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Abstract: This study assessed copper and zinc distribution in the surface layer of sediment and rhizome tissue within the saturated surface vertical flow constructed wetland of CSBP Ltd, a fertiliser and chemical manufacturer located in Western Australia. Sediment and *Schoenoplectus validus* rhizome samples were collected at various distances from the inlet pipe while water samples are routinely collected. Water samples were analysed for nutrients and metals, sediments were analysed for total and bioavailable metals and rhizomes were analysed for total metals only. Mean influent copper and zinc concentrations were 0.19mg/L and 0.24mg/L respectively. The distribution of bioavailable Cu and Zn in the top sediment layer follows a horizontal profile. Analysis of variance (ANOVA) showed that the bioavailable fraction of these metals in sediments near the inlet pipe (30.2mg/kg Cu and 60.4mg/kg Zn) is significantly higher than in sediments at the farthest location (10.3mg/kg Cu and 26.1mg/kg Zn). The average total Cu concentration in the sediment at the 2m location has reached the 65mg/kg trigger value suggested by the Interim Sediment Quality Guidelines (ANZEEC 2000). Cu and Zn concentrations in the rhizome of *S. validus* do not vary significantly among different locations. Whether Cu and Zn concentrations at the CSBP wetland may reach toxic levels to plants and bacteria is still unknown and further research is required to address this issue. The surface component of the wetland favours sedimentation and binding of metals to the organic matter on the top of the sediment, furthermore, the sediment which tends to be anoxic with reducing conditions acts as a sink for metals.

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INTRODUCTION

Constructed wetlands have proven to be a cost effective alternative for the treatment of industrial effluents (Chen et al., 2006). The robustness of wetlands systems allow them to be used for wastewaters with varying characteristics. The wetland focus of this study was designed to treat nitrogen rich wastewater from CSBP Ltd. manufacturing plant in Kwinana, Western Australia, the plant produces Ammonia, Ammonium Nitrate, Nitric Acid, Compound Nitrogen and Phosphorous Fertilisers, Superphosphate, and Chlor-alkali products. As part of their operation the plant generates different types and amounts of effluent, the main sources are cooling tower blow-down water, combined wastewater pumped from production facilities and large volumes of stormwater runoff restricted to the winter months. Wastewater originated at different locations in the plant is firstly pumped into a containment pond and from there to the constructed wetland which was built in 2004.

Because the CSBP constructed wetland displayed varying plant densities in November 2006/January 2007 with lower densities next to the inlet distribution pipe and increasing densities with distance from the inlet pipe this study was conducted to verify if heavy metal concentrations in the sediment could be affecting plant health and consequently distribution in the wetland. After this study was conducted the vegetation recovered and by March 2007 presented vigorous growth in all areas of the wetland showing that the vast die off was a mere normal seasonal effect rather than a shock load of pollutants. The sediment and plant sampling and analyses however resulted in valuable data and information about this wetland system.

In treatment wetlands it is common for pollutants, especially metals and suspended solids, to accumulate nearest the inlet pipe (Kadlec and Knight, 1996). In horizontal flow wetlands one can expect a horizontal decreasing pattern of pollutants from the inlet to the outlet (Headley et al., 2005; Cooper et al., 2005). In vertical flow (downflow) wetlands a decreasing vertical profile from top to bottom can be expected with solids usually accumulating on the surface and pollutants being converted in the first 10 cm of the medium (Molle et al., 2005; Kayser and Kunst, 2005).

