Bioconcentration of Copper in *Cyperus alternifolius* L. (Umbrella Plant) in Butuanon River

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Four sites along the Butuanon River were identified based on the presence of *Cyperus alternifolius* L. Composite samples of water, sediments and roots of *C. alternifolius* L. from each site were taken for two seasons and analyzed for copper by Flame Atomic Absorption Spectroscopy (FAAS).

For the dry season, copper in water, sediments and roots of *C. alternifolius* L. ranged from 0.007-0.016, 101.3-140.7 and 27.36 - 69.17 ppm respectively. For the wet season, copper in water, sediments and roots ranged from 0.011-0.033, 77.23-96.93 and 27.22-54.18 ppm. Pearson's *r* between copper in the roots of *C. alternifolius* L. and water are 0.86 and 0.82 while for sediments are 0.83 and 0.86 respectively. Bioconcentration factor ($BCF = Cu_{\text{roots}} / Cu_{\text{water}}$) during the dry and wet seasons ranged from 1750-9881 and 1441-2391 respectively. BCF relative to sediments ranged from 0.258-0.640 and 0.281-0.702 for dry and wet seasons, respectively.

C. alternifolius L. has the capacity to accumulate certain amounts of copper in its roots to levels exceeding those amounts present in water but not in the sediment. *C. alternifolius* L. has the potential as indicator of copper pollution in the water and sediment.

Keywords: Butuanon River, *Cyperus alternifolius* L., Umbrella Plant, bioconcentration, copper, sediment

INTRODUCTION

Heavy metal (HM) pollution in waterways was an issue of least concern in the past. Due to rapid urbanization, industrialization and technological innovations, this problem was given utmost attention by various sectors in society. One of the major waterways affected by these developments is Butuanon River, which runs through the emerging cities of Cebu and Mandaue, in the province of Cebu,

Philippines. According to old folks, river waters were used for many household activities such bathing, laundering and even for drinking purposes. Presently however, this river has become the principal disposal site for domestic sewage, industrial discharges, agricultural and farm wastes and even for defecating purposes. The Department of Environment and Natural Resources (DENR) presently classifies this river as Class D water, intended for direct agricultural irrigation,

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livestock watering and industrial processing (Nazareno, 2000).

River contamination with a negative health effect or environmental impact is one of the prime issues in fast growing cities. In most cases, water quality regulations and sanitation infrastructure in these cities are not suitably designed for unprecedented population growth and urbanization (Akoto *et al.*, 2008; Ahmad *et al.*, 2010). Contaminations due to HMs are notorious for their several health-related effects. In the environment, they usually undergo several transformations due to dissolution, adsorption and precipitation (Akçay *et al.*, 2003) which ultimately alter their nature, behavior and bioavailability (Nicolau *et al.*, 2006). The physical and chemical characteristics of sediments and river water are important factors for understanding the chemical transformation and movement of these HMs. The occurrences of enhanced concentrations of HM especially in water and sediments may be an indication of human induced perturbations rather than natural enrichment through geological weathering (Binning and Baird, 2001; Eja *et al.*, 2003). Sediments act as both carrier and sources of the contaminants in the aquatic environment (Shuhaimi, 2008). HMs produce their toxicity by forming complexes with organic compounds (Perk, 2006). Their bioavailability and toxicity depend upon its various forms and amount bound to the sediment matrices (Chukwujindu *et al.*, 2007).

Copper has been recorded as one of the metals contaminating Butuanon River (Mendoza, 1993, Suico, 1997). Its presence in the environment has attracted considerable attention and intensive researches (Biggs, 2012; Mayer-Pinto *et al.*, 2010). Increased concentrations of copper in the environment may be attributed to activities like mining (Galiulin *et al.*, 2001; Li and Zhang, 2010), industrial discharges (Kivaisi, 2001), sewage sludge disposal, fertilizer and pesticide applications. Copper compounds are commonly used for treatment of plant diseases and preservation of wood, leather and fabrics. The use of food additives, run-offs

from swimming pool waters, water supply systems (Eaton, 2005; WHO, 2004), sidings and roofs of buildings and various emissions from automobiles (Davis *et al.*, 2001) are also sources of copper.

