

Assessment of metal pollution in the Lambro Creek (Italy)

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ARTICLE INFO

Keywords:

Metals
Sediment
Water
Biota
Toxicity
Remobilisation

ABSTRACT

This study assessed the effect of metal pollution in the Lambro Creek (Southern Italy). Water, sediment and biota were collected at six sampling sites (June) for metal concentration assessment (Cr, Cu, Ni, Pb and Zn). Sequential extraction was performed to determine the distribution of metals in different geochemical sediment fractions. The influence of pH and leaching time on the release of metals from sediment to the water column was investigated via remobilization tests. A battery of toxicity tests (*Vibrio fischeri*, *Raphidocelis subcapitata*, *Phaeodactylum tricorutum*, and *Daphnia magna*) with multi-endpoints (bioluminescence, growth inhibition, and immobilization) was used to determine the overall toxicity in sediment water extracts. The results showed that metals did not exceed the probable effect concentration levels, with Cr concentration exceeding the threshold effect concentration level at all sampling points except for the one closer to the source of the creek, suggesting potential negative effect on the biota. Considering the cumulative criterion unit, sediment contamination was moderate at all sampling sites, except for L3 and L5 where biota was exposed to a very high risk. With respect to sequential analysis, the most readily available fraction of metal can be generalised as Ni > Cr > Cu > Zn > Pb. For better understanding the fate of metals in the water-sediment environment, their biogeochemical cycles should also be investigated in small creeks including both fresh (watercourse) and saltwater (river mouth) sediments.

1. Introduction

Metals enter streams through a variety of natural and anthropogenic sources (Balintova et al., 2016; Galdiero et al., 2015, 2016; Libralato et al., 2016a, 2016b; Satyro et al., 2017). Stream water quality is affected by materials from dry and wet atmospheric depositions, traffic and industry related activities, or released from roofs and building sidings, commercial and industrial discharges (Ball, 2002; Gasperi et al., 2010; Libralato et al., 2011; Race et al., 2016), including new emerging contaminants as well (Lofrano et al., 2016). Rainwater runoff discharged through the storm water drainage or combined sewer overflow systems not only influence the physico-chemical variables of the receiving water bodies, but could also adversely impact organisms living in the water column and bottom sediment (Barańkiewicz et al., 2014). Streams are also affected by metals and other pollutants from illegal discharges, such as domestic wastewater connected directly to a sewer without any legal permission (Komínková et al., 2016). Metal concentrations might serve as indicators for assessing sediment

contamination; however, it is not sufficient to assess their potential environmental impact because mobility, potential toxicity and bioavailability depend on the chemical forms in which they are present (Baran and Tarnawski, 2015). The behavior of heavy metals in urban streams is often influenced by various water and sediment characteristics such as pH, redox potential, salinity, temperature, content of organic matter, and particle size distribution (Caetano et al., 2003; Cantwell et al., 2002). A decrease in dissolved oxygen (DO) concentration and redox potential (Eh) caused by biodegradable organic substances in receiving waters may affect the release of metals from sediment to water. Changes in pH can strongly affect the remobilization of metals in water through precipitation/dissolution and adsorption/desorption processes (Ho et al., 2013; Race, 2017). Other quality indicators of freshwater ecosystem are macroinvertebrates, ensuring a wide range of sensitivities to changes in both water quality and habitats (Hussain and Pandit, 2012). Biota metal content is governed by bio-concentration dynamics (species-by-species specific) depending on seasonality, developmental stage, behavior, sex, and exposure

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conditions (Eggleton and Thomas, 2004). Accumulation of a tangible quantity of metals in aquatic biota may have dramatic consequences on the biodiversity and potential toxicological risk to human populations consuming contaminated food (Komínková et al., 2016).

Previous studies (Varol and Sen, 2012; Tang et al., 2013; Harguinteguy et al., 2013; Shafie et al., 2014) investigated the fate of toxic metals in large urban riverine systems, but their impact in small urban creeks is understudied (Hnatuková et al., 2009; Barańkiewicz et al., 2014; Komínková et al., 2016; Nábělková and Komínková, 2006).

The aim of this research was to assess Cr, Cu, Ni, Pb and Zn in water, sediment (i.e. considering various geochemical fractions), and biota (i.e. body tissues of macroinvertebrates) collected from the Lambro Creek (Italy) in order to try to fill the gaps to the knowledge about downstream transport, deposition and release under changing environmental conditions, and to look for optimal strategies for pollution control and water quality management. Remobilization tests were carried out to understand the influence of pH and leaching time variation on the release of metals from sediment to water. Toxicity tests integrated the final assessment of sediment water extracts (*Vibrio fischeri*, microalgae *Raphidocelis subcapitata* and *Phaeodactylum tricornutum*, and crustacean *Daphnia magna*).

2. Methodology

2.1. Study area

The Lambro Creek (Salerno province) flows into the Eastern Tyrrhenian Sea near Palinuro (Italy). Its catchment basin falls within Cilento e Vallo di Diano National Park. The creek is about 24 km long with a catchment area of approximately 300 km² and an average flow of 3.4 m³/s. The area could be suffering due to tourism pressure (especially during summertime), illegal dumping and residential areas, agricultural activity, and discharges from wastewater treatment plant (WWTP). Six sampling points (L1, L2, L3, L4, L5 and L6) were identified along the creek (Fig. 1) for the collection of water, sediment and macroinvertebrate specimens in June 2016.

Sampling points L1 (40°01'47.4"N 15°17'55.1"E) and L2 (40°01'46.8"N 15°17'55.3"E) are located along the coast in front of the creek mouth and in the mouth itself. L3 (40°01'59.2"N 15°17'51.9"E), L4 (40°07'36.2"N 15°19'00.0"E) and L5 (40°07'51.91"N 15°18'56.36"E) are located downstream from the local WWTP discharge. L6 (40°9'31.00"N 15°20'56.00"E) is close to the source of the creek.

