

Chemical and Microbiological Parameters in Fresh Water and Sediments to Evaluate the Pollution Risk in the Reno River Watershed (North Italy)

Chiara Ferronato, Monica Modesto, Iaria Stefanini, Gilmo Vianello,
Bruno Biavati, Livia Vittori Antisari

Department of Agricultural Science, Alma Mater Studiorum, University of Bologna, Bologna, Italy
Email: chiara.ferronato@unibo.it

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ABSTRACT

The European Water Framework (WFD) establishes a framework for the protection and the monitoring condition of all natural superficial waters of the member States. The Italian Legislative Decree n. 156/2006 implements the WFD establishing a monitoring system which foresees a detailed detection of several physical, chemical and microbiological parameters in order to assess the qualitative status of the water body. This study reports the freshwater quality in the Reno river basin (North Italy) from 2003 to 2011. The Reno superficial water was classified as “good” in the mountain stations and at the closed basin while in all the other stations of the Po plain the quality was from “mediocre” to “poor”. The decrease of water quality was due to the flowing of artificial canals that collect discharges the wastewater of sewage treatment plants, drainage and run-off from the urban, industrial and agricultural lands. In spring-summer 2011, characterized by severe drought, a study on the distribution of pollutants and nutrients in water of the Reno river and its tributaries highlight the impact of highway (Via Emilia) that closes the mountain basin of water courses. Along this street cities and industrial and craft have developed, increasing discharges of pollutants and nutrients in rivers. An increase of metals and nutrients was found from upstream to downstream, furthermore the concentration of the microbiological faecal indicators were two to three times higher than those determined in the water upstream of urban/industrial settlements. The thresholds of Italian Law for Hg and Pb were exceeding in all most rivers. The sediments analysis was also performed because they can be considered a sink and/or source for pollutants. In many monitoring sites the metals concentrations was higher than the thresholds of Italia Low (data not shown), but the availability of these metals was tested with mixtures of different strength extracting (EDTA, DTPA and water). The coefficient of partition solid/water (K_d) was calculated to evaluate the metals affinity to be in the aqueous phase and it increase as following Cr > Mn > Ni > Pb > Zn > Cu > Cd.

Keywords: Water Quality; Sediment Quality; Reno River; Italy; Pollutants

1. Introduction

The chemical, physical and biological pollution of surface water is a topic of great attention all over the world. Rivers play an important role since they collect municipal and industrial wastewaters but also they collect pollution from agricultural activities. Studies on water contamination have developed over the last 20 years with a view to monitoring and preventing water pollution. At international level there are different guideline systems for monitoring and assessing the quality of aquatic ecosystems. The European community, in the Water Frame-

work Directive 2000/60/EC (WFD) proposes an analytic method based on the detection of several physical, chemical and biological parameters in order to be summarized in a quality index. The European objective is to obtain a “good” quality status for all the natural superficial waters within the Member States by 2015. Chemical analysis of freshwater can quantify nutrients and pollutants in aquatic environments, but provides no direct indication of the potential toxic effects of these metals on aquatic biota. The excess nutrients may lead to various problems including an increase of algal bloom, loss of

oxygen, fish deaths and loss of biodiversity. Agricultural and urban activities are considered to be major sources of N and P in aquatic ecosystems [1-4], while metals are collected in rivers from a variety of sources, either natural or anthropogenic [5,6]. Pollutants may accumulate in microorganisms, aquatic flora and fauna and enter the human food chain. In the aquatic environment there is a continuous adsorption and desorption process between water column and bed sediment: studying these dynamics is the key to understanding the behavior of toxicants in a lotic system and its biological life. Because of different physico-chemical processes (e.g. adsorption, hydrolysis, co-precipitation) only a small portion of free metals are dissolved in water while a larger amount is deposited in sediments [7]. The ability of sediments to faithfully record the “environmental impact” on freshwater ecosystems over time is demonstrated [8-10]. Sediment provides habitats for benthic organisms, for microbial and fungal populations, and for all the species which reflect the quality and health of the ecosystem [11]. Contaminants are not necessarily fixed permanently by the sediments, and under changing environmental conditions they may be released into the water column by various processes of remobilization [12]. The marked tendency for heavy metals towards solid and water phase partitioning and the ability of sediments to integrate long-term information makes the sediments attractive for assessing the impact of industry and urban development on the fluvial environment [13,14]. Sediment contamination is indeed a worldwide problem, especially in industrialized countries, even though the response to this problem varies in terms of jurisdictions [15]. The Reno river is one of the most important water bodies of northern Italy to have affected the hydrographic system and development of the Po Valley (North Italy). The current configuration of the Reno basin is due to historical remediation (from the Roman Age to the early years of the last century). The protection of the hydraulic system has led to strong anthropogenic waterways to the detriment of their naturalness, and also the presence of industrial and urban settlements has similarly decreased the quality of the water. The aim of this work was therefore to evaluate the water quality of the Reno watershed from 2003 to 2008. Besides, to understand the dynamics of water pollution and sediment in the Reno basin, the Via Emilia, a main road on which the majority of civil and industrial settlements are located and which is crossed by 10^4 - 10^5 vehicles per day [16], was taken as a watershed of human activities. For this reason the variations of microbiological and chemical pollutants in the river Reno and its tributaries were studied up- and downstream of the Via Emilia during the period spring-summer 2011. In fact, in this season the rainfall is low and the rivers are fed only by wastewater from urban and industrial centers.

