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Chronic Kidney Disease of Unknown aetiology and ground-water ionicity; study based on Sri Lanka.

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Abstract High incidence of Chronic Kidney Disease of Unknown Aetiology (CKDU) in Sri Lanka is shown to correlate with the presence of irrigation works and rivers that bring-in 'nonpoint source' fertilizer runoff from intensely agricultural regions. We review previous attempts to link CKDU with As, Cd and other standard toxins. Those studies (e.g., the WHO-sponsored study), while providing a wealth of data, are inconclusive in regard to aetiology. Here we present new proposals based on increased ionicity of drinking water due to fertilizer runoff into the river system, redox processes in the soil, and features of 'tank'-cascades and aquifers. The consequent chronic exposure to high-ionicity in drinking water is proposed to debilitate the kidney via a Hofmeister-type (i.e., protein denaturing) mechanism.

Keywords Kidney disease · water quality · electrolytes · protein denaturing · fertilizers · soils · aquifers · Sri Lanka

1 Introduction

Kidney disease occurring when recognized causes (e.g., diabetes, hypertension, etc.) are absent is called 'Chronic Kidney Disease of Unknown aetiology' (CKDU). Urine-albumin excretion above standard thresholds is usually the early clinical sign of CKDU. Increasing incidence of CKDU among farmers in Sri Lanka (Ramachandran, 1994, WHO-SL-reports, 2013, Jayatilake et al, 2013), El Salvador (Gracia-Tabanino et al, 2005), China (Lin et al, 2014), South-Asia (Jessani et al, 2014) and other parts of the world have come under much scrutiny. In fact, CKDU has been a concern in many countries since the mid-twentieth century (Yusuke et al, 2010). CKDU is a serious concern in Canada (Arora et al, 2013), and also in the United States (Coresh et al, 2007). CKDU has many similarities to Balkan endemic nephropathy (Tanchev and Dorossiev, 1991). We deal mainly with CKDU in Sri Lanka while the proposed ideas may find applications elsewhere.

The North central province (NCP) of Sri Lanka was home to the ancient Anuradhapura civilization ($\sim$ 5 century BCE to 10 century CE). It is a part of the so-called "dry zone" of Sri Lanka, with a rainfall $\sim$ 1750 to 1000 mm. per annum (Agriculture Dept., 2013) and largely overlaps with the region affected by CKDU (See a national map in Fig. 1). CKDU may arise from (i) pollutants and toxins ingested from food, (ii) direct ingestion of toxins due to careless handling of agro-chemicals, (iii) prolonged exposure to toxins and pollutants from drinking water. We consider the third item, viz., drinking water, to be the main factor given that: (a) farmers in the NCP who consume spring water, but have life styles and agro-chemical usage similar to other NCP farmers have not contracted CKDU; (b) the rise of irrigation projects providing (unwitting) conduits to the excess fertilizer runoff, and (c) the co-incidence of the free-market sale of fertilizers with the rise of CKDU.

Ancient tanks (reservoirs, called "weva" in Sinhalese, the local language) were constructed in cascades along

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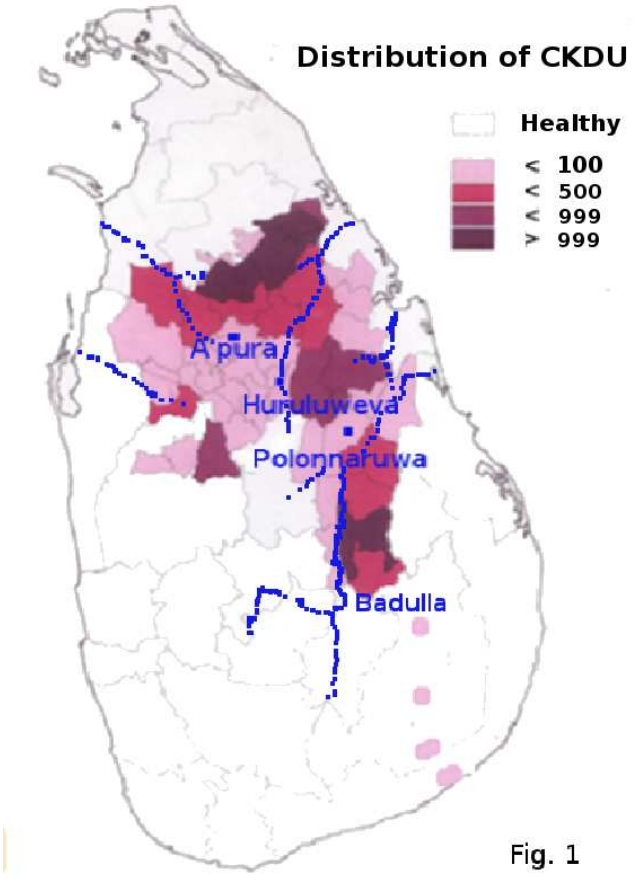

