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Water Hyacinths (*Eichhornia crassipes*) as Indicators of Heavy Metal Impact of a Large Landfill on the Almendares River near Havana, Cuba

S. Olivares-Rieumont · L. Lima · D. De la Rosa · D. W. Graham · I. Columbie · J. L. Santana · M. J. Sánchez

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Abstract The Almendares River is central to recreational and other activities in Havana, Cuba. However, monitoring indicated significant heavy metal contamination in river sediments, especially below Calle 100, the largest landfill in Havana. This work extended previous sediment studies by determining complementary Cu, Pb, Ni, Cr, Cd, and Zn levels in indigenous water hyacinths (*Eichhornia crassipes*; EC) above and below the landfill. Pb, Cu, and Zn were significantly elevated in EC roots below the landfill and also correlated with sediment data ($p < 0.05$), implying elevated levels likely result from landfill activity and might be useful biomonitors as river remediation proceeds.

Keywords Water quality · Heavy metals · Landfill leachates · Water hyacinths

Monitoring systems for assessing the accumulation and affect of heavy metal pollution in aquatic systems often rely upon living organisms (Tessier and Turner 1995). Aquatic macrophytes have particular value as monitors in river systems because they can be stationary and often abundant; their sampling are straightforward; and target plants tend to be easy to identify. Furthermore, many macrophytes innately accumulate heavy metals, and metal content in tissues often correlates with plant biomass and surrounding environmental conditions (St-Cyr et al. 1997). Among macrophytes found in tropical and subtropical regions, the water hyacinth (*Eichhornia crassipes*; EC) has particular potential as an indicator plant because it is ubiquitous and readily absorbs substances from its environment (Chigbo 1982). Furthermore, EC can accumulate metals from surrounding waters (Klumpp et al. 2002; Maddock et al. 1988; Gonzalez et al.1991, 1989) and, as such, might be a useful monitor for the effects landfill leachates that are often laden with heavy metals.

This project was initiated to evaluate EC as a possible monitor of biotic heavy metal exposure due to surface runoff from a large municipal landfill (Calle 100) near the Almendares River by Havana, Cuba. Previous monitoring had shown that Calle 100 was a major source of heavy metal pollution in the river (Olivares et al. 2005); however, this observation was based on sediment data and it was decided that supporting information was needed to confirm that elevated metals were also apparent in the resident biotic community. As background, Calle 100 is large and close to the river (it receives most of the domestic and municipal solid wastes in Havana; i.e., 1,600 ton of solid wastes per day; JICA 2005), but also has very poorly maintained drainage control systems, that has resulted in substantial quantities of untreated leachate entering the river. Although an earlier report suggested that metal levels in these leachates were low (González et al. 2005), further monitoring suggested that heavy metals must be higher than believed because metals were particularly elevated in sediments immediately downstream of the landfill (Olivares et al. 2005). Therefore, it was decided to quantify Cu,

S. Olivares-Rieumont · L. Lima · D. De la Rosa · I. Columbie · J. L. Santana
Laboratorio de Análisis Ambiental, Instituto Superior de Tecnologías y Ciencias Aplicadas, Ave. Salvador Allende y Luaces, Plaza, Ciudad Habana, Cuba

D. W. Graham (✉)
School of Civil Engineering and Geosciences, Cassie Building, Newcastle University, Newcastle Upon Tyne NE1 7RU, UK
e-mail: d.graham@ncl.ac.uk

M. J. Sánchez
Instituto de Ecología y Sistemática, Carretera de Varona Km 3 1/2, Capdevila, Boyeros, AP 10800 Ciudad Habana, Cuba

Pb, Ni, Cr, Cd, and Zn in resident EC, plants that are very abundant in the river, to determine whether leachate inputs were also producing elevated metal levels in local biota. In addition, it was desired to obtain background data (including possible metals for targeted future monitoring) to assess improvements in water quality as planned river remediation efforts proceed.

