Size fractionation of phosphorus (dissolved, colloidal and particulate) in two tributaries to Lake Lugano

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Key words: Colloids, particles, cross-flow filtration, phosphorus, bioavailability

ABSTRACT

An in-situ fractionation mainly consisting of continuous flow centrifugation and cross-flow-ultra-filtration was used to separate a particulate (particle diameter > 1 µm), a colloidal (particle diameter < 1 µm and molecular weight > 10 kD) and a dissolved fraction (molecular weight < 10 kD) of total phosphorus in river water. A similar fractionation pattern is observed in function of discharge in two tributaries to Lake Lugano, the rivers Cassarate and Vedeggio. In Cassarate river 31 % of the total P is particulate, 10 % colloidal and 59 % dissolved at low discharge, whereas at high discharge 73 % is particulate, 10 % colloidal and 17 % dissolved. 19 % of the total P is particulate, 12 % colloidal and 69 % dissolved at low discharge in Vedeggio river, and at high discharge 19 % of the total P is dissolved, 8 % colloidal and 73 % particulate. Release of ortho-phosphate (o-P) from Cassarate and Vedeggio colloids was observed at pH 10, and by the enzyme alkaline phosphatase in laboratory experiments. Calculations of a model based annual P-load into Lake Lugano showed an average total P-distribution with 60 % particulate P, 10 % colloidal P and 30 % dissolved P in Cassarate and Vedeggio river for 1996 and 1997.

Introduction

Phosphorus concentrations in rivers and lakes had increased until the late 70s in Switzerland due to industrialization, population growth and intensive agricultural development. Increased P-inputs lead to eutrophication of many lakes (BUWAL, 1994; Gächter and Stadelmann, 1993; Vollenweider, 1968). The physico-chemical evolution of nutrients, phyto- and zooplankton in Lake Lugano has been investigated (Barbieri and Mosello, 1992; Imboden, 1992; Lachavanne et al., 1992; Lami et al., 1992; Lazzaretto et al., 1992; LSA, 1998; Müller and Meng, 1992; Polli and Simona, 1992; Zamboni et al., 1992) The water quality could be improved by the P-pro-

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hibition in detergents since 1985 and the development of sewage treatment plants (Baccini, 1985; Gächter and Wüest, 1992; Siegrist and Boller, 1996; Wehrli and Wüest, 1996). However, the P-inputs into Lake Lugano are still too high, which means that the trophic status does not improve (Barbieri and Mosello, 1992; LSA, 1998). Although some authors have assumed that colloidal P was an important transient within the P-cycle (Rigler, 1964; Jackson and Schindler, 1975; Lean, 1973; Paerl and Lean, 1976), no detailed study about colloidal P in freshwater exists. In this study the P-input from two main tributaries into Lake Lugano is characterized in detail with emphasis on the role of colloids and particles.

Colloids are small particles in the size range of 1 nm to 1 μm and are important reactants in the fate of trace metals, pollutants and nutrients (Buffle and van Leeuwen, 1992; Everett, 1988). Due to their dynamic behaviour and their small amount of mass, sampling of colloids from lakes and rivers is very challenging, and various methods have been used for fractionation (e.g., Hoffmann et al., 1981; Buffle et al. 1995a; Buesseler et al., 1996). The most widely used technique for sampling colloids is cross-flow filtration, with a prefiltration or centrifugation step to remove larger particles (Buesseler et al., 1996; Gustafsson and Gschwend, 1997; Lead et al., 1997). We chose here a combination of continuous centrifugation and cross-flow ultrafiltration for sampling and fractionation of river water. The main questions in our study were: How does the P-distribution among various size fractions vary with discharge? How are the P-inputs distributed among dissolved, colloidal and particulate fractions? Which mechanisms lead to an ortho-P-release from colloidal P?

Materials and methods

Sampling site

Lake Lugano is an alpine lake in the southern part of Switzerland near Italy. Due to its morphology it is divided into a northern (270 km²) and a southern basin (290 km²). Samples were collected from two tributaries, the river Cassarate for the Northern basin and the Vedeggio for the Southern basin (Fig. 1). Both sampling sites were close to the mouth and located at water-level stations, where the discharge has been measured continuously by the authorities of Cantone Ticino (LSA).

The mean annual discharge comprises 3.87 m³ s⁻¹ (1979–1993) for the river Vedeggio and 2.57 m³ s⁻¹ (1963–1993) for the river Cassarate (LSA, 1996). Typical for both river regimes are two high flood events, one occurring in the late spring (May/June) and the other later in autumn (Oct/Nov). The discharge maxima are about 20–40 times higher than the mean annual discharge in Vedeggio and Cassarate.

Due to the crystalline background both rivers have at average discharge low alkalinity (Cassarate 1.0–1.4 mM, Vedeggio 0.8–1.4 mM), low Ca-concentrations (Cassarate 0.39–0.65 mM and Vedeggio 0.48–0.70 mM) and low ionic strength (Cassarate 3×10^{-3} M; Vedeggio 4×10^{-3} M). pH is 7.4–7.9 in Cassarate river and 7.0–7.6 in Vedeggio river. Conductivity varies with discharge, under low discharge

Figure 1. Map of Lake Lugano (Adapted from Karagounis, 1992)

it comprises between $200\text{--}400\ \mu\text{S cm}^{-1}$ in Cassarate and $240\text{--}500\ \mu\text{S cm}^{-1}$ in Veduggio and under high discharge $80\text{--}100\ \mu\text{S cm}^{-1}$ and $50\text{--}100\ \mu\text{S cm}^{-1}$ respectively.

In-situ fractionation

A separation of river water into three operationally defined size classes, a particulate, a colloidal and a dissolved fraction was performed with an in-situ fractionation device consisting of a continuous-flow centrifugation and a cross-flow ultrafiltration. The particulate fraction is defined as particles, which have a diameter $> 1\ \mu\text{m}$. Colloids are defined as submicron particles or macromolecules which have a diameter $< 1\ \mu\text{m}$ and a molecular weight $> 10\ \text{kD}$. The dissolved fraction (ultrafiltrate) is defined as the size range of molecules and ions which pass a $10\ \text{kD}$ membrane. The distinction between the colloidal and the dissolved fraction is operationally defined according to the cross-flow ultrafiltration device used and to the theoretical cut-off of $10\ \text{kD}$.

