ORIGINAL RESEARCH

Potential Link between Spatial Variation and Translocation characteristics of Heavy Metals in Paddy topsoil and Human health risks in a CKDu prevalent area of Sri Lanka

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Abstract

The chronic kidney disease of unknown aetiology (CKDu) is confined to specific geographic areas of Sri Lanka. CKDu is a deadly disease that primarily affects farming communities, mainly male farmers. Due to the precise geolocation and geologically confined spread of this disease, continuing the CKDU research investigations, we investigated the geochemistry of the soil in connection with rice in affected areas. Previously, we showed the possibility that people living in CKDu endemic areas could be at risk of adverse health impacts from excessive Pb exposure, mainly through drinking food. Furthermore, if rice, the staple diet, carries high concentrations of Pb and other heavy metals and metalloids (such as As), these could also potentially bring about adverse health outcomes for people in the affected areas.

Our current study aimed to characterise and map the spatial distribution of heavy metal(loid)s of V, Cr, Mn, Co, Cu, Zn, As, Se, Cd, and Pb in soil and rice samples. We also measured other essential soil properties, such as pH, electrical conductivity (EC), and organic matter (OM) in topsoil in Medirigiriya of the Polonnaruwa District in Sri Lanka, one of the CKDu affected areas. To better understand heavy metals exposure through the food chain, we calculated the bioaccumulation factors and transfer factors of the heavy metal(loid)s distribution among plant tissues.

Covariance analysis showed an intrinsic heterogeneity of heavy metal distribution in the paddy soil. Spatial variation maps delineated the influence of irrigation and drainage water on the distribution of heavy metals in the study area. Results showed that rice plant roots were the primary tissues, which accumulated various heavy metal(loid)s. The distribution of heavy metal(loid)s in the rice plants' edible portions (grains) was much less than in roots. We used the transfer factor (TF) to assess the concentration of metal(loid)s transported from soil to rice plants. The TF for the rice leaf to grain (TF_{L-G}) was >1.0 for Cu and Pb in our study. We conclude that elevated Pb concentrations in paddy soils may be a factor in exposing people to harmful levels of this heavy metal over a prolonged period via the food chain, which can lead to chronic human health effects.

While this study does not provide evidence that Pb causes CKDu, further research is indicated to assess the effects of excess intake of Pb and ill-health. However, our previously published research had indicated that the Pb content in rice grain exceeded WHO's permissible limit for rice of 0.2 mg/kg. Since rice generated in these regions is transported throughout the country, health recommendations must be provided to everyone on how to process the rice for safe consumption. Based on the results, we propose implementing protective measures to reduce dietary Pb intake through rice and other means, to prevent adverse chronic health effects. We also recommend monitoring both the rice-growing topsoil and rice grains for contaminant metal accumulation, as a public health and harm prevention measure.

Keywords: Bio-accumulation, Chronic kidney disease, Heavy metal(loid)s, spatial variation, Transfer factor

Introduction

Trace elements, such as zinc (Zn), copper (Cu), manganese (Mn), selenium (Se) and cobalt (Co), can play a significant role in the maintenance of plant health and yield and human health (Rahman et al., 2014). For instance, Cu, Zn, and Mn in trace levels are essential for plants, while Co, Mn, Se and Zn are vital for human health (Alloway, 2013). However, cadmium (Cd), arsenic (As), mercury (Hg), and lead (Pb) are not biologically important to living organisms and, at excessive levels, can be toxic to living organisms, including humans (Naidu et al., 2015). Excessive trace element exposure via the environment could cause brain, liver, and kidney dysfunction.

Rice consists of a substantial portion of the Sri Lankans' diet (Senanayake and Premaratne, 2016). During the main cultivation season of 2016/17, the area of paddy cultivation in Sri Lanka was 531,440 ha, which produced 1,419,253 metric tons of rice (D.C.S., 2017). It was estimated that 1.5 million farm families are currently working in the paddy-based industries (Senanayake and Premaratne, 2016). Research indicates that rice, which forms the Sri Lankan diet's staple compared to other cereal crops, has a higher potential to accumulate heavy metals and metalloids (Arsenic, As) (Zhao et al., 2012, Rahimi et al., 2017).

In Sri Lanka and elsewhere in South Asia, rice is the primary staple food, contributing most to the total daily intake of calories. As the main staple food, there is also the possibility that rice may contribute to the ingestion of toxic elements from the diet. Studies have revealed that Sri Lankan rice grains contain the second-highest level of Cd (Meharg et al., 2013) and Pb (Norton et al., 2014) in South-East Asia.

A recent study has shown increased incidents of persons with chronic kidney disease of unknown aetiology (CKDu) in the endemic regions (Ranasinghe et al. 2019). CKDu is a chronic kidney disease without a connection to the usual causative factors (Cooray et al., 2019). Though causative factors for this nephropathy were not fully identified, long-term exposure to elevated amounts of nephrotoxic heavy metal(loids) like Cd, As, and Pb has been hypothesised to cause CKDu (Kulathunga et al., 2019). The main proposed route of exposure to those elements was through food and drinking water (Kulathunga et al., 2022). Hence, quantification of dietary intake of each element is important to manage heavy metal(loids)-induced chronic kidney disease.

Environmental factors such as hard water and socio-cultural factors, including heavy alcohol consumption and inadequate nutrition, can also contribute to and worsen nephropathy.

Physiochemical factors such as water hardness and Salinity level of 50% of water samples examined had elevated compared to safe drinking water standards (0.5 g/L for salinity and 250 mg/L for total hardness) of Sri Lanka (Gobalarajah et al., 2020). In addition, alcohol consumption was reported among 40% of CKDu-affected people (Gobalarajah et al., 2020). Abeywickrama et al. (2018) reported that a diet inadequate in micronutrients could result in various adverse health outcomes.

However, based on the research on dietary exposures to metals possibly linked to CKDu, Nyachoti et al. (2022) suggested that dietary exposure to low levels of As, Pb, Cd, and inadequate Se in staple grains cannot be linked to CKDu. However, it was hypothesised that the additive or synergistic effects of combination of many factors and parameters, even at levels lower than recommended regulatory levels, together with malnutrition, harmful behaviours and toxic pollutants, can cause this CKDu epidemic (Wimalawansa, 2016).

Heavy metals, especially Pb in agricultural soil, can pose a human health risk if plants take up bioavailable forms. Pb is ranked second in the list of most hazardous heavy metals after arsenic due to its potential toxicity and global occurrence (ATSDR, 2019). Pb in agricultural soils originates mainly from the indiscriminate use of agrochemical products and procedures. These include pesticides, herbicides, fertilizers and the addition of sewage sludge to soils (Markus and Mcbratney, 1996, Johansson et al., 2009, Burguera et al., 1988). If the Pb in the soil is taken up by plants, it can end up in the crops grown in those soils and enter the food chain (Tariq and Rashid, 2013, Naidu et al., 2015).

If these crops are good at accumulating the Pb in their tissues - leaves or grains- at times, they might end up accumulating Pb at concentrations higher than that found in soils. Such plants are called metal hyper-accumulators (Naidu et al., 2003). They are usually capable of growing in soils with high concentrations of metals and can absorb these metals through their roots and concentrate in their tissues (Naidu et al., 2003). If this happens, such crops might pose an increased risk of exposure to heavy metal contaminants to animals or humans who consume those plants or parts of those plants.

This can lead to many unwanted adverse health effects in exposed animals and/or humans. Therefore, to reduce such risks due to bioaccumulation of heavy metals, the *World Health Organization* (WHO) has recommended maximum allowable limits for such heavy metals. The *Codex Alimentarius*, the international food standards commission of WHO and FAO (Food and Agriculture Organization, United Nations), has set the maximum level of inorganic arsenic in rice to be 0.2 mg/kg for trade and health protection (Alimentarius, 2019).

Kulathunga et al (2022) reported that Pb content in rice grain exceeded the permissible limit for rice of 0.2 mg/kg by the WHO's Guidelines in the CKDu-affected areas in Sri Lanka. Furthermore, a study done in Padaviya Divisional Secretariat area paddy fields reported The minimum, maximum and mean lead levels in rice were 50, 790, and 118 µg/kg, respectively (Jayalal, 2020).