STUDY SITE AND METHODS

Wetland description

The constructed wetland at CSBP is 135 m long x 95 m wide (~ 1.3 ha) it has a vertical flow design and a 1 m deep sand substrate with a void ratio of approximately 0.3. The influent distribution pipe is laid centrally on the surface of the sand and the effluent drainage pipes are laid underneath the sand column on the surface of the liner, drainage pipes are covered with gravel and wrapped in geotextile fabric. The vegetation consists of River Club-rush *(Schoenoplectus validus).* The wetland can be described as a saturated surface vertical flow system and it is operated in a sequencing batch (feed - stay - drain) mode. The sand medium is kept constantly saturated with the water level varying between the sand surface and 0.3m above it (Figure 1A). The wetland is never fully drained and draining stops when the water level reaches the surface of the sand. One batch cycle usually takes 3 days, day one - feed, day two-stay and day three – drain. It is important to highlight that batch volumes and residence time are highly variable and mainly dictated by rain events and wastewater production. Batch volumes range from 300 to 3,000m^3, with an average of 1740m^3/batch.

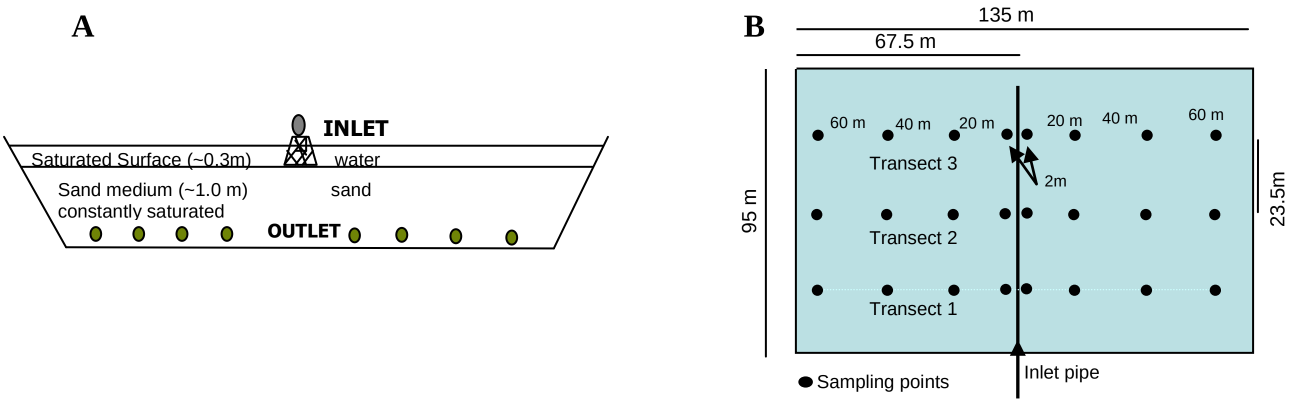

Figure 1: A- Section view of the wetland showing central inlet pipe on top of sand layer and outlet drainage pipes on the bottom of wetland. **B-** Plan view of the wetland showing transects with sediment and plant sampling locations.

The wetland is equipped with flow meters and automated composite samplers so every time the system is filled or drained samples are collected from the inlet and outlet pipes. Samples are analysed for pH, conductivity, ammonia, nitrate, total nitrogen, total phosphorous and a wide range of metals routinely (Table 1), analyses are conducted according to the Standard Methods (APHA, 1998).

Sediment and plant sampling

Sediment sampling was carried out along three transects positioned lengthwise in the wetland with the inlet pipe being located in the middle of the transect. Each transect was placed 23.5 m away from one another and from the sides of the wetland. There were 8 sampling points on each transect, two at 2 m, two at 20 m, two at 40 m and two at 60 m distance from the inlet pipe (Figure 1B). There were a total of 24 sediment samples and 18 plant samples, there were no plants located on the 2m sampling points.

On each sampling point the top 7cm of sediment was collected by means of a 50mm pipe which was introduced into the sediment to a depth of 7cm. With a shovel introduced laterally down to the bottom of the sampling pipe the sediment column was held in the pipe and transferred to plastic zip lock bags for later analysis. Before introducing the pipe into the sediment, most of the plant debris was removed from the top sediment carefully so the sludge layer was not disturbed. On the same sampling station, within a 0.2 m radius from where the sediment was collected, a live green plant sample was collected. Once collected, leaves were cut off and discarded and sediments were washed off of the root system and the rhizomes packed for later analysis. Sediment and plant samples were taken to the CSBP soil and plant laboratory for analysis immediately after collection.

