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Assessment of Soil Quality and Metal Contaminants in Paddy Soils and Water in a CKDu-Affected area in Sri Lanka

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Abstract. The proliferation of chronic kidney disease of unknown etiology (CKDu) in Sri Lanka may be attributed to exposure to trace elements of both natural and human origin in the environment, which has been extensively explored and widely debated within the scientific community. This research aims to investigate the fluctuation of soil quality in paddy fields concerning depth and to evaluate the corresponding groundwater parameters in a region identified as a high-risk area for CKDu in the North Central region via determining different parameters including trace metals in both paddy soils and groundwater. The slightly alkaline paddy soils in the study area displayed elevated electrical conductivity values in the uppermost soil layer compared to the subsoil layer. Although hazardous metals, including lead (Pb), chromium (Cr), arsenic (As), and cadmium (Cd), were detected in the paddy soil, they did not surpass the critical toxic concentrations. A statistically remarkable robust positive correlation was discovered between the concentration of Pb in the soil and its depth. Additionally, the levels of toxic metals, such as lead, arsenic, cadmium, and chromium, in the study area's groundwater were substantially lower than the safety standards set by the World Health Organization. However, some groundwater samples were found to contain anionic species, including fluoride, in concentrations that exceeded the established permissible limits. The gradual accretion of trace elements within the paddy soil presents a potential hazard for the translocation of such elements into the rice plants, thus constituting a possible threat to human health.
Key words: CKDu, drinking water, paddy soil, toxic metals, accumulation.

Introduction

In 2017, chronic kidney disease (CKD) resulted in millions of deaths, and Chronic kidney disease (CKD) was identified as the twelfth primary cause of death globally (Murray *et al*., 2020). Aging, long-lasting hypertension, obesity, diabetes, smoking, food insecurity, and well-characterized renal syndromes are the common causative factors in most globally recognized CKD patients (Wasana *et al*., 2016). In addition to the conventional factors, CKD can also be caused by glomerular and tubulointerstitial diseases resulting from various factors such as infections, nephrotoxic drugs, herbal remedies, as well as exposure to environmental and occupational toxins (Jayasumana *et al*., 2017). A novel form of CKD has been reported in several countries, including Guatemala, India, Sri Lanka, and several countries in Europe, Central America, and Asia, which do not have a relation to conventional risk factors. This new form of CKD is given the name chronic kidney disease of unknown/uncertain etiology (CKDu), because other than some plausible associations and correlations among the proposed risk factors, specific causative factor(s) for CKDu remain unrevealed across the globe even today (Wanigasuriya, 2012).

The first reports of an unknown form of chronic kidney disease (CKDu) emerged in Sri Lanka during the mid-1990s. Where it was predominantly found among farmers residing in the North Central Province (NCP). Subsequently, the prevalence of this disease has increased drastically throughout the country, including other agricultural areas such as the North,

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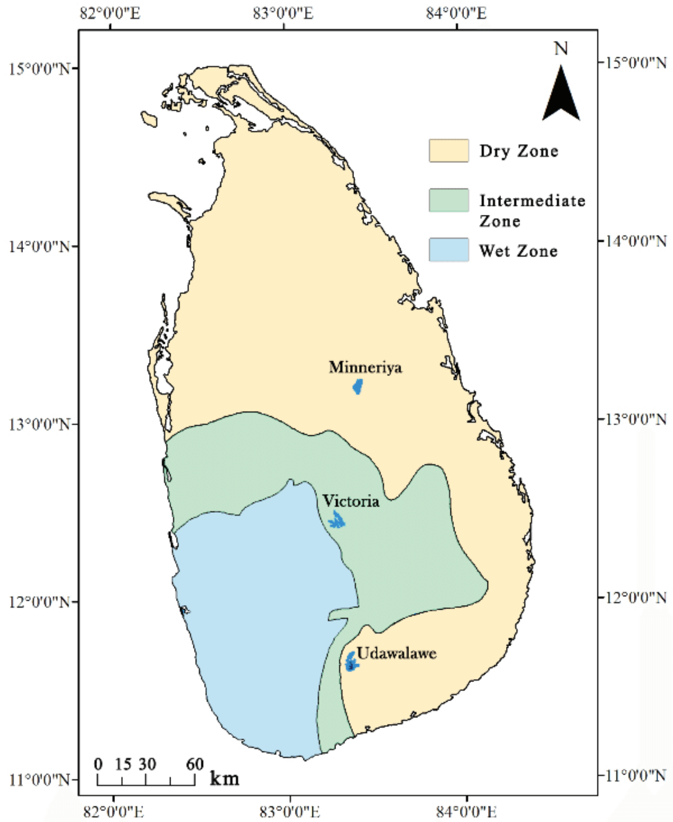

Figure 1. Climatic zones of Sri Lanka.

