

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

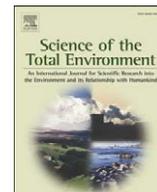
In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Science of the Total Environment

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

Measuring in-stream retention of copper by means of constant-rate additions

A. Serra^{a,*}, H. Guasch^a, E. Martí^b, A. Geiszinger^a^a Department of Environmental Sciences, Institute of Aquatic Ecology, Faculty of Sciences, University of Girona (UdG), Campus Montilivi, 17071 Girona, Spain^b Limnology group, Centre d'Estudis Avançats de Blanes (CSIC), 17300 Blanes, Spain

ARTICLE INFO

Article history:

Received 18 August 2008

Received in revised form 21 November 2008

Accepted 23 January 2009

Available online 19 March 2009

Keywords:

Artificial stream

Biofilm

Copper

Nutrient spiraling

Phosphate retention

ABSTRACT

Human practices entail inputs of nutrients and toxicants such as heavy metals to the fluvial ecosystems. While nutrient dynamics in fluvial ecosystems have been widely studied for over three decades, dynamics of toxicants still remain unclear. In this investigation, the nutrient spiraling concept and associated methodologies to quantify nutrient retention in streams were applied to study copper (Cu) dynamics in streams. The present study aimed to quantify total dissolved Cu retention using a simplified system of indoor channels colonized with fluvial biofilms. Cu retention was studied at sub-toxic concentrations to avoid negative/lethal effects on biota. In addition, Cu retention was compared with retention estimates of a macronutrient, phosphate (PO_4^{3-}), which has been widely studied within the context of the nutrient spiraling concept. The methodology used allowed a successful quantification of Cu and PO_4^{3-} retention. The results showed higher retention efficiency for PO_4^{3-} than for Cu. The biofilm played a key role in retaining both solutes. Although retention efficiency for both solutes was higher in the experiments with colonized substrata compared to uncolonized substrata, we found a positive relationship between uptake rate and chlorophyll-*a* only for PO_4^{3-} . Finally, retention efficiency for both solutes was influenced by water discharge, showing lower retention efficiencies under higher flow conditions. These results suggest that the fate and toxic effects of copper on stream biota may be strongly influenced by the prevailing environmental conditions. Our results indicate that the experimental approach considered can provide new insights into the investigation of retention of toxic compounds in fluvial systems and their controlling mechanisms.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Human activity is one of the major causes of elevated concentrations of nutrients and toxic substances in fluvial ecosystems (Cairns, 1993; Pereira and Hostettler, 1993; Ekholm et al., 2000). A wide range of organic and inorganic pollutants from industrial, urban, mining and agricultural activities in the catchments eventually reach streams and rivers through diverse surface and subsurface flowpaths (Foster and Charlesworth, 1996). Because these pollutants are transported along the fluvial networks in both particulate and dissolved forms, their effect on aquatic communities can be traced even at far distances from their input source (Johnson et al., 2005). Heavy metals are one of the most common inorganic pollutants in aquatic ecosystems (Genter, 1996). Many of the heavy metals in aquatic ecosystems, such as Cu, Mn, Fe and Zn, are essential micronutrients; therefore, their presence in water at trace concentrations is required for algal growth. However, at elevated concentrations these elements can be toxic to algae as well as to other aquatic organisms (Hall et al., 1989; Nies, 1999).

Several attempts have been made to develop mathematical models to describe the transport of toxic substances, such as heavy metals, in fluvial ecosystems. Those models are mainly based on hydrology and

on rates of sorption and desorption of metals onto sediment and suspended matter particles (Ciffroy et al., 2000; Jackman et al., 2001; Owens et al., 2001; Johnson et al., 2005). In those models, the role of biotic processes on heavy metal transport and the potential implications of toxics for biota are unconsidered. Nevertheless, several field and laboratory studies have revealed the important role of freshwater and marine algae in removing heavy metals from the water column through uptake and accumulation processes (Sunda and Huntsman, 1998; Vasconcelos and Leal, 2001; Wang and Dei, 2001; Campbell et al., 2002; Meylan et al., 2003). To our knowledge, the dynamics of heavy metals in fluvial ecosystems considering both hydrologic transport and biological processes has hardly been addressed and it is still unclear how microbenthic communities influence the downstream transport of these solutes. To fill up this gap, the conceptual context of the nutrient spiraling (Webster and Patten, 1979) and the mathematical and methodological approaches associated to it (Newbold et al., 1981; Webster and Valett, 2006) have been used in this study.

The nutrient spiraling concept was developed to describe the simultaneous processes of nutrient cycling and downstream transport, and it has significantly contributed to increase our knowledge of nutrient dynamics in stream ecosystems (Triska et al., 1983; Mulholland et al., 1985; Munn and Meyer, 1990; Martí and Sabater, 1996). This theoretical framework and all the empirical studies based on it have

* Corresponding author. Tel.: +34 972419789; fax: +34 972418150.
E-mail address: alexandra.serra@udg.edu (A. Serra).

evidenced that streams are not just transport systems, but they also have the capacity to use, transform, and retain nutrients (Bernhardt et al., 2005). Microbial communities developed on benthic stream substrata (usually referred to as biofilms) are mostly responsible for nutrient retention and transformation processes in streams (Allan, 1995). Recent research has shown the influence of physical factors such as temperature, discharge and transient storage (Valett et al., 1996; Butturini and Sabater, 1998; Peterson et al., 2001) as well as biological factors such as algal biomass (Mulholland et al., 1994; Sabater et al., 2000) on the transformation and retention of nutrients during downstream transport. These factors may likely affect downstream transport of heavy metals as well, but their relative effect is expected to differ from that on nutrient dynamics. For instance biological demand of a micronutrient as Cu is expected to be lower than the demand of a macronutrient. Some authors indicate that physical and biological factors such as water velocity and biomass accrual may influence Cu toxicity (Sabater et al. 2002) and metal sorption (Hill et al. 2000). Total metal removal is expected to increase at higher biomass concentration (Mehta and Gaur, 2005) although reduced metal sorption per unit of biomass is also expected due to decreased sorption capacity of the biofilm (Hamdy, 2000; Gong et al. 2005). However, studies focusing on the influence of physical and biological factors on the downstream transport of metals at sub-toxic concentrations are scarce.

The objectives of this study were to i) quantify the retention of dissolved copper (Cu), a potentially toxic compound, using the nutrient spiraling theoretical and methodological framework and ii) examine the influence of algal biomass and water discharge on Cu retention.

