

Chronic kidney disease of unknown etiology in Sri Lanka: The potential role of pesticides, heavy metals and drinking water as environmental risk factors

Kamani Wanigasuriya^{1,2}, Pathmalal M. Manage^{3,4},
Keith E. Levine⁵, Jill Lebov⁵, Jennifer Hoponick Redmon⁵

¹Centre for Kidney Research, ²Department of Medicine, Faculty of Medical Science, University of Sri Jayewardenepura, Sri Lanka; ³Centre for Water Quality and Algae Research, University of Sri Jayewardenepura, Sri Lanka; ⁴Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka; ⁵RTI International, RTP, NC, United States.

Introduction

Chronic Kidney Disease (CKD) is a global problem, identified as the 7th most common cause of death (WHO, 2012). In Sri Lanka, prevalence of CKD of unknown etiology (CKDu) has increased alarmingly in recent decades. The epidemic has spread among rural farming communities in Sri Lanka's North Central Dry Zone, leading to an urgent need for action. Studies conducted in recent years have failed to recognize a single etiology; evidence favors a multi-factorial etiology.

Epidemiology

In Sri Lanka, CKDu is geographically restricted, first reported in the North Central Province (NCP), later spreading to neighboring North Western, Eastern, and Uva Provinces (Chandrajith et al., 2011). Impacted areas cover ~24,000 km² and ~ 3 million people; currently ~2 million are at high risk of contracting CKDu (Wimalawansa et al., 2013). Approximately 90% of impacted families engage in paddy farming, vegetable farming, or both. Most affected people live in rural and remote communities, have little political power, and limited access to modern health care.

The WHO CKDu study (Jayathilake 2013) reported a higher age-standardized prevalence in women 16.9% (95% CI = 15.5%–18.3%) than men 12.9% (95% CI = 11.5%–14.4%), with later stage CKDu more common in men (stage 3, men 23.2%, women 7.4%; stage 4 men 22%, women 7.3%; $p < 0.001$). Many cases are young to middle-aged male farmers engaged in rice farming or other agriculture, therefore it's postulated that they are exposed to higher levels of environmental hazards than non-farming peers (Wimalawansa, 2015). Family history in 20% of CKDu cases was also noted (Jayathilake 2013).

Clinical Profile

Early stage CKDu is largely asymptomatic, so most patients present in advanced stages. Symptoms are non-specific (e.g., dysuria with back pain), 24-hour urine protein excretion is typically <1 g, and ultrasound show bilateral, small kidneys. Anemia, hypertension and edema are late features (Athuraliya 2009). Urinary tubular markers (e.g., α 1-microglobulin, NGAL) are elevated in early stage CKDu and steadily rise with progression, indicating renal tubular involvement (Nanayakkara et al., 2012). Similarly, Urinary fibrinogen and β 2-microglobulin may aid in the early diagnosis of toxin mediated tubular injury in CKDu (Wanigasuriya 2017). The reported CKDu distribution in Sri Lanka is shown in **Figure 1**.

