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Wioletta ADAMUS-BIAŁEK[1], Monika WAWSZCZAK[1] and Anna ŚWIERCZ[1]

# IMPACT OF SEWAGE TREATMENT PLANT ON LOCAL ENVIRONMENT

## WPŁYW OCZYSZCZALNI ŚCIEKÓW NA OKOLICZNE ŚRODOWISKO

**Abstract:** The aim of the study was the analysis of three wastewater treatment plants operations (Busko Zdroj, Kazimierza Wielka and Pinczow in Swietokrzyskie voivodship). The sanitary condition of sewage sludge and the management of sludge in wastewater treatment plant were investigated. These data were made available by these wastewater treatment plants. The analyses of the sewage sludge have included the sanitary basic analysis, especially the identification of *Salmonella* spp. and the present of intestinal parasites eggs. It stated, that the sanitary condition of the analyzed sewage sludge was acceptable. Microbiological purity of sewage sludge and mineral composition similar to soil organic matter (humus) allows to use them as natural fertilizers, but not for the cultivation of the plants intended for human consumption. These deposits have been widely used as a material for land reclamation in Pinczow. The further aim of the research was to investigate the microbiological purity of air near the two wastewater treatment plant (Stykow and Szczecno in Swietokrzyskie voivodship). The number of colony forming units of mannitol-positive *Staphylococcus* sp. and the total number of mesophilic and psychrophilic bacteria were analyzed in four different distance from wastewater treatment plants. Mannitolo-positive staphylococci belong to microbiological indicators of air pollution. The number of mesophilic and psychrophilic bacteria indicate the level organic matter contamination. The air pollution was observed at all measurement points. It is known, that pathogenic microorganisms can easily pass from the water to the air and next spread in the environment. This analysis shows that the use of sewage sludge to land reclamation is helpful to preserve and restore the ecological balance of mineral elements, which is an important aspect of economic and environmental protection. It is also likely, that wastewater treatment plants may have an impact on air pollution.

**Keywords:** sewage sludge, environment pollution, air pollution, fertilizers

## Introduction

There are many sources of emissions, which include also wastewater treatment plants (WWTP). Sewage treatment plants are a serious source of toxic chemical compounds, gases and biological contaminations. That may have a direct impact on the surrounding environment. A separate threat are microorganisms present in wastewater. Microorganisms gets into the wastewater and sewage sludge with humans' and animals' excrements [1]. The most frequently identified species includes *Escherichia* sp., *Salmonella* sp., *Shigella* sp., *Pseudomonas aeruginosa*, *Clostridium perfringens*, *Bacillus anthracis*, *Listeria monocytogenes*, *Vibrio cholerae*, *Mycobacterium tuberculosis*, *Streptococcus faecalis*, *Proteus vulgaris* [2, 3]. Most of the pathogenic microorganisms are removed, but some of them can get in to a sewage sludge and survive even for a few months [4-6]. The highest number of pathogens are identified in the sewage sludge arising from purification of municipal and industrial wastewater [7, 8]. The number of microorganisms in the wastewater also depends on climate, wastewater quality and the method of wastewater purification [9]. Mineral and organic composition of sludge from municipal sewage treatment plants is similar to soil organic matter (humus). Therefore, sewage sludge can be

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treated as a good organic fertilizer rich in nitrogen, phosphorus and easily degradable organic matter. This allows to use of sewage sludge to fertilize crops and soils [10-12]. Due to the economic growth in Poland, the production increase of sewage sludge is observed. The rules of the management of sewage sludge in Poland are based on European directives.

Another issue related to the wastewater treatment is microbial air pollution. Air is not an optimal environment for microorganisms [13, 14]. They form different bioaerosols to transmission from different environments (soil, water, waste, surface plants, animals and others) by wind gusts, sneezing or coughing. The source of bioaerosols is also wastewater treatment during the purification of water [15-17]. There can be found mostly *E. coli*, *Salmonella* spp., *Shigella* sp., but also *Micrococcus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Klebsiella pneumoniae*, *Neisseria meningitidis* [13]. The main place in the treatment plant, where the contamination is most likely is biological reactor chamber. Aerosols are formed during aeration of waste water. Another place, where aerosols may be formed, is the place where the vacuum trucks deliver wastewater to the treatment plant [18]. Bioaerosols spread for about 800 meters from the source of generation. Spread range is addicted to weather conditions, seasons, landform and size (range) of sewage treatment [9, 19, 20].

The aim of the study was the analysis of wastewater treatment plants operations, presence of *Salmonella* spp. and intestinal parasites eggs. The next step of the study was to investigate microbiological purity of the air near wastewater treatment plants.

## Materials and methods

The five wastewater treatment plants (Szczecno, Stykow, Pinczow, Busko Zdroj, Kazimierza Wielka) in Swietokrzyskie voivodship were analyzed in differend field.

The documentation of the analysis provided by sewage treatment plants located in Pinczow, Busko-Zdroj and Kazimierza Wielka was used as a research material. Analysis of sewage sludge management was based on data from wastewater treatment plant in Pinczow.

The total number of aerobic plate count in the air was measured based on culture settling plate technique. Open Petri dishes with the agar were distributed at the processing areas and exposed for 10 minutes. The Petri dishes were closed and incubated at 37ºC for 24-48 hours. The colony forming units (CFU) of mesophilic bacteria were counted on plates with enriched agar. The colony forming units (CFU) of mannitol-positive *Staphylococcuc* sp. were counted on the Mannitol salt agar. The degree of contamination was determined based on the total number of CFU in 1 m$^3$ of air according to Polish standard PN-89-Z-04111/02 [20]. The microbiological air pollution was carried out on a four points between the sewage treatment plant and water reservoir in Szczecno. The second place of analysis was in the six points differently distanced from the centre of sewage treatment plant in Stykow. The analyses were performed in autumn and winter.

## Results and discussion

The aim of this study was to analyze two important aspects related to the functioning of wastewater treatment plant (WWTP). The investigation of the management and the sanitary condition of sewage sludge in wastewater treatment plants was the first research. In the other hand we have analyzed the microbiological condition of the air around and near the WWTPs. The study covered the period 2008-2012. The capacity of the sewage treatment plant in Pinczow in 2010 and 2011 was about 12000 $m^3$ per day. Wastewater came mainly from private households and blocks of flats, public facilities and also from the industrial sector. Examined sewage sludge in Pinczow took the earthy form. Dry matter content was various. In 2010, the obtained dry matter was 22.5% of sludge, in 2011 - it was 14.7%. The excess sludge was generated in sewage treatment plant in Pinczow in the bioreactors and Imhoff tank. Sludge after stabilization and dehydration should be removed from the area of wastewater treatment [8]. Received data shows that precipitate was used on private lands. In 2010, 1011 mg of sludge was used on the surface of the 4.22 hectares of the soil. The precipitate was used for the reclamation of soils intended for arable land in two villages Pasturka and Szczypiec. In 2011, weight of sewage sludge used for fertilization was lower - 760 mg of sludge was spread on the surface of 25.24 hectares. In 2011, the fertilizer was used in Kije, Brzescie and Pasturka. Within this two years, the proportion between the weight of sewage sludge and the treated area were considerably different. This was mainly due to the doses limitation of sewage sludge, which was distributed on the land. The amount of sludge used for the reclamation depends on the type of soil, method of use, the quality and composition of the used sludge [14]. In 2010, 0.23 mg of the sludge was spread on 1 $m^2$ of soil, but in 2011, quantity of sewage sludge was 0.02 $mg/m^2$.

Subsequently, the provided data of microbiological analysis of sewage sludge of three sewage treatment plants (Pinczow, Busko Zdroj, Kazimierza Wielka) were compared. The aim of the study was to identify the microbial pathogens *Salmonella* spp. and the eggs of intestinal parasites *Ascaris* sp., *Trichuris* sp., *Toxocara* sp. The presence of *Salmonella* spp. was identified only in the one sample, in sewage treatment plant in Pinczow. It should be mentioned that in this WWTP the study was conducted only in 2011. These microorganisms were not detected within five years (2008-2011) in the other treatment plants. The presence of eggs of intestinal parasites was found in Busko Zdroj (two positive samples) and Kaziemierza Wielka (one positive sample). Parasites were not detected in the sewage treatment plant in Pinczow. It can be concluded that examined sewage sludge were no danger and could be used in agriculture and for land reclamation for agricultural purposes, as it was performed in the case of sewage sludge from Pinczow. Used sludge from the treatment plant did not contain elevated levels of heavy metals and microbiological contamination. Therefore, there was no risk to contamination of the environment. Data analysis leads to the conclusion, that the use of sewage sludge to soil remediation in the municipality was relevant. According to the publication, there is no universal solution to the issue of sludge management, but the solution must be appropriate to local conditions. Pinczow is a small town in which the amount of sludge formed in wastewater treatment is not big. There is also the possibility of using sludge for energy recovery by combustion, co-firing and others. Use of components of sewage sludge for soil fertilization is cost-effective. It is also beneficial to soil provided that the levels of contaminants in sludge will be acceptable. Therefore, the use of sludge as fertilizer in this case is appropriate [4, 21]. As previously mentioned, a lot of different bacterial species can be present in the savage sludge. Some of them could can be pathogenic. The use of savage sludge as an organic-mineral fertilizers and organic soil conditioners should be careful. They can be source of bacterial, viral and parasitic diseases in humans and animals [22, 23]. This fact is evidenced by descriptions of epidemics caused by infection of soils in Poland

and Europe. In Darmstadt (Germany), 89% of the population became sick for salmonellosis after eating vegetables from field irrigated with sewage sludge [3]. Also epidemic of typhoid fever was recorded in Stuttgart. During this epidemic, 600 people get sick and 10 people died. The cause of epidemic was lettuce grown on field fertilized with fresh sludge. Similar epidemics have also been reported in France after ingestion of lettuce and watercress from cultivation of contaminated sediments. In Poland, epidemics of salmonellosis and viral hepatitis A in Pisz, Braniewo, Chelm, Ustrzyki Dolne was recorded as a result of water polluted by sewage and sewage sludge. Nevertheless, the use of sewage sludge can be useful as a natural fertilizer for soil, but after a careful examination [3, 24, 25].

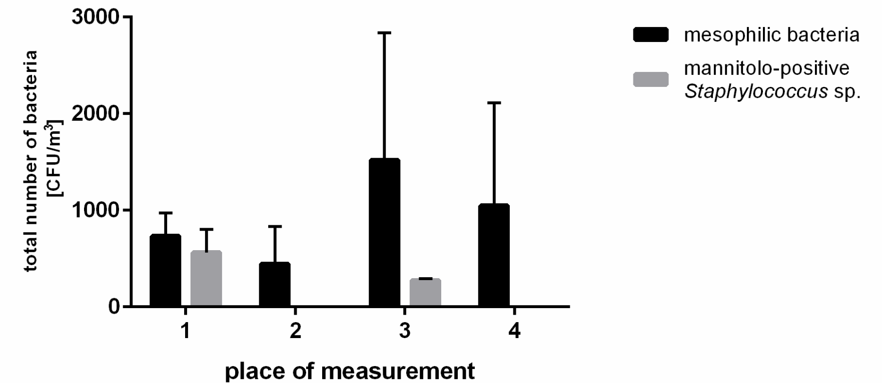

Fig. 1. Total number of bacteria in 1 m$^3$ of air in wastewater treatment plant in Szczecno. Place of measurement: 1 - area of sewage treatment plant, 2 - basin, 3 - flood area and 4 - pond

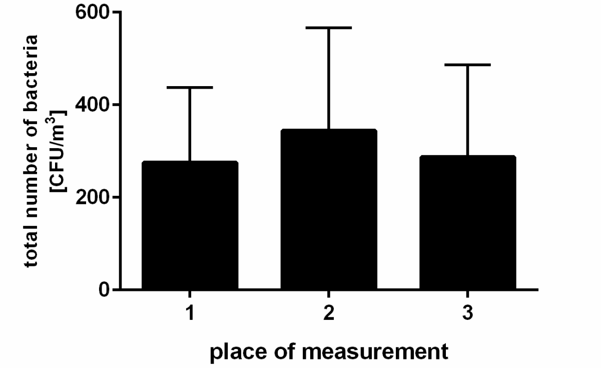

Fig. 2. Total number of bacteria in 1 m$^3$ of air in wastewater treatment plant in Stykow. Place of measurement: 1 - 3 meters from the aeration chambers, 2 - 5 meters from the border of sewage treatment, 3 - 20 meters from the compost flitch and 12 meters from the aeration chambers. All places (8) were symmetrically arranged on East and West side of WWTP

The further research included the microbiological analysis of the air near to wastewater treatment plants in Szczecno and Stykow. Studies have been conducted to determine the total number of bacteria. In the sewage treatment plant in Szczecno also identified the total number of mannitol-positive *Staphylococcus* spp. The WWTP in Szczecno was analyzed in four places: area of sewage treatment plant, basin, flood area and pond. The WWTP in Stykow was analyzed in three places, each from East and West sides first located 3 meters from the aeration chambers, second - 5 meters from the border of sewage treatment and third - 20 meters from the compost flitch and 12 meters from the aeration chambers. It was observed, that the number of bacteria was generally high and varied (Figs. 1 and 2).

The number of bacteria was not dependent on the date of sampling. The highest number of bacteria was detected in the further distance from WWTPs, but the differences wasn't significant. According to standard PN-89-Z-04111/02, in case of WWTPs in Szczecno (Fig. 1) the total number of mesophilic bacteria in the air near to sewage treatment plant and basin was not too high, but high number of *Staphylococcus* spp. in all cases classified air as contaminated. The results of microbiological analysis of the air in WWTP in Stykow were similar to the WWTP in Szczecno (Fig. 2).

It has been shown that the total number of bacteria isolated from the air from each places was acceptable. There was no relationship between the number of microorganisms in the air and the distance from the individual elements of the sewage treatment. However, it was observed the slight increase of the number of bacteria in further distance from the sewage treatment plant. It is known, the number of bacteria in the air may depend on the wind direction, temperature and intensity of operation in sewage treatment plants [15, 25]. Our results indicate high number of bacteria even in long distance from WWTPs. The low temperature of the air also didn't influence on the bacterial transport. The results confirm that the presence of sewage treatment plants can affect the air pollution in the area.

The number of detected bacteria seems to be lower in comparison to studies conducted in other WWTPs [8, 16, 18, 19, 25]. The similar studies were performed in municipal sewage treatment plants located in Kujawsko-Pomorskie. The number of microorganisms isolated from the air was two times higher than in Szczecno and nearly six times higher than in Stykow. These differences may result from the various size of the object and the amount of sewage wastewater flowing into the treatment plant. In Kujawsko-Pomorskie voivodship is about 13 000 $m^3$ of sewage wastewater. On the contrary, in Szczecno and Stykow is about 300 $m^3$ of sewage wastewater [16]. In the sewage treatment plant in Kujawsko-Pomorskie also identified a significant amount of *Pseudomonas fluorescens*, which belongs to the bacteria naturally living in the heavily polluted surface waters and wastewater [2, 16]. In our study, we analyzed only the presence of total number of bacteria and mannitol-positive *Staphylococcus* sp. This group of bacteria is epidemiologically important and it can be a more dangerous source of pathogenic bacteria for human. It is worth noting, that wastewater treatment plants may also affect the sanitary condition of the local rivers. This aspect has not been presented in the work. To fully understanding the influence of sewage treatment plants on the environment, there should be carried out the examination of the waters from local rivers. There is also another aspect of monitoring bacteria in WWTPs. On the basis of reports in the literature, along with the spread of bacteria from sewage treatment the risk of occurrence in environment of strains resistance to antibiotics is higher. To the treatment plant with sewage may get microorganisms which are multidrug-resistant [26, 27]. Tests of air and sewage sludge should be carried out more frequently. Carrying out such monitoring allows for constant control of risks of environmental pollution by treatment plants.

## Conclusions

Analysis of sanitary condition of sewage sludge and the management of sludge in wastewater treatment plant shown, that the use of sewage sludge as fertilizer is a valid option. Properly neutralized and examined sludge may become an important element in the process of land reclamation [21]. Also, based on the obtained results, there is a risk of air pollution in the vicinity of sewage treatment plants.

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## References

[1] Kuśnierz M, Świerczek E. Infrastruktura krytyczna a niezawodność systemu odprowadzania i oczyszczania ścieków w niekorzystnych warunkach pogodowych. (Critical infrastructure and reliability of wastewater drain and wastewater treatment system in adverse weather conditions). Kraków: Polska Akademia Nauk; 2010;1(1):125-135. http://dx.medra.org/10.14597/infraeco.2014.1.1.010.

[2] Salyers AA, Whitt DD. Mikrobiologia Różnorodność, chorobotwórczość i środowisko. (Microbiology. Diversity, Disease and the Environment). Warszawa: Wyd Nauk PWN; 2005.

[3] Kłapeć T, Cholewa A. Zagrożenia dla zdrowia związane ze stosowaniem nawozów organicznych i organiczno-mineralnych. (Health risk asociated with the use of organic and organic-mineral fertilizers). MONZ. 2012;18(2):131-136. http://monz.pl/fulltxt.php?ICID=1003556.

[4] Werle S, Wilk RK. Thermal utilization of sewage sludge. Materiały Pokonferencyjne, III Ogólnopolski Kongres Inżynierii Środowiska. Politechnika Śląska. 2009;1. http://wis.pol.lublin.pl/kongres3/tom1/37.pdf.

[5] Michalska-Szymaszek M. The Influence of temperature on survival, phenotype and antigen properties of *Escherichia coli* O157 strains isolated from water and clinical material. Roczn PZH. 2010;61(2):213-219. http://www.ncbi.nlm.nih.gov/pubmed/20839472.

[6] Szala B, Paluszak Z. Wykorzystanie paciorkowców kałowych w mikrobiologicznej ocenie procesu higienizacji kompostowanych osadów ściekowych. (Application of faecal stretococci in the microbiological assessment of composted sewage sludge sanitization). Water Environ Rural Areas. 2010;10(2):179-188. http://yadda.icm.edu.pl/baztech/element/bwmeta1.element.baztech-article-BATC-0004-0022.

[7] Prażmo Z, Krysińska-Traczyk E, Skórska C, Sitkowska J, Cholewa G, Dutkiewicz J. Exposure to Bioaerosols in a municipal sewage treatment plant. Ann Agric Environ Med. 2003;10:241-248. http://www.aaem.pl/pdf/10241.pdf.

[8] Okoh AI, Odjadjare EE, Igbinosa EO, Osode AN. Wastewater treatment plants as a source of microbial pathogens in receiving watersheds. Res J Chem Environ Sci. 2014;2(6):11-19. http://www.aelsindia.com/rjcesdecember2014/3.pdf.

[9] Kołwzan B, Jadczyk P, Pasternak G, Głuszczak J, Pawlik M, Krawczyńska M. et al. Ocena stanu sanitarnego powietrza w otoczeniu wybranej oczyszczalni ścieków. (Assessing air quality in the proximity of a municipal sewage treatment plant: A case study). Ochr Środ Wrocław. 2012;34(2):9-14. http://www.os.not.pl/docs/czasopismo/2012/2-2012/Kolwzan_2-2012.pdf.

[10] Rastetter N, Gerhardt A. Toxic potential of different types of sewage sludge as fertiliser in agriculture: ecotoxicological effects on aquatic and soil indicator species. J Soils Sedim. 2015;15:565-577. DOI 10.1007/s11368-014-1031-0.

[11] Heeb A, Lundegardh B, Ericsson T, Savage PG. Effects of nitrate-, ammonium-,and organic-nitrogen-based fertilizers on growth and yield of tomatoes. J Plant Nutr Soil Sci. 2005;168:123-129. DOI: 10.1002/jpln.200420420.

[12] Nowak M, Kacprzak M, Grobelak A. Osady ściekowe jako substytut glebowy w procesie remediacji i rekultywacji terenów skażonych metalami ciężkimi. (Sewage sludge as a substitute of soil in the process of remediation and reclamation of sites contaminated with heavy metals). Inż Ochr Środow. 2010;13(2):121-131. http://yadda.icm.edu.pl/yadda/element/bwmeta1.element.baztech-article-LOD7-0025-0023/c/Nowak_Kacprzak_Osady_2_2010.pdf.

[13] Gołofit-Szymczak M, Zapór L. Zagrożenia biologiczne w oczyszczalniach ścieków komunalnych. (Biological hazards in municipal wastewater treatment plants) Bezpieczeństwo Pracy. Centralny Instytut Ochrony Pracy - Państw Inst Bad. 2007:3. http://yadda.icm.edu.pl/baztech/element/bwmeta1.element.baztech-article-BPC1-0008-0063/c/Golofit-Szymczak.pdf.

[14] Papciak D, Zamorska J. Podstawy biologii i biotechnologii środowiskowej. (Basics of Biology and Environmental Biotechnology). Rzeszów: Ofic Wyd Polit Rzeszowskiej; 2005.

[15] Filipkowska Z, Janczukowicz W, Krzemieniewski M, Pesta J. Microbiological air pollution in the surroundings of wastewater treatment plant with activated-sludge tanks aerated by horizontal rotors. Pol J Environ Stud. 2000;9(4):273-280. http://www.pjoes.com/pdf/9.4/273-280.pdf.

[16] Budzińska K, Jurek A, Szejnuk B, Michalska M, Wroński G. Mikrobiologiczne zanieczyszczenie powietrza na terenie oczyszczalni ścieków komunalnych. (Microbiological air pollution in the area of municipal sewage treatment plant.). Rocz Ochr Środ. 2011:13. http://old.ros.edu.pl/text/pp_2011_098.pdf.

[17] Filipkowska Z, Janczukowicz W, Krzemieniewski M, Pesta J. Municipal wastewater treatment plant with activated sludge tanks aerated by CELPOX devices as a source of microbiological pollution of the atmosphere. Pol J Environ Stud. 2002;11(6):639-648. https://www.infona.pl/resource/bwmeta1.element.agro-article-f540b4ab-ad67-4103-9fee-5d9d2f14a77b.

[18] Breza-Boruta B. Ocena mikrobiologicznego zanieczyszczenia powietrza na terenie oczyszczalni ścieków. (An assessment of microbiological air pollution at the sewage treatment plant). Woda Środ Obsz Wiej. 2010;10(3):49-57. http://yadda.icm.edu.pl/baztech/element/bwmeta1.element.baztech-article-BATC-0004-0030.

[19] Cyprowski M, Krajewski JA. Czynniki szkodliwe dla zdrowia występujące w oczyszczalniach ścieków komunalnych. (Harmful agents in municipal wastewater treatment plants). Med Pr. 2003;54(1). http://test.imp.lodz.pl/upload/kasia/czy_szkod_kom.pdf.

[20] Norma (Polish Norm) PN-84/Z-04008/02, Ochrona czystości powietrza. Pobieranie prób. (Air purity protection. Sampling), PN-89/Z-04008/08. Ochrona czystości powietrza. Badania mikrobiologiczne. (Air purity protection. Microbiological studies).

[21] Lundin M, Olofsson M, Petterson GJ, Zetterlund H. Environmental and economic assessment of sewage sludge handling options. Resour Conserv Rec. 2004;41:255-278. DOI: 10.1016/j.resconrec.2003.10.006.

[22] Andrés P. Ecological risk of the use of sewage sludge as fertilizer in soil restoration: effects on the soil microarthropod populations. Land Degrad Dev. 1999;10:67-77. DOI: 10.1002/(SICI)1099-145X(199901/02)10:1<67::AID-LDR322>3.0.CO;2-H.

[23] Sahlström L, Aspan A, Bagge E, Denielsson-Tham ML, Albihn A. Bacterial pathogen incidents in sludge from Swedish sewage treatment plants. Water Res. 2004;38:1989-1994. DOI:10.1016/j.watres.2004.01.031.

[24] Dumonet S, Scopa A, Kerje S, Krovacek K. The importance of pathogenic organisms in sewage and sewage sludge. J Air Waste Manage. 2001;51(6):848-860. DOI: 10.1080/10473289.2001.10464313.

[25] Niazi S, Hassanvand MS, Mahvi A. Assessment of bioaerosol contamination (bacteria and fungi) in the largest urban wastewater treatment plant in the Middle East. Environ Sci Pollut Res Int. 2015;22(20):16014-21. DOI: 10.1007/s11356-015-4793-z.

[26] Li J, Zhou L, Zhang X, Xu C, Dong L, Yao M. Bioareosol emission and detection of airborne antibiotic resistance genes from a wastewater treatment plant. Atmos Environ. 2015;124(B):404-412. DOI:10.1016/j.atmosenv.2015.06.030.

[27] Huang JJ, Hu HY, Lu SQ, Li Y, Tang F, Lu Y, et al. Monitoring and evaluation of antibiotic-resistant bacteria at municipal wastewater treatment plant in China. Environ Int. 2012;42:31-36. DOI: 10.1016/j.envint.2011.03.001.

# WPŁYW OCZYSZCZALNI ŚCIEKÓW NA OKOLICZNE ŚRODOWISKO

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**Abstrakt:** Celem pracy była analiza działalności trzech oczyszczalni ścieków (Busko Zdrój, Pińczów i Kazimierza Wielka) w województwie świętokrzyskim. Materiały źródłowe zostały udostępnione dzięki uprzejmości oczyszczalni ścieków. Analizowano sposób zagospodarowania i stan sanitarny osadów ściekowych. Badania mikrobiologiczne osadu dotyczyły podstawowej analizy sanitarnej, zwłaszcza identyfikacji bakterii *Salmonella* spp. i jaj pasożytów jelitowych. Stwierdzono, że stan sanitarny analizowanych osadów ściekowych nie zagrażał środowisku. Mikrobiologiczna czystość osadów ściekowych i skład mineralny były zbliżone do materii organicznej gleby (humus) i pozwalały na wykorzystanie ich jako nawozów naturalnych, ale nie do uprawy roślin przeznaczonych do spożycia przez ludzi. Materiał ten był szeroko stosowany do rekultywacji gruntów w Pińczowie. Kolejnym celem pracy było określenie czystości mikrobiologicznej powietrza w pobliżu dwóch oczyszczalni ścieków (Szczecno i Styków w województwie świętokrzyskim). Określano ogólną liczbę gronkowców mannitolo-dodatnich oraz bakterii mezofilnych i psychrofilnych w czterech różnych odległościach od oczyszczalni ścieków. Mannitolo-dodatnie gronkowce należą do wskaźników mikrobiologicznego zanieczyszczenia powietrza. Liczba bakterii mezofilnych i psychrofilnych wskazuje na poziom zanieczyszczenia materią organiczną. We wszystkich punktach pomiarowych zaobserwowano zanieczyszczenie powietrza. Wiadomo, że patogenne mikroorganizmy mogą łatwo przedostawać się z cieczy do powietrza za pomocą bioareozoli. Analiza ta pokazuje, że wykorzystanie osadów ściekowych do melioracji jest pomocne do zachowania i przywrócenia równowagi ekologicznej, co jest ważnym aspektem ochrony ekonomicznej i środowiskowej. Jest również prawdopodobne, że oczyszczalnie ścieków mogą mieć niekorzystny wpływ na czystość powietrza.

**Słowa kluczowe:** osad ściekowy, zanieczyszczenie środowiska, zanieczyszczenie powietrza, nawozy

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# THE IMPACT OF URBAN ENVIRONMENT ON THE PHENOLOGICAL PHASES OF CRIMEAN LINDEN (*Tilia* 'Euchlora')

## WPŁYW ŚRODOWISKA MIEJSKIEGO NA PRZEBIEG FAZ FENOLOGICZNYCH LIP KRYMSKICH (*Tilia* 'Euchlora')

**Abstract:** Trees growing in urban areas are constantly exposed to the adverse conditions of the urban environment. The effect of increasing in concentration of pollutants is observed by systematic reduction in the number of trees in the streets and their poor health status, whose symptoms are discoloration and leaf necrosis occurring at the turn of May and June, and accelerated leaf fall. The subject of the study was the Crimean linden trees growing along the middle strip of Zwirki and Wigury Avenue. Control trees were growing in the park at the Cemetery of Soviet Soldiers. Phenological observations (concerning the development of the leaves) were carried out in years 2010-2011. Crimean linden trees are particularly sensitive to soil salinity. Phenological studies were complemented by visual assessment of the health status of trees. For this purpose, the degree of leaf damage was determined based on the six level scale, where "0" meant the healthy tree (no visible damage to the leaf blade). In 2011 from 128 trees - 83 trees rated as "healthy" (0-1 leaf damage index), 34 as "relatively healthy" (index of leaf damage 2-3) and 11 as "sick" (index of leaf damage 4-5). In the previous year 136 trees were observed: 60 rated as "healthy", 56 as "relatively healthy" and 20 trees were classified as "sick". For most of the surveyed trees of their health condition slightly improved. It was observed that with increasing degree of leaf damage slightly shortened the period of activity of the tested tree vegetation. There were no significant differences between the studied street trees in leaf development. Trees from the control area characterized by a longer growing season than street-trees.

**Keywords:** Crimean linden, urban trees, city environment, phenological stages

The city is a specific natural environment, transformed for the benefit of the dominance of technical elements. In the cities climatic, soil and water conditions affected by human activities have a significant impact on vegetation occurring there [1, 2]. Street trees planted in cities are exposed to unfavorable conditions for their growth and development. The factors limiting the growth and development of vegetation in cities are primarily: higher temperatures, low humidity, nutrients deficiency in the soil, high pH of the soil, limited soil volume, vandalism, mechanical damage and lack of maintenance [3-6]. The use of sodium chloride to deicing the roads in wintertime, as well as water deficiency result in a worsening condition of street trees and their withering away on a big scale [7-9]. An important factor is also the occurrence of pests with sucking mouthparts - mainly aphids and *tetranychus* [10].

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The course of the various phases of development (phenological phases) in urban trees is markedly different than in trees growing outside the cities [11]. Many studies have demonstrated that trees growing in extremely unfavorable conditions, *eg* street trees, have a much shorter growing season and less growth than their counterparts growing in park [1, 12-14]. Urban trees vegetation begin and comes to the end earlier [15]. Trees growing in the harsh urban conditions earlier reach maturity and faster get older. During the aging of the trees lose their adaptability to changing environmental conditions [1, 16, 17]. Also street trees have a much higher mortality rates than park trees [18-21]. The most vulnerable to the adverse effects of environmental stress are young trees. Life expectancy newly planted tree in urban conditions is no longer than 5 years [1]. Miller and Miller [22] found that the highest mortality is characterized by the newly planted trees and young (4-5 cm trunk circumference), within a year or two years after planting. The literature suggests that the life expectancy of trees in urban environment may be only 10 years [23, 24]. According to a study by Foster and Blaine [23] the life expectancy for linden is about 23 years.

The response of trees to stress factors can manifest itself in different ways, depending on their individual level of sensitivity and intensity, time and type of harmful effects. The reactions of trees to natural stress factors and those of anthropogenic origin are complicated and often lead to trees exclusion from many parts of urban areas [9,-10]. For many years we have seen a reduction of the number of trees in urban areas due to the progressive weakening of viability [20, 25]. This process mainly effects of street trees [26]. It is estimated that in 1971, more than 700,000 trees in Eastern Europe died [27]. In Liverpool from downtown trees planted in recent years, 39% stopped within five years [4]. Studies conducted in Warszawa by Dmuchowski et al [8] showed that within 34 years, more than half (59%) of trees along the main streets of the city center died. The greatest losses related species of trees: *Sorbus aucuparia* L. (94%), *Acer pseudoplatanus* L. (83%), *Tilia cordata* L. (65%) *Tilia* 'Euchlora' (62%). While the smallest losses showed *Tilia platyphyllos* L. (44%).

The aim of this research was the determination of the influence of urban environment on the health status of street trees - *Tilia* ‘Euchlora’and their phenological development.

## Materials and methods

The study was performed on trees belonging to one species - the Crimean linden (*Tilia* ‘Euchlora’), a sterile hybrid lime between *Tilia cordata* Mill. and *Tilia dasystyla* Steven. The species is known since the mid-nineteenth century and grown in many countries in Central and Western Europe. From the interwar period to the 70s of the last century it was often used for lining the roads, avenues, wide streets, as well as parks [28]. Currently none of street tree selection does not recommend the use of the Crimean limes for growing along the streets, due to the high sensitivity of this species to soil salinity.

The objects of research were planted in the middle strip of Zwirki and Wigury Avenue in Warszawa. Control areas was park in Cemetery of Soviet Soldiers located at the distance of 150 m from studied avenue. The leaves condition was evaluated in mid-July and mid-September using the six grade scale method of direct observation. In 2010 studies were carried out on 136 trees and in 2011 it was 128 trees. Reducing the number of trees bring about their dieback.

Phenological observation method was based on the recording date of the beginning of beginning and duration of each phenological phases. In phenological studies we used a modified summary of the descriptions offered by many authors. Phenological observations were carried out from the beginning of vegetation until its completion. Due to the different intensity of trees development processes in urban areas studies were carried out at intervals of every 2-3 days throughout the growing season.

The various development stages of leaves were described in accordance with the following principles:

- *leaf buds start to open* - visible becomes green part of the leaf on the top of bud scale;
- *the beginning of leafage* - after mining out of the bud first leaves open and both halves of the upper surfaces of the leaf blade become visible;
- *the beginning of the staining of leaves* - 10% of the leaves are discolored;
- *the beginning of the leaves fall*- the tree dropped at least 10% of the leaves;
- *the end of the leaves fall* -all the leaves have fallen.

In order to compare the average length of the individual phases of phenological stages under different health conditions, univariate analysis of variance was applied. Multiple comparisons were made using the Tukey procedure. On the basis of the analysis mentioned above the groups of means were separated. For the analysis the significance level was assumed at $\alpha = 0.05$ [29].

## Results and discussion

The health condition of trees from middle strip of Zwirki and Wigury Avenue (assessed by observing the degree of leaf damage) showed a large variation. The studies were conducted on more than 120 trees of Crimean linden in years 2010-2011. Despite the fact that the trees were growing in the same conditions (climate and habitat) and in the same distance from the roadway diverse response to environmental pressures has been observed. The health status of the surveyed trees, in the period from July to September, steadily deteriorated. In 2011 from 128 trees 83 trees were rated as “healthy” (0-1 leaf damage index), 34 as “relatively healthy” (index of leaf damage 2-3) and 11 as “sick” (index of leaf damage 4, trees with index of leaf damage noted as 5 - removed). In the previous year 136 trees were observed: 60 rated as “healthy”, 56 as “relatively healthy” and 20 trees were classified as “sick”. Increased leaf surface with characteristic necrosis were observed. Many studies have shown that main cause of leaf damage of urban trees is the adverse effects of the salt used for de-icing the streets in winter [4, 7-11]. The presence of chlorine in the leaves results in the initially invisible changes inside the plant cells, and with the increasing amounts of this ion, in morphological changes such as chlorosis visible on the leaf lamina. Research conducted in Warszawa on Crimean linden trees confirmed that even minor salinity of the soil can cause necrosis on the edges of leaves, and result in rapid withering and death of trees [7, 8, 10, 30].

Studies conducted in Warszawa by Dmuchowski et al [8, 9, 31] and Borowski [32] have shown a relationship between the degree of withering, and the conditions for growth. Relevant factors include: the type of land cover (lawn, paving, asphalt), the size of uncovered area under the tree and the distance from the roadway. Street trees growing on the wide lawns are characterized by much better state of health than those growing on narrow lawns [5, 31-33]. In July the health status of studies trees was relatively better than the health status of trees growing on other streets in the center of Warszawa [8, 31]. This may be caused by relatively better growing conditions of the Zwirki and Wigury Avenue - a wide lawn of the Avenue creates more favorable conditions for trees development than concret surfaces or narrow lawns, predominant along the street Warszawa. Changes on the leaves manifested by chlorotic and necrotic edges. Deformities and dieback part of the leaf blade was observed in early July. The comparison of tree health status recorded in July and in September (classifying trees into specific categories) revealed a general trend of significant decline in the percentage of trees categorized as 0 and 1 (leaves healthy or with minor damage) and increased share of trees in categories 3, 4, 5 with the duration of the growing season. This trend was confirmed by observations made on different species of trees [8, 9, 30]. Not only does it contribute to the deterioration of decorative qualities of the trees, but also, which is more important, it obstructs their biological functions [1].

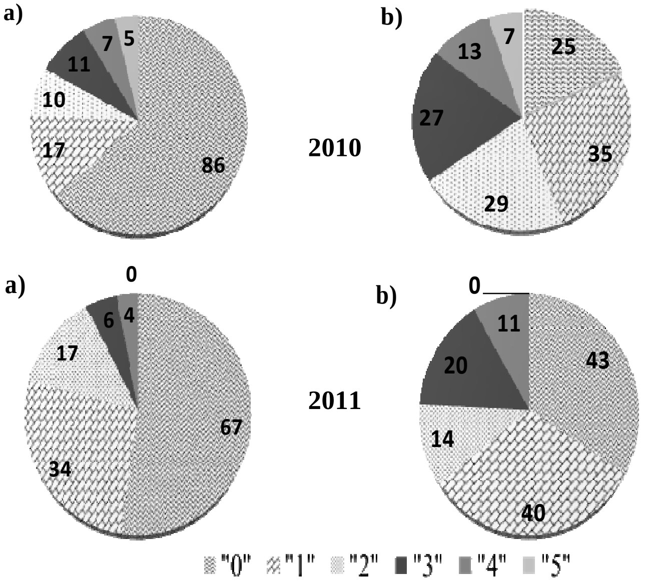

Fig. 1. The number of trees from individual health status categories based on the index of damage of the leaf blade, in two periods: a) July and b) September

Comparison of leaf state within two years of research (2010-2011) showed that in 2011 the trees were characterized by a relatively better condition both in July and in September. Probably the main reason for better state of health of studied trees were more favorable weather conditions observed in this year of the study. Summer in 2011 was rich in precipitation, while winter at the turn of 2010/2011 was characterized by a large snowfall and low temperatures. No damage was observed on the leaves of the trees from the control area (park at the Cemetery of Soviet Soldiers) throughout the growing season.

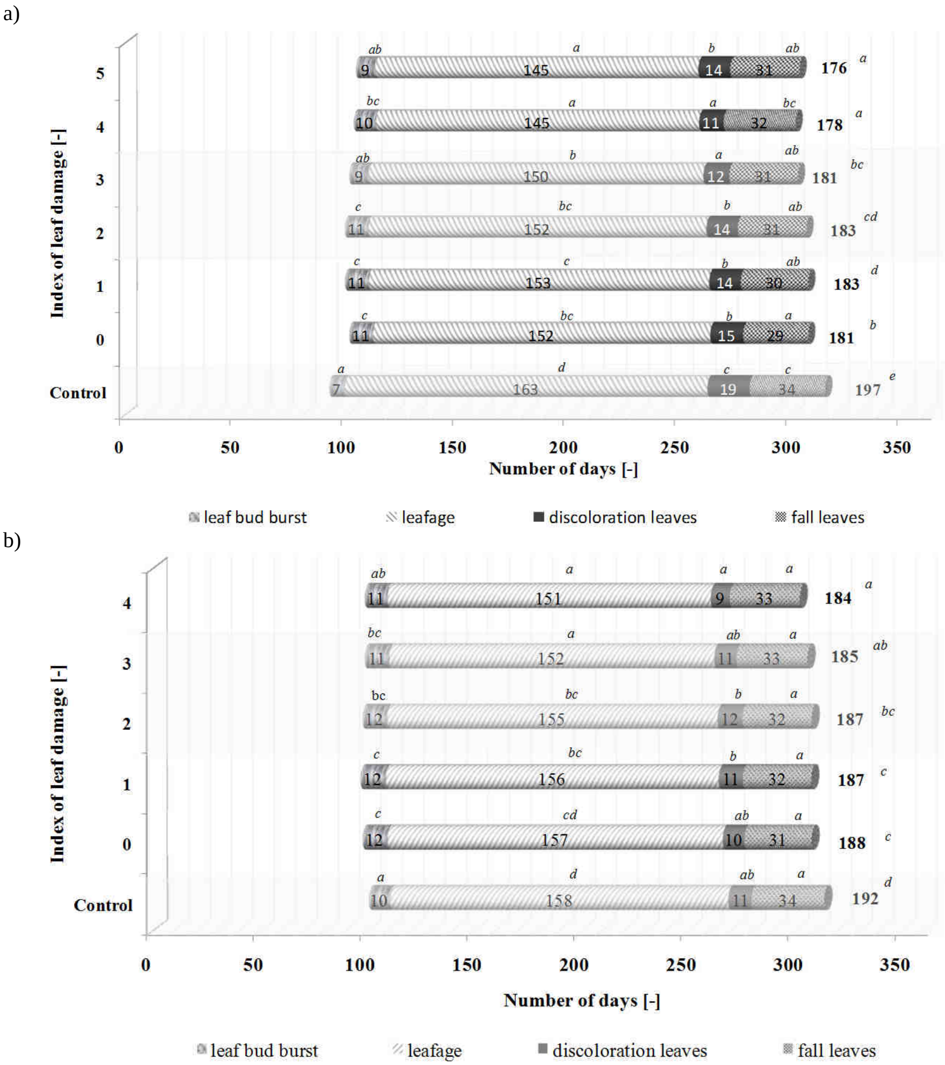

Fig. 2. The duration of the phases of development leaves of Crimean linden in: a) 2010 and b) 2011

On the basis of phenological studies we found slight differences in the time and duration of observed phases of the leaves of the trees with different health status. The phenological studies were conducted only in 2010 and 2011. This period was too short to draw definite conclusions concerning impact of the urban environment on the course of phenological phases of trees. This will be possible only after a longer period, at least a few years, of systematic research and comparison of the results with the thermal conditions prevailing during their pursuit.

Table 1

Weather conditions during the phenological observations

| **Year** | **The average monthly temperature [°C]**[*] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **I** | **II** | **III** | **IV** | **V** | **VI** | **VII** | **VIII** | **IX** | **X** | **XI** | **XII** |
| **2010** | –8.0 | –1.9 | 3.9 | 9.5 | 13.6 | 17.8 | 21.9 | 19.7 | 12.4 | 6.1 | 5.8 | –5.4 |
| **2011** | –0.6 | –3.8 | 3,3 | 11.1 | 14.4 | 19.0 | 18.1 | 18.9 | 15.1 | 8.5 | 3.0 | 2,6 |

| **Year** | **The average monthly rainfall [mm]**[*] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **I** | **II** | **III** | **IV** | **V** | **VI** | **VII** | **VIII** | **IX** | **X** | **XI** | **XII** |
| **2010** | 25 | 37 | 24 | 39 | 116 | 87 | 92 | 143 | 89 | 3 | 109 | 34 |
| **2011** | 39 | 21 | 8 | 34 | 48 | 49 | 295 | 62 | 7 | 9 | 0 | 32 |

[*] Data derived from statistical yearbook of Warszawa (2010-2012). The measurements refer to the meteorological station Warszawa - Okecie, located approximately 3 km from the surveyed trees

The length of the vegetation activity best differentiates the influence of weather - climatic conditions and habitat on urban greenery. Street trees were characterized by a shorter growing season than trees growing in control area. The length of the active period of the vegetation shortened due to degree of leaf damage of studied trees. The biggest difference concerned the trees with no apparent damage of leaves and those with the highest degree of damage (an average of 5-9 days, depending on the year of observation). There was also interference in the course of individual phenological phases. In the case of discoloration phase, there were no clear and reproducible differences in both the date and duration of this phase. Similar results were obtained among others trees [12-14].

An interesting observation related to the control area in which the phase of leaf fall lasted longer than in street trees, regardless of the degree of damage to their leaves. This can be explained based on the specific light conditions, namely: a relatively large park trees shading the leaves that may have contributed to the earlier cut-off zone produce leaves. Low light intensity or change in intensity from high to low may cause premature cut off the leaves, buds and fruits [34]. Trees growing in the vicinity of lights, much later discolor leaves than the rest on the same tree (which are not exposed to direct light).

## Conclusions

1. There was a large variation in health condition of studied street trees. In July the trees showed less damage than in September. Share of trees with average and considerable damage of leaves between July and September has almost doubled.
2. The deterioration of the health condition of trees proceedings in the course of the growing season was observed. The influence of the state of health of trees on the length of the growing season has been shown- with the increase in the degree of damage of trees, vegetation activity period was shortened by a few days (5-9 days, depending on the year of observation).
3. No significant differences were observed in the duration of the various phases of development leaves. Significant differences found only between street trees and control group.

## References

[1] Szczepanowska H. Drzewa w mieście (Trees in the city). Warszawa: Hortpress Sp. z.o.o.; 2001.

[2] Wysocki C. Miasto jako specyficzne środowisko życia roślinności. NPT. 2008;2(4):1-10. http://www.npt.up-poznan.net/pub/art_2_25.pdf.

[3] Beatty R, Heckman CT. Survey of urban tree programs in the United States. Urb Ecol. 1981;5:81-102. DOI: 10.1016/0304-4009(81)90002-4.

[4] Pauleit S, Jones N, Garcia-Martin G, Garcia-Valdecantos JL, Riviére LM, Vidal-Beaudet L, et al. Tree establishment practice in cities and towns - results from a European survey. Urb Forestry & Urb Gr. 2002;5(3):111-120. DOI: 10.1078/1618-8667-00009.

[5] Sieghardt M, Muesch-Radlgruber E, Paoletti E, Couenberg E, Dimitrakopoulus A, Rego F, et al. The abiotic urban environment: impact of urban growing conditions on urban vegetation. In: Urban Forests and Trees. Konijnendijk C, Nilsson K, Randrup TB, Schipperijn J, editors. Berlin: Springer; 2005.

[6] Sjöman H, Nielsen AB. Selecting trees for urban paved sites in Scandinavia - A review of information on stress tolerance and its relation to the requirements of tree planners. Urb Forestry & Urb Gr. 2010;9:281-293. DOI: 10.1016/j.ufug.2010.04.001.

[7] Baczewska AH, Dmuchowski W, Jozwiak A, Gozdowski D, Brągoszewska P, Dąbrowski P, et al. Effect of salt stress on prenol lipids in the leaves of Tilia 'Euchlora'. Dendrobiol. 2014;72:177-186. DOI: org/10.12657/denbio.072.015.

[8] Dmuchowski W, Baczewska AH, Gozdowski D. Reaction of street trees on the condition in Warsaw. Ecol Quest. 2011;15:97-105. DOI: 10.2478/v10090-011-0041-4.

[9] Dmuchowski W, Brogowski Z, Baczewska AH. Evaluation of vigour and health of 'street' trees using foliar ionic status. Pol J Environ Stud. 2011;20(2):489-496. http://www.pjoes.com/pdf/20.2/Pol.J.Environ.Stud.Vol.20.No.2.489-496.pdf.

[10] Baczewska AH, Dmuchowski W, Gozdowski D, Styczek M, Brągoszewska P. The influence of saline stress on the abundance of lime aphid (Eucallipterus tiliae L.) on the leaves of street trees - Crimean linden. Proc ECOpole. 2011;5(1):13-19. http://tchie.uni.opole.pl/PECO11_1/EN/BaczewskaDmuchowski_PECO11_1.pdf.

[11] Min MB. Comparison of phenological characteristics for several woody plants in urban climates. J Plant Biol. 2000;43(1):10-17. DOI: 10.1007/bf03031030.

[12] Supłat S. Straty wśród drzew ulicznych w Warszawie. Ogrodnictwo. 1989;7:9.