To avoid negative/lethal effects on biota, Cu retention was studied at a concentration $<40 \mu\text{g Cu/L}$, which is shown to be sub-toxic for periphytic biofilms under acute exposures (i.e. Navarro et al. 2002; Guasch et al. 2004). Cu retention was compared with retention estimates of a macronutrient, phosphate (PO_4^{3-}), which has been widely studied within the context of the nutrient spiraling concept. The study was conducted in an indoor artificial channel system containing colonized substrata as the biotic compartment. This system

is especially appropriated in ecotoxicological studies due to the impossibility of experimentally releasing toxicants to the natural environment. The use of artificial channels has previously been shown to be ideal to examine particular mechanisms influencing nutrient dynamics under controlled conditions (e.g., D'Angelo et al., 1991; Mulholland et al., 1991). This approach at mesocosm level contributes to increase our understanding of the fate of toxicants delivered to fluvial ecosystems.

2. Materials and methods

2.1. Experimental setup

To conduct this study, we constructed an indoor experimental system consisting of ten connected Perspex channels (each 170 cm long and 9 cm wide) (Fig. 1). Each channel unit ended with a Perspex piece to keep the water column depth at 1.5 cm. The system was supplied with dechlorinated tap water filtered through an active carbon (AC) filter. Water input at the head of the first channel unit was provided from a 10 L carboy using a centrifuge pump. The water outflow of this channel was subsequently directed to the next 10 L carboy to feed the contiguous channel units. All the carboys were placed in a water bath for water temperature control (Fig. 1). A tap located at the head of each channel allowed flow regulation to keep similar discharge among channel units. Ambient light was provided by halogen lamps ($80\text{--}100 \mu\text{mol photons/m}^2 \text{ s}$) following a 12 h/12 h light and dark cycle.

To test the influence of the stream biofilm on solute retention, the experiments in the system were conducted both with and without a biotic component. In the experiments without biota (considered as controls) 10 acid-rinsed etched glass substrata ($8.5 \times 12 \text{ cm}$) were introduced into each channel unit. These experiments were used to quantify the solute retention due to physical adsorption onto the channel and carboy walls, the tubing system and the artificial substrata. In the experiments with biota, the artificial substrata introduced into the channels (i.e., 10 etched glasses in each channel unit) were colonized. For the colonized substrata, etched glasses were

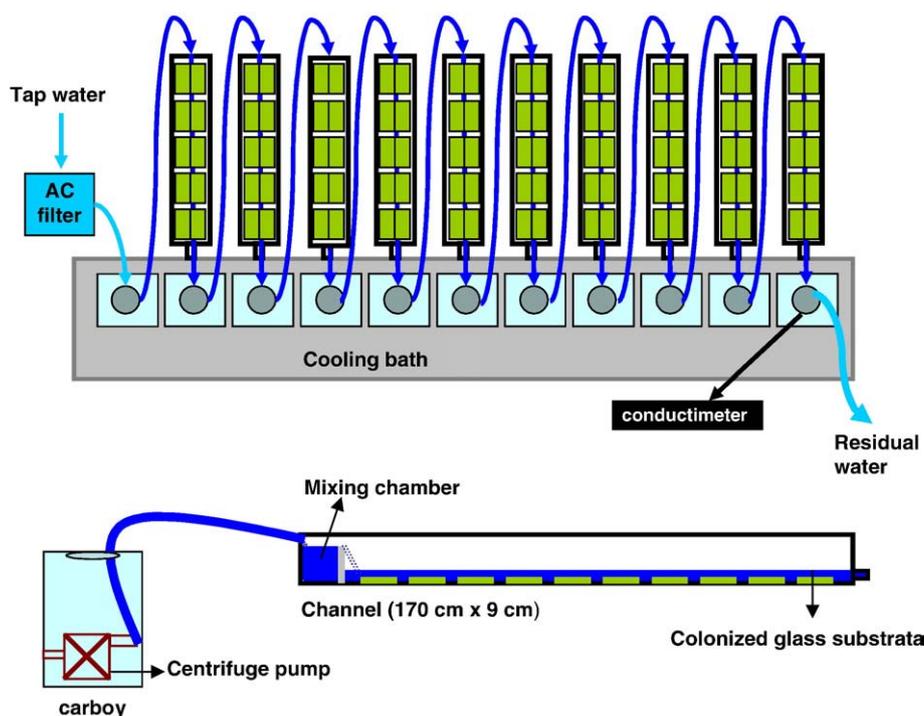


Fig. 1. Scheme of the experimental channel system showing an aerial view of the water flow connection among channel units (upper panel) and a detailed scheme of the longitudinal section of a channel unit (lower panel).

placed in an aquarium during 8 weeks to allow a mature biofilm community to be developed on them. The aquarium was filled with water from the Llémena stream, a small tributary of the River Ter (NE Spain), and contained a centrifuge pump to simulate turbulent water flow. One aliquot of the natural biofilm community scraped from the stream cobbles was used as an inoculum for the artificial substrata. Once a week, water in the aquarium was replaced with new water from the stream and reinoculated. A total of three colonizations were done over the course of the study to obtain colonized substrata for the different experiments. During the colonizations the average (\pm SEM) water temperature in the aquarium was 16.4 ± 0.7 °C, pH was 8.2 ± 0.1 , the concentration of soluble reactive phosphorus (SRP) was 36.6 ± 6.1 μ g/L, and conductivity was 504.3 ± 28.9 μ S/cm ($n = 17$).

2.2. Constant-rate solute additions

To quantify solute retention, we conducted constant-rate additions using either PO_4^{3-} or Cu in the channel system following the method described by Webster and Valett (2006). This method consists of adding a stock solution of the target compound at constant rate at the head of the channel system to raise its concentration above ambient levels and using the downstream disappearance of the added solute to quantify the retention parameters (Stream Solute Workshop, 1990).

In our experiments, phosphate was added as KH_2PO_4 and copper as CuCl_2 (copper titrisol, Merck, Darmstadt, Germany). Chloride, as NaCl, was added with both PO_4^{3-} and Cu as a conservative tracer to correct the downstream changes in the concentration of these solutes for water advection, dilution and dispersion. The nominal concentrations of the stock solution were 1663 and 618 μ g/L for PO_4^{3-} and 1000 and 415 μ g/L for Cu at high and low flow conditions respectively to achieve the desired increase in concentration. In all cases, additions of PO_4^{3-} and Cu were conducted separately to avoid possible interactive effects. The stock solution containing Cu was left to equilibrate for 24 h at room temperature prior to the additions and was set to result in sub-lethal concentrations at plateau conditions. The stock solution for both PO_4^{3-} and Cu was added at a constant rate (0.1 L/min) at the carboy of the first channel unit using a peristaltic pump. A conductivity meter with internal data logger (WTW 340i) was placed at the end of the channel system to continuously record changes in conductivity during the addition (Fig. 1). The solute addition lasted until the conductivity reached plateau conditions. This indicated that the solute added was completely mixed through the system. On average, the duration of each addition was between 1.5 and 2.5 h, depending on the water flow of each experiment.

