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Nalika Dayananda

nalika@gwu.ac.lk

Gampaha Wickramarachchi University of Indigenous Medicine

Namal Chathuranga

University of Kelaniya

Ruwan Perera

Gampaha Wickramarachchi University of Indigenous Medicine

Shermila Botheju

Gampaha Wickramarachchi University of Indigenous Medicine

Jeewantha Premaratne

University of Kelaniya

Janitha Liyanage

University of Kelaniya

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Integrated Assessment of Potentially Toxic Elements Fluxes in Paddy Agroecosystems of Dry Zone: From Fertilizer Inputs to Soil Transformation and Grain-Level Bioaccumulation

Nalika Dayananda[1]*, Namal Chathuranga[2], Ruwan Perera[1], Shermila Botheju[1], Jeewantha Premaratne[2] and Janitha Liyanage[2]

[1] Department of Indigenous Medical Resources, Faculty of Indigenous Health Sciences and Technology, Gampaha Wickramarachchi University of Indigenous Medicine, Yakkala, Sri Lanka.

[2] Department of Chemistry, Faculty of Science, University of Kelaniya, Dalugama, Kelaniya, Sri Lanka.

* Corresponding author. Tel: +94712939837

* Email address: nalika@gwu.ac.lk

ORCID iD: 0000-0002-1744-5677

Nalika Dayananda: nalika@gwu.ac.lk

Namal Chathuranga: namalchathuranga8@gmail.com

Ruwan Perera: wprtp@gwu.ac.lk

Shermila Botheju: shermila@gwu.ac.lk

Jeewantha Premaratne: jeewa@kln.ac.lk

Janitha Liyanage: janitha@kln.ac.lk

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Abstract

This study presents an integrated, multi-matrix assessment of potentially toxic element (PTE) fluxes across Chronic Kidney Disease of unknown etiology-endemic paddy agroecosystems in Sri Lanka, systematically quantifying cadmium, lead, arsenic, chromium, copper, zinc, and manganese from inorganic fertilizer inputs through soil biogeochemical transformation to grain-level bioaccumulation. Triple Superphosphate was identified as the primary PTE vector, containing Cd (6.74 mg/kg), Pb (124.34 mg/kg), and Cr (186.36 mg/kg), all exceeding safe agronomic thresholds. Girandurukotte was the most contaminated site, recording peak soil Cd (0.177), Pb (10.526), As (3.887), and Cu (38.904) in mg/kg, with hierarchical clustering confirming its geochemical distinctiveness. Cadmium demonstrated the highest bioavailability, with soil-to-root BCF values reaching 0.740 and soil-to-grain transfer factors of 0.116–0.153 across endemic sites. A statistically significant positive association was confirmed between soil and grain Cd concentrations. Root-to-grain translocation factors for Cd (0.207–0.336) substantially exceeded those of Pb (~0.05), arsenic, and chromium. Ecological risk assessment yielded PERI values of 13.01–30.70 across sites — all within the low-risk classification — yet cadmium dominated cumulative risk profiles at every location (Er up to 17.68), reflecting its disproportionate toxicological weighting. Inverse Distance Weighting spatial interpolation delineated discrete Cd and Pb hotspots geographically coinciding with documented peak CKDu prevalence zones, providing spatially explicit empirical support for a fertilizer-derived and geochemical cadmium exposure pathway underlying chronic renal injury in Sri Lanka's endemic dry zone paddy farming communities with a foundation for evidence-based fertilizer quality regulation, agricultural monitoring frameworks, and targeted public health intervention across Sri Lanka's endemic dry zone.

Keywords: Ecological risk assessment; PTEs in paddy ecosystems; Cadmium bioaccumulation; PERI; CKDu

1. Introduction

Among the most perplexing health burdens to surface from South Asia's agrarian heartlands in recent decades, Chronic Kidney Disease of uncertain aetiology (CKDu) stands as a piece of evidence to the unintended toxicological consequences of intensive food production. Unlike classical nephropathies attributable to diabetes, hypertension, or glomerulonephritis, CKDu presents as a silent, progressive, non-proteinuric tubulointerstitial nephropathy that disproportionately afflicts male paddy farming communities in the dry zone districts of Sri Lanka — most severely in Anuradhapura, Polonnaruwa, Kurunegala, Badulla, and Monaragala — with an estimated prevalence of 15–20% among adults in the most endemic localities (Athuraliya et al., 2011; Wanigasuriya et al., 2011). The disease exacts a devastating social toll: families anchored to subsistence paddy agriculture lose their primary wage earners to end-stage renal failure, while the healthcare system faces high dialysis treatment costs (Rajapaksa et al., 2016). Decades of epidemiological investigation have failed to implicate a single, discrete aetiological agent; instead, the weight of evidence increasingly implicates a syndrome of chronic, low-dose exposure to a consortium of potentially toxic elements (PTEs) — most prominently cadmium (Cd), arsenic (As), lead (Pb), chromium (Cr), and nickel (Ni) — delivered through the specific biogeochemical processes of the paddy agroecosystem that rural communities simultaneously inhabit and consume (Bandara et al., 2011; Jayasumana et al.,2014).

The paddy agroecosystem occupies a uniquely treacherous niche in the landscape of human dietary toxicology. *Oryza sativa* L. (Rice) is cultivated across more than 148 million hectares globally and constituting the caloric backbone of over three billion people (FAO, 2023) is cultivated under conditions of managed flooding that render its rhizosphere among the most biogeochemically reactive terrestrial environments on Earth. The cyclical alternation between flooded anaerobic and drained aerobic states drives perpetual oscillations in redox potential (Eh) and pH, orchestrating the reductive dissolution of iron(III) oxyhydroxides, the microbially mediated interconversions, precipitation and re-mobilization of heavy metals/metalloids — biogeochemical dynamics that collectively determine whether PTEs are sequestered in the soil matrix or liberated into porewater where they become phytoavailable (Meharg and Hartley-Whitaker, 2002; Shaheen et al., 2014). This redox-driven mobility makes paddy cultivation inherently more susceptible to PTE phytoaccumulation than dryland cropping systems operating under continuous aerobic soil conditions (Simmons et al., 2005), a vulnerability that acquires acute public health significance when the same farming communities that grow rice also consume it as their principal dietary staple.

The paddy field is not merely an agricultural system — it is an inadvertent biogeochemical refinery, concentrating potentially toxic elements from fertilizer inputs and geogenic substrates through soil transformation processes into the grain consumed daily by millions of rural households in CKDu endemic regions. The intensification of paddy agriculture across Sri Lanka's dry zone, catalyzed by post-independence agricultural policy has entailed the systematic application of phosphate-based fertilizers — triple superphosphate (TSP), diammonium phosphate (DAP), and single superphosphate (SSP) — whose manufacture from marine sedimentary phosphate rock introduces non-trivial co-contaminant loadings of Cd, uranium (U), and fluoride (F^-) with every cropping cycle (McLaughlin et al., 1996) and a fraction of which is solubilized upon soil acidification and rendered bioavailable.