Sampling was performed at least at $1\ \text{m}$ distance from the bank at a water level approximately 25% of the depth below the surface. The river water was sampled continuously with a peristaltic pump and directly centrifuged. The centrifuge (LE 402-18091, CEPA) was operated at $25000\ \text{rpm}$ with a flow rate of $1.2\ \text{l min}^{-1}$. SEM pictures and particle size analysis of the outlet from the centrifuge (not shown) defined a cut-off range of about $1\ \mu\text{m}$ as a function of the morphology and density of the particles. Particles $> 1\ \mu\text{m}$ were collected on a teflon sheet inside the centrifuge. The inlet port was made of teflon, all tubes consisted of either silicone or teflon, the retentate vessel and connecting pieces consisted of polypropylene (PPE). The outlet of the centrifuge containing the colloids and the dissolved fraction was

directly filtered with a cross-flow ultrafiltration (Filtron and Watson Marlor 701 S) equipped with two 10 kD low protein binding modified polyethersulfone membranes (Omega, 5 SQ Feet Filtron). Concentration factors for the colloidal retentate ranged between 3 and 130 depending on the river discharge. The flow rate of the ultrafiltrate ranged between 0.6–0.9 l min⁻¹.

The size distribution in the colloidal and ultrafiltrate fraction was examined by dynamic light scattering (Schurtenberger and Newman, 1993). Smooth particle size distributions in the range 5–1000 nm were observed in the colloidal fraction, with calculated mean radii of about 200 nm (Hilger, 1998). No particles larger than 5 nm were detected in the ultrafiltrate.

The cross-flow ultrafiltration was cleaned with 0.05 M NaOH suprapure for 15 min prior to use and after each filtration. The membrane was stored in 0.05 M NaOH suprapure at 4°C in the dark. After cleaning with NaOH the cross-flow ultrafiltration was rinsed with nanopure water until neutral pH was reached (10–20 l). To minimize sorption losses on tubes and vessels, 10 l centrifuged river water were also used to rinse the CFF prior to filtration. The in-situ fractionation was checked with nanopure water for blanks. All P-concentrations were below the detection limit of 0.1 µM. DOC-concentrations were < 25 µM after the centrifugation step, 25–33 µM in the ultrafiltrate and 75 µM in the retentate.

A smaller CFF (Mini-Ultrasette, Filtron, screen channel, Omega membrane, cut-off 10 kD) was used for parallel sampling from both rivers during high flood events and also for laboratory experiments, where three Mini-Ultrasettes were run in parallel with a peristaltic pump (504U SKAN AG- Smith & Nephew Watson Marlow; Masterflex model 7554-50; Ismatec SA Typ VP 46-0084). The pump speed was adjusted to an ultrafiltrate flow rate of 300 ml min⁻¹. Blanks run with the Mini-Ultrasette showed no P- and DOC-contamination both in the retentate and in the ultrafiltrate; P-concentrations were < 0.1 µM and DOC-concentrations were < 25 µM respectively. The Mini-Ultrasettes were cleaned in the same way as the CFF and stored after the NaOH-cleaning at 4°C.

Our OC-blanks are in the same order of magnitude as those reported in other studies, e.g. 25 to 33 µM C (Eyrolle et al., 1996), and < 10 µM to a maximum value of 200 µM in an intercomparison study by Buessler et al. (1996), in which 14 cross-flow filtration systems were tested, but not all systems had been optimized with respect to C-blanks. The P-blanks for the in-situ fractionation were all below the detection limit of 0.1 µM. A filtration test with KH₂PO₄-standard solution showed a deviation at the most of 19% which is comparable to the mass balance results of the in-situ fractionation with river water; no significant accumulation of o-P in the retentate was observed.

P and OC-mass balances

Mass balances for the in-situ fractionation were calculated as the sum of the concentrations in the particulate, colloidal and dissolved fraction, which were compared with the mean value of two untreated river water samples taken at the beginning and the end of each sampling campaign.

$$P_{\text{tot}} = P_{\text{part}} + P_{\text{coll}} + P_{\text{diss}}$$

$$P_{\text{part}} = \text{SS} \times \text{PP},$$

where

$$P_{\text{tot}} = \text{total P concentration } [\mu\text{M}]$$

$$P_{\text{part}} = \text{particulate P concentration } [\mu\text{M}]$$

$$P_{\text{coll}} = \text{colloidal P concentration } [\mu\text{M}]$$

$$P_{\text{diss}} = \text{dissolved P concentration } [\mu\text{M}]$$

$$\text{SS} = \text{suspended solid content } [\text{g l}^{-1}]$$

$$\text{PP} = \text{P content in particles } [\mu\text{mol g}^{-1}]$$

P_{coll} is calculated as:

$$P_{\text{coll}} = (P_{\text{tot ret}} - P_{\text{tot diss}})/\text{cf}$$

where:

$$P_{\text{tot ret}} = \text{total P concentration in retentate } [\mu\text{M}]$$

$$P_{\text{tot diss}} = \text{total P concentration in ultrafiltrate } [\mu\text{M}]$$

cf = concentration factor, calculated from the total volume filtered divided by the final retentate volume.

Calculation of the P-distribution in the annual load

The mean results of the fractionation under low and high discharge were combined with the data of a model from LSA. This model calculates monthly loads based on a discharge-concentration relation. Input data are total and dissolved (0.45- μm filtered) P-measurements based on weekly and fortnightly sampling (LSA, 1998). Months with a P-load < 8% of the annual P-load were summarized and multiplied with the proportionate P-fractionation under low discharge. Months with a P-load > 8% of the annual P-load were multiplied with the proportionate P-fractionation under high discharge.

Release of o-P from the colloidal fraction

The release of o-P from colloids, which is supposed to be directly utilisable for algae and bacteria, was examined in laboratory experiments. The influence of increased pH up to 10.2 in a colloidal river suspension was examined over time. Higher pH values (pH 10) than those encountered in lakes were used in some of the experiments to better evaluate the effects of alkaline conditions.

