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Arsenic and Cadmium Contamination in Water, Sediments and Fish is a Consequence of Paddy Cultivation: Evidence of River Pollution in Sri Lanka

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Introduction

The upper Malwathu Oya is a seasonal river. The main livelihood of people living in the imme- diate vicinity of the river is paddy cultivation, and chronic kidney disease is reported among them. Farmers utilize different types of agricultural chemicals in their ﬁelds expecting bumper harvests. Several agricultural chemicals have been reported to contain toxic trace elements in Sri Lanka. Therefore, arsenic and cadmium might end up in the river water. The presence of these trace elements in the river water and sediments can result in their bioaccumulation in ﬁsh tissues. The main purpose of this study was to investigate the presence of two trace ele- ments in water and sediments, as well as in ﬁsh tissues (gills, kidney, liver and muscle) of three food ﬁsh species, Etroplus suratensis, Anabas testudineus and Channa striata during culti- vating and non-cultivating seasons of the year. Further, the level of bioaccumulation of two trace elements in ﬁsh tissues in relation to the contamination level of water and sediments was assessed. Data were gathered for 43 months. Arsenic and cadmium concentration in water showed a signiﬁcant (P b 0.05) seasonal variation. Generally, the two trace elements in the river water were highest during the cultivating seasons than in other seasons. In all spe- cies, both trace elements in the gills highly depended on the concentration in the water. In all species, two trace elements in water and sediment did not signiﬁcantly affect the levels in muscle tissue. Therefore, the trace element levels in the edible parts of these three ﬁsh were well below the maximum permissible levels of international institutions.

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Arsenic (As) and cadmium (Cd) are widely found in the environment (Patrick, 2003) both from natural occurrence and due to anthropogenic activity (EFSA, 2009). Use of agricultural chemicals has been indicated as the main anthropogenic source of As and Cd pollution in aquatic environments of Sri Lanka (Illeperuma, 2000; Jauasumana et al., 2011). As both trace elements are poten- tially toxic (Patrick, 2003; Godt et al., 2006; Wang et al., 2012; Chen et al., 2015) at some bioavailability (Luoma and Rainbow,

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2008), their presence in aquatic environments can result in deleterious effects on aquatic organisms (Mason et al., 2000). Similar- ly, As and Cd can exist in both abiotic (water and sediments) and biotic (organisms) components of aquatic environments at different concentrations. However, toxicity occurs in aquatic animals when the rate of uptake of a trace element exceeds the com- bined rates of efﬂux and detoxiﬁcation of metal into metabolically inert forms (Luoma and Rainbow, 2005). In ﬁsh, metal uptake differs fundamentally from that of terrestrial animals as they are constantly submerged in the solution of metal ions (Perera et al.,

2015). As a result, metal distribution in ﬁsh is determined mainly by its content in water and food (Farkas et al., 2000; Mohamed,

2008). Further, ﬁsh have a tendency to accumulate heavy metals depending on their position in the food chain and their feeding habits (Houserová et al., 2007). Therefore, the concentration of As and Cd in water and bottom sediment, as well as the trophic position of ﬁsh can have signiﬁcant effects on bioaccumulation of these two trace elements in ﬁsh.

Malwathu Oya (“Oya” refers to seasonal rivers) is the second longest (164 km) river which originates from a mountain range

in the North Central Province (NCP) in Sri Lanka (The National Atlas of Sri Lanka, 2007). The upper reach of Malwathu Oya (latitude 8°15′ and 8°05′, longitude 80°26′ and 80°40′) runs across a remote area that consists of two distinct land use types, forest areas and paddy lands. People living in the upper Malwathu Oya area mainly depend on paddy cultivation as their livelihood (Perera et al., 2014). All paddy farmers in the area utilize different types of weedicides, pesticides, fungicides, rodenticides and inorganic fertilizers expecting high yield (Perera et al., 2014). The water utilized in their paddy lands diverts directly or indirectly to the upper Malwathu Oya by paddy outlet canals (POCs).

Several studies reported the prevalence of overusing agricultural chemicals by farmers in many parts of Sri Lanka (Selvarajah and Thiruchelvam, 2007; Padmajani et al., 2014). NCP of Sri Lanka is famous for chronic kidney disease (CKDu1) (Kabata et al.,

2016; Wimalawansa, 2015a, b) of which the apparent aetiology is most controversial among intellectuals. According to studies, As and Cd may be vital in spreading the disease among farmers in NCP (Jayasumana et al., 2014a, b). Many studies have been carried out in the NCP to investigate the impacts of inorganic fertilizers and other agricultural chemicals (e.g., pesticides and weedicides) that can contribute As and Cd into aquatic environment (Illeperuma, 2000; Jauasumana et al., 2011; Kumar and Singh, 2010). Paddy cultivation activities that take place in the upper Malwathu Oya catchment would also release As and Cd into the water. As a result, As and Cd can appear in water, sediment and ﬁsh tissues. Accumulation of As and Cd in ﬁsh tissues can result in deleterious effects on ﬁsh (Govind and Madhuri, 2014). However, various metals show different afﬁnities to ﬁsh tis- sues (Jezierska and Witeska, 2006a, b). For example, the non-edible tissues of ﬁsh can accumulate more metals than the edible muscle meat (Canli et al., 1998; Jezierska and Witeska, 2006a, b; Mohamed, 2008; Ambedkar and Muniyan, 2011). As freshwater ﬁsh is the cheapest source of animal protein for villagers in the NCP (Amarasinghe and Silva, 1999), the elevated levels of As and Cd in edible ﬁsh tissues can cause health risks (EFSA, 2009) as well.

Therefore, the main the purpose of this study is to investigate the effects of paddy cultivating activities (which take place in nearby river catchments) on the river water and ﬁsh. To understand the effects, the current study mainly focuses on As and Cd in water and sediments, and as the two elements have created a platform of arguments in various aspects of CKDu, in three common food ﬁsh species, Etroplus suratensis, Anabas testudineus and Channa striata (Pethiyagoda, 1991), collected from forest and paddy cultivating areas of upper Malwathu Oya. E. suratensis is mainly a herbivore (Pethiyagoda, 1991) and habitual bottom feeder (Costa, 1983; Joseph and Joseph, 1988; Vidhya and Radhakrishnan, 2012). A. testudineus is an omnivore (Pethiyagoda,

1991; Zalina et al., 2012) and C. striata is highly carnivorous (Ali, 1990; Courtenay and Williams, 2004). As the trace element accumulating tendency in different ﬁsh species and their tissues can be varied, four tissues (gill, liver, kidney and muscle) of the above-mentioned three ﬁsh species were analysed for As and Cd. Further, this study assessed the level of accumulation of As and Cd in ﬁsh tissues in relation to the contamination level of water and sediments and tried to predict the suitability of these three ﬁsh species for use as bioindicators. Finally, the human health risk due to consumption of the muscle meat of Malwathu Oya ﬁsh will be discussed.

Materials and Methods

Study Location and Sampling Sites

This study was conducted in a 30-km-long reach in upper Malwathu Oya between Ritigala Strict Nature Reserve (latitude 8°12′ and longitude 80°65′) and Nachchaduwa perennial reservoir (latitude 8°15′ longitude 80°29′) in the Anuradhapura District in the NCP of Sri Lanka (Fig. 1). The ﬁrst 17 km stretch of the river was considered as the Upper River Segment (URS) and the second 13 km stretch was considered as the Lower River Segment (LRS). Twelve sampling sites were selected from the two different types of land uses (forest and paddy lands) of the river catchment. Six sampling sites were situated in forest areas (non-paddy) while the other six sites were situated in paddy cultivating areas.

