

# Chronic renal failure among farm families in cascade irrigation systems in Sri Lanka associated with elevated dietary cadmium levels in rice and freshwater fish (*Tilapia*)

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**Abstract** Chronic renal failure (CRF), in the main agricultural region under reservoir based cascade irrigation in Sri Lanka has reached crisis proportion. Over 5,000 patients in the region are under treatment for CRF. The objective of this study is to establish the etiology of the CRF. Concentrations of nine heavy metals were determined in sediments, soils of reservoir peripheries, water and *Nelumbo nucifera* (lotus) grown in five major reservoirs that supply irrigation water. All five reservoirs carried higher levels of dissolved cadmium (Cd), iron (Fe) and lead (Pb). Dissolved Cd in reservoir water ranged from 0.03 to 0.06 mg/l. Sediment Cd concentration was 1.78–2.45 mg/kg. No arsenic (As) was detected. Cd content

in lotus rhizomes was 253.82 mg/kg. The Provisional Tolerable Weekly Intake (PTWI) of Cd based on extreme exposure of rice is 8.702–15.927 µg/kg body weight (BW) for different age groups, 5–50 years. The PTWI of Cd due to extreme exposure of fish is 6.773–12.469 µg/kg BW. The PTWI on a rice staple with fish is 15.475–28.396 µg/kg BW. The mean urinary cadmium (UCd) concentration in CRF patients of age group 40–60 years was 7.58 µg Cd/g creatinine and in asymptomatic persons UCd was 11.62 µg Cd/g creatinine, indicating a chronic exposure to Cd. The possible source of Cd in reservoir sediments and water is Cd-contaminated agrochemicals. The CRF prevalent in north central Sri Lanka is a result of chronic dietary intake of Cd, supported by high natural levels of fluoride in drinking water, coupled with neglecting of routine de-silting of reservoirs for the past 20 years.

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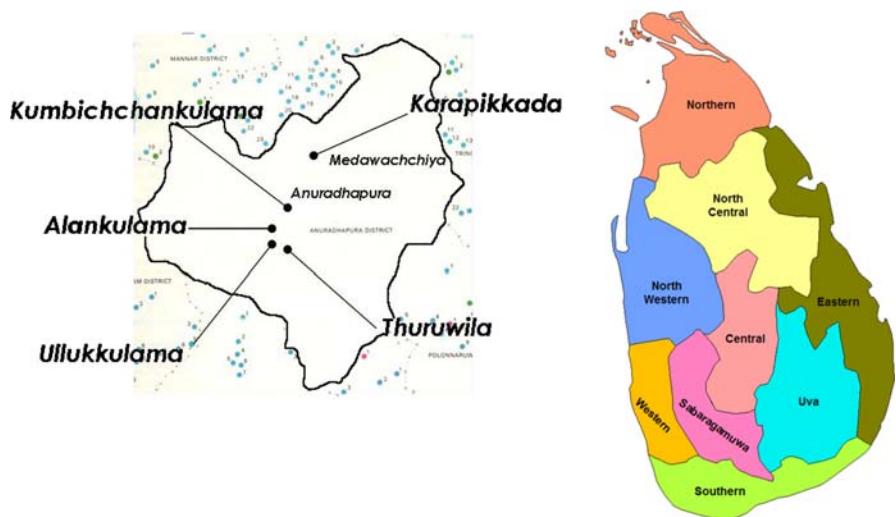
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**Keywords** Agrochemicals · Cadmium · Cd in *Tilapia* · Chronic renal failure · Reservoir sediments · cadmium · Rice grain cadmium

## Introduction

Chronic renal failure (CRF) has reached crisis proportions in the main agricultural region under reservoir-based irrigation, in the North Central Province (NCP) of Sri Lanka [commonly referred to as “Dry zone” of Sri Lanka, which is demarcated by a mean annual rainfall of 1,450 mm, mean annual runoff of

**Fig. 1** Sampling sites in the Anuradhapura district, part of the North Central Province of Sri Lanka (right)



$2.55 \times 10^6$  ha m and runoff rainfall ratio (%) of 35.8]. At present, over 5,000 patients are under treatment for renal failure (Atapattu 2006). Athuraliya et al. (2003) reported that, in a farming community in the Madawachchiya region in NCP (Fig. 1), 4.8% of the total population 38,532, amounting to 1,400 people, were affected with renal failure. The increasing trend in the reporting of patients admitted with renal failure to hospitals was however restricted to two administrative districts that came under the Mahaweli river diversion scheme for irrigation of farm lands.

The most far reaching and largest national development program ever undertaken in the modern history of Sri Lanka is the Mahaweli river diversion for irrigation and hydropower generation, commenced in 1970 and accelerated in 1977. The average annual discharge from Mahaweli River is about 8.4 billion cu m which is used to irrigate 140,000 ha in the NCP. The total extent of rice sown in 2003/2004 was 75,361 ha in the region (Mahaweli Authority 2005).

The chronic renal failure not associated with diabetes and hypertension was reported mostly among the farmers in NCP. Of the adult employed population, 5.6% of the farmers were proteinuric. Athuraliya et al. (2003) observed that 45.6% of the patients with persistent proteinuria had early renal disease, based on Creatinine clearance (Cr.cl) data (45.6% Cr.cl >60 ml/min) and 35% were in late stages of renal failure with Cr.cl <30 ml/min.

Several studies on the impact of agriculture on water quality in the dry zone of Sri Lanka have been reported. They were mostly on the occurrence of

excessive quantities of nitrates and phosphates that lead to eutrophication of reservoirs and heavy nitrate levels in ground water (Piyasiri 1995; Liyanage et al. 2000; de Zoysa 2002). Recently, in a more comprehensive study on water quality in NCP, Perera (2006) and Wickramaarachchi (2005) reported the occurrence of detectable quantities of the weedicide propanil and the insecticide chlorpyrifos in reservoirs to which surface runoff from rice fields is directed.

Occurrence of high fluoride levels in ground water in the NCP is a significant factor, but not implicated in the renal failure (Dissanayake 2006; Herath et al. 2005). However, to date, the etiology of the chronic renal failure in the farmer communities in the NCP is uncertain and the prognosis is not favorable.