Northwest, Eastern, Central, and Uva Provinces (Perera *et al*., 2021). Even though the origin of CKDu is still unclear, several potential risk factors have been identified based on different hypotheses from various investigations. Investigations have highlighted the role of nephrotoxic agents such as heavy metals (As, Cd, Pb), which may accumulate in the environment due to the extensive use of agrochemicals such as fertilizers and pesticides. These substances are thought to leach into groundwater, thereby posing health risks to the community (Jayatilake *et al*., 2013; Wanigasuriya, 2014). Moreover, other environmental factors linked to groundwater quality, such as elevated fluoride levels and water hardness, along with toxins produced by cyanobacteria, have been identified as contributing to the CKDu prevalence (Cooray *et al*., 2019). Other suggested causative factors include being in direct sunlight for extended periods, resulting in heat stress and inadequate hydration, the utilization of substandard aluminum cookware in food preparation, viral infections and the geochemical properties of the soil and drinking water, all of which affect the bioavailability and mobilization of toxic substances into groundwater (Ayala Herath *et al*., 2018). These observations highlight the complex interaction between agricultural practices and groundwater quality that potentially contribute to the CKDu. Therefore, a thorough evaluation of the quality of both drinking water and paddy soil is imperative.

In the dry zone, water availability changes depending on the dry and wet seasons of the year. Dry weather conditions are prolonged for many months of the year, and the wet season is limited to a few months of the year where the annual rainfall is less than 1000 mm in the dry zone. Surface water is readily available for the community in the NCP during the wet season. Nonetheless, surface water sources often become nearly depleted during the dry season, and people will have to rely on groundwater sources for their daily needs (Wimalawansa, 2014). For drinking purposes, 92% of people in NCP use shallow dug wells, 7% use tube wells, and only 1% use reservoirs (Jayasekara *et al*., 2013). A significant portion of the population living in the dry zone relies on groundwater as their main source of daily water requirements.

Paddy farming is the main live hood of people in the NCR. Paddy lands are scattered across the island, and farmers have been using fertilizers, pesticides, and herbicides to increase crop production. However, the amounts of fertilizers and other agrochemicals applied to paddy fields have increased exceeding the recommended levels during the past few decades (Chandrajith *et al*., 2010). The soil in paddy farming areas is therefore highly modified due to the continuous application of agrochemicals. Residues of these chemicals can persist in the environment for many years after introduction. Because, unlike other organic contaminants, these residues are difficult to break down through biodegradation or chemical degradation. The chemical composition of soil can undergo significant alterations, due to the release of heavy metals, metalloids, and other toxicants from these residues to agricultural lands, and soil plays a significant role as a repository for heavy metals and metalloids (Chandrajith *et al*., 2010). The primary objective of this investigation is to examine the impact of soil quality parameters, particularly trace metals, on the paddy soil at varying depths and groundwater quality within the same geographic region, for the purpose of evaluating potential risks associated with rice consumption and the incidence of Chronic Kidney Disease of unknown origin (CKDu) in the selected area.

Materials and Methods

Zone description and sampling

The sampling area, Maradankulama GN; (7°33'00.0"N 79°50'00.0"E) identified as a CKDu-prone area has been selected from the North Central Province in Sri Lanka for the sampling of drinking water and paddy soil. Sampling was carried out from November 2021 to December 2022, during the wet season of the year. From a paddy field, fifteen sampling sites were selected randomly based on six paddy areas, and three composite soil samples were collected from the surface, depth 1 (0-30 cm) and depth 2 (0-60 cm), separately. A total of 45 composite soil samples were collected. Each parameter in soil and water samples was analyzed in triplicate. Eighteen water samples were collected from dug wells (5-10 m deep) situated in households within the same GN division and preserved water samples in acid (HNO_3, 70%) during the transport. Acid preserved water samples were stored at 4^0C until further analysis.

Analysis of quality parameters

The experiments were conducted using chemicals and standards of analytical grade, which were obtained from Sigma-Aldrich (USA), BDH (UK), or Fluke (Switzerland). Ultrapure water was utilized throughout the experimentation process.