Sample Collection, Preparation and Analysis

Water, sediment and ﬁsh samples were collected monthly from March 2012 to September 2015 (43 months). Water samples were collected from paddy-area-sites in the URS (P1–P3) and the LRS (P4–P6) as well as from forest-area-sites in the URS (NP1– NP3) and the LRS (NP4–NP6) in upper Malwathu Oya (Fig. 1) during all four monsoon seasons (ﬁrst inter-monsoon, FIM;

1 CKDu, chronic kidney disease of unknown aetiology.

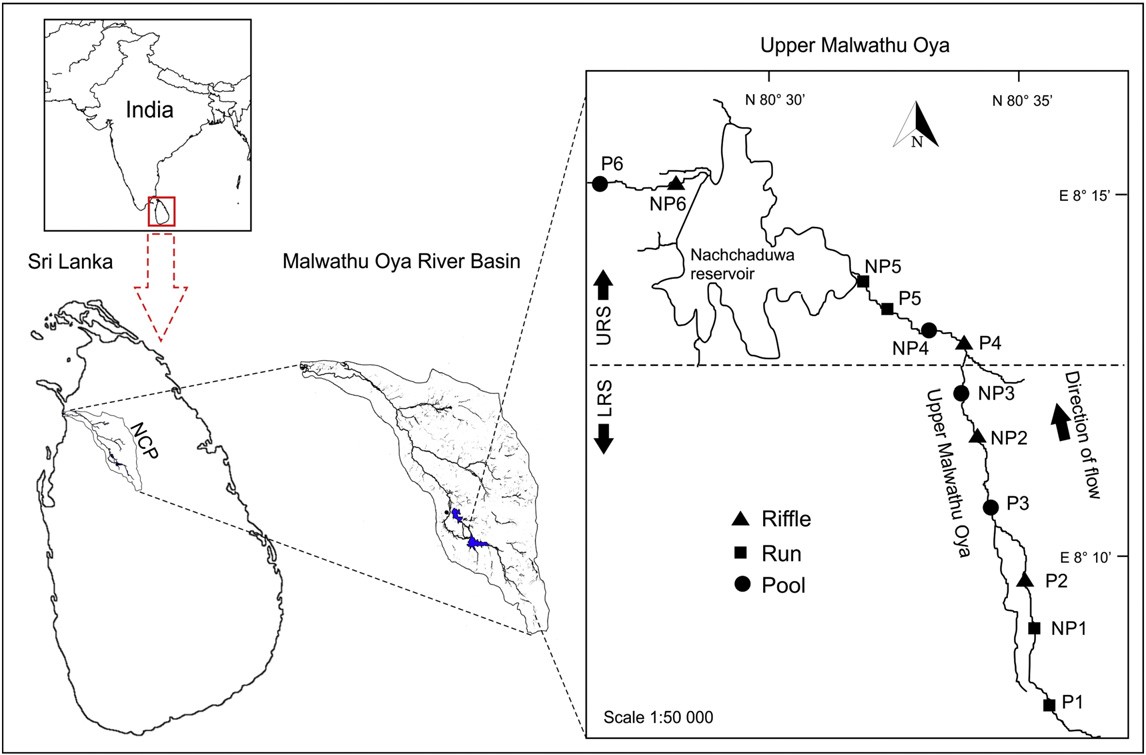


Fig. 1. Map of the study area showing locations of sampling sites.

southwest monsoon, SWM; second inter-monsoon, SIM; and northeast monsoon, NEM) of the year (Fig. 2). Water samples from POCs were collected from six sampling sites (POC1–POC6) only during FIM, SIM and NEM as water was unavailable during the SWM season. Sediment and ﬁsh were collected from only six sampling sites from the paddy-area-sites (P1, P3, P6) and forest- area-sites (NP1, NP3 and NP5) because all three ﬁsh species selected for the study were not found in other study sites during some seasons of the year.

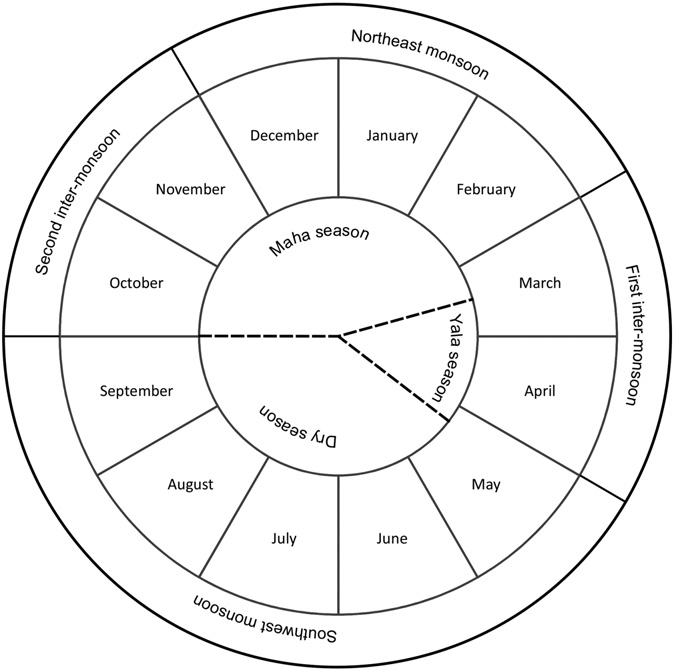


Fig. 2. Distribution of climatic seasons in the NCP.

Water

High-density polyethylene (HDPE) bottles (500 mL) were used to collect water samples. Acid-washed (10% hydrochloric acid), sampling bottles were rinsed three times with distilled water to avoid any contamination from metal and non-metal ions. Each pre-washed bottle was rinsed again (three times) with the river water at each corresponding site before collecting water samples. In deep sites (non-wadeable), surface, middle and bottom layer samples were taken. Middle and bottom layer samples were collected using a Kemmerer-type water sampler (Stednick, 1991). Surface water samples were collected using sample bottles. The value of each parameter was calculated by the mean of the three layers. In moderately deep sites (wadeable), surface and bottom water samples were collected. The value of each parameter was calculated by the mean of two layers. In shallow sites, samples were collected from midway between the surface and the bottom (EPA, 1997). Water samples from six paddy outlet ca- nals were collected from midway between the surface and the bottom using sample bottles about 10–15 m before the discharge points to assure that both water samples did not mix with each other. All water samples were collected at three different places in each study site. Duplicate water samples were collected at the same place, at the same time (EPA, 1997). The value of each parameter was calculated by the mean. In order to increase the precision of the results, all the samples were collected under clear weather conditions.

Water samples collected (to analyse total dissolved As and Cd) were preserved at 4 °C in ice and immediately transported to the laboratory. At the laboratory, all samples were ﬁltered through 0.45 μm, 30 mm diameter PTFE syringe ﬁlters (Sigma-Aldrich, USA) to remove any particulates. Subsamples (lab replicates) of 100 mL ﬁltered water samples were acidiﬁed to pH b 2 (veriﬁed with pH test strips, HACH, USA), labelled and kept at 4 °C in the refrigerator until further analysis (APHA, 2005).

Sediment

Sediments of non-wadeable sites were collected from the top 10 cm of surface with a benthic grab sampler (Ekman Grab Kit, Hoskin Scientiﬁc, Canada) and placed into a 500 mL glass jar. Sediment samples from wadeable areas were collected into polyeth- ylene bags using a Teﬂon scoop. Sediment samples were transported to the laboratory immediately and freeze-dried before analysis (Cheung et al., 2003). Sample containers and tools were cleaned as described by quality assurance and quality control speciﬁcations from the EPA (1990). In the laboratory, samples were dried at 65 °C for 24 h and ground. Powdered sediment samples were then passed through a 63 μm mesh sieve to separate large fractions (Alhashemi et al., 2012). The sieved samples were labelled and prepared for analysis.

Fish

Five to eight samples from E. suratensis, A. testudineus and C. striata were collected each month from local ﬁshers in upper Malwathu Oya. The length and weight of each ﬁsh was recorded at study sites (Table 1). Adult specimens of E. suratensis (n = 246), A. testudineus (n = 322) and C. striata (n = 218) in similar lengths were collected. Fish samples were packed in polythene bags and transported to the laboratory in an insulated box with crushed ice.

Specimens were descaled and washed thoroughly (Eneji et al., 2011). Dissections were performed on a clean bench using a titanium knife and Teﬂon forceps as described by Liao et al. (2003). The different organs (gill, kidney, liver and muscle) were care- fully dissected after rinsing with double-distilled water (Akan et al., 2009). Due to difﬁculties in obtaining sufﬁcient amounts of tissue for analysis, adequate portions of the liver and kidney from several ﬁsh specimens were collected and these samples were pooled as described by (Leeves, 2011). Each pooled sample consisted of kidney and liver tissues of ﬁve individuals of approximately similar in length (± 0.5 cm). Muscle and gill tissues were not pooled as adequate portions were available for chemical analysis. The dissected tissues samples were placed in labelled clean polyethylene bags and immediately deep-frozen at − 20 °C until prepared for analysis (Taweel et al., 2011).

