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# Papers

# Artykuły

Elena MASAROVIČOVÁ[1], Katarína KRÁĽOVÁ[1] and Matúš PEŠKO[1]

# ENERGETIC PLANT SPECIES THAT NOT COMPETE WITH CONVENTIONAL AGRICULTURE

## GATUNKI ROŚLIN ENERGETYCZNYCH, NIEKONKURUJĄCE Z ROLNICTWEM KONWENCJONALNYM

**Abstract:** The objective of this contribution is to evaluate such energetic plants that will not compete with conventional agriculture. Our analysis is based on definition of energetic plant - a plant grown as a low cost and low maintenance harvest used to make biofuels, or directly exploited for its energy content (heating or electric power production). It was emphasized that besides of woody plant species as energetic plants can be also used both crops and non-food plants. Besides switch grass (*Panicum virgatum* L), jatropha (*Jatropha curcas* L) or algae some species from family Euphorbiaceae and Asteraceae store high concentration of triacylglycerols and latex, that can be used for production of biocomponents into the fuels. Species *Amaranthus* sp., *Miscanthus sinensis* Anderss., *Euphorbia marginata* L, *Ambrosia artemisifolia* L, *Helianthus tuberosus* L, and *Solidago canadensis* L successfully grown under climatic conditions of Slovakia, are presented as a potentially used energetic plant species - herbs - that will not compete with the crops. However, it should be stressed that mentioned species are (like jatropha) invasive plants. Since production of biofuels from crops as well as from non-food plants is still actual, carbon dioxide emission and energy balance of biofuel production is presently intensively discussed. Life-cycle analysis (LCA) appeared as a useful tool to appreciate impact of biofuels on the environment. LCA is presented as a scientific method to record environmental impacts from fuel production to final disposal/recycling. This approach is also known as "well to wheel" for transport fuels or "field to wheel" for biofuels. In order to investigate the environmental impacts of bioenergy and biofuels it is necessary to account for several other problems such are acidification, nitrification, land occupation, water use or toxicological effects of fertilizers and pesticides.

**Keywords:** biofuels, biomass, conversion processes, energetic plants, invasive plants, life-cycle analysis (LCA), non-food plants

Current energetic plant species and agro-industrial biofuel production chains rely on utilization of agricultural resources that compete with traditional food production. The most important actual crops such are eg *Zea mays* L*, Brassica napus* L*, Glycine max* (L) Merr*, Triticum aestivum* L*, Helianthus annuus* L are thus used in both, food and technical industry. In our previous paper [1] we characterized energetic plants as the plants grown as a low cost and low maintenance harvest used to make biofuels, or directly exploited for its energy content (heating or electric power production). We mentioned that according to Weger [2] for the choice of suitable energetic plants following criteria could be considered: high biomass production, biomass suitability for biofuel production, manageability and economy of plant cultivation and environmental aspects. Besides crops some of non-food plants (herbs), such are *Panicum virgatum* L, *Jatropha curcas* L or some species from family Euphorbiaceae and Asteracea, store high concentration of sugars, triacylglycerols and latex, that can be used not only for production of biocomponents into the fuels, but these plants are introduced as a potentially utilized energetic plant species that will not compete with the crops. Recently was published that "life-cycle analysis" (LCA) is an useful tool to appreciate impact of biofuels on the environment. LCA could be

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characterized as a scientific method to record environmental impacts from fuel production to final disposal/recycling. This approach is also known as "well to wheel" for transport fuels or "field to wheel" for biofuels. In order to investigate the environmental impacts of bioenergy and biofuels it is necessary to account for several other problems such are acidification, nitrification, land occupation, water use or toxicological effects of fertilizers and pesticides [3, 4].

**Energetic plants as a source of feedstock and renewable energy**

Presented analysis is based on definition of energetic plant - a plant grown as a low cost and low maintenance harvest used to make biofuels, or directly exploited for its energy content (heating or electric power production). It is well known that for energetic purposes forest biomass (dendromass), energetic wastes from wood-processing industry as well as food industry and waste biomass from industrial and communal field are most frequently utilized. It is feedstock for production of second-generation biofuels. In general, besides of woody plant species as energetic plants can be also used both crops and non-food plants (eg [5-7]). We have already mentioned that from crops are exploited mainly maize, rapeseed, soybean, wheat and sunflower. From non-food plants in addition to switch grass (*Panicum virgatum* L), jatropha (*Jatropha curcas* L) or algae some species from family Euphorbiaceae and Asteraceae store high concentration of sugars, triacylglycerols and latex, that can be used for production of biocomponents into the fuels. Especially jatropha seems to be an attractive non-food plant. For example, a national mission to produce biodiesel from Jatropha (13 million tones annually by 2013), has been launched as an initiative of the Indian government to address socio-economic and environmental concerns [8].

Species *Amaranthus* sp., *Miscanthus sinensis* Anderss, *Euphorbia marginata* L, *Ambrosia artemisifolia* L, *Helianthus tuberosus* L and *Solidago canadensis* L successfully grown also under climatic conditions of Slovakia, are introduced as a potentially used energetic plant species that will not compete with the crops. However, it should be stressed that the most of mentioned species are (like jatropha) invasive plant species. These plants can be characterized as a non-indigenous or non-native species, that adversely (negatively) affect the habitats and bioregions, they invade environmentally, ecologically and economically. These plants disrupt by dominating a region, wilderness areas and wildland-urban innerface land from loss of natural controls. Some of them can adapt to the new environmental condition very fast, mainly in the case if there do not occur any adequate competitor. Under such conditions invasive plant species can form new vital and vigorous plant population and then can caused extinction of original species or even serious changes of whole ecosystem. Thus invasive plant species are one of the most important challenge for preservation of the environment. This actual topic was particularly published by Elias [9] and Cvachova and Gojdicova [10].

Very promising are results of Andrianov et al [11] with genetically modified (GM) tobacco plants (*Nicotiana tabacum* L). When grown for energy production instead for smoking, tobacco can generate a large amount of inexpensive biomass more efficiently than almost any other agricultural crop. Tobacco possesses potent oil biosynthesis machinery and can accumulate up to 40% of seed weight in oil. GM plants showed up to a 20-fold increase in triacylglycerol accumulation in tobacco leaves and about a twofold increase in

extracted fatty acids up to 5.8% of dry biomass in *Nicotiana tabacum* cv. Wisconsin. GM tobacco plants also contained elevated amounts of phospholipids and this increase in lipids was accompanied by a shift in the composition of fatty acids favourable for their utilization as biodiesel. However, it should be emphasized that enhancement of environmental tolerance in GM energetic plants likely will increase the risk of invasion into surrounding environments. Similarly, enhancement of aboveground biomass production via biotechnology could allow such cultivars to be more competitive with native vegetation or other cultivated crops. Genetic modification can change the phenotype or physiology of a plant species sufficiently to lead to alterations in plant-plant interactions and ecological functions. Thus, it is important to recognize that, like non-native species, even native plants - if modified - would pose an unknown risk of becoming invasive. This, very important aspect was analysed in several of our papers eg [1]**.**

Castor bean (*Ricinus communis* L) is a non-food plant with naturally high oil content in its seeds and its growth requires relatively low energy inputs. A life cycle assessment (LCA) of biodiesel produced from castor bean varieties being developed by Israel-based Evogene Ltd. It was found that biodiesel produced from castor bean reduces greenhouse gases (GHG) emissions by 90% compared with petroleum diesel in the US and exceeds the GHG savings achieved with soybean biodiesel, with reductions for the US of 43% compared with soybean. Based on the results of LCA it can be concluded that castor bean is a viable and sustainable second-generation source for biofuel production [12].

The agro-ecological aspects and the high yield of vegetable biomass from hemp (*Cannabis sativa* L) farming (potential production of 12 Mg of dry matter per hectare and a seed output of 0.5÷2 Mg per hectare) are the factors that indicate a possible interest in this non-food plant from the point of view of energy use. The hemp energy resources come from the vegetable biomass used as fuel, or by obtaining biodiesel from the oil derived from its seeds [13].

**Energetic plants and other feedstock for biofuel production**

Since biofuel production increased during last years, the new feedstock for processing technologies appeared. Thus, improved classification of biofuels according to the different feedstock was published (in detail [1]). **First-generation biofuels** rely on food plant species (crops) as their feedstock. *Zea mays* L*, Brassica napus* L*, Glycine max* (L) Merr*, Triticum aestivum* L*, Helianthus annuus* L all have readily accessible sugars, starches and oils. Thus to change them into biofuels simply involves either fermenting the sugars or transform the fatty oils through transesterification. **Second-generation biofuels** use lignocellulosic biomass as feedstock (mainly wood, ie trees), non-food plants and agricultural residue (as well as other organic wastes) such as corn stalks eg [4, 14].

Rather than improving the fuel-making process, **third-generation biofuels** seek to improve the feedstock using GM plants. Third generation biofuels are carbon neutral when consumed meaning that the crops consume the same amount of carbon from the atmosphere as they will release when combusted. **Fourth-generation technology** ("carbon negative" source of fuel) combines genetically optimized feedstocks, which are designed to capture large amounts of carbon, with genomically synthesized microbes, which are made to efficiently make fuels. Particular characteristics of all mentioned biofuels were published in our previously paper [1].

**Life-cycle analysis (LCA)**

Life cycle analyses look at the whole picture of how a fuel is made, from "cradle to grave". The life cycles begin with the extraction of all raw materials to make biofuel, and end with using the biofuels in engines. Understanding the benefits of biodiesel requires us to compare its life cycle emissions to those of petroleum diesel. This study examines biodiesel energy's balance, its effect on greenhouse gas emissions, and its effects on the generation of air, water, and solid waste pollutants for every operation needed to made biofuel. LCA is a scientific method to record environmental impacts "from cradle to grave", ie from production to final disposal/ recycling. Also known as "well to wheel" for transport fuels or "field to wheel" for biofuels (in detail see [15, 16]). Two of the most used types of life cycle assessment for bioenergy are those used to determine net-energy and net greenhouse gas emissions. In order to investigate the environmental impacts of bioenergy and biofuels it is absolutely necessary to account for several other problems as acidification, nitrification, land occupation, maintenance of biodiversity, water use or toxicological effects of fertilizers and pesticides (eg [3]). These authors summarized results of LCA of three liquid biofuels: FAME prepared from rapeseed and bioethanol prepared from both, wheat and sugarbeet. The analysis was based on the measurements of direct emissions from combustion of biofuels and blended fuels for the cars in Czech Republic. Comparing LCA of fossil fuels and biofuels it was found reduction of $CO_{2eqv.}$ in the case of biofuels, what corresponded with the other published foreign studies. It should be stressed that in the calculation of whole LCA of biofuel production the advanced processing technologies (including agricultural procedures) were applied. Moreover, during biofuels production some by-products originate which have to be also considered in actual calculation procedure. In another paper [17] was stressed that from the aspect of climate preservation as the most effective biofuel were found second-generation biofuels: synthetic motor fuel - BTL, bioethanol produced from lignocellulosic material - wood, straw. However, from the energy aspect these fuels have high energy cost. Based on LCA was found that production hydrogen as a motor fuel has both, high energy cost and high negative impact on the environment. These authors also compared outputs from the most important Well-to-Wheels analyses in European region focused on GHG (*Greenhouse gases*) emissions and energy production.

**Conversion processes of non-food biomass to biofuels**

Production process of second-generation biofuels utilize non-food plants (forest trees, energetic forest coppices, fast-growing trees and herbs), ligno-cellulosic residues and wastes (wood chips from forest thinning and harvest residues, surplus straw and plant residues from agriculture). Possible options for the conversion of these materials to biofuels include: thermal and catalytic cracking, hydrocracking, pyrolysis, carbonisation, catalytic and steam reforming, gasification and Fischer-Tropsch synthesis. In general, conversion of biomass to second-generation biofuels requires hard reaction conditions such are: high temperature, high pressure and presence of hydrogen. All these procedures were described in detail by Bajus [18, 19]. It should be stressed that cost of abovementioned procedures has to be also inclusive into the whole LCA.

## Conclusion

With regard to the well known fact, that already in 2009 the earth's population was estimated to be about 6.77 billion, and 850 million people is suffering from malnutrition, it is inevitable to secure primarily sufficient food for the population. Considering the increasing trend of biofuel use an important bioethical problem occurred - perplexity whether crops (eg maize, cereals, potatoes, rapeseed, and sunflower) could be used exclusively for alimentary purposes or also as an alternative energy source. Thus, energy conservation and biofuels that are not food-based are likely to be of far greater importance over the longer term. Biofuels such as synfuel hydrocarbons or cellulosic ethanol that can be produced on agriculturally marginal lands with minimal fertilizer, pesticide, and fossil energy inputs, or produced with agricultural residues have potential to provide fuel supplies with greater environmental benefits than either petroleum or current food-based biofuels [20]. The integration of plant science into energy industry research is crucial for the success and sustainability of biofuels. This integration will only be effective if the plant scientists and ecologists who work with biofuels can communicate new findings in a way that is useful to the larger interdisciplinary community. Ecological impacts associated with land conversion and the establishment of new plant species (especially non-food plants) for biofuel production are important determinates of the overall sustainability of biofuels as an energy source. Genetic modifications of plants grown for biofuel could reduce the ecological impact that biofuel agricultural systems currently incur. Inclusion of interactive climate, plant, soil and microbial controls over nutrient cycling in an LCA will provide a more realistic assessment of biofuel costs and benefits [21]. On 10 June 2010, the EC announced its scheme for certifying sustainable biofuels, part of a set of guidelines explaining how the Renewable Energy Directive, coming into effect in December 2010, should be implemented. Global-Bio-Pact, co-funded under FP7, aims to develop and harmonize global sustainability certification systems for biomass production, conversion systems and trade in order to prevent negative socio-economic impacts [22].

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## GATUNKI ROŚLIN ENERGETYCZNYCH, NIEKONKURUJĄCE Z ROLNICTWEM KONWENCJONALNYM

**Abstrakt:** Celem pracy było wytypowanie takich roślin energetycznych, które nie będą konkurować z rolnictwem konwencjonalnym. Punktem wyjścia przedstawionej analizy jest definicja roślin energetycznych - roślin uprawianych przy niskich kosztach utrzymania i zbioru, stosowanych do produkcji biopaliw lub bezpośrednio wykorzystywanych do produkcji energii (ciepła lub wytwarzania energii elektrycznej). Podkreślono, że oprócz gatunków roślin drzewiastych roślinami energetycznymi mogą być również zboża i rośliny niebędące pożywieniem. Oprócz trawy (*Panicum virgatum* L) i jatrofy (*Jatropha curcas* L), niektóre gatunki glonów z rodziny *Asteraceae* i *Euphorbiaceae* zawierające duże stężenia triacylogliceroli i lateksu, mogą być wykorzystane do produkcji biokomponentów paliw. Gatunki *Amaranthus* sp., *Anderss Miscanthus sinensis*, *Euphorbia marginata* L, *Ambrosia artemisifolia* L, *Helianthus tuberosus* L, *Solidago canadensis* L mogą być pomyślnie uprawiane w warunkach klimatycznych Słowacji. Rośliny te przedstawiane są jako potencjalnie użyteczne gatunki roślin energetycznych, niekonkurujących z uprawami roślin spożywczych. Należy jednak podkreślić, że wymienione gatunki (np. jatrofa) należą do roślin inwazyjnych. Ponieważ produkcja biopaliw zarówno z roślin uprawnych, jak też z roślin nieżywnościowych jest nadal prowadzona, dlatego emisja ditlenku węgla i bilans energii z biopaliw obecnie są intensywnie dyskutowane. Analiza cyklu życia (LCA) to użyteczne

narzędzie określania wpływu biopaliw na środowisko przyrodnicze. LCA jest przedstawiona jako metoda naukowa, pozwalająca na ocenę oddziaływania paliwa na środowisko od produkcji do ostatecznej jego likwidacji/recyklingu. Takie podejście jest również znane jako „szyb naftowy do koła" dla paliw transportowych lub „pole do koła" w odniesieniu do biopaliw. W celu zbadania wpływu bioenergii i biopaliw na środowisko należy uwzględnić kilka innych problemów, takich jak zakwaszenie, nitryfikacja, użytkowanie terenu, zużycie wody lub toksycznych nawozów i pestycydów.

**Słowa kluczowe:** biopaliwa, biomasa, procesy przetwarzania roślin energetycznych, rośliny inwazyjne, analizy cyklu życia (LCA), rośliny niespożywcze

Mariola CHOMCZYŃSKA[1], Vladimir SOLDATOV[1] and Henryk WASĄG[1]

# EFFECT OF DIFFERENT VARIANTS OF THE ION EXCHANGE SUBSTRATE ON VEGETATION OF *Dactylis glomerata* L ON THE DEGRADED SOIL

## WPŁYW RÓŻNYCH WARIANTÓW SUBSTRATU JONITOWEGO WPROWADZONYCH DO ZDEGRADOWANEJ GLEBY NA PRZEBIEG WEGETACJI *Dactylis glomerata* L

**Abstract:** Ion exchange substrates are the mixtures of cation and anion exchangers saturated with nutrient ions in the proper ratios. After introducing into degraded soil, the substrates may act as a source of macro- and micronutrients initiating plant growth that is necessary for biological restoration. In order to determine an effect of different variants of ion exchange substrate (with a different potential impact on pH of soil solution) on plant vegetation, a pot experiment with orchard grass (*Dactylis glomerata* L) as the test species was carried out. For the need of the study seven series of media were prepared: the control series (sand as a model of degraded soil) and six test series - the mixtures of sand and 2% (v/v) additions of particular variants of ion exchange substrate (each variant contained monoionic forms differing in the content of $NO_3^-$, $H_2PO_4^-$, $SO_4^{2-}$ thereby, differing in the pH of solutions equilibrated with them). The study results showed that additions of the substrate at pH = 5.5; pH = 6.0; pH = 6.5; pH = 7.0 and pH = 7.5 to sand significantly increased plant yield. The 2% addition of the substrate at pH = 6.5 caused the highest increase in wet and dry stem and root biomass, however mean values of the vegetation parameters obtained in the series with the substrate at pH = 6.5 were not significantly higher than parameters obtained in the series with the substrates at pH = 7.0 and 7.5. In the light of study results, it can be said that particular variants of substrate at pH in the range of 5.5÷7.5 are effective fertilizers intensifying plant growth during relatively short period.

**Keywords:** ion exchange substrate, soil restoration, cation exchangers, anion exchangers

Degraded areas constitute a significant problem in environmental engineering. These territories are characterized by different devastation degree and require technical and biological reclamation. Biological reclamation involves intensive cultivation of humus--productive plant mixtures. Initialization of cultivation of the mixtures mentioned requires the application of agrotechnical procedures such as i.a. mineral fertilization or introduction into recultivated soil enriched materials of organic, organic-mineral and mineral character [1]. Mineral fertilization which is recommended in biological reclamation can be realized by addition to ground synthetic ion exchangers saturated with biogenic ions. These materials can play a role of a rich source of macro- and micronutrients for plants. Moreover, they are able, to some extent, to prevent nutrient leaching from ground of weak sorption properties, which is of importance in the protection of waters from eutrophication.

The research conducted until now has proved the possibility of applying, prepared on the basis of ion exchange resins, the so-called ion exchange substrates as fertilizer add-ons [2]. In fact, they are the mixtures of cation and anion exchangers saturated in relevant proportions with macro- and micronutrient ions [3]. Owing to high exchange capacity of ionites the nutrient contents in substrates can be tenfold higher than in the best natural soils. Preparation of ion exchange substrate is based on providing the mixture of cation and anion

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exchangers with the ionic composition that assures the availability of biogenic elements to the same degree as in hydroponic nutrient solution [4, 5]. In fact, there are three basic methods for preparing the ion exchange substrate: a dynamic, static and a method of monoionic forms [5]. In the dynamic method the preparation of ion exchange substrate consists in passing the selected nutrient solution through a mixture of cation and anion exchangers placed in a column by the time an ion exchange equilibrium is achieved. The equilibrium state is set at the moment when the concentrations of biogenic ions in the effluent from the column are the same as in the solution that enters the column. Obtaining ion exchange substrate by a static method is connected with the saturation of resins with biogenic ions originating from the mixtures of reagents dissolved in water. Depending on whether the cation and anion exchangers are saturated together (salt variant of the method) or separately (acid-base variant of the method) the mixtures can contain only salts, only acids or only hydroxides. The preparation of substrate by static methods must be preceded by obtaining the sample of substrate in dynamic conditions. In the sample, ion contents of biogenic elements are determined and subsequently the quantitative proportions between them are calculated. These ratios are necessary to ensure appropriate proportions between ions in the substrate prepared in static conditions. As far as the static method is concerned, in order for the prepared substrate to have a proper ionic composition, the quantitative proportions between contained ions must correspond to the proportions that occurred in the substrate sample obtained in dynamic conditions, that is a sample remaining in the state of equilibrium with nutrient solution. The process of obtaining the substrate by a method of monoionic forms proceeds in two stages. In the first instance an individual monoionic forms containing biogenic element ions are prepared. Based on a cation exchanger, calcium, potassium and magnesium forms are prepared whereas on the basis of anion exchanger - nitrate(V), sulphate(VI) and phosphate(V) forms are prepared. Micronutrient ions are introduced into one of the forms containing a given macronutrient. Preparing monoionic forms on the basis of cation exchanger is performed by saturation of ion exchange portion with a solution of relevant salt or relevant hydroxide. On the other hand, preparing monoionic forms based on anion exchanger occurs by means of relevant acids. After the preparation monoionic forms are subject to analyses for biogenic ions content. The results of the analyses and the quantitative proportions found between macronutrient ions in a substrate sample obtained by dynamic method allow to determine weighted amounts of individual forms in order to obtain a unit mass of a complete substrate. To prepare monoionic forms and thus ion exchange substrate, basically any cation and anion exchanger can be used. The utilization of anion exchangers with weakly dissociating functional groups for the preparation of monoionic forms or also the complete substrate should take into account their influence on pH of the soil solution after the introduction into the ground. The influence depends on anion content in anion exchanger and also on the current salts concentration dissolved in soil solution. The lower the anion contents in monoionic forms and the higher salt concentrations in soil solution are, the higher pH of the solution contacting with anion exchanger being the part of the substrate. Taking into consideration the high changeability of salt concentration in soil solution in time, one should select the monoionic forms prepared on the basis of polyfunctional anion exchanger in such a way that eventual pH changes of soil solution being in the contact with the substrate, correspond to physiological requirements of the plants. In the light of the above-mentioned information it can be expected that various variants of ion exchange

substrates are able to modify the reaction of soil solution. Thus, the aim of the presented research was to verify the influence of consequent variants of ion exchange substrate, potentially differently affecting the pH of the soil solution, on the plant vegetation process.

