ARCHIVES OF ENVIRONMENTAL PROTECTION

vol. 37 no. 1 pp. 3 - 11 2011

PL ISSN 0324-8461

THE INFLUENCE OF CADMIUM AND COPPER IONS ON MICROFAUNA OF ACTIVATED SLUDGE

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Keywords: Activated sludge, BIO, heavy metals, toxicity, protozoan population, ciliate, indicator organisms.

Abstract: The prevalence of heavy metals in wastewater is the cause of death of numerous organisms which take part in biological treatment of wastewater, that is why the aim of the study has been to asses the influence of cadmium and copper ions upon the microfauna of activated sludge.
5, 10, 50, and 100 mg/l of Cd^{2+} and Cu^{2+} were added into the samples of activated sludge and then, after 24 hours, the microscopic observations of activated sludge microfauna were carried out, and all changes concerning the amount of microfauna, functional groups, and species composition were determined.
The results obtained allowed to find a high level of toxicity of Cd^{2+} and Cu^{2+} ions to activated sludge microfauna, which resulted in the changes in the value of the Sludge Biotic Index and classes of sludge, survivability of microfauna, and reduction in the number of taxonomic units. It was observed that Cu^{2+} ions are more toxic to activated sludge microfauna than Cd^{2+} ions in identical doses.
Organisms sensitive to Cd^{2+} and Cu^{2+} ions have been found to be testate amoebae, *Aspidisca sp*. and *Epistylis sp.*, as well as organisms relatively sensitive to tested metals, which turned out to be ciliates of *Opercularia* and *Vorticella convalaria* genera.

INTRODUCTION

Activated sludge is a living, flocculent mixture of microorganisms, used in biological treatment of wastewater. Almost the whole biomass of activated sludge consists of bacteria, although inside the flocs on their surface and between them numerous microfauna occurs, such as *Protozoa, Gastrotricha*, *Nematodes, Tardigrada, Oligochaeta,* and *Rotatoria* [9].

Microfauna plays an important role in development of flocculent structure of the sludge, by producing mucus and breaking too large flocs. The basic function of microfauna in activated sludge is predation of bacteria, thanks to which rejuvenation and activation of bacteria populations in flocs occur. Predators also increase the elimination efficiency of organic compounds and ammonium nitrate from wastewater [11, 20]. Through grazing swimming bacteria, microfauna plays a crucial role by improving the quality of the effluent from the wastewater treatment plant [1, 3, 7].

The process of wastewater treatment is monitored mainly by means of physical chemistry indicators, which, however, do not provide information about the reactions of living organisms upon toxic compounds often found in wastewater. The toxicity of those compounds may be determined by means of various bacterial tests [12] or on the basis of living functional groups of sludge organisms – bioindicators [17].

Toxins in the environment of a bioindicator disturb its metabolism leading to the observable morphological, biochemical, and physiological changes, and ultimately to death [17]. Individual species, groups of organisms, and even entire ecosystems are used as bioindicators. In activated sludge, the bioindicative functions, serving the purpose of assessing the functioning of a Waste Water Treatment Plant (WWTP), are performed by microfauna, mainly by protozoa [7, 14, 17].

The speed and explicit character of microfauna reactions to changes of environmental conditions make it an indicator of sludge condition, as well as efficiency of wastewater treatment [6, 11, 14]. Madoni [13] introduced a universal method, serving the purpose of assessing the efficiency of various wastewater treatment plants. He developed a ten-point Sludge Biotic Index (SBI), which uses the indicative value of several species, higher units of classification, as well as functional groups, the so-called key ones. The domination of specific key groups has determined figure values attached. The obtained value of the index (0–10) is referred to one of the four diagnostic classes of activated sludge, indicating a definite condition of sludge. Class I of sludge, with very good activity and efficiency has 10–8 points, class II, with deteriorating biological activity yet good efficiency has 7–6 points, class III with insufficient biological treatment of wastewater and poor efficiency, has 5–4 points, class IV of sludge with poor biological treatment and low efficiency has SBI 3–0 points [9, 13].

Activated sludge is sensitive to toxic agents, such as, among others, ions of heavy metals, that are frequently present in municipal wastewater [6, 11]. Exposure of protozoa to metals induces biochemical changes in their cells, similar to those in bacteria [2]. The observation of microfauna is an efficient way of monitoring the influence of even small amounts of toxins on the structure of activated sludge populations [1, 7, 16].