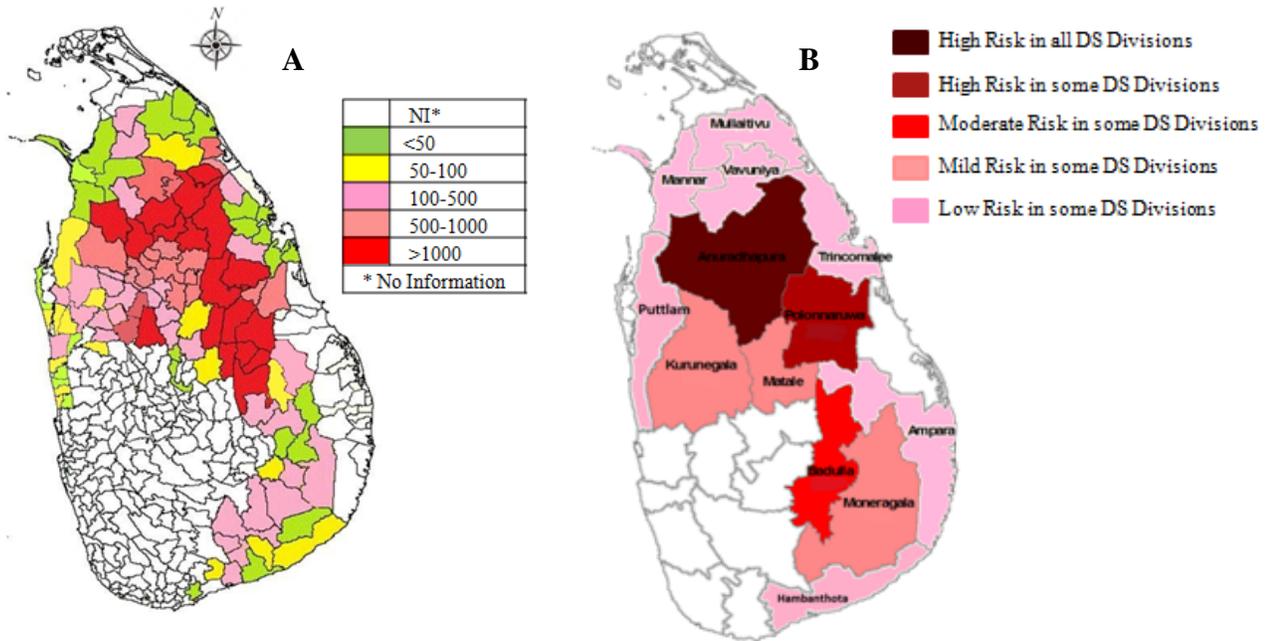


Figure 1: A. Reported distribution of CKD/CKDu Patients in Divisional Secretariats.

B. CKD/CKDu patient prevalence in Sri Lanka up to 2018

(CKDu Prevention and Research Unit, Ministry of Health, Sri Lanka, 2018)

Etiological Factors

Considering discrete geography, risk factors, clustering of cases within families, it was hypothesized that CKDu in the NCP was likely in part due to one or more environmental risk factors, with familiar clustering due to a possible genetic predisposition to environmental factor(s) (Wanigasuriya et al., 2007). To-date, the exact etiology has yet to be unraveled.

Genetic Predisposition

A mosaic of discrete regions of high prevalence could represent genetic predisposition. CKDu among other family members has repeatedly been shown (Wanigasuriya 2007, Athuraliya 2007, Jayathilake 2013). Recent sequencing revealed a possible rare variant in the KCNA10 gene, which encodes for a voltage-gated potassium channel found in proximal tubular cells that could predispose to disease (Nanayakkara et al., 2014).

Agrochemicals

Sri Lanka’s 1960s the ‘green revolution’ emphasized high-yield seeds, chemical fertilizers and pesticides. Pesticide exposure has been explored as a CKDu etiological factor of focus due to disease occurrence in farmers and heavy agrochemicals us encouraged by the green revolution. Pesticides in Sri Lanka are imported as finished products or as materials which are formulated locally. In 1973, the Pesticide Regulatory Committee was established to control import and use. The Control of Pesticides Act No.33

was passed by parliament in 1980, and the Registrar of Pesticides was appointed as a licensing authority responsible for importation control.

Due to toxicity concerns, Sri Lanka banned all class 1A and many class 1B pesticides, allowing class 2 pesticides only if there is no suitable replacement. Import of several pesticides has been banned during the last decade (**Table 1**, Appendix 1); currently imported pesticides are summarized **Tables 2 – 3**, Appendix 1. A ban on glyphosate was instituted in 2015 over CKDu concerns, but was lifted in 2018. It is estimated that ~ 0.308 kg and ~ 1.056 kg of active pesticide ingredients are used per hectare of arable lands and actively cultivated lands, respectively.

In Sri Lanka, most agrochemical importers are local agents of multinational companies, and retail traders have strong influence on farmers who depend on them for advice. Due to the large number of trade names, farmers can inadvertently overuse the same active ingredient from different products (Mohottige et al., 2002). Agrochemical adulteration or contamination with heavy metals has been suggested (Jayasumana et al., 2014, **Table 4**, Appendix 1). Furthermore, farmers do not consistently utilize personal protective equipment while engaged in application.

The earliest CKDu study in Sri Lanka evaluating pesticide exposure (Peris-John et al, 2006) consisted of 4 groups: 23 OP-exposed farmers with chronic renal failure (CRF) from an endemic area, 18 unexposed patients with CRF from a non-endemic area, 239 OP-exposed farmers without CRF, and 50 unexposed fishermen without CRF. Haemoglobin-corrected red cell AChE was measured as a surrogate marker of organophosphate pesticide exposure. AChE levels among farmers were significantly lower than in the unexposed controls ($p < 0.01$), lower in CRF patients (exposed and unexposed groups), and lower in exposed vs. unexposed group ($p < 0.05$). Authors suggested further study to evaluate possible association between pesticide use and CKDu. A hospital based case–control study (183 CKDu cases: 136 M, 47 F; 200 controls from a medical clinic) found significant association with farming and pesticide exposure with CKDu in bivariate analysis; association was not seen in multivariate analysis (Wanigasuriya 2007), and a cross-sectional population-based (886 household members age 18+: 461 M, 425 F), found a negative association with pesticide spraying and CKDu in multivariate analysis (Wanigasuriya 2011).