[13] Sæbø A, Benedikzt T, Randrup TB. Selection of trees for urban forestry in the Nordic countries. Urb for Urb Gr. 2003; 2:101-114. DOI: 10.1078/1618-8667-00027.

[14] Oleksyn J, Kloeppel BD, Łukasiewicz S, Karolewski P, Reich PB. Ecophysiology of horse chestnut (Aesculus hippocastanum L.) in degraded and restored urban sites. Pol J Ecol. 2007;55(2):245-260. http://www.srs.fs.usda.gov/pubs/ja/ja_oleksyn001.pdf.

[15] Borowski J, Latocha P. Dobór drzew i krzewów do warunków przyulicznych Warszawy i miast centralnej Polski. Rocz Dendrol. 2006; 54:83-93. http://info.botany.pl/Roczn_Dendrol/artykuly/vol54/borowski.pdf.

[16] Clark JMN. Management of mature trees. J Arboricult. 1991;17(7):173-184. http://joa.isa-arbor.com/request.asp?JournalID=1&ArticleID=2441&Type=2.

[17] Norris M. Managing and assessing aging urban trees. Trees. 2003;2004:2004b. http://treenetmedia.com/up/pdf/2005/05TS%20MANAGING%20AND%20ASSESSING%20AGING%20URBAN%20TREES_Martin%20Norris.pdf.

[18] Gilbertson P, Bradshaw AD. Tree survival in cities: the extent and nature of the problem. Arboric J: Inter J Urb Forestry. 1985;9(2):131-142. DOI: 10.1080/03071375.1985.9746706.

[19] Smith WH. Air Pollution and Forest. New York - Heidelberg - Berlin: Springer Verlag; 1988.

[20] Hauer RJ, Miller RW., Ouimet DM. Street tree decline and construction damage. J Arboric. 1994;20:94-97. http://www.uwsp.edu/forestry/Documents/hauer/Publications/Street%20Tree%20Decline%20and%20Construction%20Damage.pdf.

[21] Roman LA, Scatena FN. Street tree survival rates: Meta-analysis of previous studies and application to a field survey in Philadelphia, PA, USA. Urban Forestry Urban Greening. 2011;10:269-274. DOI: 10.1016/j.ufug.2011.05.008.

[22] Miller RH, Miller RW. Planting survival of selected street trees Taxa. J Arboric. 1991;17:185-191. http://joa.isa-arbor.com/request.asp?JournalID=1&ArticleID=2442&Type=2.

[23] Foster R, Blaine J. Urban trees survival: Trees in the side-walk. J Arboric. 1978;4:14-17.

[24] Morse SC. Trees in the town environment. J Arboric. 1978;4:1-6. http://joa.isa-arbor.com/request.asp?JournalID=1&ArticleID=1483&Type=2.

[25] Nowak DJ, Kuroda M, Crane DE. Tree mortality rates and tree population projections in Baltimore, Maryland, USA. Urb Forestry Urb Gr. 2004;2:139-147. DOI: 10.1078/1618-8667-00030.

[26] Smidt S. Schadstoffe und Nährstoffe in Blattorbanen, Natürliche Gehalte und Grenzwerte. Institut für Immissionsforschung und Forstchemie. Interner Bericht. 1988;3:1-18.

[27] Flückiger W, Braun S. Perspectives of reducing the deleterious effects of de-icing salt upon vegetation. Plant Soil. 1981;63:527-529. DOI: 10.1007/BF02370056.

[28] Dolatowski J, Seneta W. Dendrologia. Warszawa: Wyd Nauk PWN; 2008.

[29] Sokal RR, Rohlf FJ. Biometry: the principles and practice of statistics in biological research. New York: W.H. Freeman and Company; 1995.

[30] Breś W. Czynniki antropopresji powodujące zamieranie drzew w krajobrazie miejskim. NPT. 2008;2(4):1-8. http://www.npt.up-poznan.net/pub/art_2_31.pdf.

[31] Dmuchowski W, Badurek M. Chloride and sodium in the leaves of urban trees in Warsaw in connection to their health condition. Chem Inż Ekol. 2004;11(4-5):297-303.

[32] Borowski J. Wzrost rodzimych gatunków drzew przy ulicach Warszawy (rozprawa habilitacyjna). Warszawa: Szkoła Główna Gospodarstwa Wiejskiego; 2008.

[33] Quigley MF. Street trees and rural conspecifics: Will long-lived trees reach full size in urban conditions? Urb Ecosystems. 2004;7(1):29-39. http://www.ncufc.org/uploads/Lifespanoftreesinurbanenvironments.pdf.

[34] Rosenthal SI, Camm EL. Effects of air temperature, photoperiod and leaf age on foliar senescence of western larch (Larix occidentalis Nutt.) in environmentally controlled chambers. Plant Cell Environ. 1996;19:1057-1065. DOI: 10.1111/j.1365-3040.1996.tb00212.x.

# WPŁYW ŚRODOWISKA MIEJSKIEGO NA PRZEBIEG FAZ FENOLOGICZNYCH LIP KRYMSKICH (*Tilia* ‘Euchlora’)

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**Abstrakt:** Drzewa rosnące na terenach miast są narażone na nieustanne oddziaływanie zespołu niekorzystnych warunków środowiska miejskiego. W wyniku wzrostu stężenia zanieczyszczeń obserwowane jest systematyczne zmniejszanie się liczby drzew rosnących przy ulicach oraz ich zły stan zdrowotny, którego objawem są między innymi przebarwienia i nekrozy liści, występujące już na przełomie maja i czerwca, oraz wcześniejsze opadanie liści. Przedmiotem przeprowadzonych badań były drzewa lipy krymskiej rosnące wzdłuż pasa międzyjezdniowego al. Żwirki i Wigury. Kontrole stanowiły drzewa rosnące w parku przy cmentarzu Żołnierzy Radzieckich. Obserwacje fenologiczne dotyczące rozwoju liści prowadzono w latach 2010-2011. Lipy krymskie są szczególnie wrażliwe na zasolenie gleb. Badania fenologiczne uzupełniono o wizualną ocenę stanu zdrowotnego drzew. W tym celu określano stopień uszkodzenia liści na podstawie sześciostopniowej skali, gdzie „0” oznaczało drzewo zdrowe (brak widocznych uszkodzeń blaszki liściowej). W 2011 r. na 128 drzew rosnących w pasie międzyjezdniowym al. Żwirki i Wigury (od ul. Banacha do wiaduktu kolejowego) 83 drzewa oceniono jako „zdrowe” (indeks uszkodzenia liści 0-1), 34 jako „względnie zdrowe” (indeks uszkodzenia liści 2-3) oraz 11 jako „chore” (indeks uszkodzenia liści 4-5). W poprzednim roku obserwacji na 136 obserwowanych drzew 60 oceniono jako „zdrowe”, 56 jako „względnie zdrowe” oraz 20 drzew sklasyfikowano jako „chore”. W przypadku większości badanych drzew ich stan zdrowotny poprawił się nieznacznie. Zaobserwowano, że wraz ze wzrostem stopnia uszkodzenia liści nieznacznie skracał się okres aktywności wegetacyjnej badanych drzew. Drzewa z terenu kontrolnego charakteryzowały się dłuższym okresem wegetacji niż przyuliczne. Nie stwierdzono istotnych różnic między badanymi drzewami w rozwoju liści.

**Słowa kluczowe:** lipa krymska, drzewa uliczne, środowisko miejskie, fazy fenologiczne

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# CONTROLLED SYNTHESIS OF IRON OXIDE NANOPARTICLES USED AS AN EFFICIENT HEAVY METAL IONS ADSORBENT

## KONTROLOWANA SYNTEZA NANOCZĄSTEK TLENKÓW ŻELAZA STOSOWANYCH JAKO EFEKTYWNY ADSORBENT JONÓW METALI CIĘŻKICH

**Abstract:** In this paper a few attempts of iron oxide nanoparticles synthesis via chemical co-precipitation are presented. Iron(II) and (III) salts were used as a precursors and aqueous ammonia as a precipitation medium. In the literature it was proved that there are some factors, like reaction temperature, base amount (pH of the reaction mixture) or the concentration of iron salts, which affects characteristics of formed nanoparticles such as their size distribution. The aim of presented research was to select such reaction parameters which would lead to the production of the most effective sorbent for few heavy metal ions, such as: Cr(VI), Pb(II), Cr(III), Cu(II), Zn(II), Ni(II) and Cd(II). The synthesis of nanoparticles were carried out for three temperatures (30, 60, 90ºC) and three ammonia volumes (8, 10, 15 cm$^3$) for each temperature. Furthermore the influence of iron salts molar ratio Fe(II) : Fe(III) in the reaction mixture on resulting sorbent was examined. Because the syntheses were lead in the oxidizing environment, in order to compensate partial oxidation of Fe(II) to Fe(III), a few molar ratios Fe(II) : Fe(III) beyond the stoichiometric value (which is 1 : 2 for magnetite $Fe_3O_4$) were investigated. Additionally for the magnetite synthesized in the selected conditions the effect of pH on the sorption of heavy metal ions were examined. The pH conditions were estimated to not exceed 7 in order to avoid metal precipitation.

**Keywords:** adsorption, heavy metals, magnetite, nanoparticles

## Introduction

Heavy metals are used by human beings from thousands of years. Despite the awareness about their toxicity and negative impact on the living organisms the exposure to heavy metals is still significant, especially in the developing countries.

Most of heavy metals in the environment have industrial origin. The serious environmental problems constitute the industrial plants which discharge effluents containing such heavy metals as Cd, Cr, Cu, Ni, Pb or Zn. These heavy metals tend to accumulate in the living organisms, causing many health disorders like organs and nervous system damages, tumours, reduced growth and development of children and in some cases, even death [1, 2].

In the last years intensification of research on the economical and efficient technologies in order to reduce quantities and improve qualities of wastewater discharged into environment is noticeable. There are many conventional technologies for heavy metal removal such as ion exchange, chemical precipitation, flotation, chemical decomposition or adsorption [1]. Considerable attention is paid on the development of applicable and cost effective adsorbents. Among various researches on adsorbents, many works confirm that iron oxides are those which can be used for efficient heavy metal removal [3-6]. Iron oxides

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in form of magnetite, maghemite or goethite are the natural minerals which occur in earth crust. They are characterized by very reactive surface. Active sites and large surface area made them able to bound contaminants in the form of cations and anions as well [7]. Iron oxides application in wastewater treatment area is especially attractive when their size is limited to nanoscale. As nanoparticles they provide high efficiency, fast reaction kinetics and high reactivity with respect to the separated ions. These properties are caused by the extremely small particles size and a large specific surface area. Besides, those nanoparticles have also another important feature - magnetism. This feature is especially important in water and wastewater treatment systems as it provides a great convenience in the process of separation of the nanoparticles from the water after adsorption process. Separation can be carried out using a small magnetic field or even handheld magnet [8, 9].

One of the simplest and the most economical methods of the magnetite nanoparticles production is chemical co-precipitation. The method is founded on co-precipitation from Fe(II) and Fe(III) salts by addition of a base [10]. The stoichiometric reaction can be written as follows:

$$Fe^{2+} + 2Fe^{3+} + 8OH^{-} \rightarrow Fe_3O_4(\text{magnetite}) + 4H_2O \tag{1}$$

The properties of nanoparticles produced during chemical co-precipitation, such as their size, chemical composition or morphology, strongly depend on the synthesis reaction parameters. Among them one can point out such parameters as reaction temperature, pH and type of base, mixing rate, ionic strength of medium, presence of the coating agent (*eg* surfactant), reaction mixture deoxidization by for example nitrogen gas bubbling or Fe(II) : Fe(III) molar ratio value [11, 12]. For instance, it was proved that the size of the nanoparticles increase with the temperature of the synthesis reaction and decrease with the increasing amount of base [12, 13].

The aim of this study was to investigate how the synthesis conditions influence the resulting magnetite efficiency as a heavy metal sorbent. Thus syntheses reactions were led at different combinations of temperatures and base amounts. The properties of the iron oxides were examined on the basis of following ions separation efficiencies - Cu(II), Ni(II), Zn(II), Cd(II), Pb(II), Cr(III) and Cr(VI). After choosing the reaction conditions which seemed to be most reasonable for the efficient sorbent production, the pH effect of the sorption process on its efficiency regarding to four ions, *ie* Zn(II), Cu(II), Cd(II) and Pb(II), was investigated.

## Experimental

### *Chemicals and methods*

The syntheses of magnetite nanoparticles were conducted using iron salts - $FeCl_3 \cdot 6H_2O$ (Warchem), $Fe_2SO_4 \cdot 7H_2O$ (Stanlab) and base solution - $NH_3 \cdot H_2O$ 25% (Stanlab).

Heavy metal solutions at a concentration of 20 and 50 mg/dm$^3$ were prepared based on the following, mainly nitrate, salts - $Cr(NO_3)_3 \cdot 9H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $Cd(NO_3)_2 \cdot 4H_2O$, $Cu(NO_3)_2 \cdot 3H_2O$, $Pb(NO_3)_2$, $Zn(NO_3)_2 \cdot 9H_2O$ (POCH S.A.), $K_2Cr_2O_7$ (Merck). For pH correction base - NaOH (0.1M) and acid - $HNO_3$ (0.1M) solutions were used (POCH S.A.).

Atomic absorption spectrometer SepctrAA 880 (Varian) with atomization in acetylene-air flame was used to determine the metal concentration of the solution after sorption process.

*Magnetite nanoparticles preparation*

Nanoparticles syntheses were conducted following modified Liu et al method [5]. General procedure of all syntheses includes succeeding steps:

- dissolution of appropriate $FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ salts amounts in 100 $cm^3$ of distilled water,
- placing the mixture in the water bath followed by heating in order to reach required temperature,
- starting the mechanical stirring of the mixture,
- rapid addition of 50 $cm^3$ distilled water and appropriate volume of the base to initiate magnetite precipitation,
- vigorous stirring for 30 minutes whilst keeping the mixture in the same temperature conditions,
- cooling the mixture to the room temperature (optional pH measurements of mixtures after synthesis),
- washing of black precipitate of magnetite by distilled water with the help of a neodymium magnet in order to separate sorbent from unreacted residues of chemicals,
- preparation of approximately 5 $g/dm^3$ suspension of magnetite nanoparticles.

*The synthesis at different temperature and pH conditions*

In first part of the research the influence of temperature and volume of precipitating medium, which affected pH of reaction mixture, on resulting adsorbent efficiency were examined.

Table 1

Magnetite synthesis conditions at different temperature/base volumes

| **Number of syntheses/combinations** | **Temperature** | **Ammonia water volume** | **Molar ratios of chemicals $Fe^{2+} : Fe^{3+} : NH_3 \cdot H_2O$** | **Final pH after synthesis** |
|---|---|---|---|---|
| | **[°C]** | **[$cm^3$]** | | **[-]** |
| **1** | 30 | 8 | 1 : 1.5 : 3.8 | 7.31 |
| **2** | 60 | | | 5.81 |
| **3** | 90 | | | 5.31 |
| **4** | 30 | 10 | 1 : 1.5 : 4.7 | 8.84 |
| **5** | 60 | | | 8.53 |
| **6** | 90 | | | 6.95 |
| **7** | 30 | 15 | 1 : 1.5 : 7.1 | 9.37 |
| **8** | 60 | | | 9.16 |
| **9** | 90 | | | 7.92 |

The molar ratio of iron salts was according to Liu et al [1] maintained stable, equal Fe(II) : Fe(III)=1:1.5 (4.2 g $FeCl_3 \cdot 6H_2O$ and 6.1 g $Fe_2SO_4 \cdot 7H_2O$) whereas temperature of syntheses was settled at 30, 60 or 90°C and base was added in volume of 8, 10 or 15 $cm^3$.

Summarizing, nine syntheses combinations at different temperature/base volumes conditions were carried out. The amounts of chemicals used in each combination in comparison to the stoichiometric synthesis reaction, which for pure magnetite is $Fe^{2+} : Fe^{3+} : NH_3{\cdot}H_2O = 1 : 2 : 8$, and final pH of a mixtures after each synthesis were collected in Table 1.

*The synthesis at different Fe(II)/Fe(III) molar ratio conditions*

The aim of second part of the research was to investigate how the molar ratio of iron salts effects the resulting adsorbent efficiency. Because of the fact that syntheses were conducted with an oxygen access, the partial oxidation of Fe(II) to Fe(III) were predicted. Therefore a few syntheses with different Fe(II) and Fe(III) salts amounts were conducted. Four Fe(II) : Fe(III) ratios below stoichiometric one were chosen, that is 1 : 1, 1 : 1.25, 1 : 1.5, 1 : 1.75, one equal to the pure magnetite stoichiometry - 1 : 2 and one ratio above - 1 : 2.25. The molar ratios and corresponding iron salts amounts were collected in Table 2.

Table 2

Magnetite synthesis conditions at different Fe(II)/Fe(III) molar ratios

| **Number of syntheses** | **Iron salts molar ratio** | | **Iron salts amount** | |
|---|---|---|---|---|
| | **Fe(II)** | **Fe(III)** | **$FeSO_4 \cdot 7H_2O$** | **$FeCl_3 \cdot 6H_2O$** |
| | | | **[g]** | **[g]** |
| 1 | 1 | 1 | 4.20 | 4.08 |
| 2 | 1 | 1.25 | 4.20 | 5.10 |
| 3 | 1 | 1.5 | 4.20 | 6.13 |
| 4 | 1 | 1.75 | 4.20 | 7.15 |
| 5 | 1 | 2 | 4.20 | 8.17 |
| 6 | 1 | 2.25 | 4.20 | 9.19 |

All syntheses with different iron salts amounts were conducted under the most appropriate temperature/base volume conditions obtained from the first part of the research - that is at 30ºC and with 15 $cm^3$ of ammonia solution.

*Batch sorption tests*

In order to examine how the synthesis conditions of magnetite, *ie* the temperature, base volume and molar ratio of iron salts added to the reaction mixture, effect resulting adsorbent the batch adsorption tests were conducted. In every heavy metal removal procedure 5 $cm^3$ of magnetite particles suspension (5 $g/dm^3$) was placed in plastic container and poured over with 20 $cm^3$ of metal solution (Cu(II), Zn(II), Ni(II), Cd(II), Cr(VI), Cr(III) or Pb(II)) at concentration of 20 $mg/dm^3$ (for temperature/base volume testing) and 50 $mg/dm^3$ (for molar ratio testing). Every sorption experiment was performed in triplicate without pH correction. Then the reactors were placed on mechanical shaker and shaken for 8 hours at room temperature. In the next day the adsorbents were separated via external magnetic field and supernatants were collected for metal concentration measurement.

Moreover, following the procedure of sorption described above, the influence of pH during the separation process on its efficiency was examined for four metal ions, *ie* Cu(II), Zn(II), Cd(II) and Pb(II). The sorbent was prepared at 30ºC, 15 $cm^3$ of base and

Fe(II) : Fe(III) molar ratio equal to 1 : 1.5, heavy metal solutions were used in concentration of 50 mg/dm$^3$. The pH conditions during sorption process was established at different values for each test, namely 3, 4, 5, 6, 7.

## Results and discussion

The separation process efficiency $\eta$ was evaluated according to the following equation:

$$\eta = [(C_i - C_f)/C_i] \cdot 100\% \tag{2}$$

where $C_i$ and $C_f$ are metal ion concentrations before and after sorption (initial and final concentration), respectively.

*The results for synthesis at different temperature and base volume conditions*

Results of each ion separation effectiveness are shown at Figures 1-7. Analysed ions can be divided into two groups. The first group - Cu(II), Zn(II), Cd(II) and Ni(II) ions, which removal is substantially smaller than separation of the second group - Pb(II), Cr(III) and Cr(VI) ions.

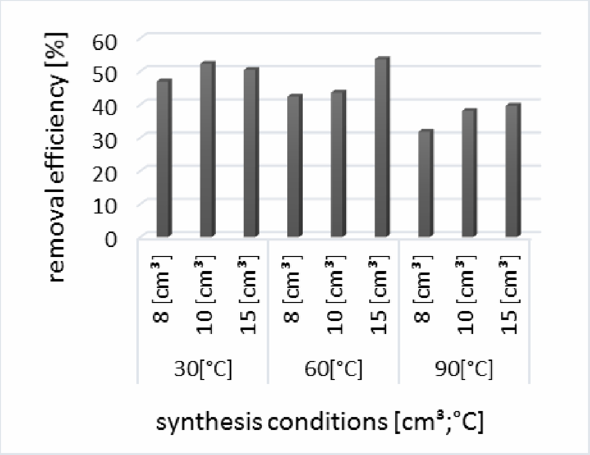

Fig. 1. Removal efficiency of Cu(II) ions by iron oxides synthesised at different ammonia volume/temperature conditions

While analysing the first group of ions it can be observed that the maximum separation efficiencies amounted to more than 50% for Cu(II) and Zn(II), over 30% for Ni(II) and about 20% for Cd(II). Concerning the effect of the base volume added during syntheses, it can be said that the smallest removal of these ions for the whole range of temperatures, were achieved using 8 cm$^3$ of ammonia solution. It is related to the too low pH of the reactions environments and thus to insufficient amounts of -OH groups required for proper synthesis of iron oxides. The pH values measured immediately after syntheses was in the range from 5 to over 7 (Table 1). Especially large diversity in separation results was obtained for Zn(II) ions. Their removal using magnetite synthesised at 30 and 60°C increased from 20% while using 8 $cm^3$ of base to over 50% while using 15 $cm^3$ of ammonia solution. Similar trend can be observed for Ni(II) ions.

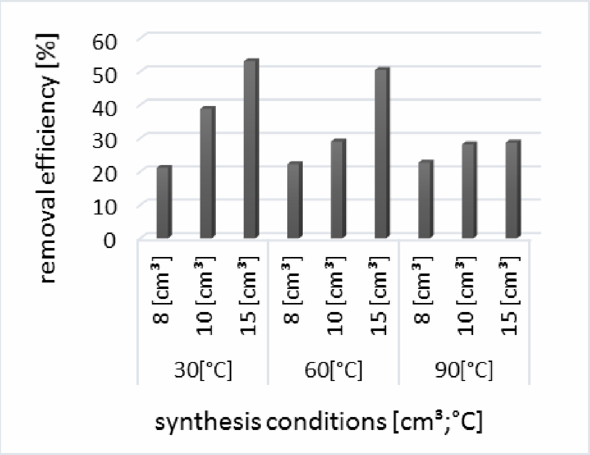

Fig. 2. Removal efficiency of Zn(II) ions by iron oxides synthesised at different ammonia volume/temperature conditions

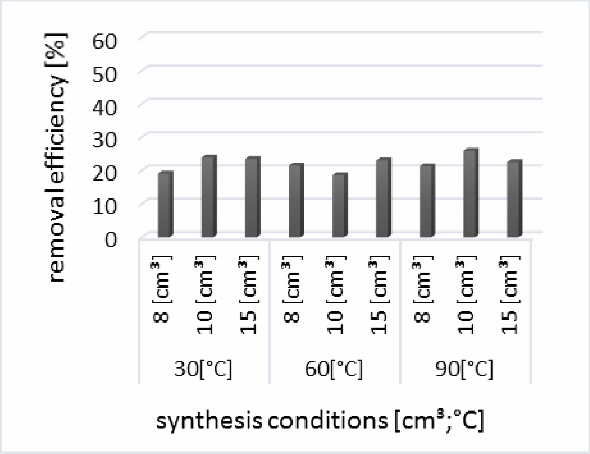

Fig. 3. Removal efficiency of Cd(II) ions by iron oxides synthesised at different ammonia volume/temperature conditions

The effect of temperature conditions on resulting sorbent separation efficiency was also most noticeable for Zn(II) ions sorption - the highest removal was obtained while using magnetite produced at 30ºC. Similar tendency was observed for Ni(II) ions with less noticeable removal efficiency decrease while using sorbent synthesised at 60ºC and more noticeable for iron oxides synthesised at 90ºC. In case of Cu(II) ions, although the biggest removal was obtained during sorption on the magnetite produced at 60ºC, the results was not so differ from those observed for material synthesised at 30ºC. In turn, the noticeable decrease of Cu(II) ions removal was observed while using sorbent made at 90ºC. The smallest differences at sorption by using sorbents obtained at different temperatures was noticed for Cd(II) ions.

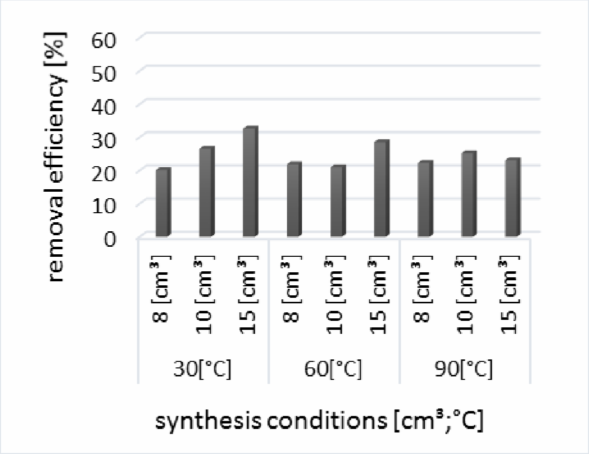

Fig. 4. Removal efficiency of Ni(II) ions by iron oxides synthesised at different ammonia volume/temperature conditions

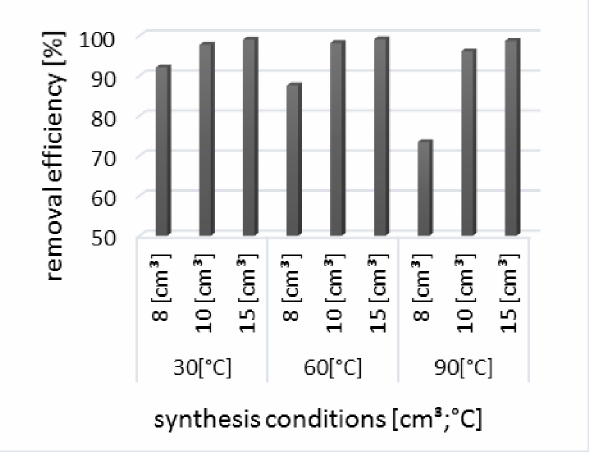

Fig. 5. Removal efficiency of Pb(II) ions by iron oxides synthesised at different ammonia volume/temperature conditions

Analysing the results for second group of ions - Cr(VI), Pb(II) and Cr(III), their high, up to 100%, removal is noticeable. Despite small differences in removal efficiencies while applying sorbents synthesised in different temperature/base amounts conditions some tendencies also can be observed. Especially with regard to the amount of base addition.

Similar as for ions from the first group, the 8 cm$^3$ of ammonia was insufficient to produce efficient sorbent. While using this amount of base at temperature increasing up to 90ºC, a clear decrease in the sorption properties of material was noticeable (due to increased evaporation, pH of the reaction mixtures during the synthesis decreased). In turn, for the majority of these metals there was no significant difference between sorbent synthesised with 10 and 15 cm$^3$ of base. The quite observable difference can be seen only for Cr(III), wherein the removal properties of the sorbent synthesized at 90ºC with 10 cm$^3$ of ammonia usage was less than with 15 cm$^3$ of base applied. Slight differences can also be noticed while analysing the effectiveness of the Pb(II) removal.

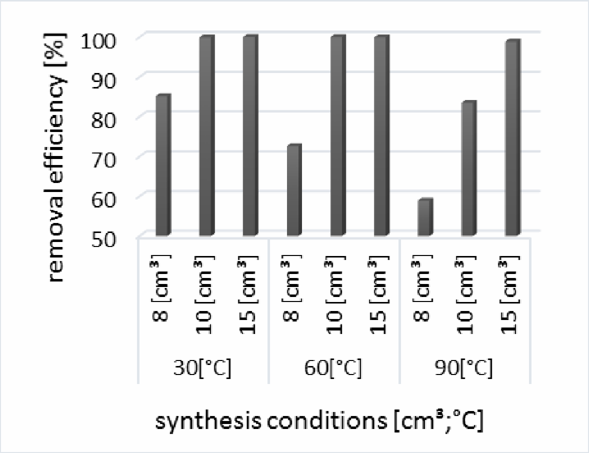

Fig. 6. Removal efficiency of Cr(III) ions by iron oxides synthesised at different ammonia volume/temperature conditions

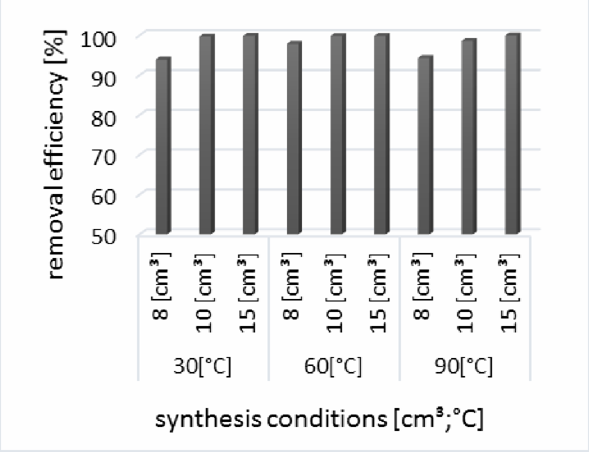

Fig. 7. Removal efficiency of Cr(VI) ions by iron oxides synthesised at different ammonia volume/temperature conditions

Given the above analyses, for further research the magnetite syntheses was carried out at 30°C with the 15 $cm^3$ of a base addition.

*The results for synthesis with different amount of Fe(II) and Fe(III) salts*

The results of ions separation using magnetite synthesized under optimal temperature and base volume conditions (selected in the previous research) but with different molar ratios of iron salts added to the reaction mixture are shown at Figure 8.

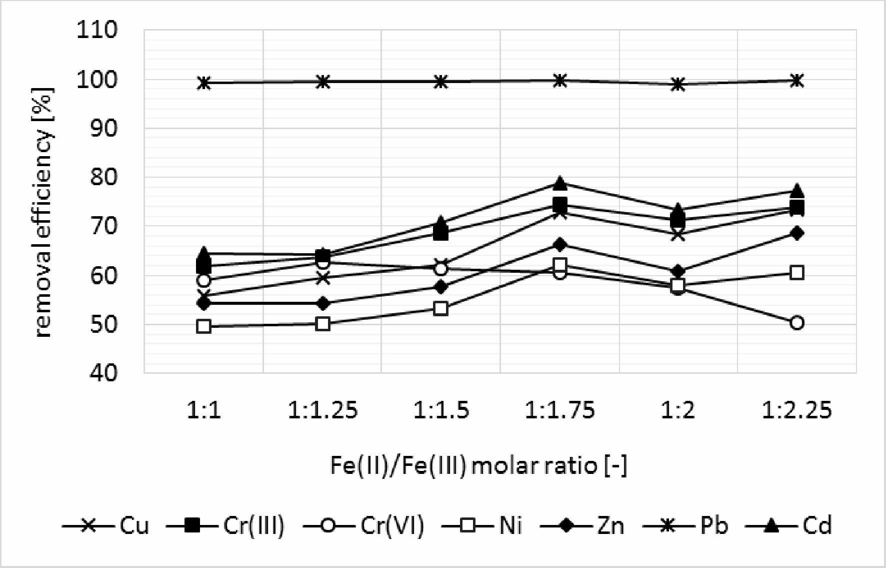

Fig. 8. Removal efficiency of heavy metal ions by iron oxides synthesised at different Fe(II) : Fe(III) molar ratios

Except Pb(II) ions, which removal regardless of the molar ratio Fe(II) : Fe(III) used during sorbent production was maintained in the range of 100%, the differences in separation efficiencies for other ions can be observed.

The least effective sorbent for most of heavy metal ions (Cu(II), Zn(II), Ni(II), Cd(II), Cr(III)) was obtained with a molar ratio Fe(II) : Fe(III) equal to 1 : 1 and 1 : 1.5. Over this ratio, while trivalent iron salt amount increased up to proportion 1 : 1.75, the sorption capacity of magnetite also increased. At Fe(II) : Fe(III) ratio equal to 1 : 2, which is stoichiometric one for pure, solid magnetite, the separation efficiencies decrease and then increase again at ratio 1 : 2.25. Considering mentioned results, Fe(II) : Fe(III) molar ratios between 1 : 1.5-1 : 1.75 seem to be the most optimal.

Although good separation effectiveness was also obtained for the ratio of 1 : 2.25, this proportion of iron salts is not recommended because of the product purity uncertainty. The aim of the study was the usage of iron oxides as the sorbent meanwhile the product obtained at 1 : 2.25 was characterized by slightly brownish colour and slower or incomplete settling on a neodymium magnet that can indicate worse magnetic proprieties. It can be the evidence of other iron forms presence such as iron hydroxides.

### *The pH of sorption process effect on Cu(II), Zn(II), Cd(II) and Pb(II) ions separation efficiency*

The results of Cu(II) Zn(II), Cd(II) and Pb(II) ions separation at different pH conditions are shown in Figure 9.

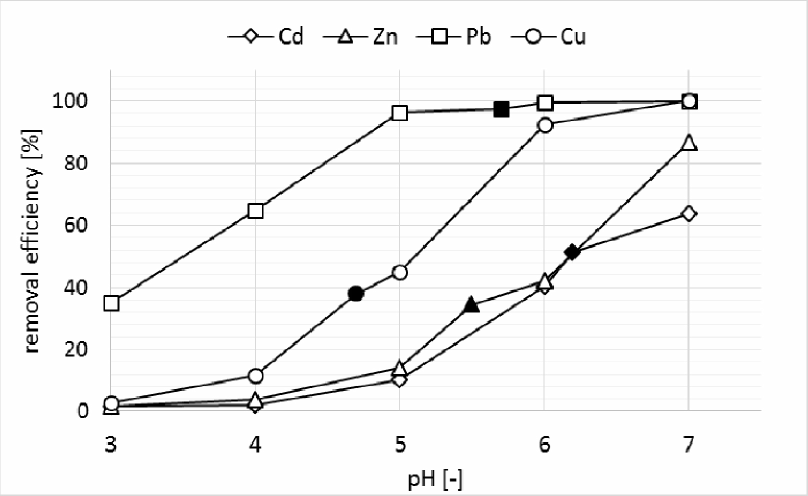

Fig. 9. The effect of pH on heavy metal ions separation effectiveness

The sorption was carried out at the following pH values: 3, 4, 5, 6, 7 and, for comparison, without pH adjustment after pouring the suspension of nanoparticles with a solution of heavy metal (marks filled in black at Fig. 9).

Analysing series of data at the graph, the close relationship between pH of the sorption process and separation efficiencies of each metal ion can be noticed.

The low pH values result in the small removal of Cd(II), Zn(II) and Cu(II) in range of 2-3% and about 35% for Pb(II). The gradual separation increase with the rise of the alkalinity was noticed for all ions. In case of Cd(II) from 3% at pH 3 to 60% at pH 7, for Zn(II) ions from 2 up to 87%, for Cu(II) ions from 3 to 92% and finally for Pb(II) ions from 35 to 100%.

Moreover, for Pb(II) ions the pH of 5 was enough to achieve almost 100% separation and more than 90% of Cu(II) ions were removed at pH 6. It is an important information, because especially in case of Pb(II) ions, the increasing pH values of its solutions above 6, cause their visible precipitation. Thus the high removal efficiency at pH 7 could be not the result of sorption by itself but also chemical precipitation process.

## Conclusions

It can be concluded that the sorbents synthesized under different temperature/amount of base conditions are characterized by relative different separation properties with respect to various ions. It turns out that too small amount of ammonia seems to be generally insufficient to produce the effective sorbent. It was proved that usage of 10 or 15 cm$^3$ of base with an indication of the latter volume is most justified. It was confirmed mainly during the Zn(II), Ni(II) and also Cr(III) ions separation analysis. Moreover, considering the effect of synthesis temperature on the magnetite efficiency, it appears to be reasonable to carry out the syntheses at the temperature of 30°C. Increasing the temperature to 60°C and 90°C does not appreciably change the sorption properties of magnetite (ions Cd(II), Cr(VI), Cd(III)) or worsen it more or less noticeably (Cu(II), Zn(II), Ni(II)).

Also the Fe(II) : Fe(III) ratio in the reaction mixture affects separation properties of the sorbent. It comes out that the most effective sorbent was synthesised while keeping Fe(II) and Fe(III) in the proportion between 1 : 1.5 and 1 : 1.75.

During the sorption process, the important element is to maintain pH at proper level. For all ions considered in this part of research, *ie* Cu(II), Zn(II), Cd(II) and Pb(II) the gradual increasing tendency in their separations was visible while increasing pH from 3 up to 7. In summary, it can be said that during the sorption the pH values should be maintained at high, efficient for the process level but also at level low enough to prevent heavy metal precipitation.

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## References

[1] Barakat M. New trends in removing heavy metals from industrial wastewater. Arabian J Chem. 2011;4:361-377. DOI: 10.1016/j.arabjc.2010.07.019.

[2] Järup L. Hazards of heavy metal contamination. Br Med Bull. 2003;68:167-182. DOI: 10.1093/bmb/ldg032.

[3] Amin M, Khodabakhsi A. Removal of Cr(VI) from simulated electroplating wastewater by megnetite nanoparticles. Environ Eng Manage J. 2010;9(7):921-927. https://www.researchgate.net/publication/266462544_Removal_of_CrVI_from_simulated_electroplating_wastewater_by_magnetite_nanoparticles.

[4] Aftabtalab A, Sadabadi H, Chakra C, Rao V, Shaker S, Mahofa E. Magnetite nanoparticles synthesis for removal of chromium(VI) from waste water. IJSER. 2014;5(1):1419-1423. https://www.researchgate.net/publication/272892639_Magnetite_nanoparticles_Fe_3_O_4_synthesis_for_removal_of_Chromium_VI_from_waste_water.

[5] Liu J, Zhao S, Jiang G. Coating $Fe_3O_4$ magnetite nanoparticles with humic acid for high efficient removal of heavy metals in water. Environ Sci Technol. 2008;42:6949-6954. DOI: 10.1021/es800924c.

[6] Predescu A, Nicolae A. Adsorption of Zn, Cu and Cd from waste waters by means of maghemite nanoparticles. Univ Politehnica Bucharest Sci Bull. 2012;74(1):255-264. http://scientificbulletin.upb.ro/rev_docs_arhiva/full907_755573.pdf.

[7] Erdemoglu M, Sarıkaya M. Effects of heavy metals and oxalate on the zeta potential of magnetite. J Colloid Interface Sci. 2006;300:795-804. DOI: 10.1016/j.jcis.2006.04.004.

[8] Tang S, Lo I. Magnetic nanopartcles: Essential factors for sustainable environmental applications. Water Res. 2013;47:2613-2632. DOI: 10.1016/j.watres.2013.02.039.

[9] Hua M, Zhang S, Pan B. Heavy metal removal from water/wastewater by nanosized metal oxides: A review. J Hazard Mater. 2012;211-212:317-331. DOI: 10.1016/j.jhazmat.2011.10.016.

[10] Maity D, Agrawal D. Synthesis of iron oxide nanoparticles under oxidizing environment and their stabilization in aqueous and non-aqueous media. J Magn Magn Mater. 2006;308:46-55. DOI: 10.1016/j.jmmm.2006.05.001.

[11] Faraji M, Yamini Y, Rezaee M. Magnetic nanoparticles: synthesis, stabilization, functionalization, characterization, and applications. J Iran Chem Soc. 2010;7(1):1-37. DOI: 10.1007/BF03245856.

[12] Petcharoen K, Sirivat A. Synthesis and characterization of magnetite nanoparticles via the chemical co-precipitation method. Mater Sci Eng B. 2012;177(5):421-427. DOI: 10.1016/j.mseb.2012.01.003.

[13] Mascolo M, Pei Y, Ring T. Room temperature co-Precipitatio synthesis of magnetite nanoparticles in large pH window with different bases. Materials. 2013;6:5549-5567. DOI: 10.3390/ma6125549.

# KONTROLOWANA SYNTEZA NANOCZĄSTEK TLENKÓW ŻELAZA STOSOWANYCH JAKO EFEKTYWNY ADSORBENT JONÓW METALI CIĘŻKICH

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**Abstrakt:** W pracy przedstawiono próby syntezy nanocząstek tlenków żelaza poprzez ich chemiczne współstrącanie. Jako medium strącające zastosowano wodę amoniakalną. W literaturze udowodniono wpływ takich czynników, jak temperatura reakcji, ilość zasady czy też stężenie soli żelaza Fe(II) i Fe(III) w mieszaninie reakcyjnej na charakterystykę, między innymi wielkość, powstających nanocząstek. Celem niniejszej pracy było dobranie takich parametrów reakcji, aby w efekcie uzyskać najbardziej efektywny sorbent względem jonów kilku metali ciężkich: Cr(VI), Pb(II), Cr(III), Cu(II), Zn(II), Ni(II) i Cd(II). Przeprowadzono syntezę nanocząstek w kombinacji trzech temperatur: (30, 60, 90ºC) oraz trzech objętości amoniaku: (8, 10, 15 cm$^3$). Ponadto sprawdzono, jaki wpływ na powstający sorbent ma stosunek molowy jonów żelaza Fe(II) : Fe(III) w mieszaninie reakcyjnej. Biorąc pod uwagę fakt, iż synteza przeprowadzana była przy dostępie tlenu, w celu kompensacji częściowego utlenienia jonów żelaza Fe(II) do Fe(III), wyjściowy stechiometryczny stosunek tych jonów, który dla magnetytu ($Fe_3O_4$) wynosi 1:2, obniżono do kilku wartości poniżej 1 : 2. Dodatkowo dla magnetytu zsyntezowanego w wybranych, optymalnych, warunkach przeprowadzono próbę wpływu pH na sorpcję jonów metali. W celu uniknięcia możliwości wytrącania się osadów maksymalne pH ustalono na nieprzekraczające wartości 7.

**Słowa kluczowe:** adsorpcja, metale ciężkie, magnetyt, nanocząstki

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# EFFECT OF BIODEGRADABLE MULCHING ON SOIL QUALITY IN STENOTERMAL VEGETABLE CROP PRODUCTION

## WPŁYW MULCZOWANIA Z WYKORZYSTANIEM WŁÓKNIN BIODEGRADOWALYCH NA JAKOŚĆ GLEBY W UPRAWIE WARZYW CIEPŁOLUBNYCH

**Abstract:** Mulching with plastic materials that cover the soil creates a physical barrier to soil water evaporation, preserves good soil structure, controls weeds and protects plants from soil contamination. The removal and disposal of non-recyclable and non-degradable plastic wastes after harvest is difficult and expensive. Using alternative biodegradable polymers as covers has been of increasing concern in recent years. This paper provides a presentation of results concerning the physical and chemical properties of soil under biodegradable nonwoven covers. Biodegradable PLA (polylactide) and Bionolle (an aliphatic polyester of butylene glycol and succinic and adipic acid) films covering the soil on tomato and cucumber fields were evaluated to estimate the changes in several physical soil properties including bulk density, water field capacity, wet soil aggregate stability and chemical soil properties including soil acidity, EC, organic matter and soil mineral status. Favourable changes in the physical structure of soil can be achieved by mulching the soil surface of biodegradable polymers. The obtained results show that using covers with PLA and Bionolle biofilm significantly increased the amount of large aggregates and decreased the percentage of the smallest size aggregates in soils; however, the observed effects were strongly affected by weather conditions. Under wet conditions, mulching increased the soil bulk density and decreased soil water capacity. The results of soil chemical analyses demonstrated the low impact of treatments on macro- and microelement concentration measured after tomato and cucumber harvesting. Soils under PLA and Bionolle covers had smaller low ion concentrations in relation to bare soils.

**Keywords:** biofilm, bulk density, water-stable aggregates, soil organic matter, tomato, cucumber

## Introduction

Plastic soil film covering has been used as mulch since the 1960s, mainly in vegetable production [1, 2]. It is well known that plastic mulch film increases the yield of many vegetables, in particularly early in the season, most likely by increasing soil temperatures and moisture and inhibiting weed growth [3-8]. However, the removal and disposal of non-degradable plastic mulch (polyethylene and polypropylene) after harvest is difficult and expensive. Disposal concerns and environmental legislation are forcing plastics manufactures to consider biodegradable polymers as an alternative material. A large amount of research is in progress to find the most suitable alternative to plastic mulch in vegetable production [9, 10]. Degradable plastics were introduced in the 1980s, though there remain many questions regarding their efficacy, degradability and potential residues [11]. After being used, photodegradable or biodegradable synthetic mulch does not need to be recollected, transported to a collection centre or landfill, or disposed through

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incineration [12]. Degradable films' breakdown is primarily affected by temperature, sunlight, moisture and microbial activity [13, 14].

An example of a successfully commercialised biodegradable polymer is poly(lactic acid), or PLA - a synthetic polymer made from lactic acid, a monomer found in nature. PLA is biodegradable and compostable [15]. Sustainability and eco-friendly characteristics make PLA an attractive polymer. PLA degrades mainly by hydrolysis, even in the absence of microorganisms. The rate of biodegradability in the composting environment depends on the size and shape of the article [16]. Another example of a completely mineralised synthetic polymer is trademarked Bionolle™, produced through the polycondensation reactions of glycols with aliphatic dicarboxylic acids such as succinic and adipic acids used as the principal raw material [17]. Aliphatic polyesters can be degraded with enzymes and microorganisms in soils [2].

The benefits of mulching on the growth and yield of vegetables have long been recognised. Mulching with organic or inorganic materials covers soils and creates a physical barrier to soil water evaporation, preserves good soil structure, control weeds and protect plants from soil contamination. Soil surface covering decreases erosion, protects against raindrop impact and increases aggregate stability [18]. In addition, mulches improve soil structure by creating favourable conditions for soil aggregation, *eg* through higher soil water content and temperature and the efficient mineralisation of soil organic matter [3]. Soil aggregates formed by the arrangement of soil particles are the fundamental components of soil structure. The linkage between soil particles depends on the interactions between primary particles and organic constituents to form stable aggregates [19]. The stability of soil structure affects soil fertility, quality and sustainability. Thus, the recognition of soil aggregate size distribution and soil aggregate stability is important to properly interpret soil structure [20]. In agricultural soils bulk density, various air and water capacity relationships and soil aggregate parameters are commonly used to indicate the physical quality of the soil. In general, soil physical quality is a central concept for developing "best management" land use practices. Mulching is known to attribute enhanced mineral nutrient availability to enhance nitrification. Mulching also improves soil aeration, creates better biological activates and thus has a consequent beneficial effect on soil fertility [11]. One of the advantages of using plastic mulches is also a reduction in fertilizer leaching. Plastic mulch covering prevents rainfall from percolating through the soil and moving nutrients. Preventing leaching improves the efficiency of plant nutrition and production. Li et al [21] demonstrated the $CO_2$ enrichment of the soil surface under plastic mulch. The metabolism, biomass, activity and diversity of the microbial community in soils can be significantly altered by $CO_2$ enrichment. It can also increase carbonate weathering, differ the rhizospheric exudation and enhance the amount of available C in the soil. $CO_2$ improvement can influence the transformation and bioavailability of metals in soils. Li et al [22] showed lower pH values and higher concentration of Cu, Pb, Zn and Cd in soil under plastic mulching. The authors also suggested that plastic mulching most likely causes the accumulation of heavy metals in surface soils by reducing metal leaching from surface runoff.

