

Microfauna as Indicator of Copper, Zinc, and Cycloheximide in Activated Sludge Processes

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ABSTRACT

Microfauna, comprising protists and little metazoan, has proved to be a useful tool for assessing the occurrence of pollution in wastewater treatment systems, namely on activated-sludge plants. In the present work, the response of the microfauna communities of activated sludge to three toxicants—copper, zinc, and cycloheximide—was studied by means of a series of assays using a bench-scale plant. Along with the community descriptive parameters such as density, taxonomic richness, and the use of biological indexes—the Shannon-Wiener Index and the Sludge Biotic Index—several operating parameters were determined in order to allow for the comparison and possible correlations between the biological and the physical-chemical parameters. The results emphasize the ability of activated sludge communities, both bacteria and microfauna, to survive and to react to toxicants. High concentrations of copper and zinc (20 and 50 mg/L) prevented the satisfactory plant efficiency and the healthy state of the microfauna, including its survival. Cycloheximide did not have important and lasting effects below 5 mg/L. Among physical-chemical parameters, removal of soluble chemical oxygen demand seems to be the only one that presents patterns revealing a cause-effect relation along toxicological assays. Biological parameters were much more sensitive and coherent. The Sludge Biotic Index was, by far, the best tool in detecting intoxicant effects in the microfauna communities. Considering all parameters studied, copper was more toxic than zinc. Cycloheximide, in the range of concentration tested, was less toxic than these metals. The work highlights the role of microfauna as an indicator of toxicants entrance, besides the well-documented indicator value of plant operation conditions and efficiency.

Key words: microfauna; activated-sludge performance; toxicants; protists; Sludge Biotic Index

INTRODUCTION

THE PRESENCE OF TOXICANTS in the aquatic environment has become, in the past years, a problem of common occurrence. As concerns to water pollution, pro-

tists seem to be an excellent tool to assess both toxicity and pollution: they are regarded as biological indicators of pollution when their presence or absence can be related to particular environmental conditions (Esteban *et al.*, 1990, 1991; Madoni, 1993, 1994a,b, 1994b; Gracia

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et al., 1994; Fernandez-Leborans and Novillo, 1995, 1996; Abraham *et al.*, 1997; Abraham-Peskir *et al.*, 1997; Chen *et al.*, 2004; Lee *et al.*, 2004; Nicolau *et al.*, 2005), and they are considered test organisms when a species or a population is used to evaluate the toxicity of relevant toxic compounds (Lynn and Gilron, 1992; Madoni *et al.*, 1992, 1994, 1996; Nalecz-Jawecki *et al.*, 1993, 2003; Schultz, 1997; Nicolau *et al.*, 1999, 2001, 2004; Sauvart *et al.*, 1999; Schlimme *et al.*, 1999).

Diversity and biotic indexes

The way biological communities are established and how their structure is affected by toxicants is a question of great interest to ecologists. This led to the appearance of methods that try to relate the effects of pollution to the community structure and to the presence of indicator species.

Diversity indexes attempt to combine both species richness and evenness in to a single value, which is assumed to reflect environmental quality. It is assumed that a well-balanced community presents, always, a high diversity, with no dominance of a particular species. It is also assumed that pollution alters the community structure by reducing diversity (Cairns and Pratt, 1987). Regardless of numerous cautionary remarks concerning their use, diversity indexes have remained very popular among ecologists (Washington, 1984).

Biotic indexes reflect in another way the environmental quality, because they use the concept of an indicator organism. These indexes are usually specific for a certain type of pollution, because indicator organisms are not simultaneously sensitive to all kinds of pollution. The concept of indicator organism is also very controversial, but there are some consensual characteristics essential to the determination of an indicator organism (James and Evisson, 1979): to have a wide geographic distribution, to be abundant in the habitat in which it occurs, to have narrow and well-defined ecological tolerances, and to be easily identified. The most frequently referred as indicator organisms are the algae, the bacteria, the protists, and the aquatic macroinvertebrates and vertebrates. In the present work, the Sludge Biotic Index (SBI), proposed by Madoni (1994a), was used to evaluate the microfauna community changes along the toxicological assays. This index is based on the abundance and diversity of the protistan community in the activated sludge of the aeration tank and on the different sensitivity revealed, by some of the microfauna groups, to physical-chemical and operational factors prevailing in the system. SBI may range from 0, indicating the poorest condition, to 10, indicating the best condition. SBI values are grouped in four classes corresponding to different quality levels: class I includes SBI values of 10, 9, and 8; class II includes 7

and 6; group III includes 5 and 4; and class IV includes the remaining values. The numerical evaluation allows for the comparison of different sludge or of sludge biological quality through time, and is based on the relationship occurring between plant performances and operating conditions on one hand, and structure of the microfauna on the other.

The protistan community of activated sludge processes

Among protists, ciliates often reach densities of about 10^7 cells/L in the aeration tank (Madoni, 1993, 1994a) and play an essential role in the purification process by removing, through grazing, the majority of dispersed bacteria, which would cause high turbidity in the final effluent.