The objective of this study is to establish the etiology of the CRF in farmer communities of north central Sri Lanka.

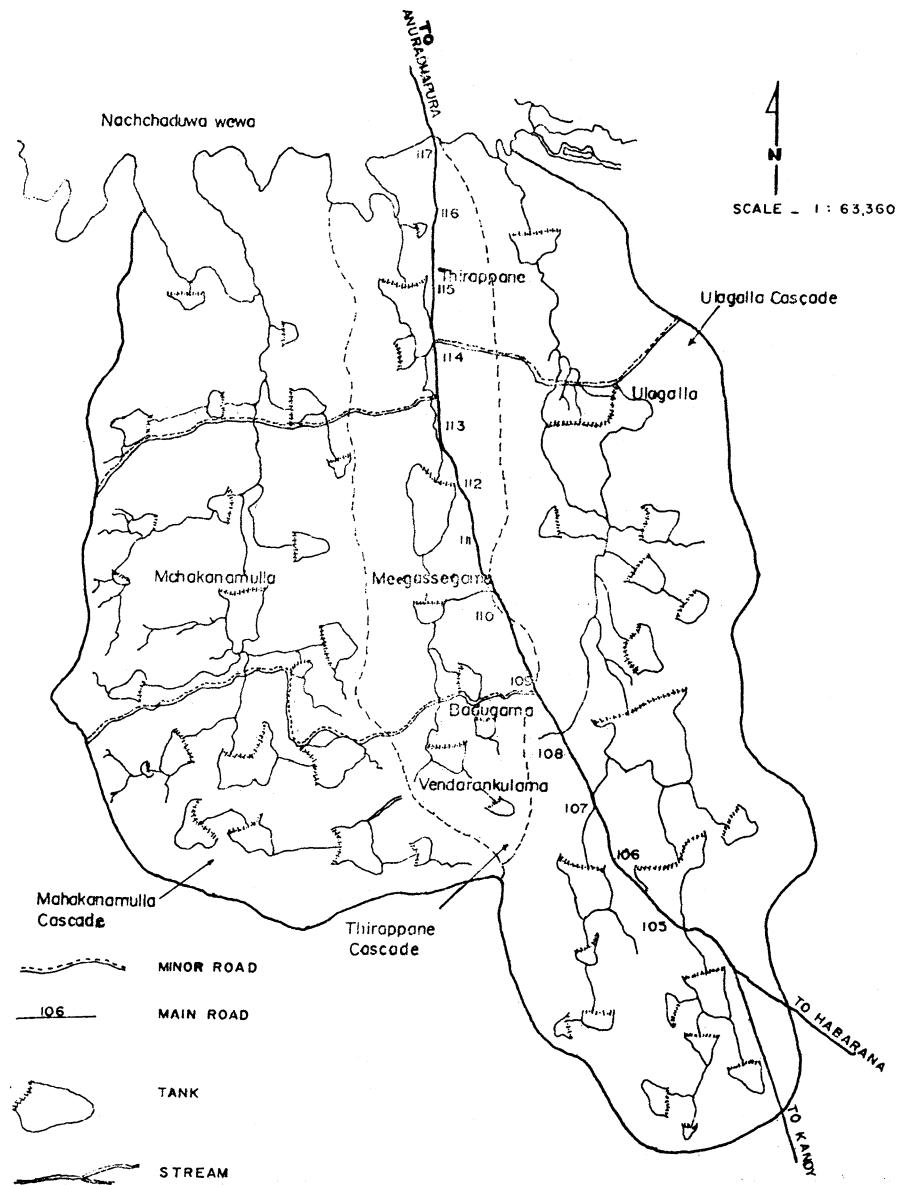
## Materials and methods

### Study area

Study area is located in the North Central Province (NCP) of Sri Lanka. This land surface is spotted with man-made reservoirs arranged in drainage patterns as a large number of micro-catchments and meso-catchments in cascades of reservoirs (Figs. 2 and 3).

All small reservoir cascades are located within the meso-catchment basin. The meso-catchment basins drain into streams that pass through the rice fields in

**Fig. 2** A tank cascade system in Anuradhapura district of the dry zone of Sri Lanka



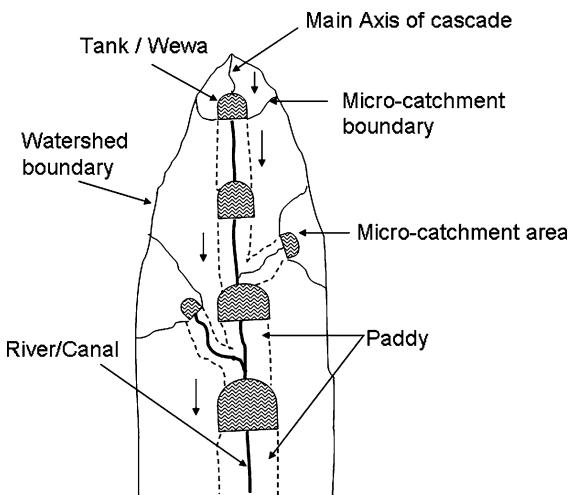
inland valleys. Drainage from the rice fields in the upper part of the cascade flow into a downstream reservoir for reuse in the rice fields that are located below the reservoir. In the Anuradhapura district alone there are 4,000 small reservoirs of which 3,000 are functioning in 280 separate cascades. Each cascade is hydrologically independent.

Four reservoirs, with independent cascades (Ullukkulama, Kumbichankulama, Karapikkada, and Alankulama) and one large reservoir linked to Mahaweli River Diversion scheme (Thuruwila wewa) were selected for sampling. Karapikkada reservoir

served the farmers in Madawachchiya where the largest number of CRF subjects was reported. Thuruwila reservoir is one of the main sources of water for the City of Anuradhapura, the administrative center of the NCP.

#### Sample collection

In order to evaluate the degree of pollution of reservoirs with heavy metals, sediments in reservoir bottoms and soil from reservoir peripheries closer to the dam were collected from the five selected reservoirs in



**Fig. 3** Schematic representation of a small tank cascade

2004. Samples from reservoir sediments were collected at random locations in an area of 50 m into the reservoir from the dam. Soil from the reservoir periphery was taken at random in an area of  $100 \times 100$  m from the water boundary and the embankment. All soil samples were collected as described by Boyd and Tucker (1992).