In three replicates of 250 ml colloidal suspension pH was stepwise adjusted to 8.3, 9.0 and 10.0–10.2 with 0.1 M NaOH suprapure under nitrogen gas atmosphere. After 15 min 5 ml aliquots were filtered with a 0.2 μm syringe filter (Sartorius Minisart-plus 178 23 K) into two 2 ml vials for direct o-P-measurements with an Autoanalyzer. Blanks were done with nanopure water to test the reagents and the o-P-concentration was followed over time in colloidal suspensions at the original pH of 7.8.

In another set of experiments, the effect of an enzyme, alkaline phosphatase derived from *Escherichia coli* (SIGMA P 4252, Type III), was investigated to release o-P from colloids. These experiments were run at 20 and 30°C. A temperature of 30°C is close to the optimum temperature of this enzyme, whereas 20°C corresponds to the summer temperatures (18–22°C) in the epilimnion of L. Lugano. Cross-flow ultrafiltration of the colloidal suspensions was performed using three Mini-Ultrasettes, with 10 kD membranes, through which the alkaline phosphatase with a molecular weight of about 160000 (Bretaudiere and Spillman, 1984) could not pass. After addition of the alkaline phosphatase prepared in a 0.1 M Tris-HCl/0.1 M NaCl-buffer solution to 150–250 ml colloidal suspension in Erlenmeyer flasks the o-P-concentration was followed over time in the ultrafiltrate. Enzyme preparation was done under sterile conditions, and enzymatic activity was tested with a standard method (E.C. 3.1.3.1). All glassware was presoaked in 0.01 M HNO₃ suprapure for 24 h prior to the experiments. As a control the o-P concentration in the ultrafiltrate was followed over time after adding the same amount of tris-buffer-solution as in the experiments without alkaline phosphatase.

Analytical methods

All samples were transported and stored at 4°C in the dark and analyzed within 48 h after sampling. Particles were lyophilized and digested in a microwave oven (Microwave Laboratory Systems, mls 1200 mega) with nitric acid (suprapure) and hydrogen peroxide prior to element analysis. Aliquots from colloidal retentate suspension and ultrafiltrate were digested in the same way prior to metal analyses. The total particulate phosphorus was analysed with a spectrophotometer using the molybdenum blue method (APHA et al., 1995). The standard reference material 2704 Buffalo River Sediment (U.S. Department of Commerce National Institute of Standards and Technology Gaithersburg, MD 20899) was used as reference material for P and metal analyses. Aliquots from untreated river water, colloidal retentate and ultrafiltrate were digested with the K₂S₂O₈-method and measured as ortho-phosphate (o-P) with the ascorbate-acid method with an autoanalyser (Technicon Autoanalyzer II and Technicon SC Colorimeter) at a wavelength of 880 nm (APHA et al., 1995). Sampling devices for metal analyses were pre-soaked with 0.01 M HNO₃. Ca, Mg, Fe and Mn were measured after digestion with HNO₃/H₂O₂ in a microwave with ICP-OES (Spectro Analytical instruments). Samples for total and dissolved organic carbon were directly acidified with HCl (pH 1) in the field. Total organic carbon (TOC) was homogenized prior to analysis. TOC, colloidal organic carbon (COC) and dissolved organic carbon (DOC) were measured with a carbon analyzer (Shimadzu Total Organic Carbon Analyzer TOC 500, ASI 502). The standard deviation for TOC-analyses ranged from 5 to 11 % with two replicates and 5 injections each. Particulate organic carbon (POC) was determined with a CHNS element analyzer in lyophilized particles (Vario EL; Elementare Analysensysteme GmbH Hanau, Germany). The suspended solid (SS) content of river water samples was determined gravimetrically. Prewashed and weighted Sartorius cellulose nitrate filters (11306-50 N) with a pore size of 0.45 µm were used for the vacuum filtration of the suspended solids. Standard deviation varied between

0–25 % ($n = 2$), for SS-content $> 5 \text{ mg l}^{-1}$, the standard deviation is $< 5 \%$. Conductivity and temperature were directly measured in the field with a Teso 240 conductivity meter. A WTW pH 323-A/set 1 pH-meter was used for direct pH-measurements. Alkalinity was determined with a Metrohm 670 automatic titrator in filtered samples.

Results

In-situ fractionation

The P- and OC-distribution over the size fractions show a clear dependence from the discharge in both rivers (Tables 1 and 2). Under low discharge the mean total P-concentration is $1.2 \text{ }\mu\text{M}$ in Cassarate river ($n = 10$). 31% of the total P is particulate, 10% colloidal and 59% dissolved. Under high discharge, the mean total P-concentration is $5.4 \text{ }\mu\text{M}$, the total P is dominated by the particulate fraction, which comprises 73% of total P, 10% is colloidal P and 17% is dissolved P (for detailed information see Table 1). The Vedeggio river shows a similar fractionation pattern: Under low discharge, the total mean P-concentration is $1.5 \text{ }\mu\text{M}$, 19% of the total P is particulate, 12% colloidal and 69% dissolved ($n = 8$). Under high discharge, the mean total P-concentration is $5.9 \text{ }\mu\text{M}$ ($n = 6$), 19% of the total P is dissolved, 8% is colloidal and 73% is particulate (for detailed information see Table 2).

