Journal of Hazardous Materials 159 (2008) 567–573

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Bioassessment of a combined chemical–biological treatment for synthetic acid mine drainage

F. Pagnanelli[a,*], I. De Michelis[b], S. Di Muzio[b], F. Ferella[b], F. Vegliò[b]

[a] Department of Chemistry, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
[b] Department of Chemistry, Chemical Engineering and Materials, University of L'Aquila, Monteluco di Roio, 67040 L'Aquila, Italy

ARTICLE INFO

Article history:
Received 10 December 2007
Received in revised form 18 February 2008
Accepted 19 February 2008
Available online 4 March 2008

Keywords:
Lepidium sativum
Daphnia magna
Ecotoxicological tests
Acid mine drainage
Heavy metals

ABSTRACT

In this work, ecotoxicological characteristics of synthetic samples of acid mine drainage (AMD) before and after a combined chemical–biological treatment were investigated by using *Lepidium sativum* and *Daphnia magna*. AMD treatment was performed in a two-column apparatus consisting of chemical precipitation by limestone and biological refinement by sulphate reducing bacteria. Synthetic samples of AMD before treatment were toxic for both *L. sativum* (germination index, *G*, lower than 10%) and *D. magna* (100% immobility) due to acid pH and presence of copper and zinc. Chemical treatment (raising pH to 5–6 and eliminating copper) generated effluents with reduced toxicity for *L. sativum* ($G = 33\%$), while 100% immobility was still observed for *D. magna*. Dynamic trends of toxicity for the first and fifth outputs of the biological column denoted a gradual improvement leading to hormesis for *Lepidium* (after the initial release of organic excess), while a constant residual toxicity remained for *Daphnia* (probably due to H_2S produced by sulphate reducing bacteria).

1. Introduction

Acid mine drainage (AMD) is the worst environmental problem associated with mining activities. AMD polluted waters are generated by the biologically enhanced oxidation of metal sulphides, which results in ore dissolution and release of sulphates, protons and toxic metals in soil solution [1]. Remediation options involve both abiotic and biological strategies. Abiotic systems promote metal precipitation by chemicals (such as limestone) that neutralise AMD pH. Biological systems exploit H_2S production by sulphate reducing bacteria (SRB) in order to precipitate metals as sulphides [2].

Biological remediation can be performed in active systems (such as off-line sulfidogenic bioreactors) or passive systems (such as biological permeable reactive barriers, PRB) both exploiting H_2S bioproduction [3]. Organic mixtures are used in PRB construction as electron donor in the dissimilatory reduction of sulphate to sulphide. Organic components used in PBR are mixtures of biological materials chosen for their local availability: biodegradable materials (mushroom compost, manure of cow, horse and sheep, municipal compost) are generally mixed with more recalcitrant ones (sawdust, peat, straw, leaf compost) to ensure long-term SRB growth [4–7]. Full-scale applications of biological PRB are also characterised by addition of gravel to improve barrier permeability, and limestone to increase pH and to favour SRB growth [8,9]. Such reducing and alkalinity-producing systems (RAPS) [2] can be inadequate for highly iron-concentrated AMD due to limestone armouring (reduction of reactivity by deposition of iron oxides precipitates) [10] and barrier plugging for iron precipitates. In such cases, an alternative engineering configuration can be adopted using a two-step procedure: firstly chemical precipitation of iron by limestone (step I, pre-treatment or chemical precipitation) and then heavy metal removal as sulphides mediated by sulphate reducing bacteria (step II, bioprecipitaton/biosorption by SRB column).

Toxic effects of AMD can be mainly related to both low pH and high concentration of heavy metals, such as copper, cadmium, lead, zinc, manganese, and arsenic.

Bioassessment of AMD toxicity has been specifically addressed using different test-organism systems such as microrganisms [11], daphnids [12–16], shrimps, fishes [17] and plants [18,19]. These studies generally investigated toxicity effects associated to AMD samples collected in different polluted sites. Nevertheless, useful information can be also obtained by studies that reported toxic effects related to pH and metallic solutions. As for heavy metals, Montvydiene and Marciulioniene [20] investigated the toxicity of different metallic solutions on *Lepidium sativum* by monitoring the inhibition of root growth in presence of these pollutants. Toxicant concentrations that induce 50% growth inhibition after 2-day experiments followed the order: Cr (1.8 mg/L) < Cu (7.6 mg/L) < Cd

* Corresponding author. Tel.: +39 06 4991333; fax: +39 06 490631.
E-mail address: francesca.pagnanelli@uniroma1.it (F. Pagnanelli).

0304-3894/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2008.02.067

Table 1
Average values of chemical composition and toxicological parameters (IC_{50}: sample dilution giving 50% germination for *L. sativum*; EC_{50}: sample dilution giving 50% immobility of *D. magna*) for synthetic AMD in the feed, after chemical pre-treatment and after biological treatment (150 days) (n.d.: not detectable, <0.02 ppm)

Monitored parameter	Feed	After chemical pre-treatment	After biological treatment
pH	3.0 ± 0.1	5.6 ± 0.2	8.1 ± 0.3
Fe (mg L^{-1})	400 ± 50	2 ± 1	n.d.
Cu (mg L^{-1})	50 ± 10	0.2 ± 0.1	n.d.
Zn (mg L^{-1})	50 ± 10	47 ± 5	n.d.
Mn (mg L^{-1})	50 ± 10	42 ± 5	n.d.
As (mg L^{-1})	2.0 ± 0.5	n.d.	n.d.
IC_{50} *L. sativum*	6.0	24.2	46.4
EC_{50} (24 h) *D. magna*	2.4	43.5	12.2
EC_{50} (48 h) *D. magna*	2.1	32.1	12.0