Ten sampling points were defined along the channel system. Each point was located at the end of each channel unit, corresponding to every 1.7 m. At these points, three water sample replicates were collected prior to each constant-rate addition for background concentrations and once the addition reached plateau conditions. Water samples were collected with 20 mL polyethylene syringes and immediately filtered with 0.2 μ m nylon filters. Samples were analyzed for PO_4^{3-} and total dissolved Cu concentrations. In each experiment, water temperature, pH and light intensity (LiCor quantum sensor, LI-192B) were measured at the beginning and at the end of the addition. In three experiments, additional water samples were taken at all sampling points before the addition in order to measure the background concentration of dissolved organic and inorganic carbon (DOC and DIC, respectively), and major cations and anions.

A total of 9 additions of PO_4^{3-} and 9 additions of Cu were conducted in this study. For each solute, the additions were done with and without biofilm communities (referred to as B and C, respectively) and at a low (1 L/min) and high (2 L/min) flow (referred to as L and H, respectively). The combination of the two factors resulted in four different treatments: LC, LB, HC, and HB. The additions with colonized substrata started after allowing the biofilm communities to adapt to the channel conditions for two days. Each set of colonized substrata

received a maximum of four constant-rate additions, which were separated by a 24-h period. These four additions followed the methodical sequence: first the high flow phosphate addition, followed by the low flow phosphate addition, the high flow copper addition and finally the low flow copper addition. This sequence was set to account for the negative effect of discharge on retention efficiency described for nutrients (Butturini and Sabater, 1998; Peterson et al. 2001) and to minimize the possible influence of previous additions on biofilm responses. Each constant-rate addition, a periphyton sample was taken to assess their physiology by measuring photosynthesis efficiency (Yield).

2.3. Biofilm measurements

Photosynthetic activity of the algal component of the biofilm communities and algal biomass were measured during the experiments. In each experiment, three colonized substrata were collected from three different channel units before the solute addition and at the plateau conditions for photosynthesis measurements. Comparison between background and plateau biofilm samples allowed examining possible effects of the solute additions on the physiologic status of the biofilm communities. We used the Pulse of Amplitude Modulated fluorometry (PHYTO-PAM Heinz Walz GmbH, Effeltrich, Germany) to measure the effective quantum yield of illuminated cells (Yield), which is an indicator of the photosynthetic efficiency of algae (Schreiber et al., 2002). As this technique is non-destructive, the same colonized substrata used for PAM fluorometry measurements were also used to measure the algal biomass, estimated as the chlorophyll-*a* concentration (chl-*a*), after extraction with 90% acetone and spectrophotometric measurements (Jeffrey and Humphrey, 1975). In the experiments performed without biofilm (referred to as C in the text), the algal biomass was not measured as empty glass substrata were used.

Observations of dominant algal groups of mature biofilms were conducted under light microscopy. In two different Cu addition experiments (at high flow and low flow conditions), colonized substrata were also collected to measure the total Cu content in the biofilms. Three samples were collected before the addition for background Cu content and three other samples were collected at the plateau conditions. Biofilm samples were scraped from the glass substrata with a microscope slide, lyophilized and weighed. Dry samples were digested with 4 mL of concentrated nitric acid (suprapure, Merck) and 1 mL of hydrogen peroxide (30%, suprapure, Merck) in a high performance microwave (Milestone, Ethos sel) and were thereafter diluted to 25 mL with milli-Q water before analysis. The concentration factor (CF) was calculated as the ratio between the increase of Cu content in the biofilm and the increase of Cu in water.

2.4. Laboratory analyses

The concentration of SRP (soluble reactive phosphorus) in water samples was analyzed by the Murphy and Riley (1962) molybdenum blue colorimetric method following APHA (1989). Concentrations of major cations and anions dissolved in water were analyzed by ion-chromatography (Metrohm Ltd., Herisau Switzerland). Anions were measured using a METROSEPA SUPP 5 column and NaHCO_3 (84 mg/L) and Na_2CO_3 (339 mg/L) as eluents. Cations were measured using a METROSEP C 2 column and tartaric acid (2,3-dihydroxybutanedioic acid; 4 mM) and dipicolinic acid (pyridine-2,6-dicarboxylic acid; 0.75 mM) as eluents.

The filtered samples for Cu analysis were acidified with 1% of nitric acid (suprapure, Merck) and stored in the refrigerator at 4 °C until analysis. Dissolved Cu concentration was analyzed using inductively coupled plasma mass spectrometry (7500c ICP-MS Agilent Technologies, Inc., Wilmington, DE). Cu content of digested biofilm samples was analyzed using the same methodology. Considering that trace

Table 1
Background concentration of major anions and cations, dissolved inorganic and organic carbon (DIC and DOC, respectively) in the water of the channel system.

Parameter	NO ₃ ⁻	NO ₂ ⁻	NH ₄ ⁺	SO ₄ ²⁻	Ca ²⁺	Mg ²⁺	Na ⁺	Cl ⁻	DIC	DOC
Concentration (mg/L)	1.68	0.07	<0.1	38.33	54.32	7.93	21.89	23.84	26.8	2.9
SEM	0.14	0.01		3.52	0.83	0.10	0.33	2.36	0.4	0.1

Values, means and standard error of the mean (SEM) of twenty samples taken before the solute additions were conducted. For DIC and DOC values are the average and standard error of the mean of three samples.

metal bioavailability to aquatic micro-organisms is dependent on metal speciation (Morel and Hering, 1993), the inorganic Cu-complexes and their solubility in the media were estimated using the chemical-equilibrium-diagram-tool Hydra/Medusa (Puigdomenech, 2002), which takes into account the concentration of the major ions in water.

2.5. Hydrology and solute retention calculations

Discharge (Q, L/min) into the channel system was determined by a mass balance approach (Gordon et al., 1992) using the conductivity data measured at the bottom of the last channel unit in the following Eq. (1):

$$Q = \frac{Q_a(T_a - T_p)}{T_p - T_b} \tag{1}$$

where Q_a is the addition flow rate (L/min), and T_a, T_b and T_p are the values of the hydrological tracer (i.e., conductivity, μS/cm) in the added solution and in the water at background and plateau conditions, respectively. Average water velocity (cm/s) along the channel system was calculated based on the total length of the system and the time required for the conductivity to reach one half of plateau value.

Removal of PO₄³⁻ and Cu from the water column was quantified using the retention metrics derived from the spiraling concept and widely used in studies of nutrient dynamics in stream ecosystems (Stream Solute Workshop, 1990; Webster and Valett, 2006). The uptake rate coefficient per unit length (K_c; m⁻¹) was measured following the Eq. (2):

$$\ln \left[\frac{C_p - C_b}{T_p - T_b} \right]_x = -K_c x \tag{2}$$

where C_b and C_p are the concentrations of the solute (Cu or PO₄³⁻) at background and plateau conditions, respectively, and x is the distance between the addition point and each sampling point. Solute uptake length (S_w; m), the negative inverse of K_c, is an indicator of the in-stream solute retention efficiency (Newbold et al., 1981). Shorter S_w indicates higher retention efficiency than longer values.

