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# CONTENT OF ZINC AND LEAD IN WATER AND IN ALGAE FROM SELECTED BLACK SEA BAYS NEAR SEVASTOPOL

## ZAWARTOŚĆ CYNKU I OŁOWIU W WODZIE I GLONACH Z WYBRANYCH ZATOK MORZA CZARNEGO W OKOLICACH SEWASTOPOLA

**Abstract:** For many years there has been intensified human pressure in the region of Sevastopol, arising out of its strategic role as the main city in the region as well as a port where the Russian or Soviet Black Sea fleet was stationed. The industry in Sevastopol, municipal sewage as well as agriculture are important sources of pollutants that enter the Black Sea in the region of this city. In terms of shaping the environmental protection policy (not only in the research region but in the whole basin), it is important to conduct monitoring research connected with the pollution of the Black Sea in regions with different levels of human pressure. The aim of this study was to assess the content of zinc and lead in water and in algae from selected Black Sea bays near Sevastopol. The samples of water and algae were collected in August 2012 from eight bays of Sevastopol (Galubaja, Kozacha, Kamyshova, Kruhla, Striletska, Pishchana, Pivdenna and the Sevastopol Bays) as well as one sample from the open sea near Fiolent. *Cystoseira barbata* and *Ulva rigida* algae were taken from the same places. The collected water samples were conserved *in situ* and after being brought to the laboratory their zinc and lead contents were determined. The collected algae were rinsed in distilled water, dried, and then homogenized and mineralized. The lead content was determined in mineralisates by AAS method with electrothermal atomization, and the zinc content was determined using the ICP-OES method.

The zinc content in water ranged from 36.43 to 233.3 μg Zn · dm$^{-3}$, and the lead content was between 1.32 and 38.32 μg Pb · dm$^{-3}$. Considerable differences in contents of the studied elements in water of individual bays were found. Variability of zinc and lead concentration in the studied water samples was 69 and 112%, respectively. The highest zinc contents were found in water from the Striletska, Kozacha, and Sevastopol Bays, and the highest lead contents from the Kozacha and Kruhla Bays. Their lowest concentration was found in the water collected in the open sea. Moreover, the lower zinc concentration was in water from Pivdenna and

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Pishchana Bays, and the lowest lead concentration was found in the Galubaja and Pishchana Bays. The zinc content in the algae ranged between 6.517 and 30.21 mg · kg$^{-1}$. The *Cystoseira barbata* algae contained over twice more zinc than the *Ulva rigida*. The lead content in the algae ranged between 0.567 and 7.692 mg Pb · kg$^{-1}$. Compared with the *Ulva rigida*, almost a half more lead was found in the *Cystoseira barbata*. No statistically significant correlation between the content of the studied elements in water and the algae biomass was observed. However, a significant positive correlation between the content of these metals in both species of algae was found. The values of the zinc bioaccumulation coefficient varied from 32 to 642, and of lead from 30 to 1,273. Contents of the studied elements, both in biotic and abiotic part of the studied ecosystems, point at anthropogenic enrichment. However, the results obtained for the Sevastopol, Kozacha, and Striletska Bays point to a danger of their excessive bioaccumulation and a potential risk to the life of aquatic organisms as well as seafood consumers.

**Keywords:** Black Sea, water, *Cystoseira barbata*, *Ulva rigida*, pollution, bioaccumulation

## Introduction

The Black Sea is a basin with strategic importance on a regional scale as well as on a world scale. Due to the location (within densely populated, urbanized and industrial terrains), considerable amounts of pollutants that worsen the water quality and developmental conditions for biological life enter this basin. In basins which are under the influence of human pressure, worsen of water quality can always be observed [1].

Many seaside cities discharge municipal sewage directly into the sea, and rivers that feed this basin carry considerable amounts of biogenic substances, heavy metals as well as durable organic compounds. The coastal zone of this basin is the most polluted, which is especially dangerous since these are reproduction regions for many species of ichthyofauna as well as zone colonized by macroalgae which play an important role in sea ecosystems. Containing the degradation of the Black Sea belongs to the priorities of the EU policy connected with an environmental protection. In order to implement effective mechanisms within this range, it is necessary to start cooperation among all the users of the Black Sea basin. Research on water quality and quantity is necessary for understanding the changes taking place in the Black Sea ecosystem and for preserving biodiversity. In recent years the importance of the Black Sea area in European Union politics has been increasing, which is a direct consequence of Romania and Bulgaria entering the Union. To meet the established goals of protecting the Black Sea environment, a number of organizations were appointed. These organizations are supposed to help strengthen the cooperation among the Black Sea countries as well as create instruments of political and economic pressure on the governments of these countries. One of them is the “Black Sea synergy – a new regional cooperation initiative” [2]. The aim of cooperation within this program is supposed to be actions that could solve the long-term conflicts, enhance trust, and later eliminate the existing obstacles. Development of cooperation among the Black Sea countries could also bring about beneficial effects that would go beyond their area. Elaboration of the environmental protection policy must, however, be preceded by accurate inventory and by indicating the most important problems. In order to do so, it is important to monitor the quality of the environment and to record any changes from the perspective of space and time. Monitoring data constitute input data for designing the environmental protection policy and are the basis for its evaluation.

Zinc and lead enter the water environment mainly from anthropogenic sources. Surface runoffs from agricultural and urbanized areas, and also sewage, both industrial and municipal, as well as combustion of fuels are the sources of these elements in the water environment. Zinc present in water is easily uptaken, both by plant and animal organisms, that is why in aquatic ecosystem where high amounts of this element can be found in abiotic parts of the environment, a phenomenon of high bioaccumulation of this element is observed. In a highly saline environment lead forms easily soluble complex compound $PbCl_3^-$. This can lead to excessive inclusion of this element into biocirculation. Under normal conditions, both studied elements that enter the water environment are very quickly bond with bottom sediments, undergoing immobilization [3]. Bioindication is an often used tool in evaluation of environmental risks of trace elements [4–6]. Determining the accumulation of harmful elements in organisms living in certain environmental conditions allows not only to evaluate the pollution degree but also to predict changes in structures of biocoenoses, both from the quantitative and qualitative perspective. Biomonitoring research results are an extremely useful tool for shaping the policy of environmental protection and nature protection. Depending on the trend in utilizing environmental resources, the level and specificity of pollutants, organisms on different levels of the trophic chain are used for biomonitoring research [7–9]. Utilize of larvae, spawn or young specimens is useful in solving problems connected with the effect of pollutants on reproductive success. For instance, research on accumulation of trace elements in adult specimens of fish for human consumption provides information on the issue of risk to humans. Utilization of macroalgae for monitoring purposes provides information on the danger coming from pollutants dissolved in water, on their resources deactivated in bottom sediments or bound with the suspension or in living organisms [7]. Based on monitoring studies with the use of autotrophic organisms it is easier to specify the effect of changes in water quality caused by periodic fluctuations or trends connected for example with the implementation of the environmental protection policy [9]. When utilizing aquatic organisms, both plants and animals, it is very important to choose the proper organ in which the content of trace elements is determined. Many authors draw attention to great differences in contents of trace elements in individual organs [10–12]. Algae are organisms of particular importance for biomonitoring due to their great affinity to heavy metals. These organisms are often use as biosorbents for heavy metals [13–15].

The aim of the research was to determine the level of pollution with zinc and lead in ecosystems of the bays in the region of Sevastopol. Content of the studied elements in water and in algae from the green and brown algae genera were the parameters used for the realization of the established goal.

## Material and methods

Due to the shape of shoreline of the studied bays as well as differences in the level of human pressure, they were treated in the research as separate ecosystems. To reach the established goal, samples of water from 8 bays in the region of Sevastopol as well as one sample from the open sea near Fiolent were collected in August 2012 (Fig. 1).

Fig. 1. Points of sampling

The samples were collected from the top layer of water (from the depth of 0–120 cm). The sampling points were selected so as to obtain a sample as representative as possible for the whole bay. In order to do so, data on water movements and on flow of sea currents were used. The samples were collected from the following bays: Galubaja, Kozacha, Kamyshova, Striletska, Kruhla, Pishchana, Pivdenna, and the Sevastopol Bay as well as from the open sea in the region of Fiolent (Fig. 1). The cumulative sample consisted of 10 initial samples with a volume of about 100 $cm^3$, collected in different points. The laboratory sample was identical with the cumulative sample. Simultaneously, samples of *Cystoseira barbata* and *Ulva rigida* algae were collected in the same points. The cumulative sample was created from 10 initial samples, each of approximately 200 g. The laboratory sample was identical with the cumulative sample. The selected species of algae are common in the studied area. Due to high capacity for accumulation of heavy metals, they are often used in the evaluation of pollution of marine ecosystems with trace elements. After being collected, the water was filtrated and conserved by adding nitric acid with the concentration of 65%, in the quantity of 2 $cm^3$ per each 100 $cm^3$ water. The algae were washed in distilled water, dried and homogenized. Such prepared samples were transported to the laboratory. Laboratory samples of the algae were subjected to wet mineralization in a closed system with the use of microwave energy. The analytical sample amounted to approximately 0.5 g. The material was digested in a mixture of $HNO_3$ and $H_2O_2$, in the 5:1, *v*/*v* ratio. Water samples to be analyzed were thickened ten times by evaporation. The lead concentration in the obtained solutions was determined by atomic absorption spectrometry with electrothermal atomization, in an M6 device manufactured by Thermo, at wavelength of 217.0 nm. The limit of determination for lead in the used method of measurement was 0.06 $\mu g \cdot dm^{-3}$. The uncertainty of measurement of the used methods was ± 8%. The limit of detection for the method to 2.7 $\mu g \cdot kg^{-1}$ d.m. of the biological material, and 0.07 μg · $dm^{-3}$ water. The zinc concentration was determined by atomic emission spectrometry, on Optima 7600 DV manufactured by Perkin Elmer, at wavelength of 206.200 nm. The limit of determination for zinc in the used method of measurement was 5.9 μg · $dm^{-3}$. The uncertainty of measurement of the used methods was ±6%. The limit of detection for the method to 0.118 μg · $kg^{-1}$ d.m. of the biological material, and 6 μg · $dm^{-3}$ water.

Certified reference material CRM 16-050 was used to check the correctness of the analyses. On the basis of the obtained results, the bioaccumulation coefficients of the studied metals in the biomass of the algae were calculated. The bioaccumulation coefficient was calculated by dividing the content of an element in the biomass by its concentration in the water. Moreover, Spearman's correlation coefficients were calculated between the concentration of the element in the water and its content in the algae biomass, and also between the element content in *Cystoseira barbata* and *Ulva rigida.*

## Results and discussion

The zinc concentration in the water collected from individual bays of Sevastopol was within the range from 36.43 to 233.3 μg · $dm^{-3}$. The average concentration of this element was 112.77 μg · $dm^{-3}$ (Fig. 2).

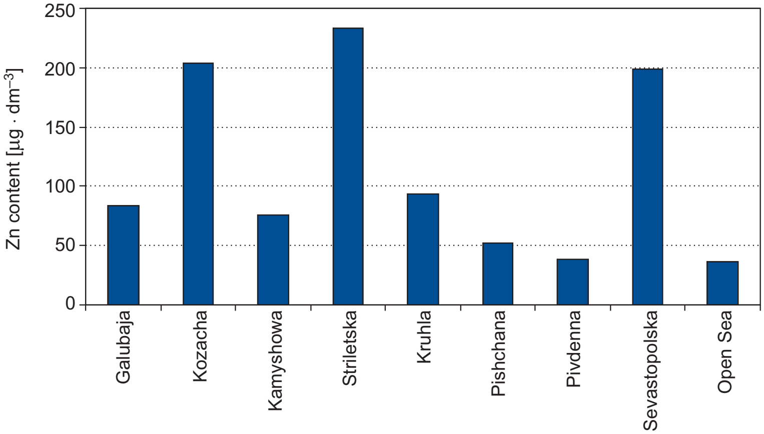

Fig. 2. Concentration of zinc in water

Considerable differences in the zinc concentration in the water in individual research sites were found. The relative standard deviation (RSD) of this element concentration was 69%. The highest zinc concentration was in the water from the Striletska, Kozacha and Sevastopol Bays, where it was: 233.3, 203.6 and 199.2 μg Zn · $dm^{-3}$, respectively. The least zinc was recorded in the water collected in the open sea and from the Pivdenna Bay. The concentrations in the water collected from these points were, 36.43 and 38.11 μg Zn · $dm^{-3}$, respectively.

The zinc concentration in the unpolluted waters of Hannah Lake was at a level of 14 μg · dm$^{-3}$, whereas in Whitson Lake it was 10 μg · dm$^{-3}$ [16]. Zinc concentration in water found by these researchers can be regarded as natural. Norris et al [17] determined the concentrations of this element in Colorado River at a level of 1090 μg · dm$^{-3}$. Kahle and Zauke [18] provide that zinc concentration in the water from the Arctic Sea amounted 0.3 μg Zn · dm$^{-3}$. Generally higher zinc concentrations are found in fresh waters, and lower ones in marine ecosystems [19].

*Ulva rigida* is one of alga from the genus Ulva suitable as bioindicator of water pollution with trace metals [20]. The zinc content in the *Ulva rigida* algae ranged between 6.517 and 15.03 mg · kg$^{-1}$ d.m., whereas in *Cystoseira barbata* between 19.083 and 30.208 mg · kg$^{-1}$ d.m. Insignificant differences in contents of this element in algae collected in individual bays were found. The relative standard deviation for zinc content in *Cystoseira barbata* was 17%, whereas in *Ulva rigida* it was 27%. On average, 2.5 times less zinc was determined in *Ulva rigida* compared with *Cystoseira barbata.* In the case of *Cystoseira barbata,* the least of this element was found in the algae collected in the open sea. Almost in each sample of *Ulva rigida* collected from the bays, a higher zinc content compared with the algae collected in the open sea was found (Fig. 3). The Kozacha Bay was the only exception.

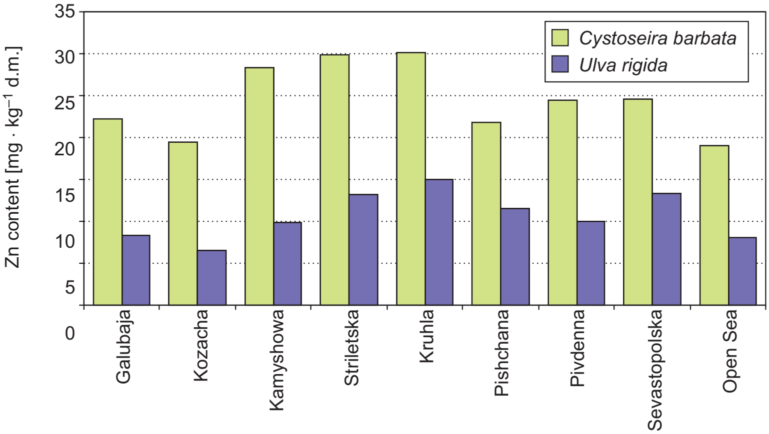

Fig. 3. Content of zinc in macroalgae

The algae collected in the Striletska and Kruhla bays contained the highest amount of zinc. Zinc contents in *Cystoseira barbata* collected in these bays were 29.84 and 30.21 mg Zn · kg$^{-1}$ d.m., respectively, and in *Ulva rigida* they were 15.03 and 13.20 mg Zn · kg$^{-1}$ d.m., respectively. The statistical data analysis did not reveal a significant correlation between the zinc content in water and algae. A statistically significant correlation ($r_{0.05}$ = 0.767) between the zinc content in both species of algae was found. Other authors also provide high correlation coefficients between zinc content in different species of algae living in certain conditions of water pollution [7, 21]. Such results confirm a similar suitability of both species in the monitoring research. Results on zinc contents in the algae, obtained in the authors' own research, are not high and close to the content of this element in algae from areas with a low human pressure index. Brito et al [7] provide results of zinc contents in algae of different species from a region with high level of human pressure in Brazil that are close to those obtained in the authors' own research. On the other hand, Caliceti et al [21] provide a much higher mean zinc contentration (64 mg Zn · $kg^{-1}$ d.m.) in algae from the Venice Lagoon, at significant differences of this element content in the algae collected in regions with a various level of human pressure. It shows high sensitivity of the bioindication methods. High contents of this element in the algae biomass are a consequence of this element concentration in water. The mentioned authors draw attention to the value of this type of research for defining the long-term trends in changes in environmental pollution, for instance in order to evaluate the environmental protection programs. Rodriguez Figueroa et al [22] recorded 63 mg Zn · $kg^{-1}$ in the seaweed *Padina durvillaei* in a copper mining region on the east coast of the Californian Peninsula. In their opinion, it is the natural content and does not prove anthropogenic enrichment, despite strong pollution of bottom sediment with this metal. Denton et al [23] determined even over 100 mg Zn · $kg^{-1}$ d.m. in the algae collected from polluted regions of the Mariana Islands, at very big differences in its concentration in samples collected from individual sites. The zinc content in the algae for consumption in Spain varied in wide limits, from 1.2 to 73 mg Zn · $kg^{-1}$ d.m., and in the case of *Ulva rigida* between 5.61 and 6.14 mg Zn · $kg^{-1}$ d.m. [24]. Similarly high concentrations of this element were found in the algae from the Azores region subjected to the effect of human pressure connected with urbanization [25]. Strezov and Nonova [26] provide similar zinc contents in *Ulva rigida* and *Cystoseira barbata* collected in the Bulgarian coastal zone of the Black Sea to the ones found in the authors' own research. These authors did not find any differences in the content of this element in either of the two species of algae.

The value of the bioaccumulation cofficient for zinc in the algae from individual bays in the region of Sevastopol varied from 32 (*Ulva rigida*) to 642 (*Cystoseira barbata*) (Table 1).

Table 1

Value of bioaccumulation coefficients of zinc and lead in algae from individual research points

| Points of sampling | Bioaccumulation coefficent of Zn | | Bioaccumulation coefficent of Pb | |
|---|---|---|---|---|
| | *Cystozeira barbata* | *Ulva rigida* | *Cystozeira barbata* | *Ulva rigida* |
| Galubaja Bay | 267 | 100 | 582 | 458 |
| Kozacha Bay | 95 | 32 | 41 | 33 |
| Kamyshowa Bay | 372 | 130 | 676 | 582 |
| Striletska Bay | 128 | 64 | 628 | 562 |
| Kruhla Bay | 324 | 141 | 39 | 30 |
| Pishchana Bay | 421 | 223 | 1273 | 775 |
| Pivdenna Bay | 642 | 263 | 401 | 303 |
| Sevastopol Bay | 124 | 67 | 569 | 383 |
| Open Sea | 524 | 221 | 688 | 429 |

Considerable differences were found in the value of this parameter, depending on the type of organism used in the research. The highest values of bioaccumulation coefficient for zinc in *Cystoseira barbata* and *Ulva rigida* were found in the Pivdenna Bay 642 and 263, respectively. The very high values of the coefficients at that research point resulted from a low content of this element in water. The zinc content in the algae of both species collected in the Pivdenna Bay points at anthropogenic enrichment. The lowest values of the zinc bioaccumulation coefficient in the studied part of biocoenoses were found in the Kozacha Bay, and then in the Sevastopol and Striletska Bays. Low values of this parameter at those research sites results from high zinc concentration in water. Melville and Pulkownik [27] provide much higher values of the zinc bioaccumulation coefficient in different species of algae collected from several estuaries of Western Australia than the ones determined in the authors' own research, reaching even 8000. The provided by these authors zinc contents in algae varied from 45 to 394 mg Zn · $kg^{-1}$ d.m.

Lead concentration in the studied water samples was in a wide range from 1.32 to 38.32 μg Pb · $dm^{-3}$ (Fig. 4), and the mean value was 11.14 μg Pb · $dm^{-3}$.

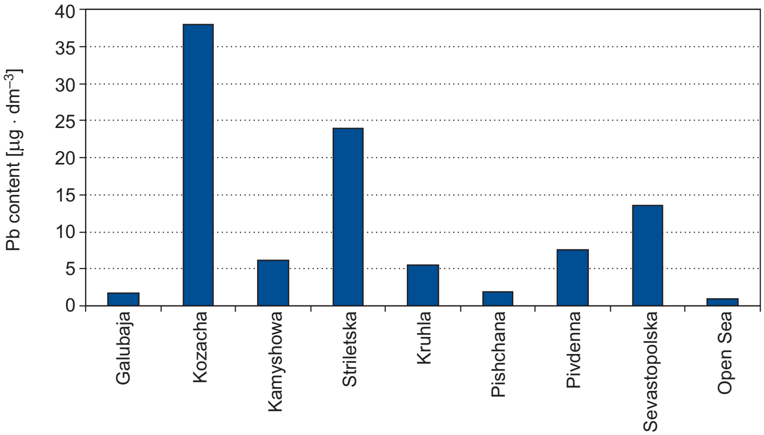

Fig. 4. Concentration of lead in water

Lead concentration in sea waters is generally lower than in fresh waters, but due to the specificity of metabolism of animals living there, high concentrations of this element in tissues of even such sea animals that lived in an unpolluted environment are seen very often. The most of this element was found in the water collected from the Kozacha Bay, and then in the Kruhla and Sevastopol Bays 38.32, 23.91 and 13.52 μg Pb · $dm^{-3}$, respectively. The lowest lead concentration was found in the water collected from the open sea and from the Pishchana and Galubaja Bays. Lead concentration in the water from the Arctic Sea was 0.6 μg Pb · $dm^{-3}$ [18]. The lead contents determined in all the samples can suggest anthropogenic enrichment of the ecosystem with this element [28, 29].

The lead content in the collected algae ranged between 0.567 mg Pb · $kg^{-1}$ d.m. (*Ulva rigida*) and 7.692 mg Pb · $kg^{-1}$ d.m. (*Cystoseira barbata*) (Fig. 5), and its mean value

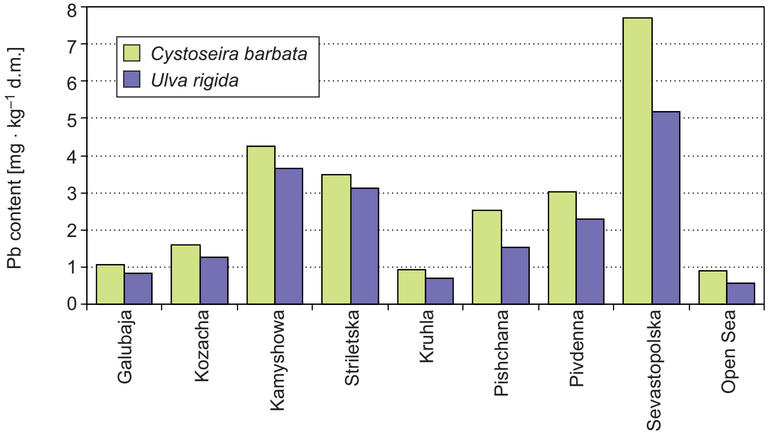

Fig. 5. Content of lead in macroalgae

reached 2.477 mg · kg$^{-1}$. No significant correlation between the content of this element in water and in the biomass of the algae that were used in the research was observed, whereas a statistically significant correlation ($r_{0.01} = 0.977$) between the lead content in both species of algae was found. The content of this element in the *Cystoseira barbata* collected in the bays ranged from 0.925 to 7.692 mg Pb · kg$^{-1}$ d.m., and in the *Ulva rigida* from 0.708 to 5.183 mg Pb · kg$^{-1}$ d.m. The most lead was found in both species of algae collected from the Sevastopol Bay, and then in the Kamyshchowa and Striletska Bays. Lead contents in the *Cystoseira barbata* from these bays were 7.692, 4.233 and 3.492 mg Pb · kg$^{-1}$ d.m., respectively, while in the *Ulva rigida* they were 5.183, 3.642 and 3.125 mg Pb · kg$^{-1}$ d.m. The lead content in the *Ulva rigida* algae was by, on average, approximately 25% lower than determined in the *Cystoseira barbata*. The lowest amount of lead was noted in the algae collected in the open sea, and then in the Kruhla and Galubaja Bays. High variability of the obtained results at individual research points indicatives of a various level of human pressure. The relative standard deviation for the lead content in the biomass of both species of algae was approximately 75%.

Results obtained in the authors' own research, in the light of literature data, are very high and indicative of possible excessive accumulation of this element in organisms of all trophic levels. In the research of Rodriguez Figueroa et al [22], the seaweed *Padina durvillaei* in a copper mining region on the east coast of the Californian Peninsula contained 7.8 mg Pb · kg$^{-1}$, which was regarded as the natural content despite a severe pollution of bottom sediments with lead. Horta-Puga et al [30] provide much lower lead contents in different species of algae collected from areas near from Veracruz coral reef in Mexico, within the range from 0.016 to 0.8 mg Pb · kg$^{-1}$ d.m. Similarly low contents were found in the algae of different species from the region of the Azores [25]. The lead content in the algae available in sales nets and intended to be used for consumption in Spain was within limits from trace quantities to 1.25 mg Pb · kg$^{-1}$ d.m., and in the *Ulva brigida* algae it was between 1 and 1.05 mg Pb · kg$^{-1}$ d.m.

Lead contents obtained in the authors' own research are comparable to the ones determined in various species of algae from areas with a high coefficient of human pressure in Brazil, amounting from 2 to 5 mg Pb · $kg^{-1}$ d.m. [7]. Lead contents similar to the ones obtained in the authors' own research were found by Denton et al [23] in the algae from polluted regions of the Mariana Islands. Caliceti et al [21] provide 3.2 mg Pb · $kg^{-1}$ d.m. as the mean lead content in the *Cystoseira barbata* algae from the Venice Lagoon, and 7.3 mg Pb · $kg^{-1}$ d.m. in the *Ulva rigida*. These authors found a considerable diversification in lead concentration in algae from individual sampling points which were located in zones with different intensity of anthropopressure. Considerably higher contents were determined in the *Ulva rigida*, which is divergent with results obtained in the authors' own research. Strezov and Nonova [26] provide similar lead contents in *Ulva rigida* and *Cystoseira barbata* collected in the Bulgarian coastal zone of the Black Sea to the ones found in this research at points of the lowest accumulation of this element. The majority of algae from the research points in the region of Sevastopol showed a much higher accumulation of lead.

Values of lead bioaccumulation coefficient in the studied algae were in a wide range from 30 (*Ulva rigida*) to 1,273 (*Cystoseira barbata*) (Table 1). The highest values of bioaccumulation factors in both species of algae, reaching 1,273 for *Cystoseira barbata* and 775 for *Ulva rigida,* were observed in the Pishchana Bay. The lowest value of the bioaccumulation coefficient in the studied organisms was recorded in the Kruhla Bay 39 and 30 for *Cystoseira barbata* and *Ulva rigida*, respectively (Table 1).

The values of the lead bioaccumulation coefficient in different species of algae collected from several estuaries of Western Australia ranged widely, from around 1,000 to over 500,000. With lead concentration in water at a level of 0.4 μg Pb · $dm^{-3}$, contents of this element in the algae varied from 0.5 to 264 mg Pb · $kg^{-1}$ d.m. [27].

## Conclusions

1. Zinc and lead contents in the water collected from individual bays of Black Sea and from the open sea in the region of Sevastopol showed considerable diversification. The determined amounts point at anthropogenic enrichment.

2. Zinc contents in the *Cystoseira barbata* and *Ulva rigida* algae differed slightly due to the location of sampling points. The determined contents of this element were low, characteristic for ecosystems unpolluted with zinc.

3. The zinc bioaccumulation coefficient took very low values. Despite considerable amounts of this element in water, there is no risk of its excessive accumulation in the parts of biocoenosis.

4. The lead content in the algae was high, comparable with literature data for ecosystems polluted with this element.

5. The lead bioaccumulation coefficient reached significantly higher values and showed greater diversity than in the case of zinc, which points out to a risk of excessive accumulation of this metal in living organisms.

6. Generally, a higher zinc and lead content was found in all the samples taken from bays (both in water and in the algae) than in those collected in the open sea.

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# ZAWARTOŚĆ CYNKU I OŁOWIU W WODZIE I GLONACH Z WYBRANYCH ZATOK MORZA CZARNEGO W OKOLICACH SEWASTOPOLA

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**Abstrakt:** Od wielu lat w rejonie Sewastopola ma miejsce nasilona antropopresja wynikająca ze strategicznej jego roli jako głównego miasta w regionie oraz portu, w którym przez wiele lat stacjonowała rosyjska lub radziecka flota czarnomorska. Ważnymi źródłami zanieczyszczeń trafiających do Morza Czarnego w rejonie Sewastopola są przemysł zlokalizowany w tym mieście, ścieki komunalne oraz rolnictwo. Prowadzenie badań monitoringowych związanych z zanieczyszczeniem Morza Czarnego w rejonach o różnym poziomie antropopresji jest istotne z punktu widzenia kształtowania polityki ochrony środowiska nie tylko w rejonie badań, ale w całym basenie tego akwenu. Celem pracy była ocena zawartości cynku i ołowiu w wodzie oraz glonach z wybranych zatok Morza Czarnego w okolicach Sewastopola. Próbki wody oraz glonów pobrano w sierpniu 2012 r. z ośmiu zatok Sewastopola (Gałubaja, Kozacha, Kamyshova, Kruhla, Striletska, Pishchana, Pivdenna i Sewastopolska) oraz jedną próbkę z otwartego morza w okolicach Fioletu. Z tych samych miejsc pobrano glony *Cystoseira barbata* i *Ulva rigida*. Pobraną wodę konserwowano na miejscu i po przewiezieniu do laboratorium oznaczono w niej zawartość cynku i ołowiu. Pobrane glony wypłukano w wodzie destylowanej, suszono, a następnie homogenizowano i mineralizowano. W mineralizatach oznaczono zawartość cynku metodą ICP-OES, a zawartość ołowiu oznaczono metodą ASA z atomizacją elektrotermiczną.