## Materials and methods

In the study sand, ion exchange resins of the KU-2 (strong acid cation exchanger) and EDE -10P (polifunctional anion exchanger) types were used. The sand was used as a model of degraded soil. It came from sand mine in Golab near Pulawy. The pH value of its water extract was 5.77.

The total ion exchange capacity of resins was 5 mval $g^{-1}$ for cation exchanger and 11 mval $g^{-1}$ for anion exchanger, respectively. Three monoionic forms: calcium, magnesium and potassium forms were prepared on the basis of cation exchanger. Anion exchanger was used for preparing six variants of nitrate, phosphate and sulphate forms differing in the pH of solutions equilibrated with them.

The preparing monoionic forms on the basis of the cation exchanger consisted in saturation of resin portions with relevant ions in water solutions containing: KOH, CaO and MgO, respectively. During preparation of monoionic forms on anion exchanger basis, resin portions were placed in solution of relevant potassium salt. Then particular acids ($HNO_3$, $H_3PO_4$, $H_2SO_4$) were added into reaction zones. The amounts of acids ensured fixed pH value of solution contacting with particular resin portion. In such way, 18 variants of monoionic forms were obtained enabling six variants of ion exchange substrates to be prepared. The variants differed in pH values of solutions equilibrated with particular monoionic forms.

In order to determine ratios between the monoionic forms in particular variants of ion exchange substrate, small amounts of cation and anion exchanger (3 g) have been saturated with nutrient solution (salt concentration about 1.65 g $dm^{-3}$) in dynamic conditions until equilibrium state was achieved. Ratios, between ions found in ion exchangers in equilibrium state, were used to calculate amounts of monoionic forms used for preparation of mass unit of particular substrate variant.

Table 1

Media series in vegetative experiment

| **Series** | **S** | **S+5.5** | **S+6.0** | **S+6.5** | **S+7.0** | **S+7.5** | **S+8.0** |
|---|---|---|---|---|---|---|---|
| Sand amount [$cm^3$] | 300 | 294 | 294 | 294 | 294 | 294 | 294 |
| Fertilizer amount [$cm^3$] | - | 6 | 6 | 6 | 6 | 6 | 6 |
| Number of pots | 9 | 9 | 9 | 9 | 9 | 9 | 9 |

Explanations: S - the control series (sand); S+5.5 - the mixture of sand and addition of the substrate containing monoionic forms equilibrated with solution at pH = 5.5; S+6.0 - the mixture of sand and addition of the substrate containing monoionic forms equilibrated with solution at pH = 6.0; S+6.5 - the mixture of sand and addition of the substrate containing monoionic forms equilibrated with solution at pH = 6.5; S+7.0 - the mixture of sand and addition of the substrate containing monoionic forms equilibrated with solution at pH = 7.0; S+7.5 - the mixture of sand and addition of the substrate containing monoionic forms equilibrated with solution at pH = 7.5; S+8.0 - the mixture of sand and addition of the substrate containing monoionic forms equilibrated with solution at pH = 8.0

The substrate variants were evaluated in a vegetation experiment. For the need of the pot test seven series of media were prepared: the control series (sand) and six test series - the mixtures of sand and 2% (v/v) additions of particular variants of ion exchange substrate

(Table 1). Then, 50 seeds of orchard grass (*Dactylis glomerata* L) - var. Amba was sown to each pot of experimental series. After 5 days since the moment of seed sowing the number of plants was standardized to 28. The experiment was carried out in a phytotron with 13/12 h light/dark. Daytime air temperature (between 7 a.m. and 8 p.m.) was 25°C. The night-time air temperature (between 8 p.m. and 7 a.m.) was 16°C. During the experiment plants were watered with distilled water. The vegetative growth period lasted 42 days. When the experiment was terminated, the aboveground shoots of plants were cut down and roots were separated. The wet and dry (dried at 105°C) biomass of shoots and roots was measured. The results obtained were used for calculation of mean values of variables characterizing plant growth in experimental series (arithmetical mean values). The significance of differences between mean values was assessed by t-Student test or v Aspin-Welch's test at confidence coefficient p = 0.95 [6-8].

## Results and discussion

The study results are presented in Table 2. The addition of all variants of ion exchange substrate to sand affected the vegetation cycle of orchard grass advantageously increasing values of vegetative parameters significantly. Specifically, wet stem biomass obtained in series: S+5.5; S+6.0; S+6.5; S+7.0; S+7.5 and S+8.0 was greater than that in the control series by six, six and half, seven and half, over seven, almost seven and over three and half times, respectively. As a rule, dry stem biomass of plants growing on sand enriched with additions of substrate variants exceeded that on sand alone by over 1000% (with the exception of series S+8.0, wherein considered variable was higher by 400% as opposed to the series S).

Table 2

Mean values of vegetative parameters in series of pot experiment [g per pot]

| Series | Wet stem biomass | Dry stem biomass | Wet root biomass | Dry root biomass |
|---|---|---|---|---|
| S | $0.298 \pm 0.021^{f}$ | $0.074 \pm 0.008^{f}$ | $0.899 \pm 0.120^{f}$ | $0.122 \pm 0.020^{f}$ |
| S+5.5 | $5.869 \pm 0.290^{af}$ | $0.918 \pm 0.081^{af}$ | $5.477 \pm 0.371^{af}$ | $0.687 \pm 0.042^{af}$ |
| S+6.0 | $5.381 \pm 0.310^{abf}$ | $0.867 \pm 0.059^{bf}$ | $5.768 \pm 0.507^{bf}$ | $0.700 \pm 0.064^{bf}$ |
| S+6.5 | $6.313 \pm 0.551^{abcf}$ | $1.000 \pm 0.093^{bcf}$ | $6.790 \pm 0.619^{abcf}$ | $0.864 \pm 0.070^{abcf}$ |
| S+7.0 | $5.998 \pm 0.482^{bdf}$ | $0.926 \pm 0.011^{df}$ | $6.562 \pm 0.558^{abdf}$ | $0.820 \pm 0.091^{abdf}$ |
| S+7.5 | $6.050 \pm 0.334^{bef}$ | $0.964 \pm 0.005^{bef}$ | $6.138 \pm 0.440^{acef}$ | $0.781 \pm 0.075^{abcef}$ |
| S+8.0 | $2.383 \pm 0.249^{abcdef}$ | $0.382 \pm 0.04^{abcdef}$ | $3.389 \pm 0.446^{abcdef}$ | $0.443 \pm 0.059^{abcdef}$ |

Explanations: S - the control series (sand); S+5.5 - the mixture of sand and addition of the substrate containing monoionic forms equilibrated with solution at pH = 5.5; S+6.0 - the mixture of sand and addition of the substrate containing monoionic forms equilibrated with solution at pH = 6.0; S+6.5 - the mixture of sand and addition of the substrate containing monoionic forms equilibrated with solution at pH = 6.5; S+7.0 - the mixture of sand and addition of the substrate containing monoionic forms equilibrated with solution at pH = 7.0; S+7.5 - the mixture of sand and addition of the substrate containing monoionic forms equilibrated with solution at pH = 7.5; S+8.0 - the mixture of sand and addition of the substrate containing monoionic forms equilibrated with solution at pH = 8.0; ± - a standard deviation; a - significant differences between series S+5.5 and the rest of series; b - significant differences between series S+6.0 and the rest of series; c - significant differences between series S+6.5 and the rest of series; d - significant differences between series S+7.0 and the rest of series; e - significant differences between series S+7.5 and series S+8.0; f - significant differences between series S and the rest of series; the results followed by the same letters are statistically different

Wet root biomass obtained on sand with fertiliser additions was greater than that obtained in the control series almost or over twenty times (again the exception was series S+8.0, wherein wet root biomass was eight times as great as that in the control S). Dry root biomass in fertilized series was in most cases almost or over six times higher than that obtained on sand only medium (this trend was not observed for series S+8.0, wherein considered variable exceeded that obtained in series S three and half times).

Among all media series supplemented with addition of ion exchange substrate the lowest values of vegetative variables were observed in series S+8.0 (Table 2). Thus, wet and dry stem and root biomass of orchard grass growing on sand enriched with addition of the substrate at 8.0 was significantly lower than those obtained on other fertilized media. Wet and dry stem and root biomass in series: S+5.5; S+6.0; S+6.5; S+7.0; S+7.5, exceeded in most cases those in series with addition of the substrate at pH = 8.0 by 150% and over 50%, respectively. Such results can be explained by abundance of substrate variant at pH = 8. In fact, the substrate at pH = 8 contained less macronutrients than other variants of ion exchange substrate (Table 3).

The addition of substrate at pH = 6.5 to sand caused the highest increases in values of vegetative parameters (Table 2). Wet and dry stem and root biomass of orchard grass growing on sand supplemented with substrate at pH = 6.5 were significantly higher than those obtained in series: S+5.5, S+6.0 and S+8.0. At the same time values of these variables did not differ significantly as opposed to values obtained in series S+7.0 (and in series S+7.5 regarding wet and dry root biomass).

Table 3

Content of ions in different variants of ion-exchange substrate [mval per 100 g]

| Substrate | $NO_3^-$ | $H_2PO_4^-$ | $SO_4^{2-}$ | $Ca^{2+}$ | $Mg^{2+}$ | $K^+$ |
|---|---|---|---|---|---|---|
| At 5.5 | 64.99 | 23.64 | 108.31 | 183.86 | 32.98 | 18.84 |
| At 6.0 | 64.58 | 23.49 | 107.63 | 182.69 | 32.80 | 18.71 |
| At 6.5 | 63.60 | 23.12 | 106.01 | 179.98 | 32.30 | 18.45 |
| At 7.0 | 62.37 | 22.67 | 103.94 | 176.44 | 31.64 | 18.09 |
| At 7.5 | 60.12 | 21.86 | 100.21 | 170.09 | 30.52 | 17.46 |
| At 8.0 | 48.12 | 14.50 | 80.21 | 136.16 | 24.45 | 13.97 |

## Summary and conclusions

The results obtained allowed the following conclusions to be formulated:

The ion exchange substrates prepared on the basis of monoionic forms equilibrated with solutions at pH in the range 5.5÷7.5 significantly increased plant biomass after introducing into sand.

Among tested substrate variants, ion exchange substrate at pH = 8.0 caused the lowest increase in biomass of orchard grass; the addition of ion exchange substrate at pH = 6.5 to sand was associated with the highest yield of *Dactylis glomerata* L.

Regarding that there were rather small differences between plant biomass for series: S+5.5; S+6.0; S+6.5; S+7.0; S+7.5 (however some of them were statistically significant), it can be said that particular variants of substrate at pH in the range of 5.5÷7.5 are effective fertilizers intensifying plant growth during the observed six weeks of vegetation period.

## Acknowledgements

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## WPŁYW RÓŻNYCH WARIANTÓW SUBSTRATU JONITOWEGO WPROWADZONYCH DO ZDEGRADOWANEJ GLEBY NA PRZEBIEG WEGETACJI *Dactylis glomerata* L

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**Abstrakt:** Substraty jonitowe są mieszaninami kationitów i anionitów nasyconych w stosownych proporcjach jonami pierwiastków odżywczych. Materiały te po wprowadzeniu do gleby zdegradowanej mogą pełnić rolę bogatego źródła makro- i mikroelementów, inicjującego rozwój pokrywy roślinnej niezbędnej w czasie biologicznej rekultywacji. Aby określić wpływ różnych wariantów substratu (o potencjalnie różnym oddziaływaniu na pH roztworu glebowego) na przebieg wegetacji roślin, przeprowadzono doświadczenie wazonowe z kupkówką pospolitą (*Dactylis glomerata* L) jako gatunkiem testowym. Na potrzeby tego eksperymentu przygotowano siedem serii podłoży: jedną serię kontrolną (piasek jako model gleby zdegradowanej) oraz sześć serii testowych będących mieszaninami piasku i 2% (objętościowo) dodatków poszczególnych wariantów substratu jonitowego (każdy wariant zawierał formy monojonowe różniące się zawartością anionów: $NO_3^-$, $H_2PO_4^-$, $SO_4^{2-}$ i tym samym różniące się wartościami pH roztworów pozostających z nimi w równowadze jonowymiennej). Rezultaty przeprowadzonego eksperymentu wykazały, że dodatki substratu o pH = 5,5; pH = 6,0; pH = 6,5; pH = 7,0 i pH = 7,5 po wprowadzeniu do piasku znacznie zwiększyły plon roślin. Dodatek substratu o pH = 6,5 spowodował największe przyrosty świeżej i suchej biomasy pędów oraz korzeni kupkówki, aczkolwiek średnie wartości parametrów wegetacyjnych uzyskane w serii z tym substratem nie były istotnie większe od wartości parametrów uzyskanych w serii z dodatkiem substratu o pH = 7,0 i 7,5. W świetle otrzymanych wyników należy stwierdzić, iż poszczególne warianty substratu o pH w zakresie 5,5÷7,5 są skutecznymi dodatkami nawozowymi intensyfikującymi rozwój roślin w stosunkowo krótkim okresie czasu.

**Słowa kluczowe:** substraty jonitowe, rekultywacja gleb, kationit, anionit

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# INFLUENCE OF YEAR SEASON AND ROOM TYPE ON INDOOR $^{222}$Rn ACTIVITY

## WPŁYW PORY ROKU I RODZAJU POMIESZCZENIA NA AKTYWNOŚĆ $^{222}$Rn

**Abstract:** In this work the monitoring of $^{222}$Rn was carried out in selected houses, located in the district of Krapkowice (it lies to the south of Opole, PL). The passive detectors LR-115 type II films (Kodak, Pathe) have been used in this survey. The measurements were carried out in single-storied residential houses in two time periods: from the end of June to the end of September and from the beginning of November to the beginning of February. The detectors were placed in kitchens, bathrooms and cellars. Our results showed that in all of the rooms examined the Rn activity did not exceed 149 kBq/m$^3$. This value is lower than the European Commission recommendations for existing buildings (Commission recommendation 2001/928/Euratom). Considering all of room types investigated, no statistically significant differences between $^{222}$Rn activities in summer and in winter were asserted. But in cellars higher than in other rooms $^{222}$Rn activities were confirmed, independently on the season of year. In most of the houses activities of $^{222}$Rn were similar, though in two of them they were significantly higher than in the others.

**Keywords:** indoor radioactivity, $^{222}$Rn, seasonal changes

Radon radioisotopes and their decay products are one of the most important contributors to human radioactive exposure from natural sources. Gaseous Rn can penetrate into living organism, especially while respiration. Remaining in close contact with tissues the radioisotopes of Rn may cause dangerous damages in human cells structure, leading to appearance of serious diseases. Because of its intermediate period of half-life (3.8 days) the main attention is usually paid to $^{222}$Rn. In 2009 an average Polish citizen received a dose of 3.19 mSv, in which the contribution of radon was 37.6% [1].

The concentrations activity of radon and their progeny are largely influenced by factors such as topography, type of house construction, building materials, temperature, pressure, humidity, ventilation, wind speed, and even the life style of the house residents [1-9].

In this work the monitoring of $^{222}$Rn was carried out in selected houses, located in the district of Krapkowice (it lies to the south of Opole, PL). The passive detectors LR-115 type II films (Kodak, Pathe) have been used in this survey. The measurements were carried out in single-storied residential houses in two time periods: from the end of June to the end of September and from the beginning of November to the beginning of February. The detectors were placed in kitchens, bathrooms and cellars.

## Materials and methods

In the present investigations the indoor $^{222}$Rn concentration was studied in nine houses. All of them were located in the district of Krapkowice. They were similar to each other and were more than 50 years old. Each of them was built of clay-bricks and has a cellar. They

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were single-family houses with kitchens, living-rooms and bathrooms. The largest distance between buildings does not exceed 1 km.

In the present investigations, a passive technique was employed using Solid State Nuclear Track Detectors (SSNTDs) which are sensitive to alpha particles in the energy range of the particle emitted by radon. SSNTDs also have the advantage of being mostly unaffected by humidity, low temperatures, moderate heating and light.

LR-115 Type 2 (12 m cellulose nitrate on 100 m polyester base) plastic track detectors each with a size of about (1.5 cm × 2 cm) were used.

The measurements were carried out in one-storey residential houses in two time periods: from the end of June to the end of September and from the beginning of November to the beginning of February. The detectors were placed in kitchens, bathrooms and cellars.

After 3 months of exposure, the detectors were subjected to chemical processing in a 10 M analytical grade sodium hydroxide solution at (60 ±1)°C, for 90 min, in a constant temperature water bath to enlarge the latent tracks produced by alpha particles from the radon decay. After the etching, the detectors were washed for 30 min with running cold water, then with distilled water and finally with a 50% water/alcohol solution. After a few minutes of drying in air, the detectors were ready for track counting. The etched tracks were counted using an optical microscope at 40 × magnification, observing 0.059 $cm^2$ of detector surface. The number of tracks was counted for each plate in 30 microscope fields of view. The $^{222}Rn$ concentration a [$Bq/m^3$] is calculated by the following formula:

$$a = (Q/t) \times 13.8 \quad (1)$$

where: Q - number of tracks per $cm^2$ plate, t - exposure time in days, 13.8 - empirical coefficient in $Bq \cdot d \cdot cm^2/m^3$, calculated from data published by Srivastava [10].

Errors σ in the track densities were calculated by multiplying the track density by $(1/N)^{1/2}$, where N is the total number of tracks counted in a sample [11]. In our measurements the background radiation from other than $^{222}Rn$ was not taken into account. However, the considerable similarity between the buildings investigated and the relatively low surface area on which they were located may indicate that the variability in track densities is a result of differences in the utilization of the rooms.

## Results and discussion

For statistical computations the R language [12] was used. R is a free software environment for statistical computing and graphics. The capabilities of R can be extended through packages, which allow specialized statistical techniques, graphical devices, programming interfaces and import/export capabilities to many external data formats.

Table 1

Statistical parameters computed from the $^{222}Rn$ activities

| Min. | $q_l$ | ME | $q_u$ | max | $a_m$ | SD |
|---|---|---|---|---|---|---|
| [$Bq/m^3$] | | | | | | |
| 8 | 27 | 49 | 57 | 78 | 148 | 36 |

It was expected that, in the winter season, the $^{222}Rn$ activity would be highest in the rooms, due to limited ventilation. In Table 1 the values of minimum min., lower quartile $q_l$, median ME, upper quartile $q_u$, maximum max, arithmetic mean $a_m$ and standard deviation

SD of $^{222}$Rn activity computed from the data are shown. The calculated σ error was high for low activities, reaching 100% for single tracks observed on a detector surface area. For the highest activities σ was 14%.

The differences between $^{222}$Rn activities in different room types can be assessed by comparing the boxplots shown in Figure 1. In these plots the lower base of the rectangle is a lower quartile, the upper base is an upper quartile and a horizontal line dividing the rectangle represents a median. Whiskers are formed by connecting the formed box with short horizontal lines drawn for quantile q = 0.95 (upper whisker) and quantile 0.05 (lower whisker). The points marked by a circle are located more than 1.5 times of interquartile range from the median. The values of $^{222}$Rn activities were similar in all of the rooms investigated. Median values are comparable and no significant differences in their values were observed. The $^{222}$Rn activities considerably exceeded the common range only in one kitchen and two living-rooms.

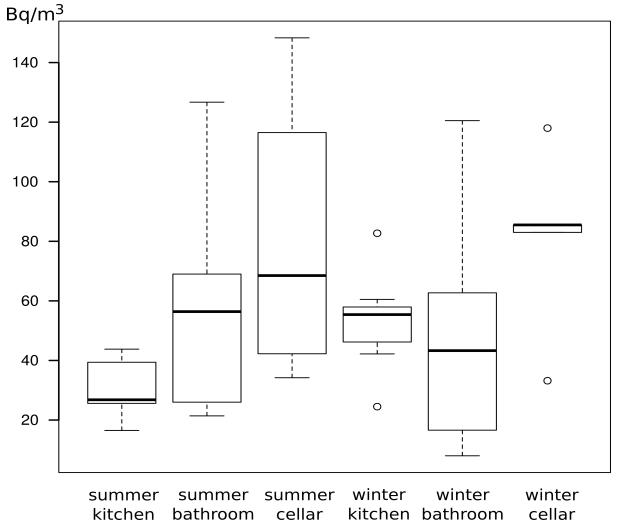


Fig. 1. The differences between $^{222}$Rn activities in different room types

All of the measurement results were lower than the limits of $^{222}$Rn activity given in the recommendations. The International Commission on Radiological Protection gives two reference values (for dwellings), above which action must be taken to reduce radon concentrations: 400 Bq/m$^3$ - for houses already built and 200 Bq/m$^3$ for newly built homes [13]. A recent recommendation of International Commission on Radiological Protection for the reference level for radon gas in dwellings is 300 Bq/m$^3$ [14].

## Conclusions

The highest $^{222}$Rn activity did not exceed the recommended value. Distribution of traces numbers was not normal (gaussian). After logarithmic data transformation they became normally distributed, what was confirmed by results of Anderson-Darling, Lilliefors and Cramer-von Mises tests. One-way ANOVA showed not statistically significant differences in means of $^{222}$Rn activities measured in summer and in winter. The

same statistical method indicated significantly higher $^{222}$Rn concentration in cellars, regardless of season of year.

The rooms investigated do not need actions reducing $^{222}$Rn concentration in air.

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## WPŁYW PORY ROKU I RODZAJU POMIESZCZENIA NA AKTYWNOŚĆ $^{222}$Rn

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**Abstrakt:** Przedstawiono wyniki badań aktywności $^{222}$Rn przeprowadzonych w wybranych budynkach znajdujących się na terenie powiatu krapkowickiego (na południe od Opola, PL). W badaniach wykorzystano pasywne detektory typu LR-115 (Kodak, Pathe). Pomiary przeprowadzono w jednorodzinnych domach mieszkalnych w dwóch okresach: od końca czerwca do końca września i od początku listopada do początku lutego. Detektory zostały umieszczone w kuchniach, łazienkach i piwnicach. Otrzymane wyniki wskazują, że w żadnym z pomieszczeń aktywność $^{222}$Rn nie przekroczyła 149 kBq/m$^3$. Wartość ta jest niższa od zalecanej przez Komisję Europejską dla budynków istniejących (zalecenie Komisji 2001/928/Euratom). Biorąc pod uwagę wszystkie rodzaje badanych pomieszczeń, nie stwierdzono statystycznie istotnych różnic między aktywnością $^{222}$Rn w okresie letnim i zimowym. W piwnicach stwierdzono wyższą niż w innych pomieszczeniach aktywność $^{222}$Rn, niezależnie od pory roku. W większości domów aktywności $^{222}$Rn były podobne, choć w dwóch z nich były one znacząco większe niż w pozostałych.