Water samples from the reservoirs were collected using a column sampler, (2.13 m height and 76.2 mm diameter) to obtain a composite water sample from each sample point. A maximum of 2 m depth was reached from the surface. Thirty samples were obtained in an S-shaped pattern (Boyd and Tucker 1992).

Fish (*Oreochromis niloticus*) samples were collected from commercial landings from Karapikkada (Madawachchiya) and Thuruwila reservoirs near the City of Anuradhapura. Well grown fish (*Oreochromis niloticus*) samples with a length of 20–22 cm with an average weight of 1 kg were used for analysis of cadmium and lead. *Oreochromis niloticus* feeds on bottom deposits derived from the plankton and other sources (Schroeder 1978). Beveridge (1984) reported that *O. niloticus* is omnivorous and can utilize blue-green algae. Jauncy and Ross (1982) observed that *O. niloticus* like most other tilapias is a herbivore, and that its diet under natural conditions is restricted to phytoplankton.

*Nelumbo nucifera* (lotus), a common food item in all Asian countries is natural vegetation in all the reservoirs in NCP. Storage rhizome samples from

mature plants (120 days old, before flowering) of *N. nucifera* were picked up randomly from each reservoir at least 10 m from the embankment.

Rice (*Oryza sativa*) samples both raw and milled were collected from households of individuals identified with chronic renal dysfunction. Coordinates of the sample sites were recorded using GPS and mapped using Google Earth browser.

Cows' milk samples were collected from milk collecting centers at Madawachchiya and Thuruwila. Milk is brought to the centers by the dairy farmers in the area of study where the herds are raised in pasture land near reservoir peripheries. Samples were taken in 20-ml acid washed glass bottles and stored at 4°C during transport and later in the laboratory until analysis was done.

Soil, lotus rhizomes, rice and fish samples were stored in polypropylene bags whereas conical flasks containing water samples were stored on dry ice to maintain 4°C while collecting samples and during transportation to the laboratory. Once the samples reached the laboratory, soil and food samples were stored under refrigerated conditions until further preparations.

Triple Superphosphate (TSP), a fertilizer produced by the action of concentrated phosphoric acid on ground phosphate (P) rock, is the most common P fertilizer used in rice cultivation in the NCP. The phosphorus content of triple superphosphate is (17–23% P; 44–52%  $P_2O_5$ ).

A formulation of weedicide that is most commonly used at least twice in the season in rice cultivation is bispyribac sodium (BPS). Samples of TSP and BPS were collected from all households of farmers visited. Cadmium content in TSP and BPS were determined by AAS as described by Loganathan and Hedley (1997).

Urine samples, from 32 CRF patients confirmed to be not associated with diabetes and hypertension by biopsy reports indicating proximal tubular sclerosis, and also from 32 asymptomatic individuals in the same village community, were collected from both Madawachchiya and Girandurukotte. Samples were collected in 20-ml acid washed glass bottles and sealed with Parafilm. Urine samples were maintained at 4°C during transport and storage.

Prior to analysis the samples were thawed and homogenized by vortexing for few seconds. Five milliliters from each sample were taken for ashing.

## Survey of food habits of CRF patients and their families

With CRF reaching crisis level in NCP, a survey of food habits was conducted in July and August 2006 to assess the relationship between food and CRF among patients and other residents of Madawachchiya in Anuradhapura district and Girandurukotte township in Pollonnaruwa district. Food and social habits and family history of renal failure was evaluated from patients reporting to Government General Hospitals.

## Analytical methods

### *Water samples*

Each water sample was well mixed and filtered through a 0.45- $\mu\text{m}$  membrane filter to determine the concentration of dissolved heavy metals. Determinations of dissolved heavy metals were done by filtering water samples through a preconditioned 0.45- $\mu\text{m}$  pore diameter cellulose acetate filter. Filters and filter device were preconditioned by rinsing with 50 ml deionized water as described by 3030B (Greenberg et al. 1992). From each filtrate, two 20-ml working samples were obtained randomly and stored in 25-ml glass vials at 4°C until analysis for heavy metals were done as described by “Standard Methods for Examination of Water and Wastewater” (Greenberg et al. 1992). Analysis was done by direct aspiration to air-acetylene flame using AAS model AA-6200 [P/N 206-50000-36].

### *Plant samples*

The lotus rhizomes as well as rice samples were thoroughly washed with deionized water several times. A sub-sample of 2 g of chopped rhizomes was taken and the percentage dry matter was estimated. A fresh sample was dried at 105°C until a constant weight was obtained. Dried samples were ground to fine powder and a sample of 0.8 g was measured into porcelain crucibles and ashed at 600°C for 4 h in a muffle furnace. Crucibles were allowed to cool overnight. Then the crucibles containing ash were washed into 250-ml conical flasks using 16 ml of HCl prepared at a ratio of 1:3. Two drops of concentrated HNO<sub>3</sub> were added to each flask and the mixture was boiled on a hotplate under a fume hood at 60°C for 10–15 min. The

mixtures were then allowed to cool, filtered through Whatman® No.40 filter papers, into 100-ml volumetric flasks, and made up to the volume using deionized water. Extracts were stored until analysis, in glass vials at 4°C.

### *Soil samples*

All soil samples were air dried on polypropylene sheets at room temperature for several days until they were deprived of moisture. Then the soil samples were well ground using porcelain mortar and pestle. Ground samples were sieved through a 250- $\mu\text{m}$  mesh sized sieve and stored in plastic containers.

From each ground soil sample, 1.2 g was measured into 60-ml graduated boiling tubes, and 15 ml of digestion acid (a standard mixture of HNO<sub>3</sub> and HCl for heavy metal extraction; Greenberg et al. 1992) was added and swirled to wet the samples. Boiling tubes were covered with watch glasses and stood overnight. The tubes were then heated at 50°C for 30 min followed by 120°C for 2 h. Once the boiling tubes were cooled, they were volumarized with 8.8% HNO<sub>3</sub>. Solutions were filtered through Whatman® No. 40 filter papers and the filtrates were stored in 25-ml glass vials as stated in water sample preparation.