Zawartość cynku w wodzie mieściła się w zakresie od 36,43 do 233,3 $\mu\text{g Zn} \cdot \text{dm}^{-3}$, a ołowiu w zakresie od 1,32 do 38,32 $\mu\text{g Pb} \cdot \text{dm}^{-3}$. Stwierdzono znaczne różnice zawartości badanych pierwiastków w wodzie z poszczególnych zatok. Względne odchylenie standardowe stężenia cynku i ołowiu w badanych próbkach wody wynosiła odpowiednio 69 i 112%. Największe zawartości cynku stwierdzono w wodzie z zatok Striletska, Kozacha i Sewastopolska, a ołowiu w zatokach Kozacha i Kruhla. Najmniejsze ich stężenie stwierdzono w wodzie pobranej na otwartym morzu, a ponadto cynku z zatok Pivdenna i Pishchana, a ołowiu z zatok Gałubaja i Pishchana. Zawartość cynku w glonach wahała się w granicach od zakresie od 6,517 do 30,21 $\text{mg} \cdot \text{kg}^{-1}$ s.m. Glony *Cystoseira barbata* zawierały ponad dwukrotnie więcej cynku w porównaniu z *Ulva rigida*. Zawartość ołowiu w glonach wahała się w zakresie 0,567 do 7,692 $\text{mg Pb} \cdot \text{kg}^{-1}$ s.m. Prawie o połowę więcej ołowiu stwierdzono w *Cystoseira barbata* w porównaniu z *Ulva rigida*. Nie stwierdzono statystycznie istotnej korelacji pomiędzy zawartością badanych pierwiastków w wodzie i w biomasie glonów. Wykazano natomiast istotną dodatnią korelację pomiędzy zawartością cynku i ołowiu w obydwu gatunkach glonów. Wartość współczynnika bioakumulacji cynku wahała się w granicach od 32 do 642, a ołowiu od 30 do 1273. Zawartości badanych pierwiastków, zarówno w biotycznej, jak i abiotycznej części badanych ekosystemów wskazują na antropogeniczne wzbogacenie, jednakże wyniki uzyskane w próbkach z zatok Sewastopolska, Kozacha i Striletska wskazują na niebezpieczeństwo nadmiernej ich bioakumulacji i potencjalne zagrożenie życia organizmów wodnych oraz konsumentów owoców morza.

**Słowa kluczowe:** Morze Czarne, woda, *Cystoseira barbata*, *Ulva rigida*, zanieczyszczenie, bioakumulacja

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# DEPENDENCE BETWEEN THE CONTENT OF COPPER AND THE CONTENT OF MAJOR ELEMENTS IN SEDIMENTS OF POLISH LAKES

## ZALEŻNOŚĆ MIĘDZY ZAWARTOŚCIĄ MIEDZI A ZAWARTOŚCIĄ PIERWIASTKÓW GŁÓWNYCH W OSADACH JEZIOR POLSKI

**Abstract:** In 416 sediment samples taken from the deepest waters of 260 lakes located within the following Lake Districts: Greater Poland, Pomeranian and Masurian, the content of Cu, Ca, Mg, Fe, Mn, K, Na, P and S was determined by the ICP-OES method and the content of the Total Organic Carbon (TOC) was determined by the coulometric titration method. The analyses revealed that in the majority of tested samples, the Cu content did not exceed 50 mg/kg and that the geometric mean of the copper content is 13 mg/kg, and thus it is much higher than the geochemical background for aquatic sediments in Poland. The high dependence determined between the Cu content in sediments and the concentration of Al, K, Mg, S and TOC and a weaker correlation between the Cu concentration and the content of P and Fe indicate that copper in sediments is primarily related to the organic matter and clay minerals and, to a lesser extent, to phosphates and iron compounds. The variation in the copper content in sediments of different Lake Districts and also the variation in the present correlations were observed.

**Keywords:** copper, lakes, correlations, clay minerals, organic matter, iron compounds, phosphates

## Introduction

Copper is considered to be one of the most toxic metals in the water environment [1, 2]. At the same time, it is an essential element for the life of many organisms. It participates in the carbohydrate metabolism and plays a role in the action of several dozen enzymes. However, excessive concentrations of copper may be toxic. Algae are

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particularly vulnerable to copper. And the fish exposed to its too high concentrations suffer from gill damage and malfunction in the transport and excretion of sodium and potassium chlorides as well as from inhibition of Na+/K+-ATPase [3–8]. Heavy metals, including copper, present in lake sediments may accumulate in a food chain to the level which is toxic for organisms, especially the ones of predators, and may also pose a risk to humans [9, 10].

Natural content of copper in lake sediments, like of other minor elements, mostly depends on the chemical composition of geological formations present within the catchment area of a given lake. In natural conditions, sediments that accumulate at the bottom of the lake are formed as a result of the accumulation of a material derived from erosion of soil, *ia* from quartz grains, feldspars, carbonate minerals, clay minerals, and of a material formed in the place of sedimentation – remains of dead plant and animal organisms and also substances that precipitate from the water, *ia* carbonates, phosphates, sulphides, hydrated iron and aluminium hydroxides [11]. The concentration of elements in lake sediments is also affected by the size of the lake catchment area, lake surface area and depth, shape of the lake basin. The speciation of copper in the water environment, and consequently its availability to aquatic organisms, is strongly affected by the alkalinity, water hardness and pH [12]. Copper in the environment is mobile under oxidative conditions, especially in the acid environment. However, it is easily bound by sediment components, mainly by the organic matter, sulphides, and also by hydrated iron oxides and phosphates [13, 14]. On the territory of Poland, the average Cu content in sediments is 7 mg/kg [15]. In view of harmful effects of copper on aquatic organisms, its permissible content in sediments was determined as 149 mg/kg [16].

An increase in the concentration of heavy metals observed nowadays in lake sediments in non-industrialised areas is often a result of their deposition from the atmosphere and of rain and thaw water runoff from urban and rural areas [17–23]. In developed areas, sediments are also contaminated by heavy metals contained in effluents discharged sometimes to surface waters. Copper, known to man for almost 6,000 years, is widely used in industry (electrical wiring, water supply, heating, electromagnets, roof covering), in alloys (tools, brake discs, heat exchangers, jewellery), and it is also used as an ingredient of algaecides, fungicides and molluscacides. It is released to the environment from many sources: *ia* as a result of coal combustion, copper ore processing, copper smelting, transport, agriculture (micro-fertiliser, plant protection products, additive in animal feed), fish culture (growth control of algae and pathogens in fish ponds), and also as a result of deterioration of buildings by atmospheric factors and use of transport means.

## Material and methods

In this paper, results of tests on aquatic sediments in Poland were used. The results were obtained from the implementation of the task of the State Environmental Monitoring – Monitoring of the quality of inland surface waters, which include the determination of the content of heavy metals and selected harmful organic compounds in sediments formed nowadays in rivers and lakes within the country. Over the period of

2010 to 2012, 416 sediment samples were taken from the deepest waters of 260 lakes (from the lakes with their surface area up to 250 ha – one sample was taken; from the lakes with their surface area from 250 to 500 ha – two samples; from the lakes with their surface area from 500 to 1,000 ha – three samples; from the lakes with their surface area from 1,000 to 5,000 ha – four samples; from the lakes with their surface area over 5,000 ha – five samples). A 5 cm thick sediment layer is taken for tests from the profundal zone of the lakes.

The content of copper and elements – calcium, magnesium, iron, potassium, manganese, sodium, phosphorus, sulphur and TOC, being part of phases (organic matter, clay minerals, carbonates, phosphates and sulphides), which may retain copper in sediments, was determined in all the samples. The content of Ca, Cu, Fe, K, Mg, Mn, Na, P and S was determined by the ICP-OES method, *ie* by the Inductively Coupled Plasma Optical Emission Spectrometry (iCAP6500 manufactured by Thermo Scientific), from the solutions obtained after digestion of sediment samples with aqua regia; the content of the Total Organic Carbon (TOC) was determined by the coulometric titration method from the solid sample (Coulomat 702 CS/LI manufactured by Strohlein). To assess the quality of made tests, a reference material (WQB-3 lake sediment) was also analysed.

## Results and discussion

Copper in sediments was found to be from 2 to 537 mg/kg. In ten out the tested samples, the copper content exceeded 50 mg/kg and in the histogram, the Cu content created a separate anomalous population of sediments contaminated with this element. These were the sediments of the Karczemne Lake, at which the town of Kartuzy is located; of the Elk Lake, at which the town of Elk is located; of the Czluchow Lake, which is located in the area of the town of Czluchow; of the Trzesiecko Lake, which is located in the area of the town of Szczecinek; of the Wasowsko-Mikorzynskie Lake and of the Patnowskie Lake, of which waters are used in the cooling cycle of the Konin Power Plant. The analysis of dependence of the copper concentration in sediments on the content of major elements does not include the results of determining elements in sediments of these lakes. In the population of sediments, taken into consideration, the copper content was found to be from 2 to 49 mg/kg, its average content was 15 mg/kg, and its geometric mean and median – 13 mg/kg. The average copper content determined in tested sediments of the profundal zone of the lakes is much higher than the average content in sediments of the littoral zone of the lakes in Poland, amounting to 3 mg/kg [15]. The variation in the copper content in sediments of different Lake Districts was observed. The geometric mean of the copper content in sediments of the Pomeranian Lake District – 11 mg/kg is slightly lower than the value calculated for sediments of the Greater Poland and Masurian Lake Districts, amounting to 13 mg/kg (Table 1).

This variation is also seen on histograms that illustrate the number of samples in different concentration classes (Fig. 1). Among the tested sediments of the lakes of the Pomeranian Lake District, the largest share has the samples containing copper within the range from 5 to 10 mg/kg; whereas among the samples taken from the lakes of the

Table 1

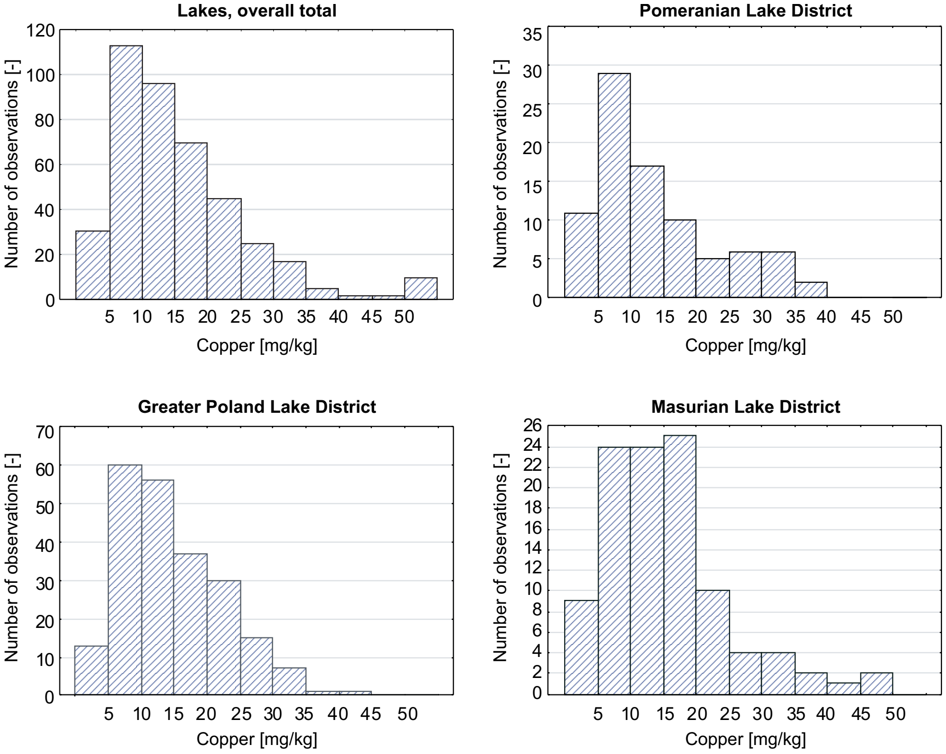
Statistic parameters of elements in lake sediments

| Element | Mean | Geometric mean | Median | Minimum | Maximum |
|---|---|---|---|---|---|
| Lakes, overall total (n = 406) | | | | | |
| Copper [mg/kg] | 15 | 13 | 13 | 2 | 49 |
| Phosphorus [%] | 0.104 | 0.086 | 0.087 | 0.005 | 0.595 |
| Aluminium [%] | 0.50 | 0.37 | 0.39 | 0.04 | 2.23 |
| Magnesium [%] | 0.32 | 0.26 | 0.27 | 0.01 | 1.25 |
| Manganese [mg/kg] | 901 | 640 | 712 | 32 | 11,770 |
| Potassium [%] | 0.112 | 0.080 | 0.086 | 0.005 | 0.510 |
| Sulphur [%] | 1.040 | 0.819 | 0.932 | 0.023 | 4.629 |
| Sodium [%] | 0.025 | 0.020 | 0.019 | 0.006 | 0.536 |
| Calcium [%] | 13.40 | 8.80 | 13.57 | 0.05 | 30.63 |
| TOC [%] | 7.14 | 5.91 | 6.11 | 0.19 | 22.90 |
| Iron [%] | 1.55 | 1.26 | 1.41 | 0.10 | 10.91 |
| Pomeranian Lake District (n = 86) | | | | | |
| Copper [mg/kg] | 14 | 11 | 10 | 2 | 38 |
| Phosphorus [%] | 0.095 | 0.075 | 0.077 | 0.011 | 0.510 |
| Aluminium [%] | 0.50 | 0.36 | 0.35 | 0.05 | 2.23 |
| Magnesium [%] | 0.26 | 0.22 | 0.24 | 0.01 | 0.69 |
| Manganese [mg/kg] | 748 | 542 | 668 | 33 | 4,999 |
| Potassium [%] | 0.108 | 0.080 | 0.086 | 0.005 | 0.330 |
| Sulphur [%] | 1.037 | 0.790 | 0.924 | 0.031 | 3.757 |
| Sodium [%] | 0.046 | 0.025 | 0.021 | 0.009 | 0.536 |
| Calcium [%] | 11.57 | 6.38 | 10.87 | 0.09 | 29.41 |
| TOC [%] | 6.43 | 4.95 | 6.08 | 0.19 | 21.00 |
| Iron [%] | 1.43 | 1.14 | 1.39 | 0.10 | 4.96 |
| Masurian Lake District (n = 210) | | | | | |
| Copper [mg/kg] | 15 | 13 | 13 | 2 | 45 |
| Phosphorus [%] | 0.115 | 0.097 | 0.094 | 0.015 | 0.60 |
| Aluminium [%] | 0.55 | 0.40 | 0.43 | 0.06 | 1.97 |
| Magnesium [%] | 0.38 | 0.32 | 0.32 | 0.023 | 1.25 |
| Manganese [mg/kg] | 997 | 698 | 726 | 73 | 11.770 |
| Potassium [%] | 0.129 | 0.091 | 0.102 | 0.011 | 0.510 |
| Sulphur [%] | 0.963 | 0.794 | 0.913 | 0.049 | 4.23 |
| Sodium [%] | 0.019 | 0.018 | 0.018 | 0.007 | 0.05 |
| Calcium [%] | 13.29 | 9.19 | 14.20 | 0.15 | 30.63 |
| TOC [%] | 7.64 | 6.58 | 6.31 | 0.48 | 21.40 |
| Iron [%] | 1.68 | 1.43 | 1.52 | 0.22 | 10.91 |

Table 1 contd.

| Element | Mean | Geometric mean | Median | Minimum | Maximum |
|---|---|---|---|---|---|
| Greater Poland Lake District (n = 102) | | | | | |
| Copper [mg/kg] | 15 | 13 | 14 | 2 | 49 |
| Phosphorus [%] | 0.086 | 0.074 | 0.076 | 0.005 | 0.437 |
| Aluminium [%] | 0.40 | 0.31 | 0.35 | 0.04 | 1.45 |
| Magnesium [%] | 0.24 | 0.20 | 0.23 | 0.01 | 0.59 |
| Manganese [mg/kg] | 843 | 627 | 706 | 32 | 4.193 |
| Potassium [%] | 0.077 | 0.060 | 0.064 | 0.005 | 0.302 |
| Sulphur [%] | 1.200 | 0.888 | 0.947 | 0.023 | 4.629 |
| Sodium [%] | 0.022 | 0.021 | 0.021 | 0.006 | 0.079 |
| Calcium [%] | 15.42 | 10.73 | 16.98 | 0.05 | 30.27 |
| TOC [%] | 6.47 | 5.33 | 5.96 | 0.19 | 15.50 |
| Iron [%] | 1.40 | 1.08 | 1.11 | 0.10 | 6.43 |

Greater Poland Lake District, the range of the most frequently observed concentrations is much wider – from 5 to 20 mg/kg; and in sediments of the Masurian Lake District, this range is from 5 to 15 mg/kg.


Lakes, overall total

Pomeranian Lake District

Greater Poland Lake District

Masurian Lake District

Fig. 1. Histograms of the copper content in lake sediments

The copper concentration in lake sediments shows a strong correlation with the aluminium content (r = 0.57) and with the potassium concentration (r = 0.50) (Table 2). Correlations with the concentration of iron (r = 0.34), magnesium (r = 0.33), sulphur (r = 0.34), TOC (r = 0.30) and phosphorus (r = 0.26) are weaker. The copper content does not show any correlation with the manganese content, and shows a negative correlation with the calcium concentration (r = 0.27). The variation in the correlation of copper with major elements may be observed in sediments of particular Lake Districts. For example, the copper concentration in sediments of the lakes of the Greater Poland Lake District is distinguished by the strongest correlation with the concentration of sulphur and TOC, and the Cu concentration in sediments of the lakes of the Pomeranian Lake District shows a strong correlation with the content of aluminium and iron.

Table 2

Correlation factors of copper with major elements

| Element | Lakes | Pomeranian Lake District | Greater Poland Lake District | Masurian Lake District |
|---|---|---|---|---|
| Aluminium | 0.57 | 0.70 | 0.47 | 0.73 |
| Calcium | –0.27 | –0.20 | –0.14 | –0.49 |
| Iron | 0.34 | 0.56 | 0.33 | 0.35 |
| Potassium | 0.50 | 0.68 | 0.31 | 0.70 |
| Magnesium | 0.33 | 0.55 | 0.24 | 0.46 |
| Manganese | 0.02 | 0.12 | 0.10 | –0.05 |
| Sodium | 0.10 | 0.15 | 0.34 | 0.37 |
| Phosphorus | 0.26 | 0.37 | 0.27 | 0.22 |
| Sulphur | 0.34 | 0.44 | 0.50 | 0.16 |
| TOC | 0.30 | 0.33 | 0.54 | 0.20 |

Scatter graphs of the dependence between the copper concentration in sediments and the content of major elements, presented in Fig. 2, illustrate a great dependence between the Cu content in sediments and the concentration of Al, K, Mg, S and TOC as well as a weaker correlation between the Cu concentration and the content of P and Fe. These dependencies show that copper in sediments is primarily related to the organic matter and clay minerals and, to a lesser extent, to phosphates and iron compounds. This confirms that the organic matter plays a major role in the retention of heavy metals, also copper [24]. Such retention of heavy metals by the organic matter is primarily related to the presence of the following function groups in its structure: R-SH, R-SS-R and R-SSH. The scatter graph for Cu and Ca illustrates a negative dependence between these elements. Probably, in conditions conducive to precipitation and accumulation of calcium carbonate, the copper concentration in formed sediments becomes relatively low. The scatter graph for Mn and Cu illustrates the lack of correlation between the concentrations of these elements in sediments. Manganese is an element of which concentration variability in sediments highly depends on the redox conditions present in the sedimentation environment. The dependence between the copper content and the

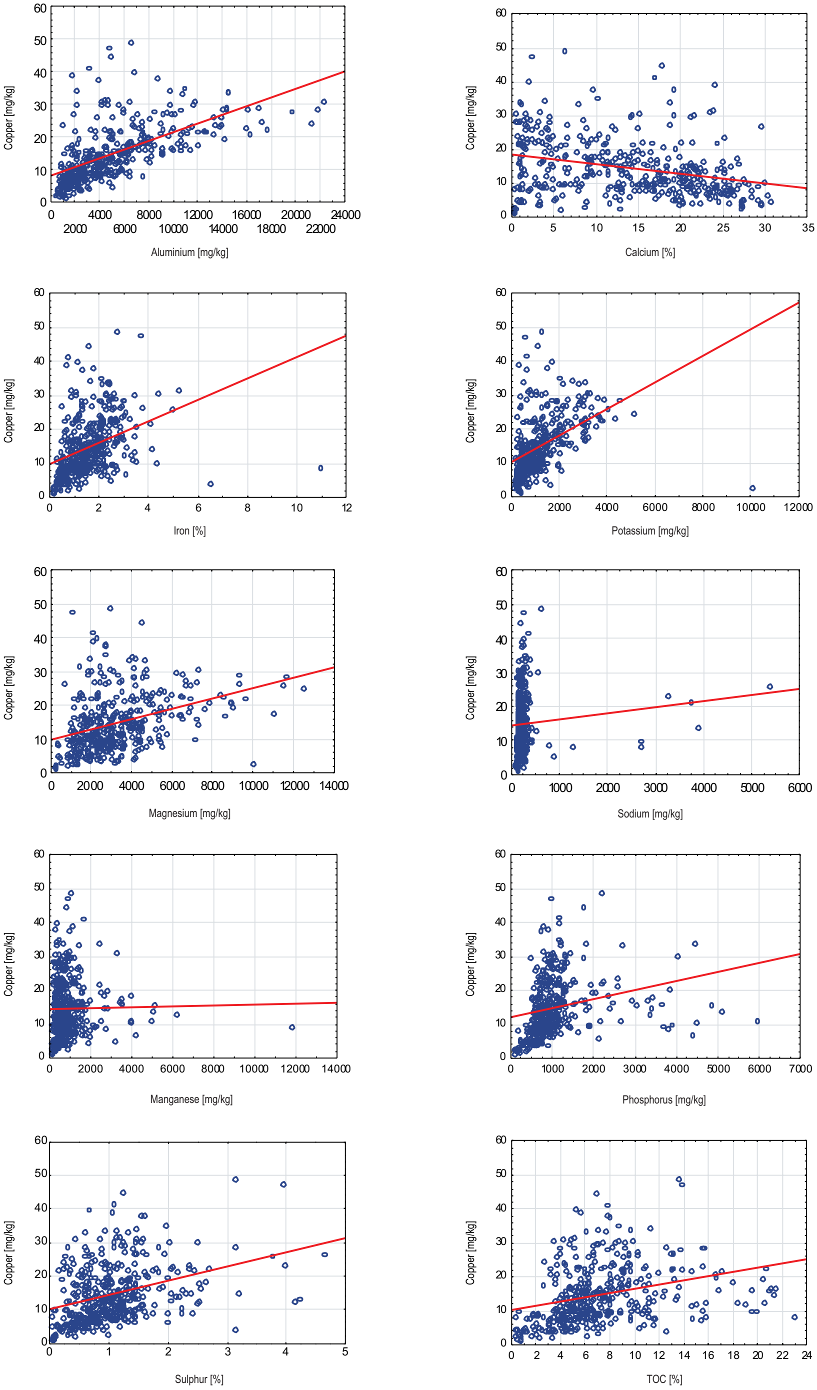


Fig. 2. Scatter graph between the copper content and the content of the major elements

phosphorus content was also observed in tested sediments. This indicates the probability of retaining this element also by phosphates. The possibility of immobilising heavy metals by apatites (calcium phosphates) is presented in many studies and such capability of apatites is used to immobilise heavy metals in contaminated environments [25, 26].

The factor analysis carried out for all the samples and for the samples of sediments taken from particular Lake Districts revealed the presence of two factors, which together explain approx. 40% of the variability (Table 3). One of them binds aluminium, potassium and magnesium (being part of minerals from the illite-smectite group) present in sediments, whereas the second factor binds sulphur and organic carbon (being part of the organic matter). Both factors have a relatively similar copper share. In sediments of the lakes of the Pomeranian Lake District, the first factor binds aluminium, potassium, copper and iron at a high share of sulphur, magnesium and TOC.

Table 3

Results of factor analysis

| Element | Overall total | | Pomeranian | | Greater Poland | | Masurian | |
|---|---|---|---|---|---|---|---|---|
| | Factor 1 | Factor 2 | Factor 1 | Factor 2 | Factor 1 | Factor 2 | Factor 1 | Factor 2 |
| Aluminium | 0.951 | 0.151 | 0.863 | –0.316 | 0.297 | 0.914 | 0.964 | 0.090 |
| Calcium | –0.650 | –0.080 | –0.470 | 0.546 | 0.133 | –0.712 | –0.654 | –0.241 |
| Copper | 0.520 | 0.640 | 0.847 | 0.169 | 0.771 | 0.272 | 0.722 | 0.544 |
| Iron | 0.419 | 0.365 | 0.741 | 0.154 | 0.513 | 0.304 | 0.457 | 0.137 |
| Potassium | 0.956 | 0.020 | 0.807 | –0.319 | 0.112 | 0.891 | 0.974 | 0.010 |
| Magnesium | 0.772 | –0.160 | 0.562 | –0.007 | 0.069 | 0.569 | 0.837 | –0.287 |
| Manganese | –0.099 | 0.137 | 0.161 | 0.675 | 0.288 | –0.210 | –0.048 | –0.057 |
| Sodium | 0.017 | 0.170 | 0.193 | 0.092 | 0.389 | –0.149 | 0.094 | 0.384 |
| Phosphorus | 0.033 | 0.387 | 0.375 | 0.599 | 0.452 | 0.015 | 0.047 | 0.263 |
| Sulphur | –0.083 | 0.710 | 0.520 | 0.334 | 0.800 | 0.044 | –0.159 | 0.638 |
| TOC | 0.053 | 0.632 | 0.496 | 0.187 | 0.728 | 0.228 | –0.062 | 0.688 |
| Output value | 7.189 | 5.910 | 10.627 | 3.860 | 8.297 | 7.068 | 8.555 | 5.859 |
| Share | 0.257 | 0.211 | 0.332 | 0.121 | 0.259 | 0.221 | 0.267 | 0.183 |

In the second factor, calcium, manganese and phosphorus have a high share. In the lakes of this Lake District, copper is primarily related to the minerals from the illite-smectite group (aluminium, potassium, magnesium) derived from glacial clays, and partly to the organic matter; whereas phosphorus compounds do not play any significant role in the binding of copper in these sediments.

In sediments of the lakes of the Greater Poland Lake District, the first factor includes copper, sulphur and TOC at a relatively high share of phosphorus and iron. The second factor binds potassium with aluminium at a high share of magnesium. In sediments of this Lake District, copper is retained primarily by the organic matter and, to a lesser extent, by phosphates, *eg* vivianite or apatites; whereas clay minerals do not play any significant role in the retaining of Cu in sediments. In sediments of the lakes of the

Masurian Lake District, the first factor groups aluminium, copper, potassium, magnesium; whereas the second factor groups sulphur and TOC. In sediments of this Lake District, copper has a relatively high share in both distinguished factors but, to a greater extent, it is retained in sediments by clay minerals, most probably from the illite-smectite group, rather than by the organic matter. This variation between the Lake Districts in the phases of sediments, which retain copper, most probably depends on the variation in the lithology of post-glacial formations related to different phases of glaciations: the Pomeranian phase (Masurian and Pomeranian Lake Districts) and the Poznan phase (Greater Poland Lake District).

## Conclusions

1. In the majority of tested samples, the copper content did not exceed the value of 50 mg/kg. Higher copper contents were found in sediments of lakes at which highly-populated towns are located or their waters are used in the cooling cycle of the power plant.
2. The geometric mean of the copper content in sediments of the profundal zone of the lakes is 13 mg/kg and is much higher than the geochemical background for aquatic sediments in Poland.
3. The high dependence determined between the Cu content in sediments and the concentration of Al, K, Mg, S and TOC and a weaker correlation between the Cu concentration and the content of P and Fe indicate that copper in sediments is primarily related to the organic matter and clay minerals and, to a lesser extent, to phosphates and iron compounds.
4. The variation in the copper content in sediments of different Lake Districts and also the variation in the present correlations, depending on the variation in the lithology of post-glacial formations related to different phases of glaciations: the Pomeranian phase (Masurian and Pomeranian Lake Districts) and the Poznan phase (Greater Poland Lake District), were observed.