Most of ciliates present in biological wastewater treatment plants feed, by filtration processes, upon dispersed populations of bacteria and can be divided into three main groups according to their feeding behavior (Madoni, 1994a): free swimmers, which swim in the sludge liquid fraction and remain in suspension in the sedimentation tank; attached ciliates, which are attached to the bacterial aggregates and settle in the sedimentation tank; and crawlers, which live in the floc surface, and settle in the sedimentation tank as well.

An efficient activated sludge plant should present in the aeration tank (Madoni, 1994a): (1) high microfauna density, at least 10^6 cells/L; (2) specific composition based in attached and crawling ciliates, with the absence of flagellates which, along with the free-swimming forms, are typical of the colonization stage; (3) a diversified community, where no group dominates numerically by a factor greater than 10. It is also considered that the dominance of swimming forms, flagellates, or sessile as *Opercularia* sp. usually indicate, for different reasons (e.g., high organic load, lack of dissolved oxygen, toxicants entrance), a lower performance of the wastewater treatment plant.

In the present work, a bench-scale plant was exposed to three toxicants—copper, zinc, and cycloheximide—in order to provide better understanding of the microfauna community changes along with the performance of the plant and the possible correlations between the former and the latter.

In what activated-sludge concerns, the relation between metals and decrease of depuration efficiency is proved, but the way they affect each of its components is still misunderstood. Metals play a dual role in the physiology of microorganisms, as some of them are vital to development and survival, while others are toxic even at low concentrations (Abraham-Peskir *et al.*, 1997). Copper and zinc were selected because of the importance

metals generally have in toxicological studies. Cycloheximide is an antibiotic that inhibits protein synthesis in eukaryotic cells, allowing for the comparison between the effects on the bacterial component and on the microfauna.

MATERIALS AND METHODS

Experimental plant

The bench-scale plant used in the present work had an aeration tank and a sedimentation tank (Fig. 1). Table 1 shows the information about several parameters in the experimental prototype. The pilot plant was continuously fed with real sewage coming from the Municipal Wastewater Treatment Plant of Braga, after primary treatment, that is, after the removal of solids, sands and grease and

primary sedimentation. Sewage was collected three times a week to prevent self-degradation in the laboratory, which could affect the results. The chemical oxygen demand (COD) of the sewage had a medium value of 450 mg/L and the medium ratio COD (soluble)/N (soluble)/P (soluble) was approximately 100/33/4, which shows that the sewage arriving to the Municipal Plant is highly rich in nitrogen and phosphorous.

The experimental plant was previously inoculated with activated sludge from the Municipal Plant, and a 3-week period elapsed until the community was considered stable, as confirmed by assessing the microfauna diversity and COD removal efficiency. Sludge age was nearly constant during the assays through daily sludge removal in the sedimentation tank. Each assay began with "fresh" sludge followed by a stabilization period to ensure the absence of acclimatization phenomena.

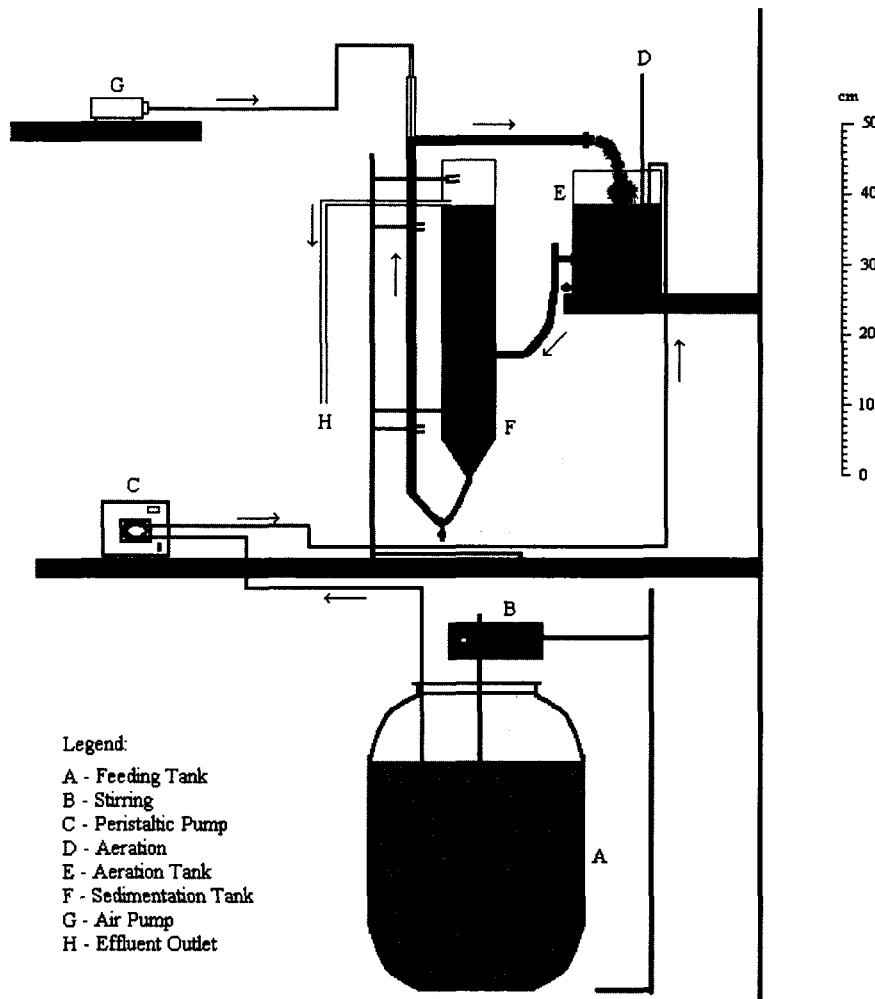


Figure 1. Experimental prototype of wastewater plant.