The translocation of PTEs from the soil solution into the rice grain proceeds through a molecularly coordinated gauntlet of membrane transport systems that simultaneously constitute the cell's nutrient acquisition machinery and its principal route of toxic contamination. Radial oxygen loss from rice aerenchyma tissue precipitates an iron oxyhydroxide plaque on the root surface — a reactive mineralogical filter of variable composition and PTE-sequestration capacity that functions as the first anatomical checkpoint governing metal entry (Liu et al., 2006). Quantifying these flux partitioning dynamics through bioconcentration factors (BCF) and translocation factors (TF) across locally cultivated rice varieties — many genotypically uncharacterized for PTE accumulation phenotype — constitutes an indispensable empirical bridge between soil contamination metrics and human dietary exposure estimates. Cadmium, arsenic, lead, and associated PTEs can converge on the rice grain through overlapping molecular transport pathways — making the grain endosperm the ultimate integrative biomonitoring of paddy ecosystem contamination and the primary dietary exposure vehicle for rural farming communities (Dayananda and Liyanage, 2021).

A transformative dimension of contemporary environmental geochemistry lies in the capacity to move beyond site-specific measurements toward spatially continuous prediction surfaces that reveal the macro-scale contamination and to overlay those surfaces upon the geographical distributions of disease to test epidemiological hypotheses with unprecedented spatial precision. Geostatistical interpolation techniques such as Inverse Distance Weighting (IDW), translate discrete sampling point concentrations into spatially continuous raster maps by exploiting the principle of spatial autocorrelation — the empirically near-universal tendency for geochemically related soils in geographic proximity to harbor more similar PTE concentrations than randomly selected pairs (Goovaerts, 1997). When spatially interpolated PTE concentration maps are subjected to contamination index calculation Enrichment Factor (EF), and the Potential Ecological Risk Index (PERI; Håkanson, 1980) — at each prediction grid node, the output transforms from a simple concentration surface into a spatially continuous risk intelligence map that renders hotspot delineation statistically defensible and visually communicable to environmental managers and public health policymakers.

This geo-epidemiological triangulation has been partially essayed in previous Sri Lankan studies but never fully realized with the spatial resolution and multi-matrix completeness that a landscape-scale, systematically sampled investigation demands. Despite a growing corpus of partial investigations, no prior study conducted within Sri Lanka's CKDu endemic landscape has achieved the integrated simultaneity of: (i) quantifying PTE loadings from all agrochemical input vectors such as fertilizers — through certified ICP-MS analysis of field-collected samples; (ii) quantification of soil PTE across the full flooding–drainage redox cycle in all CKDu endemic provinces in Sri Lanka; (iii) constructing spatially continuous Cd, Pb, As and Cr contamination surfaces at landscape scale using IDW interpolation by R software with cross-validated power parameter optimization; (iv) computing a set of contamination indices (TR, BCF, EF, PERI) and (v) performing geo-epidemiological spatial overlay of interpolated PTE hotspots against CKDu prevalence data. This investigative approach represents a decisive departure from disconnected frameworks that, while valuable individually, are epistemologically stranded — incapable of tracing PTEs through their full environmental trajectory from fertilizer bag to grain endosperm to human proximal tubule.

2. Materials and Methods

2.1. Site selection and sample collection

The rapid spread of CKDu (a non-communicable disease) observed from the north-central province (NCP) to remote communities in the Uva province of Sri Lanka, with a minimum of 15% of CKDu affected people in the age group 15-70 years (Rejomon et al., 2010) over the last decade. Hence, Girandurukotte Grama Niladhari Division (GND) [7.4629° N, 81.0175° E], Badulla district, Uva province, Ambagaswewa GND [8.1129° N, 80.9788° E], Polonnaruwa district, North Central Province, and Horowpothana GND [8.5501° N, 80.8326° E], Anuradhapura district, North Central Province were identified for the sampling as the CKDu endemic area according to the data of the Ministry of Health and Nutrition, Sri Lanka and Dambethalawa GND [7.2991° N, 81.5322° E], Ampara district was selected as the reference site where no CKDu cases were reported but having similar climate conditions which are present in the selected CKDu prevalence area (Fig 1).

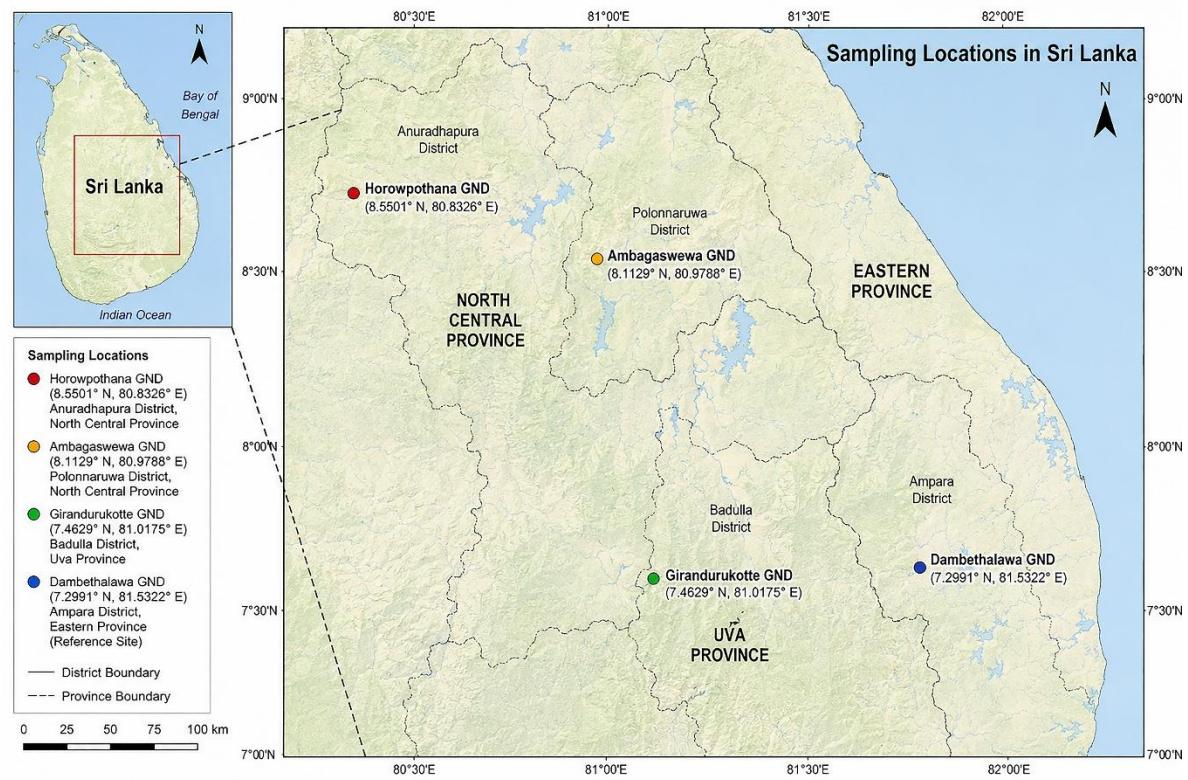


Fig 1: Geographic distribution of the selected sampling locations in Sri Lanka representing Chronic Kidney Disease of unknown etiology (CKDu) endemic and non-endemic regions.

A stratified random sampling design was implemented to ensure representative spatial coverage of paddy cultivation within each district. Regular grids were superimposed over mapped paddy extents in QGIS, with grid intersections within actively cultivated fields designated as Primary Sampling Units (PSUs). A minimum of 30 PSUs per location was targeted to provide sufficient spatial resolution for IDW interpolation. At each PSU, simultaneous collection of composite topsoil (0–20 cm), subsoil (20–40 cm), fertilizer grab samples from farmer storage sites, rice root biomass (excised approximately 10 cm from crown at panicle initiation), and mature paddy grain samples from a standardized 1 m² quadrat was undertaken.