Paddy soil samples were analyzed for pH, electrical conductivity (EC), potassium, total organic matter, phosphate, and heavy metals. To analyze the pH and EC of paddy soil, wet soil samples were used, and the rest of the sample was oven-dried (105 ^{o}C) and ground to pass through a sieve (< 2 mm) to analyze other parameters. Potentiometric method (Page *et al*., 1996c) for pH, conductivity method (Estefan *et al*., 2013) for EC, photometric method (Page *et al*., 1996a) for potassium, Walkley-Black method (Page *et al*., 1996d) for total organic matter, Troug method (Page *et al*., 1996b) for phosphate, and ICP-MS

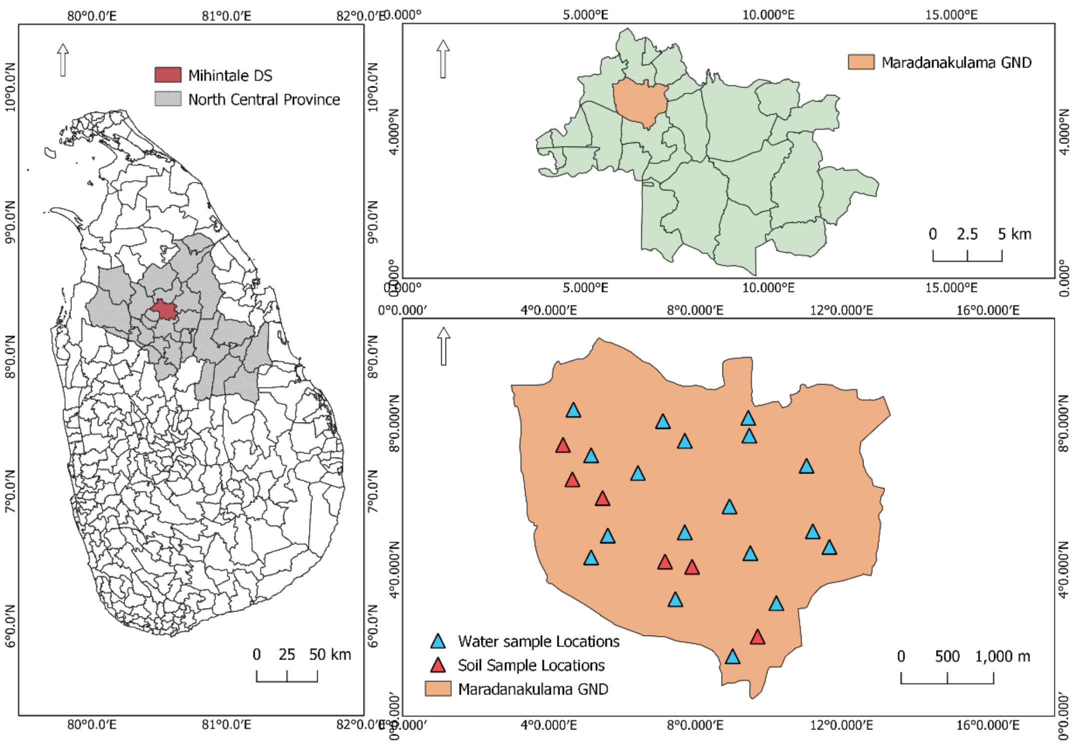

Figure 2. Sampling locations of the paddy soil and groundwater in Maradankulama GN area; Sri Lanka.

micro digestion method (APHA, 2017) for heavy metal analysis in paddy soil was used. Groundwater samples were analyzed for pH, EC, hardness, heavy metals, and anions using APHA standard methods. Each parameter was analyzed in three replicates.

pH, EC, and heavy metals in paddy soil and groundwater were analyzed using Orion star A214 pH meter, HACH HQ14d conductivity meter, and Agilent 7800 ICP-MS respectively. Potassium and phosphate in soil were analyzed using PFP7 Flame photometer, and Thermo scientific MULTISKAN GO spectrophotometer. Anions in groundwater were analyzed using Metrohm Eco IC.

