

Vermiculite bio-barriers for Cu and Zn remediation: an eco-friendly approach for freshwater and sediments protection

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Abstract The increase in heavy metal contamination in freshwater systems causes serious environmental problems in most industrialized countries, and the effort to find eco-friendly techniques for reducing water and sediment contamination is fundamental for environmental protection. Permeable barriers made of natural clays can be used as low-cost and eco-friendly materials for adsorbing heavy metals from water solution and thus reducing the sediment contamination. This study discusses the application of permeable barriers made of vermiculite clay for heavy metals remediation at the interface between water and sediments and investigates the possibility to increase their efficiency by loading the vermiculite surface with a microbial biofilm of *Pseudomonas putida*, which is well known to be a heavy metal accumulator. Some batch assays were performed to verify the uptake capacity of two systems and their adsorption kinetics, and the results indicated that the vermiculite bio-barrier system had a higher removal capacity than the vermiculite barrier (+34.4 and 22.8 % for Cu and Zn, respectively). Moreover, the presence of *P. putida* biofilm strongly contributed to fasten the kinetics of metals adsorption onto vermiculite sheets. In open-system conditions, the presence of a vermiculite barrier at the interface between water and sediment could reduce the sediment contamination up to 20 and

23 % for Cu and Zn, respectively, highlighting the efficiency of these eco-friendly materials for environmental applications. Nevertheless, the contribution of microbial biofilm in open-system setup should be optimized, and some important considerations about biofilm attachment in a continuous-flow system have been discussed.

Keywords Bio-remediation · Environment protection · Heavy metals sorption · Sediment contamination · Vermiculite clay · Wastewater

Introduction

In aquatic environment, heavy metals are subjected to various processes of adsorption/desorption and immobilization/remobilization between the water column and the bottom deposits (Sarmani et al. 1992). In many cases, sediments can act as sink of pollutants and retain contaminants by ion exchange phenomena and adsorption on clay minerals, amorphous materials or organic substances.

In a river basin, an excess of heavy metals is often found in superficial waters and in bottom sediments, and because of their persistence in the environment, they can cause different toxicity problems to living organisms, vegetables and animals (Li et al. 2001; El-Bayaa et al. 2009; Unuabonah et al. 2009). Some heavy metals, e.g., Cu and Zn, are essential micronutrients for life, but at high concentrations they can become very toxic (Malamis and Katsou 2013). For this reason, the European Framework Directive (EC 2000/60) has established some threshold limits of heavy metals concentration in all superficial and wastewaters that flow into natural water systems. Heavy metals in water, in fact, can be affected by a number of precipitation and adsorption processes, which can lead to

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sediments contamination. The management of contaminated sediments (e.g., sediment dredging) is more problematic than the management of non-contaminated sediments: the former, in fact, has to be conferred in landfill with huge costs of management, while the latter can be reused in the surrounding land, thus avoiding soil loss (Ferronato et al. 2015). For this reason, in view of a sustainable and low-cost strategy to protect these natural resources, in situ and ex situ remediation techniques are strongly incentivized (Rakowska et al. 2012).

In the last decades, a number of studies have focused on different strategies for water remediation using eco-friendly materials and avoiding the use of chemicals (Prigione et al. 2008; Quintelas et al. 2013), but to our knowledge few works focused on the possibility to extend these low-cost techniques to the prevention of sediment contamination. Among different techniques for metals remediation in canal system, the concept of metal entrapment at the interface between water and sediment through sorbent materials seems to be an interesting strategy for environmental preservation (Wang and Chen 2009).

Natural clays (e.g., vermiculite) have been studied as suitable low-cost materials to be used for wastewater treatment or as permeable barrier in landfills to avoid pollutants release (Malandrino et al. 2006; Quintelas et al. 2011; Malamis and Katsou 2013). The tetra- and octahedral structure of phyllosilicates, in fact, favors a high surface area and a high availability of negative charges. Vermiculite, between smectites, can capture heavy metals by cation exchange mechanisms between metal ions and the negative permanent charge at the planar sites (outer-sphere complexes) or by the formation of SiO-/AlO-metal bound at the clay particle edge (inner-sphere complexes) (Malandrino et al. 2006).

In recent years, many efforts have been done to increase the performances of clay adsorption, and a vast array of biological materials has been explored for heavy metals removal (Lameiras et al. 2008; Wang and Chen 2009; Costa et al. 2012; Silva et al. 2012). Many authors reported that microorganisms can promote active and passive mechanisms for metal uptake and stabilization such as native bio-sorption, enzymatic transformations, metal precipitation, synthesis of novel metal ligand and extracellular substances (Kazy et al. 2002; Valls and de Lorenzo 2002). It is well known that under stress conditions some bacteria can organize themselves in biofilm, and recent studies have demonstrated the capacity of biofilm to enhance metal removal from water solution, e.g., Ni and Cr by *Arthrobacter viscosus* (Silva et al. 2012; Quintelas et al. 2013) or Cu and Zn by *Pseudomonas putida* (Chen et al. 2005). Some studies investigated the feasibility of biofilm supported on clay

minerals and use them as permeable barriers for water remediation (Pazos et al. 2010; Cobas et al. 2013; Ferreira et al. 2013), but to our knowledge, the application of permeable barriers or bio-barriers for the protection of sediment from metals contamination has not been evaluated.