(26.5 mg/L) < Ni (73.7 mg/L) < Pb (77.8 mg/L) < Zn (149 mg/L) < Mn (300 mg/L). The same order of metal toxicity for *L. sativum* (Cu > Pb > Zn) was reported by Arambasic et al. [18]. These authors also tested metal toxicity on *Daphnia magna* after 48 h exposure observing the order Cu > Zn > Pb (EC_{50}: 0.07, 0.75 and 55.64 mg/L, respectively). Yim et al. [14] investigated the toxicity of metals on *D. magna* using single and multicomponent systems of Cd, Cu, Zn and Pb at two hardness levels. These authors found that acute toxicity increased in the order Zn < Pb < Cu < Cd, with toxicity extremely increased in soft waters (44 mg/L as $CaCO_3$). In particular, EC_{50} were 3 μg/L for Cd, 4 μg/L for Cu, 95 μg/L for Pb and 300 μg/L for Zn. They also observed that toxicity tests with metal mixtures did not show any combined effect due to simultaneous presence of different heavy metals. The data of Yim et al. [14] confirmed copper as one of the most toxic element for *D. magna*, even though a different order of toxicity for Zn and Cd was reported with respect to Arambasic et al. [18].

Gerhardt et al. [15] tested the effect of short-term exposure to AMD on *Gambusia holbrooki* (a fish) and *D. magna*. Dose–response data were modelled by using a forward multiple regression analysis: the immobility of *D. magna* resulted statistically more correlated with pH, Zn, Fe and As, while toxicity on *G. holbrooki* was mostly related to pH, Zn, Fe, Cd, Cu and Co. Isolation of pH contribute in AMD toxicity was performed by comparing AMD with a blank acid solution without metals. A forward multiple regression analysis of immobility data versus pH denoted no significant difference for pH coefficients in AMD samples and blank acid solution. The authors concluded that pH was the most determinant factor for the immobility of both species in AMD, while Zn was the metal most strongly correlated with immobility of *D. magna*.

As for pH effect, correlative studies reported by Soucek et al. [16] showed that pH was the best predictor of AMD toxicity for *D. magna* (48 h toxicity test survival) followed by Fe and Al concentrations.

Other correlative studies have been also performed in which metal toxicity for *D. magna* was addressed by considering the primary factors affecting free metal ion concentration (pH, competing ions and ligands). In particular, Heijerick et al. [21] investigated the effect of pH, hardness, dissolved organic carbon (DOC) and their interactions on Zn toxicity to *D. magna*. They found that all three parameters significantly affected metal toxicity. In particular pH and DOC increase determined a decrease of Zn toxicity. A significant positive interaction was also observed between pH and DOC due to the larger sorption of Zn on humic acids for increasing pH. Finally the increase of water hardness determined a decrease of Zn toxicity because of competition for biotic ligands among ions in solution. In Schampelaere and Janssen [22], a similar approach was used in order to assess the effect of pH, Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} on copper toxicity for *D. magna*. They found that competitive binding among Ca^{2+}, Mg^{2+}, Na^{+} and copper determined a decrease of toxicity. A non-linear relationship was observed between pH versus Cu toxicity, which was interpreted in terms of both proton competition with copper ions and toxicity of copper hydroxide complexes.

In this work, ecotoxicological characteristics of AMD samples before and after a combined chemical–biological treatment were investigated. *L. sativum* and *D. magna* were used to evaluate the abatement of toxicity after each treatment.

Analysis of literature data was integrated with experimental results here reported in order to isolate the main toxicity contributes of different metals and then the effect of each treatment in both pollution and toxicity abatement.

2. Materials and methods

2.1. Combined treatment in column reactors

Synthetic AMD samples were obtained by dissolving weighted amounts of reagent grade chemicals ($FeSO_4$, $MnSO_4$, $CuSO_4$, $ZnSO_4$, As_2O_5) in distilled water (feed composition in Table 1).

AMD was treated in a two-column apparatus by chemical treatment or pre-treatment (first step) and then by biological treatment (second step) (Fig. 1). Both columns were made of Plexiglas (height 1 m; diameter 0.2 m; column volume, $V_b = 6.65 \times 10^{-3}\ m^3$) with ten equally distant outputs (0.1 m) along the axial length, numbered from the bottom to the top of the column. For chemical precipitation tests, the first column was filled with limestone (col-

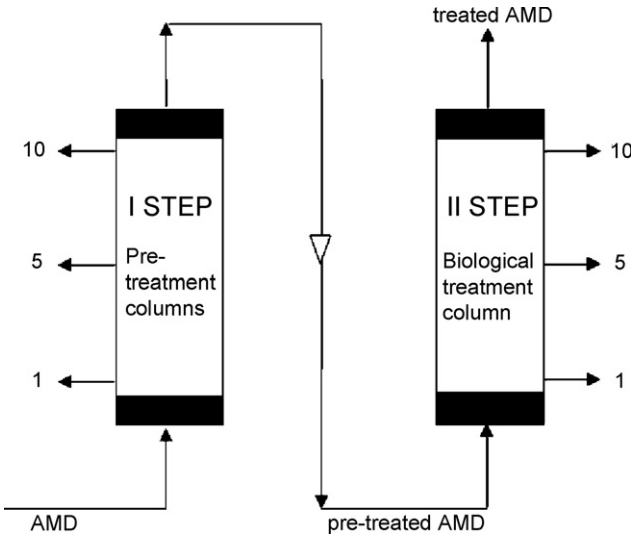

Fig. 1. Schematic representation of the two-column apparatus used for the combined treatment of synthetic AMD.

umn pore water 2 L) and fed with synthetic AMD from the bottom (30 mL/h).

