THE IMPACT OF HEAVY METALS ON BIOCENOTIC POPULATION OF WWTP'S ACTIVATED SLUDGE

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ABSTRACT

Heavy metals are toxic substances which, at certain concentrations have serious impact upon water treatment plants. This leads to impaired biological processes of activated sludge, changes in composition of biocenosis and, eventually, no biological activity.
The purpose of lab experiments was to test the influence of some heavy metals (cadmium, copper, zinc and nickel) at different concentrations, in batch bioreactors, with a retention time of wastewater of 24 hours. The inoculum was biological sludge and the bioreactors were fed with municipal wastewater supplemented with heavy metals.
The community of ciliates is an indicator of the activity of biological sludge, therefore these were predominantly monitored throughout the experiment.
The experimental results indicated that of all tested metals, cadmium at a concentration of 0.5-1 mg/l was mainly toxic, while zinc and nickel were less toxic, even at concentrations of 1.5-2.5 mg/l. The most sensitive species of protozoans to heavy metals were *Chilodonella uncinata* and *Oxytricha* sp., while the most resistant were the attached ciliates, like *Opercularia coarctata* and *Vorticella microstoma*. The attached ciliates resistant to high metal concentrations underwent morphological changes, like legthening of stem or forming buoyant individual cells or colonies.
The study emphasized the presence and survival of ciliates in activated sludge in the presence of heavy metals at concentrations exceeding the admitted limits in WWTP influents.

Keywords: heavy metals, biological sludge, ciliates

INTRODUCTION

Heavy metals are amongst the most toxic substances for living organisms. For this reason, their control and mitigation according to the national and international standards is a necessity. Some microorganisms existing in various ecosystems have the potential to reduce heavy metals through biochemical reactions.
Many studies were published in the last decades focusing on the correlation between protozoans and the treatment efficiencies. [1, 2, 3].

Madoni studied the effect of high concentrations of Cd, Cu, Cr (VI), Pb si Zn on the community of ciliates present in the activated sludge. Heavy metal toxicity in activated sludge depends mainly on two factors: metal speciation and its concentration. Other factors, like pH, sludge concentration, and influent charge affect more or less heavy metal toxicity. The studies showed that soluble ions of heavy metals are more toxic on activated sludge. The adapted sludge is more efficient in removing dissolved organics when continuously exposed to heavy metals, while high concentrations negatively affect activated sludge regardless it is adapted or not. By simultaneously testing more species – for example the community of ciliates from activated sludge – at different concentrations of metal, we can define the concentration affecting the community of microorganisms. [4]
In his studies, Gikas concluded that nickel and cobalt have an inhibitory effect on microorganisms at high concentrations. The negative effect depends on: pH, biological composition of the environment, the influence of other heavy metals, hydrostatic pressure etc. [5,6]

