ELSEVIER

Environmental Pollution 101 (1998) 107–116

ENVIRONMENTAL
POLLUTION

Toxicity of combined sewer overflows on river phytoplankton: the role of heavy metals

Martin Seidl[a], Viviane Huang[b], Jean Marie Mouchel[a,*]

[a]CERGRENE, ENPC and ENGREF, 6 et 8 avenue Blaise Pascal, Cité Descartes, Champs sur Marne, 77455 Marne-la-Vallée, Cedex 2, France
[b]IBM, URA CNRS 386, ENS Montrouge, 1 rue Maurice Arnoux, 92120 Montrouge, France

Received 25 July 1997; accepted 8 December 1997

Abstract

The toxic effect of a combined sewer overflow (CSO) on the phytoplankton community of the river Seine has been studied by means of short-term primary production measurements. As the discharged solids usually do not remain in the water column, only filtered or centrifugated fractions were tested. The collected phytoplankton were grown in the laboratory for 2 days, after addition of N, P and EDTA. Stock cultures in exponential growth were directly tested with heavy metals, but resuspended algal cells were used for effluent testing. The results show an increase of EC_{50} value for the single metal species in the order Cu$\ll$Zn$\leq$Pb. Free metal contents were calculated with the chemical speciation model Mineql+. The EC_{50} dilution of the tested CSOs effluent was 50%. This value increased by 30% after addition of 5 10^{-6} M EDTA, indicating that the effluent toxicity is only partially due to its metallic compounds.

Keywords: Phytoplankton; Heavy metals; Productivity; Combined sewer overflow; Oxygen deficit

1. Introduction

Anoxic conditions combined with high NH_3 levels due to the degradation of organic matter issued from combined sewer overflows (CSOs) are known to provoke fish kills (Boet et al., 1994; Magaud et al., 1997). This strong impact overshadows other possible toxic effects of CSOs. Beside these extreme situations, observed almost every year in the river Seine in the Paris conurbation (Boet et al., 1994) where the ecosystem degradation becomes obvious to the public, less dramatic but nevertheless significant impacts of CSOs on biological communities can be expected. However, very few publications have been devoted to the evaluation of non-lethal effects of CSOs on biological communities. Borchardt and Statzner (1990) and Borchardt (1993) have studied the complex effect of hydraulic stress and ammonia concentrations on the drift of benthic organisms. Several biological indexes have been used to demonstrate non-lethal effects of urban run off discharge onto natural biological communities. Seager and Abrahams (1989), for example, proposed the use of fish ventilation rates in addition to benthic diversity indexes. Payne and Hedges (1989) revealed that different upstream/dowstream diversity scores could be observed as soon as the catchment area was higher than 50 ha. However, such global indexes do not allow one to determine the major factors which may affect the ecosystem (shock flow, input of suspended solid, of organic matter, toxicity effect, etc.). Because of the high concentration of metals in urban run off waters (Chebbo et al., 1995), increases of metal concentrations in organisms are another sub-lethal index of biological impact. Payne and Hedges (1989) failed to show any significant difference in algae (*Cladophora*) for cadmium, lead and copper, while zinc concentrations showed a significant increase down-stream of outlets. Seidl et al. (1993) confirmed short-term increases of zinc concentrations in *Cladophora* in the river Seine after CSOs. Fraboulet et al. (1993) experienced difficulties in demonstrating any significant increases in metal concentration in caged *Asellus aquaticus*, due to changes in the animal's physiology in varying environmental conditions. However, increases of micropollutant concentrations do not necessarily prove any significant change in the given population inside the ecosystem: the influence of toxic

* Corresponding author.

0269-7491/98/$19.00
PII: S0269-7491(98)00008-6

effects on the fate of major biogeochemical components of the ecosystems is far from demonstrated.

During summer wet weather periods, the CSOs in the Paris conurbation may contribute to 30% of the flow of the river Seine, and their high metal content has already been reported (Paffoni, 1994; Estebe et al., in press). In severely eutrophicated streams, such as the river Seine, phytoplankton plays a key role in the oxygen balance. A sudden breakdown of primary production due to a phytotoxic effect would enhance oxygen depletion, while the heterotrophic bacterial activity is expected to be much less sensitive to toxic effects. We have found very little data on the ecotoxicological impacts of raw waste waters on phytoplankton communities. The aim of this study was, therefore, to evaluate the possible toxicity of waste waters to phytoplankton in the river Seine and to determine the contribution of heavy metal discharge to the overall toxic effect. These results will be further used to complete water quality models in order to simulate the impact of CSOs (Even et al., accepted).

2. Materials and methods

Toxicity of the sewage was tested under laboratory conditions on natural phytoplankton mixtures by means of dark and light bottle productivity measurements. Natural phytoplankton was collected from the river Seine and grown in the laboratory for 2–3 days to produce enough biomass in the exponential phase. Subsequently, three types of experiment were conducted: (1) tests of single metal species buffered by EDTA, (2) tests of CSO water, and (3) tests of CSO water with added EDTA.

2.1. Phytoplankton cultures

A phytoplankton community corresponding to a relatively unpolluted river Seine, was reconstituted by mixing Seine river water and Marne river water in the proportion of their flows at their confluence, usually 2:1. The sampling sites were situated in the moderately urbanised area at Noisy-le-Grand (river Marne) and at Ris Orangis (river Seine) about 25 km upstream of Paris (Fig. 1). The samples were collected from bridges in the middle of the stream, 30 cm below the surface. The average nutrient content and the composition of the river Seine water are given in Table 1. The mean daily intensity of total solar radiation during summer was about 60 W m^{-2}. The saturation value for phytoplankton of the Seine at 21°C, mentioned by Garnier et al. (1992), is about 400 μE m^{-2} (in the PAR), equivalent of 85 W m^{-2} total irradiation. This value is in the usual range given by Reynolds (1990) and far below the inhibitory level. The chlorophyll-a levels in the river Seine around Clichy were almost constant from June to the end of September 1996 around 30 μg litre^{-1}, which corresponds to an algal density of 10^7 cells litre^{-1}.

