VOL. 10, NO. 12, DECEMBER 2015 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

SEM/EDX ANALYSIS OF THE ROOTS OF WATER HYACINTHS (*Eichornia crassipes*) COLLECTED ALONG PASIG RIVER IN MANILA, PHILIPPINES

Maria Cecilia Galvez[1], June Francis De Guzman[1], Steven Doniel Gueco[1], Jeane Camelo[2], Red Castilla[1] and Edgar A. Vallar[1]
[1]Environment and Remote Sensing Research (EARTH) Laboratory, Physics Department, De La Salle University, Taft Avenue, Malate, Manila, Philippines
[2]Institute of Civil Engineering, G/f MelchorHall Osmeña Avenue, Diliman, Quezon City, Philippines
E-Mail: maria.cecilia.galvez@dlsu.edu.ph

ABSTRACT

To study the possibility of using water hyacinths (*Eichornia crassipes*) as bioindicator of water pollution along Pasig River in Manila, Philippines, elemental analysis on the roots of water hyacinths was performed using scanning electron microscope equipped with energy dispersive X-ray analysis (SEM/EDX). For water samples a Total X-ray Fluorescence Spectroscopy (T-XRF) was used. Samples of water hyacinths were collected at four well-known bridges (Jones Bridge, Mabini Bridge -formerly Nagtahan Bridge, Makati-Mandaluyong Bridge, and Bambang Bridge) from February - April 2011. For comparison, samples were also taken from a swamp at Tulay na Bato, Daet, Camarines Norte, Philippines where no known pollutants were recorded and this site is free from any anthropogenic sources. All water samples were found to contain elements like S, Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, Br, Sr, and Al. Co was detected only in Jones Bridge and the concentration of Cu in Mabini Bridge exceeded the maximum allowable limit of 0.05 ppm. Elements found in the water samples, such as Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, and Al were also present in the roots of water hyacinths. Result of elemental analysis using EDX also showed that heavy metals like Pb and Hg were not detected in the epidermis and cross-section of the samples collected in Camarines Norte site but they were detected in the roots of water hyacinth collected along Pasig River.

Keywords: water hyacinths, roots, SEM/EDX, Pasig River.

INTRODUCTION

Plants have been known to accumulate essential metal ions (Fe, Mn, Zn, Cu, Co, etc) as well as non-essential metal ions that are toxic to soil and water (Schor-Fumbarov *et al*., 2003). Large amounts of heavy metals can be detected in the roots due to absorption that commonly occurs in the root system, where it is in direct contact with pollutants (Bernd, 1993). The metal ions from the roots can either be stored in the stems or moved through the xylem to the shoot (Baker and Walker, 1990).

Water hyacinths (*Eichhornia crassipes*) popularly known in the Philippines as water lilies are very common plants found in ponds, ditches, muddy lakes, and mostly shallow water. They reproduce easily and have the capacity to release up to 2,000 seeds (Salt *et al*., 1993). During rainy season these plants pose a threat to rivers and floodways due to clogging drains. Because of this, water hyacinths are considered as invasive alien species in the Philippines (MacKinnon, 2002). Some species of these plants have been reported to grow in toxic environments such as sludge and polluted rivers. Studies have shown that water hyacinths are able to accumulate heavy metals such as Cd, Pb, Hg, Mn and Fe at substantial amounts without being toxic to the plant (Lavid, *et al*., 2001).

Figure-1. Map showing the sampling sites along Pasig River (Map data ©2015 Google).

However, a reduction of plant chlorophyll, sugar and protein contents were seen in water hyacinths exposed to Cr VI, where the roots of the water hyacinth exhibited the highest amount of accumulation followed by the leaves and the petioles (Choo *et al*., 2006). The properties exhibited by these plants make them good candidates to provide an indirect assessment of the level of contamination in the waterways where they grow. Unlike water sampling, which provides an instantaneous analysis of pollutants in the river and consequently a low concentration reading, monitoring contaminated waters using plants provides longer observations of levels of river toxicity.