### *Fish samples*

Fish samples collected from Madawachchiya-Karapikkada, Thuruwila and Nachchiyaduwa reservoirs were first washed with tap water to remove any adhered dirt followed by washing with deionized water in four changes. Offal was carefully removed with stainless steel sterile surgical blades. Muscle samples were analyzed for cadmium and lead separately. Precisely weighed quantities of sample material were taken in high silica glass (Corning) evaporating dish and subjected to ashing as described by Bureau of Nutritional Science, Ottawa (Health Canada 1985). Ash was wetted with deionized water and 2–4 ml of concentrated nitric acid. The evaporating dish was then covered with a high silica corning watch glass and refluxed by keeping on a hot plate for 1 h. Samples were then returned to the muffle furnace at 375°C for 1 h and the ash obtained was then dissolved in 9.25% hydrochloric acid and filtered.

## *Urine samples*

The cadmium concentration was analyzed by graphite furnace-AAS after wet ashing in HNO<sub>3</sub>/H<sub>2</sub>SO<sub>4</sub>/HClO<sub>4</sub> and extraction with ammonium pyrrolidine dithiocarbamate-methyl isobutyl ketone (APDC/MIBK) (Nogawa et al. 1979). Urinary cadmium levels (UCd) are reported as standardized data per gram of creatinine.

## *Atomic absorption spectrometry (AAS)*

A series of standard metal solutions were prepared for Cd, Co, Cr, Cu, Fe, Mn, Pb and Zn, using the standard solutions of 1,000 mg/l (F 1200; Fukuoka Chemical, Japan), supplied with each of the hollow cathode lamps. The number of dilutions per metal was determined according to the specified working range for that particular metal. Using the standard metal solution series, a calibration curve for each metal was obtained. Then the solutions stored in 20-ml glass vials were fed into the AAS (AAS Model: AA-6200) and aspirated into the air-acetylene flame. The detection limit of the AAS was set at 0.05 mg/l (mg/kg).

Rice, milk, fish, triple super phosphate, weedicide samples and urine samples were analyzed for cadmium and lead using AAS-graphite furnace. Analytical data were validated by further analysis at IFS, Kandy (AAS-GBC 933AA with a detection limit 0.01 mg/kg or mg/l with cadmium standard solution—Belgium 0.05–2.0 mg/kg at 228.8 nm, Graphite furnace GBC-GF 30000 with detection limit 0.015 µg/kg or µg/l).

Quality control measures were practiced for Cd extraction and analysis as described by EPA, USA (1983). Certified reference material prepared by National Institute for Environmental Studies, Japan Environmental Agency, NIES No. 10a and 10c—“Rice Flour—Unpolished”, with No. 10a rice variety Koshihikari containing  $0.023 \pm 0.003 \mu\text{g/g}$  Cd and No. 10c rice variety Toyonishiki with  $1.82 \pm 0.06 \mu\text{g/g}$  Cd, were used as the inhouse reference material. Certified reference material DORM-2 (Dogfish, *Squalus acanthias*—Muscle Certified Reference material for Trace Metals—prepared by National Research Council of Canada, Institute for national Measurement Standards, M-12, Montreal Road, Ottawa, ON, Canada, containing  $0.043 \pm 0.008 \text{ mg/kg}$  of Cd, was used for validation of Cd extraction and determination in *Oreochromis niloticus* samples.

## Statistical analysis

Variation of heavy metal concentration in water, *N. nucifera* rhizomes and soils between and within reservoir samples were evaluated by ANOVA using SAS package. Variation of data on Cadmium and Lead content in rice, milk, fish, weedicides and phosphate fertilizer was determined by two sample *t*-test. Analytical data obtained from AAS from the Animal Science laboratory and the Food Science and Technology laboratory of the faculty of Agriculture was validated with analytical data obtained from the IFS.

## Results and discussion

### Heavy metals in reservoirs

The mean concentration of dissolved heavy metals Cd, cobalt (Co), copper (Cu), Fe, manganese (Mn), Pb and zinc (Zn) in the waters of the five reservoirs tested are given in Table 1. No detectable levels of As and Cr were seen. All five reservoirs carried higher levels of cadmium, iron and lead. Dissolved cadmium in reservoir water ranged from 0.03 to 0.06 mg/l, a 19-fold increase over maximum contaminant level set by WHO. Similarly, dissolved lead content in reservoir water is higher in almost all the reservoirs. However, the cadmium content is significantly higher in Thuruwila and Karapikkada than the other reservoirs. Thuruwila is linked to the Mahaweli irrigation scheme where as Karapikkada is a reservoir in the area where large number of CRF patients is reported.

There is no significant difference in Zn content in water among the reservoirs and they are 20-fold lower than the MCL values, indicating a non-point non-industrial pollution pattern. There are no smelting industries in the area.

Table 2 represents the mean heavy metal concentration (mg/kg of soil) in reservoir bottom sediments. The total sediment cadmium concentration in reservoirs ranged from 1.78 to 2.45 mg/kg. Three of the reservoirs had relatively higher concentrations which were significantly higher than those of Karapikkada or Ullukkulama. It is a well established fact that the metals Cd, Pb, mercury (Hg) and thallium cause renal dysfunction both on acute and chronic exposure (Middendorf and Williams 2000; Donkin et al. 2000). In our study therefore, the Cd and Pb appear to be the

**Table 1** Mean dissolved heavy metal concentration in each of the subjective reservoirs

Metal	MCL <sup>a</sup> (mg/l)	Mean concentration (mg/l) <sup>b</sup>				
		1	2	3	4	5
Cd	0.003	0.05 b	0.04 c	0.06 a	0.06 a	0.03 d
Co	N/A	0.22 a	0.14 ab	0.04 c	0.15 ab	0.14 b
Cr	0.05	–	–	–	–	–
Cu	1.00	0.02 ab	0.02 ab	0.03 a	0.02 c	0.02 bc
Fe	0.30	0.20 c	0.79 b	0.47 bc	0.63 bc	1.28 a
Mn	0.05	–	0.18	–	–	–
Pb	0.01	0.03 a	0.01 bc	0.02 ab	0.03 a	–
Zn	2.00	0.10 a	0.10 a	0.06 a	0.11 a	0.10 a