That is why the aim of the study has been to determine the survivability of activated sludge microfauna in the presence of cadmium and copper ions, as well as to determine the changes in population structure, also in the bioindicative value of specific groups and species.

METHODOLOGY

The studies concerning the influence of Cd^{2+} and Cu^{2+} ions upon the activated sludge microfauna were carried out on activated sludge samples from the Wastewater Treatment Plants ”Warta” SA in Częstochowa in 2007.

A 250 ml sample of activated sludge was put in a 500 ml glass and then the salts of heavy metals were added: $CdCl_2 \times 2.5\ H_2O$, $CuSO_4 \times 5\ H_2O$ at final concentrations of 5, 10, 50, 100 mg Cd^{2+} and Cu^{2+}/l of activated sludge. The control consisted of activated sludge sample without addition of metals. All samples were placed in an incubation shaker TH15 by Edmund Bühler for 24 hours, in the temperature of 20ºC ± 2ºC. The rpm value was 120, which provided aeration by means of stirring, simultaneously not destructing the activated sludge structure [13].

The concentration of free ions of heavy metals was measured after 24 h incubation with sludge by the method of absorptive atomic spectrometry in the electro-thermal version, in graphite furnace [13]. The study was carried out on the ASA kit in the Provincial Inspectorate of Environmental Protection (Wojewódzki Inspektorat Ochrony Środowiska) in Katowice, Częstochowa branch, in accordance with the PN-EN ISO 15586:2005 norm [19].

The samples underwent microscopic analysis 24 after the introduction of the ions of metals, applying a method suggested by Madoni, which consists of identification of suitable functional groups and determination of the Sludge Biotic Index [9,13,18].

From each sample, two water preparations of activated sludge were made by means of collecting 20μl sample with micro-pipette to microscopic slide and covering it with micro-cover glass [13]. Depending on the size of a single organism or a colony, two types of magnifications were used: 100– or 400– folds. Under the microscope, at the magnification of 10 × 10, all ciliata, living amoebae, rotifers, and other *Eumetazoa* as well as large *Flagellata* were counted. *Ciliata* were classified to functional groups: crawling ciliata, attached ciliata, swimming ciliata, or predatory ciliata. In the case of ciliata colonies, all zooids were counted [18]. In each preparation, also small heterotrophic *Flagellata* were counted, using the 40 × 10 magnification, in randomly selected single stripe [9].

Using a table the Sludge Biotic Index (SBI) was determined [9]. When calculating SBI and classifying sludge, the number of species making up the activated sludge microfauna was determined, as well as the amount of individual groups of protozoans, and the total microfauna, also the number of small heterotrophic *Flagellata* was estimated [9, 18].

For determination of microfauna the following keys and atlases have been used: *A user – friendly guide to freshwater ciliates* [10], *Mikroorganizmy w osadzie czynnym (Microorganisms in activated sludge)* [5], *Podręcznik mikroskopowego badania osadu czynnego (Handbook for microscopic examination of activated sludge)* [8], *Osad czynny. Biologia i analiza mikroskopowa (Activated sludge. Biology and microscopic analysis)* [9].

The obtained results have been recalculated per 1ml and 1 litre and averaged for two preparations from one sample [9]. The results obtained from series of examinations have been averaged.

DISCUSSION

A considerable concentration reduction of metal ions in the filtrate by about 96–98% for cadmium, and 88–97% for copper has been found, in comparison to the amount of metal ions introduced to activated sludge. (Table1).

A comparable decrease in the concentration of free Cd^{2+} and Cu^{2+} ions has been observed in the studies by Madoni, in which after 24h the concentration of cadmium ions in sludge decreased in a similar way, i.e. from 50 to 1.48 mg/l and from 110 to 5.94 mg/l, and in case of copper ions from 50 to 6.12mg/l [13]. The cause of such a considerable decrease in the concentration of Cd^{2+} and Cu^{2+} is the presence of large amounts of organic and inorganic matters in wastewater, including several ligands capable of forming complexes with ions of transition metals, and the ability, possessed by active sludge biomass, of cumulating ions of metals [1].