In response to these problems, many technologies and physico-chemical methods such as coagulation, sedimentation, flotation, ionic exchange, reverse osmosis, extraction and others (Selatnia *et al.*, 2004 and Rosangela *et al.*, 2007) have been used to minimize aquatic pollution. The use of aquatic plants has been investigated for the monitoring and remediation of heavy metal-polluted water bodies. *Cyperus alternifolius* L. has a susceptibility to accumulate metals such as copper in wastewaters. It is a grass-like plant in the very large genus *Cyperus* of the sedge family, *Cyperaceae*. This umbrella plant, as it is commonly known, is a perennial exotic species that grows up to 3-5 feet, if given plenty of water. In riverine sites, the wetter the root of the umbrella plant, the better is its survival. The presence of one long-lived intercalary meristem (IM) of *C. alternifolius* produces an elongated internode which makes it ideal for developmental studies (Fisher, 1970). IMs allow rapid stem elongation, while those at the base of most grass allow damaged leaves to rapidly re-grow. The large green leaves whose edges are sharp to the touch, taper into slender petioles that form a sheath around the main stem. Its fibril roots have a life span of approximately 34.8 days (Wenyin, *et al.*, 2007).

The umbrella plant has been regarded as species that can quickly colonize polluted waters usually unsuitable for other species to survive (Soda, *et al.* 2012; Tang *et al.* 1999). The bioaccumulation of considerable amounts of heavy metals by aquatic macrophytes such as the umbrella plant, in their tissues has been reported (Peng *et al.*, 2008; Soda *et al.*, 2012; Faucon *et al.*, 2012). Uptake of copper from soil in plants through the roots is a natural and necessary process that is actively regulated by the plant (Clemens, 2001). This process is dependent on the concentration and bioavailability of copper. Pugh *et al.*, 2002

reported that the typical level of copper in plants ranges from 20 to 100 mg/kg and at this level it is phytotoxic.

The study of Johnson and Hale (2008) showed changes in trace metal concentration during root decomposition. The concentration of copper in root tissues found at lower soil depths were generally higher than expected due to reallocation of essential metals. Several other mechanisms may contribute to heavy metal tolerance depending on the type of metal and plant species (Memon et al., 2001, Mehes-Smith, M., Nkongolo, K., Chowela, E. 2013). During the root life span of *C.*

alternifolius, there are patterns of metal distribution within the plant rhizomes that are most likely a function of physiological translocation by the plants themselves (Cardwell, 2002).

This study reports on the capacity of the roots of *C. alternifolius* L. to bioconcentrate copper in the natural conditions of Butuanon River. Given that the heavy metal contamination affects the general population within the sites of Butuanon River, copper concentrations in samples of water, sediments and roots of umbrella plant were analyzed to evaluate the quality of the aquatic environment in this study area.

Figure 1. Map of Butuanon River with the Four Sampling Stations: Budla-an, Talamban, Cebu City (Station 1); Bacayan, Talamban, Cebu City (Station 2); Pilit II, Canduman, Mandaue City (Station 3); Butuanon Bridge, Mandaue City (Station 4).

METHODOLOGY

Sampling Sites. The river is situated in the northern part of Cebu and its main channel is about 10 kilometres long running through the mountainous areas of Cebu City, Mandaue City and finally draining into the Mactan Channel. Figure 1 shows the four sampling sites representing upstream, midstream and downstream of the river. Site 1 is located in Barangay Budla-an, Talamban Cebu ($10^{\circ} 23'34''$ N, $123^{\circ}52'58''$ E, elevation 182 m) and

is described as a site with no industries, grassy, deforested but with a lot of agricultural activities. Domestic wastes coupled with wastes from chicken and pig farms are also prevalent in this area. Site 2, Bacayan Bridge, Talamban Cebu ($10^{\circ} 23'42''$ N, $123^{\circ}55'7''$ E, elevation 45 m), has a quarry and stone crashing facility, furniture industry and a car wash station, including a significant number of informal settlers and a high density housing subdivision. Site 3, Pilit ($11^{\circ} 21' 31.5''$ N, $123^{\circ} 55' 35.0''$ E), has in the immediate vicinity some

