

## DISTRIBUTION OF FIVE TOXIC HEAVY METALS IN BIOTIC AND ABIOTIC CONSTITUENTS OF THE NEGOMBO LAGOON, SRI LANKA



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### Abstract

The presence of toxic metals in water has become a matter of national concern. This situation is potentially more dangerous when it occurs in lagoons and estuaries which are both highly productive and sensitive in comparison to other natural habitats. In this study, the levels of five toxic heavy metals, As, Cd, Cr, Pb and Hg, present in several abiotic and biotic constituents of the Negombo lagoon, Sri Lanka, were investigated with the objective of assessing the potential risks of accumulation. Sampling, in five locations, was conducted from December 2014 to April 2015. Water, sediment, soil, bark and leaves of the mangrove *Bruguiera gymnorhiza*, and fauna of selected taxa were collected and heavy metals were analyzed using ICP – OES following microwave digestion. Apart from Cr which was detected at low levels, none of the other metals were detected in the water. Nevertheless, the five heavy metals were present in relatively large amounts in one or more of the other tested constituents, indicating lagoon pollution, depicting an overall trend water < leaves < bark < snails < fish < crab < sediment < soil. Our investigation suggests that As and Cd, due to their high levels of accumulation in soil and sediment, impose the highest potential risks. Trends in accumulation suggest non-point sources for Cd, Cr and Pb. Findings reported here call for continual monitoring and for controlling discharge of contaminated effluents into productive lagoon ecosystems.

**Key words:** accumulation, abiotic, biotic, heavy metals, lagoon

## 1. Introduction

Heavy metal contamination in the aquatic environment is a matter of serious concern due to its adverse impact on humans and other organisms. Although heavy metals in the environment could arise from natural sources (Chibuike & Obiora, 2014), the recent increases have, to a large extent, been attributed to anthropogenic activities such as the discharge of untreated industrial effluents and the intense use of agrochemicals (Dixit *et al.*, 2015). Heavy metals that enter a water body are dispersed throughout its biotic and abiotic constituents, the levels of occurrence being influenced by the condition of the water at a given time. For instance, acidic conditions cause heavy metal ions adsorbed to soil and sediment to be released into the water increasing their bioavailability in the aquatic medium (Alghanmi, *et al.*, 2015). Similarly, other factors, such as temperature and the amount of dissolved oxygen and organic matter, also determine the fate of the heavy metals within a water body (Kuwabara *et al.*, 1996; Croteau *et al.*, 2002).

The heavy metals As, Cd, Hg, Pb and Cr (VI) are non-essential elements, and they induce toxic impacts in biota even at trace levels (Tchounwou *et al.*, 2012). The potential hazard imposed by these metals to organisms is enhanced due to the bio-accumulative and non-biodegradable nature of the elements (Cui *et al.*, 2011; Yu *et al.*, 2013). Furthermore, the potential for accumulated heavy metals to bio-magnify along food chains, ultimately reaching high concentrations in organisms at the top trophic level, has

been well documented. It has been shown that the accumulated metal concentrations in organisms generally reflect, quantitatively or semi-quantitatively, the level of environmental pollution (Dallinger, 1994), allowing them to be used as bio-indicators of metal pollution.

The Negombo lagoon in the western region of Sri Lanka (7° 09'N 79° 51' E) receives fresh water from three sources: the Ja-Ela, Dandugam Oya and the Hamilton Canal (Gammanpila, 2013). The area mainly receives rainfall from the southwest monsoon from May to September, while convectional showers occur during the remaining months of the year (CEA, 1994). The temperature varies between 24 °C to 30 °C. The Negombo lagoon is one of the most productive brackish water ecosystems in Sri Lanka in terms of mangrove vegetation, fauna and inland fishery (Samarakoon & van Zon, 1991; Jayasiri, 2004; Dahanayaka *et al.*, 2008). Previous studies have assessed the status of the Negombo lagoon through accumulation of heavy metals in selected constituents (e.g. Indrajith & Pathiratne, 2006; Indrajith *et al.*, 2008 – water, sediment and fish; Asanthi *et al.*, 2007 – water, sediment and algae; Mendis *et al.*, 2015a & b – water and fish). In this paper, the levels of the five heavy metals As, Cd, Cr, Hg and Pb were examined simultaneously, in three abiotic (water, sediment and soil) and selected biotic (mangrove, snails, fish and crabs) constituents of the Negombo lagoon. In ascertaining the levels of these elements the possibility of bio-concentration of these heavy metals was also investigated.