Statistical analysis

SPSS software version 23.0.0.0 was used to conduct all statistical analyses. Descriptive statistics were applied for the water and soil samples for the mean and standard deviation calculations. Pearson's correlation coefficients for As, Pb, Cd, Cr, Mn, Ni, Zn, Mg, K, Ca, and Fe soil collected from paddy areas were calculated. The analysis of soil samples collected from a paddy field involved performing a one-way ANOVA to assess the influence of depth on metal concentrations (Pb, As, Zn, Ni, Cr, Mg, Fe). Moreover, a multiple linear regression analysis was executed to explore the correlation between the concentration of Pb and the depth. The graphical interpretation of the relationships was generated using OriginPro software (Version 2021).

Results and Discussion

Soil quality parameters and their behavior

The pH of paddy soil ranged from 7.01 to 8.12, with an average of 7.57±0.09 (Table 1). Soil pH has a direct effect on the chemical nature of any element present in the soil (Zwolak *et al*., 2019). Paddy soil in the study area is slightly alkaline. Nonetheless, no considerable fluctuations in the average pH value were observed with regard to the soil profile depth. The soil exhibited an average electrical conductivity (EC) of 101.8 ± 10.1 µS cm^{-1}, with a range of EC values spanning from 44.8 µS cm^{-1} to 186.2 µS cm^{-1}. According to the results, the average EC decreases with depth. The number of soluble salts on the topsoil of the paddy field is usually higher than that of the bottom parts of the soil because the surface is more dynamic (Alsabhan *et al*., 2022; Othaman *et al*., 2020).

The organic matter content of the soil in the paddy fields had an average value of 1.34±0.13%, with a range of 0.41% to 2.25% observed. The average organic matter content decreases with depth. The data indicates that the upper stratum of the rice field exhibits a higher concentration of organic carbon than the subsoil. The rate of microbial decomposition is

Table 1

Statistical evaluation of soil quality parameters

Parameter	Unit	Mean	SD	Surface	Depth 1	Depth 2	Critical soil concentration*	F-value	p-value
pH	-	7.57	0.38	7.46	7.43	7.83	-	-	-
EC	µS cm^{-1}	101.8	42.9	111.2	104.6	89.7	-	-	-
Organic matter	%	1.337	0.560	2.34	1.96	0.97	-	-	-
Potassium	mg kg^{-1}	93.19	60.7	213.3	133.3	100	-	-	-
Phosphate	mg kg^{-1}	148.9	4.168	22.32	25.78	24.05	-	-	-
Pb	mg kg^{-1}	24.05	0.778	2.89^b	2.98^b	4.18^a	100-400	7.17	0.004
Cd	mg kg^{-1}	0.032	0.013	0.03	0.03	0.04	3.0-8.0	-	-
As	mg kg^{-1}	0.435	0.095	0.39^b	0.41^a	0.52^a	20-50	2.88	0.073
Zn	mg kg^{-1}	18.94	7.32	11.99^b	21.64^a	23.1^a	70-400	4.51	0.021
Ni	mg kg^{-1}	7.299	2.707	6.36	6.14	9.39	100	2.71	0.085
Mn	mg kg^{-1}	228.9	118.7	194.4^b	208.1^a	284.1^a	1500-3000	4.13	0.027
Cr	mg kg^{-1}	20.04	6.66	17.31^b	16.93^b	25.86^a	75-100	3.540	0.043
Fe	mg kg^{-1}	10899	3496	9194^b	9482^b	14021^a	-	3.83	0.034
Co	mg kg^{-1}	5.165	1.842	6.7	4.58	4.22	25-50	-	-
Cu	mg kg^{-1}	11.23	4.42	15.57	9.24	8.89	60-125	-	-

** The threshold levels that may have adverse toxic impacts on plants vary based on the speciation of the metal (Alloway, 2012)* elevated in the uppermost layer of soil, leading to a greater accumulation of organic matter in the topsoil (Cataldo & Wildung, 1978). The average potassium content in the soil was 148.9±14.3 mg kg^{-1} and the average potassium content in the samples was found to have a range of values between 80.0 and 320.0 mg kg^{-1}. Additionally, it was observed that the average potassium content decreased with depth. Fertilizers are the major sources of potassium in paddy soil. According to the results, the topsoil contains more potassium than the bottom parts. The average phosphate content in the soil was determined to be 24.05 ± 0.98 mg kg^{-1}, with values ranging from 18.12 mg kg^{-1} to 29.77 mg kg^{-1}. According to the results, there was no statistically significant variation observed in the average phosphate concentration with respect to soil depth.