Levels of contamination of heavy metals in the waters need to be monitored because exposure to high levels can result in damaged or reduced mental and central nervous function, lower and damage blood composition, lungs, kidneys, liver and other vital organs (Ostler *et al*., 1996). Heavy metals can be passed on to the human body by ingestion of contaminated plants and animals, or inhalation due to exposure by living or working near industrial areas.

Highly industrialized and densely populated areas such as Metro Manila face a problem of having highly polluted waterways running through the metropolis. The Pasig River in Manila, Philippines serves as a major trade route in transporting goods to industries built around it. Due to heavy industrialization, the river became a dumping site for factories, households, and businesses (Santelices, 2008). In 1990, the river was declared biologically dead and will not be able to support life. However local government units have been taking efforts to slowly rehabilitate the river (Santos, 2010). Pasig River is a classified as a class C river where it is designated as surface water used for raising fish and other aquatic resources, recreational water (DENR, 1990). As of 2011, the river still failed several standard quality tests such as level of dissolved oxygen content, biological oxygen demand, lead, cadmium, and coliform levels (Pasig Rehabilitation Commission, 2011). Monitoring the health of the river has been one of the primary concerns since people live and work near it. Since water hyacinths has large population along Pasig River, in this paper, the roots of water hyacinths were analyzed using scanning electron microscope equipped with energy dispersive X-ray analysis (SEM/EDX) to study the possibility of using water hyacinth as bioindicator of water pollution along this river. Samples of water hyacinths were collected at four well-known bridges (Jones Bridge, Mabini Bridge - formerly Nagtahan Bridge, Makati-Mandaluyong Bridge, and Bambang Bridge). For comparison, samples were also taken from a swamp at Tulay na Bato, Daet, Camarines Norte, Philippines where no known pollutants were recorded and this site is free from any anthropogenic sources. Elemental composition of the roots of water hyacinths and water samples were analyzed. Water samples were analyzed using a Total X-ray Fluorescence Spectroscopy (T-XRF). To our knowledge, this is the first time that this kind of study was done along Pasig River.

SITE DESCRIPTION AND METHOD

Figure-1 is a map showing the sites where the water hyacinths were collected. The Pasig River is an important river system in the Philippines since it connects Manila Bay and Laguna de Bay. It is approximately 27 km

Table-1. Elemental concentration (ppm) of water samples collected at the four sampling sites along Pasig River and at Camarines Norte.

Elements	JB	MB	MMB	BB	CB
	Jones	Mabini	Makati Mandaluyong	Bambang	Camarines Norte
S	3.7406	1.7247	1.7332	1.6494	0.7331
Cl	31.3090	1.3424	2.1298	2.8580	1.8442
K	6.1795	3.3292	4.9039	5.0441	1.9092
Ca	8.5246	6.6964	4.8636	7.5820	0.6278
Mn	0.0025	0.0107	0.0790	0.1692	0.1451
Fe	0.0023	0.0686	3.2256	3.2048	0.0399
Co	0.0041	0.0108	0.0000	0.0000	0.0000
Ni	0.0159	0.0447	0.0124	0.0128	0.0099
Cu	0.0105	0.0643	0.0290	0.0171	0.0076
Zn	0.0529	0.0919	0.0601	0.0067	0.0161
Ga	10.0000	10.0000	10.0000	10.0000	10.0000
Br	16.8960	3.1689	1.0582	1.5786	0.0286
Sr	8.4336	7.8953	0.1491	0.1645	0.0607
Al	0.0000	0.0000	0.3837	0.0000	0.0069

VOL. 10, NO. 12, DECEMBER 2015 ISSN 1990-6145
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long, with an average width of 91 m, and depths ranging from 0.5-5.5m (Gorme *et al.*, 2010). It is considered a tidal estuary in which the flow of water depends on the tide. During summer the water level in Laguna de Bay may drop below that of Manila Bay's, resulting in a reverse flow of seawater from the bay into the lake.

Between the period of February 2011 to April 2011, samples of water hyacinth roots were collected from four different locations along the Pasig River namely Jones Bridge (JB), Mabini Bridge (MB) -formerly Nagtahan Bridge, Makati- Mandaluyong Bridge (MMB), and Bambang Bridge (BB). Samples were also collected from a swamp at Tulay na Bato, Daet, Camarines Norte, Philippines, which is free from any anthropogenic sources (CB). Only natural sources and processes such as weathering of rocks, and mineral deposits were expected to produce metals in dusts, sediments and various elements in CB. The study sites along Pasig River are known for having industries such as oil depositories (in JB) and highly industrialized areas (in MMB) suspected to be discharging to the rivers.