Chemical Analysis

For chemical analysis, water, sediment and tissue samples were transported to the Analytical Chemistry Laboratory, National Aquatic Resources Research Development Agency (NARA, Sri Lanka) in an insulated box with crushed ice during night time. Tissue samples were prepared for analysis as described by Jinadasa et al. (2013). Tissues from three ﬁsh species were oven-dried at

105 °C. These dried samples were powdered and preserved in airtight polythene bags until further analysis. Tissue and sediment samples were digested using CEM XP-1500 (CEM, Matthews, USA) microwave accelerated system. Around 0.5 g of an oven-dried

Table 1

Length and weight of sampled ﬁsh and sampling sites.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sampling site | E. suratensis |  |  | A. testudineus |  |  | C. striata |  |
|  | Total length (cm) | Weight (g) |  | Total length (cm) | Weight (g) |  | Total length (cm) | Weight (g) |
| P1 | 21.3 ± .53 | 255 ± 9.1 |  | 19.4 ± .38 | 78 ± 4.4 |  | 40.4 ± .55 | 625 ± 13.1 |
| P3 | 21.5 ± .41 | 262 ± 8.6 |  | 18.7 ± .33 | 72 ± 3.5 |  | 40.8 ± .47 | 642 ± 12.0 |
| P6 | 21.2 ± .50 | 247 ± 8.1 |  | 18.9 ± .41 | 75 ± 3.8 |  | 41.3 ± .52 | 659 ± 12.6 |
| NP1 | 20.2 ± .46 | 241 ± 7.9 |  | 19.0 ± .30 | 78 ± 4.2 |  | 41.6 ± .43 | 655 ± 11.5 |
| NP3 | 21.5 ± .44 | 260 ± 4.5 |  | 19.5 ± .45 | 81 ± 4.0 |  | 40.9 ± .45 | 634 ± 14.7 |
| NP5 | 20.6 ± .38 | 242 ± 9.8 |  | 18.5 ± .24 | 69 ± 3.1 |  | 41.7 ± .50 | 647 ± 13.5 |

ﬁsh sample and powdered sediment samples were weighed accurately to four decimal places in a Teﬂon vessel. A total of 10 mL of 65% conc. HNO3 (AR, Sigma) was added and allowed to stand for 15 min in a fume hood for pre-digestion. Then, the Teﬂon vessel was connected to a microwave digester and digestion was carried out (at 200 °C for 25 min). The digested tissue and sediment samples were transferred to 50 mL volumetric ﬂasks and made up to the mark with deionized water.

A total of 100 mL from each water sample was transferred into Pyrex beakers containing 10 mL of conc. nitric acid. The samples were boiled slowly and then evaporated on a hot plate to the lowest possible volume (about 15 mL). The beakers were allowed to cool and another 5 mL of conc. nitric acid was added. Heating was continued with the addition of conc. nitric acid as necessary until digestion was complete. The samples were evaporated again to dryness and the beakers were allowed to come to room temperature followed by the addition of 5 mL of HCl solution (1:1 v/v). The solutions were warmed and

5 mL of 5 M NaOH was added, and then ﬁltered. The ﬁltrates were transferred to 100 mL volumetric ﬂasks and diluted to the mark with distilled water. These solutions were then used for the elemental analysis.

Two reagent blank samples and two spiked samples were prepared for ﬁsh, sediment and water samples using the same method. The spiked samples were routinely analysed as the method of validation procedure. The recovery limits were maintained between 75% and 125% during the analysis of all sets of samples. The limit of detection (LOD; mean blank + 3S) was calculated for all metals and results of less than LOD were considered as not detected by the instrument.

Two trace elements were determined using an atomic absorption spectrophotometer (Varian240 FS, Varian Inc., Walnut Creek, CA, USA). A graphite tube atomizer (Varian GTA-120) was used for Cd determination and a vapour generation accessory (Varian VGA 77) with open-end cell was used for As determination. The calibration curves for the absorption two trace elements were performed with a standard solution of particular elements at optimum wavelength. Subsequent to the calibration, the reagent blank samples, unknown samples and spiked samples were aspirated into the atomic absorption spectrophotometer and results were recorded.

Data Analysis

All data were analysed using SAS statistical software (SAS version 9.0). In the ﬁrst analysis, factorial experiments were used to study interactions between different factors. Land uses (paddy area and forest area), climate seasons (FIM, SWM, SIM and NEM), channel geomorphic units (CGUs; pool, rifﬂe and run) and river segments (URS and LRS) were considered as factors. For each treatment combination, three replicates were taken. To study As and Cd in abiotic components (water samples), in the SAS pro- cedure, a general linear model was used due to missing data during the drought season (SWM).

In the second analysis, a factorial experiment was used to study interaction between factors. Seasons (FIM, SWM, SIM and NEM), species (E. suratensis, A. testudineus and C. striata) and tissues (gill, kidney, liver and muscle) were considered as factors and for each treatment combination, three replicates were taken. To study As and Cd in biotic components (tissues), in the SAS procedure, proc. ANOVA was used. A smaller number of ﬁsh tissues (4) were considered in this study which resulted in a smaller number of treatment comparisons. Therefore, LSD was used.

Data of arsenic and cadmium in water and ﬁsh tissues were tested for normality. Arsenic and cadmium levels in water showed p values of 0.159 and 0.291, respectively. Tissue arsenic and cadmium levels showed p values of 0.262 and 0.181, respectively. Therefore, all data had distributed normally.

Thirdly, the correlation (Pearson) between trace metal content in water of each study site and each tissue of three ﬁsh species

found in corresponding study sites was studied. Similarly, the correlation (Pearson) between trace metal content in sediment samples of each study site and each tissue of three ﬁsh species found in corresponding study sites was studied. Correlation type (positive or negative) and signiﬁcant levels were generated. All graphs were generated using MS Excel 2013 software.

Table 2

Arsenic and cadmium concentration (μg/L) in water of POCs.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Climatic season |  | |
| FIM | SIM | NEM |
| Arsenic  POC1 | 433.2a | 385.0b | 142.0c |
| POC2  POC3  POC4  POC5  POC6 | 344.4a  243.0b  473.2a  498.2a  465.1a | 352.0a  364.2a  459.4b  277.3b  231.1b | 148.3b  140.4c  130.2c  154.2c  111.0c |
| Cadmium  POC1 | 148.5a | 126.2b | 094.0c |
| POC2  POC3  POC4  POC5  POC6 | 142.0a  243.0a  213.2a  245.1a  145.1a | 143.4a  220.1b  184.1b  192.4b  131.0b | 076.1b  151.2c  170.1c  148.2c  147.4a |

Values within rows are signiﬁcantly different at p b 0.05.

Results and Discussion

Arsenic and Cadmium in Abiotic Components

Paddy Outlet Canals

Arsenic concentrations in POCs ranged annually from 111 to 498 μg/L and Cd concentrations ranged from 76 to 245 μg/L (Table 2). The concentration of both As and Cd in POCs was higher than the corresponding study sites in upper Malwathu Oya (Tables 3 and 4). Mean As and Cd concentration in POCs signiﬁcantly (p b 0.05) changed in three seasons associated with paddy cultivation. In the majority of POCs, the concentration of both As and Cd was highest during FIM and it was always lowest during NEM. Arsenic concentrations varied in different seasons as FIM N SIM N NEM except in POC2 and POC3 where the concentration was highest during SIM. Similarly, the Cd concentration varied as FIM N SIM N NEM except in POC2 where the concentration between FIM and SIM was not signiﬁcantly different (p N 0.05).

Paddy outlet canals are small waterways constructed by farmer organizations to drain water utilized from paddy lands to the upper Malwathu Oya. Any type of application that takes place in the paddy lands affects POCs as they are directly connected to the paddy lands. For example, inorganic fertilizers and other types of chemicals applied onto paddy lands get mixed with water and drain into POCs.

In Sri Lanka, it is generally accepted that four distinct climatic seasons (Fig. 2) can be identiﬁed during a twelve month period

(Punyawardena, 2004; The National Atlas of Sri Lanka, 2007). Therefore, the rainfall seasons range from March of the current year to February of the following year (Department of Meteorology, 2014). Even if monsoonal rains account for a major share of the annual rainfall, they do not bring about homogeneous rainfall regimes over the whole island (Department of Agriculture, 2006). Therefore, out of these four monsoon seasons, two consecutive rainy seasons, SIM and NEM, make up the major growing season (Maha season) while the minor cultivating season (Yala season) is limited to the FIM and ﬁrst part of the SWM (Fig. 2). Therefore, the period between these two seasons (from mid-May to September) is rather dry.

In the upper Malwathu Oya area (which belongs to the NCP), the majority of farmers engage in tillage activities with the onset of the SIM (in Maha) than FIM (in Yala) because the extended rainfall regimes during Maha season allow farmers to cultivate more paddy lands. However, the observed concentration of As and Cd was lower during the SIM than the FIM due to dilution effects of chemical compounds as an effect of high-intensity and extended rainfall in the Maha season. Nevertheless, As and Cd distribution in POCs show a binomial distribution related to two major cultivating seasons in the study area. As rain continues during the SIM and NEM seasons without a distinct interval (Fig. 2), further dilution of trace elements in the POCs is quite pos- sible. As a result, the lowest concentrations were detected during the NEM.

However, the concentration of trace elements in POCs depends on many factors. For example, application frequency and strength can determine how much will be recovered in the POCs. Farmers (in the NCP) apply liquid and solid agro-chemicals into paddy lands in excessive quantities and in adequate frequencies (more than recommended times) expecting bumper harvests (Wimalawansa, 2015a, b). A similar situation was observed in the upper Malwathu Oya area as well. As a result of these indis- criminate applications, the surplus will be recovered in the paddy-land-soil and ﬁnally wash off into POCs. As POCs are small wa- terways, changes in application frequencies and quantities of agricultural chemicals in paddy lands would result in obvious changes in their water chemistry. As a result, the distribution of As in some POCs (e.g., As in POC2 and POC3 is higher during SIM) is different from the regular ﬂuctuation pattern.