The paddy soil samples were analyzed for the heavy metal content, with the mean concentrations in decreases of Fe (10899 mg kg^{-1}) > Mn (228.9 mg kg^{-1}) > Cr (20.04 mg kg^{-1}) > Zn (18.94 mg kg^{-1}) > Cu (11.23 mg kg^{-1}) > Ni (7.299 mg kg^{-1}) > Co (5.165 mg kg^{-1}) > Pb (0.435 mg kg^{-1}) > As (0.032 mg kg^{-1}), and Cd (0.032 mg kg^{-1}) (Table 1). Fe was predominant in paddy soil, and Pb, Cd, and As were found in significantly low concentrations. The pH and organic matter content of soil are regarded as crucial factors that impact the heavy metals in soil as well as their uptake by plants (Zwolak *et al*., 2019). The pH of the soil can have an impact on the ability of plants to absorb heavy metals, particularly in acidic conditions. Furthermore, the presence of organic matter in soil can immobilize heavy metals and decrease their availability in the soil solution, thus potentially reducing their uptake by plants. (Zwolak *et al*., 2019). Given the alkaline soil pH in the study region, the likelihood of heavy metal uptake by the rice plants is likely to have been diminished.

However, none of the analyzed toxic metals have surpassed the critical limit of soil contamination, indicating that the paddy soil in the region is unlikely to exert toxic effects on vegetation. According to the correlation matrix (Table 2), Pearson's correlation coefficients were calculated to evaluate the linear relationships between each pair of variables in soil samples. Significant positive correlations were observed between the pairs of Cr and Pb ($r = 0.812$, $p < 0.01$), As and Pb ($r = 0.802$, $p < 0.01$), as well as between Cr and As ($r = 0.768$, $p < 0.01$). It can be hypothesized that metals showing a significant positive correlation potentially originate from common sources of pollution (Zarei *et al*., 2014).

A one-way ANOVA analysis was performed to evaluate the variation in the metal content of paddy soil with respect to the depth of the paddy field (Table 1). There was a notable difference in the levels of Pb concentration at varying depths, as determined by the ANOVA test. (p-value of 0.004). The F-value for Pb was 7.17, which indicates a strong effect of depth on Pb concentration.

Furthermore, to explore the relationship between soil depth profile and lead concentration, a multiple linear regression analysis was performed. A multiple linear regression analysis was conducted to investigate the association between Pb concentration and depth. The regression equation for Pb was derived as 54.05 + 12.89 (depth), with a p-value of 0.001. The analysis indicates a robust positive correlation between Pb concentration and depth. Apart from that, a multiple linear regression analysis examines the relationship

Table 2

Pearson's correlation coefficients for metals in the paddy soil samples

	Pb	Cd	As	Zn	Ni	Mn	Cr	Mg	K	Ca
Cd	0.701									
As	**0.802**	0.605								
Zn	0.574	0.349	0.412							
Ni	0.729	0.579	0.676	0.123						
Mn	0.554	0.318	0.310	0.121	0.727					
Cr	**0.812**	0.684	**0.768**	0.214	0.970	0.692				
Mg	0.732	0.465	0.664	0.114	0.967	0.695	0.929			
K	0.485	0.518	0.493	-0.154	0.734	0.653	0.747	0.634		
Ca	0.700	0.536	0.736	0.070	0.944	0.740	0.936	0.935	0.768	
Fe	0.849	0.662	0.786	0.282	0.969	0.720	0.991	0.934	0.710	0.931

Variation of metals with the depth of the soil

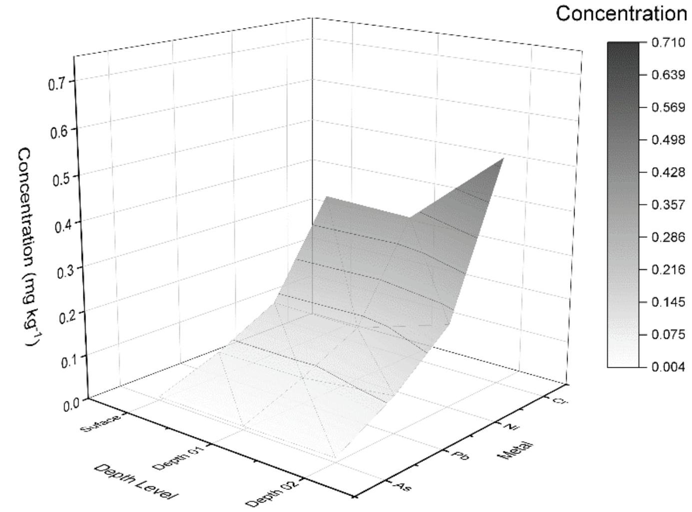

Figure 3. Variation pattern of the As, Pb, Ni, and Cr contents of the paddy soil with the depth.