Young children are particularly prone to adverse effects of Pb as they absorb 4-5 times as much ingested lead as adults from a given source (Ortega et al., 2021). This can result in neuronal damage in children leading to behavioural problems, lower I.Q., hearing loss and other hearing disabilities (Calderon et al., 2001). In adults, Pb can cause kidney damage and high blood pressure. In pregnant mothers, excessive Pb intake can cause spontaneous miscarriages (Wijayawardena et al., 2016).

Generally, in unpolluted environments, ordinary crops do not bio-accumulate enough Pb to cause adverse human health outcomes (Rahman et al., 2014). However, in Pb-contaminated soil, the uptake of Pb by the roots and accumulation of Pb in rice tissues is significantly elevated, particularly in the edible parts (Ashraf et al., 2020). This shows the potential for eventual Pb contamination of rice grain from contaminated top soils. Unfortunately, paddy rice is the staple food of Sri Lanka and many other regions. Since rice is the staple food of Sri Lanka and many other south-east Asian countries, it is vital to maintain the topsoil (0-15 cm) free of contaminants to reduce adverse health impacts through rice consumption (Wang et al., 2021).

Enrichment of topsoil, with heavy metals, especially in agricultural areas can be considered a significant source of heavy metal intake by humans that could affect their health. Besides, it can also contribute to drinking water contamination and a reserve for plant uptake (Seshadri et al., 2016, Xie et al., 2013). Heavy metals, bound in soil particles could enter the human body through the food chain. Anthropogenic activities, such as mining and smelting, also release metals into the environment and contaminants into natural ecosystems (Coelho et al., 2016); (Jung, 2008). The excessive use of inorganic and organic fertilizers (Naidu et al., 2012), pesticides, and sewage water irrigation have also contributed to heavy metal(loid)s enrichment of soil in farmlands (Wimalawansa, 2016).

Long-term intake of toxic heavy metals through food and drinking water has been postulated as a possible cause of CKDu among paddy farmers living in Sri Lanka's North Central Province (Jayasumana et al., 2015b, Jayatilake et al., 2013, Wimalawansa, 2019)

Rice-growing soils function as the primary source of heavy metals accumulating in the edible part of the rice crop (Ashraf et al., 2017). Therefore, it is essential to evaluate the heavy metal content in paddy soils and its partitioning to the edible parts (rice seed). The translocation of heavy metals from soil to rice grain is essential to understanding how they might enter the human body. Information about the spatial variation of heavy metals in soils, and their distribution among plant tissues is sparse in the Sri Lankan context.

Since rice is the staple diet among Sri Lankans, rice contaminated with heavy metals could cause harm to people. However, no credible reports exist that rice in Sri Lanka is contaminated with heavy metals or arsenic. Elements, such as Zn, Mn, Ni, and Cu in rice-growing soil do not exist in excessive (toxic) concentrations and are unlikely to harm people, through ingestion. Meanwhile, higher concentrations of Zn and Se mitigate the toxicity of other substances. Elements, such as Cd and Pb have been reported to exist in higher concentrations at specific locations, but in Sri Lanka, these have not been linked to anthropogenic causes.

The analysis reports of drinking well water in CKDu-affected regions illustrated that toxic heavy metal(loid)s (Cd, As and Pb) content was lower than human health risk levels (Kulathunga et al., 2021, Kulathunga et al., 2022). However, water hardness, electrical conductivity (Kulathunga et al., 2022, Chandrajith et al., 2011, Jayasumana et al., 2015b), and fluoride level (Levine et al., 2016) were much greater in CKDu endemic areas than in unaffected areas. However, higher levels of heavy metals (e.g., Pb) have been observed in some food items eaten by affected people (Kulathunga et al., 2020), and even in CKDu patient's biological samples (Jayasumana et al., 2015a, Levine et al., 2016, Jayatilake et al., 2013).

Given the above, the present study was undertaken in a CKDu-affected area in Sri Lanka to measure heavy metal(loid)s content in the topsoil and various parts of rice plants with field sampling. The objective was to understand the variation and concentrations of metal(loid)s in the areas sampled and the potential of those to bioaccumulate in rice.

Materials and Methods

Study area and soil sampling

This study was conducted in the Medirigiriya Divisional Secretariat area (Figure 1) in the Polonnaruwa District of Sri Lanka, extending from 7^0 40' – 8^0 21'N and 80^0 44' – 81^0 20'E with an area of 3337.9 km^2. This region reported the highest number of CKDu patients in 2017, according to the medical records and databases of the provincial Polonnaruwa District hospital. The Medirigiriya area in the District is characterised by wet and dry seasons. The north-eastern monsoon (December to February) brings higher rainfall. Annual rainfalls, typically ranging from 1000-1500 mm, and the area experiences a maximum temperature of 36 ^{0}C and a minimum of 24 ^{0}C. The main cultivated crop is rice paddy, which covers about 14,000 ha of land. Three main reservoirs supplying this area are the Minneriya, Kawdulla, and Ambagaswewa tanks, which primarily provide irrigation water.

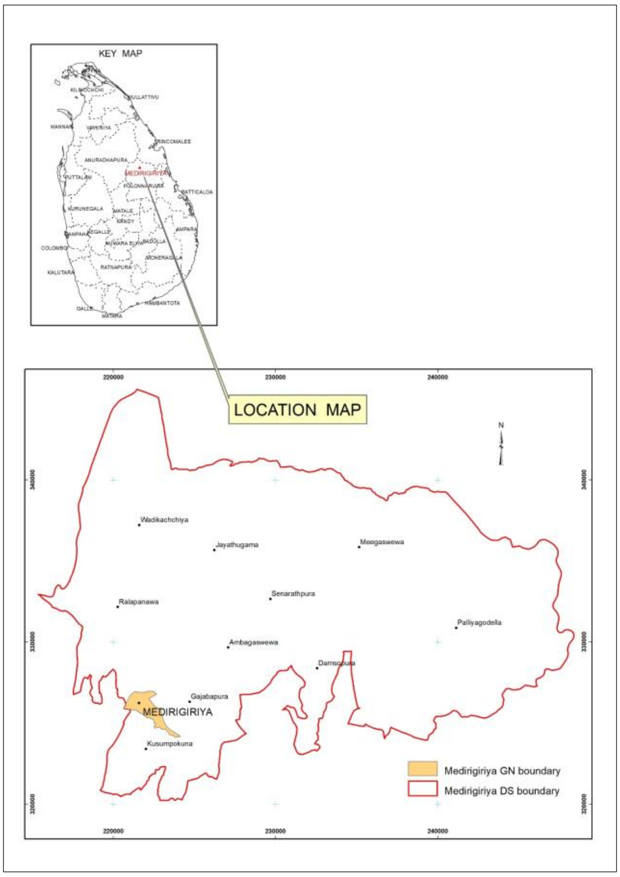

Figure 1. A schematic map of the study area

For the study, 76 soil samples were collected randomly from cultivated rice lands during March to May 2017. Using a clean stainless steel soil auger, soils from a depth of 0-15 cm were collected and put into clean polyethylene bags. Since rice is a shallow-rooted crop, soil samples from a 0-15 cm depth were collected in this study. Then, labelled samples were, transported to the soil laboratory for analyses, via sealed containers to avoid contamination.

The geographical positioning of the sampling points was recorded using Magellan eXplorist G.P.S. for spatial mapping purposes. The soil samples were homogenised by thoroughly mixing in a plastic tray and separate portions of soil samples were stored in plastic bags for processing. Soil samples were air-dried for several days in ambient conditions, and then the samples were crushed and ground using a porcelain mortar and pestle. The samples were passed through a 2 mm mesh stainless sieve to remove gravel and plant debris.

Transfer factor calculation in rice crop

As with the soil samples, mature rice plants with flowering heads and filled grains were also collected from three random locations in the study area. Rice plants were separately packed in clean polythene bags and transported to the laboratory for sample preparation and analyses. Rice plants were washed thoroughly using clean tap water several times to remove soil particles, and distilled water was used in the final washing.