Water samples were collected approximately from the midpoint of the creek, immediately below the water surface at a depth from 5 to 20 cm, according to Komínková et al. (2016), in acid pre-washed polyethylene (PE) bottles. Refrigerated samples were transported to the laboratory, filtered, acidified with HNO₃ (super grade), and stored at 4 °C for later analysis of metal content. Basic physico-chemical parameters such as pH, conductivity, DO, nitrate (N-NO₃⁻) and chemical oxygen demand (COD) were determined on the day of sampling.

Sediment sampling was carried out by collecting approximately 2 kg of bottom sediment, down to 5 cm depth, using a plastic scoop; from five to eight sub-samples were integrated in order to minimize the differences in sediment composition. Sediment samples were transported to the laboratory and air dried. Each dried specimen was homogenized and sieved into different fractions (1–2 mm, 0.5–1 mm, 0.3–0.5 mm, 0.15–0.3 mm, 0.015–0.15 mm and < 0.015 mm) through sequential sieving sessions. Considering the grain size distribution of sediment in urban creeks, the sediment fraction having < 0.5 mm size was chosen for chemical analysis, according to Race et al. (2015) and Komínková et al. (2016).

Macroinvertebrates were collected using the standard method of Three Minutes Semi-quantitative Kick Sampling (Storey et al., 1991). A hand net of 0.5 mm mesh size was placed near the bottom substrate, disturbed by the foot, and then removed from the bottom. Two different families of benthic organisms were selected from different sampling

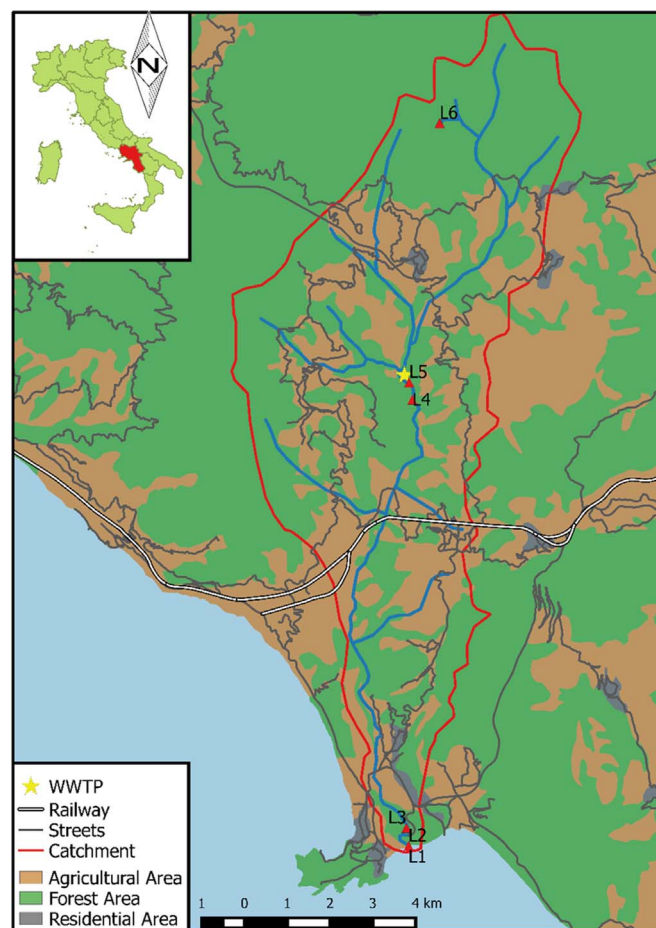


Fig. 1. Sampling points along the Lambro Creek L1–L6 and WWTP.

points according to their abundance in the creek. They were *Hydroptychidae* (Caddisfly) and *Baetidae* (Mayfly), which are filter feeders and scraper feeders (Ramirez and Gutierrez, 2014) respectively. L6 was considered as the reference site. Samples were preserved in ethanol in PE bottles sorted and freeze-dried (Christ Alpha 2–4 LSC plus).

2.2. Chemical analysis

2.2.1. Water

Water samples were filtered (0.45 µm pore size – cellulose nitrate membranes), acidified (super grade HNO₃), and stored at 4 ± 1 °C for later analysis. Samples were analyzed for Cr, Cu, Ni, Pb and Zn using an inductively coupled plasma mass spectrometer (ICP-MS) (NexION 350X, PerkinElmer, Inc.). A multi-parametric probe (Hach, Loveland, CO, USA) was used for pH, conductivity and DO field measurement. COD was determined using acidic dichromate solution followed by spectrophotometry, according to standard methods (APHA, 1998). N-NO₃⁻ was determined using ion chromatography (IC 761 compact by Metrohm Ltd., Switzerland).

2.2.2. Sediment and biota

Sediment samples were digested according to the US EPA 3051 microwave (MW) digestion method (Nábělková, 2005 mod.). A sediment mass of approximately 1 g was extracted with HNO₃ (67% v/v) and (30% v/v) in a volumetric ratio of 9:1. The mixture was digested in a microwave oven (Milestone START D) at 108 °C for 20 min. The digests were filtered after adequate cooling and diluted to 50 mL volume with deionized Milli-Q water. Filtrates were stored in PE falcons at 4 °C for later analysis. The partitioning of metals in specific geochemical fractions of sediment, which can be extracted selectively by using