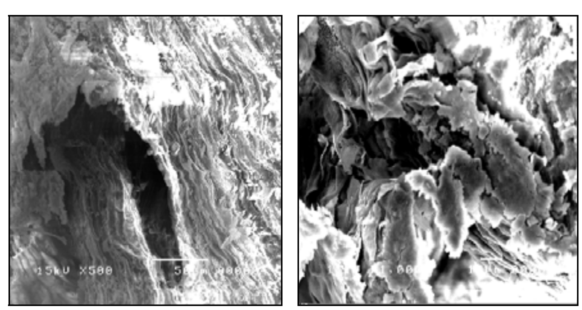
Water hyacinths that were collected at the five different sites all have the same physical size for both stems and leaves. Along with the plant samples, water was also collected at the five different locations using the grab sampling method. A disinfected polyethylene container was submerged one foot below the water surface for collection. In preparation of the solution, 100 ml of water sample was combined to known amount of standard. The standard element used was Gallium at 10 ppm. The water samples were analyzed using the T-XRF Spectrometer at the Applied Physics Laboratory of the Philippine Nuclear Research Institute (PNRI).

The root sample analysis was carried out at the Physics Department of De La Salle University Manila using a JSM-5310 Scanning Electron Microscope coupled with an Energy Dispersive X-ray Spectroscope that was operated at an acceleration voltage of 15 kV. The microscope is equipped with an Oxford energy dispersive X-ray analyzer where the elemental compositions of the particulate matters were obtained. The SEM images and analytical data of trace elements concentration were recorded with a Link Isis 3.0 software system with a SemAfore 5.0 SA20 Scan Digitizer for digital imaging. SEM/EDX was done according to the standard operating procedure for sample preparation and analysis of particulate matter samples by scanning electron microscopy. The instrument acquires either single spot spectrum or area spectrum. In this way it is possible to identify a single particle analysis and the mean values of the constituents from a group of particles. The root samples were cleaned to remove any sand, dust or other particulate matters, and then cut into 0.5 x 0.5 inches sample size. Since the samples to be analyzed were biological, further preparations were done prior to SEM analysis. The samples were subjected to freeze drying and gold sputtering to increase their ability to conduct electricity and secondary electron detection. The samples were then placed into the SEM chamber to be analyzed.

RESULTS AND DISCUSSIONS

Elemental analysis of water samples

The result of T-XRF analysis of water samples collected at the five sampling sites is shown in Table 1. The result only presented the elements that released energy from the k lines such as S, Cl, K, Ca, Mn, Fe, Ni, Co, Cu, Zn, Ga, Br, Sr, and Al. The Si and Mo elements from the spectrum were not quantified anymore, because the container of the sample contains Si and Mo. In the Philippines, the government agency that sets the maximum allowable limits for water is the Department of Environment and Natural Resources (DENR). Among the elements that we analyzed using T-XRF, it is only Cu that has an available guideline for maximum allowable limit for a Class C river under the DENR-Administrative Order 34-1990. Cu has a maximum allowable limit of 0.05 ppm. MB exceeded the limit at 0.0634 ppm during sampling time. Except for Co, most of the elements detected from the water samples collected along Pasig River were also found in the water sample in CB. Co was only detected in JB (residential and commercial, near the port area, connected to major roads, public and private vehicles) and MB (Pandacan oil depot, distillers, residential, connected to major roads). Anthropogenic contributors of Co to the aquatic environment include production of alloys and chemicals containing cobalt, sewage effluents, urban run-off, and agricultural run-off. The concentrations of most elements in the water collected along Pasig River were greater than the concentrations of elements in the water collected in CB. This is indicative of the effect of anthropogenic sources along Pasig River. The concentration of Mn, Ni, Zn, and Al from all sites were comparable to one another. The level of Fe in MMB and BB were very high compared to CB. This could be attributed to the bridge because water samples at these stations were collected near the bridge. JB contained very high concentration of Cl and Br compared to other stations. Furthermore, it also contained the highest concentration of S, K, Sr, and Ca followed by MB. Pb and



(a) (b)

Figure-2. An example of an SEM image using 500x magnification of (a) the root epidermis and (b) the root cross-section.