Each rice plant was separated into the root, sheath, blade, and rice grains. The total wet weight of plant parts was measured. Then, the plant parts were oven-dried for 24 hours at 100 ^{0}C. Dried plant portions were ground to fine particles using a stainless-steel grinder. Rice husks were removed mechanically, and the grains were ground into powder using a stainless-steel grinder. The heavy meta concentrations in various plant parts were analysed to determine the transfer factors (TFs) from roots to the leaf sheaths, sheath to leaves and leaves to grains.

Chemical analysis of soil and plant samples

Homogenised soil samples were analysed to determine electrical conductivity (EC), organic matter content (OM), pH (1:5 soil: water ratio) and trace metal contents in the soils. Soil pH and EC values were obtained using a Horiba PC 1100 benchtop pH/conductivity meter. Organic matter content was determined using a Leco Trumac CN determinator, and the total carbon content was multiplied by 1.72 to estimate the organic matter content.

After microwave acid digestion, the total amount of heavy metals in all the samples was determined. A quantity of 0.5 g of soil was weighed in pre-acid cleaned digestion tubes, and 5 mL of an acid mixture of trace metal analytical grade HCl and HNO_3 (3:1) was added for acid digestion.

Following the microwave digestion, the contents of the digestion tube were transferred to a polyethylene vessel. The digestion tube was rinsed several times with Milli-Q water and collected the rinsed solution to the same polyethylene vessel and diluted up to 50 ml by adding Milli-Q water.

Similarly, for plant materials, 0.5 g was added to acid-washed glass digestion tubes, and block digestion was completed according to the procedure described by Smith et al. (2008). Aliquots were filtered using a 0.45 µm syringe filter, and the trace metal analysis was performed using Inductively Coupled Plasma Mass Spectrometry (ICPMS 7900, Agilent Technologies, Japan).

Statistical data analysis

Descriptive statistical analysis of means and standard deviation were obtained for analytical results of the trace metals. Principal component analysis was used to evaluate the interrelationship between metal concentrations and paddy soils' characteristics.

Bioaccumulation factors (BAFs) were calculated using established procedures, i.e. by dividing the elemental concentrations of the plants by the respective elemental concentrations of each heavy metal found in the soil (Satpathy et al., 2014, Rahimi et al., 2017). Transfer factors (TFs) were calculated by the elemental concentration in the rice grains divided by the respective elemental concentration in the plant roots, sheaths or leaves (Satpathy et al., 2014, Rahimi et al., 2017).

Results and Discussion

Soil chemical characteristics

The study area's soil pH (1:5 soil: water) ranged from a quite acidic pH of 5.2 to a strongly alkaline pH of 8.4, with a geometric mean of pH 6.6. Part of the paddy lands in the selected area was not cultivated in the season (before sampling) due to the unavailability of irrigation water. Paddy soil, when submerged, becomes alkaline (Khaokaew et al., 2011, Chandrajith et al., 2010), and paddy soil pH changes depending on the wet and dry conditions encountered (Carrijo et al., 2017).

In submerged conditions, soil pH could increase by as much as 2.0 units. The alkaline conditions may decrease heavy metal solubility (Kashem and Singh, 2001, Jayawardana et al., 2014). Similar average pH values have been reported by (Sirisena et al., 2008), i.e., 6.8 and 6.3 (5.8-7.2) (Nayanaka et al., 2010) in similar geographical areas. Looking at the published literature on Sri Lanka, the pH variation found in our study is reasonably typical (Jayawardana et al., 2014, Sirisena et al., 2008).

Soil EC value varied between 0.02 and 0.88 dS/m with a geometric mean of 0.1 (mean 0.13) dS/m in the study area. A study of paddy soil in the Polonnaruwa district demonstrated that the EC value ranged from 0.03-0.16 dS/m with an average of 0.06 dS/m (Nayanaka et al., 2010).

Thus, the Medirigiriya study area had a higher soil EC value than other parts of the Polonnaruwa District. The soil salinity level is classified according to EC values (Smith and Doran, 1996), and a recording below 2.0 dS/m is denoted as non-saline soil. Therefore, paddy soils in the study area were in the non-saline category.

The organic matter (OM) content of paddy soils ranged from 0.5 to 4.6%, with a mean value of 1.5%. A previous study reported the mean OM content of 1.77% (range of 1.01-2.82%) in paddy soils located in the Polonnaruwa District (Nayanaka et al., 2010). Organic matter contents in the range of 2-4% are typical values associated with such soils.

The heavy metal(loid)s concentration of the cultivated paddy soils decreased in the following order: Mn> V> Cr > Zn> Cu> Co> Pb> As> Se, and Cd (Figure 2). The highest concentration was for Mn (801.92 mg/kg) and the lowest was for Pb (36.96 mg/kg). Pb, in particular, was low across the area and its mean concentration was 3.09 mg/kg.

Mn, Cu, Zn, Co, and Se are micronutrients for plants, including crops, whereas Cd, As, and Pb are considered toxic-heavy metals for crops and animals. The maximum allowable concentrations for agricultural soil published for Cr, Cu, Zn, As, Cd, and Pb (Alimentarius, 2019) were 300, 100, 250, 25, 0.5, and 80 mg/kg, respectively. Therefore, the metal(loid)s levels in this area were well below the permissible levels for agricultural lands (Figure 1).

Our study revealed that the soils in the studied areas are reasonably low and acceptable for agricultural purposes from the viewpoint of the heavy metal(loid)s concentrations. Previous investigations of soil heavy metals content in CKDu endemic areas have reported Mn-283, Cu-21, Cr-23 mg/kg (Chandrajith et al., 2011), Pb-10, As-7.32, and Zn-24 mg/kg (Levine et al., 2016).

Accordingly, the concentration of heavy metals in the Medirigiriya study areas' paddy soils was considerably lower than in other parts of CKDu endemic areas on the island.

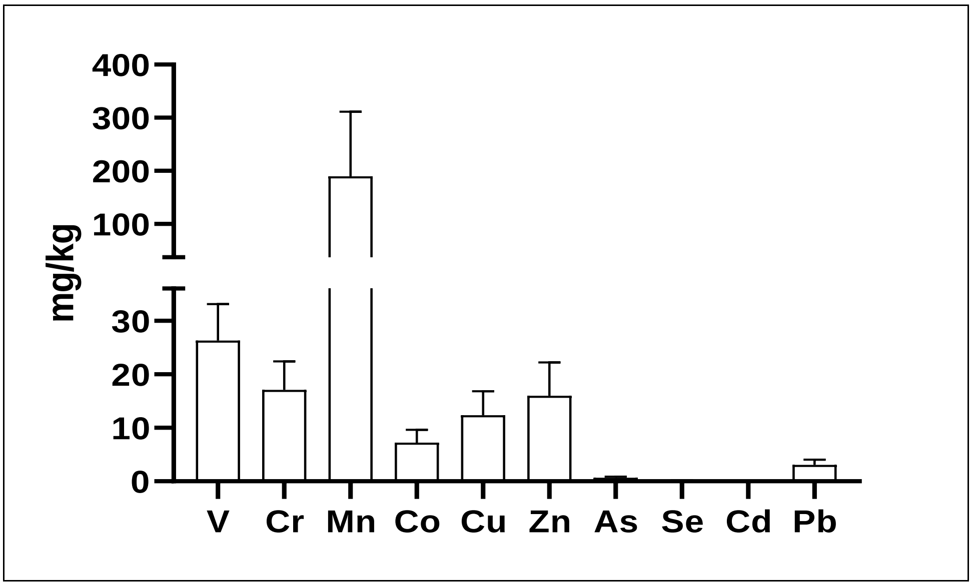

Figure 2. Paddy soil's heavy metal(loid)s content found in the study

Geographical variation in paddy land heavy metal(loid)s contamination

There was considerable heterogeneity in soil properties and metal(loid) concentrations among the study sites. One of the most consistent parameters is pH, with a coefficient of variation (CV) of 6% (pH). In contrast, the CV for EC was 103% (EC). The CVs for the series of heavy metal(loid)s measured ranged from 20-70%. To characterise how this variation in parameters and concentration varied spatially, interpolated maps were generated for each soil parameter using the geostatistical analytical tool ArcGIS Version 10. The results are presented in Figures 3, 4, and 5.

pH, EC and Organic Matter

Most soil samples in the study area had pH in the 6-7 range [Figure (3a)], with only relatively small areas outside this range. The low CV of pH proves this spatial consistency. Since most paddy fields were in the desirable pH for rice cultivation, one can assume that irrigation and other cultural management practices have exerted only a minor effect on the pH of the study area.