Fig. 1 The national distribution of CKDU (patients/district) in relation to river basins.

river tributaries, and village drinking wells depended on the water table of the tanks that feed the regolith aquifer system of the region (Panabokke, 2007). However, after the eradication of malaria in the NCP, rapidly growing populations spread into rural areas. Their drinking water is obtained from recent irrigation projects, from shallow or deep-bored wells. Today, over 15% of the NCP residents are stricken with CKDU. We review existing studies on CKDU, including the world-health-organization (WHO) study (WHO-SL-reports, 2013, Jayatilake et al, 2013). This establishes the background for our new proposals for the origin of CKDU made in this study. We propose that prolonged consumption of drinking water with high ionicity affects the kidney membrane adversely, e.g., by Hofmeister and other electrolytic mechanisms. The nature of the NCP drinking water, phosphates in reservoirs due to fertilizer runoff, regolith-fed drinking-water wells, the tank cascades and river basins etc., are discussed.

2 Commonly suggested explanations of CKDU

Early studies of chronic kidney disease looked for known nephrotoxins using limited chemical analysis of fertilizers, water, soil, food stuffs and bio-samples of patients. The accuracy of the available analytical equipment was inadequate (Mubarak, 2011), since WHO's maximum-allowed limits (MALs) for As, Cd, Pb etc., are in parts per billion. Some of these studies emphasized the hardness of NCP water as enhancing the effect of nephrotoxins although hardwater is known to act protectively in precipitating out common toxins (Gray, 2007). Genetic risk factors have also been proposed (Nanayakkara et al, 2014) as chemical analytical data for 18 metals show no enhanced levels of nephrotoxins.

2.1 **Fluoride**

Fluoride, (or fluoride conjointly with aluminum from cooking utensils) has been proposed as a cause of CKDU (Illeperuma et al, 2009, Illeperuma, 2011, Kulatunga and Illeperuma, 2013). However, other areas in Sri Lanka with high fluoride levels in the drinking water (e.g., Ampara, Huruluweva, Monaragala and Wellawaya) where similar Al-utensils are used, show no significant CKDU. Meda-wachchiya and Pulmoddai have similar amounts of Al and F (Wasana et al, 2012), but Pulmoddai has no CKDU unlike Meda-wachchiya. Hence, even if CKDU incidence overlaps the presence of fluoride, the presence of fluoride does not imply CKDU.

Studies of water samples from the Rift valley in Ethiopia (Clemens et al, 2003) found that 33% of the samples had high fluoride content. However, no associated CKDU was noted. The WHO study (Jayatilake et al, 2013) in Sri Lanka reports normal levels of serum aluminum in subjects with CKDU, suggesting no correlation with Al. The reported aluminum-leaching experiment (Illeperuma et al, 2009) used control samples containing Na^+ and F^- ions only, but not water typical of the NCP, and hence is inconclusive. Calcium, common in NCP water (17-115 mg of Ca/l), would form insoluble CaF_2 (solubility, ∼2 ppm), inhibiting fluoride uptake. In our view, F^- may contribute to CKDU by its high rank in the Hofmeister series for denaturing proteins (of the kidney membrane; see section 4).

2.2 **Cadmium.**

According to WHO guides for drinking water (Thompson et al, 2007), "Concentrations of cadmium in water are only likely to be of health concern in environments

where pH is less than 4.5", thus ruling out the Cd hypothesis, since NCP drinking water is not acidic. Nevertheless, Cadmium has been proposed as a cause of CKDU (Bandara et al, 2008, Wanigasuriya et al, 2011, Jayatilake et al, 2013). Bandara *et al.* claimed elevated levels of Cd in rice and fish. They reported Cd, Fe and Pb in five reservoirs in the high-CKDU areas. However, these results have been disputed (Chandrajith et al, 2011, Jayatilake et al, 2013, Subasinghe et al, 2012). Similarly, in a multi-variate study (Paranagama et al, 2012) from the RMIT University (Melbourne), Cd was found below the WHO-MAL of 0.005 mg/l, suggesting no role for Cd in causing CKDU in Sri Lanka.

2.3 **Arsenic.**

Kidney disease in Bangladesh is caused directly by arsenic poisoning of tube-wells (BGS, 2001). Several Kelaniya - University researchers (Jayasumana et al, 2011, 2013) proposed that arsenic (claimed to be present in fertilizers) acted in consort with hard water to cause CKDU. The main supporting evidence was biopsy data of CKDU patients showing elevated arsenic levels. However, the water analysis of the WHO study, amd independent work (Chandrajith et al, 2011, Nanayakkara et al, 2014) do not find elevated As in soil or water. Hence the biopsy data merely prove that traces of As (as found in most environments) are not flushed out but bio-accumulated by CKDU patients (since they have poor kidney function).