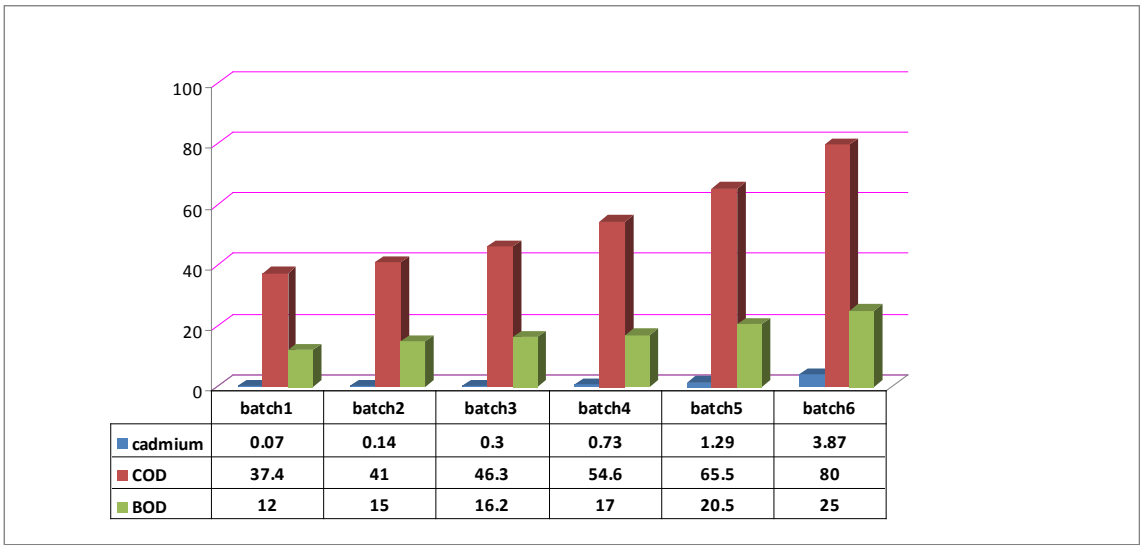
EXPERIMENTAL SETUP

The experiments were conducted in laboratory and the following heavy metals were tested: cadmium, copper, zinc and nickel. The system was discontinuous and was fed with wastewater and sludge from a municipal WWTP (Pitesti). Each metal was tested at increasing concentrations. The working conditions were: temperature 20 ± 2^0C, aeration and continuous mixing. After 24h, samples were taken from the mixed liquor and observed at microscope.
By live observation, there were identified 12 genera of ciliates in activated sludge, of which 4 of them attached (two species of *Vorticella*, *Epistylis, Opercularia*), 3 crawling hypotriche (*Euplotes, Aspidisca, Oxytricha)* and 4 free swimming holotriche *(Chilodonella, Colpidium, Litonotus, Paramecium).*

EXPERIMENTAL RESULTS

The results obtained in the four sets of batch experiments led to the following conclusions:

- **cadmium**, at concentration varying between *0.07* and 3.8 *mg/l,* affects the density and diversity of species, and a concentration of *0.07mg/l* determined the death of four of the species. At a concentration of *1.3mg/l* 8 species of ciliates died and the treatment efficiencies dropped with 10%. At the concentration of *3.87 mg/l* nine species of ciliates died and cell density diminished [photo 1]. At this concentration was registered the least treatment efficiency: 56.5% of COD and 60% of BOD [figure 1]. The most resistant species of ciliates *Colpidium*, *Vorticella* and *Opercularia*. The most resistant species were *Chilodonella* and *Oxytricha* (figure 2);



	batch1	batch2	batch3	batch4	batch5	batch6
cadmium	0.07	0.14	0.3	0.73	1.29	3.87
COD	37.4	41	46.3	54.6	65.5	80
BOD	12	15	16.2	17	20.5	25

Figure 1. Variation of biochemical parameters in batch tests-cadmium concentration (HRT – 24h)

Photo 1 - *Vorticella sp*. (stem longer than usual)

