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# Determination of Heavy Metal (Cd, Pb, Cr and Cu) Content in Selected Fruits and Vegetables Grown in CKDu Affected Puhudiwula, Mahadiwul Wewa and Unagas Wewa in Medawachchiya, Sri Lanka

A.S. Dilrukshi[1], H.K. Hiruni[1], M.K.B. Weerasooriya[1]

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## ABSTRACT

**Background:** Chronic kidney disease of unknown etiology (CKDu) is a burning health issue in the North Central province of Sri Lanka. Heavy metal contamination of food is presumed to be one of the causative factors. Hence, heavy metal level detection in fruits and vegetables grown in CKDu prevalent area was targeted.

**Methods:** Heavy metal (Cu, Cr, Pb and Cd) content of Mango, papaya, and some leafy and fruity vegetables grown in high CKDu risk Puhudiwula, Mahadiwul Wewa and low CKDu risk Unagas Wewa in Medawachchiya were detected by Flame Atomic Absorption Spectrophotometer. Information about CKDu patients in the area was also collected.

**Result:** A High level of Cd was observed in the samples at Mahadivul Wewa and Puhudivula as compared with samples at Unagas Wewa was well correlated with the number of patients noted. The contamination level of Pb and Cr was also relatively low in comparison with Cd. Hence, the present work revealed that exposure to elevated Cd levels through dietary food could be a risk factor accounts for a higher number of CKDu patients in these areas.

**Key words:** Cd, CKDu, Fruits, Heavy metal, Medawachchiya, Vegetables.

## INTRODUCTION

Chronic kidney disease with unknown etiology (CKDu) is one of the most burning environment-related health issues in North Central, North Western, and Uwa province in Sri Lanka. Medawachchiya, Padaviya, Siripura, Horowpathana, Medirigiriya, Polonnaruwa, Nikawewa, Dehiattakandiya and Girandurukotte and Wilgamuwa have been identified as the most affected regions (Fig 1). Nearly 15% of the population in Anuradhapura, Polonnaruwa and Badulla districts between the age group 15-70 years are affected by CKDu. The majority of those affected are male paddy farmers with poor socioeconomic backgrounds. Recently a growing number of women and children are also being affected by CKDu (Gunatilake *et al*., 2015).

The root of the disease is still in question and search. Contamination of Soil and water with pesticides, heavy metals such as cadmium and arsenic, and high levels of fluoride in groundwater are some of the etiological factors under investigation (Gunatilake *et al*., 2015; Wijetunge *et al*., 2015; Wanigasuriya *et al*., 2007; Ileperuma *et al*., 2009; Wasana *et al*., 2015). When the soil and water are contaminated with heavy metals, plants grown in the region also can be heavily contaminated with heavy metals. (Nagajyoti *et al*., 2010). Hence, monitoring heavy metal level (Cd, Pb, Cu, Cr) of the frequently used vegetables and fruits grown in high-risk Grama Niladhari (GN) divisions in Medawachchiya were targeted in the present study.

[1]Department of Chemistry, University of Kelaniya, Sri Lanka.

**Corresponding Author:** M.K.B. Weerasooriya, Department of Chemistry, University of Kelaniya, Sri Lanka.
Email: bandu@kln.ac.lk

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## MATERIALS AND METHODS

The research was carried out at the Department of Chemistry, University of Kelaniya, Sri Lanka during the period of 2018 May to 2018 December.

### Apparatus

Major apparatus used are Flame Atomic Absorption Spectrophotometer (FAAS)-GBC-SavantAA and Muffle furnace-Griffin electrical furnace.

### Sample collection

Fruits; mango and papaya Leafy vegetables; *Centella asiatica* (spade leaf), *Talinum fruticosum* (Jewels of opar), *Amaranthus cruentus* (Chinese spinach) and fruity vegetable; *Solanum torvum* (Turkey berry) samples were

collected from three Grama Niladhari division, named as Mahadivul Wewa, Puhudivula and Unagas Wewa in Medawachchiya, in North Central Province (NCP) of Sri Lanka. Five sites from each Grama Niladhari division (GN division) were selected. Samples were collected from ten home gardens at each site. Samples (n= 50) were collected at random locations within an area of 100 m to a nearby reservoir.