2.3. Sample Pre-treatment and Microwave-Assisted Acid Digestion

All solid matrices underwent standardised pre-treatment. Air-dried soils were disaggregated and sieved through a 2 mm stainless-steel mesh. Root samples were sequentially washed with deionised water, treated with 0.5 mM $CaCl_2$ for 30 min to desorb apoplastically bound metals, and immersed in 0.1 mM Na_2-EDTA (pH 5.7) for 10 min to remove surface-associated iron plaque. Paddy grain samples were de-husked, ground to <250 µm, and stored in HDPE vials at −20 °C.

Microwave-assisted acid digestion was performed in closed-vessel systems. Soils and fertilizers (0.250 ± 0.001 g) were digested with 7 mL trace-metal grade HNO_3 (65–70%) and 1 mL H_2O_2 (30%), ramped to 200 °C over 15 min, held for 20 min, and cooled before quantitative transfer to 50 mL volumetric flasks (USEPA Method 3051A; ISO 14869-1). Grain samples (0.500 ± 0.001 g) were digested with 8 mL HNO_3 and 2 mL H_2O_2 at 180–200 °C without HF addition, validated against NIST SRM 1568b (USEPA Method 3052). Root biomass (0.200 ± 0.001 g) was digested with 7 mL HNO_3 and 1 mL H_2O_2 at 190 °C for 15 min, validated against BCR-670.

2.3. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Analysis

Concentrations of each heavy metal element, including Cd, Pb, As, Cr, Zn, Cu, and Mn in digested food and fish samples, were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS-7800-Agilent, Germany). Multi-element ICP-MS standards (AccuStandard, USA) were used for instrumental calibration. Five standard solution series of 0.5, 1.0, .5.0, 10.0, and 20.0 µg/L were used to construct the calibration curve for the metal analysis of digested paddy plant samples. All Microwave digested samples were filtered through 0.45µm syringe filters before the insertion to the ICP-MS instrument. The instrumental minimum detection limits (MDLs) for the elements measured by using ICP-MS in µg/Lwere as follows: As (0.001), Cd (0.0005), Cr (0.0005), Cu (0.0005), Pb (0.0005), Zn (0.001), Mn (0.0005) (Dayananda and Liyanage, 2021). The calibration curve was ensured with a correlation coefficient (R^2), which fell into a range of 0.99860–0.99999. Blanks were always less than MDLs. Spike recovery rates ranged from 96 to 110% for all elements investigated. Analytical accuracy and method reliability were validated using certified reference materials (CRMs) incorporated into every digestion batch, including NIST SRM 2709a for soil matrices, NIST SRM 1568b for grain samples, and BCR-670 for root tissue validation. Data sets containing values below the limit of detection (LOD) are encountered regularly in most fields of environmental contaminant research. The current norm within environmental research is to use substitution methods when analyzing data sets that include values below the LOD, commonly replacing each LOD value with a value equal to half the LOD (LOD/2). Therefore, the same rule was applied for the censored data sets in the present study.

2.4. Statistical analysis and Spatial Interpolation

Spatially continuous PTE concentration surfaces were generated using Inverse Distance Weighting (IDW) in R software, with cross-validated power parameter optimization to minimise interpolation error. IDW exploits spatial autocorrelation — the tendency for geochemically related, proximally located soils to harbour more similar PTE concentrations than randomly selected pairs (Goovaerts, 1997). Interpolated surfaces were generated separately

for each sampling location and element to facilitate visual hotspot identification and geo-epidemiological overlay with published CKDu prevalence data.

Statistical analyses were performed using R software and SPSS Statistics. All datasets were screened for normality and homogeneity of variances ($p < 0.05$). Descriptive statistics were expressed as mean ± standard deviation. For non-normally distributed datasets, Mann–Whitney U and Kruskal-Wallis tests were applied; independent t-tests and ANOVA were used for normally distributed data. Post-hoc Tukey pairwise comparisons identified significant inter-site differences. Principal Component Analysis (PCA) was performed to characterize fertilizer heavy metal compositional profiles.

2.5. Calculation of Contamination and Pollution Indices

2.5.1. Translocation Ratio/Transfer Ratio (TR)

Translocation Ratio (TR) is an index used to evaluate the efficiency of heavy metal movement from soil into plant tissues or from one plant part to another. It indicates the capability of plants to absorb and transfer metals through biological pathways.

$$Equation: \quad TR = \frac{C_{plant}}{C_{soil}} \quad \text{---------- (1)}$$

Where: TR = Translocation ratio / transfer ratio, C_{plant} = Heavy metal concentration in plant tissue (mg/kg) and C_{soil} = Heavy metal concentration in soil (mg/kg).

2.5.2. Bioconcentration Factor (BCF)

Bioconcentration Factor (BCF) is the ratio of heavy metal concentration in plant roots to that in soil. It is used to assess the accumulation potential of plants and their ability to uptake metals from contaminated soils.

$$Equation: BCF = \frac{C_{root}}{C_{soil}} \text{---------- (2)}$$

Where: BCF = Bioconcentration factor, C_{root} = Heavy metal concentration in root (mg/kg) and C_{soil} = Heavy metal concentration in soil (mg/kg).

2.5.3. Contamination Factor (CF)

Contamination Factor (CF) is an index used to evaluate the contamination level of individual heavy metals in soil by comparing measured concentrations with background or reference concentrations.

$$Equation: CF_i = \frac{C_i}{C_{background}} \quad \text{---------- (3)}$$

Where: CF_i = Contamination factor for metal i, C_i = Measured concentration of metal i, and $C_{background}$ = Background concentration of metal i.

2.5.4. Ecological Risk Factor (E_r^i)

Ecological Risk Factor (E_r^i) is a parameter used to estimate the ecological risk posed by a specific heavy metal by integrating its contamination level with its toxic response factor.

$$Equation: E_r^i = T_r^i \times CF_i \text{ ---------- (4)}$$

Where: E_r^i = Ecological risk factor of metal *i*, T_r^i= Toxic response factor of metal *i* (Cd: 30, Pb: 5, As: 10, Cr: 2, Cu: 5, Zn: 1, and Mn: 1) and CF_i = Contamination factor.

2.5.5. Potential Ecological Risk Index (*PERI*)

Potential Ecological Risk Index (PERI) is a comprehensive ecological risk assessment tool used to evaluate the overall environmental risk posed by multiple heavy metals in soils or sediments. It combines contamination levels and toxicological sensitivity of individual metals into a single cumulative risk index.

$$Equation: PERI = \sum E_r^i \text{ ---------- (5)}$$

Where: *PERI* = Potential ecological risk index and E_r^i = Ecological risk factor for each metal. (*PERI* Classification – If *PERI* is < 150 - Low ecological risk, 150–300 - Moderate ecological risk, 300–600 - Considerable ecological risk and > 600 - Very high ecological risk)

3. Results and Discussion

3.1. Spatial variability of toxic elements in soil, grain, and root samples collected from paddy agroecosystems

3.1.1. Concentrations of toxic elements in collected paddy soils

The soil analysis revealed substantial spatial variability in potentially toxic element (PTE) concentrations across the four paddy cultivation areas. Among the investigated locations, Girandurukotte consistently exhibited the highest mean concentrations for most heavy metals, including Cd (0.177 mg/kg), Pb (10.526 mg/kg), As (3.887 mg/kg), Mn (563.446 mg/kg), Cu (38.904 mg/kg), and Zn (42.151 mg/kg), indicating comparatively elevated anthropogenic loading and long-term accumulation within this agroecosystem. In contrast, Dambethalawa recorded the lowest concentrations for all measured elements, particularly Pb (3.504 mg/kg), As (2.006 mg/kg), Cu (25.177 mg/kg), and Zn (30.328 mg/kg), suggesting relatively lower contamination pressure and reduced agricultural input intensity. Chromium concentrations remained relatively stable among Ambagaswewa, Horowpothana, and Girandurukotte (47.307–48.561 mg/kg), whereas Dambethalawa exhibited a comparatively lower mean Cr concentration (42.398 mg/kg) (Table 1).