## 2. Material and Methods

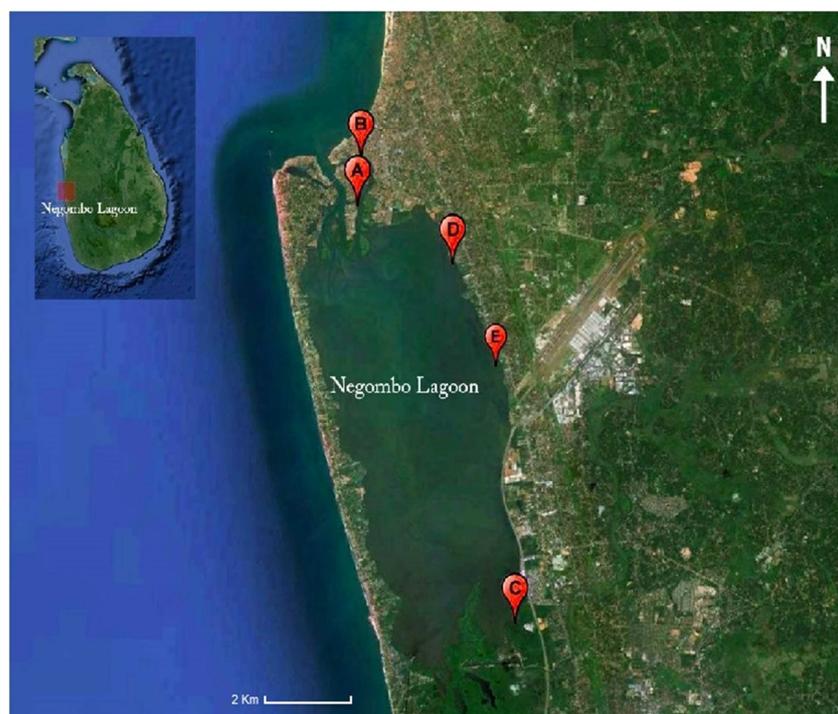
### 2.1 Collection of samples

The present study was conducted between December 2014 and April 2015 at five sampling stations: A – bordering the National Aquatic Resources, Research and Development Agency (NARA) Conservation Centre, B – bordering the main bus station in Negombo, C – Dandugam Oya inlet, D – bordering a residential area and E – in close proximity to the airport (Figure 1). At each station, samples were collected at five random points separated from each other by a distance of more than 20 m, yielding a total of 25 sampling points. At each of these points, two samples each of water, sediment and soil were collected yielding a total of 50 samples per component. The water was collected from 1 m beneath the surface with a Ruttner Sampler and stored in polypropylene containers for analysis. These containers were washed with 10 % HCl and then with de-ionized water before use. Surface sediment (down to around 3 cm from the bottom surface) was scraped off with a scoop and collected into sealable polythene bags, with excess water being drained before sealing. Soil, within 5 m away from the water and to a depth of 3 cm from the soil surface, was scooped using a deep spoon and also stored in sealable polythene bags. Obtaining soil and sediment in this manner increased the likelihood of collecting recent deposits, as opposed to mainly material of geological origin. At each point bark and mature leaves from a minimum of three trees of the mangrove *Bruguiera gymnorhiza* were collected.

Faunal species were collected at four of the stations only (other than from station C). Collection of faunal species was based on the available taxa. For instance, samples of *Hydrobiid* sp. (gastropod) were collected from station A, *Heteropneustus fossilis* (Asian stinging cat fish) from B, *Scylla serrata* (mud crab) from D and *Eutroplus suratensis* (Pearlspot Cichlid) from E. A total of ten samples each of the different taxa, which were sufficient for analysis, were collected from the four sampling stations. Snails were collected from the surface of the soil/sediment while fish and crabs were caught with the aid of fishermen in the vicinity of the sampling stations. All samples were transported to the laboratory and stored at 4°C until analyses which were carried out typically within 3 days of collection.