2. Material and Methods

2.1. Study Area

The basin of the river Reno has a total area of 4930 sq km. The territorial network composed by the river and its tributaries is the result of the countless conversions made since Roman times, in terms of extensive remediation and hydraulic protection. The reclamation of the plain has led to a radical change in the river basin where the surface water, beyond the Via Emilia, flows within artificial embankments that carry the river water to the Adriatic Sea. The mountains of Corno alle Scale (1945 m a.s.l.) and Monte Orsigna (1555 m a.s.l.) catch the watershed of the Reno river basin, with elevations varying from 1500 to 500 m a.s.l., and the territory is formed by a wide range of major and minor valleys arranged in a south-western and north-eastern direction. The hills finally meet the Via Emilia, where many towns and artisan industries have developed. The geology of the hills is characterized by gullies and outcrops of gypsum, while behind them the last stretch of the high-end plain, between 100 and 50 m a.s.l., consists of fluvial sediment that has given rise to the formation of cones. The area of the Via Emilia is characterized by alluvial deposits, formed by river waters over the centuries. The alluvial deposits (gravel, sand, silt) are of different sizes and these have formed cones in the direction of the plain. The plain itself is characterized by the vertical overlap of sedimentary bodies and was formed during the flooding of many rivers in the course of history.

The Reno river basin lies between the Apennines and the Adriatic sea, in a northern temperate zone, to the south-central border of the Po Valley.

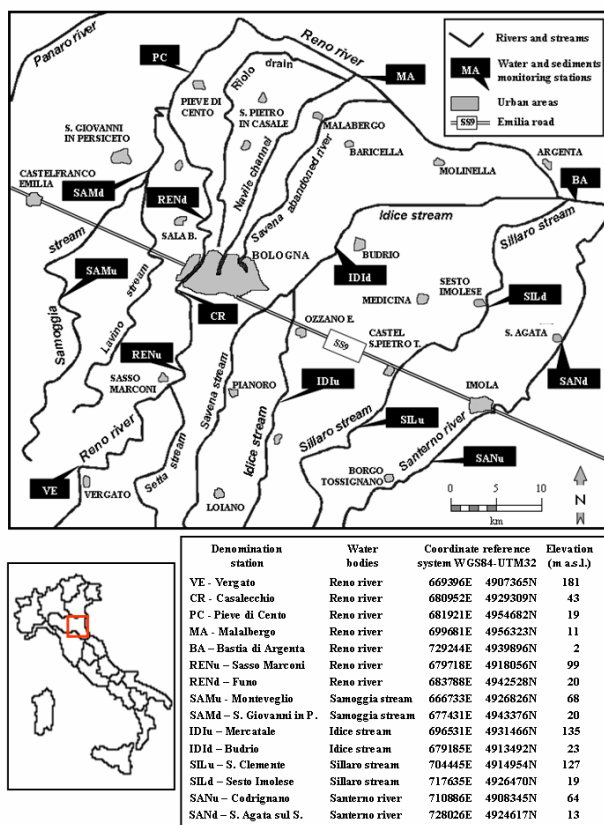
Table 1 shows the average rainfall in the mountain basin of the river Reno. The less rainy months were from May to August, while those of autumn-winter were characterized by higher rainfall.

2.2. Water and Sediment Sampling Survey

The water quality of Reno and its tributaries was monitored each month in different stations from 2003 to 2011, as established by Italian law (Legislative Decree 152/2006) by Agency of Environmental Protection of Emilia Romagna Region (ARPA-Emilia Romagna). The monitoring stations (**Figure 1**), starting from the Apennines (193 m a.s.l.) from upstream to downstream are: Vergato (VE), Casalecchio di Reno (CR, closure of the mountain basin—70 m a.s.l.), Pieve di Cento (PC, after the confluence of the artificial high water canals of the Dosolo and those of low water, and the stream Samoggia into the river Reno), Malabergo (MA, after the confluence into the river Reno of the artificial canal that passes through Bologna) and Bastia (BA, where the stream Idice and Sillaro flow into the Reno river). The Reno tributaries

Table 1. Average rainfall (1997-2010) in the mountain basin of the Reno watershed (data are expressed as mm of rainfall).

River	Pluviometric station	1	2	3	4	5	6	7	8
Reno (REN)	Porretta (352 m asl)	71	113	158	25	19	92	49	4
Samoggia (SAM)	Monte Ombraro (700 m asl)	49	67	117	28	25	110	65	0
Idice (IDI)	Loiano (741 m asl)	56	64	83	26	21	150	33	0
Sillaro (SIL)	San Clemente (166 m asl)	62	65	96	17	47	108	63	0
Santerno (SAN)	Fiorenzuola (476 m asl)	79	96	167	27	18	108	49	0

**Figure 1. Map of the Reno river basin and location of the sampling stations on Reno river and on some of its tributaries.**

flowing under the Bologna district (Samoggia (SAM), Idice (IDI), Sillaro (SIL) and Santerno (SAN)) were monitored only in one station of the Po plain upstream of the Via Emilia.

During spring-summer 2011, a new survey was performed. Water and sediment samples were collected from the stations of the river Reno and its tributaries (Figure 1) upstream and downstream of the Via Emilia. The sampling stations upstream of the road are situated within the hilly/mountainous morphology, characterized, as reported above, by evaporitic formations (Vena del gesso) and gullies. The downstream stations are characterized by alluvial gravel deposits in the western area and more silt-clay in the eastern area. On the plain human activity in-

creases and industrialization becomes heavier. The latitude and longitude coordinates of the monitoring stations are shown in Figure 1.

Water samples in 2011 survey were collected in glass bottles and kept refrigerated until analyzed and microbiological analysis was performed 24 h from the sampling. Bottles for chemical analysis were washed with diluted nitric acid to remove trace elements and then flushed with milli-Q water, while those for microbiological analysis were sterilized before use. Sediment samples (0 - 10 cm) were collected in sterilized plastic bags and kept refrigerated at 4°C until analysis. Microbiological analysis was performed 48 h from sampling, while for chemical analysis samples were first air dried. In Idice upstream station (IDIu) it was not possible to collect any sample because of the high percentage of gravel in the bed.