**Sample preparation**

Plant samples were thoroughly washed with deionized water, the consumable parts were dried under sunlight-until they got a constant weight. Then dried sample was ground well using an electric blender to get a fine powder, stored in plastic bags and freeze below -25°C till digestion.

**Digestion and metal analysis**

Sample (2.50 g) ashes in a muffle furnace, at 450°C for 4 h and allowed to cool. Deionized water (2.0 mL) was added, and the contents in the crucibles were evaporated to dryness. The remaining contents in the crucibles were again kept in a muffle furnace, ashes at 450°C for 4 h, and cooled. Thus, obtained ash was dissolved in HCl (6 M, 5.0 mL) and evaporated to dryness in a fume hood. Then, $HNO_3$ (0.1 M,15.00 mL) was added, covered, allowed to stand for 1 h, filtered and diluted to 25.00 mL with $HNO_3$ (0.1 M). A control experiment was also prepared without adding the plant sample (AOAC, 2002). Samples were analyzed for Pb, Cd, Cr and Cu using Flame Atomic Absorption Spectrophotometer (FAAS)-GBC-SavantAA and Muffle Furnace-Griffin electrical furnace. Each sample was analyzed in triplicates.

**Conditions for metal analysis**

Calibration standards for FAAS analysis were prepared by using analytical grade 1000 ppm solutions of Pb, Cd, Cr, and Cu. Samples were analyzed using air acetylene flame at a slit width of 0.2 nm for Cu, Cr, and Pb. Slit width 0.5 nm for Cd. Detected wavelengths were 324.7, 357.9, 228.7, and 283.3 nm for Cu, Cr, Cd and Pb respectively.

**Statistical analysis**

Statistical analysis was also carried out using Minitab 2017 software. Data were analyzed using Tukey pairwise comparison test.

## RESULTS AND DISCUSSION

Cu content of all the samples in three tested areas below the safe limit. As shown in Fig 2 reasonably high Cu content was observed in Turkey berry, Spade leaf, Chinese spinach, and Jewels of opar at Puhudivula and Ungas Wewa. However, the safe limit set for Cu (40 mg/kg) is well above the observed values (One-Way ANOVA, $p<0.05$). Hence, harm caused to Human beings due to Cu is negligible.

Cr content of Jewels of opar, Spade leaf, and Turkey berry in all three tested areas and Chinese spinach in Unagas Wewa exceed the safe limit for Cr (Fig 3). Cr content of Chinese spinach in Mahadiwul Wewa and Puhudivula and that of Mango in Mahadiwul Wewa and Unagas Wewa were closer to the safe limit. Cr content of Papaya in all three sites was also closer to the safe limit (One-Way ANOVA, $p<0.05$). However, literature evidence reports that elevated Cr levels in food cause no considerable damage to Kidney (Wilbur *et al*., 2012).

The highest level of Pb content which exceeds the safe limit was observed in Jewel opar at Mahadiwul Wewa. Apart from that Pb content in Chinese spinach of all 3 sites was higher and very close to the safe limit. Spade leaf of Mahadiwul Wewa and Puhudivula and Jewel of opar at Puhudivula also showed elevated Pb content, nearly closer to the safe limit. However, Pb content of turkey berries in Puhudivula is somewhat lower than the Pb content of turkey berries in Mahadivul Wewa and Uangas Wewa (Fig 4). Pb content of Papaya and Mango in all three sites was also not considerably higher (One-Way ANOVA, $p<0.05$).

As indicated in Fig 5 Chinese spinach, Jewels of Opar, Spade leaf, and Turkey berry samples in three tested areas were heavily contaminated with Cd exceeding the safe limit set by WHO. Cd content of papaya and mango was below the safe limit (One-Way ANOVA, $p<0.05$). Mango is a bigger tree with many branches. Absorbed Pb could be distributed all over the tree. Mango fruit is a very small part when compared with the size of the tree. Hence, a small portion of absorbed Pb would be seen in the fruit. Papaya is also a somewhat bigger plant concerning the other plants selected in the study. Papaya fruit is also smaller than the tree. Hence only a portion of absorbed Pb could be seen in the fruit.