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# ZALEŻNOŚĆ MIĘDZY ZAWARTOŚCIĄ MIEDZI A ZAWARTOŚCIĄ PIERWIASTKÓW GŁÓWNYCH W OSADACH JEZIOR POLSKI

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**Abstrakt:** W 416 próbkach osadów pobranych z głęboczków 260 jezior Pojezierzy: Wielkopolskiego, Pomorskiego i Mazurskiego oznaczano zawartość Cu, Ca, Mg, Fe, Mn, K, Na, P i S metodą ICP-OES oraz zawartość węgla organicznego (TOC) metodą kulometrycznego miareczkowania. Analizy wykazały, że w większości zbadanych próbek zawartość Cu nie przekraczała 50 mg/kg, a średnia geometryczna zawartość miedzi wynosi 13 m/kg i jest znacznie wyższa od tła geochemicznego dla osadów wodnych Polski. Stwierdzona wysoka zależność między zawartością Cu w osadach a stężeniem Al, K, Mg, S i węgla organicznego oraz słabsza korelacja między stężeniem Cu a zawartością P i Fe wskazuje, że miedź w osadach związana jest przede wszystkim z materią organiczną oraz minerałami ilastymi, a w mniejszym stopniu z fosforanami i związkami żelaza. Zaobserwowano zróżnicowanie w zawartości miedzi w osadach różnych pojezierzy, a także zróżnicowanie w występujących korelacjach.

**Słowa kluczowe:** miedź, jeziora, korelacje, minerały ilaste, materia organiczna, związki żelaza, fosforany

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# WATER AGE IN THE WATER SUPPLY NETWORK AS HEALTH RISK FACTOR ASSOCIATED WITH COLLECTIVE WATER SUPPLY

## WIEK WODY W SIECI WODOCIĄGOWEJ JAKO CZYNNIK RYZYKA ZDROWOTNEGO ZWIĄZANEGO ZE ZBIOROWYM ZAOPATRZENIEM W WODĘ

**Abstract:** The correct operation of collective water supply system requires continuous monitoring of water quality at various stages (collection, treatment, pumping and distribution). An important parameter associated with water quality is water age – the time from the moment of pumping the water into water supply system to its collection by people. In the case of water contamination at the exit of the water treatment plant, water age is exual to contamination time spread in the water supply network. The length of contamination time spread determines how many people will be affected by the bad water quality which can affect health or life. The paper presents a methodology for determining the risks associated with the appearance of water contamination using hydraulic model, as well as application case for selected collective water supply system.

**Keywords:** water supply network, water quality, contamination

## Introduction

The lack of drinking water supply or inadequate quality water supply are the main cause of threats for consumers using the collective water supply system (CWSS). As a result of a more rational use of water (*eg* as a result of rising prices and metering of water consumption and the use of sealed and water-saving fittings) the problem of lack of water supply is mainly caused by failures of pipelines and fittings. On the other hand, the quality of drinking water is one of the hottest topics covered also by the International Water Association (IWA). The IWA president, Glen Daigger, at the World Congress on Water, Climate and Energy in 2012, in Dublin, in his plenary speech opening mentioned above Congress pointed to 2050 as the date when the accumulated problems with the delivery of safe water, food and energy will appear.

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The collective water supply system is a set of technical objects whose purpose is to provide customers with water of the required pressure, the right quantity and quality, in accordance with the valid regulation on the quality of water intended for human consumption. The scheme of the CWSS is shown in Fig. 1.

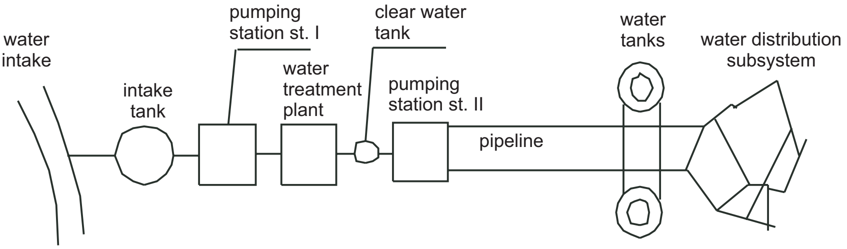

Fig.1. Collective water supply system

Water distribution subsystem (WDS) is one of the most important subsystems of the CWSS, whose aim is to provide customers with water of appropriate quality (in accordance with applicable regulations), in the right quantity and at the appropriate pressure, as well as an acceptable price. Comprehensive management of the WDS operation requires the collection of certain information relating to the operation of system's individual elements, transfer the data in real time to the operator of the system, archiving these data and alerting appropriate maintenance services about any disturbances in system operating. The subsystem operator should receive current data about potential failures, by what the time of his reaction to failure decreases [1–3].

Risk management involves three areas of studies: risk analysis, risk evaluation. risk control [4]. Risk identification generally means an analysis of risk factors, their sources, determination of the so called weak points and consequences (effects) of their occurrence. The most often this analysis concerns the undesirable events that can appear in system with a certain probability and cause certain losses, which can result in loss of the WSS safety. Analysis of the results for the possibility of a crisis can be performed on the basis of risk maps, *ie* the distribution of negligible, tolerable, controlled, unacceptable, inadmissible risks [5].

The aim of the study is to present the methodology for determining the risk associated with water age in the WDS, as a parameter affecting the safety of water consumers. A basic definition of risk, the way of determining the water age in the water pipe network, with the use of the hydraulic model, point scale for the risk parameters, are presented. The work contains an application example for the exemplary CWSS, assuming the appearance of the primary water contamination in WDS.

## Water contamination in the water supply network

Generally, contaminations in the water pipe network are divided into:

– primary – contamination occurs in the source of water (river, lake, groundwater reservoir) and despite of the treatment process the contamination enters the WDS,

– secondary – water drawn from the source is effectively treated and uncontaminated flows to the WDS, contamination occurs in the water supply network.

An example of the primary contamination in the water network is the situation, which took place at the turn of June and July 2003 in Tarnobrzeg, where in the WDS coliform bacteria was detected. The message about contamination was given to the public only after 3 days and contained the information that the water is safe for consumption after boiling. In opposition to this information commented District Sanitary Inspector, who found the water to be completely unfit for consumption. The confused residents began to buy the bottled water. The CWSS operator decided to increase the dose of chlorine in the WDS, but due to low water consumption, chlorine was not able to reach the entire water supply network. After 16 days the city authorities appealed to the residents to drain 1 $m^3$ of water on a designated day and within a specific time period. State of emergency lasted for another 4 days and then it was allowed to consume water after boiling. During the whole incident about 100 cases of poisoning were recorded.

The main mechanisms causing secondary pollution of water in the water-pipe network include [6, 7]:

– corrosion and oxidization (susceptibility of the material of the pipes),

– significant changes in speed of flow (sludge is washed out),

– low speed of water (stagnant water in water pipes, the increase in water temperature),

– rapid change in pressure resulting in local vacuum (sludge is washed out),

– poor technical and sanitary condition of pipes (corrosion of pipes, a large quantity of bio-film, pipes leak),

– corrosion caused by aggressive water,

– lack of chemical instability of the water,

– inappropriate water treatment process (causing chemical instability of water in network),

– high doses of unused disinfectant remain in water (an increase of corrosion),

– accumulation of sludge in the network,

– presence of biochemical processes in the network,

– contamination of the network during repairs, replacement of pipes and fittings (the possibility that pollutants from the ground will pass into water in water-pipe network),

– household and industrial devices directly connected to the network (pollution from the installation is sucked into water-pipe network).

An example of the secondary contamination in the water network is the situation that happened in June 2014 in Dabrowa Gornicza. Because of the momentary loss of power in Water Treatment Plant in Bedzin and Maczki, caused by the energy supplier, on June 2 the direction of water flow in the main water pipe changed and it caused that loose mineral compounds of iron and manganese have been broken off the walls of the pipeline. The consequence of these disturbances in water supply has been the secondary water contamination in several districts of the city.

## The method for determining the risks associated with the water age in the water network

According to the basic definition of risk, it was assumed that it is the product of the point weights related to the probability of threat and the consequences of threat:

$$r = P \cdot C \tag{1}$$

where: $r$ – risks associated with the consumption of the primary contaminated water,
$P$ – point weight associated with the likelihood of an event involving the appearance of the primary water contamination in the WDS,
$C$ – point weight related to the water age in the WDS.

In the proposed method the parameter $C$ associated with water age in the WDS should be equated to the time that passes between the moment when the contaminated water flows into the WDS and the moment when consumers take that water.

On the basis of own studies [8] and literature [9–21] the following point weights for the parameters $P$ and $C$ were proposed (Table 1 and 2).

Table 1

Weights point for the parameter $P$

| Likelihood | Weights point | The incidence of threat |
|---|---|---|
| Very low | 1 | every ten years |
| Low | 2 | once every five years |
| Average | 3 | every two years |
| High | 4 | once a year |
| Very high | 5 | once in six months |

Table 2

Weights point for the parameter $C$

| Weights point | The water age in WDS |
|---|---|
| 1 | > 24 h |
| 2 | from 12 to 24 h |
| 3 | from 6 to 12 h |
| 4 | from 2 to 6 h |
| 5 | < 2 h |

The shorter residence time of the contaminated water in the water supply network the greater threat to residents. Longer time gives the opportunity to inform residents through local media or text messaging from Regional Warning System, etc.

In order to determine the average water age in the given area of the water supply network the mean time for the area is calculated:

$$t = \frac{\sum_{i=1}^{5} t_i \cdot LM_i}{LM} \tag{2}$$

where: $t$ – the average water age in the studied area of water supply network,
$i$ – the time interval,
$t_i$ – the assumed average water age in the water supply network in the time interval $i$,
$LM_i$ – number of people at risk of water supply in $i$-th period of time,
$LM$ – the number of residents of the studied area of the water supply network.

For a set weight points of $P$ and $C$ in accordance with (1) risk measures were determined.

Table 3

Risk measures

| | | *C* | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| *P* | 1 | 1 | 2 | 3 | 4 | 5 |
| | 2 | 2 | 4 | 6 | 8 | 10 |
| | 3 | 3 | 6 | 9 | 12 | 15 |
| | 4 | 4 | 8 | 12 | 16 | 20 |
| | 5 | 5 | 10 | 15 | 20 | 25 |

Based on the Table 3, the following categories of risk were proposed:
- negligible for $r \in [1–3]$,
- tolerated for $r \in [4–6]$,
- controlled for $r \in [8–10]$,
- unacceptable for $r \in [12–15]$,
- impermissible for $r \in [16–25]$.

## Application case

The public water supply is Wislok river. The water treatment is on two water treatment plants – Zwieczyca I and II, located in the south-western part of the city. Water supply network (Fig. 2.) with a total length of about 878 km consists of:
- the main network – 49.8 km,
- distribution network – 504.1 km,
- house connections – 324.4 km.

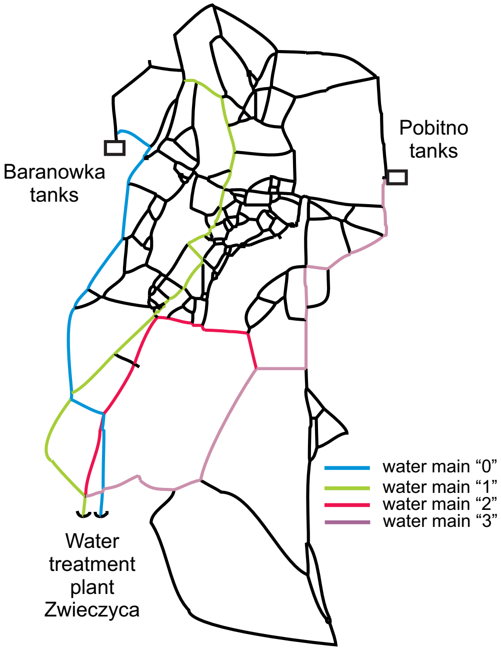

Fig. 2. CWSS of Rzeszow

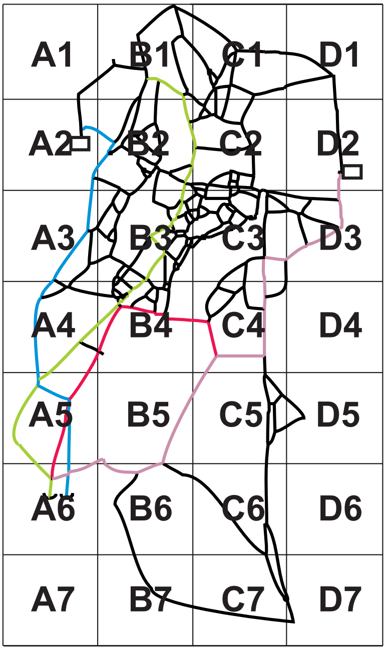

Fig. 3. Division of the CWSS of Rzeszow into areas

Main network is made of cast iron and steel pipes. The WDS was built with iron wires, steel and PE and PVC. House connections are made mainly of galvanized steel, cast iron, PE and PVC.

In order to conduct the analysis of the water age in the water supply network the city was divided into 28 areas, in accordance with Fig. 3.

To determine the water age in the WDS the hydraulic model of the Rzeszow CWSS, made in the program Epanet, was used. The input data for the model were, in addition to the spatial schema, junctions ordinates, water consumption in junctions, diameters, lengths and roughness of pipes, operating parameters of the pumping station, water tanks dimensions, daily distribution of water consumption.

The water age in the water supply network was analysed for the average daily water consumption. In Fig. 4 a stratified graph from program Epanet, showing the residence time of water in the WDS, is presented.

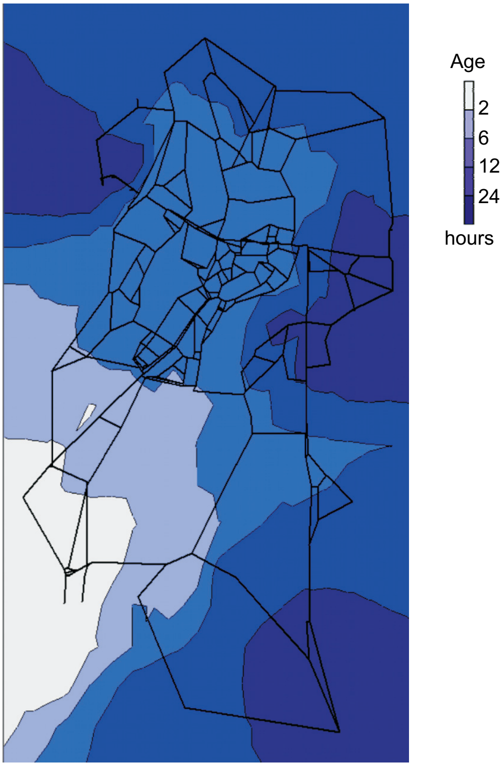

Fig. 4. A stratified graph of the water age in the water supply network

Based on the results of simulation of water age in the water supply network, for particular areas of WDS, the number of residents exposed to consumption of contaminated water for the assumed intervals, was defined. (Table 4). In the calculation of the values of t according to (4), for the subsequent time intervals, successively values

$t_i = 1, 4, 9, 18, 36$ were assumed. In the last column the point weight of the parameter *C* was defined in accordance with Table 2.

Table 4

Parameter *C* calculation

| Area | Number of residents with getting water: | | | | | *t* [h] | *C* [-] |
|---|---|---|---|---|---|---|---|
| | < 2 h | from 2 to 6 h | from 6 to 12 h | from 12 to 24 h | > 24 h | | |
| A1 | 0 | 0 | 2 690 | 1 770 | 1 050 | 17.0 | 2 |
| A2 | 0 | 0 | 0 | 4 220 | 4 080 | 26.8 | 1 |
| A3 | 0 | 8 220 | 3 260 | 520 | 0 | 6.0 | 4 |
| A4 | 0 | 15 340 | 160 | 0 | 0 | 4.1 | 4 |
| A5 | 2 240 | 460 | 0 | 0 | 0 | 1.5 | 5 |
| A6 | 5 630 | 70 | 0 | 0 | 0 | 1.0 | 5 |
| A7 | 1 190 | 530 | 1 770 | 0 | 0 | 5.5 | 4 |
| B1 | 0 | 0 | 1 180 | 320 | 0 | 10.9 | 3 |
| B2 | 0 | 0 | 6 900 | 880 | 570 | 11.8 | 3 |
| B3 | 0 | 100 | 15 400 | 0 | 0 | 9.0 | 3 |
| B4 | 0 | 9 410 | 1 940 | 0 | 0 | 4.9 | 4 |
| B5 | 660 | 10 940 | 0 | 0 | 0 | 3.8 | 4 |
| B6 | 30 | 200 | 270 | 0 | 0 | 6.5 | 3 |
| B7 | 0 | 10 | 50 | 240 | 0 | 16.0 | 2 |
| C1 | 0 | 0 | 790 | 610 | 0 | 12.9 | 2 |
| C2 | 0 | 0 | 900 | 0 | 0 | 9.0 | 3 |
| C3 | 0 | 40 | 5 950 | 570 | 330 | 11.0 | 3 |
| C4 | 0 | 3 370 | 2 420 | 1 300 | 250 | 9.2 | 3 |
| C5 | 0 | 2 730 | 7 100 | 11 980 | 0 | 13.3 | 2 |
| C6 | 0 | 70 | 860 | 4 360 | 310 | 17.4 | 2 |
| C7 | 0 | 0 | 0 | 1 750 | 4 050 | 30.6 | 1 |
| D1 | 0 | 0 | 40 | 1 560 | 0 | 17.8 | 2 |
| D2 | 0 | 0 | 320 | 1 250 | 40 | 16.7 | 2 |
| D3 | 0 | 0 | 30 | 3 530 | 2 740 | 25.8 | 1 |
| D4 | 0 | 0 | 0 | 5 960 | 2 840 | 23.8 | 2 |
| D5 | 0 | 0 | 0 | 10 | 5 790 | 36.0 | 1 |
| D6 | 0 | 0 | 0 | 1 760 | 2 340 | 28.3 | 1 |
| D7 | 0 | 0 | 0 | 0 | 5 800 | 36.0 | 1 |

Based on many years' observation of the studied CWSS and the nature of water source the weight of the parameter *P* was adopted as 2. On the basis of the value of the parameter *C* from the Table 2 and the adopted value of the parameter *P* the risk value was calculated and risk categories were defined. The results are presented as a map (Fig. 5).

Fig. 5. Map of the risk associated with the water age in WDS

On the basis of Fig. 5 it was found that:

– in 6 areas (5 of them in column D) the risk associated with the water age in the WDS is on a negligible level,

– in 7 areas (5 of them in column A) the risk associated with the water age in the WDS is on a controlled level,

– in 15 areas (mainly columns B, C and a whole line 1) the risk associated with water age in the WDS is on a tolerable level,

– the risk associated with the water age in the WDS is closely related to the hydraulics of the WDS (flow, speed, the demand for water in particular junction).

## Conclusions

The water age in the WDS is an important factor for the safety of water consumers. A short time of water inflow to the recipients can cause threat to health or life if primary water contamination appears in the WDS. A key role in ensuring water consumers safety plays water quality monitoring, from the intake through the water treatment plant until the WDS. Although the possibility of the appearance of primary contamination in the water network is small, the consequences of such an event can be tragic. Fast information to residents about the vulnerable areas quickly should be a priority for the water supply network management services. The presented methodology gives the possibility to undertake the information actions in the right order, starting with the areas with the highest threat. Information about threat can be conveyed indirectly through local media, the water supply company website, Sanitary Inspection, text messaging from the Regional Warning System or directly through stationary and mobile megaphones.

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# WIEK WODY W SIECI WODOCIĄGOWEJ JAKO CZYNNIK RYZYKA ZDROWOTNEGO ZWIĄZANEGO ZE ZBIOROWYM ZAOPATRZENIEM W WODĘ

Zakład Zaopatrzenia w Wodę i Odprowadzania Ścieków
Politechnika Rzeszowska, Rzeszów

**Abstrakt:** Poprawna eksploatacja systemu zbiorowego zaopatrzenia w wodę wymaga ciągłego monitoringu jakości wody na różnych etapach (ujmowania, uzdatniania, pompowania, dystrybucji). Istotnym parametrem związanym z jakością wody jest wiek wody, czyli czas od momentu wpompowania wody do sieci wodociągowej do jej pobrania przez odbiorców. W przypadku skażenia wody na wyjściu ze stacji uzdatniania wody wiek wody jest tożsamy z czasem rozprzestrzeniania się zanieczyszczenia w sieci wodociągowej. Od długości czasu rozprzestrzeniania zależy, ile osób korzystających z wodociągu będzie narażonych za spożycie wody o jakości zagrażającej zdrowiu lub życiu. W pracy przedstawiono metodykę określania ryzyka związanego z pojawieniem się w sieci wodociągowej zanieczyszczonej wody wykorzystującą model hydrauliczny, a także przykład aplikacyjny dla wybranego systemu zbiorowego zaopatrzenia w wodę.

**Słowa kluczowe:** sieć wodociągowa, jakość wody, zanieczyszczenie

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and Agata JABŁOŃSKA-TRYPUĆ[1]

# MONITORING OF PHARMACEUTICAL RESIDUES OF NON-STEROIDAL DRUGS WITH USE OF *Escherichia coli-GFP* BIOSENSORS

# MONITOROWANIE POZOSTAŁOŚCI FARMACEUTYCZNYCH LEKÓW NIESTERYDOWYCH Z ZASTOSOWANIEM *Escherichia coli-GFP* BIOSENSORÓW

**Abstract:** *Escherichia coli* strains containing a three different plasmid-borne transcriptional fusion between genotoxin-inducible *recA, kat G* and *sodA* promoters involved in the SOS regulon and bacteria stress response and mutated form of *gfp* reporter gene, have been used. GFP-based bacterial biosensors allowed for detection of a cytotoxic and genotoxic activity of ibuprofenum, ketoprofenum and paracetamolum – conventional non-steroidal anti-inflammatory drugs in PBS buffer and surface water. For experimental tests drugs were used at concentration of $10^{-6}$; $10^{-7}$; $10^{-8}$; $10^{-9}$ and $10^{-10}$ mg/dm$^3$, with bacteria strains time incubation of 3 and 24 hours. Experimental data indicated, that three promoters fusions with *gfp* gene as reporter were differently sensitive to applied drugs. Bacteria strains, *recA, kat G* and *sodA* promoters were a good bioindicator for cytotoxic and genotoxic effect monitoring of tested drugs in PBS buffer and surface water. The results showed, that applied in this experiment *E. coli gfp* biosensors strains could be potentially useful for environmental monitoring of cytotoxic and genotoxic effect of pharmacist residues of drugs in surface water.

**Keywords:** environmental monitoring, non-steroidal anti-inflammatory drugs, cytotoxicity, genotoxicity, *gfp* biosensors

## Introduction

Environmental pollution by human pharmaceuticals (HPs) has become a major problem in many countries worldwide. Recently, numerous scientific research have detected the residual concentrations of human pharmaceuticals and their metabolites in wastewaters and surface waters. Pharmaceutically active chemicals (PhACs) are a class of emerging contaminants, which has led to increasing concern about potential

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environmental risks [1–4]. After excretion from human body, unchanged pharmaceuticals and their metabolites are discharged into hospital, ambulatory and domestic wastewaters. Nowadays, there are no effective sewage treatment system which can remove these chemicals and as a result untreated wastewater containing these drugs enters surface waters. Pharmaceuticals and metabolites were detected worldwide at ng/dm$^3$ to μg/dm$^3$ levels in surface water in different areas. Some of them, belong to the groups of very dangerous pollutants with strong cyto- and genotoxic activity, even at trace environmental levels. In addition, the metabolites of human drugs may be even more toxic than the parent compounds. Scientific research revealed, that exposure to pharmaceutical residues has been recognized as a potential health hazard [5–8]. Currently there is a lack of information regarding how these drugs influence on condition of aquatic organisms. These products have unpredictable impacts on the aquatic ecosystems in which they enter. Of a particular importance are pharmaceutical residues causing cytotoxic, genotoxic effect and DNA damage, carcinogenesis and a number of different diseases. So, there is a very important social need for development of effecitive biosystems for monitoring and toxicity assessment of PhACs residues in environmental samples, mainly in relation to antiinflammatory drugs, which are widely used and very often detected in aquatic environments [4–10].

Nonsalicylate, conventional nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely used for human and animal therapy for decades. Among NSAIDs, ibuprofen (IB) (Fig. 1) is one of the most popular and has been shown to be more effective than acetaminophen for the treatment of pain associated with osteoarthritis (OA). IB is used in painful and inflammatory conditions. This drug was the first member of the group that came into general use [5, 8, 9, 11].

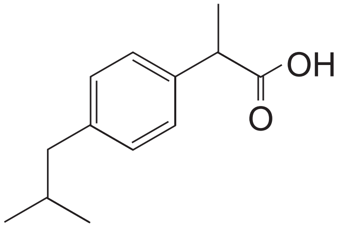

Fig. 1. Chemical structure of ibuprofenum

IB is used in rheumatoid arthritis, osteoarthritis, for relief of mild to moderate pain, primary dysmenorrhoea and reduction of fever. Ibuprofen is usually detected in influents in highest concentrations amongst the PhACs. In Europe, the fate of 13 PhACs has been traced in different municipal Wastewater Treatment Plants (WWTPs). Ibuprofen was found in effluent at maximum 3.9 mg/dm$^3$. In Poland the presence of IB in surface waters was detected at concentration of 0.05–0.1 μg/dm$^3$. Various experiments have determined that exposure to IB alone can affect aquatic organisms. IB exposure inhibits the growth of the mollusk *Planorbis carinatus*, reduces the reproductive capacity of the crustacean *Daphnia magna*, and causes abnormal behavior in *Gammarus pulex*; moreover, IB concentrations in water of greater than 100 mg/ dm$^3$ are known to be fatal to the fish *Oryzias latipes*. Ragugnetti et al [11] observed ibuprofen genotoxic effects in experimental model using *Oreochromis niloticus*. IB has been reported to induced gastrointestinal bleeding, meningitis, lymphopenia and hepatotoxicity in human bodies. It inhibits the synthesis of prostacyclin and prostaglandin of type E. Acute ingestion of IB results in nephritis, proteinuria, renal failure, adult respiratory distress syndrome and metabolic acidosis [4, 7, 9, 11–13].

Ketoprofen (KP) similarly to other non-steroidal anti-inflammatory drugs has strong antiinflammatory, analgesically and antipyretic activity. In 2007 Struwea et al [14] assessed photochemical genotoxicity of ketoprofen *in vitro* with use of photo comet assay with L5178Y mouse lymphoma cells. In 2013 Da Silveira et al [15] showed anti-cancer ability of ketoprofen, where ketoprofen-loaded polymeric nanocapsules selectively inhibit cancer cell growth *in vitro* and in preclinical model of glioblastoma multiforme (GBM) [14–16].

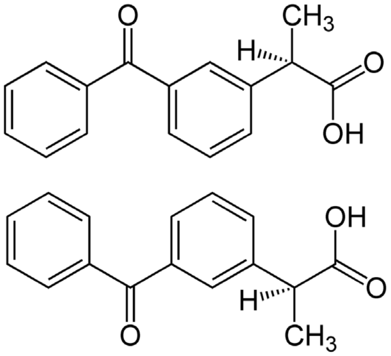

Fig. 2. Chemical structure of ketoprofenum

Paracetamol (PCM; N-(4-hydroxyphenyl) acetamide) as similar as IB and KP is a widely used analgesic and antipyretic agent that is utilized in human and animal medicine. PCM is one of the most common “first line use” drug which is present in global water bodies where it reaches concentrations up to μg /dm$^3$. Chemically, paracetamol is a phenol (Fig. 3) [17–22].

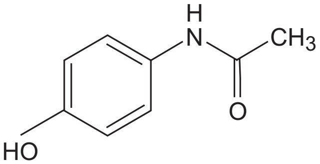

Fig. 3. Structure of paracetamol

Depending on the pharmacological mechanisms of action, NSAIDs have been divided into conventional and COX-2-selective categories. Conventional NSAIDs such as phenoprofen, ibuprofen, indomethacin, ketoprofen, keterolac, naproxen, paracetamol, piroxicam and aspirin are nonselective COX inhibitors, whereas etodolac, meloxicam, diclofenac, celecoxib, nimesulide, valdecoxib, parecoxib, rofecoxib and etoricoxib are selective COX-2 inhibitors and are also known as coxibs [19–26].

Data revealed the capacity of PCM to induce moderate genotoxicity in bivalves exposed to environmental concentrations [24]. PCM is a hepatotoxic agent and is the leading cause of acute liver failure (ALF). Research studies showed that toxic activity of PCM in living cells caused the increase in oxidative stress and/or had a direct interaction with DNA.