The claim (Jayasumana et al, 2013) that 'no reported work is available to indicate the presence of arsenic in the bedrocks of Sri Lanka', is untenable since Sri Lanka's phosphate deposits (e.g., at Eppawela) contain arsenic (e.g., 23-27 ppm of arsenic, i.e., μg/g of ore, in Eppawela (Gunawardane, 1987)). Water hardness, and traces of As are naturally present in most areas in Sri Lanka. Many agricultural hard-water areas are free of CKDU. Furthermore, according to Gray, "It is also a general rule that the toxicity of pollutants and contaminants is significantly less in hard water than in soft water" (Gray, 2007, p. 207). Hence, contrary to the claims of the Kelaniya researchers, hardness in water (<170 mg/l) is a desirable property.

2.4 **Glyphosate.**

Recently, Jayasumana *et al.* (see ITFG, 2014) proposed that glyphosate, N-(phosphonomethy)-glycine, a common herbicide ("Roundup") valued for its relative non-toxicity to animals, causes CKDU. The acute oral LD50 in the rat is 5.6 g/kg (TOXINET, 1992). Surprisingly, Jayasumana *et al.* invoke hard water to render glyphosate nephrotoxic, while also admitting its conversion to a solid complex of Mg, Ca, As and other ions. The solid forms, known for decades (Smith and Raymond, 1988)), are even less toxic, highly insoluble and hardly absorbed by organisms (Vereecken, 2005, Thelen and Jackson, 1995). Thus the glyphosate aetiology of CKDU should anti-correlate with water hardness, although Jaysasumana *et al.* claim the opposite. The ethoxylated adjuvants included in glyphosate formulations are sometimes more toxic than glyphosate itself; but this has not been the main focus of these claims.

US regulations warn that prolonged consumption of water containing glyphosate over the MAL i.e., > 700 ppb, may impair kidney or reproductive health (US-epa, 2013). The flourishing aquatic weeds in the NCP of Sri Lanka show that no significant glyphosate is present in that water. Similarly, urine analysis of patients in the NCP have not shown the presence of glyphosate (see Jayatilake et al, 2013) or metals claimed needed to from glyphosate complexes (Nanayakkara et al, 2014). Glyphosate is destroyed by the stomach's acidity (pH $\sim$ 1-2), and by gut micro-organisms (Vereecken, 2005).

The time evolution of glyphosate residues in farmers has been studied extensively. In a well-known study (Acquavella et al, 2004), urine samples from farmers in South Carolina and Minnesota had a mean glyphosate level of 3 ppb, with higher values in farmers who used no gloves, at a maximum of 233 ppb. The US-MAL (700 ppb) in drinking water, compared with the above measured residues implies that ingestion is inconsequential. No definitive evidence for CKDU arising from glyphosate has so far been presented, even in using larger dosages.

3 The WHO Study.

In 2010 the WHO sponsored an important study on CKDU in Sri Lanka (WHO-SL-reports, 2013, Jayatilake et al, 2013). Their report presented (a) a standard definition of CKDU; (b) bench-mark analytical studies of bio-samples, water, soil, and selected food stuffs, with parts per billion accuracy; (c) the population prevalence of the disease; (d) no conclusions about aetiology.

3.1 **Definitive conclusions of the WHO report.**

1. There are elevated levels of cadmium in urine, arsenic in the hair of CKDU patients, but "no significant difference in urine-arsenic and lead ... in CKDu cases compared to controls', ..96.5 % had glyphosate levels below MALs." (*n.b.*, the report uses the acronym

'CKDu' where as we use CKDU).

2. The WHO study (Jayatilake et al, 2013) using 234 water samples concluded that "Levels of cadmium, lead and uranium in sources of drinking water (see Figure 4 of the report) used by individuals with CKDu (n = 99) were within normal limits.... samples from wells, ... irrigation canals, ... , had normal arsenic, cadmium and lead levels".

Pollutant-testing is critically dependent on sample-handling (SH) methods. Poor SH leads to sample contamination and data scatter. The low toxin levels and the reported low data scatter favour confidence in the WHO-results. However, since the quality of water is highly seasonal (e.g., see Table 1), the relevant sample-collection history should have been presented.

The WHO study did not report on Cl^-, NO_3^-, CO_3^{2-}, SO_4^{2-}, PO_4^{3-}, K^+, Ca^{2+}, NH_4^+ and other commonly occurring ions, as these are usually not regarded as nephrotoxic.