paint and food processing facilities, and finally Site 4, Butuanon Bridge ($10^{\circ} 33.8' \text{ N}$, $123^{\circ} 56' 55.1'' \text{ E}$), is characterized as having domestic and industrial effluents and high density of informal settlers. Sites 2 and 3 are consistent with the sites used by Mendoza and Suico (1995), Muego (2006) and Oquiñena (2012). Figure 2 shows the four sampling sites showing the presence of *C. alternifolius* L. and their relative population density. Site 1 has a

high density of the umbrella plant on both sides of the riverbank stretching to about 5 meters, Site 2 has isolated clusters of the plant in the riverbanks and right in the middle of the riverbed, while Sites 3-4 have very low population of the plant. For all four sites, the plants are either submerged in the water especially during the wet season or partially submerged during the dry season.

Figure 2. *Cyperus alternifolius* in Budla-an, Talamban, Cebu City (A); Bacayan, Talamban Cebu City (B); Pilit II, Mandaue City (C); and Butuanon Bridge, Mandaue City (D).

Reagents and Instrumentation. All chemicals and reagents used for analyses were analytical reagent grade. Concentrated nitric acid (J.T. Baker Analyzed ACS reagent), concentrated hydrochloric acid (Univar of Ajax Fine Chemicals Pty Ltd.), 30% hydrogen peroxide (Scharlau), Whatmann filter paper #42 μm were used in the digestion. AAS-grade Merck Titrisol copper was used as the standard solution. For 100-ppm Cu standard stock solution, an aliquot of 25-mL was taken from 1000-ppm stock solution, transferred

into a 250-mL volumetric flask and diluted to the mark with distilled water. The pH, dissolved oxygen (DO) and stream flow were measured using Orion pH meter, Milwaukee DO meter and OTT for surface water flow meter (propeller type), respectively. The pH of the sediments was measured using Kelway Soil Acidity tester. Moisture determination was conducted using the Ehret TK 4067 oven. An Intertest Benelux sieve shaker and ball mill were used for the sediments. The analyses of total copper were done using the

AA-3600 Shimadzu Atomic Absorption Spectrophotometer using air-acetylene flame at a wavelength of 324.3 nm.

Collection and Analyses of Water, Sediment and Roots of *C. alternifolius*.

The collection of water, sediments and plants was done in May and July 2012 representing the dry and wet seasons respectively. The water and sediment samples were collected within the 3m x 3m stretch of the river per station. Sampling was done to ensure that the sites contain *C. alternifolius* L. growing in the river banks or riverbeds. Physico-chemical parameters such as pH, dissolved oxygen (DO) and stream flow were measured immediately prior to sample collection. Analyses of these samples for copper were done in triplicates using External Calibration (EC) and/or Multiple Standard Addition (MSA).

Water. A total of approximately 20 L pre-filtered surface river water was collected by a small dipper and placed in a clean polyethylene container. This container was pre-washed with 10% (v/v) HNO₃ and rinsed thoroughly with distilled water. The water samples were immediately transported to the laboratory and filtered using Whatman # 42. The filtered water sample measuring 500.0 mL was acidified and evaporated to approximately 10-mL, filtered and transferred to a 100-mL volumetric flask, diluted to the mark and labeled as Water Digests (WD). An aliquot of 5.00 mL WD plus varied volumes (0.00-, 25.00-, 50.00-, 75.00- and 100.00-μL) of standard copper solution was placed in separate 25-mL volumetric flasks. These calibration standards were decoded to 0.00-, 0.10-, 0.20-, 0.30-, 0.40-ppm respectively to represent MSA quantification. External calibration standards of 0.10-, 0.20-, 0.30-, 0.40-ppm were also prepared. All calibration standards and WD were subjected to Flame Atomic Absorption Spectroscopy (FAAS).

Sediments. Surface sediments were collected using a small shovel, stored in plastic bags and transported to the laboratory. These sediments were immediately air-dried for 3-5 days, screened through a sieve shaker at an

aperture of 180-um and homogenized further by a ball mill for an hour. The air-dried samples were oven dried and its moisture content determined. Oven-dried sediment samples were digested using the method by US-EPA #3050B. The resulting sediment digests (SD) were filtered and collected in 100-mL volumetric flasks, diluted to mark and run in the FAAS together with the freshly prepared calibration copper standards.