appropriate reagents, was accomplished through sequential extraction according to the procedure proposed by the Community Bureau of Reference (BCR), described by Pueyo et al. (2008) as follows: i) (exchangeable and weak acid soluble fraction) 1 g of sediment sample was extracted with 40 mL of 0.11 mol/L acetic acid solution by shaking in a mechanical shaker at 30 ± 10 rpm at 22 ± 5 °C for 16 h; the extract was separated by centrifugation at 3000 g for 20 min, collected in PE bottles and stored at 4 °C until analysis; the residue was washed by shaking for 15 min with 20 mL of DI water and then centrifuged, discarding the supernatant; ii) (reducible fraction) 40 mL of 0.5 mol/L hydroxylammonium chloride solution acidified by addition of a 2.5% (v/v) 2 mol/L HNO₃ solution was added to the residue from the first step, and the mixture was shaken at 30 ± 10 rpm at 22 ± 5 °C for 16 h; the extract was separated and the residue was washed as in the first step; iii) (oxidisable fraction) 10 mL of 8.8 mol/L hydrogen peroxide solution was carefully added to the residue from the second step; the mixture was digested for 1 h at 22 ± 5 °C and another 1 h at 85 ± 2 °C till the volume was reduced to less than 3 mL; a second aliquot of 10 mL of H₂O₂ was added and digested at 85 ± 2 °C till the volume was reduced to about 1 mL; the residue was extracted with 50 mL of 1 mol/L ammonium acetate solution, adjusted to pH 2, at 30 ± 10 rpm at 22 ± 5 °C for 16 h. The extract was separated and the residue was washed as in the previous steps. Residue from the third step (residual fraction) was digested with 9 mL of HNO₃ and 1 mL of H₂O₂, according to the US EPA 3051 microwave (MW) digestion method (Nábělková, 2005 mod.). The organic matter content of sediment samples was determined gravimetrically by loss on ignition (LOI), in accordance with Schulte et al. (1991). Sediment samples of 8 g were heated initially to 105 °C in an oven and successively to 550 °C in a muffle furnace for 4 h at least in duplicate.

Biotic samples were digested according to the method described by Komínková et al. (2016). Organisms having a mass of 1 g after freeze-drying were treated with HNO₃ and H₂O₂ (9:1 mL) and digested in a microwave oven. After cooling, the digests were filtered, adjusted to 20 mL with deionized Milli-Q water and stored at 4 °C for further analysis. The concentration of metals in all extracts was determined using ICP-MS (NexION 350X).

2.3. Remobilization test

A remobilization test was carried out according to Page (1999). A sediment sample of approximately 3 g was transferred into a falcon tube. A volume of 30 mL (solid:liquid = 1:10) of distilled water adjusted at two different pH values (2 and 6, respectively) was added separately to the sediment sample. The mixture was placed in a horizontal shaking device and shaken at 220 rpm. After 15 min and again after 3 h, the suspension was sampled to determine the changes of metal concentrations in the aqueous phase. The time intervals are intended to simulate sudden rainfall events entering the river and causing a decrease in pH in a very short-time and potential for sediments to release metals and reach equilibrium after some hours (Ho et al., 2013). Komínková and Nábělková (2007) stated that this event could be relevant in small urban creeks, where the amount of water entering the river from the drainage system could be greater than the amount flowing into the water body. The supernatant was centrifuged (3000g for 20 min) and filtered (45 µm) for the assessment of metals as described above.

2.4. Ecotoxicological assessment and metal partitioning

Fresh sediment samples were elutriated (dry weight) mixing sediment and filtered river water from the spring area in a solid to liquid ratio of 1:4 for 24 h (shaking). Elutriates were filtered (0.45 µm) and stored at 4 ± 1 °C prior to further analysis.

The acute bioluminescence inhibition assay was carried out using *V. fischeri* (NRRL-B-11177), according to ISO (2007). The luminescence

was measured with a Microtox® analyzer (Model 500, AZUR Environmental) after 30 min at 15 °C. Tests were carried out in triplicate. Data were analyzed with Microtox Omni software and the result expressed as percentage of bioluminescence inhibition (%).

The chronic growth inhibition test with *Raphidocelis subcapitata* and *Phaeodactylum tricornutum* was carried out according to ISO (2012a) and ISO (2006), respectively. The cultures were kept in Erlenmeyer flasks. The initial inoculum contained 10^4 cells mL⁻¹. The specific growth inhibition rate was calculated considering 3 replicates exposed at 20 ± 1 °C for 72 h under continuous illumination (120–60 µein/m²s). Effect data were expressed as percentage of growth inhibition (Libralato et al., 2011).

Acute toxicity tests with *D. magna* were carried out according to ISO (2012b). Newborn daphnids (< 24 h old) were exposed in four replicates for 24 and 48 h at 20 ± 1 °C under continuous illumination (1000 lx). Before starting the test, they were fed with *P. subcapitata* (300,000 cells mL⁻¹) ad libitum. Toxicity was expressed as the percentage of dead organisms (Maselli et al., 2017).

Toxicity was expressed as the percentage of effect and, whenever possible, as the median effective concentration (EC50) along with 95% confidence limit values considering parametric or non-parametric methods. Two-way analysis of variance was carried out followed by Tukey's *post hoc* analysis after testing normality and homoscedasticity of data distributions. Statistical analysis and graphs were done using GraphPad Prism (Version 6.04 for Windows, GraphPad Software, La Jolla, CA, USA).

The partitioning behavior of metals between a solid matrix and water was expressed by the distribution coefficient (K_d), defined as a logarithmic ratio between pollutant concentration in sediment and its concentration in water, indicating the medium crucial for the risk assessment (EPA, 1999). K_d values of 3 and less identify metals occurring mostly in dissolved form, while K_d values above 4 refer to metals preferentially bound to sediments (Borovec et al., 1993). The value is calculated using the following equation:

$$K_d = \log \frac{C_s}{C_w} \quad (1)$$

where, C_s is the metal concentration in sediment (mg/kg) and C_w is the metal concentration in water (mg/L) at equilibrium.