Heavy metal solubility in the soil is controlled to a large extent by pH compared to organic matter (Carrijo et al., 2017). For instance, heavy metal sorption studies showed that desorption of Cd and Pb declines when pH rises above pH 5 (Jiang et al., 2012, Loganathan et al., 2012, Zeng et al., 2011) and. As solubility decreases with increasing pH in the paddy cultivated soil (Signes-Pastor et al., 2007, Zhao et al., 2012)).

The spatial distribution of ionic concentrations (EC) in the paddy soils [Figure 3 (b)] showed a very evident spatial variation, with a relatively high CV. The EC of the study area increased from the proximity to the major irrigation tanks to places far from the irrigation tank. It is probable that irrigation and drainage, followed by high evaporation, caused the salt to concentrate in paddy lands some distance from the major irrigation tank.

The spatial variation of organic matter (OM) ranges from 0.28 to 4.62% in the study area [Figure 3(c)]. Typically, dry zone soil contains low OM in cultivated land. A study in the Polonnaruwa district reported that paddy soil OM content ranged from 0.09-2% (Sirisena et al., 2008). Further, a mean OM content of 5.8% was reported in a study of CKDu endemic areas in Sri Lanka (Balasooriya et al., 2022).

Relatively high levels of OM have been reported from other locations in Sri Lanka. For instance, in the intermediate zone (Kurunegala District), paddy soil OM varied in the range of 1.7-2.5% (Sellathurai et al., 2015) and in the wet zone (Kalutara District), paddy soil contained 7.2% of OM (Bandara, 2002, Chandrajith et al., 2005). Given that various cultivation practices influence rice paddies, OM contents tend to be highly variable (Sellathurai et al., 2015). Soil organic matter is a valuable property that controls the bio-availability of heavy metals (Hamid et al., 2018). In general, OM can form complexes with heavy metal(loid)s and increase their retention, thereby reducing bio-availability (Alloway, 2013).

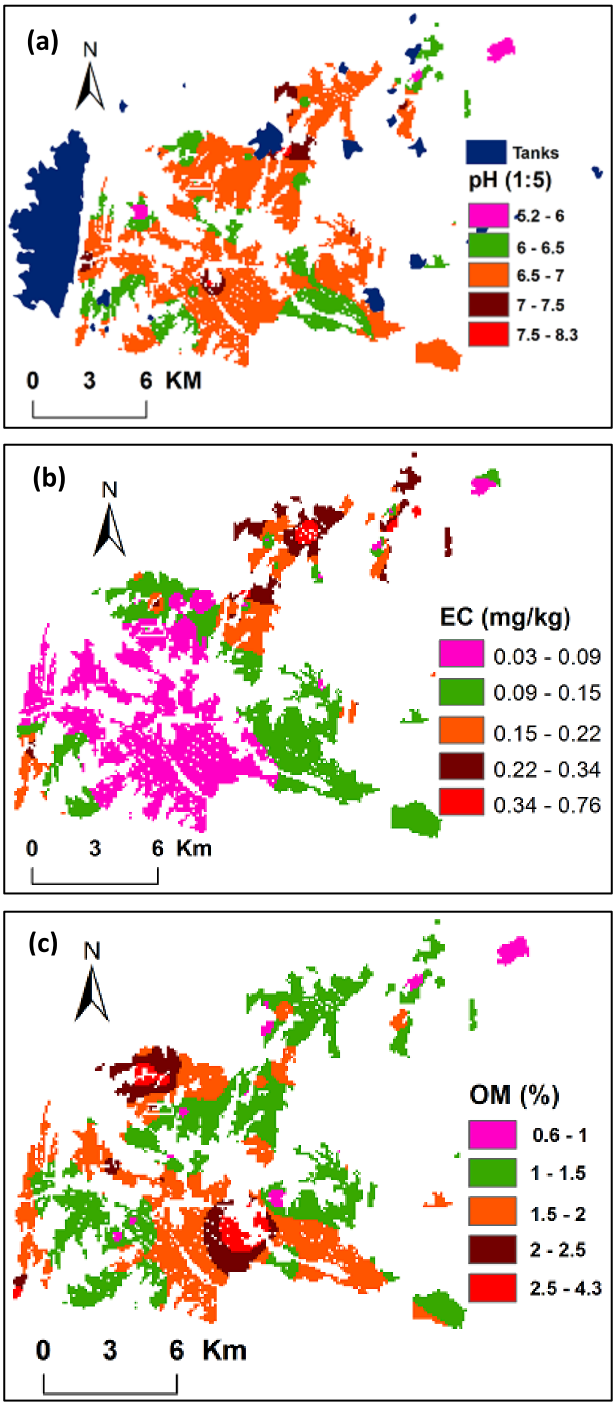

Figure 3. Spatial distribution pattern of measured parameters in the paddy soils (a) pH, (b) EC, and (c) Organic Matter (OM)

The relatively low OM content in soils in the study area can lead to the removal of measured trace metals through runoff and leaching in the soils. However, OM can also be involved in supplying heavy metal(loid)s to the soil solution, which may act as chelates and increase the heavy metal solubility in the soil solution and thereby the bioavailability of heavy metal(loid)s to the plants (Vega et al., 2004, McCauley et al., 2009)

Metals and metalloids

Environmental exposure to heavy metals has been a significant factor that was suspected to be contributing to CKDu (Kulathunga et al., 2022, Balasooriya et al., 2022). Although some essential metals such as Fe, Zn, Mn and Cu are essential for the growth of rice plants, other heavy metal(loid)s such as Cd, Cr, Pb, Hg and As are toxic and can accumulate in rice grains as well and can potentially cause adverse health risks to humans through consumption of rice and rice-based food (Diyabalanage et al., 2016).

Even essential plant nutrients like Zn, and Cu can be detrimental to plants and humans when they are present in concentrations higher than certain levels, as the excess amounts of these heavy metals can interfere with normal metabolic activities in the cells (Lidon and Henriques, 1993, Borkert et al., 1998). Therefore, paddy soils in hotspots were analysed for a series of heavy metal(loid)s, including V, Cr, Mn, Co, Cu, Zn, As, Se, Cd, and Pb.

Covariance analysis showed an intrinsic heterogeneity of paddy soil heavy metal(loid)s. The spatial variation maps (Figures 4-5) delineated the influence of irrigation and drainage water on the distribution of heavy metals in the study area. The following provides a summary of salient observations:

- The spatial distribution of V is presented in Figure 4(a). The average V concentration in the paddy soils was 24-28 mg/kg (range 28-33 mg/kg). The V spatial distribution was scattered in the paddy fields of the study area.
- The Spatial variation characteristics of Cr [Figure 4b] shared a similar distribution pattern as V. Higher Cr concentrations occurred sporadically, but in the south-east part. In contrast, a comparatively low Cr concentration distribution was evident in the north-east part.
- Compared to the spatial variation of V and Cr, Mn concentrations and spatial distribution (Figure 4c) showed low variation. Similar to EC, Mn tended to be distant from major irrigation tanks. It is possible that irrigation water transported Mn to distant paddy lands.
- The spatial variation of Co [Figure 4(e)] showed a higher concentration in the south-east direction and some notable patches found in the middle and north-east parts of the study area. Therefore, Co distribution is more heterogeneous.
- Unlike Co, the spatial distribution of Cu [Figure 4(h)] was less heterogeneous. Most of the irrigated land was characterised by 12-16 mg/kg concentrations of Cu. This lack of variation could

be more due to geographical origin than crop management practices.

- According to Zn spatial distribution pattern [Figure 4(i)], a higher concentration area (17-22 mg/kg) occurred in the south-east part, some distance from the main irrigation tank. Small patches of high concentrations of Zn existed in the middle and north sections of the study area.