Inappropriate use of agricultural chemicals is common in Sri Lanka. For example, Padmajani et al. (2014) observed that 53% of farmers in vegetable cultivating areas in the upcountry mix two or more chemicals together to make a cocktail mixture while 71% of them repeatedly apply the chemical to the same crop as they believe that the recommendations and prescriptions given in the pesticide product labels are insufﬁcient. Selvarajah and Thiruchelvam (2007) described that about 60% paddy and chilli farmers apply 30%–40% higher concentrations of agricultural chemicals in Vavuniya District in Sri Lanka. These examples stress that the quantity and frequency of application of agricultural chemicals in Sri Lanka mostly depend on the individual preference of

Table 3

Arsenic (μg/L) in upper Malwathu Oya water.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Land use | Segment | Site | CGU | FIM | SWM | SIM | NEM |
| Paddy area | URS | P1 | Run | 43.3a | 09.1c | 32.0b | 03.1d |
|  |  | P2 | Rifﬂe | 34.0a | 04.0b | 35.2a | 05.3b |
|  |  | P3 | Pool | 21.0a | 18.1a | 20.3a | 04.2b |
|  | LRS | P4 | Rifﬂe | 46.3a | 05.3c | 37.4b | 09.1c |
|  |  | P5 | Run | 48.1a | † | 41.5a | 11.3b |
|  |  | P6 | Pool | 15.3a | 09.4b | 19.0a | 02.0c |
| Forest area | URS | NP1 | Run | 14.0a | 01.1c | 10.3b | 01.1c |
|  |  | NP2 | Rifﬂe | 09.0a | † | 01.3b | 01.1b |
|  |  | NP3 | Pool | 01.1c | 08.2a | 04.4b | 01.1c |
|  | LRS | NP4 | Pool | 01.4b | 03.0b | 09.1a | 01.3b |
|  |  | NP5 | Run | 20.2a | 07.0b | 19.3a | 01.1c |
|  |  | NP6 | Rifﬂe | 15.3a | 09.4b | 17.2a | 01.1c |

a, b, c, d are based on LSMEAN (adjusted mean) separation procedure. Values within rows having different superscripts are signiﬁcantly different (P b 0.05). †, Not measured due to unavailability of water.

Table 4

Cadmium (μg/L) in upper Malwathu Oya water.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Land use | Segment | Site | CGU | FIM | SWM | SIM | NEM |
| Paddy area | URS | P1 | Run | 94.2a | 14.0d | 54.2b | 34.4c |
|  |  | P2 | Rifﬂe | 78.3a | 17.5d | 58.1b | 38.5c |
|  |  | P3 | Pool | 53.1a | 09.3c | 49.2a | 31.0b |
|  | LRS | P4 | Rifﬂe | 110.2a | 09.4d | 68.3b | 44.0c |
|  |  | P5 | Run | 134.0a | † | 92.4b | 43.2c |
|  |  | P6 | Pool | 80.1a | 03.2d | 45.2b | 25.0c |
| Forest area | URS | NP1 | Run | 35.1a | 02.5c | 27.0b | 01.1c |
|  |  | NP2 | Rifﬂe | 24.0b | † | 26.3a | 01.1c |
|  |  | NP3 | Pool | 11.2a | 01.2c | 09.0b | 02.5c |
|  | LRS | NP4 | Pool | 25.0a | 03.0c | 18.3b | 02.3c |
|  |  | NP5 | Run | 42.2a | 19.0c | 33.4b | 13.4c |
|  |  | NP6 | Rifﬂe | 46.1a | 08.1d | 30.2b | 10.4c |

a, b, c, d are based on LSMEAN (adjusted mean) separation procedure. Values within rows having different superscripts are signiﬁcantly different (P b 0.05). †, Not measured due to unavailability of water.

farmers. Therefore, chemical concentration in POCs reluctant to change according to the application practises of farmers. Further- more, Paranagama (2013) states that analysis of As content in Sri Lankan soils, particularly in agricultural areas, gradually decreases with depth, implying that it is not present naturally in soils; nevertheless, it has been introduced from the surface, most probably due to anthropogenic activities. Paranagama (2013) reported that the highest total As content was reported from imported triple super phosphate used for rice cultivation, which ranged from 25.49 mg/kg to 37.86 mg/kg in CKDu endemic areas of NCP. Similarly, Jauasumana et al. (2011) indicated that 29 out of 31 pesticide brands widely used in Sri Lanka contain As in signiﬁcant amounts ranging from 180 ± 14 μg/kg (in imidacloprid collected from Colombo) to 2586 ± 58 μg/kg (in glyphosate collected from Anuradhapura) per kg of pesticide. Wijesundara et al. (2013) observed that similar to nitrogen and phosphates, Cd concentration in reservoir water in the dry zone was elevated in the months of April and May in the Yala season, while relatively lower levels were recorded for the Maha season. They also reported that even if the other major nutrients (nitrogen, phosphorous and potassium) are added as chemical fertilizers, Cd is not added for any ﬁeld as it has no any beneﬁcial effect on crop life. Dissanayake and Chandrajith (2009) reported that phosphate fertilizers are produced by the acidulation of crushed and powdered phosphate rock. Therefore, contamination of phosphate fertilizer by toxic elements can be signiﬁcantly high in some ﬁnal fertilizer products (Dissanayake and Chandrajith, 2009).

Since As and Cd compounds are contained in agricultural chemicals, they will be recovered in POCs. Moreover, the distinct sea- sonal variability of As and Cd in POCs parallel to the major paddy cultivating seasons stresses that the origin of trace elements are rather agriculture-based. Improper cultivating practises of farmers seem to intensify the trace element levels in POCs. As POCs drain into the upper Malwathu Oya, assessment of the impacts on it is essentially important.

Upper Malwathu Oya

Seasonal Variation. Since the higher order interaction was signiﬁcant (p b 0.0001), keeping three factors (land use, CGU and river segment) constant, compared the As and Cd content between seasons. Results of the mean separation revealed that As and Cd concentrations in the upper Malwathu Oya showed a distinct seasonal variation. As concentrations in sampled waters ranged from 1 to 48 μg/L (Table 3) and Cd concentrations ranged from 1 to 134 μg/L (Table 4). Both As and Cd concentrations were higher in paddy-area-sites than forest-area-sites. Concentrations of both trace elements were higher during FIM and SIM than SWM and NEM. Mean As concentration was signiﬁcantly different (p b 0.05) between FIM and SIM in many sites (P1, P4, NP1, NP2, NP3 and NP4). Cadmium concentration was signiﬁcantly higher (p b 0.05) during FIM than SIM in many sites (except in P3 and NP2). The lowest As and Cd values were detected during NEM and SWM seasons, respectively. Further, the mean As and Cd concentration in all sampling sites during FIM and SIM were higher than the recommended values (As, 0.01 mg/L (10 μg/L); Cd, 0.003 mg/L (3 μg/L)) in drinking water (WHO, 2006).

As observed in POCs (Paddy Outlet Canals), distinct variation in two trace elements (Tables 3 & 4) in the river water is mainly associated with the paddy cultivating seasons. For example, relatively higher As and Cd concentrations observed in paddy-sites (than those in forest-sites) indicate that POCs contribute As and Cd from paddy lands. The higher As and Cd concentrations during FIM and SIM is related to Yala and Maha season cultivating activities which results water quality changes due to the contribution of polluted water from POCs. The distinct difference between trace element concentrations between FIM and SIM is associated with the similar reasons discussed under POCs in Paddy Outlet Canals. However, unlike the observations in POCs, many other fac- tors such as river hydrology (e.g., ﬂow rate and river discharge), nature of channel geomorphology (e.g., rifﬂes, runs and pools) and other water quality parameters can also alter the trace element concentrations (Meybeck et al., 1992; Zielinski et al.,

2003). Hydrological changes are mainly seasonal due to rainfall regimes during the wet season and due to higher evaporation during the dry season (Fig. 2). Extended high-intensity rainfall associated with Maha season determines the lowest trace element concentration during the NEM. Lack of inﬂow from POCs results in the lowest trace element concentration during SWM. The behaviour of Cd is more rhythmic during the two inter-monsoon seasons. Nevertheless, the behaviour of As is more irregular be- tween study sites. The highest mean concentrations of As and Cd in study sites during FIM is related to the lower dilution of trace

elements due to the less extended Yala season rains. Nevertheless, insigniﬁcant concentrations in P3 and relatively low concen- tration during FIM in NP2 stress the complex behaviour of Cd in water. However, the behaviour of As is more complicated than Cd. For example, in six study sites (exactly half of study sites), the irregular behaviour of As stresses that other than the application strength and quantities of agrochemicals many other factors are involved in the determination of concentration in river water. Interactions between chemical, hydrological and geomorphological parameters in water can alter the general seasonal rhythmic pattern of trace elements between FIM and SIM. However, the relationship between POCs and the river water with respect to the two trace elements is useful to understand the behaviour of As and Cd more closely in study sites during the FIM and SIM seasons (Correlation between POCs and River Sites).