Many tomato or/and cucumber growers in Poland are using polyethylene mulch to warm the soil to advance harvest maturity under cool soil conditions. Warm season vegetables, including tomato and cucumber have produced better quality fruit and a greater yield when grown on plastics [6, 7, 23]. Black plastic film was used for many years for its ability to absorb light and raise soil temperature, which extended the production season in a colder climate [24]. Ngouajio et al [25] and Minuto et al [26] showed that tomato growth, yield and fruit quality from black biodegradable mulch was equivalent to that in low-density polyethylene mulch. Martin-Closas [27] found similar results for organically grown tomato. In addition, Anzalone et al [8] indicated that paper, biodegradable plastic and rice straw are potential substitutes for polyethylene and herbicides. There is an increasing interest in the use of degradable mulching for protected cultivation. Unfortunately, there are few published reports on how physical and chemical soil properties change after modifications in land use. Additional information on the biochemistry and decomposition of biodegradable films and their interaction with soil properties and environmental conditions to promote aggregation would allow for the identification of more effective management practices.

Although agricultural land management is recognised to affect near-surface physical qualities of soil, little is known about how biofilm covering affects physical and chemical soil parameters. In this research, biodegradable film covering tomato and cucumber were tested to estimate the changes in several physical and chemical soil properties. Soil properties selected to indicate physical quality included bulk density, water retention and wet soil aggregate stability, whereas the chemical characteristics considered were total organic carbon, nutrients and heavy metals content.

## Material and methods

### *Soil sampling and analysis*

The field experiment was carried out at the experimental farm of the Agricultural University of Krakow in Mydlniki in 2008-2011. The effect of film covering tomato and cucumber on the physical and chemical soil properties was studied. The trials consisted of a randomised complete block design with the following treatments: biodegradable films - Bionnole, PLA and bare soil (control). Bionolle - an aliphatic polyester of butylene glycol, succinic and adipic acid - is a completely mineralised polymer. Poly(lactic acid) or polylactide (PLA) is a thermoplastic aliphatic polyester. Both polymers can biodegrade under certain conditions.

The seeds of the tomato cultivar ‘Mundi’ were sown in a greenhouse on 15 April 2008, 16 April 2009 and 31 March 2010, respectively. Seedlings were planted in the field on 29 May 2008, 27 May 2009 and 25 May 2010, respectively, at a distance of 100 x 50 cm. Black nonwoven PLA 61 $g \cdot m^{-2}$ was used for soil mulching. Plants cultivated without covers were the control.

On the cucumber experimental fields, black nonwoven Bionolle 200 $g \cdot m^{-2}$ (except 2008) and PLA 130 $g \cdot m^{-2}$ were used for soil mulching. The nonwovens were stretched before the seeds were sown. Plants cultivated without covers were the control. Experimental fields with a soil surface of 7.5 $m^2$ were established with four replications. The seeds of the cucumber cultivar ‘Mirabelle’ were sown into the field on 7 May 2008, 14 May 2009 and 8 July 2010, respectively, at a distance of 200 x 25 cm (two seeds per hole cut in the nonwoven).

The mineral fertilisation of phosphorus, potassium and magnesium was based on the results of chemical analyses of the soil samples. The content of soil P, K and Mg was supplemented before seedling planting. Nitrogen fertilizer was applied at the rate of 100 kg N · $ha^{-1}$.

Soil samples with their natural structure preserved intact were collected from the plots divided into sections differentiated by covers films. In each section, soil samples were taken at a depth of 0-20 cm after tomato and cucumber cropping. The soil samples were air-dried at room temperature and sieved.

Intact soil cores (250 $cm^3$) at a depth of 0-10 cm were sampled in four replication to measure soil bulk density (BD) and water retention parameters. The undisturbed soil samples for determining the soil bulk density were oven-dried at 105ºC to a constant weight. Volumetric (%vw) and weight (%ww) water field capacity of the soil was determined by a Kopecki-type procedure [28]. Granulometric analysis was made using the Casagrande aerometric method modified by Proszynski [29]. This procedure is regulated by the PN-R-04032 [30] standard published mostly for agricultural soil analysis in Poland.

Soil aggregates were separated by wet sieving using Yoder's procedures [31]. Previously separated out by dry sieving, a single size soil fraction (< 5 mm) was disrupted under water, where the nest of sieves was suspended in a container of water. For measurements, forty grams of air-dried aggregates in five replications were placed onto the top sieve and immersed in water for a period of time (5 min) before beginning the mechanical sieving process for 20 minutes. A motor and a mechanical arrangement lowered and raised sieves through a distance of 5 cm at a rate of 5 cycles per one minute. There were five size classes used: 0.25, 0.5, 1.0, 1.5 and 2.5 mm. The amount of soil retained on each sieve was determined by drying and weighing. The water-stable aggregate index based on the wet soil fragmentation procedure was calculated as a sum of five size classes of aggregates.

Soil pH was measured in water and 1 mol · $dm^{-3}$ KCl at a soil to solution ratio of 1:2. Soil organic carbon (SOC) was determined using the dichromate oxidation method [29]. The available form of nitrogen ($N\text{-}NO_3$ and $N\text{-}NH_4$), phosphorus, potassium, magnesium and calcium was determined by the universal method as described by Nowosielski [32]. The extractable forms of metals were measured in 1 mol · $dm^{-3}$ HCl extractant [29]. This soil extractant and procedure is currently used to estimate the availability and critical levels for soil micronutrient cations in Poland. Available N was detected using the Flow Injection Analysis (FIA) technique with spectrometric detection [33], and P, K, Mg and Ca were determined using the inductively coupled argon plasma atomic emission spectroscopy ICP-OES technique (ICP-OES Teledyne Prodigy, Leeman Labs spectrophotometer).

### *Statistical analysis*

Data collected from the study were analysed using the one-way analysis of variance test based on the ANOVA module in Statistica 8.0. Means for each treatment were separated using the Fisher test at the $p \leq 0.05$ level of significance.

### *Climatic conditions*

In 2008 and 2009 temperatures were near the average for the Krakow area from April to October (Table 1). Rainfall during the growing season in 2010 was very high, especially in May and September. This growing season was characterized as warm (July) but very wet (May and September) in compared with the years 2008-2009.

Table 1

Precipitation sums and mean monthly temperatures during the vegetation period 2008-2010

| **Year** | **April** | | **May** | | **June** | | **July** | | **September** | | **October** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **[ºC]** | **[mm]** | **[ºC]** | **[mm]** | **[ºC]** | **[mm]** | **[ºC]** | **[mm]** | **[ºC]** | **[mm]** | **[ºC]** | **[mm]** |
| 2008 | 10.1 | 35.2 | 14.4 | 26.8 | 19.5 | 26.7 | 19.0 | 142.6 | 18.0 | 41.6 | 12.5 | 96.7 |
| 2009 | 12.0 | 0.5 | 13.3 | 91.0 | 15.4 | 128.0 | 19.4 | 82.7 | 18.9 | 53.1 | 15.1 | 34.8 |
| 2010 | 8.5 | 37.5 | 12.7 | 297.9 | 17.6 | 122.0 | 21.8 | 110.4 | 18.8 | 138.2 | 12.4 | 92.4 |

## Results and discussion

Data from three tomato/cucumber growing seasons were collected. We determined several physical and chemical soil parameters as related to the impact of biofilms on soil characteristics after harvest. In the presented study, the particle size analysis showed a silty clay soil texture (14% of size particles 1.0-0.1 mm, 45% were 0.1-0.02 mm and 41% were < 0.02 mm). Soil texture has a significant influence on aggregation. Clay content affects aggregation through swelling and dispersion. Increasing clay concentration is usually associated with increased soil organic content in soil and wet soil structure stability [18].

### *Soil bulk density and water capacity*

A minor effect of the treatments on soil bulk density was observed. In 2008, in the tomato season, the bulk density (BD) measured for the control soil was 1.39 g · cm$^{-3}$ (Table 2). In the 2009-2010 seasons, the average BD for the control soil was 1.39 and 1.17 g · cm$^{-3}$, respectively. There was no significant impact of covering with biodegradable films on the soil bulk density, although soil collected from under PLA covering demonstrated a relatively low value (1.28 and 1.07 g · cm$^{-3}$ in 2009 and 2010, respectively) as compared to the control treatments. According to Reynolad [34], the optimal BD range at the field site is 1.10 to 1.23 Mg · m$^{-3}$ for medium to fine-textured soils (*ie* 18-60 wt.% particle sizes < 0.002 mm) for a soil depth of 0-10 cm. Soils often react individually to agricultural practices such as tillage, cropping system, mulching, etc. Reynolds et al [35] indicated that most of the soil physical quality indicators show complex and site-specific interactions. The nature and amount of the changes are often inconsistent, or predictable only in a general way.

In all of the tomato cropping seasons, the use of covering films did not affect the field water capacity expressed as a per cent of mass units [% ww]. Only in 2009 was the weigh water field capacity relatively higher in soil covert PLA biofilm in relation to the bare soil (34.9 and 33.0% ww, respectively). Results from a study by Ndubuisi [36] indicates that polymer film mulches improved the physical properties of the soil, such as the soil water content and the temperature in top soil layers, prompting the emergence of seedlings and greater root distribution in the soil. Martin-Closas [27], Moreno and Moreno [6] and Moreno et al [7] found similar results.

Table 2

Physical and chemical properties of soils from tomato plantations covered with biodegradable film, 2008-2010

| **Treatment** | **Bulk density [Mg · m$^{-3}$]** | **Water capacity [% ww]** | **Water capacity [% ww]** | **[% C]** |
|---|---|---|---|---|
| 2008 | | | | |
| *Before planting* | *1.33* | *39.5* | *52.5* | *2.07* |
| Control | 1.39 a | 36.0 a | 49.9 a | 2.01 a |
| PLA | 1.44 a | 35.0 a | 50.4 a | 2.25 a |
| 2009 | | | | |
| *Before planting* | *1.25* | *40.1* | *49.9* | *1.38* |
| Control | 1.39 a | 33.0 a | 46.0 a | 1.36 a |
| PLA | 1.28 a | 34.9 a | 46.0 a | 1.35 a |
| 2010 | | | | |
| *Before planting* | *1.20* | *30.8* | *43.8* | *1.68* |
| Control | 1.17 a | 40.1 a | 46.8 a | 1.63 a |
| PLA | 1.07 a | 38.7 a | 43.7 a | 1.59 a |

$\alpha = 0.05$. Fisher test - the same letter indicates no significant differences between means

In 2010, the soil bulk density differed significantly between treatments on the cucumber plantation. We measured higher BD for soil covered by biofilms than for the bare soil (Table 3). This controversial result was possibly the effect of very high precipitation in that year (798.4 mm in April-October). The increase in the soil density led to a reduction of the water capacity parameters. The some trend was observed for tomato soils. In 2008-2009, no significant effects of treatments on water retention parameters were demonstrated in the cucumber cropping seasons.

Table 3

Physical and chemical properties of soils from cucumber plantations covered with biodegradable films, 2008-2010

| **Treatment** | **Bulk density [Mg · m$^{-3}$]** | **Water capacity [% ww]** | **Water capacity [% vw]** | **[% C]** |
|---|---|---|---|---|
| 2008 | | | | |
| *Before planting* | *1.37* | *37.4* | *51.4* | *2.07* |
| Control | 1.39 a | 36.0 a | 49.9 a | 2.01 a |
| PLA | 1.44 a | 35.0 a | 50.4 a | 2.25 a |
| 2009 | | | | |
| *Before planting* | *1.30* | *39.7* | *51.6* | *1.41* |
| Control | 1.34 a | 35.7 a | 47.8 a | 1.44 a |
| PLA | 1.31 a | 38.7 a | 51.1 a | 1.36 a |
| Bionolle | 1.33 a | 37.0 a | 49.1 a | 1.37 a |
| 2010 | | | | |
| *Before planting* | *1.32* | *37.1* | *49.6* | *1.34* |
| Control | 1.11 a | 42.2 b | 46.8 a | 1.35 a |
| PLA | 1.34 b | 34.7 a | 46.6 a | 1.39 b |
| Bionolle | 1.33 b | 35.0 a | 45.0 a | 1.42 b |

$\alpha = 0.05$. Fisher test - the same letter indicate no significant differences between means

### *Soil water-stable aggregates*

High soil aggregate stability is an important factor for improving soil fertility and increasing agronomic productivity. Aggregate analysis may help to explain most aspects of soil water properties, including runoff and infiltration, as well as soil aeration and root growth [37]. Aggregates are susceptible to disruption by physical disturbances such as clay swelling, tillage and rainfall impact [18]. Mulching and covering improve soil structure and enhance soil water-stable aggregate contents by decreasing soil erosion, reducing raindrop impact and increasing the magnitude of soil organic carbon in the soil pool.

In the 2008 tomato season, there were no significant differences between wet-stable amounts of aggregates in any of the size classes under different treatments (Table 4). The sum of wet-stable aggregates in the diameters 0.25-4.0 mm ranged from 83.8% (Control) to 84.1% (PLA). In 2009, the wet-stable aggregate index was high and varied from 94.4% (Control) to 98.7% (PLA). The PLA biofilm significantly increased the amount of large aggregates 4-2.5 mm in diameter (41.8%) in comparison with the bare soil (23.6%) in 2009. An adverse effect for aggregates in the diameter of 0.50-0.25 mm was found. There is evidence that macroaggregates are very susceptible to land use modifications and agricultural practices, and are less stable than microaggregates. They are weakly cemented by SOM [38]. In extremely wet 2010, we measured from 90.8% (PLA) to 94.2% (Control) of water-stable aggregates in soil after tomato harvest. Paradoxically, the results of the 2010 season showed the highest content of large water-stable aggregates 4.0-2.5 mm in diameter (45.6%) in relation to 2009 and 2008 (32.7 and 18.0%, respectively) (Table 4). In extremely wet 2010, the lowest amount of small aggregates was measured in relation to the years 2008 and 2009 (21.5 and 14.3%, respectively). The processes responsible for forming aggregates include drying and wetting, freezing and thawing, tillage and the activity of roots and the soil biota. Soil moisture and wet-dry cycles have a variable effect on aggregation.

Table 4

Percentage of soil water-stable aggregates (means, standard deviation and *LSD* for $\alpha = 0.05$) in soil from the tomato plantation covered with biodegradable film, 2008-2010

| **Treatment** | **Aggregates in diameter of [mm]** | | | | | |
|---|---|---|---|---|---|---|
| | **4.0-2.5** | **2.5-1.5** | **1.5-1.0** | **1.0-0.50** | **0.50-0.25** | **∑ 0.25-4.0** |
| 2008 | | | | | | |
| *Before planting* | *14.4±5.9* | *6.9±2.0* | *18.8±2.8* | *27.4±2.8* | *16.2±1.8* | *83.8* |
| Control | 19.6±3.1 a | 5.3±0.5 a | 10.9±0.3 a | 25.8±1.7 a | 22.1±2.8 a | 83.8 |
| PLA | 18.0±5.0 a | 6.4±1.0 a | 11.7±1.2 a | 26.4±2.5 a | 21.5±2.1 a | 84.1 |
| *mean* | *18.8* | *5.8* | *11.3* | *26.1* | *21.8* | |
| 2009 | | | | | | |
| *Before planting* | 21.3±3.3 | 6.0±0.6 | 14.4±0.9 | 37.7±3.2 | 18.0±1.7 | 97.5 |
| Control | 23.6±1.4 a | 7.9±1.5 a | 13.6±0.2 a | 31.5±2.5 b | 18.2±2.2 b | 94.4 |
| PLA | 41.8±2.6 b | 7.6±0.2 a | 14.0±1.3 a | 25.4±1.8 a | 10.3±0.5 a | 98.7 |
| *mean* | *32.7* | *7.8* | *13.8* | *28.4* | *14.3* | |
| 2010 | | | | | | |
| *Before planting* | 36.6±3.3 | 6.7±0.5 | 13.9±0.9 | 23.8±0.6 | 11.3±2.0 | 92.4 |
| Control | 44.0±2.3 a | 9.3±0.7 a | 15.5±0.9 a | 16.2±1.3 b | 10.0±0.8 b | 94.2 |
| PLA | 41.2±4.0 a | 9.5±1.1 a | 15.3±0.8 a | 14.0±0.5 a | 7.1±0.8 a | 90.8 |
| *mean* | *42.6* | *9.4* | *15.4* | *15.1* | *8.5* | |

During wetting, clay particles tend to disperse in soils and form bridges and coatings while drying. This influence on closer contact between particles increases bridging, especially in the presence of bivalent cations ($Ca^{+2}$ and $Mg^{+2}$) in the clay colloidal complex [18].

In the 2009 and 2010 cucumber cropping seasons, a direct trend to increase the water-stable aggregate index, calculated as a sum of five size classes of aggregates, was observed for covered soil in regard to the control soil (Table 5).

Table 5

Percentage of soil water-stable aggregates (means, standard deviation and *LSD* for $\alpha = 0.05$) in soil from the cucumber plantation covered with biodegradable film, 2008-2010

| **Treatment** | **Aggregates in diameter of [mm]** | | | | | |
|---|---|---|---|---|---|---|
| | **4.0-2.5** | **2.5-1.5** | **1.5-1.0** | **1.0-0.50** | **0.50-0.25** | **∑ 0.25-4.0** |
| 2008 | | | | | | |
| *Before planting* | *22.1±7.9* | *7.2±1.1* | *22.8±1.9* | *21.3±4.8* | *11.4±2.8* | *84.8* |
| Control | 19.6±3.1 a | 5.3±0.5 a | 10.9±0.3 a | 25.8±1.7 a | 22.1±2.8 a | 83.8 |
| PLA | 18.0±5.0 a | 6.4±1.0 a | 11.7±1.2 a | 26.4±2.5 a | 21.5±2.1 a | 84.1 |
| *mean* | *18.8* | *5.8* | *11.3* | *26.1* | *21.8* | |
| 2009 | | | | | | |
| *Before planting* | *30.5±2.6* | *8.2±0.4* | *16.9±1.6* | *26.8±1.7* | *15.0±2.6* | *97.3* |
| Control | 29.0±3.3 a | 10.9±4.8 a | 16.9±3.2 a | 25.9±3.8 a | 16.9±5.3 a | 91.3 |
| PLA | 34.5±1.8 b | 8.9±0.8 a | 15.3±1.3 a | 24.0±1.1 a | 12.0±0.7 a | 94.8 |
| Bionolle | 38.4±2.7 b | 9.7±0.4 a | 15.1±1.2 a | 21.5±1.2 a | 11.6±0.8 a | 96.2 |
| *mean* | *36.2* | *9.9* | *15.2* | *21.8* | *13.2* | |
| 2010 | | | | | | |
| *Before planting* | *36.6±3.4* | *6.7±0.5* | *13.9±0.9* | *23.8±0.6* | *11.3±1.7* | *92.4* |
| Control | 44.1±4.7 a | 7.52±0.95 b | 13.4±2.3 a | 14.6±2.0 a | 10.9±0.6 a | 90.5 |
| PLA | 44.4±2.2 a | 7.77±0.21 b | 12.2±0.7 a | 15.4±0.3 a | 10.7±1.1 a | 90.6 |
| Bionolle | 48.0±4.3 b | 5.97±0.90 a | 10.7±2.0 a | 19.0±1.9 b | 11.5±0.7 a | 95.3 |
| *mean* | *45.5* | *7.1* | *12.1* | *16.3* | *10.6* | |

In 2009, 94.8 and 96.2% of the water-stable aggregate index were noted, respectively, for PLA and Bionolle biofilms in relation to the control (91.3%). In 2010 the highest value for this parameter was found for biodegradable Bionnole (95.3%) in comparison with the control soil (90.5%).

In 2010, similar to the tomato study, the highest content of water-stable aggregates in cucumber soils was measured for 4.0-2.5 mm diameters (47.3%) in relation to 2008 and 2009 (18.8 and 36.2%, respectively). Consequently, the percentage of small wet-stable aggregates (∅ 1.0-0.5 and 0.5-0.25 mm) decreased in the wetter 2010. The highest amount of the smallest aggregates were found in 2008 (21.8%) followed by 2009 (13.2%), while the lowest was in 2010 (10.6%) (Table 5).

In 2009 of the cucumber season, the PLA and Bionolle biofilms, and the Bionolle biofilm in 2010, significantly increased the percentage of large aggregates (4.0-2.5 mm in diameter) in relation to the control treatment (Table 5). The uncovered soil was directly exposed to the destructive effects of rain (raindrop splash), wind and solar radiation. The film covering (physical protection) showed a beneficial effect on the number of large soil water-stable aggregates. Indeed, the water stability of macroaggregates is known to prevent the detachment of easily transportable particles, and thereby surface clogging and runoff. It is generally considered that large aggregates are more indicative of a good soil structure for most agricultural purposes (availability of $O_2$, water and resistance to penetration by roots and shoots in seedbeds created by tillage) than small aggregates [18]. Soil structure not only affects the ability of roots to grow and to supply the leaves with water and nutrients. Macropores provide places for microorganisms, both symbiotic and pathogenic [39]. The process of soil aggregate stabilisation is complex and involves a variety of binding mechanisms. Plant roots and the organisms that live in the soil are influential both in the creation of pores and aggregates. Jastrow et al [40] demonstrated the importance of fine roots and mycorrhizal hyphae as driving factors for macroaggregate stabilisation. Under biofilms, more stable aggregates were probably formed in favourable conditions for rooting and intense soil microbial activity.

### *Soil organic carbon*

The soil organic carbon (OC) content in the analysed tomato soils ranged from 1.35 to 2.25% (Table 2). At the cucumber plantation we determined from 1.34 to 2.25% of OC (Table 3). The obtained results do not point out clear differences between treatments. However in 2010, a higher organic carbon amount than in the control treatments was noted on cucumber fields under PLA and Bionolle mulch. A similar observation was found in leak and onion soils under polypropylene and Bionolle film covering [41]. Nevertheless, it can be concluded that the use of soil film covering systems that lead to greater organic matter contents also results in higher values of soil physical quality as measured by soil structure stability. Stable microaggregates are bound to form macroaggregates with organic compounds. Soil organic matter forms complexes with primary mineral particles and secondary structural units [18]. The consequence of the high organic matter content is good soil structure and high strength in wet conditions [42]. Candan and Broquen [38] showed a significant relationship between aggregate stability and organic carbon ($r = 0.57$). Management practices include film covering and mulching a moderate moisture and temperature regime in the soil and altering the biogeochemical cycling of C, and can result in organic matter accumulation [7, 43]. The biological status of the soil depends strongly on the physical and chemical conditions of the soil.

Generally, relatively high organic matter and clay content could explain the high wet aggregate stability in arable silty clay soils from the tomato and cucumber plantations.

### *Chemical soil analyses*

The data from the soil chemical analysis are presented in Tables 6-9. In both tomato and cucumber cropping seasons, the soil pH was near 7.0 or higher, and any significant differences among the treatments were not observed (Tables 6 and 7). A high level of bivalent cations (about 1000-2000 mg Ca and 100-200 mg Mg $\cdot$ $m^{-3}$ of soil) encouraged high water-stable aggregate content irrespective of the treatments. Bivalent cations improve the soil structure by binding clay and soil organic matter particles [44, 45]. A high pH raises the negative surface charge on clay particles and flocculate dispersive clays [46]. Increased pH often resulted in increased microbial activity and higher organic matter content and consequently encouraged soil particle aggregation [45].

Table 6

Acidity, pH, electrical conductivity, *EC* and available form of macronutrients [mg · dm$^{-3}$] in soil from the tomato plantation covered with biodegradable film, 2008-2010

| **Treatment** | **pH $H_2O$ [-]** | **pH KCl [-]** | ***EC* [μS · cm$^{-1}$]** | **N-$NH_4$** | **N-$NO_3$** | **P** | **K** | **Mg** | **Ca** |
|---|---|---|---|---|---|---|---|---|---|
| 2008 | | | | | | | | | |
| *Before planting* | *7.17* | *6.35* | *620.0* | *11.8* | *70.1* | *62.1* | *73.6* | *140.3* | *1734* |
| Control | 7.27 | 6.52 | 323.0 | 0.4 | 9.5 | 7.5 | 59.3 | 93.4 | 2249 |
| PLA | 7.52 | 6.66 | 186.5 | 1.1 | 6.6 | 6.6 | 82.6 | 96.9 | 1773 |
| 2009 | | | | | | | | | |
| *Before planting* | *6.63* | *5.77* | *155.6* | *3.4* | *15.7* | *55.1* | *225* | *123* | *1164* |
| Control | 6.77 | 5.90 | 136.7 | 1.9 | 9.3 | 34.7 | 89.4 | 99 | 978 |
| PLA | 7.07 | 6.10 | 90.5 | 3.1 | 4.9 | 30.2 | 81.8 | 106 | 1025 |
| 2010 | | | | | | | | | |
| *Before planting* | *7.58* | *6.45* | *127.5* | *0.70* | *2.30* | *45.3* | *129* | *241* | *2031* |
| Control | 7.86 | 6.89 | 111.0 | 0.68 | 1.50 | 53.8 | 118 | 185 | 1736 |
| PLA | 7.84 | 6.89 | 130.0 | 1.02 | 2.20 | 69.8 | 207 | 149 | 2396 |

Electrical conductivity estimates the concentration of ions in the soil solution, and predominantly consists of the cations sodium, calcium, potassium and magnesium, and the anions chlorines, sulphates and bicarbonates. Soils under PLA and Bionolle covers had low ion concentrations in relation to the bare soils. These results were recorded for tomato (with the exception of 2010) and cucumber fields in all years.

Table 7

Acidity, pH, electrical conductivity, *EC* and available form of macronutrients [mg · dm$^{-3}$] in soil from the cucumber plantation covered with biodegradable film, 2008-2010

| **Treatment** | **pH $H_2O$ [-]** | **pH KCl [-]** | ***EC* [μS · cm$^{-1}$]** | **N-$NH_4$** | **N-$NO_3$** | **P** | **K** | **Mg** | **Ca** |
|---|---|---|---|---|---|---|---|---|---|
| 2008 | | | | | | | | | |
| *Before planting* | *7.17* | *6.35* | *620.0* | *11.8* | *70.1* | *62.1* | *73.6* | *140.3* | *1734* |
| Control | 7.27 | 6.52 | 323.0 | 0.4 | 9.5 | 7.5 | 59.3 | 93.4 | 2249 |
| PLA | 7.52 | 6.66 | 186.5 | 1.1 | 6.6 | 6.6 | 82.6 | 96.9 | 1773 |
| 2009 | | | | | | | | | |
| *Before planting* | *6.29* | *5.82* | *421.3* | *30.0* | *91.0* | *48.9* | *95* | *128* | *1097* |
| Control | 6.87 | 6.02 | 232.7 | 1.7 | 17.3 | 35.2 | 115 | 104 | 1313 |
| PLA | 6.90 | 5.96 | 87.6 | 1.6 | 9.5 | 31.8 | 69 | 142 | 1262 |
| Bionolle | 6.93 | 6.01 | 91.5 | 2.8 | 10.5 | 36.2 | 71 | 124 | 1167 |
| 2010 | | | | | | | | | |
| *Before planting* | *7.02* | *6.13* | *67.3* | *0.76* | *6.90* | *40.5* | *86.0* | *116.0* | *1075* |
| Control | 7.19 | 6.15 | 182.0 | 1.39 | 4.80 | 31.4 | 83.3 | 129.8 | 1117 |
| PLA | 7.28 | 5.88 | 91.0 | 0.84 | 9.00 | 39.5 | 89.2 | 137.0 | 1204 |
| Bionolle | 7.18 | 6.02 | 110.0 | 0.91 | 6.50 | 36.3 | 94.6 | 125.0 | 1027 |

The most probable explanation of these results is that better plant growth and higher plant biomass in places where moisture and temperature were most favourable (data not presented) resulted in higher nutrient uptake. The benefits of polyethylene mulch to crop production are well documented and include greater root growth and nutrient uptake [11].

The results of the soil chemical analysis demonstrated the low impact of treatments on macro- and microelement concentration measured after tomato and cucumber harvesting.

Mulching is known to contribute to augmenting mineral nutrient availability to enhance nitrification. Mulching creates better biological activates and thus has a consequent beneficial effect on soil fertility [11]. In our study, $NH_4$-N concentration in tomato soils after harvest ranged from 1.02 to 3.1 mg N for the PLA treatment and from 0.4-1.9 mg N for the bare soils. Nitrate concentration varied between 2.2-6.6 mg $NO_3$-N for the PLA treatment and 1.5-9.5 mg N for the control soils (Table 6). In 2008-2009, the cucumber plantation soil with PLA and Bionolle treatments had a slightly elevated $NH_4$-N concentration and lowered $NO_3$-N content (Table 7). An inverse effect of this was observed in the extremely wet 2010.

Table 8

Micronutrient and heavy metals content [mg · $kg^{-1}$ d.m. of soil] in soil from the tomato plantation covered with biodegradable films, 2008-2010

| **Treatment** | **Zn** | **Cu** | **Mn** | **Fe** | **B** | **Cd** | **Pb** | **Cr** | **Ni** |
|---|---|---|---|---|---|---|---|---|---|
| 2008 | | | | | | | | | |
| *Before planting* | *57.2* | *5.88* | *276.5* | *2428* | *1.84* | *1.06* | *33.4* | *1.38* | *2.97* |
| Control | 48.5 | 5.92 | 221.8 | 2347 | 1.33 | 0.87 | 24.9 | 1.15 | 3.19 |
| PLA | 42.6 | 4.20 | 183.2 | 2356 | 1.00 | 0.74 | 17.9 | 0.88 | 3.12 |
| 2009 | | | | | | | | | |
| *Before planting* | *42.3* | *5.81* | *182* | *1844* | *1.47* | *0.92* | *25.2* | *1.34* | *2.73* |
| Control | 51.3 | 5.33 | 161 | 1739 | 2.10 | 0.96 | 29.6 | 1.30 | 2.61 |
| PLA | 48.4 | 5.67 | 182 | 1846 | 2.57 | 0.97 | 30.4 | 1.43 | 2.82 |
| 2010 | | | | | | | | | |
| *Before planting* | *83.2* | *6.81* | *170.3* | *1467* | *2.67* | *3.53* | *36.5* | *1.04* | *1.96* |
| Control | 74.8 | 7.80 | 196.5 | 1768 | 2.77 | 1.06 | 34.6 | 1.24 | 2.24 |
| PLA | 66.9 | 8.45 | 195.9 | 1751 | 2.65 | 1.02 | 33.3 | 1.32 | 2.30 |

Table 9

Micronutrient and heavy metals content [mg · $kg^{-1}$ d.m. of soil] in soil from the cucumber plantation covered with biodegradable films, 2009-2011

| **Treatment** | **Zn** | **Cu** | **Mn** | **Fe** | **B** | **Cd** | **Pb** | **Cr** | **Ni** |
|---|---|---|---|---|---|---|---|---|---|
| 2008 | | | | | | | | | |
| *Before planting* | *47.8* | *5.12* | *247.4* | *2250* | *1.54* | *0.87* | *27.2* | *1.10* | *3.09* |
| Control | 48.5 | 5.92 | 221.8 | 2347 | 1.33 | 0.87 | 24.9 | 1.15 | 3.19 |
| PLA | 42.6 | 4.20 | 183.2 | 2356 | 1.00 | 0.74 | 17.9 | 0.88 | 3.12 |
| 2009 | | | | | | | | | |
| *Before planting* | *42.6* | *5.83* | *184* | *1841* | *1.44* | *0.89* | *24.9* | *1.32* | *2.75* |
| Control | 54.5 | 7.39 | 233 | 1878 | 2.38 | 1.03 | 32.4 | 1.33 | 2.82 |
| PLA | 61.4 | 6.70 | 230 | 2042 | 1.81 | 1.07 | 33.2 | 1.42 | 3.10 |
| Bionolle | 51.2 | 5.94 | 204 | 1984 | 1.64 | 1.07 | 31.4 | 1.38 | 2.89 |
| 2010 | | | | | | | | | |
| *Before planting* | *46.2* | *5.27* | *162.5* | *1459* | *1.63* | *0.98* | *27.1* | *1.22* | *2.66* |
| Control | 46.5 | 6.28 | 159.5 | 1520 | 1.53 | 0.99 | 26.5 | 1.20 | 2.60 |
| PLA | 47.0 | 5.75 | 138.5 | 1450 | 1.36 | 1.00 | 27.2 | 1.13 | 2.76 |
| Bionolle | 50.7 | 6.29 | 170.2 | 1619 | 1.58 | 0.97 | 28.6 | 1.25 | 2.87 |

In both tomato and cucumber cropping seasons, plant covering did not affect heavy metal concentration in soils (Tables 8 and 9). Li at al [47] studied the effect of plastic mulching on copper and zinc bioavailability in the soil in Chinese cabbage. Results showed that the mulched field had lower soil pH and $SO_4^{2-}$ contents; however, this did not have a significant effect on the distribution and translocation of Cu, while it did affect Zn.

## Conclusions

There is an increasing interest in the use of degradable plastic mulching for protected cultivation. Determining the effects of biodegradable synthetic polymer mulch in crop production with regards to microclimate modification, physical, chemical and biological properties of soil, weed control and pest and disease management requires many studies. Little is known about how biofilm covering affects physical and chemical soil parameters, however soil quality has been of increasing concern in recent years.

The soils in our study had high clay content, neutral or slight alkaline pH, and a high level of available Ca and Mg concentration. These parameters guaranteed a very good soil structure (wet-stable). The obtained results generally showed that biofilm covering with PLA increased the amount of large aggregates and decreased the percentage of the smallest size aggregates in soils in tomato and cucumber cropping, though only in 2009. In the wet year 2010, the inverse effect was observed. The Bionolle treatment in the cucumber plantation also increased large aggregates in both 2009 and 2010. During wet conditions, mulching increased bulk soil density and decreased soil water capacity in the cucumber field.

It should be emphasised that the obtained results were very dependent on weather conditions. This study only addressed arable silty clay soil managed conventionally using typical crop rotation, tillage machinery and levels of vehicular traffic. We observed a slight tendency to encourage some physical soil parameters with biofilm treatments. The investigation should be carry out in different soils and climatic conditions. As stated elsewhere, individual soils may differ significantly in behaviour from typical values and trends.

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## References

[1] Lamont WJ. Plastic mulches for the production of vegetable crops. Hort Tech. 1993;3:35-39. http://horttech.ashspublications.org/content/3/1/35.full.pdf.

[2] Krochta JM, De Mulder-Johnston CLC. Biodegradable polymers from agricultural products. Agricultural Materials as Renewable Resources. ACS Symposium Series. 1996;647:120-140. DOI: 10.1021/bk-1996-0647.ch009.

[3] Plackett D. Biopolymers: New Materials for Sustainable Films and Coatings. Wiley&Sons, United Kingdom; 2011. DOI: 10.1002/9781119994312.fmatter.

[4] Green DS, Kruger EL, Stanosz GR. Effects of polyethylene mulch in a short-rotation, poplar plantation vary with weed-control strategies, site quality and clone. Forest Ecol Manage. 2003;173:251-260. DOI: 10.1016/S0378-1127(02)00003-8.

[5] Greer L, Dole JM. Aluminum foil, aluminum-painted, plastic and degradable mulches increase yields and decrease insect-vectored viral diseases of vegetables. Hort Tech. 2003;13:276-284. http://horttech.ashspublications.org/content/13/2/276.full.pdf

[6] Moreno MM, Moreno A. Effect of different biodegradable and polyethylene mulches on soil properties and production in a tomato crop. Sci Hort. 2008;116:256-263. DOI: 10.1016/j.scienta.2008.01.007.

[7] Moreno MM, Moreno A, Mancebo I. Comparison of different mulch materials in a tomato (Solanum lycopersicum L.) crop. Spanish J Agr Res. 2009;7:454-464. DOI: 10.5424/sjar/2009072-1500.

[8] Anzalone A, Cirujeda A, Aibar J, Pardo G, Zaragoza C. Effect of biodegradable mulch materials on weed control in processing tomatoes. Weed Technol. 2010;24(3):369-377. DOI: 10.1614/WT-09-020.1.

[9] Olsen JK, Gounder RK. Alternatives to polyethylene mulch film-a field assessment of transported materials in capsicum (*Capsicum annum* L). Austral J Expt Agr. 2001;41:93-103. DOI: 10.1071/EA00077.

[10] Chiellini E, Solaro R. Recent advances in biodegradable polymers and plastics. 7thWorld Conference on Biodegradable Polymers and Plastics, Italy 2003. Book Abstracts 2003 International Union of Pure and Applied Chem. DOI: 10.1002/masy.200350701.

[11] Kasirajan S, Ngouajio M. Agron. Polyethylene and biodegradable mulches for agricultural applications: a review. Sustain Dev. 2012;32:501-529. DOI: 10.1007/s13593-012-0132-7.

[12] Brown RP. Polymers in agriculture and horticulture. iSmithers Rapra Publishing 2004. http://www.smithersrapra.com/ products/books/browse-by-category/review-reports/polymers-in-agriculture-and-horticulture.

[13] Malinconico M, Immirzi B, Santagata G, Schettini E, Vox G, Mugnozza SG. An overview on innovative biodegradable materials for agricultural applications. In: Moeller HW, editor. Progress in Polymer Degradation and Stability Research. New York: Nova Science; 2008. https://www.researchgate.net/publication/260017619_An_overview_on_innovative_biodegradable_materials_for_agricultural_applications.

[14] Miles C, Wallace R, Wszelaki A, Martin J, Cowan J, Walters T, et al. Deterioration of potentially biodegradable alternatives to black plastic mulch in three tomato production regions. Hort Sci. 2012;47(9):1270-1277. http://hortsci.ashspublications.org/content/47/9/1270.full.

[15] Ren J. Biodegradable Polylactic Acid: Synthesis, Modification, Processing and Application. London. Springer Publisher 2011. DOI: 10.1007/978-3-642-17596-1.

[16] Stevens ES. What makes green plastics green? Biocycle. 2003;44(3):24-27. http://www.bpiworld.org/resources/documents/biocycle_green_plastics.pdf.

[17] Rudnik E. Compostable Polymer Materials. Amsterdam: Elsevier Publishing; 2008. DOI: 10.1016/B978-008045371-2.50004-4.

[18] Bronic CJ, Lal R. Soil structure and management: a review. Geoderma. 2005;124:3-22. DOI: 10.1016/j.geoderma.2004.03.005.

[19] Caravaca F, Lax A, Albaladejo J. Aggregate stability and carbon characteristics of particle-size fractions in cultivated and forested soils of semiarid Spain. Soil Till Res. 2004:78:83-90. DOI: 10.1016/j.still.2004.02.010.

[20] Díaz-Zorita M, Perfect E, Grove JH. Disruptive methods for assessing soil structure. Soil Till Res. 2002;64:3-22. DOI: 10.1016/S0167-1987(01)00254-9.

[21] Li Z, Zhang RH, Wang XJ, Wang JP, Zhang CP, Tian CY. Carbon dioxide fluxes and concentrations in a cotton field in north western China: Effects of plastic mulching and drip irrigation. Pedosphere. 2011;21:178-185. DOI: 10.1016/S1002-0160(11)60116-1.

[22] Li F, Liu C, Yang Y, Bi X, Liu J. Effect of mini greenhaose on the transortation of heavy metals. Chinese J Geochem. 2006;25:212. DOI: 10.1007/BF02840154.

[23] Filippi F, Magnani G, Guerrini S, Ranghino F. Agronomic evaluation of green biodegradable mulch on melon. Italian J Agron. 2011;6:111-116. DOI: 10.4081/ija.2011.e18.

[24] Laverde G J. Plastic Film Sheeting. 2002;18(4):269-277. DOI: 10.1177/8756087902034748.

[25] Ngouajio M, Auras R, Fernandez RT, Rubino M, Counts JW, Kijchavengkul T. Field performance of aliphatic-aromatic copolyester biodegradable mulch films in a fresh market tomato production system. Hort Tech. 2008;18(4):605-610. http://horttech.ashspublications.org/content/18/4/605.full.

[26] Minuto G, Guerrini S, Versari M, Pisi L, Tinivella F, Bruzzone C, et al. Use of biodegradodable mulching in vegetable production. IFOAM Organic World Congress 2008 - Book Abstracts 2008.

[27] Martin-Closas L. Biodegradable mulching in an organic tomato production system. Acta Hort. 2008;767:267-273. DOI: 10.17660/ActaHortic.2008.767.28.

[28] Kerven GL, Menzies NW, Geyer MD. Soil carbon determination by high temperature combustion - A comparison with dichromate oxidation procedures and the influence of charcoal and carbonate carbon on the measured value. Commun. Soil Sci Plant Anal. 2000;31:1935-1939. DOI: 10.1080/00103620009370551.

[29] Ostrowska A, Gawliński S, Szczubiałka Z. Soil and Plant Analysis Procedures. Warszawa: Institute Environ Protect; 1991.

[30] PN-R-04032. 1998. Soils and mineral materials. Soil sampling and granulometric analysis. Warszawa: Polish Committee for Standarization. sklep.pkn.pl/pn-r-04032-1998p.html.

[31] Yoder RE. A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses. J Am Soc Agron. 1936;28:337-351. https://dl.sciencesocieties.org/publications/aj/abstracts/28/5/AJ0280050337?access=0&view=pdf.

[32] Nowosielski O. Fertilizer Recommendations for Horticulture Crops. Warszawa: PWRiL; 1988.

[33] PN-EN ISO 13395:2001. Water quality - Determination of nitrite and nitrate nitrogen and them sum using flow analysis (CFA and FIA) spectrometric detection. Warszawa: Polish Committee for Standarization.

[34] Reynolds WD, Drury CF, Yang XM, Tan CS. Optima soil physical quality inferred through structural regression and parameter interactions. Geoderma. 2008;146:466-474. DOI: 10.1016/j.geoderma.2008.06.017.

[35] Reynolds WD, Drury CF, Yang XM, Fox CA, Tan CS, Zhang TQ. Land management effects on the near-surface physical quality of a clay loam soil. Soil Tillage Res. 2007;96:316-330. DOI: 10.1016/j.still.2007.07.003.

[36] Ndubuisi MC. Physical properties of an Ultisol under plastic film and no-mulches and their effect on the yield of maize. J Am Sci. 2009;5:25-30. DOI: 10.7537/j.issn.1545-1003.

[37] Nimmo JR, Perkins KS. Aggregate stability and size distribution. In: Dane JH, Topp GC. Methods of Soil Analysis. Part 4 - Physical methods. Madison, Wisconsin: Soil Sci Society of America; 2002:317-328.

[38] Candan F, Broquen P. Aggregate stability and related properties in NW Patagonian Andisols. Geoderma. 2009;154:42-47. DOI: 10.1016/j.geoderma.2009.09.010.

[39] Passioura JB. Soil conditions and plant growth. Plant Cell Environ. 2002;25(2):311-318. DOI: 10.1046/j.0016-8025.2001.00802.x.

[40] Jastrow JD, Miller RM, Lussenhop J. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biol Biochem. 1998;30:905-916. DOI: 10.1016/S0038-0717(97)00207-1.

[41] Domagała-Świątkiewicz I, Siwek P. The effect of direct covering with biodegradable nonwoven film on the physical and chemical properties of soil. Pol J Environ Stud. 2013;22(3):35-42. http://www.pjoes.com/pdf/22.3/Pol.J.Environ.Stud.Vol.22.No.3.667-674.pdf

[42] Six J, Paustian K, Elliott ET, Combrink C. Soil structure and soil organic matter: I. Distribution of aggregate size classes and aggregate associated carbon. Soil Sci Soc Am J. 2000;64:681-689. DOI: 10.2136/sssaj2000.642681x.

[43] Ham JM, Huitenberg GJ, Lamont WJ. Optical properties of plastic mulches affect the field temperature regime. J Am Soc Hort Sci. 1993;118:188-193. http://journal.ashspublications.org/content/118/2/188.full.pdf+html.

[44] Zhang XC, Norton LD. Effect of exchangeable Mg on saturated hydraulic conductivity, disaggregation and clay dispersion of disturbed soils. J Hydrol. 2002;260:194-205. DOI: 10.1016/S0022-1694(01)00612-6.

[45] Briedis C, Sá JC, Caires EF, Navarro J, Inagaki TM, Boer A, et al. Soil organic matter pools and carbon-protection mechanisms in aggregate classes influenced by surface liming in a no-till system. Geoderma. 2012;170:80-88. DOI: 10.1016/j.geoderma.2011.10.011.

[46] Bolan NS, Naidu R, Syers JK, Tillman RW. Surface charge and solute interactions in soils. Adv Agron. 1999;67:87-140. DOI: 10.1016/S0065-2113(08)60514-3.

[47] Li F, Liu C, Yang Y, Chen X, Jiang Q. Effect of plastic mulching on translocation characteristics of copper and zinc in soil and Chinese cabbage (Brassica chinensis) system. J Ecol Rural Environ. 2009;25:54-58. DOI: CNKI:SUN:NCST.0.2009-02-014.

# WPŁYW MULCZOWANIA Z WYKORZYSTANIEM WŁÓKNIN BIODEGRADOWALYCH NA JAKOŚĆ GLEBY W UPRAWIE WARZYW CIEPŁOLUBNYCH

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**Abstrakt:** Mulcze z tworzyw sztucznych tworzą fizyczną barierę dla parowania wody, pozwalają zachować dobrą strukturę gleby, kontrować zachwaszczenie oraz chronią rośliny przed zanieczyszczeniami glebowymi. Usuwanie i unieszkodliwianie po zbiorach odpadów z tworzyw sztucznych nienadających się do recyklingu i niepodlegających degradacji jest trudne i kosztowne. W ostatnich latach stosowanie alternatywnych włóknin biodegradowalnych jako mulczy lub okryć w uprawie roślin cieszy się coraz większym zainteresowaniem. Praca jest prezentacją wyników dotyczących właściwości fizycznych i chemicznych gleb mulczowanych biodegradowalnymi włókninami. W badaniach oceniano zmiany niektórych fizycznych właściwości gleby, takich jak: gęstość objętościowa, pojemność wodna i wodoodporność agregatów glebowych po uprawie pomidora i ogórka. Do ściółkowania gleby wykorzystano włókninę PLA (polilaktyd) i Bionolle (poliester butylenu, alifatycznego glikolu, kwasu bursztynowego i adypinowego). Chemiczne właściwości gleby oceniano, oznaczając w nich odczyn, stężenie soli, zawartość węgla organicznego, dostępne dla roślin składniki pokarmowe oraz zawartość metali ciężkich. Okrywanie powierzchni gleby włókninami z polimerów biodegradowalnych wpłynęło korzystnie na strukturę gleby. Biowłókniny PLA i Bionolle zwiększały ilość makroagregatów wodoodpornych, a zmniejszały odsetek najmniejszych agregatów w glebie, jednak obserwowane zmiany były silnie modyfikowane poprzez warunki atmosferyczne w poszczególnych latach prowadzenia badań. W bardzo mokrym roku okrywanie gleby zwiększało jej gęstość objętościową oraz zmniejszało pojemność wodną. Mulczowanie gleby z użyciem biodegradowalnych włóknin nie wpływało znacząco na zawartość składników pokarmowych i metali ciężkich w glebie. Obserwowano nieznaczny spadek EC w glebach pod ściółkami w porównaniu do gleby nieokrywanej.