Although low birth-weight, nephron number and kidney disease have been correlated (Luyckx and Brenner, 2005), this has not been considered in the WHO and any other studies, even though malnutrition is common in the NCP. This is a possible aetiology for CKDU and could be mistaken as a genetic correlation.

Furthermore, the validity of the widely used publicly available data (e.g., Fig. 1) and other alternative data on the incidence of CKDU has not been critically examined against hospital records in the WHO-study.

4 Nephrotoxicity of strong electrolytes.

The kidney needs 'good' drinking water (Thompson et al, 2007, CEA-SL, 2013, US-epa, 2013). We consider the prolonged exposure of the kidney to many ionic species, all below MALs, but adding to a high ionicity. Such exposure is termed 'chronic exposure'. We suggest that chronic exposure leads to CKDU, noting recent claims that CKDU in Sri Lanka is associated with tubulointerstitial damage (Nanayakkara et al, 2014).

Ions have the capacity to denature proteins in cell membranes. The well-known Hofmeister (Baldwin, 1996) "salting-out" occurs at higher concentrations, but the effect occurs at the molecular level, and accumulates statistically over time, even at low concentrations.

The filtration membrane of the nephron is made up of the endothelium, the 'basement membrane', and the outer epithelium. Ions and molecules being filtered out of the blood pass through fenestrae in the endothelium, and across the basement membrane (a network of proteins). Negatively charged proteoglycans in the pore regions control filtrant movement through electrostatic action. The podocytes on the outer epithelium actively intervene by synthesizing and manipulating proteins that arch filtration slits, using up adenosine triphosphate (ATP). The filtration concentrates filtrants, working against a gradient and an osmotic pressure. Part of this pressure is provided by the blood, and fine-tuned by *in situ* degrading of ATP. Hence these processes are affected by ions in the medium in accordance with their Hofmeister activity as well as electrolytic activity. Furthermore, the epithelial- to- mesenchymal transition that generates fibrosis is known to involve electrolyte-sensitive phosphorylation steps.

When the filtration membrane is impaired by high load and mechanisms causing protein denaturing, the pore size in the membrane is not maintained, and protein molecules pass into the urine. Toxins are not filtered out and accumulate in the body. High osmolarity (high pressure) in the glomerular capillaries progressively damages the filtration membrane. Negatively charged proteoglycans may also be affected by higher concentrations of cations. Interestingly, K^+ and PO_4^{3-} are the main ions found in the intracellular fluid and in water with fertilizer runoff.

Among the effects of increased ionicity are (i) the depletion of water molecules near the kidney membrane, (ii) changes in water activity and ion activity, (iii) osmotic activity, (iv) hydrophobic interactions. Hofmeister ranked the ions by their capacity to denature proteins. The ranking is protein specific, but a 'typical' Hofmeister series is:
Cations: $NH_4^+ > K^+ > Na^+ > Mg^{2+} > Ca^{2+}$; Anions: $F^- > H_2PO_4^- \geq SO_4^{2-} > HCO_3^- > Cl^- > NO_3^- >$.
The ionic content of the water, and enzyme systems control the folding and unfolding of proteins. Solutes contributed by fertilizer runoff (e.g, K^+, HPO_4^{2-}, NH_4^+ ion and even urea) have a high rank in the Hofmeister series. Similarly, among anions, fluorides, phosphates and sulphates are the most active in protein denaturing, while nitrates are least effective. Hence the chronic exposure of the kidney membrane to Hofmeister-active ions may be very relevant to the onset of CKDU. The electrical conductivity (EC) correlates with the total dissolved ionic content and the protein denaturing capacity of electrolytes, but EC itself does not adequately weight the ions in conformity with Hofmeister activity.

While ionic mixtures have not been studied, specific solutes acting individually on the kidney, e.g., high NaCl, high phosphate or acid-base imbalance, serum Cl^-, etc., have been studied (Zhang et al, 2013). Similarly, the electrolyte and acid-base disorders in CKDU are well documented (Alcźar Arroyo, Nefrologia). High phosphate levels cause hyper-phosphatemia (Thatte et al, 1995). The needed phosphate levels are rarely found in

water bodies. On the other hand, another phosphate-centric paradigm has been proposed very recently (Kuroo, 2013), but will not be examined here.

The role of phosphates in causing algal bloom, deteriorating water quality etc., is recognized. The guidelines (US-epa, 2013, Thompson et al, 2007) on drinking water mention nitrates (and nitrites) from fertilizer use and specify their MALs. Phosphates, even used in water purification (to remove heavy metals), are usually considered harmless, with an MAL of 2mg/l, i.e, within the same magnitude as the MAL for glyphosate (0.7mg/l). But unlike glyphosate which is not present in the NCP water, PO_4^{3-} is abundant (Table 1).