The aim of this study was to perform some batch and open-system experiments in order (1) to study the efficiency of vermiculite clay for Cu and Zn removal from water solution and test its beneficial effect for sediment protection; (2) to compare the efficiency of vermiculite barrier system with a bio-barrier one, where a biofilm of *P. putida* had been previously attached on the vermiculite surface.

This study was carried out during a visiting scholar period in 2013–2014 at the CEB, University of Minho (Portugal), and it was performed in collaboration with Dip.SA, University of Bologna.

Materials and methods

Materials and reagents

Vermiculite clay (Sigma-Aldrich; specific surface area $39 \text{ m}^2 \text{ g}^{-1}$; mean particle ϕ 0.5 mm; porosity 10 %) was selected as adsorbent material for batch and open-system assays due to its low cost and eco-friendly characteristics. *P. putida* strain was obtained from the Spanish Type Culture Collection of the University of Valencia. Nitrate salts of Cu and Zn (Panreac) were tested separately in each assay in order to avoid metal competition which could affect the interpretation of the data (Fonseca et al. 2012).

Vermiculite characterization

Vermiculite point of zero charge (pH_{pzc}) was determined in order to evaluate the pH value below which vermiculite negative charges get saturated and cannot retain any other cations. One gram of vermiculite was added to 50 ml of NaCl 0.1 M and the initial pH was adjusted in the range 1–9. The mixtures were shaken at 140 rpm for 24 h in an orbital shaker, and the initial pH values were plotted against the final one. The pH_{pzc} was defined as the pH value at which the $\text{pH}_{\text{initial}}$ and the pH_{final} did not change during time and cross the theoretical line that fits the points $\text{pH}_{\text{initial}} = \text{pH}_{\text{final}}$ (Rivera-Utrilla et al. 2001).

The elemental composition of vermiculite was determined by inductively coupled plasma optical emission spectroscopy (ICP–OES, Spectro, Arcos) after acid digestion of samples with HCl and HNO_3 suprapure (3:1 w:w, Carlo Erba).

The characterization of the functional groups was performed by Fourier transform infrared spectroscopy (FT-IR, BOMEM MB 104) on pressed KBr pellets. The pellet was obtained by grounding 1 mg of each sample and 100 mg of KBr in an agate mortar. Background correction for atmospheric air was used, and spectra were obtained in the range 500–4000 cm^{-1} with a minimum of 30 scans and a resolution of 4 cm^{-1} .

Biofilm preparation

In order to correlate the optical density (OD) of *P. putida* culture with its concentration (g L^{-1}), the microorganism was grown until its exponential phase at 26 °C in Luria–Bertani broth (LB: 10 g L^{-1} tryptone; 5 g L^{-1} yeast extract; 5 g L^{-1} NaCl). The OD of the suspension and of its scalar dilutions (up to 1:50) was measured with a spectrophotometer at 620 λ (T60 UV–Visible Spectrophotometer, PG Instruments), and simultaneously, 10 ml of each measured sample was filtered on pre-weight nitrocellulose filters ($\phi = 45 \mu\text{m}$) and oven-dried at 105 °C in order to collect all the microbial cells. The dry mass of the filtered culture was then related to its OD through a linear regression. The regression equation ($y = 0,9091x + 0,0029$) was consequently applied to correlate the OD measurement (x) to the mass of the culture (y).

For the biofilm preparation, *P. putida* strain was cultivated for 24 h and refreshed two times in order to obtain a pure culture with a final concentration of 3 g L^{-1} . The bacterial suspension (500 ml) was centrifuged in sterilized polypropylene centrifuge tubes at 7000 rpm for 15 min and subsequently resuspended in 30 ml of three different media (Luria–Bertani broth: LB, Diluted Luria–Bertani broth: DLB, water: W), in order to obtain a very concentrated inoculum and to refresh it in different stress-living conditions. Vermiculite (1 g) was added to the concentrated suspension, and the system was left to equilibrate for 48 h with moderate agitation speed in order to promote microbial adhesion on vermiculite sheets. At the end of this equilibration period, the biofilm structure was observed by scanning electron microscope (SEM–Leica Cambridge S360) after treating samples of vermiculite sheets through a graded ethanol series (10, 25, 50, 80, 100 %) and gold covering (Chen et al. 2009).