In the laboratory, detritus and large zooplankton specimens were removed by a 100-μm nylon mesh. The following salts, Pro-Analysis, were added to the river water: 0.5 mg litre^{-1} NH_4–N (NH_4Cl) and 0.05 mg litre^{-1} PO_4–P (Na_2HPO_4) and 5 10^{-6} M EDTA (Na_2EDTA). The growth took place in 2-litre polycarbonate Erlenmeyer flasks with untied caps (Nalgene) in a thermostated incubator (20 ± 1°C) under gyratory conditions (90 rpm) and continuous day light (40 ± 5 W m^{-2} Sun-Glo®). Two days after the beginning of incubation, the culture was directly used for single metal testing or used after careful filtration and resuspension of biomass in mineral water (Evian) for effluent testing (Table 2). We have chosen this commercial mineral water for dilution because of its very low micropollutants level and its stable composition close to that of river Seine (Table 1).

2.2. Experiments

Sublethal effects on primary production will not necessarily be accompanied by a reduction in cell number (De Filippis et al., 1991). More sensitive techniques are needed. Determination of photosynthesis by means of oxygen production in light and dark bottles has been an approved method for a long time. Primary production determined as the rate of carbon uptake, measured by ^{14}C incorporation, is another newer technique. Both methods are in good agreement, whereas the differences between *in situ* and *in vitro* measurements fall into the range of methodological errors (Williams et al., 1979; Davies and Williams, 1984). It should be noted that

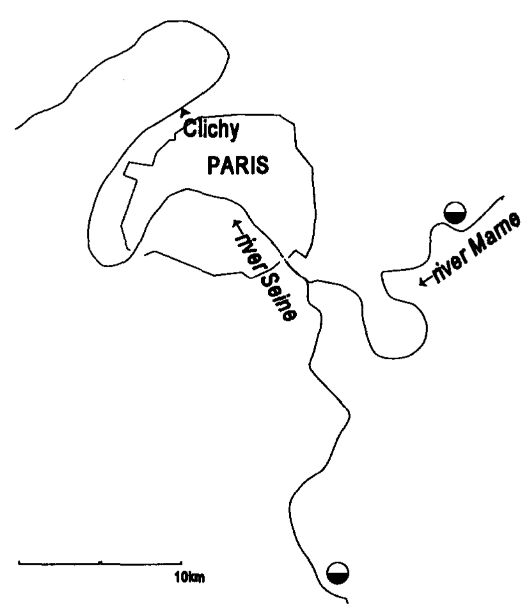

Fig. 1. Sketch of the area (◒, sampling stations).

these methods measure different, although closely linked, processes: ^{14}C determines the carbon flux, whereas the oxygen method is more associated with energy transfer. The ^{14}C method was used to evaluate the effect of heavy metals on photosynthesis by different authors (Azeez and Banerjee, 1986; Wallen, 1990; Wong and Chau, 1990; Thomson and Couture, 1993). Compared to growth measurements, productivity measurements are fast (several hours instead of several days) and allow one to investigate a specific process rather than an overall effect. Because of its simplicity and close relation to the problem of oxygen depletion after CSOs, we have chosen the oxygen method.

The main criterion for the design of experiments was the choice of optimal oxygen difference. Measured concentration had to be higher than 2 mg litre^{-1} and lower than the saturation value, with a high enough difference between the initial and final concentrations, in order to limit measurement errors. CO_2 consumption during photosynthesis should not induce a rise in alkalinity higher than 0.5 pH units, to avoid a strong modification of metal speciation. The optimum conditions were found by diluting the stock culture two or three times with mineral water to give a blank production between 0.5 and 1 mg litre^{-1} h^{-1}. Oxygen was measured with an accuracy of 0.03 mg litre^{-1} (Orbisphere 2607 oxygen metre), which led to an average error of 3.2% in the measured effect. The reproducibility of duplicates was always better than 7%.

The toxic mixtures were transferred in glass Biochemical Oxygen Demand (BOD) bottles with one or two small glass spheres for better mixing. Blanks were run with mineral water only. BOD bottles for light exposition were bubbled for a few minutes with nitrogen (Pro-Analysis, filtered through 0.2-μm PTFE membrane) in order to lower the initial oxygen content. The initial oxygen content was controlled and after an incubation period of generally 6 h, oxygen was measured again, pH and temperature were checked, and samples eventually preserved for metal analysis. Incubation conditions were the same as mentioned for the stock culture.

2.3. *Metals and effluent*

The heavy metals used were added as slightly acidified (1‰ HNO_3, Merck ultrapure) stock solutions of $ZnSO_4 \cdot 7H_2O$, $Cu(II)SO_4$ and $Pb(NO_3)_2$ (Merck or Sigma Pro-Analysis). The effluents were collected during two rain events at the most important CSO outlet of Paris at Clichy. The solid and liquid phases were separated in the laboratory immediately after collection, by means of centrifugation (4500 rpm, 15 min) followed by a double filtration for optimum elimination of native microorganisms and transparency of the sample (Whatman GF/F and Nuclepore polycarbonate 0.2-μm porosity filters). Filtered solutions were used directly or kept frozen for further toxicity tests.

2.4. *Calculations*

The results were converted to net production by adding gross production (measured in light bottles) and respiration (measured in dark bottles), expressed per unit of time (mg O_2 litre^{-1} h^{-1}). Differences in temperature of more than 0.5°C between bottles of the same test, due to slight day and night variations in the incubator, were corrected with the Arrhenius equation. Temperature effects on photosynthesis of natural phytoplankton communities can be described by a Q_{10} of 2.2, whereas respiration is much less influenced by temperature (Reynolds, 1990). The toxic effect was described as inhibition of the net production rate, compared to the blank. The concentration giving a 50% effect (EC_{50}),

Table 1
Typical mean values for physico-chemical data of the river Seine (Huang, 1994) during summer 1993 in the Paris area and for Evian mineral water (IPL, 1996)

	pH	NH_4 (mM)	NO_3 (mM)	PO_4 (μM)	Ca (mM)	Mg (mM)	Fe (μM)	HCO_3 (mM)	Cl (mM)	SO_4 (mM)
Seine	7.8	0.1	0.33	5	3	0.2	0.1	3.7	0.7	0.3
Evian	7.3	< 0.003	0.05	< 0.1[a]	2	1	< 0.1	5.8	0.14	0.12

[a] Values measured in our laboratory.