Roots of *C. alternifolius* L. A whole cluster of randomly selected *C. alternifolius* was uprooted using a 2-ft metal bar previously rinsed with water. The plant samples were placed in large plastic bags and transported to the laboratory. Specimen sample of the umbrella plant (Code No. 2013-1) was deposited at the Biology Stockroom of the University of San Carlos, Cebu City, Philippines. The roots were separated from the other plant parts and gently washed with tap water and distilled water to remove all adhering soil particles. The roots were air-dried for about 48-72 hours, oven dried and its moisture content determined. Oven-dried roots were osteorized using a blender and three replicate samples were used for digestion (AOAC 16th edition, Vol 1 Method # 975.03). The resulting root digests (RD) were analyzed by FAAS coupled with EC quantification using 0.00-, 0.10-, 0.20-, 0.30- and 0.40-ppm copper standards.

From the equation of the EC and/or MSA calibration curves and using the appropriate dilution factors, the concentration of total copper in the river water, sediment and root samples were computed.

Limit of Detection and Method Validation. The Limit of Detection (LOD = 3s) was determined by running the reagent blank and a 0.20-ppm copper standard 30 times in the FAAS. Percentage recoveries for water samples were determined and calculated using Equation 1.

Bioconcentration Factor. Bioconcentration is a process by which the concentration of a chemical in an organism becomes higher than its concentration in the air or water around

$$\% \text{ recovery} = \frac{A_{\text{WD+Cu standard}} - A_{\text{WD}}}{A_{\text{Cu standard}}} \times 100 \quad (\text{Equation 1})$$

where: $A_{\text{WD+Cu standard}}$ = absorbance of WD + Cu standard
 A_{WD} = absorbance of WD only
 $A_{\text{Cu standard}}$ = absorbance of Cu standard only

the organism. Although the process is the same for both natural and manmade chemicals, the term bio-concentration usually refers to chemicals foreign to the organism. From the results of the analysis of the amount of copper in the water, sediment and roots, the bioconcentration factor (BCF) was calculated as the ratio of the trace element (copper) concentration in the plant tissues at harvest (mg kg^{-1} dry-wt) to the concentration of the element (copper) in the external environment (Zayed *et al.* 1998). Thus to solve for BCF in this study, Equations 2 and 3 are used.

$$\text{BCF} = \text{Cu}_{\text{roots}} / \text{Cu}_{\text{water}} \quad (\text{Equation 2})$$

$$\text{BCF} = \text{Cu}_{\text{roots}} / \text{Cu}_{\text{sediments}} \quad (\text{Equation 3})$$

Treatment of Data. Correlation coefficient using Pearson's r was calculated to determine the relationships between (a) Cu in roots and water and (b) copper in roots and sediments, in the four sampling stations. The data of the mean of the replicates and the evaluation of significance differences among stations, seasonal variation and by interaction were processed using multiple analyses of variance (MANOVA) through Microsoft Excel Version 2007 and Graph Pad Prism Version 6.0 Free trial version (Graph Pad Software, Inc. San Diego, California).

RESULTS AND DISCUSSION

The Study Site. Butuanon River is a small river running through two emerging cities of Cebu and Mandaue of the Province of Cebu, Philippines. Four sampling stations were used to represent the upstream (Station 1), midstream (Stations 2 and 3) and downstream (Station 4) regions. Table 1 summarizes the physico-chemical characteristics of water and sediments for the dry and wet seasons. During the dry season, the river water was clear in Station 1, slightly turbid in Stations 2 and 3 and dark brown to black in Station 4. The downstream site (Station 4), which is approaching the mouth of the river showed zero stream flow and noticeable signs of gas formation at the surface of the water. This is attributed to the release of CH_4 or H_2S gases arising from anaerobic digestion of organic matter. During the wet season, waters were all turbid following a heavy downpour prior to sampling and actual drizzles on the day of sample collection. Generally, the sediments prevalent in Station 1 consisted mostly of sand, while Stations 2, 3 and 4 were mostly silt and clay. The pH of the water ranges from very acidic (2.85) to slightly basic (8.56). Based on the DAO 34, the pH range of Class D waters like Butuanon River should range from 6.00-9.00. The results showed that it is within

Table 1. Physico-Chemical Parameters of River Water and Sediments in the Four Stations.