### 2.2 Analysis of heavy metals

The procedure employed for analysis of heavy metals in water was according to APHA 3120 – B (US EPA, 2007). Samples of the water were digested with conc. HNO<sub>3</sub> (10 cm<sup>3</sup> conc. HNO<sub>3</sub> for 50 cm<sup>3</sup> of each sample) and further concentration was achieved by evaporation. In cases where NaCl precipitated before the concentration process, the solutions were topped to different volumes using de-ionized water. After filtration the solutes were analyzed using ICP – OES and tested for heavy metals. Whenever necessary, the solute was diluted. Soil and sediment (500 g each) and 20 g of each biotic constituent, i.e. bark, leaves and faunal tissue, were also digested for analysis, as described in US EPA (2007).



**Figure 1.** Map of the Negombo lagoon showing the five sampling stations

Following microwave digestion, the concentrations of As, Cd, Cr, Pb and Hg in each sample were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Varian 720-ES, Australia). Recovery studies were done with spiked samples and certified reference materials (CRM). Analyses were conducted in duplicate, and repeated if the error exceeded 10 %. For the recovery procedure, a known amount of the standard mixture of the five heavy metals was spiked into conc.  $\text{HNO}_3$  ( $10 \text{ cm}^3$ ) (blank sample) and digested. The CRMs used were 2711a Montana II, 2976 muscle tissue, 1570a spinach and TMDA 64.2. ICP-OES and VGA. Calibration check solutions were analyzed to ensure instrument performance and to validate calibration, using the same acid matrix.

Calibration checks ( $20 \mu\text{g}/\text{dm}^3$  for Hg and  $1.00 \text{ mg}/\text{dm}^3$  for the other metals) were used for every 20 samples. Certified aqueous reference standards of TMDA 64.2 and ICP-OES wavelength calibration solutions were obtained from an outside source and analyzed according to instructions provided. The generated results were checked to ensure that they were within the acceptable range.

### 2.3 Potential risk factors

The Contamination Factor (Cf) which describes the contamination of a given toxic substance in a water body as suggested by Håkanson (1980) is useful for assessing the potential dangers imposed by the heavy metal loads in a water body and is derived using the

formula  $C_f = C_{\text{Current}} / C_{\text{Pre-ind}}$ . These indices were calculated for the Negombo lagoon. Since data on pre-industrial levels are lacking for any of the tested metals for Sri Lankan systems, the values obtained for a range of European and American water bodies prior to industrialization, which have been also widely applied even for tropical estuaries (e.g Vowotor *et al.*, 2014), were used for this purpose. Additionally, the Ecological Risk Factor (Er) based on the Toxicity Response Factor (Tr) assigned to each of the metals (Håkanson 1980) was calculated using  $Er = Tr \times C_f$ .

### 3. Results

The analyses revealed the presence of all five toxic heavy metals i.e. As, Cd, Cr, Pb and Hg in at least one of the biotic or abiotic constituents analyzed from each of the five sampling stations in the Negombo lagoon. Figure 2 shows the mean levels ( $\pm$  standard errors) of the metals recorded in the different constituents, whilst the minimum- maximum ranges detected for each of the metals are shown in Table 1. Some of the salient features evident from the results are set out below.