Table 1. Operational parameters of the bench-scale plant.

Capacity of the aerated tank	2 L
Total capacity of the system	5 L
Feeding	370 mL/h
Residence time in the aerated tank	5.5 h
Residence time in the system	13.0 h
Dissolved oxygen in the aerated tank	3.6–4.8 mg/L
Mean temperature	19°C
pH of the sewage	6.5–7.5
pH of mixed liquor	5.8–6.3

Toxicant concentrations

Copper, as $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, and zinc, as ZnCl_2 , were used to obtain different final concentrations of metal: 4 (only for zinc), 8, 20, and 50 mg/L, and were added in one single dose to the aeration tank. Copper and zinc chlorides were previously dissolved in 10 mL of mixed liquor. Considering that only the soluble fraction of metals is toxic to organisms (Madoni *et al.*, 1996), soluble copper and zinc were measured in the mixed liquor of the aeration tank just before and 24 h after the adding of the copper and zinc chloride solutions. The soluble copper and zinc concentrations were determined, after filtration and digestion with nitric acid (65%), by photometric Merck Spectroquant Methods.

Cycloheximide was added, also in one single dose, to obtain three final concentrations: 1, 5, and 20 mg/L.

Physical–chemical parameters

During the period of assays, suspended solids (MLSS), volatile suspended solids (MLVSS), sludge volume index (SVI), temperature (T), dissolved oxygen (DO), and pH were measured in the aeration tank, following Standard Methods (APHA, 1985). Determinations of soluble COD, total N, nitrate, and ammonia (all soluble) of the influent and of the effluent were carried on (using also photometric Merck Spectroquant Methods) to calculate COD removal efficiency and the possible occurrence of nitrification and/or denitrification in the system. The parameters were determined daily during the assays, by calculating the average of two samples taken at the same time.

Biological parameters

Microscopic analysis was performed using bright-field and, whenever necessary, phase-contrast illumination to inspect two subsamples of 25 μL taken from the aeration tank according to the subsampling technique described by Madoni (1984). Protists were identified according to Madoni (1994b) and Foissner *et al.* (1991,

1992, 1994, 1995). Each sample was studied according to the method proposed by Madoni (1994a) and the correspondent functional groups and SBI were achieved. To calculate the SBI, it is necessary to previously find the number of species (or supraspecific level whenever species identification could not be achieved), the abundance of each species and of total microfauna, and to estimate the number of small flagellates. For each sample, the Shannon-Wiener Diversity Index (Shannon and Weaver, 1949) was also determined. Based on the Information Theory, this index is a measure of the average degree of uncertainty in predicting to what species (or other taxonomic category) an individual, chosen at random from a collection of S species and n individuals, will belong. The equation used for the Shannon function was:

$$H' = -\sum_{i=1}^S (P_i \times \log_2 P_i), \text{ where } P_i = n_i/n$$

Toxicological assays

The assays lasted, in most cases, 7 days, that is, 168 h: hour 0 (zero) corresponds to the hour just before the adding of the toxicant; along each assay, the already mentioned physical–chemical and biological parameters were assessed at the beginning of the assays, just before the adding of the toxicant, at 24, 48, at 72 h, and at the end of the assay. As said above, soluble metal content was determined just before and 24 hours after the adding of copper and zinc chlorides.

RESULTS

It is known that organic matter can, in a variable degree, complex metal ions, making them less toxic for aquatic life (Sujarittanonta and Sherrard, 1981; Madoni *et al.*, 1996). The results of the determination of soluble metals after the adding of the copper and zinc chloride solutions are shown in Table 2. The results of soluble metal concentration after 24 h did not reflect the added feed concentration; therefore, results are discussed upon the initial concentration of each toxicant.

The patterns obtained for the variation of MLSS in the experiments varied a lot. The initial values were around 1,250 mg/L in the copper and cycloheximide assays and around 1,500 mg/L in the zinc assays, with the exception of the assay with 8 mg/L (MLSS 1,815 mg/L). The oscillations during the assays, along with the variation of COD in the effluent, led to complex patterns of variation of other parameters such as organic load, for example. Anyway, the MLVSS presented the same patterns of variation of MLSS in all assays and the ratio VSS/TSS was always around 0.8.

Table 2. Mixed liquor metals concentrations.

Compound	Nominal concentration		Concentration before adding (mg/L) ^a	Increase 24 h after adding (mg/L) ^a
	mg/L	mM		
Copper	8	0.126	0.12 ± 0.01	0.35 ± 0.05
	20	0.317	0.11 ± 0.03	0.58 ± 0.06
	50	0.794	0.13 ± 0.02	3.44 ± 0.13
Zinc	4	0.062	0.47 ± 0.05	0.59 ± 0.09
	8	0.123	0.55 ± 0.06	0.63 ± 0.06
	20	0.308	0.46 ± 0.05	1.79 ± 0.05
	50	0.769	0.37 ± 0.05	2.30 ± 0.02

^aArithmetic media of three dosing ± standard deviation.

