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IMPACT OF HEAVY METALS ON THE VIABILITY OF ACTIVATED SLUDGE

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Abstract. With an ever increasing population, biological wastewater treatment process has a crucial importance in the world. Activated sludge can be defined as a microbial community and consists of free, flocculated and filamentous bacteria, protozoa, rotifers and other invertebrates. Many studies on the relationships between protozoa and physicochemical and operational parameters have showed that species of these communities represent an important indicator of the wastewater treatment process efficiency. At a certain concentration, heavy metals and other pollutants are toxic to most microorganisms. The protozoa community is represented by interacting organisms, including species that are sensitive, intermediate or resistant in their tolerance to pollutants. The focus of the research was to determine the effect of 3 metals (copper, chromium and zinc) on the activated sludge viability. Experiments were conducted in a continuous flow bioreactor (simulating the conditions of a WWTP) fed with real wastewater and inoculated with activated sludge obtained from a local wastewater treatment plant. Microscopic determinations were used to evaluate the diversity and dynamics of the activated sludge biocenosis community. The study emphasised that all three metals had a more or less impact on the protozoa community highlighting both the most sensitive and the most resistant species.

Keywords: activated sludge, wastewater treatment, heavy metals, biocenosis.

AIMS AND BACKGROUND

Heavy metals and other pollutants[1] are toxic to most microorganisms at a certain concentration. The presence of heavy metals in water is of interest because of their known toxic effects on the environment[2,3] and also on the performance of biological wastewater treatment process. The mechanisms by which metals are eliminated in wastewater treatment process has been widely reported[4].

Heavy metals such as copper, zinc, and chromium are commonly present in untreated wastewaters coming from households and from industries like mining, metallurgy, electroplating, and chemical production. The deleterious effects of heavy metals is directly related to its solubility in the presence of the activated sludge[5]. The term 'activated' comes from the fact that the sludge particles teem

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with bacteria, fungi and protozoa. Heavy metals incline to affect the metabolic functions of microorganisms in activated sludge and lower the effectiveness of the biological processes in wastewater treatment plants[6]. Studies have shown that adapted sludge maintains a high removal efficiency of dissolved organic matter although exposed to constant input of heavy metals[7]. This suggests that adaptation can reduce the negative effects of toxic substances on biological reactions, and then some microbial groups can become predominant.

Protozoa and other higher life forms constitute approximately 5% of the activated sludge biomass. Taxonomic classification of these organisms is based primarily on motility. The protozoa in the activated sludge treatment process fall into four main classes: amoebae, flagellates and ciliates (free-swimming, crawling and stalked) and metazoa[8,9]. Many studies conducted on the relationships between protozoa and physicochemical and operational parameters have shown that species of these communities represent an important bioindicator of a wastewater treatment process efficiency, their presence being related to effluent quality[10, 11]. The composition of protozoa community may be affected by several parameters such as the type of biological treatment and the influent characteristics, sludge loading and sludge age, organic loading rate[12, 13]. Furthermore, protozoa populations can change rapidly under circumstances of toxic upset making them useful for the assessment of heavy metals toxicity.

The aim of the research was to determine the effect of 3 metals (copper, chromium and zinc) on the activated sludge viability.

EXPERIMENTAL

Experiments were conducted in a continuous flow bioreactor (Fig. 1), simulating the conditions of a WWTP, with the following configuration: anoxic reactor (C1), aerobic reactor (C2), settling tank (D), feeding pump (P1), recirculation pump (P2), activated sludge recirculation pump (P3), influent vessel (V1) and effluent vessel (V2).

Operating parameters: hydraulic retention time of 3 h in anoxic conditions and 9 h in aerobic conditions; room temperature (22–28°C); dissolved oxygen concentration in the anoxic reactor between 0.06–0.22 mg/l; dissolved oxygen concentration in the aerobic reactor ranged between 2.7 and 5.2 mg/l. The sludge concentration of the bioreactor was approximately 3 g/l. Internal recirculation and sludge recirculation summed up to 300 % of the influent flow.