The CKDu National Research Project Team study (WHO study) provided evidence of nephrotoxic pesticide exposure in CKDu patients (Jayathilake, 2013). Residues were detected in urine of CKDu patients ($n = 57$) with frequency of 2,4-D, 3,5,6-trichloropyridinol, p-nitrophenol, 1-naphthol, 2-naphthol, glyphosate, and AMPA as 33%, 70%, 58%, 100%, 100%, 65%, and 28% respectively. Urinary levels of isopropoxyphenol, 2,4,5-trichlorophenol and entachlorophenol were below detection limits. A major limitation of this study was lack of a control population. Redmon et al. (2014) discusses published WHO findings and presents additional considerations that may enhance subsequent investigations designed to identify CKDu risk factors. Glyphosate-metal complexes have been hypothesized as contributing to CKDu etiology (Jayasumana et al., 2014). Those who oppose this theory claim hard water in endemic areas would convert glyphosate to solid complexes of magnesium and calcium that are highly insoluble and poorly absorbed (Dharmawardana et al., 2014). A hospital-based case-control study (125 cases, 180 controls, Jayasumana et al. 2015) found significantly increased ORs for farming, use of fertilizers, and use of organophosphates, paraquat, MCPA, glyphosate, bispyribac and mancozeb with bivariate logistic regression analysis. They reported significantly higher glyphosate levels in water from abandoned wells compared to active wells (median 3.2 $\mu\text{g/L}$ and 0.6 $\mu\text{g/L}$, respectively). Further positive associations were found with pesticide applications and glyphosate in drinking well-water, especially from abandoned wells with the hardest water glyphosate levels.

To date, studies do not provide conclusive evidence on the etiological association between agrochemicals and CKDu. Limitations include a lack of data connecting exposure levels and the role of potential confounders. Therefore, there is a need for a more robust research agenda to establish the potential relationship between agrochemicals and CKDu.

Heavy Metals

A limited geochemical laboratory analysis to determine the concentration of a suite of elements in biological (whole blood and hair) and environmental (drinking water, rice, soil, and freshwater fish) samples from two towns within endemic NCP region was reported (Levine et al., 2016). A broad panel, metallomics/mineralomics approach was used, and basic statistical measurements were used to compare media against applicable benchmarks (e.g., US soil screening levels). Cadmium (Cd), lead (Pb), and mercury were detected at concentrations exceeding US reference values in many of the biological samples, suggesting that study participants are subjected to chronic, low level exposure to these elements. Within the limited number of environmental samples, arsenic (As) was determined to exceed initial risk screening and background concentration values in soil, while drinking water samples reflected the unique trigonal hydrogeochemistry, including prevalence of hard water, and fluoride (F), iron, manganese, sodium, and Pb exceeding applicable drinking water standards. Collected data were intended to be used in the subsequent study design of a comprehensive and multifactorial etiological study of CKDu risk factors.

Cadmium-Specific Studies

In 2008, Bandara et al. reported high Cd levels in reservoir water serving CKDu households and paddy fields, as well as in soil, rhizomes, rice and inland fish. Subsequent studies failed to reproduce these findings. In other studies (Chandrajith et al. 2011; Levine et al. 2016), the maximum measured Cd rice concentration was reported as below the Codex Alimentarius Commission reference level (2009) and MAC for Chinese rice. However, given the importance of rice to the Sri Lankan diet, it is possible that chronic exposure to Cd below reference levels could act as an environmental nephrotoxin, especially if other risk factors are present. Cd levels in drinking water were consistently found to be in keeping with International Drinking Water Standards (WHO 2011).

Nanayakkara et al. (2014) showed urinary Cd excretion to be lower in CKDu patients and their unaffected relatives compared to controls. These findings corroborate results reported by Wanigasuriya et al. (2017), where urinary Cd was significantly lower in CKDu patients compared to farmer and non-farmer controls. Nevertheless, results of the CKDu National Research Project Team study were interpreted to include Cd as a CKDu etiological factor. They found increased urinary Cd in CKDu patients compared to healthy controls from both endemic and non-endemic areas, demonstrating a dose-effect relationship between urinary Cd and CKD stage (Jayathilake et al. 2013). Food items from endemic areas contained Cd and Pb above reference levels suggesting chronic ingestion. Levine et al. (2016) reported determined Cd and Pb blood concentrations in CKDu patients exceeded mean US reference values from healthy nonsmokers for 68.7 and 89.2% of the samples, respectively (CDC 2015). Tables 1 and 2, Appendix 2, summarize urinary Cd and As from various studies. In conclusion, studies related to Cd and CKDu were based on cross-sectional studies where samples were obtained at different times and different locations. The results remain inconsistent, making it difficult to establish a clear link.

Arsenic-Specific Studies

Some researchers have reported widespread As contamination of drinking water, food and soil, and have inferred that pesticides and/or groundwater were the sources (Jayasumana et al., 2013). Wasana et al. (2012) assessed well water quality from high and low CKDu-prevalence areas and from four springs in the Kebithigollewa DSD. As and Cd levels were found to be well below the WHO standard of 10 µg/L and 3 µg/L, respectively (WHO, 2004). Although the As theory led to a vigorous public debate, currently none of the researchers promote it as a CKDu causative factor. As content of pesticides available in Sri Lanka is summarized in Table 5, Appendix 1 (Jayasumana et al., 2015).