#### 3.1 Apportionment among constituents

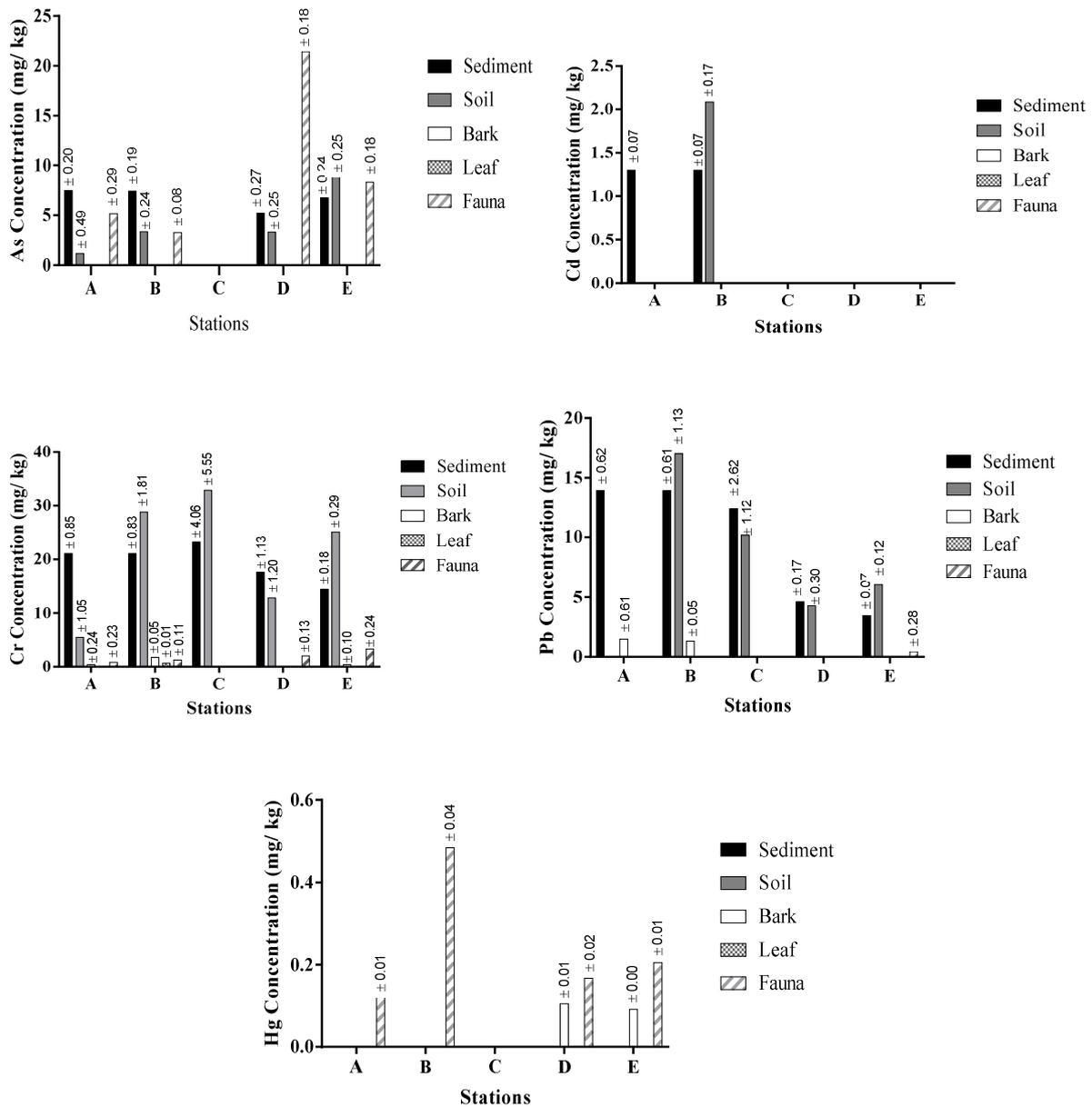
The mean  $\pm$  standard error of the pH values in the water samples recorded for the five sampling stations were A:  $7.79 \pm 0.24$ , B:  $6.55 \pm 0.14$ , C:  $5.65 \pm 0.10$ , D:  $7.78 \pm 0.04$  and E:  $7.85 \pm 0.12$ . The one way ANOVA and Tukey pairwise comparison test revealed significant station-wise differences in pH ( $p \leq 0.05$ ), with station C having a significantly lower pH. With respect to the heavy metals, it is