254 **Table 1**: Mean concentrations and Tukey pairwise comparison results for potentially toxic elements in soil, grain, and root samples collected from four paddy-cultivating
255 regions.

Potentially Toxic Element	Sampling Location	Soil samples					Grain samples					Root samples				
				at 95% Confidence Interval					*at 95% Confidence Interval*					*at 95% Confidence Interval*		
		Mean	±SD	Lower Bound	Upper Bound	p-value	Mean	±SD	Lower Bound	Upper Bound	p-value	Mean	±SD	Lower Bound	Upper Bound	p-value
Cadmium	Ambethalawa	0.15	0.05	0.13	0.17	0.000	0.020	0.011	0.016	0.024	0.000	0.079	0.044	0.063	0.095	0.006
	Horowpothana	0.13	0.05	0.11	0.15	0.000	0.018	0.010	0.015	0.022	0.003	0.055	0.037	0.041	0.069	0.605
	Girandurukotte	0.18	0.06	0.16	0.20	0.000	0.021	0.013	0.016	0.025	0.000	0.067	0.053	0.047	0.086	0.108
	Dambethalawa	0.06	0.04	0.04	0.07	-	0.008	0.008	0.005	0.012	-	0.041	0.039	0.026	0.056	-
	Total	0.13	0.07	0.12	0.14	-	0.017	0.012	0.015	0.019	-	0.060	0.045	0.052	0.069	-
Lead	Ambethalawa	8.47	1.13	8.04	8.89	0.000	0.086	0.010	0.083	0.090	0.000	1.695	0.241	1.605	1.784	0.000
	Horowpothana	7.57	1.53	7.00	8.14	0.000	0.080	0.018	0.073	0.086	0.000	1.510	0.311	1.394	1.626	0.000
	Girandurukotte	10.53	1.21	10.08	10.98	0.000	0.106	0.015	0.101	0.112	0.000	2.096	0.246	2.004	2.188	0.000
	Dambethalawa	3.50	1.27	3.03	3.98	-	0.035	0.016	0.029	0.041	-	0.715	0.265	0.616	0.814	-
	Total	7.52	2.86	7.00	8.03	-	0.077	0.030	0.071	0.082	-	1.504	0.569	1.401	1.607	-
Arsenic	Ambethalawa	3.52	0.49	3.34	3.71	0.000	0.004	0.006	0.002	0.006	1.000	0.189	0.068	0.164	0.214	0.000
	Horowpothana	3.23	0.55	3.03	3.44	0.000	0.006	0.008	0.003	0.010	0.562	0.164	0.055	0.144	0.185	0.001
	Girandurukotte	3.89	0.57	3.67	4.10	0.000	0.006	0.007	0.003	0.008	0.735	0.199	0.062	0.175	0.222	0.000
	Dambethalawa	2.01	0.58	1.79	2.22	-	0.004	0.006	0.002	0.006	-	0.102	0.068	0.077	0.128	-
	Total	3.16	0.89	3.00	3.32	-	0.005	0.007	0.004	0.006	-	0.164	0.073	0.150	0.177	-
Chromium	Ambethalawa	48.56	2.51	47.62	49.50	0.000	0.149	0.012	0.144	0.153	0.000	1.457	0.096	1.421	1.493	0.000
	Horowpothana	47.31	2.63	46.33	48.29	0.000	0.139	0.013	0.134	0.144	0.012	1.420	0.110	1.379	1.461	0.000
	Girandurukotte	48.25	3.22	47.05	49.45	0.000	0.145	0.016	0.139	0.151	0.000	1.436	0.103	1.397	1.474	0.000
	Dambethalawa	42.40	2.70	41.39	43.41	-	0.128	0.012	0.124	0.132	-	1.279	0.093	1.245	1.314	-
	Total	46.63	3.71	45.96	47.30	-	0.140	0.015	0.137	0.143	-	1.398	0.122	1.376	1.420	-
	Ambethalawa	538.15	34.64	525.22	551.08	0.000	10.759	0.693	10.501	11.018	0.000	161.433	10.405	157.547	165.318	0.000

Manganese	Horowpothana	526.74	38.34	512.42	541.06	0.000	10.534	0.769	10.247	10.821	0.000	158.018	11.504	153.722	162.314	0.000
	Girandurukotte	563.45	31.16	551.81	575.08	0.000	11.267	0.623	11.034	11.500	0.000	169.053	9.345	165.564	172.543	0.000
	Dambethalawa	447.40	40.75	432.19	462.62	-	8.948	0.813	8.645	9.251	-	134.222	12.220	129.659	138.785	-
	Total	518.93	56.48	508.73	529.14	-	10.377	1.129	10.173	10.581	-	155.681	16.947	152.618	158.745	-
Copper	Ambethalawa	36.24	2.12	35.45	37.04	0.000	1.816	0.104	1.778	1.855	0.000	9.052	0.519	8.858	9.245	0.000
	Horowpothana	35.22	2.01	34.47	35.97	0.000	1.764	0.100	1.727	1.802	0.000	8.804	0.498	8.618	8.990	0.000
	Girandurukotte	38.90	1.64	38.29	39.52	0.000	1.946	0.088	1.914	1.979	0.000	9.714	0.409	9.562	9.867	0.000
	Dambethalawa	25.18	1.80	24.50	25.85	-	1.256	0.092	1.222	1.291	-	6.294	0.446	6.128	6.461	-
	Total	33.89	5.55	32.88	34.89	-	1.696	0.280	1.645	1.747	-	8.466	1.383	8.216	8.716	-
Zinc	Ambethalawa	40.84	2.01	40.08	41.59	0.000	4.084	0.202	4.008	4.159	0.000	14.298	0.713	14.032	14.565	0.000
	Horowpothana	39.88	1.99	39.13	40.62	0.000	3.988	0.201	3.913	4.063	0.000	13.941	0.697	13.681	14.201	0.000
	Girandurukotte	42.15	1.60	41.55	42.75	0.000	4.212	0.158	4.153	4.271	0.000	14.743	0.552	14.536	14.949	0.000
	Dambethalawa	30.33	2.39	29.43	31.22	-	3.030	0.239	2.941	3.119	-	10.621	0.842	10.306	10.935	-
	Total	38.30	5.10	37.38	39.22	-	3.829	0.511	3.736	3.921	-	13.401	1.780	13.079	13.722	-

NB: p-values from Tukey pair-wise comparison with reference at 95% confident interval and values are indicated in mg/kg.