The bioreactor was fed with real municipal wastewater and inoculated with activated sludge sampled from a local wastewater treatment plant.

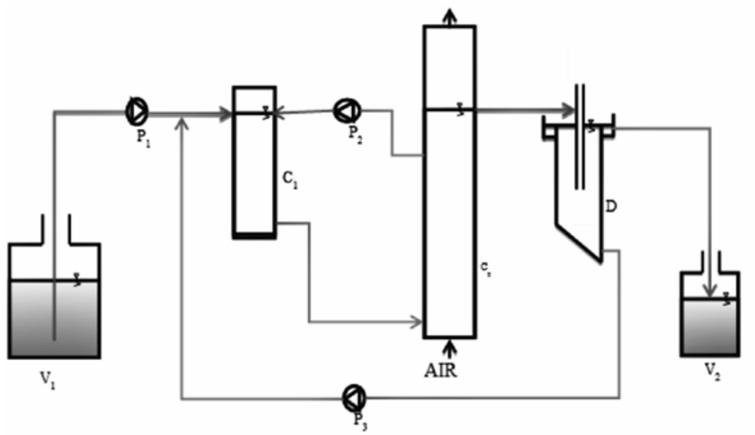

Fig. 1. Schematic representation of the experimental set-up

The experiments were performed in series of 2 weeks consecutively in order to evaluate the heavy metals impact on the sludge biocenosis diversity. The experimental protocol consisted of two weeks operation with municipal wastewater only (as control) followed by two weeks of operation with municipal wastewater enriched with heavy metals. The metals salts used in the experiment were: potassium chromate (K_2CrO_4), copper sulphate ($CuSO_4$) and zinc sulphate ($ZnSO_4$). The same protocol was used for all tested metals. The tested concentrations were: copper 200 and 400 μg/l, chromium 200 and 400 μg/l, zinc 1000 and 2000 μg/l.

The heavy metals effects on the viability of the biocenotic communities of the sludge were evaluated by microscopic investigations (trinocular Optech microscope with built-in camera).

RESULTS AND DISCUSSION

In order to determine the effects induced by heavy metals (copper, chromium and zinc) on the protozoa community, the activated sludge was investigated periodically during each testing period – control (without the addition of metals in the influent) and during testing period (with heavy metal addition). The general aspect of the sludge is presented in Fig. 2. The sludge flocs are characterised by an average degree of compaction, a slightly difuse structure and a specific brownish colour.

The trophic network of the activated sludge during control testing period was characterised by the following protozoa categories: testaceous rhizopods (*Euglypha* sp., *Arcella* sp., *Trinema* sp., *Cyclopyxis* sp.), nude rhizopods (*Amoeba* sp.), crawling ciliates (*Aspidisca* sp., *Chilodonella* sp., *Oxytricha* sp.), solitary stalked ciliates (*Vorticella* sp., *Acineta* sp.), colonial stalked ciliates (*Epistylis* sp.), predator ciliates (*Trachelophyllum* sp., *Amphileptus* sp., *Litonotus* sp., *Rotaria* sp.) and nemathods (Table 1).

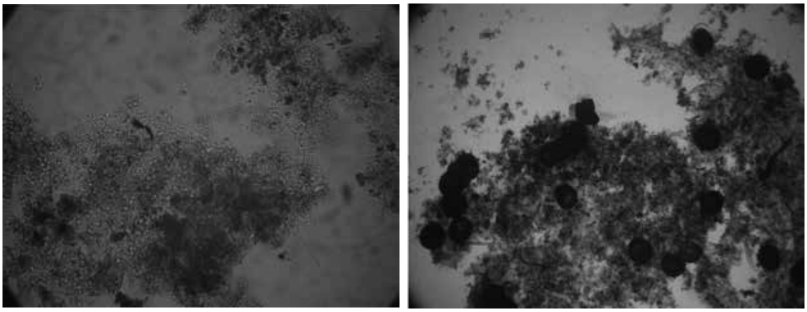

Fig. 2. General morphological aspect of the activated sludge during control period (left: ob. 20×; right: ob. 40×)

The presence of rotifers indicates a stable sludge with plenty of oxygen and good wastewater treatment performances, underlining the high nitrification rate (conclusion supported also by the high frequency of testaceous rhizopods) and high organic matter removal efficiency (correlated with the existence of the vast diversity of protozoa). The trophic structure indicates a good quality of the sludge.