For biological precipitation tests, the second column was filled with a solid medium containing 80% (v/v) of compost, 15% (v/v) of cow manure, 5% (v/v) of straw and traces of limestone (column pore water 0.9 L). Bacteria for column start-up were propagated by an inoculum kindly furnished by the research group of Professor Groudev (Department of Engineering Geoecology, University of Mining and Geology, Sofia, Bulgaria), who collected it in the Curilo mine district located near Sofia [23]. Bacteria were preliminary cultivated in closed shaken flasks using standard procedures for SRB reported in the literature [24]. In particular cultivation medium defined as C Medium was used: KH_2PO_4 0.5 g L^{-1}; NH_4Cl 1 g L^{-1}; Na_2SO_4 4.5 g L^{-1}; $CaCl_2{\cdot}6H_2O$ 0.06 g L^{-1}; $MgSO_4{\cdot}7H_2O$ 0.06 g L^{-1}; sodium lactate 6 g L^{-1}; yeast extract 1 g L^{-1}; $FeSO_4{\cdot}7H_2O$ 0.004 g L^{-1}; sodium citrate$\cdot 2H_2O$ 0.3 g L^{-1} [24]. SRB were inoculated along the column length through the ten outputs. After inoculation and bacteria acclimatisation, pre-treated sample of AMD coming from the tenth output of the first column were fed to the second column (10 mL/h).

AMD treatment in the two-column apparatus was carried-out for 150 days.

Samples from the different outputs of the first and second columns were collected during time and used for analytical determinations (pH and residual metals by an Inductively Coupled Plasma Spectrophotometer) and for ecotoxicological tests.

2.2. Toxicity tests with L. sativum

Toxicity tests were evaluated by monitoring germination and radical growth of *L. sativum*. For each test 4 replicates were performed and compared with a control in deionised water. For each replicate 10 seeds were placed in a Petri dish, on filter paper moistened with 5 mL of the tested solution. Dishes were closed in plastic bags to maintain humidity and kept at 25 °C without light for 72 h. After this time, germination of seeds in each dish was checked and root length measured: a minimum length of 1 mm was considered for germination. A test was considered valid if the control presented at least 80% of germinated seeds with 14 mm root length.

Phytotoxicity of synthetic AMD was evaluated before and after each treatment by two end-points: the number of germinated seeds and their root length.

The inhibition index (I) estimates toxic effects of tested samples on root growth:

$$I = \frac{L_C - L_S}{L_C} \times 100 \quad (1)$$

where L_C and L_S are the average root length for control and sample, respectively.

The germination index (G) comprehends simultaneously the toxic effects exerted on both germination and root length:

$$G = \frac{G_S L_S}{G_C L_C} \times 100 \quad (2)$$

where G_S and G_C are the number of the germinated seeds for sample and control, respectively.

2.3. Toxicity tests with D. magna

Toxicity tests with *D. magna* were performed by using Daphtoxkit F Trade Mark magna according to the manufacture's instructions. Each test plate have 6 rows and 5 columns allowing the analysis of 5 toxic conditions plus a control (one test for each row). Twenty neonates, hatched from ephippia, were used for each test condition replicated 4 times in the wells of the row (5 neonates in each well). The plates were incubated at 20 °C, in darkness, for 24 and 48 h and then immobility was recorded.

A test was considered valid if the control presented an immobility below 20%.

2.4. Liquid samples tested in ecotoxicological tests

Toxicity tests were performed on liquid effluents collected during time from the different outputs of both columns. Toxicity tests were also performed on cultivation medium of sulphate reducing bacteria (C Medium and C Medium without lactate) and on liquid samples containing organics released from second column filling: 50 g of solid mixture were suspended in 100 mL of deionised water, mixed for 48 h, centrifuged at 4000 rpm and then used for ecotoxicological tests.

3. Results and discussion

The combined chemical/biological treatment here proposed allowed the complete abatement of the contaminants contained in the synthetic AMD (iron, copper, arsenic, zinc and manganese) by different chemico-physical mechanisms: chemical precipitation and adsorption in the first column, bioprecipitation and biosorption (sorption onto biological matrices and bacterial biomass) in the second column. In particular iron, copper and arsenic were completely removed in the first step (mainly by chemical precipitation), while the second step of treatment was necessary for manganese and zinc abatement (by bioprecipitation and biosorption). The average residual concentrations of each pollutant after 150-day treatment were reported in Table 1.

3.1. Toxicity tests with L. sativum

Dynamic trends of inhibition (I) and germination (G) indices were reported in Fig. 2 (output 10) and Fig. 3 (outputs 1 and 5) for both columns. Quantitative comparisons among different liquid samples were performed considering mean values and standard deviations for both indices calculated by these dynamic trends.

In order to assess the attenuation related to each treatment, phytotoxicity of synthetic AMD was determined firstly. AMD presented very low germination indices, $G = 5 \pm 2$, and high inhibition, $I = 94 \pm 2$ (Fig. 2). These phytotoxic effects can be associated both to the acidic pH of AMD and to the presence of heavy metals. As for pH, synthetic AMD was characterised by constant pH during time (3.0 ± 0.1) (Fig. 4), which is quite low with respect to the optimal pH range of growth of *L. sativum*, i.e. 5.5–8.0 [25]. Regarding metals, copper and zinc were identified among the most inhibiting heavy metals for *Lepidium* [18,20].