The Tukey pairwise comparison results demonstrated statistically significant differences ($p < 0.05$) among sampling locations for nearly all soil metals, confirming strong spatial heterogeneity in soil contamination patterns (Table 1). Particularly, Cd, Pb, Cu, Zn, and Mn showed highly significant differences between Dambethalawa and the remaining locations, emphasizing the comparatively lower contamination status of Dambethalawa soils. The elevated Pb and Cd concentrations observed in Girandurukotte and Ambagaswewa may be associated with intensive fertilizer application, agrochemical usage, irrigation water contamination, and cumulative agricultural deposition processes. Furthermore, the relatively high Mn concentrations observed across all sites indicate the strong geogenic contribution of manganese-rich parent materials in addition to agricultural inputs. The results also indicate that Girandurukotte may represent the most environmentally vulnerable site among the studied agroecosystems with respect to cumulative heavy metal loading.

Color intensity of Fig 2 represents the relative enrichment levels of each metal, where darker red shades indicate comparatively higher concentrations and lighter shades indicate lower concentrations. Dendrogram clustering illustrates the similarity relationships among sampling locations and metal accumulation profiles.

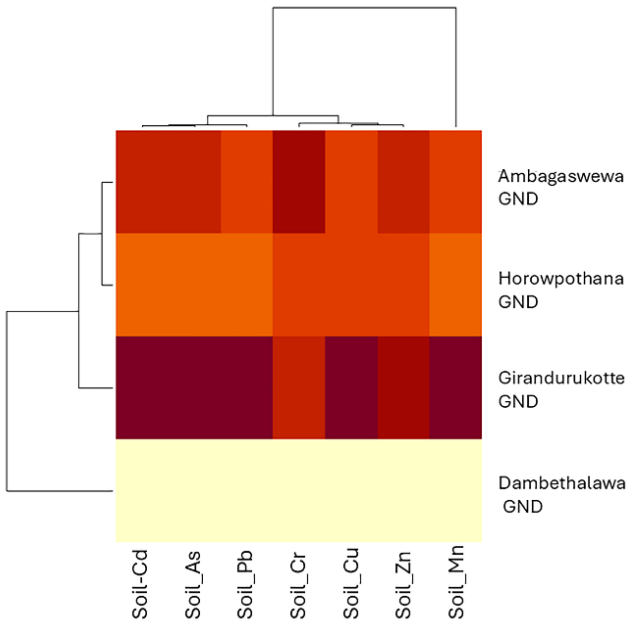


Fig 2: Hierarchical clustering heatmap illustrating the spatial distribution and similarity patterns of soil heavy metal concentrations across four CKDu hotspot-associated paddy agroecosystems in Sri Lanka

The clustering structure clearly separated Girandurukotte from the other sampling regions, indicating that this agroecosystem possessed a substantially different and comparatively enriched heavy metal signature. Girandurukotte exhibited the highest relative intensities for Cd, As, Cu, Zn, and Mn, as reflected by the darker red coloration across most investigated metals (Fig 2). This enrichment pattern suggests intensified anthropogenic loading, potentially associated with long-term fertilizer application, agrochemical usage, irrigation-mediated sediment deposition, and enhanced trace metal accumulation within intensively cultivated paddy soils. The strong clustering of Cd, Cu, Zn, and Mn additionally indicates that these metals may share common geochemical sources or similar accumulation pathways within the agroecosystem. Ambagaswewa and Horowpothana formed an intermediate cluster characterized by moderate heavy metal accumulation levels. Although both sites demonstrated elevated concentrations relative to Dambethalawa (the reference), Ambagaswewa generally exhibited slightly greater enrichment for Cd, Cr, and Zn, suggesting stronger anthropogenic influence or differences in soil geochemical behavior. The close clustering between these two agroecosystems indicates comparable contamination dynamics and environmental conditions governing metal retention and mobility.

However, Dambethalawa; the reference site, formed a clearly distinct cluster characterized by uniformly low heavy metal concentrations, as indicated by the pale coloration across all investigated metals. This pattern suggests comparatively minimal contaminant loading and relatively lower anthropogenic pressure within this agroecosystem. The distinct separation of Dambethalawa from the CKDu hotspot regions further supports the hypothesis that localized agricultural intensification and agrochemical inputs contribute substantially to heavy metal enrichment in endemic dry-zone paddy systems. The dendrogram associated with the metals demonstrated close clustering among Cd, As, Pb, and Cu, indicating potential similarities in their environmental behavior, transport mechanisms, or agricultural sources. Such clustering patterns may reflect co-introduction through phosphate fertilizers, pesticide formulations, irrigation water contamination, or geogenic contributions from parent soil materials.

3.1.2. Concentrations of toxic elements in collected Oryza sativa L. grain samples

Grain PTE concentrations were substantially lower than soil and root concentrations, indicating restricted translocation into edible tissues. Nevertheless, distinct spatial variations were evident. Girandurukotte consistently recorded the highest grain concentrations for Pb (0.106 mg/kg), Mn (11.267 mg/kg), Cu (1.946 mg/kg), and Zn (4.212 mg/kg). Cadmium concentrations ranged from 0.008 mg/kg in Dambethalawa to 0.021 mg/kg in Girandurukotte. Arsenic concentrations remained low across all sites (0.004–0.006 mg/kg), and chromium concentrations were minimal (0.128–0.149 mg/kg) (Table 1), indicating limited Cr translocation into grain despite its relatively high soil abundance.

Tukey pairwise comparisons revealed significant site-dependent accumulation for Pb, Mn, Cu, and Zn ($p < 0.05$), reflecting variations in soil bioavailability, rhizosphere chemistry, and irrigation practices. The elevated grain Pb in Girandurukotte is of particular concern given the direct dietary exposure pathway. Although overall grain concentrations were relatively low, chronic rice-based dietary exposure in populations consuming predominantly local paddy could yield cumulative health risks. The comparatively low grain Cd and As concentrations suggest partial physiological barriers to translocation from roots to grain; nevertheless, measurable concentrations at all sites confirm continuous bioaccumulation within the soil–plant system.

3.1.2. Concentrations of toxic elements in collected paddy root samples

Root samples exhibited substantially higher PTE concentrations than grain tissues, confirming the role of roots as the primary accumulation compartment. Girandurukotte again showed the highest root concentrations for Pb (2.096 mg/kg), As (0.199 mg/kg), Mn (169.053 mg/kg), Cu (9.714 mg/kg), and Zn (14.743 mg/kg). Dambethalawa consistently showed the lowest root concentrations. Cd in roots ranged from 0.041 mg/kg (Dambethalawa) to 0.079 mg/kg (Ambagaswewa), while Cr remained relatively stable across sites (1.279–1.457 mg/kg) (Table 1).

The strong statistical significance observed for most root metal concentrations demonstrates high sensitivity of root accumulation to site-specific soil chemistry. Elevated Pb, Mn, Cu, and Zn root concentrations reflect efficient uptake followed by partial restriction of upward translocation — consistent with known plant detoxification mechanisms whereby roots immobilize toxic metals within root tissues, limiting transfer to edible parts. The progressive attenuation of metal concentrations from soil to roots to grain indicates physiological control over metal translocation, though elevated root concentrations in Girandurukotte and Ambagaswewa imply long-term environmental exposure and continued metal cycling within these agroecosystems.