All these findings revealed that tender leafy vegetables that grow as small plants were heavily contaminated with tested heavy metals Pb, Cr, and Cd. Turkey berry, a fruity vegetable of the small shrub-like plant also been

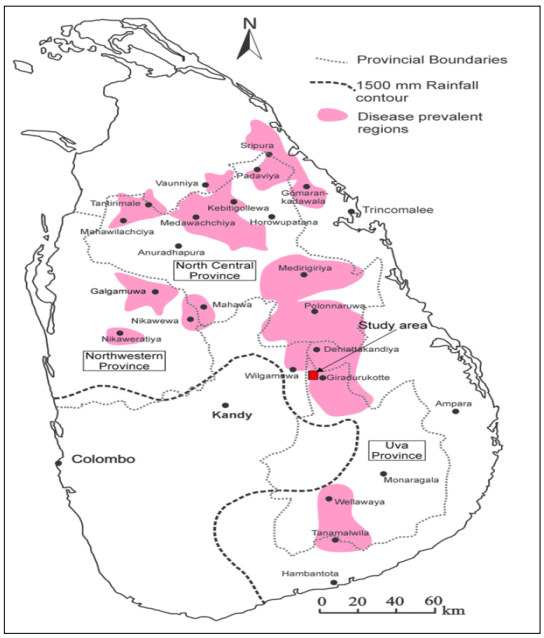
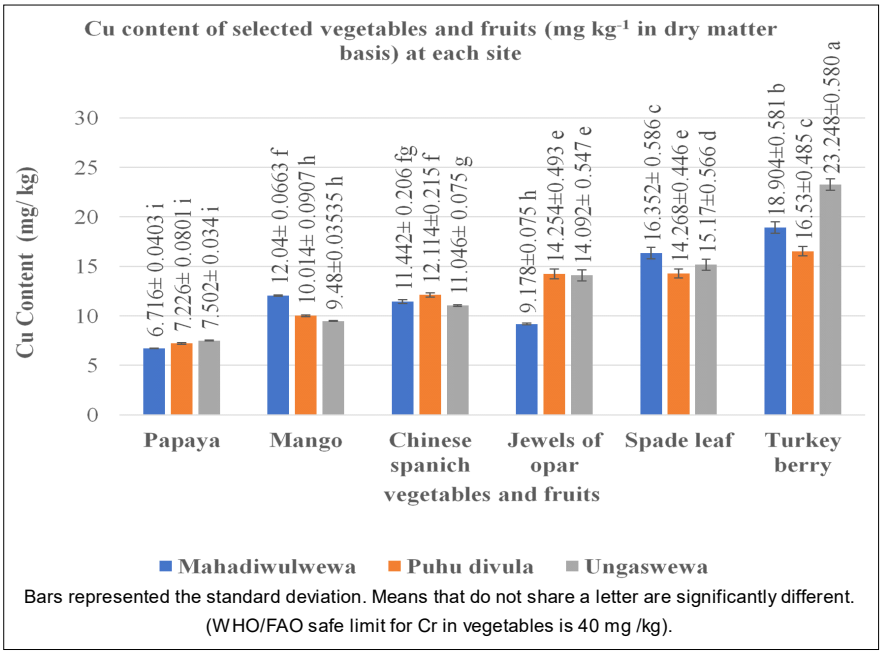