A partial abatement of AMD toxicity was obtained after chemical treatment, whose tenth output was characterised by $G = 33 \pm 2$ and $I = 72 \pm 5$ (Fig. 2). This improvement can be related to pH rise (from 3.0 ± 0.1 to 5.6 ± 0.5), which determined the complete abatement of the most toxic heavy metal, copper (Table 1). In fact, a previous study about pH effect on *L. sativum* did not denote any significant inhibition of root growth for pH above 5 [25]. Consequently, remaining toxicity can be mainly related to Zn, which was not removed during the first column treatment (Table 1).

Toxicity indices for the last output (tenth) of biological treatment were quite surprising showing 0% germination indices (Fig. 2). Effluent pH (8.1 ± 0.3) (Fig. 4), and complete abatement of toxic metals in the second step (Table 1) suggested other causes for such finding. In particular, an explanation can be found by analysing the toxicity dynamics of the first and fifth outputs of the second

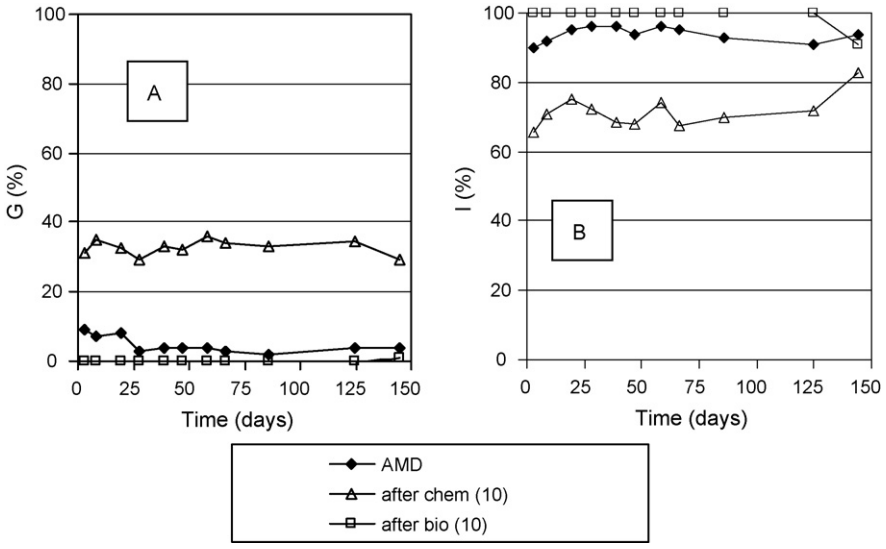

Fig. 2. Germination index (A) and inhibition index (B) obtained by ecotoxicity tests on *L. sativum* using synthetic AMD, AMD after chemical treatment and AMD after biological treatment (tenth output).

column (Fig. 3). It is possible to see that, for increasing values of treated volumes, the toxicity of the first output tended to decrease up to negative inhibition, meaning a promotion of radical growth (hormesis: germination index larger than the control). A similar trend can be observed also for the fifth output, but shifted towards larger volumes.

These data can be explained considering that the initial phase of II column running was characterised by the gradual release of both cultivation medium (fed during start-up of the column for SRB acclimatisation) and water-leachable organic compounds of the solid filling. This chromatographic effect led to temporary toxicity of the effluents, excessively rich of organic substances and nutrients. In particular for the first output, located 0.1 m from the feed inlet, a 40-day treatment was necessary in order to obtain control-like indices. It can be reasonably assumed that this time corresponds to the volume necessary for the elution of organics in 0.1 m of column length. According to this assumption, organics would be absent in the effluents of the fifth output (located 0.5 m from the inlet section) only after 200-day treatment (40×5). Even if this datum is not available, a verification of such assumption can be made in the following way. After 144 days, the effluent of the fifth output was characterised by $G = 64\%$ and $I = 31\%$. Analogously the effluent of the first output collected after $144/5 = 28.8$ days presented very similar values, $G = 70\%$ and $I = 29\%$.

Toxic effects of SRB culture medium, culture medium without lactate and II column filling mixture were then specifically tested to verify this aspect (Fig. 5). Germination and inhibition indices associated to culture medium denoted its significant toxicity against *L. sativum* (Fig. 5A). Ecotoxicological tests performed on diluted samples (1:10) at the same pH, denoted a substantial improvement of both *G* and *I*, indicating that the high concentration of organics was mainly responsible for *Lepidium* inhibition (Fig. 5B). In fact, cultivation medium without lactate (the organic substrate) did not show any significant toxic effects for *Lepidium* (Fig. 5A). Even liquid samples contacted with II column filling denoted the toxic effects due to organic release (Fig. 5A), but also the growth improvement (hormesis) associated to diluted samples (Fig. 5B). Such stimulating effects on root growth of *L. sativum* have been already observed by Hoekstra et al. [26], who tested the effect of low-concentrated dung extracts from different farms.