The high concentration of DO allowed for a wide nitrification in the aeration tank, well shown in the content of ammonia and nitrates in the influent and the effluent (Table 3). The values presented are referred to soluble concentrations. Experimental results do not allow for the establishment of a cause-effect relation between them and the adding of toxicants. The content of nitrite was always below 2 mg/L.

Among physical-chemical parameters, removal of soluble COD seems to be the only one that presents patterns revealing a cause-effect relation along toxicological assays. Among biological parameters, and in a general way, the toxic compounds caused a decrease of species richness and of population density. In the case of cycloheximide, however, the patterns of variation are less consistent. Figures 2, 3 and 4 present the most significant results of the assays with copper, zinc and cycloheximide, respectively: the removal of COD, the taxonomic richness, the Shannon-Wiener Index, the SBI, the relative abundance of functional groups according to Madoni (1994a), and the abundances of crawlers and the genus *Opercularia*, as they are often related to opposite environmental conditions in plants, favorable and prejudicial, respectively.

In Figure 2, the initial degradation of the system in the assay with 8 mg/L of copper is clear for the COD removal and the biological parameters as the SBI and the

species richness, but not for the Shannon-Wiener Index. The distribution of functional groups and the ratio *Opercularia* sp./crawlers do not reflect per se the disturbance of the community: there was a significant stimulation of the crawlers due to the growth of *Aspidisca cicada*. By the end of the assay, the system was apparently completely recovered. The adding of 20 mg/L of copper had not significantly stronger consequences on most of the parameters, but the sessile clearly dominated the samples and *Opercularia* sp. prevailed upon crawlers. Recovery was possible in 7 days for the species richness and the Shannon-Wiener Index. The highest concentration of copper, 50 mg/L, was significantly more toxic than 8 mg/L considering all the parameters, and did not allow for the recover in 7 days, with the exception of species richness that reached 10 by the end of the assay. In conclusion, the higher concentrations of copper caused disturbances that, by the end of the assay, did not disappear completely, and the lower concentration of copper caused changes that could be recovered from in 1 week and, in this case, a significant increase of the crawler *Aspidisca cicada* could be observed.

Figure 3 allows for the conclusion that all concentrations of zinc affected the biological communities in the aeration tank. This was less apparent in the case of 4 mg/L, because, in this case, the disturbance was abrupt only in the first 24 h for the soluble COD removal effi-

Table 3. Concentrations of soluble nitrogen compounds in the influent and in the final effluent (no relevant differences between assays were found).

Nitrogen compound	Conc. in the influent (mg/L)	Conc. in the effluent (mg/L) ^a
Total nitrogen	62.0–81.5	54.5–73.0 (–10 to –12%)
Nitrate	0.3–1.4	10.0–50.4 (+2,500 to +3,500%)
Ammonia	61.5–75.8	32.5–38.0 (–42 to –52%)

^aBetween (), the relative decrease (–) or increase (+).