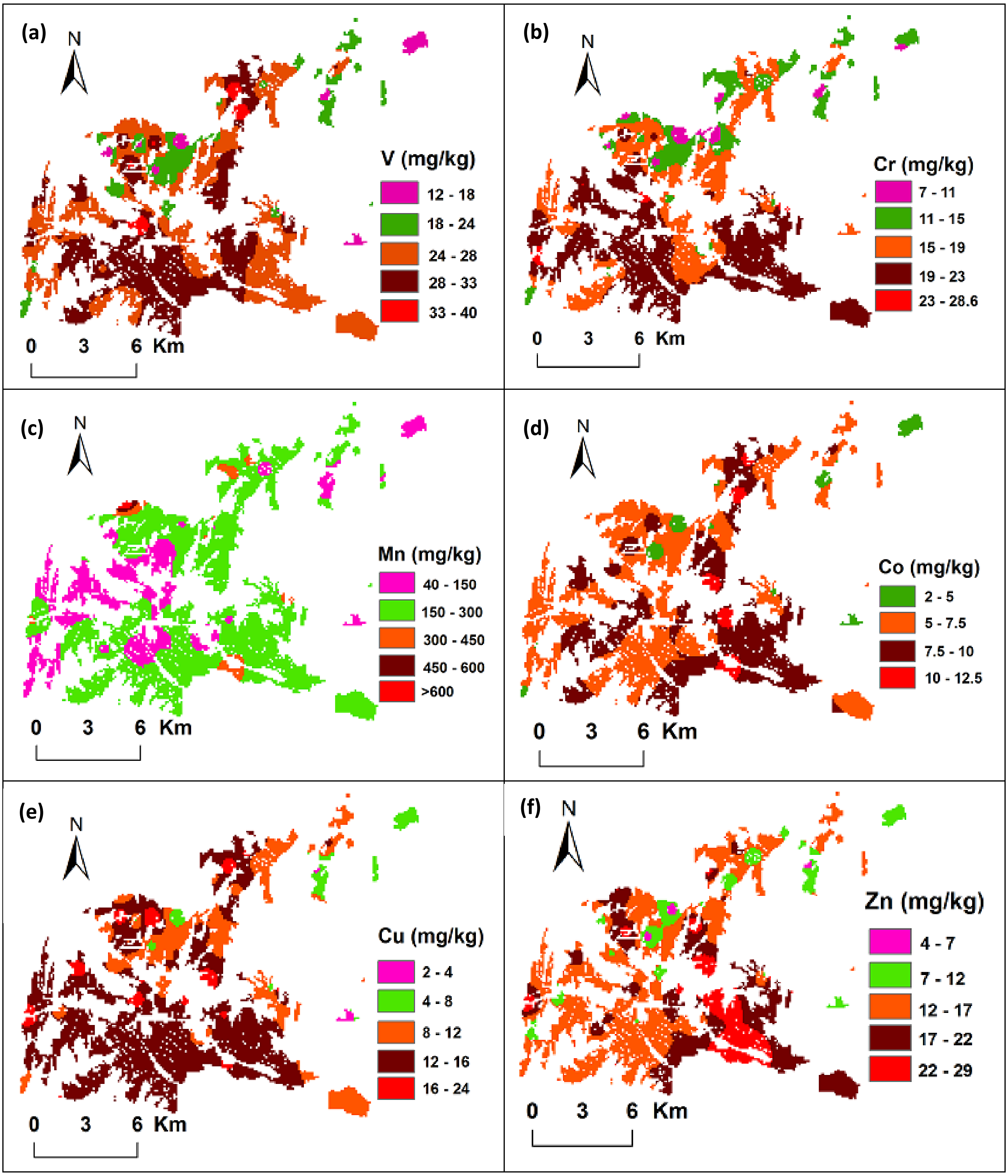

Figure 4. Spatial distribution pattern of measured heavy metals in paddy soils: (a) V (b) Cr (c) Mn (d) Co (e) Cu and (f) Zn

The spatial distribution of As [Figure 5(a)] also showed the As content increased further away from the main irrigation tank, mainly toward the south-east direction. The spatial distribution of Se [Figure 5(b)] has more spatial heterogeneity.

A low concentration level is observed in the north-east study area, while high concentration patches can be observed close to or further away from the main irrigation tank. Therefore, Se spatial distribution was not influenced by irrigation.

More Cd concentrated paddy lands were in the south part [Figure 5(c)] than the north. Lead [Figure 5(d)] was more evenly distributed in the study area, and most paddy lands contained Pb in amounts ranging from 2.5 to 3.5 mg/kg.

Previously published research carried out in dry zone area reported the Pb content in paddy cultivated lands were in the range of 2.9-9.3 mg/kg (mean Pb content 6.05 mg/kg) and vegetable cultivated lands were in the range of 3.29-10.35 (mean 7.75 mg/kg) (Rosemary et al., 2014). Such results were higher concentrations than in our study. Paddy soil samples obtained from CKDu endemic areas have found Pb content in the ranges of 1.52-7.71 mg/kg (mean Pb content of 3.76 mg/kg) (Balasooriya et al., 2022).

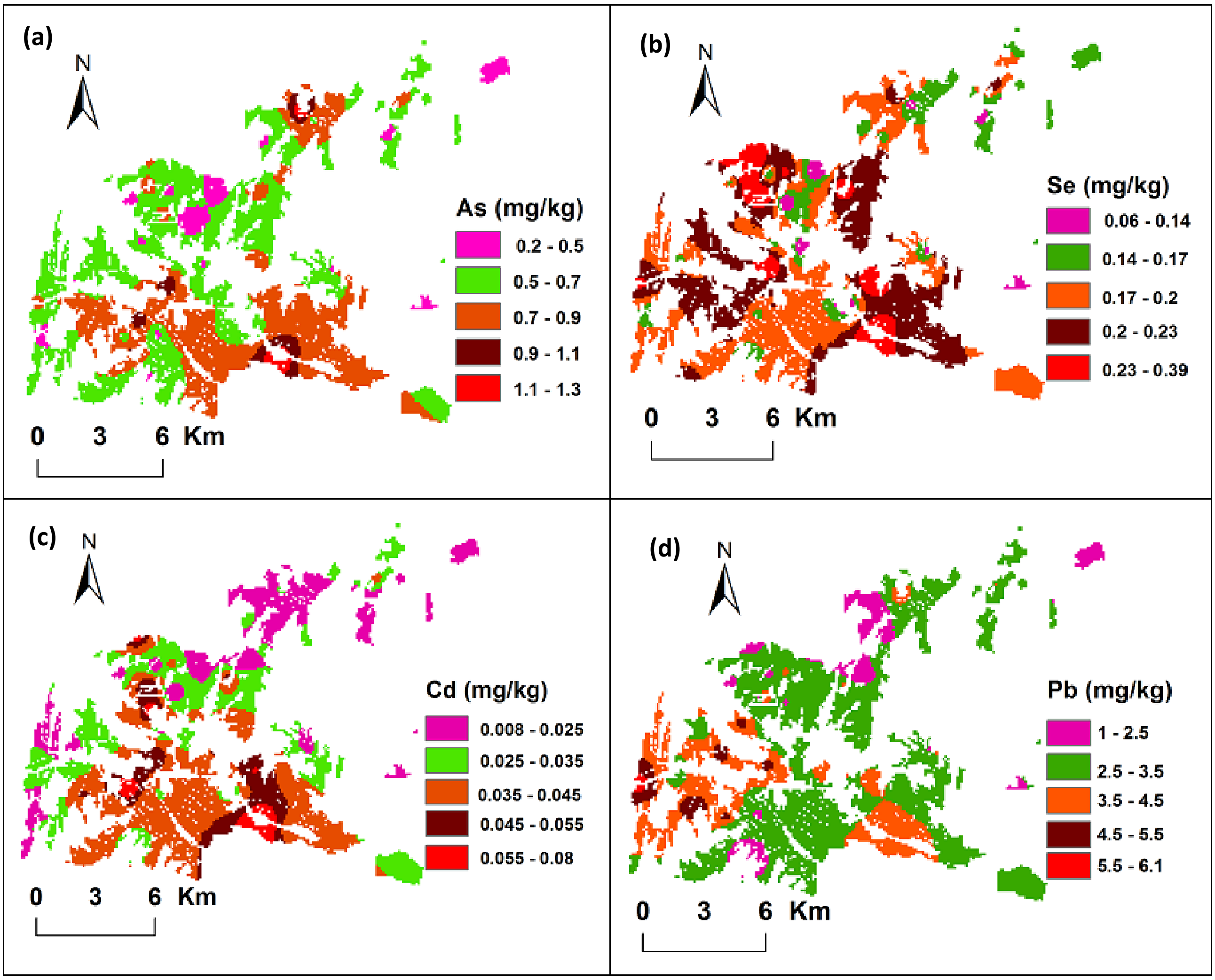

Figure 5. Spatial distribution pattern of measured heavy metals in paddy soils: (a) As (b) Se (c) Cd and (d) Pb

Heavy metal(loid)s transfer characteristic in paddy field

The bioaccumulation factor is a critical measurement of a plant's ability to acquire a particular metal from its growth media (Payus and Talip, 2014, Hadif et al., 2015, Tang et al., 2019). It also quantifies the bioavailability of that metal (Rahimi et al., 2017). When the plant exhibits a BAF ≥ 1.0, this indicates the hyper-accumulation of the specified metal (Callahan et al., 2006).