Microorganisms

Ochratoxin A, a mycotoxin known to cause interstitial fibrosis, has been identified in many foods in the NCP, but at levels below European safety limits. Higher urinary Ochratoxin A levels found in CKDu patients and their unaffected relatives compared with Japanese controls are of uncertain significance (Desalegn et al., 2011). Leptospirosis is endemic in Sri Lanka and has been linked to CKDu (Gamage & Sarathkumara, 2016). Hantavirus is another important zoonotic disease that present with similar clinical features to leptospirosis; flu-like illness and fever and is known to cause acute kidney injury. Although implicated, progression to CKDu has not been proven (Rajapurkar et al., 2012).

Drinking water

Fluoride, hardness, and cyanotoxins are currently under investigation as causative factors.

Fluoride and Hardness

Endemic CKDu areas are known to overlap with a hydrogeochemical region that contains elevated ground F levels and hard water. Water sources for drinking and cooking in these areas are dug, shallow or deep wells. Chandrajith et al (2011) studied geochemical properties of well water randomly collected from endemic and non-endemic regions and found F in well water from endemic areas exceeded the WHO recommended level (0.6 mg/L). It was also noted that Ca-bicarbonate type water is predominant in endemic regions and that hard water could enhance cytotoxic F properties. According to Levine et al. (2016), water samples in the region did contain elevated F levels and a large percentage of samples had hard or very hard water, as estimated from calcium and magnesium levels.

Wasana et al (2016) studied synergic effects of Cd, F and water hardness in conditions that mimicked the natural environmental. Sprague–Dawley male rats were dosed with Cd (50 mg/L) and F (100 mg/L) alone or in combination in drinking water for twelve weeks, and increased liver and kidney damage was noted from Cd and F co-exposure. Therefore, synergic effects of heavy metals and other anions present in natural water could be a factor in CKDu etiology and warrants further investigation.

Cyanotoxins

Cyanobacteria are present in many water ecosystems (blue-green algae), and blooms can produce cyanotoxins which are diverse in chemical structure and toxicity. Cyanotoxins are classified as hepatotoxins (microcystins, nodularin, cylindrospermopsin), neurotoxins (saxitoxin, anatoxin), cytotoxin (aplysiatoxin,) and skin and gastrointestinal irritants (Carneiro et al., 2015; Liang et al., 2007). The most common cyanotoxin groups are microcystins (MCs), while microcystin-LR (MC-LR) is their most toxic structural variant (Idroos et al., 2015, Manage et al., 2009). There is some emerging evidence that may support cyanotoxins in the etiology of CKDu. Drinking from shallow wells increases CKDu risk, whereas consumption of spring water lowers prevalence (Jayasekara et al., 2013). Well water levels fluctuate

with those of nearby canals, suggesting the ground water table is recharged from potentially contaminated irrigation and reservoir systems. Mice fed with extracts of cyanobacteria that grow in reservoirs in endemic area developed acute tubular necrosis, but not interstitial nephritis (Dissananyake et al. 2010).

Recent research identified cyanobacteria in 75% of freshwater bodies tested in CKDu endemic areas compared to 40% of freshwater bodies in non-endemic areas (Manage et al., 2012). Recent findings also reveal presence of toxin-producing cyanobacteria (*Microcystis aeruginosa*, *Cylindrospermopsis* sp. and *Anabaena* sp.) in many drinking water reservoirs. It has been reported that human exposure to toxins produced by freshwater cyanobacteria may be a possible cause of the emerging renal disease (Piyathilaka et al., 2015).

Health effects can occur from ingestion of water containing a high level of microcystin, dermal contact from swimming, wading or showering, or inhalation of airborne droplets (Lawton et al., 1999). Cyanotoxins are also environmentally persistent long after the bloom has lysed (Manage et al., 1999; Idroos et al., 2014; Sethunga and Manage., 2010). The occurrence of microcystins are a serious health hazard and acute doses have been responsible for illness and fatalities in animals and humans, leading the WHO to set a guideline limit of 1 µg/L microcystin-LR (MC-LR) as the maximum allowable concentration in drinking water (WHO, 1998). Moreover, chronic exposure has been associated with primary liver cancer in epidemiological studies (Fisher and Dietrich., 2000). In sum, cyanotoxins and potential synergetic interaction with other environmental compounds could be a factor in CKDu etiology and warrant further investigation.

Preventive Action

Specific CKDu interventions are challenging due to lack of clear understanding of the etiology. Presently, provision of safe drinking water is the major intervention priority taking place in Sri Lanka. Various technologies (e.g., reverse osmosis, rain harvesting, filtration) are being used in endemic areas, but further research is needed to identify cost-effective, sustainable technologies. Several agrochemicals have been proactively banned, but it is not clear if they contribute to the genesis or progression of the disease. Early detection and treatment is desperately needed to prevent CKDu progression to advanced stages. Community awareness is high, and there are comprehensive screening programs underway.