2.3. Chemical and Microbiological Analysis of Fresh Water Samples

Water samples were processed for the following analysis: electrical conductivity (EC), temperature and pH were measured in the field (Hach-Lange probe). The concentration of carbonate ion (HCO_3^-) was performed in laboratory by titration with 0.02 N HCl at the end point of pH 4.4. Dissolved organic C and N (DOC and DON) were determined by TOC analyzer (TOC-UV series, Shimadzu Instruments) on unfiltered samples.

The detection limits was 0.5 ppb for both total C and N. Major and trace elements were determined by *Inductive Coupled Plasma-Optical Emission Spectroscopy* (ICP-OES) (Ametek, Spectro) [17].

Anions (NO_3^- , NO_2^- , Cl^- , SO_4^{2-} , PO_4^{3-}) were determined by *Integrated Capillary High-Pressure Ionic Chromatography* (Dionex, ICS 4000 Thermo Scientific).

For microbiological analysis samples were first diluted (until 1:10000) in Phosphate Buffered Saline (PBS buffer, BR0014G, Oxoid) and filtered through nitrocellulose membranes (0.45 µm pore size, 47 mm diameter, Sartorius). Filters were placed on solid selective media for the detection and enumeration of fecal indicators (*E. coli*, *Enterococcus* spp.). Chromcult Coliformen Agar (1.00850, Merck) was used for *Escherichia coli* detection after in-

incubation in aerobic conditions at 44°C for 24 h. *E. coli* typically appears as blue/purple colonies whereas coliforms appear as red/rose colonies. Testing for indole production and citochrome oxidase activity gave further confirmation of the microorganism identity.

Slanetz & Bartley (1.05262, Merck), selective agar Wa used for *Enterococcus* spp. detection after incubation in aerobic conditions for 48 h at 37°C. Membranes with red-maroon or pink colonies were then transferred to plates with Bile Aesculine Azide Agar (100072, Merck) and incubated for 2 h at 44°C. Colonies that turned dark brown to black with a typical dark halo were considered to be fecal enterococci colonies.

Colonies were enumerated and the results were expressed in CFU/100mL, according to the following equation:

$$C = (A * N * V_s * F) / (B * V_t)$$

where $C = n.$ of colonies confirmed in 100 ml; $A = n.$ of colonies confirmed; $B = n.$ of colonies to confirm; $N = n.$ of colonies suspected; $T =$ volume of sample analyzed; $V_s =$ reference volume (100 ml); $F =$ dilution factor.

2.4. Chemical and Microbiological Analysis of Sediment Samples

Sediment samples were air-dried and sieved to 2 mm. Electrical conductivity (EC, Orion) was performed on the 1:2.5 ratio w:v with distilled and on the same ratio pH was determined by potenziometric pHmeter (pH metro, Crison). Total carbonate (CaCO_3) were quantified by volumetric method, according to Dietrich-Fruehing. Total C (TOC) was determined according to Springer and Klee (1954) methodology [18] while Total Nitrogen (TN) with Kjeldahl method [19]. The major and trace elements concentrations were carried out in aqua regia where 250 mg of sample, finely ground in agate mortar, were digested in microwave oven (Milestone, 1200) with 6 mL HCl and 2 mL HNO_3 , suprapure (Carlo Erba), brought to 20 ml with milli-Q water, filtered on Wathmann 42. The solution was detected by ICP-OES. For the available metals fraction, 2.5 g of sediment sample was extracted in 25 ml EDTA (ethylenediaminetetraacetic acid buffered to pH 4.65 with ammonium acetate and acetic acid) and 10 g were extracted in 20 ml DTPA (diethylenetriamine pentaacetic acid + TEA buffered to pH 7.3) [20]. The suspensions were shaken for 1 h and, after filtration with Wathman 42, the solution was detected for Cd, Co, Cr, Cu, Ni, Pb, Zn by ICP-OES. 10 g of sediment sample were extracted with distilled water (1:10 w:v) shaken for 16 h, centrifugated and filtrated with Millipore system at 0.45 μm ; the solution was analyzed by ICP-OES. The partitioning coefficient K_d (l/kg, [21]) was calculated according to the following equation:

$$K_d = C_s / C_w$$

where C_s is the pseudo total metal concentration extracted with aqua regia ($\text{mg}\cdot\text{kg}^{-1}$), and C_w is the dissolved metal concentration extracted with deionized water ($\text{mg}\cdot\text{kg}^{-1}$). Results were then expressed as log value.

Spores of *Clostridium* spp. were detected according to [22] with slight modifications. Briefly, 15 g of sediment sample were first placed in 135 ml of sterile Phosphate Buffered Saline (PBS buffer, BR0014G, Oxoid) plus Tween 80 (0.1%, V/V) and then stirred for 30 min to standardize the mixing. A further serial dilution (1:100) of each samples was heated at 85°C for 10 min to facilitate the sporulation. Each dilution (10^{-1} and 10^{-2}) was tested in quintuplicate by seeding 0.5 mL of the suspension in Sulfite Polymyxin Sulfadiazine Agar (110,235, Merck) and incubated in anaerobic conditions at 37°C for 24 ± 1 h. Suspected *Clostridium perfringens* black colonies were purified in Tryptone Soya Agar (1.05458, Merck) and identified by catalase production and biochemical profile (API 20 A, Biomerieux).