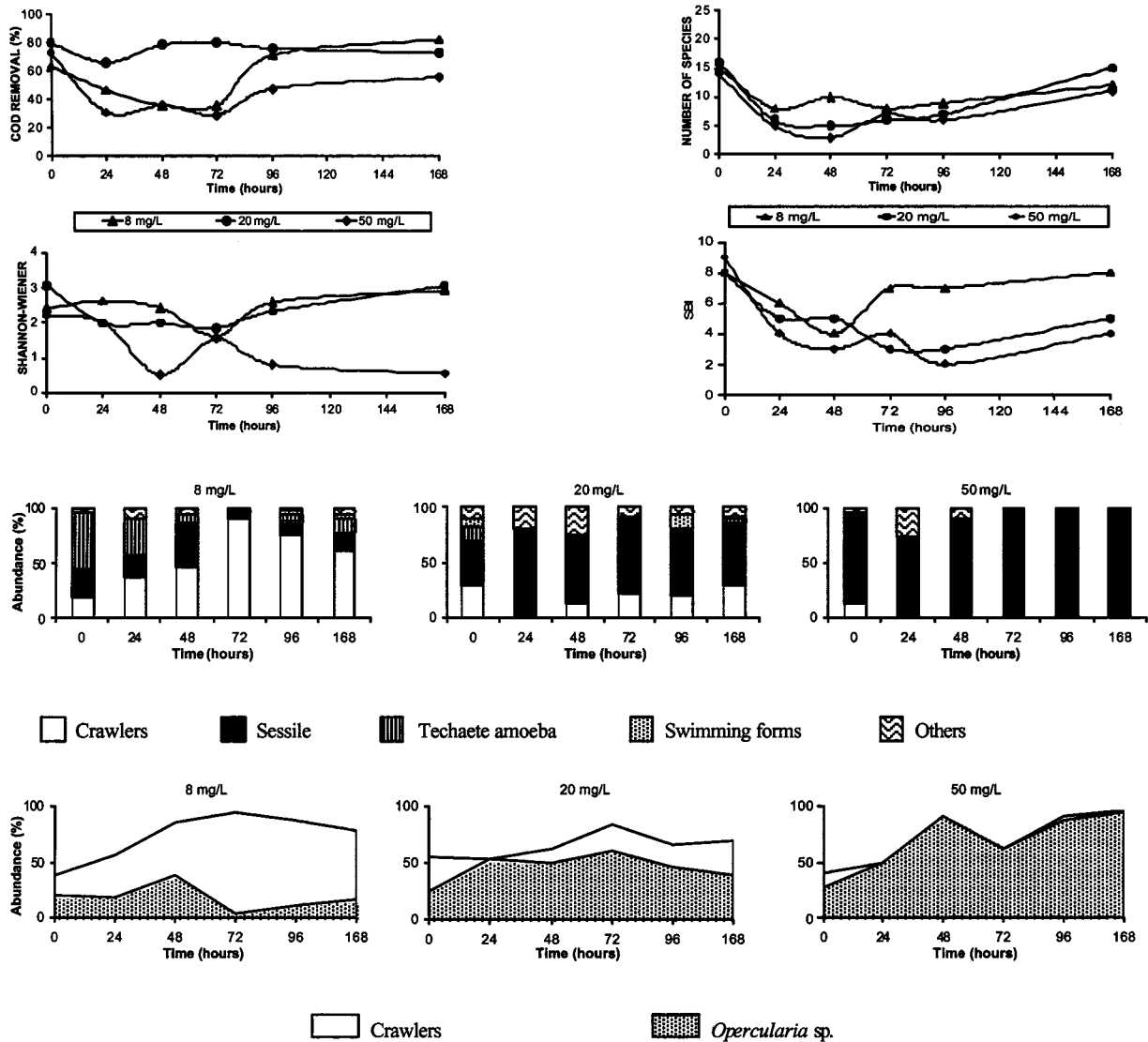


Figure 2. Variation of COD removal, taxonomic richness, Shannon-Wiener Index, Sludge Biotic Index (SBI), relative abundance of functional groups, and ratio crawlers/*Opercularia* sp. in the assays with copper.

ciency or when the ratio *Opercularia* sp./crawlers is considered. On the other hand, it seems that there was a wider recovering in the case of 50 mg/L than in the case of 20 mg/L of zinc, except for the SBI value. The relatively high value of the Shannon-Wiener Index by the end of the assay with 50 mg/L was due to a substantial improvement on the species richness, not so reflected in the SBI: the SBI is not as sensitive as the Shannon-Wiener Index to changes in the species richness. The concentration of 20 mg/L of zinc only allowed for the recovering of the soluble COD removal efficiency and the species richness, but it was especially toxicant considering the Shannon-Wiener Index, the description of functional groups and the ratio *Opercularia* sp./crawlers. The con-

sequences of the adding of 8 mg/L of zinc seem less drastic than in the case of 4 mg/L, and the main effects in the microfauna were observed only 48 h after the adding of zinc. The unusually high organic load determined in the beginning of the 8 mg/L assay can account for this fact.

Figure 4 shows that cycloheximide affected all the parameters in a much smoother way than metals did. Even the highest concentration, 20 mg/L, allowed for the recovering of all the parameters, except for the SBI, that was still 4 by the end of the assay; this was due to the supremacy of swimming forms. For the higher concentrations of cycloheximide, a stronger stimulation of the growth of the swimming forms was always observed.