Table 2
Work scheme for the different tests

Test	Treatment of stock culture	Dilution water	Toxic substance
Single metal species	clearing by 100 μm	mineral water + 5×10^{-6} M EDTA	metal
Waste effluent	filtration 0.4 μm + resuspension of retained biomass in mineral water	mineral water	CSO effluent centrifuged and/or filtered at 0.2 μm

Metals were added as $ZnSO_4$, $CuSO_4$ or $Pb(NO_3)_2$ (Merck Pro-Analysis). A stock solution of 0.15 M metal + 1‰ HNO_3 was diluted 50 times before use.

was calculated by linear regression of log–logit plots as described by Litchfield and Wilcoxon (1949), using Sigma Plot® software. EC_{50}s for trace metals are given in concentration units, while EC_{50}s for effluents are expressed as dilutions.

2.5. Metal analysis

All material used for culture handling or for mixture preparation was made of polycarbonate, polysulfone or polyethylene, previously cleaned in 2% nitric acid and washed six times with ultrapure (18 MΩ) water. We verified that the BOD bottles did not release any significant metal during 24 h of incubation, compared to the rather high metal levels used during the experiments. BOD bottles were cleaned separately following the same procedure as for the plastic-ware. Metals were analysed in 0.2- or 0.4-μm syringe-filtered fractions (Nuclepore) and kept frozen until analysis. The analysis of total dissolved trace metals was performed in a clean environment by Graphite Furnace Atomic Spectrometry (Varian 800-GTA100). The analysis was verified with a NRCC dissolved trace metal reference SLRS-3.

As stated by the free-ion model, the real toxic agent is the free metal ion and not the metal complex (Tessier and Turner, 1995). This model is generally accepted today, although several exceptions have been reported (Guy and Kean, 1980; Campbell, 1995; Errecalde et al., 1998). What is not contested is that metals bound to strong ligands such as EDTA or NTA are no longer available nor toxic to phytoplankton. The concentration of antagonists to heavy metals, such as Ca and Mg, can be an important factor, as they modify the physiological action of metals. Interspecies differences in toxicity response to a given metal may vary by a factor of 10 and the change of toxicity due to adaptation to the metal may be almost of the same order of magnitude (Niedrelehner and Cairns, 1992; Loez et al., 1995).

The free metal-ion concentration in river water, for the single metal-species test, was calculated with the Mineql+ program (Schescher and McAvoy, 1992) and NIST equilibrium constants (NIST, 1993) using mean river-water composition. Such a model can be used only if the composition of the medium is well known and relatively stable during the exposition. In particular, the evaluation of relevant complexation constants for natural organic matter is extremely difficult. Due to progress in computer technology, calculations of metal speciation in toxicity testing are more frequent (Allen et al., 1980; Guy and Kean, 1980; Wolterbeek et al., 1995; Errecalde et al., 1998). The addition of a strong and stable metal complexant, such as EDTA, to the culture medium optimises growth (Huebert and Shay, 1992), but also, as a powerful complexant with known characteristics, it enables one to calculate accurately the free ion concentrations. The slight difference between Seine water and mineral water did not give any significant divergence in speciation results.

3. Results and discussion

3.1. Growth

Laboratory cultures showed the usual exponential growth curve, with a productivity maximum after 2 days of incubation. Cultures issued from low phytoplankton concentrations (autumn) reached, half a day later, a lower maximum than the cultures started with high phytoplankton concentrations (spring). The mean growth rate was 1.4 day^{-1}. This value is in the high range of growth rates reported in the literature (Dauta, 1982). The gross production/respiration ratio reflects the physiological state of the population; it was about 5 during the exponential phase and decreased rapidly after the collapse of the culture. We have observed that the culture grown with added EDTA grew slightly faster, but also collapsed faster (Fig. 2). A good correlation was found between net productivity and chlorophyll-a concentration with an assimilation ratio of 0.44 μmol $O_2\,\mu g^{-1}$ Chl-a h^{-1} (Fig. 3). Since the same ratio was found for stock cultures and for blanks in mineral water, the low phosphorus content of mineral water did not influence the short-term productivity measurements. Instead, nutrient availability would appear to modify long-term processes like growth. The intra- or extracellular nutrient stocks, built up in the culture medium, proved to be more than sufficient for the very short incubation period we used. The maximum chlorophyll-a concentration measured in stock cultures was about 200 $\mu g\, litre^{-1}$, similar to the maximum of algal blooms in spring downstream of Paris. The populations of stock cultures were mainly composed of Chlorophyceae species: *Chlorella* and *Scenedesmus* and to a lesser extent of *Selenastrum* and *Chlamydomonas*. The diatoms, mostly *Tabellaria* and *Asterionella*, were never dominant and represented less than 10% of the total population. The different cultures used for assays surprisingly showed

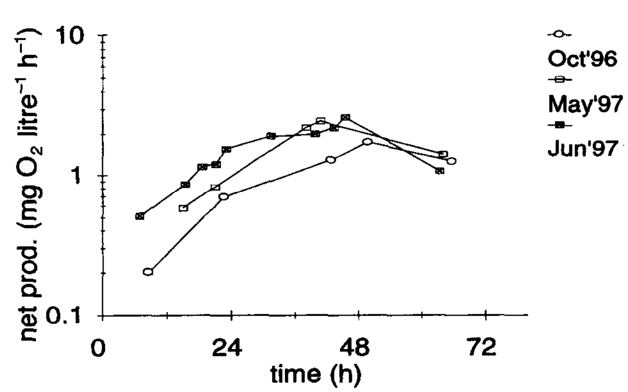

Fig. 2. Growth curves for stock cultures of Seine phytoplankton.

only slight variations of the composition. No daphnides and only sporadic flagellates have been observed. A bacterial density of 9×10^9 cells litre^{-1}, comparable to that of river Seine during dry summer periods, was found in non-filtered blanks. Despite a small bacterial biomass compared to the phytoplankton, the bacterial respiration may be in the same range as that of phytoplankton.

No metal contamination was revealed. The concentrations for stock culture and blanks, given in Table 3, are representative for all tests. Concentrations were slightly elevated in flasks spiked with EDTA, due to extraction of adsorbed metals.