3. Results

3.1. Water Quality

The water quality of Reno river from 2003 to 2011 was classified as “good” in the mountain stations and at the closed basin (VE and CR, respectively) while in all the other stations of the plain the quality was “mediocre” and “poor”. This trend is clearly evident in the box plot constructed from the chemical and physical data of the time series (Figure 2). The deterioration of the water quality followed the increase in electrical conductivity (EC) and nutrient load and the first critical point was the PC station, after the closure of the basin, where Samoggia (SAM) river and the network of artificial drainage canals with a high pollutants load [23] flow into Reno. Similarly, in Bastia station (BA) high pollutants load were found after confluence of Sillaro (SIL), Idice (IDI) and artificial canals (Canale Navile and Savena Abbandonato) which cross the Bologna city and collect its municipal and industrial wastewater. The nutrients concentration were high with an average amount of NH_4^+ and PO_4^{3-} of 6 and 3 $\text{mg}\cdot\text{L}^{-1}$ respectively, showing an endpoint of 12 mg $\text{NH}_4^+\text{L}^{-1}$ and 7 mg $\text{PO}_4^{3-}\text{L}^{-1}$ (data not shown, authors’ communication). The percentage of dissolved oxygen decreases to 4% - 5% so that the quality of water in Reno was compromised. From station PC to BA Reno river was able to self-purify by pollutants, decreasing their concentrations at the Malabergo (MA) station as shown in Figure 2.

The self-purification capacity of Reno was compromised by the poor water quality of its tributaries than that of the artificial canals network (Table 2). The water quality of tributaries was classified “poor” every years according to Italian Law (D. lgs 152/2006). The values of EC in the water of the tributaries increase as follows:

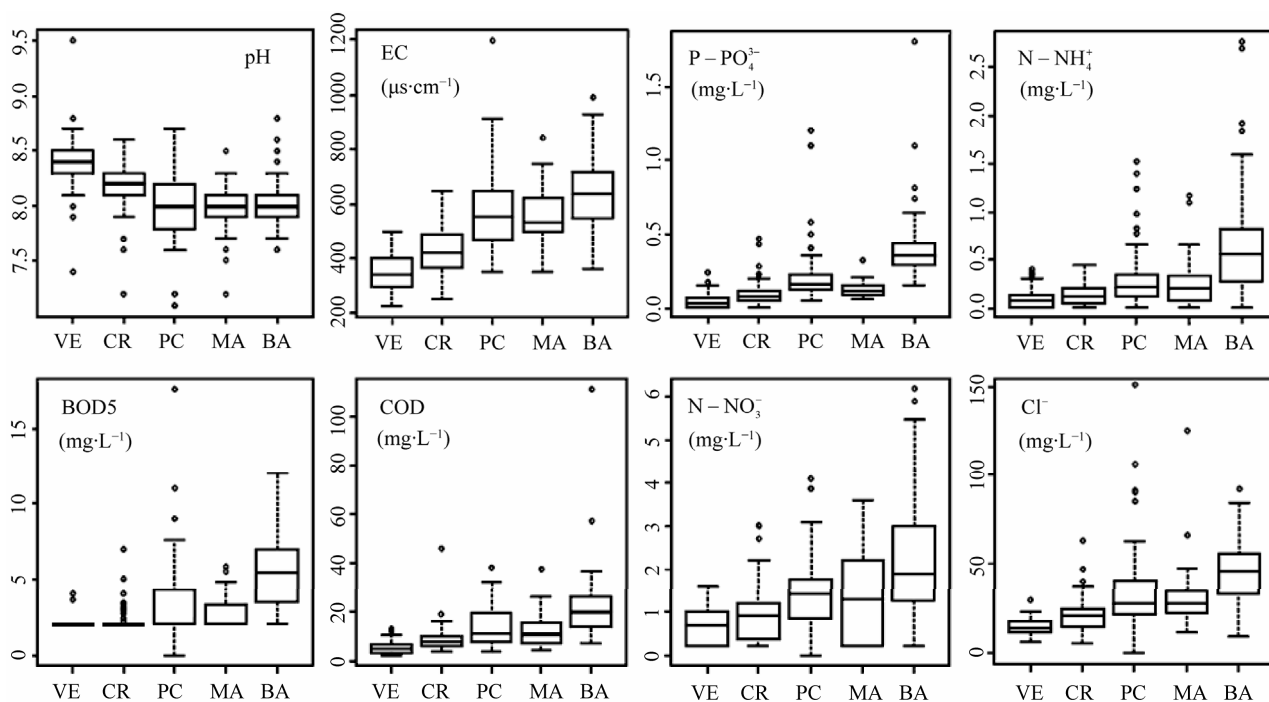


Figure 2. Boxplot of the historical trend in Reno river and its tridutaries. Data present mean, maximum and minimum value of some fundamnental parameters on water.

Table 2. Average, maximum and minimum concentration of water quality parameters of the Reno tributaries (rivers Samoggia, Idice, Sillaro and Santerno). Suspended solids (SS), total nitrogen (TN), ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), dissolved oxygen (DO), biochemical and chemical oxygen demand (BOD5 and CO5, respectively), total phosphorus (TP), orthophosphate (ORT-P), chloride and sulphate (Cl and SO_4^{2-}) are expressed as $\text{mg}\cdot\text{L}^{-1}$, Electrical conductivity (EC) as $\mu\text{S}\cdot\text{cm}^{-1}$. Data of microbiological parameters are expressed as log *Escherichia coli* (log ES) and *Enterococci* (log EN).