Sampling Station	Stream Flow (m/s)		Water pH		Sediment pH		Dissolved Oxygen (DO) (mg/L)	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
1	0.307	4.82	8.20	8.56	7.40	7.55	5.20	7.73
2	0.451	2.36	7.79	8.34	7.20	7.00	4.35	7.60
3	0.109	4.40	8.37	8.48	7.00	6.75	2.15	7.30
4	0.000	10.68	6.63	2.85	7.20	7.00	0.00	5.60

the DENR standard value, except for Station 4 (pH = 2.85) during the wet season. The high pH values were attributed to sewage water discharges from the surrounding areas (Malik et al. 2010), while the low pH can be a result of the decay of both domestic and industrial waste litter in the downstream site. The pH of the sediments ranges from neutral to very slightly basic values except for Station 3 (pH = 6.75) during the wet season. DO in the upstream site is high (10.68 mg/L) due to turbulent water flow. Turbulence causes aeration of the waters, consequently, a rapid uptake of oxygen is also expected. Interestingly, during the dry season, the downstream site registered a very low DO, (ca. 0.00 ppm) and significant amount of solids are evident causing a very slow stream flow. The DO reading in this site is similar to the results of Abowei (2010) who reported that at high temperature, typical during a dry season, the solubility of oxygen decreases.

Notably, this site approaches the mouth of the river which happens to drain eventually into the Mactan Channel.

Chemical Analyses of River Water, Sediments and Root Samples.

Dry Season. The concentration of copper in water, sediments and plant roots are shown in Figure 3 for the dry and wet seasons respectively. The concentration of copper in the river water is extremely low compared to that in the sediments and roots. This means that the water is relatively clean and the sediments are the repositories of these metal contaminants (Horsfall and Spiff, 1999). Table 2 shows the actual concentration of copper in the water for both seasons. The high amount of copper in water for Station 3 during the dry season can be attributed to upstream discharges of untreated domestic and industrial wastewaters.

Figure 3. Copper in River Water, Sediments and Roots of *C. alternifolius* L. in the Four Stations during the Dry and Wet Seasons.

Potential sources of copper like electrical wirings, electroplating wastes, plumbing pipes, industrial chemicals, dyes, paints and pharmaceutical wastes are potentially transported by the river flow. A dilution effect might bring about low levels of copper in

Station 4 due to its downstream topography. These copper levels in water during the dry season also showed comparable results from the study of Oquiñena (2012). All sampling stations do not exceed the standard of 0.05 mg/L copper for Class D waters.

Table 2. Copper in River Water for the Dry and Wet Seasons in the Four Stations.

Sampling Station	Copper (ppm) in River Water	
	Dry Season	Wet Season
1	0.007 ± 0.000	0.011 ± 0.002
2	0.006 ± 0.001	0.033 ± 0.007
3	0.016 ± 0.013	0.021 ± 0.006
4	0.007 ± 0.001	0.016 ± 0.006

On the other hand, Figure 3 also shows that Stations 1, 2 and 3 have roughly the same amount of copper in the sediments during the dry season. However, the copper content found in the roots of *C. alternifolius* L. collected from Station 1 was slightly higher than Stations 2 and 3, presumably due to the application of copper-based pesticides in mango plantations. Copper in runoffs resulting from natural weathering or released from disturbed soils contribute 68% of copper release in the waterways (Georgopoulos et al., 2001). Station 4 which is a downstream site and approaching the mouth of the river is characterized with silt and clay sediments. These two types of sediments inherently have a high tendency of absorbing metals (Mohiuddin, et al., 2012). This would explain the high levels of copper content in this area. This result is comparable to the results of Chiu-Wen Chen et al (2012) who reported that copper concentration in sediments is relatively high in the mouth of the Salt River and gradually diminishes toward the harbour region. Chiu-Wen Chen et al., (2012) also concluded that the upstream industrial and municipal wastewater discharges along the river bank are the major sources of copper pollution. According to the Philippines' DENR and Klope, 1980 conservative and maximum tolerable levels of copper in sediments are 20-ppm and 100-ppm respectively. All sampling stations exceeded the maximum tolerable level for copper in sediments.