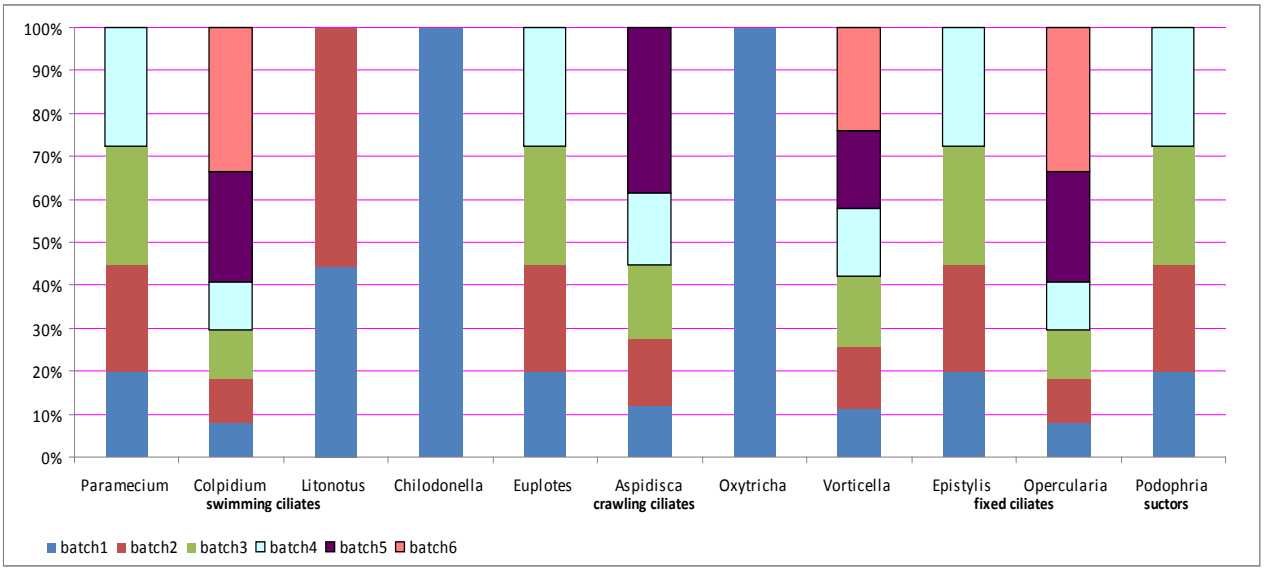
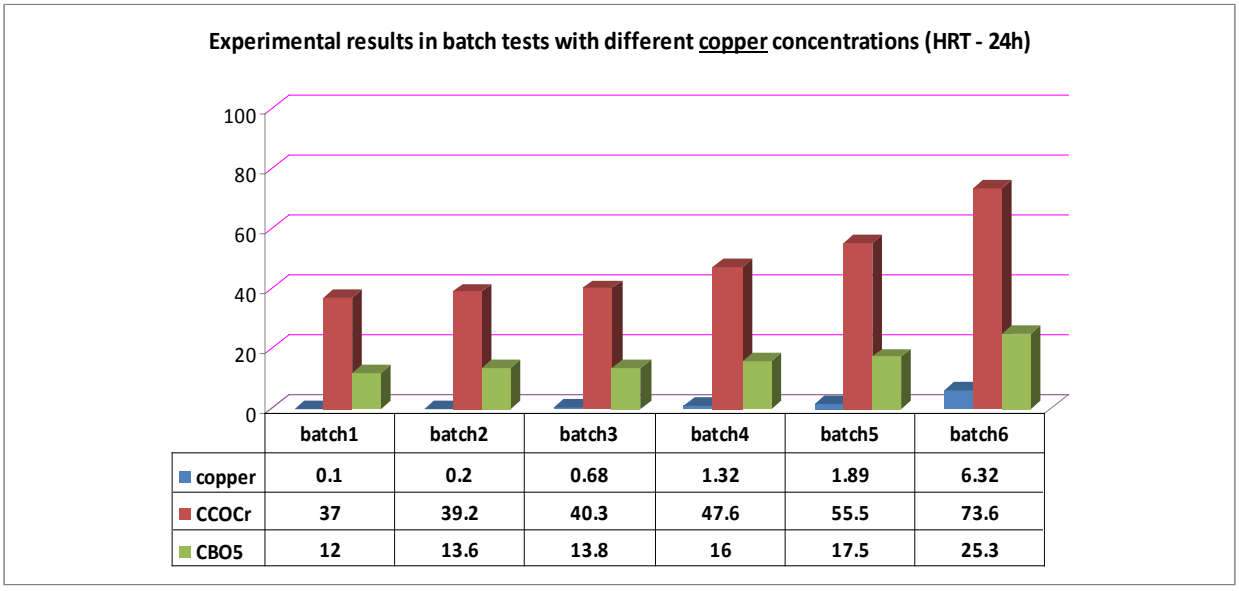


Figure 2. Distribution of ciliates in batch tests treated with cadmium

- **copper -** *0.1 and 0.2 mg/l* did not affect the constitution of biocenosis in general, while at concentrations of *0.68 and 1.32 mg/l Chilodonella* disappeared. At *1.89 mg/l*, four of the ciliate genera disappeared and the treatment efficiencies dropped slightly. At *6.32 mg/l* seven genera disappeared [photo.2] and the treatment efficiency decreased with 60% by COD and 62% by BOD (figure 3). Only V*orticella* si *Opercularia* survived after addition of 6.32mg/l Cu. The most sensitive was C*hilodonella* sp. (figure 4).



	batch1	batch2	batch3	batch4	batch5	batch6
copper	0.1	0.2	0.68	1.32	1.89	6.32
CCOCr	37	39.2	40.3	47.6	55.5	73.6
CBO5	12	13.6	13.8	16	17.5	25.3

Figure 3

Photo 2. *Vorticella* and holotriches in presence of 6.32 mg/l Cu.