The OC-concentrations in the three defined fractions show a similar behaviour in dependence from the discharge, where the particulate and dissolved fraction are strongly influenced by the discharge. Under low discharge conditions, the dissolved ultrafiltered organic carbon (UF-DOC) and colloidal organic carbon (COC) comprise between 80 and 95% of the TOC, with 1–17% being colloidal OC. Under higher discharge, the UF-DOC comprises between 19–54% of the TOC, the COC between 4–16% and the particulate organic carbon (POC) 41–79%. Tables 3 and 4 show the OC-distribution over size fractions of Cassarate and Vedeggio river samples and the mass balances of the in-situ fractionation. In general, the sum of the POC, COC and UF-DOC from the in-situ fractionation comprise $116 \pm 13 \%$ of the TOC ($n = 10$) compared with the measured TOC in an unfractionated Cassarate sample, taken as a reference value. Additionally the sum of the COC and UF-DOC was compared with measurements of DOC in a directly in the field $0.45\text{-}\mu\text{m}$ -filtered sample. The sum of ultrafiltered DOC and COC comprise $99 \pm 18 \%$ ($n = 8$) of the DOC in the $0.45\text{-}\mu\text{m}$ -filtered Cassarate river sample. In Vedeggio river, the sum of UF-DOC, COC and POC comprises $114 \pm 12 \%$ ($n = 7$) of the TOC of a unfractionated reference river sample. Some samples at high discharge (nr. 12–14, Table 4) show much higher discrepancies between the sum of the size fractions and the measured TOC, which are due to TOC losses by sedimentation in samples with high particle concentrations. The sum of ultrafiltered DOC and COC also shows a reasonable mass balance comprising $100 \pm 14 \%$ ($n = 9$) of the DOC of a $0.45 \text{ }\mu\text{m}$ -filtered river sample.

Table 1. Particulate, colloidal and dissolved P and P-mass balances in Cassarate river

Nr.	Date	Time [central Europ. time]	Q [m ³ s ⁻¹]	Water temp. [°C]	SS [mg l ⁻¹]	Cond. [μS cm ⁻¹]	Total P-concentrations [μM]						% Σ of T
							cf	p ^a	c ^b	d ^c	Σ ^d	T ^e	
1	13.06.95	14.30–21.00	9.2	12.0	18.4	227	100	0.6	0.04	0.9	1.6	1.3	125
2	05.09.95	09.00–11.00	0.6	11.9–12.1	3.1	349–351	52	0.2	0.08	0.4	0.7	0.9	82
3	05.09.95	12.00–13.30	0.6	14.2–13.3	3.9	402–347	37	0.4	0.19	0.4	1.0	1.0	99
4	05.09.95	16.10–17.10	0.5	14.4–14.9	3.6	341–374	31	0.4	0.20	0.5	1.1	1.1	102
5	05.09.95	19.00–20.10	0.6	14.8–14.7	3.5	405–409	40	0.3	0.19	0.5	1.0	1.1	94
6	09.05.96	11.00–13.30	3.8–3.9	10.8	16.2	230	63	0.9	0.10	0.7	1.7	1.8	93
7	24.07.96	09.15–14.30	1.7	15.0	5.8	264	67	0.3	0.06	1.0	1.3	1.5	88
8	14.10.96	14.00–16.00	1.1–0.9	11.8	1.4	108–115	14	0.2	0.15	1.0	1.3	1.3	102
9	15.10.96	08.40–10.00	4.6–5.3	12.1–11.9	135	83	3	8.3	1.88	2.2	12.4	7.5	165
10	15.10.96	13.00–14.00	7.4	11.9–11.6	95	88–92	3	5.2	0.85	1.2	7.2	5.9	121
11	04.06.97	09.00–12.35	0.6	12.1–14.5	5.0	112–98	40	0.3	0.04	0.6	1.0	1.1	88
12	05.06.97	09.15–10.30	3.9	nd	59	nd	14	4.5	0.40	1.7	6.6	10.7	62
13	05.06.97	10.40–12.00	3.3	13.0	37	89	19	3.1	0.41	1.1	4.6	5.7	80
14	05.06.97	12.40–16.00	6.6	14.0	210	96	34	9.4	0.15	1.2	10.7	9.5	113
15	05.06.97	19.10–23.00	3.6	14.1	53	95	31	5.0	0.15	1.1	6.2	2.7	225
16	06.06.97	11.00–15.00	3.1	13.5	6.2	100	42	0.4	0.03	0.6	1.0	0.8	125
17	01.10.97	11.30–17.30	0.5	16.0	5.1	175	40	0.7	0.10	2.0	2.9	2.6	109
18	20.11.97	15.15–17.00	0.4	6.0	2.7	198	28	0.4	0.09	0.5	1.0	0.92	106

^a p = particulate; ^b c = colloidal; ^c d = dissolved;
^d Σ = Sum of the particulate, colloidal and dissolved total phosphorus;
^e T = mean value of total P in untreated river water sampled at beginning and end of each sampling.
nd = not determined; SS = suspended solids; cf = concentration factor.

Table 2. Particulate, colloidal and dissolved P and P-mass balances in Vedeggio river

Nr.	Date	Time [central Europ. time]	Q [m ³ s ⁻¹]	Water temp. [°C]	SS [mg l ⁻¹]	Cond. [μS cm ⁻¹]	Total P-concentrations [μM]					% Σ of T	
							cf	p ^a	c ^b	d ^c	Σ ^d		T ^e
1	14.06.95	09.20–15.00	9.7	11.9	3.0	224	130	0.2	0.1	0.5	0.8	0.6	137
2	06.09.95	07.50–08.55	1.2	15.9–16.7	3.4	457–505	55	0.5	0.3	2.6	3.3	2.9	116
3	06.09.95	11.00–11.55	1.2	17.5	3.4	490	38	0.4	0.3	2.2	2.9	2.6	112
4	06.09.95	14.00–15.00	1.2	18.7–19.1	3.7	498	40	0.4	0.4	2.2	3.0	1.9	158
5	12.02.96	16.00–18.05	2.2	6.1	0.8	260	42	0.1	0.2	0.5	0.8	1.0	84
6	08.05.96	10.15–12.00	4.4	13.4	2.2	240	44	0.2	0.1	0.5	0.8	0.8	100
7	08.05.96	21.20–21.55	5.2	nd	24	283	7	1.7	0.7	0.8	3.2	3.3	98
8	23.07.96	15.40–17.45	2.0	20.8–20.4	1.2	458–480	50	0.2	0.1	1.0	1.4	1.2	116
9	14.10.96	15.15–18.00	1.8	nd	0.6	284	67	0.3	0.1	0.6	0.9	1.1	85
10	15.10.96	09.30–11.40	8.7–10.5	nd	43	192	21	6.3	0.5	2.0	8.8	8.1	109
11	15.10.96	11.30–12.40	10.8–13.1	nd	36	146	27	5.0	1.0	3.6	9.6	10.4	92
12	13.11.96	09.00–11.00	22.0	nd	43	nd	20	3.4	0.2	0.7	4.3	2.9	147
13	13.11.96	12.00–14.00	26.0	11.5	97	48	24	6.0	0.1	0.6	6.7	5.0	134
14	13.11.96	15.10–17.00	30.0	nd	138	nd	14	10.8	0.2	0.4	11.4	5.5	207
15	20.11.97	11.15–12.35	3.1	10.7	1.5	236	36	0.3	0.1	0.6	0.9	0.7	128

^a p = particulate; ^b c = colloidal; ^c d = dissolved;
^d Σ = Sum of the particulate, colloidal and dissolved total phosphorus;
^e T = mean value of total P in untreated river water sampled at beginning and end of each sampling.
nd = not determined; SS = suspended solids; cf = concentration factor.