The health effects of soft- and hard water (Ca, Mg, as bi/carbonates, and sulphate) are well known (Gray, 2007). Stroke, ischaemic heart disease, and gastric cancer are inversely correlated with hardness (i.e., up to moderate levels of hardness $\tilde{H} < 170$ mg/l). An $\tilde{H} \sim$ 100-150 mg/l is recommended for drinking water. Gray remarks (Gray, 2007, sec. 10.5) that "both Ca and Mg are common in ... foods, with only 5-20% of the daily intake ... coming from water. So why these relationships exist remains unclear". In our view, Ca, Mg, and bicarbonate are at the lower end of the Hofmeister series, while the "fertilizer ions" K^+, phosphate, NH_4^+ are at the high end. This suggests that while the increase of hardness (below a threshold) correlates with good health, the increasing fertilizer ionicity (above a threshold) would correlate with increased ill health.

5 Ionicity and fertilizer-runoff into the NCP.

The notable CKDU areas, viz., Anuradhapura, Polonnaruwa, and Badulla lie along the Mahaweli river (Fig. 1), or near other irrigation works (e.g., Padaviya area). The 'accelerated Mahaweli project' linked the Mahaweli ganga ('ganga' = river), the Elahara canal, Sudu ganga, Jaya ganga and 'tanks' in the Anuradhapura area. Thus the extended Mahaweli basin covers most of the CKDU-stricken areas. Most people in these "endemic" areas use water from four sources (i) some 3000 medium and large tanks, over 15,000 small tanks, rivers, irrigation streams etc. (ii) Ground wells dependent on the shallow regolith aquifers supported by the tank cascades (Panabokke, 2007). (iii) Deep tube wells etc., that may reach the deeper 'fracture zone' aquifers. (iv) Water-bearing quartzite strata that give rise to natural springs (Panabokke, 2007, Ch. 11)). The Kebithigollewa springs provide perennial spring water to a rural population free of CKDU, but face health risks from soft water.

Although there are many studies of the water in the NCP (Nilusha, 2012, Paranagama et al, 2012, Yatigammana, 2012), a comprehensive study similar to that for the Rift valley (Clemens et al, 2003) is not available. In a multi-variate study (Paranagama et al, 2012) by RMIT-University scientists in Melbourne, an eigenvector analysis of the correlation matrices was conducted. We regard this as a significant study using good methodology. They showed that Na, Cl, Mg, F and Ca fall into one factor group showing strong inter-dependency. The soluble N and P fall together, while Cd is singled out at concentrations < 0.005 mg/l (the WHO MAL). The first set is clearly from salts causing hardness. Nitrogen and phosphorus are not correlated in the non-stricken areas, while they are strongly correlated in the CKDU-ares. When N and P are strongly correlated, this is most easily understood in a common origin in typical N, P, K fertilizer runoffs that are "nonpoint source" pollutants. This supports our hypothesis that CKDU in the NCP is linked with fertilizer runoff into the river systems from agricultural activity in the central hills.

Five CKDU-villages, *viz.*, Medawachchiya, Padaviya, Girandurukotte, Medirigirya and Nikaweva were identified *circa* 1990, 1992, 2000, 2004, 2007 respectively (Jayasekara et al, 2013). Thus the environmental cause of CKDU is gradually spreading, and retained once established. Jayasekera *et al.* have reported detailed incidence maps for these areas. These maps (Jayasekara et al, 2013) report CKDU data on a more microscopic basis than Fig.1 for each locality (e.g., Fig. 2 for the Girandurukotte - Pollonnauwa region). They illustrate the proximity of these CKDU areas to the Mahaweli river, and support our hypothesis that fertilizer-runoff ionicity is linked to kidney disease.

Table 1 presents data for the active dissolved Phosphorous in the main water reservoirs ('tanks') of the NCP, for 2012, as reported by the National Water Supply and Drainage Board of Sri Lanka (NWSDB-SL). The boldface entries indicate hypereutrophic lakes (using the 2002 classification, Joint Research Centre, European Union). Such high levels of phosphate correlate with the dry period of the NCP. The phosphate-rich water seeps to the shallow aquifers which sustain the drinking-water wells. This water has a high ionicity from fertilizer runoff. When compared to municipal water, 'chronic exposure' to this water is proposed to carry an increased capacity for denaturing proteins. According to the US-EPA, 'Chronic exposure' is when exposure occurs over many years at concentrations that display no outward effects, and hence falling below the usual MALs.