Materials and Methods

Samples were collected at five locations between February and April (the dry season, when river flow is lowest and most steady) in 2003, 2004, and 2005 along a 10 km reach of the Almendares River (Fig. 1). This reach was above the tidal zone (the bottom station, station 2, was > 5 km from the coastline), which was verified by both conductivity and chloride analyses (Mora 2004). Each sampling site was located above, below, or parallel to Calle 100 and covered an area of about 80 m^2. Specifically, stations 2 and 3 were well below the landfill, station 4 was just below the entrance point of the main leachate channel, and stations 5 and 6 were located either parallel or well upstream of the landfill.

About 10–12 EC were collected manually per sampling station from quiescent zones outside of the main current from water depths between 0.25 and 0.5 m. Typically, streamflow velocities were between 0.25 and 1.0 m/s at each sampling site that allowed many EC to anchor to the sediment bed, which is very common in the Almendares River during the dry season; i.e., EC develop longer petioles, become bottom-fixed, and presumptively obtain more nutrients from the sediment zone (Niño-Sulkowska and Lot 1983). Thus, EC can be “emergent” plants in the

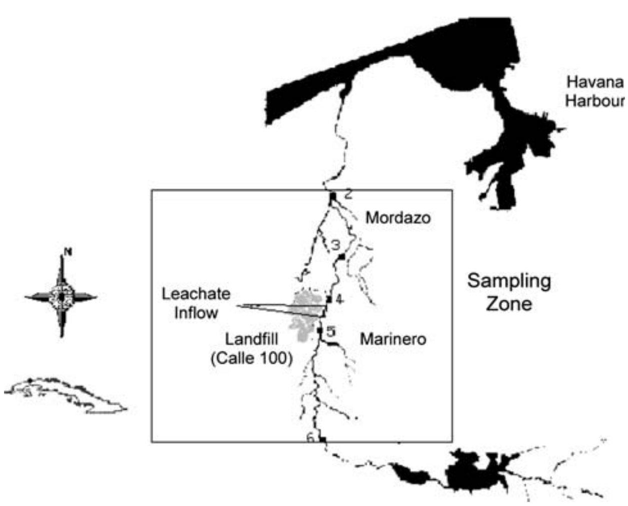

Fig. 1 Sampling stations relative to the landfill (Calle 100) and the coastline

Almendares River, although the stationary period only lasts for 6 months (in the winter and spring). Once the wet season commences in May, the stationary EC are uprooted and washed downstream from their point of origin. However, data suggest that this seasonal exposure is adequate to accumulate heavy metals that are reflective of their local area (see later).

Physical sampling was straightforward. Collected plants were returned to the laboratory immediately; carefully washed twice with distilled water; fractionated between leaf and radicular sources; and composite samples were created from leaf and root fractions, using similar masses per plant per station. The composite samples were dried at 60°C for 48 h, sub-sampled in smaller portions, and macerated within an agate mortar, periodically adding liquid nitrogen to enhance the grinding process. It should be noted that composite samples were used here because they provide a reflective, albeit non-statistical, description of plants at each site; the method has been used previously successfully (e.g., Boswell et al. 1996); and it reduced costs, which was very important in this sampling program. However, to confirm the merit of composite sampling, roots from sets of ten individual plants from different stations were collected and analyzed to estimate variability among plants that might comprise a “typical” composite sample. Mean masses and standard deviations were determined for each metal, and coefficient of variations calculated (CV; standard deviation divided the mean) to describe variance. Except for Cd that was present at very low levels (CV = 0.3), CVs for the metals were always lower than 0.2, which Tessier and Turner (1995) define as acceptable for comparing living organisms in monitoring. Therefore, composite samples were deemed reasonable for subsequent comparisons among stations.