Table 3. Particulate, colloidal and dissolved organic carbon in Cassarate river. Colloidal OC-mass-balances refer to untreated water sample and to 0.45 µm filtrate

Nr.	date	TOC [µM]							% (c+d) of filt.	% Σ of T
		p ^a	c ^b	d ^c	c + d	filt.	Σ ^d	T ^e		
1	13.06.95	75	6	133	140	136	215	129	103	166
2	05.09.95	22	13	153	166	134	188	210	124	90
3	05.09.95	39	33	171	203	203	242	242	100	100
4	05.09.95	35	20	108	127	110	162	110	115	148
5	05.09.95	36	17	127	145	nd	180	153	nd	118
6	09.05.96	69	9	92	101	142	170	133	71	127
7	24.07.96	41	7	108	111	150	157	150	74	104
8	14.10.96	11	31	140	171	nd	182	148	nd	123
9	15.10.96	581	77	141	218	nd	799	622	nd	128
10	15.10.96	664	157	189	218	210	1010	808	104	125
11	04.06.97	23	7	83	90	nd	113	175	nd	65
15	05.06.97	616	18	150	168	nd	784	462	nd	170
18	01.10.97	74	13	103	105	108	190	154	97	123
19	20.11.97	27	15	50	65	nd	93	77	nd	120

^a p = particulate; ^b c = colloidal; ^c d = dissolved.
^d Σ = Sum of the particulate, colloidal and dissolved organic carbon;
^e T = TOC in untreated water sample.
nd = not determined; filt = 0.45 µm filtered.

Table 4. Particulate, colloidal and dissolved organic carbon in Vedeggio river. Colloidal OC-mass-balances refer to untreated water sample and to 0.45 µm filtrate

Nr.	Sampling date	TOC [µM]							% (c+d) of filt.	% Σ of T
		p ^a	c ^b	d ^c	c + d	filt.	Σ ^d	T ^e		
1	14.06.95*	28	7	178	185	178	213	176	104	121
2	06.09.95	38	33	198	231	242	270	212	95	127
3	06.09.95	35	31	242	274	244	308	290	112	106
4	06.09.95	42	38	197	235	191	278	210	123	132
6	08.05.96	320	70	125	195	233	515	483	83	107
8	23.07.96	nd	12	125	137	158	nd	nd	86	nd
10	15.10.96*	637	37	182	220	nd	857	693	nd	124
11	15.10.96*	474	56	157	212	nd	686	728	nd	94
12	13.11.96*	469	43	117	159	142	629	191	113	328
13	13.11.96*	1140	23	134	157	191	1297	283	82	458
14	13.11.96*	1621	61	125	185	183	1807	283	101	638
15	20.11.97	12	14	118	132	nd	144	119	nd	122

* = sampling under high discharge.
^a p = particulate; ^b c = colloidal; ^c d = dissolved;
^d Σ = Sum of the particulate, colloidal and dissolved organic carbon;
^e T = TOC in untreated water sample.
nd = not determined; filt = 0.45 µm filtered.

Calculated annual P-loads and depth of intrusion

The results of the calculated annual P-load with respect to the fractionation for the years 1996 and 1997 are listed in Tables 5 and 6. In general, about 60 % of the annual P-load is particulate, ca. 10% is colloidal and 30% is dissolved. The depth of intrusion was calculated as the depth, where the density of the river water is equal to that of the lake water (Wüest, 1996). Temperature and conductivity data profiles from the southern and northern basin of Lake Lugano were taken into account with the water temperature, the conductivity and the suspended solids content and the particle density of the time corresponding river water samples. The calculation showed that the depth of intrusion generally is in the range of the metalimnion to the epilimnion.

o-P-release from colloidal P

Release of o-P was observed from both river colloidal suspensions at pH 10.0–10.2 (Figs. 2 and 3). An increase of the pH of a colloidal suspension from Cassarate to 8.3 and 9.0 did not show any o-P-release from Cassarate colloids within 15 min. As shown in Figure 2, at a pH of 10.0–10.2, a significant o-P release takes place within hours. After 24 h, the o-P concentration is about 2.6 times higher compared to the original o-P concentration at a pH of 7.9. Neither the control of the reagents nor the control of the colloidal suspension at the original pH showed a significant increase of o-P within 24 h. o-P-concentrations varied within a standard deviation of $\pm 5\%$. Within 4 h, the same amount of colloidal P, about 4–5 %, was released as o-P from both river colloids. Figure 3 shows the increase of o-P concentration in Vedeggio colloids at a pH of 10.2.