References

01. Athuraliya, T. N. C., et al. (2009). Prevalence of chronic kidney disease in two tertiary care hospitals: high proportion of cases with uncertain aetiology. *Ceylon Medical Journal*, 54(1).
02. Bandara, J.M.R.S., et al. (2008). 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). *Environ Geochem Health*, 30(5), 465-478.
03. Centers for Disease Control and Prevention (CDC). (2015). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Data Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention [http://www.cdc.gov/nchs/nhanes.htm].
04. Chandrajith, R., et al. (2011). Chronic kidney diseases of uncertain etiology (CKDu) in Sri Lanka: geographic distribution and environmental. *Environ Geochem Health* 33, 267–278.
05. Chandrajith, R., et al. (2011). Chronic kidney diseases of uncertain etiology (CKDu) in Sri Lanka: geographic distribution and environmental. *Environ Geochem Health* 33, 267–278.
06. Codex Alimentarius Commission (2009). Joint FAO/WHO Food Standards Programme (<http://www.fao.org/fao-who-codexalimentarius/sh-roxy/es/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-701-32%252Fal32REPe.pdf>, accessed 14 April 2016).
07. Desalegn, B., et al. (2011). Mycotoxin detection in urine samples from patients with chronic kidney disease of uncertain etiology in Sri Lanka. *Bull Environ Contam Toxicol*, 87(1), 6.
08. Dharmawardhana, C.C., et al. (2014). Quantum mechanical metric for internal cohesion in cement crystals. *Scientific Reports*, 4, p.7332.
09. Dissanayake, D. M. (2010). The cyanobacterial toxins: a hidden health hazard. Selected invited speaker abstracts from 9th Asia Pacific Association of Medical Toxicology, Hanoi, Vietnam, 16-19.
10. Edition, F. (2011). Guidelines for drinking-water quality. *WHO Chronicle*, 38(4), pp.104-8.
11. Fischer, W. J. & Dietrich, D. R. (2000). Pathological and biochemical characterization of microcystin-induced hepatopancreas and kidney damage in carp (*Cyprinus carpio*). *Toxicol Appl Pharmacol* 164, 73–81.
12. Gamage, C.D. & Sarathkumara, Y.D. (2016). Chronic kidney disease of uncertain etiology in Sri Lanka: Are leptospirosis and Hantaviral infection likely causes? *Medical hypotheses*, 91, pp.16-19.
13. Idroos, S. & Manage, P.M. (2015). Toxin Producing Cyanobacteria in Labugama, Kalatuwawa Drinking Water Reservoirs. In Proceedings of International Forestry and Environment Symposium, 20.
14. Idroos, S.F., et al. (2014). Development of an efficient extraction method to Quantify Microcystin-LR from Natural microcystin bloom samples from Colombo Lake Sri Lanka (International Environmental Symposium, January 2014).
15. Jayasekara, J. M. K. B., et al. (2013). Prevalence of G6PD deficiency in patients with chronic kidney disease of unknown origin in North Central Region of Sri Lanka: case control study. *International Journal of Recent Scientific Research*, 4, 455–458.
16. Jayasumana C, et al. (2015). Drinking well water and occupational exposure to Herbicides is associated with chronic kidney disease, in Padavi-Sripura, Sri Lanka *Environmental Health* 14:6 <https://doi.org/10.1186/1476-069X-14-6>.
17. Jayasumana, C., et al. (2015). Phosphate fertilizer is a main source of arsenic in areas affected with chronic kidney disease of unknown etiology in Sri Lanka. *SpringerPlus*, 4(1), p.90.
18. Jayasumana, C., et al. (2014). Glyphosate, hard water and nephrotoxic metals: are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in Sri Lanka? *International Journal of Environmental Research and Public Health*, 11(2), 2125-2147.
19. Jayasumana, M. A. C. S., et al. (2013). Possible link of chronic arsenic toxicity with chronic kidney disease of unknown etiology in Sri Lanka. *Journal of Natural Science Research*, 3, 64–73.