For metal analysis, 500 mg of dry sample was weighed into a Teflon beaker and 5 mL of HF (23 mol/L) was added to each sample. Mixtures were then boiled gently on a hot plate in reflux for 20 min, and 5 mL of HNO_3 (14 mol/L), 5 mL of $HClO_4$ (12 mol/L), and 5 mL HCl (12.5 mol/L) were sequentially added until the sample was fully digested. The digested remains were then transferred to a volumetric flask and were diluted with distilled water to a final volume of 25 mL. Cu, Pb, Ni, Cr, Cd, and Zn levels in the re-suspended digests were determined by atomic absorption spectrometry (Buck Scientific 210VGP) with D_2 background correction and air/acetylene flame. All glassware was acid washed for 24 h in 1.5 M HNO_3 and rinsed several times with twice distilled water before use.

For the quality assurance, three separate digestions and analyses were performed per composite sample and compared with Certified Reference Materials (CRM). BCR No. 60 (i.e., trace elements in the aquatic plant *Lagarosiphon major*) was processed and analyzed to assess analytical precision in Cu, Pb, and Cd, and Zn analysis. Alternately, BCR-596 (the aquatic plant *Trapa natans)* and BCR-145 (sewage sludge) were used for Cr and Ni, respectively. Extraction efficiency for metals ranged from 96 to 110% relative to CRM, and analytical precision was always between 0.035 and 0.089 (as CV). The detection limits for analyzed metals were Cu (0.9 mg/kg), Pb (7.1 mg/kg), Ni (1.9 mg/kg), Cr (5.2 mg/kg), Cd (0.4 mg/kg), and Zn (0.5 mg/kg).

The Wilcoxon ranked-sum test was used to assess the statistical significance of differences between individual means ($p < 0.05$). All data were tested for normality using the Shapiro–Wilk test before analysis. Data were also examined using hierarchical cluster analysis via the complete linkage method (as an amalgamation rule) and Euclidean distances were estimated to determine stations that had similar or different contamination patterns (Miller and Miller 2002).

Results and Discussion

Cu, Pb, Ni, Cr, Cd, and Zn levels were quantified in the tissues of resident EC in the Almendares River in the dry seasons of 2003, 2004, and 2005. Both leaf and root tissues were assessed; however, metal levels were much higher in the roots versus the leaves, which is consistent with results of others (Maddock et al. 1988; Vesk et al. 1999). For example, Fig. 2 shows relative Pb and Zn levels in roots versus leaves in stations sampled in 2004 (similar distributions were seen in other years for these metals). Unfortunately, similar quantitative root–leaf comparisons were not possible for the other metals because leaf metal levels were often below detection limits. However, given that leaf metal levels were consistently low, and root and leaf metal levels often correlated (where leaf data were available), root levels were subsequently used for comparisons among stations in the study. Root metal analysis was further justified because metal transport to upper plant tissues in resident plants in "contaminated" sediments is often via the root system rather than aerosol deposition (Lasat et al. 2000; St-Cyr et al. 1997). Therefore, although translocation of metals from roots to leaves has only been documented for some metals (e.g., Pb, Cd, and Zn; Lu et al. 2004; Vesk et al. 1999), root metal levels were used for monitoring here because signal sizes were large, and analyses were precise and straightforward.

Table 1 summarizes heavy metal levels in EC roots for the different stations and years in the study. These data were compared from three perspectives, detected levels relative to quasi-background levels; statistically, comparing measured levels above and below the landfill; and via cluster analysis of contamination patterns among the five sampling stations.

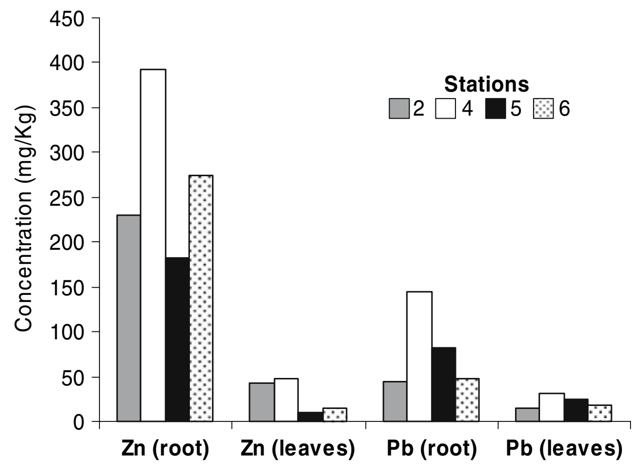

Fig. 2 Pb and Zn levels in radicular and foliar systems from EC collected in 2004 in stations 2, 4, 5, and 6