**Słowa kluczowe:** radioaktywność w pomieszczeniach zamkniętych, $^{222}$Rn, zmiany sezonowe

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# EVALUATION OF PROTEOLYTIC ACTIVITY OF *Bacillus mycoides* STRAINS

## OCENA AKTYWNOŚCI PROTEOLITYCZNEJ SZCZEPÓW *Bacillus mycoides*

**Abstract:** The research has focused on evaluation the capability of *Bacillus mycoides* strain isolated from soil (designated as A134) and from water (designated as G3) to secrete proteolytic enzymes. The effect of temperatures (30÷60ºC) and medium composition (albumin, casein, skim milk) on protease production was evaluated. The proteolytic activity was determined by using the spectrophotometric method and expressed as amounts of μmol tyrosine liberated by 1 $cm^3$ crude enzyme. In conducted research, the amount of tyrosine depended on the temperature, medium formulation and strain of *Bacillus mycoides.* It was found that milk and albumin media were proved to be best for protease production by soil strain A134 at 30ºC (14.00 and 13.72 μmol of tyrosine, respectively), while casein medium at 60ºC (11.44 μmol). Strain *B. mycoides* isolated from water G3 behaved differently from strain A134. The best temperature for enzyme production was 60ºC but milk and albumin media (16.2 and 17.0 μmol, respectively), whereas 30ºC on casein medium. Moreover, it was observed that this strain did not exhibit any enzyme expression on casein medium at 50 and 60ºC.

**Keywords:** *Bacillus mycoides,* proteolytic activity, temperature

Proteases are enzymes that catalyse hydrolytic reactions in which protein molecules are degraded to peptides and amino acids [1, 2]. They constitute one of the most important groups of industrial enzymes and have applications in different industries such as detergent, food, feed, pharmaceutical, leather and waste processing. Microbial proteases account for approximately 60% of the total worldwide enzyme sales and are preferred to the enzymes from plant and animal sources, since they possess almost all the desired characteristics for biotechnological applications [1, 3]. Most commercial proteases, mainly neutral and alkaline are produced by organisms belonging to the genus Bacillus [4]. They are specific producers of extracellular proteases and attractive industrial tools for a variety of reasons, including their high growth rates leading to short fermentation cycle times, their capacity to secrete proteins into the extracellular media [5]. All these bacteria are easily isolated from natural environment.

Various nutritional, physical and chemical factors such as media composition, temperature, pH, aeration, incubation times, inoculum concentration significantly affect protease production. Interactions of these parameters are reported to have a significant influence on the production of the enzyme [3, 6, 7]. Bacterium from *Bacillus cereus* "group" such as *Bacillus mycoides* and *Bacillus cereus* were reported to produce proteolytic enzymes however, few studies have been done on proteolytic enzymes from *B. mycoides* [8].

The aim of presented research was the evaluation of the ability to synthesize protease by selected *Bacillus mycoides* strains, isolated from natural environment, depend on the temperature and medium formulation.

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## Materials and methods

The bacterial strains used in this study were *Bacillus mycoides* strains, isolated from soil samples - *B. mycoides* marked as A134 and from water of Turawa lake - *B. mycoides* marked as G3.

The growth media for protease production consisted of a basal salt solution containing [g · $dm^{-3}$]: $(NH_4)_2SO_4$ - 2.0; $K_2HPO_4$ - 3.0; $KH_2PO_4$ - 2.0; $MgSO_4x7H_2O$ - 0.5 that was enriched appropriate for albumin, casein and skim milk (1%). The pH of the medium was adjusted to 7.0. The cultures were maintained at 30ºC for 48 h in a shaking incubator (110 rpm). At the end of incubation culture were centrifuged at 4000 rpm for 20 min then obtained cell-free supernatants were used as crude enzyme to determine proteolytic activity. The effect of temperature on protease production was defined by incubating the reaction mixtures at different temperatures (30, 40, 50 and 60°C). Protease activity was determined in the presence of 1% casein hydrolysate solution using the spectrophotometric method. For these studies protease activity was expressed as amounts of tyrosine liberated from casein per 10 min by 1 $cm^3$ enzyme solution under the assay conditions. The amount of tyrosine was calculated according to Tyrosine Standard Curve [9].

## Results and discussion

In conducted research, it was found that each strain has its own special conditions for maximum enzyme production and the amount of released micromoles of tyrosyne dependent on the temperature and the medium composition. The ability of *Bacillus mycoides* strains to protease production at the varied range temperature in different medium formulation are presented in the Figures 1-3.

Strain *Bacillus mycoides* G3, isolated from water maximum activity obtained at 60ºC on albumin medium (17.00 µmol) and 4-fold lesser at 30ºC (4.4 µmol). In contrast to this strain *Bacillus mycoides* A134 isolated from soil, showed the highest level of protease activity at 30ºC (13.72 µmol) and to a lesser extent at 60ºC (10.52 µmol). However this strain did not exhibit any activity at 40ºC (Fig. 1).

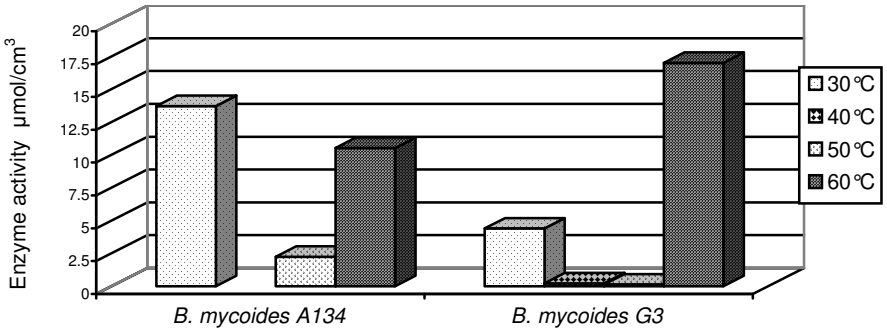


Fig. 1. Effect different temperature on protease activity of *Bacillus mycoides* strains on albumin medium

Casein medium was proved to be good medium for protease production by *Bacillus mycoides* A 134 that showed high level of released micromoles of tyrosine at 60ºC

(11.44 µmol) followed 2-fold lower activity at 50ºC than found in case 60ºC. Lower temperature was found to have adverse effect on the metabolic activities of the microorganisms. In the remainder temperatures 30÷40ºC the amount of tyrosine released was lower from 86.36% till 91.96% respectively, compared with 60ºC. The second strain designated as G3 showed high protease expression at 30ºC but merely 4.12 µmol of tyrosine but did not secrete enzymes in higher temperatures 50÷60ºC (Fig. 2).

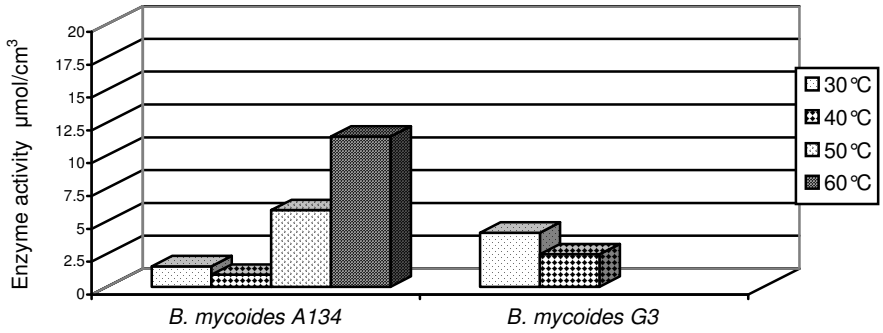


Fig. 2. Effect of temperature on protease activity of *Bacillus mycoides* strains on casein medium

The least analysed medium with skim milk, showed that extracellular secretion of proteases was most intense by *Bacillus mycoides* marked as G3 at 60ºC (16.2 µmol) and lower by 37.37% at 30ºC. It was found that temperature 50ºC did not affect protease production. The high protease activity was found in case of *B. mycoides* A134 at 30ºC (14.00 µmol) and less in the others temperatures from 82.3 till 95.8%, compared with the lowest temperature (Fig. 3).

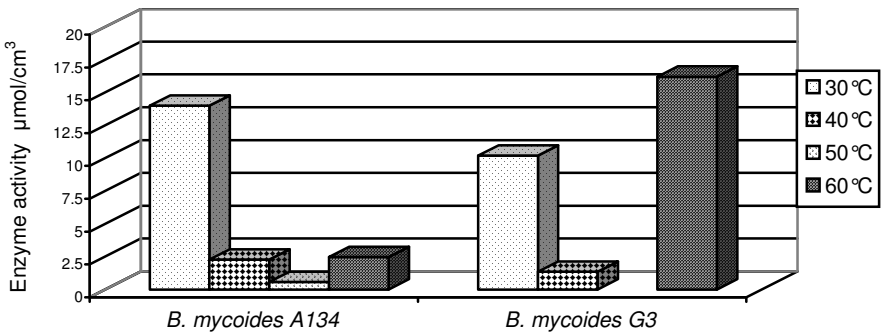


Fig. 3. Effect of temperature on protease activity of *Bacillus mycoides* strains on milk medium

## Conclusions

Conducted research proved diversity between *Bacillus mycoides* strains in terms of their protease production, when different temperature and media composition were applied and enabled to draw the conclusions:

1. Biosynthesis of protease catalysed by *Bacillus mycoides* at different temperatures was the most diversified in depending of strain and media formulation.
2. The highest amount of tyrosine was released by *Bacillus mycoides* G3 at 60ºC on albumin and milk medium.
3. Biosynthesis of proteases catalysed by *Bacillus mycoides* A134 was the most intense at 30ºC on albumin and milk medium.

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## OCENA AKTYWNOŚCI PROTEOLITYCZNEJ SZCZEPÓW *Bacillus mycoides*

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**Abstrakt:** Celem badań była ocena potencjalnej zdolności proteolitycznej szczepu *Bacillus mycoides* A134 wyizolowanego z gleby i szczepu G3 wyizolowanego z wody. Oceniono wpływ temperatury (30÷60ºC) i składu podłoża zawierającego albuminę, kazeinę lub odtłuszczone mleko na aktywność proteolityczną. Oznaczenie aktywności proteolitycznej przeprowadzono metodą spektrofotometryczną, a wyniki podano jako ilość uwolnionych mikromoli tyrozyny. W przeprowadzonym doświadczeniu ilość mikromoli tyrozyny uzależniona była od temperatury i składu podłoża, a także szczepu. Stwierdzono, że *Bacillus mycoides* A134 uwolnił największą ilość w temperaturze 30°C na podłożach z mlekiem odtłuszczonym lub albuminą (14,00 oraz 13,72 µmoli tyrozyny, odpowiednio), natomiast w 60ºC na podłożu z kazeiną (11,44 µmol). W porównaniu do niego szczep wyizolowany z wody G3 wykazywał odmienną aktywność. Największą ilość mikromoli tyrozyny stwierdzono w temperaturze 60ºC na podłożach z mlekiem lub albuminą (16,2 oraz 17,0 µmoli odpowiednio), a 30°C na podłożu z kazeiną. Ponadto nie stwierdzono żadnej aktywności proteolitycznej na podłożu z kazeiną w 50 i 60ºC.

**Słowa kluczowe:** *Bacillus mycoides,* aktywność proteolityczna, temperatura

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# CADMIUM AND MERCURY RAT BODY BURDEN FOLLOWING COPPER SUPPLEMENTS

## RETENCJA KADMU I RTĘCI W ORGANIZMIE SZCZURA KARMIONEGO PASZĄ Z DODATKIEM MIEDZI

**Abstract: S**tudies involved the influence of a diet containing 10-times increased copper concentrations (50 µg/kg) in comparison with a standard diet on the absorption of mercury and cadmium (mercury-203 and cadmium-109) given intragastrically for 28 days to Wistar male rats at doses corresponding to 10 mg of cadmium or mercury/kg of feedstuff. Concentrations of radiomercury and radiocadmium were determined in the carcass 1 h, 3 h, 12 h, 1 d, 2 d, 4 d, 8 d and 16 d postdosing. Moreover, feed intake and body weight gains were evaluated during the experimental period. A ten-time increase in copper dietary intake did not produce statistically significant differences in the distribution of cadmium in the carcass of rats. On the other hand, copper reduced the retention of mercury in the carcass within the experimental period and significant differences were found 1 d and 2 d postdosing. Treatment of the rats with cadmium and mercury decreased body weight gains in comparison with those reported in normal rats. The supplementation of dietary copper reduced decreases in body gains when compared with those in the rats exposed only to mercury and or cadmium. The results indicate a beneficial role of supplements of dietary copper in decreasing the bioavailability of inorganic mercury *via* the gastrointestinal tract. Lack of differences in the bioavailability of cadmium in the presence of supplemental copper suggests that the mechanisms of mercury-copper and cadmium-copper interactions may vary. Increased body weight gains in rats intoxicated with mercury or cadmium and fed the copper supplemented diet suggested that copper may diminish toxic actions of the two heavy metals.

**Keywords:** cadmium, mercury, copper, rat, interaction

Cadmium and mercury are natural chemical compounds occurring as residues in food and feedstuffs because of their presence in the environment resulting from human activities such as farming, industry, car exhausts or from contamination during food and feedstuffs processing and storage. Contamination of food and feed by mercury and cadmium cannot be entirely avoided. Current reports of the European Food Safety Authority stress a potential risk of exposure to enhanced levels of cadmium and mercury in food and feedstuffs [1, 2]. The presence of cadmium and mercury in feedstuffs poses a serious toxicological problem even though the influx of these metals into the environment is being limited. People and animals may be exposed during their entire lifespan to dietary cadmium and mercury. It is believed that a frequent consumption of some feeds and feedstuffs may contribute significantly to the overall human and animal exposure to cadmium and mercury. Cadmium and mercury may affect the metabolism of trace elements including copper [3-7]. On the other hand, it seems reasonable that copper supplementation of feeds for animals may influence cadmium and mercury accumulation in the body. The aim of this study was to find the effect of dietary supplements of copper on the body retention of mercury and cadmium given via the gastrointestinal tract.

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## Material and methods

Two-month old male Wistar rats from a commercial breeding station (Kozlowska Breeding Station, Warsaw) were used. The rats were kept in group of 5 in stainless steel cages.

Animals were acclimatized under standard laboratory conditions for a one week and then were randomly assigned into four treatment groups comprising 40 rats each: Group 1 and 2 (LSM), the controls, fed a standard pelleted diet LSM for rodents (Fodder Manufacture at Motycz Poland) and were given intragastrically a water solution of cadmium chloride or mercury chloride, respectively, daily for 28 days except weekends at a dose corresponding to 10 mg of cadmium or mercury/kg of feedstuff. Rats in groups 3 and 4 were treated similarly to those in groups 1 and 2 except for a diet which was supplemented with copper chloride 10-times the recommended level in the LSM diet fed in groups 1 and 2. Cadmium and mercury chloride given to rats in all groups were labelled with cadmium-109 and mercury-203, respectively. Daily feed and water consumption was evaluated weekly throughout the experiment. An initial and weekly body gains were evaluated.

All rats were killed by immersion in gaseous carbon dioxide 1 h, 3 h, 6 h, 12 h, 1 d, 2 d, 4 d, 8 d, and 16 d postdosing. The content of cadmium-109 or mercury-203 in the carcass (whole body without the stomach and intestines) was measured in a whole-body counter ZM 701 (Polon, Poland). Reference standards for quantification of carcass were prepared by intraperitoneal injection of the appropriate solution of cadmium-109 or mercury-203 to rats which were killed 30 min thereafter. The area under the curves (AUC) of radiocadmium or radiomercury retention versus time points was calculated by the trapezoidal rule. Data were analysed statistically using Student's *t*-test at $p<0.05$. The experiments were approved by the Local Ethics Committee for Animal Experiments in Lublin, Poland.

## Results

Food and water intake was similar in all examined rats although rats intoxicated with mercury or cadmium and fed the copper supplemented diet demonstrated a higher growth rate in comparison with those fed a standard laboratory diet. No visible sign of cadmium or mercury toxicity except some uneasiness noticed at the beginning of cadmium or mercury exposure were found.

The carcass retention of mercury is presented in Figure 1. Results indicate that copper supplemented diet decreased mercury retention in the body of rats throughout the whole experimental period. Significant decreases were found 1 d and 2 d after mercury-203 administration. The AUC values for mercury and mercury plus copper treated rats were 595 (100%) and 473 (79%), respectively. This indicates that mercury retention in rats fed the copper supplemented diet was lower by about 21% as compared with that in rats fed a standard laboratory diet.

The carcass retention of cadmium was show in Figure 2. Data indicate that the retention of cadmium in rats fed the copper fortified diet was not significantly affected by increased intake of dietary copper. Moreover, the AUC values in the controls and copper treated rats were similar, 320 and 340, respectively.

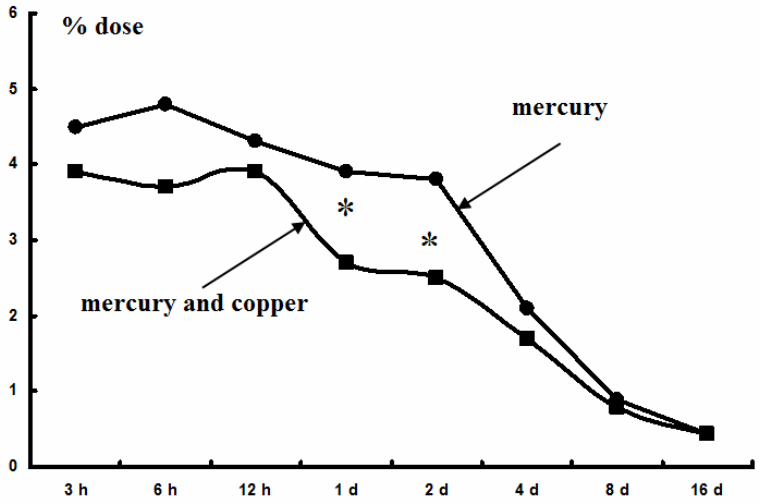


Fig. 1 Carcass retention of mercury (Hg-203) in rats. * - indicates statistically significant differences at $p<0.05$

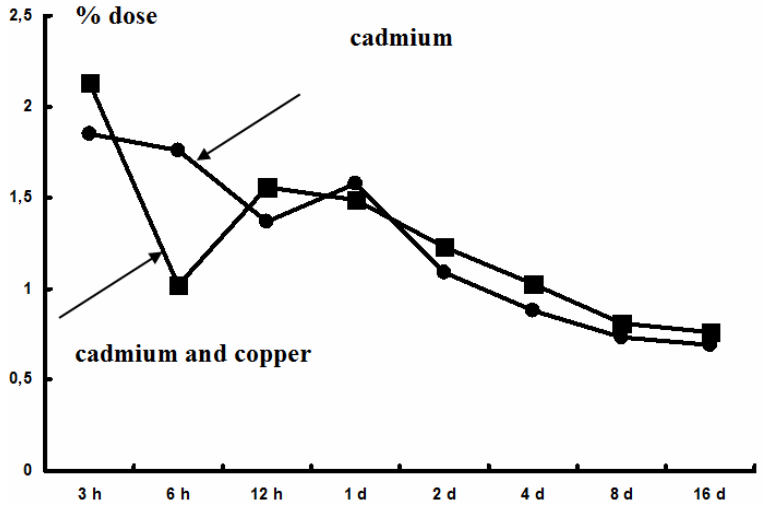


Fig. 2. Carcass retention of cadmium (Cd-109) in rats

## Discussion

The bioavailability, retention and consequently toxicity of the metals are affected by several factors such as nutritional status including body trace element stores. Mercury and copper interaction is not well understood. It was found that mercury can replace metallothionein (Mt) bound Cu in *in vitro* studies [9]. Oral intoxication with mercuric chloride increased copper retention in the kidneys [6]. There are not reports which suggest that copper may affect mercury retention in the body. The data presented here show that supplements of copper reduced significantly mercury retention in the body. It may be

postulated that copper forming a stable complex with Mt replaces mercury bound to this protein. As a result, mercury may be released into the circulation and then excreted. It was found that cadmium administered at toxic levels decreased copper concentration in several organs except for kidneys [7]. On the other hand, Toshiyuki et al [8] reported that copper given with cadmium increased cadmium retention and toxic action within the body, whereas copper level was unaffected. Based on the current results it was found that copper failed to influence significantly the body burden of cadmium although a higher body rate growth may suggested that that copper reduced a toxic action of cadmium within the body. Thus, these results may support findings presented by Peraza et al [10] who reported that supplements with copper reduced mortality rate and severity of anemia in animals receiving large doses of cadmium. The data presented here indicate that the mechanism of interaction between copper and mercury or copper and cadmium varies with regard to the metal involved. It is worth stressing that copper supplements increased the body weight gains of rats which may suggest a beneficial role of this nutrient in the case of mercury and cadmium intoxication.

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## RETENCJA KADMU I RTĘCI W ORGANIZMIE SZCZURA KARMIONEGO PASZĄ Z DODATKIEM MIEDZI

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**Abstrakt:** Obecność kadmu i rtęci w paszach stanowi poważny problem w toksykologii mimo znacznego ograniczenia dopływu tych metali do środowiska. Z raportu Europejskiego Urzędu do Spraw Bezpieczeństwa Żywności z ostatnich lat wynika, że istnieje potencjalne ryzyko narażenia na zwiększone ilości tych metali zawartych w paszach i żywności pochodzenia zwierzęcego. Dane z piśmiennictwa wskazują, że wchłanianie kadmu i rtęci z przewodu pokarmowego zależne jest od składu diety, w tym zawartości składników mineralnych. W niniejszych badaniach uwzględniono wpływ diety, zawierającej zwiększoną dziesięciokrotnie ilość miedzi (50 µg/kg) w odniesieniu do paszy standardowej na przyswajanie chlorków rtęci i kadmu (znakowanych rtęcią-203 i kadmem-109) podawanych dożołądkowo przez 28 dni szczurom samcom szczepu Wistar w ilościach odpowiadających 10 mg Cd lub Hg/kg paszy. Zawartość radiokadmu i radiortęci oznaczano w korpusie zwierząt

po uprzednim usunięciu przewodu pokarmowego z treścią po 1 h, 3 h, 12 h, 2 d i 4 d, 8 d i 16 d od zakończeniu aplikacji radioizotopów. W trakcie doświadczenia określano dodatkowo spożycie paszy i przyrosty masy ciała. Dziesięciokrotny wzrost zawartości miedzi w paszy nie powodował statystycznie istotnych zmian w rozmieszczeniu radiokadmu w korpusie zwierząt, aczkolwiek obserwowano zauważalne zwiększenie ilości kadmu przez cały okres badań. W przypadku rtęci dodatek miedzi do diety powodował obniżenie zawartości tego metalu w korpusie przez cały okres badań, a różnice statystycznie istotne notowano po 1 d i 2 d od zakończeniu podawania rtęci. Podawanie szczurom rtęci i kadmu zmniejszało przyrosty masy ciała w porównaniu do odpowiednich danych uzyskanych u zwierząt niezatruwanych tymi metalami, nieeksponowanych na te metale ciężkie. Dodatek miedzi do paszy łagodził spadki przyrostów masy ciała u zwierząt zatruwanych rtęcią i kadmem. Uzyskane wyniki wskazują na korzystne oddziaływanie zwiększonych ilości miedzi w paszy, wyrażające się ograniczeniem przyswajania rtęci nieorganicznej podawanej drogą pokarmową. Brak znacznych zmian w biodostępności kadmu w obecności zwiększonych ilości miedzi wskazuje, że mechanizmy interakcji rtęć-miedź i kadm-miedź mogą być zróżnicowane. Warto jednak podkreślić, że dodatek miedzi do paszy powodował ponadto zwiększone przyrosty masy ciała u zwierząt zatruwanych rtęcią i kadmem, co może sugerować ochronne właściwości miedzi w przypadku zatruć testowanymi metalami ciężkimi.