**Słowa kluczowe:** biowłókniny, gęstość objętościowa, agregaty wodoodporne, materia organiczna, pomidor, ogórek

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# ENVIRONMENTAL EFFECTS OF TANNERY WASTE INCINERATION IN A TUNNEL FURNACE SYSTEM

## EFEKTY ŚRODOWISKOWE PROCESU SPALANIA ODPADÓW GARBARSKICH W PIECU TUNELOWYM

**Abstract:** Processes applied commonly in the leather tanning industry pose a serious impact on the environment. Leather processing requires a substantial input of energy, water and chemicals. Moreover, it generates significant amounts of liquid and solid waste. Processing of 1 Mg raw hides gives up to 600-700 kg waste, the considerable amounts of which are solid tannery waste generated during or after the proper tanning process. Such waste contains chromium compounds, which are commonly used as tanning agents. Utilization of solid tannery waste is a severe problem for tanneries, the main method applied practically is landfilling. Solid tannery waste exhibits a relatively high heating value (approx. 12-14 MJ/kg), but also a considerable water content (for some types of waste up to 40-50%) as well as a chromium content reaching approx. 3%. Incineration of such waste is possible, it requires maintaining the proper process conditions in order to minimize the negative impact on the environment, however. The research was carried out using a prototype tunnel furnace designed especially for solid tannery waste incineration. The article presents a brief overview of the experimental installation and the research conditions as well as environmental effects of the process in the aspect of flue-gas composition, energy and mass balance, regarding particularly the chromium content in the process residues. As the research revealed, by the right process conditions setting it is possible to obtain chromium(III) oxide in the ash, which can be used as a chromium ore substitute in the chemical or metallurgical industry.

**Keywords:** tannery waste, tunnel furnace, waste incineration, chromium(III) oxide

## Introduction

Processing of raw hides into leather is one of the oldest and most important industrial activity in the history of the mankind. It can be summarized as the conversion of raw hides or skins, which are prone to natural decay processes, into the stable and durable material, namely the leather [1]. Further on, the leather can be used for manufacturing a wide range of products. Through the ages the technology of tanning was changing, especially in the aspect of tanning agents which varied form urine to natural substances extracted from plants. The currently applied technology is based upon the process developed in the 19th century, in which chromium(III) salts are used as the tanning agents. Nowadays more than 90% tanneries worldwide apply chromium salts during the tanning process [1]. This technology allows to produce products of high quality, it brings about a severe burden for the environment, however. The leather production requires a significant input of energy, process water and chemicals. There is a huge amount of dangerous by-products generated as well, including wastewater (with high COD and inorganic salts concentration), solid waste and odours [1, 2]. Figure 1 presents the input/output overview of the process.

In recent years many developments have been presented in aspect of tannery wastewater treatment, including biological oxidation, membrane processes and others [3].

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One of the main problem for tanneries is the proper solid waste management, however. As presented in Figure 1, processing of 1 Mg rawhide generates up to 730 kg of solid waste. There are different types of such waste, depending on the operations, during which waste is generated. In many cases the only technology applied for waste treatment is landfilling, which has a negative impact on the environment [1, 2].

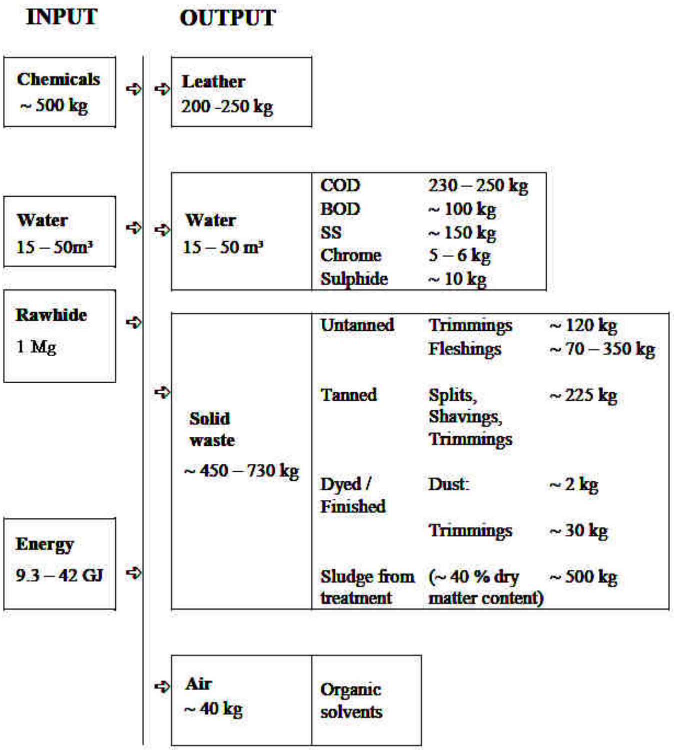

Fig. 1. Input/output overview for a conventional chrome-tanning process for bovine salted hides per 1 Mg of raw hide processed [4]

The main aim of this work was to present the technology of solid tannery waste incineration developed in cooperation with Faculty of Chemical Engineering and Technology, Krakow University of Technology, as well as to discuss the results of pilot tests and the environmental effects of the process.

## Tannery waste

### *Types of tannery waste*

The production of leather involves many single operations. The most important one is the proper tanning during which tanning agent compounds crosslink collagen fibres of hides. It assures the biological stability of the final product. In the group of operation prior to the proper tanning (knows as beamhouse operations) there are generated such types of waste as hide trimmings (unused pieces of hides) and fleshings (rest of the tissues scrapped mechanically off). They are biologically unstable and prone to decay processes. The types of waste generated during the operations conducted after the proper tanning, namely leather trimmings, splits, shavings and dust, are of the same biological stability as the leather itself, as they contain the same chromium compounds in their inner structure. Solid waste comes also form wastewater sludge [1, 2, 4, 5].

### *Amounts o tannery waste*

As the FAO statistics reveal [6], the worldwide annual production of bovine hides and skins amounts to about 6 million Mg (wet salted weight) - see Table 1. Sheepskins, lambskins, goatskins and kidskins are processed for 600 thousand Mg (dry weight). It means that, roughly calculating, about 3.5-4.0 million Mg solid waste is generated annually. In Poland the production of bovine hides in recent years is stable and amounts to approx. 28 thousand Mg. Other sources give information on the amounts of leather produced in Poland - in years 2012 and 2013 it was 14.4 and 13.9 thousand Mg, respectively [7]. Based upon these values the amounts of solid tannery waste generated annually in Poland can be estimated giving not less than 15 thousand Mg waste.

Table 1

Production of bovine hides and skins (wet salted weight [thousand Mg]) [6]

| | **2001-2003 average** | **2007** | **2010** | **2011** | **2012** | **2013** | **2014 (prel.)** |
|---|---|---|---|---|---|---|---|
| World | 5 934.0 | 6 777.0 | 6 416.5 | 6 390.2 | 6 400.3 | 6 574.4 | 6 617.1 |
| Europe | 1 149.0 | 1 030.2 | 1 012.1 | 989.1 | 959.1 | 965.5 | 977.7 |
| EU (27) | 668.7* | 702.2 | 700.6 | 699.2 | 670.3 | 668.2 | 669.3 |
| Poland | 30.7 | 24.6 | 26.6 | 33.8 | 28.3 | 28.2 | 28.3 |

* EU (15)

### *Characteristics of tannery waste*

Tannery wastes consist mostly of organic matter, which indicates that its incineration is feasible. Table 2 presents the characteristics of tannery waste which is of importance in the aspect thermal treatment.

Table 2

Characteristics of tannery waste [5, 8]

| **Types of waste** | | **Lower heating value** | **Water content** | **Combustion residue - ash** | **Total organic substances content in dry matter** |
|---|---|---|---|---|---|
| | | **[kJ/kg]** | **[%w/w]** | **[%w/w]** | **[%w/w]** |
| Untanned | Hide trimmings | 7 753 | 59.9 | 4.7 | 86.9 |
| | Fleshings | 8 952 | 59.5 | 4.6 | 91.4 |
| Tanned | Shavings | 6 663 | 53.6 | 7.8 | 87.5 |
| | Buffing dust | 16 953 | 14.3 | 6.2 | 87.4 |
| | Leather trimmings | 19 772 | 10.2 | 4.7 | 87.7 |
| Sludge | | 716 | 54.7 | 24.3 | 65.5 |

Worth noticing are: relatively high heating values, small amounts of inorganic matter (apart from sludge), as well as high water content rates in cases of some waste types, which implies the necessity of waste drying prior to the incineration process. The tanned waste (trimmings, shavings, dust) contains approx. 3-4% chromium, which comes from the proper tanning operation [1, 8].

## Experimental

### *The installation*

In [8] the concept of tannery waste incineration was described. The idea was to use a tunnel furnace system to enable the drying of waste in the first zone and assure proper conditions of combustion. In order to carry out research an experimental installation was built. In a tunnel furnace it is possible to maintain thermal conditions which continuously change along the length of the tunnel. It enables a virtual division of the tunnel into a few sections, in which successive processes take place: waste drying, volatilization of organic matter and combustion.

The system was manufactured by Firma Czylok, Poland. The tunnel is approx. 7 m long. The waste is put in special containers with perforated bottoms (to allow air circulation), which are transmitted through the tunnel by a roller system. Preheating and maintaining the temperature during the process is possible due to the three sections of electric heaters. The nominal power of the total system is 78 kW. The system is equipped with temperature, pressure and oxygen sensors, which give information about temperature at several points of the system, pressure at the entrance and in the combustion section, as well as oxygen concentration in the flue gas. The heat exchanger enables cooling the flue gas, which eventually goes out through a chimney equipped with measurements inlets for flue gas analyzers. The detailed information on the system can be found in [7].

### *Flue gas analysing*

In order to measure the concentration of contaminants in the flue gas following instruments were applied:

- AWE-PW analyzer (producer, ZAM Kety sp. z o.o., Poland) for analysis of VOC concentration in the flue gas;
- Ecom-Sg Plus analyzer (producer: rbr Computertechnik GmbH, Germany) for measurements of $SO_2$, NO, $NO_2$ and CO in the flue gas;
- P-10ZA analyzer (producer, ZAM Kety sp. z o.o., Poland) for the determination of flue-ash concentration in the flue gas.

### *Process conditions*

There is a wide range of process conditions settings, which vary depending on types and amounts of waste processed, amounts of process air, temperatures in the successive zones of the tunnel, process air temperature, velocity of transmitting containers with waste along the tunnel, etc. For the purpose of the aim of this work such parameters were set which enable the stable and controllable work of the installation. It was possible due to long period of initial tests which helped the staff to learn how the process varies depending on changes of the input parameters.

The incinerated waste was: leather trimmings, shavings and buffing dust, mixed together, in a weight ratio of 2:2:1. The average heating value of the mixture was approx. 13 900 kJ/kg, the average water content - approx. 28% w/w. Each container consisted 3 kg (±0.1 kg) of the waste mixture. In one series of experiment about 27-30 containers of waste were submitted to the process (approx. 81-90 kg). The waste flow was 15 kg/h (5 containers in an hour), which gives 12 min of time interval between the introduction of successive containers. The temperature of the preheated process air was 350ºC. The air was introduced right after the entrance door of the tunnel. The temperature in the combustion section was set at 875ºC in order to exceed 850ºC - the minimum temperature of waste incineration allowed by EU regulations [9]. The introduction of containers started when the temperature in the combustion section approached 650ºC. The total time of container transmission through the tunnel was 1 h 45 min. The temperatures, pressures, and oxygen content in the flue gas were recorded.

## Results

As the result of the process the waste was incinerated. The residues were flue gas and ash. After the initial time of stabilizing the temperature in the tunnel it reached the level of 860-870ºC in the combustion section of the tunnel. The temperature changes along the tunnel are periodic and correspond to the introduction and ignition of successive portions of waste.

### *Process balancing*

In order to describe environmental effects the energy input/output ratio as well as mass fluxes have to be known. The balance was calculated for 1 hour of incineration process in the most stable conditions which could be obtained during the experiment.

#### Mass balance

To calculate the input mass fluxes the waste as well as the process air were taken into consideration. The average composition of tannery waste as given in [2, 10] enabled to calculate the fluxes of C, N, O, S, H and inorganic compounds in the waste. The mass flux of air was calculated thanks to indications of volume flux measurements enabled by the measurement equipments installed in the experimental tunnel furnace system.

To calculate the output mass fluxes, the ash and the flue gas were taken into consideration. According to ash analysis the dry ash contains 56.4% w/w chromium [5]. This allows to establish the mass flux of chromium in the output. The mass fluxes of flue gas components were either calculated based on the input mass fluxes (nitrogen mass flux), combustion stechiometry (carbon dioxide and water mass fluxes) or analyzes carried out during the process (oxygen and contaminants mass fluxes). The average concentration of contaminants is shown in Table 3. The details on the calculating procedure are presented in [5]. The mass balance of the tannery waste incineration process are presented in Table 4. There is a slight difference in input and output values which undoubtfully is related to measurements errors and is negligible.

Table 3

Concentrations* and mass fluxes of measured contaminants in the flue gas

| Contaminant | Average concentration | Mass flux |
|---|---|---|
| | [mg/m$^3$] | [g/h] |
| VOC | 10.7 | 2.53 |
| Flue-ash | 69.5 | 16.4 |
| $SO_2$ | 753.2 | 178.1 |
| $NO_x$ | 502.1 | 118.7 |
| CO | 296.4 | 70.1 |
| Sum | | 385.8 |

* in the process conditions, without reference to standardized oxygen concentration in the flue gas

Table 4

Mass balance of the tannery waste incineration process [kg/h]

| | Input | | Output | |
|---|---|---|---|---|
| | **1. Waste** | **15.0** | **1. Ash** | **1.10** |
| | • Water | 4.20 | • Chromium in ash | 0.62 |
| | • Carbon | 5.08 | **2. Flue gas** | **302.0** |
| | • Nitrogen | 1.51 | • Nitrogen | 218.5 |
| | • Sulphur | 0.10 | • Carbon dioxide | 18.6 |
| | • Hydrogen | 0.65 | • Water | 12.85 |
| | • Mineral content | 1.08 | • Oxygen | 51.7 |
| | • Chromium | 0.59 | • Contaminants | 0.39 |
| | **2. Combustion air mass flux** | **287.7** | | |
| | • Water | 2.8 | | |
| | • Nitrogen | 218.5 | | |
| | • Oxygen | 66.4 | | |
| **Sum** | | **302.7** | | **303.1** |

## Energy balance

The energy fluxes which enter the system are: chemical energy of the waste and electrical energy required by the installation to maintain the appropriate temperature in the combustion section and to drive rollers, fans and other system elements. To assess the output fluxes the energy exchanged in the heat exchanger as well as energy losses in the flue-gas and in form of heat radiation have to be taken into consideration.

Electrical energy consumption and the energy flux exchanged in the heat exchanger are recorder by the installation controller. The energy flux which enters the system with the waste (chemical energy) was calculated on the basis of heating value. The values are presented in the Table 5. It has to be emphasised that due to the technical limitations (necessity to use electrical heaters instead of preferable gas burners, inability to increase heat recovery rate in a given heat exchange system) the efficiency rate is low (21%). The calculations were presented here in order to give the complete overview of the process. They show that more than 100 kW is lost or partially accumulated in the furnace walls. The low efficiency rate is an important indication for the further upgrading of the system into the full-scale.

Table 5

Energy fluxes input/output overview

| **Flux type** | **Value** |
|---|---|
| | **[kW]** |
| Input | |
| Chemical energy flux (in waste) | 57.9 |
| Electrical energy flux | 74.0 |
| Sum | 131.9 |
| Output | |
| Exchanged heat flux | 28.0 |

## Environmental effects of the process

To describe the impact of the tannery waste incineration process on the environment one important aspect has to be take into account, namely the comparison of the presented treatment method with the one applied so far. The waste used in the research were bound to be landfilled as landfilling is the most commonly applied treatment method for such waste in Poland [1].

Tannery waste landfilling requires minimal energy input as the disposal yards are most commonly located on the sites of tanning facilities. In most cases, there are no or little safety measures to protect the sites from emitting substances into ground waters (leaching through atmospheric fall) or atmosphere (odours, dust emissions). The main risk is connected with probability of chromium extraction from waste into environment, especially through leaching. Solubility of chromium(III) compounds, which predominate in tanned leather and leather waste, is very slight, but still possible. The pH is of importance - in the slightly basic conditions a redox reactions may take place, giving as a result much more soluble chromium(VI) compounds. Furthermore, organic matter originated form hide material processing is effective in forming soluble organic Cr(III) complexes [11, 12]. In case of uncontrolled chromium extraction from waste to I or II class surface waters for each single Mg waste approx. $10^4$ $m^3$ water may become polluted (meaning the chromium concentration exceed 0.05 $mg/dm^3$ [13]). For this rough calculations the extraction rate on the level of 3-5% was assumed. The environmental effect of tannery waste landfilling is therefore definitely negative, unless precautions have been taken to ensure the proper bottom and cover sealing of the landfill site.

The presented incineration method for tannery waste treatment affects the environment mainly in two fields. The first effect comes form that fact that the technology generates waste itself, namely the flue-gas and the ash. Both residues contains contaminants which may affect negatively the environment.

In case of the ash it is noticeable that this residue amounts to less than 1/10 of the waste input mass and that it consist mostly of chromium(III) compounds. As the preliminary tests reveals [5, 8], the process temperature is too low for the chromium oxidation to take place, so nearly all chromium in the ash is the chromium(III). With relatively simple means it is possible not only to collect the ash in an environmental safe way, but also to reuse the ash in such applications, which require high chromium(III) concentration. It could be the chemical industry (production of sodium chromate(VI), the basic chromium substrate for a wide range of industrial processes) or metallurgy (the ash as a substitute of chromite ore for stainless steel production).

The emission to atmosphere includes carbon dioxide and contaminants. The analyzes of the basic gaseous contaminants show that there is a need for the flue gas cleaning system to be installed in the case of implementing the technology to industrial applications. This means additional costs and also waste generated during the flue gas cleaning (collected ash, absorbed sulphur compounds, etc.).

The second aspect of the process contributing to its environmental impact is the energy consumption. As the rough calculations show the energy uptake is too high in comparison to the energy reused. The low heat exchange rate, lack of gas burners (instead electrical preheating system was applied), as well as the long period of heat accumulation in the tunnel walls were these factors which lowered the energy efficiency of the whole system. In the optimal case, it should be possible to regain energy from the process, which would limit the negative environmental impact. As the experiments show, the additional energy input (apart from the chemical energy of the waste) can be significant and has to be taken into consideration.

## Conclusions

Without full data on costs, emissions factors and energy uptake the comparison between waste incineration process in a tunnel furnace system and waste landfilling, which in most cases takes place so far, could be only qualitative. Even facing the fact that the incineration process requires some energy input and gives out emission of the substances which are not neutral to the environment, tanned waste landfilling seems to pose more potential threat to the environment components (ground and surface water, soil and living organisms) than any thermal treatment technology, mostly due to the risk of chromium penetration outside the waste disposal sites. The technology of tannery waste incineration presented in this paper is an attempt to develop such treatment method which would be implementable in industrial practise and safe to the environment. The research so far proved that incineration of tanned waste is possible, the tunnel system can be applied for such a process and that the combustion is complete, leaving no or little organic matter in the ash, which can be treated as the chromium concentrate to various applications.

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## References

[1] Famielec S, Wieczorek-Ciurowa K, Waste form leather industry - threats to the environment. Technical Transactions PK. 2011;1-Ch:43-48. http://suw.biblos.pk.edu.pl/resources/i4/i5/i4/i6/r4546/FamielecS_WasteLeather.pdf.

[2] Kanagaraj J, Velappan KC, Chandra Babu NK, Sadulla S. Solid wastes generation in the leather industry and its utilization for cleaner environment. J Sci Industrial Res. 2006;65:541-548. http://nopr.niscair.res.in/bitstream/123456789/4856/1/JSIR%2065%287%29%20541-548.pdf.

[3] Lofrano G, Meric S, Zengin GE, Orhon D. Chemical and biological treatment technologies for leather tannery chemicals and wastewaters: a review. Sci Total Environ. 2013;461-462:265-281. DOI: 10.1016/j.scitotenv.2013.05.004.
[4] Reference Document on Best Available Techniques for the Tanning of Hides and Skins. Seville: European ICCP Bureau; 2013. http://eippcb.jrc.ec.europa.eu/reference/BREF/TAN_Adopted552013.pdf.
[5] Famielec S. Proces spalania odpadów garbarskich w piecu tunelowym jako metoda ich unieszkodliwiania [rozprawa doktorska] (The process of tannery waste incineration in a tunel furnace as a metod of its utilization – doctoral dissertation). Kraków: Politechnika Krakowska; 2014. https://suw.biblos.pk.edu.pl/resources/i4/i7/i2/i1/i6/r47216/FamielecS_ProcesSpalania.pdf.
[6] World statistical compendium for raw hides and skins, leather and leather footwear 1998-2014, Rome: Food and Agriculture Organization of the United Nations; 2015. www.fao.org/3/a-i4651e.pdf.
[7] Rocznik Statystyczny Polski 2014 (Statistical Yearbook of Poland 2014). Warszawa: GUS; 2015. http://stat.gov.pl/files/gfx/portalinformacyjny/pl/defaultaktualnosci/5515/2/9/1/rocznik_statystyczny_rp_2014.pdf.
[8] Famielec S, Wieczorek-Ciurowa K, Incineration of tannery waste in a tunnel furnace system. Civil Environ Eng Reports. 2013;10:63-72. http://www.ceer.uz.zgora.pl/CR_filearchive/da5668eebc7152cfac7156b658bd7cd8.pdf.
[9] Rozporządzenie Ministra Gospodarki z dnia 21 marca 2002 r. w sprawie wymagań dotyczących prowadzenia procesu termicznego przekształcania odpadów (DzU 2002 nr 37 poz. 339 z późn. zm.) (The Regulation of the Ministry of Economy concerning requirements for thermal waste processing). http://isap.sejm.gov.pl/Download?id=WDU20020370339&type=2.
[10] Fela K, Wieczorek-Ciurowa K, Konopka M, Wożny Z, Present and prospective leather industry waste disposal. Polish Journal of Chemical Technology. 2011;13:53-55. DOI: 10.2478/v10026-011-0037-2.
[11] Kotaś J, Stasicka Z. Chromium occurrence in the environment and methods of its speciation. Environ Pollut. 2000;107:263-283. DOI: 10.1016/S0269-7491(99)00168-2.
[12] Prokisch J, Katz SA, Kovacs B, Gyori Z. Speciation of chromium form industrial waste and incinerated sludges. J Chromat A. 1997;774:363-371. DOI: 10.1016/S0021-9673(97)00334-8.
[13] Rozporządzenie Ministra Środowiska z dnia 22 października 2014 r. w sprawie sposobu klasyfikacji stanu jednolitych części wód powierzchniowych oraz środowiskowych norm jakości dla substancji priorytetowych (DzU 2014 poz. 1482) (The Regulation of the Ministry of Environment concerning classification methods of surface waters homogenous parts and environmental quality standards for priority substances). http://isap.sejm.gov.pl/Download?id=WDU20140001482&type=2.

# EFEKTY ŚRODOWISKOWE PROCESU SPALANIA ODPADÓW GARBARSKICH W PIECU TUNELOWYM

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**Abstrakt:** Stosowane powszechnie w przemyśle garbarskim technologie garbowania i wyprawy skór są istotnym obciążeniem dla środowiska. Procesy te wymagają znacznych nakładów energii, wody oraz chemikaliów, powstają również duże ilości odpadów, zarówno stałych, jak i ciekłych. Szacuje się, że podczas procesu obróbki 1 Mg skór surowych garbarnia generuje ok. 600-700 kg odpadów. Znaczna część tych odpadów to odpady stałe, powstałe podczas lub po procesie garbowania właściwego, dlatego też zawierają w swoim składzie związki chromu, stosowane powszechnie jako garbnik. Unieszkodliwianie tych odpadów jest dla garbarni uciążliwe, najczęściej są one składowane na przyzakładowych składowiskach. Stałe odpady garbarskie charakteryzują się stosunkowo wysoką wartością opałową (ok. 12-14 MJ/kg), ale też znaczną zawartością wilgoci (dla niektórych rodzajów odpadów nawet do 40-50%) oraz udziałem chromu wynoszącym ok. 3%. Unieszkodliwianie tych odpadów poprzez ich spalanie jest możliwe, wymaga jednak zachowania odpowiednich warunków prowadzenia procesu, by nie stwarzać zagrożenia dla środowiska. Badania prowadzono z wykorzystaniem prototypowego pieca tunelowego zaprojektowanego do prowadzenia procesu termicznego unieszkodliwiania odpadów garbarskich. W artykule przedstawiono krótką charakterystykę instalacji, przebieg badań oraz efekty środowiskowe procesu spalania odpadów garbarskich w aspekcie składu gazów odlotowych, bilansu energetycznego oraz bilansu masowego dla procesu ze szczególnym uwzględnieniem zawartości chromu w pozostałościach poprocesowych.

Jak wykazały badania, przy odpowiednim doborze parametrów procesu chrom występuje w popiołach w postaci tlenku chromu(III), możliwe jest więc ich wykorzystanie jako substytutu rud chromowych w procesach chemicznych czy metalurgicznych.

**Słowa kluczowe:** odpady garbarskie, piec tunelowy, spalanie odpadów, tlenek chromu(III)

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# EFFECT OF AGEING ON THE STRUCTURE AND THERMAL PROPERTIES OF POLYMER COMPOSITES WITH CARDBOARD FIBER

## WPŁYW STARZENIA NA STRUKTURĘ I WŁAŚCIWOŚCI TERMICZNE KOMPOZYTÓW POLIMEROWYCH Z WŁÓKNEM TEKTUROWYM

**Abstract:** In this work the results of investigations of thermal properties and structure PE-HD with addition of cardboard fibre before and after UV radiation ageing were presented. In this work composites with content of 1, 3, 5% of cardboard fibre in relation to the polyethylene have been produced. The investigations for the influence of the fillers on the properties of prepared composites before and after ultraviolet radiation ageing have been conducted. Investigations of crystallinity degree by means of DSC method as well as investigations of the structure using optical microscope have been conducted. The DSC investigations prove the decrease in the crystallinity degree of PE-HD during addition of cardboard fibre and increase for the samples after ultraviolet radiation ageing. While increasing the amount of cardboard fibre in the composite the Vicat softening temperature and absorptivity of water decreases for the samples before ultraviolet radiation ageing.

**Keywords:** composites, thermal properties, cardboard fibre, structure, ultraviolet radiation ageing, recycling

In recent years, in various industries, polymer composites are in demand as a group of materials with interesting, sometimes very specific properties. Properties of polymer composites depend on structural factors of the polymer and the conditions of use. Structural factors include: molecular weight, chemical structure of the macromolecules chain constitution, crystallinity, molecular orientation, and the presence of additional components. However, among the conditions of utility it can be distinguished: temperature, time loading, pressure, type of deformation, etc. [1-9]. Modification of polymers is very important, as to obtain the best properties of the modified polymer. Polymers can be modified by introducing various kinds of polymer modifiers, fillers or stabilizers. There are three methods of modification: physical, chemical and physicochemical. Modification of natural polymers include changing their structure due to the forced orientation, mixing the polymer with modifiers, polymer complex formation, changing the degree of dispersion and the construction of supramolecular individual phases, changing the structure and nature of the interactions in the interfacial zones. The advantage of physical modification is a chance to fast implementation of research results and the ability to carry it as a part of the production process, which allows you to adjust - to some extent - their characteristics to the requirements of customers. To obtain composites with high homogeneity and necessary properties depends on the mixing conditions and physicochemical properties, thermodynamic and rheological characteristics of the starting polymer [4, 5]. The aim of the research was to determine the effect of UV aging on the thermal, utility properties and structure of the composite produced by using polyethylene (PE-HD) and fiber cardboard. The choice of fiber cardboard as a filler is justified by the possibility of changing specific

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properties of PE-HD for specific applications, for recycled paperboard and reduce the cost of developing a new polymer material.

## Materials, apparatus and methods

Polyethylene PE-HD, produced by “CHEMO PETROL, a.s”, cardboard and fiber with an irregular shape and length of 1-2 mm obtained from the packaging by mechanical fragmentation, have been used for study. The drying process of cardboard fibers has been carried in the dryer Zelmet with heat chamber KC-100/200 at 80ºC for 12 hours. In order to obtain composite, polymer was blended mechanically with fiber carton. Then plasticized and extruded in injection molding machine Krauss Maffei KM65 - 160C, using the following parameters: rotational speed of the screw 260 mm/s; each barrel zone temperature: $T_1$ = 180ºC, $T_2$ = 190ºC, $T_3$ = 200ºC, $T_4$ = 210ºC; die temperature $T_5$ = 220ºC. Follow this method, composites were prepared with the following composition: PE-HD + 1 wt.% fiber cardboard, PE-HD + 3 wt.% fiber cardboard, PE-HD + 5% by weight fiber cardboard. Samples for testing of PE-HD and composites made by injection molding were made on injection molding machine KRAUSS MAFFEI KM65 - 160C1. The parameters of the injection molding, which were obtained at the optimal values of the characteristics were as follows: maximum permissible pressure in the plasticizing 60 MPa, injection time 0.7 s, the contact pressure 30 MPa, holding time 30 s, cooling time 20 s, dosage time 6.8 s, mold closing force 650 kN, mold temperature 50ºC, barrel zone temperatures: $t_1$ = 160ºC, $t_2$ = 170ºC, $t_3$ = 180ºC, $t_4$ = 195ºC, die temperature $t_5$ = 205°C. Samples for testing of composites with different percentage compositions were prepared with the same parameters of the injection. Differential scanning calorimetry studies were performed using a scanning microcalorimeter type PC 200 NETZSCH 200. DSC curves were recorded during the heating rate of 10ºC/min in a temperature range from 20 to 190ºC. Preparations for DSC test were cut perpendicular to the direction of flow of the samples obtained by injection molding. To determine the degree of crystallinity, the software PC 200 NETZSCH was used. This program allows examination of the course of melting of the sample at a given temperature range and the designation of the area between the thermographic curve and the base line on the occurrence of reflexes endothermic. Indium was used as the pattern, sample weight was ranged from 7 to 10 mg.

The samples were weighed by scale, produced by SARTORIUS company, with an accuracy of 0.01 mg, and internal calibration and measurement enclosed space. The structure was observed under an optical microscope Nikon Eclipse E 200. Test samples were applied to a thickness of 20 microns which were cut by microtome Thermo Electron Corporation of core samples used for DSC studies. Determination of Vicat softening temperature brought by a device HAAKE. The study of water absorption was carried out for samples weighing from 0.8 to 2.5 g in accordance with applicable standards. The ageing process was performed in a test chamber using a UV arc tube high-pressure mercury discharge lamp. Time of the ageing process in the chamber calculated assuming the literature data 1000 kWh/m$^2$ as the power of global solar radiation during the year and amounted to 223 hours, which using a discharge lamp with specified capacity equivalent to 4 years of solar radiation.

## Results and discussion

In Figures 1-3 is shown a DSC thermogram of polyethylene and composites with cardboard fiber before and after UV ageing. Table 1 summarizes the sizes of determined on the basis registered thermography DSC curves. A polyethylene sample by subjecting the radiation, the energy absorbed by the polymer were decreased. The lowest values of the melting enthalpy obtained after the process of radiation.

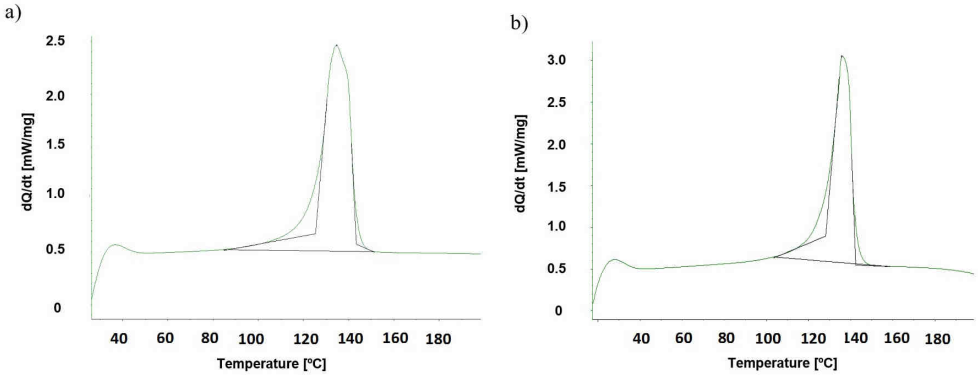

Fig. 1. DSC thermograms of polyethylene: a) before, b) after UV ageing

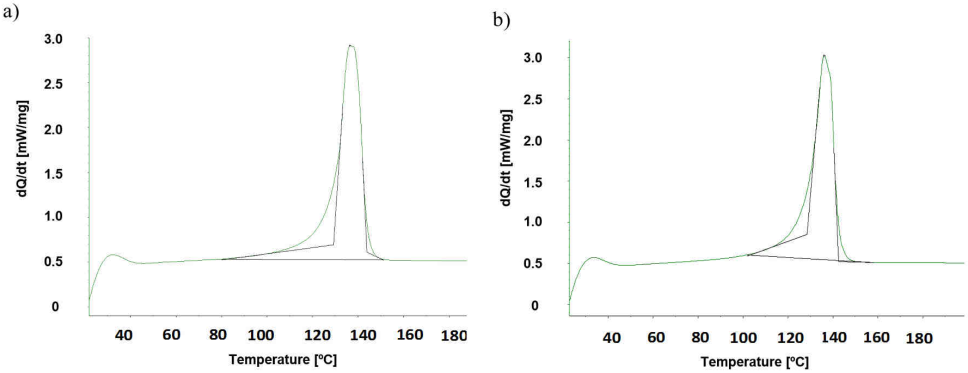

Fig. 2. DSC thermograms of polyethylene + 1% of cardboard fibers: a) before, b) after UV ageing

In proportion to changes in the enthalpy of fusion, start and stop temperature of convertion agent were changing. As a result of subjecting the reflexes of ageing has been narrowed and shifted to higher temperature range. As a result of subjecting the composite polyethylene of 1% cardboard fiber ageing, the energy absorbed by the sample is increasing. As a result of the ageing process reflexes has been narrowed and shifted to the higher temperature range, and the value of the degree of crystallinity increased (Fig. 2). For polyethylene with 3 and 5% of fiber carding after ageing showed an increase of enthalpy of fusion. The average value of the crystalline melting point has increased and reflexes has narrowed (Fig. 3). From the analysis of the study by DSC shows a significant effect of ageing UV rays on the thermal properties of polyethylene and cardboard fiber composites. In the case of test materials, the method of preparation and thermal history of the samples affect to the mobility of macromolecular segments, nucleation, growth and orientation of the crystallites.

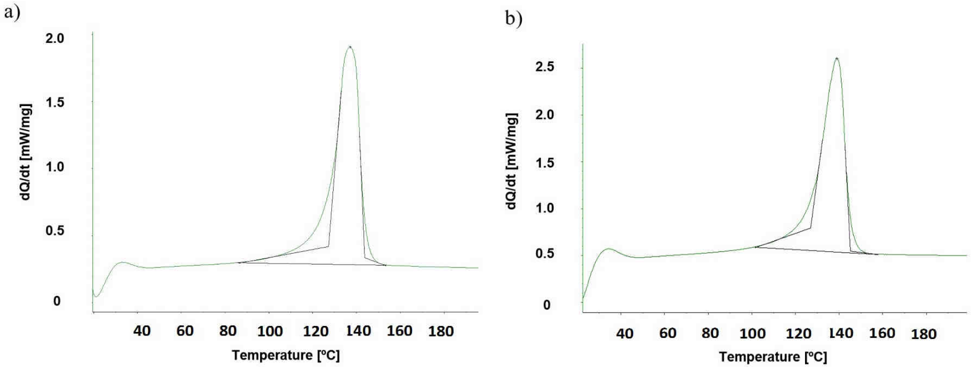

Fig. 3. DSC thermograms of polyethylene +5% of cardboard fibers: a) before, b) after UV ageing

Table 1

The results of DSC tests

| Composition of the sample | The degree of crystallinity [%] | Melting range [°C] | The melting point of max. reflexes [°C] |
|---|---|---|---|
| PE | 64.5 | 125.4-143.5 | 134.6 |
| PE + 1% cardboard fibers | 67.1 | 129.2-143.6 | 136.4 |
| PE + 3% cardboard fibers | 52.4 | 125.4-144.5 | 137.2 |
| PE + 5% cardboard fibers | 49.6 | 127.3-143.8 | 137 |
| PE aged | 61.9 | 128.2-142.4 | 135 |
| PE + 1% cardboard fibers aged | 61.1 | 128.6-142.6 | 136 |
| PE + 3% cardboard fibers aged | 60.4 | 128.3-145.2 | 134.8 |
| PE + 5% cardboard fibers aged | 58.8 | 127-145.2 | 138.8 |

In studies on the structure of the polyethylene on optical microscope after ageing the growth of crystalline structure was found. There are clearly visible shapes of spherulites with large dimensions. The structure is more visible and organized (Fig. 4).

Based on the analysis of the DSC thermograms revealed changes in the melting range of the crystalline phase, the temperature at which crystallization takes place at a maximum rate and the crystallization start temperature. This direction of changes reflects the capacity of the crystallization of polyethylene in the composite, which also affects to higher degree of crystallinity UV aged composite. A small amount of fiber carding, its specific shape and arrangement along the direction of flow may affect the increase in the degree of crystallinity (Table 1, Fig. 5). It follows to higher probability of intermolecular interactions in the polymer, leading to the creation of centers of crystallization upon cooling of the composite PE + 1% fiber.

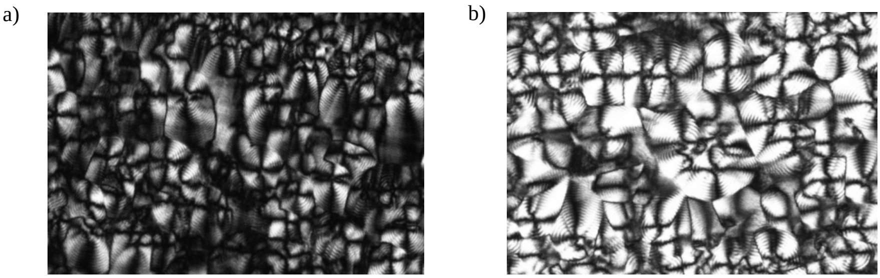

Fig. 4. Structure observed under an optical microscope at a magnification of 400x: a) PE, b) PE after UV ageing

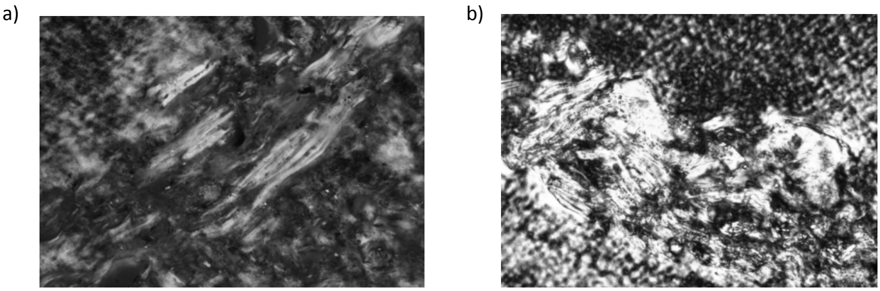

Fig. 5. Structure observed under an optical microscope at a magnification of 400x: a) PE/1% cardboard fiber, b) PE/1% cardboard fiber after UV ageing

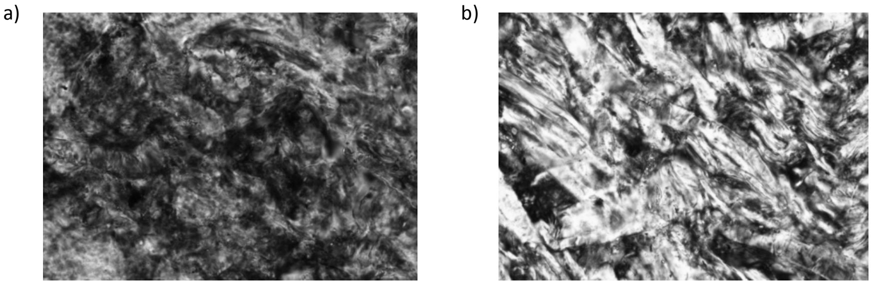

Fig. 6. Structure observed under an optical microscope at a magnification of 400x: a) PE/5% cardboard fiber, b) PE/5% cardboard fiber after UV ageing

The results of DSC tests show a decrease in the degree of crystallinity for composites with fiber 3 and 5%, which alters the performance of composites. The reason for the decrease in the degree of crystallinity may be changing the structure system: the fiber orientation along the direction of the polymer flow in the injection mold cavity, however, small disturbances at 3% of the fiber board and then the entanglement of fibers together in the fiber content of 5% (Fig. 6).

The study shows that with increasing filler content in the composite increases the value of Vicat softening temperature. The same increase in temperature followed by a sample after ageing. In the case of samples with the same content of the filler, the softening point was lower after aging. The higher the filler content is in the composite, the greater is the water absorption (Table 2).

Table 2

The results of Vicat softening temperature and water absorption tests

| Composition of the sample | Vicat softening temperature [ºC] | Water absorption [%] |
|---|---|---|
| PE | 115 | 0.01 |
| PE + 1% cardboard fibers | 116 | 0.02 |
| PE + 3% cardboard fibers | 117 | 0.11 |
| PE + 5% cardboard fibers | 118 | 0.13 |
| PE aged | 113 | 0.01 |
| PE + 1% cardboard fibers aged | 114 | 0.01 |
| PE + 3% cardboard fibers aged | 115 | 0.17 |
| PE + 5% cardboard fibers aged | 115 | 0.25 |

## Conclusions

On the basis of DSC tests, it was found that the content of filler fibers in the form of a cardboard a composite of polyethylene influences the crystalline melting point. The influence of the degree of crystallinity of the filler whose value decreases with the increase of its content in the composite. The properties of the polyethylene as well as a test of the composite are significantly affected by UV ageing. After UV ageing decreases the degree of crystallinity, however, an increased with increased fiber content compared to the paperboard samples instead of the aged. There were significant changes in the structure during the test composites under an optical microscope. At higher fiber content in the composite cardboard are arranged in clusters, and there is no visible direction along the direction of the flow lines. With an increase in the filler content, the softening temperature increases. In the case of samples with the same content of the filler, the softening point was lower after ageing.

## References

[l] Rosato DV, Rosato MG, Schott NR. Plastics Technology. Handbook. Exeter: Momentum Press; 2011.

[2] Chibani N, Djidjelli H, Dufresne A, Boukerrou A, Nedjma S. Study of effect of old corrugated cardboard in properties of polypropylene composites: study of mechanical properties, thermal behavior, and morphological properties. J Vinyl Addit Techn. 2014;1:1-8. DOI: 10.1002/vnl.21437.

[3] Salit, MS, Jawaid M, Yusoff NB, Hoque ME. Manufacturing of Natural Fibre Reinforced Polymer Composites. Switzerland: Springer; 2015. DOI 10.1007/978-3-319-07944-8.

[4] Gnatowski A, Koszkul J. Investigations of the influence of compatibilizer and filler type on the properties of chosen polymer blends. J Mater Process Technol. 2005;162-163:52-58. DOI: 10.1016/j.jmatprotec.2005.02.240.

[5] Jurkowski B, Jurkowska B. Sporządzanie kompozycji polimerowych. (Preparation of polymer compositions). Warszawa: WNT; 1995.

[6] Fraser WA, Ancker FH, Dibenedetto AT, Elbirli B. Evaluation of surface treatments for fibers in composite materials. Polym Composite. 2004;4(4):238-248. DOI: 10.1002/pc.750040409.
[7] Noritake A, Hori M, Shigematsu M, Tanahashi M. Recycling of polyethylene terephthalate using high-pressure steam treatment. Polymer J. 2008;40:498-502. DOI: 10.1295/polymj.PJ2007237.
[8] Gnatowski A, Ziewiec A, Gnatowska R. Impact analysis performance injection in polymer composites processing shrinkage. AIP Conf. Proc. 2015. DOI: 10.1063/1.4913182.
[9] Matabola KP, de Vries AR, Luyt AS, Kumar R. Studies on single polymer composites of poly(methyl methacrylate) reinforced with electrospun nanofibers with a focus on their dynamic mechanical properties. Express Polym Lett. 2011;7:635-642. DOI: 10.3144/expresspolymlett.2011.61.

## WPŁYW STARZENIA NA STRUKTURĘ I WŁAŚCIWOŚCI TERMICZNE KOMPOZYTÓW POLIMEROWYCH Z WŁÓKNEM TEKTUROWYM

Politechnika Częstochowska

**Abstrakt:** W pracy przedstawiono wyniki badań właściwości termicznych oraz struktury polietylenu o dużej gęstości i kompozytów polietylenu z włóknem tekturowym przed i po procesie starzenia promieniami UV. Zbadano stopień krystaliczności metodą DSC oraz strukturę za pomocą mikroskopu optycznego w świetle przechodzącym. Określono również temperaturę mięknienia wg Vicata oraz chłonność wody. Badaniom poddano kompozyty polietylenu o zawartości włókna: 1, 3 i 5%. Stwierdzono wpływ napełniacza na stopień krystaliczności, którego wartość maleje ze wzrostem zawartości włókna tekturowego zarówno przed, jak i po starzeniu promieniami UV. W przeprowadzonych badaniach zarejestrowano niższe wartości temperatury mięknienia wg Vicata i większą chłonność wody dla kompozytów polietylenu z włóknem tekturowym po starzeniu promieniami UV.

**Słowa kluczowe:** kompozyty, właściwości cieplne, włókno tekturowe, struktura, starzenie promieniami UV, recykling

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Katarzyna GRATA[1] and Małgorzata NABRDALIK[1]

# ASSESSMENT OF THE ANTIFUNGAL PROPERTIES OF NETTLE EXTRACTS AGAINST *Fusarium proliferatum*

## OCENA WŁAŚCIWOŚCI PRZECIWGRZYBOWYCH POKRZYWY ZWYCZAJNEJ WOBEC *Fusarium proliferatum*

**Abstract:** The aim of conducted research was to determine the antifungal properties of the leaf and nettle rot extracts on the growth of *Fusarium proliferatum*. The antagonistic properties of nettle extracts at 2.5, 5.0, 10, 20 and 40% concentrations were assayed with a dual culture plate method on PDA and Tomato media. The culturing process was conducted at 26ºC for 9 days and the fungal linear growth was measured every 1-2 days. The positive control in the experiment was Topsin M 500 SC whereas negative the distilled water. The fungistatic activity of nettle extracts was determined against the conidia germination rate index, the growth rate index and the rate of mycelial growth inhibition of *Fusarium proliferatum*. Obtained results shows that the nettle rot extracts inhibited the spore germination (from 7.5 to 47.3%) at all tested concentrations and nettle leave extracts (from 5.9 to 54.0%, respectively) towards negative control, while stimulated compared to positive control. The highest inhibition of the linear growth of mycelium has been observed both for nettle rot and leaf extracts on Tomato medium (9.8 and 11.2%, respectively) and on PDA medium (5.5 and 3.2%, respectively) at 40% concentration towards negative control, while stimulated compared to positive control.