We also calculated the areal uptake rate at background concentrations (U; μg/m² min) for the two solutes, following Eq. (3) (Stream Solute Workshop 1990):

$$U = \frac{C_b Q}{S_w w} \tag{3}$$

where w is the width of the channel. U is the mass of a solute that is removed from the water column per unit time and benthic area. Several biotic and abiotic processes, such as biological uptake, adsorption, precipitation and complexation, contribute to this removal (e.g., Triska et al., 2006). As this parameter corrects S_w for hydrology and solute concentration, we use it in this study to explore the influence of algal biomass on PO₄³⁻ and Cu retention including data from all the additions with biota together.

2.6. Statistical analyses

Possible effects of solute additions, especially those of Cu, on the photosynthetic activity of the biofilm were evaluated using paired T-test (StatSoft, Inc., 1999) on Yield values measured before the additions and at plateau. The influence of water discharge and algal biomass on PO₄³⁻ and Cu retention was examined by means of linear regression analyses. The relationship between solute retention and discharge was examined using data from additions with and without biota separately. For algal biomass, this relationship was examined using only data from additions with biota.

3. Results

3.1. Characterization of the experimental conditions

During the constant-rate solute additions, average ± SEM discharge and water velocity were 0.83 ± 0.05 L/min and 0.59 ± 0.08 cm/s, respectively, at low flow conditions (n = 10), and 2.11 ± 0.05 L/min and 0.89 ± 0.03 cm/s, respectively, at high flow conditions (n = 8). Water column depth was on average 2.83 ± 0.21 cm under low flow conditions (n = 10) and 4.45 ± 0.20 cm under high flow conditions (n = 8). Water temperature, pH and conductivity varied in a narrow

Table 2
Discharge (Q), chlorophyll content (chl-a), uptake length (S_w) and areal uptake rate (U) obtained for each PO₄³⁻ and Cu addition experiment conducted at high (H) and low (L) flow, and with (B) and without (C) biofilm.

Treatment	Cu additions					PO ₄ ³⁻ additions				
	Q	Time plat	Chl-a	S _w -Cu	U-Cu	Q	Time plat	Chl-a	S _w -PO ₄ ³⁻	U-PO ₄ ³⁻
	L/min	min	μg/cm ²	m	μgCu/m ² min	L/min	min	μg/cm ²	m	μgPO ₄ ³⁻ /m ² min
LC	0.68	137	ND	89.3	0.01	0.82	96	ND	NS	NS
	0.73	108	ND	91.7	0.02	0.72	112	ND	NS	NS
HC	2.27	69	ND	163.9	0.04	2.13	59	ND	NS	NS
	2.36	48	ND	161.3	0.23	1.91	48	ND	NS	NS
LB	1.13	106	1.6 ± 0.7	15.2	0.29	1.02	107	2.6 ± 0.1	18.0	10.38
	0.79	121	11.8 ± 1.5	21.3	1.51	0.7	70	7.0 ± 2.3	13.3	12.13
	0.76	13	14.1 ± 1.6	19.3	0.34	0.95	98	12.8 ± 1.7	8.5	21.28
HB	1.97	49	9.8 ± 1.4	67.6	1.11	2.05	54	4.5 ± 1.0	30.8	13.54
	2.09	65	11.9 ± 4.7	39.4	0.41	2.12	54	7.0 ± 1.0	25.6	14.71

Values of chl-a are means ± standard error of the mean (SEM) of three replicates taken at plateau conditions. NS: not significant. ND: no data.

range on all experiments and averaged 15.3 ± 0.6 °C, 8.04 ± 0.37 and 484 ± 2 $\mu\text{S}/\text{cm}$, respectively ($n = 18$). A more detailed description of the average water chemical composition for all the experiments is summarized in Table 1.

The average background PO_4^{3-} and Cu concentrations were 18.1 ± 0.6 $\mu\text{g}/\text{L}$ (C.V. = 10.61%) and 1.2 ± 0.5 $\mu\text{g}/\text{L}$ (C.V. = 113.19%), respectively. At plateau conditions, the concentrations were increased up to 96.4 ± 8.7 $\mu\text{g}/\text{L}$ (C.V. = 27.18%) for PO_4^{3-} and 34.8 ± 2.3 $\mu\text{g}/\text{L}$ (C.V. = 19.69%) for Cu ($n = 9$ for each solute). Inorganic Cu speciation measured for the pH range of the experiments showed that dissolved $\text{Cu}(\text{OH})_2$ was the predominant Cu-complex (>90% of the total copper) in the water during the experiments. The results presented correspond always to total dissolved Cu concentration.

The algal component of the biofilm was dominated by diatoms and filamentous green algae (*Cladophora* sp. and *Spirogyra* sp.). Biofilm chlorophyll-*a* content varied one order of magnitude among the different experiments and ranged from 1.62 to 14.1 $\mu\text{g chl-}a/\text{cm}^2$ (Table 2). The average \pm SEM ($n = 5$) photosynthetic yield (Yield) of the biofilm before the solute additions was 0.51 ± 0.08 after 1 day, 0.52 ± 0.03 after two days and 0.51 ± 0.08 after 3 days in the channels system, indicating that the physiological state of the community was not changing in the course of the experiments. In addition, it did not change during the course of the additions. It was 0.51 ± 0.03 and 0.50 ± 0.02 before the Cu additions and at plateau conditions, respectively. For PO_4^{3-} additions, Yield average values were 0.54 ± 0.03 and 0.52 ± 0.02 before the additions and at plateau conditions, respectively. Results from the *T*-test analysis did not show any

significant difference in Yield between the two sampling times on each of the additions performed.

3.2. Copper and phosphate retention metrics

In the addition experiments with uncolonized substrata (i.e. control experiments), Cu concentration showed a significant decline along the channels at plateau conditions, whereas PO_4^{3-} concentration did not show any significant decline (Fig. 2). All the addition experiments performed in channels with colonized substrata showed a significant longitudinal decline for both PO_4^{3-} and Cu concentrations at plateau conditions (Fig. 2).