3.2. Complexation

The proportion of free ions for the metals we tested were very different. The maximum concentration of lead effectively tested was 24 mg litre^{-1}. An increase of the total lead concentration caused the precipitation of lead carbonates and/or hydroxides and thus no increase in free metal ions. Copper is complexed far more by EDTA than by other organic chelators. The river Seine around Paris has a complexing capacity of about 1.6×10^{-7} M Cu-equivalents (Huang, 1994), probably originating from industrial and domestic sources of EDTA and NTA and is comparable to concentrations found in other European rivers (Frimmel 1989; Xue et al., 1995). Assuming that half of this amount was EDTA (strong complexant) and a half was NTA (weak complexant), the dissolved copper of the Seine water would be complexed half by EDTA and half by hydroxides. Lead will be bound mostly to carbonates, and zinc will be only slightly bound by chelators with more than 60% remaining as free ions.

The fraction of metals bound to algal surfaces will be dependent on algal density. Hart et al. (1992) describe the adsorption of copper and zinc to algal surfaces in artificially enriched lake water with a Langmuir equation. They determined a conditional stability constant of 10^9 M^{-1} for Cu and 10^6 M^{-1} for Zn, and a total concentration of algal binding sites between 6 and 10×10^{-8} M for an algal density of 5×10^6 cells litre^{-1}. Bates et al. (1982) used phytoplankton monocultures (3×10^9 cells litre^{-1}) and showed that 28% of added zinc (15 μM) would bind to algal surfaces within less than 4 h. EDTA addition to a mixture of CSO and phytoplankton (about 1 μM Zn and 10^8 cells litre^{-1}) could desorb about the same amount of the initial dissolved zinc concentration. This distribution of zinc between algal surfaces and water phase is in good agreement with the constants measured by Hart et al. (1992).

3.3. Toxicity of single metal species

Figure 4 gives an example of oxygen production test results after copper addition. The ratio of gross production over respiration for the blank is about 6, slightly higher than in stock cultures, probably because of the removal of dead cells before testing. The ratio decreases as the inhibition effect increases. Variations in the composition of the phytoplankton inoculum, variations in the physiology of the used culture (gross production to respiration ratio ranged from 3 to 7) as well as test durations varying from 4 to 20 h do not seem to influence significantly the obtained toxicity results. Figure 5 gives the result of three separate tests (zinc) under different conditions with no significant (<99%) difference of EC_{50} values.

Table 3
Total dissolved metal content in blanks, cultures (5×10^{-6} M EDTA) and CSO effluents

	Lead (μM)	Zinc (μM)	Copper (μM)
Blanks in Evian water	0	0.011	<0.003
Cultures (EDTA)	0.0008	0.11	0.011
Seine river[a]	0.0022	0.082	0.019
CSO 20-08-96	—	1.1	0.93
CSO 28-10-96	0.0038	0.57	0.08

The analyses of blanks were performed on filtered samples at the end of incubation.
[a] Mean values for the Seine water <0.4 μm, in August, downstream of Paris (Huang, 1994).

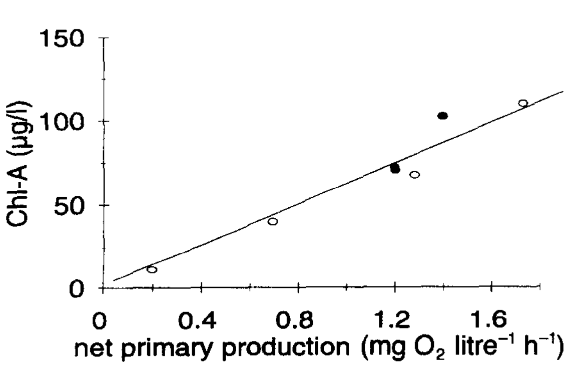

Fig. 3. Relation between net primary production and chlorophyll content for stock cultures (○) and blanks (●).

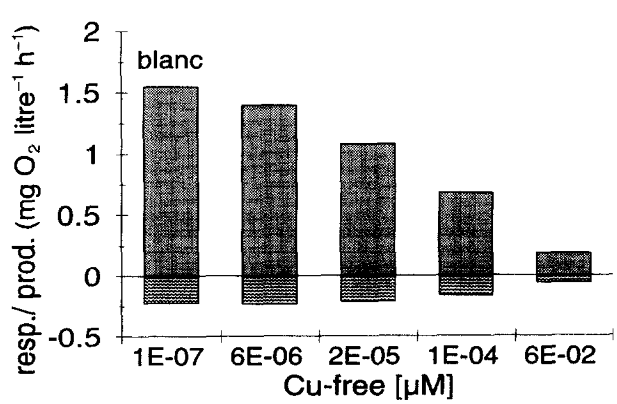

Fig. 4. Example of productivity measurements with copper.

For relevant comparisons, the total metal concentrations have been transformed to free ion concentration using Mineql+. The proportion of free ion and thus the EC_{50} (Figs 6 and 7) will be dependent on the physicochemical properties of each metal. The usual toxicity ranking is observed: lead$\leq$zinc$\ll$copper. The EC_{50} value of lead will be reached only after lowering the pH or in solutions containing less carbonates. Free-ion EC_{50}s computed from total metal EC_{50}s are identical to EC_{50}s obtained directly from the plot of free-ion concentrations. Photosynthesis was affected much more than respiration for all substances tested (Fig. 4). Photosynthesis is indeed a complex process based on some metal-sensitive enzyme systems like NADP oxidoreductase (De Filippis et al., 1981; Karez, 1989).

The EC_{50} value of 5.2 μM free zinc obtained during this study (Table 4) is in good agreement with values found for growth inhibition of *Selenastrum*, 1.1 μM (Errecalde et al., 1998) and 1.8 μM (Bartlett et al., 1974) or 7.9 μM found for *Scenedesmus* (Peterson, 1982). Wong and Chau (1990) gave an approximative EC_{50} value of 0.4 μM free zinc for inhibition of primary productivity of a phytoplankton community of lake Ontario, with no difference between green algae and diatoms. It has to be emphasized that these values were obtained under different laboratory conditions and estimated by chemical equilibrium calculations. As the EC_{50} decreases with increasing exposure, it may be expected that EC_{50} based on growth-rate measurements would lead to lower values than tests based on short-term production tests.