Table 1. Concentration of cadmium and copper ions in activated sludge samples after 24 hours

Metal	Amount of metal introduced to sample (mg/l)	Activated sludge samples	
		Metal concentration after 24 h incubation	
		(mg/l)	%
Cadmium	5	0.073	1.46
	10	0.120	1.20
	50	1.100	2.20
	100	3.100	3.10
Copper	5	0.150	3.00
	10	0.290	2.90

Well-digested types of sludge, with good quality of the effluent, generally have a widely differentiated species composition of protozoa [4]. In the activated sludge of the WWTP "Warta" the following representatives of microfauna, identified and qualified into functional groups, have been found:

1. Group of crowling ciliata: *Aspidisca lynceus* (Mueller, 1773; Ehrenberg, 1830), *Aspidisca cicada (costata)* (Mueller,1786; Claparede, Lachmann, 1858), *Acineria uncinata* (Tucolesco,1962), *Euplotes moebiusi* (Kahl, 1932).
2. Group of attached ciliata: *Epistylis coronata* (Nusch, 1970), *Epistylis plicatilis* (Ehrenberg, 1831), *Opercularia sp.* (Fig.1.), *Vorticella convalaria* (Linnaeus, 1758) (Fig.2.), *Vorticella infusionum* (Dujardin,1841), *Vorticella microstoma* (Ehrenberg, 1830).
3. Group of predatory ciliata: *Acineta sp., Holophyra discolor* (Ehrenberg, 1833) *Litonotus sp.*
4. Group of amoebae: *Arcella sp., Centropyxis sp.*
5. Group of large *Flagellata*
6. Group of *Rotatoria*
7. Group of small heterotrophic *Flagellata* (HNF – *Heterotrophic Nanoflagellata)*

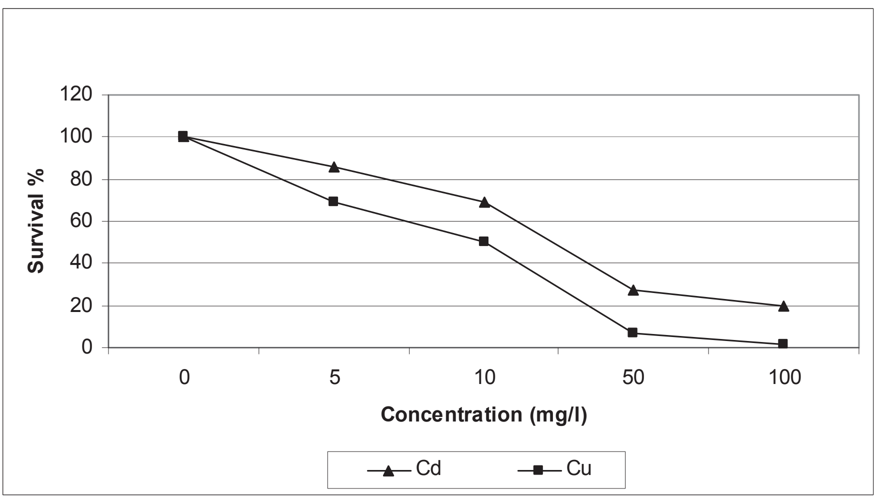

Fig. 1. Survivability of activated sludge microfauna in the presence of Cd^{2+} and Cu^{2+} ions

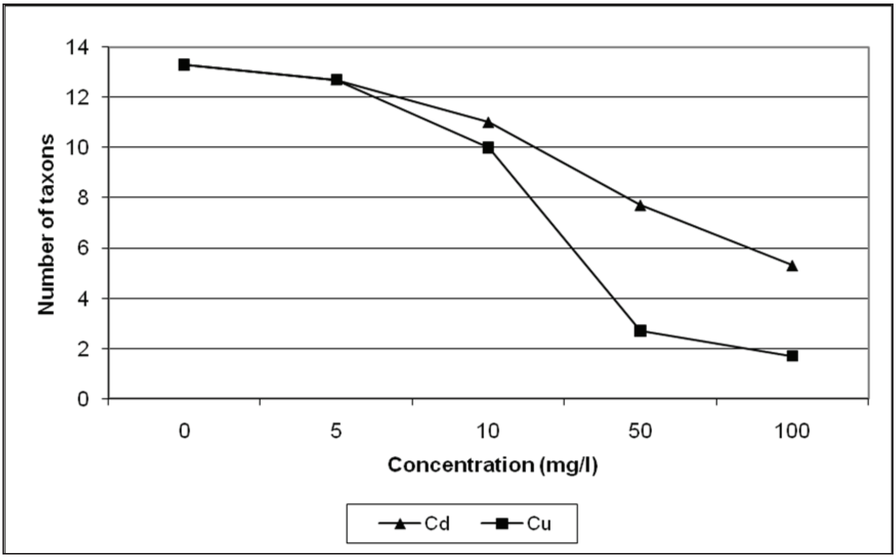

Fig. 2. Changes in the number of taxons of activated sludge microfauna under the influence of Cd^{2+} and Cu^{2+} ions

Such a population of microfauna is typical for majority of WWTPs with activated sludge [4, 9, 13, 14].