Retention metrics for Cu and PO_4^{3-} obtained from the addition experiments are summarized in Table 2. Uptake length of Cu ($S_w\text{-Cu}$) ranged from 89.3 to 163.9 m in control experiments and from 15.2 to 67.6 m in channels with colonized substrata. Therefore, $S_w\text{-Cu}$ was from 2 to 6 times shorter in the experiments with colonized substrata. Uptake length of PO_4^{3-} ($S_w\text{-PO}_4^{3-}$) was measurable only in channels with colonized substrata where it ranged from 8.5 to 30.8 m. $S_w\text{-Cu}$ from control experiments was positively related to water discharge ($R^2 = 0.996$, $P < 0.01$; $n = 4$). Although a positive trend was observed between $S_w\text{-Cu}$ and discharge in the experiments with colonized substrata, the relationship was not statistically significant ($R^2 = 0.65$, $P = 0.1$, $n = 5$). $S_w\text{-PO}_4^{3-}$ from experiments with colonized substrata were positively related to water discharge ($R^2 = 0.80$, $P < 0.05$, $n = 5$).

In the experiments performed with biofilm, $U\text{-Cu}$ ranged from 0.29 to 1.5 $\mu\text{gCu}/\text{m}^2 \text{ min}$ and $U\text{-PO}_4^{3-}$ from 10.3 to 21.3 $\mu\text{gPO}_4^{3-}/\text{m}^2 \text{ min}$. $U\text{-Cu}$ in

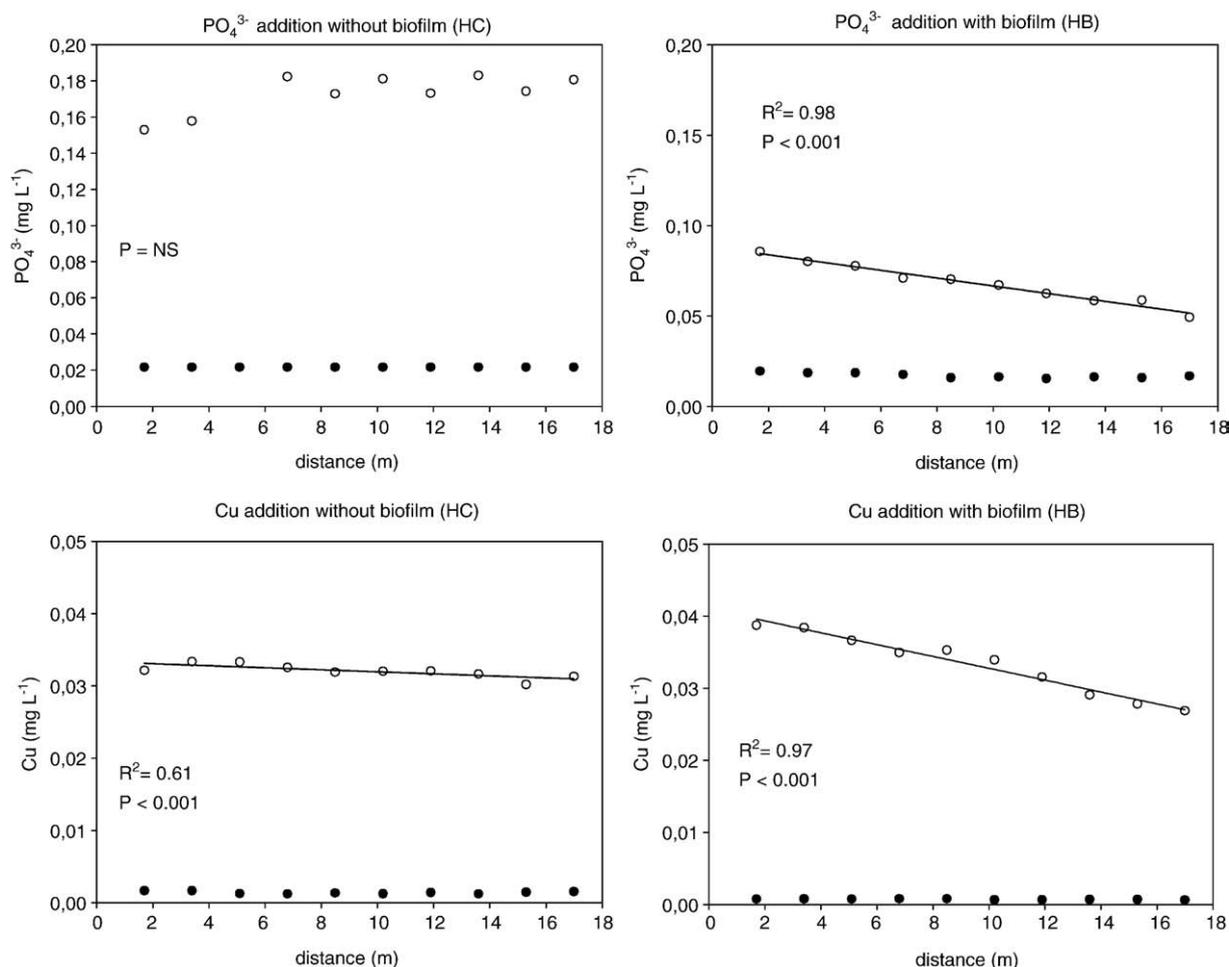


Fig. 2. Illustrative examples of results from solute additions showing longitudinal variation of Cu and PO_4^{3-} concentrations along the channel system at background (solid circles) and plateau (open circles) conditions. Adjust (R^2) and significance (P) of the linear regression are also indicated.

control experiments ranged from 0.01 to 0.23 and was on average c.a. 10 times lower than in experiments with colonized substrata. The highest $U-Cu$ value measured in control experiments coincided with a 5 times increase in ambient Cu concentration (i.e., from <0.27 to $1.4 \mu gCu/L$). $U-Cu$ did not show any significant relationship with $chl-a$ content, whereas $U-PO_4^{3-}$ was positively related with this parameter ($R^2 = 0.87$; $P < 0.05$; $n = 5$). Measurements of Cu accumulation in the biofilm showed higher concentration factors in the experiments performed under low flow ($CF = 4.48 \pm 0.20$) than in those under high flow conditions ($CF = 1.28 \pm 2.23$) (average \pm SEM; $n = 3$).

4. Discussion

The experimental design used in the present study was addressed to quantify copper retention at mesocosm scale considering both, downstream transport and the influence of fluvial communities on the retention of this element. To our knowledge, this ecological perspective has not been previously considered in copper retention studies.

Retention of copper was quantified by applying the concepts and methodologies comprised in the nutrient spiraling theory (Newbold et al., 1981; Stream Solute Workshop, 1990; Webster and Valett, 2006), and results were compared to retention measurements of phosphate, a macronutrient used as a reference. The concentrations of both solutes resulting from the constant-rate additions were high enough to detect longitudinal changes along the channel distance, but sufficiently low to avoid either positive or negative effects on the biota as indicated by the lack of significant effects on the photosynthetic activity of the biofilms during the additions.