	Samoggia (SAM)			Idice (IDI)			Sillaro (SIL)			Santerno (SAN)		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
SS	147.9	2290.0	6.4	125.8	1362.0	7.0	1170.3	64632.0	31.0	50.0	460.0	4.0
CE	898.1	1305.0	570.0	805.5	1878.0	531.0	1120.8	1930.0	338.0	670.6	963.0	350.0
pH	8.1	8.7	7.3	8.2	8.8	7.8	8.2	8.6	7.3	8.2	8.7	6.9
TN	5.2	12.5	1.6	4.0	8.2	1.3	6.2	13.5	2.5	2.5	6.7	1.3
NH_4^+	0.8	3.7	0.0	0.3	1.3	0.0	0.4	3.1	0.0	0.6	5.8	0.0
NO_3^-	3.0	10.0	0.2	2.4	6.7	0.0	4.1	8.7	0.3	1.5	5.0	0.2
NO_2^-	0.1	0.5	0.0	0.1	1.9	0.0	0.1	1.0	0.0	0.1	0.8	0.0
DO	7.1	15.9	2.2	8.2	13.0	2.3	7.6	13.0	2.0	7.1	18.4	1.7
O_2	66.3	120.0	23.0	81.4	134.0	27.0	72.1	142.0	23.0	63.6	143.0	10.0
BOD5	6.2	32.0	2.0	4.0	12.0	2.0	2.7	11.0	2.0	3.4	13.5	2.0
COD5	22.2	70.6	6.0	18.1	54.0	8.0	14.5	87.0	4.0	15.6	77.0	5.0
ORT-P	0.2	0.7	0.0	0.1	0.4	0.0	0.0	0.3	0.0	0.1	0.6	0.0
PT	0.4	1.0	0.1	0.3	0.9	0.0	0.3	1.9	0.1	0.2	1.0	0.0
Cl	56.6	152.0	18.0	52.1	216.0	2.6	94.3	429.0	12.0	26.9	62.0	10.0
SO_4^{2-}	130.3	243.0	64.5	138.8	303.0	9.6	230.1	533.0	20.6	123.7	306.0	48.0
Log ES	3.9	5.0	0.0	4.0	5.3	0.0	3.7	5.1	0.0	4.5	5.7	0.0
Log EN	3.5	4.5	0.0	3.6	4.9	0.0	3.8	5.5	0.0	4.0	5.1	0.0

SIL > SAM > ISI > SAN while the highest nutrient load was detected in water of SIL and SAM streams.

The dissolved oxygen varied from an average percentage of 90 in SAN to 66 to 75% for the other tributaries. BOD5 and COD in SAM water were two and three times higher than the other fresh waters while high amount of chlorides and sulfates was detected in SIL stream. The water quality of both Reno and its tributaries was compromised by a high number of colonies of pathogen microorganisms. In particular the maximum number of colonies of both *Escherichia coli* and *Enterococcus* spp. in Reno was of 15,000 and 10,000 CFU 100 mL⁻¹ in the VE and CR stations, respectively, increasing to 68,000 and 63,000 CFU 100 mL⁻¹ in the stations on the plain (e.g. PC and BA). The presence of *E. coli* colo-

nies varies as follows: IDI (220,000 CFU 100 mL⁻¹) > SIL (127,000 CFU 100 mL⁻¹) >> SAM (11,800 CFU 100 mL⁻¹) > SAN (8000 CFU 100 mL⁻¹), while the average of the *Enterococcus* spp. colonies are 300,000 CFU 100 mL⁻¹ in SIL and 73,000 CFU 100 mL⁻¹ in IDI; lower values are found for SAM and SAN (7,800 and 600 CFU 100 mL⁻¹ respectively). The percentage of *Salmonella* spp. in the Reno water ranges from 21 to 31% of positive cases of pathogen presence, while in the tributaries it is as follows: SIL 20%, IDI 28%, SAM 31% and SAN 37%.

The results obtained by Spring-Summer 2011 survey were showed in **Table 3**. High amount of major anion (e.g. NO₃⁻, Cl⁻, SO₄²⁻ and HCO₃⁻), nutrient load (dissolved organic C, N, S and PO₄³⁻) and pathogen micro-

Table 3. Mean concentration of chemical-physical and microbiological elements in up- and downstream stations. Chemical parameters are expressed as mg·L⁻¹, CE is expressed as μS·cm⁻¹ and microbiological parameters are expressed as CFU 100 mL⁻¹.