Sediment and plant analysis

For plant analysis rhizome material was digested in nitric acid using a Milestone microwave. Sediment analysis was carried out with the DTPA extraction method (Lindsay and Norvell, 1978) and with the Aqua Regia digestion method. The Aqua Regia extraction method (digestion with 3:1 mixture of hydrochloric and nitric acids) is a stronger method of extraction than the DTPA and it corresponds to the total fraction of metals in the sediment, the DTPA method corresponds to the parcel of metals that are potentially bioavailable. Copper, zinc, manganese, calcium, magnesium, sodium, iron, potassium, phosphorus, sulphur and boron were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (McQuaker *et al*., 1979).

Data analysis

Data from the different locations were grouped in 2m, 20m, 40m and 60m distances from the inlet, for plants the 2m location was not present. One way analyses of variance (ANOVA) with distance from the inlet pipe were used to assess the significance of spatial differences in sediment and rhizome Cu and Zn concentration. Tukey’s post multiple comparison was chosen to verify differences between pairs. If the raw concentration data did not satisfy the homoscedasticity (homogeneity of variance) and normality requirements for ANOVA then the concentration data were log10 transformed to meet the assumptions of homoscedasticity and normality. If still after transformation data did not meet ANOVA requirements, then the nonparametric statistic test Kruskal-Wallis was performed with the Man-Whitney test performed to identify differences between pairs. The critical level for all statistical tests was $P \leq 0.05$. Statistical analyses were conducted with Minitab (Minitab software, 2006).

RESULTS AND DISCUSSION

Water quality

Influent and Effluent quality parameters from August 2004 to June 2007 are briefly described in Table 1 below. These results are based on 436 influent and 427 effluent samples collected and analysed by CSBP during the period. The estimated detection limits (DL) are given in the table. Metals such as Cd, Co, Cr and Pb had concentrations below the detection limits (ICP-AES) in more than 90% of influent and effluent samples and therefore are not presented.

It is evident from the table that the average percentage removal for metals is just an unrefined indication of performance; the true performance is higher than the one shown as it can be seen by the high percentage of effluent samples whose metal concentrations were brought to below detection limits (e.g. in 88% of the 427 effluent samples analysed Cu concentrations were below the detection limit).

Table 1: Water quality parameters in and out of the constructed wetland at CSBP. Only values > DL included.

Influent	**pH**	**NH3-N**	**NO3-N**	**N tot**	**P tot**	**Al**	**Cu**	**Fe**	**Mn**	**Mo**	**Ni**	**Zn**
		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Average	7.54	43.8	13.6	56.9	11.3	0.202	0.186	1.100	0.096	0.036	0.050	0.242
Minimum	2.54	2.3	0.4	6.2	1.3	0.042	0.021	0.050	0.021	0.026	0.026	0.024
Maximum	9.07	192.0	146.1	336.1	101.0	1.830	1.540	4.900	0.728	0.091	0.110	1.524
Median	7.55	36.0	8.7	46.2	6.6	0.138	0.168	0.964	0.068	0.033	0.050	0.194
Effluent												
average	7.53	36.1	11.5	47.7	7.2	0.059	0.070	0.236	0.047	0.031	0.050	0.085
Minimum	6.00	0.2	0.5	4.6	0.2	0.026	0.022	0.040	0.021	0.026	0.050	0.021
Maximum	8.29	135.0	99.6	185.6	25.6	0.548	0.238	1.610	0.157	0.057	0.050	0.510
Median	7.53	32.0	8.1	42.1	5.2	0.050	0.044	0.149	0.046	0.030	0.050	0.056
Avg removal (%)		17.4	15.9	16.1	36.7	70.7	62.1	78.5	50.5	12.0	1.0	64.9
DL	-	0.05	0.1	0.1	0.1	0.020	0.020	0.020	0.020	0.020	0.020	0.020
Effluent samples below DL (%)		0	0	0	0	12	88	0	4	26	32	70

