $20 \pm 2^\circ$C, where they were kept for 4 hours, then taken out and placed in water for the next 4 hours in order to defrost them. 25 cycles of freezing and thawing were conducted.

### Leaching tests

The tests of leaching hazardous substances from waste were conducted by means of Swiss method TVA. AS. 1991. A test according to this method was conducted in two 24-hour leaching phases with water saturated with carbon dioxide in water mass proportion to a prism 10:1, while the tested material was not crumbled. Each phase of leaching was conducted with a fresh portion of water saturated with CO$_2$. After filtering, hazardous substances in the leachates were analyzed. Average normalized concentration was counted according a formula:

$$[\text{mg/dm}^3] = \frac{(C_1 + C_2)M_1}{2M_2},$$

where:

- $(C_1 + C_2)$ - sum of measured concentrations of eluats after 24 and 48 hours in [mg/dm$^3$],
- $M_1$ - mass of a sample [g],
- $M_2$ - dry mass of a sample after leaching [g].

For leaching tests by means of TCLP method, crumbled samples, separated with a sieve (with sieve meshes of 10 mm) were used. After the introductory test, for the right leaching, solution with pH $= 2.88 \pm 0.5$ was used. After leaching and filtering the solution was analyzed by means of absorptive atomic spectrometry method to see whether any metals were present.

### Analytical methods

The determination of the concentration of metals was done according to analytical curves with appropriate lengths of waves using atomic absorption spectroscopy (AAS) Carl Zeiss Jena.
RESULTS AND DISCUSSION

Tests of physicomechanical parameters

The tests of the initial setting time showed that in the case of cement with additives more water was necessary in order to get the norm consistence than in the case of ordinary cement. The initial setting time was various depending on the kind of cement additive (Fig. 1). Cement with the waste addition had longer setting time and the bigger amount of additive was, the longer the setting time was (by 42 minutes for 10% of addition in comparison to the reference sample).

![Fig. 1. Setting times of prisms with various sludge additions](image)

The setting time in the case of cement with silica fume addition started 55 minutes earlier than in the case of ordinary cement. Additions of waste to cement with pozzolans retarded this moment and made it close to setting times reached for cement without pozzolans.

The results of absorbability, as well as compression and flexural strength determination are presented in Table 3. The highest absorbability was noted for prisms made of cement without additives (5.22%). The absorbability of remaining samples was between 2.19 and 4.14%.

The compression strength was highest in the case of cement with 10% pozzolan addition (56.1 MPa) – it is resistance that classifies it a class higher than cement without additive, which belongs to II class – 42.5 MPa. Waste additions lowered the compression strength, but did not lower the class of cement. The lowest compression strength was showed in prisms with the highest sludge addition to cement – 10% waste addition to cement without pozzolans caused lowering of the compression strength by about 24% in
comparison to cement without waste addition. Also 10% waste addition to cement with
pozzolan addition significantly lowered the strength – to 35.8 MPa. This parameter was
reduced by 36% in comparison to the strength of cement-pozzolan samples and by almost
29% in comparison to cement without additives. Anyway, all prisms with waste addition
to cement and cement with pozzolans reached compression strength classifying them to
32.5 MPa class.

After 25 cycles of freezing and thawing there were no chips or cracks on the prisms
– all samples were resistant to low temperatures. The change of prism mass after these
processes was slight. In order to check the influence of freezing and thawing on the
strength of samples, strength tests after the conducted cycles of freezing/thawing were
performed (Fig. 2).

Tests showed that all prisms conditioned in water had compression strength higher
than prisms after 28 days of hardening. The processes of freezing and thawing had some
impact on compression strength increase (in comparison to strength after 4 weeks of

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbability [%]</th>
<th>Flexural strength [MPa]</th>
<th>Compression strength [MPa]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>5.22</td>
<td>9.3</td>
<td>50.4</td>
</tr>
<tr>
<td>CP</td>
<td>3.07</td>
<td>9.8</td>
<td>56.1</td>
</tr>
<tr>
<td>C2.5</td>
<td>4.14</td>
<td>8.7</td>
<td>38.6</td>
</tr>
<tr>
<td>C5</td>
<td>3.33</td>
<td>8.7</td>
<td>39.8</td>
</tr>
<tr>
<td>C10</td>
<td>4.51</td>
<td>8.9</td>
<td>38.3</td>
</tr>
<tr>
<td>CP2.5</td>
<td>2.19</td>
<td>9.9</td>
<td>42.2</td>
</tr>
<tr>
<td>CP5</td>
<td>2.34</td>
<td>9.2</td>
<td>41.9</td>
</tr>
<tr>
<td>CP10</td>
<td>3.93</td>
<td>9.1</td>
<td>35.8</td>
</tr>
</tbody>
</table>

Table 3. Results of absorbability tests and tests of compression and flexural strength of cement mortars with various waste additions

Fig. 2. Compression strength of prisms before and after freeze-thaw resistance tests
hardening) for all prisms with waste addition, apart from the prism with the highest, 10% waste addition to cement mortar without pozzolans.

For all prisms compression strength in relation to reference prisms, i.e. conditioned in water during freeze-thaw resistance tests, was lowered. The biggest strength drop after freeze-thaw resistance tests (in relation to reference prisms) was noted for cement with 10% of waste addition.