Table 5. Calculated annual P-load from Cassarate River to Lake Lugano

Low discharge	Average P-distribution %		1996 P-input [t P a ⁻¹]	1997 P-input [t P a ⁻¹]
dissolved	59		1.6	0.7
colloidal	10		0.3	0.1
particulate	31		0.9	0.3
High discharge				
dissolved	17		0.8	1.0
colloidal	10		0.5	0.6
particulate	73		3.5	4.3
Annual average	1996	1997	1996	1997
dissolved	32	24	2.4	1.7
colloidal	10	10	0.75	0.7
particulate	58	66	4.35	4.6
total annual P-load [t P a ⁻¹]			7.5	7.0

Table 6. Calculated annual P-load from Vedeggio River to Lake Lugano

Low discharge	Average P-distribution %		1996 P-input [t P a ⁻¹]	1997 P-input [t P a ⁻¹]
dissolved	68		1.0	1.2
colloidal	12		0.2	0.3
particulate	19		0.3	0.3
High discharge				
dissolved	19		0.7	1.3
colloidal	8		0.3	0.5
particulate	73		2.8	4.8
Annual average	1996	1997	1996	1997
dissolved	30	30	1.6	2.5
colloidal	8	9	0.4	0.8
particulate	62	61	3.3	5.1
total annual P-load [t P a ⁻¹]			5.3	8.4

Figure 2. o-P-release at a pH of 10 (filled squares) and 10.2 (filled triangles) from Cassarate colloids. Means and standard deviation of the o-P-concentrations in 0.2 μm filtered samples are shown (n = 3) at 20 °C. Control at pH 10 is the o-P-concentration in demineralized water at pH 10 and under nitrogen gas atmosphere (open squares). The o-P-concentration in 0.2 μm-filtered colloidal retentate at the original pH of 7.9 is also shown (open triangles)

Release of o-P from both river colloids in the presence of alkaline phosphatase is shown in Figures 4a and b. The results with low enzyme concentrations of 3 and 20 u l⁻¹ did not show a clear trend in the o-P release. This might be due to enzyme flocculation or an enzyme inhibition through o-P (Olsson, 1988). However, with higher enzyme concentrations of 114 and 230 u l⁻¹ the o-P concentration in the ultrafiltrate nearly doubled after 1 h. At the most 17–19% of the colloidal P was released as o-P with 230 u l⁻¹ enzyme concentration from both river colloids. The control showed a constant o-P concentration within 5 h with a standard deviation of ± 2.7% (n = 2). Measurements of the enzyme activity in the colloidal suspension

Figure 3. o-P-release at a pH of 10.2 from Vedeggio colloids. Means and standard deviation of the o-P-concentrations in 0.2 μm filtered samples are shown (n = 3) at 20°C (filled triangles). Control shows the o-P-concentration in 0.2 μm-filtered colloidal retentate at the original pH of 7.9 (open circles)

Figure 4. Enzymatic o-P release from colloids with alkaline phosphatase. Means and standard deviations are shown (n = 2). Controls shown are o-P-concentration in the ultrafiltrate from Cassarate and Vedeggio colloids without alkaline phosphatase. **a** o-P concentration in the ultrafiltrate from Cassarate colloids (3 days after sampling) at 30°C, with 114 or 230 u l⁻¹ alkaline phosphatase added. **b** o-P concentration in the ultrafiltrate from Vedeggio colloids (1 and 2 days after sampling) at 30°C, with 230 u l⁻¹ alkaline phosphatase added

showed an activity of only 4% of the enzyme specific activity value in the experiments with low enzyme concentrations. In the experiments with 114 u l⁻¹, the activity was 26 ± 4% and in the experiments with 230 u l⁻¹, the enzyme activity comprised 52 ± 10%.

Discussion

In-situ fractionation

A similar dependence of the P-concentrations and P size distributions on discharge was observed in both rivers. No seasonal effects were observed. The distribution of dissolved and particulate P is strongly influenced by the discharge. Under high discharge, the particulate fraction is dominant, whereas under low discharge the dissolved fraction is the dominating one.

The mass balance approach showed good results with a mean of 100% and a standard deviation of 14–20%. Only few examples showed a larger difference, which can be explained with the uncertainty of the analysis of the reference samples taken at the beginning and the end of each sampling. Variations in the suspended solid content strongly influence the mass balance and also the total P-content in the unfractionated reference sample. Bauer et al. (1996) showed that the P-concentration in the ultrafiltrate did not vary over filtration time. We also assumed that the P-concentration in the ultrafiltrate stays constant over the filtration time.

An important question is the source of colloidal and particulate P, as both concentrations increase with raising discharge. Due to the morphology of both catchment areas, soil erosion is one important diffuse source for the increasing particle and colloid input under storm events. Resuspension of river sediment is another important source for particles and colloids. After longer dry periods, the wash out from sewers lead to an high P-input, which mostly consists of colloids and particles. Another P-source is the sewage treatment plant in Bioggio where incompletely treated waste water, bulking sludge and scumming sludge entered the Vedeggio river during heavy rain fall events lasting more than 2 days.

Interestingly, the colloidal P-fraction shows an average amount of 10% of total P independently of discharge, while the dissolved fraction is dominating under low discharge and the particulate one dominates under high discharge conditions. This leads to the conclusion that colloidal P-pools from either point or diffuse sources must become active with rain and increasing discharge, because the colloidal P-concentration increases under high discharge. However, with an increasing number of colloids per volume, aggregation is strongly enhanced, which would lead to a relative loss of colloidal P. On the other hand, the conductivity diminishes by about 2–8 times. Therefore, the lower ionic strength increases the colloidal stability.

Our results can be compared with only few other studies about colloidal P in aquatic systems. Bauer et al. (1996) defined the colloidal fraction in the size range of $< 0.2 \mu\text{m}$ membrane filtered and $> 1 \text{ kD}$ cross-flow-filtered. The colloidal P-fraction of different seawater samples made 1 to 20% of the total P (1–3 $\mu\text{M P}$), which is in agreement with the results of this work. A wider range for the colloidal P fraction of 0–48% in creek water samples was reported by Mayer and Jarrell (1995). They define the colloidal fraction as the difference between 1.0- μm - and 0.05- μm -membrane filtered samples.