The evaluation of metal concentrations in sediment was carried out by the cumulative criterion unit (CCU), evaluated as the following equation (Clements et al., 2000):

$$CCU = \sum \frac{C_s}{C_i} \quad (2)$$

where, C_s is the total recoverable metal concentration after *aqua regia* digestion of sediment. In more detail, calculation after digestion by HNO₃ and H₂O₂ was used (Florian et al., 1998; Nábělková, 2011) because the obtained concentrations (C_s) were comparable with differences up to 10%. The consensus based sediment quality guidelines (SQGs) for fresh ecosystems (MacDonald et al., 2000) benchmark criterion threshold effect concentration (TEC) was used as the criterion (C_i). This relation was applied for evaluation of levels of metal pollution at the sites: background (CCU < 1), low (CCU 1–2), medium (CCU 2–10), and high (CCU > 10) metal concentrations (Clements et al., 2000).

The evaluation of metal concentrations in the tissue of macro-invertebrates was assessed by the biota sediment accumulation factor (BSAF), according to Rand (1995), using the following equation:

$$BSAF = \frac{C_o}{C_s} \quad (3)$$

where, C_o is the metal concentration in tissue (mg/kg) and C_s is the metal concentration in sediment (mg/kg).

Table 1
Physico-chemical parameters of water samples from the Lambro Creek.

Sampling points	pH	Conductivity $\mu\text{S}/\text{cm}$	DO mg/L	COD mg/L	N-NO ₃ ⁻ mg/L
L6	8.2	271	8.8	15.5	0.70
L5	8.5	318	10.26	50.5	1.27
L4	8.9	318	9.97	34	0.99
L3	8.9	489	13.01	31	0.98
L2	7.9	565	10.02	24	2.02
L1	8.2	539	9.94	–	–

3. Results and discussion

3.1. Water

Water quality data are summarised in Table 1. Conductivity was within a range of 271–567 $\mu\text{S}/\text{cm}$ and showed an increasing trend along the creek from the source to the mouth. The lowest value of conductivity was at the source of the creek (L6) while the highest value was measured at the mouth, mainly due to saline water intrusion. DO values ranged from 8.8 to 13.01 mg/L. The lowest value was obtained in the source area, despite the absence of evident indications of anthropic contamination, but in the presence of high rates of decomposing vegetation (namely natural organic matter, NOM). COD was within the range of 15.5–50.5 mg/L. The highest concentration of COD (50.5 mg/L) was measured at the sampling point immediately below the WWTP discharge (L5) and decreased downstream. N-NO₃⁻, in turns, ranged from 0.7 mg/L to 2.02 mg/L: the highest value was obtained at the mouth, possibly due to the presence of NOM and anthropogenic pollution, the high value of N-NO₃⁻ at the creek mouth allowed this zone to be classified at risk of eutrophication (Paerl, 1997).

The amount of metals (Cr, Cu, Ni, Pb and Zn) in water is shown in Fig. S1 (Supplementary Materials). The values were analyzed considering, as a reference, the background median concentration of Italian stream water (De Vivo et al., 2008). The results revealed that Cr in L1–6, Ni in L2, L3 and L5, Pb in L5, and Zn in L3, L4 and L5 could pose an acute hazard to the aquatic biota, its concentration being above the background levels. The discharges of local WWTP and storm water runoff from a busy road and parking area near a recreational area might have increased Pb and Zn concentrations in L5 and L3, as suggested in Polkowska et al. (2001) and Fu and Wang (2011).

3.2. Sediment

3.2.1. Characterization

The grain size distribution of bottom sediment from the Lambro at various sampling points is shown in Fig. S2 (Supplementary Materials). The results showed that the coarser fractions (1–2 mm and 0.5–1 mm) were dominant at all sites. The fine sand fraction (0.15–0.3 mm) was the highest at sampling points L5 (5.45%) and L6 (9.8%); however both sites had sediment mostly containing coarse fraction within a range of 85.3–92.9%. Various authors (Fernandes et al., 2011; Saeedi et al., 2013; Race et al., 2015) reported the increase in binding capacity of metals in sediment fractions with decreasing grain size. The finest fractions are the main support for metal binding to sediment and the presence of OM can influence their mobility due to the adsorption of cations onto negatively charged groups (Lin et al., 2002; Du Laing et al., 2009; Baran and Tarnawski, 2015).

Sediment OM content was found within a range of 0.5–4.6% (Table 2) showing high correlations with clay and silt fractions ($r = 0.954$, $p < 0.01$) considering that the concentration of OM can be significantly affected by local conditions such as morphology and hydrology of the creek as well as the presence of vegetation (Komínková et al., 2016).

The total concentration of metals in sediment samples is presented

Table 2
Mean values of toxic metals (in mg/kg), OM content (in %) in sediments from the Lambro Creek and metals concentration expressed in CCU.