1 = Kumbichchankulama, 2 = Alankulama, 3 = Thuruwila, 4 = Karapikkada, 5 = Ullukkulama reservoirs

N/A, Data not available; –, indicate insignificant value or zero

<sup>a</sup> Maximum contamination levels defined by the World Health Organization for drinking water<sup>b</sup> Mean value of thirty samples for each sampling sitea,b,c = Values followed by the same letter in each row are not significantly different at  $P = 0.05$  based on *t*-test**Table 2** Mean heavy metal concentration in mg/kg dry weight in reservoir bottom sediments in selected water reservoirs in NCP in Sri Lanka

Metal	Mean concentration (mg/kg) <sup>a</sup>				
	1	2	3	4	5
Cd	2.42 a	2.45 a	2.18 ab	1.78 b	1.87 b
Co	36.42 a	32.76 a	36.81 a	32.07 a	15.19 b
Cr	52.71 a	3.60 c	21.14 b	42.79 a	12.32 bc
Cu	27.34 b	24.19 b	49.63 a	26.52 b	17.92 b
Fe	2,075.11 b	1,977.35 c	2,144.82 b	2,279.91 a	1,777.12 d
Mn	277.20 a	127.69 bc	130.81 bc	164.01 b	77.33 c
Pb	11.45 a	8.81 a	8.79 a	10.79 a	2.51 b
Zn	58.83 ab	54.88 ab	66.72 a	47.29 b	45.38 b

1 = Kumbichchankulama, 2 = Alankulama, 3 = Thuruwila, 4 = Karapikkada, 5 = Ullukkulama reservoirs

<sup>a</sup> Mean value of 30 for each sampling site.a,b,c, = Values followed by the same letter in each row are not significantly different at  $P = 0.05$  based on *t*-test

most critical heavy metals that would relate to the existing renal dysfunction in the region.

Table 1 shows that the dissolved Cd and Pb content in reservoir water are higher than the MCL. The human health Risk Assessment Studies by Curtis and Smith (2002) showed that the metal solubility in soil water is a key determinant of metal accumulation in soil, water and plants/fishes. The ratio of metal concentration in soil to metal concentration in soil water ( $K_d$ ) estimates solubility of the metal. Cadmium tends to be more mobile in the soil system and therefore

more available to plants than many other heavy metals. Cadmium in solution is found mostly as  $Cd^{2+}$  (Alloway 1995). Absorption/desorption of cadmium is about 10-fold more rapid than Pb. Based on dissolved metal level in reservoir water and the amount found in sediment soil, the estimated  $K_d$  values for Cd and Pb for the five reservoirs tested are given in Table 3.

Lowest  $K_d$  values were reported in Karapikkada although the sediment Cd concentration is the lowest. This is probably due to the fact that the Cd solubility

**Table 3**  $K_d$  values for cadmium and Lead in five reservoirs

Metal	Ratio of metal conc. in soil to metal conc. in water, $K_d$				
	1	2	3	4	5
$K_d$ Cd	47.76	61.81	38.26	31.66	58.38
$K_d$ Pb	385.95	1,509.51	396.67	332.09	—

$K_d$  = The ratio of metal concentration in soil to metal concentration in water an estimate of solubility

1 = Kumbichchankulama, 2 = Alankulama, 3 = Thuruwila, 4 = Karapikkada, 5 = Ullukkulama reservoirs

Mean value of 30 samples for each sampling site

also depends on the soil properties. It is also noteworthy that the highest CRF occurrence was reported in the neighborhood of the Karapikkada reservoir. Bates and Sharp (1983) reported  $K_d$  values for Cd and Pb, at a soil pH range 4.5–9.0, as 1.3–26.8 and 4.5–7,640 respectively. The plant transfer coefficient for Cd and Pb under these conditions were 1–10 and 0.01–0.10, respectively. It is therefore evident that Cd is more mobile and could be taken up by plants or any aquatic flora easily.

#### Heavy metals in *Nelumbo nucifera* and rice

The most common large plants found in all the dry zone reservoirs are the species of *N. nucifera* which is traditionally cultivated in reservoirs over the past 2,000 years of irrigated agriculture for phytoremediation purposes and never for food (Bandara 2007). In the present day, however, the lotus storage rhizome (root) is a popular food item among Sri Lankans.

The type and quantity of heavy metals taken up by lotus storage roots collected from the selected reservoirs are given in Table 4.

*Nelumbo nucifera* stored seven of the heavy metals tested. However, no Cr was detected in *Nelumbo* storage rhizomes. *Nelumbo nucifera* is known to produce Cd-induced phytochelatin (Locus et al. 2006; Baycu 2002) that chelate with cadmium and store in the storage rhizomes. Lotus root is used as a vegetable along with rice by Sri Lankans. An estimate of total intake of Cd on the consumption of 100 g of the lotus storage rhizome is 4.53 mg, which is more than 90-fold increase over the recommended dietary allowance (0.03 mg/day). The plant uptake of dissolved Cd appears to be very considerable, and the effect is visible in rice grown under irrigation with reservoir water, in their grain cadmium level. Table 5 represents the rice grain cadmium level in Anuradhapura,

**Table 4** Mean ( $n = 14$ ) concentrations of heavy metals accumulated in *Nelumbo nucifera* storage rhizomes

Metal	Mean concentration in <i>Nelumbo nucifera</i> rhizome <sup>a</sup>	
	mg/kg	Standard deviation
Cd	252.82	12.49
Co	237.73	19.57
Cr	—	
Cu	47.86	4.04
Fe	7,975.00	522.14
Mn	579.44	128.49
Pb	16.32	3.54
Zn	1461.79	221.62

<sup>a</sup> Storage rhizomes were collected from approximately 120 days old *Nelumbo nucifera* plants