**Keywords:** stinging nettle, antifungal activity, *Fusarium proliferatum*

*Fusarium* are ubiquitous in soil and can exist as saprophytes or pathogens in plant tissues. They belong to the most frequent pathogens causing disease and losses of agricultural crops around the world. Particularly the *F. oxysporum, F. solani, F. culmorum and F. proliferatum* are causing severe damage to agriculture in pre- and post-harvest. *Fusarium* spp. belong to the most dangerous pathogens of cereals, tomatoes, corn, asparagus. In addition, they have the ability to produce highly toxic to humans and animals with differentiated structure mycotoxins such as trichothecenes, zearalenone, fumonisin [1-4]. The most important method of protecting the plants against phtytopathogens is the use of fungicides. However, many of which are toxic, non-biodegradable and have undesirable effects on other organisms present in the environment. Therefore, there is a need to find alternative agents for the control of pathogenic fungal diseases in plants. There is a good reason to suppose that the secondary metabolites of plants have evolved to protect them from attack by microbial pathogens. So, natural products from plants (*eg* essential oils, extracts) have great potential as novel fungicide sources for controlling phytopathogens in agriculture [5, 6].

*Urtica dioica* L. belongs to the family Uriticaceae, commonly called as stinging nettle, is a perennial plant growing in temperate and tropical wasteland areas around the world [5, 7]. The different parts used include leaf, root, stem, flower and modified plant organs. Phytochemical studies revealed the presence of many different valuable classes of organic compounds. The compounds which are reported from the nettle are flavonoids, phytosterols, tannins, proteins, polysaccharydes, essential oil, fatty acids, volatile

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[*] Contribution was presented during ECOpole'15 Conference, Jarnoltowek, 14-16.10.2015

compounds, vitamins and minerals in this plant [7-10]. For this reason U. *dioica* has been reported to have various activities like antioxidant, insecticide, antimirobial antiviral. The effect of nettle extracts on microorganism has been studied by a very large number of researches that indicate activity of the nettle extracts against of bacteria (*eg Bacillus* spp., *E. coli*, *S. aureus*, *P. aeruginosa*) [8, 9, 11, 12] and fungi (*Candida albicans*, *Aspergillus flavus*, *Alternaria alternata*, *Alternaria solani*, *Curvularia lunata*, *Botritis cinerea* and *Rhizoctonia solani* [5, 8, 11-14]. The antifungal effects of the phyto-extracts indicate the potential of selected plant species as a source of natural fungicidal material [5, 13].

The main purpose of this study was to evaluate the antifungal properties of the leaf and root extracts of *Urtica dioica* on the growth and spore germination of *Fusarium proliferatum*.

## Materials and methods

In this study the fungistatic properties of the nettle extracts at 2.5, 5.0, 10, 20 and 40% concentrations were determined against the conidia germination, the growth rate index and the rate of mycelial growth inhibition of *Fusarium proliferatum.*

***Conidial germination.*** Analysis of effect the nettle extracts on spore germination was performed with the modifying slide germination method. Fungal cultures were cultivated on PDA medium slants at 25 ± 2ºC for 7 days and the well-developed fungal culture was rinsed with 10 cm$^3$ of sterile water containing 0.05% Tween 80. Mycelium was filtred from sterile gazue and suspension was adjusted to 10$^6$ conidia/cm$^3$ in hemocytemetr. Subsequently 30 mm$^3$ of nettle extracts and 30 mm$^3$ of fungal spores were transfered on 1 cm$^3$ sterile, clean glass slide. Control treatments were prepared by substitution of nettle extracts by Topsin M 500 SC (positive control - PC) and distilled water (negative control - NC). The slides were incubated in humid chambers in triplicate at 25 ± 2ºC for 24 hours. After this period the number of germinated and non-germinated conidia were counted under microscope using x200 magnification. Conidia germination was presented as the germination rate index [15].

***Mycelium growth***. The antagonistic activity of the nettle extracts were assayed *in vitro* tests with a dual culture plate method using two different media: PDA and Tomato media. First, on this media were inoculated with 0.5 cm$^3$ of the appropriate concentrations of nettle leaf and nettle root extracts. Next, the media were inoculated with fungal mycelial-disks (diameter of 10 mm) obtained from growing cultures of tested *F. proliferatum* strains. The positive control in the experiment was Topsin M 500 SC whereas the negative one the distilled water. The culturing process was conducted at 25 ± 2ºC for 9 days and the fungal linear growth were measured in two directions on each plate every 1-2 days. Each experiment was run in four replicates, where one was represented by one culturing plate with the growth medium and the mycelial disc. The antagonistic activity of this extracts on the growth of *F. proliferatum* was estimated as the growth rate index [16].

The fungistatic properties of the nettle extracts have been designated on the basis of the percentage reduction of the linear growth of the *F. proliferatum.*

## Results and discussion

The plants are the source of many important potential bioactive metabolites used in the limiting the growth of microorganisms. Certain plants extracts act in many ways on various fungi and may be applied to the crop as agricultural chemicals. *Urtica dioica* may also be leading factor in wide range of activities against many phytopathogens, where these pathogens have developed resistance against specific fungicides [5, 8]. Therefore, the antifungal activity extracts of nettle leaf and nettle root of *Urtica dioica* on the conidia germination and linear was evaluated toward tested *F. proliferatum.* Conducted studies have shown differences in fungistatic activity of extracts, depending on theirs concentrations and the stage of the fungus. The potential of infectious the pathogenic fungi largely depends on their ability to produce spores. Usually they make the infection of plant organs, and less frequently the perpetrator of the first stage of the disease are hyphae. The extracts of stinging leaf and nettle root showed the capacity to inhibit the spore germination and linear growth of the *F. proliferatum*.

The degree of fungal conidia germination was different, depending on the kind of extracts and also its concentrations. The results are shown in Figure 1.

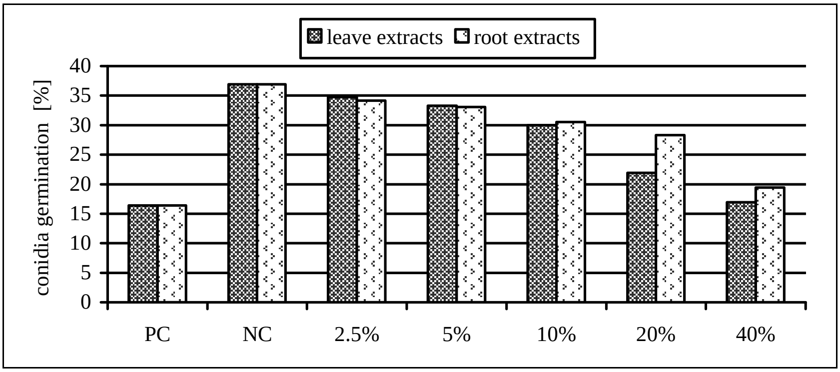

Fig. 1. Influence of the nettle extracts on the conidia germination of *Fusarium proliferatum*. PC - positive control, NC - negative control

The value of the conidia germination was at a level of from 34.15 to 19.43% when the extracts of nettle leaves were used, and from 34.73 to 16.95% after application of the extracts of nettle root. However, Topsin M 500SC used as the positive control inhibit the spore germination at the level 16.4%, while 36.9% in case of the negative control. For the both extracts, it has been observed that with the increase of theirs concentrations, the higher inhibition of the conidia germination was obtained (Fig. 1).

The percentage of noted reduction of the conidia germination amounted between 47.4% when applying 40% extract of nettle root extract to 7.5% (for 2.5% root extract) and 54.1% for 40% of the nettle leaves extracts to 5.9% (for 2.5% leaves extract) towards to the negative control, while stimulated compared to the positive control. However, the strongest inhibition of the spore germination of *F. proliferatum* was observed when Topsin M 500 SC was used.

Moreover the antifungal activity of the leaves and nettle root extracts towards *F. proliferatum* cultured on 2 different media was evaluated by the values of the growth rate index of the mycelium and the degree of the linear growth inhibition of fungi.

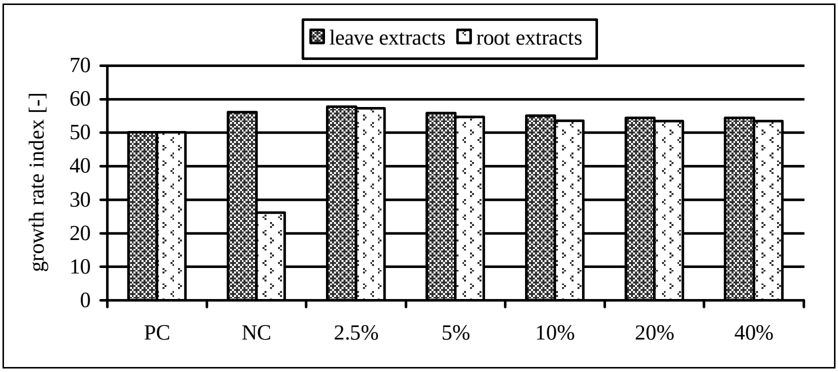

Fig. 2. Influence of the nettle extracts on the growth rate index of *F. proliferatum* on PDA medium. The symbols are in Figure 1

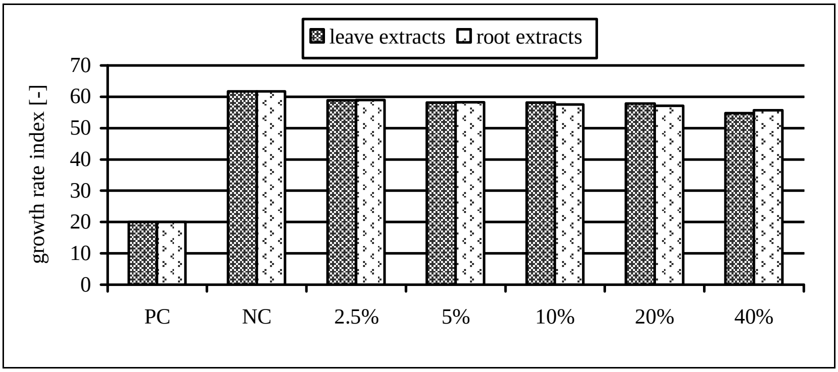

Fig. 3. Influence of the nettle extracts on the growth rate index of *F. proliferatum* on Tomato medium. The symbols as in Figure 1

The value of the growth rate index of *F. proliferatum* on PDA medium amounted from 53.46 to 57.31 for extracts of nettle root and from 54.39 to 57.79 for extracts of nettle leaves. The antagonistic activity of the both extracts were most effective on PDA medium when the nettle root extract at 40% concentration was applied. The percentage decrease was about 4.8% compared with the negative control. In contrast, the results obtained with nettle leave extract at the same concentration was lower and the percentage reduction was about 3.2% (Fig. 2). Similar activity of nettle extracts against *F. proliferatum* were obtained on Tomato medium, while the inhibitory efficacy of *U. dioica* was slightly higher. The value of the growth rate index amounted between 59.0-55.6 (for extracts of nettle root) and 58.8-54.8 (for extracts of nettle leaves). The decrease in the growth rate index was from 4.4 to 9.8% and from 4.6 to 11.2% compared with positive control, respectively. The highest measured value of the growth inhibition was noted at 40% concentration both the extract of nettle root and extract of nettle leaves (Fig. 3).

Selective activity of *U.dioica* against the phytopathogenic fungi has been described in many research papers [5, 8, 13, 14, 17]. It has been proved that antifungal activity of nettle extracts is closely related to composition and concentrations of extracts, the sensitivity of spores and mycelium of the fungus. Hadizadeh et al [5] sugested that extract of nettle at the highest concentration of 0.9% markedly inhibited the mycelium growth of *A. radicina* completely (100%) or almost completely (97%) of *R. solani* and showed a relatively high degree (80%) of *F. oxysporum* and *F. solani*. Similarly Tapwal et al [13] found that the *U.dioica* can restrict the growth of *A. radicina* (41.6%), *R. solani* (18.8%) and *F. oxysporum* (20.1%) but at a higher concentration the leave extract *ie* 20%. Other studies [17] report that the ethanolic extract and their compounds had inhibitory activities on the growth rate (from 3.17 to 8.97%) and mycelial weight of *P. digitatum*.

On the other hand nettle herb extracts stimulated linear growth of colonies, biomass increment and formation of spores in *A. sclerotiorum* and *A. alternata*. In the case of *F. oxysporum* increasing the number of macroconidia was recorded only in the highest concentration of nettle extract, although the inhibition of the linear mycelial growth was observed [14]. Similarly Modarresi-Chahardehi et al [11] observed a different effect of nettle extract on the fungi, notably inhibition the germination of conidia *A. flavus*, *A. fumigatus*, *Penicillium* sp. and *Rhizopus* sp., while found no effects on mycelium. The resistance of fungal species against crude extracts of *U. dioica* could be due to their morphological structure, fungi have thicker cell walls and contain higher percentage of chitin. Furthermore inhibition of mycelial growth and production of morphological elements may significantly reduce the survival of pathogens in the soil [11, 18].

## Conclusions

On the basis of conducted research one can draw the following observations:

1. Increased concentration the extracts of nettle leaves and nettle root inhibit linear growth of mycelium and spore germination of *F. proliferatum* compared to negative control, but increased compared to Topsin M 500 SC (positive control).
2. Nettle root extracts has demonstrated 2 fold more potent on the mycelium growth than the extract from the nettle leaves.
3. Much more intense effect (3-4 fold higher) compared to the linear growth revealed extracts on spore germination.
4. On the tomato medium inhibiting the linear growth of *Fusarium proliferatum* was 2-3 fold higher than on the PDA medium.
5. Due to the stronger effects of the Topsin M 500 SC should be carried out studies with higher concentrations of the nettle leaves and nettle root extracts of *U.dioica*.

## References

[1] Andrzejak R, Werner M. Progress Plant Protect. 2006;46(2):700-703. http://www.researchgate.net/publication/242516602_WPYW_WYBRANYCH_PREPARATW_NA_WZROST_IN_VITRO_GRZYBW_RODZAJU_FUSARIUM_USZKADZAJCYCH_WYPUSTKI_SZPARAGW.

[2] Kuzdraliński A, Paterek A, Gierasimuk N. Nauki Przyrodnicze. 2014;4(2):4-18. http://www.naukiprzyrodnicze.ssnp.org.pl/wpcontent/uploads/2014/09/Czasopismo2014nr2.pdf.

[3] Ślusarski C. Zesz Probl Postęp Nauk Roln. 2008;529:219-226. http://www.zeszytyproblemowe.pan.pl/images/stories/Zeszyty/2008/529/31-25slu.kal.pdf.

[4] Tekiela A. Wieś Jutra. 2010;3:219-226.

[5] Hadizadeh I, Peivastegan B, Kolahi M. Pak J Biol Sci. 2009;12(1):58-63. http://docsdrive.com/pdfs/ansinet/pjbs/2009/58-63.pdf.

[6] Fletcher JC, Bender B, Budowle WT. Microbiol Mol Biol Rev. 2006;70(2):450-471. DOI: 10.1128/MMBR.00022-05.

[7] Joshi BC, Mukhija M, Kalia AN. Int J Green Pharm. 2014;8(4):201-209. DOI: 10.4103/0973-8258.142669.

[8] Saklani S, Chandra S. Int J Pharm Rev Res. 2012;12(2):57-60. http://globalresearchonline.net/journalcontents/v12-2/010.pdf.

[9] Gülçin I, Küfrevioglu ÖI, Oktay M, Büyükokuroglu ME. J Ethnopharmacology. 2004;90:205-215. DOI: 10.1016/j.jep.2003.09.028.

[10] Sidaoui F, Belghith IS, Barth D, Trabelsi-Ayadi M, Cherif JK. Internat J Pharmacognosy Phytochem Res. 2015;7(4):707-713. http://ijppr.com/PDF/7/IJPPR,Vol7,Issue4,Article14.pdf.

[11] Modarresi-Chahardehi A, Ibrahim D, Fariza-Sulaiman S, Mousavi L. Int J Tro Biol. 2012;60(4):1567-1576. http://www.revistas.ucr.ac.cr/index.php/rbt/article/viewFile/2074/2037.

[12] Sanchez DOS, Najera GLA, Rivera IL, Ramirez OD, Cisneros GV, Garcia VMN. Polibtanica. 2009;28:23.

[13] Tapwal NA, Garg S, Gautam N, Kumar R. Braz Arch Biol Technol. 2011;54(6):1093-1098. http://www.scielo.br/pdf/babt/v54n6/03.pdf.

[14] Gleń K, Boligłowa E. J Res Appl Agric Eng. 2012;57(3):104109. http://www.pimr.poznan.pl/biul/2012_3_19GB2.pdf.

[15] Burgieł Z, Kończyk D. Acta Agraris et Silvestria. 1989;XXVIII:5-31.

[16] Burgieł Z, Acta Agraris et Silvestria. 1984;XXIII:187-195.

[17] Hadi M, Sorkhi MRA, Kashefi B, Sobhanipur A, Shamsi MH. Middle-East J Sci Res. 2013;17(12):1701-1708. DOI: 10.5829/idosi.mejsr.2013.17.12.1250.

[18] Madigan MT, Martinko JM, Brock TD. Brock Biology of Microorganisms. New Jersey, USA: Upper Saddle River, Pearson-Prentice Hall; 2006.

# OCENA WŁAŚCIWOŚCI PRZECIWGRZYBOWYCH POKRZYWY ZWYCZAJNEJ WOBEC *Fusarium proliferatum*

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**Abstract:** Celem przeprowadzonych badań była ocena właściwości przeciwgrzybowych ekstraktów z liści i korzenia pokrzywy zwyczajnej wobec *Fusarium proliferatum*. Ocenę właściwości antagonistycznych ekstraktów w stężeniach 2,5, 5,0, 10, 20 i 40% przeprowadzono metodą hodowlano-płytkową z zastosowaniem podłoży PDA pomidorowego. Hodowlę prowadzono w temp. 26ºC przez 9 dni, dokonując pomiarów co 1-2 dni. Kontrolę pozytywną stanowił Topsin M 500 S.C, a kontrolę negatywną woda destylowana. Określono indeks kiełkowania zarodników, indeks tempa wzrostu oraz stopień redukcji liniowego wzrostu *Fusarium* sp. Uzyskane wyniki badań wykazały zahamowanie kiełkowania zarodników przez wszystkie zastosowane stężenia ekstraktów z korzenia pokrzywy (od 7,5 do 47,3%) oraz stężenia ekstraktów z liści pokrzywy (od 5,9 do 54,0%) w porównaniu do próby negatywnej, lecz stymulujące działanie w porównaniu do próby pozytywnej. Najwyższy stopień zahamowania liniowego tempa wzrostu obserwowano zarówno po zastosowaniu ekstraktu z korzenia, jak i liści na podłożu pomidorowym odpowiednio o 9,8 i 11,2%, a na podłożu PDA odpowiednio o 5,5 i 3,2% przy stężeniu 40% w porównaniu do próby negatywnej, lecz stymulujące działanie w porównaniu do próby pozytywnej.

**Słowa kluczowe:** pokrzywa zwyczajna, aktywność przeciwgrzybowa, *Fusarium proliferatum*

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# APPLICATIONS OF NANOFORMULATIONS IN AGRICULTURAL PRODUCTION AND THEIR IMPACT ON FOOD AND HUMAN HEALTH

## ZASTOSOWANIA NANOZWIĄZKÓW W PRODUKCJI ROLNEJ I ICH WPŁYW NA ŻYWNOŚĆ I ZDROWIE CZŁOWIEKA

**Abstract:** In last years, an increasingly growing number of nanoagrochemicals such as nanopesticides, nanofertilizers or plant growth stimulating nanosystems is put into the practice. This is connected with the demand to decrease the dose-dependent toxicity of pesticides by reduction of the amount of applied active ingredients in nanoscale formulations, securing not only their increased aqueous solubility resulting in better bioavailability but also targeted delivery, controlled release and/or protection against degradation. Application of nanofertilizers enables more effective supply of nutrient to plants. The contribution is focused on nanoformulations applied in agriculture enabling controlled release of the active ingredient into weeds and the body of pests and nutrients to plants as well as on benefits of the use of nanoformulations in food industry related to food packaging, food security, encapsulation of nutrients and development of new functional products. The environmental impact of nanoagrochemicals and related health risks are highlighted as well.

**Keywords:** nanoinsecticides, nanoherbicides, nanofungicides, controlled release and targeted delivery, environmental impact, health risks

## Introduction

Nanotechnology, one of the key technologies of the 21st century, can improve current agricultural practices through the enhancement of management and conservation of inputs in crops, animal production and fisheries [1, 2] and to secure "sustainable intensification" of agricultural production resulting in increasing global food production, improved food quality and reduction of waste [3, 4].

Formulations containing nanoparticles (NPs) or encapsulated nanoscale materials with various coatings are suitable for improving the solubility and permeability, *ie* the bioavailability of an active ingredient (AI), reducing the dose of the AI and its dose-dependent toxicity, securing controlled release and targeted biodistribution of the AI and alleviating its impact on the environment [5-7].

Nanoscale herbicides, insecticides and fungicides can help to attenuate environmental pollution, loss of biodiversity and emergence of agricultural pests and pathogens. Moreover, the use of NPs in food sector can contribute to improvement of food safety by pathogen detection, design of smart food packaging systems and enrichment of food with nutraceuticals [2, 8, 9]. The increasingly widespread use of NPs in various areas of anthropogenic activities requires a thorough investigation of their environmental impacts and possible negative effects on human health.

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## Nanopesticides

Nanoscale herbicides, insecticides and fungicides were designed for better protection of plants from damaging influences such as weeds, insects or plant diseases in comparison with the application of bulk active ingredients. Methods of preparation of NPs and nanoformulations were summarized in [10]. Encapsulation of pesticides in polymeric core shells of NPs can result in safer and more convenient management of pesticides that promises environmental safety [11]. The control of parasitic weeds with nanoencapsulated herbicides was found to reduce the phytotoxicity of herbicides on crops [12]. The application of nanoherbicides is connected with a reduced environmental impact with simultaneous preservation of herbicidal effectiveness. Foliar application of nanoemulsion systems can be used to increase penetration and uptake of an active ingredient by weeds. Nanoformulations of a herbicide with polymer coating enable its controlled and slower release due to potential interactions between the herbicide and the polymer. CuO NPs were found to be more toxic than bulk CuO or dissolved Cu(II) ions to *Landoltia* (*Spirodela*) *punctata* [13] and *Zea mays* [14]; the activity of antioxidant enzymes increased after the treatment of *Elodea densa* plants with CuNPs [15]; CuO NPs induced also DNA damage in several plants.

Problems connected with plant fungal diseases can be alleviated by design and preparation of effective nanofungicides that can control fungal diseases by specifically inhibiting or killing the fungi that cause the diseases. Several metals (*eg*, Ag, Cu) or metal oxides (*eg*, CuO, ZnO) NPs were found to be effective fungicides. Well-dispersed and stabilized AgNPs solution can act as an excellent fungicide due to good adhesion on bacterial and fungal cell surface [16], and biosynthesized AgNP-based biopesticides can be used in the future as nanoweapon against phytopathogens [17]. Also the antifungal activity of CuNPs is connected mainly with NPs adhesion to the bacterial cell surface because of their opposite electrical charges, resulting in a reduction reaction at the bacterial cell wall [18]. Hexaconazole NPs (100 nm) stabilized by polyethylene glycol (PEG) were more potent than bulk hexaconazole and were found to be a safe nanofungicide [19]. The controlled release of carbendazim from nanoformulations of PEG-based functionalized amphiphilic copolymers was observed, and the efficacy of these nanoformulations against plant pathogenic fungi *Rhizoctonia solani* expressed by $ED_{50}$ values varied from 0.40 to 0.74 $mg/dm^3$ [20]. However, in the case of application on nanofungicide formulations in field, possible interactions of nanofungicides with non-target organisms affecting directly or indirectly the maintenance of soil fertility could be always considered [21].

Nanoinsecticides are agents of chemical or biological origin that control insects in more effective manner than the bulk insecticides. The insecticidal efficacy of liposome-based formulations was described also by Hwang et al [22] and Kang et al [23], and the chitosan coated nanoliposomes exhibited sustainable slower release of the entrapped core material (etofenprox and alpha-cypermethrin) due to thicker coating layer [24]. Pyridalyl nanosuspension prepared using sodium alginate was 2- and 6-fold more effective as stomach poison against *Helicoverpa armigera* than the technical product and the commercial formulation, respectively [25]. Also some nanosized inorganic materials (*eg* $Al_2O_3$, Fe(0), $TiO_2$, ZnO, Ag, $SiO_2$) were found to be good insecticides (in detail see in [10]).

A comprehensive survey on nanoherbicide, nanofungicide and nanoinsecticide formulations and their biological effects was published by Jampilek and Kralova [10].

## Nanofertilizers

Fertilizers play a pivotal role in increasing the agricultural production by up to 35-40%. Nanofertilizers represent an efficient means to distribute fertilizers in a controlled fashion with high site specificity, thus reducing collateral damage. They can reduce the dosage of nutrient, ensure its controlled slow delivery resulting in the increased efficacy of the fertilizer and thus overcome the problem of eutrophication. In nanofertilizers, the nutrients can be capsulated inside nanomaterials, coated with thin protective polymer film and delivered as particles or emulsions of nanoscale dimensions [26]. Fertilizers formulations of chitosan NPs for controlled release of NPK [27, 28], hydroxyapatite NPs for the supply of P nutrients to crops [29] and fertilizer loaded nanoclay/superabsorbent polymer composites [30] were used. Foliar application of nano-potassium and nano-calcium chelated fertilizers on *Ocimum basilicum* had beneficial effect on production characteristics of plants [31]; calcium phosphate nano gel fertilizer composites were found to increase the germination in *Oryza sativa*, *Arachis hypogea* and *Amaranthus spinosus* plants [32].

A significant improvement of some production characteristics of *Pennisetum americanum* L. plants due to application of ZnO NPs was observed by Tarafdar et al [33], while Pandey et al [34] reported about the enhanced activity of some enzymes after application of Zn nanofertilizer. Nano-$Al_2O_3$ enhanced root elongation of *Arabidopsis thaliana* [35] and *Lemna minor* [36] and increased the quantum yield of photosystem II [37]. Production characteristics of plants significantly increased also after the application of iron fertilizers [38, 39]. Nano-iron oxide was also found to facilitate the photosynthate and iron transferring to the leaves of peanut [40]. The single-walled carbon nanotubes (SWCNTs) were found to activate seed germination and enhance growth of different organs of several plants [41]. A beneficial effect on the growth of plants after application of multi-walled carbon nanotubes (MWCNTs) was observed as well [42-44]. Using nanotechnology, intelligent nanofertilizers can be designed in which extremely thin layer of polymer would be placed over the fertilizer granules and extremely tiny biological sensors situated in this polymer would be able to detect “signals” sent naturally by plant roots, which would essentially tell the polymer when to dissolve and release the nutrients into the soil [45].

## Nanotechnology in food industry

Important areas of nanotechnology in food sector are, for example, food safety (through the use of nanosensors for pathogen detection), smart packaging and valorisation of food products by nanoencapsulation/nanodelivery of food ingredients (*eg* flavours) [8, 46]. Nanostructured lipid carriers may improve the bioavailability and stability of bioactive compounds, consumer acceptability, functionality, nutritional value and safety of food systems, prolong shelf-life and provide controlled release of encapsulated materials [47]. Novel functional foods developed using nanotechnology may have physiological benefits or reduce risks of diseases. Vitamin D3 in whey protein isolate NPs was found to be suitable for use in clear or non-clear beverages as enriching agent [48].

The function of packaging is to protect the packed food and to maintain its integrity and quality. The package should hinder gain or loss of moisture, prevent microbial contamination and act as a barrier against transfer of oxygen, carbon dioxide and aromatic compounds. The packaging material itself should not promote deteriorative food quality changes or endanger the health of the consumer of the packed food as a consequence of uncontrolled migration of any chemical substances from packaging into food [49]. The use of NPs in food packaging can improve protection of foods, for example, by reducing permeation of gases, minimizing odour loss and increasing mechanical strength and thermal stability. Nanoscale food packaging materials may extend food life, improve food safety, alert consumers that food is contaminated or spoiled, repair tears in packaging and even release preservatives to extend the life of the food in the package [50]. Intelligent packaging is an emerging technology that uses the communication function of the package to facilitate decision making to achieve the benefits of enhanced food quality and safety [51]. Current nanocomposite technologies suitable to enhance the mechanical and barrier properties of synthetic polymers and biopolymers for food packaging, the development of intelligent packaging with enhanced communication function focusing mainly on oxygen, humidity and freshness indicators and nanostructured coatings that enhance the barrier properties of packaging films were summarized in a review paper of Mihindukulasuriya and Lim [52].

## Environmental impact of nanoparticles

Most hazardous chemicals applied in agriculture exert unwarranted toxicity and lethal effects on non-target organisms, develop physiological resistance in target and cause adverse environmental effect. By the use of green and efficient alternatives for the management of insect pests in agriculture, toxic effects on the environment could be alleviated [53, 54]. As favourable could be considered formulations that target the pests specifically and in turn, prevent pollution, for example, the “gut buster” (*ie* the encapsulated product) breaks open only when it comes in contact with the alkaline environment like the gut of the insects [55]. The application of such smart pesticides results in more precise, controlled and effective use of pesticides enabling potential reduction of the overall quantities of applied pesticides [56].

On the other hand, some NPs, for example, AgNPs, are toxic because they can destroy bacteria and other microorganisms, and their entry in the environment represents a risk that useful bacteria and aquatic organisms will be destroyed as well [57]. Consequently, although AgNP-based biopesticides could revolutionize the agricultural sector in the future, increased attention must be given to the impact of risk factors associated with their usage on the environment.

The toxicity of NPs depends on the shape, size, surface area and surface charge of NPs as well as on several abiotic factors such as pH, ionic strength, water hardness, presence of organic matter. The most frequent mechanisms of toxic effects exhibited by NPs are damage of membranes, generation of reactive oxygen species (ROS) and genotoxicity. Increased attention must be devoted to toxicological investigation of non-biodegradable materials due to risks connected with their accumulation and persistence in soil, plants and mammals, which may subsequently result in various pathological processes [58, 59]. The most important regulations related to the application of NPs especially in relation to nature, environment and health are discussed in [10].

## Health risks of nanoparticles

NPs can enter the human body in several ways: (i) via the lungs where a rapid translocation through the blood stream to vital organs is possible, including crossing the blood brain barrier; (ii) absorption by the intestinal tract; and (iii) absorption by the skin [60]. Toxic effects of nanomaterials are closely connected with physicochemical properties of NPs such as particle size and size distribution, agglomeration state, shape, crystal structure, chemical composition, surface area, surface chemistry, surface charge, and porosity [61].

Small sized (1-100 nm) NPs derived from Ag, Cu, Al, Si, carbon or metal oxides were found to easily cross the blood-brain barrier and/or produce damage to the barrier integrity by altering endothelial cell membrane permeability [62]. According to Cho et al [63], metal oxide NPs induce unique inflammatory footprints in the lung with a subsequent increase of the risk of asthma attacks and caused DNA damage in the human lung epithelial cell line A549 [64, 65]. A comprehensive critical review paper about applications of AgNPs and human health was presented by Ahamed et al [66]. Disruption of the mitochondrial respiratory chain by AgNPs resulting in increased ROS production and interruption of ATP synthesis and leading to DNA damage was observed [67]. AgNPs exhibited also geno- and cytotoxic effects in human mesenchymal stem cells (hMSCs) at high exposure concentrations [68] and reduced the cell viability of alveolar macrophages and lung epithelial cells [69].

Zero-valent iron NPs induced oxidative stress resulting in the damage of lung cells [70]. After exposure to SWCNTs, pulmonary granulomas were observed in rats [71], and functionalization of SWCNTs played an important role in their cytotoxicity [72]. Dermal exposure to unrefined SWCNTs led to dermal toxicity due to accelerated oxidative stress in the skin of exposed workers [73]. On the other hand, the diameter of MWCNTs was found to be a critical factor for inflammogenicity and the subsequent mesothelial carcinogenesis [74, 75]. Oxidized MWCNTs were found to induce massive loss of the viability of human T cells through programmed cell death at doses of 400 ng/dm$^3$, which corresponds to approximately 10 million carbon nanotubes per cell [76].

## Conclusion

The application of nanoscale science and nanotechnology in agricultural and food industry/production demonstrated to have a great potential in providing various innovations and improved solutions. In this contribution, the most important and frequent applications of nanotechnology in agricultural and food production are summarized. NPs and/or controlled release and targeted delivery nanoformulations are broadly used for agrochemicals (*eg*, nanopesticides, nanofertilizers) and were primarily designed to reduce the amount of applied active ingredients by means of their enhanced bioavailability and protection against degradation, which finally resulted in a decrease of dose-dependent toxicity for non-target organisms and environmental burden. The application of nanotechnology in the areas such as food packaging, food security, detection of pathogens and contaminants by using nanosensors and indicators, encapsulation of nutrients and development of new functional products is growing rapidly. Nanoscale food packaging materials help to extend food life. Nano-size materials change their physical and chemical properties in comparison with bulk materials and can become toxic when reaching nano-size. Therefore increased attention must be devoted to the impact of risk factors associated with their usage on the environment and possible adverse effects on non-target organisms and mammals, especially humans.

## References

[1] Thornton PK. Phil Trans R Soc B. 2010;365:2853-2867. DOI: 10.1098/rstb.2010.0134.

[2] Sekhon BS. Nanotechnol Sci Appl. 2014;7:31-53. DOI: 10.2147/NSA.S39406.

[3] Sonkaria S, Ahn SH, Khare V. Recent Pat Food Nutr Agric. 2012;4:8-18. DOI: 10.2174/2212798411204010008.

[4] Pérez-de-Luque A, Hermosín MC. Nanotechnology and its use in agriculture. In: Bagchi D, Bagchi M, Moriyama H, Shahidi F, editors. Bio-nanotechnology: A Revolution in Food, Biomedical and Health Sciences. West Sussex: Wiley-Blackwell; 2013:299-405.

[5] Nalwa HS. Encyclopedia of Nanoscience and Nanotechnology. Valencia, CA, USA: American Scientific Publisher; 2004-2011.

[6] Bhushan B. Handbook of Nanotechnology. Berlin-Heidelberg: Springer-Verlag; 2004.

[7] Rai M, Ribeiro C, Mattoso L, Duran N. Nanotechnologies in Food and Agriculture. Heidelberg: Springer; 2015.

[8] Lopes CM, Fernandes JR, Martins-Lopes P. Food Technol Biotechnol. 2013;51:183-197. http://www.ftb.com.hr/images/pdfarticles/2013/April-June/ftb_51-2_183-197.pdf.

[9] Knauer K, Bucheli TD. Nano-materials: Research needs in agriculture. Rev Suisse Agric. 2009;41:341-345.

[10] Jampílek J, Kráľová K. Ecol Chem Eng S. 2015;22:321-361. DOI: 10.1515/eces-2015-0018.

[11] Kumar S, Bhanjana G, Sharma A, Sarita, Sidhu MC, Dilbaghi N. Sci Adv Mater. 2015;7:1143-1148. DOI: 10.1166/sam.2015.2243.

[12] Pérez-de-Luque A, Rubiales D. Pest Manag Sci. 2009;65:540-545. DOI: 10.1002/ps.1732.

[13] Shi JY, Abid AD, Kennedy IM, Hristova KR, Silk WK. Environ Pollut. (Oxford, U.K.). 2011;159:1277-1282. DOI: 10.1016/j.envpol.2011.01.028.

[14] Wang ZY, Xie XY, Zhao J, Liu XY, Feng WQ, White JC, et al. Environ Sci Technol. 2012;46:4434-4441. DOI: 10.1021/es204212z.

[15] Nekrasova GF, Ushakova OS, Ermakov AE, Uimin MA, Byzov IV. Russ J Ecol. 2011;42:458-463. DOI: 10.1134/S1067413611060117.

[16] Kim HS, Kang HS, Chu GJ, Byun HS. Solid State Phenom. 2008;135:15-18. DOI: 10.4028/www.scientific.net/SSP.135.15.

[17] Mishra S, Singh HB. Appl Microbiol Biotechnol. 2015;99:1097-1107. DOI: 10.1007/s00253-014-6296-0.

[18] Mondal KK, Mani C. Ann Microbiol. 2012;62:889-893. DOI: 10.1007/s13213-011-0382-7.

[19] Kumar R, Nair KK, Alam MI, Gogoi R, Singh PK, Srivastava C, et al. J Nanosci Nanotechnol. 2015;15:1350-1356. DOI: 10.1166/jnn.2015.9088.

[20] Koli P, Singh BB, Shakil NA, Kumar J, Kamil D. J Environ Sci Health B Pestic Food Contam Agric Wastes. 2015;50:674-681. DOI: 10.1080/03601234.2015.1038961.

[21] Yadav A, Aggarwal A. NY Sci J. 2014;7:7-15. http://www.sciencepub.net/newyork/ny0711/002_27311ny071114_7_15.pdf.

[22] Hwang IC, Kim TH, Bang SH, Kim KS, Kwon HR, Seo MJ, et al. J Fac Agric Kyushu Univ. 2011;56:33-40, http://catalog.lib.kyushu-u.ac.jp/handle/2324/19633/p033.pdf.

[23] Kang MA, Seo MJ, Hwang IC, Jang C, Park HJ, Yu YM, et al. J Asia Pac Entomol. 2012;15:533-541. DOI: 10.1016/j.aspen.2012.05.015.

[24] Suresh Kumar RS, Shiny PJ, Anjali CH, Jerobin J, Goshen KM, Magdassi S, et al. Environ Sci Pollut Res. 2013;20:2593-2602. DOI: 10.1007/s11356-012-1161-0.

[25] Saini P, Gopal M, Kumar R, Srivastava C. J Environ Sci Health B. 2014;49:344-351. DOI: 10.1080/03601234.2014.882168.

[26] Rai V, Acharya S, Dey N. J Biomater Nanobiotechnol. 2012;3:315-324. DOI: 10.4236/jbnb.2012.322039.

[27] Corradini E, De Moura M, Mattoso L. eXpress Polym Lett. 2010;4:509-515. DOI: dx.doi.org/10.3144/expresspolymlett.2010.64.

[28] Hasaneen MNA, Abdel-Aziz HMM, El-Bialy DMA, Omer AM. Afr J Biotechnol. 2014;13:3158-3164. DOI: 10.5897/AJB2014.13699.

[29] Sarkar S, Datta SC, Biswas DR. Proc Natl Acad Sci B Biol Sci. 2015;85:415-421. DOI: 10.1007/s40011-014-0371-2.

[30] Liu RQ, Lal R. Sci Rep. 2014;4:Article number 5686. DOI: 10.1038/srep05686.

[31] Ghahremani A, Akbari K, Yousefpour M, Ardalani H. Int J Pharm Res Schol. 2014;3:235-241. http://ijprs.com/abstract.php?id=404&aid=417.

[32] Umarani R, Mala R. Asian J Microbiol Biotechnol Environ Sci. 2013;15:327-331.

[33] Tarafdar JC, Raliya R, Mahawar H, Rathore I. Agric Res. 2014;3:257-262. DOI: 10.1007/s40003-014-0113-y.

[34] Pandey AC, Sanjay SS, Yadav RS. J Exp Nanosci. 2010;6:488-497. DOI: 10.1080/ 17458081003649648.

[35] Lee CW, Mahendra S, Zodrow K, Li D, Tsai YC, Braam J, et al. Environ Toxicol Chem. 2010;29:669-675. DOI: 10.1002/etc.58.

[36] Juhel G, Batisse E, Hugues Q, Daly D, van Pelt FN, O´Halloran J, et al. Aquatic Toxicol. 2011;105:328-336. DOI: 10.1016/j.aquatox.2011.06.019.

[37] Nel A, Xia T, Madler L, Li N. Science. 2006;311:622-627. DOI: 10.1126/science.1114397.

[38] Askari M, Amirjani M, Saberi T. J Plant Process Function. 2014;3:43-56. http://jispp.iut.ac.ir/browse.php?a_id=129&sid=1&slc_lang=en.

[39] Baghai N, Sayide MF. J Saffron Res. 2014;1:156-169. http://en.journals.sid.ir/ViewPaper.aspx?ID=374410.

[40] Liu XM, Zhang FD, Zhang SQ, He XS, Wang RF, Wang YJ. Plant Nutr Fertilizer Sci. 2005;11:14-18.

[41] Lahiani MH, Dervishi E, Chen J, Nima Z, Gaume A, Biris AS, et al. ACS Appl Mater Interfaces. 2013;5:7965-7973. DOI: 10.1021/am402052x.

[42] Wang XP, Han HY, Liu XQ, Gu XX, Chen K, Lu DL. J Nanopart Res. 2012;14:Article Number 841. DOI: 10.1007/s11051-012-0841-5.

[43] Mondal A, Basu R, Das S, Nandy P. J Nanopart Res. 2011;13:4519-4528. DOI: 10.1007/s11051-011-0406-z.

[44] Tripathi S, Sonkar SK, Sarkar S. Nanoscale. 2011;3:1176-1181. DOI: 10.1039/c0nr00722f.

[45] Monreal CM. Intelligent NanoFertilizers. AAFC & Carleton University; 2012, http://bio.albertainnovates.ca/media/60531/monreal.pdf.

[46] Neethirajan S, Jayas DS. Food Bioprocess Technol. 2011;4:39-47. DOI: 10.1007/s11947-010-0328-2.

[47] Tamjidi F, Shahedi M, Varshosaz J, Nasirpour A. Innov Food Sci Emerg Technol. 2013;19:29-43. DOI: 10.1016/j.ifset.2013.03.002.

[48] Abbasi A, Emam-Djomeh Z, Mousavi MAE, Davoodi D. Food Chem. 2014;143:379-383. DOI: 10.1016/j.foodchem.2013.08.018.

[49] Johansson C. Functional barriers against migration for food packaging. In: Lagaron JM, editor. Multifunctional and Nanoreinforced Polymers for Food Packing. Woodhead Publishing in Materials; 2011:316-344.

[50] Ajaykumar MT. Res J Biotechnol. 2014;9:12-24.

[51] Yam KL, Takhistov PT, Miltz J. J Food Sci. 2005;70:R1-R10. DOI: 10.1111/j.1365-2621.2005.tb09052.x.

[52] Mihindukulasuriya SDF, Lim LT. Trends Food Sci Technol. 2014;40:149-167. DOI: 10.1016/j.tifs.2014.09.009.

[53] Bhattacharyya A, Bhaumik A, Rani PU, Mandal S, Epidi T. Afr J Biotechnol. 2010;9:3489-3493.

[54] Rai M, Ingle A. Appl Microbiol Biotechnol. 2012;94:287-293. DOI: 10.1007/s00253-012-3969-4.

[55] Prasad R, Kumar V, Prasad KS. Afr J Biotechnol. 2014;13:705-713. DOI: 10.5897/AJBX2013.13554.

[56] Lyons K, Scrinis G. Under the regulatory radar? Nanotechnologies and their impacts for rural Australia. In: Merlan F, Raftery D, editors. Tracking Rural Change: Community, Policy and Technology in Australia, New Zeeland and Europe. Abilene: ANU Press; 2009:151-171, http://press.anu.edu.au/tracking/pdf/ch08.pdf.

[57] Masarovičová E, Kráľová K. Ecol Chem Eng S. 2013;20:9-22. DOI: 10.2478/eces-2013-0001.

[58] Handy RD, Owen R, Valsami-Jones E. Ecotoxicology. 2008;17:315-325. DOI: 10.1007/s10646-008-0206-0.

[59] Vaculíková E, Plachá D, Čech-Barabaszová K, Jampílek J. Adv Sci Eng Med. 2014;6:477-485. DOI: 10.1166/asem.2014.1529.

[60] Hoet PHM, Brüske-Hohlfeld I, Salata OV. J. Nanobiotechnol. 2004;2:12. DOI:10.1186/1477-3155-2-12.

[61] Obersdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, et al. Part Fibre Toxicol. 2005;2:8. DOI: 10.1186/1743-8977-2-8.

[62] Sharma HS, Hussain S, Schlager J, Ali SF, Sharma A. Acta Neurochir Suppl 2010;106:359-364. DOI: 10.1007/978-3-211-98811-4_65.
[63] Cho WS, Duffin R, Poland CA, Howie SEM, MacNee W, Bradley M, et al. Environ Health Perspect. 2010;118:1699-1706. DOI: 10.1289/ehp.1002201.
[64] Karlsson HL, Cronholm P, Gustafsson J, Möller L. Chem Res Toxicol. 2008;21:1726-1732. DOI: 10.1021/tx800064j.
[65] Wang Z, Li N, Zhao J, White JC, Qu P, Xing B. Chem Res Toxicol. 2012;25:1512-1521. DOI: 10.1021/tx3002093.
[66] Ahamed M, AlSalhi MS, Siddiqui MKJ. Clin Chim Acta. 2010;411:1841-1848. DOI: 10.1016/j.cca.2010.08.016.
[67] AshaRani PV, Mun GK, Hande MP, Valiyaveettil S. ACS Nano 2009;3:279-290. DOI: 10.1021/nn800596w.
[68] Hackenberg S, Scherzed A, Kessler M, Hummel S, Technau A, Froelich K, et al. Toxicol Lett. 2011;201:27-33. DOI: 10.1016/j.toxlet.2010.12.001.
[69] Soto K, Garza KM, Murr LE. Acta Biomater. 2007;3:351-358. DOI: 10.1016/j.actbio.2006.11.004.
[70] Keenan CR, Goth-Goldstein R, Lucas D, Sedlak DL. Environ Sci Technol. 2009;3:4555-4560. DOI: 10.1021/es9006383.
[71] Warheit D, Laurence B, Reed K, Roach D, Reynolds G, Webb T. Toxicol Sci. 2004;77:117-125. DOI: 10.1093/toxsci/kfg228.
[72] Sayes C, Liang F, Hudson J, Mendez J, Guo W, Beach J, et al. Toxicol Lett. 2006;161:135-142. DOI:10.1016/j.toxlet.2005.08.011.
[73] Shvedova A, Castranova V, Kisin E, Schwegler-Berry D, Murray AR, Gandelsman VZ, et al. J Toxicol Environ Health. 2003;66:1909-1926. DOI: 10.1080/713853956.
[74] Nagai H, Okazaki Y, Chew S, Misawa N, Yamashita Y, Akatsuka S, et al. Proc Natl Acad Sci USA. 2011;108:E1330-1338. DOI: 10.1073/pnas.1110013108.
[75] Poland C, Duffin R, Kinloch I, Maynard A, Wallace W, Seaton A, et al. Nat Nanotechnol. 2008;3:423-428. DOI: 10.1038/nnano.2008.111.
[76] Bottini M, Bruckner S, Nika K, Bottini N, Bellucci S, Magrini A, et al. Toxicol Lett. 2006;160:121-126. DOI: 10.1016/j.toxlet.2005.06.020.