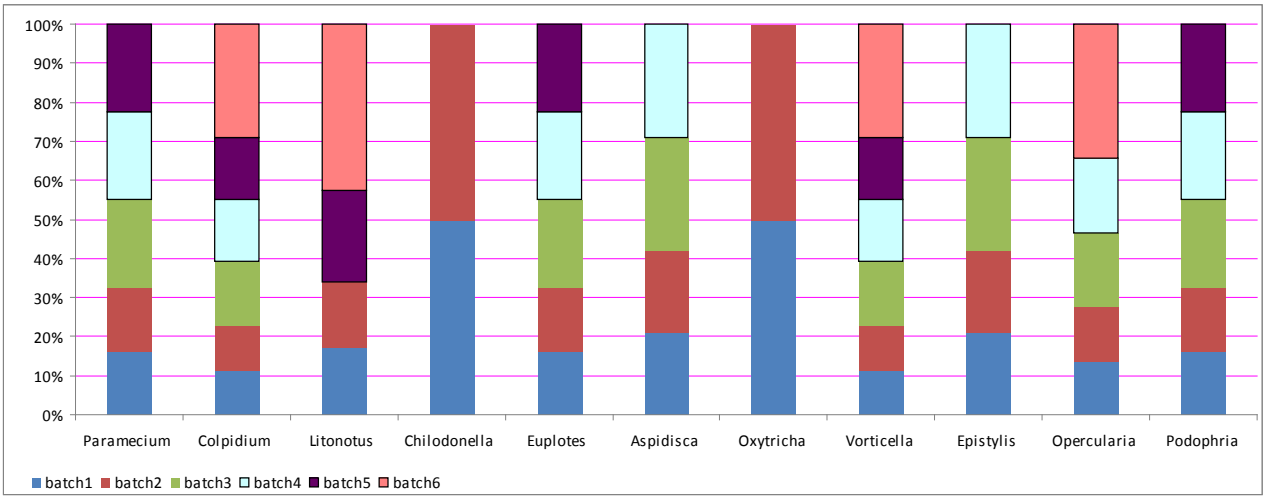


Figure 4. Distribution of ciliates in batch tests treated with copper

- **zinc** in concentration of *0.14-0.54 mg/l* does not affect density and diversity of microorganisms and at *1.23mg/l* only one genus disappeared. At *2.32 mg/l* two genera disappeared, while at *8.7 mg/l* only six genera survived [photo 3]. The treatment efficiencies decreased with ~10% [figure 5]. At high concentration, the most affected microorganisms were *Chilodonella* sp. and *Oxytricha* sp. and the most resistant were peritriches [figure 6].