Sludge Biotic Index serves the purpose of monitoring the course and results of the WWTP functioning [7], provides the information on protozoa diversity, attributing more points to sludge types with greater diversity [4].

Reduction of values of Sludge Biotic Index results from reduced density of microfauna, and lower number of taxons, change of the dominating groups that one proper for well functioning sludge, to another dominating group [3, 9, 13]. Changes in the value of Sludge Biotic Index (SBI) after introduction of metal ions allowed to determine the change of sludge class under the influence of metal ions for each sample (Table 2).

Table 2. Changes of sludge class due to metal ions, on the basis of the SBI indicator

Number of introduced ions of metal mg/l	Cadmium		Copper	
	SBI value	Respective class	SBI value	Respective class
0	10	I	10	I
5	10	I	10	I
10	10	I	9	I
50	7	II	5	III
100	4	III	5	III

Change of sludge class under the influence of metal ions occurred only after the introduction of 50 mg Cd^{2+}/l to class II and 100 mg to class III. In the case of copper ions, already after the introduction of 50 mg/l Cu^{2+} the sludge class was reduced to class III (Table 2). Those changes indicate the reduction of efficiency of the studied sludge, as well as insufficient biological treatment of wastewater [9].

The metal ions introduced appeared to bring about a significant decrease in the amount of activated sludge microfauna, even under the influence of as little as 10 mg/l of metal, and a substantial reduction of that amount under the influence of 50 and 100 mg of the tested metals (Fig. 3). When determining the toxicity of chemical substances, the composition of the examined biocenosis is of importance. An increased share of sensi-

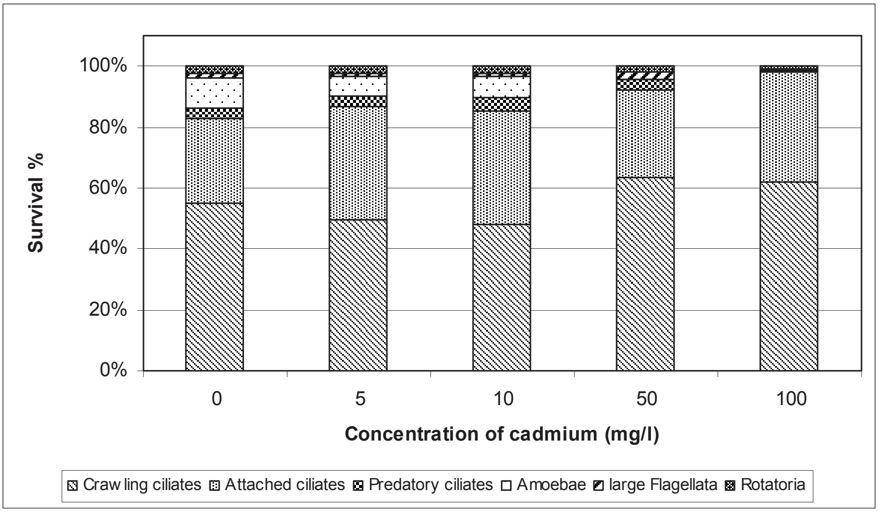

Fig. 3. Functional groups of activated sludge microfauna in the presence of Cd^{2+} ions