# ZASTOSOWANIA NANOZWIĄZKÓW W PRODUKCJI ROLNEJ I ICH WPŁYW NA ŻYWNOŚĆ I ZDROWIE CZŁOWIEKA

**Abstrakt:** W ostatnich latach coraz bardziej rośnie liczba nanochemikaliów stosowanych w rolnictwie, takich jak: nanopestycydy, nanonawozy lub stymulujące wzrost roślin nanosystemy. Jest to związane z potrzebą ograniczenia, zależnej od dawki, toksyczności pestycydów poprzez zmniejszenie ilości stosowanych składników aktywnych w nanopreparatach, zapewniając nie tylko ich zwiększoną rozpuszczalność w wodzie, co gwarantuje lepszą biodostępność, ale również kontrolowane uwalnianie i/lub ochronę przed degradacją. Zastosowanie nanonawozów umożliwia bardziej efektywne dostarczanie składników odżywczych roślinom. W pracy przedstawiono nanozwiązki stosowane w rolnictwie, umożliwiające kontrolowane uwalnianie składnika czynnego do chwastów i szkodników oraz składników odżywczych dla roślin, jak również korzyści wynikające z użycia nanozwiązków w przemyśle spożywczym związanym z pakowaniem żywności, bezpieczeństwem żywności, kapsułkowaniem składników odżywczych i rozwojem nowych produktów funkcjonalnych. Omówiono również wpływ nanoagrochemikaliów na środowisko i związane z nimi zagrożenia dla zdrowia.

**Słowa kluczowe:** nanoinsektycydy, nanoherbicydy, nanofungicydy, kontrolowane uwalnianie i ukierunkowana dostawa, wpływ na środowisko, zagrożenie dla zdrowia

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# ASSESSMENT OF ANTIFUNGAL ACTIVITY OF EXTRACTS FROM NETTLE (*Urtica dioica* L.) AGAINST *Alternaria solani*

## OCENA AKTYWNOŚĆ PRZECIWGRZYBOWEJ EKSTRAKTÓW Z POKRZYWY ZWYCZAJNEJ (*Urtica dioica* L.) WOBEC *Alternaria solani*

**Abstract:** The aim of research was to assess antifungal activity of nettle extracts at 2.5, 5.0, 10.0, 20.0, 40.0% concentrations obtained from a root and leaves against phytopathogenic strain *Alternaria solani*. Their antifungal activity was assessed on the basis of the growth rate index of mycelium and the spore germination index. It has also been assessed how different sterilisation techniques affect the properties of extracts under study. It has been proved, on the basis of obtained results, that extracts sterilised with saturated steam under pressure did not show antifungal activity. For the root extract, which showed the highest antifungal activity, only 7% of the mycelial growth inhibition have been obtained. Whereas, the root extracts sterilised by filtration limited the mycelial growth by 75% and spore germination of *A. solani* by 38%.

**Keywords:** antifungal activity, *Urtica dioica*, *Alternaria solani*, growth rate index, spores germination index

## Introduction

Tomatoes grown in field conditions are very often infested by *Alternaria* fungi, which cause alternaria diseases. The crops may be decreased due to leaves damage and the tomatoes damage. The pathogen may cause the tomato seedling blight and fruit rot [1]. The protection of plants is mainly based on the proper agricultural technology, an application of resistant plant varieties and the use of conventional chemical fungicides. However, the current schemes of plant disease control are being verified more frequently and natural substances *eg* plant extracts, are introduced as a possibility of plant protection [2]. In ecological cultivations of tomatoes, it is recommended to perform copper-sprayings alternately with grapefruit extracts, once the risk of plant disease occurs. The results of recently conducted research have proved that natural sources of compounds with antibacterial, antifungal and antioxidant properties are herbal plants including *Urtica dioica* L. often called common nettle or stinging nettle [3, 4].

*Urtica dioica* is widespread through Europe and North America, and also occurs in North Africa and parts of Asia. There are naturalised populations in several other parts of the world. The plant has a long history of use as a source of medicine, food and fibre [3, 5, 6].

The commonly known phytochemical compounds from *U. dioica* are lectins, sterols, terpenes, volatile compounds and fatty acids, polysaccharides, protein, vitamins, minerals and flavonoids [3, 6]. The flavonoids are mainly kaempferol, isorhamnetin, quercetin, isoquercitrin and rutin. The shikimic acid derivatives like phenylpropanes, caffeic acid and various esters of this acid such as chlorogenic acid and caffeoyl malic acid have been identified. The leaves are rich in vitamins B, C, K (phylloquinone), carotenoids and

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minerals such as calcium, iron, magnesium, phosphorus, potassium and sodium [6, 7]. Other main constituents present are amino acids, tannins, glucokinnins and chlorophyll. GC-MS analysis of *U. dioica* essential oil by Gul et al [8] identified 43 compounds. The main components of essential oil of *U. dioica* are carvacrol, carvone, naphthalene, (E)-anethol, hexahydrofarnesyl acetone, (E)-geranyl acetone, (E)-β-ionone and phytol [8].

Due to varied phytochemical composition, the common nettle has been universally applied, however, among herbal plants it has been most underrated. Not many research papers describe its potential application when fighting against phytopathogenic moulds, focusing on its medical properties instead. In folk medicine, nettle plants have been used as a diuretic, antibleeding, stimulating blood circulation, nutritional, anticancer, antiatherosclerotic, antiasthmatic, antidandruff, hemostatic and hypoglycemic. For a long time nettle has been used to treat arthritis and rheumatism. It also inhibits inflammation of the urinary tract and the digestive tract, improves digestion and an absorption of nutrients. It stimulates the immune system, increasing the resistance against infections (immunostimulant) [3, 6, 9, 10].

The raw materials collected for the research are leaves, stem, root with rhizomes and seeds. Most often, however, leaves and roots are used [3, 6]. The raw material is very often microbiologically infested and may be an additional source of contamination. Therefore, prior to its application, it is recommended to perform a sterilisation process, which in consequence, as it a thermal process, may lead to changes in natural compounds content and their activity. The above mentioned changes depend on the processing conditions of the plant, mainly the length of time and the temperature of heating.

The aim of the research was to assess the antifungal activity of nettle extracts (*U. dioica*) subject different sterilisation processes against phytopathogenic strain *Alternaria solani*.

## Materials and methods

In the research, the antifungal activity of commercial extracts from the root and leaf subject to sterilisation process have been assessed. The dry root extract containing 0.8% of phytosterols contained less than 300 CFU/g of microorganisms and the leaf extract of about 100 CFU/g. The analysed extracts were used to prepare aqueous solutions at the following concentrations: 2.5, 5.0, 10.0, 20.0 and 40.0%. The solutions were left for several hours at the room temperature and sterilised afterwards. The sterilisation process was carried out with saturated steam pressure in an autoclave for 15 minutes at 121°C and the pressure of 2 bars. The other sterilisation method employed vacuum filtration with Sartorius membrane filters with pore size of 0.2 μm. Obtained extracts were marked as STA (sterilisation in the autoclave) and STF (filtration sterilisation) respectively.

In the experiment, an indicator strain was *Alternaria solani* - strain isolated from the surface of the tomato fruit infested with alternaria disease.

A fungistatic activity of the extracts under study was assessed with the culture-plate method on PDA medium containing [g/dm$^3$]: glucose 20.0, potato extract 4.0, agar 15.0. In test trials, certain concentrations of root and leaf extracts were added on the culturing medium and then the plates were inoculated with fungi discs of 10 mm diameter. The control trial contained cultures of *A. solani* with sterile water instead of the supernatant. All plates were incubated at 25±2°C for 10 days. Every 1-2 days the measurements of the fungi discs diameter were noted until the mycelium of *A. solani* in the control trial overgrown the plate. All measurements were conducted in 3 replicates, where one replicate was the plate with the culturing medium and one mycelial disc. The activity of nettle extracts on the growth of *A. solani* was assessed against the growth rate index, calculated according to the formula [11]:

$$GRI = \frac{A}{D} + \frac{b_1}{d_1} + \frac{b_2}{d_2} + \ldots + \frac{b_x}{d_x}$$

where GRI represents the growth rate index, $A$ is a mean value of diameter measurements [mm], $D$ is the length of the experiment (number of days), $b_1$, $b_2$, $b_x$ denote an increase in a diameter size since the last measurement and $d_1$, $d_2$, $d_x$ are the number of days since the last measurement.

The evaluation of fungistatic activity was also carried out on the basis of spores germination of *A. solani* in the presence of tested concentrations of nettle root and leaf extracts. In the control trial the spores were suspended in water. All tests were run in 3 replicates. Microscopic observations were completed after 24 hours of the extracts activity, and the spores germination evaluation was based on the following scale [11]:

0 - non-germinatin conidia,
1 - germ tube shorter than the length of the spore,
2 - the length of germ tube equal to the length of the spore,
3 - germ tube twice as long as the length of the spore,
4 - germ tube branched and many times longer than the spore.

The effect of tested extracts on the spore germination has been evaluated on the basis of germination index, calculated according to the formula [11]:

$$SGI = \frac{\sum (n \cdot a) \cdot 100}{N \cdot 4}$$

where: *SGI* represents the spores germination index, $n$ - number of the spores in the specific grade on the scale, $a$ - grade on the scale, $N$ - general number of the counted spores, 4 - the highest grade of the scale.

The toxicity of the nettle was measured as a percentage of the colony growth inhibition or the germination ability showed in the presence of the analysed extracts against control trials containing water (controls).

The research has not included tests on the control trials which were not sterile, as preliminary analysis showed that plant material was heavily contaminated microbiologically, which prevented the measurement of the mycelium and conidia observation.

In order to determine the significant statistical differences ($p < 0.05$) between *U. dioica* extracts sterilised with different methods, ANOVA analysis has been applied with Tukey’s HSD test.

## Results and discussion

Presented papers show a pilot research, in which fungistatic activity of *U. dioica* root and leaf extracts against pathogenic strain *A. solani* have been evaluated. Conducted tests allowed to determine how sterilisation methods of the extracts affect their activity and the growth pace of tested strain.

Table 1

Influence of extracts of *U. dioica* sterilised in the autoclave (STA) and by filtration (STF) on the mycelial growth rate index of *A. solani*

| | **STA** | | | | **STF** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Root extract** | | **Leaf extract** | | **Root extract** | | **Leaf extract** | |
| | **mean** | **SD** | **mean** | **SD** | **mean** | **SD** | **mean** | **SD** |
| Control | $57.5^{a}$ | ±2.2 | $58.2^{a}$ | ±2.1 | $58.8^{a}$ | ±1.2 | $58.8^{a}$ | ±1.2 |
| 2.5% | $55.54^{a}$ | ±0.64 | $55.4^{a}$ | ±1.6 | $15.14^{b}$ | ±0.81 | $15.80^{b}$ | ±0.69 |
| 5.0% | $54.7^{a}$ | ±1.3 | $54.9^{a}$ | ±1.4 | $19.5^{c}$ | ±2.8 | $16.47^{b}$ | ±0.82 |
| 10.0% | $54.6^{a}$ | ±3.8 | $55.9^{a}$ | ±3.2 | $15.60^{bc}$ | ±0.45 | $16.80^{b}$ | ±0.86 |
| 20.0% | $54.9^{a}$ | ±1.9 | $53.9^{b}$ | ±1.6 | $17.97^{bc}$ | ±4.3 | $15.5^{b}$ | ±2.0 |
| 40.0% | $53.3^{a}$ | ±2.4 | $55.6^{a}$ | ±3.2 | $14.7^{bd}$ | ±1.4 | $17.09^{b}$ | ±0.86 |

Different letters indicate significant differences (ANOVA, $p < 0.05$, Tukey's HSD test)

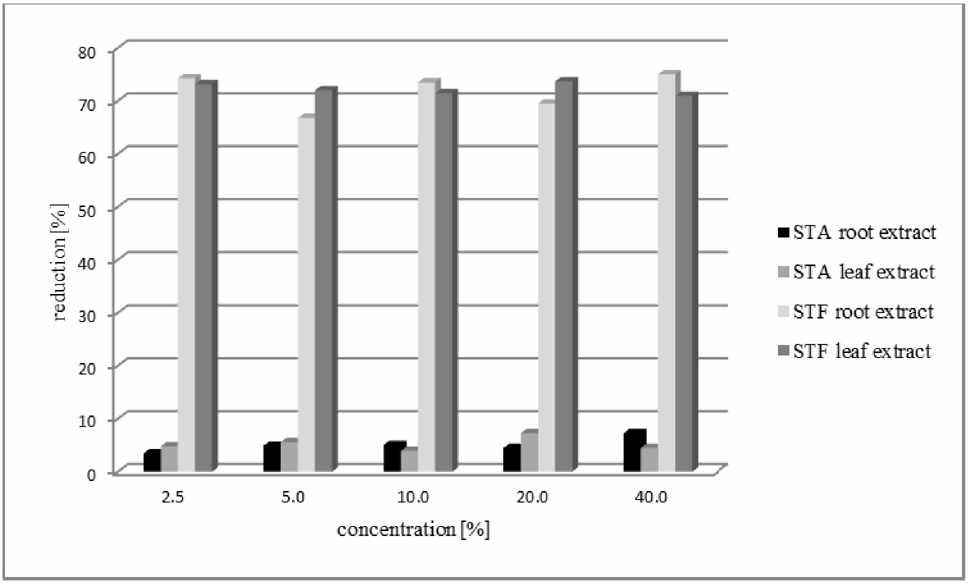

Fig. 1. The reduction of the mycelial growth rate index of *A. solani* induced by *U. dioica* extracts sterilised in the autoclave (STA) and by filtration (STF)

The values of GRI characterising the mycelial growth pace of *A. solani*, for the consecutive concentrations of the roof and leaf extracts sterilized in autoclave (STA) and by filtration (STF) have been presented in Table 1. The table presents arithmetic mean values of GRI and standard deviation for the trial. The highest noted values of GRI, not exceeding

58 units, were obtained in control trials. Plant extracts sterilized by saturated steam under pressure did not inhibit the mycelial growth of *A. solani* and no statistically significant differences were noted for the root and leaf extracts in comparison with the control trials. The opposite has been observed when sterilisation applied filtration method. In this case statistically significant differences were obtained between tested and control trials. However, no clear correlation has been found between the type of the extract, its concentration and GRI. The highest value of GRI has been noted for the root extract at 0.5% concentration and the lowest at 40.0% concentration obtaining the growth reduction of 75% (Table 1, Fig. 1). For the remaining extracts under study and their concentrations, measured values of GRI fluctuated at a similar level and obtained differences were not statistically significant. Antifungal activity of the nettle extracts have also been proved by other researchers [12, 13]. Hadizadeh et al [12] obtained full inhibition of the mycelial growth of *A. alternata* after the application of 0.9% nettle extract. It should be noted, however, that applied extract was the ethanol extract not the aqueous extract used in the presented paper. The same results were obtained by Hadizadeh et al [13] with the application of 1500 and 2000 ppm essential nettle oil. Similar results were noted by Tapwal et al [14], who observed the highest mycelial growth inhibition of *A. solani* and *A. zinniae* (41.67 and 29.87% respectively) when 20.0% aqueous nettle extract was applied.

In conducted studies, it has also been assessed how *U. dioica* extracts affect spore germination abilities of *A. solani* depending on the concentration of the extracts. As shown in Table 2 the extracts sterilised in the autoclave did not show antifungal activity. Although no statistically significant differences have been noted between the control trial and tested trial, it should be noted that SGI values in tested trials were higher than the values obtained in the control trials. On the basis of microscopic observations, it has been noted that in the control trials dominated germinating spores of the 4th type on the scale and single non-germinating spores were present. In the test trials only spores constructing very long and multi-branched tubes were visible. It has been assumed that all spores germinated as type 4 and the value of SGI amounted 100. The above results show, that extracts sterilised in the autoclave simulated germination of *A. solani* only slightly. Therefore Figure 2 does not include any data concerning values of SGI obtained when sterilisation in the autoclave was applied. However, extracts sterilized with filtration inhibited spores germination. Siginificant statistical differences in values of SGI have been noted for all concentrations of the root extracts and for 5.0% concentration of the extract from the nettle leaf. For the root extracts, the lowest value of SGI amounting 60.21 units have been obtained for 40.0% extract, which caused reduction higher that 37% (Table 2, Fig. 2). However, according to other research papers, the spores transported with the rain drops or by wind are responsible for spreading the pathogen and their growth reduction would allow to inhibit the alternaria diseases [1, 15]. For this reason, 37% reduction obtained in own research might not be sufficient in practice, as proved by research of Pati and Kolte [16], who obtained 86.6% reduction of the germinating spores of *A. brassicae* after the application of *U. dioica* extract and described it as a poor result. The essential nettle oil used by Hadizadeh et al [13] did not allow to completely reduce germinating spores either. The authors obtained the dicrease in germinating spores of 93.9% and the reduction in the length of germinating hyphae. The above show that spores of *Alternaria* spp. are less sensitive to the nettle extracts than the mycelium.

Table 2

The effect of *U. dioica* extracts sterilised in the autoclave (STA) and by filtration (STF) on the spore germination index of *A. solani*

| | **STA** | | | | **SF** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Root extract** | | **Leaf extract** | | **Root extract** | | **Leaf extract** | |
| | **mean** | **SD** | **mean** | **SD** | **mean** | **SD** | **mean** | **SD** |
| Control | $96.2^{a}$ | ±3.9 | $96.2^{a}$ | ±3.9 | $97.09^{a}$ | ±0.61 | $97.09^{a}$ | ±0.61 |
| 2.5% | $100^{a}$ | ±0 | $100^{a}$ | ±0 | $82.03^{bc}$ | ±0.78 | $94.8^{a}$ | ±3.2 |
| 5.0% | $100^{a}$ | ±0 | $100^{a}$ | ±0 | $89.72^{b}$ | ±0.14 | $74.0^{b}$ | ±2.5 |
| 10.0% | $100^{a}$ | ±0 | $100^{a}$ | ±0 | $92.2^{b}$ | ±1.2 | $96.4^{a}$ | ±1.7 |
| 20.0% | $100^{a}$ | ±0 | $100^{a}$ | ±0 | $91.3^{b}$ | ±1.3 | $95.7^{a}$ | ±1.7 |
| 40.0% | $100^{a}$ | ±0 | $100^{a}$ | ±0 | $60.2^{bd}$ | ±1.3 | $94.3^{a}$ | ±1.6 |

Different letters indicate significant differences (ANOVA, $p < 0.05$, Tukey's HSD test)

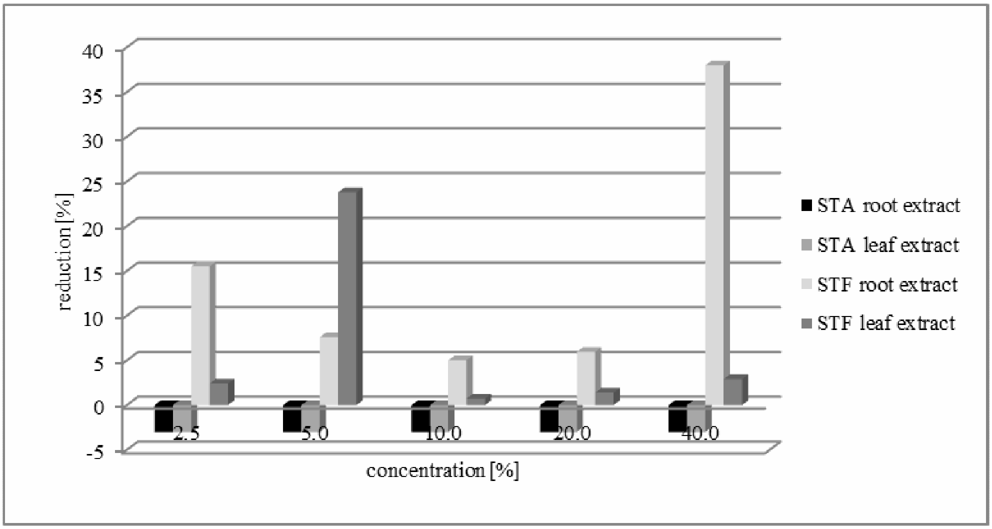

Fig. 2. Reduction of the spore germination index of *A. solani* induced by *U. dioica* extracts sterilized by filtration (STF)

In Poland, the tomato field plantations are protected against alternaria disease with numerous conventional fungicides. In order to reduce the risks associated with the application of pesticides and their effects on people's health it is advised to develop and introduce an integrated plant protection or techniques, which aim at reducing the amount of applied pesticides. A good solution might be extracts prepared on the basis of common herbal plants. However, natural extracts used in plant protection, may be microbiologically contaminated and become a new source of contamination. The contamination may be primary and may depend on the natural environment or secondary, caused for example by an improper storage of plants, their packaging or transportation. In order to avoid the contamination of herbal material, the plants are sterilised. It should be noted, that it is a thermal process and may lead to change in the content of natural ingredients, which will directly affect their properties. These changes depend largely on the conditions of the process, that is the time and the temperature of warming the plant material, which has been proved by the authors' own research. It has been proved that extracts of *U. dioica* under study, sterilised with saturated steam under pressure do not show antifungal properties against phytopatogenic strain *A. solani* contrary to extracts sterilized with the use of filters. Furthermore, in the research, the root extract of *U. dioica* was more active than the extract from the leaf. This is probably associated with the differences in chemical content of the extracts and further changes in this content which may occur under the high temperature. The influence of the temperature on antimicrobial activity of plant extracts is not clear though. It may be stated, referring to other research papers, that the activity is closely connected with chemical composition of the extract. In most cases, extracts sterilised with the filtration method show higher inhibitory activity against fungi than extracts sterilized in the autoclave [17]. For example, Venturoso et al [18] proved high antifungal activity of garlic extract sterilised with filtration method, whereas sterilisation method applied to cinnamon and clove extracts had no effect on their activity. In the research of both Abimbola [19] and Hashemi et al [20] plant extracts sterilised in the autoclave showed lower antimicrobial activity in comparison with the filtered ones. The authors explained that the differences in the antimicrobial activity induced by high temperature may be the result of many factors involved. The heating process and higher pressure may cause damage to the cell wall and thus increase the bioavailability of antimicrobial substances. Moreover, during the thermal process new compounds with antifungal properties may be formed. Furthermore, filtration is a mechanical or physical operation. Mechanical filtration is typically achieved by passing water or solvent through materials which act as a sieve, physically trapping the particulate matter. Contaminants or bacteria are removed by filter through a membrane having microscopic holes that allow water or solvent molecules, but not larger compounds, to pass through. It is also possible that some phytochemical compounds cannot pass through the filter [20]. The results obtained in Kim et al [21] study seem to suggest that any loss in the antimicrobial activity of a compound which had undergone hydrolysis is being compensated by the increase in activity of some other compounds as a result of the high temperature.

Chemical analyses of *U. dioica* revealed the presence of many valuable chemical compounds like phytosterols, saponins, flavonoids and tannins [6]. Some of these compounds have been reported to be hydrolysable at high temperatures either into more active compounds or less active compounds. In conducted research the extracts sterilised in the autoclave have not shown antifungal properties, therefore there is high probability that inhibitors in the nettle are thermolabile. High temperature caused damage in their structure, which led to either complete or partial loss of antifungal activity.

## Conclusions

The paper presents obtained results in conducted research on the assessment of antifungal activity of extracts from *U. dioica*, which were subject to the sterilisation process in the autoclave and with the use of filters. The following has been concluded:

- root extract from *U. dioica* shows higher antifungal activity in comparison with leaf extract. An application of 40.0% aqueous root extract caused 75% mycelial growth inhibition and 38% inhibition of spores germination of *A. solani*;

- antifungal activity of nettle extracts is not inhibited when extracts are sterilized with filtration; it may be an indication that filtration is a suitable choice for the sterilisation of *U. dioica* root extract;
- sterilisation with the saturated steam under pressure caused a complete loss of inhibitory properties of extracts under study;
- extracts from nettle have the potential application in the protection of tomato plant against *A. solani*. The future for using plant extracts and plant products is promising, because they are less expensive and less hazardous to the environments.

## References

[1] Kozik EU, Ostrzyżek I, Szczechura W. Alternaria solani - tomato pathogen and perspectives of breeding resistant cultivars. Post Nauk Roln. 2010;4:55-65. http://www.instytucja.pan.pl/images/stories/pliki/wydzialy/wydzial_v/dwum_pnr/2010/PNR4-10.pdf.

[2] Sobolewski J, Robak J, Czajka A. Element of integrated pest management of tomato in the field, against the early and late blight with the use of natural products. Prog Plant Prot. 2014;54(3):363-367. DOI: 10.14199/ppp-2014-059.

[3] Asgarpanah J, Mohajerani R. Phytochemistry and pharmacologic properties of Urtica dioica L. J Med Plants Res. 2012;6(46):5714-5719. DOI: 10.5897/JMPR12.540.

[4] Bisht S, Bhandari S, Bisht NS. Urtica dioica (L): an undervalued, economically important plant. Agric Sci Res J. 2012;2(5):250-252.

[5] Taylor K. Biological flora of the British isles: Urtica dioica L. J Ecol. 2009;97:1436-1458. DOI: 10.1111/j.1365-2745.2009.01575.x.

[6] Joshi BC, Mukhija M, Kalia AN. Pharmacognostical review of Urtica dioica L. Int J Green Pharm. 2014;8:201-209. http://greenpharmacy.info/index.php/ijgp/article/view/414.

[7] Kukrića ZK, Topalić-Trivunovića LN, Kukavicab BM, Matoša SB, Pavičića SS, Borojab MM, et al. Characterization of antioxidant and antimicrobial activities of nettle leaves (Urtica dioica L.). APTEFF 2012;43:257-272. DOI: 10.2298/APT1243257K.

[8] Gül S, Demirci B, Hüsnü Can Baser K, Akpulat HA, Aksu P. Chemical composition and in vitro cytotoxic, genotoxic effects of essential oil from Urtica dioica L. Bull Environ Contam Toxicol. 2012;88:666-671. DOI 10.1007/s00128-012-0535-9.

[9] Gutowska I, Jakubczyk K, Dec K, Baranowska-Bosiacka I, Drozd A, Janda K, et al. Effect of the extract from nettle (Urtica dioica L.) fruit cluster on the synthesis of pro-inflammatory agents in hepatocytes treated with fluoride. Fluoride. 2014;47(2):109-118.

[10] Gülçin I, Küfrevioglu OI, Oktay M, Büyükokuroglu ME. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.). J Ethnopharmacol. 2004;90:205-215. DOI:10.1016/j.jep.2003.09.028

[11] Nabrdalik M. Disinfective activity of 8-hydroxyquinoline sulfate on moulds. Ecol Chem Eng A. 2012;19(8):905-913. DOI: 10.2428/ecea.2012.19(08)088.

[12] Hadizadeh I, Peivastegan B, Kolahi M. Antifungal activity of nettle (Urtica dioica L.), colocynth (Citrullus colocynthis L. Schrad), oleander (Nerium oleander L.) and konar (Ziziphus spina-christi L.) extracts on plants pathogenic fungi. Pakistan J Biolog Sci. 2009;12(1):58-63. DOI: 10.3923/pjbs.2009.58.63.

[13] Hadizadeh I, Peivastegan B, Hamzehzarghani H. Antifungal activity of essential oils from some medicinal plants of Iran against Alternaria alternate. Am J Appl Sci. 2009;6(5):744-748. DOI: 10.3844/ajas.2009.857.861.

[14] Tapwal A, Nisha, Garg S, Gautam N, Kumar R. In vitro antifungal potency of plant extracts against five phytopathogens. Braz Arch Biol Technol. 2011;54(6):1093-1098. DOI: 10.1590/S1516-89132011000600003.

[15] Chaerani R, Voorrips RE. Tomato early blight (Alternaria solani): the pathogen, genetics, and breeding for resistance. J Gen Plant Pathol. 2006;72:335-347. DOI 10.1007/s10327-006-0299-3.

[16] Patni CS, Kolte SJ. Effect of some botanicals in management of Alternaria blight of rapeseed-mustard. Ann Pl Prot Sci. 2006;1:151-156. http://www.cabdirect.org/abstracts/20063109266.html.

[17] Ke-Qiang CAO, van Bruggen AHC. Inhibitory efficacy of several plant extracts and plant products on Phytophthora infestans. J Agricult Univ Hebei. 2001;24:108-116.

https://research.cip.cgiar.org/confluence/download/attachments/37192421/caokeqiang_21_.pdf?version=1&modificationDate=1274361052000.
[18] Venturoso LR, Bacchi LMA, Gavassoni WL, Pontim BCA, Conus LA. Influence of different sterilization methods on the antifungal activity of aqueous extracts of medicinal plants. Rev Bras Plantas Med. 2010;12(4):499-505. DOI: 10.1590/S1516-05722010000400014.
[19] Abimbola IO. The effect of autoclaving and membrane filtration on the antimicrobial activities of Alchornea cordifolia leaf extract. Glo Res J Micro. 2014;4(1):6-9.
[20] Hashemi SR, Zulkifli I, Zunita Z, Somchit MN. The Effect of selected sterilization methods on antibacterial activity of aqueous extract of herbal plants. J Biol Sci. 2008;8:1072-1076. DOI: 10.3923/jbs.2008.1072.1076.
[21] Kim TJ, Silva JL, Jung YS. Enhanced functional properties of tannic acid after thermal hydrolysis. Food Chem. 2011;126:116-120. DOI: 10.1016/j.foodchem.2010.10.086.

# OCENA AKTYWNOŚĆ PRZECIWGRZYBOWEJ EKSTRAKTÓW Z POKRZYWY ZWYCZAJNEJ (*Urtica dioica* L.) WOBEC *Alternaria solani*

Samodzielna Katedra Biotechnologii i Biologii Molekularnej, Uniwersytet Opolski

**Abstrakt:** Celem pracy była ocena aktywności przeciwgrzybowej ekstraktów z korzenia oraz z liścia pokrzywy zwyczajnej (*Urtica dioica* L.) w stężeniach 2,5, 5,0, 10,0, 20,0, 40,0% wobec fitopatogennego szczepu *Alternaria solani*. Aktywność przeciwgrzybową ekstraktów z pokrzywy określono w oparciu o indeks tempa wzrostu grzybni oraz indeks kiełkowania zarodników. W badaniach określono również wpływ metod sterylizacji testowanych wyciągów na ich aktywność. Na podstawie uzyskanych wyników wykazano, iż ekstrakty sterylizowane parą wodną pod ciśnieniem nie wykazują aktywności przeciwgrzybowej. W przypadku ekstraktu z korzenia, który charakteryzował się większą aktywnością inhibicyjną, uzyskano zaledwie 7% redukcję tempa wzrostu grzybni. Natomiast ekstrakt z korzenia poddany sterylizacji przez sączenie ograniczał o 75% wzrost grzybni i o 38% kiełkowanie zarodników *A. solani*.

**Słowa kluczowe:** aktywność przeciwgrzybowa, *Urtica dioica, Alternaria solani,* indeks tempa wzrostu, indeks kiełkowania zarodników

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Damian PANASIUK[1]

# INVENTORY OF MERCURY EMISSION TO AIR, WATER AND SOIL IN POLAND FOR YEAR 2013

## INWENTARYZACJA EMISJI RTĘCI DO POWIETRZA, WÓD I GLEBY W POLSCE W 2013 ROKU

**Abstract:** Poland is one of major mercury emitter to air in Europe but this emission is constantly decreasing from level 33.3 Mg in 1990. Newest official inventory defined mercury emission to air from industrial processes and fuel combustion in residential sector on level 10.4 Mg annually. Additionally estimation of emission from the use of mercury-containing products was based on model for distribution and emission (0.46 Mg). Dental practice (0.29 Mg) and bodies cremation (0.06 Mg) are next sources of mercury emission to air. Totally 11.2 Mg mercury annually is emitted to air. According to E-PRTR data for 2013, mercury discharge to water was 2.99 Mg with majority of 2.75 Mg from large and medium industrial facilities as well as rest from municipal waste water plants in large towns. Sewage sludge from waste water plants transferred to agriculture is source of mercury emission to soil (0.31 Mg). Mercury discharges to water and soil from dental amalgam in buried bodies (0.16 Mg) are other emission source. In sum mercury emission to air, water and soil in Poland in year 2013 can be estimated on level 14.7 Mg annually. Significant load of 10.4 Mg mercury annually is safely stored in wastes from the use of mercury-containing products and from dental practice.

**Keywords:** emission, mercury, air, water, soil

## Introduction

Poland is one of major mercury emitter to air in Europe. There are two countries in official EMEP inventory for year 2013 [1] with annual emission on level 10 Mg: Germany and Poland. Next countries are Italy (8.1 Mg), Ukraine (6.8 Mg), the United Kingdom (6.1 Mg), Spain (5.4 Mg) and France (3.8 Mg). The Russian Federation not report own mercury emission but its last available data on emission was 14 Mg in 2006.

This ranking mainly is result of coal combustion in power and heating plants with mercury emission in countries: Germany (6.6 Mg), Poland (5.9 Mg), Spain (2.5 Mg), the Czech Republic (1.7 Mg) and the United Kingdom (1.6 Mg), not data for Ukraine. Industry and other stationary combustion are next important sources of mercury emission to air in Europe.

## Historical inventories of mercury emission

Mercury emission in Poland is constantly decreasing from 1990. Inventories for EMEP are prepared with delay of 2 years, for example in March 2015 for year 2013. In first inventories prepared until 2008, officially reported annual mercury emission decreased from 33.3 Mg for 1990 to 19.8 Mg for 2002 and increased to 21.3 Mg for 2006 (36% decrease in 16 years), see Figure 1. In 2009 mercury emissions for period 2005-2007 were recalculated to level 15.4-16.1 Mg [2]. Next inventories for years 2008-2010 maintained reported emission on level 14.5-15.7 Mg.

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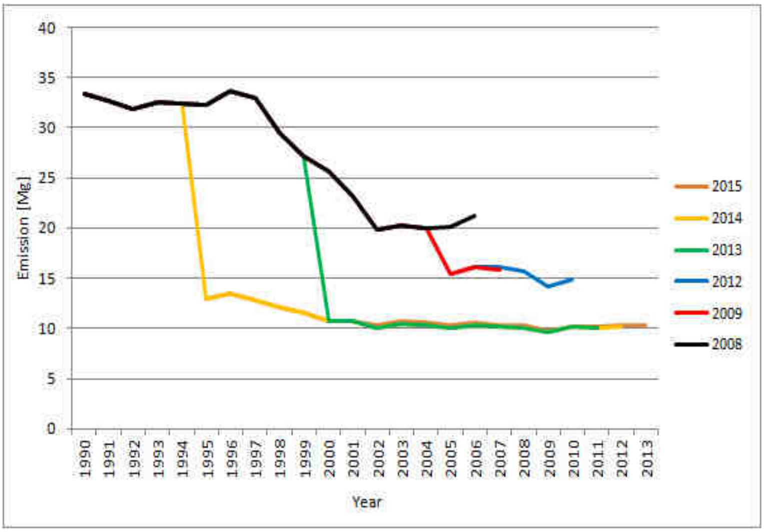

Fig. 1. Changes of inventories of mercury emission to air in Poland for years 1990-2013

In 2013 Polish National Centre for Emissions Management (KOBIZE) [3] decided to change reported emissions from year 2000 for data completeness and consistency. Applied emission factors for public power sector have been based on a country study conducted by Polish energy group PGE. Mercury emissions were recalculated to level 9.6-10.7 Mg. In next year inventories from year 1995 were additionally recalculated. Reported emission decreased from 13.0 Mg for 1995 and 13.4 Mg for 1996 to 11.6 Mg for 1999. In future recalculations for period 1990-94 are also expected but there are significant lacks of statistical data. Now only inventories for period 1995-2013 are comparable [4]. Due to new assumptions mercury emission in Poland decreased by 20% in last 18 years. Comparison of inventory for 1990 and 2013 shows bigger 3-times decrease of this emission.

## Emission to air

Newest official inventory for 2013 [5] estimated mercury emission to air on level 10.4 Mg annually. National inventory covers emissions from following SNAP sectors: energy industry (5.8 Mg), commercial and residential combustion plants (1.7 Mg), other industries (2.9 Mg) and incineration of municipal wastes (0.1 Mg). Detailed data for emissions from industrial branches are available for NFR sectors [4]: non-ferrous metals production (1.4 Mg), iron and steel production (0.5 Mg), cement production (0.3 Mg) and chemical industry (0.2 Mg). E-PRTR database [6] is incomplete source. It reported only emission 3.3 Mg of mercury to air with majority from energy sector (2.6 Mg).

Estimation of additional mercury emissions to air was prepared by Panasiuk and Glodek [7]. Mercury is intentionally used for production of batteries, measuring and control equipment, light sources as well as other electrical and electronic equipment. Emission from the use of these products was assessed [8] on the basis of model for distribution and emissions [9]. Consumption of mercury for production of mercury-containing products launched annually to Polish market (9.4 Mg) was based on data for EU-25 [10] with assumptions of population proportion and smaller batteries consumption in Poland. Mercury emission to air from one year's mercury consumed in products (initial and later within 10 years) was estimated on level 0.46 Mg. The biggest emission originates from steel scrap and landfills. Smaller shares are released by breaking and waste incineration. The other 2.90 Mg Hg contained in products is re-collected and stored safely. Mercury stream in wastes of mercury-containing products transported to municipal landfills was estimated on level 4.84 Mg. Remaining 1.20 Mg of mercury is still accumulated in products used to 10 years after production [11].

Dental practice and bodies cremation are next sources of mercury emission to air. Amount of mercury in dental materials launched annually to Polish market (10 Mg) was estimated on the basis of the Polish Ministry of Health data (13 million of amalgam fillings per year) and Maxson's study [10]. It was assumed that 2.2 Mg of mercury is accumulated in society as new dental fillings and 7.8 Mg is passed to solid waste. From this amount, the excess of prepared mixture (2.0 Mg) is recollected as hazardous waste. Remaining 5.8 Mg of mercury in old amalgam wastes is in half collected and later treated as hazardous waste. However second half (2.9 Mg) is transported to infectious wastes and incinerated with average 90% emission reduction (currently only in industrial plants, earlier also in hospital plants without proper equipment). These process causes emission of 0.29 Mg mercury to air.

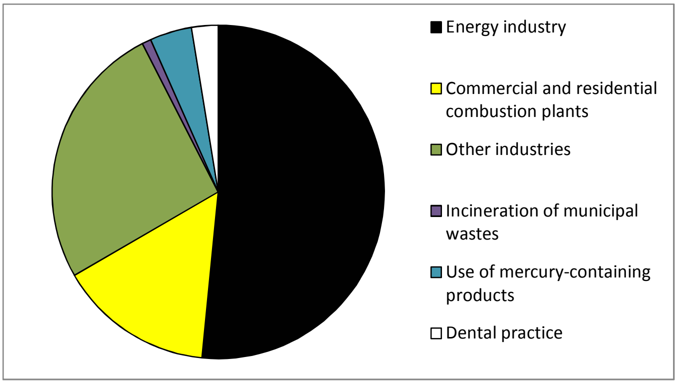

Fig. 2. Main sources of mercury emission to air in Poland in 2013

Mercury emission to air from bodies cremation in Poland was reported by EC [12] on level 0.06 Mg. It was assumed that 9% of corpse was cremated in Poland in 2011, there are 3 crematoria applying mercury removal techniques (with 50% Hg capture) and 10 crematoria not applying these techniques. Totally 11.2 Mg mercury is annually emitted to air from industry, products and dental practice.

## Emission to water and soil

Data on mercury discharges to water was used from E-PRTR database [6] for year 2013. These direct and non-direct discharges (releases and transfers) from Poland were

2.99 Mg. Majority of mercury discharge (2.75 Mg) originates from large and medium industrial facilities. The biggest discharges to water in Poland were from production and processing of metals (1.82 Mg), landfills (0.51 Mg) and mining (0.23 Mg). Among smaller sources of releases and transfers are independently operated industrial waste water treatment plants (0.10 Mg), industrial scale production of basic inorganic chemicals (0.05 Mg) and basic organic chemicals (0.02 Mg) as well as energy sector (0.01 Mg).

In Europe chlor-alkali industry was using mercury cell electrolysis for many years. This process was source of mercury emission to air and water. Last Polish electrolyzer for chlor-alkali production was working in PCC Rokita factory in Brzeg Dolny. For year 2013 this factory reported release of 0.06 Mg mercury from independently operated industrial WWTP. This electrolyzer was closed in the beginning of 2015.

Mercury discharges to water were also reported for municipal waste water plants in 20 Polish large towns. These discharges from residential sector, hospitals and dental clinics as well as small industrial facilities in 2013 were 0.25 Mg. The biggest mercury releases were reported for plants in Poznan (78 kg), Gdansk (35 kg), Sosnowiec (27 kg), Szczecin (22 kg) and Lodz (21 kg).

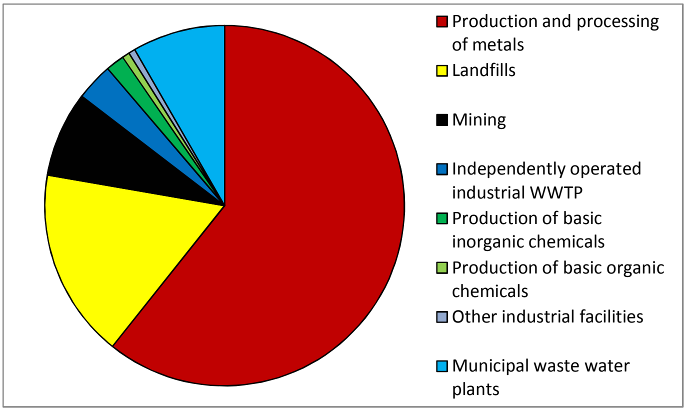

Fig. 3. Main sources of mercury discharges to water in Poland in 2013

Sewage sludge from municipal waste water plants transferred to agriculture is source of mercury emission to soil. This emission was estimated on level 0.31 Mg [13]. Mercury discharges to water and soil from dental amalgam in buried bodies are other emission source. These discharges was calculated on level 0.16 Mg on the basis of burial data, assuming that 20% of Hg is reaching groundwater and the rest is chemically bounded. Any releases to soil from Polish industry are reported in E-PRTR.

## Conclusions

In sum mercury emission to air, water and soil in Poland in year 2013 can be estimated on level 14.7 Mg annually. The biggest inventoried load of mercury is emitted to air, see Table 1.

Table 1

Main mercury flows in Poland in 2013

| | **Emission/storage [Mg/year]** |
|---|---|
| Emission to air | 11.2 |
| Discharges to water | 3.0 |
| Identified emission to soil | 0.5 |
| Safe storage of hazardous wastes | 10.4 |

Significant load of 10.4 Mg mercury annually is safely stored in hazardous wastes. This load covers 2.9 Mg contained in recycled wastes of batteries, measuring and control equipment, light sources as well as other electrical and electronic equipment and from dental practice, 4.9 Mg collected in dental clinics and 2.6 Mg captured from incineration of dental wastes.

## Acknowledgments

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## References

[l] Centre on Emission Inventories and Projections. European Monitoring and Evaluation Programme; 2015. http://www.ceip.at/.

[2] Inwentaryzacja emisji do powietrza $SO_2$, $NO_2$, CO, $NH_3$, pyłów, metali ciężkich, NMLZO i TZO w Polsce za rok 2005 (Inventory of $SO_2$, $NO_2$, CO, $NH_3$, particulates, HMs, NMVOCs and POPs emission to air in Poland for year 2005). Warszawa: Inst Ochr Środ; 2009.

[3] Poland's Informative Inventory Reports 2013-2014. Warszawa: National Centre for Emissions Management; 2013, 2014. http://webdab.umweltbundesamt.at/download/submissions2014/PL_IIR2004.zip?cgiproxy_skip=1.

[4] Poland. National sector emissions. NFR 2014.11.30 files for years 1995-2013. European Monitoring and Evaluation Programme; 2015. http://www.ceip.at/ms/ceip_home1/ceip_home/status_reporting/2015_submissions/.

[5] Krajowy bilans emisji $SO_2$, $NO_X$, CO, $NH_3$, NMLZO, pyłów, metali ciężkich i TZO w układzie klasyfikacji SNAP i NFR (National balance of $SO_2$, NOx, CO, $NH_3$, NMVOC, particulates, HMs and POPs emission in SNAP and NFR classification). Warszawa: KOBiZE; 2015. http://www.kobize.pl/uploads/materialy/Inwentaryzacje_krajowe/2015/Bilans%20emisji%20-%20raport%20podstawowy_2013.pdf.

[6] The European Pollutant Release and Transfer Register. European Environment Agency; 2015. http://prtr.ec.europa.eu/.

[7] Panasiuk D, Głodek A. Substance flow analysis for mercury emission in Poland. Web of Conferences 2013;1,38001. DOI: 10.1051/e3sconf/20130138001.

[8] NILU Polska. Szacowanie kosztów zanieczyszczenia rtęcią dla scenariusza status-quo, MERCPOL project stage I, Report for Polish Ministry of Environment. Katowice: 2009. http://www.gios.gov.pl/images/dokumenty/raporty/etap1_20101022.pdf.

[9] Kindbom K, Munthe J. Product-related emissions of mercury to air in the European Union. IVL Swedish Environmental Research Institute; 2007. https://www.unece.org/fileadmin/DAM/env/lrtap/TaskForce/tfhm/fourth%20meetingdocs/Products_Hg_Emissions.pdf.

[10] Maxson P. Mercury flows and safe storage of surplus mercury. Report by Concorde East/West Sprl for DG Environment of the European Commission; 2006. http://ec.europa.eu/environment/chemicals/mercury/pdf/hg_flows_safe_storage.pdf.

[11] Panasiuk D, Głodek A. Mercury emission to air, water and soil in Poland. In: Nriagu J, et al, editors. Heavy Metals in the Environment: Selected Papers from the ICHMET-15 Conference, Progress in Environmental Science, Technology and Management, Maralte BV; 2012. DOI: 10.5645/b.2.27.

[12] Study on the potential for reducing mercury pollution from dental amalgam and batteries. BIO Intelligence Service. Final report prepared for the European Commission - DG ENV; 2012. http://bookshop.europa.eu/

en/study-on-the-potential-for-reducing-mercury-pollution-from-dental-amalgam-and-batteries-pbKH3013440/.

[13] COHIBA. Work Package 4. Summary report Poland. Katowice: IETU Institute for Ecology of Industrial Areas; 2011. http://www.cohiba-project.net/publications/en_GB/publications/_files/87106044606875184/default/Poland%20National%20WP4%20report%20Final.pdf.

## INWENTARYZACJA EMISJI RTĘCI DO POWIETRZA, WÓD I GLEBY W POLSCE W 2013 ROKU

Wydział Biologii i Nauk o Środowisku, Uniwersytet Kardynała Stefana Wyszyńskiego w Warszawie

**Abstrakt:** Polska jest jednym z głównych emitorów rtęci do powietrza w Europie, ale emisja ta stale spada z poziomu 33,3 Mg w 1990 r. Najnowsza krajowa inwentaryzacja określiła emisję rtęci do powietrza z procesów przemysłowych i spalania paliw w sektorze mieszkaniowym na poziomie 10,4 Mg rocznie. Dodatkowo oszacowanie emisji z użytkowania produktów zawierających rtęć zostało oparte o model dystrybucji i emisji (0,46 Mg). Kolejnymi źródłami emisji rtęci do powietrza są praktyka dentystyczna (0,29 Mg) i kremacja zwłok (0,06 Mg). Łącznie 11,2 Mg rtęci rocznie jest emitowanych do powietrza. Na podstawie danych E-PRTR dla roku 2013 zrzuty rtęci do wód wyniosły 2,99 Mg z większością 2,75 Mg z dużych i średnich zakładów przemysłowych oraz resztą z komunalnych oczyszczalni ścieków w dużych miastach. Osady ściekowe z oczyszczalni ścieków przekazywane do rolnictwa są źródłem emisji rtęci do gleby (0,31 Mg). Innym źródłem emisji są uwolnienia rtęci do wód i gleby z wypełnień amalgamatowych w grzebanych zwłokach (0,16 Mg). W sumie emisja rtęci do powietrza, wód i gleby w Polsce w 2013 r. może być oszacowana na poziomie 14,7 Mg rocznie. Znaczący ładunek 10,4 Mg rtęci rocznie jest bezpiecznie składowany w odpadach z użytkowania produktów zawierających rtęć oraz z praktyki dentystycznej.