C-fractionations and mass-balances

The OC-distribution of the different fractions are in agreement with those of the P-distribution. The particulate and dissolved, ultrafiltered OC are also strongly dependent from discharge. The results of the COC-fractions (comprising 1–17% of TOC) are in good agreement with the results of Rostad et al. (1997), who found between 1.1–17% of COC in the Mississippi River and its tributaries. Buesseler et al. (1996) showed with an intercomparison study that there are huge differences of 4–67% of COC between various manufacturers of cross-flow filtration systems. However, good results for OC with a reproducibility of 91–106% using a cross-flow filtration with a 10 kD cut-off and 1 kD with different seawater samples and total

DOC and COC-concentrations of 50–250 μM C were observed by Whitehouse et al. (1990) and Guo and Santschi (1996). In a colloidal size range between 0.4 μm and 10 kD 82–98% of the 0.4 μm DOC were in the colloidal fraction in samples of the Venice Lagoon (Martin et al., 1995). Most workers do not take into account the particulate organic fraction in the mass balance approach, but only compare the sum of colloidal OC and ultrafiltered DOC with a reference sample, which has been membrane filtered (mostly 0.45 μm). Our comparison of the sum of COC and ultrafiltered DOC also shows reasonable agreement with a 0.45- μm filtered water sample with a standard deviation of 25 %, which is comparable to the cited authors.

Availability of colloidal P in Lake Lugano

Calculation of the size distribution of the average annual P-loads showed that approximately 60% of the total P-input is particulate, 10% colloidal and 30% dissolved. Most of the dissolved P is o-P which is directly usable for bacteria and algae growth (Boström et al., 1988). As colloids have a lower density and a smaller diameter than particles, their residence times in the epilimnion or in the water column may be quite long. As the calculated depth of intrusion lays within the productive zone, colloids can be considered as a potentially available P-pool. A significant amount of o-P release from river colloids could be observed at increased pH of 10.0–10.2 within few hours, and also in the presence of alkaline phosphatase, which is excreted by algae and bacteria (Francko and Heath, 1979; Olsson, 1988; Yiyong, 1996). pH in the epilimnion of Lake Lugano reaches values between 8.7 (June/July) and 9.4 (August), whereas the pH of both rivers are between 7.0 and 7.9 (LSA, 1996). Therefore the hydrolysis of organic phosphorus-esters could be a mechanism for the o-P-supply under o-P-deficiency, especially when o-P is depleted from the euphotic layers in summer. The availability of P from the particulate fraction is less likely to play a relevant role, since the particles are quickly transported to the deeper water layers. In contrast, the colloidal P-fraction with a longer residence time in the euphotic layers may contribute to the available P.

ACKNOWLEDGEMENTS

We thank David Kistler, Daniel Kobler, René Schönenberger, Adrian Amman, Hans-Ruedi Zweifel for field work. Thomas Rüttimann and Antonin Mares are acknowledged for the TOC- and POC-analyses. We also thank Christoph Mattenberger for calculation support. The authorities of Cantone Ticino are acknowledged for data exchange and field support. This work was supported by the Swiss National Science Foundation, within the SPPU program.

REFERENCES

- APHA, AWWA and WEF, 1995. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington.
- Baccini, P., 1985. Einschränkung des Phosphatverbrauches – Ein Beitrag zum Gewässerschutz. GWA, 1: 1–6.
- Barbieri, A. and R. Mosello, 1992. Chemistry and trophic evolution of Lake Lugano in relation to nutrient budget. Aquat. sci. 54(3–4): 219–237.

- Bauer, J.E., K.C. Ruttenberg, D.M. Wolgast, E. Monaghan and M.K. Schrope, 1996. Cross flow filtration of dissolved and colloidal nitrogen and phosphorus in seawater: results from an intercomparison study. *Mar. Chem.* 55(1-2): 33-52.
- Boström, B., G. Persson and B. Broberg, 1988. Bioavailability of different phosphorus forms in freshwater systems. *Hydrobiologia* 170: 133-155.
- Bretaudiere, J.P. and T. Spillman, 1984. *Methods of Enzymatic Analysis – Enzymes 2: Esterases, Glycosidases, Lyases, Ligases*, IV. Verlag Chemie, Weinheim, Basel.
- Buesseler, K.O., J.E. Bauer, R.F. Chen, T.I. Eglinton, O. Gustafsson, W. Landing, K. Mopper, S.B. Moran, P.H. Santschi, R. Vernonclark and M.L. Wells, 1996. An intercomparison of cross flow filtration techniques used for sampling marine colloids: overview and organic carbon results. *Mar. Chem.* 55(1-2): 1-31.
- Buffle, J. and G.G. Leppard, 1995. Characterization of Aquatic Colloids and Macromolecules. 1. Structure and Behaviour of Colloidal Material. *Environ. Sci. Technol.* 29(9): 2169-2175.
- Buffle, J. and H.P. van Leeuwen, 1992. *Environmental particles. Environmental analytical and physical chemistry series*. Lewis Publishers, Boca Raton/FL, USA.
- BUWAL, 1994. *Der Zustand der Schweizer Seen*, Bern.
- Everett, D.H., 1988. *Basic principles of colloid chemistry*. Royal Society of Chemistry, London.
- Eyrolle, F., M.F. Benedetti, J.Y. Benaim and D. Fevrier, 1996. The distribution of colloidal and dissolved organic carbon, major elements, and trace elements in small tropical catchments. *Geochim. Cosmochim. Acta* 60(19): 3643-3656.
- Francko, D.A. and R.T. Heath, 1979. Functionally distinct classes of complex phosphorus compounds in lake water. *Limnol. Oceanogr.* 24(3): 463-473.
- Gächter, R. and P. Stadelmann, 1993. *Gewässerschutz und Seenforschung*. In: E. Rouss (ed.), *Der Sempachersee*. Mitt. Naturforsch. Ges. Luzern, pp. 343.
- Gächter, R. and A. Wüest, 1992. Auswirkungen von Sanierungsmassnahmen auf den Trophiegrad und Sauerstoffhaushalt von Seen. *EAWAG News*, 34D: 27-32.
- Guo, L.D. and P.H. Santschi, 1996. A critical evaluation of the cross flow ultrafiltration technique for sampling colloidal organic carbon in seawater. *Mar. Chem.* 55(1-2): 113-127.
- Gustafsson, Ö. and P.M. Gschwend, 1997. Aquatic colloids: Concepts, definitions, and current challenges. *Limnol. Oceanogr.* 42(3): 519-528.
- Hilger, S., 1998. Characterization of colloidal and particulate phosphorus in two Lake Lugano tributaries. Ph. D. Thesis, ETH, Zürich, 140 pp.
- Hoffmann, M.R., E.C. Yost, S.J. Eisenreich and W.J. Maier, 1981. Characterization of Soluble and Colloidal-Phase Metal Complexes in River Water by Ultrafiltration. A Mass-Balance Approach. *Environ. Sci. Technol.* 15(6): 655-661.
- Imboden, D., 1992. Possibilities and limitations of lake restoration: Conclusions for Lake Lugano. *Aquat. sci.* 54(3-4): 381-390.
- Jackson, T.A. and D.W. Schindler, 1975. The biogeochemistry of phosphorus in an experimental lake environment: evidence for the formation of humic-metal-phosphate complexes. *Verh. int. Ver. Limnol.* 19: 211-221.
- Karagounis, I., 1992. Ein physikalisch-biochemisches Seemodell – Anwendung auf das Nordbecken des Luganer Sees. VAW Mitteilungen, 116. Versuchsanstalt für Wasserbau, Hydrologie und Glaziologie der ETH Zürich, Zürich, 1-156 pp.
- Lachavanne, J.B., J. Perfetta and R. Juge, 1992. Influence of water eutrophication on the macrophytic vegetation of Lake Lugano. *Aquat. sci.* 54(3-4): 351-363.
- Lami, A., P. Guilizzoni, D. Ruggiu, B. Polli, M. Simona and A. Barbieri, 1992. Role of pigments on algal communities and photosynthesis. *Aquat. sci.* 54(3-4): 321-330.
- Lazzaretti, M.A., K.W. Hanselmann, H. Brandl, D. Span and R. Bachofen, 1992. The role of sediments in the phosphorus cycle in Lake Lugano: II. Seasonal and spatial variability of microbiological processes at the sediment-water interface. *Aquat. sci.* 54(3-4): 285-299.
- Lead, J.R., W. Davison, J. Hamilton-Taylor and J. Buffle, 1997. Characterizing Colloidal Material in Natural Waters. *Aquatic Geochemistry*, 3: 213-232.
- Lean, D.R.S., 1973. Phosphorus dynamics in lakewater. *Science*, 179: 768-680.
- LSA, 1996. „Ricerche sull evoluzione del Lago di Lugano“-Aspetti limnologici. Programma quinquennale 1993-1997 (campagna 1993). ISSN 1013-8080, Laboratorio Studi Ambientali (LSA). Commissione Internazionale per la protezione delle Acque Italo-Svizzere, Cantone Ticino.