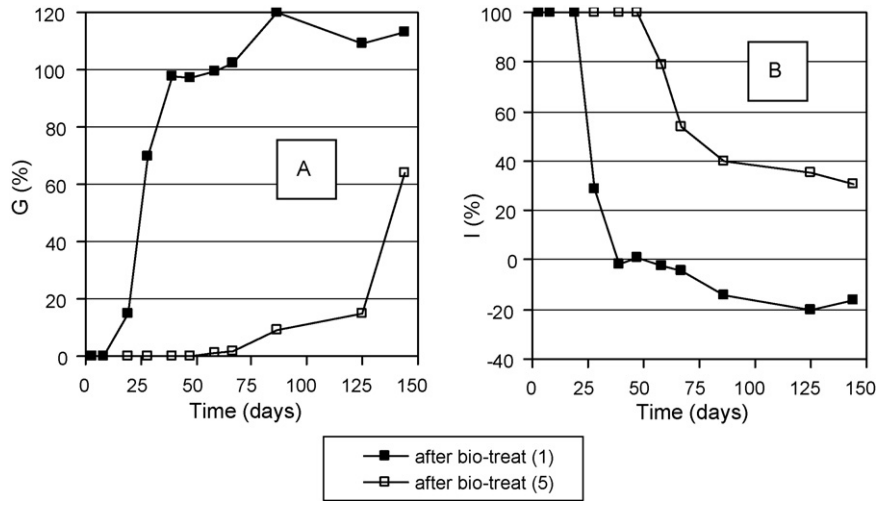

Fig. 3. Germination index (A) and inhibition index (B) obtained by ecotoxicity tests on *L. sativum* using AMD after biological treatment (first and fifth outputs).

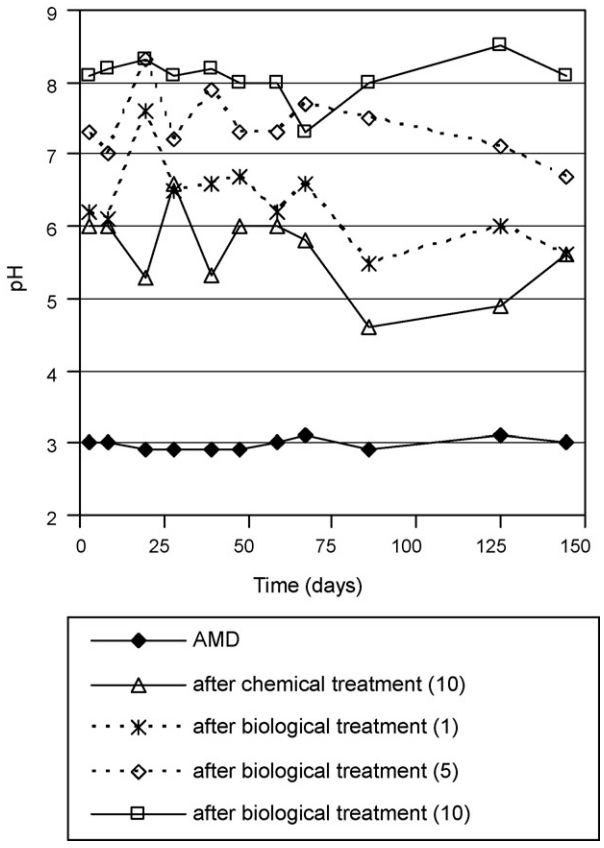

Fig. 4. pH of synthetic AMD, of AMD after chemical treatment (tenth output) and AMD after biological treatment (first, fifth and tenth outputs).

Further details about inhibition effects of AMD can be obtained by ecotoxicological tests on polluted samples diluted with distilled water (dosage-effect diagrams) (Fig. 6).

Both germination and inhibition indices of diluted samples of synthetic AMD presented a double-slope trend (Fig. 6A): slight improvements of AMD characteristics from 100% to 10% of AMD concentration (*x*-axis), and steep amendments below 10%, leading to hormesis ($G > 100$ and $I < 0$ for AMD concentration <4%). The significant improvements in this range should be mainly due to dilution of toxic metals with the most toxic metal, copper, presenting concentration lower than 2 ppm for AMD concentration below 4%.

The observed improvement for AMD concentration <4% cannot be related to pH, which did not present inhibiting values in this range (4.7 for 4% and 5 for 1% AMD concentration).

The dosage-effect diagram of pre-treated AMD presented a sigmoid trend for both germination and inhibition index (Fig. 6B). Chemical treatment in the first column determined pH rise (to 5.6 ± 0.2) and copper removal to non toxic concentration (0.2 ppm) (Fig. 6A). As a consequence, pre-treated AMD required a lower dilution degree to attenuate toxic effects than AMD before treatment. AMD concentrations for which $G = 50\%$ were 6.0 and 24.2 for AMD and pre-treated AMD, respectively (IC_{50} values in Table 1).

Residual toxicity of pre-treated AMD can be mainly related to zinc (the second most toxic metal for *L. sativum*), which was quite completely refractory to chemical treatment in the first column (Table 1). Dosage-effect diagram showed that zinc concentration giving control-like indices was 8 ppm calculated for 16% concentration of pre-treated AMD. For larger dilution degrees hormesis was observed.

Dilution of tenth output of bio-treatment column denoted a linear correlation between % concentration of bio-treated AMD and both indices of toxicity (Fig. 6C). This dosage-effect diagram confirmed that residual toxicity of such effluents was mainly due to high concentration of organics. In fact, as dilution increased, the toxic effects of bio-treated AMD decreased.

IC_{50} value calculated for bio-treated samples denoted a further improvement of such effluents with respect to pre-treated samples (Table 1). This means that, even if toxicity of bio-treated AMD was larger than that of pre-treated AMD (Fig. 2), the cause of such toxicity (organic excess) can be attenuated by a lower degree of dilution with respect to residual toxicity of chemical treatment (Fig. 6). This observation along with the transient characteristics of organic excess (Fig. 3), denoted that biological treatment was a necessary step not only for eliminating metals that are refractory to chemical precipitation, but also to improve phytotoxic characteristics of the final effluents.