Sampling points	Cr	Cu	Ni	Pb	Zn	OM	CCU
L6	33	5	38	3	62	4.6	3.2
L5	48	10	43	4	51	1.5	3.8
L4	71	16	45	5	65	2.2	4.8
L3	99	15	44	4	56	0.9	5.3
L2	95	14	40	3	47	0.5	4.9
L1	107	14	37	4	40	0.8	5.0
TEC	43.4	31.6	22.7	35.8	121	–	–
PEC	111	149	48.6	128	459	–	–
BV	28	58	20	51	82	–	–

TEC-Threshold effect concentration, PEC-Probable effect concentration, CCU-Cumulative criterion unit, BV-Geochemical background values for stream sediments of Campania region, Southern Italy (Albanese et al., 2007).

in Table 2. Detected metals were tallied with the consensus based sediment quality guidelines (SQGs) for fresh ecosystems (MacDonald et al., 2000) to estimate the degree of contamination. Threshold effect concentration (TEC) and probable effect concentration (PEC) are the indices of SQGs describing the contaminant concentration below which no adverse effects are expected to occur and above which adverse effects are expected to occur more often. The measured concentrations of studied metals did not exceed PEC levels. However, the concentration of Cr exceeded TEC levels at all sampling points, except for L6, indicating potential negative effects on the biota. Marine sediment showed higher concentrations of Cr and Ni compared to TEC levels. This result was probably related to the contribution from the washout of Cr and Ni released by wear from vehicle brake lining and asphalt road surfaces ending up in the sea (Ball et al., 1998). Sprovieri et al. (2006) reported a concentration of 31.7 and 29.1 mg/kg for Cr and Ni, respectively, for marine sediment collected in the southern area of Capo Palinuro (Italy), which is in the proximity of L1. Cr and Ni were higher than the background level at all sampling points. The creek presented low concentrations of metals that are generally widespread in other urban streams, such as Cu, Pb and Zn. The concentration of Cu, Pb and Zn was always below the background level established for stream sediments of the Campania region (Albanese et al., 2007). The results of CCU (Table 2) demonstrated the medium level of sediment contamination at all sampling points according to the classification by Clements et al. (2000). At L3, biota was exposed to a higher risk compared to all other sampling sites. The medium level is presumably characterized by higher mortality of sensitive species and changes in macroinvertebrate diversity (Clements et al., 2000).

The percentage distribution of metals in the different sediment fractions are presented in Fig. 2. The bioavailability and toxicity of metals can be derived from the specific chemical forms in which they are present and their binding state. Metals bound to the first three fractions (exchangeable, reducible, and oxidisable) of the sequential extraction analysis are susceptible to release from sediment to water in the case of variation in environmental conditions (Race et al., 2015; Baran and Tarnawski, 2015).

The (bio-)availability of metals in the most readily available fraction can be generalised as Ni > Cr > Cu > Zn > Pb. The exchangeable fraction of Ni was almost constant downstream in the creek. Cr bound to exchangeable and acid soluble fraction showed a drastic increase in L5 and L6. The distribution of Cr in marine sediments demonstrated the highest concentration in the oxidisable fraction, in contrast with the results obtained by Usero et al. (1998), which have the highest Cr (more than 79%) bound to the residual fraction of sediment from Huelva estuary (Spain). For comparison, Soliman (2012) reported from sediment collected from the Egyptian Mediterranean coastal area with values of 2%, 8.1%, 3.6% and 86.1% of Cr bound to exchangeable and acid soluble, reducible, oxidisable, and residual fractions, respectively.