Because of the activity of pharmaceutical residues of conventional NSAIDs and their impact on the environment and the public health, it is mandatory to provide highly sensitive and robust analytical methodologies to control them and their active metabolites, at trace levels [25–30].

Although classical analytical methods can detect most chemicals in environmental sample with great precision, they are elaborate and expensive and also do not differentiate between the unavailable and bio-available fractions. Biological assays with use of living cells are able to showing the bioavailability and ecotoxicological effects of compounds [31–38]. Nowadays, various *in vitro* biotest systems have been developed for genotoxic risk assessment of pharmaceuticals in environmental samples. Several genotoxin-specific recombinant bacterial biosensors have been constructed to determine genotoxic potency of analytes. These tests are based on the ability of compounds to induce DNA damage, oxidative stress formation, enhance the formation of mutations or chromosomal aberrations or initiate a cellular stress response. To establish genotoxicity, various genetically modified bacteria-based assays have been developed. The DNA – damage and oxidative stress formation assays are based on activation of bacterial SOS and oxidative stress response upon exposure to genotoxic compounds. The SOS, SoxRS and OxyR regulons with *recA*, *sodA* and *katG* promoters are one of the most thoroughly studied genotoxic stress regulons for bacteria. These specific genes are induced in response to reactive oxygene species (superoxide anion, hydrogen peroxide and hydroxyl radicals) and other DNA-damaging (*eg* alkylating) agents, such as anticancer drugs and different chemicals [38–46]. The genotoxin-sensitive *recA*, *katG* and *sodA* promoters transcription is induced upon DNA damage (genotoxic and mutagenic effect). The application of these promoters in order to create some effective genotoxicity bacteria biosensors is connected with broad involvement of RecA protein and *katG* and *sodA* expression products in several DNA repair pathways, including the repair of daughter-strand gaps and double-strand breaks, as well as in an error prone damage tolerance mechanisms called SOS mutagenesis and stress oxidation response [37, 39, 41, 45].

The green fluorescent protein (*gfp*) reporter gene is one of the common used in microbial biosensors creation, because it is stable, direct and convenient tracing tool, without external substrate. *Gfg* expression can be observed in living cells [39].

Among the many classes of pharmaceuticals, the conventional non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most important groups with an annual production of several kilotons. NSAIDs are the sixth most sold drugs worldwide. Many authors have reported levels of these drugs exceeding 1 $\mu g/dm^3$ in wastewaters and in the effluents of sewage treatment plants (STP), while lower concentrations have been found in surface waters. Due to the biological activity of these drugs, there are considered to be hazardous to living organisms and human health. There is a need to monitor of their presence in environmental samples and assess their cytotoxic and genotoxic risk to living organisms [1–10]. Thus, the present study showed the possibility of application of *E. coli* K-12 *gfp* microbial biosensor strains for cytotoxic and genotoxic effects monitoring of ibuprofen, ketoprofen and paracetamol – one of the most popular drugs use in human and veterinary medicine.

## Methodology

### Chemicals

Ibuprofen, ketoprofen and paracetamol were commercially obtained from Bialystok pharmacy. These drugs had been dissolved in PBS buffer (1.44 g $Na_2HPO_4$, 0.24 g $KH_2PO_4$, 0.2 g KCl, 8 g NaCl per $dm^3$ of destilled water, pH = 7) at determined experimental concentrations before they were used.

### Bacteria strain and plasmid

In the experiment *Escherichia coli* K-12 MG1655 stationary phase cells: *Escherichia coli* K-12 *recA*::*gfpmut2*, *Escherichia coli* K-12 *katG*::*gfpmut2*, *Escherichia coli* K-12 *sodA*::*gfpmut2* and *Escherichia coli* K-12 *promoterless*::*gfpmut2*, genetically modified were used. They contained a pUA66 plasmid-borne transcriptional fusion between DNA-damage inducible, oxidative stress *recA*, *katG* and *sodA* promoters involved in the SOS regulon and oxidative stress response and fast folding GFP variant reporter gene-*gfpmut2*. The genetic structure of pUA66 plasmid is described in the work of Zaslaver et al [44]. In the present work a more stable and fast folding mutant of *gfp* gene – *gfpmut2* with excitation and emission wavelengths of 485 and 507 nm was used [44].

### Bacteria growth condition

*Escherichia coli* K-12 MG1655 strains: *Escherichia coli* K-12 *recA*::*gfpmut2*, *Escherichia coli* K-12 *katG*::*gfpmut2*, *Escherichia coli* K-12 *sodA*::*gfpmut2* and *Escherichia coli* K-12 *promoterless*::*gfpmut2* were cultured overnight in LB agar medium (Merck, Germany) at 30°C supplemented with $10^4$ $\mu g/dm^3$ of kanamycin (Sigma-Aldrich, Germany). Colonies were carried to LB broth medium (10 g NaCl, 10 g tryptone and 5 g yeast extract per 1 $dm^3$ of destilled water) with 100 $\mu g/cm^3$ of kanamycin and incubated overnight at 30°C. Afterwards the cells were washed with PBS buffer.

### Monitoring of bacteria growth and concentration

At the beginning of the experiment the initial bacteria cells density was standardized to OD (Optical Density) value by using spectrophotometer (Multi Detection System, Promega) at the wavelength of 600 nm. The concentration of bacteria cells per $cm^3$ of PBS was assessed by series dilutions system and expressed as $CFU/dm^3$ values.

Dynamic growth of bacteria strains treated with drugs was monitored by the use of standard spectrophotometer analysis of Optical Density values at the wavelength of 600 nm.

## Bacteria cells treatment with ibuprofen, ketoprofen and paracetamol in PBS buffer

$10^{-4}$ $dm^3$ of stationary phase bacteria cells ($2 \cdot 10^{11}$ $CFU/dm^3$) were suspended in $40^{-4}$ $dm^3$ of PBS buffer and the following drugs were used for genotoxicity testing: ibuprofen (PBS buffer solution of 200 mg of ibuprofenum) (IB), ketoprofen (PBS buffer solution of 50 mg of ketoprofenum) (KP) and paracetamol (PBS buffer solution of 500 mg of paracetamolum) (PCM). Drugs were applied at five different concentrations: $10^{-6}$; $10^{-7}$; $10^{-8}$; $10^{-9}$ and $10^{-10}$ $mg/dm^3$. The concentration range of the drugs analysed in research was selected experimentally from the minimum level of *recA::gfp*, *katG::gfp* and *sodA::gfp* constructs sensitivity and according to the reviewed references recommendation, which indicated the concentrations observed in the environment [26]. The time of bacteria incubation with drugs (3 h and 24 h) was estimated for monitoring of sensitivity of genetic constructs for quick (3 h) and later (24 h) response. The control samples – *Escherichia coli* K-12 *recA*::*gfpmut2*, *Escherichia coli* K-12 *katG*::*gfpmut2* and *Escherichia coli* K-12 *sodA*:*gfpmut2* strains in PBS buffer were not treated with drugs. For verification the correct activity of *recA*, *katG* and *sodA* promoters, *Escherichia coli* K-12 strain containing pUA66 plasmid without the promoter – *Escherichia coli* K-12 *promoterless*::*gfpmut2* – was used as the control one. Additionally, for assessment of genotoxic sentivity of *recA::gfp* construct, 4% acetone was used as the negative control and 50 μM methylnitronitrosoguanidine (MNNG, known genotoxin) as the positive control.

## Bacteria cells treatment with drugs in surface water

Surface water samples were collected in sterile flasks from the Bialka river. The samples were sterilized by filtration. $10^{-4}$ $dm^3$ of stationary phase bacteria cells ($2 \cdot 10^{11}$ $CFU/dm^3$) were suspended in $40^{-4}$ $dm^3$ of surface water and the following drugs were used for genotoxicity testing: ibuprofen and ketoprofen at concentration of $10^{-8}$ $mg/dm^3$ for IB and $10^{-7}$ $mg/dm^3$ for KP with use of *recA::gfp* and *katG::gfp* genetic constructs. Drugs concentrations were selected for the highest stimulation of *gfp* gene expression in PBS buffer (for IF = 8.01 and IF = 8.37, respectively).

The conditions of bacteria incubations and the control protocols were the same as above.

## Analytical method for the intensity of *gfp* gene fluorescence (IF) analysis

After exposition of bacteria cultures to tested drugs, the strains were washed with PBS buffer and the intensity of fluorescence of *gfp* gene in the volume of $10^{-4}$ $dm^3$ of bacteria cells suspension ($1 \cdot 10^7$ CFU/dm$^3$) in PBS buffer was measured with the spectrofluorometer (Multi Detection System, Promega). The measurements were done at excitation and emission wavelengths of 485 and 507 nm.

## Assessment of SFI values

The specific fluorescence intensity (*SFI*) value which is defined as the raw fluorescence intensity (*IF*) divided by the optical density (*OD*) measured at each time point at 600 nm was calculated according to the below formula for monitoring the dynamic of *gfp* expression after bacteria treatment with drugs:

$$SFI = \frac{IF}{OD} \tag{1}$$

where: $SFI$ – Specific Fluorescence Intensity,
$IF$ – The raw fluorescence intensity of the strains at excitation and emission wavelengths of 485 and 507 nm,
$OD$ – Optical Density at 600 nm of the strains.

## Detection of $S_{gfpexp.}$ value

For the increased *SFI* values with the level of *gfp* expression in comparison with the control sample the percentage stimulation of *gfp* ($S_{gfpexp.}$) was calculated according to the formulas:

$$S_{gfpexp.}(\%) = I_{TS}\,(\%) - SFI_{CS}\,(\%) \tag{2}$$

where: $I_{TS}$ (%) – the increase for SFI values for tested drugs sample in comparison with the control sample,
$SFI_{CS}$ (%) – *SFI* for the control sample = 100%.

## Assessment of $F_I$ values

For each concentration of tested drugs the induction factors ($F_I$) were calculated.

$$F_I = (Fl_I / OD_0) / (Fl_0 / OD_I) \tag{3}$$

where: $Fl_I$ – the raw fluorescence of the culture treated with DNA – damaging compound,
$Fl_0$ – the raw fluorescence of the control sample without genotoxin,
$OD_I$ – the optical density at 600 nm of treated culture,
$OD_0$ – the optical density of the control sample.

The *SFI*, $S_{gfpexp.}$ and $F_{I,}$ values express the potency of influence of tested drugs on the sensitivity of genotoxicity and oxidative stress *recA::gfp*, *katG::gfp* and *sodA::gfp* constructs.

## Classification of tested drugs as genotoxins

The $F_I$ values were calculated for classification of tested drugs as genotoxins. According to Ptitsyn et al [45] and Kostrzynska et al [39] a chemical was identified as a genotoxin if its induction factor was 2 or more ($F_I \geq 2$).

## Statistical analysis

Statistical data obtained in this study are expressed as mean ± standard deviation (SD) for $n = 8$. The data were analyzed by the use of standard statistical analyses, including one-way Student's test for multiple comparisons to determine the significance between different groups. The values for $P < 0.05$ were considered significant.

# Results

In the experiment the positive fluorescence reactivity of *Escherichia coli* K-12 was obtained for each tested genetic constructs with three different promoters *recA*, *katG* and *sodA* and for all tested drugs, especially at concentration of $10^{-6}$; $10^{-7}$; $10^{-8}$ mg/dm$^3$

*Escherichia coli* K-12 MG1655 *recA*::*gfpmut2* strain treatment with ibuprofen, ketoprofen and paracetamol showed that administration of three drugs caused a significant increase ($p \leq 0.05$) in *SFI*, $F_I$ and $S_{gfpexp.}$ values compared to non-treated cells (Table 1). Bacteria cells reacted with different efficiency in *gfp* expression after incubation with drugs which possessed different chemical structure.

Longer treatment of *recA::gfp* bacteria strain with KP and PCM (up to 24 h) intensified *SFI*, $F_I$ and $S_{gfpexp}$ values at concentration of $10^{-8}$ mg/dm$^3$. Bacteria cells incubation with IB caused the strongest stimulation of *gfp* expression for 3 h incubation at concentrations of $10^{-7}$; $10^{-8}$ mg/dm$^3$, compared to the control sample. A maximum point of *recA::gfp* stimulation (about 701% higher *gfp* stimulation comparable to control sample) was observed for KP at concentration of $10^{-8}$ mg/dm$^3$ and 24 h incubation with drug (Table 1).

*E. coli* K-12 *katG::gfp* treatment with IB, KP and PCM resulted in a progressive significant stimulation of *SFI* values for IB and PCM at concentration of $10^{-7}$; $10^{-8}$ mg/dm$^3$ and for KP at concentration of $10^{-6}$; $10^{-7}$; $10^{-8}$ mg/dm$^3$ for 3 and 24 h incubation with drugs compared to the control sample (Table 2). The maximum point for *SFI* value ($S_{gfpexp.}$= 737%) was for KP at the concentration of $10^{-7}$ mg/dm$^3$ and 24 h of incubation time.

*E. coli* K-12 *sodA::gfp* cells administrated with IB, KP and PCM exerted some influence on *SFI* and the parameters with the maximum point for *SFI* ($S_{gfpexp.}$ = 599%) were for $10^{-7}$ mg/dm$^3$ of KP after 24 h incubation with drug. KP almost at each concentration significantly modulated (in 80% of cases) *gfp* expression. Only in the case of $10^{-9}$ mg/dm$^3$ KP concentration no significant differences in *SFI* between KP and control sample were observed (Table 3).

The monitoring of bacteria cultures density as optical density value (*OD*) of *E. coli recA::gfp* and *E. coli katG::gfp* and *E. coli sodA::gfp* at the start of bacteria treatment (time 0) and after 3 and 24 h of incubation with drugs indicated a significant decrease in

Table 1

*SFI* values for *E. coli* K-12 *recA::gfp mut2* treated with ibuprofen, ketoprofen and paracetamol in PBS buffer in comparison with the control sample (bacteria strain in PBS buffer), *T* – time of bacteria strain incubation with drugs, $F_I$ – induction factor values, $S_{gfpexp.}$ [%] – the percent of stimulation of *gfp* expression after treatment of bacteria cells with drugs in comparison with the control sample [100%]

| *C* [mg/dm$^3$] | *T* [h] | Control sample *SFI* ± SD | Ibuprofen *SFI* ± SD | $F_I$ | $S_{gfpexp}$ [%] | Ketoprofen *SFI* ± SD | $F_I$ | $S_{gfpexp.}$ [%] | Paracetamol *SFI* ± SD | $F_I$ | $S_{gfpexp.}$ [%] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-6}$ | 3 | 26.5 ± 3.4 | 24.3 ± 3.6 | — | — | 27.6 ± 4.3 | — | — | 32.6 ±3 .4 | — | — |
| | 24 | 41.0 ± 5.4 | 36.4 ±2 .8 | — | — | 49.8 ± 4.1 | — | — | 65.4 ± 3.8 | — | — |
| $10^{-7}$ | 3 | 26.5 ± 3.4 | 26.3 ± 3.1 | — | — | 30.0 ± 3.4 | — | — | 27.3 ± 3.6 | — | — |
| | 24 | 41.0 ± 5.3 | 39.2 ± 3.0 | — | — | 56.4 ± 5.3$^{ab}$ | — | — | 140.3 ± 5.2$^{abc}$ | 2.42 | 142 |
| $10^{-8}$ | 3 | 26.5 ± 3.4 | 45.7 ± 4.6$^{a}$ | — | 72 | 33.3 ± 4.6 | — | — | 90.4 ± 6.4$^{abc}$ | 2.41 | 141 |
| | 24 | 41.0 ± 5.4 | 283.0 ± 7.2$^{a}$ | 6.89 | 589 | 356.0 ± 8.3$^{ab}$ | 7.70 | 670 | 114.0 ± 7.1$^{abc}$ | 2.78 | 178 |
| $10^{-9}$ | 3 | 26.5 ± 3.4 | 59.4 ± 3.6$^{a}$ | 2.24 | 124 | 64.4 ± 4.2$^{ab}$ | 2.43 | 143 | 105.5 ± 6.3$^{abc}$ | 2.98 | 198 |
| | 24 | 41.0 ± 5.3 | 298.0 ± 8.3$^{a}$ | 7.27 | 627 | 370.0 ± 6.4$^{ab}$ | 8.01 | 701 | 176.0 ± 6.8$^{abc}$ | 3.30 | 230 |
| $10^{-10}$ | 3 | 26.5 ± 3.4 | 29.4 ± 3.5 | — | — | 29.4 ± 3.4 | — | — | 32.4 ± 4.1 | — | — |
| | 24 | 41.0 ± 5.3 | 43.5 ± 4.0 | — | — | 53.5 ± 4.9 | — | — | 72.4 ± 5.4$^{abc}$ | — | — |

Mean values ± SD; $n = 8$; a – significantly different from control ($p < 0.05$); b – significantly different from ibuprofen (IB) group ($p < 0.05$); c – significantly different from ketoprofen (KP) group ($p < 0.05$).

Table 2

*SFI* values for *E. coli* K-12 *katG::gfp mut2* treated with ibuprofen, ketoprofen and paracetamol in PBS buffer in comparison with the control sample (bacteria strain in PBS buffer), *T* – time of bacteria strain incubation with drugs, $F_I$ – induction factor values, $S_{gfpexp.}$ [%] – the percent of stimulation of *gfp* expression after treatment of bacteria cells with drugs in comparison with the control sample [100%]

| *C* [mg/dm$^3$] | *T* [h] | Control sample *SFI* ± SD | Ibuprofen *SFI* ± SD | $F_I$ | $S_{gfpexp.}$ [%] | Ketoprofen *SFI* ± SD | $F_I$ | $S_{gfpexp.}$ [%] | Paracetamol *SFI* ± SD | $F_I$ | $S_{gfpexp.}$ [%] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-6}$ | 3 | 20.4 ± 2.8 | 31.3 ± 3.9 | — | — | 24.3 ± 3.9 | — | — | 24.8 ± 3.7 | — | — |
| | 24 | 35.6 ± 3.5 | 39.6 ± 3.4 | — | — | 45.0 ± 4.6$^{a}$ | — | — | 42.6 ± 4.2 | — | — |
| $10^{-7}$ | 3 | 20.4 ± 2.8 | 32.6 ± 3.8 | — | — | 120.6 ± 7.6$^{ab}$ | 5.9 | 491 | 29.8 ± 3.2 | — | — |
| | 24 | 35.6 ± 3.5 | 49.8 ± 4.3 | — | — | 280.0 ± 8.4$^{ab}$ | 7.8 | 686 | 88.0 ± 5.3$^{abc}$ | 2.5 | 147 |
| $10^{-8}$ | 3 | 20.4 ± 2.8 | 85.4 ± 5.5$^{a}$ | 4.2 | 318 | 124.0 ± 6.3$^{ab}$ | 6.1 | 507 | 70.8 ± 4.6$^{ac}$ | 3.4 | 247 |
| | 24 | 35.6 ± 3.5 | 210.0 ± 7.50 | 5.9 | 490 | 298.0 ± 8.5 | 8.4 | 737 | 110.0 ± 7.4 | 3.1 | 209 |
| $10^{-9}$ | 3 | 20.4 ± 2.8 | 14.0 ± 8.0$^{a}$ | 6.8 | 586 | 99.0 ± 5.3$^{a}$ | 4.8 | 385 | 102.0 ± 7.2$^{ab}$ | 5.0 | 400 |
| | 24 | 35.6 ± 3.5 | 240.0 ± 7.6$^{a}$ | 6.7 | 574 | 167.0 ± 7.3$^{ab}$ | 4.7 | 370 | 123.0 ± 8.1$^{abc}$ | 3.4 | 245 |
| $10^{-10}$ | 3 | 20.4 ± 2.8 | 31.4 ± 4.0 | — | — | 26.0 ± 3.4 | — | — | 31.2 ± 5.1 | — | — |
| | 24 | 35.6 ± 3.5 | 46.8 ± 4.3 | — | — | 49.0 ± 3.8 | — | — | 43.3 ± 5.6 | — | — |

Mean values ± SD; *n* = 8; a – significantly different from control ($p < 0.05$); b – significantly different from ibuprofen (IB) group ($p < 0.05$); c – significantly different from ketoprofen (KP) group ($p < 0.05$).

Table 3

*SFI* values for *E. coli* K-12 *sodA::gfp mut2* treated with ibuprofen, ketoprofen and paracetamol in PBS buffer in comparison with the control sample (bacteria strain in PBS buffer), *T* – time of bacteria strain incubation with drugs, $F_I$ – induction factor values, $S_{gfpexp.}$ [%] – the percent of stimulation of *gfp* expression after treatment of bacteria cells with drugs in comparison with the control sample [100%]

| *C* [mg/dm$^3$] | *T* [h] | Control sample *SFI* ± SD | Ibuprofen *SFI* ± SD | $F_I$ | $S_{gfpexp.}$ [%] | Ketoprofen *SFI* ± SD | $F_I$ | $S_{gfpexp.}$ [%] | Paracetamol *SFI* ± SD | $F_I$ | $S_{gfpexp.}$ [%] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{-6}$ | 3 | 22.7 ± 3.1 | 29.5 ± 3.4 | — | — | 70.3 ± 7.6$^{ab}$ | 2.1 | 110 | 29.6 ± 3.8 | — | — |
|  | 24 | 38.3 ± 3.9 | 41.0 ± 3.6 | — | — | 93.2 ± 8.2$^{ab}$ | — | 43 | 45.7 ± 4.6 | — | — |
| $10^{-7}$ | 3 | 22.7 ± 3.1 | 34.6 ± 3.6 | — | — | 126.0 ± 8.1$^{ab}$ | 4.5 | 355 | 32.0 ± 3.9$^{c}$ | — | — |
|  | 24 | 38.3 ± 3.9 | 52.5 ± 5.2$^{a}$ | — | — | 301.0 ± 9.0$^{ab}$ | 6.8 | 586 | 85.0 ± 6.2$^{abc}$ | 2.22 | 122 |
| $10^{-8}$ | 3 | 22.7 ± 3.1 | 74.3 ± 5.3$^{a}$ | 2.3 | 127 | 156.0 ± 7.4$^{ab}$ | 5.8 | 487 | 82.0 ± 5.2$^{ac}$ | 2.6 | 161 |
|  | 24 | 38.3 ± 3.9 | 175.0 ± 6.8$^{a}$ | 3.6 | 257 | 306.0 ± 8.9$^{ab}$ | 6.9 | 599 | 134.0 ± 8.2$^{ac}$ | 2.5 | 150 |
| $10^{-9}$ | 3 | 22.7 ± 3.1 | 98.0 ± 7.4$^{a}$ | 3.3 | 232 | 85.0 ± 5.7$^{a}$ | 2.7 | 174 | 111.0 ± 8.3$^{a}$ | 3.9 | 290 |
|  | 24 | 38.3 ± 3.9 | 190.0 ± 7.4$^{a}$ | 3.9 | 296 | 133.0 ± 7.6$^{ab}$ | 2.5 | 147 | 133.0 ± 8.9$^{ab}$ | 2.4 | 147 |
| $10^{-10}$ | 3 | 22.7 ± 3.1 | 34.6 ± 4.5 | — | — | 29.0 ± 3.7 | — | — | 33.4 ± 6.2 | — | — |
|  | 24 | 38.3 ± 3.9 | 64.5 ± 5.3$^{a}$ | — | — | 52.0 ± 4.3 | — | — | 42.7 ± 5.6 | — | — |

Mean values ± SD; $n = 8$; a – significantly different from control ($p < 0.05$); b – significantly different from ibuprofen (IB) group ($p < 0.05$); c – significantly different from ketoprofen (KP) group ($p < 0.05$).

*OD* (growth inhibition) values for $10^{-6}$, $10^{-7}$, $10^{-8}$ mg/dm$^3$ tested concentrations of ketoprofen for 24 h treatment and for IB at concentrations of $10^{-6}$, $10^{-7}$ mg/dm$^3$ for 24 h incubation. There were no statistical differences in the case of paracetamol and a shorter time (3 h) of IB and KP influence on bacteria cells. Prolonged treatment (up to 24 h) of bacteria cells with IB and KP significantly influenced the *OD* value of bacteria strains, especially at higher concentration. The *OD* inhibition values after treatment of bacteria strains with IB and KP were very similar for the three strains with *recA*, *katG* and *sodA* promoters.

Bacteria incubation with PBS buffer (the control sample) without any drugs addition resulted in no statistically differences in *OD* value from 0 to 24 hours continuous cultivation.

Treatment of *E. coli recA::gfp* and *E. coli katG::gfp* biosensor bacteria strains in surface water enhanced the sensitivity of *recA::gfpmut2* and *katG::gfp* genotoxic system and increased the stimulation of *gfp* expression and *SFI* value in comparison to incubation in PBS buffer (Table 4). Prolonged treatment (up to 24 h) of bacteria cells with the ketoprofen significantly influenced *gfp* expression with the maximum values for $F_I$ = 9.06 for *recA::gfp* genetic construct and $F_I$ = 11.41 for *katG::gfp* genetic construct comparable to the control sample.

In this experiment, for assessment of genotoxic sensitivity of a *recA::gfp* genetic biosensing system, 4% acetone was tested as the negative control. In the case of this chemical there was no increased in $F_I$ values for 3 h and 24 h of incubation. Methylnitronitrosoguanidine (MNNG) – known genotoxin at the concentration of 50 µM – was used as the positive control. For this analyte $F_I = 8.4$ for 24 h incubation time and $F_I = 2.8$ for 3h (data not shown). These results proved stronger sensitivity of a *recA::gfp* biosensing system for MNNG than for an acetone stressor.

Table 4

*SFI* values for *E. coli* K-12 *recA::gfp mut2* and *E. coli* K-12 *katG::gfp mut2* treated with ketoprofen at concentration of $10^{-7}$ and $10^{-8}$ mg/dm$^3$ in comparison with the control sample (bacteria strain in surface water), *T* – time of bacteria strain incubation with drugs, $F_I$ – induction factor values, $S_{gfpexp.}$ [%] – the percent of stimulation of *gfp* expression after treatment of bacteria cells with drug in comparison with the control sample [100%]

| Strain | *C* [mg/dm$^3$] | *T* [h] | Control sample *SFI* ± SD | Ketoprofen *SFI* ± SD | $F_I$ | $S_{gfpexp.}$ [%] |
|---|---|---|---|---|---|---|
| *E. coli* K-12 *recA::gfp* | $10^{-8}$ | 24 | 44.5 ± 5.4 | 403.0 ± 10$^{a\ A}$ | 9.06 | 806 |
| *E. coli* K-12 *katG::gfp* | $10^{-7}$ | 24 | 39.8 ± 6.2 | 454.0 ± 12$^{aB}$ | 11.41 | 1041 |

Mean values ± SD; n = 8; a – significantly different from control ($p < 0.05$); A – significantly different from ketoprofen group in PBS buffer for *E. coli* K-12 *recA::gfp* ($p < 0.05$); B – significantly different from ketoprofen group in PBS buffer for *E. coli* K-12 *katG::gfp* ($p < 0.05$).

## Discussion

The results of this study indicate that treatment of bacteria cells with ibuprofen, ketoprofen and paracetamol lead to over 6- and 8-fold stimulation ($F_I$ = 8.01 in the case of ibuprofen, $F_I$ = 8.37 in the case of ketoprofen and $F_I$ = 6.99 in the case of paracetamol) of bacteria genotoxin-sensitive *recA*, *katG* and *sodA* promoters and *gfp* gene expression.

The results obtained in the experiment are in agreement with the studies of Belkin et al [31]; Ptitsyn et al [45]; Albano et al [32]; Kostrzynska et al [39]; Norman et al [37]; Alhadrami and Paton [38] and some others [31–46], who presented data, that genetic constructs with *recA*, *katG* and *sodA* genes in transcriptional fusion with reporter gene systems (with *gfp* and *lux* reporters) were sensitive and useful for measurement of cyto- and genotoxicity of anticancer drugs and various chemicals in environmental studies.

According to the results obtained in this experiment IB, KP and PMC modulate and increase the reactivity of *recA*-, *katG*- and *sodA*-genotoxin sensitive, oxidative stress promoters in relation to control sample.

Most biotests have usually described the effects of medicine concentrations from micrograms per liter to milligrams per liter on aquatic organisms. Ibuprofen as a drug is very popular all over the world and has been detected in rivers [48].

Some laboratory studies on ecotoxicological influence of ibuprofen with use of tests with *Daphnia magna*, amphibians *Hyalille Azteca* and *O. niloticus* has revealed the genotoxic effect of ibuprofen at concentration of 300 ng/dm$^3$ [11, 49–50].