Calculated BAF values for heavy metal(loid)s of V, Cr, Cu, As, Se, Cd, and Pb were 1.56, 1.5, 2.77, 6.86, 2.52, 7.23, and 1.15, respectively. For all metal(loid)s, the BAF values were greater than 1.0. Therefore, it is possible to conclude that the paddy variety cultivated in this area could be hyper-accumulators for these metal(loid)s.

Higher BAF values for rice crops have been reported in South Africa for Cd (4.12), Cr (4.00), Pb (1.28), and Cu (1.03) (Payus and Talip, 2014). The observed higher BAF values in this study were mainly due to higher metal accumulations in the roots of the rice plants. This hyper-bioaccumulation of heavy metal(loid)s due to the rice roots' ability to retain metals, possibly through chelation and adsorption (Du et al., 2018).

Table 1. Transfer Factor (TF) of Heavy Metals and Metalloid (As)

Parameters	V	Cr	Cu	As	Se	Cd	Pb
TFR-S	.004	.33	.24	.01	.07	.2	.3
TFS-L	1.91	2.45	4.25	3.45	1.92	.63	2.34
TFL-G	.11	-	1.33	.35	.53	-	1.29

TF_{R-S} = transfer root to the sheath, TF_{S-L} = transfer sheath to leaf, TF_{L-G} = transfer leaf to grain

Transfer factor for root to leaf sheath (TF_{R-S}) of rice plant was <1 for all metals and followed the ascending order as follows: V < As < Se < Cd < Cu < Pb < Cr (Table 1).

However, except Cd, other measured heavy metals exhibit a greater transfer factor (>1) for the sheath to leaf (TF_{S-L}) (Table 1). Thus, the absorbed heavy metal(loid)s were highly mobile within the plant. In contrast, the transfer factor for the leaf to grain (TF_{L-G}) was lower than one, except in Cu and Pb (Table 1). Further, Cr and Cd did not show any detectable transfer to the rice grain. Cu, Pb, and Se had superior accumulation ability in the study area's rice grains.

Studies in Sri Lanka (Diyabalanage et al., 2016), including our study published in a companion paper (Kulathunga et al. 2022), have indicated the Pb content in rice grain tend to exceed WHO's permissible limit for rice of 0.2 mg/kg (Alimentarius, 2019). Lower transfer factors for V and Cd among plant tissues would limit their mobility inside the rice plant, reducing the risks of those metals entering the human body via eating rice.

The TF of heavy metals and the heavy metalloid AS varied in the order of TF_{R-S} < TF_{L-G} < TF_{S-L} in this study and was consistent with other research findings (Udousoro et al., 2013, Rahimi et al., 2017, Tang et al., 2019). The transported heavy metals within the rice plant were largely localised at the nodes on the stems, and leaf sheaths, which resulted in a reduction of transport to grains from leaf and sheath, consistent with Song et al. (2014). Further, the efficacy of heavy metals accumulation differed among different organs of the rice plant (Su, et al., 2010). Those elements may be the reasons for the variation of TFs.

Spatial distribution of heavy metals and relationship with CKDu

People living in CKDu-affected areas consume locally grown rice throughout their lives. This study examined the spatial soil characteristics of paddy soils and heavy metal(loid)s transfer characteristics to the edible part of rice to understand the possible implications for the spread of CKDu disease. An important observation of this study was the greater translocation ability of Pb to rice grain compared to other measured heavy metals.

The greater contribution of rice to the total daily intake of Pb (Kulathunga et al., 2021) was evident in the greater BAF for Pb reported in this study. This analysis should be considered the first of its kind, providing an entry point for identifying the importance of heavy metal(loid)s and their possible linkage to CKDu prevalence in an affected geographical area in Sri Lanka. Further studies are underway to investigate whether there is a potential link between heavy metals and metalloids (such as Arsenic) in the prevalence of CKDu.

Knowledge of the spatial variations of heavy metal(loid)s in topsoils of different rice-growing areas, and the extent of contribution to rice grans could provide a base for developing mitigation strategies to lower health risks through the food chain.

Conclusions

The heavy metal and As concentrations of the paddy topsoil in the study area were well below the maximum allowable limits of agricultural soils. Our study results demonstrated that Cd was the least abundant metal, while Mn was the most abundant in the paddy soil of the study area. The spatial assessment revealed that the north-east and south-east sections of the paddy fields, which are furthest from the main irrigation tank, have a higher concentration of heavy metals.

This difference in heavy metal distribution, based on the unique variation of the study area, may be influenced by factors such as land use pattern, irrigation, agrochemical application, or drainage. The present study was limited in scope and was, therefore, unable to discern the influence of such factors. According to the results for BAF and TF, the root of a rice plant can absorb and retain much of the heavy metal(loid)s. Relatively small amounts are translocated to other tissues, including the grain.

Among toxic metals, Pb can be more easily translocated to edible parts. Therefore, there is a possibility that rice cultivars grown in Sri Lanka may be more able to translocate Pb into the grain, which, in turn, may cause health risks. However, this aspect needs to be more thoroughly investigated.

In our study, the TF for the rice leaf to grain (TF_{L-G}) was >1.0 for Cu and Pb. It means that both Cu and Pb, absorbed by rice plants through the puddled soils, would be easily transferred and bio-accumulated in the grains. It should also be noted that Pb content in rice grain exceeded the permissible limit for rice of 0.2 mg/kg by the WHO's Guidelines (Alimentarius, 2019).

There are many factors postulated in previous studies of this research group and by many other researchers as potential causative factors of CKDu. Chronic exposure to heavy metals, alcohol consumption, malnutrition, and hardness of water can result in additive or synergistic effects, possibly contributing to CKDu. Since this disease can potentially be triggered by the collective effect of multiple factors, CKDu could occur even when the chronic exposure to multiple elements, but at below the levels than recommended individual elements.

Considering the above, necessary health advice should be issued to reduce the exposure to CKDu risk factors in affected regions. For example, health advice can be issued on how to process the rice for safe dietary intake and how much to consume to avoid any adverse health effects from excessive exposure to Pb through rice consumption. Therefore, periodic monitoring of the heavy metal concentrations in soil, as well as in rice grains is recommended to reduce future health risks.

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References

Abeywickrama, H. M., et al. (2018). Micronutrient Status in Sri Lanka: A Review. *Nutrients,* 10**:** 1583.

Alimentarius, C. (2019). *General Standard for Contaminants and Toxins in Foods*. (**https://www.fao.org/fao-who-codexalimentarius/thematic-areas/contaminants/en/)** (Accessed 30 May 2022).

Alloway, B. J. (2013). *Heavy Metals in Soils; Trace Metals and Metalloids in Soils and Their Bioavailability.* Springer Netherlands.

Ashraf, U., et al. (2017). Lead (Pb) Toxicity; Physio-Biochemical Mechanisms, Grain Yield, Quality, and Pb Distribution Proportions in Scented Rice. *Frontiers in Plant Science,* 8**:** 259 (**https://doi: 10.3389/fpls.2017.00259**).

Ashraf, U., et al. (2020). Lead (Pb) Distribution and Accumulation in Different Plant Parts and Its Associations with Grain Pb Contents in Fragrant Rice. *Chemosphere,* 248**:** 126003 (**https://doi:org/10.1016/j)**.