Cu within the exchangeable fraction increased from a negligible

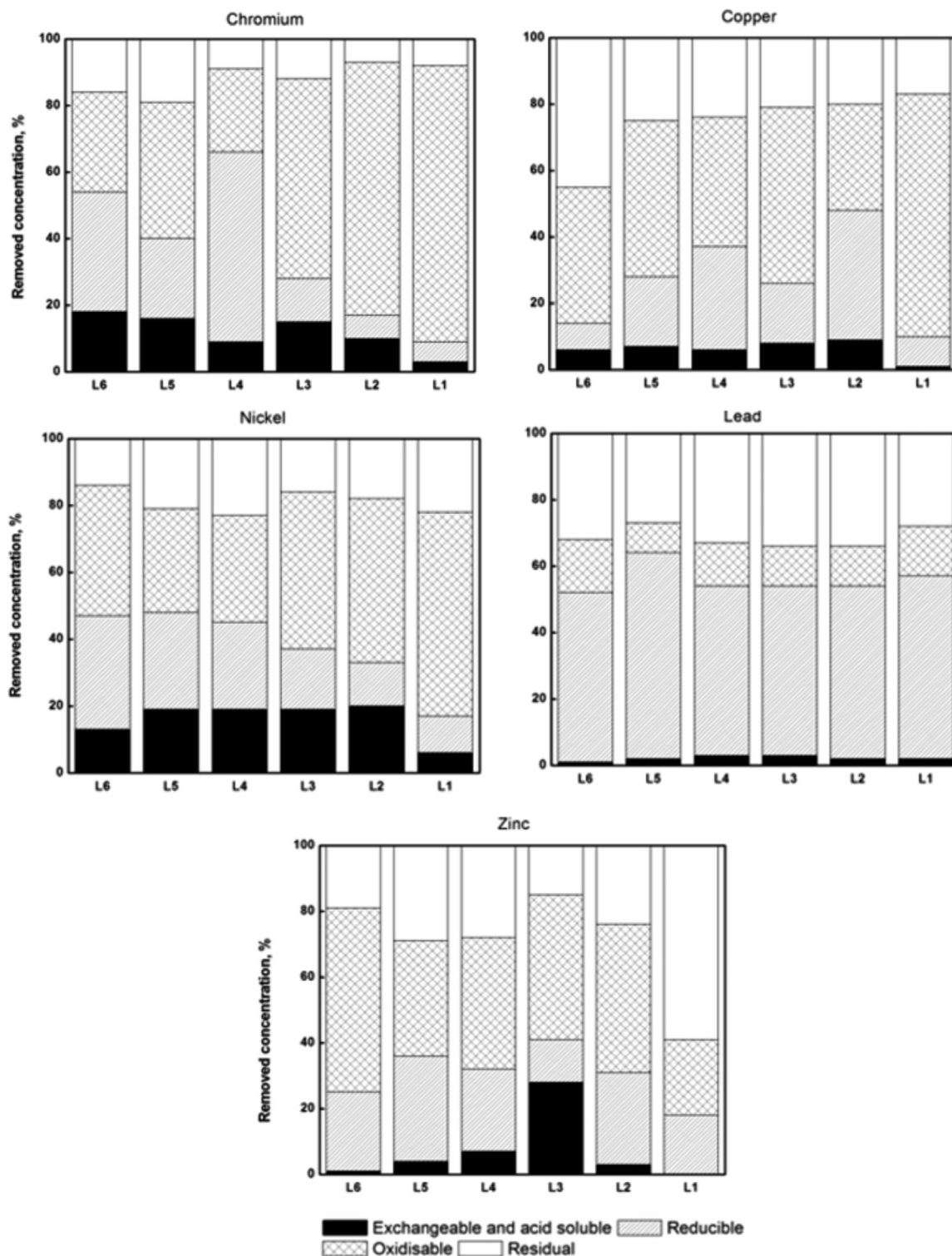


Fig. 2. Proportions of metals bound to geochemical fractions of sediment from study creek.

amount (below the detection limit) up to 9% of the total concentration. Cu is predominantly found retained in sediment fractions with a high amount of organic matter because of the high stability constant of organic Cu compounds (Morillo et al., 2004; Reis et al., 2013; Komínková et al., 2016). Zn was the most available metal with the highest proportion bound to the most readily available exchangeable fraction at L3. Higher Zn concentration can be assigned to the higher frequency of human and vehicular activity near L3, as is located near the recreational park purposes. Zn associated with oxidisable and residual

fractions constituted 23–56% and 15–59%, respectively. The exchangeable amount of Zn in marine sediment was almost negligible, while 59% of total concentration was associated with the residual fraction. Similarly, Pb was associated predominantly with a reducible fraction ranging from 51% to 62% of the total concentration due to a high tendency to form complex compounds with Fe/Mn oxides in sediment (Xiangdong et al., 2001).

The analysis of distribution coefficient (K_d) revealed that L3 and L5 had the highest concentration of Zn in the dissolved form with K_d values

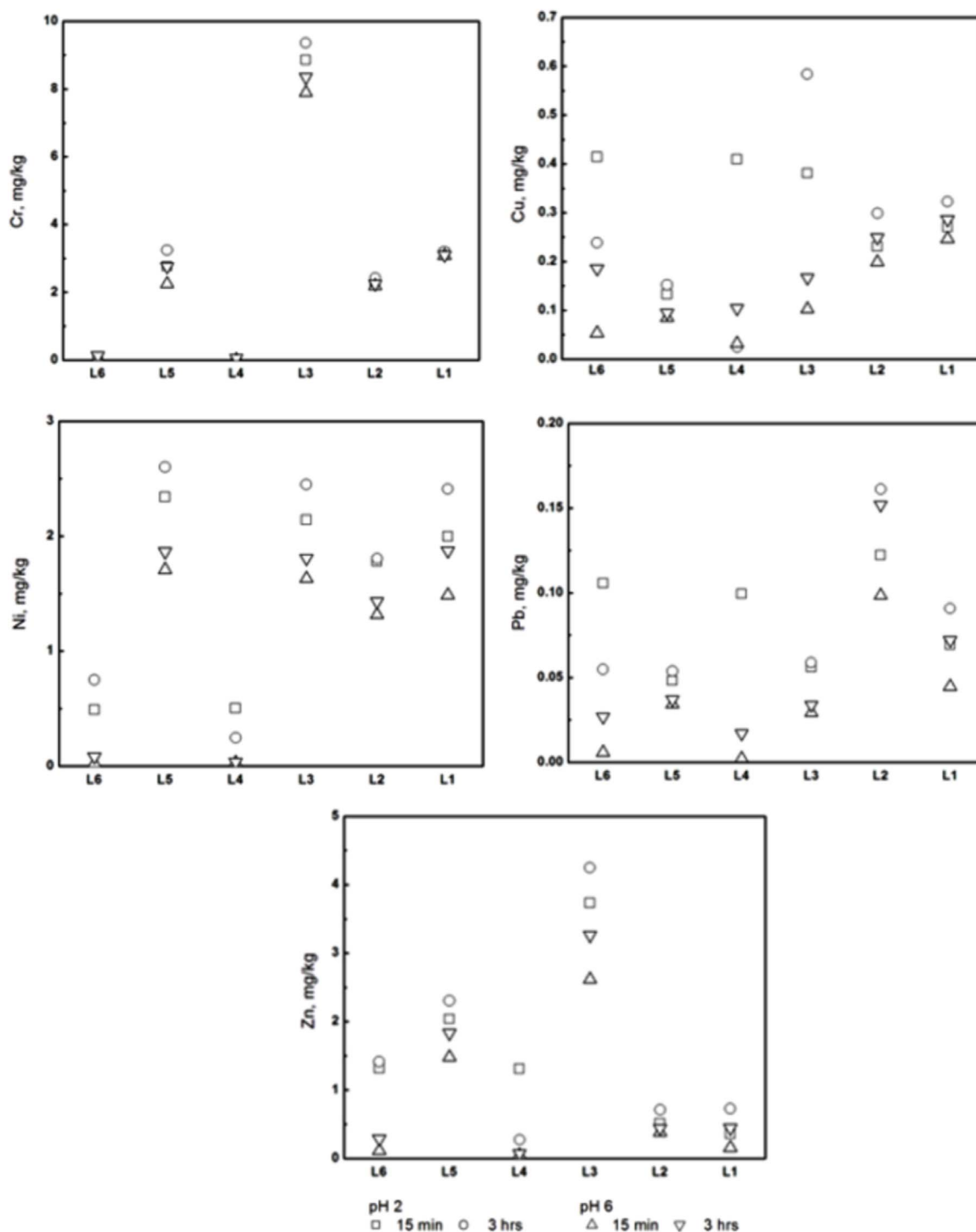


Fig. 3. Remobilization of metals from contaminated sediment samples.

of 2.9 and 3.2, respectively. The values of K_d obtained for Cu, Cr and Ni indicated that these metals are preferentially bound to sediments. Similarly, K_d value of 3.3 for Pb at L5 indicated a preferential partitioning into the water compartment, posing potential risks to the biota.