Table 4 presents the *taxa* observed during all the as-

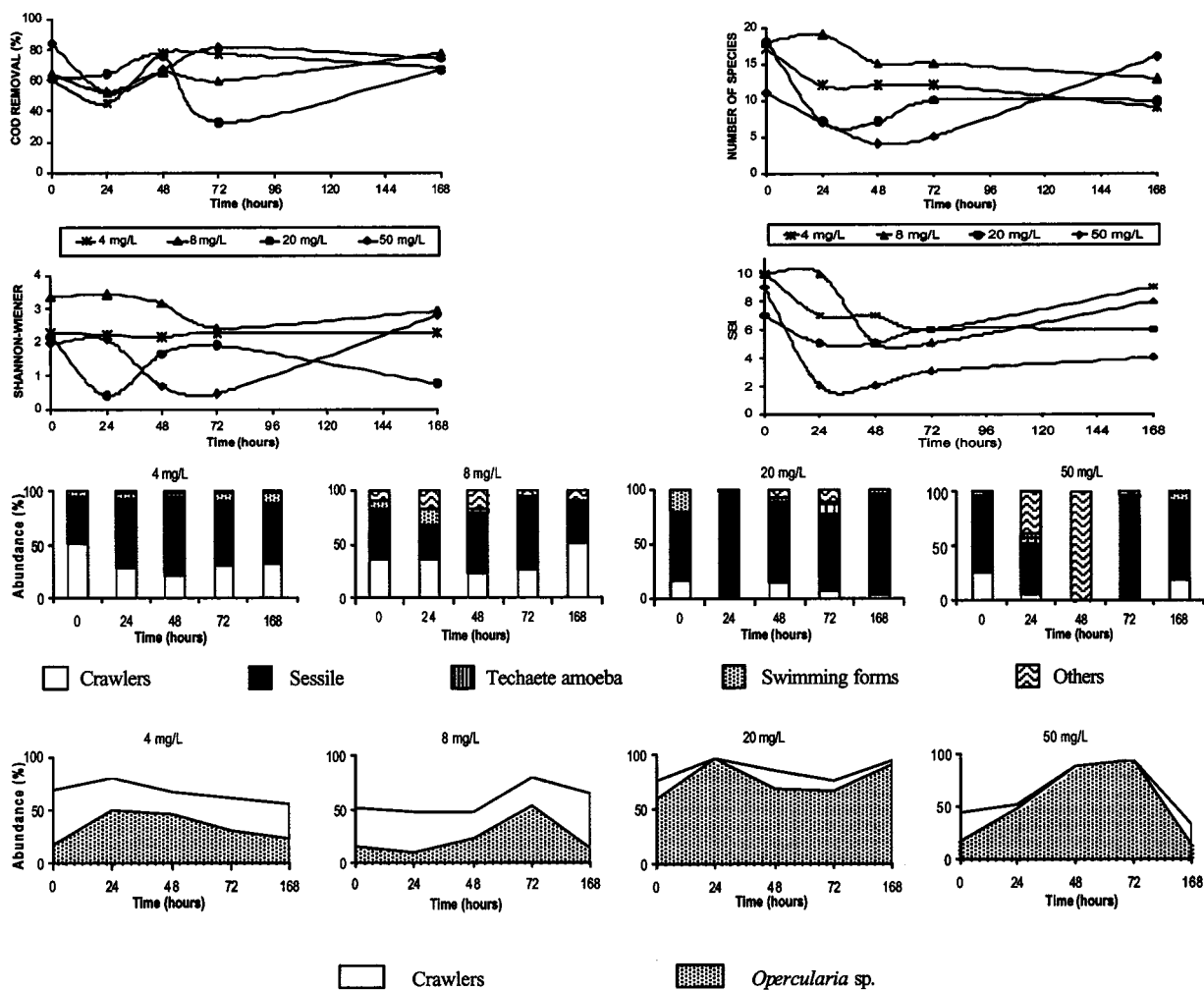


Figure 3. Variation of COD removal, taxonomic richness, Shannon-Wiener Index, Sludge Biotic Index (SBI), relative abundance of functional groups, and *ratio crawlers/Opercularia sp.* in the assays with zinc.

says. Some of the organisms were not identified to the species level, but different species were distinguished as species 1, species 2, etc.

DISCUSSION

Authors usually refer to soluble concentrations, namely when metals are considered, as organics matter can, in a variable degree, complex ions, thus reducing its toxicity. In the present work, an attempt was made to relate soluble concentrations, determined 24 h after the adding of metals, to the other results: as said before, the results do not reflect, in any case, these soluble concentrations, and sometimes even contradict them. Moreover, these soluble concentrations do not reflect the feed added concentrations. The major effects were probably inflicted

in the first 24-h period. Despite the variations potentially introduced by the oscillations of the organic and volumetric load or the suspended solids in the toxicant soluble fraction, it was decided to present and discuss the results in terms of the concentrations of metals added to the aeration tank (nominal concentrations).

The high concentration of dissolved oxygen in the aeration tank and the high amount of ammonium in the influent had notorious consequences in the general work of the plant, the more evident being the nonexpectable high rate of nitrification, considering the prevalence of low pH values in the aeration tank (Bitton, 1994) and the presence of toxicants (Eckenfelder, 1992; Bitton, 1994). Madoni *et al.* (1999) states that nitrifying micro-organisms of activated sludge are, in a general way, less affected than heterotrophic by metals as copper, lead, and cadmium, but not in the case of zinc or chromium. Stasi-