Table 1. Activated sludge species diversity before metal testing

Group	Species	Initial	After 14 days
Testaceous rhizopods	*Euglypha* sp.	1467	1230
	Arcella sp.		67
	Trinema sp.		958
	Cyclopyxis sp.		867
	Centropyxis sp.		269
Nude rhizopods	*Amoeba* sp.		67
Free swimming ciliates	*Cinetochillum* sp.	200	
	Cyclidium sp.	67	
	Uronema sp.	34	
Large ciliates	*Paramaecium* sp.		
Crawling ciliates	*Aspidisca* sp.		200
	Chilodonella sp.	67	133
	Oxytricha sp.	93	67
Solitary stalked ciliates	*Vorticella* sp.	67	67
	Acineta sp.		93
Colonial stalked ciliates	*Epistylis* sp.		67
Predator ciliates	*Trachelophyllum* sp.	400	67
	Amphileptus sp.		133
	Litonotus sp.		67
Rotifers	*Rotaria* sp.		200
Nemathods	–	34	67
Total individuals (ml)		2429	4557
Total number of species		9	18

Copper influence on the biological diversity of activated sludge. Investigations showed that copper in concentration of 200 μg/l did not affect the protozoa community of the activated sludge (Table 2).

Tabel 2. Copper influence on the activated sludge protozoa community

Group	Species	Cu^{2+} 200 μg/l		Cu^{2+} 400 μg/l
		after 3 days (ind./ml)	after 14 days (ind./ml)	after 14 days (ind./ml)
Testaceous rhizopods	*Euglypha sp.*		135	
	Arcella sp.	135	67	135
	Trinema sp.	135	256	186
	Cyclopyxis sp.	5	135	135
	Centropyxis sp.	230	649	453
Flagellate	*Peranema* sp.		13	26
Nude rhizopods	*Amoeba* sp.		13	13
Free swimming ciliates	*Cinetochillum* sp.		67	67
	Cyclidium sp.			
	Uronema sp.			
Large ciliates	*Paramaecium* sp.	13		
	Stentor sp.		13	13
Crawling ciliates	*Aspidisca* sp.	435	586	446
	Euplotes sp.		53	13
	Chilodonella sp.			
	Oxytricha sp.	375	67	27
Solitary stalked ciliates	*Vorticella* sp.		67	67
	Acineta sp.			
	Podophrya sp.		67	53
	Paradophrya sp.		32	13
Colonial stalked ciliates	*Epistylis* sp.			
Predator ciliates	*Trachelophyllum* sp.		67	
	Amphileptus sp.			
	Litonotus sp.		67	13
Rotifers	*Rotaria* sp.		67	27
Nematods	–	67	67	13
Total individuals (ml)		1395	2488	1379
Number of species		9	19	17

An increase in the species number (from 9 to 19 species) was registered highlighting the existence of an unstable development phase of the taxa. In particular, an 82% decrease of the crawling ciliates *Oxytricha* sp. was observed, emphasising the bioindicator quality of these species in the presence of copper. Also the testaceous rhizopods, *Euglypha* sp. showed sensitivity to high concentration of copper.

Doubling the copper concentration (from 200 to 400 μg/l) in the influent has not generated a strong effect on the sludge trophic network, but the species number was adversely affected. Ciliates usually occur under good floc formation and generally indicate good activated sludge operation so the presence of crawling ciliates *Aspidisca* sp. and *Euplotes* sp. indicated good wastewater treatment performances.

Chromium influence on the biological diversity of activated sludge. In the case of chromium, unlike the other heavy metals tested, doubling the concentration (from 200 to 400 μg/l) had little to no effect on the sludge biocenosis.