3.3. Remobilization of metals

The influence of pH variation on the mobility of metals in the water-sediment environment was tested through lab-based remobilization experiments. The time-dependent leaching behavior of studied metals at two different pH values and time intervals is shown in Fig. 3. The

results showed that Cr partitioned into water in given conditions. The highest Cr release of 9.3 mg/kg was obtained at pH 2 after 3 h in L3 sediment. A similar concentration of Cr was bound to the exchangeable fraction of sediment from L3, further supporting this result. Cr release from sediment of creek mouth (L2) and marine coast (L1) showed the negligible influence of pH and leaching period. Similarly, negligible amounts of released Cr (< 0.02%) were evident for sediment from L4 and L6. Li et al. (2013) reported a decrease in the release of Cr with increasing leaching time after the first 30 min. In the present study, a higher release of Cr (up to 9.45%) was evident from sediment leaching up to 3 h.

The release of Cu was evidently higher at L3 after 3 h of leaching at pH 2; increasing the leaching time from 15 min to 3 h showed a decreased release of Cu at pH 2 for sediment from L4 and L6. Ho et al. (2013) reported higher mobility of Cu at pH 2–8 as a result of significant transfer from the oxidisable fraction to reducible fraction under the effects of oxidation. The highest release of Zn was observed from sediment of L3 at pH 2 leached up to 3 h. Lower release of Zn was observed in L1 by a negligible amount bound to exchangeable fraction. Komínková and Nábělková (2006) reported that the highest release of Zn occurred at decreasing pH values up to 2, which is in agreement with the present study. Similarly, Ho et al. (2013) reported the highest leachability of Cd, Cu, Pb, and Zn at pH 2, while minimum amounts were released at pH 6 for Cu (< 0.15%) and Pb (< 0.003%). A decreasing concentration of Zn in surface water of the Smolnik Creek (Slovakia) as pH increases to 6 was reported by Balintova et al. (2012).

The highest Ni release was observed at L1, L3 and L5 for 3 h leaching at both pH 2 and 6. Ni release from sediment into water at most of the sampling points is supported by almost constant concentration of Ni bound to exchangeable fraction of sediment. Concentration of Ni within a range of 1.4–2.4 mg/kg was leached from marine sediment. The highest concentration of Pb released was observed at L2, whereas the lowest release was observed at L3. From Fig. 3, the release of Pb was dependent on pH and leaching time in L1, L2 and L6, which is in agreement with the results from Centioli et al. (2008) who evidenced a decreasing pattern of Pb release with increasing pH value.

Sediment content of metals was shown to be mainly related to grain size, pH, EC and OM (Table 3). Inter-elemental association of Cu/Cr ($r = 0.815$, $p < 0.05$) showed significant correlation. Elemental association revealed that the paired elements had a similar source or common sink in the stream sediments (Sekabira et al., 2010). Association between metals and physico-chemical parameters highlighted significant correlation between EC/Cr ($r = 0.920$, $p < 0.01$) and pH/Ni pair ($r = 0.823$, $p < 0.05$), whereas the rest were not significantly correlated. The results showed that OM was significantly negatively correlated to Cr ($r = 0.834$, $p < 0.05$). Likewise, Cr and Cu were negatively correlated to the silt and clay fraction of sediment (Cr with $r = 0.915$, $p < 0.01$ and Cu with $r = 0.923$, $p < 0.01$). Silt and clay fractions were positively correlated to OM ($r = 0.954$, $p < 0.01$) and negatively correlated to EC ($r = 0.809$, $p = 0.05$).

3.4. Biota

Metal accumulation in macroinvertebrates is presented as BSAF in Fig. S3 (Supplementary Materials). BSAF greater than 1 indicated bioaccumulation of metals to levels that are higher than sediment ones. The results showed that *Hydropsychidae* accumulated a higher level of Zn at L5 (site just below the municipal WWTP), in agreement with the results from Komínková and Nábělková (2006) who reported BSAF for Zn > 2 for *Hydropsychidae* found in a creek affected by combined sewer

overflows. A significant decrease in BSAF for Cu was observed at L5, while an increase in BSAF for Zn at L3 was observed, possibly due to the impacts of human activity (i.e. parking area). Higher accumulation of Cu in L4 could be related to the content of sediment OM compared to other sampling sites, except for L6 (Table 2). Similarly, the increase in accumulation of Zn at L3 and decrease in accumulation of Cu at L5 could be addressed to the presence of higher [Zn] and lower [Cu], respectively, in the exchangeable sediment fraction. Moreover, K_d values determined above and below 4 for Cu and Zn at L5 and L3, indicated the possibility of the mentioned accumulation pattern for Cu and Zn.

Another organism sampled was *Baetidae* (Mayfly), which belongs to the scraper feeding group and consumes periphyton (Ramirez and Gutierrez, 2014). The highest accumulation of Cr in tissues of *Baetidae* was observed at L5, followed by Zn and Cu. Canivet et al. (2001) explained higher accumulation of Cr as the result of its ability to reduce from Cr^{6+} to Cr^{2+} , which tends to bond to biological macromolecules as it gets absorbed by organisms, and which becomes difficult to excrete again and remains accumulated in organisms. Moreover, BSAF of Zn was found to be higher than 1 at L4.