Batch system for water remediation

A first batch system experiment aimed to evaluate the vermiculite uptake and removal percentage in a short time (which was assumed to be 24 h, based on equilibria determinations) and at different sorbate/sorbent ratios.

The contaminated water (150 ml) was additionate with 100 mg L^{-1} of Cu or Zn to different vermiculite amounts from 0.5 to 2 g and stirred at 140 rpm. The pH of the solution was periodically monitored and eventually adjusted in order to avoid metals precipitation and keep it constant to the initial values ($\text{pH } 6 \pm 0.5$ for both Cu and Zn system). Cu and Zn concentrations were periodically measured by ICP-OES (PerkinElmer), and certified reference materials (Optima Multi-Element Standard, PerkinElmer Pure and BCR-320) were used to verify the analytical accuracy (typically $< \pm 5 \%$). Metal uptake (Q , mg kg^{-1}) and removal percentage (D , %) of metals by vermiculite were calculated according to Rosales et al. (2012).

A second batch system experiment aimed to compare the adsorption kinetics of vermiculite (vermiculite permeable barrier: VPB) with that of vermiculite loaded with a biofilm of *P. putida* as described in the previous section (vermiculite permeable bio-barrier: VPB-Bio). Moreover, each assay was performed in three different media (LB, DLB and W) contaminated with 100 mg L^{-1} of Cu or Zn, in order to evaluate the microbial contribution in metal binding under different C-source deficiency conditions. To 150 ml of contaminated solution, 1 g of VPB or VPB-Bio was added and stirred at 140 rpm and 26 °C for 96 h in order to reach the equilibrium uptake. Periodically, the supernatant was collected, and the concentration of Cu and Zn was monitored by ICP-OES as described for the first batch experiment.

Pseudo-first-order and pseudo-second-order models were applied according to Rosales et al. (2012) to compare the kinetics of metal sorption at different conditions, through the following equations:

Pseudo-first-order (Lagergren 1907):

$$dq/dt = k1 \times (qe - qt) \quad (1)$$

Pseudo-second-order (Ho and McKay 1999):

$$dq/dt = k2 \times (qe - qt)^2 \quad (2)$$

where qt is the mass of metal adsorbed at time t (mg g^{-1}); qe is the mass of metal adsorbed at equilibrium (mg g^{-1}); $k1$ (L/min) and $k2$ ($\text{g}_{\text{verm}}/(\text{mg}_{\text{metal}}\text{t})$) are the pseudo-first and pseudo-second-order constants, respectively. Both equations were derived in linear form according to Azizian (2004), and qe and $k2$ were related to the slope and to the intercept, respectively, of the produced graph.

Open-system assay

An open-system assay was performed with the aim to evaluate the efficiency of vermiculite permeable barriers



(VPB) and bio-barriers (VPB-Bio) to prevent Cu and Zn contamination of sediments in continuous flow.

The open-system setup was made of a Plexiglas column (height: 33 cm, internal ϕ 3.5 cm) filled with 96 cm³ of VPB or VPB-Bio and 192 cm³ of fresh sediment from Dosolo canal (Bologna, IT), while a control assay column was filled with only sediment (Ct). The biofilm attachment on VPB-Bio was promoted in batch as previously described for 48 h in DLB medium and left to stand in the column for 2 h. Before starting the experiment, the excess of medium present in the column was extracted with a proper pipette.

Distinct water solutions containing 10 mg L⁻¹ of Cu or Zn were fluxed upwards through the system using a peristaltic pump (flow rate 0.5 L h⁻¹) for 24 h, and the metals concentration in the outflow was periodically monitored by ICP-OES as previously described.

Samples of sediment, VPB and VPB-Bio were collected from each column before and after the experiment for the analysis of Cu and Zn concentration. Each sample was dried at 60 °C, finely grounded, digested with aqua regia (suprapure HCl and HNO₃ 3:1 w:w) in a microwave oven (Milestone 1200) and analyzed by ICP-OES (Arcos, Spectro) according to Antisari et al. (2011). A mass balance approach was used to evaluate the metal partitioning in each component of the column setup. Total Cu and Zn amount released in outflow water was calculated as residual part of the total metal flowed into the system.

The biofilm evolution throughout the metal treatment was observed in VPB-Bio subsamples through SEM (Chen et al. 2009), while the difference between surface functional groups before and after metal treatment was observed on both VPB and VPB-Bio samples by Fourier transmission infrared spectroscopy (FTIR BOMEM MB 104).