20. Jayatilake, N., et al. (2013). Chronic kidney disease of uncertain aetiology: prevalence and causative factors in a developing country. *BMC Nephrology*, 14(1), 180.
21. Lawton, L.A., et al. (1999). Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters. *The Analyst*, 119: 1525-1530.
22. Levine, K. E., et al. (2016). Quest to identify geochemical risk factors associated with chronic kidney disease of unknown etiology (CKDu) in an endemic region of Sri Lanka-a multimedia laboratory analysis of biological, food, and environmental samples. *Environmental Monitoring and Assessment*, 188(10), 1-16. [548]. <https://doi.org/10.1007/s10661-016-5524-8>
23. Liang, C.C., et al. (2007). In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nature protocols*, 2(2), p.329.
24. Manage, P. M., et al. (2009a). Isolation and identification of novel microcystin-degrading bacteria. *Appl. Environ. Microbiol.*, 75(21), 6924-6928.
25. Manage, P. M., et al. (1999). Seasonal changes in densities of cyanophage infectious to *Microcystis aeruginosa* in a hypereutrophic pond. *Hydrobiologia*, 411, 211-216.
26. Manage, P.M. (2012). Study on algal and cyanobacteria identification, species composition, determination of concentrations of N, P, Mn, Fe, COD, TOC (Total Organic Carbon) and screening of hepatotoxin Microcystin-LR in some selected drinking and irrigation water bodies in Sri Lanka. Final report submitted to National Water Supply and Drainage Board.
27. Marambe, K.N., et al. (2007). A comparison of learning strategies, orientations and conceptions of learning of first-year medical students in a traditional and an innovative curriculum.
28. Mohottige N S, et al. (2002) Agro-pesticide use in Sri Lanka Major policy issues. Department of Agricultural Extension, Faculty of Agriculture University of Peradeniya accessed from [https://www.ctc-n.org/sites/www.ctc-n.org/files/resources/agro-pesticide use in sri lanka - major policy issues.pdf](https://www.ctc-n.org/sites/www.ctc-n.org/files/resources/agro-pesticide_use_in_sri_lanka_-_major_policy_issues.pdf)
29. Nanayakkara, S., et al. (2012). Tubulointerstitial damage as the major pathological lesion in endemic chronic kidney disease among farmers in North Central Province of Sri Lanka. *Environmental health and preventive medicine*, 17(3), p.213.
30. Nanayakkara, S., et al. (2014). An integrative study of the genetic, social and environmental determinants of chronic kidney disease characterized by tubulointerstitial damages in the North Central Region of Sri Lanka. *Journal of Occupational Health*.
31. Pathmalal M. Manage, et al.. (2019). A Descriptive Survey in an Endemic Area of Sri Lanka – Characterizing Sociodemographic, Consumption, and Agrochemical Exposure Patterns Associated with Chronic Kidney Disease of Uncertain Aetiology (CKDu). Presented at Third International Workshop on Chronic Kidney Diseases of Uncertain/Non-traditional Etiology in Mesoamerica and Other Regions, San José, Costa Rica, 20-22 March 2019. https://tools.niehs.nih.gov/conference/ckd_2019/index.cfm.
32. Manage, P.M, et al. (2009b). Hepatotoxic effects of *Microcystis aeruginosa* (PCC7820) on Wister Rates, Accepted for Golden Jubilee Special Issue of the Vidyodaya Journal.
33. Peiris-John R.J., et al. (2006) Exposure to acetylcholinesterase-inhibiting pesticides and chronic renal failure. *Ceylon Med J.*, 51(1), 42–3.
34. Piyathilaka, M. A. P. C., et al. (2015). Microcystin-LR-induced cytotoxicity and apoptosis in human embryonic kidney and human kidney adenocarcinoma cell lines. *Microbiology* (2015), 161, 819–828.
35. Rajapurkar, M.M., et al.. (2012). What do we know about chronic kidney disease in India: first report of the Indian CKD registry. *BMC nephrology*, 13(1), p.10.
36. Redmon, J., et al. (2014). Additional perspectives on chronic kidney disease of unknown aetiology (CKDu) in Sri Lanka - lessons learned from the WHO CKDu population prevalence study. *BMC Nephrology*, 15, 125. <https://doi.org/10.1186/1471-2369-15-125>.
37. Sethunge, S., et al. (2010) Nuisance algae in water supply projects in Sri Lanka. *Sustainable Built Environments*. pp. 62-69.

38. Sethunga. H. and Manage, P.M. (2014). Contamination status of algae toxins microcystins in some selected water bodies in Sri Lanka, International Environmental Symposium, 25.
39. van der Post, R.S., et al. (2015). Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. *Journal of medical genetics*, 52(6), pp.361-374.
40. Wanigasuriya K., et al. A Descriptive Survey in an Endemic Area of Sri Lanka – Characterizing Sociodemographic, Consumption, and Agrochemical Exposure Patterns Associated with Chronic Kidney Disease of Uncertain Aetiology (CKDu) (Unpublished data)
41. Wanigasuriya K., et al. (2017) Novel urinary biomarkers and their association with urinary heavy metals in chronic kidney disease of unknown aetiology in Sri Lanka: a pilot study *Ceylon Medical Journal*; 62: 210-17.
42. Wanigasuriya K.P., et al. (2011) Chronic kidney disease of unknown aetiology in Sri Lanka: is cadmium a likely cause? *BMC Nephrol*; 12:32.
43. Wanigasuriya, K P, et al. (2007). Chronic renal failure in North Central Province of Sri Lanka: an environmentally induced disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 101(10), 1013-1017.
44. Wasana, H. M., et al. (2017). WHO water quality standards Vs Synergic effect (s) of fluoride, heavy metals and hardness in drinking water on kidney tissues. *Scientific reports*, 7, 42516.
45. Wasana, H.M., et al. (2016). Drinking water quality and chronic kidney disease of unknown etiology (CKDu): synergic effects of fluoride, cadmium and hardness of water. *Environmental geochemistry and health*, 38(1), pp.157-168.
46. WHO (2012). Chronic Kidney Disease of Unknown Origin (CKDu). www.whosrilanka.org. Accessed 19 November 2014.
47. Wimalawansa, S.J. (2013). Purification of contaminated water with reverse osmosis: effective solution of providing clean water for human needs in developing countries. *J Emerg Technol Adv Eng*, 3(12), pp.75-89.
48. Wimalawansa, S.J. (2015). Escalating chronic kidney diseases of multi-factorial origin (CKD-mfo) in Sri Lanka: causes, solutions, and recommendations—update and responses. *Environmental health and preventive medicine*, 20(2), p.152.
49. World Health Organization, 2004. Cadmium in drinking-water: background document for development of WHO guidelines for drinking-water quality (No. WHO/SDE/WSH/03.04/80). World Health Organization.