**Leaching tests**

In the respect of the environmental protection it was important to check if the solidified waste in cement mortar is safe for the environment, and whether heavy metals are bound with cement firmly enough and, for example, atmospheric falls will not lead to gradual leaching of metals to the environment. Metal concentrations after leaching of cement and cement-pozzolan prisms by means of TVA. AS. 1991 method are presented in Table 4.

In eluates from leaching rollers with additions of waste to cement mortar, only zinc was present, but in concentration not exceeding the acceptable value. For rollers made with addition of waste to cement with pozzolans, the acceptable concentration of copper ions in leachates for the sample with the highest amount of additive was exceeded. The concentrations of the remaining metals present in the solution did not exceed the acceptable norms. Eluates from leaching tests by means of TCLP method did not contain cobalt, cadmium, or lead. Chromium was present in leachates only at the highest – 10% addition of waste to cement without pozzolans (Tab. 5). However, highest concentrations of iron and zinc in leachates were detected, but the acceptable concentration was not exceeded [6].

### Table 4. Concentration of contaminants in eluats after leaching by means of TVA. AS. method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cu</th>
<th>Mn</th>
<th>Fe</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.29</td>
<td>ND</td>
<td>0.67</td>
<td>ND</td>
</tr>
<tr>
<td>CP</td>
<td>0.21</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C2.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.30</td>
</tr>
<tr>
<td>C10</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.0</td>
</tr>
<tr>
<td>CP2.5</td>
<td>0.50</td>
<td>ND</td>
<td>ND</td>
<td>0.37</td>
</tr>
<tr>
<td>CP5</td>
<td>0.31</td>
<td>ND</td>
<td>ND</td>
<td>0.62</td>
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<tr>
<td>CP10</td>
<td>1.12</td>
<td>0.08</td>
<td>ND</td>
<td>0.88</td>
</tr>
</tbody>
</table>

ND – not detected

### Table 5. Concentration of contaminants in eluats after leaching by means of TCLP method

<table>
<thead>
<tr>
<th>Type of contaminant</th>
<th>Ni</th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
<th>Zn</th>
<th>Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>CP</td>
<td>C2.5</td>
<td>C5</td>
<td>C10</td>
<td>C2.5</td>
</tr>
<tr>
<td>Ni</td>
<td>0</td>
<td>0</td>
<td>0.28</td>
<td>0.28</td>
<td>0.57</td>
<td>0</td>
</tr>
<tr>
<td>Cu</td>
<td>0.81</td>
<td>0.15</td>
<td>0.15</td>
<td>0.31</td>
<td>0.46</td>
<td>0.15</td>
</tr>
<tr>
<td>Mn</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.31</td>
<td>0.46</td>
<td>0.15</td>
</tr>
<tr>
<td>Zn</td>
<td>1.30</td>
<td>0.87</td>
<td>2.83</td>
<td>3.91</td>
<td>6.09</td>
<td>2.39</td>
</tr>
<tr>
<td>Cr</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.66</td>
<td>0</td>
</tr>
</tbody>
</table>

STABILIZATION OF POST-GALVANIC SLUDGE USING PORTLAND CEMENT AND ...
CONCLUSION

The introduction of silica fume addition to cement mortar improved the physicomechanical parameters of cement.

Tests showed that post-galvanic sludge additive, created during the neutralization of used etching baths and diluted with washings from the processes of zinc and copper plating of wire, to cement mortar with and without pozzolans decreased mechanical parameters of mortars, however not to such an extent as to disqualify the received products.

10% of waste addition to cement mortar with pozzolans was not safe from the environmental point of view – copper was not firmly bound in the matrix material. With such an addition of waste in the leachate (from the leaching tests by means of TVA. AS. method), the acceptable concentration of copper was exceeded. In eluates from leaching tests by means of TCLP method the concentration of tested metals was not exceeded. It seems that the stabilization of this kind of waste in cement with pozzolan additive is not safe ecologically. In this case expected effect of the immobilization of metals in the cement matrix was not reached. It is possible that some waste components retard cement hydration, which was also observed in the case of the solidification of waste containing Fe, Cu, Ni, Zn in cement with fly ash additions [7].

The tested kind of waste can be safely used as an addition to cement mortar in the amount of 5%. Such an amount of waste in cement does not disqualify received products with respect to tested physicomechanical parameters and is ecologically safe.

REFERENCES

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STABILIZATION OF POST-GALVANIC SLUDGE USING PORTLAND CEMENT AND CRUSHED MINE DEBRIS

Odpady pogalwaniczne zaliczane są do odpadów niebezpiecznych, dlatego muszą być unieszkodliwiane. Jedną z najprostszych metod unieszkodliwiania jest zestalanie w spoiwach mineralnych. W niniejszej pracy przeprowadzono próby zestalania w zaprawie cementowej oraz w zaprawie cementowej z dodatkiem pyłów krzemionkowych odpadu powstałego po neutralizacji zużytych kąpieli do trawienia, rozcieńczonych wodami popłucznymi, pochodzących z procesu cynkowania oraz miedziowania drutu. Badania wykazały, że dodatek odpadów w ilości do 5% jest bezpieczny pod względem ekologicznym. Przy takiej ilości dodatku odpadów do cementu następuje nieznaczne obniżenie parametrów fizykomechanicznych, a metale są trwale immobilizowane w matrycy. Dodatek pyłów krzemionkowych w ilości 10% do cementu poprawiał cechy wytrzymałościowe wyrobów, ale wprowadzenie odpadów obniżało te parametry.