noteworthy that, with the exception of Cr (recording a maximum of  $0.09 \text{ mg/dm}^3$ ), none of the other toxic heavy metals (As, Cd, Pb, and Hg) were detected in the water. Nevertheless, the detected levels of the five metals in the other constituents i.e. sediment, soil and the biota, are indicative of heavy metal contamination of the Negombo lagoon (Figure 3 and Table 1). Considering sediment and soil, the highest metal levels recorded were for Cr ( $34.3$  and  $49.8 \text{ mg/kg}$  respectively). Both these constituents were also relatively rich in Pb, whilst As and Cd were present at low levels. It is significant that Hg was not recorded in sediment or soil at any of the sampling stations. Considering heavy metal concentrations recorded in the flora and fauna, apart from Cd, all the other toxic heavy metals were recorded in one or more of the biotic constituents. From among these, as although not recorded in the mangrove, was seen to reach high levels, with a maximum of  $22.5 \text{ mg/kg}$ , in crabs. Cr, Hg and Pb were recorded in comparatively high concentrations in both floral and faunal components (Figure 2 and Table 1).

The overall trends of the mean levels of accumulation of the five metals recorded in water, sediment, soil, flora and fauna are shown in Figures 3 (a) and (b). The graphs show that, apart from subtle differences, there is a similar overall pattern of accumulation for As, Cd, Cr and Pb with concentrations increasing in the order water < leaves < bark < snails < fish < crab < sediment < soil. Hg was only recorded from the biotic constituents. Soil and sediment evidently function as sinks of these heavy metals.

The  $C_{Pre-ind}$  and  $T_r$  factors as well as the generated  $C_f$  and  $E_r$  values are given in Table 2. Accordingly, both concentration factors for sediment and soil are generally

low (i.e. < 1) and in the order of  $Pb < Cr < As < Cd$ . The Ecological Risk Factors increase in the overall order of  $Cr < Pb < As < Cd$ .



**Figure 2.** Mean concentrations ( $\pm$  standard error) of the five tested heavy metals in the different biotic and abiotic constituents in the five sampling stations (A to E). (Note: results for water, where only Cr was detected at low levels, are not shown)

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**Table 1.** The minimum and maximum values of the five selected heavy metals in the different biotic and abiotic constituents in the Negombo lagoon.

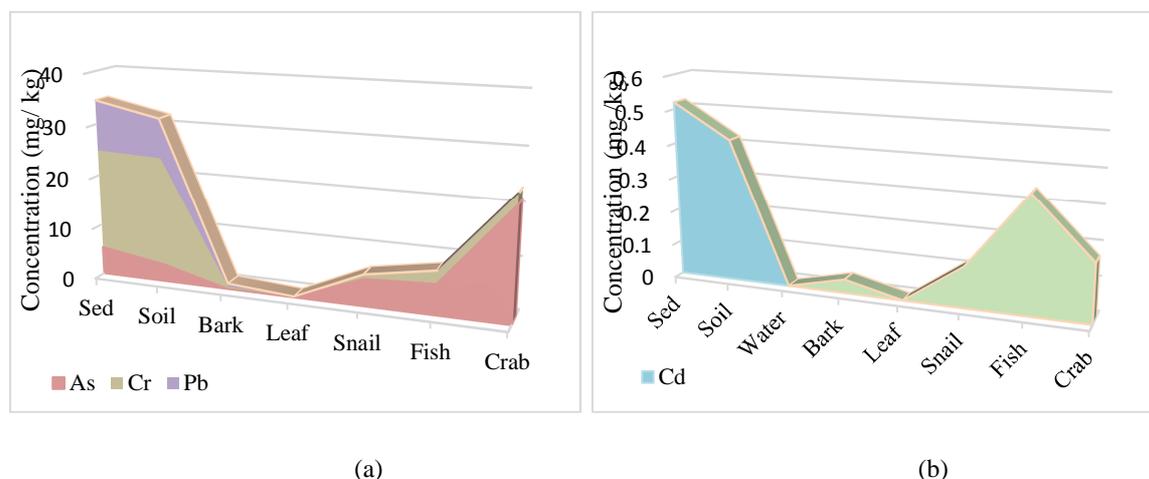
Constituent	As	Cd	Cr	Hg	Pb
Water (mg /dm <sup>3</sup> ) (n=50)	ND	ND	ND - 0.09	ND	ND
Sediment (mg /kg) (n=50)	ND - 8.65	ND - 1.67	7.64 - 34.30	ND	2.53 - 19.61
Soil (mg /kg) (n=50)	ND - 9.89	ND - 2.63	2.40 - 49.80	ND	ND - 20.26
<i>Bruguiera</i>					
Bark (mg /kg) (n=50)	ND	ND	ND - 1.93	ND - 0.15	ND - 4.09
Leaves (n=50)	ND	ND	ND - 0.69	ND	ND
Snails (mg /kg) (n=10)	3.46 - 6.25	ND	ND - 1.76	0.07 - 0.22	ND
Fish (mg /kg) (n=20)	2.90 - 9.23	ND	0.92 - 4.69	0.15 - 0.61	ND - 2.34
Crabs (mg /kg) (n=10)	20.51 - 22.46	ND	1.41 - 2.48	0.05 - 0.26	ND