Appendix 1

Table 1: Banned pesticides in Sri Lanka 2011-2017*

2011	Paraquat Dimethoate Fenthion Cyromazine	
2012	Alachlor	
2013	Chlorpyrifos Carbaryl Carbofuran	
2017	2,4-D Dicofol 1,2-Dibromoethane Azinphos-methyl Benomyl Binapacryl Chlorobenzilate Dinitro- <i>ortho</i> -cresol & its salts	Dinoseb & its salts Ethylenedichloride Fluoroacetamide Hexachlorobenzene Oxydemeton-methyl Toxaphene Tributyl-tin compound Trichlorfon Tridemorph

*Department of Agriculture, Sri Lanka (Personal communication)

Table 2. Leading Pesticides imported to Sri Lanka in 2012**

Rank Pesticide	kg or L Approved for Import
Glyphosate (acid equivalent)	5,295,082
Propanil	995,310
MCPA	686,375
Mancozeb	671,504
Chlorpyrifos	420,008
Carbofuran	299,000
Diazinon	196,735
Profenofos	140,768
Carbosulfan	107,000
Pretilachlor + Pyribenzoxim	102,297

**Pesticide Importation Data; Office of the Registrar of Pesticides: Gannoruwa, Sri Lanka, 2012

Table 3: Registered insecticide list 2018*

Common Name	Strength	Class II	Class III	Class IV (U)
abamectin	18g/l EC	*		
acephate	75% SC	*		
acetamiprid	20% SP		*	
bifenthrin	100 g/l EC	*		
bistrifluron	100g/IEC		*	
buprofezin	10% WP		*	
carbosulfan	200 g/L SC	*		
chlorfluazuron	50g/IEC			*
chlorantraniliprole	200 g/L SC			*

chromafenozide	50g/l SC			*
Chlorantraniliprole 20% + Thiomethoxam 20%	20+20 % WG		*	
deltamethrin	25g/l EC	*		
diazinon	5% GR	*		
diazinon	500g/l EC	*		
emamectin benzoate	5% w/w SG		*	
ethiprole	100g/l SC		*	
etofenprox	100g/l EC			*
fenobucarb	500g/l EC	*		
fenpyroximate	50g/l EC	*		
fipronil	50g/l SC	*		
fipronil	0.3% GR	*		
flubendiamide	24% WG		*	
hexythiazox	50g/l EC		*	
imidacloprid	70% WDG	*		
imidacloprid	70% WG	*		
imidacloprid	70% WS	*		
imidacloprid	200g/l SC	*		
indoxacarb	150g/l EC		*	
lambda-cyhalothrin	50g/l SC	*		
lufenuron	5% EC			*
metaldehyde	5% GR	*		
methoxyfenozide	240g/l SL			*
novaluron	100g/l EC			*
phenthoate	500g/l EC	*		
profenofos	500g/l EC	*		
pymetrozine	50%WG			*
quinalphos	250g/l EC	*		
spinosad	25g/l SC		*	
spinetoram	25%WG			*
sulfloxaflor	50% WG			*
thiacloprid	240g/l SC	*		
thiamethoxam	70% WS		*	
thiamethoxam	25% WG		*	
thiocyclam hydrogen oxalate	50% SC		*	
thiocyclam hydrogen oxalate	4% GR		*	
thiodicarb	375g/l SC	*		
tebufenozide	200g/l SC			*

*Department of Agriculture, Sri Lanka (Personal communication)

Table 4. Trace metal profiles of commonly used fertilizers in Sri Lanka (mg/kg) (Jayasumana et al., 2015)