Madawachchiya and Girandurukotte (Fig. 1). The cadmium content in rice grains collected from the farms of CRF patients in Madawachchiya, ranged from 0.001 to 0.093 mg/kg dry weight with a mean value  $0.0444 \text{ mg/kg} \pm 0.0165$ . The Cd content in rice grain in the Anuradhapura–Thuruwila area is 0.001–0.194 mg/kg, with a mean value of  $0.0404 \pm 0.0196$ . All the samples collected were positive for Cd content. We observed that the background value for rice grain Cd in Sri Lanka was found to be 0.001 mg/kg. The maximum permissible level of cadmium in rice grain according to Codex committee (2002) is 0.2 mg/kg. The amounts of Cd in rice grain harvested from fields irrigated with Thuruwila reservoir water are not sufficient to cause acute toxicity. However, in polluted areas of Ishikawa, Nagasaki and Akita prefectures in Japan, the tubular dysfunction was diagnosed due to dietary exposure to cadmium, where mean Cd content in rice is 0.44, 0.53 and 0.58 mg/kg, respectively. In comparison, no CRF was detected in non-polluted areas of the same prefecture where Cd

**Table 5** Estimated weekly Intake of Cd and Pb from contaminated rice samples collected from rice fields irrigated with Karapikkada and Thuruwila reservoir water

Age group (years)	4–5	14	20–29	30–39	40–49
Body weight (kg) <sup>a</sup>	16.2	42.4	56.2	57.8	59.3
Daily rice intake (kg) <sup>b</sup>	0.19	0.38	0.38	0.38	0.38
Karapikkada reservoir irrigation area					
WI-Cd µg/kg BW <sup>c</sup>	7.64	5.84	4.40	4.28	4.17
WI-Pb µg/kg BW <sup>d</sup>	87.76	67.07	50.59	49.19	47.95
Thuruwila reservoir irrigation area					
WI-Cd µg/kg BW <sup>e</sup>	15.93	12.17	9.18	8.93	8.70
WI-Pb µg/kg BW <sup>f</sup>	110.83	84.69	63.89	62.13	60.56

<sup>a</sup> Age group and body weight; Simmons et al. (2005)<sup>b</sup> FAO (2006)<sup>c</sup> Based on rice grain Cd, 0.093 mg/kg<sup>d</sup> Based on rice grain Pb, 1.069 mg/kg<sup>e</sup> Based on rice grain Cd, 0.194 mg/kg<sup>f</sup> Based on rice grain Pb, 1.350 mg/kg

content of rice was <0.3, <0.10 and 0.07 mg Cd/kg, respectively (Shigematsu et al. 1979). Similarly, the mean Cd concentration in rice samples obtained from endemic Jinzu river basin in Japan where itai itai was reported in 1967 was 0.02–1.06 mg/kg (Matsuda et al. 2003; Watanabe et al. 2002), which is far in excess of what is reported in NCP. However, the Provisional Tolerable Weekly Intake of rice under different body masses indicates the degree of chronic intake and its potential impact on health of the farmers. Tables 5 and 6 represent the weekly mean intake of cadmium and lead through the staple rice based on body weight groups.

Although the weekly intake of Cd from irrigated rice grains from Karapikkada reservoir water is below the MCL 7 µg/kg Body weight (JEFCA 2003), the children up to 5 years are subjected to higher levels of Cd that can cause renal dysfunction. However, long term dietary exposure over the years results in accumulation of Cd levels which lead to chronic renal failure. The exposure due to consumption of rice irrigated with Thuruwila reservoir is well above the MCL throughout the different age groups.

Lead is ubiquitous in soil. Levels of 8–20 mg/kg found in non-cultivated soils indicate that it has always been present in man's environment, although not necessarily at such high levels. In cultivated soils, Pb levels up to 360 mg/kg have been reported.

**Table 6** Estimated weekly Intake of Cd from samples of fish collected from Karapikkada and Thuruwila reservoir

Age group (years)	4–5	14	20–29	30–39	40–49
Body weight (kg) <sup>a</sup>	16.2	42.4	56.2	57.8	59.3
Daily intake—Fish (kg) <sup>b</sup>	0.068	0.14	0.14	0.14	0.14
Karapikkada reservoir					
WI-Cd µg/kg BW <sup>c</sup>	3.37	2.56	1.93	1.88	1.83
WI-Cd Rice + Fish <sup>d</sup>	11.10	8.40	6.34	6.16	6.01
Thuruwila reservoir					
WI-Cd µg/kg BW <sup>e</sup>	12.47	9.47	7.15	6.95	6.77
WI-Rice + Fish <sup>d</sup>	28.39	21.64	16.34	15.88	15.48

<sup>a</sup> Age group and body weight; Simmons et al. (2005)<sup>b</sup> FAO (2006)<sup>c</sup> Based on fish Cd, 0.115 mg/kg body weight (BW)<sup>d</sup> µg of Cd/kg BW<sup>e</sup> Based on fish Cd, 0.425 mg/kg

Considering the extreme exposure level of 1.35 mg of Pb/kg of rice (Table 5), the weekly intake 60.55–110.83 µg of Pb/kg body weight is far below the maximum permissible intake of 100 µg/day and the maximum permissible level of 5 mg/kg of rice (WHO 1972).

However, Herath et al. (2005) reported that the renal failure in the Padaviya area, north-east of Madawachchiya in the NCP is probably due to aluminum and fluoride complexes ingested with ground water used in cooking in aluminum utensils. Although chemical evidence for the interaction is available, no clinical evidence is presented by the authors for the cause of renal failure. The experimental sites where reservoirs are located in our study are in the same region with the same level of fluoride content in ground water ranging from 1 to 4 mg/l. The authors based their argument on F content in ground water assuming that the residents depend totally on well water for drinking purposes. Figure 4 shows the distribution of fluoride in ground water in Sri Lanka. The more dental fluorosis cases were reported in the north-western province where the mean F level in ground water ranges from 0.5 to 3.0 ppm. The highest number of renal patients in NCP was first reported during the period January 2001–December 2002 (Athuraliya et al. 2003). It is from the region where the F content in ground water is 1.0–3.00 mg/l. Pharmacokinetic analysis of the net plasma F concentrations showed that the apparent plasma half-life of F



**Fig. 4** Distribution of fluoride in ground water in Sri Lanka.  
Source: Dissanayake (2006)

was  $4.3 \pm 0.6$  h when urine was acid and  $2.4 \pm 0.4$  h when alkaline (Ekstrand et al. 1980).