**Słowa kluczowe:** emisja, rtęć, powietrze, woda, gleba

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Terese RAUCKYTE-ŻAK[1]

# COMPARISON OF THE SEQUENTIAL EXTRACTION METHODS FOR SOIL SUBJECTED TO THE LONG-TERM EFFECT OF SEWAGE

## PORÓWNANIE METOD EKSTRAKCJI SEKWENCYJNEJ GLEBY PODDANEJ WIELOLETNIEMU DZIAŁANIU ŚCIEKÓW

**Abstract:** The collation and comparison of two sequential extraction procedures: 5-stage Tessier's and less-known 4-stage Sposito's for samples of soil treated with wastewaters from the low-tonnage vegetable and animals fats production were presented. The subject production of technological fats was carried out in a small oleochemical plant and the presented results are the continuation of the previous papers. The doses of wastewaters directed to fields used for testing were limited by total nitrogen concentration in the total batches not exceeding the threshold value of 170 kgN/ha · year. The experimental plots were sown in the consecutive years with vegetation such as: oat, rape, triticale and mustard, which were introduced to the examined soils in bulk in a form of a green fertilizer. In order to compare the changes taking place in time, the acreage of the fields used for testing was fertilized with the separately mixed raw wastewaters after their defatting and physicochemical pretreatement. Fertilization processes were carried out on regular basis once a year within the period of four years. The soil samples used for the analyses were taken from the surface layer from the experimental fields. The obtained results indicate the significant variability of changeability levels concerning exchangeable and residual fractions determined in accordance with the selected sequential extraction methods.

**Keywords:** sequential extraction procedures, wastewaters from oleochemical production, exchangeable fractions, residual fractions

## Introduction

Pretreated industrial wastewaters from the production of industrial fats of vegetable and/or animal origin produced in small oleochemical plants have the beneficial N:P:K ratio for the fertilization needs [1]. The essential condition for the agricultural use of such wastewaters is their effective defatting and the elimination of colloidal and suspension systems [2, 3]. In specialist literature, there are some examples of papers concerning the issue of agricultural utilization of wastewaters from the production of vegetable or animal fats production including the aspect of total changes in heavy metal concentrations being the result of agricultural utilization [4-10]. However, there have been only few reports on changeability of the individual fraction fields observed during many years of carrying out experiments concerning soil fertilization with such wastewaters. This issue seems to be particularly essential because the total metals concentrations do not reflect the changes taking place with their participation and the participation of wastewaters pollutants' load components during many years of providing the soil with these substances.

The aim of the paper was the quantitative identification of the dynamics of fractional changeability taking place in the surface layer of soil in case of two fractions: the exchangeable one and the residual one for the selected heavy metals during the period of

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four-year agricultural utilization of defattened raw and physicochemically pretreated wastewaters from the low tonnage oleochemical production. In order to achieve this aim, one of the frequently used for soil assessment sequential extraction methods, that is the 5-stage Tessier's procedure [11] and the less known 4-stage Sposito's procedure [12] were applied.

## Materials and methods

### *Study area and soil sampling*

Soil from the experimental fields was rated as the light soils category with sandy granulometric composition - sandy clay loam in plough and decayed material layer. The examined soils were produced from glacial till and they have the genetic material sequences typical of the category of brown soil, soil profile: Ap-Bw-Ck [13]. Before the experiment, the soil reaction was acidic, pH in 1.0 M KCl 3.7 ± 0.2 and the active acidity (in distilled $H_2O$) measured potentiometrically was pH 6.7 ± 0.2. The average decay content in the accumulation layers was 13.21 g/kg and the organic carbon 7.66 g/kg, and the total nitrogen content was 0.70 g/kg, plant assimilable forms of phosphorus amounted 52.0 mg $P_2O_5$/kg, potassium 27.0 mg $K_2O$/kg and magnesium - 14.0 mg/kg.

Doses of wastewaters directed to fields used for testing were limited with total nitrogen concentration, in portions not exceeding the amount of 170 kg N/ha·year. The experimental plots were sown every year in the consecutive years with vegetation such as: oat, rape, triticale and mustard, which were introduced to the examined soils in bulk in a form of green fertilizer. Soil samples from the fields used for testing (50.0 acres each) fertilized with mixed defattened raw wastewaters and the pretreated ones were taken in accordance with the Polish standard PN-ISO 10381-2 from the surface layer 0.0-20.0 cm. The soil reaction sample was determined in accordance with the methods given in PN-ISO 10390:1997. The soil material for the analyses of the total heavy metals contents was taken within 4 years in equal monthly intervals.

### *Soil analysis*

Soil samples collected from the areas of experimental plots were sequentially extracted acc. to Sposito's and Tessier's methods [11, 12], by using air dried analytical samples (40.0 g each) which were treated with extracting solutions of increasing extraction power (Table 1).

After filtering solid parts, the filtrate was analyzed by using AAS (apparatus: BUCK Scientific 210 VGP and Spectr AA Varian 220 SS). The soil specimens, washed with water and dried, were used at the next stage of the extraction - at I (Tessier and Sposito), IV (Sposito) and V (Tessier) fractions. The results of the filtrate analyses for the contents of Cd and Zn in first and fifth (fourth) speciation fractions during four experimental years are presented in Figures 1 and 2.

Table 1

Sposito's and Tessier's procedures used for fractionation analysis

| No | Fraction | Extraction steps a) |
|---|---|---|
| | | Sposito's procedure [12, 14, 15] |
| 1 | I Exchangeable | a-1) 400 $cm^3$ 0.5 M $KNO_3$, shaken at 25°C (16.0 h).<br>a-1.1) 400 $cm^3$ deionized water, shaken at 25°C (2.0 h). |
| | II Sorbed | b-1) 400 $cm^3$ 0.5 M NaOH, shaken at 25°C (16.0 h). |
| | III Organic | c-1) 400 $cm^3$ 0.05 M $Na_2EDTA$, shaken at 25°C (6.0 h). |
| | IV Carbonate and sulfide forms | d-1) 400 $cm^3$ 4.0 M $HNO_3$, shaken at 80°C (16.0 h). |
| | | Tessier's procedure [11, 14-16] |
| 2 | I Exchangeable | a-2) 320 $cm^3$ 1.0 M $CaCl_2$ (pH 7), shaken at room temperature (1.0 h). |
| | II Carbonate | b-2) 320 $cm^3$ 1.0 M $CH_3COONH_4$ acidifying with 80% $CH_3COOH$ up to pH 7 and shaken at room temperature (5.0 h). |
| | III Bound to Fe and Mn oxides | c-2) 800 $cm^3$ 0.04 M $NH_2OH \cdot HCl$ in 25% $CH_3COOH$ (pH 2) shaken at 96 ± 3°C (5.0 h). |
| | IV Organic | d-2) 120 $cm^3$ 0.02 M $HNO_3$ and 200 $cm^3$ 30% $H_2O_2$ (pH 2) shaken at 85 ± 2°C (3.0 h).<br>d-2.1) 200 $cm^3$ 3.0 M $CH_3COONH_4$ in 20% $HNO_3$ (v/v) was added and shaken at room temperature (pH 2) (0.5 h). |
| | V Residual | e-2) 120 $cm^3$ $HNO_3$ and 200 $cm^3$ 30% $H_2O_2$ were added and mixed (pH 2). The mixture was shaken at boiling point and at that time it was treated twice with $HNO_3$ and $H_2O_2$ up to the moment of releasing white fumes (1.0 h). 200 $cm^3$ 30% $H_2O_2$ was added and was shaken at boiling point (0.5 h). |

a) sequential extractions were carried out using 40.0 g dried at room temperature soil samples

### *Physicochemical method for pretreatment of wastewaters from the oleochemical production*

The simplified technological scheme of physicochemical installation in the wastewaters treatment plant where the pretreatment of mixed wastewaters from the subject oleochemical production was carried out was presented with the detailed description in paper [1]. Post production wastewaters from the production of fats of vegetable and animals origin were directed to the preliminary chamber to be mixed and averaged and then pumped into the aerated chamber at the first stage of pretreatment where hydrogen peroxide was additionally introduced in doses 150.0-175.0 g $H_2O_2/m^3$ of wastewaters in order to separate the protein and fat flotate. Pretreatment at the second, chemical stage was carried out with lime milk (in unit doses 1.25-1.50 kg $CaO/m^3$ of the defattened wastewaters after the first stage of pretreatment) with the final flocculation with aqueous 0.3% flocculant solution (Praestol 859 BC Stockhausen dosed in the amount of pure component 50.0 $g/m^3$of wastewaters). The process was additionally aided with dissolved air flotation (DAF) (basic flotation parameters: saturation time 10.0 min, saturation pressure 500 kPa, the level of pretreated wastewaters recirculation 20%) and oxidized with hydrogen peroxide in doses 235.0-250.0 g $H_2O_2/m^3$ of wastewaters. After oxidation and separation of phases, the flotate was directed to the tank to be additionally condensed and next to the plate and frame filter press in order to be dehydrated, while the pretreated wastewaters were directed to the storage tank from where they were directed to experimental fields.

## Results and discussion

The subject production of technological fats of vegetable and animal origin was carried out in a small oleochemical plant located in the rural area, and the presented results of the research are the continuation of papers [1, 2]. The aim of this study was the quantitative identification and the comparison of the dynamics of changes in two fractions, that is the exchangeable one and the residual one, obtained after the application of Tessier's extraction procedures (fractions I and V) and Sposito's extraction procedures (fractions I and IV) for such heavy metals as Cd and Zn. The dynamics of changes was analyzed in the surface soil layer (0.0-20.0 cm) during the period of four-year agricultural utilization of the defatted raw and physico-chemically pretreated, mixed wastewaters from the subject production. In order to compare the changes taking place over time, the acreage of the experimental fields was fertilized with the separately mixed raw wastewaters after their defatting and with the ones which were physico-chemically pretreated. During the experimental period, the ranges of basic parameters values for the mixed raw wastewaters after their defatting and the ones which were physico-chemically pretreated were in accordance with the data given in Table 2.

Table 2

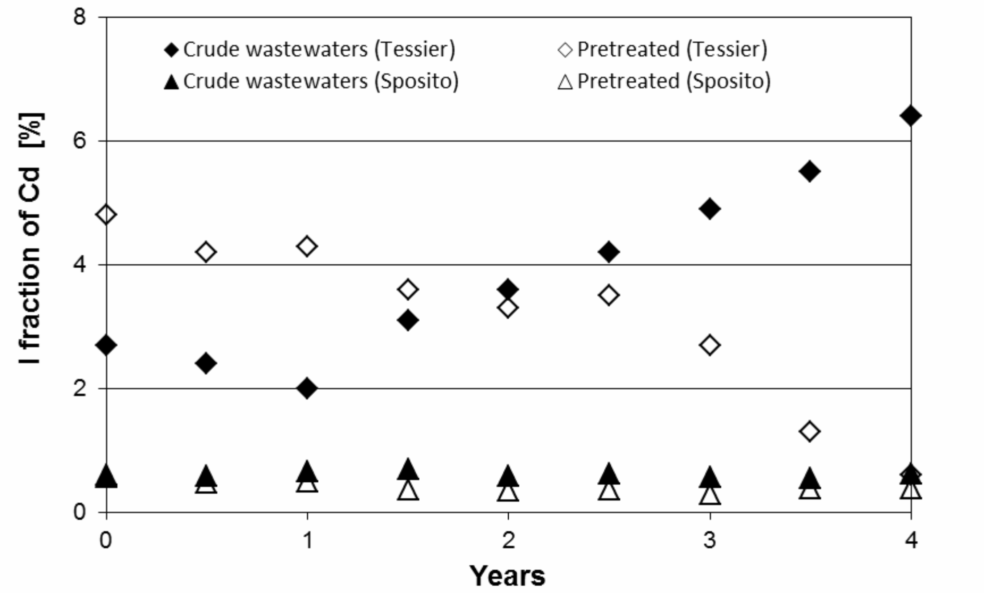
Compilation of ranges of basic parameters values for the raw and physico-chemically pretreated wastewaters directed to the experimental plots [1, 2]

| **Item** | **Parameter** | **Unit** | **Crude wastewater** | **Pretreated wastewater** [a)] |
|---|---|---|---|---|
| 1 | Reaction (pH) | [-] | 3.9-5.0 | 8.4-9.3 |
| 2 | Total suspended solids (TSS) | [mg/dm$^3$] | 477.3-2530.0 | 18.9-42.7 |
| 3 | Chemical oxygen demand (COD$_r$) [b)] | [mg O$_2$/dm$^3$] | 3348.9-10201.6 | 809.6-4405.7 [b)] |
| 4 | Biochemical oxygen demand (BOD$_5$) | [mg O$_2$/dm$^3$] | 905.8-4593.9 | 467.9-2067.8 |
| 5 | Total nitrogen (TN) | [mg N/dm$^3$] | 175.3-720.9 | 88.8-170.7 |
| 6 | Total phosphorus (TP) | [mg P/dm$^3$] | 60.7-220.8 | 11.3-40.8 |
| 7 | Ether extract (EE) | [mg/dm$^3$] | 703.3-5999.0 | 3.7-27.3 |
| 8 | Potassium (K) | [mg/dm$^3$] | 149.4-606.2 | 66.9-483.7 |

[a)] range of values after two stages of pretreatment; [b)] using $H_2O_2$, the values of COD were given as corrected ones: $COD_r = COD_p - f \cdot c$ ($f = 0.25$ based on the data from the study [17, 18])

In case of soil samples being subject to raw defatted wastewaters, their acidity over time was found from the initial level of $pH_0 \approx 6.7 \pm 0.3$ to the final value $pH_K \approx 5.9 \pm 0.4$ (there was no liming of this experimental plot, and the raw wastewaters after the defatting process have the reaction pH ≈ 3.9-5.0). In soil samples, where pretreated wastewaters with reaction pH = 7.8-8.8 were applied during the four years of experiments, no significant change of this parameter was found because its level was within pH ≈ 6.7 ± 0.2, during the whole experimental period. On the basis of the carried out analyses of the soil samples it was found that cadmium and zinc concentrations remained at the level of natural background in case of the subject soils. There was no significant influence of dosing wastewaters on the level of the registered values of these metals. The total load did not undergo any changes during the experimental period and was roughly in accordance with the polynomial equation $C_M(t) = at^2 + bt + c$ (where: $C_M$ - total Cd or Zn concentration determined with the use of AAS, and parameter $t$ means experimental time expressed in monthly units) [2].

a)



b)

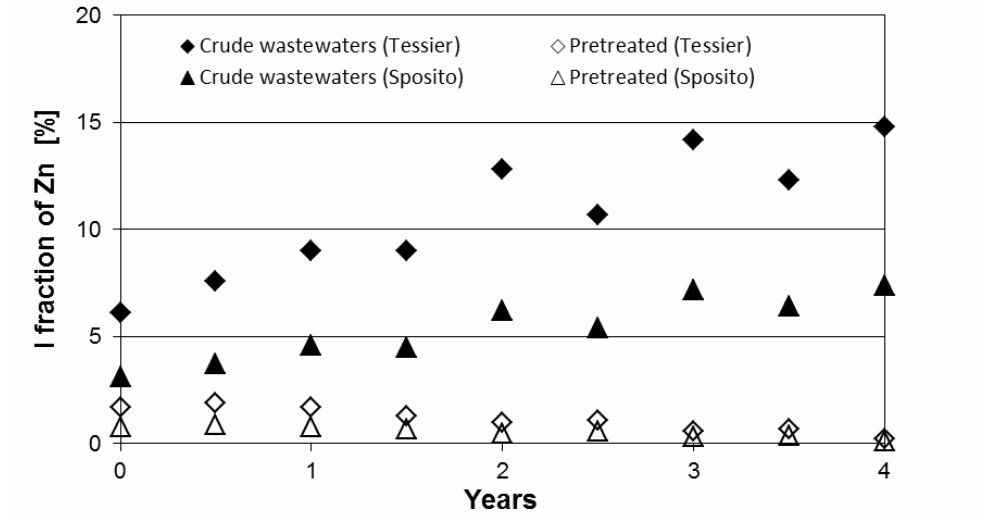
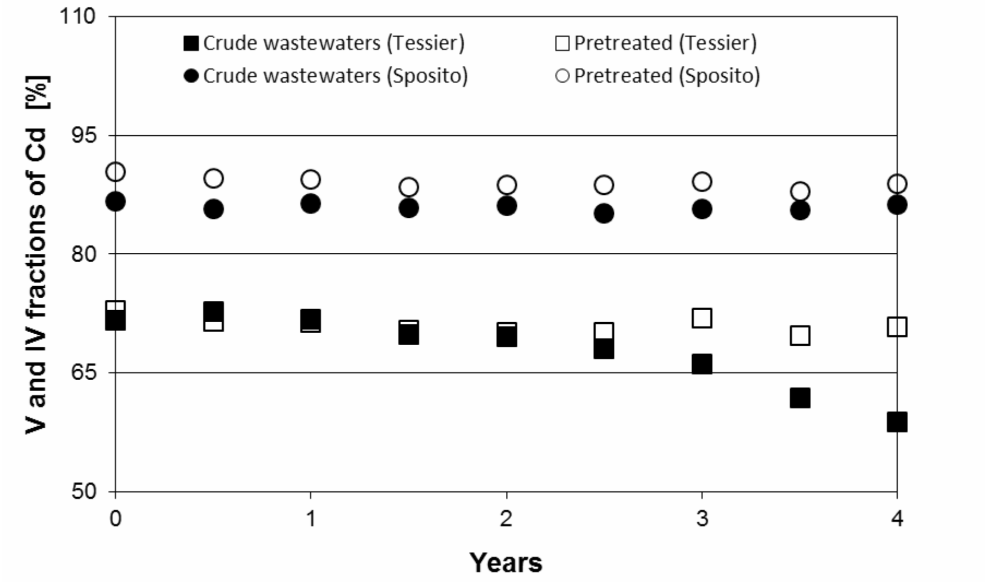


Fig. 1. Comparison of changes in exchangeable fraction contents (fraction I) for: a) cadmium and b) zinc within the period of four-year fertilization with raw defatted wastewaters and the ones which were physico-chemically pretreated and originated from oleochemical production

a)



b)

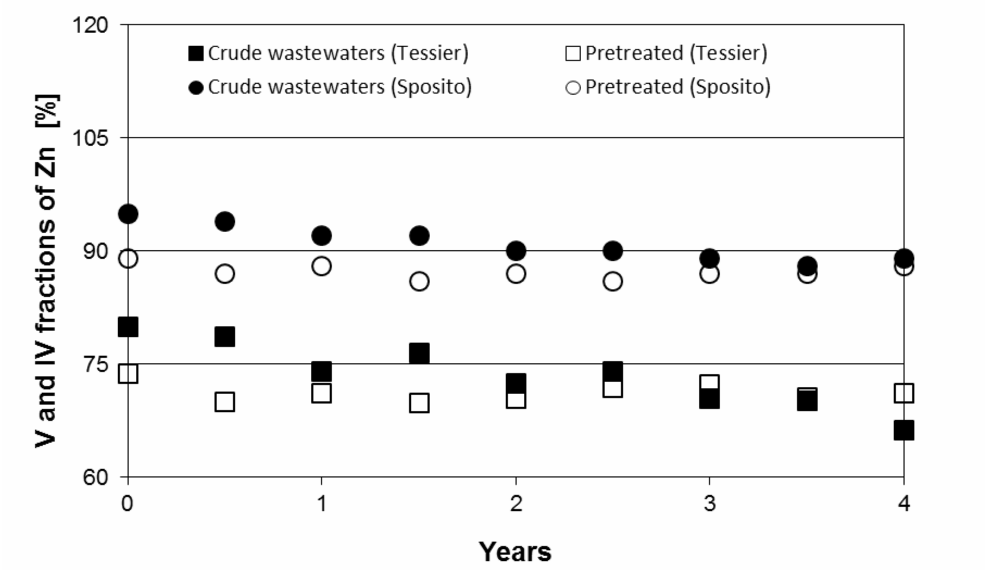


Fig. 2. Comparison of changes in the residuals fraction (fraction IV in Sposito's procedure and fraction V in Tessier's procedure (Table 1)) for: a) cadmium and b) zinc within the period of four-year fertilization with raw defatted wastewaters and the ones which were physico-chemically pretreated and originated from oleochemical production

Cadmium concentrations registered during the experimental period did not exceed the level of 1.0 mg/kg dry mass and they were around the values of 0.6 (± 0.2) mg/kg dry mass. The zinc contents in the determined samples respectively fluctuated around 49.7 ± 2.7 mg/kg dry mass, not exceeding the values for such type of soils applied to agricultural utilization [3]. In case of fraction I determined for cadmium and zinc, the results obtained with the use of Tessier's and Sposito's extraction procedures differed both with regard to results obtained for soil samples treated with raw, defatted as well as physico-chemically pretreated wastewaters (Fig. 1a and b).

In case of cadmium, the higher percentage content of leachable fraction determined with Tessier's method was obtained with the increasing trend in its quantity in line with the time of long-term dosing of raw defatted wastewaters. However, in case of Sposito's procedure, no evident tendencies or trends were registered over the time of dosing during the whole experimental period (Fig. 1a). In case of this fraction's content, determined in soils treated with physico-chemically pretreated wastewaters, the application of Tessier's procedure allowed to determine the content with the evident tendency of its successive decrease over time and the stable level in case of content determined in accordance with Sposito's procedure (Fig. 1a). In case of zinc, the determined contents of fraction I for soil samples treated with raw defatted wastewaters differed in values - the ones obtained in accordance with Tessier's procedure contained higher levels of values (from 6 to 14.9%) than the ones received with the use of Sposito's procedure (from 3 to 7%) (Fig. 1b). In case of contents analyses of this fraction for the soil samples treated with physico-chemically pretreated wastewaters equal stable values were obtained in the experimental period (Fig. 1b). Therefore, the thesis can be suggested that in case of soil being subject to long-term treatment with raw defatted wastewaters but without lime there are transformations leading to the increase in cadmium ion-exchange resin. This parameter functionally depends on a range of factors including the reaction value concerning the soil environment [19-22]. The labile forms of metal fractions which are present in soils significantly influence Cd and Zn mobility, bioaccessibility and toxicity [15]. Definitely, the highest percentage participation was registered in case of so called residual fraction (fraction V in Tessier's method and fraction IV in Sposito's procedure). In case of soil samples treated with pretreated wastewaters, cadmium and zinc contents in this fraction were registered at the level around 70% (Tessier's method) and above 85% (Sposito's method). In case of fraction V, being the residual load for soil samples treated with defatted raw wastewaters, the decrease in the qualitative content registered over time was observed in case of any metal (Fig. 2a and b). The lowest one was in case of zinc - around 10-15%. The residual fraction is the load which is the least determined with regard to its qualitative and structural content and its properties significantly depend on local soil formation and environmental conditions, which at the same time makes it difficult to describe and interpret it fully and only on the basis of quantitative changes taking place over time [1, 19, 23].

## Conclusions

Analyzing the fractional content with the use of two extraction procedures during the experimental period, different percentage levels of the determined fractions are obtained and often the different trends during the time of long-term treatment of the examined soils with wastewaters. On the basis of the comparison of results obtained for an exchangeable fraction and the so called residual fraction, it is difficult to explicitly characterize the direction of changes taking place between the fraction loads in soils being subject to long-term treatment with wastewaters from oleochemical production. In case of cadmium, the phenomenon of significant differences in percentage participation of fractional loads over time should be also assessed with regard to random scattering of the received values, considering its relatively low concentration in soil (for $Cd_{(0)} = 0.6$ (± 0.2) mg/kg dry mass), and at the same time the statistical assessment of the obtained results.

## References

[1] Żak S, Rauckyte-Żak T, Laurinavičius A. The influence of treated oleo-chemical wastewater applications on the metal speciation forms in soils. J Environ Eng Landsc. 2013;21(2):85-95. DOI: 10.3846/16486897.2013.773259.

[2] Żak S, Rauckyte T. Research on the contents of heavy metals in soils subjected to many-year agroutilization of wastewaters from low tonnage oleochemical production. Ecol Chem Eng A. 2008;15(9):997-1003.

[3] Rozporządzenie Ministra Środowiska z dnia 18 listopada 2014 r. w sprawie warunków, jakie należy spełnić przy wprowadzaniu ścieków do wód lub do ziemi, oraz w sprawie substancji szczególnie szkodliwych dla środowiska wodnego. Dz.U. 2014, poz. 1800. [Regulation of the Minister of the Environment of 18 November 2014 on required quality standards for introducing sewage to water or soil and on extremely hazardous substances for the water environment (Journal of Laws 2014, item 1800)]. http://isap.sejm.gov.pl/DetailsServlet?id=WDU20140001800.

[4] Paredes MJ, Moreno E, Ramos-Cormenzana A, Martinez J. Characteristics of soil after pollution with waste waters from olive oil extraction plants. Chemosphere. 1987;16(7):1557-1564. DOI: 10.1016/0045-6535(87)90096-8.

[5] Sierra J, Marti E, Montserrat G, Cruañas R, Garau MA. Characterisation and evolution of a soil affected by olive oil mill wastewater disposal. Sci Total Environ. 2001;279(1-3):207-214. DOI: 10.1016/S0048-9697(01)00783-5.

[6] Tardioli S, Bànnè ETG, Santori F. Species-specific selection on soil fungal population after olive mill waste-water treatment. Chemosphere. 1997;34(11):2329-2333. DOI: 10.1016/S0045-6535(97)00044-1.

[7] Paredes MJ, Monteoliva-Sanchez M, Moreno E, Perez J, Ramos-Cormenzana A, Martinez J. Effect of waste waters from olive oil extraction plants on the bacterial population of soil. Chemosphere. 1986;15(5):659-664. DOI: 10.1016/0045-6535(86)90015-9.

[8] Russell JM, Cooper RN, Lindsey SB. Soil denitrification rates at wastewater irrigation sites receiving primary-treated and anaerobically treated meat-processing effluent. Bioresour Technol. 1993;43(1):41. DOI: 10.1016/0960-8524(93)90080-U.

[9] Russell JM. Irrigation of primary treated and anaerobically treated meat-processing wastes onto pasture: Lysimeter trials. Agr Wastes. 1986;18(4):257-268. DOI: 10.1016/0141-4607(86)90071-5.

[10] Russell JM, Cooper WC. Irrigation of pasture with meat processing plant effluent. Proc. 42nd Purdue Industrial Waste Conf. Lewis, Chelsea MI, 1987; 491-497. http://e-archives.lib.purdue.edu/cdm/ref/collection/engext/id/39303.

[11] Tessier A, Campbel P, Bisson M. Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem. 1979;51(7):844-851. DOI: 10.1021/ac50043a017.

[12] Sposito G, Lund L, Chang A. Trace metal chemistry in arid-zone field soils amended with sewage sludge: I. Fractionation of Ni, Cu, Zn, Cd, and Pb in solid phases. Soil Sci Soc Am J. 1982;46(2):260-264. DOI: 10.2136/sssaj1982.03615995004600020009x.

[13] IUSS WRB 2006 (IUSS Working Group WRB. (2006). World reference base for soil resources 2006. 2nd edition. World Soil Resources Reports No. 103. FAO, Rome. http://www.fao.org/ag/agl/agll/wrb/doc/wrb2006final.pdf).

[14] He Q, Ren Y, Mohamed I, Ali M, Hassan W, Zeng F. Assessment of trace and heavy metal distribution by four sequential extraction procedures in a contaminated soil. Soil Water Res. 2013;8(2):71-76. http://www.agriculturejournals.cz/publicFiles/91845.pdf.

[15] Hass A, Fine P. Sequential selective extraction procedures for the study of heavy metals in soils, sediments, and waste materials - a critical Review. Crit Rev Environ Sci Technol. 2010;40(5):365-399. DOI: 10.1080/10643380802377992.

[16] Gleyzes C, Tellier S, Astruc M. Fractionation studies of trace elements in contaminated soils and sediments: a review of sequential extraction procedures. Trends Anal Chem. 2002;21:451-467. DOI: 10.1016/S0165-9936(02)00603-9.

[17] Talinli I, Anderson GK. Interference of hydrogen peroxide on the standard COD test. Water Res. 1992;26:107-110. DOI: 10.1016/0043-1354(92)90118-N.

[18] Kang YW, Cho MJ, Hwang KY. Correction of hydrogen peroxide interference on standard chemical oxygen demand test. Water Res. 1999;33:1247-1251. DOI: 10.1016/S0043-1354(98)00315-7).

[19] Lepp NW. Effects of heavy metals pollution in plants. Effects of heavy metals on plant function (ed. LEPP N. W.). London: Applied Science Publishers, 1981;1:111-143.

[20] Rattan RK, Datta SP, Chhonkar PK, Suribabu K, Singh AK. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater - a case study. Agric Ecosyst Environ. 2005;109(3-4):310-322. DOI: 10.1016/j.agee.2005.02.025.

[21] Lassat MM. Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual. 2002;31(1):109-120. https://clu-in.org/download/remed/lasat_article.pdf.

[22] Fitamo D, Itana F, Olsson M. Total contents and sequential extraction of heavy metals in soils irrigated with wastewater, Akaki, Ethiopia. Environ Manage. 2007;39:178-193. DOI: 10.1007/s00267-006-0074-4.

[23] Krishnamurti GSR, Naidu R. Speciation and phytoavailability of cadmium in selected surface soils of South Australia. Aust J Soil Res. 2000;38:991-1004. DOI: 10.1071/SR99129.

# PORÓWNANIE METOD EKSTRAKCJI SEKWENCYJNEJ GLEBY PODDANEJ WIELOLETNIEMU DZIAŁANIU ŚCIEKÓW

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**Abstrakt:** Przedstawiono zestawienie i porównanie dwóch procedur ekstrakcji sekwencyjnej: 5-etapową Tessiera oraz mniej znaną 4-etapową Sposito dla próbek gleby traktowanej ściekami z małotonażowej produkcji tłuszczów roślinnych i zwierzęcych. Przedmiotowa produkcja technicznych tłuszczów była prowadzona przez mały zakład oleochemiczny, a prezentowane wyniki są kontynuacją dotychczasowych prac. Dawki ścieków kierowanych na poletka doświadczalne limitowano stężeniem azotu ogółem w łącznych porcjach nieprzekraczających granicznej ilości 170 kg N/ha·rok. Poletka testowe corocznie były obsiewane roślinnością, w kolejnych latach: owsem, rzepakiem, pszenżytem i gorczycą, które w formie zielonego nawozu były w całej masie wprowadzane do badanych gleb. Dla porównania zachodzących zmian w czasie, eksperymentalne areały nawożono oddzielnie mieszanymi ściekami surowymi po ich odtłuszczeniu i podczyszczonymi fizykochemicznie. Nawożenie prowadzono regularnie raz w roku w okresie czterech lat. Próbki gleby do analiz pobierano z powierzchniowej warstwy z poletek doświadczalnych. Uzyskane wyniki wskazują na znaczące zróżnicowanie poziomów zmienności frakcji jonowymiennych i pozostałych oznaczanych zgodnie z wytypowanymi metodykami ekstrakcji sekwencyjnej.

**Słowa kluczowe:** procedury ekstrakcji sekwencyjnej, ścieki z produkcji oleochemicznej, frakcje wymienna oraz pozostałość

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# EFFECT OF FERTILISATION ON THE CHANGES OF SOIL pH AND EXCHANGEABLE ALUMINIUM CONTENT IN SOIL

## WPŁYW NAWOŻENIA NA ZMIANY ODCZYNU ORAZ ZAWARTOŚCI GLINU WYMIENNEGO W GLEBIE

**Abstract:** We assessed the level of acidification of the soil and the content of exchangeable aluminium in relation to the type and dose of mineral fertiliser. The study was based on a vase experiment with barley of the brewery variety Propino. The results showed that mineral fertilizing with NPK combined with ammonium nitrate gave the highest acidification and the highest contents of exchangeable aluminium. Calcium sulphate applied in different doses did not change soil reaction, and the content of exchangeable aluminium was lower than when combination of NKP fertilizing and ammonium nitrate was applied.

**Keywords:** acidification, exchangeable aluminium, mineral fertilization, *Hordeum vulgare*

One of the most important problems of agricultural production in the world is extensive and retaining acidification of soils [1]. In conditions of acidic soils typical for Poland the content of exchangeable aluminium in soil, which causes decrease in the quality of cultivated plants [2, 3], is one of the main factors that affect crops. Aluminium occurs in its insoluble forms in soils of the neutral and acidic reaction. With a decrease of pH aluminium changes into forms that dissolve in soil solution and are toxic for plants. The most toxic forms for plants are $AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{+7}$ (commonly denoted as $Al_{13}$) and $Al(H_2O)_6^{+3}$ (denoted as $Al^{+3}$) [4]. Unbalanced fertilizing without application of lime leads to strong acidification of soil, which causes considerable decrease in crops of cultivated plants, including barley.

This study was aimed to determine the effect of the fertilizer dose on the soil reaction and the content of exchangeable aluminium. In the experiment we used calcium sulphate ($CaSO_4 \cdot 2H_2O$) which is a waste during gas desulphurisation in heat and power stations.

## Material and methods

We conducted the vase experiment with barley of the brewery variety Propino in 2014. The experiment was designed with the method of independent series in three repetitions and five variants. Dosing of fertiliser was conducted according to the methods of vase experiments:

- variant I: control cultivation without fertilizing;
- variant II: mineral fertilizing NPK at a dose of 20 g/12 $dm^3$;
- variant III: mineral fertilizing NPK at a dose of 20 g/12 $dm^3$, ammonium nitrate at two doses of 6 g/12 $dm^3$;

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- variant IV: mineral fertilizing NPK at a dose of 20 g/12 $dm^3$, ammonium nitrate at two doses of 6 g/12 $dm^3$ and calcium sulphate at a dose of 15 g/12 $dm^3$;
- variant V: mineral fertilizing NPK at a dose of 20 g/12 $dm^3$, ammonium nitrate at two doses of 6 g/12 $dm^3$ and calcium sulphate at a dose of 30 g/12 $dm^3$.

Soil used in the experiment was prepared earlier and fitted into 15 vases. The initial pH of the soil was measured. After four months of growth we again measured pH of soil and checked exchangeable aluminium by the method of Sokolow [5]. During growth period we determined the relative content of chlorophyll using chlorophyll meter CCM 200.

## Results and discussion

In Poland at present 20% of arable soils have strongly acidic reaction (below 4.5 pH). Acidic and strongly acidic soils form 50% of arable land [6]. Optimum pH of soil enables proper growth and function of the root system that supplies plants with sufficient in water and nutrients, which provides high crop of good quality with efficient use of fertilizers [7]. Outwashing of calcium by rainwater, about 140 kg CaO/ha during a year, is the main cause for strong acidification of soils retained on a large area of Poland. Light and very light soils are particularly susceptible to washing out of calcium [8]. Retaining low pH (very acidic) in soils leads to their chemical degradation. This includes increase of concentrations of aluminium and manganese which are toxic for plants, and freeing heavy metals, mostly cadmium, zinc, lead and nickel, alongside with depletion of magnesium available for plants in soil. Highly acidic heavy soils deteriorate in structure which creates hydrological and aerial conditions unfavourable for plants.

Table 1

Reaction of soil used in the experiment

| **Repetitions** | **pH [-]** | |
|---|---|---|
| | **$H_2O$** | **KCl** |
| 1 | 4.98 | 4.50 |
| 2 | 4.99 | 4.59 |
| 3 | 5.04 | 4.82 |
| Means (pH) | 5.00 | 4.64 |

Table 2

Reaction of soil after the experiment in different variants

| **Repetitions** | **pH [-]** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **I** | | **II** | | **III** | | **IV** | | **V** | |
| | $H_2O$ | KCl | $H_2O$ | KCl | $H_2O$ | KCl | $H_2O$ | KCl | $H_2O$ | KCl |
| 1 | 4.98 | 4.93 | 5.06 | 4.71 | 4.22 | 4.05 | 4.59 | 4.41 | 4.45 | 4.05 |
| 2 | 5.05 | 4.69 | 5.00 | 4.61 | 4.44 | 4.12 | 4.47 | 4.40 | 4.25 | 4.27 |
| 3 | 5.10 | 5.01 | 4.94 | 4.61 | 4.30 | 4.10 | 4.55 | 4.33 | 4.53 | 4.38 |
| Means (pH) | 5.04 | 4.88 | 5.00 | 4.64 | 4.32 | 4.09 | 4.54 | 4.38 | 4.41 | 4.23 |

Soil reaction measured before and after the experiment was acidic (Tables 1 and 2). Calcium sulphate did not change pH of soil despite it contains calcium. Thus it is unsuitable for improvement of reaction of acidic soils, but might be good fertilizer for soils of alkaline reaction with a deficiency of calcium. In such soils availability of calcium from chalk or dolomite would be too low for plants. Thus application of calcium sulphate might give good results in soils where calcium occurs in soil in a form temporarily unavailable for plants.

In our study the control variant had low content of aluminium - 0.39 mg/100 g of soil, and variant I, where only NPK fertilizing was applied - 0.55 mg/100 g of soil (Table 3). Similarly low content of exchangeable aluminium at 0.4 mmol(+)·kg$^{-1}$ (0.36 mg/100 g) was noted in studies of Rutkowska et al [3], also for the control sample with no mineral fertilization. In our study the highest content of aluminium occurred with the combination of NPK fertilizing and ammonium nitrate (1.9-2.2 mg/100 g of soil) (Table 3, Fig. 1). The mean content of exchangeable aluminium was in this variant more than five times higher than in the control sample. The literature shows that ammonium nitrate causes acidification [9], which favours increase of the content of exchangeable aluminium in soil [10].

Table 3

Effect of fertilizing on changes in the content of exchangeable aluminium in soil

| | **Content of exchangeable aluminium $Al_w^{3+}$ [mg/100 g]** | | | | |
|---|---|---|---|---|---|
| | **Variant** | | | | |
| | **I** | **II** | **III** | **IV** | **V** |
| | 0.433 | 0.276 | 2.244 | 1.536 | 1.457 |
| | 0.354 | 1.062 | 2.087 | 0.827 | 1.575 |
| | 0.394 | 0.315 | 1.969 | 1.221 | 1.457 |
| $\bar{x}$ | 0.394 | 0.551 | 2.100 | 1.194 | 1.496 |

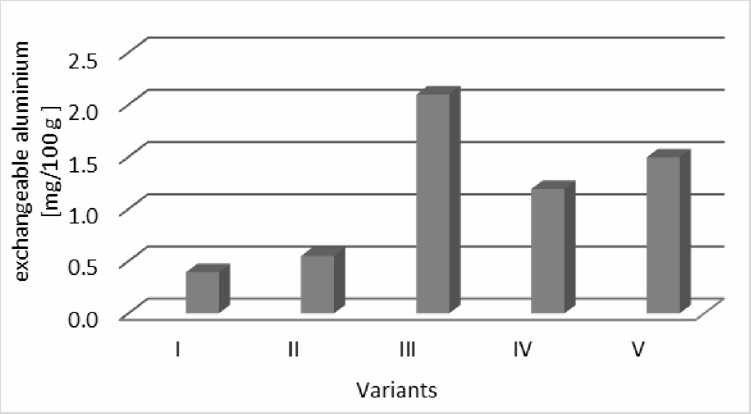

Fig. 1. Changes in exchangeable aluminium

Calcium sulphate used in different doses (variant IV and V) did not cause any change of the initial reaction of soil (Tables 1 and 2). We recorded three times higher content of exchangeable aluminium in the variant IV (1.2 mg/100 g of soil with the dose of calcium sulphate at 250 kg/ha) and more than three times higher in variant V (1.5 mg/100 g of soil with the dose of calcium sulphate 500 kg/ha). But in these variants also NPK with ammonium nitrate were applied, besides calcium sulphate. Ammonium nitrate causes decrease of soil pH and increase of the content of exchangeable aluminium, as shown in variant III. Additional applying of calcium sulphate in the soil did not increase its reaction, but caused higher concentrations of exchangeable aluminium than in variant III.

The content of chlorophyll estimated in tissue of leaves using chlorophyll meter CCM 200 indicates varied in relation to applied fertilizing. This meter uses two wave length to determine absorbance. One wave length increases within the range of the absorbance by chlorophyll, while the other one is used to compensate for mechanical changes, such as thickness of the leaf. The meter measures transmittance of both wave lengths and calculates the CCI (Chlorophyll Content Index), which is proportional to the content of chlorophyll in a sample. Thus the value of the CCI is an index of a relative content of chlorophyll. The lowest CCI was recorded in the control variant. NPK fertilizing (variant II) or the combination of NPK with ammonium nitrate (variant III) cause small increase of the chlorophyll content in leaves of barley. Introducing calcium sulphate in the soil doubled the content of chlorophyll in relation to the control variant (Fig. 2).

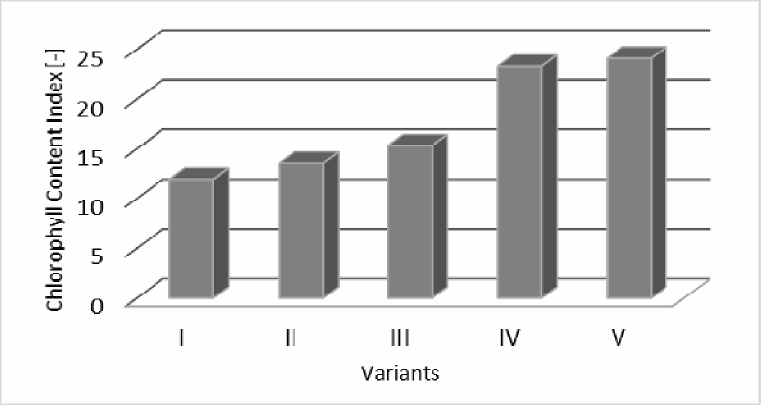

Fig. 2. Relative content of chlorophyll in leaves of barley

Mineral fertilizing in one of the most important factors that affect the amount of crop [11]. Application of calcium sulphate ($CaSO_4 \cdot 2H_2O$) in agriculture and gardening in Poland is not a common practice.

Studies in this direction should be continued with extended diagnostics including the value of crop, the content of protein in crop, and the control of the chemical content of barley. Preliminary results in this direction show that it is rather the chemical content of the waste from gas desulphurisation will be decisive for its commercial application in agriculture [12].

## Conclusions

Based on the study the we drew the following conclusions:

1. NPK fertilising with ammonium nitrate increased acidic reaction and the content of exchangeable aluminium in soil.
2. Calcium sulphate did not cause change of soil pH, thus it can be used as fertilizers on soils of alkaline reaction with a deficit of calcium.

3. Application of calcium sulphate had a positive effect in on the content of chlorophyll in leaves of barley.

## References

[1] Stevens CJ. Dise NB. Gowing, DJ. Regional trends in soil acidification and exchangeable metal concentrations in relation to acid deposition rates. Environ Pollut. 2009;157(1):313-319. DOI: 10.1016/j.envpol.2008.06.033.

[2] Brzeziński M, Barszczak T. Zawartość glinu wymiennego w glebie w świetle trwałych doświadczeń nawozowych w Skierniewicach [The content of exchangeable aluminium in soil in view of long-term fertilization experiments in Skierniewice]. Zesz Probl Post Nauk Roln. 2009;541:67-72. http://omega.sggw.waw.pl/sk/media/publikacje/BRZEZ_BARSZCZ_2009.pdf.

[3] Rutkowska B, Szulc W, Twardowska A. Wpływ niezrównoważonego nawożenia na zmiany odczynu oraz zawartości glinu wymiennego w glebie lekkiej. [Effect of unbalanced fertilization on the changes of soil pH and exchangeable aluminium content in sandy soil]. Zesz. Probl. Post. Nauk Roln. 2010;556:923-928. http://omega.sggw.waw.pl/sk/media/PUBLIKACJE_3/rutk_szulc_tward_2010.pdf.

[4] Kinraide TB. Identity of the rhizotoxic aluminium species. Plant Soil. 1991;134:167-178. DOI: 10.1007/978-94-011-3438-5_80.

[5] Ostrowska A, Gawliński S, Szczubiałka Z. Metody analizy i oceny właściwości gleb i roślin. [Methods of analysis and assessment of the soil and plants]. Warszawa: Instytut Ochrony Środowiska; 1991.

[6] Siuta J, Żukowski B. Rozwój i potencjalne zagrożenie agroekosystemów. Część I. Uwarunkowania agroekologicznego rozwoju gleb mineralnych. [Development and potential threats to agro-ecosystems. Part I. Agro-environmental conditions of the development of mineral soils]. Ochr Środ Zasobów Natur. 2009;39:115-125. http://www.ios.edu.pl/pol/pliki/nr39/nr39-115-125.pdf.

[7] Raun WR, Jonson GV. Inproving nitrogen use efficiency for cereal production. Agron J. 1999;3(91):357-363. DOI: 10.2134/agronj1999.00021962009100030001.

[8] Łabętowicz J, Rutkowska B, Szulc W, Sosulski T. Ocena wpływu wapnowania oraz gipsowania na zawartość glinu wymiennego w glebie lekkiej. [Estimation of liming and gypsum application on the content of exchangeable aluminium in sandy soil]. Ann UMCS, Sec. E. 2004;59(2):631-637. http://wydawnictwo.up.lublin.pl/annales/Agricultura/2004/076.pdf.

[9] Malhi SS, Harpiak JT, Nyborg M, Wiśniowska-Kiellan B. Effect of long-term application of different nitrogen forms on soil reaction and some microelements contents in soil land bromegrass hay. Zesz Probl Post Nauk Roln. 1999;465:547-558.

[10] Filipek T. Występowanie glinu ruchomego w glebie i jego oddziaływanie na rośliny. [The Effect on Plants of Mobile Aluminium in Soil]. Post Nauk Roln. 1989;4/5/6:3-14.

[11] Liszewski M. Reakcja dwóch form jęczmienia jarego pastewnego na zróżnicowane technologie uprawy. [The response of two forms of feed spring barley to different cultivation systems]. Zesz. Nauk. UWP Wrocław. 2008;565. http://www.dbc.wroc.pl/dlibra/doccontent?id=2675.

[12] Plaskacz-Dziuba M, Buczkowski R, Igliński B, Cichosz M, Dziuba P. Analiza fizykochemiczna odpadu z instalacji odsiarczania spalin metodą półsuchą z elektrociepłowni przemysłowej w Janikowie. [Physico-chemical analysis of the waste from installation of semi-dry flue gas desulfurization of industrial CHP plant in Janikowo]. Inż Ekol. 2014;38:91-105: DOI: 10.12912/2081139X.36.