3.5. Ecotoxicity

All toxicity data are reported in Fig. 4. No significant toxicity was measured in the marine sediment samples (L1 and L2) with *P. tricornutum* and *V. fischeri*. L2 showed biostimulation effects in *P. tricornutum* ($p < 0.05$ compared to the control groups) indicating potential eutrophication (Libralato et al., 2016a, 2016b). After 30 min of exposure, *V. fischeri* test revealed that bioluminescence inhibition tended to increase immediately above and below the WWTP discharge point, with the maximum effect detected in L6 (40%). The differences in bacterial toxicity may be related to the particular composition of sediment and different chemical association of sediment particles. In this respect, organic matter content can be traced as a possible candidate for sediment toxicity (Jarque et al., 2016). Higher organic matter content at L4–6 could be a possible reason for increase in bioluminescence inhibition at these sites. Reduction in *R. subcapitata* growth followed the same trend of luminescence results, but toxicity was more pronounced: L5 was the most toxic (96% of growth inhibition), while L4 (downstream from the WWTP discharge) showed < 64% of the effect. The finding that the maximum toxicity to algae was at L5 suggests that the discharge of toxic metals from WWTP caused the growth inhibition. Similarly, exposure to L6 elutriate showed an algal growth inhibition of 79%. Outcomes from *D. magna* tests highlighted no immobilization for L3, L4 and L5 elutriates, except for L6 (30%). Significant correlations were observed between bioavailability of metals, OM, and toxicity of elutriates. Toxicity of L6 sediment elutriate could be associated to the high Cr mobility.

Table 3
Pearson correlation coefficient for toxic metals and physico-chemical parameters.

	Zn	Cr	Cu	Pb	Ni	pH	EC	DO	OM	SC
Zn	1									
Cr	-0.593	1								
Cu	-0.211	<i>0.815</i>	1							
Pb	0.268	0.163	0.600	1						
Ni	0.536	0.041	0.462	0.655	1					
pH	0.573	-0.013	0.383	0.802	<i>0.823</i>	1				
EC	-0.753	0.920	0.594	-0.209	-0.270	-0.352	1			
DO	-0.045	0.535	0.551	0.279	0.554	0.588	0.403	1		
OM	0.683	<i>-0.834</i>	<i>-0.782</i>	<i>-0.200</i>	-0.188	0.006	<i>-0.814</i>	-0.562	1	
SC	-0.543	-0.915	-0.923	-0.352	-0.247	-0.118	<i>-0.809</i>	-0.568	0.954	1

R values derived from Pearson's correlation are reported in bold (with $P < 0.01$) and italic (with $P < 0.05$); SC-silt and clay fraction; EC-electrical conductivity, DO-dissolved oxygen; OM-organic matter.

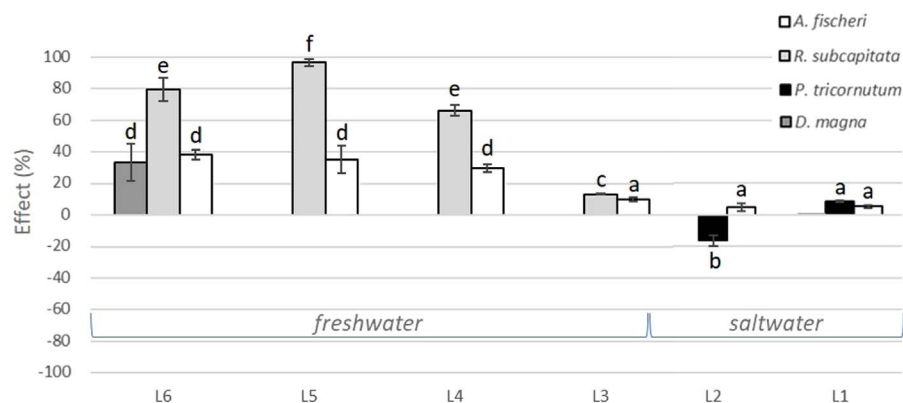


Fig. 4. Toxicity effects of L1-L6 samples; L1-2: toxicity on saltwater species (*V. fischeri* and *P. tricornutum*); L3-6: toxicity on freshwater species (*V. fischeri* (i.e. procedures for salinity adjustment), *R. subcapitata* and *D. magna*); samples with equal letters presented toxicity effects not significantly different ($p < 0.05$).

4. Conclusions

This study showed the impact of municipal WWTP discharge on mobility and bioavailability of metals in the Lambro Creek. Metal contamination of water compared to Italian background levels for stream water in the Campania region showed potential threats from Pb and Zn to the aquatic biota at sites downstream from the WWTP discharge point and from recreational areas. Water contamination by Ni and Cr was highlighted at the creek mouth. The results demonstrated that all sites were characterized by medium levels of the cumulative criterion for toxic metals, which may cause negative effects on the benthic community. Similarly, sediment toxicity was more pronounced at the site below the WWTP discharge point. In contrast, the creek mouth showed biostimulation effects on diatoms, indicating potential eutrophication. The results of metal accumulation in biota showed higher bioavailability of Zn at the sites below the WWTP discharge and near the recreational area. It can be concluded that traditional activated WWTPs may not be enough to efficiently treat metals, thus not preventing their accumulation in the receiving aquatic environment, and subsequent accumulation in biota.

For better understanding of the fate of metals in the water-sediment environment, a thorough study of small creeks is vital to understand their biogeochemical cycles in ideally low impacted areas, including both fresh (watercourse) and saltwater (river mouth) sediments.

Acknowledgement

This work was supported by project CIGA (Project no. 42220/1313/423107) and IGA (Project no. 42220/1312/423132) of Czech University of Life Sciences, Prague. Professor Massimiliano Fabbicino and Marco Race wish to acknowledge Alfredo Ponzio for his support in the experimental activity.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.ecoenv.2017.11.041>.

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