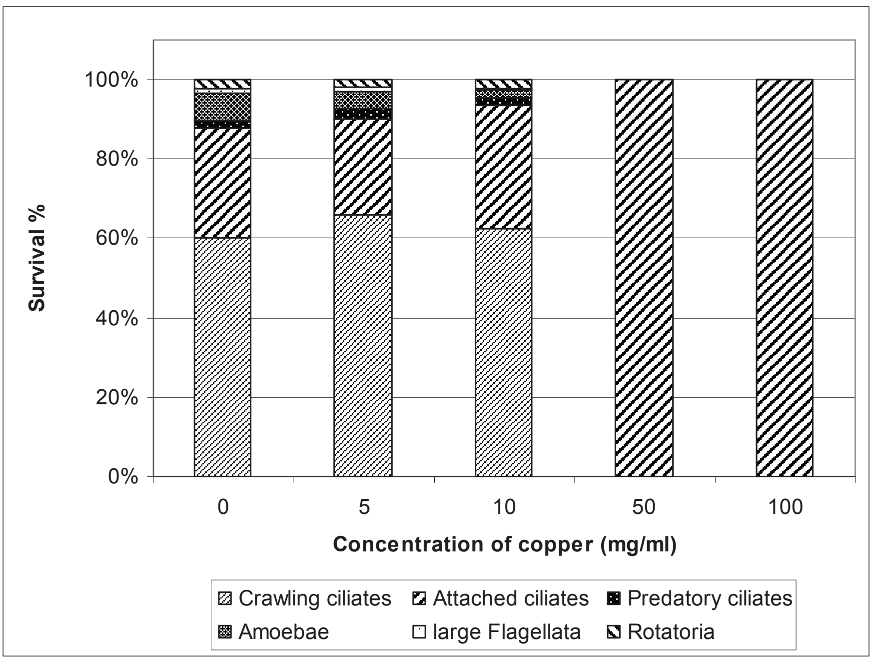

Fig. 4. Functional groups of activated sludge microfauna in the presence of Cu^{2+} ions.

tive populations causes the toxicity of a given substance to increase towards the entire biocenosis [13]. That is why, in the studies of Madoni [13] the survivability of activated sludge microfauna subjected to the activity of metal ions, was higher. 90 mg of cadmium ions /l resulted in the death of 50% of living cells of protozoans. For the concentration of copper ions which equals to 50 mg/l the protozoans mortality amounted to 90%.

Under the influence of heavy metals, decrease of species' diversity in activated sludge microfauna occurs [4]. In the studies performed, the number of taxons became reduced due to the introduction of metal ions (Fig. 4). The noticeable decrease in the number of taxons has been noted even at the concentration of 10 mg/l for both cadmium and copper. A further increase in the concentration of metal ions resulted in a more profound decrease of the number of classification units, while copper ions demonstrated more intense activity than cadmium ions.

Differences may indicate the occurrence of a larger number of classification units sensitive to copper ions, but may also be related to a greater toxicity of copper ions for microfauna than that of cadmium ions. The absence of cell walls and envelopes in protozoa explains their sensitivity and fast reaction to contamination of the environment with metals. Toxic ions dissolved in water react directly with the semi-permeable cell membranes and, because of that, they affect more intensely the protozoa cells, in comparison to cells of microflora representatives [15, 16].

Quantitative changes being a result of introduction of metals entail qualitative changes, reconstruction of microfauna population structure has been observed (Figs. 5–6). Biocenosis of sludge underwent reconstruction; with the increase of cadmium concentration (50 mg/l and 100 mg/l) a decrease of the number of predatory ciliata, amoebae, *Flagellata*, and *Rotatoria* has been observed. Also in the case of cadmium ions in concentrations 50 and 100 mg/l Cu^{2+} amoebae, large *Flagellata, Rotatoria*, and crawling ciliata were eliminated, while attached ciliata remained (Fig. 6.) The occurrence of spe-

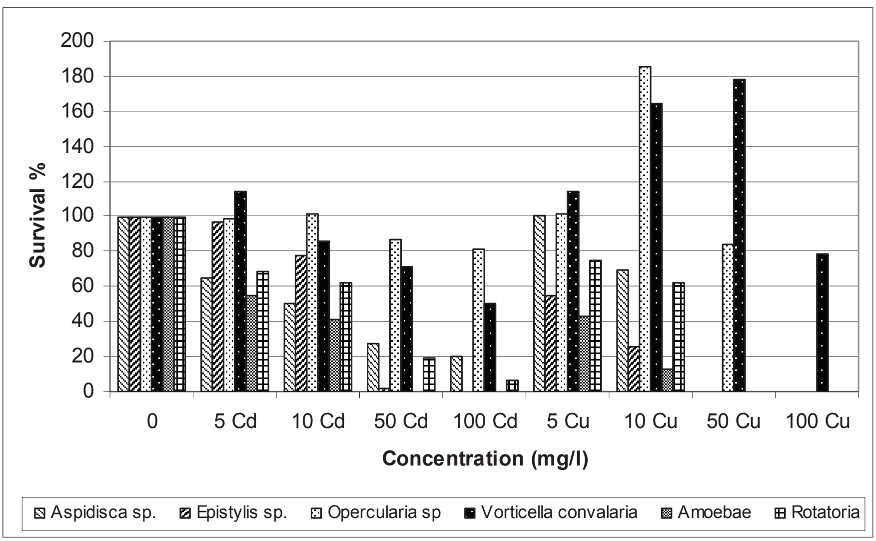

Fig.5. Comparison of survivability of dominating of activated sludge microfauna species, depending upon the concentration of Cd^{2+} and Cu^{2+} ions, in reference to control