**Słowa kluczowe:** kadm, rtęć, miedź, szczur, interakcja

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# QUALITY INSPECTION OF NAIL POLISH REMOVERS AND ASSESSMENT OF HEALTH HAZARD ASSOCIATED WITH THE USE OF THE REMOVERS

## KONTROLA JAKOŚCI ZMYWACZY DO PAZNOKCI ORAZ OCENA ZAGROŻENIA ZDROWIA PRZY ICH STOSOWANIU

**Abstract:** The volatile organic compounds content in nail polish removers available on the home market was determined. Qualitative and quantitative analyses of acetone, methanol, ethanol, isopropanol and ethyl acetate were performed. Theoretical VOCs concentrations in the standard room and time for reduction to low values were calculated.

**Keywords:** nail polish removers, volatile organic compounds, indoor air quality

According to accessible literature, people spend about 80% of the time in the indoor areas [1-3]. For reasons of safety, the indoor air quality (IAQ) is important. Nitric and carbon oxides, volatile organic compounds (VOCs) and particulates are the most common indoor air pollutants [3]. One of sources of VOCs are consumer products, such as paints, polishes, cleaning products, deodorizers, glues, sealants and cosmetics.

Components of nail polish removers directly react on a nail plate. Due to this fact, in recent years producers have limited use of acetone in nail polish removers. Acetone dries a nail plate when it is often used. Other solvents, eg ethyl acetate, are gentler [4].

Taking into account the hazardous effects of acetone, consumers, examining the declared by producers ingredients used to production, nowadays choose acetone free products more often than they used to. Considering the cost of chemical agents, production of acetone free products is obviously more cost-consuming than production of the removers containing acetone. This fact may be used by unfair producers to reduce costs. The goal of this work was studies of available on the home market nail polish removers and determination of VOCs emission to indoor air.

## Materials and methods

Nail polish removers available on the home market where used as the samples. The products were grouped into two classes: 10 removers with declared acetone content (class A) and 7 acetone-free removers (class B).

The studies of VOCs content in nail polish removers were performed by gas chromatography method. The applied method enables a selective determination of organic solvents in consumer products.

The samples of analyzed product (5 $mm^3$ of nail polish removers) were injected into 1114 $cm^3$ glass containers, tightly closed with the screw caps containing the silicon membranes. After equilibration the gas samples were drawn with a Hamilton gas-tight syringe and analyzed on a gas chromatograph. The qualitative and quantitative analyses of

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polish removers components were performed using a CHROM 5 gas chromatograph with FID detectors. The chromatograph was provided with two steel chromatographic columns. Column I was 2 meter long of 3 mm inside diameter packed with 8% SE-30 + 0.21% Carbowax 20M on Chromosorb W NAW (60÷80 mesh). Column II was 1 meter long of 3 mm inside diameter packed with Chromosorb 102 (80÷100 mesh). The following temperatures were applied: column I 50ºC, column II 100ºC, injectors 120ºC, detectors 150ºC. Nitrogen (40 $cm^3 \cdot min^{-1}$) was used as the carrier gas. Identification of removers' ingredients was performed on the both columns. Quantitative analyses of ethyl acetate and *n*-butyl acetate were performed on column I. Analyses of acetone, methanol, ethanol and isopropanol were performed on column II. The apparatus was calibrated using standard gas mixtures prepared in the same containers in which the measurements were carried out.

## Results and discussion

17 samples of nail polish removers were examined. Results of the analyses of VOCs content in the examined samples are given in Table 1.

Table 1

Contents of volatile organic compounds in the nail polish removers

| Product | Substance | Content in product [g·100 $cm^{-3}$] | Summary VOCs content in product [g·100 $cm^{-3}$] |
|---|---|---|---|
| A1 | acetone | 18.62 | 18.62 |
| A2 | acetone | 76.75 | 76.75 |
| A3 | acetone | 58.80 | 70.32 |
| | ethanol | 11.52 | |
| A4 | acetone | 75.88 | 75.88 |
| A5 | acetone | 66.80 | 66.80 |
| A6 | acetone | 70.83 | 70.83 |
| A7 | acetone | 64.65 | 64.65 |
| A8 | acetone | 59.17 | 65.82 |
| | ethanol | 6.65 | |
| A9 | acetone | 71.63 | 71.63 |
| A10 | acetone | 67.35 | 67.35 |
| B1 | acetone | 50.42 | 72.21 |
| | isopropanol | 21.24 | |
| | ethyl acetate | 0.55 | |
| B2 | acetone | 23.05 | 40.69 |
| | methanol | 17.64 | |
| B3 | methanol | 5.64 | 71.15 |
| | ethanol | 23.76 | |
| | ethyl acetate | 41.75 | |
| B4 | acetone | 17.39 | 84.12 |
| | methanol | 66.73 | |
| B5 | acetone | 1.67 | 94.66 |
| | methanol | 0.30 | |
| | ethanol | 9.06 | |
| | isopropanol | 31.94 | |
| | ethyl acetate | 51.69 | |
| B6 | acetone | 45.01 | 65.49 |
| | isopropanol | 20.48 | |
| B7 | methanol | 0.07 | 87.39 |
| | isopropanol | 2.26 | |
| | ethyl acetate | 85.06 | |

Substances contained in nail polish removers may significantly affect indoor air quality. For that reason an attempt to determine concentration of those substances in indoor air was undertaken.

In order to determine the average use of nail polish removers a group of 10 women was questioned. The average use was determined to be 2.5÷4 $cm^3$ of product. Considering very high volatility of nail polish removers ingredients it may be assumed that all the ingredients are emitted into the air in very short time being a source of VOCs emission, the initial concentrations of VOCs in the standard room of volume 17.4 $m^3$ [5] were calculated. Results are presented in the Table 2. VOCs concentrations were strongly high (> 3 $mg \cdot m^{-3}$) [6] and were in the majority of cases over 100 $mg \cdot m^{-3}$.

Table 2

The initial concentrations of VOCs in the standard room and the time to reduce of concentrations to low values

| **Product** | **VOCs emission [g]** | **Initial average concentration of VOCs [$mg \cdot m^{-3}$]** | **Time to reduce of VOCs concentration to 0.25 $mg/m^3$ [h]** |
|---|---|---|---|
| A1 | 0.47÷0.74 | 34.78 | 7.12 |
| A2 | 1.92÷3.07 | 143.35 | 9.16 |
| A3 | 1.76÷2.81 | 131.34 | 9.04 |
| A4 | 1.90÷3.04 | 141.73 | 9.15 |
| A5 | 1.67÷2.67 | 124.77 | 8.96 |
| A6 | 1.77÷2.83 | 132.30 | 9.05 |
| A7 | 1.62÷2.59 | 120.75 | 8.92 |
| A8 | 1.65÷2.63 | 122.94 | 8.94 |
| A9 | 1.79÷2.87 | 133.79 | 9.06 |
| A10 | 1.68÷2.69 | 125.80 | 8.97 |
| B1 | 1.81÷2.89 | 134.88 | 9.08 |
| B2 | 1.02÷1.63 | 76.00 | 8.25 |
| B3 | 1.78÷2.85 | 132.90 | 9.05 |
| B4 | 2.10÷3.36 | 157.12 | 9.30 |
| B5 | 2.37÷3.79 | 176.81 | 9.47 |
| B6 | 1.64÷2.62 | 122.32 | 8.93 |
| B7 | 2.18÷3.50 | 163.23 | 9.35 |

The time necessary to reduce the concentration of VOCs in the standard room to low concentration level (0.25 $mg \cdot m^{-3}$) was determined [6]. Changes of VOCs concentration in the standard room may be presented in a geometric progression:

$$C_t = C_0 \cdot N^t$$

where: $C_0$ - initial concentration, $C_t$ - concentration at the time t, N - air exchange rate, t - time.

Concentration of volatiles in indoor air is regarded as a low, when its values is below 0.25 $mg \cdot m^{-3}$ [6]. Transforming the above relation we obtain relation from which we may calculate the time necessary to reach the assumed concentration:

$$t > \frac{\log 0.25 - \log C_0}{\log N}$$

To estimate the necessary time to obtain concentrations below 0.25 $mg \cdot m^{-3}$, an average air exchange rate N of 0.5 $h^{-1}$ was applied [5]. Results of calculations are presented in

Table 2. The time to reduce of VOCs concentrations to low values appears to be in the range of 7÷9.5 hours.

## Conclusions

Analyses of 17 nail polish removers available on the home market were performed. In the products there were identified very volatile organic compounds such as acetone, methanol, ethanol, isopropyl alcohol and ethyl acetate.

Calculated VOCs concentrations in the standard room during application of the tested products are strongly increased. Nail polish removers are a significant source of VOCs emission into the indoor air. The time to reduce of VOCs concentrations to low values appears to be in the range of 7÷9.5 hours.

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## KONTROLA JAKOŚCI ZMYWACZY DO PAZNOKCI ORAZ OCENA ZAGROŻENIA ZDROWIA PRZY ICH STOSOWANIU

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**Abstrakt:** Przeprowadzono badania zawartości lotnych składników zmywaczy do paznokci dostępnych na rynku krajowym. W badanych produktach zidentyfikowano i oznaczono ilościowo aceton, metanol, etanol, izopropanol i octan etylu. Określono teoretyczne stężenia lotnych związków organicznych wydzielających się ze zmywaczy do paznokci w pomieszczeniu standardowym i wyznaczono czas niezbędny do uzyskania niskich wartości tych stężeń.

**Słowa kluczowe:** zmywacze do paznokci, lotne związki organiczne, jakość powietrza wewnętrznego

Paweł MAŁAGOCKI[1] and Jadwiga PISKORSKA-PLISZCZYŃSKA[1]

# BIOTEST AS AN INDICATOR OF DIOXIN-LIKE PCBs PRESENCE

## ZASTOSOWANIE BIOTESTU JAKO INDYKATORA OBECNOŚCI DIOKSYNOPODOBNYCH PCB

**Abstract:** Dioxins (*polychlorinated dibenzo-p-dioxins, PCDDs, polychlorinated dibenzofurans*, PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) are toxic compounds which are commonly present in the food chain. According to the European Union regulations the presence of these compounds in food may be determined by complementary and screening and methods considered confirmatory. The combination of the two methods allows a relatively fast selection of food and feed samples containing dioxins at concentrations exceeding acceptable levels and permits an unequivocal confirmation of tested compounds. The aim of the study was to evaluate the usefulness of the biotest based on genetically modified cell lines sensitive to PCDDs and PCDFs for the simultaneous detection and quantitative determination of dl-PCBs in food samples. Mouse hepatoma cell lines (Hepa1L6.1c3) with the luciferase reporter gene were used. The principle of the biotest involving the dioxin mechanism of action includes the presence of receptor Ah agonist Hepa1L6.1c3 cell line synthesize luciferase at concentrations proportional to the agonist dose. The measurement of luciferase activity for various 2,3,7,8-TCDD concentrations (calibration curve) permits quantitative measurements of the Ah receptor antagonist content in examined food samples. The extraction of dl-PCBs from matrixes (salmon, herring and sprat muscles) was performed using the extraction and purification procedure for PCDDs/PCDFs analysis. Separation of dl-PCB from PCDD/PCDF was done by a column chromatography. The concentration of dl-PCBs was determined using the biotest. The results involving the same samples were compared with those obtained by the HRGC/HRMS method regarded as confirmatory. The results confirm the biotest as a useful method for dl-PCBs determinations; the results obtained by the two methods are parallel and meets the criteria defined by the Commission Regulation (EC) No. 1881/2006.

**Keywords:** dioxins, dioxin-like PCB, bioassay, detection method

Polychlorinated biphenyls consist of 209 congeners with a varied number and position of chlorine atoms in the aromatic rings. These compounds found numerous industrial applications including flame retardants, hydraulic fluids and transformer heat exchangers. Many reports concerning toxicity of technical PCB mixtures involved the implementation of regulations that limit or ban production in majority of developed countries [1].

Investigations concerning the mechanism of toxic action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD and PCDF) indicate that 12 PCB congeners (IUPAC numbers 77, 81, 105, 114, 118, 126, 156, 157, 167, 169 and 189) are able to activate AhR and cause dioxin-like toxic effects (dioxin-like PCBs, dl-PCBs). The most toxic congener among dl-PCBs is PCB-126 (WHO-TEF equal 0.1) [2].

AhR is a cytoplasmic receptor, which after binding the agonist forms a complex with the protein AhR nuclear translocator (ARNT) and then subsequently migrates to the nucleus. AhR:ligand complex can specifically bind to dioxin responsive elements (DRE) in nuclear DNA sequences present in the promoter regions of numerous genes including P450 1A1 and 1A2 cytochromes, cytosol aldehyde dehydrogenase 3, NAD(P)H:quinine oxidase, and other enzymes required for xenobiotic metabolism.

The knowledge of the dioxin mechanism of action mediated by AhR allowed the development of many bioassays used for detection of dioxins and dioxin-like substances.

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*In vitro* cell cultures were transformed using plasmids containing a reporter gene under the transcriptional control of AhR. The activation of the receptor in the presence of AhR agionists involved the activation of transcription and translation of AhR-dependent genes including the reporter gene. The XDS-CALUX (Chemically Activated Luciferase Expression made by Xenobiotic Detection Systems, Inc., Durham, USA) is a biotest which utilizes the luciferase reporter gene in mouse hepatoma cell line (H1L6.1c3). This test has been used in the National Veterinary Research Institute in Pulawy (Poland) for the determination of PCDD and PCDF content in food and feedstuffs since 2005.

The goal of this work was to adopt the XDS-CALUX bioassay to determine dioxin-like PCBs in fish tissues.

## Material and methods

**Principle of dl-PCBs content determination method**. The method involved both chemical and biological steps. The chemical step included coextraction of dl-PCBs and lipid from a sample. The extract is cleaned using $H_2SO_4$-modified silica and activated carbon columns followed by elution of dl-PCBs from the carbon column with hexane:toluene:ethyl acetate (8:1:1, V:V:V). After evaporation, the dl-PCBs content is determined by the XDS-CALUX. The activation of AhR by dl-PCBs present in the extract triggers luciferase expression in a dose-dependent manner. The measurement of luciferase activity according to a 2,3,7,8-TCDD calibration curve allows the determination of dl-PCBs content and expressing the result in WHO-TEQ [2].

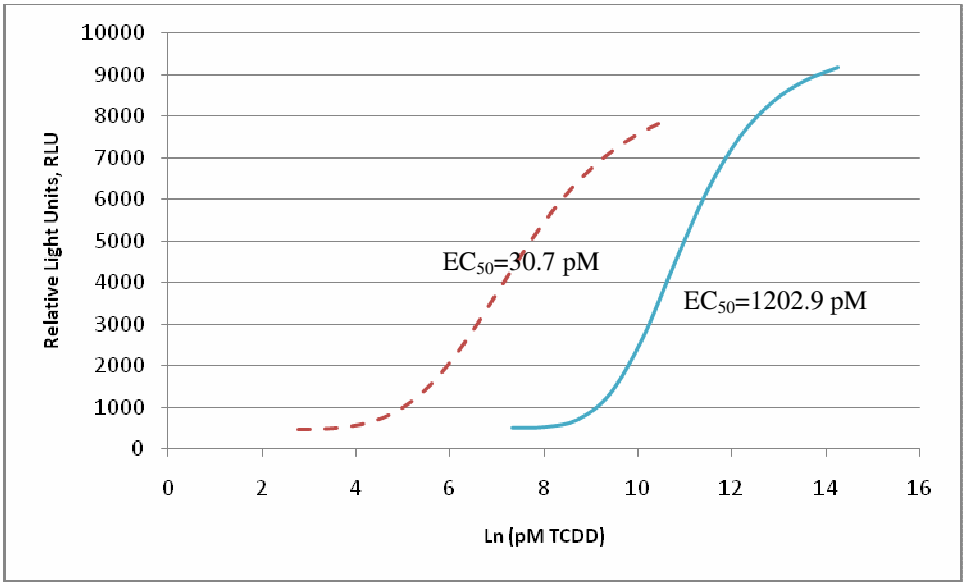


Fig. 1. Dose-response curves of 2,3,7,8-TCDD (dashed line) and PCB-126 (solid line). Cells were exposed to TCDD at a concentration of 0.8 to 776.4 pM and PCB-126 at a concentration of 14.1 to 30674.8 pM. Measured luminescence (in RLU, *Relative Light Units*) of reporter gene product (luciferase) is drawn on the *y* axis, and the natural logarithm of PCB-126 and 2,3,7,8-TCDD molar concentrations are drawn on the *x* axis

**Adaptation of the CALUX bioassay for dl-PCBs detection**. PCB-126 has the highest biological activity among all dl-PCBs congeners and is responsible for about 90% of dl-PCB-derived toxicity of food and feed. However, this congener is a weaker AhR activator in comparison with the most potent 2,3,7,8-TCDD dioxin. Dose response curves for both 2,3,7,8-TCDD and PCB-126 are shown in Figure 1. For each dl-PCB congener, the relative potency factor (REP) expressing its activity related to 2,3,7,8-TCDD in given biological setup can be calculated. The REP is considered as the ratio of the $EC_{50}$ of 2,3,7,8-TCDD to the $EC_{50}$ of an examined congener (PCB-126).

In this study, $EC_{50}$ values were 30.7 pM and 1202.9 pM for 2,3,7,8-TCDD and PCB-126, respectively. The calculated $REP_{PCB\text{-}126}$ value was 0.026 indicating that PCB-126 is about 39 times less potent than 2,3,7,8-TCDD in the XDS-CALUX, thus the dl-PCB results should be corrected by this factor in the case of using the 2,3,7,8-TCDD calibration curve for dl-PCB determination. Moreover, it was necessary to increase the sample amount to achieve a low detection limit of the method. On the basis of analysis of numerous dose-response curves, we assessed the lowest detectable amount of PCB-126 at a level of about 3.5 pg (ie 0.35 pg WHO-TEQ). To obtain the limit of detection (about 0.5 pg WHO-TEQ/g matrix) it was necessary to use 20 g of sample (fish muscles). An increased amount of matrix caused an enlarged extracted fat and needed to modify the clean-up process. Routine analyses involved a column with modified silica (33% $H_2SO_4$). This method produced high background values, so we performed experiment to check the efficiency of 22, 33, 44, 55 and 66% $H_2SO_4$ modified silica in clean-up of 5 g fish oil. The 44% $H_2SO_4$ modified silica was used for further experiments, because it gave both sufficient clean-up and failed to clog columns.

**Method of dl-PCB determination in fish muscles**. Fat is extracted from 10 g of lyophilized sample by shaking with hexane:dichloromethane (1:2, V:V). The extract is evaporated to dryness and the obtained fat is weighted. After adding 44% $H_2SO_4$ modified silica analytes are extracted three times with hexane (30, 10 and 10 $cm^3$) and spread to two columns, the first with 44% $H_2SO_4$ modified silica and the second with a mixture of activated carbon:celite 545 (1:99, w:w). dl-PCBs are eluted with hexane:toluene:ethyl acetate (8:1:1, V:V:V), evaporated to dryness and measured using XDS-CALUX bioassay.

**Bioassay**. The cells are grown in RPMI 1640 with L-glutamine medium enriched with 8% of fetal calf serum and 1% of penicillin/streptomycin in a temperature of 37°C, 5% of $CO_2$ concentration and relative humidity higher than 97%. Before exposition cells are transferred to a 96-well plate, 1.5 x $10^5$ cells/well. After 20 to 24 h incubation, cells are exposed to extracts or standard solutions and once again incubated for 20 to 24 h. After incubation, the cells are lysed and the luciferase activity in the lysate is measured in the presence of luciferine. Concomitant measurements of a 2,3,7,8-TCDD dose-response relationship permitted the quantitative evaluation of dl-PCB presence in examined extract.

**Checking the bioassay ability to analyze dl-PCB content**. The method was validated according to the 1881/2006/EC [3]. All parameters obtained fulfilled the requirements of the methods used in dioxins and dl-PCB analysis. This method was used to analyze dl-PCB content in fish muscle samples, among them 11 herring, 11 sprat, and 6 salmon samples. Results were compared with those obtained by the HRGC/HRMS method considered the golden standard in dioxin and dioxin-like compounds analysis [4].

## Results and discussion

Results of dl-PCB content in fish samples obtained by the bioassay and instrumental HRGC/HRMS method are shown in Table 1. The closest agreement of the bioassay (an average of 2.54) and HRMS (2.29 pg WHO-PCB-TEQ/g; $t_{observed} = 5.74$, $t_{critical\ 0.001} = 4.59$) results were found in herring samples. In the case of sprat and salmon the values were 2.29 and 3.33 pg WHO-PCB-TEQ/g ($t_{observed} = 17.25$, $t_{critical\ 0.001} = 4.59$) and 3.80 and 5.79 pg WHO-PCB-TEQ/g ($t_{observed} = 6.17$, $t_{critical\ 0.001} = 5.41$), respectively. The bioassay results were underestimated with regard to the dl-PCB content by 30% on average. Differences between the means obtained by the two methods are statistically significant for all matrices. One of possible explanations of this finding is that the HRGC/HRMS method determines concentrations of only 12 congeners of dl-PCB, and the final result (in WHO-TEQ, Toxic Equivalent) is obtained by adding quotients of congener concentrations and congener WHO-TEF. The CALUX bioassay measures overall activity of all dioxin-like compounds present in the matrix. Disagreement between WHO-TEF and REP values can be to some extent responsible for differences in results.

It is worth noting that the correlation of the bioassay and HRGC/HRMS methods should never attain 1, thus, Pearson's correlation coefficient r = 0.82 (Fig. 2) obtained in our analysis seems to be quite satisfactory and similar to the literature data involving the correlation of PCDD/PCDF results.