Medical studies established that nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of promising cancer chemopreventive agents and antineoplastic agents. It was observed that long-term use of NSAIDs has been shown to reduce the risk of cancer of the colon and other gastrointestinal organs as well as of cancer of the breast, prostate, lung, and skin. These very useful activity of NSAIDs is connected with its ability for restoring normal apoptosis and reducing cell proliferation in human adenomatous colorectal polyps, experimental colonic tumors, and in various cancer cell lines that have lost critical genes required for normal function. NSAIDs, particularly selective cyclooxygenase-2 (COX-2) inhibitors such as celecoxib, have been shown to inhibit angiogenesis in cell culture and in rodent models of angiogenesis. Probably, the main molecular mechanism of NSAIDs act is related to its down-regulation of pro-inflammatory cytokines and/or growth factors and its influence on transcription factors, the mechanism of which is not well understood [51–54].

The results of the above experiment provided the conformation of the possible influence of ibuprofen, ketoprofen and paracetamol on the genes expression, similarly as Kanwar et al [52]; Rayburn et al [53] and Vaish et al [51], In 40% for *recA* promoter, 43,33% for *katG* and 63,33% for *sodA* promoter of the cases there were significant differences (comparable to the control sample) regarding the level of promoters sensitivity and *gfp* expression after bacteria treatment with all applied concentrations of tested NSAIDs for both short (3 h) and longer time of incubation (up to 24 h).

Some authors [15, 17, 18, 21, 51–54] studies indicated that the activity of NSAIDs are dose- and time-dependent. It was also confirmed by data obtained in our studies. Longer bacteria exposure (up to 24 h) on IB, KP and PMC resulted in a progressive stimulation of promoters activity and *gfp* gene expression. Higher values of $F_I$ factor were obtained for IB, KP and PMC after 24 hours incubation than after 3 h. Generally, the strongest stimulation of *gfp* expression was possessed after bacteria treatment with IB and KP than PCM ($F_I$ = 8.01; $F_I$ = 8.37; and $F_I$ = 6.99, respectively).

The main molecular mechanisms of action of NSAIDs act by suppressing cyclooxygenase (COX)-1 and COX-2 enzymes or tumour necrosis factor (TNF)-α and inducible nitric oxide synthase (iNOS). Phospholipase $A_2$ ($PLA_2$) or phospholipase C (PLC) catalyses liberation of arachidonic acid from membrane phospholipids, which is further oxygenated into PGs by the action of lipoxygenase (LOX) and COX enzymes. By blocking the action of COX, NSAIDs can relieve inflammation by reducing vasodilatation and pain, which is produced mainly by prostaglandin E (PGE)2 and prostacyclin (PGI2). Most of the NSAIDs block synthesis of PGs by inhibiting COX enzymes nonselectively [4–9, 18, 21, 55].

Our results showed that IB and KP *E. coli* K-12 longer (up to 24 h) treatment significantly inhibited bacteria cells growth. Bacteria incubation with IB and KP importantly, dose-dependently intensified their cytotoxic effect on living bacteria cells after 24 hours incubation. Our results are in agreement with earlier empirical studies of other authors who demonstrated cyto- and genotoxic effect of IB and KP on living cells. Vaish et al [51], Kanwar et al [52] and Rayburn et al [53] have demonstrated that NSAIDs reduced cell proliferation in human cells and in various cancer cell lines. This group of drugs influenced the activity of DNA of the cells and genes expression and it could be the main mechanisms of its cyto- and genotoxicity.

We obtained stronger reactivity of *E. coli* K-12 *recA::gfp mut2* and *E. coli* K-12 *katG::gfp mut2* in surface water for ketoprofen at concentration of $10^{-8}$ and $10^{-7}$ mg/dm$^3$ treated samples. It was possibly due to the different chemical composition and pH value of PBS buffer and tested sample of surface water. Additionally the presence of the other chemicals in surface water which could influence *gfp* expression in bacteria strains maybe occured. It is important, therefore, to check all river's water (specially in the hospital's surroundings) for the presence of drugs belonging to these groups of chemicals.

The cyto- and genotoxicity studies of NSAIDs are being conducted for some years on bacteria, human cells and other organisms [51–53]. In above experiment we applied *E. coli* K-12 bacteria cells with three different genetic constructs with *recA*, *katG* and *sod*A promoters and *gfp* gene as reporter as a model organism for genotoxic studies. Obtained data, are generally in agreement with other results which were previously obtained in *in vivo* and *in vitro* tests of higher organisms, including human cells.

## Conclusions

1. The results of the presented study indicated that *recA::gfpmut2*, *katG::gfpmut2* and *sodA::gfpmut2* genetic systems were sensitive to IB, KP and PCM applied in the experiment.

2. Experimental data indicated that three promoters fusions with *gfp* gene as reporter were differently sensitive to applied drugs. For applied drugs the highest sensitivity was observed for *sodA* promoter and IB and KP.

3. The results indicated that *gfp E. coli* strains with *recA*, *katG* and *sodA* could be potentially useful for monitoring of cyto- and genotoxic effect of pharmacist residues in water.

4. The validation of used in this work genetic systems in *E. coli* demands more experimental analysis, which should be focused on the assessment of their sensitivity on drugs with different chemical structure and mechanisms of biological activity.

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# MONITOROWANIE POZOSTAŁOŚCI FARMACEUTYCZNYCH NIESTERYDOWYCH LEKÓW Z ZASTOSOWANIEM *Escherichia coli-GFP* BIOSENSORA

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**Abstrakt:** W pracy wykorzystano szczepy *Escherichia coli* zawierające plazmidowe, trzy różne konstrukty genowe indukowalnych genotoksynami promotorów *recA, kat G* i *sodA* pochodzących z regulonu SOS oraz szlaków bakteryjnej odpowiedzi stresowej w fuzji z genem reporterowym *gfp*. GFP-bakteryjny biosensor pozwolił na detekcję cyto- i genotoksycznej aktywności ibuprofenu, ketoprofenu i paracetamolu – konwencjonalnych niesterydowych leków przeciwzapalnych w buforze PBS oraz wodzie powierzchniowej. Leki stosowano w stężeniach $10^{-6}$; $10^{-7}$; $10^{-8}$; $10^{-9}$ and $10^{-10}$ mg/dm$^3$, z czasem inkubacji bakterii 3 i 24 godziny. Wyniki eksperymentu wykazały zróżnicowaną wrażliwość trzech różnych konstrukcji genowych na badane leki. Szczepy bakterii oraz *RecA, kat G* i *sodA* promotory okazały się dobrymi bioindykatorami monitorowanego cyto- i genotoksycznego efektu testowanych leków w buforze PBS i wodzie powierzchniowej. Uzyskane rezultaty wskazują na potencjalną użyteczność stosowanych w pracy bakteryjnych biosensorów w monitorowaniu pozostałości farmaceutycznych leków w środowisku.

**Słowa kluczowe:** monitoring środowiskowy, niesterydowe przeciwzapalne leki, cytotoksyczność, genotoksyczność, *gfp* biosensory

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# FRACTIONATION OF HEAVY METALS IN BOTTOM SEDIMENTS AND SEWAGE SLUDGES USING SEQUENTIAL EXTRACTION

## FRAKCJONOWANIE METALI CIĘŻKICH W OSADACH DENNYCH I ŚCIEKOWYCH Z UŻYCIEM EKSTRAKCJI SEKWENCYJNEJ

**Abstract:** In order to determine the forms of heavy metals in bottom sediment or sewage sludge the speciation analysis is performed. The analysis is based on the sequential extraction of metals with increasingly aggressive solvents. The five steps extraction proposed by Tessier et al gained wide recognition. It extracts metals in following groups: exchangeable, associated with carbonates, with hydrated iron oxides and manganese oxides, with organic matter and metals that can be found in the residual fraction. Metals that can be found in the two first fractions (exchangeable and carbonate) are believed to be mobile. As a result of research carried out by the Standards, Measurements and Testing Programme of the European Commission, formerly the Community Bureau of Reference, a shorter, three-stage extraction procedure known as the BCR procedure was accepted.

Comparison of heavy metal (Zn, Cu, Ni, Pb, Cd, Cr) fractionation methods was done. Two different sequential extraction methods were investigated: Tessier, and BCR method. For the experiment following materials were used: certified reference material LGC6181, sewage sludge were collected from mechanical-biological municipal wastewater treatment plant located in Czestochowa, and bottom sediment from the Poraj Reservoir. After results comparison, it was stated that content of particular chemical forms of heavy metals in total amount in certified material, sewage sludge and bottom sediment vary depending on used extraction method. In case of certified material differences referred to cadmium concentrations in exchangeable-carbonate, iron and manganese oxides, and zinc in residual (insoluble compounds) fractions. Also in sewage sludge and bottom sediment cadmium concentrations measured in exchangeable-carbonate, in organic-sulfide, and in residual (insoluble compounds) fractions after extraction according to Tessier method, did not equal to values obtained after use of BCR extraction method. This also applied to zinc and lead concentrations in iron and manganese oxides fraction. The discrepancy between the results could be explained with both: used extractants, and extraction conditions (different reagents, temperature, and time). The results point out how important is the choice of a proper extraction method depending on the aim of speciation analysis but also depending on the analyzed chemical forms of heavy metals.

**Keywords:** heavy metals, sequential extraction procedures, bottom sediment, sewage sludge

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## Introduction

In order to determine heavy metal forms in soils, bottom sediments or sewage sludge, which affect mobility and bioavailability of metals, speciation analysis is performed, based on sequential extraction. It is based on gradual release of metals from soil or sludge by solutions with increasing aggressiveness [1, 2]. For each step reagents are chosen, which are able to extract a group of metal connections with known properties. Specific chemical elements in a particular fraction are not identified, only the fraction as a whole. Most often this fractions are distinguished: exchangeable, carbonate, iron and manganese oxides, organic and sulfide, residue (metals embedded in the crystalline network of primary and secondary minerals, particularly silicates). Metals found in the first two fractions are considered to be mobile, those in the two following – temporarily immobilized. The iron and manganese oxides fraction is sensitive to changes in redox potential, whereas metals bound to organic matter are released during the mineralization process of this substrate.

First complex and still utilized procedure of sequential extraction of heavy metals from bottom sediment samples taken from natural water environment (river) was developed by Tessier, Campbell and Bisson [3]. This procedure was repeatedly discussed and underwent numerous modifications. The modifications were mainly related to the used reagents and the extraction conditions. Modification of the Tessier procedure proposed by Zerbe et al [4] consisted in the introduction of different reagent for extracting metals from exchangeable fraction. It was 1 M $CH_3COONH_4$. The modification proposed by Perez Cid et al [5] involved the usage of microwaves to support the extraction. In this way the time of the process was reduced, with the use of the same reagents, from 17 hours and 56 minutes down to 30 minutes. Different modification was presented by Gomez Ariza et al [6] introducing higher concentration of the reducing agent – 0.4 M $NH_2OH{\cdot}HCl$. A method with an entirely different procedure was proposed by Stover et al [7], a six-step extraction designed for metal fractionation in anaerobically stabilised sewage sludge, which enabled the determination of metals separately in organic and sulfide fraction. After the extraction of metals from exchangeable fraction (1 M $KNO_3$, pH = 7) and adsorbed (0.5 M KF, pH = 6.5) the Authors proposed extraction from organic fraction (0.1 M $Na_4P_2O_7$), carbonate (0.1 M EDTA, pH = 6.5), and then from sulfide (1 M $HNO_3$) and residue ($HNO_3$ + HCl). The Authors did not include the stage of oxides fraction metal separation. For a complete dissolution of resistant sulfides Rudd et al [7] performed a modification of Stover method by increasing the concentration of $HNO_3$ acid from 1 M to 6 M.

As a result of the work of the Standards, Measurements and Testing Programme of the European Union Commission, a shortened, three-stage extraction was adopted, known as the BCR procedure (from previous name of this Commission – Community Bureau of Reference) [8–10]. The modification of this procedure was to increase the concentration of the $NH_2OH{\cdot}HCl$ reagent solution from 0.1 to 0.5 M for the metal extraction from reducible fraction – iron and manganese oxides. For a more complete extraction of metals from this fraction not only an increase in reagent concentration was proposed, but also lowering the pH from 2 down to 1.5 with the usage of $HNO_3$ [11, 12].

In the literature there are also BCR procedure modifications, which include the application of microwave energy or ultrasonic waves for intensification of the extraction process [13, 14].

The course of extraction is influenced by many factors: type of examined sample, its pH, degree of fragmentation, extraction period, solid mass to solution ratio, temperature, chemical properties and selectivity of chosen extractants, order of particular extraction steps.

Table 1

Application of sequential extraction procedures to sediment and sewage sludge (selected examples)

| Procedure | Matrix | Elements determined | Reference |
|---|---|---|---|
| Tessier | Sediments of the Nile River (Egypt) | Cd, Cu, Cr, Fe, Pb, Mn, Ni, Zn | [18] |
| BCR | Sediments of Jinjiang River (China) | Cr, Ni, Cu, Zn, Cd, Pb | [19] |
| BCR | Sediments of the Deule River (France) | Cd, Co, Cu, Fe, Ni, Pb, Sr, Zn | [20] |
| Tessier | Sediments of Bharali River (India) | Zn, Cu, Ni, Pb, Cd, Fe, Mn | [21] |
| BCR | Sediments of Jarama River (Spain) | Zn, Cu, Ni, Pb, Cd, Fe, Mn | [22] |
| BCR | Sediments of Titicaca Lake (Bolivia) | Cu, Fe, Ni, Co, Mn, Cd, Pb, Zn | [23] |
| BCR | Sediments of Nashina Lake (China) | Cd, Cr, Cu, Mn, Ni, Pb, Zn | [24] |
| Tessier | Sediments of Qarun Lake (Egypt) | Fe, Mn, Zn, and Cu | [25] |
| BCR | Sediments of Quamzhou Bay (China) | Cu, Zn, Cr, Co, Fe, Mn, V, Pb | [26] |
| BCR | Sediments of Xiamen Bay (China) | Cu, Pb, Zn, Cd, Cr | [27] |
| BCR | Sediments of Gowatr Bay (Iran) | Cu, Pb, Zn, Mn, Ni, Co, Cr, V | [28] |
| BCR | Sediments of Akyatan Lagoon (Turkey) | Cd, Cr, Cu, Mn, Ni, Pb, Zn | [29] |
| BCR | Six different sewage sludges (Spain) | Cu, Cr, Ni, Fe, Zn, Pb, Cd | [30] |
| BCR | Five different sewage sludges (Spain) | Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb | [31] |
| BCR | Five different sewage sludges (China) | Cu, Zn, Pb, Cd | [32] |
| BCR | Anaerobic sewage sludge (Turkey) | Cd, Cr, Cu, Mn, Pb, Ni, Zn, Fe | [33] |
| Tessier | Five different sewage sludges (Egypt) | Cd, Cu, Cr, Fe, Mn, Ni, Zn | [34] |
| BCR | Liquefaction residues of sludge (China) | Cu, Zn, Pb, Cd, Cr, Ni | [35] |
| BCR | Sewage sludge treatment wetlands (Italy) | Cr, Cu, Ni, Cd, Pb, Zn | [36] |
| Tessier | Composting of sewage sludge (Greece) | Cu, Ni, Pb, Zn, Mn, Fe | [37] |

Since the development of the sequential extraction procedures, they were and still are subject to criticism, mostly for the lack of certainty as to the selectivity of the applied reagents, the possibility of metal readsorption, the utilization of different sample preparation methods, *ia* the way and time of drying, grinding, homogenising the material, as well as conditions under which the extraction is conducted [9]. Presented in the literature criticism of the procedures proves that there is no extraction scheme which could be applied to every speciation study of heavy metals. It is common to obtain different results after applying different extraction schemes [15, 16]. Studies on the extraction procedures are being continued, aiming to improve the selectivity of the eluents, and the quality and repeatability of the results. Despite numerous disadvantages, the sequential extraction method is an important source of information about activity or stability of heavy metals in fractions of examined material, which reflects on the assessment of metal behaviour mechanisms in the environment, *ia* their mobility, moving, bioavailability [2, 17]. Examples of application of the Tessier and the BCR sequential extraction in research on heavy metal fractionation in bottom sediment and sewage sludge are shown in Table 1.

## Materiałs and methods

As a research material, bottom sediment and sewage sludge were used. The bottom sediment was collected from the Poraj Dam Reservoir fed by the Warta River. The sewage sludge originated from municipal wastewater treatment plant, and it was dewatered mechanically on a belt press, after being biochemically stabilized during mesophilic digestion process. The sludge and the sediment were dried in the laboratory in a dried (105°C), grinded and sieved through a 0.4 mm mesh. In the research certified reference material LGC6181 (sewage sludge) was also used.

In order to determine total heavy metal content, the sludge, the sediment and the reference material mineralization was conducted, using a mixture of concentrated acids: nitric and hydrochloric (1 + 3). The mineralization was conducted for 2 hours in the temperature of 120$^o$C (Vario compact thermostat produced by Machery Nagel). For quantitative determination of heavy metals occurring in particular chemical forms in sludge, sediment, and certified material, sequential extraction according to the Tessier and the BCR procedures were applied – Table 2.

Table 2

Sequential extraction procedures

| Chemical reagents and conditions (Tessier procedure) | Chemical reagents and conditions (BCR procedure) | Forms of metals |
|---|---|---|
| 8 cm$^3$ 1 M $MgCl_2$, pH = 7, temp. 22$^o$C, shaking 1 h | 40 cm$^3$ 0.11 M $CH_3COOH$, temp. 22$^o$C, shaking 16 h | Exchangeable |
| 8 cm$^3$ 1 M $CH_3COONa$, pH = 5 with $CH_3COOH$, temp. 22$^o$C, shaking 5 h | | Bounded with carbonates |
| 20 cm$^3$ 0.04 M $NH_2OH \cdot HCl$ in 25% (v/v) $CH_3COOH$, temp. 96$^o$C, shaking 6 h | 40 cm$^3$ 0.5 M $NH_2OH{\cdot}HCl$, temp. 22$^o$C, shaking 16 h | Bounded with Fe and Mn oxides |
| 3 cm$^3$ 0.02 M $HNO_3$ i 5 cm$^3$ 30% $H_2O_2$, pH = 2, temp. 85$^o$C, shaking 2 h,<br>3 cm$^3$ 30% $H_2O_2$, temp. 85$^o$C, shaking 3 h<br>5 cm$^3$ 3.2 M $CH_3COONH_4$ in 20% (v/v) $HNO_3$, temp. 22$^o$C, shaking 0.5 h | 10 cm$^3$ 8.8 M $H_2O_2$, temp. 22$^o$C, 1 h; temp. 85$^o$C, 1 h<br>10 cm$^3$ 8.8 M $H_2O_2$, temp. 85$^o$C, 1 h<br>50 cm$^3$ 1 M $CH_3COONH_4$, temp. 22$^o$C, shaking 16 h | Bounded with organic matter – sulfides |
| 2 cm$^3$ 65% $HNO_3$ + 6 cm$^3$ 36% HCl, temp. 120$^o$C, 2 h | 2 cm$^3$ 65% $HNO_3$ + 6 cm$^3$ 36% HCl, temp. 120$^o$C, 2 h | Residual |

The preparation of the necessary reagents and the extraction procedure was carried out according to [8]. In case of the Tessier procedure, unlike described in [3], for metal extraction from residue fraction concentrated acids $HNO_3$ and HCl were used, instead of HF and $HClO_4$.

Concentration of heavy metals: zinc, copper, nickel, cadmium, lead, and chromium, in obtained eluents, were determined by means of atomic absorption spectrometry (novAA 400 spectrometer produced by Analytic Jena). The analyses were carried out in triplicate.

The method for result quality control is to compare the analyses results for particular elements content to the total metal content, determined independently [38]. The overall concentration of metals in the sludge (Total) determined after mineralization with aqua regia was compared with the sum of metal concentrations in the extracted fractions ($F1 + F2 + F3 + F4$). Recovery in the sequential extraction procedure was calculated as follows:

$$\text{Recovery} = \frac{F1+F2+F3+F4}{\text{Total}} \cdot 100\%$$

## Results and discussion

It was stated that depending on the applied extraction method, the portion of analyzed chemical forms of particular heavy metal in the total content alike in the bottom sediment, in the sewage sludge, and in the certified material differed considerably – Table 3.

The biggest disparity in results was obtained with respect to cadmium content. In case of the bottom sediment, differences were regarding the amount of this metal in exchangeable-carbonate and organic-sulfide fraction, in the certified material – in exchangeable-carbonate and iron and manganese oxides fraction, while in the sewage sludge they included content in all fractions. Applying the extraction according to Tessier, the highest cadmium content in the bottom sediment was determined in exchangeable-carbonate fraction (52% of total content), whereas using the BCR method in residue fraction (33%). In case of the sewage sludge, the highest cadmium content was observed in iron and manganese oxides fraction (39%) and organic-sulfide (49%), using the Tessier and the BCR procedure respectively.

Zinc content in exchangeable-carbonate and organic-sulfide fraction of the bottom sediment had similar values obtained using both procedures, equal respectively to 27 and 30%, and 11 and 13% of total amount, while in the sewage sludge both in those fractions and in iron and manganese oxides fraction different results were obtained. In the sewage sludge, the highest zinc content was obtained in iron and manganese oxides fraction (45% of total content) using the procedure according to Tessier, while in organic-sulfide fraction (50%) using the BCR procedure. Only in residue fraction of the sewage sludge, similar content of this metal was obtained using both procedures (17 and 18%). In the certified material, the highest correspondence occurred in exchangeable-carbonate fraction, while the largest differences in residue fraction.

In case of copper and chromium content determination, in all fractions of the bottom sediment and the sewage sludge, while nickel and chromium in fractions of the certified material, consistence of the results were obtained using both procedures. However different nickel and lead content in exchangeable-carbonate fraction of the bottom

Table 3

Content of heavy metals in chemical fractions of bottom sediment, sewage sludge and certified reference material LGC6181

| Metal | Fraction | Content in bottom sediment | | | | Content in sewage sludge | | | | Content in reference material | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | “Tessier” | | extraction “BCR” | | extraction “Tessier” | | extraction “BCR” | | extraction “Tessier” | | extraction “BCR” | |
| | | [mg/kg] | [%] | [mg/kg] | [%] | [mg/kg] | [%] | [mg/kg] | [%] | [mg/kg] | [%] | [mg/kg] | [%] |
| Zn | *F*1 | 16.0 ± 1.2 | 26.6 | 17.2 ± 0.9 | 29.9 | 432 ± 5 | 16.4 | 146 ± 4 | 5.4 | 284 ± 4 | 26.4 | 258 ± 4 | 24.4 |
| | *F*2 | 17.8 ± 0.8 | 29.6 | 11.5 ± 0.5 | 20.1 | 1180 ± 12 | 44.7 | 704 ± 7 | 26.0 | 326 ± 6 | 30.3 | 390 ± 5 | 36.9 |
| | *F*3 | 6.8 ± 0.6 | 11.3 | 7.7 ± 0.4 | 13.4 | 576 ± 4 | 21.8 | 1360 ± 9 | 50.3 | 165 ± 3 | 15.3 | 232 ± 2 | 22.0 |
| | *F*4 | 19.6 ± 0.7 | 32.5 | 21.0 ± 1.4 | 36.6 | 452 ± 7 | 17.1 | 494 ± 6 | 18.3 | 302 ± 5 | 28.0 | 176 ± 3 | 16.7 |
| Cu | *F*1 | 1.3 ± 0.2 | 30.9 | 1.1 ± 0.1 | 25.0 | 7.2 ± 0.4 | 2.4 | 5.1 ± 0.3 | 1.7 | 29.5 ± 0.5 | 9.2 | 16.2 ± 0.9 | 5.0 |
| | *F*2 | 1.0 ± 0.1 | 23.8 | 1.1 ± 0.2 | 25.0 | 9.4 ± 0.7 | 3.1 | 8.2 ± 0.2 | 2.7 | 11.8 ± 0.3 | 3.7 | 7.1 ± 1.2 | 2.2 |
| | *F*3 | 0.8 ± 0.1 | 19.1 | 0.9 ± 0.1 | 20.5 | 234 ± 9 | 78.4 | 248 ± 8 | 81.5 | 216 ± 5 | 67.6 | 243 ± 8 | 75.2 |
| | *F*4 | 1.1 ± 0.2 | 26.2 | 1.3 ± 0.1 | 29.5 | 48.0 ± 1.1 | 16.1 | 43.1 ± 0.7 | 14.1 | 62.1 ± 1.6 | 19.5 | 56.7 ± 2.3 | 17.6 |
| Ni | *F*1 | 5.3 ± 0.3 | 23.1 | 6.8 ± 0.3 | 29.3 | 66.2 ± 5.0 | 34.5 | 60.3 ± 6.2 | 32.3 | 14.4 ± 0.4 | 30.0 | 9.5 ± 0.1 | 22.3 |
| | *F*2 | 9.2 ± 0.4 | 40.2 | 9.7 ± 0.3 | 41.8 | 50.7 ± 1.3 | 26.4 | 42.4 ± 0.9 | 22.7 | 7.2 ± 0.2 | 15.0 | 7.3 ± 0.2 | 17.1 |
| | *F*3 | 4.4 ± 0.2 | 19.2 | 2.9 ± 0.1 | 12.5 | 49.1 ± 0.8 | 25.6 | 57.5 ± 2.3 | 30.7 | 12.6 ± 0.2 | 26.3 | 12.9 ± 0.2 | 30.2 |
| | *F*4 | 4.0 ± 0.3 | 17.5 | 3.8 ± 0.2 | 16.4 | 26.0 ± 0.5 | 13.5 | 26.7 ± 0.4 | 14.3 | 13.8 ± 0.3 | 28.7 | 13.0 ± 0.3 | 30.4 |
| Pb | *F*1 | 9.4 ± 0.4 | 45.2 | 8.3 ± 0.5 | 39.0 | 10.4 ± 0.3 | 10.3 | 9.8 ± 1.1 | 9.6 | 15.7 ± 0.4 | 14.5 | 9.3 ± 0.5 | 8.7 |
| | *F*2 | 2.5 ± 0.2 | 12.0 | 3.4 ± 0.2 | 15.9 | 5.8 ± 0.3 | 5.7 | 7.2 ± 0.2 | 7.0 | 3.5 ± 0.3 | 3.3 | 6.1 ± 0.4 | 5.7 |
| | *F*3 | 1.7 ± 0.2 | 8.2 | 2.0 ± 0.1 | 9.4 | 4.2 ± 0.2 | 4.2 | 7.4 ± 0.3 | 7.2 | 3.8 ± 0.1 | 3.5 | 14.7 ± 0.3 | 13.7 |
| | *F*4 | 7.2 ± 0.4 | 34.6 | 7.6 ± 0.3 | 35.7 | 80.5 ± 1.5 | 79.8 | 78.1 ± 2.3 | 76.2 | 85.1 ± 1.1 | 78.7 | 77.2 ± 0.7 | 71.9 |

Table 3 contd.

| Metal | Fraction | Content in bottom sediment | | | | Content in sewage sludge | | | | Content in reference material | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | “Tessier” | | extraction “BCR” | | extraction “Tessier” | | extraction “BCR” | | extraction “Tessier” | | extraction “BCR” | |
| | | [mg/kg] | [%] | [mg/kg] | [%] | [mg/kg] | [%] | [mg/kg] | [%] | [mg/kg] | [%] | [mg/kg] | [%] |
| Cd | *F*1 | 1.3 ± 0.2 | 52.0 | 0.7 ± 0.2 | 29.2 | 1.7 ± 0.1 | 20.8 | 0.9 ± 0.1 | 11.8 | 2.7 ± 0.2 | 44.3 | 1.1 ± 0.1 | 18.6 |
| | *F*2 | 0.4 ± 0.1 | 16.0 | 0.3 ± 0.1 | 12.5 | 3.2 ± 0.3 | 39.0 | 1.4 ± 0.2 | 18.4 | 1.8 ± 0.1 | 29.5 | 2.9 ± 0.2 | 49.2 |
| | *F*3 | 0.3 ± 0.1 | 12.0 | 0.6 ± 0.1 | 25.0 | 1.1 ± 0.1 | 13.4 | 3.7 ± 0.3 | 48.7 | 0.7 ± 0.1 | 11.5 | 1.2 ± 0.3 | 20.3 |
| | *F*4 | 0.5 ± 0.1 | 20.0 | 0.8 ± 0.2 | 33.3 | 2.2 ± 0.2 | 26.8 | 1.6 ± 0.1 | 21.1 | 0.9 ± 0.2 | 14.7 | 0.7 ± 0.1 | 11.9 |
| Cr | *F*1 | 0.3 ± 0.1 | 14.3 | 0.4 ± 0.1 | 16.0 | 3.8 ± 0.2 | 1.0 | 4.2 ± 0.4 | 1.1 | 5.4 ± 0.3 | 7.7 | 3.4 ± 0.2 | 4.8 |
| | *F*2 | 0.5 ± 0.1 | 23.8 | 0.4 ± 0.1 | 16.0 | 10.6 ± 0.3 | 2.7 | 9.0 ± 0.3 | 2.2 | 8.1 ± 0.2 | 11.5 | 4.5 ± 0.1 | 6.4 |
| | *F*3 | 0.7 ± 0.2 | 33.3 | 0.9 ± 0.1 | 36.0 | 272 ± 4 | 69.8 | 302 ± 9 | 74.3 | 37.3 ± 0.3 | 53.1 | 42.5 ± 0.5 | 60.3 |
| | *F*4 | 0.6 ± 0.1 | 28.6 | 0.8 ± 0.1 | 32.0 | 103.1 ± 5.2 | 26.5 | 91.1 ± 3.2 | 22.4 | 19.4 ± 0.4 | 37.7 | 20.1 ± 0.7 | 28.5 |

Fraction: *F*1– exchangeable and carbonates-bound, *F*2 – Fe/Mn oxides-bound, *F*3 – organic matter/sulfides-bound, *F*4 – residual.

sediment were obtained, as well as in iron and manganese oxides and organic-sulfide fraction of the sewage sludge.