Correlation between POCs and River Sites. If paddy cultivating activities affected the river water quality, the concentration of As and Cd in paddy-sites (P1–P6) should increase according to the concentration of those trace elements in corresponding POCs. However, the strength of the above relationships can vary seasonally according to the nature of the cultivating activities and rainfall regimes. For exam- ple, As (r2 = 0.59, p = .001) and Cd (r2 = 0.42, p = .01) levels between POCs and paddy-sites are highly correlated during the FIM (Fig. 3). Nevertheless, it is poorly correlated during SIM (r2 = 0.20, p = .07 and r2 = 0.11, p = .17 for As and Cd, respectively) and NEM (r2 = 0.05, p = .34 and r2 = 0.04, p = .41 for As and Cd, respectively).

Pollutants that enter the ﬂowing waters travel some distance before they are thoroughly mixed throughout the ﬂow (EPA,

1997). The same phenomenon was observed with respect to trace element pollution in upper Malwathu Oya as well. Less extend- ed and low-intensity rainfall regimes during the FIM do not encourage the mixing process as it does during the SIM and NEM (more extended and high-intensity rainfall) seasons. Therefore, the chemicals that brings by POCs can be recovered at relatively higher concentrations at the study sites (P1–P6). However, the strength of As and Cd that diverts through POCs can be weakened more easily due to relatively higher ﬂow rates and river discharge during SIM and NEM. Further, the chemical concentration is

|  |  |
| --- | --- |
| Arsenic - FIM y = 0.0021 + 0.0844x  0.06 R² = 0.59  0.04  0.02  0  0.2 0.3 0.4 0.5 0.6 | Cadmium - FIM y = 0.0251 + 0.3679x  0.16 R² = 0.42  0.12  0.08  0.04  0  0.12 0.17 0.22 0.27 |
| Arsenic - SIM y = 0.0113 + 0.0504x  R² = 0.20  0.05  0.04  0.03  0.02  0.01  0  0.2 0.3 0.4 0.5 | Cadmium - SIM y = 0.0356 + 0.1459x  R² = 0.11  0.1  0.08  0.06  0.04  0.02  0  0.11 0.16 0.21 0.26 |
| Arsenic - NEM y = 0.0017 + 0.0287x  R² = 0.05  0.012  0.008  0.004  0  0.1 0.15 0.2 | Cadmium - NEM y = 0.0306 + 0.0385x  R² = 0.04  0.05  0.04  0.03  0.02  0.01  0  0.06 0.11 0.16 0.21 |

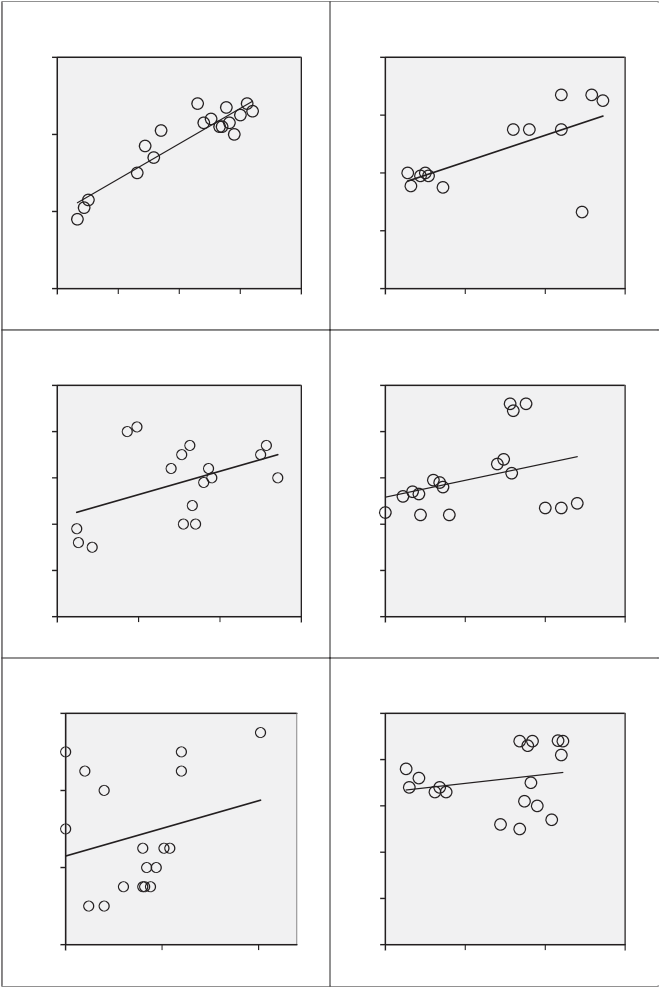


Fig. 3. Relationship between trace metal levels (mg/L) in POCs (X axis) and paddy-sites (Y axis).

relatively higher in POCs during the FIM than in the SIM and NEM (Table 2). Therefore, relatively higher contributions of trace elements from POCs and less efﬁcient mixing process determines the strong correlation between POCS and river water.

Studies related to the seasonal trace element variations in aquatic environments in the NCP of Sri Lanka are rare. Many studies basically address the levels of As and Cd in different reservoirs without continuous assessments. However, according to Wijesundara et al. (2013), Cd concentration in tank water (in a tank cascade close to upper Malwathu Oya) was elevated from April to May in the Yala season while relatively lower concentrations were recorded during the Maha season. This result is similar to the observations of the current study.

Arsenic and Cadmium in Biotic Components

Arsenic level in four tissues of each species varied as A. testudineus N C. striata N E. suratensis and Cd level varied as C. striata N A. testudineus N E. suratensis (Fig. 4). Since the three-way interaction between seasons, species and tissues is signiﬁcant (p b 0.0001), keeping two factors (season and species) constant, As (Table 5) and Cd (Table 6) between tissues were compared. As level between tissues is signiﬁcantly different (p b 0.05) in all seasons. As accumulation pattern in tissues is similar in all ﬁsh in all seasons (kidney N liver N gill N muscle) (Table 5). Nevertheless, similar tissue level Cd accumulation patterns were observed only in A. testudineus and C. striata (liver N gill N kidney N muscle); it is different and irregular in E. suratensis (Table 6).

Species Level Arsenic and Cadmium Accumulation

E. suratensis is omnivorous but mainly depend on plant matter (Pethiyagoda, 1991). It feeds mainly on a wide variety of plant matter (from unicellular algae to macrophytes), dead organic matter and some animal matter (ﬁsh eggs, copepod, ﬁsh scales) (Vidhya and Nair, 2012). A. testudineus is an omnivore (Pethiyagoda, 1991; Zalina et al., 2012) and C. striata is highly carnivorous (Ali, 1990; Courtenay and Williams, 2004). If the feeding habits altered As bioaccumulation, a variation in species level accumu- lation might be observed. Nevertheless, similar As accumulation tendencies in the tissues of the three species stresses that the afﬁnity of ﬁsh towards arsenic does not change according to the feeding habits of ﬁsh. However, the similar afﬁnities showed towards Cd by A. testudineus and C. striata, but not by E. suratensis, stresses that their feeding habits and habitat preferences affect bioaccumulation. As an air-breathing ﬁsh, C. striata prefers stagnant, slow-running and shallow water (Mat Jais, 2007) while A. testudineus is mainly associated with turbid, stagnant habitats (Pethiyagoda, 1991). C. striata mainly feed on ﬁsh and crusta- ceans while A. testudineus prefers macrophytes with shrimp and ﬁsh fry (Pethiyagoda, 1991). Although the trophic positions of these two species are different, their habitat and feeding preferences (interest towards animal matter) are more or less similar. However, the seasonal changes in food availability, disparity in feeding habits and habitat preferences (benthopelagic) of E. suratensis might change the accumulation pattern of Cd in them.

Tissue Level Arsenic and Cadmium Accumulation

Trace elements (e.g., As, Cd) unlike organic pollutants, cannot be chemically degraded (Hooda, 2010) or biodegraded by microorgan- isms (Mermut et al., 1996). Therefore, these chemicals accumulate in living tissues any time they are taken up (Edem et al., 2008) and are stored faster than they are broken down (metabolized) or excreted (Extension Toxicology Network, 1993). As a consequence, bioaccu- mulation can develop at any concentration without minimum level requirements (Amiard et al., 1987; Marcovecchio, 2004), and the capacity of bioaccumulation can be different according to the target tissues (Alhashemi et al., 2012; El-Moselhy et al., 2014).