Metal distribution in the sediment

Due to their higher concentrations in the sediment when compared to other elements analysed and possible toxic effect on the wetland plants the focus in this study is primarily on copper and zinc. Copper and zinc concentrations are higher near the inlet pipe (2m) and decrease outwards, with the lowest concentrations at the 60m location (Table 2). The DTPA is a weaker method of extraction than the Aqua Regia method, while the prior is used for estimating the potential bioavailability of metals in sediments the latter is more representative of the total concentration of metals in the sediment.

Table 2: Mean (standard deviation) metal concentration in the sediment -dry weight- sampled at different distances from the inlet pipe within CSBP constructed wetland. Means are based on six observations for each location. Samples were taken in January 2007.

Method	**Metal**		**2 m**		**20 m**		**40 m**		**60 m**	
DTPA extraction	**Cu**	**(mg/kg)**	30.2	(17.1)	19.6	(6.5)	11.2	(3.8)	10.3	(10.1)
	Zn	**(mg/kg)**	60.4	(33.7)	47.4	(9.0)	40.3	(5.8)	26.1	(21.9)
	Mn	**(mg/kg)**	3.6	(1.9)	2.2	(0.8)	1.7	(0.3)	2.5	(1.8)
	Fe	**(mg/kg)**	25.5	(14.2)	19.9	(6.6)	14.9	(3.8)	29.1	(8.8)
AQUA REGIA extraction	**Cu**	**ppm**	92.7	(71.8)	28.4	(7.1)	22.4	(6.3)	25.7	(26.6)
	Zn	**ppm**	198.5	(143.8)	77.8	(14.6)	78.6	(17.1)	52.6	(56.3)
	Cd	***ppb***	499.8	(361.7)	196.0	(26.2)	200.4	(32.0)	168.4	(136.4)
	Co	***ppb***	1226.0	(603.0)	552.5	(209.2)	435.4	(57.7)	581.0	(177.5)
	Ni	***ppb***	6555.7	(3681.8)	2887.0	(294.5)	2651.3	(264.5)	3747.0	(1367.4)
	As	***ppb***	4192.2	(1523.2)	2423.0	(260.3)	2332.7	(209.6)	2322.2	(614.0)
	Mo	***ppb***	940.0	(646.7)	196.5	(39.9)	174.0	(48.7)	416.8	(297.2)
	Se	***ppb***	165.5	(78.3)	106.5	(20.8)	107.5	(40.5)	120.6	(55.8)
	Pb	***ppb***	4366.4	(3337.8)	1203.1	(256.5)	1052.7	(103.8)	1531.9	(1142.7)

Bioavailable copper and zinc: Copper concentration data had to be $\log_{10}$ transformed to satisfy ANOVA requirements. One-way ANOVA indicated significant differences among distances ($P<0.05$) with Tukey's post multiple comparison test showing that the difference was between the 2m and the 60m locations.

Zinc concentration data did not meet ANOVA requirements even after $\log_{10}$ transformation therefore the nonparametric Kruskal-Wallis test for medians was used. No statistical difference ($P>0.05$) was found in zinc concentrations among the locations studied. However, when the Mann-Whitney test was performed between the 2m and 60m locations the result was that medians differed significantly ($P= 0.453$).

These results imply that the significant higher concentrations of bioavailable copper in the sediment near the inlet pipe might represent a more stressful environment for the plants which could in turn result in the evident pattern of lower density, and smaller size of plants near the inlet pipe and higher density and bigger size of plants around the 60m location.