Summing up, the bioassay can be a useful tool for a screening analysis assessing overall toxicity of sample, but the official control required the confirmation of positive results by the HRGC/HRMS method.

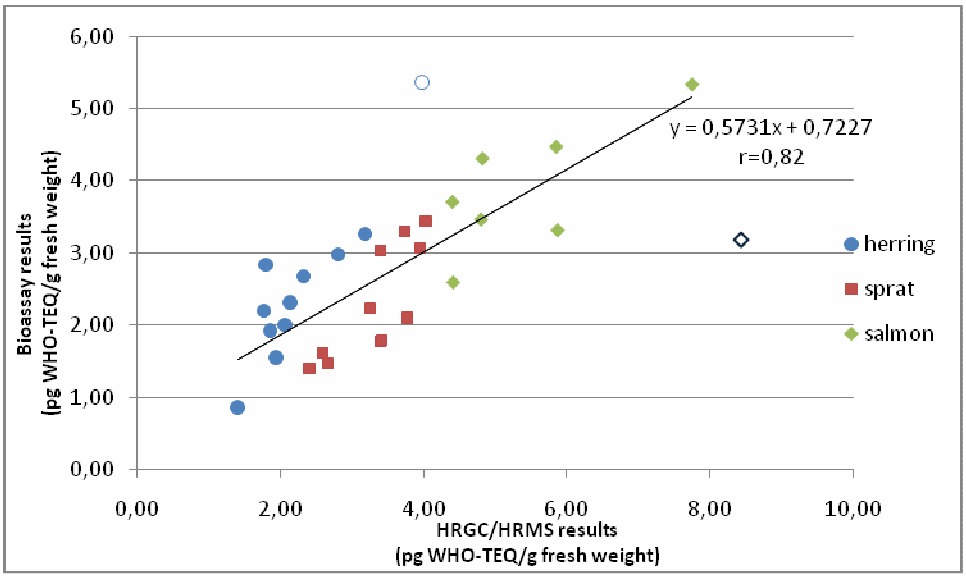


Fig. 2. Bioassay (XDS-CALUX) and chemical analysis (HRGC-HRMS) results correlation. Empty symbols are outliers not used in correlation analysis. Pearson correlation coefficient r = 0.82 (n = 26)

Table 1

Comparison of dl-PCB results obtained using bioassay and HRGC/HRMS methods

| Matrix | Sample no. | Bioassay | HRMS |
|---|---|---|---|
| | | [pg PCB-WHO-TEQ/g fresh weight] | |
| Herring | 002 | 0.86 | 1.40 |
| | 005 | 2.84 | 1.79 |
| | 008 | 2.01 | 2.06 |
| | 020 | 2.68 | 2.32 |
| | 027 | 5.36 | 3.98 |
| | 028 | 2.31 | 2.13 |
| | 046 | 1.93 | 1.86 |
| | 064 | 2.98 | 2.80 |
| | 066 | 2.20 | 1.77 |
| | 073 | 1.55 | 1.94 |
| | 067 | 3.26 | 3.18 |
| **Mean ± standard deviation** | | **2.54 ± 1.16** | **2.29 ± 0.75** |
| Salmon | 003 | 3.29 | 3.74 |
| | 004 | 1.78 | 3.39 |
| | 007 | 1.62 | 2.59 |
| | 023 | 1.80 | 3.41 |
| | 024 | 1.47 | 2.66 |
| | 025 | 1.41 | 2.40 |
| | 045 | 2.23 | 3.25 |
| | 065 | 2.10 | 3.77 |
| | 068 | 3.44 | 4.03 |
| | 069 | 3.07 | 3.95 |
| | 072 | 3.03 | 3.40 |
| **Mean ± standard deviation** | | **2.29 ± 0.77** | **3.33 ± 0.56** |
| Sprat | 001 | 3.72 | 4.40 |
| | 006 | 5.34 | 7.75 |
| | 019 | 4.48 | 5.85 |
| | 026 | 3.18 | 8.44 |
| | 063 | 4.32 | 4.82 |
| | 070 | 2.60 | 4.41 |
| | 071 | 3.47 | 4.80 |
| | 086 | 3.32 | 5.87 |
| **Mean ± standard deviation** | | **3.80 ± 0.87** | **5.79 ± 1.54** |

In the case of sprat and salmon samples (Table 1), the losses of dl-PCB may take place during the evaporation stage so it needs optimization. In addition, it is believed that the cleaning-up should be improved especially in the case of the adsorption and elution of dl-PCB fraction. Modification of these processes should significantly increase the recoveries of analytes and make a closer agreement between the bioassay and HRGC/HRMS results. At present, improvements of the method are being continued.

## Conclusions

1. The method used is sensitive, repeatable, and permits a relatively fast determination of dl-PCB in samples of fish muscles.
2. The method is suitable for screening analysis.
3. The adsorption and elution of dl-PCB needs optimization.

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## ZASTOSOWANIE BIOTESTU JAKO INDYKATORA OBECNOŚCI DIOKSYNOPODOBNYCH PCB

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**Abstrakt:** Dioksyny (polichlorowane dibenzo-*p*-doksyny (PCDD) i polichlorowane dibenzofurany (PCDF)) oraz dioksynopodobne polichlorowane bifenyle (dl-PCB)) są związkami toksycznymi występującymi m.in. w łańcuchu żywieniowym. Zgodnie z przepisami Unii Europejskiej, do oznaczania ich zawartości w żywności można zastosować metody komplementarne: przesiewową i potwierdzającą. Ich połączenie pozwala na stosunkowo szybką selekcję próbek żywności i pasz zawierających dioksyny w stężeniach przekraczających dopuszczalne poziomy oraz pozwala na jednoznaczne potwierdzenie obecności badanych związków. Celem pracy było określenie przydatności biotestu bazującego na genetycznie zmodyfikowanej linii komórek wrażliwych na działanie PCDD i PCDF do równoczesnego wykrywania i ilościowego oznaczania dl-PCB w próbkach żywności. W badaniach zastosowano linię komórkową hepatomy mysiej (Hepa1L6.1c3) z wprowadzonym transgenem lucyferazy pod kontrolą receptora Ah. Zasada biotestu wykorzystującego mechanizm działania dioksyn jest następująca: w obecności agonistów receptora Ah komórki Hepa1L6.1c3 syntezują enzym lucyferazę w stężeniu proporcjonalnym do dawki agonisty. Pomiar aktywności lucyferazy wobec serii stężeń 2,3,7,8-TCDD (krzywa kalibracyjna) pozwala na ilościową ocenę zawartości agonistów receptora Ah w badanym ekstrakcie próbki żywności. Do ekstrakcji dl-PCB z matrycy (mięśnie łososia, śledzia, szprota) użyto zoptymalizowanej dla PCDD/PCDF metody ekstrakcji i oczyszczania. Na kolumnach chromatograficznych oddzielano dl-PCB od PCDD/PCDF. Zawartość dl-PCB oznaczano, stosując biotest. Wyniki uzyskane biotestem porównywano z rezultatami oznaczeń chemiczną metodą potwierdzającą HRGC/HRMS, wykonaną w tych samych próbkach. Uzyskane dane pozwalają ocenić biotest jako przydatne narzędzie w badaniach zawartości dl-PCB, ponieważ wyniki uzyskane obydwoma metodami są porównywalne, a metoda spełnia kryteria określone przepisami prawa wspólnotowego (Rozp. 1881/2006/WE).

**Słowa kluczowe:** dioksyny, dioksynopodobne PCB, biotest, metoda detekcji

Małgorzata NABRDALIK[1], Katarzyna GRATA[1] and Adam LATAŁA[1]

# PROTEOLYTIC ACTIVITY OF *Bacillus cereus* STRAINS

## AKTYWNOŚĆ PROTEOLITYCZNA SZCZEPÓW *Bacillus cereus*

**Abstract:** The aim of conducted research was to assess proteolytic activity of *Bacillus cereus* strains, depending on the source of proteins in a growth medium and temperature. Two *Bacillus cereus* strains: G10 and A96, isolated from soil and water respectively, were applied in the research. The source of carbon in the growth media was provided by the protein substrates as follows: albumin, casein and skimmed milk. The temperature ranged between 30 and 60ºC. The proteolytic activity was determined with the use of a spectrophotometric method with 2% casein as a substrate, at the wavelength $\lambda$ = 560 nm. In the experiment, the proteolytic activity depended on the type of protein substrate in the growth medium and the temperature. *B. cereus* G10 strain showed the highest activity at 30ºC in case of albumin and the lowest in the growth medium with skimmed milk. At temperatures 40 and 50ºC the most favourable medium was with an addition of skimmed milk and the least favourable with albumin. Proteolytic activity was not recorded when the temperature was the highest and the medium contained skimmed milk, whereas at the same temperature the lowest activity was noted again in the medium with albumin. *Bacillus cereus* A96 strain showed, however, different activity. The highest activity was noted at tempertaures of 30 and 50ºC in the medium with skimmed milk, and the lowest in the medium with casein. The opposite was noted at 40ºC, as no proteolytic activity was recorded in the presence of skimmed milk while the highest was recorded in the presence of casein. When analysing the influence of the temperature on the proteolytic activity, it has been noted that strains of *Bacillus cereus* are the most vigorous at the temperature of 30 and 40ºC, and the least vigorous at 50 and 60ºC. The highest recorded values for the screened strains have been obtained at 30 and 40ºC in the medium with skimmed milk.

**Keywords:** *Bacillus cereus*, proteolytic activity, albumin, casein, skimmed milk

Proteases are an important group of enzymes both physiologically and commercially. Proteases constitute nearly 65% of the global industrial enzyme market most of which are alkaline proteases [1]. Most of these find applications in the food industry, in the meat tenderization process, peptide synthesis, for infant formula preparations, baking and brewing. Furthermore, they are used in pharmaceuticals and medical diagnosis, in the detergent industry as additives, as well as in textile industry in the process of dehairing and leather and silk processing [2].

The genus *Bacillus* contains a number of industrially important species and an approximately half of the present commercial production of bulk enzymes derives from the strains of *Bacillus* spp. The *Bacillus cereus* group is the major source of commercial alkaline protease production worldwide [3, 4].

The aim of conducted research was to assess proteolytic activity of *Bacillus cereus* strains, depending on the source of proteins in a growth medium and temperature.

## Materials and methods

In the research the supernates of *B. cereus* strains marked as G10 and A96, isolated prior to the experiment from water and soil respectively, were used. The supernates had been obtained from the bacterial culture grown in the medium containing [g/dm$^3$]: $(NH_4)_2SO_4$ 2.0, $K_2HPO_4$ 3.0, $KH_2PO_4$ 2.0, $MgSO_4 \times 7H_2O$ 0.5. The basic culture medium

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was supplemented with 1% source of carbon in the form of protein substrates such as: albumin, casein and skimmed milk. Incubation was carried out at 30ºC for 48 hour.

Proteolytic activity was determined in the presence of 2% casein as a substrate. The mixture of supernate and casein was incubated at temperature range between 30 and 60ºC for 10 min. The reaction was stopped by adding 5% trichloroacetic acid and the mixture was then left for 15 min and centrifuged at 10 000 rpm for 10 min. Obtained supernates were mixed with 6% $Na_2CO_3$ and Folin's reagent. The absorbance was measured at the wavelength $\lambda = 560$ nm. Enzymatic activity was expressed as the number of micromoles of tyrosin literated by 1 $cm^3$ of the bacterial culture.

## Results

In presented paper, *Bacillus cereus* strains G10 and A96, were screened for production of proteolytic enzymes in culture media containing different sources of protein and at temperature range between 30 and 60ºC.

In conducted research, the number of liberated micromoles of tyrosine depended on the type of protein substrate in the culture medium and the temperature.

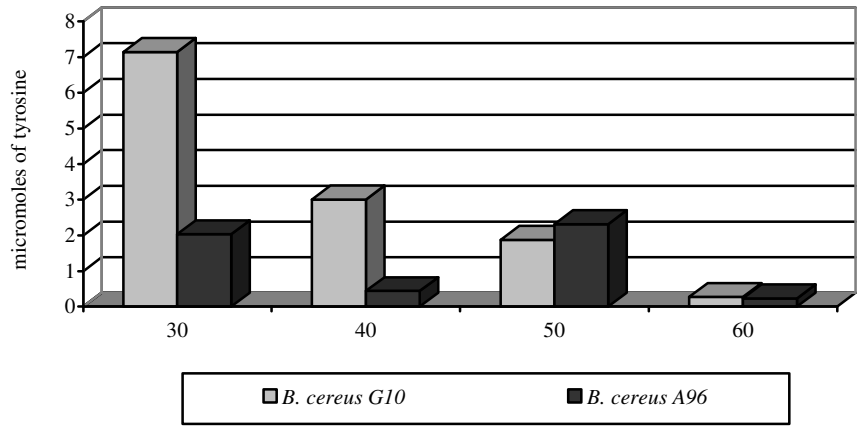


Fig. 1. Proteolytic activity of *B. cereus* strains in the medium with albumin

As shown in Figure 1, it can be stated that *B. cereus* G10 strain isolated from water showed higher proteolytic activity in the medium with albumin when compared with the soil strain *B. cereus* A96. The strain liberated over three times more tyrosine, that is 7.114 micromoles, at 30ºC than the other bacterial strain under study. *B. cereus* A96 soil strain was slightly more active only at 50ºC and liberated 2.316 micromoles of tyrosine. The lowest activity for both of the strains was noted at 60ºC and obtained results were similar: 0.269 and 0.232 micromoles of tyrosine, respectively.

In case of medium with casein as the source of carbon (Fig. 2) it was noted that 30ºC was the most favourable temperature for *B. cereus* G10 strain isolated from water and 40ºC was the most favourable for the soil strain *B. cereus* A96. In the optimal temperature conditions, *B. cereus* G10 strain liberated 6.9 micromoles of tyrosine, which is 11-fold

higher value when compared with *B. cereus* A96 strain. It should be highlighted, that at 40ºC *B. cereus* A96 strain liberated the highest amount of tyrosine of all recorded values in the experiment, which amounted 8.836 micromoles. Obtained value was twice higher than the value obtained in case of *B. cereus* G10 strain isolated from water. Similarly to the culture medium with albumin, also in the medium with casein proteolytic activity was the lowest at 60ºC.

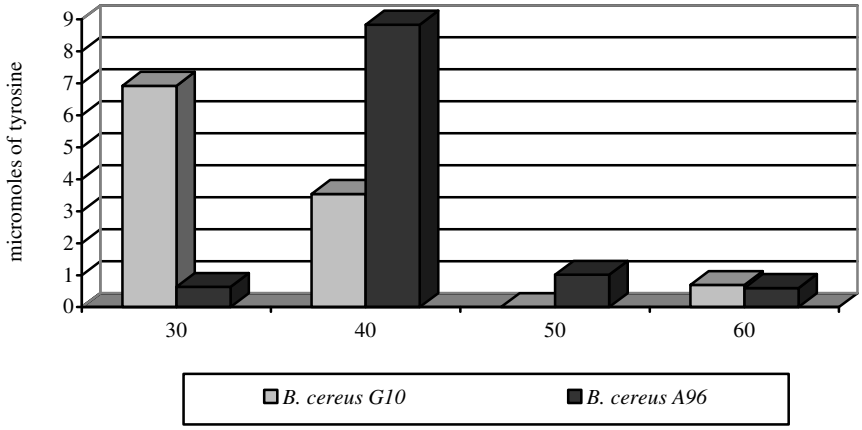


Fig. 2. Proteolytic activity of *B. cereus* strains in the medium with casein

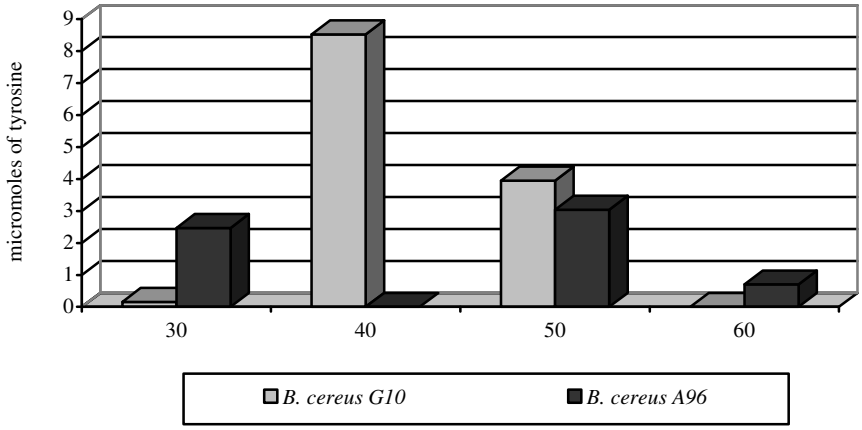


Fig. 3. Proteolytic activity of *B. cereus* strains in the medium with skimmed milk

The last analysed source of carbon was skimmed milk (Fig. 3), which turned out to be the most favourable for *B. cereus* G10 strain at 40ºC. The obtained amount of micromoles of tyrosine was the highest for this culture medium and amounted 8.52 micromoles. The highest amount of tyrosine in case of *B. cereus* A96 was liberated at 50ºC and amounted

3.036 micromoles, which was lower in comparison with the results noted for *B. cereus* G10 under the same conditions. Again, the lowest proteolytic activity was observed at 60ºC.

## Summary and conclusion

The results obtained in the present study showed significant variety of proteolytic activity of individual *B. cereus* strains, in terms of different sources of protein substrate applied in the research and allowed to conclude the following:

1. There has been no distinct correlation between the temperature of incubation and proteolytic activity noted, which proves that analysed ability is specific for individual strains and depends on the protein substrate in the culture medium as well as the place the strain had been isolated from.
2. High specificity of exocellular proteases liberated by the strains under study, in relation to analysed protein substrates, proves that the process of their biosynthesis is induced by proteins.
3. Analysing the influence of temperature on proteolytic activity, it has been found that *B. cereus* strains showed the highest activity at 30 and 40ºC, and the lowest at 50 and 60ºC.
4. The highest recorded values for the strain isolated from water have been obtained in the medium with albumin and casein at 30ºC and in the medium with skimmed milk at 40ºC.
5. The strain isolated from soil showed the highest activity at 40ºC in the medium with skimmed milk.

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## AKTYWNOŚĆ PROTEOLITYCZNA SZCZEPÓW *Bacillus cereus*

Samodzielna Katedra Biotechnologii i Biologii Molekularnej, Uniwersytet Opolski

**Abstrakt:** Celem podjętych badań była ocena aktywności proteolitycznej szczepów *Bacillus cereus* w zależności od źródła białka zawartego w podłożu oraz temperatury. Do badań wykorzystano 2 szczepy *Bacillus cereus* G10 oraz A96 wyizolowane odpowiednio z gleby i wody. Źródłem węgla w pożywkach były substraty białkowe: albumina, kazeina oraz odtłuszczone mleko. Aktywność proteolityczną oznaczono w zakresie temperatur od 30 do 60ºC. Oznaczenia aktywności proteolitycznej przeprowadzono metodą spektrofotometryczną, używając jako substratu 2% kazeiny po 10-minutowej inkubacji, przy długości fali $\lambda$ = 560 nm. W przeprowadzonym doświadczeniu aktywność proteolityczna uzależniona była od rodzaju substancji białkowej zawartej w podłożu oraz temperatury. I tak, badany szczep *B. cereus* G10 w temperaturze 30ºC największą aktywność proteolityczną wykazywał w obecności albuminy, a najniższą w obecności odtłuszczonego mleka. W temperaturach 40 i 50ºC najkorzystniejszym podłożem okazało się podłoże zawierające odtłuszczone mleko, a najmniej korzystne zawierające albuminę. W najwyższej temperaturze nie stwierdzono aktywności proteolitycznej w obecności odtłuszczonego mleka, zaś najniższą aktywność odnotowano ponownie w środowisku albuminy. Z kolei drugi

z badanych szczepów, *Bacillus cereus* A96, charakteryzował się odmienną aktywnością. W temperaturach 30 i 50ºC najwyższą aktywność proteolityczną odnotowano w podłożu zawierającym odtłuszczone mleko, a najniższą w środowisku kazeiny. Natomiast w temperaturze 40ºC zaobserwowano odwrotną sytuację, gdyż nie stwierdzono aktywności proteolitycznej w obecności odtłuszczonego mleka, a najwyższą aktywność uzyskano w obecności kazeiny. Analizując wpływ temperatury na aktywność proteolityczną, stwierdzono, iż największą aktywność badane szczepy *Bacillus cereus* wykazują w temperaturach 30 i 40ºC, a najmniejszą w 50 i 60ºC. Najwyższe wartości dla badanych szczepów uzyskano w temperaturach 30 i 40ºC na podłożu z dodatkiem odtłuszczonego mleka.

**Słowa kluczowe:** *Bacillus cereus*, aktywność proteolityczna, albumina, kazeina, odtłuszczone mleko

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# LEACHABILITY OF HEAVY METALS (Fe, Zn and Ni) FROM COAL MINE ROCKS

## WYPŁUKIWANIE METALI CIĘŻKICH (Fe, Zn i Ni) ZE SKAŁ PRZYWĘGLOWYCH

**Abstract:** The majority of heat and energy delivered to Polish houses derive from coal combustion. The exploitation of underground resources is connected with excavation of huge quantities of metal-rich waste rocks. These metals may be released to the environment and contribute to the pollution of water and soil systems which is dangerous for biota and human health. The mobility and bioavailability of metals depend on their chemical form. In present work, we determined geochemical speciation of Fe, Ni and Zn in samples of waste rocks from five coal mines from both USCB and LCB. The most abundant metal in easily-extractable phases (water-soluble, exchangeable, acid-soluble) was Zn ($MF_{Zn}$ = 24.4÷53.4) followed by Ni ($MF_{Ni}$ = 5.1÷19.2) and Fe ($MF_{Fe}$ = 0.2÷3.6). The mobility of Fe was similar in rocks originating from both coal basins (K-W, $p = 0.2253$), Ni was higher in LCB whereas Zn in USCB rocks (K-W, $p < 0.05$). It was also found that during first years of storage and exposure to natural weathering, only a small portion of metals was released to the environment as the total concentrations and fractionation of Fe, Ni and Zn were similar in fresh and weathered coal waste rocks from Wesola and Murcki coal mines, up to 3 and 15 years of weathering, respectively.

**Keywords:** waste rocks, heavy metal, geochemical speciation

In Poland, in spite of the changes in energy sector that took place during last decades, the majority of electricity and heat still derive from coal combustion (more than 90% in 2005) [1]. Almost all hard coal production in Poland is concentrated in the Upper Silesian Coal Basin (USCB) (Southern Poland). The only active coal mine (CM) outside USCB - Bogdanka CM is located in South-Eastern Poland and is the only CM exploiting resources of the Lublin Coal Basin (LCB). Coal mining activities is inevitably connected with the excavation of huge amounts of dump rocks [2, 3]. The disposal of waste rock originating from coal production is an important environmental issue due to the potential production of acidic and metal-rich drainage (AMD) [4]. Following transport of heavy metals to the soil and water system [5-10] results in growing hazard to living organisms, including people.