between metal concentrations and depth. The findings of this analysis revealed a strong positive correlation between the concentrations of Zn, Cr, Mg, and Fe metals and the depth of the soil profile. The statistical analysis showed that the relationship between metal concentrations and depth is significant, as the p-values for Zn, Cr, Mg, and Fe were all below 0.05. According to the previous studies performed in Sri Lanka related to the toxic metal contents in fertilizers, notably high concentrations of toxic metals, such as cadmium (Cd), arsenic (As), chromium (Cr), lead (Pb), zinc (Zn), and copper (Cu), have been detected in urea, a commonly used fertilizer in paddy and other agricultural operations in Sri Lanka (Perera *et al*., 2021). Contaminated toxic metals, including Pb, can be transported into the lower layers of the soil profile (Dowdy and Volk., 1983, Sun *et al*., 2018). The metal contents of paddy soil may increase with depth due to a variety of factors. One possible explanation is that metal ions may be more likely to be transported and deposited in lower layers of soil due to factors such as gravity or water flow. Another possibility is that metal ions may be more strongly adsorbed or complexed to soil particles in lower layers, leading to increased accumulation. Additionally, the process of soil formation may lead to the precipitation of metal ions in lower soil layers (Schmidt *et al*., 2003). Figure 3 illustrates unequivocally the fluctuation of chosen toxic metals with the progression of soil depth.

Groundwater quality parameters.

Cd, As, Cr, and Pb are highly suspected as major causative factors for CKDu (Rango *et al*., 2015). However, none of the samples analyzed in this study contained detectable levels of Pb. The instrument used in this study was not able to detect lead in samples of groundwater from the study area, suggesting that lead concentrations were below the lower detection limit, which was 1 ng L^{-1} (2% HNO_3 + 0.5% HCl, matrix). The heavy metal content in the investigated samples exhibited the following order, Zn > Mn > Ni > Fe > Co > Cu > Cr > As > Cd, with mean concentrations of 51.9 µg L^{-1}, 32.5 µg L^{-1}, 10.04 µg L^{-1}, 6.7 µg L^{-1}, 0.280 µg L^{-1}, 0.322 µg L^{-1}, 0.244 µg L^{-1}, 0.373 µg L^{-1}, and 0.003 µg L^{-1} for each respective metal. (Table 3)

The levels of Pb, Cr, Cd, and As, which are toxic heavy metals, were all found to be well below the recommended guidelines set by the World Health Organization (WHO) and Sri Lanka standards (SLS). Direct consumption of this water may not cause any health-related issues in humans, but chronic exposure to low concentrations might influence CKDu. The potential relationship of exposure to heavy metals, specifically cadmium, with water hardness and fluoride, and its influence on the manifestation of CKDu has been recognized by previous researchers (Edirisinghe *et al*., 2018).

Among the common cations Ca^{2+}, Mg^{2+}, and Na^{+} were found to be dominant and the cation content varies as $Ca^{2+} > Mg^{2+} > Na^{+} > K^{+} > Al^{3+}$ with a mean concentration of 52.74 mg L^{-1}, 40.34 mg L^{-1}, 28.77 mg L^{-1}, 0.424 mg L^{-1}, and 3.53 µg L^{-1} respectively (Table 3). The pH of the groundwater lies within the WHO and Sri Lanka Standards. However, EC, hardness, and the concentration of some anions including fluoride, chloride, nitrite, and bromide groundwater samples have exceeded permissible limits. Groundwater in the study area can be categorized as "very hard" as the average hardness is greater than 180 mg $CaCO_3$ L^{-1} (Wasana *et al*., 2016). High EC and elevated hardness of water make the groundwater unpalatable. Therefore,