The variations in protozoa community diversity during the experimental period with chromium addition is presented in Table 3. A high trophic network diversity was observed throughout the entire investigation period. The simultaneous maintenance in the system of the protozoa community with the influent contamination highlight their high tolerance. Also, the maintanance of the ptotozoa community after a testing period of 14 days demonstrates the adaptive capacity, the system becoming stable. Thus, the diversity of the protozoa community identified was characterised by the presence of: testaceous rhizopods (*Arcella* sp., *Trinema* sp., *Cyclopyxis* sp., *Centropyxis* sp.), flagellate (*Peranem*a sp.), nude rhizopods (*Amoeba* sp.), free swimming ciliates (Cinetochill*um* sp.), large ciliates (*Aspidisca* sp., *Euplotes* sp., *Oxytricha* sp.), solitary stalked ciliates (*Vorticella* sp., *Paradophrya* sp.) predator ciliates (*Litonotus* sp.) and rotifers (*Rotaria* sp.).

In addition, throughout the investigation period, the development of zooglea formations (Fig. 3) was noticed. This underlines the low toxicity of chromium over bacterial colonies.

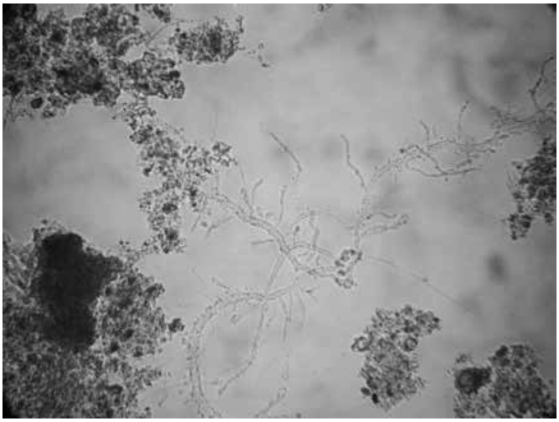

Fig. 3. Zooglea formations (ob. 200×)

Tabel 3. Chromium influence on the activated sludge protozoa community

Group	Species	Cr(VI) 200 μg/l		Cr(VI) 400 μg/l
		after 3 days (ind./ml)	after 14 days (ind./ml)	after 14 days (ind./ml)
Testaceous rhizopods	*Euglypha* sp.		67	67
	Arcella sp.	135	210	185
	Trinema sp.	135	186	186
	Cyclopyxis sp.	148	198	210
	Centropyxis sp.	398	410	368
Flagellate	*Peranema* sp.	13	26	26
Nude rhizopods	*Amoeba* sp.	13	67	67
Free swimming ciliates	*Cinetochillum* sp.	67	132	113
	Cyclidium sp.			
	Uronema sp.			
Large ciliates	*Paramaecium* sp.			
	Stentor sp.	13	27	27
Crawling ciliates	*Aspidisca* sp.	435	789	930
	Euplotes sp.	13	27	67
	Chilodonella sp.	35	8	
	Oxytricha sp.	37	67	67
Solitary stalked ciliates	*Vorticella* sp.	67	67	67
	Acineta sp.			
	Podophrya sp.	67	35	35
	Paradophrya sp.	13		
Colonial stalked ciliates	*Epistylis* sp.			
Predator ciliates	*Trachelophyllum* sp.			
	Amphileptus sp.			
	Litonotus sp.	13	67	13
Rotifers	*Rotaria* sp.	35	67	67
Nematods	–	23	36	36
Total individuals (ml)		1660	2478	2531
Number of species		18	17	17

Zinc influence on the biological diversity of activated sludge. During the sludge biocenosis monitoring exposed to zinc concentration of 1000 μg/l, a slight decrease of the population was recorded for the identified species (Table 4).

The increase of zinc concentration in the influent helped reduce population levels by approximately 50%, without evidence of a specific effect on a species. For example, no alteration was registered in the case of the crawling ciliates *Oxytricha* sp. (Fig. 4), the population remaining constant during the investigation. Instead, a decrease of *Podophrya* sp. and *Euplotes* sp. representatives was noticed. This feature is not associated with metal toxicity, but with the stability trend of the system.