The threat posed by metals depends strongly on their mobility and bioavailability [11-13] which is different depending on metal geochemical form. Water-soluble and exchangeable forms are considered readily mobile and available to biota. Carbonate bound, occlusion in Fe and Mn oxides, or complexes with organic matter OM have been found to be released due to changes in pH and redox conditions and be responsible for long-term effects [14, 15] whereas metals incorporated in the crystalline lattices appear relatively stable.

The aim of the present study was to determine chemical portioning of Fe, Ni and Zn in coal waste rocks from both Polish coal basins and evaluate the amount of metals that may be released from the investigated material in short and long-term perspective. We also wanted to assess changes in chemical fractionation of Fe, Ni and Zn with the course of time.

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## Material and method

The mine tailings used used for this work originated from Upper Silesian (USCB) and Lublin Coal Basins (LCB). USCB: samples were collected from currently exploited Murcki, Wesola and Wujek coal mines (M1, WU1, WU2, WE1) as well as the dumping areas (M2-M4, WE2, WE3). LCB: samples originated from Bogdanka (B1-B4), the only coal mine exploiting resources of the area. Fractionation of Fe, Zn and Ni was carried out following the sequential partial dissolution scheme proposed by Tessier [13] and following modifications proposed by Yu et al [16] the water soluble fraction step was added before the extraction of exchangeable fraction.

The relative index of metal mobility is calculated as a "mobility factor" [17-19] on the basis of the equation:

$$MF = \frac{\text{water} - \text{soluble} + \text{exchangeable} + \text{acid} - \text{soluble}}{\text{water} - \text{soluble} + \text{exchangeable} + \text{acid} - \text{soluble} + \text{reducible} + \text{oxidizable} + \text{residual}} x100$$

## Statistical analysis

Due to the non-parametrical character of the data, Kruskall-Wallis and U Mann--Whitney tests were applied and the significance level of $p < 0.05$ was accepted. Statistical tests were performed with Statistica 8 software.

## Results and discussion

In fresh rock samples Fe was the most abundant metal of all investigated (av. 2468.22 mg/kg) (Fig. 1), which was present mostly in residual phase (60.8% [WU2] - 82.1% [B2]) (Fig. 2). Fe was followed by Zn (552.92 mg/kg) and Ni (356.04 mg/kg) (Fig. 1). Similarly to Fe, the majority of Ni in the investigated fresh rocks was bound with residual fraction (60.7% [B4] - 77.5% [M1]) (Fig. 2).

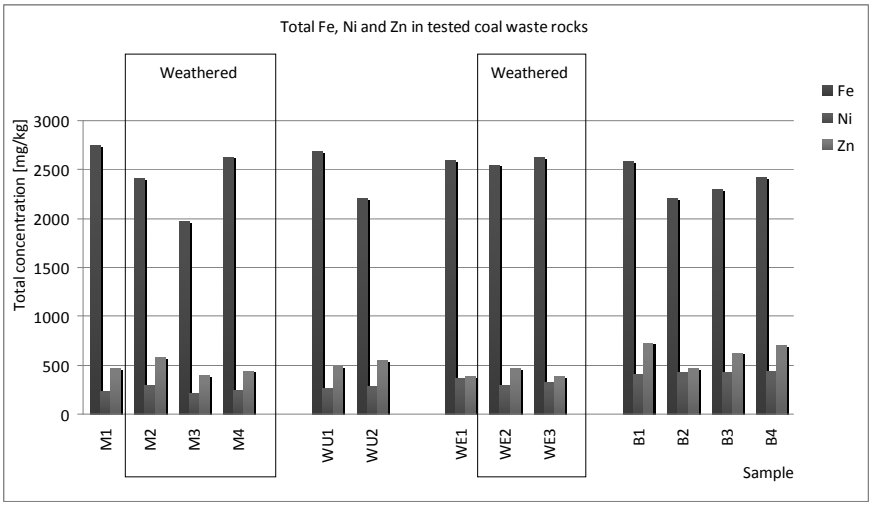


Fig. 1. Total concentrations of Fe, Ni and Zn in waste rocks originating from different Polish coal mines

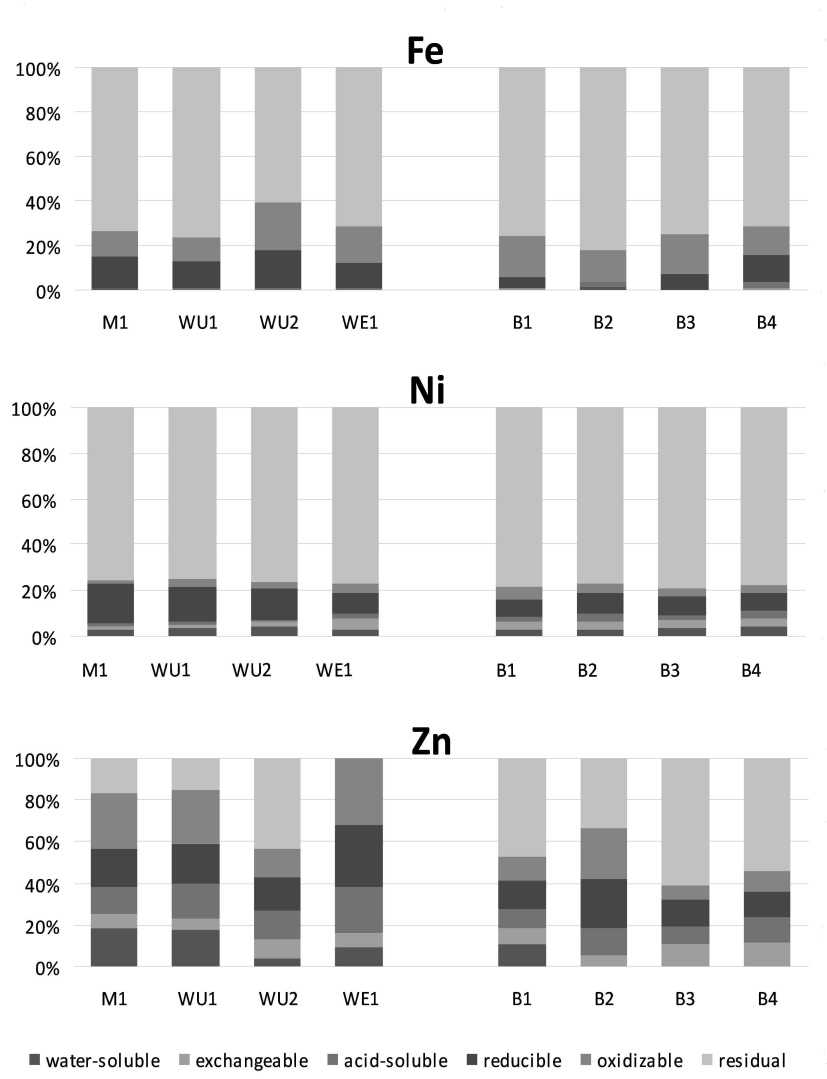


Fig. 2. Proportional distribution of Fe, Ni and Zn between fractions in fresh rock samples

The distribution of Zn between fractions in fresh rock samples differed from reported for Fe and Ni. The results show that the pool of labile metal (water-soluble+exchangeable+acid-soluble) does not depend on its total concentration. In spite of the fact that the total highest concentrations between investigated metals were found for Fe, the most abundant metal in labile phase was Zn (high MF values) (Tab. 1). Statistical analyses showed that the mobility of Fe was similar in rocks originating from both coal basins (K-W, $p = 0.2253$), Ni was higher in LCB whereas Zn in USCB rocks (K-W, $p < 0.05$). However, the pool of easily leachable Fe and Ni was higher in rocks

collected from LCB (K-W; $p < 0.05$) whereas the Zn was comparable in rocks from both coal basins.

Table 1

Mobility factors and selected properties of the investigated coal waste rocks

| Sample | $MF_{Fe}$ | $MF_{Ni}$ | $MF_{Zn}$ |
|---|---|---|---|
| M1 | 0.8 | 5.1 | 43.7 |
| M2 | 2.8 | 7.1 | 46.5 |
| M3 | 0.5 | 6.4 | 41.4 |
| M4 | 1.2 | 6.4 | 53.4 |
| WU1 | 0.6 | 6.5 | 45.0 |
| WU2 | 0.5 | 8.0 | 41.3 |
| WE1 | 0.6 | 14.3 | 38.5 |
| WE2 | 1.1 | 8.6 | 43.2 |
| WE3 | 2.8 | 10.3 | 48.1 |
| B1 | 0.7 | 13.4 | 46.3 |
| B2 | 3.6 | 16.0 | 24.4 |
| B3 | 0.2 | 14.9 | 37.3 |
| B4 | 3.6 | 19.2 | 43.3 |

In the weathered rocks [M2-M4, WU2-WU3] total concentrations of the metals investigated were similar to those reported for fresh rock originating from an appropriate coal mine. The sequential extraction of samples subjected to natural weathering revealed that there were only minor changes in proportional distribution of those metals between geochemical forms including the increase of water-soluble (Fe and Ni) and exchangeable (Ni and Zn) metals (Fig. 3). Similar phenomena have recently been described for waste material from surface coal mine sites in Southern Wales [6]. It can be therefore assumed that in short-terme perspective the environmental hazard due to Fe, Ni and Zn leaching from it is of minor importance. However, with the course of time the decrease in rock pH may appear and following mobilization of metals bound to other geochemical fractions, especially carbonates. Additionally, waste rocks from both coal basins contain substantial amounts of metals, especially Fe and Zn, bound to phases susceptible to oxidation (Figs 2 and 3). Waste rocks are often used for civil engineering purposes which causes an increase in the exposed area of waste rock subjected to weathering and may contribute to the intensification of heavy metals elution and transport to water and soil system.

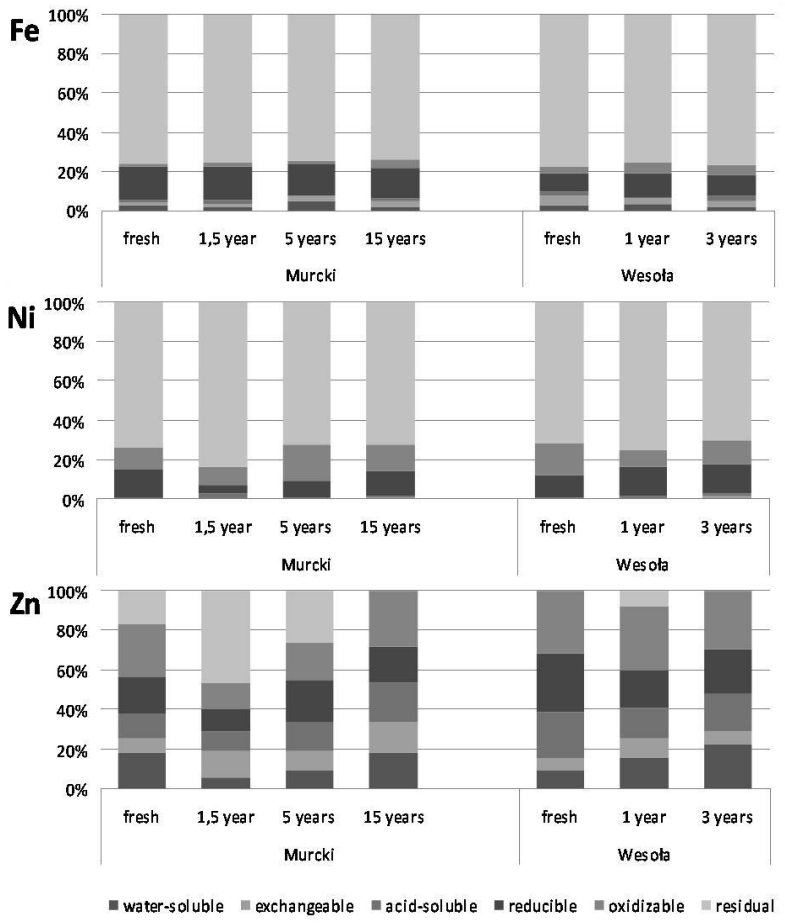


Fig. 3. Proportional distribution of Fe, Ni and Zn between fractions in fresh rock samples

## Abbreviations

LCB - Lublin Coal basin, USCB - Upper Silesian Coal Basin, capacity, MF - mobility factor, AMD - acid mine drainage, CM - coal mine, W-S - water soluble, EX - exchangeable, A-S - acid-soluble, RD - reducible, OX - oxidizable, RS - residual.

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## WYPŁUKIWANIE METALI CIĘŻKICH (Fe, Zn i Ni) ZE SKAŁ PRZYWĘGLOWYCH

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**Abstrakt:** Większość ciepła i energii dostarczanej do polskich domostw pochodzi ze spalania węgla kamiennego. Podziemna eksploatacja tego surowca związana jest z wydobyciem na powierzchnię dużych ilości bogatych w metale skał przywęglowych. Metale te mogą zostać uwolnione do środowiska, przyczyniając się tym samym do zanieczyszczenia gleby i wody, które jest niebezpieczne dla organizmów żywych i zdrowia ludzi. Mobilność i biodostępność metali zależy od ich chemicznej postaci. W bieżącej pracy przedstawiono wyniki geochemicznej specjacji Fe, Ni oraz Zn w próbkach odpadowych skał przywęglowych z pięciu kopalni GZW i LZW. Wykazano, że w łatwo wypłukiwanej formie (wodno-rozpuszczalnej, wymiennej, kwaso-rozpuszczalnej) największe stężenia osiągał Zn ($MF_{Zn}$ = 24,4÷53,4), następnie Ni ($MF_{Ni}$ = 5,1÷19,2) i Fe ($MF_{Fe}$ = 0,2÷3,6). Mobilność Fe była porównywalna w skałach pochodzących z obu Zagłębi (K-W, p = 0,2253), Ni większa z LCB, natomiast Zn z USCB (K-W, p < 0,05). Przeprowadzone badania wskazują, że przez pierwsze lata składowania jedynie niewielka część metali zostaje uwolniona do środowiska, ponieważ zarówno stężenia całkowite, jak i specjacja Fe, Ni i Zn były podobne w świeżych i zwietrzałych skałach kopalni Wesoła i Murcki (odpowiednio do 3 i 15 lat wietrzenia).

**Słowa kluczowe:** skały odpadowe, metale ciężkie, geochemiczna specjacja

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and Henryk SOBCZUK[1]

# CAPILLARY RISE PHENOMENON IN AERATED CONCRETE. MONITORING AND SIMULATIONS

## ZJAWISKO PODCIĄGANIA KAPILARNEGO W BETONIE KOMÓRKOWYM. MONITORING I SYMULACJE

**Abstract:** Capillary rise is a dangerous phenomenon touching many objects built of aerated concrete which is a porous material and highly prone to water influence in case of any horizontal water insulation failures. Aerated concrete is the artificial building material offering both suitable strength parameters and heat insulation properties. Water migrating from the ground to the walls of the building negatively influences not only its construction but also increases heat loses and decreases indoor air comfort (low temperatures, fungi and bacteria development) which is in the World's literature called SBS (*Sick Building Syndrome*). All above presented facts connected with water migration through the aerated concrete walls underline the need to monitor and simulate capillary rise phenomenon in building envelopes made of aerated concrete. The paper presents the simulation of capillary rise in a model aerated concrete sample. The model applied for simulations will be based on the Richards equation for water flow in unsaturated conditions. The model is commonly used in agrophysics and this work is an attempt to use it for building materials. The simulations results will be verified with laboratory experiment of capillary rise in the real samples of aerated concrete equipped with Time Domain Reflactometry (TDR) probes which will enable to monitor the phenomenon propagation.

**Keywords:** capillary rise, monitoring, simulations, TDR

Water, which is a necessary substance for all living organisms causes many problems during buildings exploitation. Unfortunately in moderate climate countries it is a common problem and should be considered from both engineering and the ecological point of view. Water contents exceeding acceptable states are the most common in historical buildings made of stone or red brick without suitable water proof insulations but also they occur in many new buildings, sometimes built of modern materials like aerated concrete. Aerated concrete is one of the most popular building materials in Polish market [1]. It is not natural material and its cellular structure was intentionally designed to obtain suitable heat insulation parameters. Method of aerated concrete production was invented at the end of XIX-th century.

Basic assortment of aerated concrete consists of the following apparent densities: 400, 500, 600 and 700 kg/m$^3$. Two first variants have weaker strength parameters, but better insulation parameters. The other are better from the constructional point of view with still satisfactory heat insulation parameters.

From that point of view it should be underlined that aerated concrete is a particularly interesting solution from the point of view of ecology and environment engineering, especially that there are production technologies providing ashes utilization from powerhouses [2].

The major reason for buildings destruction by water influence is caused by capillary structure of the material (Fig. 1). This causes the building barriers to absorb water from the

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ground, water falls or sanitary installations fails in the common phenomenon called capillary rise.

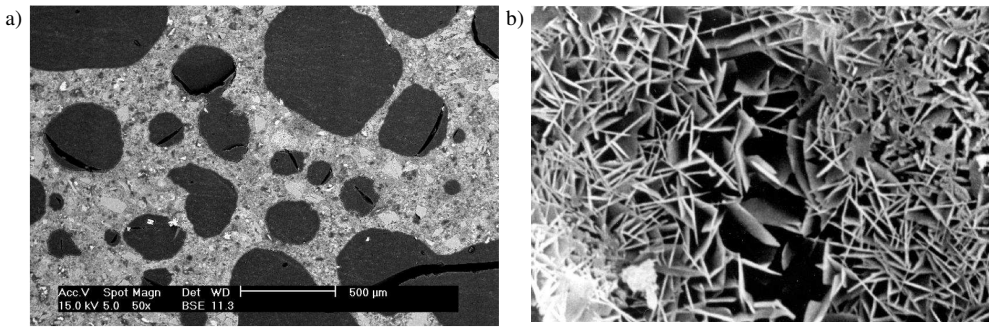


Fig. 1. Scanning Electron Microscope photography of aerated concrete: a) porous structure (spherical macro-pores in black color), b) micro-capillars with flat, needle-shaped crystals, and macro-capillar [3]

Sometimes the damages are caused by phase changes during winter season (congealing and thawing) or transportation of salts which during evaporation crystallize and destroy the porous structure of the material, but especially external plasters.

All above-mentioned exploitation problems indicate the necessity to predict and monitor the behavior of building barriers from that point of view.

## Materials and methods

The studies presented in this work are separated into two parts. The first part is modeling of capillary rise phenomenon in aerated concrete sample, second part is the laboratory experiment of capillary rise with constant moisture monitoring, which from that point of view, should be treated as the applied model verification.

Numerical simulations were conducted using the FEFLOW 5.2 program (*Finite Element subsurface FLOW systems*). The software by Wasy Ltd, Germany [4], based on the finite element method was used in the studies. FEFLOW is a popularly used and repeatedly, successfully verified model [5-9] applied in numerical calculations of groundwater movement, pollutants and heat transport in saturated or unsaturated porous media.

Mathematical description of water movement in the porous medium applied in the FEFLOW model is based on Darcy's law [1]:

$$\vec{q} = -k\nabla\Psi$$

and Richards equation [4]:

$$C(\Psi)\frac{\partial\Psi}{\partial t} = \nabla \cdot k(\Psi)\nabla\Psi - S(\Psi)$$

where: q - water flux, Ψ - hydraulic potential, k - Darcy's constant (water permeability coefficient), C - differential water capacity of the material, t - time.

Above-mentioned equations are solved basing on sample parameterization (initial and boundary conditions).

The sample model was of the following dimensions: 22×16×6 cm. Initial condition was moisture value equal 0.1 $cm^3/cm^3$ (10% vol.) and the boundary conditions were the following:

- vertical left and right, horizontal top - constant flux value equal zero (water insulation),
- horizontal bottom - water.

The reference points were set of the following positions above water level: 5, 10, 15 and 20 cm. Duration of the simulated process was set for 20 days.

To measure the capillary rise phenomenon a technique of constant, quantitative monitoring TDR (*Time Domain Reflectometry*) was applied [11-15].

For that aim a sample of aerated concrete with the following dimensions 24×16×6 cm was prepared. The sample moisture was set to 10% vol.Then the sample were isolated with bitumen mass to minimize any ambient air influence of moisture properties of the sample (sorption from the air). Bottom sample surface was left without water isolation. The experiment was conducted in isothermal conditions - 23°C (±0.5°C).

In such a prepared sample four Time Domain Reflectometry LP/mts (Easy Test) probes were inserted in regular distribution of 5 cm (mostly determined by the area of TDR probe influence range) on the following attitudes - 7, 12, 17 and 22 cm.

In a water container, on a special holder a measured sample was set to provide the maximal contact of the sample with water environment. The container was filled with water 2 cm above the bottom edge of the sample and the TDR probes were consequently placed 5, 10, 15 and 20 cm above the water level (Fig. 2). Constant water level was kept by especially prepared device.

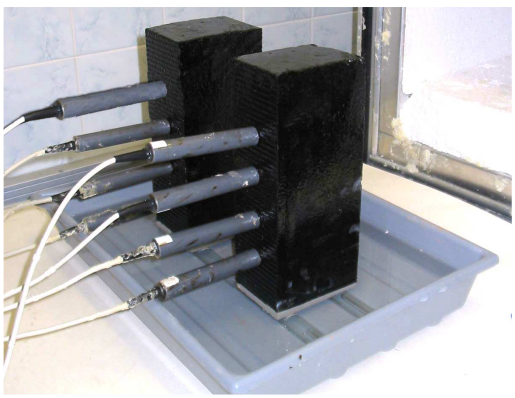

Fig. 2. Experimental setup for capillary rise determination

The measurement was conducted during the period of 20 days until the saturation state (34% vol.) was obtained.

## Results

The simulations results (Fig. 3) are presented in the form of changing moisture on the different levels of the sample. Initial moisture of the material was 0.1 $cm^3/cm^3$ (10% vol.). The first increase of moisture occurred on the height of 5 cm above water level after 6 hours of simulation. Since then the moisture increase slope was steep, and after 2 days it

reached 30% vol., which was close to state of saturation (occurred after 5 days of the process) and in case of this concrete was 34% vol. At the level of 10 cm the water increase was noticed after 2.5 days of simulated process, at 15 cm after about 5 days of simulated time. The increase of moisture at the height of 20 cm occurred later (after 10 days of simulated time).