Element	Urea		MOP		TSP	
	Mean	Range	Mean	Range	Mean	Range
Al	2.6	1.0-3.3	151.3	97.9-231.1	9939.0	8923.0-11290.0
Sb	0.1	0.1-0.2	0.1	0.1-0.1	6.0	5.7-6.0
As	0.1	ND-0.3	0.3	0.2-0.4	28.9	26.5-31.9
Ba	0.1	0.1-0.1	1.1	1.0-1.3	79.1	77.6-83.4
Be	0.1	0.1-0.1	0.1	0.1-0.2	2.2	2.1-2.3
Cd	ND	ND	0.1	0.1-0.2	2.0	1.9-2.0
Cr	ND	ND	1.2	0.4-2.7	29.3	22.6-33.7
Co	0.1	0.1-0.1	0.2	0.2-0.3	6.0	5.9-6.4
Cu	0.2	0.1-0.4	0.3	0.3-0.4	15.0	14.2-16.0
Fe	1.0	ND-1.7	2371.3	2252.3-2634.3	11215.3	10910.3-11760.3
Pb	0.2	0.2	0.8	0.8-0.9	252.5	251.7-263.9
Mn	0.3	0.1-0.4	12.3	11.6-13.7	1948.0	1886.0-2034.0
Ni	1.0	0.2-3.7	0.3	0.2-0.5	25.0	23.9-27.1
Se	0.2	ND-0.5	1.7	1.4-2.1	2.0	1.2-2.5
Ag	0.1	0.1-0.1	0.1	0.1-0.1	0.3	0.1-0.3
Sr	0.1	ND-0.1	10.2	9.8-10.6	245	230-277.9
Tl	0.1	ND-0.1	0.1	ND-0.2	0.5	0.5-0.5
Sn	0.1	0.1-0.2	0.2	0.2-0.3	0.7	0.7-0.7
Ti	ND	ND	4.1	2.6-5.0	439.6	379.0-496.3
V	0.2	0.2-0.4	0.3	0.2-0.5	37.1	34.9-39.3
Zn	ND	ND	0.8	0.2-1.3	489.8	443.6-544.0

Table 5. Arsenic content of the pesticides available in Sri Lanka** (Jayasumana et al., 2015)

Active ingredient	Type	No of samples	As Range (µg/kg)	Mean As (µg/Kg)	2012 Imported quantity (MT)
Dimethoate	I	12	965-2457	1957	NA
Glyphosate	H	18	858-2586	1896	5295
Fenoxaprop-p-ethyl	H	12	1254-2578	1835	41
Mancozeb	F	15	458-2478	1680	692
Carbofuran	I	18	831-2458	1578	299
Propanil	H	12	512-2584	1324	1094
Methomyl	I	12	1112-1458	1279	09
Quinalphos	I	12	928-1893	1278	08
Carbendazim	F	12	1163-1458	1278	20
Profenofos	I	12	458-1452	968	141
MCPA	H	18	458-1496	967	686
Bispyribac Na	H	12	721-1458	923	50
Methoxyfenozide	I	12	872-911	902	04
Thiamethoxam	I	12	542-1024	874	08
Chlopyriphos	I	18	654-1365	804	420
Phenthoate	I	12	565-1258	785	32
Diazinon	I	12	625-995	708	197
Oxyfluorfen	H	06	423-788	602	33
Pretilachlor + Pyribenzoxim	H	12	415-655	530	102
Tebuconazole	F	12	288-680	420	19
Imidacloprid	I	12	180-359	239	33

F = fungicide H= herbicide I = insecticide

Annexure 2

Table 1: Urinary Cd excretion $\mu\text{g/g Cr}$

CKDu patients Mean Median (Range)	Controls in endemic areas Mean Median (Range)	Controls in non endemic areas Mean Median (Range)	Non farmer controls in non-endemic Mean Median (Range)	
(n=495) 1.039, 0.695 (0.005 to 8.93)	N=132 0.646, 0.18, (0.005 to 5.13)a	N=250 0.345, 0.265 (0.005 to 2.079)b		Jayathilake et al (2013)
(n= 18) 0.788 (0.549)	(n=18) 0.571 (0.289)	(n=8) 0.390 (0.172)		Chandrajith et al (2010)
(n=35) 0.68 0.57 (21.25-58.09)		(n=37) 1.30 p<0.001 0.91 (0.73-1.69)	(n=35) 0.56 0.48 (0.32-0.68)	Wanigasuriya et al (2018)

A Urine cadmium concentration of cases compared to controls from endemic area P < 0.001.

b Urine cadmium concentration of cases compared to controls from non-endemic area P < 0.05.

Table 2 Urinary As excretion $\mu\text{g/g Cr}$

	CKDu patients Mean, Median (Range)	Controls from endemic areas Mean, Median (Range)	Controls from non-endemic areas Mean, Median (Range)	Non farmer controls in nonendemic Mean, Median (Range)
Jayathilake (2013)	n=495 45.447, 26.3 (0.4-616.6)	N=132 92.443, 6.99 (0.2-966.29)	N=250 56.572, 42.025 (5.38-50.28)	
Wanigasuriya et al (2018) (n=35)	59.0, 33.76 (21.25-58.09)		51.4 33.19 (22.32-56.24)	47.2(53.0) 34.11 (22.93-48.18)

Acceptable level :Western countries 10-15 $\mu\text{g/g Cr}$, Japan 50 $\mu\text{g/g Cr}$

Urinary As <100 $\mu\text{g/L}$ as unexposed by the United States Department of Health and Human Services Public Health Service Agency for Toxic Substance and Disease Registry .