In contrast, the level of copper in roots of *C. alternifolius* showed significant differences in all sites. During the dry season, the level of copper in Station 1 is high, considering that this site has no industrial activities and no visible signs of informal settlers. This implies

that copper is primarily contributed by runoffs from agricultural activities. The popular concept of industrial discharges being the primary source of all pollution is misguided because virtually every form of human activity is a potential source of pollution (Reeve, 2002). Station 3 has low levels of copper for the reason that the plant sample was not directly exposed to the river water.

Wet Season. Station 2 registers a high concentration of copper in water during the wet season as shown in Table 2. Mainly domestic activities and a car wash station were observed in this site. However, recent anthropogenic activities such as treating of wood furniture using copper-based pesticides were evident and there is a strong link that this activity is the source of the elevated levels of copper in this area. In addition, copper from storm water runoff originating from the sidings and roofs of building, various emissions from automobiles and depositional processes also contribute to the level of metal in this area (Davis, et al., 2001). Furthermore, with a basic pH in this site, the copper complexes are formed and predominate in aerated natural waters and these are slightly soluble in water (Zuehlke and Kester, 1983). In the study of Gaur (2005) high concentrations of metals, like copper, were detected in water in the rainy season compared to the summer season. According to his report, this is because during the rainy season, runoff from open contaminated sites, agricultural field and industries directly comes into the river without any treatment. Station 1 has low levels of copper due to heavy downpour of rain which washed away the waters to the lower zone of the river. Copper levels detected during the wet season were roughly two to six times greater compared to the dry season.

In contrast, Stations 2 and 4 during the wet season have roughly the same amount of copper in sediments but slightly lower than Stations 1 and 3. Silt and clay sediments have an effect on the solubility, distribution and bioavailability of copper in the environment. The turbulence caused by heavy rainfall guaranteed that there is more uptake of oxygen, formation of precipitates arising from formation of copper complexes. These complexes may settle in the sediments but because of the heavy rains there is subsequent dissolution. Station 1 however was higher because of the anthropogenic activity such as treatment of agricultural land with copper-based pesticides. In general, the concentration of copper in sediments are very much higher (20,000 x) than those in water. This manifests the accumulated contamination in the river sediments over the years. The result showed contrast to the study of Peng (2008) who found increase in the copper concentration in waters than in the sediments. This reveals that copper is mostly organic in this study and that of Peng (2008) is mostly inorganic in nature.

The concentration of copper in the roots from Stations 2 and 4 were almost twice of those

found in Stations 1 and 3. The roots collected from Stations 1 and 3 were entirely exposed to the overflowing water caused by heavy rainfall. This suggests a dilution of the copper content in this area. Copper in the roots of *C. alternifolius* L. in the downstream region is considerably higher than the upstream region during the wet season. The results showed that *C. alternifolius* can concentrate certain amount of copper in their roots to levels exceeding those present in water but not in sediment (Zayed et al., 1998; Sinha, 1999; Qian et al., 1999). According to Galiulin (2001), plants that are completely submerged in water accumulated more metals than floating and partially submerged plants. This confirms the role of the contact area between the plant and the aqueous environment. Varying soil characteristics can influence the uptake of copper by the roots of plants (Lepp, 1981).

Relationships of Copper Concentration in the Roots, Water and Sediments.

Correlation using Pearson's r between copper in (a) roots and water and (b) roots and sediments are shown in Figure 4 (A-D) respectively. There is a positive relationship between the copper in the roots of *C.*

Figure 4. Copper concentration in the roots with respect to the water during the wet (A) and dry (B) seasons; with respect to the sediment during the wet (C) and dry (D) seasons.

alternifolius L. with those in the water ($r^2 = 0.87$ and 0.82) and sediment ($r^2 = 0.83$ and 0.86). These results show the combined effects of the environmental pollution from water and sediment in the river. These results were also comparable to the study of Bonanno (2010) who reported a Pearson's r of 0.76 and 0.81 for copper in the roots of *Phragmites australis* with respect to water and copper in the roots of *Phragmites australis* with respect to the sediments, respectively.