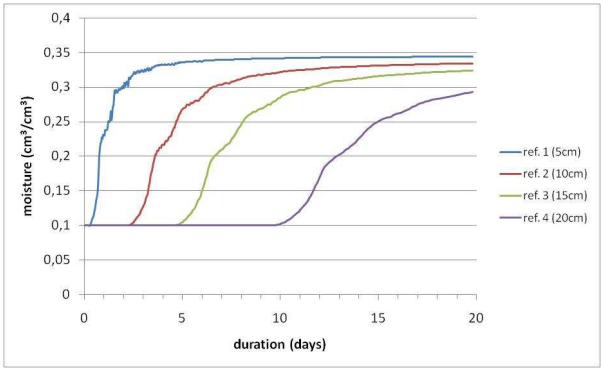


Fig. 3. Moisture changes in the reference points of the modeled sample

The result of the laboratory experiment represents the comparable moisture changes which are presented in Figure 4. TDR sensors installed on the level of 5 cm initially showed moisture about 0.1 $cm^3/cm^3$ and the first increase was observed about six hours later. After 2 days moisture value was about 30% vol. and was increasing gently during the whole experiment to reach the value of 34% vol. which was nearly saturation. At the height of 10 cm moisture increase appeared after 2.5 day of the experiment which is similar to the simulated trend. At the level of 15 cm moisture increase readouts were observed about fifth day of the experiment and the probe 20 cm above water level during seventh day of the experiment.

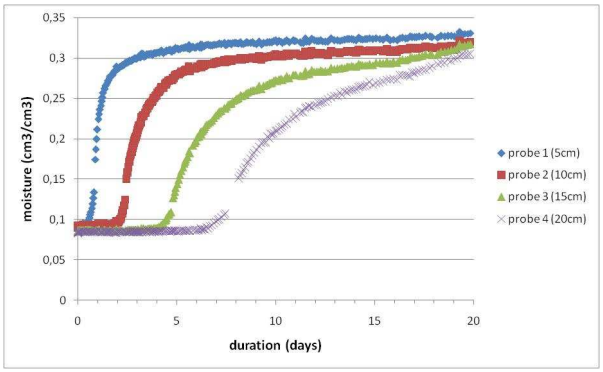


Fig. 4. Moisture changes in sample determined experimentally

Laboratory experiment confirmed the efficacy of the applied model to simulate the capillary rise phenomenon. Moisture changes have similar progresses. Correlation

coefficients between particular probes readouts and reference points on the model sample are the following: 0.992 for the probe at a level of 5 cm; 0.956 for 10 cm probe; 0.978 third probe (15 cm) and finally 0.877 for the probe set 20 cm above water level. Weaker correlation between simulation results and the laboratory experiment (probe 4) may be explained by the insulation from the above which also prevented air permeation and thus slowed water rise. In case of laboratory experiment the insulation allowed the air to escape and the capillary rise was more regular.

## Conclusions

Both computer modeling but also laboratory experiment confirm strong capillary properties of aerated concrete which is not a good property of the material, especially when suffers rainfalls, floods or moisture insulation fails.

Modeling software (FEFLOW) applied for simulation of groundwater movement can be successfully used for modeling of capillary rise phenomenon in building materials when suitable parameterization and discretization is done.

Time Domain Reflectometry (TDR) is a good method for quantitative monitoring of water movement in building materials and can be successfully applied for laboratory or *in situ* verifications of water movement models.

## Acknowledgements

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# ZJAWISKO PODCIĄGANIA KAPILARNEGO W BETONIE KOMÓRKOWYM. MONITORING I SYMULACJE

Politechnika Lubelska

**Abstrakt:** Podciąganie kapilarne to niebezpieczne zjawisko dotyczące wielu obiektów wymurowanych z betonu komórkowego, będącego materiałem porowatym, podatnym na wpływ wody w przypadku zniszczeń poziomych izolacji przeciwwilgociowych. Beton komórkowy to materiał pochodzenia sztucznego, zapewniający zarówno wysokie właściwości wytrzymałościowe, jak i cieplne. Woda migrująca z gruntu do przegród budynku wpływa negatywnie nie tylko na jego konstrukcję, lecz również zwiększa straty ciepła, obniża parametry komfortu cieplnego (niskie temperatury, rozwój grzybów i bakterii), prowadząc do tak zwanego w literaturze Syndromu Chorego Budynku SBS (*Sick Building Syndrome*). Wszystkie powyżej opisane fakty dotyczące migracji wody w ścianach z betonu komórkowego podkreślają potrzebę monitoringu i symulacji zjawiska podciągania kapilarnego w przegrodach budowlanych z betonu komórkowego. Artykuł przedstawia symulację zjawiska podciągania kapilarnego w modelowej próbce betonu komórkowego. Model zastosowany do symulacji oparto na równaniu Richardsa dla przepływu wody w warunkach nienasyconych. Model jest w powszechnym zastosowaniu w dziedzinie gruntoznawstwa, a niniejsza praca jest próbą zastosowania go dla materiałów budowlanych. Wyniki symulacji zostaną zweryfikowane za pomocą badań laboratoryjnych zjawiska podciągania kapilarnego w próbkach rzeczywistych z betonu komórkowego z zainstalowanymi sondami TDR (*Time Domain Reflectometry*), które umożliwią pełny monitoring zjawiska.

**Słowa kluczowe:** podciąganie kapilarne, monitoring, symulacje, TDR

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# ANTIOXIDANTS AND ENVIRONMENTAL STRESS: EFFECT OF LOW-MOLECULAR COMPOUNDS ON MOLECULE OF PROTEIN

## ANTYUTLENIACZE I ICH AKTYWNOŚĆ ŚRODOWISKOWA. WPŁYW ZWIĄZKÓW DROBNOMOLEKULARNYCH NA PROTEINĘ

**Abstract:** The aim of this study was to evaluate the relationships between the chemical structure of tea catechins and their binding affinities to HSA (*Human Serum Albumin*) by fluorescence quenching method at pH 7.4 and 37°C. The quenching constants ($K_q$), binding constants ($K_b$), and free energy changes ($\Delta G^0$) were determined for the tested systems. The presented study contributes to the current knowledge in the area of protein-ligand binding, particularly tea catechin-HSA interactions.

**Keywords:** environmental oxidative stress, antioxidant, tea catechin, serum albumin, protein-ligand binding

Human organism is constantly exposed to undesirable effects of various environmental pollutants including free radicals. Although their properties vary markedly, all free radicals have one common feature - causing oxidative stress (also known as environmental stress) [1]. Oxidative stress plays an important role in the pathogenesis of many human diseases and in the physiological process of aging [2]. Therefore, considerable attention has been focused on the study of naturally occurring substances with antioxidant activity, which could protect human organism against environmental pollutants [3]. Tea catechins are ranged among these outstanding compounds and exert a broad spectrum of biological activities including antioxidant properties [4]. Serum albumin is one of the most abundant proteins in circulatory system and possesses a wide range of physiological functions involving the binding, transport and deposition of many endogenous and exogenous ligands.

It is well known that many low-molecular drugs are bound to serum albumin and their effectiveness thus depends on their binding ability [5]. On the other hand, drugs can cause various changes in protein conformation which may influence its physiological function. Such impaired proteins may be consequently accumulated in body tissues. Spectroscopic techniques including fluorescence spectroscopy represent an ideal tool for studying conformational changes in protein structure since they allow non-destructive measurements of compounds present at low concentration under various conditions [6]. The aim of this study was to evaluate the relationships between the chemical structure of tea catechins and their binding affinities to HSA (*Human Serum Albumin*) in detail using fluorescence quenching method at pH 7.4 and 37°C.

## Materials and methods

Human serum albumin and tea catechins (Fig. 1) were obtained from Sigma-Aldrich GmbH, Germany. All other chemicals were of analytical grade.

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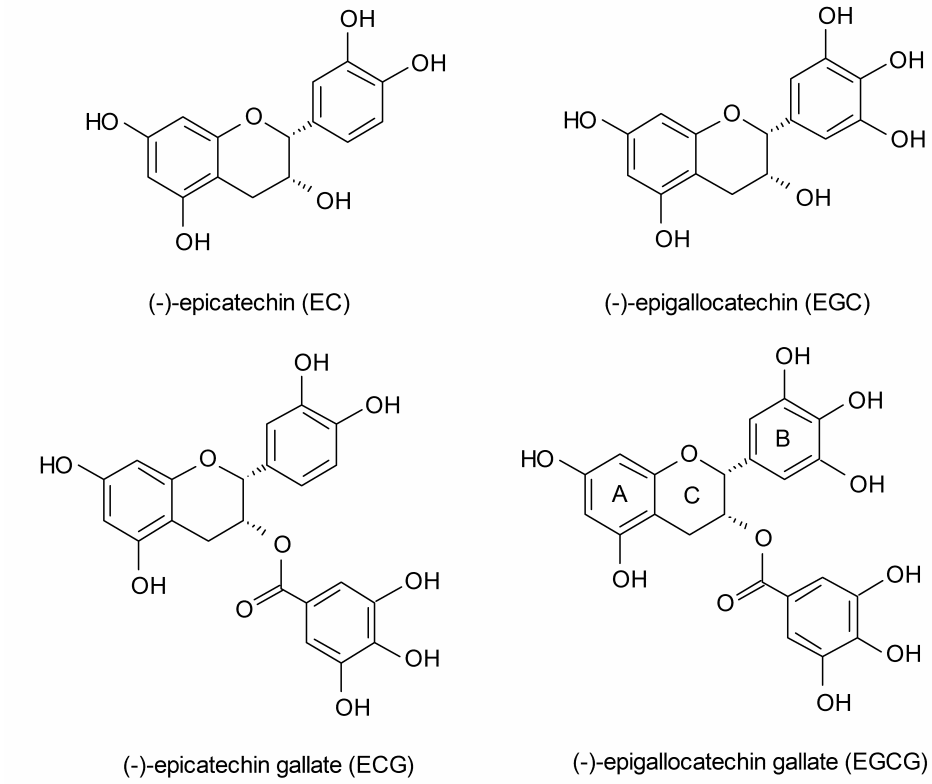


Fig. 1. Structures of tested catechins

Human serum albumin was dissolved in sodium phosphate buffer (pH 7.4; 0.1 M; 0.05% sodium azide) in order to yield solution with concentration of 4 x $10^{-6}$ M. Catechins were dissolved in anhydrous methanol in order to yield 1 x $10^{-2}$ M stock solutions.

Fluorescence spectra were recorded using a luminescence spectrometer LS 50B (Perkin Elmer) with a 10 mm quartz Suprasil cuvette. Quantitative analysis of the potential interaction between catechins and HSA was performed by the fluorimetric titration. Briefly, solution of HSA (4 x $10^{-6}$ M) was titrated in cuvette by successive additions of catechin solution (1 x $10^{-2}$ M) to a final concentration of 4 x $10^{-5}$ M. Fluorescence emission spectra were recorded from 300 to 530 nm with excitation at $\lambda$ = 295 nm while stirring. The excitation and emission slits were both set to 5 nm and scanning speed to 200 nm/min. All experiments were carried out at 37°C. Appropriate blanks were subtracted to correct the fluorescence background. Fluorescence intensity was read at emission wavelength of 348 nm which corresponds with the emission maximum of HSA.

All measurements were performed three times. The mean values of constants and standard deviations were calculated. Standard deviations were always lower than 10%.

## Results and discussion

*Fluorescence quenching mechanism*

Quenching of protein intrinsic (tryptophan) fluorescence was employed for more detailed study of tea catechin-HSA interactions. Fluorescence intensity of HSA gradually decreased with increasing concentration of all tested catechins (Fig. 2). Tea catechins with the galloyl group on the C-ring (ECG and EGCG) caused more pronounced decrease in the tryptophan fluorescence than the non-galloylated catechins (EC and EGC). Moreover, the galloylated catechins induced red shift in the protein emission maximum (shift to longer wavelength about 20 and 25 nm for EGCG-HSA and ECG-HSA system, respectively). The red shift is caused by decrease in a hydrophobic property of binding cavity near sole tryptophan (Trp-214) in HSA molecule suggesting that this tryptophan residue has been brought to a more hydrophilic environment and protein secondary structure has been changed [7]. Significant red shift in EGCG-HSA system was observed also by Maiti et al [8]. No shift in the emission maximum of HSA was observed in the case of the non-galloylated catechins.

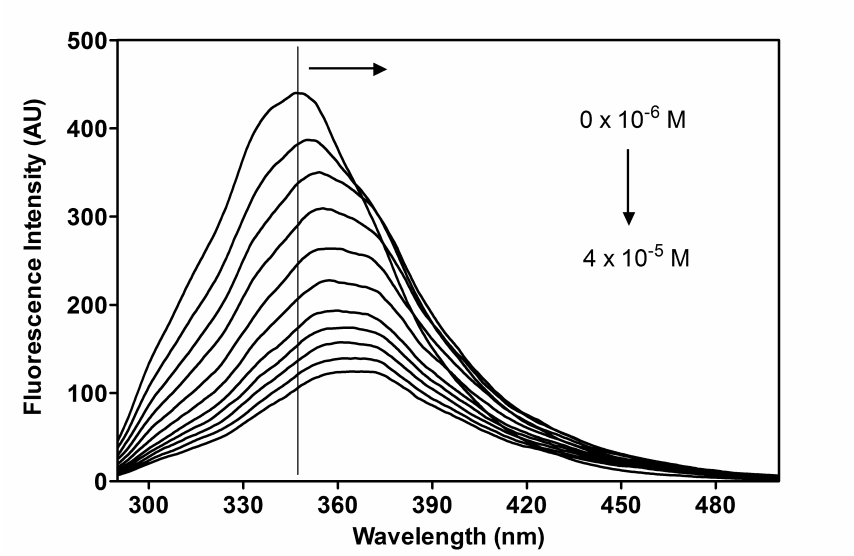


Fig. 2. Fluorescence quenching spectra of HSA (4 x $10^{-6}$ M) in the increasing concentration of epigallocatechin gallate (0-4 x $10^{-5}$ M) in sodium phosphate buffer (pH 7.4, 0.1 M, 0.05% sodium azide) at $\lambda_{ex}$ = 295 m and 37°C

In order to clarify the fluorescence quenching mechanism induced by the tested catechins, the fluorescence quenching data were analyzed using the Stern-Volmer equation:

$$F_0/F = 1 + K_q\tau_0[Q] = 1 + K_{SV}[Q] \quad (1)$$

where $F_0$ and F are the fluorescence intensities of HSA in the absence and presence of the quencher, respectively. [Q] is the quencher concentration, $K_{SV}$ is the Stern-Volmer

constant, $K_q$ is the bimolecular quenching rate constant and $\tau_0$ is the lifetime of the fluorophore in the absence of the quencher ($\tau_0$ is about 5 ns) [9].

The Stern-Volmer constants ($K_{SV}$) and bimolecular quenching rate constants ($K_q$) were determined from the eq. (1) using linear regression of the plot of $F_0/F$ versus [Q] (Table 1). The representative Stern-Volmer plot of EC-HSA system is displayed in Figure 3. All values of $K_q$ were much greater than the diffusion-limited rate constant of the biomolecule ($K_{diff}$ = 1.0 x $10^{10}$ l $mol^{-1}s^{-1}$) which suggested that the static quenching mechanism is dominant in the studied interactions [9]. The highest value of $K_q$ was determined for epicatechin gallate and further decreased in the order ECG > EGCG >> EC > EGC. The importance of the galloyl moiety on the C-ring was evident because quenching constants for the catechin gallates (ECG and EGCG) were significantly higher than those of catechins lacking the galloyl group (EC and EGC). Catechins with the catechol group on the B-ring (EC and ECG) showed more pronounced quenching effect than their analogs with the pyragollol group (EGC and EGCG).

Table 1

The Stern-Volmer constants ($K_{SV}$), bimolecular quenching rate constants ($K_q$), binding constants ($K_b$), and free energy changes ($\Delta G^0$) of the catechin-HSA systems (pH 7.4, 37°C)

| Tested compound | $K_{SV}$ [$10^4$ $M^{-1}$] | $K_q$ [$10^{12}$ $M^{-1}s^{-1}$] | $K_b$ [$10^4$ $M^{-1}$] | $\Delta G^0$ [kJ·$mol^{-1}$] |
|---|---|---|---|---|
| Epicatechin | 1.19 | 2.38 | 2.58 | –26.18 |
| Epigallocatechin | 0.46 | 0.93 | 1.92 | –25.42 |
| Epicatechin gallate | 11.62 | 23.24 | 6.86 | –28.70 |
| Epigallocatechin gallate | 9.19 | 18.39 | 6.80 | –28.68 |

$K_q = K_{SV}/\tau_0$; $\tau_0$ = 5 x $10^{-9}$ s [9]. All correlation coefficients (R) were higher than 0.990. Standard deviations (mean value of three independent experiments) were lower than 10%.

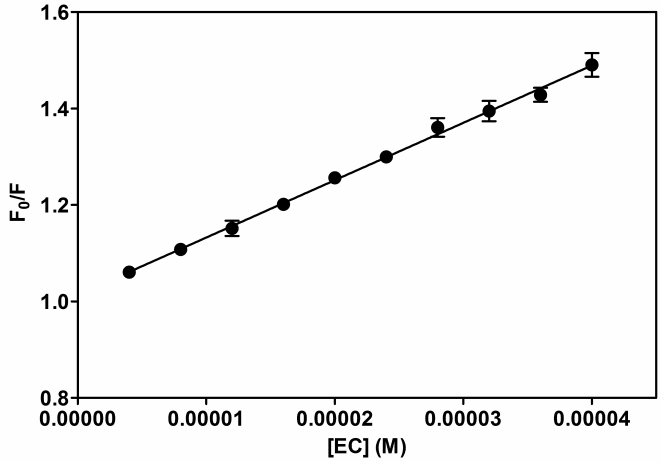


Fig. 3. The Stern-Volmer plots of epicatechin-HSA system at pH 7.4 and 37°C. [HSA] = 4 x $10^{-6}$ M, [EC] = 0-4 x $10^{-5}$ M, $\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 348 nm, y = 11890x + 1.014, and R = 0.995

*Binding parameters*

The binding constants ($K_b$) of catechin-HSA systems were calculated using the following Lineweaver-Burk equation [10]:

$$1/(F_0 - F) = 1/F_0 + 1/(K_b F_0[Q]) \quad (2)$$

where $F_0$ and F are the fluorescence intensities before and after the addition of the quencher, [Q] is the quencher concentration. The free energy change ($\Delta G^0$) value was estimated from the following equation [11]:

$$\Delta G^0 = -RT\ln K_b \quad (3)$$

where R is the gas constant (8.314 J $K^{-1}$ $mol^{-1}$) and T is temperature (T = 310 K).

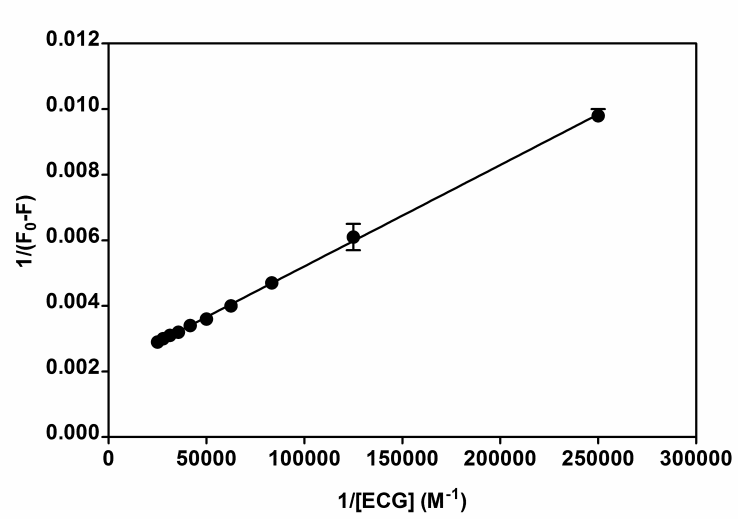


Fig. 4. The Lineweaver-Burk plots for epicatechin gallate-HSA system at pH 7.4 and 37°C. [HSA] = 4 x $10^{-6}$ M, [ECG] = 0-4 x $10^{-5}$ M, $\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 348 nm, y = 3.09 x $10^{-8}$x + 2.23 x $10^{-3}$, and R = 0.998

The binding constants ($K_b$) were obtained by plotting of $1/(F_0 - F)$ versus 1/[Q] (Fig. 4). The binding affinity was the strongest for epicatechin gallate and decreased in the order ECG > EGCG >> EC > EGC. The free energy changes were calculated using the eq. (3). The obtained results are summarized in the Table 1. The galloylated catechins (ECG and EGCG) showed significantly higher binding ability than the non-galloylated catechins (EC and EGC) due to the presence of the galloyl group on the C-ring, ie the additional aromatic ring and three hydroxyl groups which can establish hydrophobic interactions and hydrogen bonds, respectively. Our results are consistent with earlier obtained data by high-performance affinity chromatography with immobilized albumin column [12]. In addition, catechol-type catechins (EC and ECG) possessed stronger binding affinity than pyragollol-type catechins (ECG and EGCG) which suggests that the insertion of an additional hydroxyl group on the B-ring does not contribute to their binding affinities as was described in literature [12]. One of the most studied tea catechin is epigallocatechin gallate and the results obtained for the EGCG-HSA system by Maiti et al [8] are in good

agreement with our data although the experimental conditions were slightly different. The spontaneity of HSA-catechin interactions was confirmed by the negative values of $\Delta G^0$.

## Conclusions

The interactions of tea catechins and HSA were investigated by fluorescence quenching method. Tea catechins quenched tryptophan fluorescence of HSA mainly by static quenching mechanism in the studied range of concentrations (0-40 x $10^{-6}$ M). Thus, the non-fluorescent complexes were formed. All tested catechins bound spontaneously to the molecule of HSA with different binding affinity. The binding constant was the strongest for epicatechin gallate and decreased in the order ECG > EGCG >> EC > EGC. The most important structural feature of the tested catechins contributing to HSA binding was the galloyl group on the C-ring. The presented study contributes to the current knowledge in the area of protein-ligand binding, particularly tea catechin-HSA interactions.

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## ANTYUTLENIACZE I ICH AKTYWNOŚĆ ŚRODOWISKOWA. WPŁYW ZWIĄZKÓW DROBNOMOLEKULARNYCH NA PROTEINĘ

**Abstrakt:** Celem pracy była ocena zależności między strukturą chemiczną katechin herbaty i ich powinowactwem do HSA (*albumina ludzkiej surowicy*) za pomocą metody wygaszania fluorescencji przy pH 7,4 i w temperaturze 37°C. Dla badanych układów wyznaczono stałą szybkości wygaszania ($K_q$), stałą wiązania ($K_b$) i zmiany entalpii swobodnej ($\Delta G^0$). Prezentowana praca poszerza aktualny stan wiedzy na temat wiązania białko - ligand, zwłaszcza interakcji katechiny - HSA.