Upstream stations		HCO ₃ ⁻	NO ₃ ⁻	NO ₂ ⁻	SO ₄ ²⁻	Cl ⁻	TDS	pH	CE	FC	<i>E. coli</i>	EN	DOM	DON	DOS	PO ₄ ³⁻
IDI u	Mean	112.9	2.19	0.12	28.73	54.41	339	8.2	644	246	598	242	2.64	0.58	4.02	0.44
	Max	137.2	3.69	0.44	47.85	60.33	437	8.4	830	1100	2000	540	3.93	0.73	11.49	1.04
	Min	91.8	1.04	0.01	13.14	42.14	192	7.9	365	10	130	76	1.73	0.33	0.10	0.01
REN u	Mean	71.7	0.98	0.02	15.41	17.06	168	8.1	319	44	512	69	2.22	1.40	2.55	0.04
	Max	76.3	2.08	0.03	25.15	29.56	242	8.5	460	100	2000	110	2.79	2.65	5.82	0.07
	Min	68.6	0.22	0.01	8.47	7.56	123	7.7	234	0	60	20	1.82	0.50	0.10	0.01
SAM u	Mean	106.9	6.55	0.03	33.66	51.50	392	8.1	745	1566	452	452	2.22	0.76	8.45	0.04
	Max	127.8	14.13	0.06	42.71	63.91	655	8.2	1245	7000	700	1270	2.83	1.05	32.09	0.08
	Min	87.2	1.28	0.01	24.11	45.18	241	7.8	458	80	90	112	1.53	0.38	0.10	0.01
SAN u	Mean	107.0	1.04	0.01	65.31	38.20	334	8.0	634	35	72	30	3.29	0.96	15.64	0.04
	Max	156.8	2.78	0.03	145.34	54.90	537	8.4	1020	90	200	80	4.39	1.22	41.40	0.07
	Min	75.4	0.01	0.00	11.36	22.96	225	7.3	429	0	3	11	2.74	0.67	5.29	0.01
SIL u	Mean	96.7	1.82	0.12	29.79	81.52	455	8.0	865	107	113	72	3.14	0.83	33.25	0.04
	Max	108.9	4.46	0.54	53.18	117.29	855	8.5	1625	400	200	103	5.00	1.27	107.35	0.05
	Min	76.9	0.01	0.00	14.14	17.28	183	7.6	347	12	21	11	2.22	0.30	0.10	0.02
Downstream stations																
IDI d	Mean	117.5	4.83	0.17	42.36	62.07	375	8.0	713	314	1471	739	3.14	2.58	6.95	0.19
	Max	151.4	7.14	0.38	86.18	66.83	484	8.1	920	1400	7000	3300	5.27	6.04	22.01	0.43
	Min	93.6	3.67	0.03	20.15	56.52	184	7.9	349	0	0	45	2.43	0.64	0.10	0.04
REN d	Mean	77.0	3.99	0.04	28.50	34.56	227	8.0	431	563	721	594	3.86	1.64	2.25	0.05
	Max	112.5	16.51	0.06	38.53	67.83	295	8.4	561	2200	2200	2000	5.63	2.91	7.41	0.09
	Min	61.5	0.47	0.00	21.72	22.10	132	7.4	250	20	7	0	2.04	0.57	0.10	0.02
SAM d	Mean	136.2	5.51	0.05	47.58	49.56	421	8.1	801	2780	2931	525	2.90	1.10	25.00	0.24
	Max	178.8	13.49	0.18	67.21	75.77	537	8.4	1020	13,000	13,000	2000	5.04	1.70	104.56	0.45
	Min	86.3	0.17	0.00	13.57	15.97	236	7.7	449	20	176	80	1.26	0.27	0.10	0.10
SAN d	Mean	109.3	3.89	0.06	73.83	51.11	410	8.0	780	1064	1280	199	3.90	1.75	9.30	0.25
	Max	150.2	5.19	0.19	143.82	69.99	650	8.5	1235	5000	5000	660	5.08	2.04	15.73	0.54
	Min	69.6	2.24	0.00	16.22	24.03	220	7.5	418	40	48	14	2.81	1.19	0.10	0.01
SIL d	Mean	135.7	22.21	0.17	112.65	111.57	657	8.1	1250	1720	12,772	3272	3.78	3.12	9.71	0.27
	Max	196.9	34.46	0.44	260.04	138.23	972	8.4	1848	6000	41,000	12,800	4.90	5.00	48.13	0.47
	Min	77.0	6.66	0.00	20.76	95.20	404	7.8	768	40	220	60	3.22	1.06	0.10	0.08

organisms (fecal coliforms, *E. coli*, *Enterococcus* spp.) as well as metals concentrations (Table 4) was clearly detected in downstream stations. The concentration of the microbiological fecal indicators was two to three times higher than those determined in the water upstream of urban/industrial settlements. The Reno river had the nutrients load lower than the other rivers while SIL had the higher load. In these two rivers Hg concentration higher than threshold of Italian law ($1 \mu\text{g}\cdot\text{L}^{-1}$), compromising their water quality which were classified “poor”. In all samples the Pb concentration in fresh waters were higher than legal threshold ($10 \mu\text{g}\cdot\text{L}^{-1}$).

3.2. Sediment Quality

The sediments of IDI, SAM and SAN had higher percentages of skeleton upstream than the plain stations, while these of REN and SIL had an homogeneous fine texture (Table 5). Total organic C (TOC) and total N (TN) range from 0.4 to $2.4 \text{ g}\cdot\text{kg}^{-1}$ and from 0.1 to $1.4 \text{ g}\cdot\text{kg}^{-1}$, respectively, whereby the C/N ratio was low (8 - 12), except for Reno (32 and 22). pH values range from 8.1 to 9.3 and the highest values are found in SAN and SAM downstream stations. The fecal contamination in sediments has been estimated by the content of *Clostridium* spp. spores

Table 4. Mean, minimum and maximum value of trace elements concentration on water in up and downstream stations. Data are expressed in $\mu\text{g}\cdot\text{L}^{-1}$.