However, no uniformity was observed in the occurrence of CRF in the regions containing 1–3.0 mg/l F in ground water, and especially in the southern and south eastern coastal belt where the food habits are very different to those in the NCP. The coastal population depends on sea fish rather than freshwater fish for proteins. Dental fluorosis is a common problem in Hambantota area in the Southern Province. The survey on food habits in NCP done in July–August 2006 reveals that farmers in the region use both well (ground) and surface water for drinking and cooking. All surveyed individuals were *Tilapia* fish eaters.

#### Provisional weekly intake of Cd through *Tilapia* consumption

The Cd content in muscles of the freshwater fish *Oreochromis niloticus* collected from two of the reservoirs, Karapikkada and Thuruwila, showed a mean  $0.0575 \pm 0.0216$  Cd content in fish in Karapikkada, with a maximum value  $0.1150$  mg/kg, and a mean value of  $0.2022 \pm 0.0535$  and a maximum value of  $0.4250$  mg/kg at Thuruwila reservoir. The chronic accumulation of cadmium through intake from gastrointestinal tract exceeds (Table 6) the MCL recommended by Codex committee, which is  $7 \mu\text{g}/\text{kg}$  body weight. Therefore, it is evident that the average weekly intake of Cd in residents in a region irrigated with a reservoir carrying heavy levels of Cd in sediments, and in the fish reared in those reservoirs, is higher than the maximum contaminant level of  $7 \mu\text{g}/\text{kg}$  body weight, purely based only on rice and fish intake in the average meal.

Bioaccumulation of heavy metals such as Cd and Pb by fish has been extensively studied (Benoit et al. 1976; Holcombe et al. 1976; Murphy et al. 1978; Kaviraj and Ghosal 1998; Kong et al. 2005; El-Demerdash and Elagamy 1999).

#### Overall Cd body burden

It was also observed that the mean Cd content in the milk samples (composite samples) in the NCP is  $0.10 \text{ mg/l}$  (range  $0.05$ – $0.15 \text{ mg/l}$ ), indicating a much higher potential exposure of consumers in the region. The elevated Cd level in cow's milk is expected with the high levels of Cd in reservoir periphery soils (RPS) ranging from  $0.56$  to  $1.05 \text{ mg/kg}$  of soil, which are the main pasture land for the livestock. The situation that prevails in the NCP appears to meet the requirement of critical threshold level of Cd (Ikeda et al. 2003) that must be accumulated in the kidney before the onset of renal dysfunction.

It was evident that the NCP residents are exposed to high Cd levels from reservoir water (many use it as drinking water), irrigated rice (staple), the freshwater fish (dominant type and apparently in certain areas the only fish) *Oreochromis niloticus*, the only protein source among farmer families, and milk from their own cows reared on Cd-contaminated pasture.

The exposure to cadmium over a long period, probably over 20 years, could be the main cause of

the present increase in trend in CRF in the NCP. The Cd levels in urine samples of patients reported with CRF and residents in the same regions when tested showed that both are exposed to Cd although the actual values are highly variable. The mean UCd values of CRF patients were  $7.58 \mu\text{g Cd/g creatinine} \pm 6.18$  in comparison to  $11.62 \mu\text{g Cd/g creatinine} \pm 8.45$  in asymptomatic individuals. The CRF patients in Girandurukotte (Pollonnaruwa under Mahaweli irrigation scheme in NCP) had a mean urine Cd level of  $5.65 \mu\text{g Cd/g creatinine} \pm 5.46$  which was significantly different from urine Cd levels in asymptomatic residents (with  $13.92 \mu\text{g Cd/g creatinine} \pm 11.40$ ) where the subjects were in the age group 40–60 years. Similar exposure to Cd was seen in CRF patients in Madawachchiya, with urinary cadmium  $10.57 \mu\text{g Cd/g creatinine} \pm 0.85$  and asymptomatic residents with  $9.52 \mu\text{g Cd/g creatinine} \pm 3.84$  in the neighborhood of Karapikkada reservoir. The amount of Cd excreted by the CRF patients were significantly different to that of asymptomatic residents in the same area. The UCd levels in both CRF patients and asymptomatic individuals were higher than  $5.00 \mu\text{g Cd/g creatinine}$ . WHO standards stipulate that a urinary excretion of  $2 \mu\text{g Cd/g creatinine}$  is normal while  $10 \mu\text{g Cd/g creatinine}$  is indicating an irreversible situation of chronic exposure and potential renal dysfunction (WHO 2000). In our study, the subjects tested had UCd levels above  $5 \mu\text{g Cd/g creatinine}$ . Mueller et al. (1998) also reported similar observations where elevated levels of low molecular weight proteinuria occur in cases where the UCd level exceeds  $10 \mu\text{g Cd/g creatinine}$  and the situation is irreversible. Although the variation among the patients and asymptomatic individuals are greater, the amounts excreted are significantly different among patients and asymptomatic individuals. However, the UCd levels in Madawachchiya reflect an excessive and chronic exposure to cadmium. It is noteworthy that the UCd values increases at the early period Cd accumulation and begins to decline at later stages of renal failure. The mean urinary cadmium concentration bound to a metallothioneine healthy population is considered to be less than  $0.5\text{--}2.0 \mu\text{g/l}$ . Both asymptomatic and CRF patients had relatively high levels indicating a chronic exposure. Similarly, Jin et al. (2002) reported that, in the highly exposed areas, most of the blood and urine Cd values were higher than  $5 \mu\text{g/l}$ . Watanabe et al. (2002) stressed that it is reasonable to accept

that the allowable level of Cd concentration in rice should be lowered as age progresses. Therefore, the decreasing exposure as PTWI (Table 6) does not reduce the body burden effectively. This is further confirmed by the continuing dietary exposure to cadmium evident by the high level of urine Cd observed among the residents, although the daily excretion per day is approximately 0.001% (Satarug and Moore 2004).

However, the asymptomatic residents excreted significantly higher levels of cadmium with urine bound to chelating protein metallothioneine. The possible inability to express metallothioneine protein synthesis probably makes them more prone to tubular dysfunction. Athuraliya et al. (2003), working in NCP, reported that the CRF patients did show tubular interstitial type renal disease, which is an indication of involvement of Cd. Renal biopsy reports of CRF patients at Madawachchiya in 2007 revealed that endocytosis and proximal tubular sclerosis was predominant and no glomerular renal dysfunction was observed, suggesting a possible involvement of chronic exposure to Cd.