**Fig 1:** CKDu prevalence regions in Sri Lanka (Balasooriya *et al*., 2020).

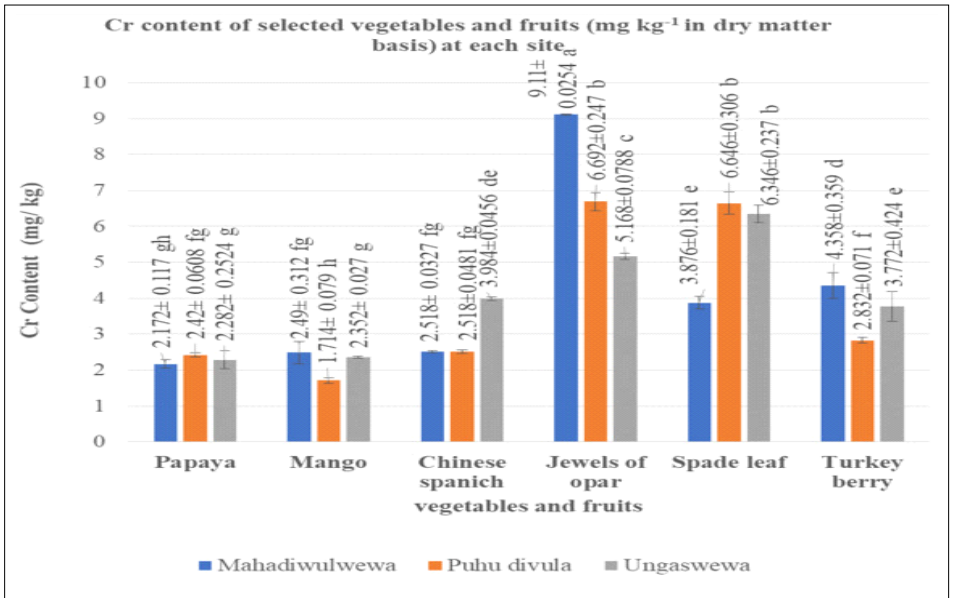
contaminated to a considerable level. Of these contaminants, Cr cause no considerable damage to Kidney (Wilbur *et al.*, 2012).

Mahadiwul Wewa, Puhudiwula, and Unagas Wewa selected in the present study were located, within a 100 m area of three reservoirs, which are used for farming. Bandara *et al.* (2008) also claimed elevated Cd and Pb levels in the soil at a 50 m area of another two reservoirs in Medawachchiya. In agreement with Bandara's work, in the present study higher-level Cd and Pb, which exceed WHO limit, were detected in leafy and fruity vegetables grown within a 100 m area of above three reservoirs located in CKDu-prone Medawachchiya. As reported in Bandara *et al.* (2009) average Cd level in cultivated vs. uncultivated soils in Medawachchiya, has been recorded as 0.02±0.01 vs. 0.11±0.19 mg/kg. As indicated by them Cd pollution




Bars represented the standard deviation. Means that do not share a letter are significantly different. (WHO/FAO safe limit for Cr in vegetables is 40 mg /kg).