Table 1 Characterization of vermiculite (Sigma-Aldrich)

Surface area	m ² g ⁻¹	39.0
Mean diameter	mm	0.5
ZPC	pH	1.9
Mg	mg g ⁻¹	99.73
Al		45.82
Fe		37.72
K		20.54
Ca		19.76
P		3.27
Na		0.77
Mn		0.28
Cu		0.03
Zn		0.07

Results and discussion

Vermiculite characterization and uptake

The summary of vermiculite physicochemical characterization is given in Table 1, and it highlights the high concentration of Mg²⁺ and Fe²⁺ as a result of Al³⁺ substitution in the octahedral positions of the vermiculite sheets, while the moderate concentration of Al³⁺ can be

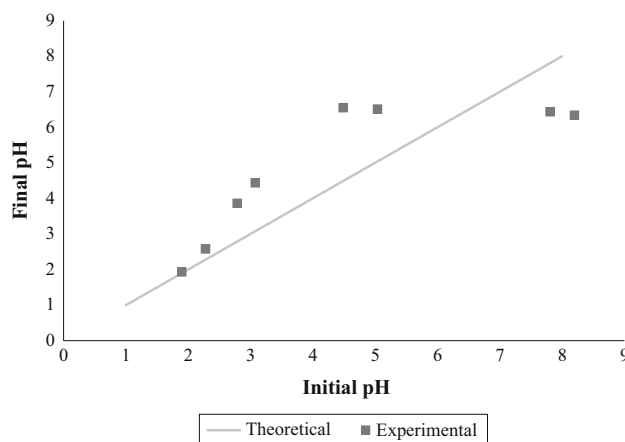


Fig. 1 Representation of the pH_{pzc} of vermiculite using pH drift method

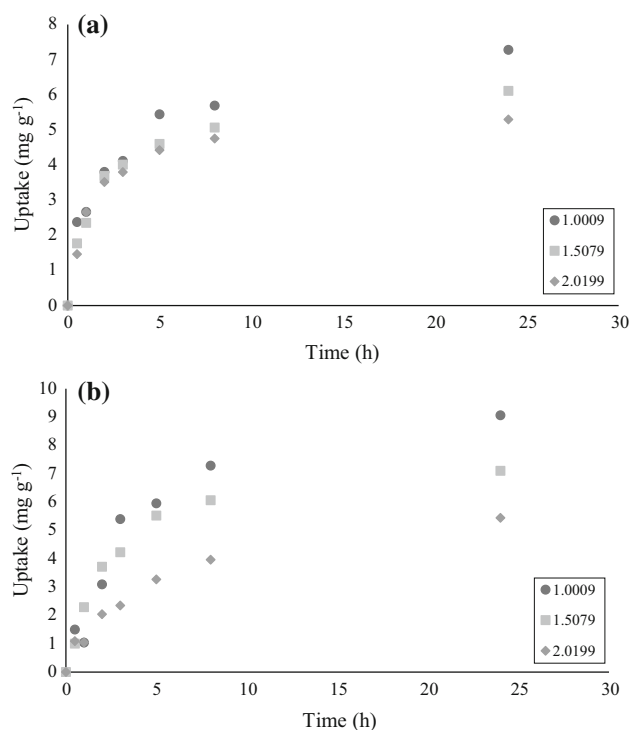


Fig. 2 Uptake of Cu (a) and Zn (b) by different amounts of vermiculite



Table 2 Cu and Zn uptake (Q , mg g⁻¹) and removal (D , %) and standard deviation (SD) by 1 ± 0.3 g of vermiculite barrier (VPB) and bio-barrier (VPB-Bio)