	Idice (IDI)			Reno (REN)			Samoggia (SAM)			Santerno (SAN)			Sillaro (SIL)		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Upstream stations															
Al	4.9	14.9	0.3	84.5	285.0	1.4	4.9	14.2	0.3	11.9	23.2	0.3	30.2	87.8	0.3
As	3.0	7.9	0.1	1.7	5.0	0.1	1.4	6.6	0.1	3.0	9.6	0.1	2.1	5.2	0.1
B	175.3	237.0	87.5	66.7	130.0	41.4	225.0	305.0	142.0	260.9	462.0	62.4	371.6	634.0	175.0
Ba	53.7	84.5	23.4	43.9	68.2	19.5	33.1	47.7	16.3	56.8	89.0	25.9	48.2	92.3	14.5
Cu	6.4	16.3	1.0	4.7	9.1	1.0	5.0	9.2	1.0	4.6	8.9	1.0	4.5	9.1	1.0
Fe	15.7	32.3	2.2	15.4	34.8	4.0	7.7	20.0	1.3	24.0	53.8	3.6	21.0	51.5	2.3
Hg	1.0	1.6	0.5	1.1	3.5	0.0	1.0	1.7	0.0	0.4	0.7	0.0	4.6	17.2	0.4
Li	38.9	51.2	20.0	11.1	21.6	6.4	42.3	53.4	28.1	27.5	40.3	11.8	70.0	108.0	34.1
Mn	10.8	33.2	1.3	6.2	13.4	1.7	2.3	4.8	0.6	8.8	25.1	2.0	4.2	6.8	1.3
Ni	2.2	3.6	0.0	1.4	2.6	0.0	1.7	2.8	0.0	2.1	4.1	0.0	2.4	3.2	1.6
Pb	50.5	122.0	5.8	33.2	74.2	4.5	31.4	84.8	7.1	46.7	122.0	5.6	42.8	97.3	2.4
Se	5.5	12.4	1.6	3.3	6.5	1.6	3.0	9.1	1.3	4.6	11.8	1.6	5.3	9.3	1.6
Sr	780.2	1089.0	435.0	333.0	573.0	248.0	727.6	874.0	504.0	747.8	1067.0	398.0	977.0	1349.0	560.0
Zn	28.2	68.3	3.7	11.9	27.8	0.1	15.5	43.9	0.1	95.5	441.0	3.3	12.7	23.4	2.3
Downstream stations															
Al	5.5	13.9	0.3	39.7	80.9	8.6	6.9	17.5	0.3	15.1	34.3	2.5	6.5	17.8	0.3
As	3.5	8.9	0.1	3.1	6.1	0.1	5.8	11.0	0.1	4.5	10.4	0.1	4.2	12.5	0.1
B	167.4	201.0	101.0	84.4	129.0	47.9	279.4	340.0	199.0	153.0	204.0	66.2	319.6	393.0	206.0
Ba	51.5	75.2	17.5	53.5	86.5	25.7	59.0	89.1	25.6	56.6	98.0	18.9	58.4	86.1	22.4
Cu	7.9	15.7	1.0	4.1	6.4	1.0	4.2	8.2	1.0	6.2	14.5	1.0	4.7	8.6	1.0
Fe	31.6	58.8	2.4	28.6	47.4	7.5	20.3	32.4	5.5	30.6	52.7	4.8	12.9	25.7	3.0
Hg	0.9	1.3	0.4	1.0	2.5	0.3	0.9	1.5	0.5	0.4	0.5	0.0	2.0	6.3	0.6
Li	40.8	46.9	28.8	14.5	21.9	7.9	45.1	50.5	39.0	19.0	27.4	11.1	67.1	84.1	43.0
Mn	40.9	152.0	4.3	8.5	16.7	1.3	141.6	412.0	7.4	25.7	57.1	1.3	111.7	254.0	16.1
Ni	4.4	6.8	2.1	1.7	2.8	0.0	5.4	7.6	2.9	2.6	4.4	0.0	4.6	8.8	1.7
Pb	49.4	112.0	7.5	39.4	88.2	6.2	75.7	150.0	11.6	50.2	112.0	9.0	70.5	173.0	0.0
Se	5.0	11.0	1.6	4.5	7.7	1.6	7.1	13.7	1.6	4.7	8.8	1.6	6.7	16.0	1.6
Sr	851.4	1074.0	579.0	430.0	657.0	295.0	897.2	1135.0	640.0	596.4	889.0	349.0	1075.6	1384.0	694.0
Zn	18.6	30.9	3.3	12.7	19.0	3.3	17.5	24.0	8.3	18.8	51.9	0.1	11.6	17.5	0.1

Table 5. Sediment characterization by Skeleton (Sk) and fine earth percentage and some macro descriptors. EC is expressed as $\mu\text{S}\cdot\text{cm}^{-1}$, CaCO_3 and Total Organic Carbon (TOC) are expressed as percentage value. Total Nitrogen (TN), Total Phosphorous (TP) and Total Sulfur (TS) are expressed as $\text{mg}\cdot\text{kg}^{-1}$. *Clostridium* spp. (Clos) are expressed as $\text{CFU}\cdot\text{g}^{-1}$.

	Sk	Fine earth	pH	EC	CaCO_3	TOC	TN	TP	TS	Clos
IDI d	81.3	18.7	8.99	175	18.4	0.4	0.5	413	708	400
REN u	1.6	95.4	8.16	379	25.5	2.4	0.6	433	969	8000
REN d	5.8	94.2	9.22	140	20.5	0.6	0.3	216	362	700
SAM u	95.2	4.8	8.84	259	37.2	0.5	0.4	278	1382	800
SAM d	0.4	99.6	9.32	113	17.3	1.5	1.4	350	612	400
SAN u	59.5	40.5	8.54	334	31.3	0.3	0.3	334	629	1800
SAN d	4.3	95.6	9.32	113	17.3	1.5	1.4	764	910	400
SIL u	0	100	9.04	142	12.9	0.7	0.8	343	664	560
SIL d	4.1	95.9	8.1	539	20.1	0.1	0.1	343	1152	5000

which were higher in upstream Reno and SAN than the other river, an increasing trend was found in SIL. The coefficient of correlation (data not shown) showed that enrichment of *Clostridium* spores were influenced by increase of EC and total organic C and S and by decrease of pH value.

The metals concentration in sediments increased from upstream to downstream and in SAN they exceeded the threshold value for Pb and Sn (100 and $1 \text{ mg}\cdot\text{kg}^{-1}$, respectively) (Table 6). The percentage of available metals on total aqua regia determination decreased as a function of extracting solutions (Figure 3). As expected EDTA, an acid chelate agent (pH 4.65), extracted a greater amount than DTPA (pH 7.3), while deionized water was the weakest extract agent. The mobility of metals in the aquatic system was usually studied by the partitioning coefficient (K_d) from liquid phase and sediments. The logarithmic values of this coefficient ranged from 1.9 for Cd to 4.3 for Cr whereby the K_d decreased as follows: $\text{Cr} > \text{Mn} > \text{Ni} > \text{Pb} > \text{Zn} > \text{Cu} > \text{Cd}$.