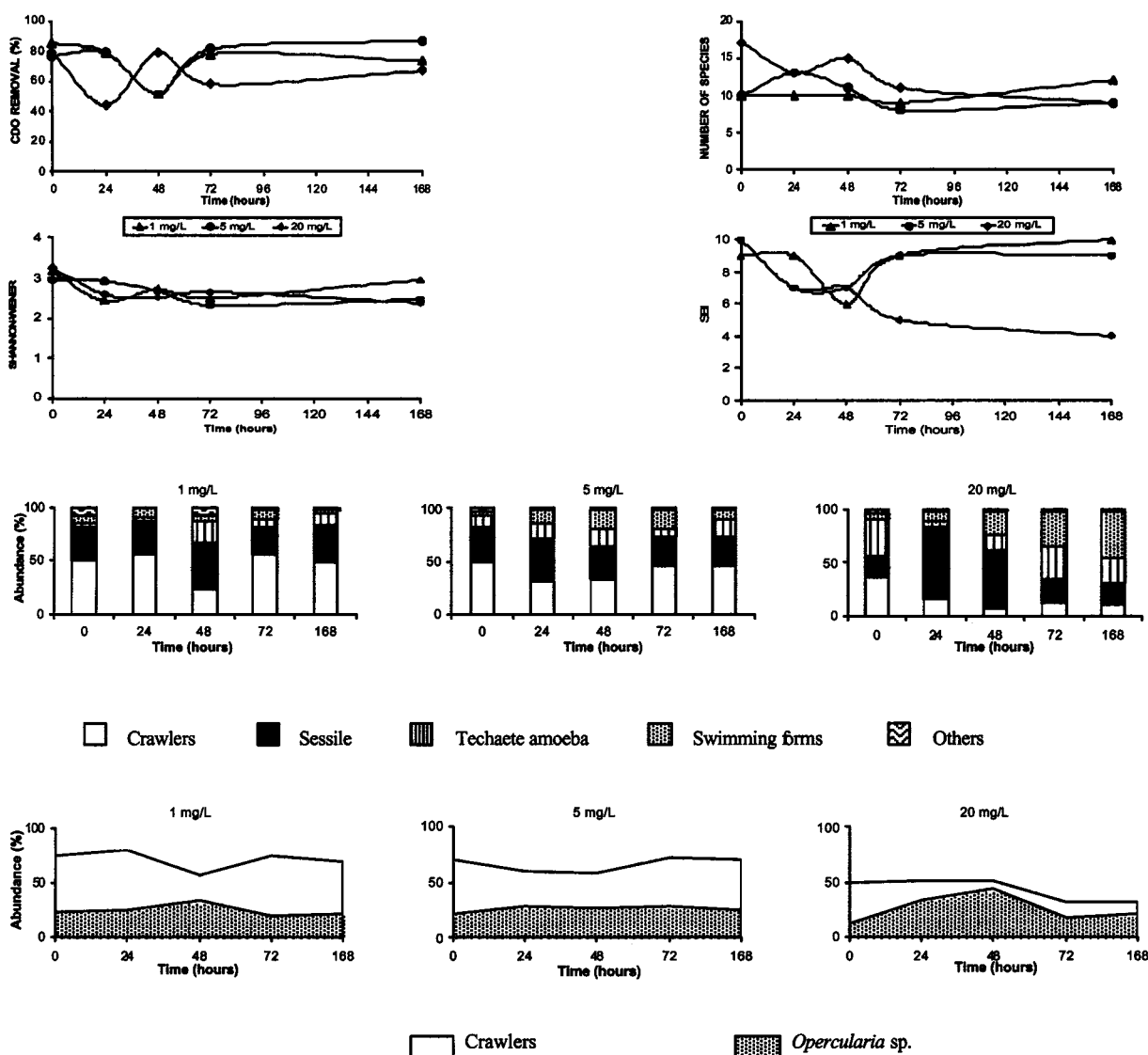


Figure 4. Variation of COD removal, taxonomic richness, Shannon-Wiener Index, Sludge Biotic Index (SBI), relative abundance of functional groups, and *ratio crawlers/Opercularia sp.* in the assays with cycloheximide.

nakis *et al.* (2003) corroborated this, in what chromium (IV) concerns. In the present work, no significant differences were detected between the assays with copper and zinc, in what nitrification respects. The high concentrations of oxygen and ammonium had apparently overlapped the inhibitory factors, allowing for the nitrification to occur. Nitrification had, on the other hand, contributed to the low values of pH in the aeration tank.

The soluble COD removal was inhibited by all toxicants, especially by copper. Cycloheximide was not expected to have such a strong effect: cycloheximide is known for its effects on the eukaryotes (Frankel, 1970; Heyer and Frankel, 1971, Robert and Orias, 1973), and COD removal is mainly assigned to prokaryotes. Effects

on this component could be indirect: cycloheximide may have affected the bacterivorous protists, and this could have been reflected either on the amount of dispersed bacteria in the effluent or on the health state of the bacteria, turning COD removal less effective.

The effects of toxicants on the microfauna were relevant in the variation of both indexes, but the SBI appears to be more sensitive than the Shannon-Wiener Index: at the end of the assays, there were communities which seemed recovered when the Shannon-Wiener Index was considered, but not in the case of the SBI. Furthermore, the disturbance following the adding of the toxicants was frequently more evident in the case of the SBI than in the case of the Shannon-Wiener Index, and this was espe-

Table 4. Taxa of microfauna found in the tested mixed liquor during the assays.

Crawling ciliates
<i>Acineria uncinata</i>
<i>Aspidisca cicada</i>
<i>Aspidisca lynceus</i>
<i>Chilodonella uncinata</i>
<i>Drepanomonas revoluta</i>
<i>Euplotes patella</i>
<i>Euplotes affinis</i>
<i>Stylonychia</i> sp.
<i>Trachaelophyllum pusillum</i>
<i>Trithigmostoma cucullulus</i>
<i>Trochilia minuta</i>
Testate amoebae
<i>Arcella</i> sp.
<i>Diffugia</i> sp.
<i>Euglypha</i> sp.
Swimming ciliates
<i>Cinetochilum margaritaceum</i>
<i>Cyclidium</i> sp.
<i>Colpidium</i> sp.
<i>Paramecium caudatum</i>
<i>Paramecium putrinum</i>
<i>Spirostomum teres</i>
<i>Uronema nigricans</i>
Sessile ciliates
<i>Carchesium</i> sp.
<i>Epistylis</i> sp.
<i>Opercularia</i> sp. 1
<i>Opercularia</i> sp. 2
<i>Vorticella</i> sp.
<i>Vorticella convallaria</i>
<i>Vorticella microstoma</i>
<i>Vorticella aquadulcis</i>
<i>Zoothamnium</i> sp.
Carnivorous ciliates
<i>Amphileptus</i> sp.
<i>Coleps</i> sp.
<i>Litonotus</i> sp.
<i>Prorodon</i> sp.
Suctorina
<i>Podophrya</i> sp.
<i>Tokophrya</i> sp.
Metazoans
Nematode sp. 1
Rotifer sp. 1
Rotifer sp. 2
Rotifer sp. 3
Rotifer sp. 4
Oligochaeta sp. 1
Oligochaeta sp. 2

cially true in the assays with cycloheximide. Both indexes warranted, as expected, more information than taxonomic richness itself.