The relatively high correlation suggests that *Cyperus alternifolius* L. can be regarded as indicator of metal pollution of the water body and sediments. It can also be employed as biomonitors to provide quantitative assessment of the Butuanon River. The use of biomonitors that are living and growing in a given area could yield valuable information not only on the presence of anthropogenic stressors, but more importantly, on the adverse impact the stressors are having on the environment (Wang et al., 1997; Chang et al., 2009; Ngayila et al., 2009).

Bioconcentration Factor for Roots of *C. alternifolius* L. The bioconcentration factors (BCF) are shown in Figures 5A and 5B for both sampling seasons and for the copper in roots with respect to the water and sediments. Figure 5A presents a high BCF for copper in the roots with respect to water during the dry season. This may be attributed to the low stream flow during this season which enhances the process of natural bioconcentration of the copper metal in the roots of the plant. The BCF values are within the bioaccumulation endpoint set by the U.S

EPA (1976) which is within the range of 1000 to 5000. During the wet season, the BCF is low which may be attributed to the fast and turbulent flow of the river water. The results showed that *C. alternifolius* can bioconcentrate high amounts of copper on its roots. The highest amount that it can bioconcentrate is ca. 70-ppm. The copper accumulated in its roots exceeds those present in water but not in the sediment suggesting that the main biosorption/accumulation of the metal by the *Cyperus alternifolius* is through the water. The BCF of this plant reaches as high as 9881 and is slightly higher than the study of Soda et al (2012) done in constructed wetlands. Based on the study of Ali et.al, (2002) the BCF of copper in roots of *Phragmites australis* ranged from 612 to 1592 while in the roots of *Zea mays*, BCF ranges from 349 to 1931.

Figure 5B shows the low BCF values for copper in plant with respect to the sediments for both seasons. Clay and silt are lighter compared to sandy sediments; in effect metals can easily diffuse along the whole stretch of the river. This type of sediment has a high affinity to absorb more metals, but because of the fast turbulent river water, sediments are rapidly displaced from one site to another site. The BCF values of the roots of *C. alternifolius* L. for copper with respect to sediments were exceptionally low but comparable to the study of Yoon (2006) using *Cyperus esculentus* L. The BCF value for copper with respect to sediment is 0.4800 to 0.5000 (Yoon, 2006) and the study of Ashraf (2011) on *Cyperus rotundus* L. reported a BCF value for copper with respect to sediments of 0.5623 . The low BCF values of the roots *C. alternifolius* with respect

Figure 5. BCF Cu in plant and Cu in water (A); BCF Cu in plant and Cu in sediments (B). Blue bars correspond to the dry season while red bars correspond to the wet season.

to the sediments may be attributed to the fact that at slightly basic soil pH (Table 1), the bioavailable fractions of copper in the sediment is less accessible for plant uptake (Cardwell, 2002).

Accumulation of copper in plants including *C. alternifolius* L. are oftentimes reported to be predominant in the roots (Tang et al., 1999; Cheng et al., 2002 ; Soda et al., 2012 ; Farrag et al., 2012). Consequently, an accurate estimate of the metal content in the plant's environment is best reflected by the amount of metal detected in the roots. Cheng et al (2002) reported that the amount of copper and other heavy metals accumulated by *Cyperus alternifolius* L. in a constructed wetland, occurred in the following rank: highest in lateral roots, main roots, rhizomes, leaves and lowest in shoots. The roots accumulated 466 mg/kg of copper and were considerably higher in the roots than in the shoots. These findings revealed that the mobility of different metals was less within the umbrella plant as shown by its translocation factor (TF) < 1.0. In a study by Pugh et al (2002), 20-100 mg/kg of the metal in a plant (leaves) is a level that is considered phytotoxic.

Baker and Brooks (1989) as cited by Mokhtar et al (2011) reported that hyper accumulation of heavy metals is said to occur when the plants are able to accumulate more than 1000 mg/L of the heavy metals into the plant system, either accumulating in the roots or shoots. A number of plants have been shown to accumulate metals in their shoots and show an exceptionally high tolerance to them while showing phytotoxic effects to others (Pandey, 2006; Akinola and Ekiyoyo, 2006). *Thlaspi caerulescens* is a hyper accumulator of Cd and Zn but is very sensitive to toxic concentrations of Cu (Lombi et al., 2001). On the other hand, Nazir et al (2011) reported that 88% of the plant samples they studied showed that copper concentration were greater in the roots than those in the shoots, indicating low mobility of copper from the roots to the shoots, and hence immobilization of copper in the roots. Farrag et al., 2012 reported that the TF of 9 metals including

copper in *C. articulatus* was found to be less than 1 for all metals, confirming the significance of below-ground biomass (roots) as a more effective heavy metal accumulator than the above ground biomass (stems and leaves).