		Time (h)	LB				DLB				W			
			D (%)	SD	Q (mg g ⁻¹)	SD	D (%)	SD	Q (mg g ⁻¹)	SD	D (%)	SD	Q (mg g ⁻¹)	SD
Cu treatment														
VPB	0	0,00	0,0			0,00		0,00		0,00	0,0	0,00	0,0	
	1	2,06	0,1	0,34	0,0	9,37	3,9	1,73	0,7	12,83	0,8	1,91	0,1	
	3	3,77	0,2	0,62	0,0	10,58	4,6	1,95	0,8	24,02	1,3	3,58	0,2	
	5	4,10	0,2	0,67	0,0	11,73	4,7	2,16	0,8	29,68	0,7	4,42	0,1	
	8	4,15	0,2	0,68	0,0	12,42	4,9	2,29	0,9	35,33	1,3	5,26	0,2	
	24	7,47	0,4	1,22	0,1	17,08	5,5	3,15	1,0	49,63	0,3	7,39	0,1	
	48	nd	nd	nd	nd	17,42	4,1	3,21	0,7	56,34	1,9	8,39	0,4	
	96	nd	nd	nd	nd	21,37	11,8	3,84	2,1	61,57	0,3	9,17	0,1	
VPB-Bio	0	0,00	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00	0,0	
	1	1,95	0,1	0,32	0,0	28,38	2,9	5,24	0,6	79,07	0,5	8,36	0,1	
	3	4,95	0,2	0,81	0,0	28,75	2,5	5,30	0,5	82,70	0,9	nd	0,1	
	5	5,41	0,3	0,88	0,0	32,67	4,3	6,03	0,8	76,49	3,3	9,26	0,4	
	8	8,99	0,4	1,47	0,1	36,77	8,2	6,78	1,6	80,50	0,8	9,75	0,1	
	24	12,82	0,6	2,09	0,1	35,81	5,9	6,61	1,1	84,04	0,8	10,18	0,1	
	48	nd	nd	nd	nd	32,67	3,6	6,03	0,7	85,61	0,9	10,37	0,1	
	96	nd	nd	nd	nd	35,56	11,7	6,56	2,2	91,01	2,9	11,02	0,3	
Zn treatment														
VPB	0	0,00	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00	0,0	
	1	0,01	0,0	0,00	0,0	19,43	2,9	3,75	0,8	22,37	0,2	3,39	0,0	
	3	1,47	0,1	0,26	0,0	21,24	4,7	3,86	1,1	28,96	0,1	4,39	0,0	
	5	4,42	0,2	0,78	0,0	25,38	6,2	4,48	0,8	36,27	1,3	5,50	0,2	
	8	1,56	0,1	0,28	0,0	28,99	4,5	5,12	1,0	40,55	1,3	6,15	0,2	
	24	2,29	0,1	0,40	0,0	41,42	5,7	7,32	1,5	51,97	0,7	7,89	0,1	
	48	nd	nd	nd	nd	49,29	8,9	8,71	3,0	55,67	2,3	8,45	0,3	
	96	nd	nd	nd	nd	nd	nd	nd	nd	60,69	1,7	9,21	0,2	
VPB-Bio	0	0,00	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00	0,0	
	1	0,00	0,0	0,00	0,0	24,55	2,2	4,22	0,5	61,06	0,6	9,23	0,1	
	3	11,12	0,6	1,93	0,1	27,18	0,9	4,67	0,3	67,81	1,4	10,25	0,2	
	5	12,22	0,6	2,12	0,1	33,30	3,4	5,73	0,8	72,87	6,8	11,02	1,0	
	8	9,05	0,5	1,57	0,1	35,80	6,5	6,16	1,3	69,73	2,2	10,54	0,3	
	24	9,74	0,5	1,69	0,1	39,89	2,8	6,86	0,7	74,81	3,3	11,31	0,5	
	48	nd	nd	nd	nd	42,22	3,4	7,26	0,8	78,09	1,6	11,81	0,2	
	96	nd	nd	nd	nd	45,75	4,9	7,87	1,1	85,94	0,9	13,00	0,1	

related to Si⁴⁺ substitutions in tetrahedral position (Malandrino et al. 2006).

The evaluation of the pH_{pzc} is of fundamental importance in surface science, because it determines how easily a sorbent material may capture harmful ions, such as heavy metals. The pH_{pzc} was 1.9 (Fig. 1), and it confirms that vermiculite, as the majority of natural clays, has net negative charge in almost all natural conditions. Only at pH lower than 1.9, in fact, the adsorbent surface is positively charged (repelling cations), while at pH higher than 1.9 the surface is always negatively charged (attracting cations/repelling anions).

The uptake of both metals is shown in Fig. 2, and it indicates that uptake increases with decreasing the dose of adsorbent; for each assay the uptake increases rapidly during the first 8 h and it starts to reach equilibrium after 24 h. It is well known that the dynamics of metal cations adsorption on clays is fast, due to the high cation exchange capacity of these minerals and to their high specific surface area associated with the small particle size (Abollino et al. 2006).

The maximum Cu and Zn uptake after 24 h was 7.28 and 9.06 mg g⁻¹, respectively, confirming that metal