Obtainment of different results depending on the applied method is also confirmed by other research [10, 15, 39]. The reason for discrepancies in the obtained results can be both used different extractants, and conditions of conducting the extraction (temperature and time period). The characteristics of reagents used in the procedures of sequential extraction with critical justification for their utilization were presented by Gleyzes et al [9]. The Authors stated among others that due to different solubility of each metal carbonates, their release may be incomplete and continued in next step. In the procedure according to Tessier, this inconvenience can be corrected with the usage of sodium acetate solution with pH = 4.74, and ensuring the material-solution ratio 1:25, moreover by increasing the time of the extraction. In case of iron and manganese oxides fraction, with high iron content, there may occur incomplete dissolution of oxides (lowering the metal content in this fraction), or organic metal complexes may be extracted, which cover iron oxides (raising the content). Some of the sequential extraction procedures enable dividing this fraction into: easily reducible (Mn(III)/(IV) oxides), medium-reducible (amorphous Fe(III) oxides), and hardly reducible (crystalline Fe(III) oxides) [2]. The efficiency of the extractant depends on its reducing capabilities expressed by the value of redox potential ($E_h$). Hydroxylamine is a preferred extractant, with $E_h = -1.87$ V as a solution in 25% acetic acid.

Oxidizing reagents used for heavy metal extraction from organic fraction may also lead to sulfide oxidation, which is why this fraction is also often called organic-sulfide (introduction of ammonium acetate prevents readsorption of released metal ions). However oxidation of organic matter resistant to high temperatures may not occur. As the oxidant, 30% $H_2O_2$ is the most often used, acidified to pH = 2.0 with nitric acid. This reagent additionally causes the oxidation of metal sulfides. Elevated temperature favours the decomposition of organic matter. Solution of sodium chlorate (I) NaClO (pH = 8.5–9.5) or potassium pyrophosphate $K_4P_2O_7$ (pH = 10) is less commonly used.

The fraction of residue metals includes mainly metals embedded into the crystal lattice of primary and secondary minerals included in sludge. There are mainly metals of silicate and aluminosilicate minerals, as well as metals which have not been extracted in earlier stages of the sequential extraction. Under natural conditions, this metals can be considered as permanently immobilized.

In order to extract the metals, the crystal lattice of stable minerals must be destroyed by means of concentrated solutions of inorganic acids ($HClO_4$, $HNO_3$, HCl) or their mixtures at elevated temperature, however the decomposition of silicate minerals takes place in the presence of HF acid.

Total heavy metal content determined after mineralization of the analysed bottom sediment and sewage sludge using a mixture of concentrated acids: nitric and hydrochloric, and the total calculated based on determined quantities in each chemical fractions are presented in Table 4.

The heavy metal content obtained by adding their amount in each chemical fractions of the bottom sediment, the sewage sludge and the certified material, determined both after the extraction with the procedure according to the Tessier, and the BCR procedure,

Table 4

Total content of heavy metals in sewage sludge, bottom sediment and certified reference material

| Material | Metal | Total content [mg/kg] | Σ F1 + F2 + F3 + F4 (extraction "Tessier") | | Σ F1 + F2 + F3 + F4 (extraction "BCR") | |
|---|---|---|---|---|---|---|
| | | | Content [mg/kg] | Recovery [%] | Content [mg/kg] | Recovery [%] |
| Bottom sediment | Zn | 55.9 ± 2.1 | 60.2 | 107.7 | 57.4 | 102.7 |
| | Cu | 4.6 ± 0.5 | 4.2 | 91.3 | 4.4 | 95.6 |
| | Ni | 21.4 ± 0.3 | 22.9 | 107.0 | 23.2 | 108.4 |
| | Pb | 22.2 ± 0.4 | 20.8 | 93.7 | 21.3 | 95.9 |
| | Cd | 2.1 ± 0.2 | 2.5 | 119.0 | 2.4 | 114.3 |
| | Cr | 2.2 ± 0.3 | 2.1 | 95.5 | 2.5 | 113.6 |
| Sewage sludge | Zn | 2863 ± 22 | 2640 | 92.2 | 2704 | 94.4 |
| | Cu | 309.0 ± 8.1 | 298.6 | 96.6 | 304.4 | 98.5 |
| | Ni | 204.0 ± 7.4 | 192.0 | 94.1 | 186.9 | 91.6 |
| | Pb | 109.1 ± 5.2 | 100.9 | 92.5 | 102.5 | 94.0 |
| | Cd | 7.7 ± 0.5 | 8.2 | 106.5 | 7.6 | 98.7 |
| | Cr | 431.0 ± 11.2 | 389.5 | 90.4 | 406.3 | 94.3 |
| Certified reference material LGC6181 | Zn | 1100 ± 50 | 1077 | 97.9 | 1056 | 96.0 |
| | Cu | 354.0 ± 18.0 | 319.4 | 90.2 | 323.0 | 91.2 |
| | Ni | 45.0 ± 3.0 | 48.0 | 106.7 | 42.7 | 94.9 |
| | Pb | 105.0 ± 8.0 | 108.1 | 102.9 | 107.3 | 102.2 |
| | Cd | 5.8 ± 0.3 | 6.1 | 105.2 | 5.9 | 101.7 |
| | Cr | 78.0 ± 8.0 | 70.2 | 90.0 | 70.5 | 90.4 |

did not differ significantly from content determined after direct mineralization with a mixture of concentrated acids $HNO_3$ and HCl. Total content of zinc, copper, nickel and lead in four analyzed fractions amounted to 91–108%, whereas cadmium and chromium 90–119% of their total quantity. This proves the correctness of applied research methodology and the credibility of the obtained results [32, 40]. The best compatibility of total metal content and the sum in individual fractions was obtained for lead in the bottom sediment, copper in the sewage sludge, and zinc and lead in the certified material.

## Conclusions

Analysis of heavy metal chemical forms in an examined material depends on the amount of stages of used method, type and concentration of reagents, extraction conditions (temperature, time period, pH). Used sequential extraction procedures refer to group metal discharge and not always are selective with regard to particular heavy metals. The choice of suitable extraction method is important, depending on the purpose of conducted speciation analysis and analyzed chemical forms of heavy metals. It is also crucial to strictly follow the extraction conditions and to compare the results obtained with the same extraction procedure for heavy metal fractionation.

During the conducted research, different proportion of certain chemical forms of heavy metals in their total content in the bottom sediment, the sewage sludge and the certified material, was obtained, depending on the applied extraction procedure (Tessier, BCR). The discrepancy of the obtained results referred mainly to zinc and cadmium, to a lesser extent to copper and chromium. While a high correspondence of the total metal content in particular fractions with total content determined after direct mineralization was observed.

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## FRAKCJONOWANIE METALI CIĘŻKICH W OSADACH DENNYCH I ŚCIEKOWYCH Z UŻYCIEM EKSTRAKCJI SEKWENCYJNEJ

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**Abstrakt:** W celu określenia form chemicznych metali ciężkich w osadach dennych, czy też w osadach ściekowych wykonuje się analizę specjacyjną opartą na ekstrakcji sekwencyjnej, która polega na stopniowym wydzielaniu metali z osadów roztworami o wzrastającej agresywności. Szerokie uznanie zdobyła pięciostopniowa ekstrakcja zaproponowana przez Tessiera i współpracowników, której zastosowanie umożliwia wydzielenie metali wymienialnych, związanych z węglanami, z uwodnionymi tlenkami żelaza i manganu, z materią organiczną oraz pozostałych. Za mobilne uważa się metale występujące w dwóch pierwszych frakcjach (wymiennej i węglanowej). W wyniku prowadzonych prac w ramach Programu Pomiarów i Testowania w Komisji Unii Europejskiej przyjęto skróconą, trzy etapową ekstrakcję, znaną jako procedura BCR.

Przeprowadzono porównawcze badania frakcjonowania metali ciężkich (Zn, Cu, Ni, Pb, Cd, Cr), wykorzystując ekstrakcję sekwencyjną stosowaną przez Tessiera oraz procedurę BCR. Materiałem badawczym były: materiał certyfikowany LGC 6181, osad ściekowy pochodzący z mechaniczno-biologicznej oczyszczalni ścieków komunalnych w Częstochowie oraz osad denny pobrany ze zbiornika zaporowego Poraj. Porównując wyniki, stwierdzono różny udział określonych form chemicznych metali ciężkich w całkowitej ich zawartości w badanych próbkach w zależności od zastosowanej procedury ekstrakcji. Dla materiału certyfikowanego rozbieżności dotyczyły głównie zawartości kadmu we frakcji wymienno-węglanowej oraz tlenków żelaza i manganu, cynku we frakcji pozostałościowej (związków praktycznie nierozpuszczalnych). Również w osadzie ściekowym i osadzie dennym zawartość kadmu oznaczona we frakcjach wymienno-

-węglanowej, organiczno-siarczkowej oraz związków praktycznie nierozpuszczalnych po ekstrakcji metodą Tessiera nie pokryła się z wartościami uzyskanymi po ekstrakcji metodą BCR. Dotyczyło to także zawartości cynku i ołowiu we frakcji tlenków żelaza i manganu. Przyczynami rozbieżności uzyskanych wyników mogły być zarówno użyte ekstrahenty, jak i warunki prowadzenia ekstrakcji (różne reagenty, temperatura i czas). Potwierdza to, jak ważny jest dobór odpowiedniej metody ekstrakcji w zależności od celu prowadzonej analizy specjacyjnej i analizowanych form chemicznych metali ciężkich.

**Słowa kluczowe:** metale ciężkie, procedury ekstrakcji sekwencyjnej, osad denny, osad ściekowy

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# THE SULFUR CONTENT IN SOIL AFTER APPLICATION OF COMPOSTED MATERIALS CONTAINING FOILS

## ZAWARTOŚĆ SIARKI W GLEBACH PO APLIKACJI PRZEKOMPOSTOWANYCH MATERIAŁÓW Z DODATKIEM FOLII

**Abstract:** The aim of the research was to assess the content of total and assimilable forms of sulphur in soil after application of composted materials with the addition of polyethylene and corn starch foils. The experimental design consisted of 7 treatments carried out in 3 replications on two soils: 0 – non-fertilized soil, NPK – soil fertilized with mineral fertilizers, K1 – soil fertilized with composted material without the addition of foil, K2 – soil fertilized with composted material with the addition of foil A (which included 47.5% polyethylene C + 45% corn starch + 7.5% compatibilizer), K3 – soil fertilized with composted material with the addition of foil B (which included 65% polyethylene C + 30% corn starch + 5% compatibilizer), K4 – soil fertilized with composted material with the addition of foil C (which included 65% polyethylene C + 30% corn starch + 5% compatibilizer and copolymer), and K5 – soil fertilized with composted material with the addition of foil C and microbiological inoculum. The experiments were conducted in soils with the granulometric composition of light loam and medium loam. Cock's-foot was the test plant. The mean yield of *Dactylis glomerata* L., collected from the treatments located in soil with the granulometric composition of light loam, was between 1.4 and 2.4 Mg d.m. $\cdot$ ha$^{-1}$ and between 1.8 and 3.6 Mg d.m. $\cdot$ ha$^{-1}$ for treatments conducted in soil with the granulometric composition of medium loam. Total S content was determined after sample mineralization in a chamber furnace at 450°C for 8 h, after prior binding of sulphur sulfate $Mg(NO_3)_2$. Assimilable forms of S were extracted with solution of 0.03 mol $\cdot$ dm$^{-3}$ $CH_3COOH$. The S content in the obtained solutions and extracts was determined using the ICP-OES method. The highest content of total forms of sulphur was determined in soil with the granulometric composition of medium loam, to which composted materials K3 and K4 (220 mgS $\cdot$ kg$^{-1}$ d.m. of soil) were introduced. The content of assimilable forms of sulphur ranged from 8.3 to 12.9 mgS $\cdot$ kg$^{-1}$ d.m. of soil in the case of treatments located in soil with the granulometric composition of light loam, and from 13.1 to 17.4 mgS $\cdot$ kg$^{-1}$ d.m. of soil for medium loam treatments.

**Keywords:** soil, compost, sulphur, polyethylene, corn starch, *Dactylis glomerata* L.

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## Introduction

As a result of pro-environmental actions (aiming at restricting the emission of sulphur compounds to the atmosphere by industry), limiting the use of natural and organic fertilizers, and increasing the acreage of plants with high demands for sulphur, an increasing sulphur deficiency in soil has been observed in many countries (including Poland) [1–3]. Forecasts made the Sulphur Institute indicate that the global deficit of this element in 2015 will amount to 12.5 mln Mg [4]. Taking into account the problem of sulphur deficiency in soils, the need for fertilization with this element is becoming more and more important [5]. It is necessary to take innovative and effective actions to improve the balance of this element in Polish soils. There is also a real need to provide plants with adequate amounts of sulphur (through fertilization).

A way to improve the nutrient balance in soil, including sulphur, may be to apply composts (among other things), and using biodegradable waste for composting may solve many problems connected with waste management [6]. Mineralization and humification of organic matter, which take place during biological processing of biomass, lead to significant changes in physical, chemical, and biological properties of the composted biomass [7]. An addition of structure-forming materials (*eg* post-use polymeric materials) may have a positive effect on the rate and direction of the process. Plastics waste constitutes a serious problem (both ecological and economic) many EU Member States have been wrestling with for many years [8], and composting of this waste may find wide application in decomposition of such materials [9].

Composting of polymeric materials is an alternative solution which will undoubtedly contribute to reducing the stream of waste getting into landfills [10]. However, the rate of degradation of such materials is influenced by a lot of factors, such as their chemical structure, molecular weight, supermolecular structure, physical properties, and the degree of size reduction [11]. In recent years there have been many studies on developing polymeric materials that would undergo biodegradation in the natural environment. One way is to diversify their chemical composition by addition of a biocomponent which can significantly diversify properties of polymers and in consequence decide on their susceptibility to disintegration and biodegradation [12].

Taking into account the possibility of natural use of products (obtained in the composting process) with the addition of polyethylene and corn starch foils, in terms of improving the balance of nutrients (including sulphur) in soil, studies have been conducted aiming at evaluating the content of selected sulphur forms in soil.

## Material and methods

The assessment of the effect of application of composted plant materials with the addition of polyethylene and corn starch foils to soil on the content of total and assimilable forms of sulphur in the soil was conducted in field experiment conditions. The experiments were located at the Experimental Station of the Faculty of Agriculture and Economics in Krakow in Mydlniki. The research was conducted in 2013 in soil with the granulometric composition of light loam (hereinafter referred to as light soil) and of medium loam (hereinafter referred to as medium soil). Selected properties of the soil material prior to the commencement of the research are presented in Table 1.

Table 1

Some chemical properties of soils material before establishment of experiments (0–20 cm)

| Determination | Unit | Light loam | Medium loam |
|---|---|---|---|
| pH in $H_2O$ | — | 7.03 ± 0.02* | 6.84 ± 0.02 |
| pH in KCl | — | 5.60 ± 0.51 | 5.95 ± 0.40 |
| Electrolytic conductivity | $[mS \cdot cm^{-1}]$ | 0.05 ± 0.01 | 0.02 ± 0.01 |
| Organic C | $[g \cdot kg^{-1}$ d.m.] | 9.23 ± 0.01 | 9.24 ± 0.02 |
| Total N | | 0.84 ± 0.03 | 1.02 ± 0.01 |
| Total S | | 0.10 ± 0.02 | 0.15 ± 0.07 |

* ± standard deviation, n = 3.

The micro-plot experiments were set up with the randomized blocks method. The area of a plot was 1 $m^2$ (which was dictated by a reduced amount of produced materials). Doses of the composted materials were calculated based on nitrogen content in them (Table 4). Phosphorus and potassium were topped up to the highest amounts amended with composted material. These two elements were applied in the form of enriched triple superphosphate and potassium salt. Mineral components in the treatment marked as NPK were used in equivalent doses to those in treatments where composted materials were used but in the form of mineral fertilizers (N – ammonium nitrate, P – enriched triple superphosphate, and K – potassium salt). In all the treatments (except the control treatment), total NPK doses introduced before sowing (with composted materials and with mineral fertilizers) and for top dressing after harvest of the first and second cuts (mineral fertilization) were: 170 $kgN \cdot ha^{-1}$, 40 $kgP \cdot ha^{-1}$, and 120 $kgK \cdot ha^{-1}$. After application of composted materials and mineral fertilizers, followed by mixing them with soil, *Dactylis glomerata* L. seeds were sown, and then rolling was conducted.

The experimental design consisted of 7 treatments carried out in three replications:
– 0 – soil without fertilization,
– NPK – soil fertilized with mineral fertilizers (NPK),
– K1 – soil fertilized with composted material I without the addition of foil,
– K2 – soil fertilized with composted material II with the addition of foil A,
– K3 – soil fertilized with composted material III with the addition of foil B,
– K4 – soil fertilized with composted material IV with the addition of foil C,
– K5 – soil fertilized with composted material V with the addition of foil C and microbiological inoculum.

The biomass for composting was prepared from rape straw, wheat straw, freshly chipped corn, and from waste generated during cleaning of pea seeds. The mixture of the crushed and moistened components was prepared assuming a value of the C:N ratio ~ 30:1 as optimal for the conditions of the composting process. The assumed C:N value was obtained at the following proportion of biomass components: corn chips – 13.1 kg d.m., rape straw – 4.3 kg d.m., wheat straw – 8.3 kg d.m., and waste from cleaning of pea seeds – 2.8 kg d.m. After mixing, the materials were moistened to approximately 45%. Selected properties of the components used for biomass preparation are presented in Table 2.

Table 2

Some chemical properties of raw materials used to prepare a mixture of composts

| Material | Dry matter | Organic matter | Total N | Total S |
|---|---|---|---|---|
| | $[g \cdot kg^{-1}]$ | $[g \cdot kg^{-1}$ d.m.] | | |
| Wheat straw | *941.5 ± 1.3* | 996.7 ± 4.3 | 5.2 ± 0.8 | 0.26 ± 0.01 |
| Rape straw | 945.0 ± 2.0 | 990.0 ± 2.0 | 9.2 ± 0.3 | 0.28 ± 0.00 |
| Waste from the cleaning of pea seeds | 905.2 ± 1.9 | 944.3 ± 20.0 | 35.6 ± 0.8 | 0.90 ± 0.01 |
| Corn chips | 932.2 ± 0.6 | 960.1 ± 0.5 | 7.0 ± 0.3 | 0.89 ± 0.07 |

* ± standard deviation, n = 3.

8 per cent (in relation to dry matter of the mixture) of crushed polymeric materials (foils) which had been produced at the Central Mining Institute in Katowice was added to such prepared biomass. The foils used in the research differed in density, share of polyethylene and of corn starch (Table 3). The percentage of polymeric materials introduced to the composted biomass was limited not only due to physical parameters of the used foils, but also due to technological restrictions. Foils F(B) and F(C) had the highest (65%) content of polyethylene C. The foils which were subjected to composting contained 30% corn starch and 5% compatibilizer. On the other hand, foil F(A) contained 45% corn starch and 7.5% compatibilizer.

Table 3

Selected compositions of polymeric films used for composting

| Foil | Polyethylene C | Corn starch | Compatibilizer |
|---|---|---|---|
| | [% d.m.] | | |
| F(A) | 47.5 | 45.0 | 7.5 |
| F(B) | 65.0 | 30.0 | 5.0 |
| F(C) | 65.0 | 30.0 | 5.0 + copolymer |

The following were determined in the materials used for the research, in the soil material before commencement of the field experiments, and in the soil material collected after harvest of the second cut: total nitrogen content by Kjeldahl method after prior $N\text{-}NO_3$ reduction with Devarda's alloy and mineralization of the material sample in concentrated sulphuric acid in an open system; organic carbon content by oxidation-titration method; and total sulphur content was determined after sample mineralization in a chamber furnace at $450^{o}C$ for 12 hours, after prior binding of sulphate sulphur with $Mg(NO_3)_2$ solution. Assimilable forms of sulphur were determined after extraction with $CH_3COOH$ with the concentration of $0.03 \text{ mol} \cdot dm^3$. Sulphur content in the solutions and extracts was determined using the ICP-OES method on a Perkin Elmer Optima 7300 DV instrument (Table 4). The presented results of the analyses are an arithmetic mean from 3 replications. Standard deviations (SD) were computed for the arithmetic mean values presented in the tables. The significance of differences between arithmetic means was verified on the basis of homogenous groups determined by Tukey's test at the significance level α ≤ 0.05. All statistical computations were conducted using Statistica PL package (version 12.5).

Table 4

The nitrogen and sulfur content in the composted materials used in the experiment

| Determination | Composted material | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| | $[g \cdot kg^{-1}$ d.m.] | | | | |
| Total N | 28.5 ± 0.1* | 27.7 ± 0.1 | 23.3 ± 0.3 | 23.0 ± 0.2 | 27.1 ± 0.7 |
| Total S | 3.4 ± 0.2 | 2.8 ± 0.5 | 2.9 ± 0.1 | 3.8 ± 0.2 | 3.5 ± 0.2 |

* ± standard deviation, n = 3.

The course of weather conditions during the experiment varied especially between individual months. The greatest amount of precipitation (total from April to September) was recorded in June, and the smallest in April. The highest mean annual air temperature was recorded in July. Meteorological conditions (precipitation, temperature) during the experiment are shown in Fig. 1.

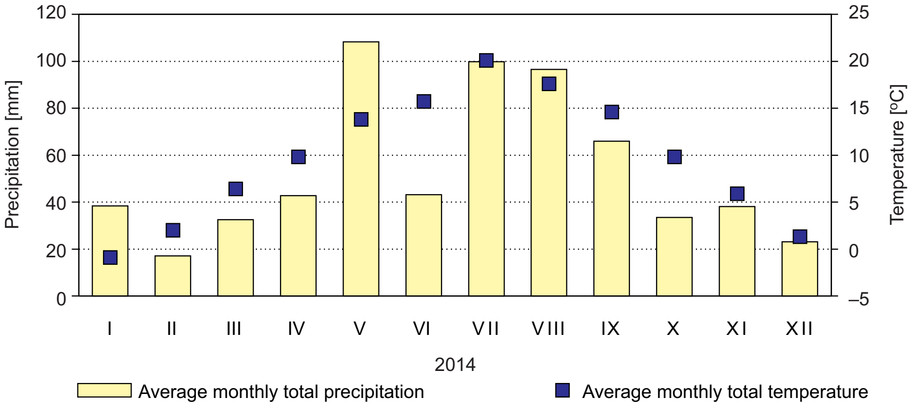

Fig. 1. Monthly and periodic total precipitation and mean daily air temperature in study year

## Results and discussion

The ability to use the yield-forming role of sulphur remains an important part of agricultural science. Undoubtedly one of the actions whose purpose is to improve the balance of sulphur in soil is to add this element to multi-component mineral fertilizers (which in recent years have become more and more popular among farmers) [13–14]. Organic materials generated from waste substances might be another alternative source of this element. Reuse of waste organic matter for plant and soil fertilization now appears to be one of the most rational ways of its management.

The amount and rate of uptake of sulphate anions depend on many factors, the most important of which are: soil reaction, atmospheric conditions, content of organic matter, and type of flora. Based on the authors' own research it was established that soil fertilization with composted materials with the addition of foil did not have a significant effect on the change in soil reaction (Table 5). Treatments K2 and K5, conducted in light soil, were an exception – a significant increase (by 0.8 unit) in pH value took place there. The pH values of the treatments conducted on medium soil, with respect to the control treatment, increased by 0.2 to 0.4 unit depending on the applied fertilization. Similarly to the case of light soil, the highest pH value was found in the soil to which composted material with the addition of foil C and microbiological inoculum (K5) was added.

Table 5

pH, nitrogen and organic carbon content in soil (0–10 cm)

| Object | Light loam | | | Medium loam | | |
|---|---|---|---|---|---|---|
| | pH KCl [-] | Total N | Organic C | pH KCl [-] | Organic C | Total N |
| | | $[\mathrm{g \cdot kg^{-1}\ d.m.}]$ | | | $[\mathrm{g \cdot kg^{-1}\ d.m.}]$ | |
| 0 | $5.5^{a} \pm 0.2$* | $0.80^{a} \pm 0.09$ | $8.5^{a} \pm 1.2$ | $5.5^{a} \pm 0.3$ | $10.7^{ab} \pm 0.3$ | $1.06^{abcd} \pm 0.03$ |
| NPK | $5.9^{abc} \pm 0.5$ | $0.82^{a} \pm 0.08$ | $8.4^{a} \pm 0.7$ | $5.7^{abc} \pm 0.4$ | $11.6^{ab} \pm 1.2$ | $1.05^{abcd} \pm 0.03$ |
| K1 | $5.4^{a} \pm 0.3$ | $1.01^{abcd} \pm 0.16$ | $11.6^{ab} \pm 2.0$ | $5.5^{a} \pm 0.2$ | $12.9^{ab} \pm 0.9$ | $1.20^{cd} \pm 0.06$ |
| K2 | $6.3^{bc} \pm 0.2$ | $0.93^{abc} \pm 0.14$ | $10.9^{ab} \pm 1.4$ | $5.8^{abc} \pm 0.1$ | $12.6^{ab} \pm 1.2$ | $1.16^{bcd} \pm 0.07$ |
| K3 | $5.5^{a} \pm 0.4$ | $1.05^{abcd} \pm 0.14$ | $10.9^{ab} \pm 1.1$ | $5.7^{abc} \pm 0.2$ | $14.3^{b} \pm 0.6$ | $1.28^{d} \pm 0.09$ |
| K4 | $5.5^{a} \pm 0.2$ | $0.97^{abc} \pm 0.13$ | $10.8^{ab} \pm 1.8$ | $5.7^{abc} \pm 0.1$ | $13.2^{ab} \pm 1.3$ | $1.21^{cd} \pm 0.10$ |
| K5 | $6.3^{bc} \pm 0.3$ | $0.87^{ab} \pm 0.11$ | $10.2^{ab} \pm 2.0$ | $5.9^{abc} \pm 0.1$ | $12.4^{ab} \pm 1.9$ | $1.14^{bcd} \pm 0.13$ |

* ± standard deviation, $n = 3$; Means followed by the same letters in columns did not differ significantly at $\alpha \le 0.05$ according to the t-Tukey test.

The organic carbon content in the soil material ranged from 8.42 to 10.9 $\mathrm{g \cdot kg^{-1}}$ d.m. for the treatments conducted on light soil, and from 10.7 to 14.3 $\mathrm{g \cdot kg^{-1}}$ d.m. for treatments conducted on medium soil (Table 5). A beneficial effect of application of composted materials on the increase in organic carbon content in both soils was recorded. Compared to the organic carbon content in the soil material taken from the control treatments (0), application of composted materials with the addition of foil marked F(B) had a significant and beneficial effect on the content of this element, regardless of soil type. Soil fertilization with composted materials with the addition of foil F(C) and microbiological inoculum (K5), regardless of soil type, caused a reduction of organic C content in comparison with the soil to which composted material with the addition of the same foil F(C) but without microbiological inoculum was introduced (K4).

When analyzing total nitrogen content in the soils fertilized with composted materials with the addition of foil it was found that the content of this element was varied, depending on applied fertilization and soil granulometric composition. Total nitrogen content in light soil, regardless of treatment, ranged from 0.80 to 1.05 gN · kg$^{-1}$ d.m. (Table 5). Total nitrogen contents in medium soil were higher and ranged from 1.05 to 1.28 gN · kg$^{-1}$ d.m. Regardless of soil granulometric composition, the highest nitrogen content was determined in the treatment into which composted material with the addition of foil F(B) was applied (K3).