The indoor channel system was sensitive enough to measure retention of the two solutes and to detect differences in their retention according to slight variations in hydrological and biological conditions. Phosphate uptake length was only measurable in experiments with colonized substrata evidencing the relevance of biofilms in controlling retention of this element. Values of phosphate uptake length were short and within the range of other phosphate dynamics studies performed in artificial channels (D'Angelo et al., 1991). This indicates that the system was highly efficient retaining phosphate. We were also able to quantify copper retention, the main aim of this investigation. In contrast to phosphate, copper uptake lengths were measurable in experiments performed with and without biota. However, values of copper uptake length were shorter in the presence of biofilms, indicating that retention efficiency for this element was enhanced by the presence of stream-bed biota (microbenthic biofilms). This increase may be related to a greater availability of metal binding sites and also to the indirect effects of biotic activity (photosynthesis), since photosynthesis causes and increase in the local pH which can modify metal solubility and bioavailability (Guasch et al. 2002). The relatively low DOC concentration of the water used for the experiments, suggests that precipitation of Cu-organic complexes was unlikely under our experimental conditions (Breault et al. 1996) thus it is not expected to contribute in the removal of Cu from the water column.

The abiotic retention differences between Cu and phosphate may be due a) to their anionic and cationic behaviour, b) to specific binding sites for Cu in the channel system, or a combination of both. The role of the cationic/anionic behaviour cannot be derived from our results. This could be addressed with abiotic experiments using other metallic anions (chromium).

In fact, using results of copper uptake rates from experiments with and without biota we estimated that 89.8% of the retention was driven by the biofilm while the remaining 10.2% could be attributed to physical adsorption onto the walls of the channels, carboys and tubes. Peña-Castro et al. (2004) measured the capacity of heavy metal removal by the microalga *Scenedesmus incrustatus* in continuous culture assays and found similar results relative to biotic copper removal in their experiments, accounting for 72% of the total copper

removal contrasting with the 28% attributed to abiotic factors. These results together with ours indicate the relevance of the biotic control on dynamics of copper under sub-lethal concentrations in aquatic systems. Nevertheless, retention efficiency for copper was 1.7 times lower than that for phosphate in the experiments with colonized substrata. A different demand of the stream-bed biota for the two solutes may explain this difference. Phosphate is a macronutrient, and it is well known that algae have mechanisms to retain it very efficiently (Doods, 2003), whereas copper is a micronutrient. Therefore, biological requirements for phosphate are expected to be higher than those for copper. Although differences between Cu and P demand have not been directly assessed in periphyton, marine plankton studies reported average Cu:P ratio between 0.4 and 0.5 mmol/mol (Martin et al., 1976; Ho et al., 2003). This low value of the ratio supports our statement since Cu requirement of algae is about 1000 times lower than the requirements for phosphorus.

The experimental setup also allowed examining the influence of hydrologic (water discharge) and biological factors (algal biomass) on the retention of the studied solutes. Retention efficiencies for both solutes were influenced by water discharge, being 2 and 3 times lower for phosphate and copper respectively, in the experiments conducted under higher flow conditions. These results agree with previous findings from stream nutrient retention studies (Butturini and Sabater, 1998; Peterson et al., 2001; Hall et al., 2002). Most biological activity in small streams is associated to the stream benthos where a higher discharge reduces the surface-volume ratio and decreases nutrient exchange with benthic microbial communities; thus, reducing nutrient retention efficiencies (Martí et al., 2004). Our results indicate that copper retention may also be subjected to this hydrological control in streams. The lack of relationship found between $U-Cu$ and algal biomass combined with the lower Cu retention found under high flow conditions and the higher accumulation of copper (as measured by the concentration factor) found in the biofilm exposed to lower flow conditions supports this hypothesis.

In the range of algal biomass measured in the experiments, phosphate uptake rates increased with increasing $chl-a$ content. This finding agrees with previous studies and demonstrates the important role of algal activity in controlling nutrient dynamics (Grimm et al., 1981; Triska et al., 1983; Tate, 1990; Grimm, 1992). As it is reviewed in Doods (2003), phosphate uptake is influenced by periphyton biomass and metabolic activity, and advective transport of phosphorus to periphyton assemblages. Sabater et al. (2000) found that phosphate mass-transfer velocity (which also indicates nutrient retention efficiency) was positively correlated with primary production in a forested Mediterranean stream. In addition, in calcareous streams periphyton activity can enhance phosphate retention by increasing pH and thus inducing phosphate co-precipitation with calcium carbonate (Martí and Sabater, 1996). Finally, Mulholland et al. (1994) documented that thick periphyton masses increased 6 times phosphate retention compared to thin biofilms by increasing the relative area of dead zones and enhancing internal cycling and retention. All these above mentioned processes contribute to an increase of the short-term phosphorus retention; and thus, it is not surprising that phosphate uptake from our mesocosm experiments gradually increased with algal biomass.

Despite the difference in copper retention efficiency between the colonized and uncolonized experiments, we did not find any relationship between copper uptake rates and algal biomass.

Some studies report the influence of biomass concentration on metal removal, however no consistent results are found in this issue (Mehta and Gaur, 2005). Reduced metal adsorption per unit of biomass was found when algal biomass was higher, and was related to a decreased sorption capacity of the biofilm (Hamdy, 2000; Gong et al., 2005). However other studies reported increased metal removal at higher biomass concentration (Hill et al., 2000; Mehta and Gaur, 2001).

The duration of the copper additions in our experiments may have been too short to observe the diffusion effect, limiting copper uptake mostly to the surface layer of the biofilm regardless of its thickness. This phenomenon has been described in other studies (Barranguet et al., 2002) and would explain the lack of relationship between copper uptake rate and algal biomass in our experiments.

The use of biofilm communities in our study offers a more realistic view of the processes involved in the downstream transport of heavy metals compared to the commonly used laboratory experiments focused on estimations of metal uptake rates or bioaccumulation kinetics by algal cultures and periphyton (e.g. Campbell, 1995; Vigneault et al., 2000; Meylan et al. 2003; Morin et al., 2008). The results obtained evidence that this experimental design provides a relatively simple but ecologically relevant approach to investigate dynamics of toxicants in fluvial systems and the influence of biofilm as well as other ecologically relevant factors on them.

The quantification of biotic and abiotic metal retention in a simplified river system, including its downstream transport, provides an ecological perspective based on fluvial ecology concepts. This approach complements the information obtained from existing studies addressing the transport of metals in fluvial systems (e.g. Ciffroy et al., 2000; Runkel, 1995) and the retention of metals by algae (e.g. Sunda and Huntsman, 1998; Knauer et al., 1997). We foresee future applications of the conceptual and experimental approach considered to further investigate the influence of different environmental factors on copper dynamics, the effects on the benthic microbial communities and the dynamics of other toxic compounds.