3.4. *Effluents and EDTA*

3.4.1. *Tolerance to metals*

We have observed only a small difference (5%) in EC_{50} obtained on phytoplankton populations grown in stock cultures, with and without EDTA addition. The non metal-buffered cultures were less sensitive to waste effluent than the buffered cultures, which would suggest

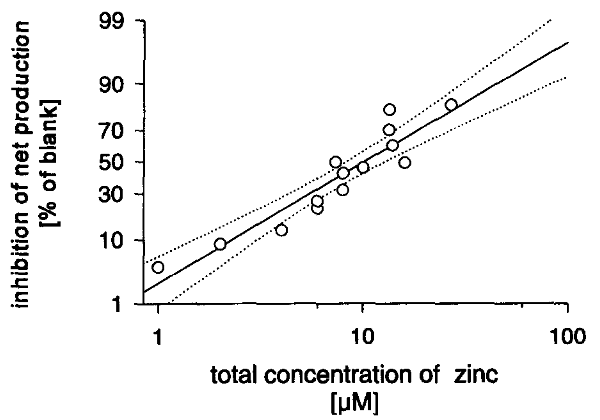

Fig. 5. Intertest variability for zinc assays. Result of three different zinc tests with regression line and 95% confidence limits.

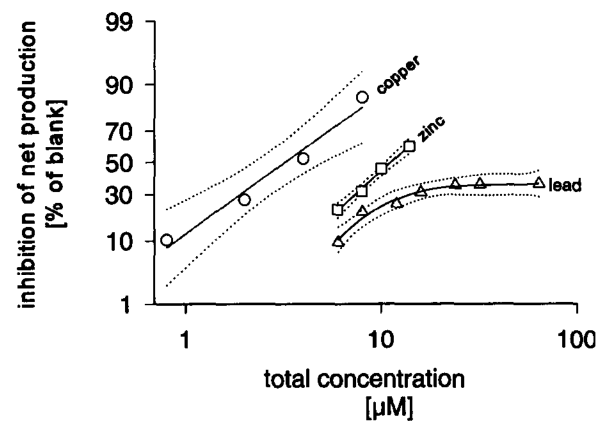

Fig. 6. Probability–log plots for the heavy metals tested, concentration of total metal. Each line is the result of two different tests.

Table 4
EC_{50} values and their 95% confidence limits, for single metal species, in the presence of 5×10^{-6} M EDTA

Metal	Total metal (μM)			Free metal ion (μM)		
	EC_{50}	$95\%_{low}$	$95\%_{up}$	EC_{50}	$95\%_{low}$	$95\%_{up}$
Zinc	10.5	7.3	15	5.23	2.8	9.7
Copper	3.15	2.4	4.2	3.57×10^{-4}	0.94×10^{-4}	14×10^{-4}
Lead	—	—	—	40.6	13	100

Table 5
Evaluation of toxicity of 0.2-μm filtered CSO effluent in the absence and presence of 5×10^{-6} EDTA

CSO Clichy	Normal			EDTA added		
	EC_{50}	$95\%_{low}$	$95\%_{up}$	EC_{50}	$95\%_{low}$	$95\%_{up}$
20-8-96	0.47	0.4	0.6	0.68	0.6	0.8
28-10-96	0.43	0.3	0.7	0.88	0.6	>1

EC_{50} and their confidence limits are given as effluent dilutions.

a slightly higher tolerance of species grown in a medium with higher free-metal concentrations. However, the small difference we found, although systematic, is not significant as the mean error for inhibition data was about 4%. A 10-fold artificial rise of background concentration of zinc, manganese or copper in stock cultures caused modifications of the phytoplankton species, but not of the total number of cells. These modifications were of the same amplitude as the *in situ* variations (Hart et al., 1992). The adaptability of algae to high metal levels was demonstrated by Niedrelehner and Cairns (1992). Most organisms possess a mechanism to regulate the intracellular concentration of metals needed for growth. In the case of the Seine river, the residence time and the metal content were probably not high enough to observe such an effect, neither in the laboratory nor *in situ* (Huang, 1994).

3.4.2. CSO

Effluents issued from Clichy CSO during two different rain events have been tested against several samples of natural Seine river phytoplankton. For both events, the dissolved phase of the sewer effluent was shown to be toxic. Although both CSO samples did not contain the same amount of metals (Table 3) nor probably of organic micropollutants, the difference between EC_{50}s of the two events was not significant (Table 5). However, the acuteness of the toxicity effect, represented by the slope of the line in Fig. 8, was significantly different. The confidence limits for the EC_{50} of the 20-8-96 sample are larger than those of the 28-10-96 sample, because of the significantly lower slope of the former. After heavy rain storms in Paris, with 1–2 months return periods, we observed large patches of polluted waters, several kilometres long, with a low oxygen content (Seidl et al., accepted). The proportion of CSO effluent in these patches was in the range 15–25%. Depending on the characteristics of the discharged water, we might expect a reduction in the primary production of 10–30% in such patches.

The addition of 5 10^{-6}M EDTA significantly decreased the effluent toxicity. As shown in Table 5, 50–100% more effluent was needed in solutions where EDTA had been added to obtain the same inhibition effect (namely 50%). This suggests that a fraction of CSO toxicity, but not all, is due to heavy metals. The added EDTA was sufficient to complex all heavy metals in the solution and eventually extract metals bound to solid surfaces. With higher EDTA concentrations, Carlson and Morisson (1992) showed that the EC_{50} value for raw wastewater sludge of Göteborg, measured with Microtox (600 mg SS $litre^{-1}$), was almost four times higher after addition of 10^{-2}M EDTA. The waste waters discharged during the events that we sampled contained 0.6–1.1 μM of total dissolved zinc, with probably 80% in free-ion form. At the EC_{50} dilutions, the concentrations of free Zn were about 0.2–0.4 μM, which can explain only a little less than 10% inhibition of photosynthesis (Fig. 6). If the toxic effects can be considered as additive, inhibition due to zinc would explain more than half of the reduction in toxicity at EC_{50} after EDTA addition. Lead and copper will most probably not contribute to the toxicity, due to their low concentration in the waste waters and the high amount of complexing agents. However, evaluating the contribution of specific pollutants in a global toxic effect is not an easy task. The additive model seems acceptable for the 20-8-96 event, since the inhibition due to 60% of CSO water is twice as high as the inhibition measured in the solution containing 30% CSO water, but it is clearly not acceptable for the effluent collected during the 29-10-96 event (Fig. 9). Once toxic effects cannot be added, the concept of contribution should not be used. The absolute net production enhancement after EDTA addition seems almost constant for both events and whatever the dilution used. If we assume that EDTA addition only prevents the interaction of toxic metal ions with biochemical processes in algal cells, the conclusion would be that the toxic effect of metals