Motowicka-Terelak and Terelak [15] and Kabata-Pendias et al [16] state that total sulphur content in Polish soils is varied and depends on organic matter content, soil granulometric composition, and on the level of industrial emissions. Based on the authors' own research it was found that the content of total forms of sulphur was between 150 and 180 mgS · kg$^{-1}$ d.m. in the treatments located on light soil, and between 190 and 220 gS · kg$^{-1}$ d.m. in the treatments located on medium soil (Table 6). Kulczycki and Spiak [17] also state that agriculturally used soils of south-western Poland contain between 72 and 490 gS · kg$^{-1}$ d.m. The contents of total sulphur determined in the soil material coming from the field experiments are within the range given by the quoted authors and do not indicate contamination with this element. Assessment of the content of total sulphur in the examined soil material, taking into account the agronomic category of the soil and conducted according to the elaboration by Kabata-Pendias et al [16], showed that the content of total forms of this element determined in all the treatments fertilized with composted materials with the addition of foil (introduced on light and medium soil) corresponded with mean content. Low total sulphur content was found in both soils in the treatment without fertilization (0) and in the treatment fertilized with mineral fertilizers (NPK).

Table 6

Total and assimilable forms of sulphur content in soil

| Object | Light loam | | Medium loam | |
|---|---|---|---|---|
| | Total S | Assimilable S | Total S | Assimilable S |
| | [mg · kg$^{-1}$ d.m.] | | [mg · kg$^{-1}$ d.m.] | |
| 0 | $148.04^{ab} \pm 0.02$ | $9.22^{abc} \pm 0.76$ | $195.80^{b} \pm 0.01$ | $13.1^{abcd} \pm 1.8$ |
| NPK | $153.18^{ab} \pm 0.02$ | $12.9^{abcd} \pm 2.9$ | $192.20^{b} \pm 0.01$ | $13.1^{abcd} \pm 1.1$ |
| K1 | $183.86^{b} \pm 0.03$ | $12.7^{abcd} \pm 4.7$ | $211.49^{b} \pm 0.01$ | $16.6^{cd} \pm 2.7$ |
| K2 | $182.51^{b} \pm 0.03$ | $10.2^{abcd} \pm 2.0$ | $208.19^{b} \pm 0.03$ | $16.6^{cd} \pm 3.0$ |
| K3 | $182.47^{b} \pm 0.02$ | $8.97^{ab} \pm 0.39$ | $219.73^{b} \pm 0.02$ | $12.5^{abcd} \pm 1.0$ |
| K4 | $181.21^{b} \pm 0.03$ | $12.0^{abcd} \pm 4.3$ | $216.57^{b} \pm 0.02$ | $17.4^{d} \pm 3.7$ |
| K5 | $158.40^{ab} \pm 0.03$ | $8.3^{ab} \pm 1.1$ | $203.51^{b} \pm 0.02$ | $16.2^{bcd} \pm 1.4$ |

In the experiment located on light soil, application of composted organic materials with the addition of foil caused a 20% increase in total S content in comparison to the control treatment (without fertilization) and the treatment where fertilization with mineral fertilizers was applied (Table 6). In the case of the treatments located on medium soil, the most beneficial effect was recorded after application of composted materials with the addition of foil F(B) and F(C) in treatments K3 and K4. It was also found that soil fertilization with composted plant materials with the addition of foil and microbiological inoculum (K5) contributed to the decrease in the content of total forms of sulphur, as compared with the treatment in which the same composted material, but without the addition microbiological inoculum, was applied (K5). A similar dependence was observed in the case of analogical treatments located on light soil. However, a statistical analysis of the obtained results did not confirm the significant differences.

In the majority of agriculturally used soils in Poland the content of assimilable forms of sulphur does not exceed 25 mg $\cdot$ kg$^{-1}$ soil, and in approximately 70% of the area of agricultural lands this content is within a range from 5 to 20 mg $\cdot$ kg$^{-1}$ soil [18]. Based on the authors' own research it was found that the content of assimilable forms of sulphur was much more diversified than in the case of total forms and was between 8.28 and 12.89 mgS $\cdot$ kg$^{-1}$ d.m. for the treatments located on light soil, and between 12.5 and 17.4 mgS $\cdot$ kg$^{-1}$ d.m. for the treatments on medium soil (Table 6). According to the limit values proposed by Lipinski et al [18], the analyzed light soil was classified into soils of average content of this element (10.1–15.0 mgS $\cdot$ kg$^{-1}$ d.m.), whereas the medium soil had a high content of assimilable forms of sulphur (15.1–20 mgS $\cdot$ kg$^{-1}$ d.m.). According to Terelak et al [19], the mean sulphate content in sandy soils amounts to 15 mg $\cdot$ kg$^{-1}$ d.m., whereas in heavy loam soils it is 20 mg $\cdot$ kg$^{-1}$ d.m. In the authors' own research the content of assimilable forms of sulphur was determined in medium soil in treatment K4, in which fertilization with composted material with the addition of foil F(C) (17.4 mgS $\cdot$ kg$^{-1}$ d.m.) was applied. This may have been directly influenced by the content of this element amended with the composted material IV (Table 4). Attention should also be drawn to the fact that both light and medium soil (to which the same composted material but with the addition of microbiological inoculum was added) had a lower content of assimilable forms of sulphur.

The average percentage of assimilable forms of sulphur in the total content of this element for the treatments located on light soil was 6.3%, whereas for the treatments on medium soil it was 7.3%. A similar percentage of sulphate sulphur in the total content of this element was shown in the research by Kulczycki and Spiak [17]. According to Kalembasa and Godlewska [20], the content of assimilable forms of sulphur is directly associated with the content of total forms of this element in soil, and soil fertilization with composts does not cause larger changes in the content of assimilable forms of S in soil. Szulc et al [1] highlight that changes in the content of sulphates in soil are conditioned mainly by transformations of organic matter.

Despite little diversification in the content of individual forms of sulphur in soil depending on applied fertilization, the carbon to sulphur ratio indicates an advantage of mineralization processes of sulphur-containing organic compounds in all the analyzed treatments. As numerous studies show, at C:S ratio < 200 mineralization is predominant, and immobilization of sulphur compounds is predominant only at C:S ratio >

400 [21, 22]. Rejman-Czarnecka [23] states that at C:S ratio below 50 relative sulphur deficiency in soil takes place, which may result in reduction of plant yield and deterioration of yield quality. Relatively low values of the C:S ratio (light soil: between 57 and 64; medium soil: between 56 and 65) were found in this research. Similar values of the C:S ratio (between 53 and 58) were found in the research of Kulczycki and Spiak [17].

## Conclusions

1. In comparison to the soils fertilized with mineral fertilizers, the most beneficial effect on the increase in content of total forms of sulphur was found after application of composted plant materials with the addition of foils (F(B) and F(C)) with 30% corn starch, regardless of soil.
2. The average percentage of assimilable forms of sulphur in the total content of this element for the treatments located on light and medium soil did not exceed 10%.
3. The content of assimilable forms of sulphur in the soil with the granulometric composition of medium loam, after fertilization with composted materials with the addition of foil, increased by, on average, 21% compared with the soil fertilized only with mineral fertilizers. A reverse dependence was found in the case of light soil, where a decrease in content of assimilable forms of sulphur by, on average, 19% took place.

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# ZAWARTOŚĆ SIARKI W GLEBACH PO APLIKACJI PRZEKOMPOSTOWANYCH MATERIAŁÓW Z DODATKIEM FOLII

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**Abstrakt:** Celem badań była ocena zawartości form ogólnych oraz przyswajalnych siarki w glebie po aplikacji materiałów przekompostowanych z dodatkiem folii otrzymanych z polietylenu i skrobi kukurydzianej. Schemat doświadczenia obejmował 7 obiektów prowadzonych w 3 powtórzeniach na dwóch glebach: 0 – gleba nienawożona, NPK – gleba nawożona nawozami mineralnymi, K1 – gleba nawożona materiałem przekompostowanym bez dodatku folii, K2 – gleba nawożona materiałem przekompostowanym z dodatkiem folii A zawierającej 47,5% PE C + 45% skrobi kukurydzianej + 7,5% kompatybilizatora, K3 – gleba nawożona materiałem przekompostowanym z dodatkiem folii B zawierającej 65% PE C + 30% skrobi kukurydzianej + 5% kompatybilizatora, K4 – gleba nawożona materiałem przekompostowanym z dodatkiem folii C zawierającej 65% PE C + 30% skrobi kukurydzianej + 5% kompatybilizatora i kopolimer oraz K5 – gleba nawożona materiałem przekompostowanym z dodatkiem folii C i szczepionki mikrobiologicznej. Eksperymenty przeprowadzono na glebach o składzie granulometrycznym gliny lekkiej oraz gliny średniej. Rośliną testową była kupkówka pospolita. Średni plon biomasy kupkówki pospolitej zebrany z obiektów zlokalizowanych na glebie o składzie granulometrycznym gliny lekkiej wynosił od 1,4 do 2,4 Mg s.m. · ha$^{-1}$ oraz od 1,8 do 3,6 Mg s.m. · ha$^{-1}$ dla obiektów prowadzonych na glebie o składzie granulometrycznym gliny średniej. Zawartość S ogólnej oznaczono po mineralizacji próbki w piecu komorowym w temperaturze 450$^{o}$C przez 8 godzin, po uprzednim związaniu siarki siarczanowej $Mg(NO_3)_2$. Przyswajalne formy S wyekstrahowano roztworem 0,03 mol·dm$^3$ $CH_3COOH$. W uzyskanych roztworach i ekstraktach zawartość S oznaczono metodą ICP-OES. Największa zawartość form ogólnych siarki oznaczono w glebie o składzie granulometrycznym gliny średniej, do której wprowadzono przekompostowany materiał K3 i K4 (220 mgS · kg$^{-1}$ s.m. gleby). Zawartość przyswajalnych form siarki mieściła się w przedziale od 8,3 do 12,9 mgS · kg$^{-1}$ s.m. gleby w przypadku obiektów zlokalizowanych na glebie o składzie granulometrycznym gliny lekkiej oraz od 13,1 do 17,4 mgS · kg$^{-1}$ s.m. gleby dla obiektów na glinie średniej.

**Słowa kluczowe:** gleba, kompost, siarka, polietylen, skrobia kukurydziana, kupkówka pospolita

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# EFFECT OF PACKAGES ON NITRATES AND NITRITES CONTENTS IN SAUERKRAUTS

## WPŁYW OPAKOWANIA NA ZAWARTOŚĆ AZOTANÓW(V) I (III) W KAPUSTACH KISZONYCH, CHODNICZO SKADOWANYCH

**Abstract:** Nitrites are thought to be ten times more toxic to humans and animals than nitrates. Nitrites are able to form nitrosamines, stable, strongly toxic, mutagenic, teratogenic and carcinogenic compounds. The presented paper investigates the effect of packaging types and length of chilled storage on changes in nitrates and nitrites contents in white and red cabbage sauerkraut. Two types of bags were used for packing the product: one made from low density polyethylene (PE-LD) and the other from the metalized polyethylene terephthalate (PET met/PE). Vegetables were analysed before and after packaging and after 1, 2, 3, and 4 months of chilled storage in two types of packaging. It has been observed in this work that nitrate contents in cold-stored sauerkrauts fluctuated in subsequent four months and the values found were generally significant. A type of packaging did not have a significant effect on the levels of nitrites in the sauerkrauts analyzed.

**Keywords:** cold stored; nitrates; nitrites; packaging; sour white cabbage, sour red cabbage

## Introduction

High atmospheric concentration of nitric oxides consequent to industrial emission can be a cause of elevated concentrations of nitrates and nitrites in plants [1]. Nitrites, and indirectly nitrates, can be hazardous for the health, when consumed in food in too much amounts. Nitrites are thought to be ten times more toxic to humans and animals than nitrates. Nitrites toxicity is caused, among other things, by methemoglobinemia (cyanosis). Nitrite ion formed by nitrate reduction is responsible for the hemoglobin's $Fe^{2+}$ ion oxidation to $Fe^{3+}$ [2, 3]. Nitrites are able to form nitrosamines, stable, strongly toxic, mutagenic, teratogenic and carcinogenic compounds [2, 4].

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On the other hand, several clinical trials are being performed to determine the broad therapeutic potential of increasing nitrite bioavailability on human health and disease, including studies related to vascular aging. Inorganic nitrite, as well as dietary nitrate supplementation, represents a promising therapy for treatment of arterial aging and prevention of age-associated CVD in humans [5, 6].

Nitrates (75–87% of total content) and nitrites (16–43%) in human body derive principally from vegetables [7, 8]. Among vegetables, the Cruciferous are characterized by a medium (white cabbage) or low (cauliflower, Brussels sprouts) accumulation of these compounds, but due to a large mass consumed, they can be a significant component of daily food intake. These are seasonal vegetables. Some of them may be consumed raw or preserved by souring or freezing [9]. The content of nitrates and nitrites in the plant raw material can not reflect the real intake since both pre-treatment (washing, peeling) and culinary and technological treatments may lead to changes in their levels [10, 11].

Biological value of food is a wide notion which includes a nutritive value, sensory attributes, being also significant for the consumer health. Hence, biological value is understood to be not only the content of valuable vitamins, mineral compounds, and pro-health substances but is also considered with regard to the level of contaminants which have a negative effect for the health. Food contaminants include, among other substances, nitrates and nitrites [12].

Cruciferous are abundant in several biologically active substances such as, among other, vitamins C and E, carotenoids, polyphenols, and glucosinolates. They owe their anti-inflammatory and antimutagenic properties to the presence of these substances, which also affect expression of the gene responsible for cell proliferation in the development of cancer through modulation of cell redox homeostasis [13, 14]. Moreover, some polyphenol groups exhibit anti-inflammatory, anti-allergic, anti-clotting, anti-virus, as well as anti-cancerogenic properties [15].

The process of sauerkraut fermentation, known from the ancient times, is one of the biological methods of food preservation based on lacto-fermentation. The products obtained due to this process are characterized by extended shelf life and by microbiological safety. They are better digestible and their properties are different from the initial material [16, 17].

The raw material used in the sauerkraut fermentation is cabbage both white and red. A main constituent of sauerkraut is lactic acid, the product of sugars' fermentation conducted by such lactic acid bacteria as, *eg Lactobacillus acidophilus*. The identical process runs in the large intestine, where the bacteria colonizing it are employed to ferment probiotics. The lactic acid formed is a constituent of skin protective layer (epidermis), playing also a substantial protective role in the mucous membrane. The consumption of sauerkraut enables easier colonization of the large intestine by the LAB species, which are responsible for crucial functions in human body. They produce vitamins and enzymes, inhibits the formation of putrefactive products, improve the peristalsis of gastrointestinal tract, and are also found to compete with such pathogenic bacteria as, for example, *E. coli* or *Candida albicans* [18].

The level of nitrates in vegetables changes depending on the intensity of fertilization, climatic conditions, plant species, and plant maturity at harvest [19]. The presence of some amounts of nitrates in plants is a consequence of the natural cycle of nitrogen absorbed by the plants to synthesize protein. The remaining quantity is a result of the excessive soil fertilization, infiltration of soil surface layer with sewages or the process of leaching running in lode. Nitrates can also be added as preservatives to several products. The content of nitrates tended to rise in plants at the early stage of growth, inadequate exposure to the sun's rays, acidic pH of soil, low humidity, deficiency of such nutrients as molybdenum and magnesium, and when herbicides were used [20].

The obligatory EC Regulation of 2005 sets maximum acceptable levels for nitrates only in certain vegetables such as fresh, preserved, and frozen spinach, fresh lettuce, and Iceberg-type lettuce. The Regulation of the Minister of Health of 2003, which was obligatory until 30 April 2004, defined the maximum nitrates contamination of cabbage at the level of 750 mg $NO_3$/kg of fresh weight; the level of nitrites was not set. The human intake of nitrate(V) should not however exceed the acceptable daily intake of 3.7 mg/kg body weight (b.w.) and in the case of nitrates(III) of 0.07 mg $NO_2$/kg b.w. [20–22].

Vegetables which are self-produced by farmers can be cultivated and sell on the market and there is no an obligation to control their production [23]. In view if the above, a contemporary man is searching for effective methods of food storage to minimize losses occurring during this process. Hence, studies are conducted all over the world to find a method which will be beneficial from both nutritional and economic point of view and the use of which is inexpensive but efficient enough to maintain a natural composition of the stored product.

Therefore, vegetables can contain excessive amounts of nitrates that may have a negative heath effect on a consumer. Food containers are multifunctional and one of their fundamental function is to protect the product against mechanical damages as well as external factors. With regard to the sour products, barrels are commonly used for their storage. On the other hand, characteristic feature of the unit packaging is its small volume and the fact that such packaging should provide specific microclimate for the product to maintain high quality as long as possible in appropriate storage condition [24].

The aim of this paper was to examine and compare sour white and red cabbage, which was chilled stored for four subsequent months, in terms of changes in the nitrates and nitrites content. The experimental material was packed in two ways: in low density polyethylene (PE-LD) bags and in metalized polyethylene terephthalate (PET met/PE) bags.

To our best knowledge this is the first study determining the effect of cold storage in different packaging type (especially innovative is one of presented packaging type – metalized foil made from PET met/PE) on selected contaminants of the chilled stored white and red sauerkraut.

This study aimed also at increasing the consumer knowledge about biological value of the sour cabbage: white and red, particularly with regard to the presence of such contaminants as nitrates and nitrites as well as at contributing in the selection of an optimal packaging intended for the chilled storage of such a product.

## Material and methods

### Material

The experimental material comprised sour white cabbage and sour red cabbage, which were freshly soured and purchased in 5 selected stands of direct selling in Krakow. Vegetables were analysed before and after packaging and after 1-, 2-, 3- and 4-month periods of chilled storage. First analyses were done immediately before packaging. The remaining material was divided into two batches, one was packed in low density polyethylene (PE-LD) bags with the zipper closure (0.91–0.92 g/cm$^3$; size: 230 x 320 mm) and the other in bags of the similar size made of the laminate: metalized polyethylene terephthalate (PET met/PE) (a polymer from the polyester group, obtained through a polycondensation reaction between dimethyl terephthalate (DMT) and ethylene glycol (GE), CAS number: 25038-59-9, density 1.370 g/cm$^3$) with polyethylene. The representative samples obtained were then stored at chilled conditions (4–5$^o$C) in a fridge for four subsequent months.

### Analytical methods

The adequately prepared mean and representative samples of vegetables were analyzed for the levels of contaminants: nitrates and nitrites according to the Polish Standard PN-92/A-75112 [25]. Samples were analyzed prior to their packaging and after the established periods of chilled storage.

Colorimetric method using to determinate this contaminants based on nitrites coloured reaction with Griess I, II reagent. Previously nitrates must be reduced to nitrites.

Nitrates content was assessed using Griess I (sulfanilamide, Sigma-Aldrich) and Griess II (n-(1-Naphtyl)ethylene-diamine dihydrochloride, water solution, Sigma-Aldrich). The principle of this method is to cause a colour reaction of nitrate(III) with n-(1-Naphtyl)ethylene-diamine dihydrochloride in acidic conditions, and to measure absorbance at wavelenght 538 nm. Nitrates had tobe reduced to nitrites before to beginning of colour reaction.

The described research method is recognized and widely used in assays to determine the content of nitrates and nitrites in vegetables.

### Statistical analysis

All analyses were carried out in three parallel replications (n = 3) and for all the mean values obtained, standard deviations (SD) were calculated. In the vegetables investigated, single- and two-factor analysis of variance was used to establish the level of significance for the differences in the levels of nitrates and nitrites depending on the processes applied (chilled storage) and type of packaging. The single-factor analysis of variance was employed to determine how significant were differences between mean parameter values with regard to two different packaging types used for the storage of vegetables. In two-factor analysis of variance, significance of differences was found between values of the parameters evaluated in the chilled stored vegetable depending on the packaging type. The Statistica 9.1. PL program was applied for all the calculations made. The Duncan's multiple range test was used to assess significance of differences at the critical significance level of $p \le 0.05$.

## Results

As the dry matter content in the vegetable varies depending on the process applied and the container used, all the results presented below along with conclusions have been discussed basing on the results calculated per the dry matter unit. In consequence, only an effect of the process applied was shown.

### Nitrates and nitrites

The nitrate levels in white and red cabbage sauerkraut were expressed as the amount of $NO_3^-$ nitrate ions per kg of dry matter (Tables 1 and 2).

Table 1

The content of nitrates and nitrites in cold-stored sour white cabbage under the influence of packaging type and storage time [mg/kg d.m.]

| The kind of processing | Nitrates $[NO_3^-]$ | | Nitrites $[NO_2^-]$ | |
|---|---|---|---|---|
| Before storage | $522^{b} \pm 21$ | | $0.60^{cd} \pm 0.04$ | |
| Cool storage | The kind of packaging | | | |
| | Zipper seal bags (PE-LD) | | Bags (PET met/PE) | |
| | Nitrates | Nitrites | Nitrates | Nitrites |
| 1 month | $84.9^{e} \pm 4.0$ | $0.70^{cd} \pm 0.11$ | $139^{de} \pm 19$ | $0.80^{bc} \pm 0.00$ |
| 2 months | $247 \pm 26$ | $1.10^{b} \pm 0.04$ | $520^{b} \pm 15$ | $1.80^{a} \pm 0.37$ |
| 3 months | $176^{d} \pm 29$ | $0.60^{cd} \pm 0.04$ | $156^{d} \pm 70$ | $1.10^{b} \pm 0.04$ |
| 4 months | $576.7^{ab} \pm 8.4$ | $0.50^{d} \pm 0.04$ | $624^{a} \pm 16$ | $0.70^{cd} \pm 0.19$ |
| Mean value for packaging | $321^{A} \pm 200$ | $0.70^{A} \pm 0.24$ | $392^{A} \pm 220$ | $1.00^{A} \pm 0.51$ |

Values are presented as mean value ± standard deviation (n = 3). The values denoted with the same small or capital letters don't differ statistically significantly at $p < 0.05$.

After 1-, 2- and 3-month chilled storage, the content of these substances in the sauerkrauts stored in the PE-LD bags decreased significantly ($p \le 0.05$) by 83.7, 52.7, and 66.3%. respectively, compared with the values before packaging (Table 1). With regard to the sauerkraut packed in the PET met/PE bags, 1- and 3-months' chilled storage caused statistically significant reductions in the nitrate levels of 73.4 and 70.1%

respectively; after 4-months of storage, a significant increase was noted of 19.6% compared with the unpacked sauerkraut; while after 2 months of storage in such bags, the nitrate level corresponded ($p>0.05$) to the level determined prior to packaging.

After 1-, 2-, and 3-month chilled storage of the red cabbage sauerkraut, the level of these substances fell significantly ($p \leq 0.05$) in the products packed in the PE-LD bags (48.1, 13.1, and 39% respectively) as well as in those kept in the PET met/PE bags (56.7, 19.8, and 49.5% respectively), compared with the product before packaging (Table 2). In comparison with the unpacked sauerkraut, after 4 month of storage there were no statistically significant ($p > 0.05$) changes in the content of these substances, regardless of the type of packaging applied.

Table 2

The content of nitrates and nitrites in cold-stored sour red cabbage under the influence of packaging type and storage time [mg/kg d.m.]

| The kind of processing | Nitrates [$NO_3^-$] | | Nitrites [$NO_2^-$] | |
|---|---|---|---|---|
| Before storage | $1042.3^{ab} \pm 0.7$ | | $12.20^{c} \pm 0.42$ | |
| Cool storage | The kind of packaging | | | |
| | Zipper seal bags (PE-LD) | | Bags (PET met/PE) | |
| | Nitrates | Nitrites | Nitrates | Nitrites |
| 1 month | $541^{de} \pm 92$ | $13.50^{bc} \pm 0.46$ | $451^{e} \pm 11$ | $12.20^{c} \pm 0.45$ |
| 2 months | $906^{c} \pm 33$ | $19.90^{a} \pm 0.22$ | $836^{c} \pm 26$ | $14.50^{b} \pm 0.33$ |
| 3 months | $635.3^{d} \pm 6.5$ | $9.00^{d} \pm 0.30$ | $525.9^{de} \pm 4.5$ | $4.90^{e} \pm 0.54$ |
| 4 months | $1151^{a} \pm 29$ | $4.70^{e} \pm 0.32$ | $958^{bc} \pm 54$ | $6.1^{e} \pm 1.2$ |
| Mean value for packaging | $855^{A} \pm 240$ | $11.9^{A} \pm 5.0$ | $763^{A} \pm 240$ | $10.0^{A} \pm 3.8$ |

Values are presented as mean value ± standard deviation ($n = 3$). The values denoted with the same small or capital letters don't differ statistically significantly at $p < 0.05$.

A type of the packaging used was found to have no statistically significant ($p > 0.05$) effect on the level of nitrates in the sauerkrauts stored in chilling conditions.

The nitrite levels in white and red cabbage sauerkraut were expressed as the amount of $NO_2^-$ nitrate ions per kg of dry matter (Tables 1 and 2).

In the case of white cabbage sauerkraut chilled stored in the zipped PE-LD bags, only in the samples stored for 2 months was the content of nitrites significantly higher ($p \leq 0.05$) (of 122.4%); whereas, in those kept in the another packaging type, after 2 month and 3 month of chilled storage increased by 293.1% and 138.0% respectively, compared to the levels determined in the vegetables prior to packaging (Table 1).

Two-month chilled storage led to a significant increase ($p \leq 0.05$) in the level of nitrites in the red cabbage sauerkraut stored in the zipped PE-LD and PET met/PE bags of 63% and 18.9% respectively; although, after 3- and 4-month periods of storage the significant reductions were observed of respectively 26.2 and 61.5% (PE-LD bags) as well as 59.8 and 50.0% (PET met/PE bags), compared to the unpacked product (Table 2).

It has been proved that a type of the packaging used had no significant effect ($p > 0.05$) on the level of nitrites in the cold-stored sauerkrauts (Tables 1 and 2).

## Discussion

### Nitrates and nitrites

The results obtained in this work for the nitrate levels in the sauerkrauts not being packed, generally correspond to the literature data concerning their levels, however, in the raw (not soured) red and white cabbage. Gajewska et al [23] proved that white cabbage harvested in the Spring/Summer season contained 75.0–915.2 mg $NaNO_3$/kg fresh weight, while the cabbage originated from the Autumn/Winter season had 30.5–655.4 mg $NaNO_3$/kg fresh weight. The sour white cabbage examined in this work had 50.6 mg nitrates per kg fresh weight, when calculated per $NO_3^-$ ions, and 69.3 mg/kg fresh weight, expressing the result as the amount of $NaNO_3$. The amounts of nitrates reported by Du et al [26] in white cabbage were 259–1250 mg $NO_3^-$/kg fresh weight, exceeding the results obtained in the present work.

According to Santamaria [27], vegetables from *Cruciferae* family, depending on species, belong to the vegetable group characterized by either low or moderate level of nitrates, being respectively: 200–500 mg/kg fresh weight in broccoli and cauliflower; and 500–1000 mg/kg fresh weight in cabbage and Savoy cabbage. Compared to the value obtained in this work, the level of nitrates in fresh red cabbage (958.7 mg $NO_3^-$/kg fresh weight) reported by Wojciechowska et al [28] was lower. Such a large discrepancy in the values reported by various authors may be due to the fact that in the case of vegetables, their ability to accumulate nitrates may result from genetic factors and is a characteristic feature attributed to the specific plant species or individual cultivar [23].

Nitrates level in vegetables is contingent not only on the cultivation conditions but also on biological features of these plants. Different plant parts accumulate different contents of nitrates. They are transported from roots principally to leaves where they undergo biotransformation. For this reason, leafy vegetables usually contain more these compounds than other vegetable types. Nitrates accumulation is the largest in the first days after nitrogen fertilization and also in plants in which photosynthesis is limited. Therefore, nitrates content is higher in the morning than in the afternoon. Nitrates and nitrites can also be formed by transformation of nitrogen compounds during storage of products (vegetables) in coolers and freezers [8].

It has been observed in this work that nitrate contents in cold-stored sauerkrauts fluctuated in subsequent four months and the values found were generally significant. However, after this period of storage, the level of this constituent was similar to the value determined prior to packaging, regardless of the packaging type. Tendencies presented by the authors with regard to several vegetable species are similar and not so clear-cut. Wojciechowska and Rozek [29] noted that red cabbage which was chilled stored for four months had 22.3% more nitrates and these results are much more higher than those reported in this paper. As for the butterhead lettuce stored for 14 days in the PE bags, after 7 days, a great increase (of 36.4%) in these substances was observed and then, after 14-days' storage, a fall of 6.8%, compared to the vegetable not being packed [30]. A large fall in the levels of these substances (from 46 to 49%) was observed by Chew et al [31] in various cold-stored (4°C) Amaranthus species. A reduction in nitrate content in the stored vegetables could result from their conversion into nitrites. Storing of the plant raw material under inappropriate conditions (in the temperature higher than recommended) along with the lack of oxygen may lead to undesirable biochemical processes, which in turn may affect the levels of nitrates [32].

The results obtained cannot be fully verified by the findings of other authors, since, particularly with regard to the products stored in various types of packaging, there is no data on an effect of chilled storage on changes in nitrate contents.