Results indicate (Table 5) that the accumulation tendency of As in different tissues does not change according to the concentration in the medium. Therefore, in all ﬁsh species during all seasons (at different concentrations in water), arsenic level changed as kidney b liver b gill b muscle. However, the behaviour of Cd was different; the tissue level accumulation tendency in A. testudineus and C. striata was liver b gill b kidney b muscle in all seasons except in A. testudineus during SWM (Table 6). The observed difference in A. testudineus is mainly associated with its response to dry weather conditions. Upper Malwathu Oya is a seasonal river, thus, during

2.5

Arsenic Cadmium

0.35

2

Arsenic (mg/L)

1.5

1

0.5

0.3

Cadmium (mg/L)

0.25

0.2

0.15

0.1

0.05

0 0

ES AT CS

Fig. 4. Arsenic and cadmum level in three ﬁsh species; ES, E. suratensis; AT, A. testudineus; CS, C. striata.

Table 5

Seasonal variation of arsenic (mg/kg) in ﬁsh tissues.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Species | Tissue | FIM | SWM | SIM | NEM |
| E. suratensis | Gill | 0.462c | 0.364c | 0.406c | 0.357c |
|  | Kidney | 3.438a | 3.080a | 3.213a | 3.155a |
|  | Liver | 2.670b | 2.735b | 2.675b | 2.590b |
|  | Muscle | 0.096d | 0.027d | 0.034d | 0.026d |
| A. testudineus | Gill | 1.389c | 1.232c | 1.340c | 1.286c |
|  | Kidney | 4.223a | 4.116a | 4.732a | 4.490a |
|  | Liver | 2.193b | 2.233b | 1.946b | 1.919b |
|  | Muscle | 0.082d | 0.067d | 0.064d | 0.082d |
| C. striata | Gill | 1.213c | 1.082c | 1.157c | 1.154c |
|  | Kidney | 4.184a | 4.321a | 4.071a | 4.303a |
|  | Liver | 2.186b | 1.979b | 2.142b | 2.035b |
|  | Muscle | 0.102d | 0.099d | 0.073d | 0.072d |

Values within columns are signiﬁcantly different at p b 0.05 (mean comparison made column-wise for each species).

the dry season, in the latter part of the SWM, many areas of the river tend to dry up leaving water in pools and some runs. Because of their unique air-breathing ability, A. testudineus prefer to burrow into the mud to survive (Pethiyagoda, 1991). This adaption might allow more contact with sediment and mud than other species, which can result in more Cd in their gills. On the other hand, the second highest level of Cd in the gill of A. testudineus and C. striata during other seasons (FIM, SIM and NEM) is mainly associated with its habitual bottom access as a feeding habit. For example, during feeding times, they access benthic animals which results in agitation of bottom sediment. Sediments contain higher content of Cd (Fig. 5). Bioturbation (the mechanical disturbance of benthic sediments) by benthic organisms disrupts the water–sediment interface (Sandnes et al., 2000) and brings oxygen into the sediment, thus enabling Cd to re-dissolve (Delmotte et al., 2007). Repeating the same process by bottom-living ﬁsh tends to bathe their gills with Cd-rich water, which results in more Cd in the gill tissue. As benthopelagics, similar results were observed in E. suratensis as well. However, their accumulation pattern is different during the FIM (Table 6); therefore, they might show different feeding habits; accessing more macrophytes and algae due to the availability of more plant matter during the FIM.

Fish uptake As (McIntyre and Linton, 2011) and Cd (Kamunde and MacPhail, 2011) directly from exposure to contaminated water or indirectly by consumption of food containing the chemical (Drexler et al., 2003). The difference in the levels of accumu- lation in different organs or tissues of ﬁsh is primarily attributed to the various physiological roles carried out in each organ (Kotze, 1997; Senthil et al., 2004). Canli and Atli (2003) described that the ﬁsh's behaviour and feeding preferences along with some abiotic factors determine the bioaccumulation of trace elements in different tissues. Further, the accumulation of As and Cd in ﬁsh tissues strongly reﬂect the exposure pathways. For example, Szebedinszky et al. (2001) and Franklin et al. (2005) ex- plained that in diet-exposed ﬁsh, Cd concentration is generally higher in the gastrointestinal tract than in the gills, whereas in the case of waterborne exposure, Cd concentration is generally higher in gills and kidneys. Similarly, the gills are the prime site for arsenic accumulation and reﬂects the excretory roles of the organs (Oladimeji et al., 1984). Moreover, the external position of the gills in ﬁsh and its well-branched structural organization, which results in a large volume of water passing through the gill surface, make the gills a prime site for trace element accumulation (Subathra and Karuppasamy, 2007). As explained by Szebedinszky et al. (2001), more Cd may enter through the dietary paths which results in high Cd levels in the liver in all the ﬁsh species. Therefore, the order of arsenic accumulation kidney N liver N gill N muscle stresses that a waterborne pathway is more important in As bioaccumulation while the order liver N kidney N gill N muscle reﬂects the importance of dietary pathways in Cd bioaccumulation.

The highest arsenic level found (than other species) in the liver of E. suratensis and an irregular accumulation pattern was observed between A. testudineus and C. striata, which implies that the accumulation tendency does not increase with the trophic position of ﬁsh. If the arsenic was concentrated along the food chains, the relevant levels should be high at higher trophic positions. However, the

Table 6

Seasonal variation of cadmium (mg/kg) in ﬁsh tissues.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Species | Tissue | FIM | SWM | SIM | NEM |
| E. suratensis | Gill | 0.056c | 0.034b | 0.080b | 0.030b |
|  | Kidney | 0.085a | 0.064a | 0.110a | 0.080a |
|  | Liver | 0.075b | 0.065a | 0.087b | 0.081a |
|  | Muscle | 0.001d | 0.001c | 0.001c | 0.001c |
| A. testudineus | Gill | 0.327b | 0.344a | 0.347b | 0.307b |
|  | Kidney | 0.311c | 0.298b | 0.314c | 0.271c |
|  | Liver | 0.395a | 0.340a | 0.386a | 0.360a |
|  | Muscle | 0.004d | 0.022c | 0.018d | 0.018d |
| C. striata | Gill | 0.386b | 0.486b | 0.459b | 0.374b |
|  | Kidney | 0.249c | 0.265c | 0.258c | 0.240c |
|  | Liver | 0.521a | 0.500a | 0.527a | 0.536a |
|  | Muscle | 0.005d | 0.025d | 0.013d | 0.015d |

Values within columns are signiﬁcantly different at p b 0.05 (mean comparison made column-wise for each species).

20

18

NP1, 4.98± .54

NP3, 9.23± 1.05

NP5, 4.15± .65

P1, 4.83± .033

P3, 14.51± 1.94

P6, 17.34± 1.17

16

Concentration mg/kg

14

12

NP1, 1.98± .02

NP3, 3.56± .07

NP5, 2.17± .01

P1, 2.66± .11

P3, 3.46± .05

P6, 4.22± .20

10

8

6

4

2

0

Arsenic Cadmium

Fig. 5. Arsenic and cadmium levels in sediments.

highest level of As at lower trophic positions (E. suratensis) compared to the higher trophic positions (represented by A. testudineus and C. striata) indicated that although bioaccumulation occurs, there is no indication of biomagniﬁcation of arsenic along the aquatic food chains in upper Malwathu Oya. Therefore, As pollution is more severe in lower trophic positions. By contrast, Cd level in ﬁsh liver has varied as C. striata N A. testudineus N E. suratensis. The concentration of Cd in the liver is very low (0.065–0.087 mg/kg) at lower trophic positions and it is relatively higher (0.500–0.536 mg/kg) at higher trophic positions. This indicates an increase in dietary Cd level towards the higher trophic position reﬂecting the possibility of bioaccumulation of more Cd at higher trophic positions. It also suggests the pos- sibilities of biomagniﬁcation of Cd along aquatic food chains. Therefore, Cd pollution is more severe in higher trophic positions.

Previous studies have reported similar observations with respect to As and Cd in ﬁsh tissues and stresses that As is bioconcentrated by ﬁsh but not biomagniﬁed through the food chain (Eisler, 1988; Maeda, 1994; Mason et al., 2000; Barwick and Maher, 2003; Culioli et al., 2009; Leeves, 2011). However, Cd shows biomagniﬁcation (Croteau et al., 2005; Abdelghani and Elchaghaby, 2007). Therefore, the results of the current study are similar to the above results. Several studies have demonstrated the accumulation tendency of As and Cd in freshwater ﬁsh. Reviewing information from thirty-two studies related to Cd accumu- lation in natural environments, Perera et al. (2015) reported that the liver shows higher afﬁnity (24 studies out of 32) towards Cd rather than kidneys (5 out of 32) and gills (7 out of 32). If As is considered, the accumulation tendency might be highest in the gill (Akan et al., 2009; Petkovšek et al., 2012), kidney (Palaniappan and Vijayasundaram, 2009), liver (Culioli et al., 2009; Dsikowitzky et al., 2013) or muscle (Squadrone et al., 2013). Therefore, it is apparent that the accumulation pattern of As and Cd in ﬁsh tissues can be varied because many other factors in the water can affect bioaccumulation in ﬁsh (Iivonen et al.,

1992; Mason et al., 2000). However, the presence of As and Cd in river water affects its bioaccumulation in ﬁsh.