The Shannon-Wiener Index is considered by several authors and despite many criticisms, the best diversity index (Hulbert, 1971; Gray and Pearson, 1982; Washington, 1984; Ludwig and Reynolds, 1988; Kingston and Riddle, 1989). The relation between the structure of the microfauna communities and the performance of the wastewater plants is strongly established, so some authors have emphasized the advantage of using diversity indexes in these studies (Morishita, 1976). However, other have commented on the problem of using directly the diversity indexes and the biological indicators of the saprobic system in the assessment of the wastewater treatment processes, commenting that those indexes lead to an excessive simplification of the extreme complexity of these systems. This could be true in the case of the Shannon-Wiener Index, but not for the SBI. The fact of having been created specifically for the evaluation of these complex ecosystems, and a deep knowledge of the communities of the activated-sludge processes, accounted for the applicability of the SBI in the present study. Even when crawlers were stimulated by the lower concentration of copper, the SBI reflected the worsening of the environmental conditions. The association between the concept of indicator species, taxonomic richness, and abundance appears to be the basis of the success of this index.

As said by Abraham *et al.* (1997), the lack of correlations between the protistan populations and the prevalent physical-chemical parameters in the wastewater treatment plants is expectable, as the determination of these populations in activated-sludge systems is complex and multifactorial. Gracia *et al.* (1994) suggested that many factors beyond concentration and time of exposure must be taken in consideration in the study of toxicant effects in these studies.

On the other hand, it is true that the toxicants can influence other variables beyond those strictly correlated with the microfauna, thus creating a complex web of causes and effects and turning difficult to distinguish between direct and indirect effects. The lower concentrations of toxicants limit the interpretation of the results, more than the higher concentrations of toxicants. The exception was the assay with 50 mg/L of zinc, where a sudden recovery of the system took place.

Aspidisca cicada showed a remarkable tolerance to the lowest concentration of copper, in accordance to what happened in other works (Madoni *et al.*, 1992, 1994; Gracia *et al.*, 1994; Abraham *et al.*, 1997). In addition to *Aspidisca cicada*, other works point out other crawlers resistant to the exposure to metals: *Euplotes* sp. (Madoni

et al., 1992, 1994; Cingolani, 1997), *Chilodonella uncinata* (Abraham *et al.*, 1997), and *Acinertia uncinata* (Gracia *et al.*, 1994). Abraham-Peskir *et al.* (1997) highlight the possibility of *Aspidisca cicada*, beyond vorticellids, to tolerate soluble metal; they show the potential resistance of *Aspidisca cicada* to zinc, which is not corroborated by the present study. On the other hand, the tolerance of *Opercularia* sp., namely *O. coarctata* and *O. minima*, to toxic compounds (Esteban *et al.*, 1991; Gracia *et al.*, 1994; Madoni *et al.*, 1994, 1996) and its intimate relation with low-quality influents (Curds and Cockburn, 1970; Curds, 1975; Salvadó *et al.*, 1995) is well established and documented. In the present work, this was especially shown in the assays with copper and zinc. Stasinakis *et al.* (2003) reported the degradation of the microfauna from 1 mg/L of chromium (VI) and did not refer to any stimulation of the community.

The highest concentration of cycloheximide, 20 mg/L, caused a slight increase of *Opercularia* sp. 24 h after the adding, but the stimulation of swimming forms was more consistent in this case.

CONCLUSIONS

In this study some relations could be obtained through the observation of microfauna during toxicants entrance. Copper, at 8 mg/L, stimulated the crawler fraction, namely *Aspidisca cicada*, known for its well-established relationship with good performance parameters. That must prevent the generalization of the concept of indicators of good quality of water treatment whenever there is the possibility of contamination by metals, namely copper. Zinc did not allow for the same observations. In a general way, zinc and copper at 4 (only for zinc) and 8 mg/L caused little effects and allowed for the recovery of the microfauna within a week. Concentrations of 20 and 50 mg/L caused much more drastic effects and microfauna could not recover completely in the period of the assays. Cycloheximide had smoother effects and only a concentration of 20 mg/L did not allow for the recovery of the system. Remarkable effects were the stimulation of swimming forms, and the fact that, despite being an antibiotic that inhibits the metabolism of eukaryotic cells, it also affected the COD removal ability of the system; the possibility of being a direct or an indirect effect was not clear.

Toxicants modify the structure of the protists community by altering the taxonomic richness or the density, although some authors emphasize the former and others the latter. In the present study, the taxonomic richness was more consistently affected than density. At any rate, the SBI correlated better to the alterations of the microfauna

than the Shannon-Wiener Index, perhaps due to the fact that associates the concept of indicator species to taxonomic richness and to overall density.

ACKNOWLEDGMENT

Ana Nicolau was supported by grant BPD/11594/02 from FCT-Portugal.

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