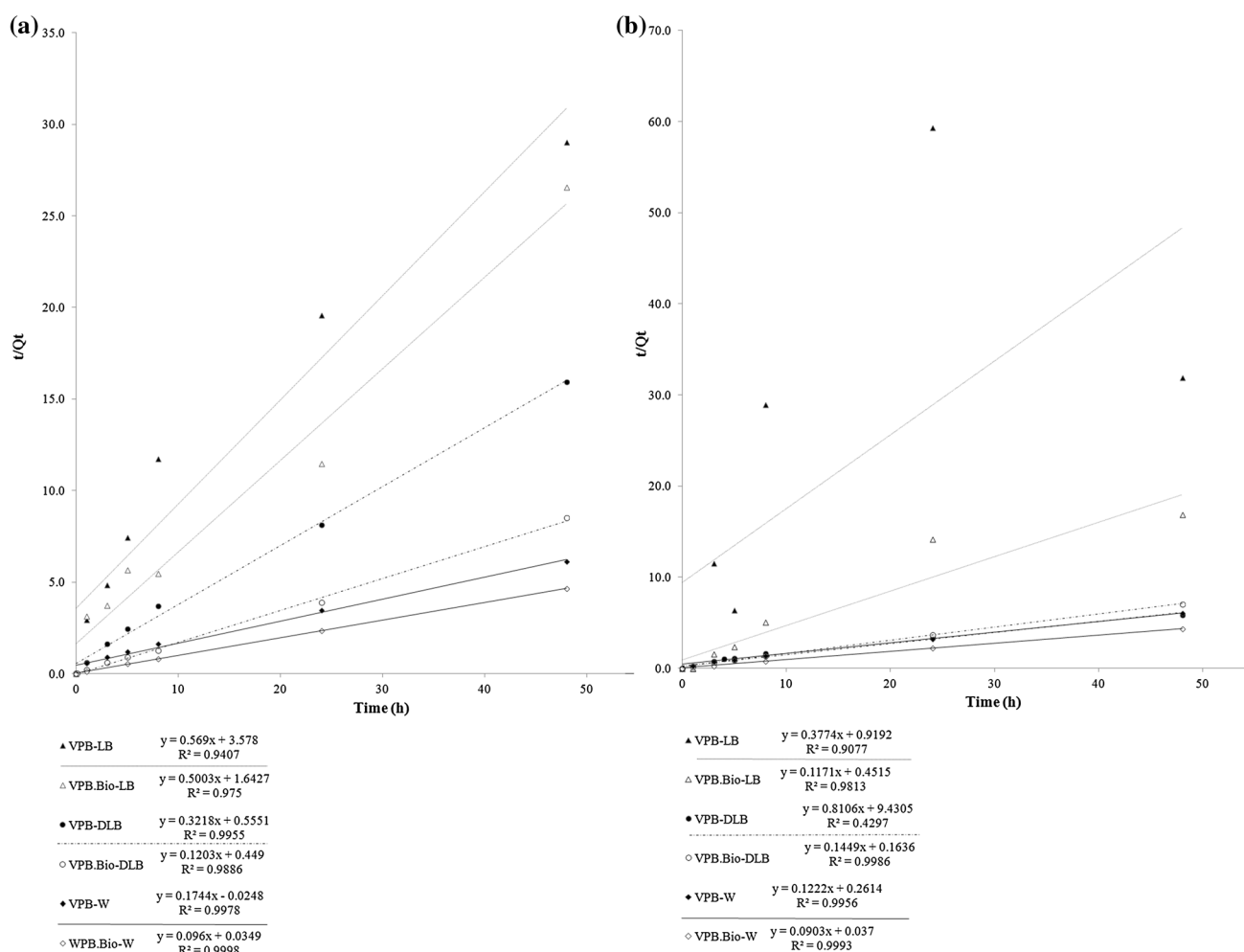


Fig. 3 Adsorption kinetic of Cu (a) and Zn (b) on vermiculite permeable barrier (VPB) and bio-barrier (VPB-Bio). Standard deviation was always $<\pm 5\%$

removal is linked to a high number of sorption sites available on the surface (Hameed 2009; Unuabonah et al. 2009) and suggesting that these metals have different affinities with the vermiculite surface. In fact, Zn ion has higher electrostatic attraction than Cu and can be easily adsorbed into the surface sites. On the contrary, Cu ion has tetragonal distortion and its adsorption is more difficult in the intra-lamellar sites (Abollino et al. 2006; Vieira dos Santos and Masini 2007). The lowest vermiculite amount tested showed the best performances in terms of uptake capacity and velocity of adsorption, confirming that this material is very suitable for metal remediation in fresh-water open systems.

Batch assays for water remediation

Batch assays tested the possibility to increase metal adsorption on clay from aqueous solutions by exploiting *P. putida* ability to entrap Cu and Zn ions.

Copper and zinc removal (D , %) and uptake (Q , %) of each assay are given in Table 2, and it shows that the removal efficiency increases as follows: $W > DLB > LB$. These results suggest that the growth of the surface-attached microbial biofilm predominates in low-nutrient media (Soini et al. 2002). In each assay, after 24 h of contact, the equilibrium state was reached and the presence of *P. putida* increased vermiculite barrier performances by enhancing the remediation efficiency of vermiculite. The contribution of *P. putida* biomass on water remediation was appreciable only during nutrient starvation conditions (W medium): In this case, the metal uptake increased by 2.79 and 3.42 mg g^{-1} for Cu and Zn, respectively (Table 2).