Relationship between Arsenic and Cadmium in Fish Species and Environmental Compartments

Gill and Kidney

Signiﬁcant (p b 0.05) positive correlations were observed between water-As and gill-As as well as between water-Cd and gill- Cd levels in all three ﬁsh species (Table 7). Correlations between sediment-As level and gills of E. suratensis was poor but positive

Table 7

Pearson correlation coefﬁcients (r) between arsenic and cadmium in ﬁsh and environmental compartments.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Species | Tissue | Arsenic |  |  |  |  |  |  | Cadmium |  | | | | |
|  |  | Water |  |  |  | Sediment |  |  | Water |  |  |  | Sediment |  |
|  |  | n | r | p |  | r | p |  | n | r | p |  | r | p |
| E. suratensis | Gill | 24 | .508† | .011 |  | .027 | .899 |  | 24 | .475† | .019 |  | .014 | .947 |
|  | Kidney | 27 | .068 | .751 |  | .150 | .486 |  | 24 | .226 | .289 |  | .132 | .538 |
|  | Liver | 27 | .133 | .536 |  | .336 | .108 |  | 24 | .058 | .788 |  | .331 | .114 |
|  | Muscle | 24 | −.272 | .198 |  | .203 | .340 |  | 24 | −.189 | .377 |  | .058 | .789 |
| A. testudineus | Gill | 24 | .521‡ | .009 |  | .433† | .035 |  | 25 | .482† | .017 |  | .497‡ | .007 |
|  | Kidney | 27 | .096 | .654 |  | .118 | .582 |  | 25 | .256 | .227 |  | .025 | .909 |
|  | Liver | 24 | .193 | .366 |  | .269 | .204 |  | 24 | .376 | .070 |  | .536‡ | .007 |
|  | Muscle | 24 | .142 | .507 |  | .265 | .211 |  | 24 | .170 | .427 |  | −.039 | .858 |
| C. striata | Gill | 24 | .507† | .011 |  | .457† | .025 |  | 28 | .652‡ | .001 |  | .449† | .028 |
|  | Kidney | 26 | .161 | .452 |  | .301 | .153 |  | 26 | .258 | .224 |  | .451 | .027 |
|  | Liver | 24 | .230 | .281 |  | .276 | .192 |  | 24 | .288 | .173 |  | .511† | .011 |
|  | Muscle | 24 | .050 | .818 |  | .400 | .053 |  | 24 | .351 | .092 |  | −.038 | .861 |

p values for each pair of variables are reported; correlation is signiﬁcant at the 0.05 level (†) and at the 0.01 level (‡).

(r = .027, p = 0.899). However, sediment-As level was signiﬁcantly positively correlated with the gill tissue of A. testudineus (r = .433, p = 0.035) and C. striata (r = .457, p = 0.025). Sediment-Cd level was poorly positively correlated in E. suratensis (r = .014, p = 0.947). The correlation between sediment-Cd level and gill-Cd level in A. testudineus (r = .497, p = 0.007) and C. striata (r = .449, p = 0.028) was signiﬁcantly positively correlated. The correlation between trace element level between abi- otic factors (water and sediment) and kidney tissue showed similar results. Water-As and water-Cd level was poorly positively correlated with As and Cd in the kidneys of all three ﬁsh species. Similarly, a poorly positive correlation was observed between sediment-As and sediment-Cd level and kidney of all ﬁsh species.

Arsenic and cadmium was present in variable concentrations at different study sites of the upper Malwathu Oya (Tables 3 and 4). According to the present data, the level of As and Cd in the gills highly depend on the concentrations of the two elements in the water. Fish do not cohabit at one site but they habitually move from one site to the other. Their gills are continuously exposed to different concentrations of arsenic and cadmium while incessantly moving between sites (i.e., from paddy sites to forest sites and vice versa). Therefore, trace element levels in the gills change according to the available concentrations in the water.

Their gills are continuously exposed to different concentrations of arsenic and cadmium while incessantly moving between sites from paddy sites to forest sites and vice versa. Fish can up-take trace metals by two main routes (Farkas et al., 2000; Rozon-Ramilo et al., 2011), either from water through the gills or through the digestive tract. However, the signiﬁcant correlation between sediment trace element levels and gills of A. testudineus and C. striata (Table 7) are related to the feeding habits of these two ﬁsh species. A. testudineus (Riede, 2004) and C. striata (Rahman et al., 2012) prefer to access the river bottom. Because of their preference towards animal matter, these two species access the bottom to eat up bottom-living invertebrates (Pethiyagoda, 1991). As a result, the bottom sediment, which contains more arsenic and cadmium (Fig. 5), becomes agitated. Therefore, when ﬁsh are feeding, their gills come into direct contact with arsenic- and cadmium-rich water, which resulted in having more trace elements in the gill tissue. However, a poor correlation between sediment As level and E. suratensis gill As level was observed and it is also associated with the habitat preference of the ﬁsh. They prefer to live and feed near the bottom as well as in midwaters or near the surface (benthopelagic). Therefore, the habitat preferences as well as the bioturbation of bottom sediment by ﬁsh for diet require- ments mix more As and Cd with water, which possibly passes through their gills resulting in more trace elements in the gill tis- sue. Therefore, the positive relationship between the gill and sediment trace element level is understandable. The behaviour of As and Cd in the kidney seems to be more or less similar. As waterborne exposure results in more As and Cd in the gills and kidneys (Oladimeji et al., 1984; Szebedinszky et al., 2001; Franklin et al., 2005), their deposition and excretions rates might not be diverse with respect to kidney tissue. Therefore, a similar correlation was observed in all ﬁsh species.

Liver

Arsenic and cadmium levels between water and the liver poorly but positively correlated in all three species (Table 7). Sim- ilarly, sediment-As level was poorly positively correlated with the liver in all species. However, the behaviour of sediment-Cd was rather different; although sediment-Cd level was poorly correlated with the liver-Cd level of E. suratensis (r = 331, p = .114), it showed a signiﬁcantly positive correlation in A. testudineus (r = 536, p = .007) and C. striata (r = 511, p = .011).

Arsenic and cadmium in water did not signiﬁcantly affect the level of those in the liver with respect to all three ﬁsh species.

Similarly, sediment-As content has not signiﬁcantly affected the liver-As level in E. suratensis, probably due to the fact that E. suratensis mainly prefers plant matter as their food (Pethiyagoda, 1991). Although trace metal accumulation is possible in plant matter, being at the lowest level in aquatic food chains, the available levels might be much lower. In contrast, strong cor- relation between sediment-Cd level and the liver tissue reﬂects the feeding habit of A. testudineus and C. striata. They represent higher trophic levels in aquatic food chains and consume worms, crustaceans and molluscs from the river bottom. Therefore, since sediments contains Cd, the potential effect of bioaccumulation is quite possible when consumer organisms subsequently ingest metals bioaccumulated in organisms at a lower trophic levels (Drexler et al., 2003). Therefore, the accumulated rate of Cd in A. testudineus and C. striata might be higher than the depuration rates which resulted in a strong correlation between sediment-Cd level and liver.

Muscle

Arsenic and cadmium levels between water and muscle tissue did not show a signiﬁcantly positive correlation with all three species (Table 7). Similarly, sediment-As level did not positively correlate with the muscle tissue in all species.

Water-As and water-Cd levels did not affect As and Cd levels in the muscle tissue in all ﬁsh species. As the trace element level in the muscle tissue is comparatively very low (Tables 5 and 6), the effect of As and Cd on the muscle tissue can also be low. Poor relationship between sediment-As levels and the muscle tissue indicates that the effect of sediment on muscle-As level in all spe- cies is at a minimum level. Similarly, poor correlation between sediment-Cd levels and the muscle tissue of E. suratensis indicates that the effect of sediment on the accumulation of Cd in it is at a minimum level.

Rosso et al. (2013) reported that out of nine ﬁsh species, two detritivore ﬁsh species (Cyphocharax voga and Astyanax

eigenmanniorum) showed signiﬁcant correlation coefﬁcients with sediment-As. However, in the same study, they have not observed signiﬁcant correlation between water and ﬁsh. Another study reported that a correlation was not found between Cd concentrations in water and sediment or between Cd concentrations in water and muscle and gills of the ﬁsh Leuciscus cephalus (Demirak et al., 2006). Therefore, the negative relationship might be associated with the physiological adaptations of the two ﬁsh species. Further, release of trace elements from sediments into the water body and, consequently, to the ﬁsh, depends on the

chemical fractionation of metals and other factors such as sediment pH and other physical and chemical characteristics of the aquatic system (Morgan and Stumm, 1991).