Total copper and zinc: The Aqua Regia extraction method resulted in higher concentrations of Cu and Zn. In this case no statistical analysis was conducted but the same pattern of higher mean concentrations near the inlet pipe and lower mean concentrations towards the 60m location was verified (Figures 2 and 3)

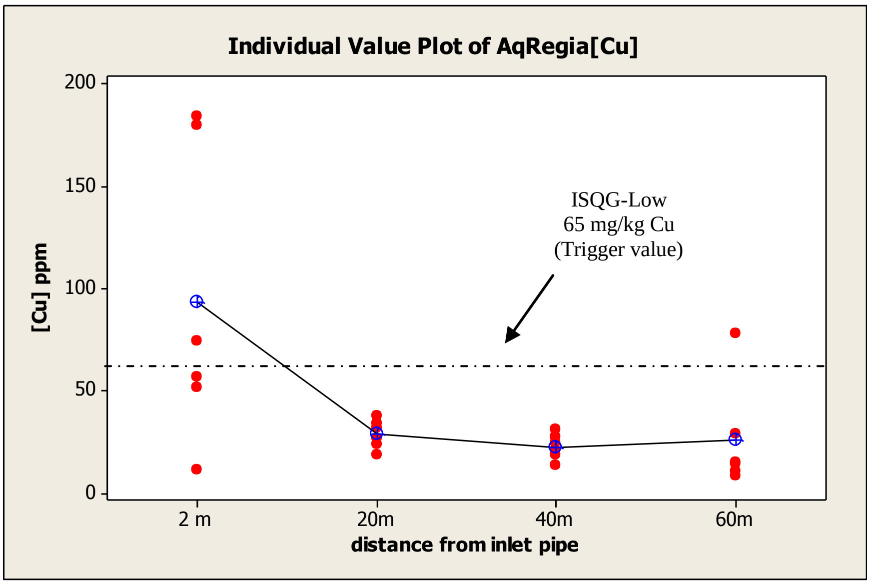

Figure 2: Individual concentration values of total Cu in the wetland sediment extracted by the Aqua Regia method. The solid line is connecting mean values from each location. The dashed line represents the ANZECC (2000) ISQG Low (trigger value) for copper in sediments.

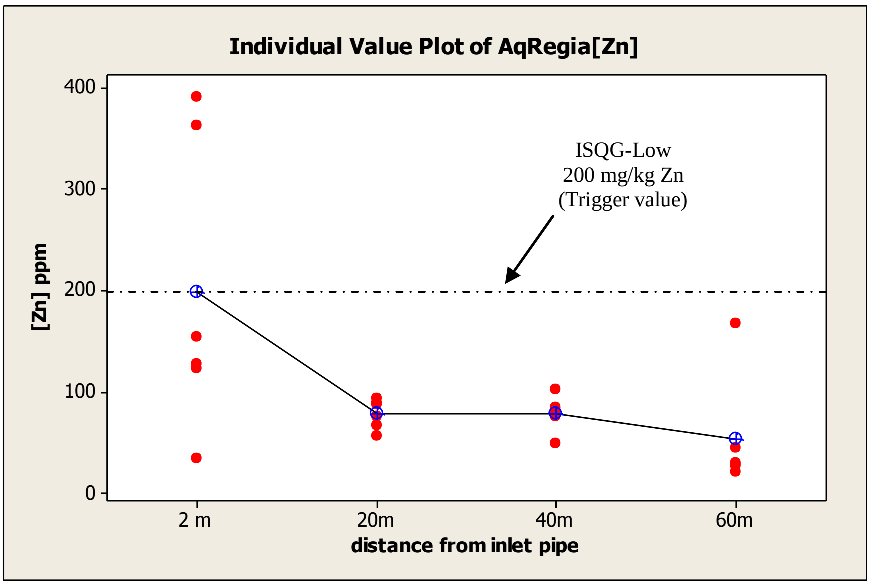

Figure 3: Individual concentration values of total Zn in the wetland sediment extracted by the Aqua Regia method. The solid line is connecting mean values from each location. The dashed line represents the ANZECC (2000) ISQG Low (trigger value) for zinc in sediments.