Hg were not detected in the sampled water because of the detection limit of the spectrometer. JB, the one near

Manila Bay, contains the most number of elements with high concentrations as compared to CB.

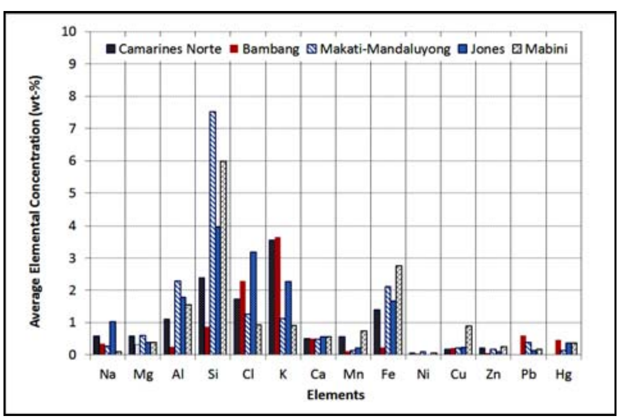
SEM/EDX analysis of roots

A sample of an SEM image of the root epidermis and cross-section can be seen in Figure-2. The images were observed under the SEM at 500x magnification. The average elemental concentrations of the different heavy metals detected in the root epidermis of the 50 water hyacinths collected at the 5 different sites are presented in Figure-3a while Figure-3b shows the average elemental concentrations of the different heavy metals in the root cross-section. These heavy metals are suspected to have been in contact with the plants from the water. As can be seen from Figure-3 heavy metals like Pb and Hg were not detected in the epidermis and cross-section of the samples collected in Camarines Norte site but they were detected in the roots of water hyacinths collected along Pasig River.

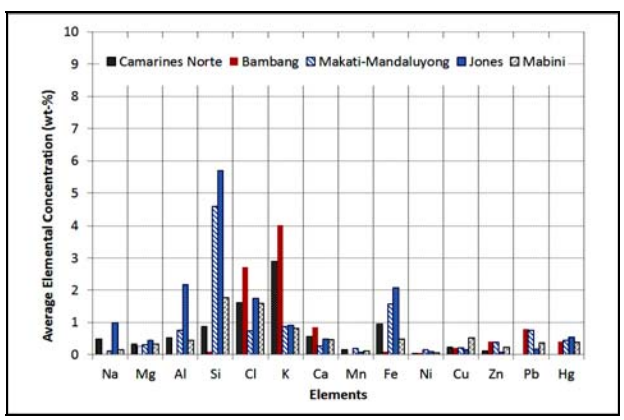
A one way analysis of variance was performed to analyze the differences in percent mean elemental concentrations among different locations using a 5% probability level. Table-2 shows the summary of the result for elements that showed significant differences. Locations belonging to the same subset have no significant difference. Elements like Mg, Ca, Ni, and Zn have no significant differences in percent mean elemental concentration in all sampling locations. On the other hand, Na, Al, Si, Cl, K, Mn, Fe, Cu, Hg, and Pb resulted in p-values lower than 0.05. A Tukey-Kramer test was done on each element to know where the highest or lowest concentrations of heavy metals were detected. For Na, JB and CB have no significant difference but the elemental concentration of Na for JB was slightly higher compared to CB, and was significantly higher compared to BB. Al was highest in JB and MMB, and comparing it with the T-XRF result for water, the concentration of Al in MMB was the highest among all locations. Si was highest in MMB and JB, and lowest at BB and CB. Roots of water hyacinths at BB, JB, and CB had no significant difference in the elemental concentration of Cl however it was highest in BB then JB. Water sampled at JB had the highest Cl concentration. K was highest in BB but comparable to CB and lowest in MB. The significant result from the SEM/EDX analysis of the roots was that for Cu, Pb, and Hg. Tukey-Kramer analysis revealed that in the case of Cu, the percent elemental concentration of Cu found in the roots of water hyacinths collected along MB was the highest and significantly different from all other locations. Cu concentration from T-XRF analysis of water sampled in MB exceeded the guideline limit at the time of sampling. Pb and Hg was not found in the CB but was detected in all the roots of water hyacinths collected along Pasig River. This was an indication of the effect of anthropogenic activities happening within the vicinity of Pasig River.