Although the seasonal behaviour of regolith aquifers in a CKDU area is unavailable, the study of aquifers in the Malal Oya Basin (Panabokke, 2007) can be used. The total hardness (mg/l of $CaCO_3$) exceeded 140 mg/l in 23 of the 24 wells studied (range: 94 mg/l to 2304

Table 1 Dissolved reactive phosphorous concentration (mg/l.) in some reservoirs during the dry season in the NCP in 2012, Sri Lanka. The high concentrations are due to fertilizer runoff into the rivers during February to August from rain in the central hills (P-concentrations return to near normal by November during the rainy season of the dry zone (Nilusha, 2012, Paranagama et al, 2012, Yatigammana, 2012)). **Boldface** indicates hyper-eutrophic conditions. Source: NWSDB-SL.

Reservoir (waeva)	Feb	Mar	Apr	May	Jun	Jul	Aug
Thuruwila w.	**0.10**	**0.15**	**0.11**	**0.21**	**0.18**	**0.19**	0.09
Tissa w.	**0.13**	**0.11**	**0.19**	**0.13**	**0.07**	0.07	0.07
Nuwara w.	0.02	**0.15**	**0.12**	**0.12**	**0.11**	**0.10**	**0.11**
Nallachchiya w.	0.09	0.08	**0.18**	**0.18**	**0.28**	**0.11**	**0.10**
Galnewa w.	**0.13**	**0.11**	**0.18**	**0.21**	**0.36**	**0.17**	**0.16**
Eppawela w.	0.01	0.07	**0.19**	**0.20**	**0.33**	**0.20**	**0.18**
Kala w.	**0.11**	**0.14**	**0.14**	**0.15**	**0.15**	0.05	0.15
Mahakandarawa w.	**0.12**	**0.14**	0.03	**0.17**	**0.19**	**0.17**	**0.20**
Habarana w.	**0.16**	0.03	0.08	0.07	**0.18**	**0.11**	0.06
Kiriwaduna w.	0.03	0.08	**0.14**	0.06	**0.20**	**0.14**	**0.16**
Padaviya w.	0.06	0.09	**0.14**	0.07	**0.14**	**0.13**	0.03
Jayanthi w.	**0.14**	0.09	**0.15**	**0.15**	**0.15**	**0.13**	0.08
Wahalkada w.	**0.11**	**0.13**	0.06	0.08	0.07	**0.10**	0.08
Kebitigollawa w.	0.07	**0.10**	0.08	0.07	0.06	0.05	0.05
Parakrama Samud.	**0.20**	0.06	**0.13**	0.08	**0.14**	**0.13**	**0.23**
Minneriya w.	**0.43**	**0.16**	0.05	**0.28**	0.06	0.06	0.09

mg/l). The average electrical conductivity was over 400 μS/cm for 22 out of 24 wells (range: 276 to 12,386 μS/cm). These results confirm the high ionicity of these water sources, but their Hosmeister activity needs to be determined.

The hydrology of the soil may lead to increased ionicity. Panabokke studied a typical land unit in the NCP, and described five soil profiles (A-E). "Fluctuations in the water table occur most frequently in the locations of profiles C, D, and E. The alternating reducing and oxidizing conditions that arise as a result ... promote the solution of the iron and manganese which would get partly leached out in the receding water table, and partly precipitated in the form of concretions" (Panabokke, 1959). This type of redox fluctuations are important to many other non-equilibrium ionic processes, e.g., conversion of carbonate to bicarbonate and reaction with ambient CO_2, the balance between, e.g., HPO_4^{-2}, $H_2PO_4^-$ and the pH of the soil, NO_3^-, NO_2^- and ambient oxygen in interactions with fertilizer runoff and other environmental aspects of ionic balance. This redox mechanism suggests that soil hydrology may create ionicity conditions needed for CKDU, even without fertilizer runoff inputs.

The pollution of lakes ("weva" or tanks) due to fertilizer runoff is demonstrated *via* their biological response. Algae inhabiting tanks in the early 20-th century produced no toxins (Kulasooriya, 2011), but the ecological stresses of phosphates (where farmers use even 5-10 times the optimal amounts of phosphate), increasing ionicity etc., have generated toxic cynobacteria in all NCP tanks (Perera et al, 2013). The degraded water quality further overloads the kidneys of NCP residents. While such studies of the effect of excess phosphate on microorganisms are available for aquatic bodies, little is known about the impact of fertilizer runoff on the soil microorganisms and their evolution under such stress.

An agricultural region with risk of high ionicity and CKDU is the Jaffna peninsula, already reporting some CKDU cases (Gunatilleke, 2013). However, according to Joshua *et al.* (Joshua et al, 2013), the trend is to convert irrigated farms into residential areas, reducing the use of water and fertilizer usage for agriculture.