ND indicates metal levels below limits of detection; n indicates number of samples analyzed

### 3.2 Inter-site comparisons

To interpret inter-site differences the combined results for water, sediment, soil, and mangrove bark and leaves for each of the metals were considered. Results for fauna were not included due to the disparity in sample sizes between sites. A two way ANOVA indicated that, overall in the Negombo lagoon, there is a significant metal-wise difference, with loads of some metals being markedly

higher than the others (metals:  $p < 0.001$ ). Nevertheless, the inter-site differences in total metal levels were not significant (sites:  $p > 0.05$ ), indicating that the total metal loads between sites were somewhat comparable. The interaction between sites and metals was also not significant (metals x sites:  $p > 0.05$ ) suggesting that the trends of accumulation of each of the metals in the different sites were nearly consistent.



**Figure 3.** The patterns of accumulation of heavy metals (means) in the different biotic and abiotic constituents of the Negombo lagoon, depicted in two concentration ranges (a) Pb, Cr, As and (b) Cd, Hg.

The extent of consistency between sites suggests similarity in the sources of contamination and this was examined using the Pearson's correlation coefficient matrix, following Vowotor *et al.* (2014). The correlation matrix for the five heavy metals under consideration is given in Table 3. Interestingly, the significant and strong positive correlations between the three contaminants; Cd, Cr and Pb, points

to similar sources of inputs suggesting the likelihood of non-point sources for these metals. On the other hand, As and Hg are not significantly correlated with any of the other metals, nor with each other, suggesting that these two metals most likely arise from different sources. The localization of Hg also suggests a point source of input.

**Table 2.** Concentration Pre-industrial ( $C_{Pre-ind.}$ ), Toxic response factor ( $T_r$ ), Contamination Factor ( $C_f$ ) and Ecological Risk Factor ( $E_r$ ) values for As, Cd, Cr and Pb. Note: Hg was not recorded in sediment and soil.

		As	Cd	Cr	Pb
$T_r^*$		10	30	2	5
$C_f$	Sediment	0.4	0.5	0.2	0.1
	Soil	0.2	0.4	0.2	0.1
$E_r$	Sediment	4.0	15.0	0.4	0.5
	Soil	2.0	12.0	0.4	0.5

\*Source - Håkanson (1980)

**Table 3.** Pearson's Correlation Coefficients showing the consistency in occurrence of the five tested heavy metals in the five sampling sites.

	Cd	Cr	Pb	As
Cr	0.55* $p < 0.01$			
Pb	0.79* $p < 0.001$	0.90* $p < 0.001$		
As	0.20 $p > 0.05$	0.25 $p > 0.05$	0.19 $p > 0.05$	
Hg	-0.11 $p > 0.05$	0.18 $p > 0.05$	0.20 $p > 0.05$	0.36 $p = 0.05$

\*significant association ( $p < 0.01$ )