5. Conclusions

The methodological approach used in this study allowed the quantification of copper retention at mesocosms scale, considering the downstream transport of the metal and the influence of the fluvial biofilm on its retention. The system retained copper in a lower efficiency than phosphate, this may reflect the different biological demand for both solutes. Retention of both solutes resulted negatively influenced by water discharge. Although copper retention was enhanced by the presence of biofilm, it was not significantly correlated with algal biomass. Conversely, phosphate uptake was positively related with algal biomass of the biofilm.

Having into account the observations of this study, which have been done in an artificial river, we can tentatively extrapolate them to a real field situation. Overall it is expected that biofilms will have an important role on Cu retention and that this will be increased under low flow conditions.

This approach may contribute notably to increase our understanding on the fate of toxicants entering the stream ecosystem.

Acknowledgments

This study was supported by two Spanish Ministry projects (REN2003-07702 and CGL2006-12785) and the EC project KEY-BIOEFFECTS (MRTN-CT-2006-035695).

A. Serra was supported by FPI pre-doctoral scholarship BES-2004-5527, from the Spanish Ministry of Science and Education.

References

- Allan JD. Stream ecology: structure and function of running waters. London, U.K.: Chapman & Hall; 1995. 388 pp.
- APHA. Standard methods for the examination of water and waste water. 17th edition. Washington, D.C., USA: American Public Health Association; 1989.
- Barranguet C, Plans M, Van der Grinten E, Sinke JJ, Admiraal W. Development of photosynthetic biofilms affected by dissolved and sorbed copper in a eutrophic river. *Environ Toxicol Chem* 2002;21:1955–66.
- Bernhardt ES, Likens GE, Hall JR, Buso DC, Fisher SG, Burton TM, et al. Can't see the forest for the stream? The capacity of instream processing to modify terrestrial nitrogen exports. *Bioscience* 2005;55:219–30.
- Breault RF, Colman JA, Aiken GR, McKnight DM. Copper speciation and binding by organic matter in stream water. *Environ Sci Technol* 1996;30:3477–86.
- Butturini A, Sabater F. Ammonium and phosphate retention in a Mediterranean stream: hydrological versus temperature control. *Can J Fish Aquat Sci* 1998;55:1938–45.
- Cairns Jr J. Environmental science and resource management in the 21st century: scientific perspective. *Environ Toxicol Chem* 1993;12:1321–9.
- Campbell PGC. Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. In: Tessier A, Turner DR, editors. Metal speciation and bioavailability in aquatic systems, IUPAC Series. Chichester: John Wiley and Sons; 1995. p. 45–102.
- Campbell PGC, Errécalde O, Fortin, Hiriart-Baer V, Vigneault B. Metal bioavailability to phytoplankton — applicability of the biotic ligand model. *Comp Biochem Physiol* 2002;133:189–206.
- Ciffroy P, Moulin C, Gailhard J. A model simulating the transport of dissolved and particulate copper in the Seine river. *Ecol Model* 2000;127:99–117.
- D'Angelo DJ, Webster JR, Benfield EF. Mechanisms of stream phosphorus retention: an experimental study. *J North Am Benthol Soc* 1991;10:225–37.
- Doods WK. The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. *J Phycol* 2003;39:840–9.
- Eklholm P, Kallio K, Salo S, Pietiläinen OP, Rekolainen S, Laine Y, et al. Relationship between catchment characteristics and nutrient concentrations in an agricultural river system. *Water Res* 2000;15:3709–16.
- Foster IDL, Charlesworth SM. Heavy metals in the hydrological cycle: trends and explanation. *Hydrol Process* 1996;10:227–61.
- Genter RB. Ecotoxicology of inorganic chemical stress to algae. In: Stevenson RJ, Bothwell ML, Lowe RL, editors. Algal ecology freshwater benthic ecosystems. San Diego, CA: Academic Press; 1996. p. 403–68.
- Gong R, Ding Y, Liu H, Chen Q, Liu Z. Lead biosorption and desorption by intact and pretreated *Spirulina maxima* biomass. *Chemosphere* 2005;58:125–30.
- Gordon ND, McMahon TA, Finlayson BL. Stream hydrology: an introduction for ecologists. West Sussex, England: John Wiley and Sons; 1992.
- Grimm NB. Biogeochemistry of nitrogen in arid-land stream ecosystems. *J Ariz Nev Acad Sci* 1992;26:130–46.
- Grimm NB, Fisher SG, Minckley WL. Nitrogen and phosphorus dynamics in hot desert streams of southwestern USA. *Hydrobiologia* 1981;83:303–12.
- Guasch H, Paulsson M, Sabater S. Effect of copper on algal communities from oligotrophic calcareous streams. *J Phycol* 2002;38:241–8.
- Guasch H, Navarro E, Serra A, Sabater S. Phosphate limitation influences the sensitivity to copper in periphytic algae. *Freshw Biol* 2004;49:463–73.
- Hall J, Healey FP, Robinson GGC. The interaction of chronic copper toxicity with nutrient limitation in two chlorophytes in batch culture. *Aquat toxicol* 1989;14:1–14.
- Hall RO, Bernhardt ES, Likens GE. Relating nutrient uptake with transient storage in forested mountain streams. *Limnol Oceanogr* 2002;47:255–65.
- Hamdy AA. Biosorption of heavy metals by marine algae. *Curr Microbiol* 2000;41:232–8.
- Hill WR, Bednarek AT, Larsen IL. Cadmium sorption and toxicity in autotrophic biofilms. *Can J Fish Aquat Sci* 2000;57:530–7.
- Ho TY, Quigg A, Finkel ZV, Milligan AJ, Wyman K, Falkowski PG, et al. The elemental composition of some marine phytoplankton. *J Phycol* 2003;39:1145–59.
- Jackman AP, Kennedy VC, Bhatia N. Interparticle migration of metal cations in stream sediments as a factor in toxics transport. *J Hazard Mater* 2001;82:27–41.
- Jeffrey SW, Humphrey G. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem Physiol Pflanzen* 1975;167:191–4.
- Johnson VG, Peterson RE, Olsen KB. Heavy metal transport and behaviour in the lower Columbia river, USA. *Environ Monit Assess* 2005;110:271–89.
- Knauer K, Behra R, Sigg L. Adsorption and uptake of copper by the green alga *Scenedesmus subspicatus* (chlorophyta). *J Phycol* 1997;33:596–601.
- Martí E, Sabater F. High variability in temporal and spatial nutrient retention in Mediterranean streams. *Ecology* 1996;77:854–69.
- Martí E, Aumatell J, Godé L, Poch M, Sabater F. Nutrient retention efficiency in streams receiving inputs from wastewater treatment plants. *J Environ Qual* 2004;33:285–93.
- Martin JH, Bruland KW, Broenkow WW. Cadmium transport in the California Current. In: Windom HL, Duce RA, editors. Marine pollutant transfer. Heath; 1976. p. 159–84.
- Mehta SK, Gaur JP. Removal of Ni and Cu from single and binary metal solutions by free and immobilized *Chlorella vulgaris*. *Eur J Protistol* 2001;37:261–71.
- Mehta SK, Gaur JP. Use of algae for removing heavy metal ions from wastewater: progress and prospects. *Crit Rev Biotechnol* 2005;25:113–52.
- Meylan S, Behra R, Sigg L. Accumulation of copper and zinc in periphyton in response to dynamic variations of metal speciation in freshwater. *Environ Sci Technol* 2003;37:5204–12.
- Morel FMM, Hering JG. Principles and applications of aquatic chemistry. New York: John Wiley and Sons; 1993.
- Morin S, Duong TT, Herlory O, Feurtet-Mazel A, Coste M. Cadmium toxicity and bioaccumulation in freshwater biofilms. *Arch Environ Contam Toxicol* 2008;54:173–86.
- Mulholland PJ, Newbold JD, Elwood JW, Ferren LA, Webster JR. Phosphorus spiraling in woodland streams: seasonal variations. *Ecology* 1985;66:1012–23.
- Mulholland PJ, Steinman AD, Palumbo AV, Elwood JW, Kirschtel DB. Role of nutrient cycling and herbivory in regulating periphyton communities in laboratory streams. *Ecology* 1991;72:966–82.
- Mulholland PJ, Steinman AD, Marzolf ER, Hart DR, DeAngelis DL. Effect of periphyton on hydraulic characteristics and nutrient cycling in streams. *Oecologia* 1994;98:40–7.
- Munn NL, Meyer JL. Habitat-specific solute retention in two small streams: an intersite comparison. *Ecology* 1990;71:2069–82.

- Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 1962;27:31–6.
- Navarro E, Guasch H, Sabater S. Use of microbenthic algal communities on ecotoxicological tests for the assessment of water quality. *J Appl Phycol* 2002;14: 41–8.
- Newbold JD, Elwood JW, O'Neill RV, VanWinkle W. Measuring nutrient spiralling in streams. *Can J Fish Aquat Sci* 1981;38:860–3.
- Nies DH. Microbial heavy metal resistance. *Appl Microbiol Biotechnol* 1999;51:730–50.
- Owens PN, Walling DE, Carton J, Meharg AA, Wright J, Leeks GJL. Downstream changes in the transport and storage of sediment-associated contaminants_P, Cr and PCBs in agricultural and industrialized drainage basins. *Sci Total Environ* 2001;266:177–86.
- Peña-Castro JM, Martínez-Jerónimo F, Esperanza-García F, Cañizares-Villanueva RO. Heavy metals removal by the microalga *Scenedesmus incrassatulus* in continuous cultures. *Bioresour Technol* 2004;94:219–22.
- Pereira WE, Hostettler FD. Nonpoint source contamination of the Mississippi River and its tributaries by herbicides. *Environ Sci Technol* 1993;27:542–1552.
- Peterson BJ, Wollheim WM, Mulholland PJ, Webster JR, Meyer JL, Tank JL, et al. Control of nitrogen export from watersheds by headwater streams. *Science* 2001;292:86–90.
- Puigdomenech I. HYDRA/MEDUSA: make equilibrium diagrams using sophisticated algorithms. Royal Institute of Technology in Stockholm; <http://www.kemi.kth.se/inorg/medusa/> (latest database update January 2005). 2002.
- Runkel RL. Simulation models for conservative and nonconservative solute transport in streams. In: Osterkamp WR, editor. Effects of scale on interpretation and management of sediment and water quality, proceedings of the 1995 meeting of the International Association of Hydrological Sciences, Boulder, Colorado, July 3–14, vol. 226. IAHS publication; 1995. p. 153–9.
- Sabater F, Butturini A, Marti E, Munoz I, Romani A, Wray J, et al. Effects of riparian vegetation removal on nutrient retention in a Mediterranean stream. *J North Am Benthol Soc* 2000;19:609–20.
- Sabater S, Navarro E, Guasch H. Effects of copper on algal communities at different current velocities. *J Appl Phycol* 2002;14:391–8.
- Schreiber U, Gademann R, Bird P, Ralph PJ, Larkum AWD, Kühl M. Apparent light requirement for activation of photosynthesis upon rehydration of desiccated beachrock microbial mats. *J Phycol* 2002;38:125–34.
- StatSoft, Inc. STATISTICA for windows [Computer program manual]. Tulsa, OK: StatSoft, Inc., 2300 East 14th Street, Tulsa, OK 747104. 1999.
- Stream Solute Workshop. Concepts and methods for assessing solute dynamics in stream ecosystems. *J North Am Benthol Soc* 1990;9:95–119.
- Sunda WG, Huntsman SA. Processes regulating cellular metal accumulation and physiological effects: phytoplankton as model systems. *Sci Total Environ* 1998;219:165–81.
- Tate CM. Patterns and controls of nitrogen in tallgrass prairie streams. *Ecology* 1990;71: 2007–19.
- Triska FJ, Kennedy VC, Avanzino RJ, Reilly BN. Effect of simulated canopy cover on regulation of nitrate uptake and primary production by natural periphyton assemblages. In: Fontaine FD, Bartell SM, editors. Dynamics of lotic ecosystems. Ann Arbor, Michigan, USA: Ann Arbor Science; 1983. p. 129–60.
- Triska F, Pringle CM, Duff HH, Avanzino RJ, Zellweger G. Soluble reactive phosphorus (SRP) transport and retention in tropical, rain forest streams draining a volcanic landscape in Costa Rica: in situ SRP amendment to streams and laboratory studies. *Biogeochemistry* 2006;81:145–57.
- Valett HM, Morrice JA, Dahm CN, Campana ME. Parent lithology, surface-groundwater exchange, and nitrate retention in headwater streams. *Limnol Oceanogr* 1996;41:333–45.
- Vasconcelos MT, Leal MF. Seasonal variability in the kinetics of Cu, Pb, Cd and Hg accumulation by macroalgae. *Mar Chem* 2001;74:65–85.
- Vigneault B, Percot A, Lafleur M, Campbell PFC. Permeability changes in model and phytoplankton membranes in the presence of aquatic humic substances. *Environ Sci Technol* 2000;34:3907–13.
- Wang WX, Dei RCH. Effects of major nutrient additions on metal uptake in phytoplankton. *Environ Pollut* 2001;111:233–40.
- Webster JR, Patten BC. Effects of watershed perturbation on stream potassium and calcium dynamics. *Ecol Monogr* 1979;49:51–72.
- Webster JR, Valett HM. Solute dynamics. In: Hauer FR, Lamberti GA, editors. Methods in stream ecology. 2nd ed. San Diego: Academic Press; 2006. p. 169–85.