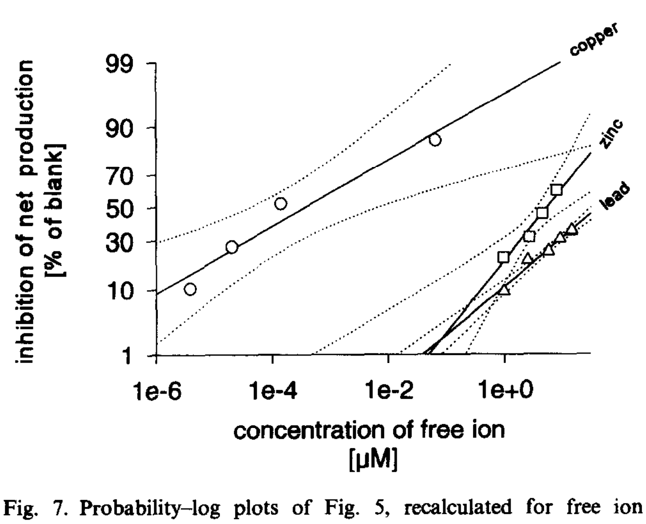

Fig. 7. Probability–log plots of Fig. 5, recalculated for free ion concentrations.

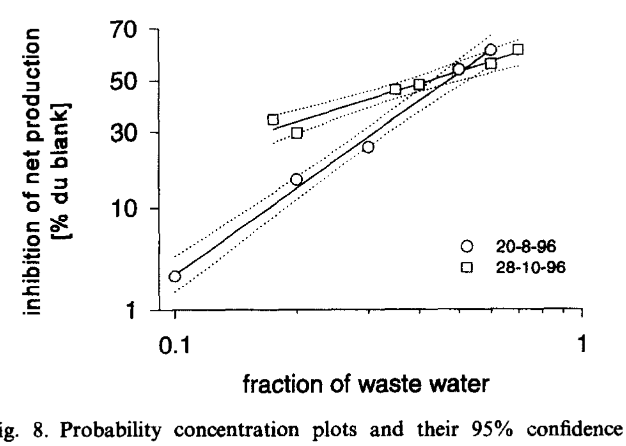

Fig. 8. Probability concentration plots and their 95% confidence limits for the two effluents tested.

contained in CSO waters does not depend on their concentration. Once again, this invalidates the additive or linear model.

Many other types of toxic substance can be found in CSO waters. Marchand et al. (1989) gives values of about 10 μg litre^{-1} of chlorinated solvents and anionic surfactants and 25 ng litre^{-1} of Lindane in the dissolved fraction of urban sludges. Chevreuil et al. (1990) report 290 ng litre^{-1} of PCB in Parisian sewage. Even if toxic effects of single compounds are known, the complexity of waste effluents makes it hardly possible to determine individual contributions or interactions. Since it is not possible to reconstitute CSO waters step by step to specifically study synergic or threshold effects, conclusions regarding contributions of specific pollutants should be as cautious as possible. In particular, the presence of toxic metal complexes in polluted river water has already been reported (Tubbing et al., 1992).

3.4.3. *Suspended matter*

The metal load of a CSO consists of a particulate and a dissolved fraction. Recent investigations (Seidl et al., accepted) showed a fast deposition of the discharged solids in the river. The pollutants affecting the phytoplankton directly should, therefore, be mostly found in the dissolved fraction and possibly in the exchangeable

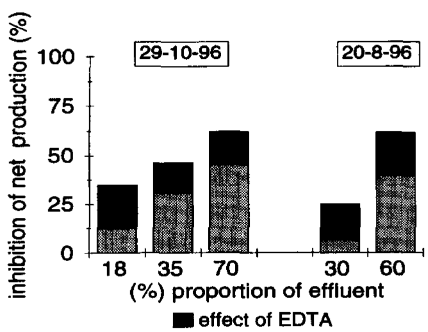

Fig. 9. Summary of effluent tests with and without EDTA. Total bars show the toxicity of mixtures of CSO and Evian water; lightly shaded bars show the toxicity of the mixtures after EDTA addition (5×10^{-6} μM). The darkly shaded areas indicate the effect of EDTA addition.

or colloidal part of the solid fraction. The dissolved fraction, arbitrarily defined as <0.4 μm contains complexed and free metal ions, as well as metals adsorbed onto colloidal matter.

Preliminary results obtained with centrifuged, but not filtered, CSO waters (Table 6) indicate that the suspended, not settleable, matter contains large amounts of weakly bound heavy metals, able to increase the toxicity. The EC_{50} of centrifuged effluent (0.21) was significantly different from the EC_{50} of centrifuged and filtered effluent (0.45). The change in light attenuation, due to suspended matter, was less than 5%, and did not significantly modify oxygen production. Analysis of CSOs mentioned elsewhere (Morisson et al., 1989; Chebbo and Bachoc, 1992), confirm that the very fine particles in CSOs are extremely polluted and that they contain a large amount of extractible metals. The labile part is comparable to the total dissolved fraction and may be responsible in our tests for the increase in toxicity.

4. Conclusions

The set up for BOD measurements may be used, after minimal adaptation, as a simple, fast and low-cost method for the evaluation of toxicity to phytoplankton.

The Parisian CSOs contain important quantities of loosely complexed zinc, able to inhibit primary production. If some lead is present it will be probably be bound as a non-toxic aqueous carbonate complex, subject to precipitation. Copper is potentially toxic, even if its concentrations in the CSO effluents are low. A 35-fold increase of the background level showed an inhibitory effect. However, copper is also strongly complexed by natural or anthropogenic ligands. The way the copper is complexed will greatly determine its contribution to overall effluent toxicity. If EDTA significantly contributes to the total complexation capacity for copper measured in the river Seine, we would expect a very low toxicity due to copper.