#### 4. Discussion

In the current investigation, the five tested heavy metals (As, Cd, Cr, Pb and Hg) were recorded at relatively high levels in one or more of the biotic or abiotic constituents in the Negombo lagoon. In the water, however, none of the elements, with the exception of Cr, was recorded, and that too at relatively low levels. The absence/low concentration of the heavy metals in the water reported here is in conflict with previously documented values for the Negombo lagoon. For instance, values reported by Indrajith & Pathiratne (2006) and Indrajith *et al.* (2008) for Cd and Pb, and by Asanthi *et al.* (2007) for arsenic, in water, were as high as 5.70, 2.10 and 2.16  $\mu\text{g/L}$ , respectively. The seemingly contradictory results obtained in the present study could be viewed in the light of the conditions and processes that prevail in the lagoon. One of the most important processes controlling the

transport of heavy metals is the adsorption onto surfaces of solid particulate matter, which results in lowering levels in the water medium (Rieuwerts *et al.*, 1998). Usually in lagoons, triggered by acidic conditions, heavy metals tend to precipitate with sulphide, lowering bioavailability in the water (Simpson & Spadaro, 2016). Such processes give the water body the ability to regulate toxic metals between the biotic and abiotic components. In the present study there is some evidence for variability in pH levels across the sampling stations which may have contributed to some extent to the observed differences in the metal loads between the sites, although such differences were not significant. Two other factors that would seriously affect concentrations of heavy metals and other contaminants in the water at a given time is the rate of flushing and the residence time of water, both of which would have a major impact on the rate of sedimentation as well as on the water chemistry. The

rate of water exchange between the lagoon and the adjacent ocean occurs mainly through tidal action and the quantity of freshwater discharge. The Negombo lagoon is reported to feature a weak tide (maximum range is about 10 cm) but it has a strong and variable freshwater discharge resulting in a residence time of between 2-14 days (Rydberg & Wickbom, 1996; Rajapaksha, 1997). The intermittent showers in the area during the study period and the high freshwater discharge into the lagoon would have facilitated some flushing which most likely resulted in low accumulation of heavy metals in the water as observed here.

In the present study the heavy metal loads recorded in sediment and soil were much higher than those in water, with soil and sediment being near equally polluted, reiterating the function of these two constituents as heavy metal sinks. Soil and sediment generally act as sinks of heavy metals primarily due to the organic fraction that has the capacity to adsorb these elements, the process being considerably influenced by factors such as pH, metal speciation, ionic strength, and other properties that are inherent to the target metal (Lin & Chen, 1998). Flushing and the re-suspension of metals through the disturbance of sediment or soil would temporarily cause elevation of metal loads in water at a particular site (Eisenreich *et al.*, 1980).

Heavy metal levels observed in the present study for sediment in the Negombo lagoon are similar for Pb and Cr, whilst Cd values are higher than previously recorded values (Indrajith *et al.*, 2008). It is also noteworthy that

although Hg has been previously recorded in sediment from the Negombo lagoon (Indrajith *et al.*, 2008), the present study did not detect this metal in any of the soil or sediment samples. Studies have demonstrated that metal ions with similar valance states compete for metal binding sites, i.e. in this case for surfaces of organic matter. Thus, there is a possibility that Hg was displaced upon additional loadings of other metals such as Pb (Holmberg, 2006). The weak association noted between Hg and Pb observed in the present study, however, does not provide persuasive evidence that displacement occurred. The present investigation highlights As and Cd as metals with the highest potential to concentrate in soil or sediment whilst Cd, due to its high prevailing levels and high potency to cause adverse health impacts, shows up as the metal with the higher ecological risk. Although the risks associated with individual metals are low accordingly to the stated threshold values (see Hakanson, 1980), the 'true risk' to biota may arise from the summation of risks posed by all metals (as well as other contaminants) present in the ecosystem (Hakanson, 1980).

What is of considerable interest is that, despite concentrations in water being beyond detectable limits for most of the metals and for the majority of the sampling sites, there were relatively high levels of accumulation of those metals in the biotic elements. Usually living organisms take up heavy metals from abiotic constituents, i.e. from soil, water and sediment. The level of accumulation also depends on the particular trophic position each organism occupies in the food chain. The heavy metals that are