Table 1 Metal levels (mg/kg dry weight) in EC roots in the Almendares River

Year	Station 2	Station 3	Station 4	Station 5	Station 6	Background levels
Cu						
2003	43	54	65	40	32	15 ± 8[a]
2004	53	–	74	73	42	
2005	40	61	55	11	12	
Pb						
2003	37	39	75	21	22	16[a]
2004	45	–	144	82	48	
2005	60	59	53	12	12	
Ni						
2003	–	14	21	40	25	37–43[b]
2004	39	–	25	53	62	
2005	15	12	15	<1.9	<1.9	
Cr						
2003	–	132	164	281	45	38 ± 28[a]
2004	50	–	51	129	32	
2005	18	45	58	–	–	
Cd						
2003	<1	<1	<1	<1	<1	
2004	1.3	–	1.2	1.5	3.9	
2005	2.8	2.5	2.0	1.7	2.7	
Zn						
2003	133	191	186	135	142	78 ± 40[a]
2004	229	–	392	182	274	
2005	111	129	127	49	35	

[a] Gonzalez et al. 1989

[b] Maddock et al. 1988

First, a semi-quantitative comparison was made between root metal levels from EC in the Almendares River and a less industrially-impacted Cuban river (Gonzalez et al. 1989), and it was found that metal levels were generally higher in the Almendares River relative to the background river. As an example (except for stations 5 and 6 in 2005), Cu, Pb, and Zn levels in EC roots were about 2–5 times higher in Almendares River plants relative to plants in the background river. Furthermore, Pb levels in the Almendares River were in the "high" range for metal pollution according to definitions of Vesk and Allaway (1997), and were similar to Pb levels observed in EC roots in another river that was heavily impacted by industrial pollution (Maddock et al. 1988). Also, EC root levels of Cr and Ni were often above background levels, although the highest levels were usually observed above the landfill. In contrast, Cd levels were consistently low in all samples and no background information was available for comparison.

A second assessment of the possible influence landfill leachates on the river was by statistical comparison of EC root metal levels above (stations 5 and 6) and below (stations 3 and 4) the landfill. These four stations were chosen for comparison because previous data showed that metals in the river tend to precipitate rapidly due to high pH levels, therefore only the most local stations were used for statistical analysis. Figure 3 presents mean metal levels and 95% confidence intervals from the two pairs of stations. Zn, Cu, and Pb levels were all statistically significantly higher below the landfill than above ($p < 0.05$). In contrast, Ni, Cr, and Cd levels did not differ significantly around landfill, although mean Ni and Cr levels were higher above the landfill than below.

The final assessment of effects of the landfill on EC root contamination patterns was performed via hierarchical cluster analysis using the complete linkage method.

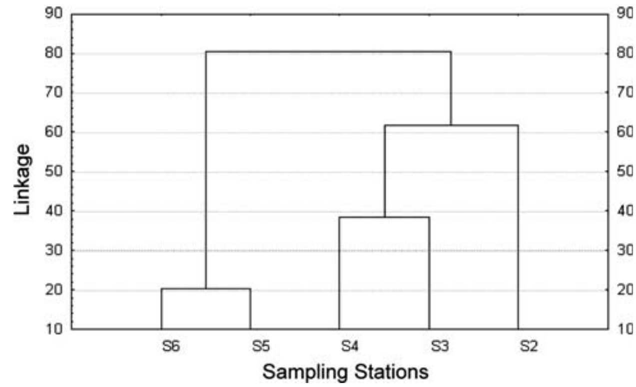

Fig. 4 Dendogram showing relatedness of stations based on root metal levels

Figure 4 presents a dendogram of station clustering based on EC root metal levels over the 3-year study, which indicates two main clusters of related stations. The first cluster includes stations 5 and 6, both above landfill, and the second cluster includes stations 2, 3, and 4, all below the landfill. Although this cluster pattern is consistent with previous cluster analysis using sediment data (Olivares et al. 2005) and confirms the significance of the landfill, elevated Cr and Ni levels above the landfill suggests that additional metal pollutant sources (other than the landfill) also must contribute to metal contamination in the river.