Fish as Water Pollution Indicators

Accumulation of As and Cd in ﬁsh tissues can be related to contamination level in water and sediments. In freshwater ﬁsh, the gills are the main point of entry for dissolved metals (Li et al., 2009) and metalloids (Ananth et al., 2014). Therefore, arsenic and cadmium level in gills essentially reﬂect their potency in the water. It is further emphasized by the signiﬁcant (p b 0.05) corre- lation between water and gill arsenic level of all species in the present study as well. Signiﬁcant (p b 0.01) correlation between water-As and gill-As level of A. testudineus as well as between water-Cd and gill-Cd level of C. striata (Table 7) stresses the afﬁnity of the gill tissue of those two species towards waterborne arsenic and cadmium. If the quantity of a pollutant in the environment or its change can be determined by using a species, it reﬂects the possibility of that species being used as a biomonitor species (Markert et al., 1997). However, considering the present data, except for the gills, all of the tissues studied in the three species do not reﬂect the trace element proﬁle in water,; thus, only the gills can be considered as a possible bioindicator tissue. Moreover, the gills of A. testudineus and C. striata reﬂects the pollution level (as correlation was highly signiﬁcant) of As and Cd, respectively. However, not all species are suitable for use in biomonitoring programs because some species can efﬁciently regulate their body trace element levels (Bryan, 1984). Exposing the ﬁsh to variable levels of the two trace elements, the possibility of their reﬂecting the quality of the environment should be monitored. Therefore, further studies are required to understand the suitability of these two species in biomonitoring programs.

Suitability of Fish for Human Consumption

E. suratensis, A. testudineus and C. striata are more common local food ﬁshes. Overall As and Cd levels in these ﬁsh species are shown in Fig. 6. In all four tissues, As concentration ranged from 0.026 to 3.438 mg/kg, 0.064 to 4.732 mg/kg and 0.072 to

4.321 mg/kg in E. suratensis, A. testudineus and C. striata, respectively (Table 5). Cadmium varies from 0.001 to 0.110 mg/kg,

0.004 to 0.395 mg/kg and 0.005 to 0.536 mg/kg in E. suratensis, A. testudineus and C. striata, respectively (Table 6). Nevertheless, being the consumable portion of the ﬁsh, identiﬁcation of trace metal levels in muscle meat is essentially useful.

According to the above results, As and Cd concentrations in the muscle meat of all three ﬁsh species were well below the max- imum permissible levels of many recognized international institutions (Fig. 6). For example, according to Australia New Zealand Food Authority (1999) the recommended arsenic level is 2.0 μg/g and it is 1.2 μg/g for the USEPA (2000). According to the European Union (2006) and (MOFAR (1996) of Sri Lanka, the maximum suggested level of cadmium for human consumption is 0.05 mg/kg in wet weight basis of the ﬁsh muscles.

Arsenic occurs in different inorganic and organic forms in the environment, both from natural occurrence and from anthropo- genic sources. The inorganic forms of arsenic are more toxic as compared to the organic arsenic (EFSA, 2009) and are mostly found in surface waters as oxyanions of trivalent arsenite (As(III)) or pentavalent arsenate (As(V)) (Smedley and Kinniburgh,

2002). Organic forms of arsenic (e.g., monomethylarsonic acid and dimethylarsinic acid) are usually minor in surface waters but proportions of organic forms of arsenic can increase as a result of methylation reactions catalysed by microbial activity (IPCS, 2001; Smedley and Kinniburgh, 2002). In the environment, As behaves in similar ways to nitrogen and phosphorus (Smedley and Kinniburgh, 2002). As a result of these similarities, arsenic gets taken into the biochemical pathways of nitrogen and phosphorus, resulting in the formation of compounds such as arsenobetaine in ﬁsh (Wahlen, 2004). Higher percentage of As in ﬁsh is present as organic As (Mandal and Suzuki, 2002; Ciardullo et al., 2010). These organic forms become toxic after

2.040

1.840

1.640

Arsenic (mg/kg)

1.440

1.240

1.040

0.840

0.640

0.440

0.240

0.040

Arsenic Cadmium

2.00

0.05

ES AT CS

0.055

0.050

0.045

Cadmium (mg/kg)

0.040

0.035

0.030

0.025

0.020

0.015

0.010

0.005

0.000

Fig. 6. Arsenic and cadmium level in the muscle tissue (horizontal dotted lines indicate maximum permissible levels).

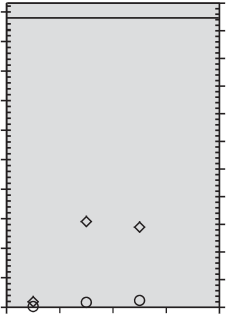


Table 8

Quantity of arsenic and cadmium receive due to per capita consumption (36 g/day) of ﬁsh.

|  |  |  |
| --- | --- | --- |
| Fish species | Arsenic (μg/day) | Cadmium (μg/day) |
| E. suratensis | 1.620 | 0.036 |
| A. testudineus | 2.628 | 0.540 |
| C. striata | 3.096 | 0.504 |

metabolic conversion to the trivalent form of arsenic (IPCS, 2001). However, inorganic arsenic compounds are also found in ﬁsh

(Ciardullo et al., 2010).

The FAO/WHO Expert Committee on Food Additives (JECFA, 2010) derived an inorganic arsenic BMDL (benchmark dose) for a

0.5% increased incidence of lung cancer, which was computed to be 3.0 μg/kg body weight per day. Average human body mass globally was 62.0 kg and 57.7 kg in Asia (Walpole et al., 2012), thus the average body mass of a Sri Lankan is considered as

60 kg. Therefore, 180 μg of arsenic per day is allowable in an adult person. As the per capita ﬁsh consumption in Sri Lanka is

36 g per day (Amarasinghe, 2013), by consumption of E. suratensis (mean arsenic level is 0.045 mg/kg), a person can receive a maximum of 1.62 μg of arsenic per day (Table 8). Therefore, the level derived from the current study with respect to E. suratensis (1.62 mg/day) is well below the recommended levels of (JECFA, 2010). Similarly, JECFA (2010) derived BMDL for cadmium to be 0.80 μg/kg body weight per day (or 25 μg/kg body weight per month (PTMI).2 By consuming E. suratensis (mean cadmium level is

0.001 mg/kg), a person receives 0.036 μg of cadmium per day. The estimation of arsenic and cadmium for other species is given in Table 8.

A limited number of other studies have demonstrated As and Cd in the muscle meat of different freshwater ﬁsh species in Sri Lanka. Jinadasa et al. (2013) reported very low concentrations (b 0.05 mg/kg) of Cd in the muscle meat of common freshwater food ﬁshes (Oreochromis spp. and C. striata) collected from the NCP. However, a considerable level of As was not detected in the above study. Indrajith et al. (2010) reported 0.002–0.048 mg/kg in the muscle tissue of E. suratensis. The ﬁrst study was con- ducted with specimens collected during eight months from six reservoirs of the Anuradhapura District (in NCP) while the latter was restricted to specimens collected from several locations within several months (exact duration not given). Out of the limited number of studies conducted with respect to the bioavailability of trace elements in freshwater ﬁshes in the NCP, being the most marketable and abundant species, a majority of studies address arsenic and cadmium in exotic species (Oreochromis mossambicus and Oreochromis niloticus). For example, Rajapaksha et al. (2008) reported 0.06–0.20 mg/kg cadmium in the muscle tissue of O. niloticus collected from two reservoirs in Anuradhapura District in 2004 (exact duration not given). Chronic kidney disease with uncertain aetiology being one of the most controversial current health topics in the country, and some schools of thought indicate possible correlation with trace elements and CKDu, assessment of tissue level As bioaccumulation should be closely mon- itored. The reported negative results of arsenic might be associated with the restriction of sampling for several months without continuous monitoring of specimens. In contrast, the current study continued for 43 months (in a single reservoir) by collecting ﬁsh samples (from three ﬁsh species) on a monthly basis (ﬁsh). Therefore, the observed results of the current study might be different from others. However, the observed trace element levels of the current study are within the suggested safe range. It emphasizes that the muscle meat of the three ﬁsh species found in upper Malwathu Oya is safe for human consumption.

Conclusions

Paddy cultivation has been identiﬁed as the main anthropogenic source that affects river water quality. Arsenic and cadmium concentration in water showed a bimodal pattern which coincided with the bimodal rainfall of the dry zone and the paddy cultivation pattern in the area. The presence of arsenic and cadmium in sediments and water affect bioaccumulation in ﬁsh. However, the trace element levels in ﬁsh varied as C. striata N A. testudineus N E. suratensis. Accumulation of As does not change according to the feeding habits of ﬁsh. Nevertheless, the feeding habits and habitat preferences of ﬁsh affect the accumulation tendency of Cd. Arsenic accumula- tion pattern in different organs (i.e., kidney N liver N gill N muscle) stresses that the waterborne pathway is more important and Cd ac- cumulation pattern (i.e., liver N kidney N gill N muscle) reﬂects the importance of dietary pathways. Further comprehensive assessment on the gills of A. testudineus and C. striata is necessary to understand the suitability of the species for biomonitoring studies. Water and sediment trace element levels do not considerably affect the muscle tissue. Therefore, ﬁsh consumption is not affected by paddy cultivation.

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