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taken up by the biotic elements may later return to the abiotic media through senescence, i.e. the shedding of leaves or death, and through the addition of faecal matter (James & Bartlett, 1984). In the present study, from among the biotic components, leaves of *Bruguiera gymnorhiza* accumulated the least amounts of a given heavy metal, which is consistent with reported trends in other studies, for instance in the mangrove *Avicennia marina* in the Persian Gulf (Amoozadeh *et al.*, 2014). Thus, a lower amount of pollutants in the leaves of a plant, in comparison to the bark, as observed in the present study, is acceptable given its much shorter life span. The capacity of heavy metals to concentrate in bark and leaves indicates that organisms consuming these components, directly or indirectly, would be exposed to heavy metal toxicity. Although the leaves of *B. gymnorhiza* are not consumed by people, it is used as fodder for cattle, while many herbivorous invertebrates and fish consume both leaves and bark of the plant. The values for Cr and Pb in mangroves observed in the present study exceed the values for algae in the same lagoon reported by Asanthi *et al.* (2007), whilst the Cd and As levels are lower. The recorded levels here for Hg cannot be compared as no previous records are available.

The tested fauna, i.e. fish, snails and crabs, recorded varying but relatively high levels of the five toxic heavy metals. The rate of accumulation of a particular heavy metal within a given species is associated with the rates of uptake and excretion as well as the storage potential for the metal (Abdullah *et al.*, 2007). Additionally, it has been shown that, because sediment has a

high capacity to accumulate heavy metals in a given ecosystem, the taxa associated with detritus food chains may show higher levels of concentration of heavy metals in comparison to those associated with grazing food chains in the same ecosystem (Ireland & Richards, 1977, Athalye, 2000, Cui *et al.*, 2011). One of the fish species *Heteropneustes fossilis* is a fresh water fish but also inhabits the lagoon. This species is a bottom dweller and hence would have had exposure to high levels of the heavy metals in the sediment. It should also be kept in mind that the high levels of heavy metals recorded in fish and crabs may not be entirely due to acquisition in the lagoon, since their mobility may have resulted in exposure to heavy metals elsewhere. It is also documented that crustaceans and molluscs are able to fix heavy metals, particularly lead, in their shells (Sultana *et al.*, 2015) which would not have shown up in the present study where only the flesh was considered. The concentrations reported here for fish exceed levels previously reported for Cr, Pb and Hg (Indrajith *et al.*, 2008; Mendis *et al.*, 2015a), although Cd levels were lower. Straightforward comparisons are, however, not ideal since there is considerable inter-specific variation in heavy metal accumulation rates that is influenced by the physiology of the species concerned (Sultana *et al.*, 2015).

Interpreting site-wise disparities, the total metal loads in the present study revealed that sampling stations overall did not differ from each other hinting at the possibility of non-point sources. This was particularly so for Cd, Cr and Pb where significant and positive associations were evident between sites

indicating an overall similarity in occurrence and hence common sources of input. The heaviest levels of most heavy metals (with the exception of Hg), was at station B which was adjacent to the main bus stand and the city centre, indicating possible contamination from motor fuel, garages as well as from other urban sources. Despite Station A being adjacent to a conservation site, it was seen to be contaminated to some extent, possibly resulting from the accumulation of garbage at its fringes brought about by tidal currents. It is interesting that Station C which is the Dadugam Oya inlet is contaminated with Cr and Pb. The absence of a marked correlation of As and Hg with other metals suggests the likelihood of point sources, the former being relatively high in Station D which is a residential area. The absence of Hg in the Dadugam Oya inlet indicates that it is not brought into the lagoon through this freshwater inlet channel. Station E which lies in close proximity to the airport did not particularly stand out as a heavily metal-polluted site.

Biological systems are highly complex, and relationships between pollutant sources and the levels of accumulation in biotic or abiotic elements may not be straightforward. However, more importantly, the present study demonstrates the potential dangers of heavy metal accumulation in the biotic components in the Negombo lagoon. The dangers resulting from exposure to high concentrations of toxic heavy metals is well known, but of major concern is the possibility of concentration from continued exposure to even relatively low levels of these heavy metals. This is particularly a matter of serious concern

since the flesh of crabs and fish, which are consumed by people, had high levels of accumulation of the different metals. Mussels are also frequently harvested from the lagoon for human consumption. Thus, a seemingly harmless, or in this case non-detectable level of toxic waste in water, may mask accumulation in other constituents of the environment which could endanger the sustenance of the entire ecosystem. This highlights that safety measures must be in place to control the discharge of heavy metal contaminated effluents into such productive lagoon ecosystems.

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