5.1 **Safe-water ionicity.**

Water hardness $\tilde{H}$ is used to indicate the Ca and Mg content with $\tilde{H} = 2.5[Ca^{2+}(ppm)] + 4.1[Mg^{2+}(ppm)]$. Ionicity is also related to the electrical conductivity (EC). Activity coefficients and other electrolytic properties like the conductivity can be related to the ionic strength $I = (1/2)\sum_i c_i z_i^2$, where z_i is the charge of the ionic species i with concentration c_i. However, a more detailed picture is given by the ionicity vector $\mathbf{c}$ made up of all the relevant c_i. In the following we use the notation c_i for the concentration of the i-th substance, while more specific symbols (e.g., b_i for their values in blood) will be used for specific vectors. Thus the MALs in water of the ion i will be denoted by d_i rather than c_i. We have also constructed a CKDU-safe-water ionicity (SWI), D_i, by studying the (CKDU-free) Anuradhapura municipal water and the Kebithigollewa spring-water values (using available or estimated data).

Table 2 The ionicity vectors **b** for blood, the drinking-water MAL vector **d**, safe-water ionicity **D**, and 'typical' ionic compositions **c*** in a CKDU area. See sec. 5.1 for discussion.

item i	Ca	Cl^-	HCO_3	K mg/l	Mg ± 8%	Na	NO_3	PO_4	SO_4	pH	EC (S/m)
b_i	50	3600	1586	156	15	3243	1.9	32	24	7.4	0.65
d_i	200	250	600	20	150	200	10	0.5	250	7.5	0.8
D_i	30	32	20	2	20	25	5	0.03	55	7	na
c_i^*	100	110	50	8	22	3.5	8.7	0.3	101	7.6	1.1

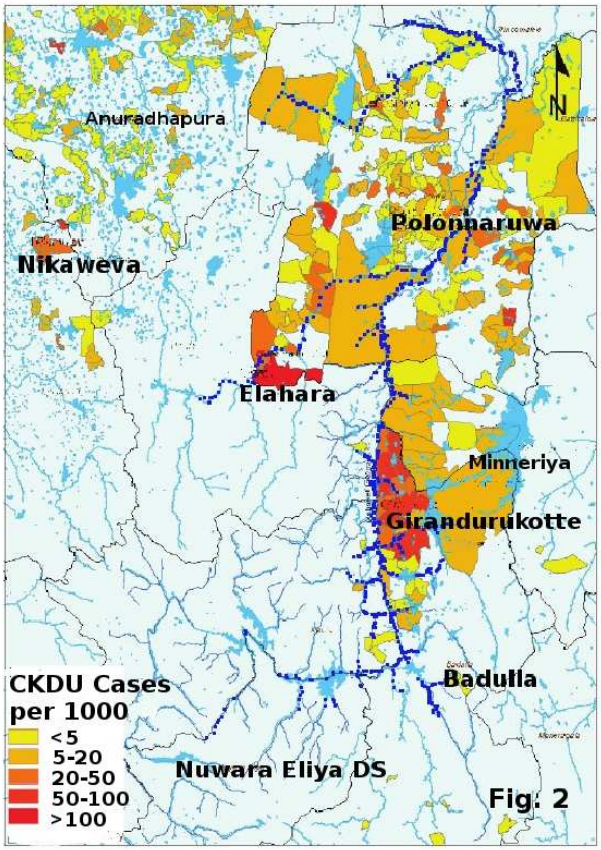

Fig. 2 A detailed map of a CKDU area by the Mahaweli river, with the number of CKDU patients per 1000 inhabitants indicated. (*n.b.*, Fig. 1 uses patients per district). This map has been adapted from published work (Jayasekara et al, 2013) where maps for additional areas are given.

That is, the known composition of two CKDU free areas in the NCP, namely, Anuradhapura-city where municipal water is used, and Kebithigollewa (natural spring water) provide a range of values for c_i, within which we deem to have a mean safe ionicity denoted by D_i. We take the phosphate level in uncontaminated lakes ($\leq$0.03 mg/l) as the SWI value (i.e, $D_{\rm PO_4}$).

The blood contains many ions, some in mmol/liter, while others are in μmol/l, and yet they play an important physiological role. We consider the following ions written alphabetically.

Al, Ca, Cl, Cu, F, Fe, H, HCO_3, K, Mg, Na, NO_3, PO_4, SO_4, Zn.