3.2. Toxicity tests with D. magna

Ecotoxicological tests with *D. magna* were performed considering the effect of the exposure time (24 and 48 h) (Fig. 7). All untreated samples of synthetic AMD were characterised by 100% immobility of daphnids. Same results were obtained for AMD after chemical treatment regardless the exposure time. The analysis of chemical characteristics of pre-treated AMD (Table 1) along with literature data about heavy metal toxicity suggested that Zn is the primary responsible for *D. magna* immobility [14,18,21]. In fact, previous data considering inhibition effects of acid solution denoted 50% immobility for pH lower than 4.9 [15].

As in the case of phytotoxicological tests, *D. magna* immobility presented time-depending profiles for the first and the fifth outputs of biological treatment (Fig. 7).

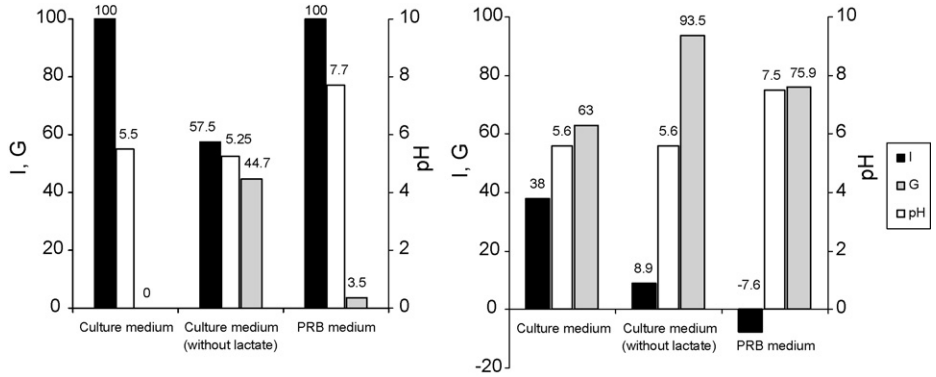

Fig. 5. Germination index, inhibition index and pH obtained for toxicity tests on *L. sativum* using culture medium, culture medium without lactate and liquid samples contacted with solid filling of the second column (PRB medium) (A: undiluted samples; B: 1:10 diluted samples).

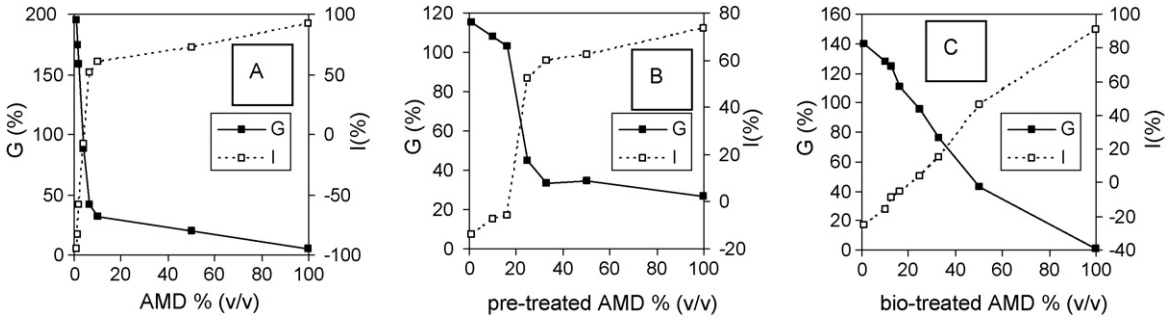

Fig. 6. Dosage–response diagrams obtained for toxicity tests on *L. sativum* using synthetic AMD (A), AMD after chemical treatment (B) and AMD after biological treatment (C) (tenth output after 150-day treatment).

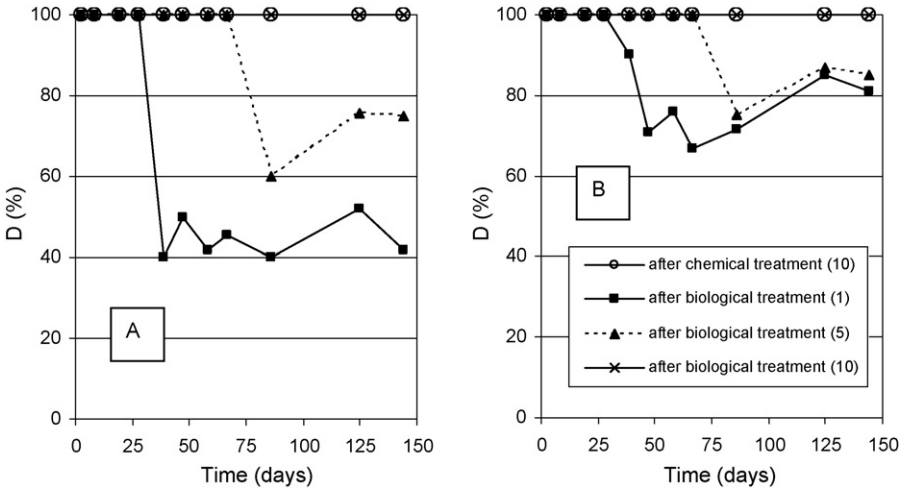

Fig. 7. % Immobility (*D*) of *D. magna* using AMD after chemical treatment (tenth output) and AMD after biological treatment (first, fifth and tenth outputs; A: 24 h exposure; B: 48 h exposure).