The presence of solitary stalked ciliates is generally a sign of stable and healty activated sludge. The presence of *Vorticella* sp. in the activated sludge can be correlated with a good system operation because these species tend to leave their stalks under bad treatment conditions such as low DO levels or toxicity.

Table 4. Zinc influence on the activated sludge protozoa community

Group	Species	Zn^{2+} 1000 μg/l		Zn^{2+} 2000 μg/l
		after 3 days (ind./ml)	after 14 days (ind./ml)	after 14 days (ind./ml)
Testaceous rhizopods	*Euglypha* sp.	67	134	67
	Arcella sp.	163	287	96
	Trinema sp.	135	430	67
	Cyclopyxis sp.	134	332	143
	Centropyxis sp.	258	234	67
Flagellate	*Peranema* sp.	26	13	13
Nude rhizopods	*Amoeba* sp.	5		
Free swimming ciliates	*Cinetochillum* sp.	134	154	67
	Cyclidium sp.			
	Uronema sp.			
Large ciliates	*Paramaecium* sp.			
	Stentor sp.	13		
Crawling ciliates	*Aspidisca* sp.	1161	945	674
	Euplotes sp.	67	13	5
	Chilodonella sp.			
	Oxytricha sp.	67	67	67
Solitary stalked ciliates	*Vorticella* sp.	43	67	67
	Acineta sp.			
	Podophrya sp.	67	13	5
	Paradophrya sp.			
Colonial stalked ciliates	*Epistylis* sp.			
Predator ciliates	*Trachelophyllum* sp.			
	Amphileptus sp.			
	Litonotus sp.	13	5	
Rotifers	*Rotaria* sp.	35	13	3
Nematods	–	5		
Total individuals (ml)		2393	2707	1341
Number of species		17	14	13

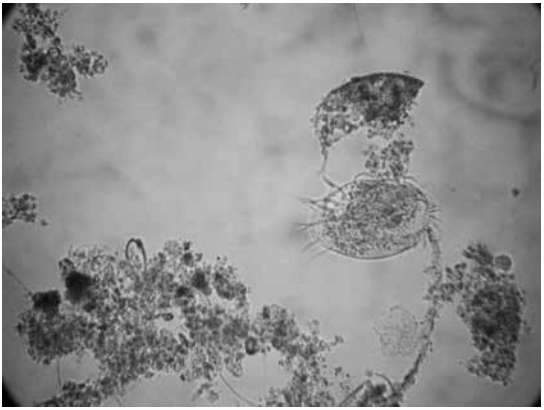

Fig. 4. *Oxytricha* sp. (ob. 200×)

CONCLUSIONS

The focus of the research was to determine the effect of 3 metals (copper, chromium and zinc), in different concentrations on the activated sludge viability. The results have permitted the establishment of the following toxicity sequence: Cu > Zn > Cr.

Investigations showed that copper (200 and 400 μg/l) did not affect the protozoa comunity of the activated sludge. A decrease of *Oxytricha* sp. and *Euglypha* sp. was registered to do toxic upset (bioaccumulation/bioconcentartion of the metal in the sludge) underlining the bioidicator quality of the species in the presence of copper.

A concentration of 2000 μg/l of zinc has affected the activated sludge biocenosis by reducing the population levels by approximately 50%. Nevertheless, no evidence of a specific effect on a species was registered. The population of *Oxytricha* sp. remained constant during the investigation highlighting the species high tollerance for zinc.

Chromium (200 and 400 μg/l) had little or no effect on the activated sludge biocenosis demonstating the protozoa community adaptive capacity. The low toxicity of chromium(VI) over bacterial colonies was emphasised with the development of zoogleal formation throughout the hole testing period.

Compared to the control during the whole investigation period for the metals tested the presence of flagellates was notted. These organisms feed on soluable organic matter and their presence can indicate a high soluble BOD level. Flagellates are usually found during recovery from a toxic discharge to the wastewater treatment plant underling the heavy metals negative action due to their bioconcentration/bioaccumulation in the activated sludge.

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