A recent study reported by Ahmadpour et al (2014) reported that copper concentration among plant parts of *Jatropha curcas* were in the following trend; roots > stems > leaves. Although copper accumulation by the plant did not reach the criteria of copper hyper accumulators, this species showed a potential to be used in phytostabilization (reduction of metal mobility) of mildly copper contaminated areas. However the plant cannot be used for phytoextraction of copper contaminated soils.

In order for the plant to be used for phytoextraction, it has to be confirmed to be a hyperaccumulator (Terry and Banuelos, 2000). Van der Ent et al (2013) redefined the term hyperaccumulation and reported that in order for a plant to be a hyperaccumulator, the presence of high concentration of copper in the foliage (leaves) should be considered diagnostic of hyperaccumulation. Hence, high concentrations of copper in the roots or shoots are not a gauge for considering the plant as hyperaccumulator. In his study, Van der Ent et al (2013) proposed a threshold concentration of 300 µg/g (mg/kg) for copper, although at the moment, there has been no confirmed report of any plant to be hyperaccumulator of copper.

In the light of these studies, *C. alternifolius* L. is not considered a hyperaccumulator according to the definition of Van der Ent et al (2013). Root bioconcentration of copper (ca 70 mg/kg) in field conditions of Butuanon River is reported and although Cheng et al (2002) reported 466 mg/kg of copper in the roots of umbrella plant in a constructed wetland, this paper has not confirmed that this amount will also be possible under field conditions. A continuing study is in progress and the results will be reported elsewhere. The umbrella plant however has shown potential as indicator of copper pollution in river water and sediments

and may also be used for possible phytostabilization.

Limit of Detection, Method Validation and Statistical Evaluation of Data. The Limit of Detection (LOD = 3s) is 0.006 while recovery tests for water ranged from 107 - 112 %. The analysis between stations, seasons and interaction was interpreted using MANOVA through Microsoft Excel/ GraphPad v 6.0. Table 3 shows the statistical analysis of the

results of the chemical analyses of the roots, water and sediments collected from Butuanon River. The *p* values show significant difference for water for season, location and interaction. There is significant difference for sediments based on season and interaction while there was no significant difference for location (*p* = 0.054). The results of the roots of *C. alternifolius* L. show no significant difference by season (*p* = 0.121) while there was significant difference for location and interaction.

Table 3. Summary of the Statistical Data for Water, Sediments and Roots.

Matrix	Factor	<i>p</i> value (<i>p</i> critical=0.05)	Significance
Water	Season	0.0002	Yes
	Location	0.0173	Yes
	Interaction	0.0096	Yes
Sediments	Season	<0.0001	Yes
	Location	0.054	No
	Interaction	0.0069	Yes
Roots of Plant	Season	0.121	No
	Location	0.0004	Yes
	Interaction	<0.0001	Yes

CONCLUSION

The level of copper in the river water is below the criteria set by DENR which is 0.05 ppm while that in sediments exceeded the conservative limit of 20-ppm and beyond the maximum tolerable limit of 100-ppm (DENR) for both seasons. There is no significant difference in the copper concentrations of the sediments which implies that the sediments have accumulated the maximum amount of copper it can contain over the years. The aquatic plant *Cyperus alternifolius* L. has the capacity to bioconcentrate relatively high amounts of copper in its roots, ca.70 mg/kg, to levels exceeding those present in water but not in the sediment. This suggests that the main biosorption/accumulation of the metal by the *Cyperus alternifolius* L. is through the water. The capacity of *Cyperus alternifolius* L. to accumulate copper is not affected by the season. It can be used as indicator of copper pollution in river water and sediment.

Further studies involving an extended period beyond wet and dry seasons under Philippine conditions are in progress. Copper levels in the rhizomes, sources of copper contamination in these sites and speciation of copper are planned in the next study and will be reported elsewhere.

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