**Fig 2:** Cu content in fruits and vegetables at CKDu-prone sites in Medawachchiya.

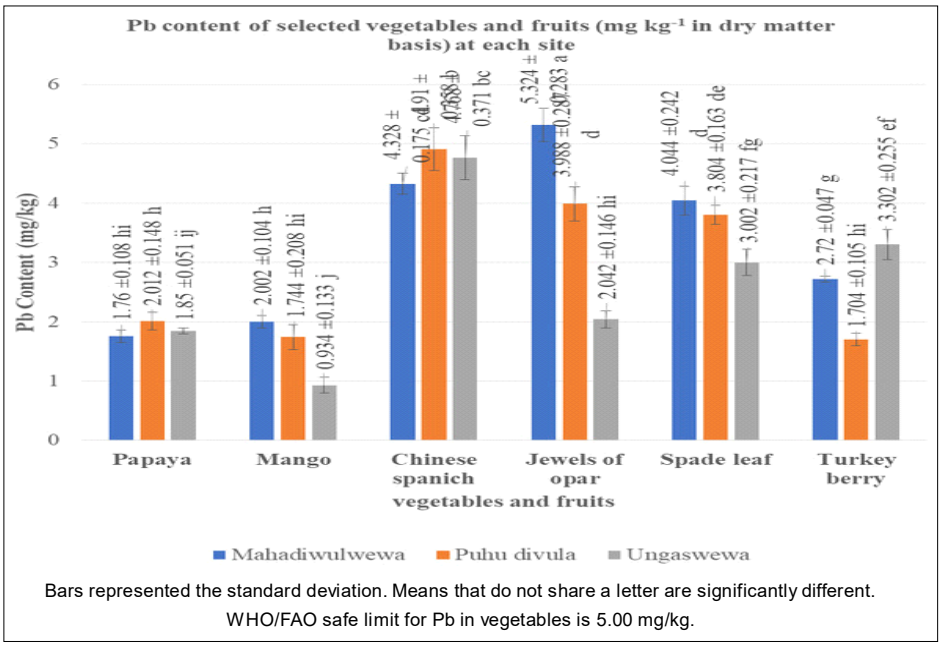



Bars represented the standard deviation. (WHO/FAO safe limit for Cr in vegetables is 2.3 mg / kg). Means that do not share a letter are significantly different.

**Fig 3:** Comparison of Cr content in fruits and vegetables among three study sites.

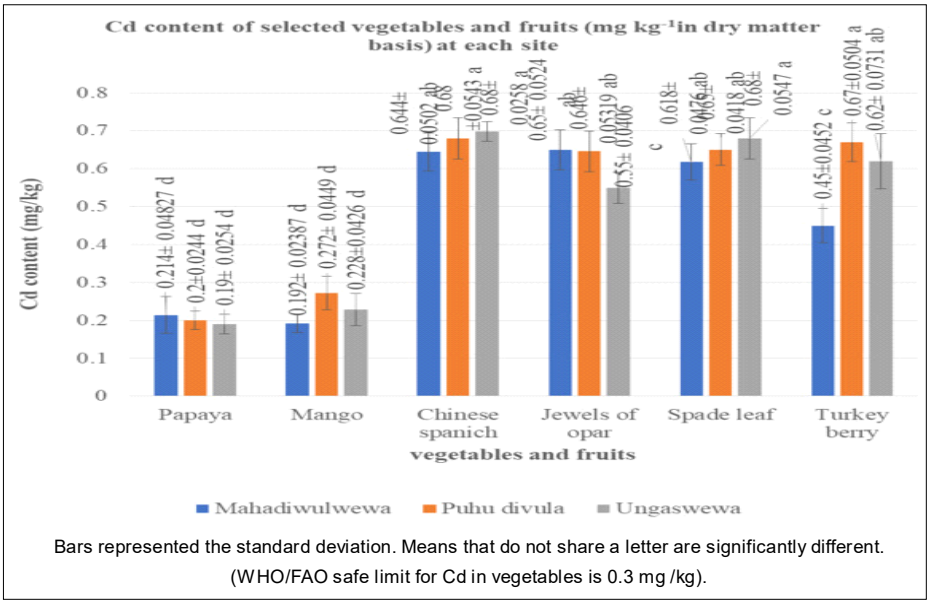
has occurred through contaminated phosphate fertilizers and pesticides used in agriculture. Cd level of Triple superphosphate (TSP) fertilizer has been reported as 23.5-71.7 mg /kg.

Chandrajith *et al*. (2009) also reported that the application of fertilizer in the long run led to a significant level of accumulation of Cd and Pb in agricultural soil in NCP. Compared with other heavy metals, Cd tends to be more mobile in the soil, can easily enter from soil to plant, and accumulate in cultivated crops and vegetables (Curtis and Smith, 2002). Bandara *et al.* (2009) also revealed that most human exposure to Cd occurs due to high levels of dietary Cd which comes through agriculture. Provisional tolerable weekly intake for Cd at all ages for rice, pulses, fish, breast milk, and cow's milk has exceeded the safe level recommended by Codex (2009). Acien *et al*. (2009) also revealed that renal dysfunction can often be caused due to chronic exposure to Cd. Renal tubular damage caused due to Cd is known to be irreversible. Chronic exposure to Pb is reported to cause hematological, cardiovascular, neurological and progressive tubulointerstitial nephropathy that leads to kidney failure (Acien *et al.,* 2009).




Bars represented the standard deviation. Means that do not share a letter are significantly different.
WHO/FAO safe limit for Pb in vegetables is 5.00 mg/kg.