Nevertheless, comparing these dynamic trends with those obtained for *L. sativum*, a constant residual toxicity remained for *D. magna* even increasing the treated effluent volume. Considering the complete abatement of toxic metals after biological treatment and the values of the final pH, other causes have to be found for *Daphnia* immobility. On the other hand, toxicity tests by using an extract of the II column filling material did not evidence significant negative effects on *D. magna* (5% and 30% immobility for 24 and 48 h tests, respectively).

Toxic effects observed in bio-treated effluents can be then reasonably assumed to be due to H_2S gas produced by SRB throughout the column, but mainly concentrated in the top part of the apparatus. Acid digestion of solid samples taken after the dismantlement of biological column (experimental data not reported here) denoted that metal abatement occurred preferentially in the top section of the column where gaseous sulphide was concentrated. The metabolic product of sulphate reduction could be then the main cause of toxicity of treated AMD for *D. magna*, and also contribute to the observed inhibition of *L. sativum*.

Dosage-effect diagrams obtained for AMD samples before and after treatment were reported in Fig. 8. These diagrams showed similar sigmoid trends for synthetic AMD before and after each treatment. Liquid concentration giving 50% immobility (EC_{50}) were determined by probit analysis for both time exposures (Table 1)

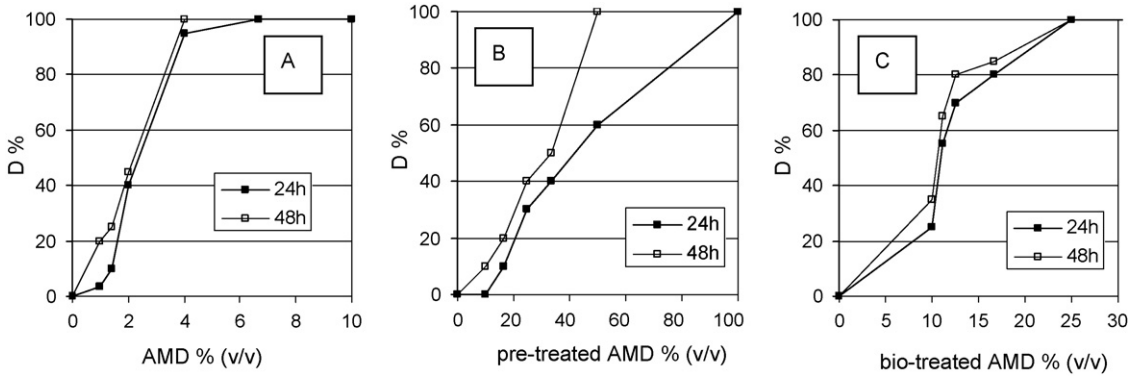

Fig. 8. Dosage–response diagrams obtained for toxicity tests on *D. magna* (immobility (*D*) after 24 and 48 h exposure) using synthetic AMD (A), AMD after chemical treatment (B) and AMD after biological treatment (C) (tenth output after 150-day treatment).

showing a significant abatement of AMD toxicity only after the chemical treatment.

4. Conclusions

In this work, the efficiency of a combined chemical/biological treatment for AMD was assessed in terms of ecotoxicological effects on *L. sativum* and *D. magna*.

Synthetic samples of AMD before treatment resulted toxic for both *L. sativum* (*G* lower than 10%) and *D. magna* (100% immobility).

Chemical treatment generated effluents with reduced toxicity for *L. sativum* (*G* = 33%); 100% immobility was observed for *D. magna* even though dosage-effect diagrams denoted an increase of EC_{50} with respect to untreated samples.

Effluents from the final output of bio-treatment column denoted high toxicity for both organisms. Nevertheless, an evolution of toxic effects can be observed for the different outputs of the second column: first and fifth column effluents denoted a gradual improvement in *Lepidium* tests, while a constant residual toxicity was observed for *D. magna*.

Chemical composition of AMD samples before and after each treatment allowed isolating the main contributes in AMD toxicity and the effect of each treatment in pollution and toxicity abatement.

In particular, according to previous studies reported in the literature, the synthetic AMD used in this study presented three main contributors to toxicity: pH, Cu and Zn. Chemical treatment (raising pH to 5–6 and eliminating copper) generated effluents whose toxicity was mainly due to Zn for *Lepidium* and also to pH for *D. magna*. Biological treatment (raising pH to 8 and eliminating all metals) was characterised by initial elevated concentration of organics which resulted toxic for *Lepidium*, while sulphide gas accumulating in the top section of the column could be reasonably responsible (but not experimentally proven) for the residual toxicity observed for *Daphnia*.

The combined action of chemical and biological precipitation was necessary in order to eliminate the different heavy metals as insoluble precipitates (hydroxides and carbonates in the first step, and sulphides in the second step).

Toxicity tests on column effluents (as simulation of full-scale permeable reactive barriers) gave preliminary information about the potential impact of treated effluents in the environment using different test-organisms.

Experimental data here reported showed that, after an initial transient, treated effluents can be suitable for vegetable growth while residual toxicity was observed for more sensitive organisms such as daphnids.