ATSDR. (2019). *ATSDR'S Substance Priority List*. (**https://www.atsdr.cdc.gov/spl/index.html**) (Accessed 24 May 2022) .

Balasooriya, S., et al. (2022). Major and Trace Elements in Rice Paddy Soils in Sri Lanka with Special Emphasis on Regions with Endemic Chronic Kidney Disease of Undetermined Origin. *Environmental Geochemistry and Health,* 44**:** 1841-1855.

Bandara, W. (2002). Performance of Some Vegetable Crops on Dykes in Low-Lying Lands in the Low Country Wet Zone. *Annals of the Sri Lanka Department of Agriculture,* 4**:** 55-64.

Borkert, C., Cox, F. and Tucker, M. (1998). Zinc and Copper Toxicity in Peanut, Soybean, Rice, and Corn in Soil Mixtures. *Communications in Soil Science and Plant Analysis,* 29**:** 2991-3005.

Burguera, J., Burguera, M. and Rondon, C. (1988). Lead in Roadside Soils of Merida City, Venezuela. *Science of the Total Environment,* 77**:** 45-49.

Calderon, J., et al. (2001). Exposure to Arsenic and Lead and Neuropsychological Development in Mexican Children. *Environmental Research,* 85**:** 69-76.

Callahan, D. L., Baker, A. J., Kolev, S. D. and Wedd, A. G. (2006). Metal Ion Ligands in Hyperaccumulating Plants. *JBIC Journal of Biological Inorganic Chemistry,* 11**:** 2-12.

Carrijo, D.R., Lundy, M.E. and Linquist, B.A. (2017). Rice Yields and Water Use under Alternate Wetting and Drying Irrigation: A Meta-Analysis. *Field Crops Research,* 203**:** 173-180.

Chandrajith, R., Dissanayake, C. B and Tobschall, H. J. (2005). Geochemistry of Trace Elements in Paddy (Rice) Soils of Sri Lanka–Implications for Iodine Deficiency Disorders (Idd). *Environmental Geochemistry and Health,* 27**:** 55-64.

Chandrajith, R., et al. (2010). Natural Radionuclides and Trace Elements in Rice Field Soils in Relation to Fertilizer Application: Study of a Chronic Kidney Disease Area in Sri Lanka. *Environmental Earth Sciences,* 60**:** 193-201.

Chandrajith, R., et al. (2011). Chronic Kidney Diseases of Uncertain Etiology (Ckdue) in Sri Lanka: Geographic Distribution and Environmental Implications. *Environmental geochemistry and health,* 33**:** 267-278.

Coelho, S. D., et al. (2016). Lead in Duplicate Diet Samples from an Academic Community. *Science of The Total Environment,* 573**:** 603-607.

Cooray, T., et al. (2019). Assessment of Groundwater Quality in Ckdu Affected Areas of Sri Lanka: Implications for Drinking Water Treatment. *International journal of environmental research and public health,* 16**:** 1698.

DCS (2017). *Paddy Average Yield and Production by District*. (**http://www.statistics.gov.lk/Agriculture/StatisticalInformation/rubpaddy**) (Accessed 03 June 2019).

Diyabalanage, S., et al. (2016). Has Irrigated Water from Mahaweli River Contributed to the Kidney Disease of Uncertain Etiology in the Dry Zone of Sri Lanka? *Environmental Geochemistry and Health,* 38**:** 679-690.

Du, F., Yang, Z., Liu, P. and Wang, L. (2018). Accumulation, Translocation, and Assessment of Heavy Metals in the Soil-Rice Systems near a Mine-Impacted Region. *Environmental Science and Pollution Research,* 25**:** 32221-32230.

Gobalarajah, K., et al. (2020). Impact of Water Quality on Chronic Kidney Disease of Unknown Etiology (Ckdu) in Thunukkai Division in Mullaitivu District, Sri Lanka. *B.M.C. nephrology,* 21**:** 1-11.

Hadif, W. M., et al. (2015). *Heavy Metals Accumulation in Parts of Paddy Oryza Sativa L. Grown in Paddy Field Adjacent to Ultrabasic Soil*. A.I.P. *Conference Proceedings*, 020036, A.I.P. Publishing. 020036.

Hamid, Y., et al. (2018). Immobilization of Cadmium and Lead in Contaminated Paddy Field Using Inorganic and Organic Additives. *Scientific reports,* 8**:** 17839.

Jayalal, A. (2020). Mapping of the Cadmium Lead and Mercury in Rice of Paddy Fields within a Ckdu Hotspot in Sri Lanka. *Journal of Agricultural Sciences – Sri Lanka,* 5(3): 328–335 (**http://doi.org/10.4038/jas.v15i3.9025**).

Jayasumana, C., et al. (2015a). Phosphate Fertilizer Is a Main Source of Arsenic in Areas Affected with Chronic Kidney Disease of Unknown Etiology in Sri Lanka. *Springer Plus,* 4**:** 1-8.

Jayasumana, C., Gunatilake, S. and Siribaddana, S. (2015b). Simultaneous Exposure to Multiple Heavy Metals and Glyphosate May Contribute to Sri Lankan Agricultural Nephropathy. *B.M.C. Nephrology,* 16**:** 103 (**https://www.researchgate.net/publication/280007753**).

Jayatilake, N., Mendis, S., Maheepala, P. and Mehta, F.R. (2013). Chronic Kidney Disease of Uncertain Aetiology: Prevalence and Causative Factors in a Developing Country. *B.M.C. Nephrology,* 14**:** 180 (**https://www.researchgate.net/publication/265602836**).

Jayawardana, D., Pitawala, H. and Ishiga, H. (2014). Assessment of Soil Geochemistry around Some Selected Agricultural Sites of Sri Lanka. *Environmental Earth Sciences,* 71**:** 4097-4106.

Jiang, H., et al. (2012). Effects of pH and Low Molecular Weight Organic Acids on Competitive Adsorption and Desorption of Cadmium and Lead in Paddy Soils. *Environmental monitoring and assessment,* 184**:** 6325-6335.

Johansson, C., Norman, M. and Burman, L. (2009). Road Traffic Emission Factors for Heavy Metals. *Atmospheric Environment,* 43**:** 4681-4688.

Jung, M. (2008). Heavy Metal Concentrations in Soils and Factors Affecting Metal Uptake by Plants in the Vicinity of a Korean Cu-W Mine. *Sensors,* 8**:** 2413-2423.

Kashem, M. A. and Singh, B. R. (2001). Metal Availability in Contaminated Soils: I. Effects of Flooding and Organic Matter on Changes in Eh, pH and Solubility of Cd, Ni and Zn. *Nutrient Cycling in Agroecosystems,* 61**:** 247-255.

Khaokaew, S., et al. (2011). Speciation and Release Kinetics of Cadmium in an Alkaline Paddy Soil under Various Flooding Periods and Draining Conditions. *Environmental Science and Technology,* 45**:** 4249-4255.

Kulathunga, M., Wijayawardena, A., Naidu, R. and Wijeratne, A. (2019). Chronic Kidney Disease of Unknown Aetiology in Sri Lanka and the Exposure to Environmental Chemicals: A Review of Literature. *Environmental Geochemistry and Health,* 41**:** 2329-2338.

Kulathunga, M., Wijayawardena, M. and Naidu, R. (2021). Dietary Heavy Metal (Loid) S Exposure and Prevalence of Chronic Kidney Disease of Unknown Aetiology (Ckdu) in Sri Lanka. *Environmental Geochemistry and Health***:** 1-12.

Kulathunga, M. R. D.L., Wijayawardena, M. A. A., Naidu, R., Wimalawansa, S. J. and Rahman, M. M. (2022). Health Risk Assessment from Heavy Metals Derived from Drinking Water and Rice, and Correlation with Ckdu. *Frontiers in. Wate*r, 3: 786487 (**https://www.researchgate.net/publication/358312092**).

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**:** 548 (**https://doi: 10.1007/s10661-016-5524-8**).

Lidon, F. C. and Henriques, F. S. (1993). Effects of Copper Toxicity on Growth and the Uptake and Translocation of Metals in Rice Plants. *Journal of plant nutrition,* 16**:** 1449-1464.