Sediment quality guidelines

Because of the difference in the extraction capacity of metal from the sediment given by different methods it is important that before any comparison be made an evaluation of the extraction methods used to derive the values of sediment quality guidelines must be carried out. Current sediment quality guidelines use values that are based on the total concentration of metals rather than their bioavailable fractions; such values are comparable to the Aqua Regia values presented in this study. Background concentrations of Cu and Zn in the local beach sand are expected to be low but no sampling occurred in the surrounding of the wetland.

For Australia and New Zealand the trigger values for Cu and Zn in sediments are based on modified values presented by Long et al. (1995) from the US National Oceanic and Atmospheric Administration (NOAA) database. The sediment quality guidelines were published as part of the Australia and New Zealand guidelines for fresh and marine water quality, ANZECC (2000). The Low (trigger-value) and High Interim Sediment Quality Guidelines (ISQG) values for Cu and Zn are 65-270 mg/kg and 200-410 mg/kg respectively .The mean total concentrations of Cu and Zn for the 2m location are 92.7 ppm and 198.5 ppm respectively, so we can conclude that Cu concentrations at the 2m location are of greater concern than Zn, as Cu concentrations have already past the guidelines' trigger value while Zn is still, but just, below the trigger value set by the ANZECC (2000) guidelines for sediment quality.

The Canadian Sediment Quality Guidelines for Protection of Aquatic Life, CCME (2001) has more conservative values, suggesting for Cu an interim freshwater sediment quality guideline (ISQG) value of 35.7 mg/kg and a probable effect level (PEL) of 197 mg/kg. For Zn these values are ISQG = 123 mg/kg and PEL = 315 mg/kg.

Although heavy metal values for the CSBP wetland are not above the ISQG high values, attention should be paid to the rate that metals accumulate in the sediment so high ISQG values are not overtaken. It is also important to note that there is a great variability in metal tolerance among plant species, with some species presenting toxicity symptoms at lower concentrations than others. *Schoenoplectus* and *Typha* spp can be more tolerant than other species (Dunbabin and Bower, 1992).

The same pattern of higher values near the inlet pipe and lower values on the other locations was observed for Cd, Ni, As, Mo, Se and Pb. However, the concentrations of these elements are low and far below the trigger values - low ISQG, proposed by the ANZECC (2000) guidelines.

Premi and Cornfield (1969) verified no impact of copper and zinc on ammonification and nitrification in an experiment with soil incubation even when these elements were applied in water at the highest concentrations of 10,000 ppm. Cela and Summer (2002), however, found that more than 3.8mg water-extractable Cu/Kg soil was inhibitory for nitrification, whereas less than 2mg/kg was safe for nitrification to occur. The values obtained from the water extraction method for metal used by Cela and Summer are not comparable to the ones obtained here since the extraction methods are totally different. The impact of metals on nitrification was not quantified here.

Metal distribution in the rhizome tissue

Results of the plant tissue analysis are presented in Table 3. Only healthy plants were collected for analysis. The overall average concentration of copper in rhizome tissue for the wetland was 793.7 (± 554.1) mg/kg and for zinc it was 571.2 (± 221.1) mg/kg.

The nonparametric Kruskal-Wallis test was used to test the hypothesis of different rhizome copper and zinc concentrations in the wetland. Copper and zinc concentrations in the rhizome tissue did not vary significantly ($P > 0.05$) among different locations in the wetland.

Table 3: Mean (standard deviation) metal concentration in the rhizome tissue sampled at different distances from the inlet pipe within CSBP constructed wetland. Means are based on 4 observations for each location. Samples were taken in January 2007.