Table 6
Total dissolved metals in assays with centrifuged (C) and centrifuged plus filtered (CF) effluent collected on 28-10-96 at Clichy

	Lead (nM)		Zinc (μM)		Inhibition (%)	
	CF	C	CF	C	CF	C
10% Clichy	0.53	0	0.03	0.05	7.6	21
20% Clichy	1.06	3.8	0.05	0.08	30	61
40% Clichy	1.3	7.8	0.11	0.19	48	67
60% Clichy	2.2		0.22		56	
CSO extractible	3.8	27 62%	0.57	1.03 84%		

Blanks contained 10 ppm of suspended solids (algae) and the 60% dilution about 32.

The filtered fraction of CSO waters may still decrease the net productivity after 5-fold dilution in the river. In such conditions 10–30% inhibition of production is expected. However, we could also demonstrate that the non-settleable fraction of the particulate fraction for CSOs would increase the inhibitory effect, up to 60%, for the CSO sample we studied. Given the importance of photosynthesis in the oxygen balance of man-affected, generally eutrophicated, streams, such toxicity effects should be included in models describing CSO impacts.

We found that EDTA addition significantly enhanced photosynthesis in solutions containing dilutions of CSO waters. Our results also show that free zinc ions contained in CSO waters produced an inhibition effect of about half of the total reduction of inhibition due to EDTA addition at the EC_{50} CSO dilution. This suggests that zinc may contribute significantly to the toxicity towards phytoplankton. Speciation computations suggest that lead and copper would not contribute much to the effluent's toxicity. However, given the extreme complexity of toxic effects in pollutant mixtures, contributions of individual chemicals to the total toxic effect should not be quantified before a very complete set of experiments are performed to assess pollutant interactions.

Acknowledgements

This study was funded in the frame of the PIREN-Seine program. Chlorophyll-a data have been kindly provided by SIAAP. The contribution of one of the anonymous referees to improving the English of the former manuscript is gratefully acknowledged.

References

Allen, H.E., Hall, R.H., Brisbin, T.D., 1980. Metal speciation, effects on aquatic toxicity. Environmental Science and Technology 14, 441–442.

Azeez, P.A., Banerjee, K., 1986. Effect of copper and cadmium on carbon assimilation and uptake of metals by algae. Toxicology and Environmental Chemistry 12, 77–86.

Bartlett, L., Rabe, F.W., Funk, W.H., 1974. Effects of copper, zinc and cadmium on *Selenastrum capricornutum*. Water Research 8, 179–185.

Bates, S.S., Tessier, A., Campbell, P.C.G., Buffle, J., 1982. Zinc adsorption and transport by *Chlamydomonas variabilis* and *Scenedesmus subspicatus* (Chlorophyceae) grown in semicontinuous culture. Journal of Phycology 18, 521–529.

Boet, P., Duvoux, B., Allardi, J., Belliard, J., 1994. Impact des orages estivaux sur le peuplement piscicole de la Seine à l'aval de l'agglomération parisienne. La Houille Blanche 1/2, 141–147.

Borchardt, D., Statzner, B., 1990. Ecological impact of urban stormwater runoff studied in experimental flumes: population loss by drift and availability of refugial space. Aquatic Sciences 52, 299–314.

Borchardt, D., 1993. A framework for the evaluation of ecological impacts of sewer overflows discharges in running waters. In: Proceedings of the 6th International Conference on Urban Storm Drainage, Vol. 1, 42–47. Niagara Falls, Canada, September 12–17, pp. 494–499, 1AWQ-1AHS.

Campbell, P.G.C., 1995. Interactions between trace metals and aquatic organisms: a critique of the Free-ion Activity Model. In: Tessier, A., Turner, D.R. (Eds.). Metal Speciation and Bioavailability in Aquatic Systems. John Wiley and Sons, New York, pp. 45–102.

Carlson, C.E.A., Morisson, G.M., 1992. Fractionation and toxicity of metals in sewage sludge. Environmental Technology 13, 751–759.

Chebbo, G., Bachoc, A., 1992. Characterization of suspended solids in urban wet weather discharges. Water Science and Technology 25, 171–179.

Chebbo, G., Mouchel, J.M., Saget, A., Gousailles, M., 1995. La pollution des rejets urbains par temps de pluie: flux, nature et impacts. Techniques, Sciences et Méthodes 11, 796–806.

Chevreuil, M., Granier, L., Chesterikoff, A., Letolle, R., 1990. PCB partitioning in waters from river, filtration plant and wastewater plant: the case for Paris (France). Water Research 24, 1325–1333.

Dauta, A., 1982. Condition de developpement du phytoplacton. Etude comparative du comportement de huit espèces en culture. 2. Rôle des nutriments. Annales de Limnologie 18, 263–292.

Davies, J.M., Williams, P.B., 1984. Verification of ^{14}C derived primary organic production measurements using an enclosed ecosystem. Journal of Plankton Research 6, 457–474.

De Filippis, L.F., Hamp, R., Ziegler, H., 1981. The effects of sublethal concentrations of zinc, cadmium and mercury on *Euglena*. Archives of Microbiology 128, 407–411.

Estebe, A., Mouchel, J.M., Thevenot, D., in press. Urban runoff impacts on particulate metal concentration in river Seine. Water, Air, Soil Pollution.

Errecalde, O., Seidl, M., Campbell, P.G.C., 1998. Influence of a low molecular weight metabolite (citrate) on the toxicity of cadmium and zinc to the unicellular green alga *Selenastrum capricornutum*: an exception to the free-ion model. Water Research 321, 419–429.

Even, S., Mouchel, J.M., Seidl, M., Servais, P., Poulin, P., accepted. Simulation des déficits d'oxygène dissous dans la Seine en aval de déversoirs d'orage à l'aide du modèle Prose. Annales de Limnologie.

Fraboulet, S., Mulliss, R., Flores-Rodriguez, J., Mouchel, J.M., Revitt, M., Garnier-Zarli, E., Thévenot, D.R., 1993. The use of metal bioindicators to assess the impact of combined sewer overflows on the river Seine. In: Proceedings of the 6th International Conference on Urban Storm Drainage, Vol. 1, Niagara Falls, Canada, September 12–17, pp. 500–505, 1AWQ-1AHS.