# WPŁYW NAWOŻENIA NA ZMIANY ODCZYNU ORAZ ZAWARTOŚCI GLINU WYMIENNEGO W GLEBIE

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**Abstrakt:** W pracy oceniono stopień zakwaszenia gleby oraz zawartość glinu wymiennego w zależności od rodzaju zastosowanego nawozu mineralnego oraz jego dawki. Badania prowadzono w oparciu o doświadczenie wazonowe z jęczmieniem jarym odmiany browarniczej Propino. Uzyskane wyniki wskazują, że największe zakwaszenie oraz największe zawartości glinu wymiennego stwierdza się w warunkach stosowania nawożenia w kombinacji NPK wraz z saletrą amonową. Zastosowany w różnych dawkach siarczan wapnia dwuwodny nie wpłynął na zmianę odczynu gleby, a odnotowana zawartość glinu wymiennego była niższa niż w kombinacji NPK wraz z saletrą amonową.

**Słowa kluczowe:** zakwaszenie, glin wymienny, nawożenie mineralne, jęczmień jary

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# REMOVAL OF Cu(II) AND Pb(II) FROM AQUEOUS SOLUTIONS BY LACTIC ACID BACTERIA

## USUWANIE Cu(II) I Pb(II) Z ROZTWORÓW WODNYCH PRZEZ BAKTERIE KWASU MLEKOWEGO

**Abstract:** The aim of the study was to compare the removal of Cu(II) and Pb(II) from aqueous solution by lactic acid bacteria (LAB). Effect of various process parameters, viz., initial metal ions concentration, pH, and contact time has been studied for the removal of copper and lead ions. Langmuir and Freundlich models were applied to describe the biosorption isotherm of the metal ions by LAB biomass. Langmuir model fitted the equilibrium data better than the Freundlich isotherm. The sorbent showed the maximum sorptive capacity amounting to be 11.07 and 10.51 mg · g$^{-1}$ for Cu(II) and Pb(II) ions, respectively. The optimum conditions were pH 6.0 with equilibrium time of 40 min for both metal ions. The involvement of functional groups on the surface of dried biomass in biosorption process is also discussed.

**Keywords:** biosorption, Cu(II), Pb(II), isotherm, lactic acid bacteria

Lactic acid bacteria (LAB) are nonpathogenic, food safe bacteria that are used in the production of many fermented food products. They ferment glucose into lactic and acetic acid, ethanol and $CO_2$, which contributes to better quality, texture and aroma of fermented products. LAB also produces bacteriocins and other compounds that inhibit the growth of undesirable microorganisms present in food. They are a part of the microbial population of the digestive tract of healthy humans and animals, and are involved in their metabolism [1]. Microbial cell is the natural adsorbent for metal ions due to its nature and composition of the cellular membrane [2]. Application of LAB in the process of metal ions binding has started recently, although studies that examined the binding ability of metal ions by other microorganisms were carried out. Most of the studies have used readily available waste microbial biomass from the pharmaceutical or food industries, in order to remove heavy metals from industrial wastewater. However, the investigated waste microbial biomass cannot be used in the field of food technology. In drinking water and food processing only food safe microorganisms can be used. Among all of them, LAB has the added advantage because of probiotic properties of some strains. Food and water contamination by heavy metals continues to increase due to the increasing environmental pollution. Heavy metals in food, even at a very low concentration, may lead to development of adverse health effects. If they enter the body, they are not degradable and tend to form complexes and accumulate in the tissues [3]. There are various conventional physical and chemical methods for removing heavy metals from water as chemical precipitation, ion exchange, membrane technologies etc. These methods are effective, but often dependent on the metal ion concentration, expensive and not environment friendly. Binding of heavy metals on the LAB could be a promising solution for heavy metal removal from water, liquid food and from the body [1].

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[*] Contribution was presented during ECOpole'15 Conference, Jarnoltowek, 14-16.10.2015

The objective of the present work is to investigate the biosorption potential of LAB in the removal of Cu(II) and Pb(II) from aqueous solution. The influence of different parameters on biosorption, such as pH, initial metal concentration and contact time, were performed. Different equilibrium and kinetic models were applied to describe the biosorption process. The desorption experiments were implemented to identify the release of metal ions and recovery of biosorbents, while competitive biosorption and the Fourier transform infrared (FTIR) analysis were also used in this study to look at potential binding sites and possible functional groups of LAB.

## Material and methods

The bacteria used in this work were lyophilized cultures of *Lactobacillus rhamnosus, L. casei* and *L. plantarum* (Avet PHARMA, Warszawa, Poland). The bacteria transferred to MRS plates and cultured anaerobically for 71 h at 37°C. A colony from the plate was transferred to MRS broth and incubated under anaerobic conditions for 48 h at 37°C. The cultured biomass was washed twice with deionized water and lyophilized.

The concentrations of Cu(II) and Pb(II) in the biosorption experiments were determined spectrophotometrically (Photolab Spectral, WTW, Germany). Before measuring the samples were passed through a Whatman filter (pore size 0.45 μm) and then diluted with deionized water. The initial and the final concentrations of heavy metals used in batch mode studies were estimated spectrophotometrically. The removal efficiency of the microorganisms was calculated from the difference between initial and final concentrations.

Experimental tests were conducted in 250 $cm^3$ Erlenmeyer flasks with of 50 $cm^3$ of metal solution at constant level of biomass (0.1 g), at 37°C and agitation of 120 rpm. Biosorption experiments were carried out to investigate the effects of pH, contact duration and initial metal concentration. The pH values were adjusted between 2.0-8.0 by adding 0.1 M NaOH or 0.1 M $HNO_3$. The contact durations ranged from 0-120 min. The initial Cu(II) and Pb(II) concentrations varied from 8.0 to 150.5 $g \cdot dm^{-3}$ and 9.5 to 154.6 $g \cdot dm^{-3}$, respectively. The functional groups present in the biomass, before and after interaction with Cu(II) and Pb(II) were detected using a Fourier transform infrared (FTIR) spectrometer. The spectrum was recorded in the range of 4000-500 $cm^{-1}$.

The metal uptake [mg metal $\cdot$ $g^{-1}$ dry biomass] was calculated:

$$q_e = \frac{(C_0 - C_e) \cdot V}{M} \tag{1}$$

where $C_0$ and $C_e$ are the respective initial and equilibrium metal concentrations in the solution [$mg \cdot dm^{-3}$], $V$ is the volume of the solution [$dm^3$], and $M$ is the dry weight of the biomass [g]. The metal sorption ability of the biomass was determined by the above-mentioned procedure in all the following experiments unless stated otherwise.

Heavy metals biosorption isotherms were obtained at constant pH and ionic strength. To test the fit of data, Langmuir and Freundlich isotherm models were applied to this study. The Langmuir isotherm model is valid for monolayer sorption onto a surface and a finite number of identical sites, and is given by:

$$q_{eq} = \frac{q_{\max} K_L C_{eq}}{1 + K_L C_{eq}} \tag{2}$$

or presented in linear form as follows:

$$\frac{1}{q_{eq}} = \frac{1}{q_{\max}} + \frac{1}{K_L \cdot q_{\max} \cdot C_{eq}} \tag{3}$$

where $q_{\max}$ [mg · g$^{-1}$] is the maximum amount of the metal ion per unit weight of the cell to form a complete monolayer on the surface bound at a high $C_{eq}$ [mg · dm$^{-3}$] and $K_L$ a constant related to the affinity of the binding sites, $q_{\max}$ represents a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in the comparison of adsorption performance, particularly in cases where the sorbent did not reach full saturation in experiments.

The empirical Freundlich isotherm model based on a heterogeneous surface is given by:

$$q_{eq} = K_F C_{eq}^{1/n} \tag{4}$$

where $K_F$ and $n$ are Freundlich constants characteristic of the system, $K_F$ and $n$ are indicators of adsorption capacity and intensity, respectively. Freundlich parameters can be determined from the linear form of the eq. (4) by plotting the ln $q_{eq}$ versus ln $C_{eq}$ the slope is the value of $1/n$ and the intercept is equal to ln $K_F$. The Freundlich isotherm is also more widely used and provides information on the monolayer adsorption capacity, in contrast to the Langmuir model. All data shown are the mean values of three replicate experiments, and error bars are indicated wherever necessary.

## Results and discussion

The pH of the solution is one of the most important parameters affecting the biosorption process due to its impact on both the solubility of metal ions and the ionization states of surface functional groups of the biosorbent system [4].

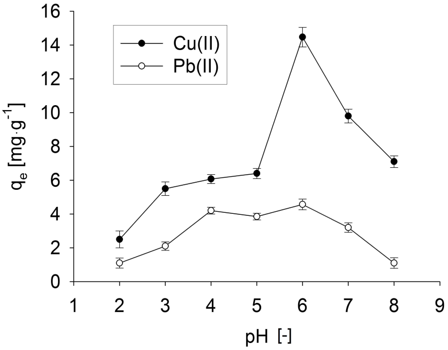

Fig. 1. Initial pH effect on Cu(II) and Pb(II) removal conditions by LAB (metals concentration 78.0 and 92.0 mg · dm$^{-3}$ respectively, contact time 60 min, biomass 0.1 g, temperature 37°C, agitation rate 120 rpm)

As depicted in Figure 1, the metal ions uptake ions was strongly affected by the pH of the medium as it increased with solution pH increase. The sharpest increase in maximum capacity ($q_e$) was obtained between pH values of 2 and 6, corresponding for the latter pH value to capacities of 14.47 and 4.57 $\text{mg} \cdot \text{g}^{-1}$ for copper and lead, respectively. Further lead and copper biosorption studies were carried out at pH 6. At low pH values, the high concentration of $H^+$ protons competes with and decreases the adsorption of positively charged metals ions [5, 6] but an increase in the pH causes the ionization of the biosorbent surface functional groups and thus, increases the total negative charge which increases biosorption. At pH values higher than 6, precipitation of insoluble metals hydroxides may be formed and the concentration of free Cu(II) and Pb(II) ions decreases, thereby the adsorption capacity of Cu(II) and Pb(II) decreases [7].

The biosorption rates of Cu(II) and Pb(II) ions on LAB biomass were shown in Figure 2. Rapid uptake of metal species was occurred within 20 min and an equilibrium was reached in 40 min. After this equilibrium period the amount of adsorbed metal ions did not change significantly with contact time. Such a phenomenon of rapid removal of Cu(II) and Pb(II) ions has also been reported earlier for biosorbents such as *Bacillus thioparans*, green algae *Cladophora fascicularis, Neurospora crassa* and *Candida ablicans* [8-11].

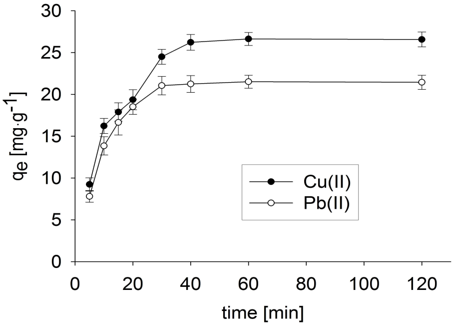

Fig. 2. Biosorption of Cu(II) and Pb(II) by LAB as a function of time (metals concentration 101.5 and 102.7 $\text{mg} \cdot \text{dm}^{-3}$ respectively, pH 6.0, biomass 0.1 g, temperature 37ºC, agitation rate 120 rpm)

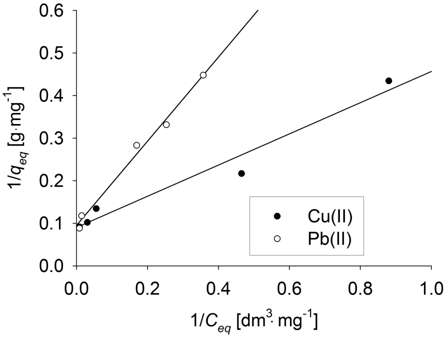

Fig. 3. Langmuir isotherms for Cu(II) and Pb(II) biosorption by LAB (initial pH 6.0, biomass 0.1 g, temperature 37ºC, agitation rate 120 rpm)

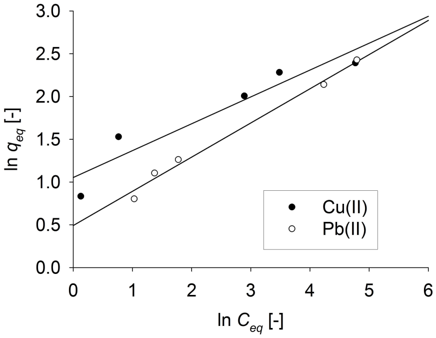

Fig. 4. Freundlich isotherms for Cu(II) and Pb(II) biosorption by LAB (initial pH 6.0, biomass 0.1 g, temperature 37°C, agitation rate 120 rpm)

The linearized Langmuir and Freundlich isotherms of Cu(II) and Pb(II) ions are given in Figures 3 and 4. The isotherm constants and correlation coefficients are given in Table 1. Langmuir sorption model served to estimate the maximum metal uptake values where they could not be reached in the experiments. In general, for good biosorbents, high $q_{\max}$ and high $K_L$ are desirable [12]. However, biosorbent with a low $q_{\max}$ and a high $K_L$ could outperform a biosorbent with high $q_{\max}$ and a low $K_L$ [13]. In this study, $K_L$ and $q_{\max}$ value were found out 0.197 and 11.07 mg · g$^{-1}$ for Cu(II) and 0.088 and 10.51 mg · g$^{-1}$ for Pb(II) which indicated that LAB is an encouraging biosorbent for the tested metal ions (Table 1). The correlation coefficients (R$^2$) were found to be 0.96 and 0.99 for Cu(II) and Pb(II) biosorption, respectively. Mrvčić et al [1] reported a $q_{\max}$ value of 15.53 mg · g$^{-1}$ for Cu(II) biosorption by *Lactobacillus plantarum*.

Fitting of the biosorption data of Cu(II) and Pb(II) to Langmuir isotherm showed that the binding energy on the whole surface of the LAB was uniform. Fitting adsorption data to Langmuir isotherms also showed that the adsorbed metal ions do not interact or compete with each other and they are adsorbed by forming a monolayer. This phenomenon, at the same time, indicated that chemosorption was the principal removal mechanism in biosorption [14].

Table 1

Adsorption isotherm parameters for Cu(II) and Pb(II) ions on LAB

| **Metal ions** | **Langmuir model** | | | **Freudlich model** | | |
|---|---|---|---|---|---|---|
| | **$K_L$** | **$q_{\max}$ [mg · g$^{-1}$]** | **$R^2$** | **$n$** | **$K_F$** | **$R^2$** |
| | $q_{eq} = q_{\max}K_LC_{eq}/(1+K_LC_{eq})$ | | | $q_{eq} = K_FC_{eq}^{1/n}$ | | |
| Cu(II) | 0.197 | 11.07 | 0.96 | 0.73 | 2.87 | 0.90 |
| Pb(II) | 0.088 | 10.51 | 0.99 | 1.12 | 1.63 | 0.98 |

Table 1 suggests that metal biosorption is less confidentially explained by Freundlich model, on the basis of $R^2$, than Langmuir model.

The FTIR spectra of LAB before and after interaction with heavy metals (copper and lead) are shown in Figures 5-7. The sharp peak at 3378 cm$^{-1}$ was characteristic of hydroxyl groups bound to alcohol, and strong CH$_2$ stretching frequency gave rise to peak at 2926 cm$^{-1}$. Differences were also noted at 1634 cm$^{-1}$ (C═O, stretching vibrations). Some differences in the spectra of the 3 samples were observed in the broad range of 1218 to 1413 cm$^{-1}$ indicating the presence of ─OH groups, COO− anions, and C═O groups (deformation and stretching vibrations). A clear difference in signal intensity was also found at 1028 cm$^{-1}$ (C─O groups).

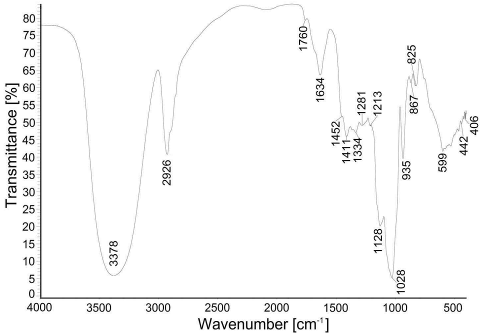

Fig. 5. FTIR spectrum of native LAB

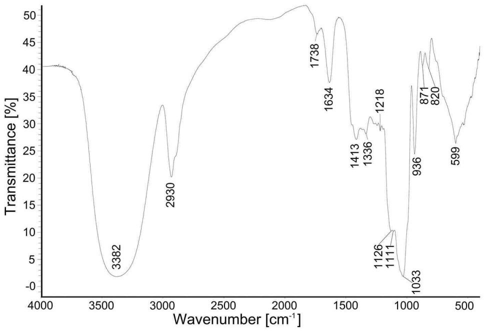

Fig. 6. FTIR spectra of LAB after treatment with Cu(II)

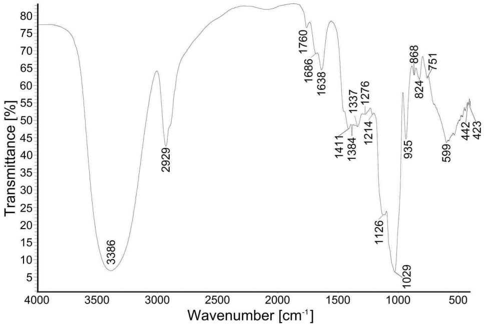

Fig. 7. FTIR spectra of LAB after treatment with Pb(II)

The analysis of the FTIR spectrum confirmed the presence of many ionizable functional groups, which are able to react with Cu(II) and Pb(II) ions. Carboxyl or hydroxyl groups are known to be involved in metal binding by forming coordination bonds that facilitate the stability of the metal complexes [15]. The results of FTIR analysis obtained in this study demonstrated that ─OH, C═O, and COO− groups played an important role in the binding of Cu(II) and Pb(II) ions onto the LAB biosorbent.

## Conclusions

In this study, the LAB were used as effective biosorbents of Cu(II) and Pb(II) from waters. The biosorption performances were strongly affected by parameters such as pH, contact duration and heavy metal concentration. The optimum pH for the biosorption of Cu(II) and Pb(II) by LAB at pH 6.0. The uptake of metals was very fast. Adsorption equilibrium was reached within 40 min of biomass addition. The batch experimental results fitted well to the Langmuir isotherm model. The maximum adsorption uptake ($q_{\max}$) of respectively Cu(II) and Pb(II) calculated from the Langmuir equation for biosorption by LAB were 11.07 and 9.79 mg · g$^{-1}$. The FTIR analysis also suggests the involvement of some functional groups (─OH, C═O, and COO−) in Cu(II) and Pb(II) biosorption.

## References

[1] Mrvčić J, Stanzer D, Šolić E, Stehlik-Tomas V. Interaction of lactic acid bacteria with metal ions: opportunities for improving food safety and quality. World J Microbiol Biotechnol. 2012;28:2771-2782. DOI: 10.1007/s11274-012-1094-2.

[2] Blackwell KJ, Singleton I, Tobin. Metal cation uptake by yeast: a review. Appl Microbiol Biotechnol. 1995;43(4):579-584. DOI: 10.1007/BF00164757.

[3] Vinodhini R, Narayanan M. Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (common carp). Int J Environ Sci Tech. 2008;5(2):179-182. DOI: 10.1007/BF03326011.

[4] Halttunen T, Salminen S, Tahvonen R. Rapid removal of lead and cadmium from water by specific lactic acid bacteria. Int J Food Microbiol. 2007;114:30-35. DOI: 10.1016/j.ijfoodmicro.2006.10.040.

[5] Akar T, Tunali S. Biosorption performance of Botrytis cinerea fungal by-products for removal of Cd(II) and Cu(II) ions from aqueous solutions. Miner Eng. 2005;18:1099-1109. DOI: 10.1016/j.mineng.2005.03.002.

[6] Sassi M, Bestani B, Said AH, Benderdouche N, Guibal E. Removal of heavy metal ions from aqueous solutions by a local dairy sludge as a biosorbant. Desalination. 2010;262:243-250. DOI: 10.1016/j.desal.2010.06.022.

[7] Liu Y, Cao Q, Luo F, Chen J. Biosorption of $Cd^{2+}$, $Cu^{2+}$, $Ni^{2=}$, and $Zn^{2+}$ ions from aqueous solutionscby pretreated biomass of brown algae. J Hazard Mater. 2009;163:931-938. DOI: 10.1016/j.hazmat.2008.07.046.

[8] Rodriguez-Tirado V, Green-Ruiz C, Gómez-Gil B. Cu and Pb biosorption on Bacillus thioparans strain U3 in aqueous solution: Kinetic and equilibrium studies. Chem Eng J. 2012;181-182:352-359. DOI: 10.1016/j.cej.2011.11.091.

[9] Deng L, Su Y, Su H, Wang X, Zhu X. Biosorption of copper(II) and lead(II) from aqueous solutions by nonliving green algae Cladophora fascicularis: Equilibrium, kinetics and environmental effects. Adsorption. 2006;12:267-277. DOI: 10.1007/s10450-006-0503-y.

[10] Kiran J, Akar T, Tunali S. Biosorption of Pb(II) and Cu(II) from aqueous solutions by pretreated biomass of Neurospora crassa. Process Biochem. 2005;40:3550-3558. DOI: 10.1016/j.procbio.2005.03.051.

[11] Baysal Z, Çinar E, Bulut Y, Alkan H, Mehmet D. Equilibrium and thermodynamic studies on biosorption of Pb(II) onto Candida albicans biomass. J Hazard Mater. 2009;161:62-67. DOI: 10.1016/j.jhazmat.2008.02.12.

[12] Davis TA, Volesky B, Mucci A, A review of the biochemistry of heavy metal biosorption by brown algae. Water Res. 2003;37:4311-4330. DOI: 10.1016/S0043-1354(03)00293-8.

[13] Hashim MA, Chu KH. Biosorption of cadmium by brown, green and red seaweeds. Chem Eng J. 2004;97:249-255. DOI: 10.1016/S1385-8947(03)00216-X.

[14] Kiliç M, Keskin ME, Mazlum S, Mazlum N. Effect of conditioning for Pb(II) and Hg(II) biosorption on waste activated sludge. Chem Eng Process. 2008;47:31-40. DOI: 10.1016/j.cep.2007.07.019.

[15] Polak-Berecka M, Szwajgier D, Waśko A. Biosorption of $Al^{+3}$and $Cd^{+2}$by an exopolysaccharide from Lactobacillus rhamnosus. J Food Sci. 2014;79(11):2404-2408. DOI: 10.1111/1750-3841.12674.

# USUWANIE Pb(II) I Cu(II) Z ROZTWORÓW WODNYCH PRZEZ BAKTERIE KWASU MLEKOWEGO

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**Abstrakt:** Celem pracy było porównanie zdolności usuwania Cu(II) i Pb(II) z roztworu wodnego przez bakterie kwasu mlekowego (LAB). Badano wpływ różnych parametrów, tj. stężenia jonów metali, pH i czas kontaktu, na proces usuwania jonów miedzi i ołowiu. Do opisu izoterm adsorpcji jonów metali przez biomasę LAB zastosowano modele Langmuira i Freundlicha. Uzyskane dane doświadczalne były lepiej dopasowane do modelu Langmuira niż Freundlicha. Sorbent wykazał maksymalną zdolność sorpcyjną, wynoszącą 11,07 i 10.51 mg · $g^{-1}$ odpowiednio dla jonów Cu(II) i Pb(II). Optymalne warunki biosorpcji obu jonów metali wynosiły: pH 6,0 i czas równowagi 40 minut. Omówiono również udział grup funkcyjnych na powierzchni biomasy w procesie biosorpcji.

**Słowa kluczowe:** biosorpcja, Cu(II), Pb(II), izoterma, bakterie kwasu mlekowego

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# APPLICATION OF THE TRIAL RESEARCH SET FOR PRETREATMENT OF CASINGS PROCESSING WASTEWATERS

## WYKORZYSTANIE PILOTAŻOWEGO ZESTAWU BADAWCZEGO DO PODCZYSZCZANIA ŚCIEKÓW Z JELICIARNI

**Abstract:** The designed research set of capacity up to 1.0 $m^3$/h for testing physicochemical methods of raw industrial wastewaters pretreatment directly at the source of their origin or storage was presented. The installation built on the mobile pallet is equipped with the cylinder-conical central flotation chamber with the surface flotation fodder, pipe reactors, where the chemical reagents are dosed from the preparation and dosing stations and processing units where the dispersed air flotation or dissolved air flotation or oxidation aided with hydrogen peroxide in accordance with own invention. The subject set can be used to optimize different physicochemical pretreatment methods, including coagulation, chemical precipitation and final separation of dispersed phases with the use of the mentioned above flotation methods. The experimental conditions and the obtained reductions of indicator values with the application of the subject research installation were set for the selected wastewaters from casings processing.

**Keywords:** mobile research installation, pretreatment of wastewaters from casings processing department, flotation

## Introduction

Technological wastewaters generated by a conventional casings processing department mainly originate from the degumming process on frames, during which grinding, softening in wash water, burnishing, final grinding, degumming and debonding are frequently used [1-4]. The second important process generating the essential pollutants load is intestines sorting which includes the quality control from the point of view of the occurrence of perforation, measurements and sorting in accordance with the size, arrangement in bunches and salting as well as final packaging [1-4]. Also, general technological wastewaters may sometimes contain the pollutants load originating from the incomplete or inadequately run process of their preliminary treatment and cleaning of animal intestines from the remains of the easily decomposed feed, the remains of fat and entrails which should be removed at the stage of slaughtering [1, 4]. The typical pollutants load in general technological wastewaters generated by casings processing departments contains significant amounts of salts (mainly NaCl), total suspended solids (TSS), etheric extract (EE) and significant concentrations of loads of chemical and biochemical oxygen demand (COD & $BOD_5$), mainly originating from intestinal mucus [1, 2]. The known methods of physicochemical pretreatment of technological wastewaters from such production are mainly based on the application of coagulation [5] and microcoagulation methods induced by electrolysis [6, 7], the application of membrane processes [8, 9] or these methods additionally aided with oxidation [10-13]. However, the known methods of full treatment generally consist in physicochemical stages of preliminary pretreatment as the ones mentioned above, and at the

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second stage in the application of biological methods based on for example the activated sludge with the low concentration [14] or other active sludge variants cleaning in the conditions with the high concentrations of chlorides [4, 9]. The other known method of neutralizing are agroutilization processes which consist in their application in order to hydrate and to a smaller extent for the fertilization features of the subject wastewaters [15]. In order to work out the detailed wastewaters treatment technologies from such productions, apart from choosing the optimum method, the key aspect is the determination of sequence of work of particular processing bonds and their efficiency. Many operations and unit processes applied to processing of wastewaters from the tested casings processing department in laboratory scale, undergo the problem of scale - up during their practical use - at the stage of implementation. In case of low-tonnage quantities of wastewaters, to which wastewaters generated by casings processing departments belong, the key issue before the stage of project work is to assume the treatment conditions selected in laboratory scale and verified during the next processing scales.

The aim was to work out the effective method of physicochemical pretreatment of wastewaters generated from the degumming and sorting processes of animal intestines as well as the processing conditions on the self - constructed experimental installation for testing the methods, including the ones used in conditions concerning the flow technology [16, 17].

## Experimental part

### *Construction of experimental installation*

The mobile experimental installation for testing physicochemical methods of industrial wastewaters pretreatment was presented in a detailed form in paper [17]. The experimental set was constructed on the moveable palette and consisted in the following processing bonds: a) central air dispersed flotation chamber equipped with surface flotation fodder and two pipe reactors, b) the self-invented saturation - dispersion scheme [16] and the pressure saturation chamber, c) the operating panel for steering and process optimization, d) stations for preparing and dosing chemical reagents. In case of the variant with continuous work of the experimental installation, the raw wastewaters after exact averaging were directed to the pipe reactor (or two pipe reactors), where reagents were introduced in a form of flow. In case of such mode of work, the cylinder - conical volume functions as a dispersed chamber for the flotation scheme at the same mechanically separating of the concentrated by flotation dispersed phases with the use of surface flotation fodder. The flocculant in this option is dosed at the intake to the dispersed chamber. Depending on the applied flotation technique, the saturation tank is used (DAF technology) or the scheme of connected in rows aeration modes (to the volumes to which air is pumped under pressure) consisting in permanently concentrically arranged cylindrical membrane aeration devices and external cylindrical pump chambers, to which wastewaters are introduced with the linear speeds enabling optimum air saturation (IAF technique) [16]. The exemplary experimentally set parameters regarding physicochemical pretreatment with the use of the mentioned above flotation techniques were set in Tables 1 and 2.

### *Analytical part*

The elimination efficiency of particular loads was determined in accordance with standards for water and wastewaters: reaction (pH) (PN-EN ISO 10523:2012), total suspended solids (TSS) (PN-EN 872:2007), determination of chemical oxygen demand COD by dichromate method (PN-ISO 15705:2005 and PN-ISO 6060:2006), determination of biochemical oxygen demand $BOD_n$ by the dillution method (PN-EN 1899-1:2002 and PN-EN 1899-2:2002), determination of total nitrogen (TN) (PN-EN 25663:2001), total phosphorus (TP) (PN-EN ISO 6678:2006) and etheric extracte (EE) PN-ISO (PN-86/C-04573.01). In case of application of hydrogen peroxide, the value correction was introduced for the determination of COD. The value of real chemical oxygen demand was given after correcting its value by subtracting the mass participation introduced by the residual hydrogen peroxide on the basis of the following relation $COD_r = COD_p - f \cdot c$ ($COD_r$ - real, $COD_p$ - determined in the sample after the reaction is over, $c$ - $H_2O_2$ concentration in a sample determined iodometrically, $f = 0.25$ - correction ratio, assumed on the basis of literature data [18, 19]).

## Results and discussion

During the period of time of carrying out tests, basic parameters concerning raw wastewaters from degumming and sorting processes were registered at typical levels of values (Tables 1 and 2) comparable with the available literature data [1, 15]. The aim was to find out such a physicochemical method of pretreatment for the flow conditions, which apart from high efficiency of reduction of the basic TSS, EE, COD & $BOD_5$ and TN & TP loads would allow to eliminate the potential loads of microbiological charges, which presence in such streams cannot be excluded. On the basis of preliminary samples, one-stage coagulation with final flocculation with the use of flocculant Praestol 859 BC (Stockhausen) was selected for the physicochemical pretreatment of raw wastewaters. The application of flocculation before the stage of separation by flotation results in measureable reductions in basic indicator values. However, they are at relatively low level and within the wide range of percentage reductions (Tables 1 and 2). It is induced mainly by condensation of the dispersed phases of colloidal and suspension protein and fatty complexes as well as oligo- and/or polysaccharides, especially in the form of components of raw intestines mucus and the remains of viscera. In case of such type of pretreatment, the differences in the reduction levels of particular parameters according to the applied flotation technique are vague for wastewaters from the process of degumming or sorting. Additional aiding the flotation with aggregated hydrogen peroxide pollutants results in a slight increase in effectiveness of the separation level of TSS, EE, COD & $BOD_5$ loads. Separated flotates are resistant to gravitational and pressurized dehydration. Lime milk in form of 7.5% solution was selected from the group of coagulants tested in a laboratory as the most effective one to accomplish the assumed processing and sanitary aims. The application of this coagulant and flocculation with the mentioned polyelectrolyte results in significant improvement in quality parameters at the installation outlet and the evident increase in the reduction level of the indicator values (Tables 1 and 2) while the deposits sediments can be distinguished by the beneficial susceptibility to their gravitational or pressurized dehydration, which is connected with the increase in CaO, $Ca_3(PO_4)_2$ and lime soaps participation in these flotates' dry mass.

Table 1

Examples of wastewaters from the casing separating plant for which methods of physicochemical pretreatment have been developed to be applied for verifying the process in semi-technical scale of the presented here testing set

| No. | Pretreatment method | Raw effluent parameters (concentration) | Reduction levels [%] | Used flotation type[b] |
|---|---|---|---|---|
| 1 | final flocculation (Praestol 859 BC) | COD (2650-6740 mg/dm$^3$)<br>$BOD_5$ (1070-2590 mg/dm$^3$)<br>EE (149-310 mg/dm$^3$)<br>TN (173-283 mg/dm$^3$)<br>TP (22-58 mg/dm$^3$) | 19-32<br>15-27<br>57-74<br>6-14<br>2-6 | IAF (pH = 6.69-7.33) (air pressure: 200-210 kPa, recirculation level 10%) |
| 2 | final flocculation (Praestol 859 BC) | COD (3440-5370 mg/dm$^3$)<br>$BOD_5$ (1460-2140 mg/dm$^3$)<br>EE (117-299 mg/dm$^3$)<br>TN (132-201 mg/dm$^3$)<br>TP (11-48 mg/dm$^3$) | 23-34<br>16-30<br>65-84<br>5-10<br>3-7 | OxIAF (pH = 6.80-7.40) [a] (air pressure: 200-220 kPa, $H_2O_2$ dose - 120-140 g/m$^3$, recirculation level 5%) |
| 3 | final flocculation (Praestol 859 BC) | COD (2960-4680 mg/dm$^3$)<br>$BOD_5$ (1220-1840 mg/dm$^3$)<br>EE (88-303 mg/dm$^3$)<br>TN (92-227 mg/dm$^3$)<br>TP (17-55 mg/dm$^3$) | 24-35<br>19-28<br>71-86<br>4-9<br>3-7 | DAF (pH = 6.73-7.27) (saturation pressure: 300 kPa, recirculation level 5%) |
| 4 | final flocculation (Praestol 859 BC) | COD (2450-3820 mg/dm$^3$)<br>$BOD_5$ (980-1570 mg/dm$^3$)<br>EE (126-211 mg/dm$^3$)<br>TN (109-166 mg/dm$^3$)<br>TP (23-39 mg/dm$^3$) | 29-35<br>26-31<br>77-88<br>6-12<br>3-8 | OxDAF (pH = 6.94-7.51) [a] (saturation pressure: 300 kPa, $H_2O_2$ dose - 90-110 g/m$^3$, recirculation level 5%) |
| 5 | coagulation and precipitation with lime milk and final flocculation (Praestol 859 BC) | COD (3070-4310 mg/dm$^3$)<br>$BOD_5$ (1280-1770 mg/dm$^3$)<br>EE (137-244 mg/dm$^3$)<br>TN (64-199 mg/dm$^3$)<br>TP (26-38 mg/dm$^3$) | 60-66<br>48-59<br>> 98<br>9-17<br>76-80 | OxIAF (pH = 8.75-9.14) [a] (air pressure: 200 kPa, $H_2O_2$ dose - 50-65 g/m$^3$, recirculation level 10%) |
| 6 | coagulation and precipitation with lime milk and final flocculation (Praestol 859 BC) | COD (2790-4010 mg/dm$^3$)<br>$BOD_5$ (1070-1620 mg/dm$^3$)<br>EE (141-341 mg/dm$^3$)<br>TN (92-227 mg/dm$^3$)<br>TP (14-54 mg/dm$^3$) | 67-71<br>51-63<br>> 98<br>12-20<br>78-81 | OxDAF (pH = 8.64-9.05) [a] (saturation pressure: 300 kPa, $H_2O_2$ dose - 50-55 g/m$^3$, recirculation level 5%) |

[a] flotation technique acc. to Author's solution [16]

[b] different types of applied flotation techniques for final separation of the dispersed phases are marked as follows: IAF - induced air flotation, DAF - dissolved air flotation, OxIAF or OxDAF - flotation using induced air and dissolved air enhanced by the oxidation with hydrogen peroxide

Aiding the flotation with hydrogen peroxide does not significantly influence the efficiency of the processes of charges' reduction but it only allows to change the process parameters (for instance lower levels of saturation pressure, lower recirculation levels). However, this aiding reagent needs to be dosed within the concentrations range and the range of reaction values preventing its sudden decomposition together with the liberation of molecular oxygen. Lime milk components and hydrogen peroxide are the reagents significantly increasing the sanitary safety of industrial wastewaters processing, which is also the notable aspect concerning such productions that must satisfy the determined veterinary criteria.

Table 2

Examples of wastewaters from the mucous stripper plant for which methods of physicochemical pretreatment have been developed to be applied for verifying the process in semi-technical scale of the presented here testing set

| No. | Pretreatment method | Raw effluent parameters (concentration) | Reduction levels [%] | Used flotation type[b] |
|---|---|---|---|---|
| 1 | final flocculation (Praestol 859 BC) | COD (1560-2770 mg/dm$^3$)<br>BOD$_5$ (850-1430 mg/dm$^3$)<br>EE (97-211 mg/dm$^3$)<br>TN (214-297 mg/dm$^3$)<br>TP (25-38 mg/dm$^3$) | 18-26<br>12-21<br>49-78<br>6-15<br>2-5 | IAF (pH = 7.15-7.63) (air pressure: 200-220 kPa, recirculation level 10%) |
| 2 | final flocculation (Praestol 859 BC) | COD (1830-2910 mg/dm$^3$)<br>BOD$_5$ (1120-1750 mg/dm$^3$)<br>EE (132-187 mg/dm$^3$)<br>TN (155-198 mg/dm$^3$)<br>TP (8-31 mg/dm$^3$) | 22-31<br>14-24<br>76-85<br>5-14<br>3-6 | OxIAF (pH = 7.27-7.77) [a] (air pressure: 300-310 kPa, $H_2O_2$ dose - 210-220 g/m$^3$, recirculation level 5%) |
| 3 | final flocculation (Praestol 859 BC) | COD (2210-2630 mg/dm$^3$)<br>BOD$_5$ (1180-1480 mg/dm$^3$)<br>EE (140-170 mg/dm$^3$)<br>TN (277-349 mg/dm$^3$)<br>TP (34-43 mg/dm$^3$) | 22-32<br>12-25<br>81-93<br>6-11<br>2-6 | DAF (pH = 7.70-8.21) (saturation pressure: 300 kPa, recirculation level 10%) |
| 4 | final flocculation (Praestol 859 BC) | COD (1870-2730 mg/dm$^3$)<br>BOD$_5$ (980-1610 mg/dm$^3$)<br>EE (249-407 mg/dm$^3$)<br>TN (264-295 mg/dm$^3$)<br>TP (14-39 mg/dm$^3$) | 24-32<br>16-23<br>89-95<br>8-13<br>3-7 | OxDAF (pH = 7.06-7.38) [a] (saturation pressure: 300 kPa, $H_2O_2$ dose - 90-110 g/m$^3$, recirculation level 5%) |
| 5 | coagulation and precipitation with lime milk and final flocculation (Praestol 859 BC) | COD (1880-2540 mg/dm$^3$)<br>BOD$_5$ (940-1510 mg/dm$^3$)<br>EE (130-244 mg/dm$^3$)<br>TN (187-203 mg/dm$^3$)<br>TP (21-38 mg/dm$^3$) | 52-64<br>45-53<br>79-91<br>8-15<br>63-78 | OxIAF (pH = 8.80-9.33) [a] (air pressure: 200 kPa, $H_2O_2$ dose - 50-60 g/m$^3$, recirculation level 10%) |
| 6 | coagulation and precipitation with lime milk and final flocculation (Praestol 859 BC) | COD (2380-2660 mg/dm$^3$)<br>BOD$_5$ (1270-1470 mg/dm$^3$)<br>EE (177-431 mg/dm$^3$)<br>TN (152-201 mg/dm$^3$)<br>TP (12-34 mg/dm$^3$) | 60-73<br>54-69<br>82-96<br>10-16<br>76-80 | OxDAF (pH = 8.81-9.23) [a] (saturation pressure: 300 kPa, $H_2O_2$ dose - 50-60 g/m$^3$, recirculation level 10%) |

[a] flotation technique acc. to Author's solution [16]

[b] different types of applied flotation techniques for final separation of the dispersed phases are marked as follows: IAF - induced air flotation, DAF - dissolved air flotation, OxIAF or OxDAF - flotation using induced air and dissolved air enhanced by the oxidation with hydrogen peroxide

The mixed wastewaters from the process of degumming and the process of sorting were subject to physicochemical processing with lime milk with final flocculation at the comparable level of reduction of the basic indicator values, where the increase in the participation of the wastewaters volume from the sorting process indicated the tendency to lower the reduction decrease levels of the analyzed indicator values. The deciding factors were also the intensity of mixing and the influence of salts concentration on the efficiency of the separation processes - the significant influence of these parameters on the phenomenon and intensity of foaming during the flotation release of the condensed pollutants were observed. The increase in salts concentration and the intensity of mixing (for example due to pumping through the system consisting in two series of pipe reactors) probably resulted in the increase in the concentration of sodium soaps in the volume of the treated wastewater, which resulted in periodic disturbances in the coagulation process and precipitation of insoluble lime soaps.

## Conclusions

Physicochemical pretreatment of technological wastewaters generated by conventional casings processing department may consist in the application of coagulation based on lime milk combined with flocculation with the extremely cationic flocculant. Then at the doses above 50 g $H_2O_2/m^3$, the process of separation of the aggregated pollutants by flotation can be additionally aided with this reagent. The additional, beneficial result of applying the alcaic coagulation reagent and the oxidant in form of hydrogen peroxide is hygienisation of the pretreated stream, which can significantly eliminate or reduce the incidental microbiological infections [20].

## References

[1] Rüffer H, Rosenwinkel KH. Taschenbuch der Industrieabwasserreinigung. (Handbook of Industrial Wastewater). München Wien: R. Oldenbourg Verlag; 1991, 131-134.

[2] Shammas NK, Wasowski JP, Wang LK, Selke WA. Pretreatment of Meat-Processing Waste. In: Flotation Technology Volume 12 of the series Handbook of Environmental Engineering, Humana Press; 2010; 503-565. DOI: 10.1007/978-1-60327-133-2_16.

[3] Cleaner production assessment in meat processing. United Nations Environment Programme, Division of Technology, Industry and Economics (UNEP DTIE). Danish Environment Protection Agency; 2000, 38-40. https://books.google.pl/books?isbn=9280718444.

[4] Industrial Waste Treatment Handbook (Second edition). Woodard & Curran Inc., Elsevier; 2006;149-334. DOI: 10.1016/B978-075067963-3/50009-6, 409-496. DOI: 10.1016/B978-075067963-3/50012-6.

[5] Konieczny P, Ekner E, Uchman W, Kufel B. Effective use of ferric sulfate in treatment of different food industry wastewater. Acta Sci Pol Technol Aliment. 2005;4(1):123-132. http://www.food.actapol.net/pub/12_1_2005.pdf.

[6] Songjiang L, Bo Z, Yu W. Micro-electrolysis coagulation and biological treatment of casings processing wastewater. Environ Sci Manage. 2009;34(4):102-105. DOI: 10.3969/j.issn.1673-1212.2009.04.028.

[7] Sahu OP, Chaudhari PK. Review on chemical treatment of industrial waste water. J Appl Sci Environ Manage. 2013;17(2):241-257. DOI: 10.4314/jasem.v17i2.8.

[8] Xiong Q, Zhang F, Yao Z. Ultrafiltration and nanofiltration for advanced treatment of casing wastewater. Food Machinery. 2012;1:59-61. http://caod.oriprobe.com/articles/29438331/Ultrafiltration_and_nanofiltration_for_advanced_tr.htm.

[9] Dan Z, Yanmao D. Technical progress in the treatment of casing wastewater. Industrial Water Treat. 2012;32(7):18-20. http://www.ecojournals.org/cn/simple_view_abstract.aspx?pcid.

[10] Benli H, Hafley BS, Keeton JT, Lucia LM, Cabrera-Diaz E, Acuff GR. Biomechanical and microbiological changes in natural hog casings treated with ozone. Meat Sci. 2008;79:155-162. DOI: 10.1016/j.meatsci.2007.08.013.

[11] Guzel-Seydim ZB, Greene AK, Seydim AC. Use of ozone in the food industry. Lebensmittel-Wissenschaft und-Technologie. 2004;37(4):453-460. DOI:10.1016/j.lwt.2003.10.014.

[12] Kim JG, Yousef AE, Dave S. Application of ozone for enhancing the microbiological safety and quality of foods: A review. J Food Protect. 1999;62(9):1071-1087.

[13] Kim JG, Yousef AE, Khadre M A. Ozone and its current and future application in the food industry. Adv Food Nutr Res. 2003;45:167-218. DOI:10.1016/S1043-4526(03)45005-5

[14] Neumann H, Voighr HP. Erfahrungen aus Niedersachsen über aerobe Stabilisierung konzentriererter gewerblicher industrieller Abwässer. [Experiences from Lower Saxony over aerobic stabilization of concentrated commercial industrial wastewater] Berichte aus Wassergütewirtschaft und Gesundheitsingenieurwesen. München: Technische Universität München; 1981;33;20-56.

[15] Luo J, Lindsey S, Xue J. Irrigation of meat processing wastewater onto land. Agr Ecosyst Environ. 2004;103:123-148. DOI: 10.1016/j.agee.2003.10.008.
[16] Żak S, Zabłocki L. Method of flotation conduct. 2012. Polish Patent 211742 B1 [in Polish].
[17] Żak S, Zabłocki L. Experimental set for testing methods of industrial wastewaters treatment with the use of flotation techniques, 9th International Conference "Environmental Engineering", May 22-23, 2014, Vilnius, Lithuania, selected papers. Vilnius: Technika, 2014, 1-5. DOI: 10.3846/enviro.2014.101.
[18] Talinli I, Anderson GK. Interference of hydrogen peroxide on the standard COD test. Water Res. 1992;26(1):107-110. DOI: 10.1016/0043-1354(92)90118-N.
[19] Żak S. Problem of correction of the chemical oxygen demand values determined in wastewaters treated by methods with hydrogen peroxide. Proc ECOpole. 2008,2(2):409-414. http://tchie.uni.opole.pl/ecoproc08b/Zak_08b.pdf
[20] http://www.h2o2.com/industrial/applications.

# WYKORZYSTANIE PILOTAŻOWEGO ZESTAWU BADAWCZEGO DO PODCZYSZCZANIA ŚCIEKÓW Z JELICIARNI

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**Abstrakt:** Przedstawiono skonstruowany zestaw badawczy o przepustowości do 1,0 m$^3$/h do testowania fizykochemicznych metod podczyszczania surowych ścieków przemysłowych bezpośrednio u źródła ich powstawania lub gromadzenia. Zbudowana na mobilnej palecie instalacja jest wyposażona w cylindryczno-stożkową centralną komorę flotacji z powierzchniowym zgarniaczem flotatu, reaktory rurowe, do których dozowane są reagenty chemiczne ze stacji ich przygotowania i dozowania, oraz zespoły procesowe do prowadzenia flotacji technikami zdyspergowanym lub rozpuszczonym powietrzem albo z zastosowaniem wspomagania utlenianiem nadtlenkiem wodoru według własnego wynalazku. Za pomocą przedmiotowego zestawu można optymalizować różne metody fizykochemicznego podczyszczania z zastosowaniem między innymi koagulacji, strącania chemicznego oraz do finalnego wydzielania faz zdyspergowanych wymienionymi technikami flotacji. Dla wytypowanych ścieków powstających podczas przetwórstwa jelit przedstawiono ustalone doświadczalnie warunki oraz uzyskiwane wyniki poziomów redukcji wielkości wskaźnikowych z zastosowaniem omawianej instalacji badawczej.

**Słowa kluczowe:** mobilna instalacja badawcza, podczyszczanie ścieków z jeliciarni, flotacja