The nitrite levels determined prior to packaging in the sauerkrauts investigated generally do not agree with the values reported by other authors. A mean value declared by Hou et al [33] in white sour cabbage was over five times higher, being 3.08 mg/kg fresh weight for the packed product; and 6.41 mg/kg fresh weight for the unpacked sauerkraut. The aforementioned authors presented also such values for the marinated cucumber (2.62 mg/kg fresh weight) and turnip (2.68 mg/kg fresh weight).

Nitrite contents in the raw vegetables from Cruciferae family fluctuated from 1.47 mg/kg fresh weight in green cauliflower to 3.49 mg/kg in white cauliflower, as was reported by Leszczynska et al [32]. The amounts reported by Smiechowska [34] (0.2–3.3 mg/kg fresh weight) were similar to those found in this work; although, slightly higher levels of 0.9 mg $NaNO_2$/kg fresh weight in the Spring/Summer season and 1.1 in the Autumn/Winter season were recorded by Gajewska et al [23]. According to Du et al [26], in white cabbage nitrite content ranged from 0.00 to 0.41 mg $NO_2^-$/kg fresh weight, being minimally lower than the value obtained in this work.

Greater amounts of these substances were determined in lettuce and beetroot: in the Spring/Summer season: respectively 2.3 mg $NaNO_2$/kg and 1.5 mg $NaNO_2$/kg fresh weight; and in the Autumn/Winter season: respectively 2.9 mg $NaNO_2$/kg and 1.8 mg $NaNO_2$/kg fresh weight fresh weight [23]. On the other hand, it has been revealed that tomato, carrot or cucumber possess low tendency to accumulate these substances (about 0.6 mg $NaNO_2$/kg fresh weight). The authors of certain papers, did not state the presence of nitrites in the vegetables examined, at all; these were findings of Hsu et al [35] with regard to English spinach, Chinese cabbage and Iceberg-type lettuce as well as Huarte-Mendicoa et al [36] referring broccoli.

The nitrite level in fresh healthy and undamaged vegetables which were stored properly is low probably due to retaining the balance between nitrate and nitrite reductases. During the process of fermentation, nitrite concentration rises as a result of microbiological decomposition of nitrates and activity of endogenous nitrate reductase. The level of these substances is affected by a number and type of LAB species. Throughout the process of storage, accumulation of nitrites can be inhibited [33].

Four-month chilled storage resulted in reductions in this parameter in white and red sour cabbage stored in the PE-LD bags of 16.7 and 6.3% respectively, compared to the product not being packed.

In the case of white sour cabbage kept in the PET met/PE bags under chilled conditions, the content of these substances was increasing at each stage of investigation and after 4 months of storage increased by 16.7% compared to the unpacked product. It is worth noting that such tendency was not observed for the sauerkraut obtained from red cabbage, in which a rapid statistically significant decrease of 50% was recorded, compared to sauerkraut not being packed. Generally, it has been revealed that a type of packaging did not have a significant effect on the levels of nitrites in the sauerkrauts analyzed; however, there is no data in the available literature confirming this thesis.

It has been found that losses noted in this parameter were smaller of 27.5% in Butter-head lettuce, which was chilled stored for 14 days [30]. On the other hand, Chew et al [31] observed an increase in nitrite content within the range 54–70% in the blanched Amaranthus after its 4-day chilled storage. In turn, the presence of packaging had a substantial effect on the levels of the aforementioned substances, as was observed by Hou et al [33]. Packed vegetables, compared to those unpacked, exhibited a 2-fold decrease in their content, which agrees with the findings obtained for the sour red cabbage.

## Conclusions

It has been observed in this work that nitrate and nitrite contents in cold-stored sauerkrauts fluctuated in subsequent four months and the values found were generally significant.

Tendencies presented by this paper are not clear-cut, but after this period of storage, the level of nitrates and nitrites (excluding red cabbage sauerkraut) were generally similar to the value determined prior to packaging, regardless of the packaging type.

A type of the packaging used was found to have no statistically significant effect on the level of nitrates and nitrites in the sauerkrauts stored in chilling conditions.

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## WPŁYW OPAKOWANIA NA ZAWARTOŚĆ AZOTANÓW(V) I (III) W KAPUSTACH KISZONYCH, CHODNICZO SKADOWANYCH

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**Abstrakt:** Azotany(III) są dużo bardziej toksyczne dla zdrowia człowieka niż azotany(V). Związki te są prekursorami N-nitrozozwiązków, które charakteryzują się właściwościami kancerogennymi, mutagennymi i embriotoksycznymi. Celem niniejszej pracy było zbadanie wpływu rodzaju opakowania (torebki wykonane z folii polietylenowej o małej gęstości (PE-LD) oraz torebki z metalizowanego politereftalanu etylenu (PET met/PE) i czasu chłodniczego składowania na zmiany zawartości azotanów(V) i azotanów(III) w kapuście kiszonej białej i kapuście kiszonej czerwonej. Analizy były wykonywane w warzywach przed zapakowaniem oraz po 1-, 2-, -3 i 4-miesięcznym okresie chłodniczego składowania w dwóch rodzajach opakowań. W toku pracy zaobserwowano na ogół istotne statystycznie zmiany w zawartości azotanów(V). Badania nie wykazały istotnego statystycznie ($p > 0,05$) wpływu rodzaju użytego opakowania na zawartość azotanów(III) w przechowywanych chłodniczo kiszonkach.

**Słowa kluczowe:** przechowywanie chłodnicze; azotany(V), azotany(III), opakowania strunowe z PE-LD, opakowania metalizowane PET/met/PE, kapusta kiszona biała, kapusta kiszona czerwona

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# THE RELEASE OF LEACHABLE CONSTITUENTS FROM COPPER SLAG DEPENDING ON CONDITIONS OF THE LEACHING PROCESS

## UWALNIANIE WYMYWALNYCH SKŁADNIKÓW Z ŻUŻLA POMIEDZIOWEGO W ZALEŻNOŚCI OD WARUNKÓW PROCESU WYMYWANIA

**Abstract:** The research on leaching of waste components is one of the methods for assessing the level of their contamination by soluble forms of heavy metals. The process of leaching depends on several factors, which change can cause a release of contaminants at different levels. The study presents the results on leaching of $Cl^-$, $SO_4^{2-}$, Fe, Na, K and heavy metals (Pb, Cd, Ni, Zn, Cu, Cr) from copper slag, which depends on the pH level of the eluent being used in the research. The research on leaching of heavy metals was also performed at various ratio of liquid to solid (L/S = 10 and 100 $dm^3$/kg) and with using a waste with different sizes of the fraction. There was also analysed the total content of heavy metals in the waste. The highest leaching of $Cl^-$, Cr, Ni, Cu, Pb and Zn was observed with the eluent pH level of 13. Waste grain reduction to the size of < 0.125 mm has caused an increase in the release of copper and zinc into the aqueous phase.

**Keywords:** cooper slag, leaching, heavy metals

# Introduction

Mining of copper ore and its processing are inseparably associated with the impact on the natural environment. Copper metallurgy is a branch of industry, producing a diverse range of wastes. There can be included, among others, flotation tailings wastes, smelter slags, dusts from dedusting dust-containing gases and sludge from wastewater treatments. Certain wastes are reused in a production process, and some of

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them are used in various sectors of the economy. The waste, which cannot be reused in any other manner is deposited in waste landfills, which are specially designated for this purpose. Such action is within the line of the concept of sustainable development, and safety for environment is one of the principles for rational management of industrial wastes.

During copper smelting in electric or shaft kilns, the so-called (lump or granulated) copper slag is formed. For more than 40 years, the lump copper slag is used for production of road aggregates. This waste is poured on heaps, where after crystallization is processed into aggregate that is used for substructures and grits for upper layers of roads. Granulated copper slag is used as an abrasive to clean metal parts and as a material for backfilling former mine excavations. Due to the works, leading to introduce technologies of (electric) suspension roasters instead of shaft kilns, undertaken by the Polish steel industry, there will cause a raise in copper production, and thus, an increase of the quantity of slag being produced from electric furnace [1, 2]. Therefore, some attempts were made to use this waste as a raw material for production of hydraulic binders [3, 4].

Industrial waste placed in slag heaps or landfills can affect the soil-water environment. Contaminants contained therein can be leached into the soil and then get into the surface and underground waters. Leaching of constituent from waste may depend on the influence of different weather conditions [5]. The mobility of harmful substances is most frequently determined by the fundamental leaching test comprising of a single 24-hour extraction with a ration of liquid to solid applied in the test, amounting to L/S = 10 $dm^3$/kg [6]. This method is utilized in Poland, inter alia, for the purpose of identifying the possibilities of disposing wastes in landfills of a given type. The criteria for waste acceptance at landfills are governed by the Council Decision of 19 December 2002 (2003/33/EC) [7]. This document specifies admissible limit values of leaching particular waste components. When performing the test on leaching contaminants from waste materials, the conditions in which the process is carried out are of special meaning. A change made in the laboratory conditions can impact on the level of contaminants' release. In case of heavy metals, their solubility depends on the pH of the eluent with which the waste is in contact [8]. Zink, nickel and lead are generally characterized by the lowest leachability at the pH ranging from 7 to 10, depending on the test material. Chromium occurring in the form of anion has the lowest leachability at the pH within the range of 5–7. This is caused by the fact that the test materials have varying capacities to neutralize the acids or alkalis.

The paper presents test results on the leachability of contaminants from lump copper slag in terms of its impact on the environment. There were implemented changes in conditions for performing the process of leaching, involving the application of leachant having various pH levels. Additionally, there was performed analysis of concentrations of selected heavy metals (Pb, Cd, Ni, Zn, Cu, and Cr) in the waste and in water extracts prepared from two slag fractions with grain size of < 10 mm and < 0.125 mm and at the ratio of L/S = 10 and 100 $dm^3$/kg.

## Material and methods

As the research material was used lump copper slag from smelting copper with code 10 06 80 (lump and granular copper slags) [9]. Lump copper slag extracted for the research tests was as fraction (lumps of irregular shapes and sizes). This slag is obtained in the process of smelting briquetted copper concentrates in a shaft kiln. Due to its low copper content, it constitutes the final waste in the smelting process. In the liquid state and at a temperature of approx. 1200°C, it is transported to the place of deposition – on a heap, where after being poured out, the process of solidification and slow cooling are taking place under atmospheric conditions. Chemical and mineral compositions of the tested slag are demonstrated in Table 1 and in Fig. 1.

Table 1

Chemical composition of copper slag

| Properties | Content [wt.%] |
|---|---|
| Loss on ignition* | 0.00 |
| $Fe_2O_3$ | 17.38 |
| $Al_2O_3$ | 15.56 |
| Total $SiO_2$ | 42.76 |
| CaO | 11.88 |
| MgO | 7.29 |
| $SO_3$ | 0.12 |
| $Cl^-$ | 0.015 |
| $Na_2O$ | 1.09 |
| $K_2O$ | 3.38 |

* With the correction associated to the oxidation of sulfides.

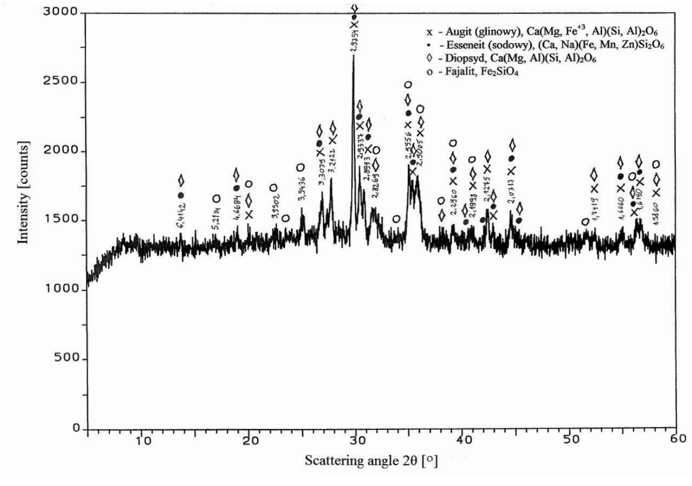

Fig. 1. XRD pattern of lump copper slag

The tested slag was characterized by the highest percentage content of $SiO_2$, and the lowest content of $Cl^-$. On the diffractogram characterizing the lump copper slag (Fig. 1) can be observed peaks demonstrating the contents of its crystalline phases. These are mainly augite, diopside and esseneit – minerals included in the pyroxene group. Table 2 presents results determining the total content of heavy metals in the waste. The tested slag contains mostly: zinc (7833 mg/kg d.m.), copper (4679 mg/kg d.m.) and lead (2364 mg/kg d.m.). The lowest concentration was observed for nickel – 41.5 mg/kg d.m..

Table 2

Total content of heavy metals in the waste

| Heavy metal | Total content [mg/kg d.m.] |
|---|---|
| Cu | 4679 |
| Pb | 2364 |
| Zn | 7833 |
| Cd | 68.6 |
| Ni | 41.5 |
| Cr | 595 |

Chemical composition was designated according to the procedures outlined in European Standard EN 196-2:2013 [10]. Designation of the mineral composition was performed by using X-ray power diffraction (XRD). In order to tailor water extracts from waste, a laboratory sample was prepared with grain size of < 10 mm in accordance to requirements specified in EN 12457-4:2002 [6]. The research test on leaching contaminants was performed under the impact of eluent with different pH levels of: 4, 7 and 13. This liquid consisted of deionized water, the pH level of which was reduced by adding nitric acid(V) and raised by applying sodium hydroxide. The course of the study on leaching contaminants involved 24-hour extraction [6]. The test on leaching heavy metals from waste fraction of < 10 mm was also performed at the liquid to solid ratio of L/S = 100. Additionally, the waste was shredded into the fraction size of < 0.125 mm and it was checked to what extent the reduction of waste grain size impacts the leachability of selected heavy metals. The waste sample were also subjected to mineralization to determine the total content of heavy metals. Waste digestion was performed using aqua regina by mineralization in a closed system. Concentrations of individual components of eluents after being filtrated were determined by the method of atomic absorption spectrometry.

## Results and discussion

The concentrations of leachable forms of waste components in water extracts are presented in Figs. 2–5. The obtained research test results demonstrate the release of such parameters as: chlorides ($Cl^-$), sulphates ($SO_4^{2-}$), iron (Fe), potassium (K), sodium (Na), heavy metals, including: cadmium (Cd), general chrome (Cr), nickel (Ni), copper

(Cu), lead (Pb), zinc (Zn), caused by changes in the pH level of the eluent. Analysis of concentrations of selected contaminants in water extracts from wastes is required to be performed for the purpose of determining the feasibility of storing wastes at landfills of a given type, and at the same time, to assess the degree of contamination of the waste. Therefore, the results of analyses of individual parameters were compared with the permissible leaching limit values for inert waste landfills [7] (Figs. 2–4).

On the basis of the test results, the dependency between the release level of particular components and the pH of the eluent was observed. The concentrations of chlorides with the pH of 4 were designated at the same level. While, this indicator was characterized by 15 times higher leachability then it has in an alkaline environment. The leachability of sulphates was characterized differently. The lowest concentration was determined at the pH of 4, and the highest one at the pH of 7. For chlorides and sulphates the leaching limit values were not exceeded. This indicates a low rate of contamination of the water extracts by these compounds, and thus on their negligible salinity.

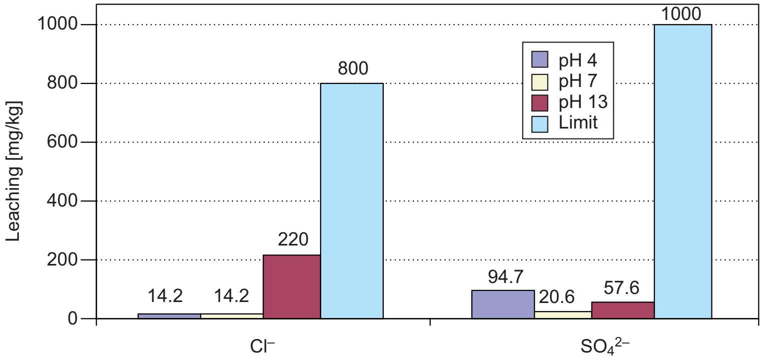

Fig. 2. Leaching of $Cl^-$ and $SO_4^{2-}$ depending on the pH of the eluent, compared to the permissible limit value for inert waste landfills

Water extracts collected from the test waste were characterized by a low content of leachable forms of heavy metals. However, the effect of changes in the pH of the eluent on the derived values of concentrations can be observed. The slag being subject of the study was characterized by a higher tendency for the release of Cr, Ni, Cu, Pb, and Zn under the impact of the acting liquid with the pH of 13 (Figs. 3, 4). In all of the samples, concentrations of cadmium and manganese were below the limit of quantification. Chromium and nickel are being leached above the limit of quantification solely under the alkaline conditions. The lowest leaching of Cu, Pb and Zn was observed in the pH-neutral environment. As the acidity of the eluent increased, the content of these elements in the water extracts increased. The greatest difference in the eluent's pH-dependent release rate can be observed for lead. Concentration of this element was almost three times higher at the pH of 3 and thirteen times higher at the pH of 13, when compared to the value obtained at neutral pH of the eluent.

The dependency between the leachability of heavy metals from waste materials and the pH of the eluent are also confirmed by research tests performed on the basis of other

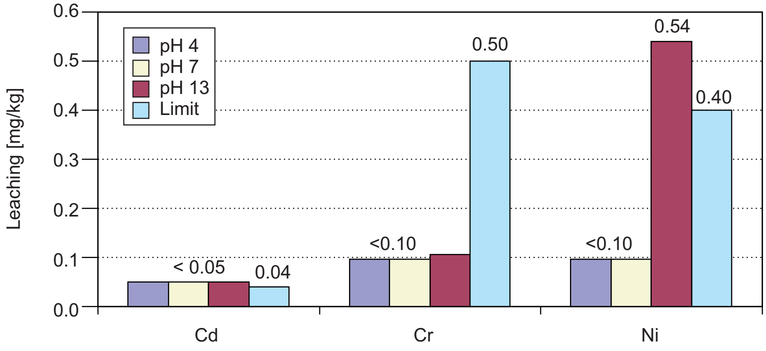

Fig. 3. Leaching of Cd, Cr, Ni depending on the pH of the eluent, compared to the permissible limit value for inert waste landfills

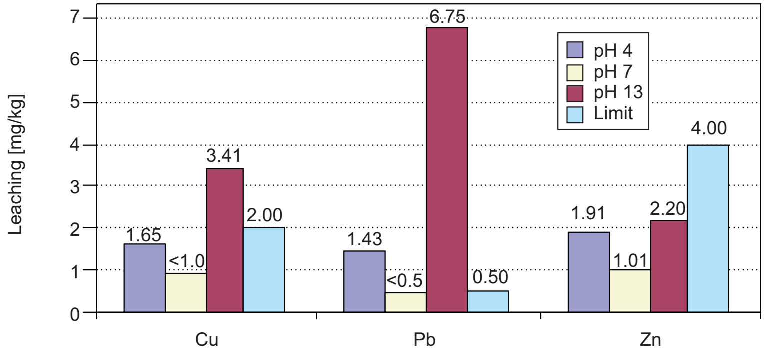

Fig. 4. Leaching of Cu, Pb, Zn depending on the pH of the eluent, compared to the permissible limit value for inert waste landfills

industrial waste [11] and conducted by other authors [12–13]. Due to changes in the pH of the environment, where the waste is placed, heavy metals are characterized by a raised tendency to release. This is significant, when waste are deposited in landfills and under the impact of the weather conditions. At such situation, it can lead to an increase in the release of leachable forms of contaminants from wastes. In accordance to legal regulations being in force in Poland, the permissible leaching limit values are being compared with results on concentrations of contaminants determined in the standard leaching test. This test is based on pouring the waste with water having a neutral pH in the proportion of 10 : 1 (water : waste).

Based on the obtained test results, it can be observed that none of these parameters at a pH of 7 does not exceed the limit concentration for inert waste landfills. However, in the case of leaching of Ni, Cu and Pb at a pH of 13, and for Pb at a pH of 4, the permissible leaching limits for this type of landfills have been exceeded. As a consequence, this can lead to pollution of the natural environment caused by heavy metals.

In the water extracts from waste the contents of Fe, K and Na (Fig. 5) were also determined. Potassium and sodium as alkali metals were characterized by another

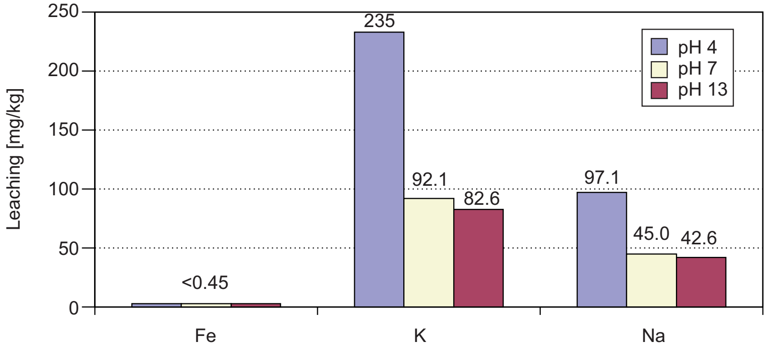

Fig. 5. Leaching of Fe, K, Na depending on the pH of the eluent

leaching tendency. The highest concentrations of these elements were designated in an acid environment, and the lowest ones in strongly alkaline environment. Concentrations of Fe in all specimens were determined below the limit of quantification.

In the study was also performed an analysis on the level of leaching of heavy metals from the waste. For this purpose, the overall content of heavy metals in digest solutions prepared from the waste specimens was determined (Table 3). The slag was characterized by marginal leaching level of heavy metals (within the limits ranging from 0.01 to 1.3%). The lowest leachability demonstrated chromium and also copper, zinc and lead, when the liquid with the pH of 7 was applied. Nickel, despite the fact that it had the lowest leachability in acidic and neutral environments, was characterized by an increased level of leaching in strongly alkaline environment.

Table 3

Percentage level of heavy metals leaching

| Heavy metal | Total content [mg/kg d.m.] | Level of leaching [%] | | |
|---|---|---|---|---|
| | | pH 4 | pH 7 | pH 13 |
| Cu | 4679 | 0.04 | 0.01 | 0.07 |
| Pb | 2364 | 0.06 | < 0.02 | 0.29 |
| Zn | 7833 | 0.02 | 0.01 | 0.03 |
| Cd | 68.6 | < 0.07 | < 0.07 | < 0.07 |
| Ni | 41.5 | < 0.24 | < 0.24 | 1.30 |
| Cr | 595 | < 0.02 | < 0.02 | 0.02 |

The process of leaching contaminants also depends on the form in which the waste is available. In Table 4 are summarized results of leaching heavy metals from the waste with a grain size of < 10 mm (unground waste) and after its particles are grinded to the size of < 0.125 mm. The analysis of heavy metals leaching from waste with grinded particles was performed by applying an eluent with the pH levels of 4 and 7.

Table 4

Leaching of heavy metals depending on the particle size of the waste

| Heavy metal | Leaching at pH 4 [mg/kg d.m.] | | Leaching at pH 7 [mg/kg d.m.] | |
|---|---|---|---|---|
| | particle size < 0.125 mm | particle size < 10 mm | particle size < 0.125 mm | particle size < 10 mm |
| Cu | 1.74 | 1.65 | < 1.00 | < 1.00 |
| Pb | 1.18 | 1.43 | < 0.50 | < 0.50 |
| Zn | 3.72 | 1.91 | 1.95 | 1.01 |
| Cr | 0.14 | < 0.10 | < 0.10 | < 0.10 |
| Cd | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| Ni | < 0.10 | < 0.10 | < 0.10 | < 0.10 |

Reduction of waste particle size caused a slight increase in the release rate of Cu, Zn and Cr during the leaching by using a liquid having a pH of 4. If case of liquid with a pH of 7, higher concentrations in water extracts were observed for zinc. Designated concentrations of this metal in ground waste were almost doubled (both, for the pH levels of 4 and 7). Lead was the only element characterized by higher leaching from uncrushed faction of the waste. On the basis of results listed in the table, it can be noted that an acidic pH of the eluent caused a rise in the release rate of Cu, Pb and Zn from each fraction of the waste under tests.

The leaching of heavy metals is also affected by the ratio of liquid volume to the mass of a solid that is used in the test (L/S) and by the period for which the waste is shaken. Results of the analysed heavy metal concentrations derived in the test at L/S = 10 (fundamental test) and L/S = 100 dm$^3$/kg are presented in Table 5. The values expressed there are in mg/dm$^3$ and also calculated per kg dry mass, obtained in the test at the pH level of 4 for the eluent. For the sample with the ratio of L/S = 100, the test was also performed based on EN 12457-4:2002 standard, except that the period during which the waste was shaken amounted to 5 h.

Table 5

Concentration of heavy metals obtained with L/S = 10 and 100 dm$^3$/kg

| Heavy metal | Leaching at pH 4 | | | |
|---|---|---|---|---|
| | L/S = 10<br>24 hours shaking | | L/S = 100<br>5 hours shaking | |
| | [mg/dm$^3$] | [mg/kg d.m.] | [mg/dm$^3$] | [mg/kg d.m.] |
| Cu | 0.18 | 1.65 | 0.45 | 43.6 |
| Pb | 0.15 | 1.43 | 0.17 | 16.5 |
| Zn | 0.21 | 1.91 | 1.05 | 102 |
| Cr | < 0.01 | < 0.10 | < 0.01 | < 0.10 |
| Cd | < 0.005 | < 0.05 | < 0.005 | < 0.05 |
| Ni | < 0.01 | < 0.10 | 0.02 | 1.94 |

Adding a larger volume of water during the test (L/S = 100), despite the fact that the sample was shaken for a shorter period of time, caused an incensement in the release level of Cu, Pb, Zn, and Ni into the aqueous phase, greater than for the ratio of L/S = 10. The largest difference could be observed in a concentration of zinc, which in the water extract at L/S = 100 was 5 times higher than at L/S = 10. There were also observed higher concentrations for nickel (above the limit of quantification). During the test, at L/S = 100 heavy metals were also characterized by a higher leachability than at lower ratio of L/S, per one kg waste dry weight. This leads to this that in the test 10 times higher volume of water was used at the same weight of the waste sample. However, the impact of the ratio of liquid to the waste mass on the degree of leaching of cadmium and chromium was not observed. These metals, despite their high content in the waste do not exhibited the tendency to release.

## Conclusions

The copper slag was characterized by a very low release of contaminants. According to the results of leaching being performed on the basis of the fundamental test, it was classified to the category of inert wastes. As it was presented in the study, the slag was characterized by various tendency to leaching contaminants due to changes in leaching conditions and in the waste grain size. Application of eluent at pH level of 13 caused an increase in the rate of leaching of $Cl^-$, Cr, Ni, Cu, Pb, and Zn. Heavy metals, such as Cu, Pb and Zn are also characterized by a higher leachability, when the pH of the liquid is at the level of 4, rather than in an inert environment. However, due to an extensive application of copper slag in road construction and cement industry, this waste is temporarily disposed in heaps. Therefore, it does not pose a high risk to the ground-water environment. Solely in the case, when the environment is strongly alkaline, the amount of leaching of certain heavy metals can exceed the acceptable levels. Mineral construction materials used in road construction can contribute to increasing the pH of the surrounding environment. Therefore, the research on level of contaminants release from cooper slag should be continued in the context of their safe use to the substructure roads in order to reduce the possible leaching of heavy metals.

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# UWALNIANIE WYMYWALNYCH SKŁADNIKÓW Z ŻUŻLA POMIEDZIOWEGO W ZALEŻNOŚCI OD WARUNKÓW PROCESU WYMYWANIA

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**Abstrakt:** Badanie wymywalności składników odpadów jest jedną z metod oceny poziomu ich zanieczyszczenia rozpuszczalnymi formami metali ciężkich. Przebieg procesu wymywania uzależniony jest od kilku czynników, których zmiana może powodować uwalnianie zanieczyszczeń na różnych poziomach. W pracy przedstawiono wyniki wymywalności $Cl^-$, $SO_4^{2-}$, Fe, Na, K oraz metali ciężkich (Pb, Cd, Ni, Zn, Cu, Cr) z żużla pomiedziowego w zależności od pH cieczy wymywającej użytej w badaniu. Badanie wymywalności metali ciężkich przeprowadzono także przy różnym stosunku cieczy do ciała stałego (L/S = 10 i 100 $dm^3$/kg) oraz z wykorzystaniem odpadu o różnej wielkości frakcji. Dokonano także analizy zawartości ogólnej metali ciężkich w odpadzie. Zaobserwowano najwyższą wymywalność $Cl^-$, Cr, Ni, Cu, Pb i Zn przy pH cieczy wymywającej na poziomie 13. Redukcja ziaren odpadu do wartości < 0,125 mm spowodowała wzrost uwalniania miedzi i cynku do fazy wodnej.

**Słowa kluczowe:** żużel pomiedziowy, wymywalność, metale ciężkie

# Varia

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