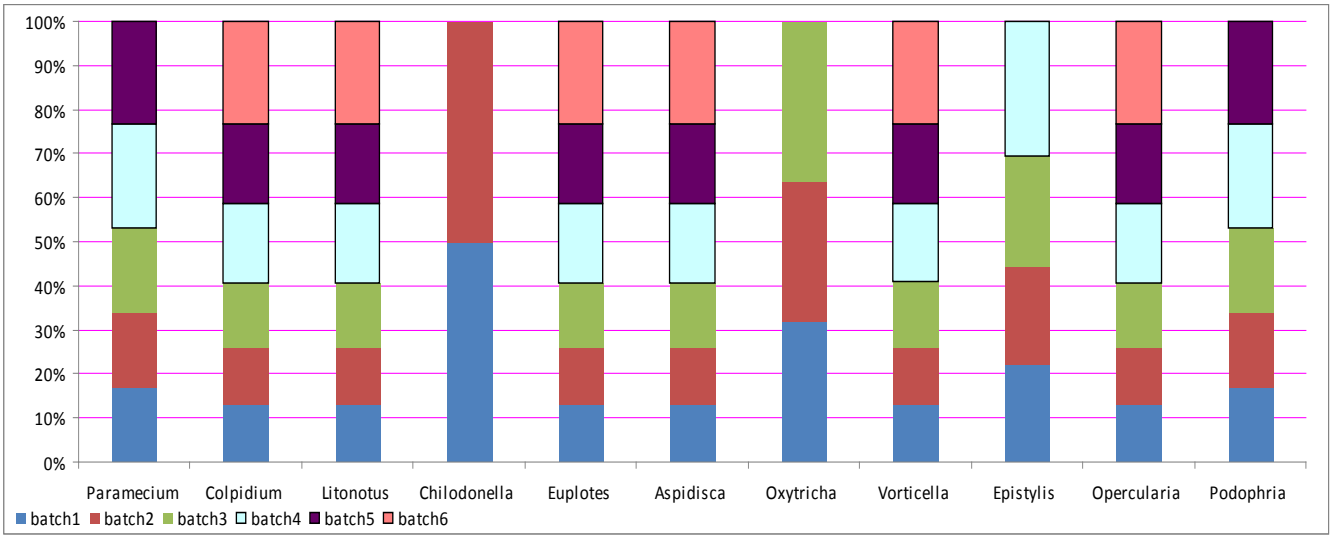


Figure 5. Experimental results in batch tests with different zinc concentrations (HRT – 24h)

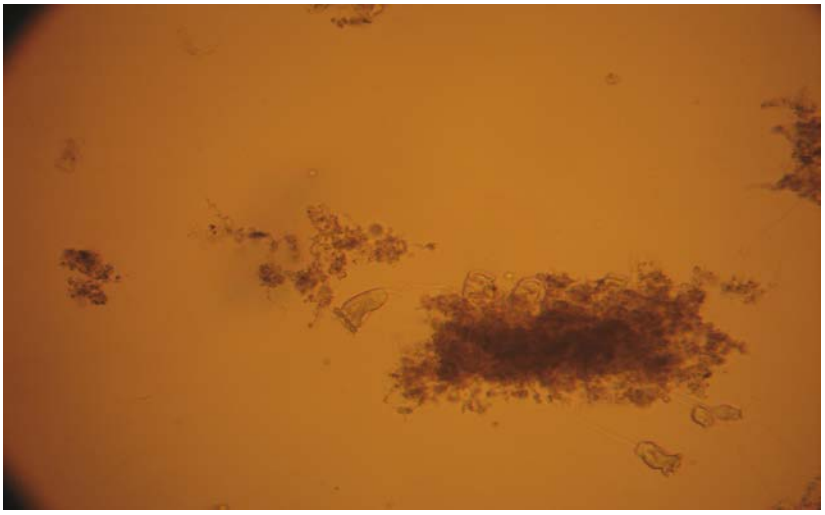


Photo 3. Activated sludge with fixed ciliates at 8.7mg/l Zn.

- **nickel concentrations of** *0.18 and 0.62 mg/l* did not affect the aspect of biocenosis in general but at concentrations of *1.27 and 2.58 mg/l* two and three genera disappeared. The most sensitive ciliates to nickel were *Chilodonella and Oxytricha.* At *6.13mg/l* four genera disappeared and the treatment efficiencies of activated sludge had a less significant decrease. At *38.4 mg/l*, nine genera disappeared [photo 4] and the efficiency of COD removal decreased with 50% [figure 7]. The most resistant ciliates to nickel were *Vorticella*, *Opercularia* and *Euplotes* which survived at *38.4mg/l* Ni. *Euplotes* is a bioindicator of wastewater polluted with nickel [figure 8].