Ionic concentrations satisfy certain constraints due to their interactions and mass-action effects. Thus, e.g., HCO_3 and CO_3 ions are linked by the concentration of hydrogen ions. However, chemical equilibrium is rarely attained in nature. This justifies treating them as approximately independent variables represented by a direction vector c_i, orthogonal to others when $i \neq j$ (i.e., for example, c_1 is a variable independent of c_2). If chemical or physiological interactions make the two variables non-orthogonal, we may include such effects *via* the overlaps S_{ij} of the two vectors c_i, c_j and handle the equations via the technique of Lagrange multipliers. However, here we neglect them by setting $S_{ij} \to \delta_{ij}$. If we associate a Hofmeister activity a_i with the i-th ionic species, then we can also define a Hofmeister-weighted ionicity $I_h = \sum_i a_i c_i$ as a single number characterizing the action of a mixture of ions.

Urea, used in fertilizers, plays a role in denaturing proteins, and in kidney function. It exists in water in a quasi-ionic state due to association with H^+ or OH^-, and contributes to the ionicity.

In tables 2 and 3 typical values of b_i for blood serum, d_i for MAL in water, D_i for SWI, and c_i^* of a 'typical' NCP-water sample are given. This is a 'model water sample' for CKDU areas, constructed by reviewing many published reports (Wasana et al, 2012, Nilusha, 2012, Paranagama et al, 2012, Lasantha et al, 2008, Yatigammana, 2012).

The ionicity of drinking water is significantly different from that of the blood. The blood obtains its salts largely from food. The ionic composition of plants, meat and fish are strictly controlled by various physiological processes; in contrast, biological species cannot adequately control trace toxins which may bio-accumulate. However, the WHO study (Jayatilake et al, 2013), and other studies (Chandrajith et al, 2011, Yatigammana, 2012, Paranagama et al, 2012, Nilusha, 2012) show that metal toxins in food are negligible except for cases like lotus root. The principal components of the NCP-diet are rice, vegetables, curried fish or meat, and lentils

Table 3 This table reports ionicity data equivalent to those in Table 2, for trace ions, viz., Aluminum to Zinc.

item i	Al	As	Cd	Cu μ g/l	F^- $\pm 40\%$	Fe	Pb	Zn
b_i	<10	0	.2	125	30	115	80	900
d_i	200	10	5	1130	2000	300	15	5000
D_i	200	2	3	na	400	na	na	na
c_i^*	50	4	1.5	na	800	na	na	na

(*Lens culinaris*). Chemical analyses show that the NCP-fish are safe for consumption. (Subasinghe et al, 2012, WHO-SL-reports, 2013) Lentils remain safe even when grown in toxic soil (Ismael et al, 2012). Hence CKDU is, on balance, likely to be linked to the quality of drinking water and not food.

The MALs used in different contexts differ; e.g., CODEX allows 50mg/l of Nitrate in bottled water, but not in municipal water. The MALs d_i given in regulatory documents have not been constructed taking account of the consorted effect of several ionic species, and recognizing CKDU as a possible outcome of such water ionicity. WHO regulatory documents (WHO-guidelines, 2011) discuss studies on Ca, Mg and other ions in relation to cardio-vascular diseases, but not kidney disease. Experimental data regarding how high-ionicity water affects the proteins in the kidney membrane (or related model proteins) are needed to clarify the questions raised here. The trace-ions included in Table 3 are not a primary reason for CKDU. They become relevant (and toxic) when the kidney is debilitated and fails to eliminate them. It should be noted that fertilizer runoff may contain some of these trace ions in MAL-conforming amounts, although their joint effect is unknown. This leads us to consider that a repeat of the WHO-type study (sec. 3), extended to be more comprehensive, as in the Ethiopian study (Clemens et al, 2003). It should include common 'safe' ions as well; such a study is necessary to review the findings of the WHO-study where no conclusive nephrotoxins were found.

It would be useful to set definitive upper thresholds for ionicity when CKDU becomes very likely. However, we currently have no empirical estimates, although a model based on the Hofmeister ionicity I_h of the ions found in the fertilizer-runoff could be formulated.

Legislating agrochemical use to follow chemistry (not sales push and subsidies), funding research for reducing the need for agrochemicals (Waidyanatha, 2013, Waidyanatha et al, 1979), use of precision-farming methods with *in situ* soil analysis during farming (US-EPA, 2003), are needed to optimize agricultural practices as well as environmental health.

6 Conclusion

In this study we noted the correlation (both in space and time) of the incidence of CKDU in Sri Lanka with the ecological consequences of (i) the onset of excess fertilizer use, often as much as 5-10 times the recommended amounts, (ii) the unwitting creation of a conduit moving excess-fertilizer to the North-central province by irrigation systems, adding to the ionicity of the water, (iii) prolonged use of excessively ionic water is suggested to cause CKDU by a Hofmeister-type protein-denaturing mechanism in the kidney.

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