References

[1] A.S. Sheoran, V. Sheoran, Heavy metal removal mechanism of acid mine drainage in wetlands: a critical review, Miner. Eng. 19 (2006) 105–116.
[2] D.B. Johnson, K.B. Hallberg, Acid mine drainage remediation options: a review, Sci. Total Environ. 338 (2005) 3–14.
[3] D.B. Johnson, Biohydrometallurgy and the environment: intimate and important interplay, Hydrometallurgy 83 (2006) 153–166.
[4] I.S. Chang, P.K. Shin, B.H. Kim, Biological treatment of acid mine drainage under sulfate-reducing conditions with solid waste materials as substrate, Water Res. 34 (2000) 1269–1277.
[5] B. Christensen, M. Laake, T. Lien, Treatment of acid mine water by sulfate-reducing bacteria; results from a bench scale experiment, Water Res. 30 (1996) 1617–1624.
[6] O. Gibert, J. de Pablo, J.L. Cortina, C. Ayora, Treatment of acid mine drainage by sulfate-reducing bacteria using permeable reactive barriers: a review from laboratory to full-scale experiments, Rev. Environ. Sci. Bio/Technol. 1 (2002) 327–333.
[7] K.R. Waybrant, C.J. Ptacek, D.W. Blowes, Treatment of mine drainage using permeable reactive barrers: column experiments, Environ. Sci. Technol. 36 (2002) 1349–1356.
[8] S.G. Benner, D.W. Blowes, W.D. Gould, R.B. Herbert, C.J. Ptacek, Geochemistry of a permeable reactive barrier for metals and acid mine drainage, Environ. Sci. Technol. 33 (1999) 2793–2799.
[9] R.D. Ludwig, R.G. McGregor, D.W. Blowes, S.G. Benner, K. Mountjoy, A permeable reactive barrier for treatment of heavy metals, Ground Water 40 (2002) 59–66.
[10] M. Simon, F. Martın, I. Garcıa, P. Bouza, C. Dorronsoro, J. Aguilar, Interaction of limestone grains and acidic solutions from the oxidation of pyrite tailings, Environ. Pollut. 135 (2005) 65–72.
[11] N.F. Gray, C. O'Neill, Acid mine-drainage toxicity testings, Environ. Geochem. Health 19 (1997) 165–171.
[12] K.T. Kim, Y.G. Lee, S.D. Kim, Combined toxicity of copper and phenol derivatives to Daphnia magna: effect of complexation reaction, Environ. Int. 32 (2006) 487–492.
[13] I. Lopes, D.J. Baird, R. Ribeiro, Genetic adaptation to metal stress by natural populations of Daphnia longispina, Ecotox. Environ. Saf. 63 (2006) 275–285.
[14] J.H. Yim, K.W. Kim, S.D. Kim, Effect of hardness on acute toxicity of metal mixtures using Daphnia magna: prediction of acid mine drainage toxicity, J. Hazard. Mater. 138 (2006) 16–21.
[15] A. Gerhardt, L. Janssens de Bisthoven, A.M.V. Soares, Evidence for the stepwise stress model: Gambusia holbrooki and Daphnia magna under acid mine drainage and acidified reference water stress, Environ. Sci. Technol. 39 (2005) 4150–4158.
[16] D.J. Soucek, D.S. Cherry, G.C. Trent, Relative acute toxicity of acid mine drainage water column and sediments to Daphnia magna in the Puckett's Creek Watershed, Virginia, USA, Arch. Environ. Contam. Toxicol. 38 (2000) 305–310.
[17] L. Janssens de Bisthoven, A. Gerhardt, K. Guhr, A.M.V. Soares, Behavioral changes and acute toxicity to the freshwater shrimp *Atyaephyra desmaresti* Millet (Decapoda Natantia) from exposure to acid mine drainage, Ecotoxicology 15 (2006) 215–227.
[18] M.B. Arambasic, S. Bjelic, G. Subakov, Acute toxicity of heavy metals (copper, lead, zinc), phenol and sodium on *Allium cepa l.*, *Lepidium sativum l.* and *Daphnia magna St.*: comparative investigations and the practical applications, Water Res. 29 (1995) 497–503.
[19] M.B. Cole, D.E. Arnold, B.J. Watten, Physiological and behavioural responses of stonefly nymphs to enhanced limestone treatment of acid mine drainage, Water Res. 35 (2001) 625–632.
[20] D. Montvydiene, D. Marciulioniene, Assessment of toxic interactions of heavy metals in a multicomponent mixture using Lepidium sativum and Spirodela polyrrhiza, Environ. Toxicol. 19 (2004) 351–358.
[21] D.G. Heijerick, C.R. Janssen, W.M. De Coen, The combined effects of hardness, pH and dissolved organic carbon on the chronic toxicity of Zn to *Daphnia magna*: development of a surface response model, Arch. Environ. Contam. Toxicol. 44 (2003) 210–217.
[22] K.A.C. Schampelaere, C.R. Janssen, A biotic ligand model predicting acute copper toxicity for Daphnia magna: the effects of calcium, magnesium, potassium and pH, Environ. Sci. Technol. 36 (2002) 48–54.
[23] S.N. Groudev, I.I. Spasova, P.S. Georgiev, In situ bioremediation of soils contaminated with radioactive elements and toxic heavy metals, Int. J. Miner. Process. 62 (2001) 301–308.
[24] J.R. Postgate, The Sulfate-Reducing Bacteria, Cambridge University Press, 1979.
[25] L.J. Audus, Studies on the pH-relationships of root growth and its inhibition by 2:4-chlorophenoxyacetic acid and coumarin, New Phytol. 48 (1949) 97–114.
[26] N.J. Hoekstra, T. Bosker, E.A. Lantinga, Effects of cattle dung from farms with different feeling strategies on germination and initial root growth of cress (Lepidium sativum L.), Agric., Ecosyst. Environ. 93 (2002) 189–196.