**Fig 4:** Comparison of Pb content in fruits and vegetables among three study sites.




Bars represented the standard deviation. Means that do not share a letter are significantly different.
(WHO/FAO safe limit for Cd in vegetables is 0.3 mg /kg).

**Fig 5:** Comparison of Cd content in fruits and vegetables in three study sites

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Based on the survey we conducted, 19, 13 and 7 CKDu patients were recorded at Mahadiwul Wewa, Puhudivula and Unagas Wewa respectively. Of all the patients, 12 had both diabetes and hypertension. Ten had diabetes. Seven had hypertension. Others had no diabetes or hypertension. Supporting the above evidence Young *et al*. (2015) also suggested that Cd is a possible contributor to the prevalence of CKD and exerts a detrimental effect on the kidneys in diabetic or hypertensive patients (Young *et al*., 2015). Even at low Cd levels, chronic Cd exposure can lead to renal damage, particularly in patients who suffer from diabetes or hypertension (Akesson *et al.,* 2005). This risk may contribute to a synergic effect in the development and progression of CKD. Consequently, diabetic or hypertensive subjects become more susceptible to the diseased condition.

Bandara *et al*. (2008) also noted high levels of Cd pollution in water, freshwater fish, their staple diet rice and other food items such as milk and vegetables. Further, they pointed out that the existence of a high level of fluoride in ground water which is used as drinking water increases Cd absorption through the gastrointestinal tract as fluoride compounds. Apart from that, Cd absorption through the human gastrointestinal tract has been reported to increase, in the subject with low proteins and iron status (Bandar *et al.* 2008). Pre-school children (1-4 years) in the NCP are under-nourished and underweight with a Z score of 0.208 (Rathnayake and Weerahewa, 2005). Those children at a very young age are also highly vulnerable to the current crisis in the NCP.

As pointed out by Bandara *et al.* (2008), if the situation doesn't reverse human dietary Cd exposure continuously to increase, the diseased condition will be more prevalent in the next few decades, especially in high-risk groups with diabetes, hypertension, and those undernourished in protein and iron.

## RECOMMENDATIONS

**Early disease detection**

- **Screening**

The asymptomatic nature of early-stage kidney disease highlights the need for intensive screening. Detecting kidney issues early can prevent the acceleration of disease progression.

- **Potential biomarker**

The use of Cystatin C in urine as a biomarker for chronic kidney disease (CKD) has been suggested for dogs (Shivasakthi *et al*., 2019), with the possibility of adopting this approach for human CKD diagnosis after further research.

**Environmental remediation**

- **Reduce heavy metal toxicity**

Efforts should focus on rehabilitating contaminated environments, especially by regulating Cd levels and removing Cd from soil. Eco-friendly and low-cost alternatives for remediation, such as phyto-extraction, bioremediation and the use of nanostructured materials, are essential (Paul *et al*., 2021).

- **Bio-adsorbents**

Agricultural waste (*e.g*., rice husk, coconut husk, wheat bran, *etc*.) can be used as bio-adsorbents to effectively remove toxic metals, including Cd, from polluted water. These materials are cost-effective, readily available, and have proven to be effective in removing heavy metals (Monica *et al*., 2023).

- ***Moringa oleifera***

This plant has biosorbent properties that allow it to absorb heavy metals, making it a promising solution for both environmental and health sectors, especially due to its ease of cultivation and cost-effectiveness (Kanitta and Wanne, 2016).

## CONCLUSION

The escalating trend of chronic renal failure in the North Central Province of Sri Lanka is primarily linked to long-term dietary exposure to cadmium, especially from contaminated agricultural fertilizers. Immediate measures, such as early disease screening and environmentally-friendly remediation strategies, are necessary to curb this growing health issue.

Above recommendations offer a comprehensive approach to tackling the problem at multiple levels: Improving health screening and diagnosis, rehabilitating the environment, and exploring cost-effective, sustainable methods to reduce heavy metal exposure.

**Conflict of Interest**

No conflict of interest among all authors.

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