3.1.2. Assessment of Spatial Variability and Pollution Risk of Cadmium and Lead using Spatial Interpolation

The spatial interpolation maps (Fig 3 and Fig 4) illustrate the geographical distribution and intensity of cadmium (Cd) concentrations in paddy soils using the Inverse Distance Weighting (IDW) geostatistical technique. Fig 3 clearly demonstrates considerable spatial heterogeneity in Cd accumulation among the four agricultural regions. Among the four study regions, Girandurukotte exhibits the highest spatial intensity of Cd accumulation, with several hotspots distributed throughout the cultivated landscape. These elevated zones suggest localized

enrichment likely associated with long-term fertilizer application, irrigation water inputs, and intensive agricultural activities. Ambagaswewa also demonstrates relatively high Cd accumulation, though the contaminated zones appear moderately dispersed compared with Girandurukotte. The clustering of higher Cd concentrations in these two regions aligns with the elevated mean Cd concentrations reported in the statistical analysis, confirming the reliability of the spatial interpolation results.

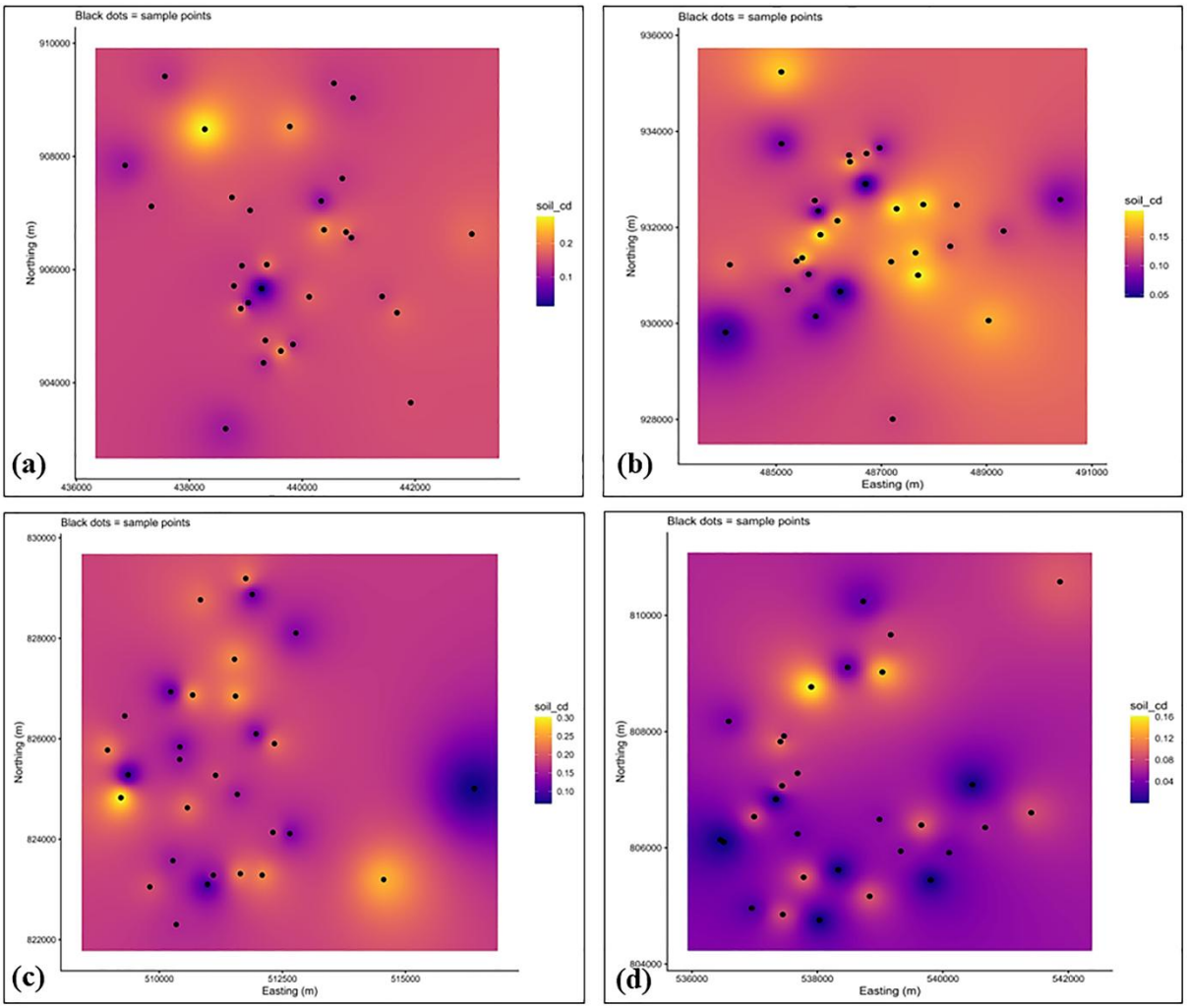


Fig 3: Inverse Distance Weighting (IDW)-based spatial interpolation map showing the distribution of Cadmium in paddy soils across the four sampling locations: (a) Ambagaswewa, (b) Horowpothana, (c) Girandurukotte, and (d) Dambethalawa.

Horowpothana presents intermediate Cd distribution patterns, characterized by moderate concentration gradients and fewer pronounced hotspots. The contamination appears more spatially uniform, suggesting a comparatively stable distribution of Cd within the agricultural soils. In contrast, Dambethalawa exhibits the lowest Cd concentrations across the study area, with predominantly cooler color zones indicating minimal contamination levels. The relatively low spatial variability observed in reference further supports the interpretation that this region experiences lower anthropogenic heavy metal loading compared with the other CKDu-endemic regions.