Analyte		**20m**		**40m**		**60m**	
NITROGEN	%	1.21	(0.72)	1.69	(0.65)	1.86	(0.71)
PHOSPHORUS	%	0.37	(0.10)	0.36	(0.04)	0.48	(0.21)
COPPER	mg/kg	541.98	(310.24)	564.20	(134.04)	1102.00	(1008.62)
ZINC	mg/kg	513.96	(104.68)	576.62	(116.43)	519.56	(322.21)
MANGANESE	mg/kg	44.38	(12.19)	45.30	(4.11)	52.05	(15.42)
IRON	mg/kg	2559.08	(1301.97)	2393.33	(481.07)	3840.78	(2939.96)
NITRATE	mg/kg	42.00	(3.92)	54.50	(18.34)	52.00	(44.42)
BORON	mg/kg	16.80	(2.50)	16.73	(1.50)	16.75	(3.31)

On a dry weight basis, macrophyte roots and rhizomes can have a much higher concentration than the sediments where they are located. It is also known that in emergent macrophytes, as it is the case of *S. validus*, the roots and rhizomes accumulate significantly greater concentrations of metals than stems and leaves (Cardwell et al., 2002). When the concentrations of Cu and Zn were analysed in rhizome and leaf tissues of S. validus (data not shown) sampled from the CSBP treatment wetland and a non-treatment wetland located at the Environmental Technology Centre/Murdoch University no significant difference was found between concentrations of Cu and Zn in rhizome and leaf tissue from the CSBP plants, however the rhizomes from the non-treatment wetland plants presented significant higher concentrations of Cu and Zn than the leaves.

In a study conducted by Dunbain and Bowmer (1992) using constructed wetlands to treat industrial wastewaters containing metals it was found that *Schoenoplectus* and *Typha* spp. can be more tolerant than other species and that metal tolerance is a fuction of plant phenology, vigour and growth as well as metal speciation and aquatic chemistry. Also, high nutrient concentrations, as found in the CSBP wetland, could increase the toxicity tolerance of macrophytes (Manios et al., 2003).

Copper rhizome concentrations here are much higher than those reported by Murray-Gulde *et al.* (2005) for *Schoenoplectus californicus* planted in a wetland receiving copper contaminated wastewater, in her study copper concentrations in the roots ranged from 9.34 (±5.14) to 51.32 (±32.13) mg/kg.

The significant higher concentrations of bioavailable metals in the sediment where plants are not present (2m) compared to the lower concentrations in sediment where plants grow more vigorously (60m) suggest that plants uptake and store considerable amounts of metals from the sediment indicating that *S. validus* plays an important role in removing and immobilizing heavy metals at the CSBP wetland. The rate which these metals leach out of the decaying plant matter and re-enter the water however is unknown and would be focus of further investigation.

Ebbs and Kochian (1997) working with *Brassica sp.* in phytoremediation for contaminated soils report concentration in roots in excess of 10 000 mg/kg Zn and for Cu ranging from 750 to 1500 mg/kg. Studying metal accumulation in some macrophytes from polluted urban streams in Southeast Queensland Cardwell *et al.* (2002) verified that roots of S. validus contained 76.6 mg/kg of Cu and up to 1568 mg/kg of Zn, on a dry weight basis. In this study, the overall concentration of Cu is similar to that of Zn in the rhizome, with higher values for Cu than for Zn being observed; these results differ from the pattern of higher Zn accumulation reported in the literature.

CONCLUSIONS

Whether Cu and Zn concentrations at the CSBP wetland may reach toxic levels to plants and bacteria is still unknown. Further research is being conducted to address this issue. What has been verified is that the surface component of the wetland favours sedimentation and binding of metals to the organic matter and the sediment which tend to be anoxic with reducing conditions act as a sink for metals. The distributions of bioavailable Cu and Zn in the top sediment layer follow a horizontal profile. Concentrations of these metals in sediments near the inlet pipe (2m) are significantly higher than in sediments at the farthest location (60m).

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