**Słowa kluczowe:** środowiskowy stres oksydacyjny, antyutleniacze, katechiny herbaty, albuminy surowicy, wiązanie białko - ligand

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# EVALUATION OF THE CONTENT OF CHLOROPHENOLS AND THEIR DERIVATIVES IN WATER SUPPLY SYSTEMS

## OCENA ZAWARTOŚCI CHLOROFENOLI I ICH POCHODNYCH W WODACH WODOCIĄGOWYCH I RZECZNYCH

**Abstract:** There is currently great environmental interest in chlorinated phenol derivatives on account of their high toxicity and wide industrial use. Surface water contains many inorganic and organic compounds both of natural and anthropogenic origin. The composition of the specified compounds in aquatic ecosystems is related to the influence of different natural factors or a variety of human activity. Aromatic compounds, due to their toxicity and persistency in the environment, establish serious danger not only for living organisms but also for the biocenosis. One of the most important group of ecotoxins are chlorophenols. They exhibit high toxicity including genotoxic, mutagenic and cancerogenic activity. Moreover, substitution of these compounds by chlorine atoms may increase their toxic action and prolong the period of their bioaccumulation in living organisms. Chlorination is one of the method which lets to remove pathogens, organic matter and xenobiotic from water. This process uses oxidants like chlorine or chlorine dioxide. Chlorination leads to transformation part of recycled compounds in forms which can be more toxic and dangerous than their precursors. This process leads also to formation of new chloroorganic compounds like halomethanes, chlorinated aromatic hydrocarbons and a lot of other organic compounds which can be toxic. Their concentration in drinking water depends on the concentration of the precursors and dose of chlorine which is used in the water purification process. The aim of this work was to determine the occurrence of phenol, chlorophenols, chlorocatechols and chlorinated metoxyphenols both in drinking water of Warsaw and Tomaszow Mazowiecki, and surface water taking from Vistula and Pilica Rivers. The chromatographic analyses were made using a gas chromatograph connected with a mass spectrometer.

**Keywords:** phenol, chlorophenols, chlorination, chlorine oxidants

Water is one of the main elements of the environment which determine the existence of life on the Earth, affect the climate and limit the development of civilization.

Water resources management requires constant monitoring in terms of its qualitative-quantitative values. Proper assessment of the degree of water pollution is the basis for conservation and rational utilization of water resources.

Aromatic compounds, due to their toxicity and persistence in the environment, establish serious danger towards living organisms, including humans. One of the most important group of ecotoxins are phenol and chlorophenols [1, 2]. The presence of phenol compounds in water significantly impairs the taste and smell of the water. Thus, phenol can be organoleptically detected in water at concentration of 10÷30 μg/dm$^3$ [3].

Phenol (1-hydroxybenzene) is a colorless, crystalline substance of characteristic odour, soluble in water and organic solvents [4]. Phenol was one of the first compounds inscribed into The List of Priority Pollutants by the US Environmental Protection Agency (US EPA). Phenol is synthesized on an industrial scale by extraction from coal tar as it is formed by transformation of high quantities of cumene present in plants that were used for tar production. Hydroxybenzene is also obtained in a reaction between chlorobenzene and sodium hydroxide, toluene oxidation and synthesis from benzene and propylene [5, 6].

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Chlorophenols are the most widespread and the largest group of phenols, that are used in a number of industries and products. Those compounds are formed as a result of use and degradation of phenolic pesticides such as 2-chlorophenol, 4-chlorophenol, phenoxyherbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D). Exposure to high levels can cause damage to the liver and immune system. These compounds are also formed in the environment by chlorination of mono- and polyaromatic compounds present in soil and water. In drinking water there are present as a result of the chlorination of phenols during disinfection, as by-products of the reaction of hypochlorite with phenolic acids, as biocides, or as degradation products of phenoxy herbicides. Those most likely to occur in drinking water as by-products of chlorination are 2-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol.
Chlorophenols are also present in drinking water due to the substitution of organic matter and low molecular weight compounds (present in purified water) with chlorine atoms derived from inorganic chlorine oxidants [7].

The appearance of metoxyphenols including those substituted with metoxy residues in the second (guaiacols) and in the second and sixth positions (syringols) is related with plant decay in which lignin contained in cell's walls transformed. In a few last decades the intensive usage of wood in combustion processes also led to the excessive emission of metoxyphenols to the atmosphere and thus to other systems of the environment. The processes of wood pulp chlorination employed during paper production are a very important source of these compounds (including chlorinated) in water. This type of activity generates many chlorinated compounds including high substituted chlorinated metoxyphenols. Phenols, chlorophenols, and chlorocatechols are usually used as a precursors and components of many chemicals including dyes, plastics, resins and pharmaceuticals. The compounds are also formed as a result of use and degradation of pesticides and biphenyls. Some of described compounds have natural origin. Phenol is formed during decomposition of organic matter, catechol is biosynthetized by plants and fungi create chlorinated metoxyphenols [8].

Phenol, catechol, chlorophenols and metoxyphenols exhibit high toxicity including mutagenicity and carcinogenicity [9, 10]. Most of these compounds reveal acute toxicity as they easily penetrate cell membrane and finally exert necrosis of skin and organ within the body. Clinical investigation showed that people exposed to drinking water contaminated with chlorophenols suffer from infections, dermatitis, irritation of digestive tract and strong exhaustion. Moreover, substitution of these compounds by chlorine atoms may increase their toxic action and prolong the period of their bioaccumulation in living organisms.

Described compounds may be transformed in water as they undergo the influence of many abiotic and biotic factors including UV irradiation, metal oxides and temperature influence and microbes and plants activity. The transformation processes lead to increase in the diversity of phenols and some of them are characterized by strong toxicity (stronger, than their precursors) towards water organisms.

**Collecting samples**

Samples of drinking water and river water were collected in Warsaw and Tomaszow Mazowiecki six times within a year (in period of January, March, May, July, September and November 2008).

In Warsaw tap water was collected from the area supplied by Central Water Supply that pumps it from the Vistula River. Drinking water in Tomaszow Mazowiecki was collected from the area supplied by Water Supply "Tomaszow" that pumps it from the Pilica River.

Tap water was collected after final purification process-chlorination which uses:

- chlorine dioxide (0.3÷0.4 active chlorine/$m^3$ water) in Warsaw,
- chlorine (0.3÷0.4 active chlorine/$m^3$ water) in Tomaszow Mazowiecki.

Selected areas of the cities were supplied with water exposed to the strongest anthropogenic contamination. The water taken from these sources potentially should have contained the highest amounts and the diversity of compounds analyzed and their precursors.

## Materials and methods

The standards of these compounds (purity: 98÷99%) were bought from Fluka AG: 1-hydroxybenzene (phenol), 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2,3,6-trichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 2,3,4,5-tetrachlorophenol, pentachlorophenol, 4-metylphenol, 4-chlorocatechol, 3,4,5-trichlorocatechol, tetrachlorocatechol, 2-metoxyphenol (guaiacol), 4,6-dichloroguaiacol, 3-chlorosyringol, trichlorosyringol, 3,4,5-trichloroveratrole. Methanol, dichloromethane, diethyl ether, methylene chloride, acetone, hexane and phosphoric acid of HPLC purity were obtained from Baker JT, USA.

## Preparation of samples

Samples were collected in 1 $dm^3$ volumes in dull HD polyethylene bottles. To inhibit microbes development and oxidation of phenols, 10 $cm^3$ of methanol and 0.1 g of ascorbic acid were added to the samples. Next, 200 g of sodium chloride was added and samples were acidified with phosphoric acid to pH 2.0 to decrease solubility of investigated compounds in water (higher effectiveness of adsorption of phenols by octadecyl layer). Then, the inner standard was added (1.5 μg of 2,3,6-trichlorophenol in 1 $cm^3$ of acetone). Finally, samples were mixed using an electromagnetic stirrer (750 r.p.m.) for 30 minutes.

## Solid-Phase Extraction

Adsorption of chlorophenols and their derivatives was performed on "EMPORE Extraction Disks" in a Baker Separex system. The system "EMPORE spe" of extraction filters (disks) was equipped with its binding phase octadecyl C18, diameter of 50 mm and layer thickness of 1.0 mm. The filters were used in a special vacuum extraction apparatus "Baker Separex" made of borosilicate glass. The octadecyl layer was conditioned using 10 $cm^3$ volumes of diethyl ether, methylene chloride, methanol and bidistilled water. Elution was made with two 5 $cm^3$ volumes of diethyl ether and methylene chloride, respectively. Eluent was evaporated, individual phenols and their derivatives were dissolved in hexane, acetylated (derivatized) and concentrated down to a volume of 0.1 $cm^3$ for gas chromatography separation.

## Gas Chromatography - Mass Spectrometry

Chlorophenols are usually determined by use of chromatographic techniques such as HPLC [11] or GC [12]. However, because of their high polarity, they give broad, tailed peaks if separated directly (without prior derivatization) by GC.

The chromatographic analysis ware made using a gas chromatograph (Hewlett-Packard type 5890) connected with a quadruple mass spectrometer type 5972 (temperature MS - 162ºC) equipped with capillary column HP 5 (60 m x 0.25 mm). Chromatographic separations were led in programmed heating conditions in the range of the temperature from 60ºC which was keeping by 1 min then increased to 80ºC at the rate of 5ºC/min and to 210ºC at the rate of 10ºC/min. Finally increased to 260ºC. The temperature of the splitless injector was 260ºC, the carrier gas was helium (rate of flow - 1cm$^3$/min). Qualitative estimation was made in SCAN system (identification of individual compounds by comparison with standards of spectra from spectra library type NBS 75KL) and quantitative estimation was done in SIM system (identification by comparison with calibrated standards).

## Results and discussion

Surface water contains many organic compounds both of natural and anthropogenic origin. The composition of the particular compounds in water is related to the influence of different natural factors or a kind of human activity on the aqueous ecosystems. It is considered that the occurrence of chlorophenols and their derivatives in the environment is strictly related to human activity including mainly industry, production and degradation of pesticides and disposal of the communal sewages to open waters [13].

Obtained results evidenced the presence in collected samples of phenol, chlorophenols (4-chlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, tetra- and pentachlorophenol), 4-chlorocatechol, 4,6-dichloroguaiacol, 3-chlorosyringol, trichlorosyringol.

Results of analyses have exerted, that water of Vistula River is significantly contaminated with phenolic compounds.

The highest concentrations were noted for: 4,6-dichloroguaiacol (2.83 μg/dm$^3$), phenol (1.67÷2.70 μg/dm$^3$), 2,4,5-trichlorophenol (0.25÷2.72 μg/dm$^3$), in particular for the pentachlorophenol (0.71÷641.3 μg/dm$^3$). The lowest concentrations were observed for the: 2,4-dichlorophenol (0.02÷0.03 μg/dm$^3$), trichlorosyringol (0.03 μg/dm$^3$) and 4-metylophenol (0.04 μg/dm$^3$) (Tab. 2).

Smaller number of organic compounds and lower concentration of phenol identify in samples which were taken from the Pilica River. Following compounds were detected: phenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, tetrachlorophenol, pentachlorophenol, 4-metylophenol and guaiacol. The highest concentrations were observed for: pentachlorophenol (5.60 μg/dm$^3$) and 2,4,5-trichlorophenol (2.49 μg/dm$^3$), in turn the lowest for 4-metylophenol (0.03 μg/dm$^3$) (Table 1).

In tap water (treated water) destined for the city of Warsaw following compounds were noted: phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, tetrachlorophenol, pentachlorophenol, 4-metylophenol, 4-chlorocatechol, 3,4,5-trichlorokatechol, tetrachlorokatechol and 4,6-dichloroguaiacol. The highest concentration was noted for pentachlorophenol (3.27 μg/dm$^3$), phenol (0.43÷1.86 μg/dm$^3$) and 2,4,6-trichlorophenol (1.01÷1.14 μg/dm$^3$), whereas the lowest

established for 4-metylophenol (0.02÷0.09 μg/dm$^3$), tetrachlorocatechol (0.03 μg/dm$^3$) and 2,4,5-trichlorophenol (0.03÷0.04 μg/dm$^3$) (Table 2).

Table 1

The means concentrations [μg/dm$^3$] and their standard deviation obtained for phenol, chlorophenols, chlorocatechols and guaiacols determined in the drinking water of Tomaszów Mazowiecki and surface water from Pilica River six times during 2008

| Determined compounds | January | | March | | May | |
|---|---|---|---|---|---|---|
| | Pilica River | Tomaszow Mazowiecki | River Pilica | Tomaszow Mazowiecki | Pilica River | Tomaszow Mazowiecki |
| phenol | 1.37±0.252 | 1.20±0.361 | 0.05±0.021 | 2.23±0.252 | 0.15±0.025 | 1.80±0.400 |
| 2,4-dichlorophenol | 0.04±0.012 | - | - | - | - | - |
| 2,4,5-trichlorophenol | 2.49±0.170 | - | 0.53±0.252 | | 0.62±0.104 | - |
| 2,4,6-trichlorophenol | 0.06±0.015 | 0.83±0.252 | 0.89±0.259 | 0.62±0.076 | 0.19±0.051 | 0.10±0.015 |
| tetrachlorophenol | - | - | - | - | 0.28±0.057 | 0.35±0.058 |
| pentachlorophenol | 0.03±0.000 | - | - | - | 5.60±1.253 | 0.70±0.100 |
| 4-methylphenol | - | - | - | 0.38±0.040 | - | - |
| 4-chlorocatechol | - | - | - | - | - | - |
| 3,4,5-trichlorocatechol | - | 0.12±0.031 | - | - | - | - |
| tetrachlorocatechol | - | - | - | - | - | - |
| guaiacol | - | - | 0.59±0.036 | - | - | - |
| 4,5,6-trichloroguaiacol | - | - | - | - | - | 0.48±0.076 |
| 3-chlorosyringol | - | - | - | - | - | - |

| Determined compounds | July | | September | | November | |
|---|---|---|---|---|---|---|
| | Pilica River | Tomaszow Mazowiecki | Pilica River | Tomaszow Mazowiecki | Pilica River | Tomaszow Mazowiecki |
| phenol | 0.05± 0.010 | 7.07±1.172 | 1.13±0.252 | - | 0.15±0.014 | 1.25±0.071 |
| 2,4-dichlorophenol | - | 0.22±0.085 | 1.03±0.153 | - | - | - |
| 2,4,5-trichlorophenol | - | 0.53±0.153 | - | 0.13±0.038 | 0.05±0.007 | - |
| 2,4,6-trichlorophenol | - | 0.24±0.125 | - | 0.16±0.015 | 0.03±0.000 | 0.09±0.014 |
| tetrachlorophenol | - | 0.33±0.058 | 0.57±0.153 | 0.79±0.110 | - | 0.04±0.000 |
| pentachlorophenol | - | - | 2.35±0.304 | - | - | - |
| 4-methylphenol | - | - | - | - | 0.03±0.000 | 0.03±0.000 |
| 4-chlorocatechol | - | - | - | 0.70±0.100 | - | - |
| 3,4,5-trichlorocatechol | - | - | - | 0.43±0.058 | - | - |
| tetrachlorocatechol | - | - | - | 0.67±0.058 | - | - |
| guaiacol | - | 1.37±0.321 | - | 0.44±0.032 | - | - |
| 4,5,6-trichloroguaiacol | - | - | - | - | - | - |
| 3-chlorosyringol | - | 0.33±0.115 | - | - | - | - |

Presence of numerous phenolic derivatives evidenced also in samples from tap water from Tomaszów Mazowiecki. For example: phenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, tetrachlorophenol, pentachlorophenol, 4-metylophenol, 4-chlorocatechol, 3,4,5-trichlorocatechol, tetrachlorocatechol, guaiacol, 4,5,6-trichloroguaiacol and 3-chlorosyringol. The highest concentrations among the phenolic derivatives were noted for phenol (1.20÷7.07 μg/dm$^3$), guaiacol (0.44÷1.37 μg/dm$^3$) and 2,4,6-trichlorophenol (0.09÷0.83 μg/dm$^3$) (Table 1).

The presence of phenol of natural origin is related with photochemical decomposition of different natural substances that are contained in cell walls of plants under the influence of UV irradiation. For example, aromatic amino acids - tryptophan and tyrosine are transformed to phenol and lignin (natural biopolymer present in cell walls of plants) is

decomposed to metoxyphenols such as guaiacols and dimethoxyphenols included syringols. High concentration of phenol in surface water may be related to their resuspension from sediments as this compound is intensively incorporated into humus substances [14]. Chlorinated compounds may be formed *de novo* from simple organic and inorganic compounds and also from aromatic compounds that are present in the environment.

Table 2

The means concentrations [μg/dm$^3$] and their standard deviation obtained for phenol, chlorophenols, chlorocatechols and guaiacols determined in the drinking water of Warsaw and surface water from Vistula River six times during 2008

| Determined compounds | January | | March | | May | |
|---|---|---|---|---|---|---|
| | Vistula River | Warsaw | Vistula River | Warsaw | Vistula River | Warsaw |
| phenol | 1.67±0.306 | 1.53±0.208 | - | 1.86±0.350 | 2.10±0.656 | 0.05±0.015 |
| 2-chlorophenol | - | - | - | - | - | - |
| 2,4-dichlorophenol | - | 0.08±0.015 | - | - | - | - |
| 2,4,5-trichlorophenol | 0.25±0.050 | - | 2.72±0.501 | - | 1.76±0.251 | - |
| 2,4,6-trichlorophenol | - | 1.01±0.101 | - | 1.14±0.053 | 0.06±0.010 | 0.04±0.000 |
| tetrachlorophenol | 1.33±0.306 | 0.05±0.010 | 0.68±0.104 | - | - | - |
| pentachlorophenol | 641.3±61.655 | 3.27±0.252 | 6.87±0.666 | - | 0.71±0.056 | - |
| 4-methylphenol | - | - | - | 0.09±0.020 | - | - |
| 4-chlorocatechol | - | - | 0.65±0.150 | - | - | - |
| 3,4,5-trichlorocatechol | 0.20±0.021 | 0.08±0.006 | - | - | - | - |
| tetrachlorocatechol | - | - | 0.20±0.035 | - | - | - |
| guaiacol | - | - | 0.63±0.205 | - | - | - |
| 4,6-dichloroguaiacol | - | - | 2.83±0.289 | - | - | - |
| trichlorosyringol | - | - | - | - | - | - |

| Determined compounds | July | | September | | November | |
|---|---|---|---|---|---|---|
| | Vistula River | Warsaw | Vistula River | Warsaw | Vistula River | Warsaw |
| phenol | 2.70±0,100 | 0.45±0.050 | 2.34 ±0,060 | 0.43±0.058 | 1.70±0,141 | 0.06±0.007 |
| 2-chlorophenol | - | 0.03±0.000 | - | | - | - |
| 2,4-dichlorophenol | - | - | 0.03± 0,000 | 0.03±0000 | 0.02 ±0,000 | 0.03±0.000 |
| 2,4,5-trichlorophenol | - | 0.04±0.006 | 0.27± 0,058 | 0.03±0.000 | 0.75±0,071 | - |
| 2,4,6-trichlorophenol | - | - | 0.08± 0,000 | - | - | - |
| tetrachlorophenol | - | - | 0.50± 0,100 | - | - | - |
| pentachlorophenol | - | - | - | - | - | - |
| 4-methylphenol | - | - | - | 0.03±0.000 | 0.04±0,014 | 0.02±0.000 |
| 4-chlorocatechol | - | 0.04±0.000 | - | - | - | - |
| 3,4,5-trichlorocatechol | - | - | - | - | - | - |
| tetrachlorocatechol | - | - | - | 0.03±0.000 | - | - |
| guaiacol | - | - | 0.37± 0,058 | - | - | - |
| 4,6-dichloroguaiacol | - | - | - | 0.14±0.010 | - | - |
| trichlorosyringol | - | - | 0.03± 0,006 | - | - | - |
| 3,4,5-trichloroveratrole | - | - | - | - | - | - |

In water, chlorinated metoxyphenols may be dechlorinated or O-demethylated to form chlorophenols or phenol molecules [15].

The chlorination processes have very important meaning in elimination of organic matter, xenobiotics and some other pathogens from drinking water. However their use may cause risk for the formation of new, strongly toxic, mutagenic and carcirogenic compounds.

Phenolic derivatives were observed in water from Vistula River. The most often noted compound was phenol (in each samples except March). Similar in the Pilica River phenol was observed in each month. Beside of the fact that in surface water from Vistula river concentrations of phenol were higher than in water from Pilica River. After disinfection opposing correlation had occurred - higher concentrations of phenol were in tap water from Tomaszów Mazowiecki.

Analysis of the literature data confirm that a lot of low-molecular compounds can develop in treated water through degradation of dissolved natural organic matter.

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## OCENA ZAWARTOŚCI CHLOROFENOLI I ICH POCHODNYCH W WODACH WODOCIĄGOWYCH I RZECZNYCH

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**Abstrakt:** Wody powierzchniowe zawierają wiele związków organicznych zarówno pochodzenia naturalnego, jak i antropogennego. Związki aromatyczne ze względu na ich toksyczność i obecność w środowisku stanowią poważne zagrożenie nie tylko dla żyjących organizmów, ale również biocenoz przyrodniczych. Jedną z najważniejszych grup ektotoksyn są chlorofenole. Stosuje się je jako prekursory i składniki wielu substancji chemicznych, w tym polimerów, barwników oraz leków. Chlorofenole są silnie toksyczne, wykazują działanie genotoksyczne, mutagenne i rakotwórcze. Ponadto podstawienie tych związków dodatkowymi atomami chloru

powoduje wzrost ich toksyczności i wydłuża czas akumulacji w organizmach żywych. Metodą, która pozwala pozbyć się patogenów, nadmiernej ilości materii organicznej oraz ksenobiotyków z wody, jest jej chlorowanie. Proces ten wykorzystuje oksydanty chlorowe, wśród których chlor gazowy oraz ditlenek chloru mają bardzo ważne znaczenie. Skutkiem ubocznym stosowania chloru jest tworzenie nowych związków chloroorganicznych, takich jak halometany, chlorofenole, chlorowane węglowodory aromatyczne oraz wiele innych związków organicznych. Ich stężenia w wodzie pitnej zależą w głównej mierze od stężeń ich organicznych prekursorów oraz od dawki chloru zastosowanej w procesie uzdatniania wody. Celem pracy było określenie zawartości fenolu, chlorofenoli, chlorokatecholi oraz chlorowanych metoksyfenoli w wodzie pitnej z Warszawy i Tomaszowa Mazowieckiego oraz w wodzie pobranej z rzek Wisły i Pilicy, wykorzystując do pomiaru metodę chromatografii gazowej.

**Słowa kluczowe:** fenol, chlorofenole, chlorowanie, oksydanty chlorowe