Despite this slight masking of the impact of the landfill by other possible metal sources, Cu, Pb, and Zn levels in EC roots are still significantly higher below the landfill, consistent with earlier work (Olivares et al. 2005), and suggests that levels of these metals in EC roots are impacted by landfill releases (Gonçalves et al. 2004). In fact, Fig. 5 plots sediment data from Olivares et al. (2005) with metal levels for EC roots provided here, and significant correlations are apparent between EC roots and

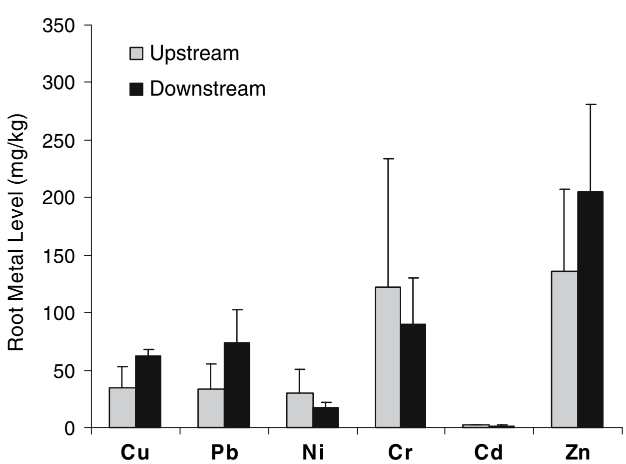

Fig. 3 Root metal levels from stations upstream (5 and 6) and downstream (3 and 4) of Calle 100. Error bars refer to 95% confidence intervals

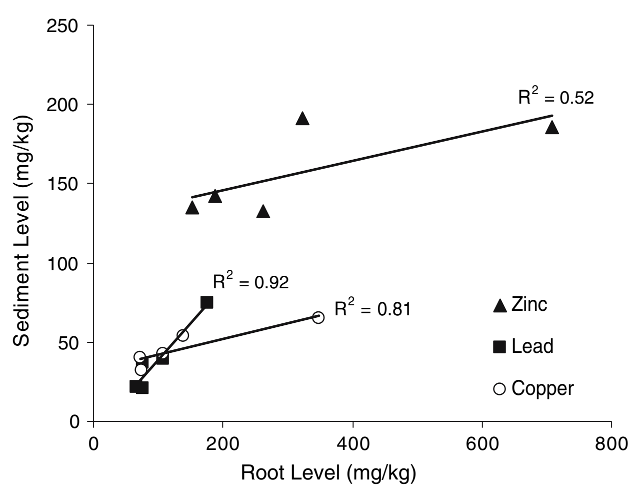

Fig. 5 Relationship between root and sediment levels of Cu, Pb, and Zn around Calle 100

sediments at the five sampling stations ($p < 0.05$). This presents further validation of the EC root composite data, which although only from seasonally rooted plants, provides a useful ancillary measure (with sediment metal analysis) for gauging remediation efforts in the river.

In summary, analysis of accumulated heavy metals in EC roots proved useful for identifying bioavailable metals in the Almendares River associated with Calle 100. The landfill clearly impacts metal levels in local aquatic macrophytes, especially the levels of Cu, Pb, and Zn. Further, elevated Cu, Pb, and Zn were also found in sediments, which imply that these metals should be useful for future river monitoring related planned clean-up efforts at the landfill. Regardless, more study is needed to determine the full affect of Calle 100 on river water quality. Specifically, work is needed to establish best practices for remediation and also establish achievable targets for developing the river as a future recreational site.

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