Frimmel, H., 1989. NTA und EDTA in Fliessgewaessern der Bundesrepublik Deutchland. Vom Wasser 72, 175–184.

Garnier, J., Billen, G., Hanset, P., Coste, M., 1992. Développement algal et eutrophisation dans le réseau hydrographique de la Seine. In: Rapport de Synthèse 1989–1992, Vol. I, CNRS PIREN Seine.

Guy, R.D., Kean, A.R., 1980. Algae as a chemical speciation monitor I. A comparison of algal growth and computer calculated speciation. Water Research 14, 891–899.

Hart, B.T., Currey, N.A., Jones, M.J., 1992. Biogeochemistry and effects of copper, manganese and zinc added to enclosures in Island Billabong Magela Creek, northern Australia. Hydrobiologia 230, 93–134.

Huang, V.W., 1994. Evolutions des métaux dissous dans la Seine. In: Rapport 1993/III Bassins versants urbains, CNRS PIREN Seine.

Huebert, D.B., Shay, J.M., 1992. The effect of EDTA on cadmium and zinc uptake and toxicity in *Lemna trisulca*. Archives of Environmental Contamination and Toxicology 22, 313–318.

IPL, 1996. Institut Pasteur de Lyon, Analyses physico chimique des sources Evian.

Karez, S.C., 1989. Les métaux et les algues marines: toxicité et accumulation du zinc et du cadmium chez *Crisophaera elongata* et

Acetabularia acetabulum. These de doctorat en toxicologie fondamentale et appliquée Université Paris VII.

Litchfield, J.T., Wilcoxon, F., 1949. A simple method of evaluating dose effect experiments. Pharmacology and Experimental Therapy 96, 99–113.

Loez, C.R., Topalian, M.L., Salibian, A., 1995. Effects of zinc to the structure and growth dynamics of a natural freshwater phytoplankton assemblage reared in the laboratory. Environmental Pollution 88, 275–281.

Magaud, H., Migeon, B., Morifin, P., Garric, J., Vindimian, E., 1997. Modelling fish mortality due to urban storm runoff: interacting effects of hypoxia and un-ionized ammonia. Water Research 31, 211–218.

Marchand, M., Caprais, J.C., Pignet, P., Porot, V., 1989. Organic pollutants in urban sewage and pollutant inputs to the marine environment. Water Research 23, 461–470.

Morisson, M.P., Revitt, D.M., Ellis, J.B., 1989. Metal speciation in separate storm water system USWQ conference. September 1989, Wageningen.

Niedrelehner, B.R., Cairns, J., 1992. Community response to cumulative toxic impacts: effects of acclimation on zinc tolerance of Aufwuchs. Canadian Journal of Fisheries and Aquatic Sciences 46, 2155–2163.

NIST, 1993. NIST Critical stability constants of metal complexes data base. In: Martell, A.E., Smith, R.M. (Eds.). National Institute of Standards and Technology, Standard Reference Database 46, Gaithersburg.

Paffoni, C., 1994. Caractérisation des eaux déversées par temps de pluie à l'usine de Clichy. La Houille Blanche 1, 33–38.

Payne, J.A., Hedges, P.D., 1989. An evaluation of the impacts of discharges from surface water sewer outfalls. Proceedings of the 2nd Wageningen Conference on 'Urban Storm Water Quality and Ecological Effects upon Receiving Waters', Wageningen University Press.

Peterson, R., 1982. Influence of copper and zinc on growth of a freshwater alga, *Scenedesmus quadricauda*: the significance of chemical speciation. Environmental Science and Technology 16, 443–447.

Reynolds, C.S., 1990. The Ecology of Freshwater Phytoplankton. Cambridge University Press, Cambridge.

Schescher, W.D., McAvoy, D.C., 1992. MINEQL$^+$: a software environment for chemical equilibrium modelling. Computers, Environment and Urban Systems 16, 65–76.

Seager, J., Abrahams, R.G., 1989. The impact of storm sewage discharges on the ecology of a small urban river. In: Proceedings of the 2nd Wageningen Conference on 'Urban Storm Water Quality and Ecological Effects upon Receiving Waters', Wageningen University Press.

Seidl, M., Servais, P., Mouchel, J.M., accepted. Organic matter transport and degradation in the river Seine (France) after combined sewer overflows. Water Research.

Seidl, M., Bourdier, C., Mouchel, J.M., 1993. Evolution des teneurs en métaux dans *Cladophora glomerata*. Mise en évidence de l'influence des déversements. in PIREN-Seine Report. 1992—Vol. IV. CNRS, France.

Tessier, A. and Turner, D.R. (Eds.), 1995. Metal Speciation and Bioavailability in Aquatic Systems. Wiley, New York.

Thomson, P.A., Couture, P., 1993. Physiology of carbon assimilation in green alga during exposure to and recovery from cadmium. Ecotoxicology and Environmental Safety 26, 205–215.

Tubbing, D.M.J., Admiraal, W. and Van der Meent, W., 1992. Complexes of copper inhibit bacterial metabolism. Second European Conference on Ecotoxicology (SECOTOX), Amsterdam, May 1992.

Wallen, D.G., 1990. The toxicity of chromium to photosynthesis of the phytoplankton assemblage of lake Erie. Aquatic Botany 38, 331–340.

Williams, P.B., Raine, R.C.T., Bryan, J.R., 1979. Agreement between the ^{14}C and oxygen methods of measuring phytoplankton production. Oceanologica Acta 4, 411–416.

Wolterbeek, H.T., Viragh, A., Sloof, J.E., Bolier, G., Van Der Veer, B., Kok, J., 1995. On the uptake and release of zinc in the growing alga *Selenastrum capricornutum* Printz. Environmental Pollution 88, 85–90.

Wong, P.T.S., Chau, Y.K., 1990. Zinc toxicity to freshwater algae. International Journal of Toxicity Assessment 5, 167–177.

Xue, H., Sigg, L., Kari, F.G., 1995. Speciation of EDTA in natural waters: exchange kinetics of Fe-EDTA in river water. Environmental Science and Technology 29, 59–68.