Pseudo-first-order model did not fit the experimental data and presented low R^2 values (data not shown), while the pseudo-second-order model could better describe the adsorption kinetic of the system (Fig. 3). According to Azizian (2004), it should be noticed that



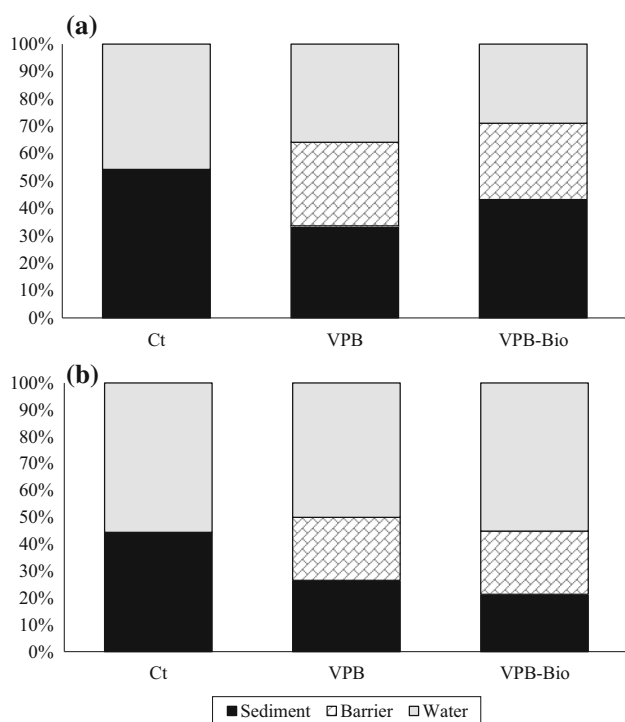


Fig. 4 Distribution of Cu (a) and Zn (b) between sediment, barrier and water in open-system assay. VPB stands for vermiculite barrier, while VPB-Bio stands for vermiculite bio-barrier system. Standard deviation was always $< \pm 5$ %

generally the sorption process obeys to pseudo-first-order kinetic at high solute concentration, while it obeys to a pseudo-second-order model at lower initial concentration of solute. Moreover, by deriving the pseudo-second-order equation, the lower is the slope of the curve, the faster is the kinetic (Azizian 2004). In both Cu and Zn adsorption kinetics, VBP-Bio systems showed lower k_2 than VPB for each experimental condition and the lowest k_2 was obtained in the assays performed in water. These results suggest that the presence of microbial biofilm on vermiculite barrier acts as a catalyst in the adsorption process.

The presence of Na^+ and phosphate groups in LB medium may interfere with vermiculite negative charges reducing the layer distances of vermiculite (Müller and Défago 2006) and therefore the available sites for metal entrapment. Bacteria can partially inhibit this exchange mechanism by metabolic path, but the adsorption kinetic of the system is low. LB medium favors the growth of *P. putida* in batch, but probably it does not allow the production of exopolysaccharides molecules (EPS) on the vermiculite particles, which are crucial substances contributing to bind significant amounts of heavy metals (Pal and Paul 2008; Gadd 2009). Many authors, in fact, confirm that EPS production by several bacteria species normally increase according to their nutrients starvation

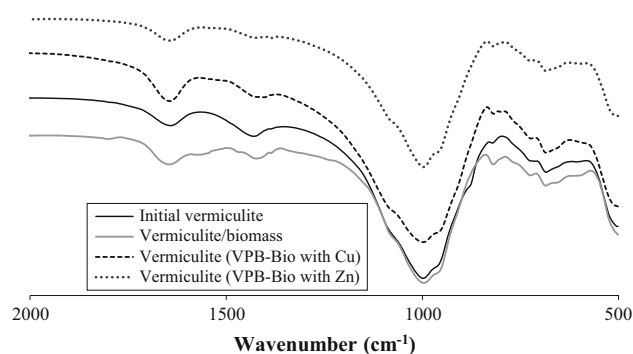


Fig. 5 FT-IR spectra of characteristic functional groups of pure vermiculite (VPB) and vermiculite loaded with biomass (VPB-Bio) before and after metal treatment

and environmental stress conditions (Sutherland 2001; González et al. 2010; Fang et al. 2011; Quintelas et al. 2011).

Open-system assay

In a continuous system, VPB and VPB-Bio were placed at the interface between water and sediment to test their efficiency in reducing metal adsorption from water into bottom sediments, and Fig. 4 displays the percentage of metal distributed in water, VPB or VPB-Bio and sediment for each column.

After 24-h treatment, Cu concentration in sediments varied as follows: $\text{Ct} > \text{VPB-Bio} > \text{VPB}$. In control column, 45 % of Cu was released in the water outflow, while in VPB and VPB-Bio columns the metal released was 35 and 28 %, respectively. These results confirm that the use of a permeable barrier at the interface between water and sediment can reduce up to 20 % the Cu adsorption into sediments and up to 10 % the amount of metal from water out flow.