The soils in the periphery of reservoirs are usually submerged during the rainy season (September–December) from the surface runoff from rice and other farm fields. Compared to the background soil cadmium levels of  $0.39\text{--}0.51 \text{ mg/kg}$  in uncultivated soil (Premarathna et al. 2005), the high levels of Cd in reservoir sediment soils and periphery soils shows that general pollution from agrochemicals could be the main reason for accumulation of Cd in them. In 1974, the Sri Lankan agriculture sector utilized 15,300 metric tons of  $\text{P}_2\text{O}_5$ -phosphate compared to 36,200 metric tons in 1984. The total amount of  $\text{P}_2\text{O}_5$  used to date since 1973 is 957,200 metric tons (Fertilizer secretariat 2006). We observed that the TSP used by the Sri Lankan farmers carried  $71.739 \text{ mg Cd/kg}$  of  $\text{P}_2\text{O}_5$ . At this contamination level at least 68.9 metric tons of Cd has been added to agricultural land since 1973.

With the heavy rainfall of 2,500 mm spread over a period of 9 months, the surface runoff from the wet zone upcountry where the soil is acidic (below  $\text{pH} = 6.5$ ) increases the mobility of Cd. Cadmium that leaches into waterways eventually ends up in the large reservoirs in the NCP through the linkage provided by the Mahaweli River Diversion Scheme in 1983. Loganathan and Hedley (1997) reported that

93% of the Cd applied in phosphate fertilizer remained within the 0–120 mm soil depth. Approximately 90% of the applied Cd would be removed from the soil by leaching. Dissanayake and Weerasooriya (1986) reported the presence of 1 ppb Cd in suspension in Mahaweli river waters. The sedimentation of NCP reservoirs are considered to be very high as reported by Tennakoon, based on a case study of Kallanchiya reservoir in NCP (Tennakoon 2006). He is of the view that most reservoirs that have a depth of water column of 6 m today would have only 1.5 m in 2008, with a sedimentation rate of 3.6 m per 50 years. In Sri Lanka, especially the reservoirs in the NCP have not been de-silted in an organized manner since 1983. Tables 1–3 show the possible release of Cd sorbed in organic material in sediments to water as dissolved Cd which would be available for plant uptake when irrigated.

Furthermore, regular pollution of the NCP and reservoirs in the Mahaweli system with phosphate and Nitrate fertilizer (Piyasiri 1995; Liyanage et al. 2000; de Zoysa 2002) has been reported earlier. In addition to Cd pollution with phosphate fertilizer, the most commonly used weedicide in rice cultivation in Sri Lanka, a formulation based on bispyribac sodium, was also found to contain 0.5 mg Cd/l  $\pm 0.1$ . By and large, all agrochemicals used in rice farming, with regular cultivation over a period of 30 years, would have contributed with time to the accumulation of the large quantities of cadmium that eventually settle in reservoir sediments (1.78–2.45 mg Cd/kg).

The dry zone environment of the NCP remains comparatively dry, averaging 1,500 mm rainfall mostly in the October–January period, and remains dry for the rest of the year, compared to the wet zone which receives 2,500 mm of rainfall throughout the year (Natural Resources of Sri Lanka 1991). The prevalent low rainfall and subsequent intermittent wetting and drying of sediments at the top boundary surface of water in reservoirs, expose the sediments to air. This weather pattern will shift sediments from the reduced to oxidized conditions altering the mobility of cadmium ions. In addition, the drainage pattern practiced in rice farming would facilitate uptake of Cd by the rice plant.

Cadmium absorption through the human gastrointestinal tract tends to increase when the subject is undernourished especially in proteins and iron. Rathnayake and Weerahewa (2005) reported that pre-

school children (1–4 years) in the NCP are undernourished and underweight with a Z score of  $-0.208$ . The overall pollution (especially with Cd) of water, freshwater fish (the only protein-rich food source cheaply available to NCP farm families), as well as their staple rice and other food items such as milk and vegetables would be the main reason for the present CRF crisis in the NCP. The youngest CRF patient reported so far is 9 years old (Athuraliya et al. 2003). They also reported that, in the Madawachchiya region, Persistent Proteinuric patients among habitual alcohol consumers and tobacco smokers were 10.1% (62/613) compared to the 2.5% (13/529) among non-consumers of alcohol and nonsmokers. It appears that use of alcohol and tobacco favors CRF occurrence, which is probably due to the association of Cd intake through tobacco use. However, they also reported that among the total adult with Persistent Proteinuria, 75 out of 127 (59.05%) were males and 52 out of 127 (40.94%) were females. We learned during the food habit survey that, among the farmer families, the traditional “priority serving of food” is practiced. As such, females in the house (mother and the daughters) are usually served only with the gravy of fish curry while fish portions are served very rarely, i.e. if there is any left over after males are served. Females tend to consume a lesser quantity of rice. This possible higher PTWI would routinely contribute to the relative higher frequency of occurrence of CRF among males. However, this priority serving is not visible with the increase in economic status of families. Therefore, it demands further investigation into the apparent susceptibility of male farmers to CRF. The prevalent high fluoride content in ground water that is used as drinking water by some farmers would also appear to facilitate Cd absorption through the gastrointestinal tract as fluoride (JEFCA 2004) compounds of Cd, leading to the present outbreak of CRF in the NCP. The existing situation demands more intensive protective measures in view of the observations made by Satarug et al. (2000): “If Cd pollution continuous to increase, so will human dietary Cd exposure, and renal tubular dysfunction is likely to become more prevalent in human populations in the next 10–20 years, particularly in high-risk groups such as those with diabetics and those with poor vitamin C status, low body Fe stores.”

Cd-induced CRF is a difficult problem that may not have an overnight solution. However, an immediate

preventive measure of regulating Cd addition to the environment and removing Cd polluted sediments in water reservoirs by resumption of regular de-silting programs would eliminate entry of elevated levels of Cd into the food chain.

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