cific indicative species is usually explained by their sensitivity to specific environmental factors, such as, e.g. temperature, oxygen, pH, or definite toxic substances [4]. In the presence of huge concentrations of copper ions in activated sludge, attached ciliata accounted for 100% of microfauna, from which it results that only in that behaviour group species resistant to copper ions occur; likewise in the study carried out by Nicolau, after introduction of 50 mg of Cu^{2+}ions/l of activated sludge, also the attached species became the dominating group of ciliata [18].

In the research carried out, the survivability of the most frequently occurring classification units in the studied activated sludge was compared, namely of: *Aspidisca sp., Epistylis sp., Opercularia sp., Vorticella convalaria,* amoebae, and *Rotatoria,* in the presence of Cd^{2+} and Cu^{2+} ions (Fig. 7). With the increasing concentration of Cd^{2+} and Cu^{2+} ions, a gradual reduction in the amounts of *Aspidisca sp., Epistylis sp.,* amoebae, and *Rotatoria* was observed, while high concentrations of copper eliminated those organisms altogether. Amoebae, due to their high sensitivity to all changes of environmental factors, testify about good sludge fermentation, that is why their extinction is a sign of disturbances in activated sludge [4].

The study demonstrated that organisms resistant to metal ions were found to be from the genus *Opercularia*, as well as the *Vorticella convalaria*, whereas the greatest tolerance to increasing concentrations of both ions has been observed in *Vorticella convalaria.* Those species are able to survive in extreme conditions, which suggested indicative poor condition of activated sludge [4]. The occurrence of *Opercularia sp.* was observed in bad conditions of activated sludge described by Szyłak-Szydłowki and Grabińska-Łoniewska [21]. It is known that *Opercularia* as one of ciliates occurred in the wastewater treatment plants and sludge containing toxins and heavy metals [9].

On the basis of the studies performed it can be stated that:

– copper ions are more toxic to the activated sludge microfauna than cadmium ions
– determination of Sludge Biotic Index allows for quick assessment of the quality of activate sludge
– reconstruction of multispecies population of sludge microfauna into a monospecies one indicates the presence of toxic substances in wastewater
– *Opercularia sp.* and *Vorticella convalaria* may be good indicators efficiency of poor sludge.

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Received: July 26, 2010, accepted: January 15, 2011.

WPŁYW JONÓW KADMU I MIEDZI NA MIKROFAUNĘ OSADU CZYNNEGO

Powszechne występowanie metali ciężkich w ściekach jest przyczyną obumierania wielu organizmów biorących udział w biologicznym oczyszczaniu ścieków, dlatego celem pracy była ocena wpływu jonów kadmu i miedzi na mikrofaunę osadu czynnego. Do prób osadu czynnego wprowadzano 5, 10, 50 i 100 mg/l jonów Cd^{2+} oraz Cu^{2+} a następnie po 24 godzinach dokonywano obserwacji mikroskopowych mikrofauny osadu czynnego i określano wszelkie zachodzące zmiany dotyczące liczebności organizmów, składu gatunkowego i grup funkcyjnych. W uzyskanych wynikach stwierdzono wysoką toksyczność jonów Cd^{2+} i Cu^{2+} wobec mikrofauny osadu czynnego. Jony te powodowały zmiany wartości Biotycznego Indeksu Osadu i klas osadu, spadek przeżywalności mikrofauny oraz spadek ilości jednostek taksonomicznych, przy czym zaobserwowano, iż jony Cu^{2+} są bardziej toksyczne dla mikrofauny osadu niż jony Cd^{2+} w tych samych dawkach. W populacji osadu czynnego organizmami wrażliwymi na obecność jonów Cd^{2+} i Cu^{2+}, są ameby domkowe *(Testacea),* orzęski *Aspidisca sp*. i *Epistylis sp.*. Zaobserwowano również występowanie w osadzie czynnym gatunków mało wrażliwych na testowane metale ciężkie, którymi okazały się orzęski osiadłe *Opercularia sp.* i *Vorticella convalaria.*