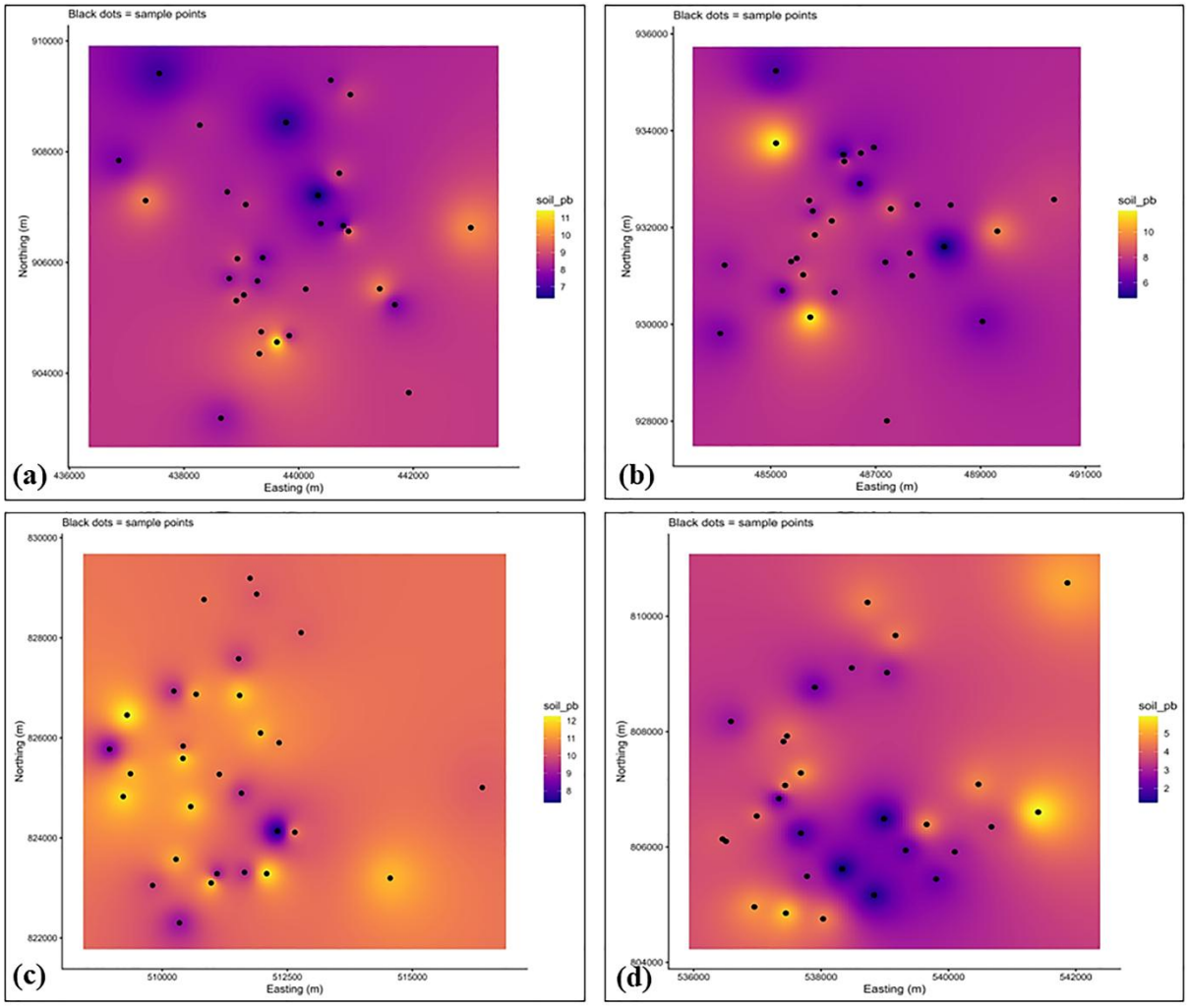


Fig 4: IDW-based spatial interpolation mapping for Lead concentrations in paddy soils across the sampling locations: (a) Ambagaswewa, (b) Horowpothana, (c) Girandurukotte, and (d) Dambethalawa.

In the Ambagaswewa sampling area, Pb concentrations exhibited moderate spatial heterogeneity with several localized hotspots distributed mainly in the central and eastern parts of the study region. Lower Pb concentrations were observed in scattered patches toward the northern and southeastern boundaries. The interpolated surface suggests that Pb accumulation in this area is unevenly distributed, possibly influenced by localized agricultural inputs, irrigation practices, or soil physicochemical characteristics. The Horowpothana site demonstrated distinct spatial variability with pronounced high-concentration zones located in the western and south-central regions. Comparatively lower Pb levels were concentrated around the central-eastern section of the map. The occurrence of isolated hotspots surrounded by moderate concentration zones indicates localized contamination sources or variable retention capacities of the soil matrix within the sampling landscape.

In Girandurukotte, the spatial distribution pattern showed relatively elevated Pb concentrations over a larger proportion of the study area compared to the other locations. Several hotspots were concentrated in the western and southern sections, while localized lower concentration pockets appeared in the central region. The broader spread of higher Pb concentrations may indicate more extensive anthropogenic influences such as prolonged agrochemical usage, irrigation water quality, or sediment deposition processes associated with intensive paddy

cultivation. The Dambethalawa reference site exhibited comparatively lower overall Pb concentrations, although some isolated elevated zones were observed toward the northeastern and eastern portions of the study area.

3.2. Potentially toxic element concentrations in commonly applied inorganic fertilizers used in paddy agroecosystems

The analysis of inorganic fertilizers revealed substantial variability in heavy metal concentrations among fertilizer categories, indicating that agricultural inputs may serve as important anthropogenic pathways for trace metal enrichment within paddy agroecosystems. Among the analyzed fertilizers, Triple Superphosphate exhibited the highest concentrations of Cd, Pb, Cr, and Cu, while Urea showed markedly elevated concentrations of As and Zn (Table 2).

Table 2: Selected toxic metal/metalloid concentrations in commonly applied inorganic fertilizers used in paddy agroecosystems

Metal/Element (mg/kg)	Urea	Muriate of Potash (MOP)	Triple Superphosphate (TSP)
Cd	1.74 ± 0.14	0.04 ± 0.02	6.74 ± 0.37
Pb	89.22 ± 9.78	6.34 ± 1.64	124.34 ± 14.58
As	9.90 ± 2.72	0.24 ± 0.03	1.88 ± 0.10
Cr	102.00 ± 4.17	23.61 ± 4.45	186.36 ± 44.89
Mn	211.24 ± 128.10	4.20 ± 0.75	234.00 ± 55.84
Cu	21.32 ± 1.10	0.58 ± 0.17	141.64 ± 15.90
Zn	723.00 ± 96.50	93.82 ± 20.42	348.70 ± 21.93

The exceptionally high cadmium concentration detected in Triple Superphosphate (6.74 ± 0.37 mg/kg) is environmentally significant because phosphate fertilizers are widely recognized as major anthropogenic sources of Cd contamination in agricultural soils. Cadmium impurities in phosphate fertilizers originate primarily from phosphate rock deposits used during fertilizer manufacture. Continuous long-term application of phosphate fertilizers may therefore progressively elevate soil Cd concentrations, particularly in intensively cultivated ecosystems where fertilizer inputs are repeatedly applied over multiple growing seasons.

Similarly, the elevated Pb concentrations observed in both Triple Superphosphate (124.34 ± 14.58 mg/kg) and Urea (89.22 ± 9.78 mg/kg) indicate substantial lead-associated contamination potential. Lead accumulation in agricultural soils is particularly concerning because Pb is persistent, non-biodegradable, and capable of long-term environmental retention through adsorption onto soil particles and organic matter. Chromium concentrations were also notably elevated in Triple Superphosphate (186.36 ± 44.89 mg/kg) and Urea (102.00 ± 4.17 mg/kg), suggesting that fertilizer-derived Cr inputs may contribute to gradual soil enrichment (Table 2). Although chromium mobility in soils is often limited, prolonged accumulation can increase ecological and toxicological risks depending on soil redox conditions and chromium speciation.