- LSA, 1998. "Ricerche sull'evoluzione del Lago di Lugano"-Aspetti limnologici. Programma quinquennale 1993–1997 (campagna 1996 e 1997), Laboratorio Studi Ambientali (LSA). Commissione Internazionale per la protezione delle Acque Italo-Svizzere, Cantone Ticino.
- Martin, J.M., M.H. Dai and G. Cauwet, 1995. Significance of colloids in the biogeochemical cycling of organic carbon and trace metals in the Venice Lagoon (Italy). *Limnol. Oceanogr.* 40(1): 119–131.
- Mayer, T.D. and W.M. Jarrell, 1995. Assessing Colloidal Forms of Phosphorus and Iron in the Tualatin River Basin. *J. Environ. Qual.* 24: 1117–1124.
- Müller, R. and H.J. Meng, 1992. Past and present state of the ichthyofauna of Lake Lugano. *Aquat. sci.* 54(3–4): 338–350.
- Olsson, H., 1988. Phosphatases in Lakes- Characterization, Activity and Ecological Implications, Uppsala University, Uppsala.
- Paerl, H.W. and D.R.S. Lean, 1976. Visual Observations of Phosphorus Movement between algae, bacteria and abiotic particles in Lake Waters. *J. Fish. Res. Board Can.* 33: 2805–2813.
- Polli, B. and M. Simona, 1992. Qualitative and quantitative aspects of the evolution of the planktonic populations in Lake Lugano. *Aquat. sci.* 54(3–4): 303–320.
- Rigler, F.H., 1964. The phosphorus fractions and the turnover time of inorganic phosphorus in different types of lakes. *Limnol. Oceanogr.* 9: 511–518.
- Rostad, C.E., J.A. Leenheer and S.R. Daniel, 1997. Organic Carbon and Nitrogen Content Associated with Colloids and Suspended Particulates from the Mississippi River and Some of Its Tributaries. *Environ. Sci. Technol.* 31: 3218–3225.
- Schurtenberger, P. and M.E. Newman, 1993. Characterization of Biological and Environmental Particles using Static and Dynamic Light Scattering. In: J. Buffle and H.P.v. Leeuwen (eds.), *Environmental Particles. Environmental Analytical and Physical Chemistry Series*. Lewis Publishers, Boca Raton/FL, USA.
- Siegrist, H. and M. Boller, 1996. Auswirkungen des Phosphatverbots auf die Abwasserreinigung, EAWAG News, 42 D: 9–11.
- Vollenweider, R.A., 1968. Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication, 27, OECD, Paris, 159 pp.
- Wehrli, B. and A. Wüest, 1996. 10 Jahre Seenbelüftung: Erfahrungen und Optionen. Schriftenreihe der EAWAG, Nr. 9., EAWAG, Dübendorf.
- Whitehouse, B.G., P.A. Yeats and P.M. Strain, 1990. Cross-flow filtration of colloids from aquatic environments. *Limnol. Oceanogr.* 35(6): 1368–1375.
- Wüest, A., 1996. Einschichtungen partikelbeladener Zuflüsse in Seen, Peak-Kurs, EAWAG, Dübendorf.
- Yiyong, Z., 1996. UV-sensitive P compounds: release mechanism, seasonal fluctuation and inhibitory effects on alkaline phosphatase activity in a shallow Chinese freshwater lake (Donghu Lake). *Hydrobiologia*, 335: 55–62.
- Zamboni, F., A. Barbieri, B. Polli, G. Salvade and M. Simona, 1992. The dynamic model Seemod applied to the southern basin of Lake Lugano. *Aquat. sci.* 54(3–4): 367–380.

Received 19 October 1998;

revised manuscript accepted 6 May 1999.