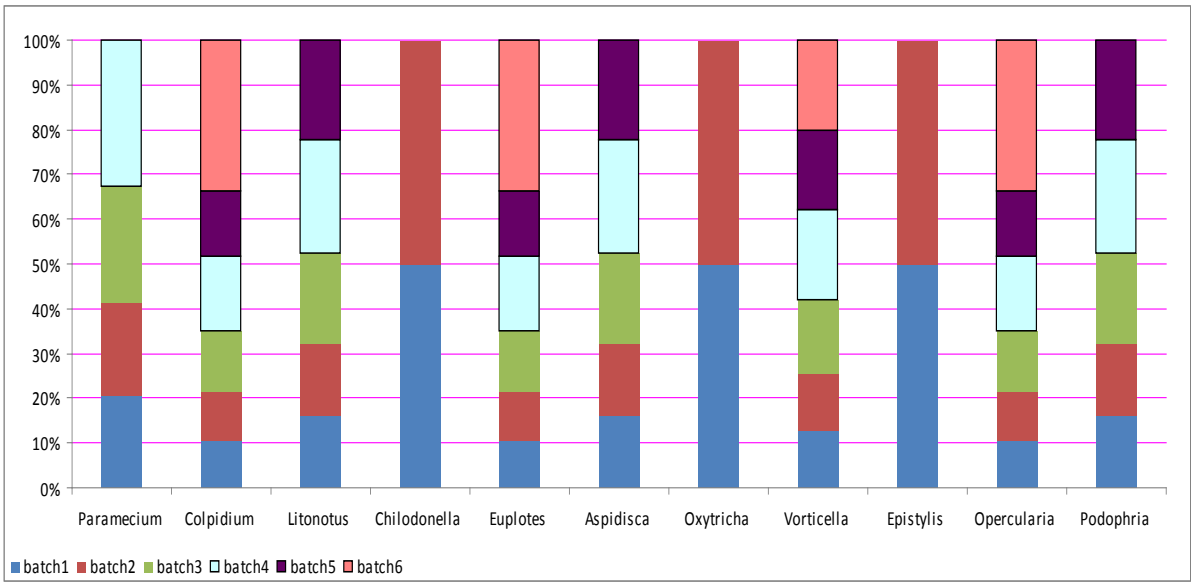


Figure 7. Experimental results of batch tests with different nickel concentrations

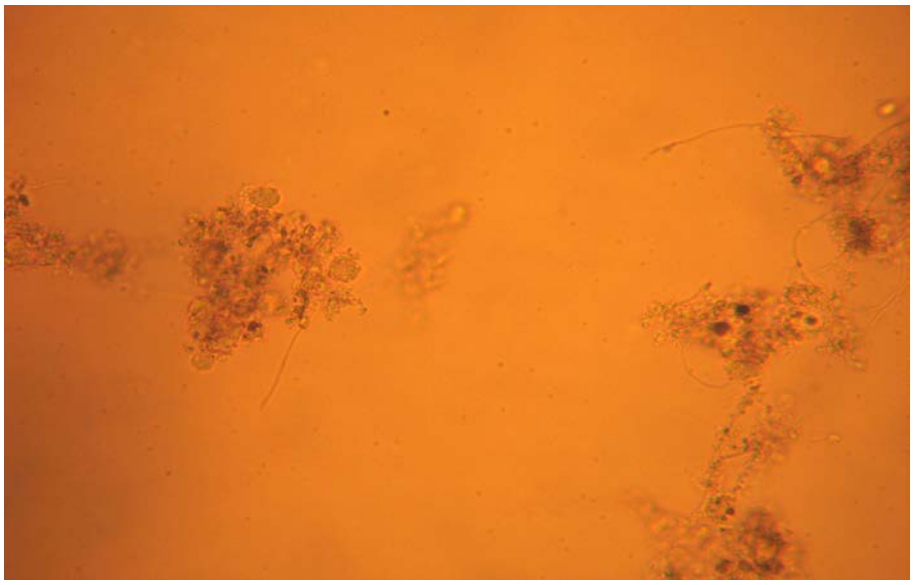


Photo 4. At high concentrations of nickel – 38.4mg/l
- dispersed, nonflocculating activated sludge

CONCLUSIONS

Studying the effect of heavy metals on activated sludge performance, we came up to the following conclusions:

- the most toxic of all tested metals was cadmium at the concentration of 0.5-1.0 mg/l while the least toxic was zinc (1.23-2.32 mg/l) and nickel (1.27-2.58mg/l);
- the toxicity of heavy metals was in general: Cd> Cu>Zn>Ni;
- the most sensitive microorganisms to tested heavy metals were *Chilodonella* sp and *Oxytricha* sp*.* The most resistant ciliates were *Opercularia* sp. and *Vorticella* sp. which survived at highest concentrations of Zn and Cu: 8.7mg/l respectively 6.32 mg/l. Resistance of *Euplotes* sp. at high nickel concentrations is an indication of waters strongly polluted with metals.

This work showed that microorganisms of activated sludge from municipal treatment plants can adapt and survive in the presence of high metal concentrations.

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