Loganathan, P., Vigneswaran, S., Kandasamy, J. and Naidu, R. (2012). Cadmium Sorption and Desorption in Soils: A Review. *Critical Reviews in Environmental Science and Technology,* 42**:** 489-533.

Markus, J. and Mcbratney, A. (1996). An Urban Soil Study: Heavy Metals in Glebe, Australia. *Australian Journal of Soil Research,* 34**:** 453-465.

McCauley, A., Jones, C. and Jacobsen, J. (2009). Soil Ph and Organic Matter. *Nutrient Management Module,* 8**:** 1-12.

Meharg, A. A., et al. (2013). Variation in Rice Cadmium Related to Human Exposure. *Environmental Science and Technology,* 47**:** 5613-5618.

Naidu, R., et al. (2012). Contaminants and the Soil Environment in the Australasia-Pacific Region: *Proceedings of the First Australasia-Pacific Conference on Contaminants and Soil Environment in the Australasia-Pacific Region,* Held in Adelaide, Australia, 18–23 February 1996. Springer Science & Business Media.

Naidu, R., et al. (2003). Bioavailability of Metals in the Soil Plant Environment and Its Potential Role in Risk Assessment. Bioavailability, toxicity and risk relationships in ecosystems*. 4th International Symposium on the Biogeochemistry of Trace Elements,* Berkeley, California, U.S.A., 23-26 June 1997., 21-57, Science Publishers, Inc. 21-57.

Naidu, R., Wong, M. H. and Nathanail, P. (2015). *Bioavailability—the Underlying Basis for Risk-Based Land Management*. Springer.

Nayanaka, V., Vitharana, W. and Mapa, R. (2010). Geostatistical Analysis of Soil Properties to Support Spatial Sampling in a Paddy Growing Alfisol.

Norton, G. J., et al. (2014). Lead in Rice: Analysis of Baseline Lead Levels in Market and Field Collected Rice Grains. *Science of the Total Environment,* 485**:** 428-434.

Nyachoti, S., et al. (2022). Dietary Exposures to Metals in Relation to Chronic Kidney Disease of Unknown Cause (Ckdu) in Sri Lanka. *Exposure and Health,* 14**:** 63-73.

Ortega, D. R., et al. (2021). Cognitive Impairment Induced by Lead Exposure During Lifespan: Mechanisms of Lead Neurotoxicity. *Toxics,* 9.

Payus, C. and Talip, A.F.A. (2014). Assessment of Heavy Metals Accumulation in Paddy Rice (Oryza Sativa). *African Journal of Agricultural Research,* 9**:** 3082-3090.

Rahimi, G., Kolahchi, Z. and Charkhabi, A. (2017). Uptake and Translocation of Some Heavy Metals by Rice Crop (*Oryza Sativa*) in Paddy Soils. *Agriculture (Pol'nohospodárstvo),* 63**:** 163-175.

Rahman, M. A., et al. (2014). Heavy Metals in Australian Grown and Imported Rice and Vegetables on Sale in Australia: Health Hazard. *Ecotoxicology and environmental safety,* 100**:** 53-60.

Satpathy, D., Reddy, M. V. and Dhal, S. P. (2014). Risk Assessment of Heavy Metals Contamination in Paddy Soil, Plants, and Grains (Oryza Sativa L.) at the East Coast of India. *BioMed research international,* 2014.

Sellathurai, T., Sivananthawerl, T., Galagedara, L. and Mowjood, M. (2015). Organic Matter Content of a Lowland Paddy Soil as Affected by Plant Growth and Urea Fertilization. *Tropical Agricultural Research*, 26 (2): 415 – 421.

Senanayake, S. and Premaratne, S. (2016). An Analysis of the Paddy/Rice Value Chains in Sri Lanka. *Australia South Asia Research Centre Working Paper,* 4 (**https://journals.sagepub.com/doi/abs/10.1177/1018529120160104**).

Seshadri, B., et al. (2016). Phosphorus–Cadmium Interactions in Paddy Soils. *Geoderma,* 270**:** 43-59.

Signes-Pastor, A., Burló, F., Mitra, K. and Carbonell-Barrachina, A. (2007). Arsenic Bio-geochemistry as Affected by Phosphorus Fertilizer Addition, Redox Potential and pH in a West Bengal (India) Soil. *Geoderma,* 137**:** 504-510.

Sirisena, D. N., et al. (2008). Spatial Variability of Some Soil Fertility Parameters in Rice Fields of Girithale Irrigation Scheme. *Annals of Sri Lanka Department of Agriculture,* 10**:** 199-210.

Smith, E., Juhasz, A., Weber, J. and Naidu, R. (2008). Arsenic Uptake and Speciation in Rice Plants Grown under Greenhouse Conditions with Arsenic Contaminated Irrigation Water. *Science of the Total Environment,* 392**:** 277-283.

Smith, J. and Doran, J. (1996). Measurement and Use of pH and Electrical Conductivity for Soil Quality Analysis. *Methods for Assessing Soil Quality,* 49, Chapter 10: 169-185.

Song, W. Y., Yamaki, T. Y., Yamaji, N., Ko, D., Jung, K. H., et al. (2014). A rice ABC transporter, OsABCC1, reduces arsenic accumulation in the grain. *PNAS*, 111(44): 15699-15704 (**https://www.pnas.org/doi/full/10.1073/pnas.1414968111**).

Su, Y. H., McGrath, S. P. and Zhao, F. J. (20100. Rice is more efficient in arsenite uptake and translocation than wheat and barley. *Plant Soil*, 328: 27–34.

Tang, L., et al. (2019). Heavy Metal Distribution, Translocation, and Human Health Risk Assessment in the Soil-Rice System around Dongting Lake Area, China. *Environmental Science and Pollution Research***:** 1-11.

Tariq, S. R. and Rashid, N. (2013). Multivariate Analysis of Metal Levels in Paddy Soil, Rice Plants, and Rice Grains: A Case Study from Shakargarh, Pakistan. *Journal of Chemistry,* Article ID 539251 (**https://www.researchgate.net/publication/258395080**).

Udousoro, I. I., Umoren, I. and Udoh, A. (2013). Translocation and Accumulation of Trace Metals in Rice Plants in Nsit Ubium, Akwa Ibom State of Nigeria. *Geosystem Engineering,* 16**:** 129-138.

Vega, F., Covelo, E., Andrade, M. and Marcet, P. (2004). Relationships between Heavy Metals Content and Soil Properties in Minesoils. *Analytica Chimica Acta,* 524**:** 141-150.

Wang, F., et al. (2021). Typical Sources of Cd to Paddy Fields in Different Contaminated Areas and Their Impacts on Cd Accumulation in Topsoil and Rice in Changzhutan, China. *Environmental Research,* 193**:** 110523.

Wijayawardena, M. A., Megharaj, M. and Naidu, R. (2016). Exposure, Toxicity, Health Impacts, and Bioavailability of Heavy Metal Mixtures. *Advances in Agronomy*, 138: 175-234.

Wimalawansa, S. J. (2016). The Role of Ions, Heavy Metals, Fluoride, and Agrochemicals: Critical Evaluation of Potential Aetiological Factors of Chronic Kidney Disease of Multifactorial Origin (CKDmfo/CKDu) and Recommendations for Its Eradication. *Environment, Geochemistry and Health,* 38**:** 639-678 (**https://www.researchgate.net/publication/282873549**).

Wimalawansa, S. J., Dissanayake, C. B (2019). Factors Affecting the Environmentally Induced, Chronic Kidney Disease of Unknown Aetiology in Dry Zonal Regions in Tropical Countries—Novel Findings. *Environments,* 7**:** 1-26.

Xie, Z. M., Chen, J. and Naidu, R. (2013). Not All Phosphate Fertilizers Immobilize Lead in Soils. *Water, Air and Soil Pollution,* 224**:** 1712-1719.

Zeng, F., et al. (2011). The Influence of pH and Organic Matter Content in Paddy Soil on Heavy Metal Availability and Their Uptake by Rice Plants. *Environmental pollution,* 159**:** 84-91.

Zhao, F-J., Stroud, J. L., Khan, M.A. and McGrath, S. P. (2012). Arsenic Translocation in Rice Investigated Using Radioactive 73 as Tracer. *Plant and Soil,* 350**:** 413-420.