CONCLUSION AND RECOMMENDATIONS

The presence of heavy metals was detected on the water hyacinth roots using SEM/EDX techniques. All water samples were found to contain elements like S, Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, Br, Sr, and Al. Co was detected only in Jones Bridge and the concentration of Cu in Mabini Bridge exceeded the maximum allowable limit of 0.05 ppm. Elements found in the water samples, such as Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, and Al were also present in the roots of water hyacinths. Result of elemental analysis using EDX also showed that heavy metals like Pb and Hg were not detected in the epidermis and cross-section of the samples collected in Camarines Norte site but they were detected in the roots of water hyacinths collected along Pasig River. Roots of water hyacinths and water collected near Jones Bridge and Mabini Bridge were found to have high concentrations of heavy metals. The most hazardous heavy metals Pb were found highest in Bambang Bridge and Hg were found highest in Jones Bridge. The result of analysis of heavy metals that was absorbed by the water hyacinth in the epidermis and cross-section parts reflected and indicates the influence of polluted effluents released in the different locations in the Pasig River. Elements found in the water samples, such as Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, and Al were also present in the roots of water hyacinths.



(a)



(b)

Figure-3. Average elemental concentrations found in the (a) epidermis of the roots of water hyacinths and (b) Cross-section of the roots of water hyacinths collected at different locations along Pasig River.

Table-2. Result of Tukey-Kramer test for elements with p-values less than 5%.

Element	a		b		c		d	
Na	BB	4	CB	2				
	MB	3	JB	1				
	MMB	2						
	CB	1						
Al	BB	3	CB	3	MMB	2		
	CB	2	MB	2	JB	1		
	MB	1	MMB	1				
Si	BB	2	CB	2	MB	2	JB	2
	CB	1	MB	1	JB	1	MMB	1
Cl	MMB	3	MB	3	CB	3		
	MB	2	CB	2	JB	2		
	CB	1	JB	1	BB	1		
K	MB	3	CB	2				
	MMB	2	BB	1				
	JB	1						
Fe	BB	3	CB	4				
	CB	2	MB	3				
	MB	1	MMB	2				
			JB	1				
Cu	JB	4	MB	1				
	CB	3						
	MMB	2						
	BB	1						
Pb	JB	3	JB	3	MB	3		
	MB	2	MB	2	MMB	2		
		1	MMB	1	BB	1		
Hg	MMB	4	MMB	4				
	MB	3	MB	3				
	BB	2	BB	2				
		1	JB	1				

Legends: letters a < b < c < d (ranking among subsets); numbers 5 < 4 < 3 < 2 < 1 (ranking within a subset)

However since the water analysis did not include most of the heavy metals that were in the government regulations, a different kind of testing should be done to detect their levels. With these findings, aquatic plants such as water hyacinth can serve as a bioindicator of the environment. This indicator tells us the degree of contamination or level of water pollution in Pasig River. Water hyacinth can considered a good accumulator of heavy metals, based on the data it accumulated more than 0.1% of heavy metals in the epidermis part and cross section part on its plant parts (Abdul, 2000). The presence of these elements has significant impact in the animals, humans and the environment. The high concentration of these heavy metals present in the environment could cause hazardous effect; especially with people health's living nearby that are directly affected. The studies of heavy metals and continue monitoring of these elements in the environment is essential for the benefit of human beings, animals and the environment. Further research is needed to strengthen the understanding about the accumulation of heavy metals in the water hyacinth and potential use as bioindicator for water pollution. Also, for possible use of water hyacinth for to help remediate the pollution caused by the heavy metals in the river since it has the capacity of storing these without being hazardous to the plant. The study could be extended to analyzing the different parts of water hyacinths from leaves to stems to roots.

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