Table 3

Statistical evaluation of water quality parameters

Parameter	Unit	Mean	SD	Guideline value	
				WHO	SLS
pH	-	7.42	0.56	6.5-8.5	6.5-8.5
EC	$\mu S\ cm^{-1}$	913	495	N/A	750
Hardness	$mg\ CaCO_3\ L^{-1}$	395.0	209.8	N/A	250
Phosphorus	$mg\ L^{-1}$	ND	ND	N/A	2
Pb	$\mu g\ L^{-1}$	ND	ND	10	10
Cd	$\mu g\ L^{-1}$	0.003	0.005	3	3
As	$\mu g\ L^{-1}$	0.373	0.312	10	10
Ni	$\mu g\ L^{-1}$	10.04	7.07	70	20
Fe	$\mu g\ L^{-1}$	6.70	8.15	300	300
Cr	$\mu g\ L^{-1}$	0.244	0.176	50	50
Co	$\mu g\ L^{-1}$	0.28	0.57	N/A	N/A
Cu	$\mu g\ L^{-1}$	0.322	0.242	2000	1000
Zn	$\mu g\ L^{-1}$	51.9	113.0	3000	3000
Mn	$\mu g\ L^{-1}$	32.5	83.9	400	100
Al	$\mu g\ L^{-1}$	3.53	5.36	200	200
Na	$mg\ L^{-1}$	28.77	18.78	200	200
K	$mg\ L^{-1}$	0.424	0.52	N/A	N/A
Mg	$mg\ L^{-1}$	40.34	30.61	N/A	30
Ca	$mg\ L^{-1}$	52.74	26.23	N/A	100
Fluoride	$mg\ L^{-1}$	0.753	0.414	1.5	1
Chloride	$mg\ L^{-1}$	180.8	166.5	250	250
Nitrite	$mg\ L^{-1}$	5.98	9.23	3	3
Bromide	$mg\ L^{-1}$	0.495	0.41	0.5	N/A
Phosphate	$mg\ L^{-1}$	0.024	0.103	N/A	2
Sulfate	$mg\ L^{-1}$	46.5	46.3	250	250
Nitrate	$mg\ L^{-1}$	2.217	2.941	50	50

groundwater in the study area is not suitable for direct consumption. This water must undergo suitable pretreatment processes before consuming for drinking purposes. Groundwater analyses indicate that the most abundant anion in the environment is chloride, present at concentrations of 180.8 mg L^{-1} on average. This is followed by sulfate at 46.5 mg L^{-1}, nitrite at 5.98 mg L^{-1}, nitrate at 2.22 mg L^{-1}, fluoride at 0.753 mg L^{-1}, and bromide at 0.495 mg L^{-1}. Phosphate was not present in any of the samples. Fluoride, chloride, nitrite, and bromide were found in 17%, 22%, 44%, and 33% of the water samples, respectively, exceeding the established guidelines. Anions are more likely to cause problems with the taste, but when present in markedly higher concentrations, anions are considered to cause health concerns. In most of the areas affected by CKDu, results from recent studies suggest a relationship between high levels of fluoride in groundwater and the occurrence of CKDu (Wanigasuriya, 2012). Several studies have suggested the combined effect of fluoride together with water hardness or aluminum as contributing factors for CKDu (Wickramarathna *et al*., 2017).

Conclusion

This study was designed to conduct a comprehensive analysis of various parameters, including groundwater and paddy soil, to evaluate the status of drinking water and variation of soil parameters in paddy fields with the depth in a selected CKDu-prone area within the Mihintale Divisional Secretariat of the North Central Province, Sri Lanka. Analysis of the paddy soil in the study area revealed a slight alkalinity, with an average pH that is suitable for agricultural practices. Analysis of soil samples revealed with increasing soil depth, there was a decrease in the electrical conductivity, organic matter, and potassium content of soil samples, whereas heavy metal concentrations, including Pb, Zn, Mn, As, and Fe, increased. Apart from that, there was evidence of a statistically significant positive correlation between Lead (Pb) concentrations and soil depth. The upper soil layers, characterized by elevated levels of organic matter, potassium, electrical conductivity, and a slightly alkaline pH, are deemed more fertile than the deeper soil layers. Nonetheless, the deeper soil layers, which exhibit relatively elevated concentrations of hazardous metals, are also noteworthy as these toxic elements may leach into the lower layers of the soil and accumulate due to prolonged and excessive utilization of fertilizers. According to the results, the levels of toxic metal in most groundwater samples were significantly low and thus unlikely to result in acute toxicity in humans. Nevertheless, ongoing monitoring and evaluation of commonly used agrochemicals, suspected as significant contributors to heavy metal/metalloid contamination in paddy soil, is necessary to ascertain their quality and safety for agricultural applications.

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