After Zn treatment, the metal concentration in sediment was $\text{Ct} > \text{VPB} > \text{VPB-Bio}$, while in water no significant difference between the systems was noticed. The application of the permeable barrier could reduce 18 % the metal contamination in sediments, and the use of VPB-Bio successfully contributed to sediment mitigation up to 23 %.

The FTIR spectra of VPB-Bio before and after metal treatment are presented in Fig. 5, and it shows the alteration that surface chemical groups suffered before and after the metals flow. The presence of bacteria could only be detected by a weak band at 1550 cm^{-1} which corresponds to the vibration of the amide II, N–H or C–N from proteins, where vermiculite does not adsorb (Rong et al. 2010). No characteristic bands of organic matter were found on VPB-Bio samples, probably due to the low amount of biomass.



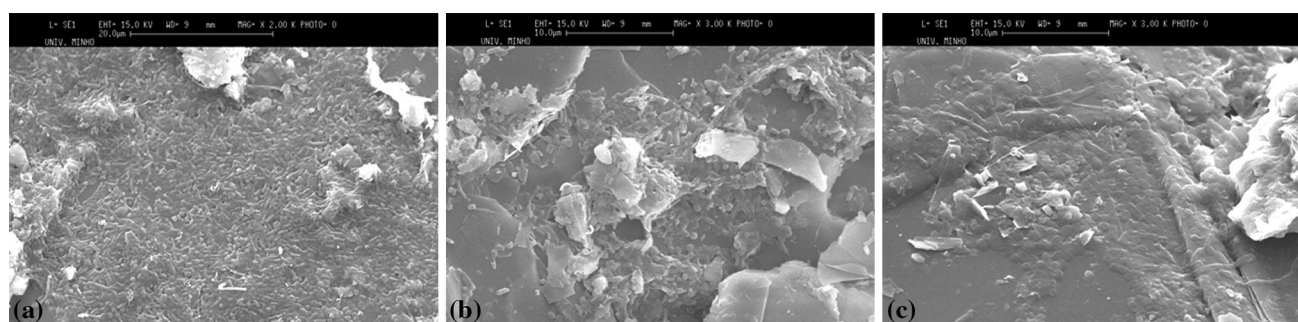


Fig. 6 SEM image of the biofilm grown on vermiculite sheets before the open-system assay (a) and after Cu (b) and Zn (c) treatment

Moreover, SEM images showed that the biofilm density was strongly reduced after the metal treatment (Fig. 6), and this hypothesis was confirmed from metals quantification by ICP analyses of vermiculite, where no significant differences between the VPB and VPB-Bio were detected (Fig. 4).

In both cases, the immobilization of the biofilm was not strong enough to avoid microbial migration through the column. Some authors indicates that time, nutrients availability and hydraulic stress can deeply affect the stability of biofilms on a surface (Percival et al. 1999; Ollos et al. 2003) by influencing the electrostatic forces which are essential to keep the biofilm attached to a solid surface. In our study, the low flow rate and the low contact time may have affected both thickness and resistance of the biofilm on VPB-Bio, resulting in a partial detachment of microbial biomass during the flow as shown in Fig. 6 (Soini et al. 2002). Therefore, metals retained by the biomass could migrate through the column and be subjected to a number of chemical and biochemical processes which typically occur in soil and sediment such as complexation and leaching (Kashem and Singh 2001; Stietiya and Wang 2006; Fonseca et al. 2009).

In this study, the application of VPB successfully proved their ability to protect both water and sediment from metal contamination. Moreover, the positive contribution of biofilm supported on vermiculite has been confirmed in batch system assays and needs to be optimized for open systems. Studying bio-recovery of metals from a complex system such as water and sediments will require more experimentation on biofilm immobilization by modeling different flow rates and contact time conditions and by monitoring their diffusion on the sediment layer.

Conclusion

Vermiculite is an interesting eco-friendly material suitable for water remediation. The batch experiments confirmed the positive contribution of *P. putida* supported on

vermiculite surface to metal sorption processes and could therefore be used as low-cost bio-remediation technique for water remediation.

The application of VPB and VPB-Bio at the interface between water and sediments in open system is a promising solution for preventing metal contamination of sediments. The continuous-flow studies, in fact, showed that vermiculite can be applied as permeable barrier in natural systems with a moderate capacity to limit the spread of contamination. However, some limitations in using the bio-barrier systems were highlighted and further efforts should be done to optimize the efficiency of the open setup, e.g., increasing the hydraulic stress for promoting the stability of the biofilm attachment, in order to gain the best benefit from *P. putida* biomass.

The optimization of such systems is very important because it could represent a very eco-friendly technique for preventing the contamination of the watercourses and therefore for improving the environmental safety of the territory.

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