4. Discussions

The water quality of the Reno basin is strongly influenced by human impact related to the very high density of inhabitants. When Reno river flows in the plain its bed is hanging and its river banks manifest a reduced biodiversity vegetation [23]; despite this, Reno river is able to implement processes of self-purification. The type of land cover can influence the water quality, which can improve greatly in forest areas [24] compared to agricultural land, where pollution is widespread. The riparian vegetation of Reno is able to decrease the pollutants and nutrients load between PC and BA stations, when no natural tributaries or artificial canals flow into its water, despite that the land-use of the plain is prevalently agricultural.

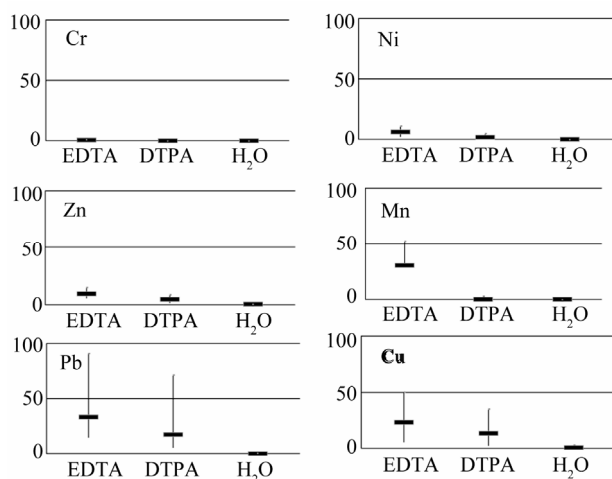
The Spring-Summer 2011 survey highlights how the settlements of the Via Emilia are the main cause of pol-

lution due to an increase of nutrients load, pathogens and contaminants [25]. The high concentrations of N and P downstream are due to urban wastewater discharge [7] rather than runoff from agricultural land [25]. In this recent years, a severe droughty in Spring and Summer time is characterized by low rainfall and increasing of the evaporation process, while the minimum vital outflow of rivers decreases and consequently the concentrations of pollutants increase. Indeed, under this latter condition the increase in EC was correlated to an increase in the nutrient load [26]. In this season only episodic storms that increase the soil losses due to soil erosion were observed, that fail to dilute the content of water pollutants. Temperature and rainfall can affect the some microorganisms growth and permanence such as *E. coli* and *Enterococcus* spp. [27,28]. The increase of pollutants load (e.g. Pb and Zn) in water can be expected with the reduction of flow, whereby the decrease of pathogens concentration can be due to high concentrations of metals (e.g. Hg and Pb) discharges in rivers. The enrichment of metals and nutrients concentrations in sediments, especially in those with the fine texture, highlights that the adsorption process is the prevailing self-purification mechanism [29]. As well as the sediments represent a memory of aquatic ecosystem [30], their role of sink of pollutants and nutrients is correlated to fine size (silt and clay) and iron and manganese oxides. The *Clostridium* spores in sediments indicate a fecal contamination [22] and their growth depends on the S and C content because they are sulphur reductive bacteria involving in the sulphur-compound demolition in anoxic environment [31].

The high concentration of metals in sediments can compromise the life of the aquatic ecosystem [11], but the pollution risk from metals depends on their chemical speciation rather their total elemental contents [32]. The extraction with EDTA and DTPA solutions reveals a different percentage of availability of the metals in sediments and the high amount extracted by EDTA is due to

Table 6. Total trace element concentration in sediment. Data are expressed in mg·kg⁻¹ on dry weight.

	As	B	Ba	Cd	Co	Cr	Cu	Mn	Ni	Pb	Sb	Sn	V	Zn
RE u	2.9	38	139	0.2	24	73	36	782	47	41	1.2	0.7	42	74
RE d	2.0	12	96	0.1	10	81	13	674	30	84	1.4	0.4	17	36
SAM u	3.0	35	207	0.1	20	54	26	1472	26	84	1.1	0.4	34	57
SAM d	4.0	36	191	0.1	23	102	27	817	47	41	1.5	0.7	43	62
SAN u	5.2	13	80	0.1	15	71	14	852	27	94	1.1	0.4	26	43
SAN d	4.3	48	300	0.2	32	111	31	847	38	171	4.7	2.0	40	108
SILL u	3.6	116	202	0.1	34	84	50	546	50	78	1.1	0.7	84	95
SILL d	4.2	36	275	0.1	22	45	30	905	35	98	1.1	0.2	36	71

**Figure 3. Mean, min and max value of some metal availability among EDTA, DTPA and water. Data are expressed as percentage on the total fraction determined by aqua regia.**

the acid pH [33,34], while the DTPA with neutral pH does not extract the metals immobilized in sediments [34,35]. High percentage of Pb and Cu are extracted by both solutions highlighting a greater pollution risk than the other metals which are poorly extractable. The same trends are found by the water-sediment partitioning coefficient (K_d) [21], in which relatively low values of $\log K_d$ of Cd (1.85), Cu (2.07), Pb (2.76) and Zn (2.35) suggesting that these metals are less likely associated with sediments and more free for transportation and mobilization in water, K_d higher than 2.8 suggesting a low geochemical mobility in water.

5. Conclusion

The mediocre and poor water quality determined in the Reno watershed is due to the anthropogenic impact due to the municipal and industrial waters discharged into fresh water rivers. The nutrient and pollutant loads affect the self-purification capacity of the Reno river. Adsorption of pollutants in sediments seems to be the main self-

purification mechanism while the low pathogenic contamination is related to severe drought during the spring-summer period that, lowering the river flow, will increase the concentration of pollutants in water.

The study of the release of pollutants in sediment-water interface is therefore a very important goal to increase the self-purification capacity. Sediment is the sink of nutrients and pollutants and their hazard can be evaluated by metals speciation and by their availability. The partition coefficient (K_d) of metals from water to sediment seems to be a good source of information about pollution risks. Therefore, the impact of anthropogenic activities on the fluvial ecosystems should be studied taking into account the water-sediment interface.

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