Urea demonstrated remarkably high Zn concentrations (723.00 ± 96.50 mg/kg), substantially exceeding those observed in the other fertilizer types (Fig 5). Zinc is an essential micronutrient for plant metabolism and is

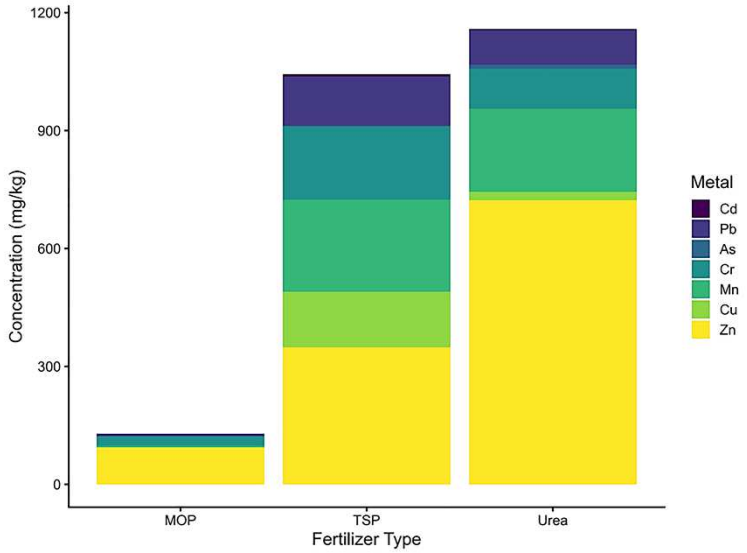


Fig 5: Total burden in fertilizers comparing the cumulative concentrations of selected potentially toxic metals in three common fertilizer types: MOP, TSP, and Urea.

often intentionally added to fertilizers to improve crop productivity. However, excessive Zn accumulation may alter soil microbial processes and interfere with nutrient cycling within agroecosystems. The elevated Cu concentration in Triple Superphosphate (141.64 ± 15.90 mg/kg) may reflect contamination associated with raw material impurities or fertilizer manufacturing processes. Copper-containing agrochemicals and fungicides may additionally contribute to cumulative Cu enrichment within intensively cultivated paddy soils.

Additionally, Muriate of Potash consistently exhibited the lowest concentrations for most investigated metals, suggesting comparatively lower contamination potential relative to the other fertilizer types. Nevertheless, even low-level chronic inputs may contribute to long-term accumulation within poorly drained paddy environments characterized by slow contaminant turnover. The findings demonstrate that inorganic fertilizers represent a significant pathway for heavy metal/metalloid introduction into rice-based agroecosystems. The repeated application of contaminated fertilizers may progressively enhance soil metal accumulation, increase plant uptake potential, and ultimately facilitate trophic transfer into edible rice grains.

The PCA biplot (Fig 6) explains approximately 89.9% of the total variance, with Principal Component 1 (Dim1) accounting for 69.1% and Principal Component 2 (Dim2) explaining 20.8% of the variation. The separation of fertilizer types across the PCA space indicates substantial compositional differences in heavy metal content. TSP is positioned strongly on the positive side of Dim1, indicating a strong association with elevated concentrations of Cd, Cr, Cu, Mn, Pb, and Zn. This confirms that TSP acts as the major contributor of multiple heavy metals among the analyzed fertilizers (Fig 6). Urea is located in the upper positive quadrant and aligns more closely with As and Zn vectors, suggesting comparatively greater enrichment of these elements relative to the other fertilizers. In contrast, MOP is clearly separated on the negative side of Dim1, demonstrating substantially lower overall element concentrations and indicating a comparatively cleaner fertilizer profile.

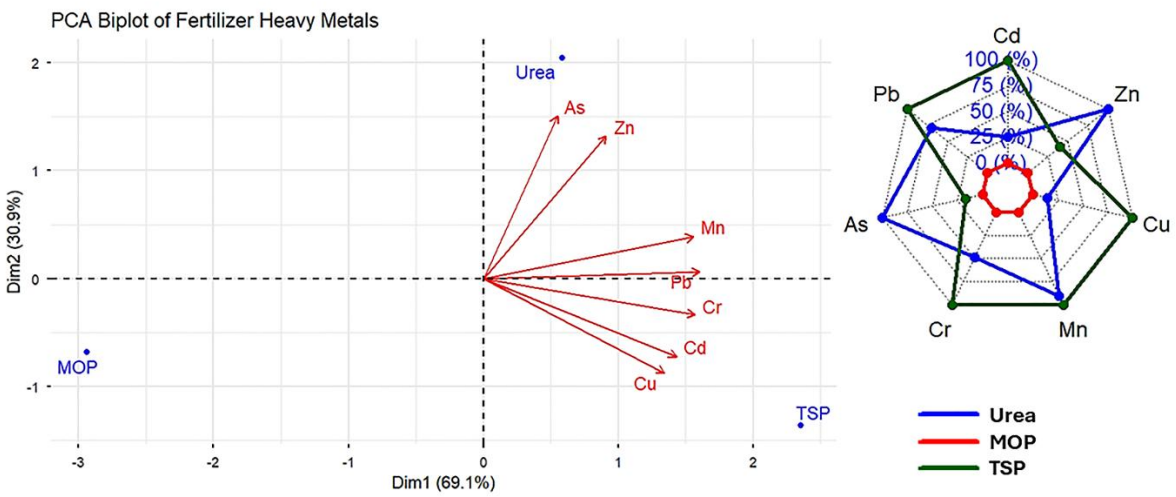


Fig 6: Multivariate visualizations describing the heavy metal composition of the analyzed fertilizers: a Principal Component Analysis (PCA) biplot and a radar chart.

The vector directions and lengths in the PCA further illustrate relationships among metals. Metals such as Cd, Cu, Cr, Pb, and Mn cluster closely together and point toward the TSP region, indicating strong positive correlations and common enrichment sources (Fig 6). Zn and As show slightly different orientations, suggesting somewhat distinct distribution patterns among fertilizers. Longer vectors represent stronger contributions to total variability, highlighting Mn, Zn, and Cd as key discriminating elements influencing fertilizer differentiation.

According to the radar chart, TSP exhibits the highest concentrations for most analyzed metals, particularly Cd, Pb, Cr, Cu, and Mn, confirming its dominant contribution to metal loading in agricultural soils (Fig 6). The broader spatial coverage of the TSP polygon reflects its overall higher contamination burden compared with the other fertilizers. These findings support concerns regarding the long-term accumulation of toxic metals in agricultural soils through repeated fertilizer application and highlight the importance of monitoring fertilizer quality in CKDu-endemic agricultural regions.

3.3. Indexed-based Evaluation of Heavy Metal Contamination, Enrichment, and Ecological Risk in Paddy Soils

3.3.1. Contamination factor (CF) values of heavy metals in paddy soils

CF values for all metals were below unity, indicating low contamination intensity relative to background reference concentrations. However, distinct spatial differences in enrichment patterns were observed among the study locations. Girandurukotte exhibited the highest CF values for Cd (0.589), Pb (0.526), Cu (0.865), Zn (0.444), and Mn (0.663), suggesting enhanced metal accumulation within this agroecosystem (Table 3). These findings are particularly important because Cd enrichment in paddy soils has frequently been associated with phosphate fertilizer applications, which constitute a recognized anthropogenic source of cadmium in agricultural systems.

Similarly, the relatively elevated Cu and Zn values may reflect cumulative agrochemical applications and micronutrient amendments used to sustain intensive rice cultivation. Meanwhile, the reference showed substantially lower CF values for most metals, indicating comparatively limited anthropogenic enrichment.

Table 3: Contamination factor (CF) values considered metals/metalloids in paddy soils from four CKDu hotspot agroecosystems.

Sampling site	CF values						
	Cd	**Pb**	**As**	**Cr**	**Mn**	**Cu**	**Zn**
Ambagaswewa	0.499	0.423	0.352	0.540	0.633	0.805	0.430
Horowpothana	0.431	0.378	0.323	0.526	0.620	0.783	0.420
Girandurukotte	0.589	0.526	0.389	0.536	0.663	0.865	0.444
Dambethalawa	0.185	0.175	0.201	0.471	0.526	0.559	0.319

Although CF values remained below the critical contamination threshold, the observed spatial enrichment patterns demonstrate that certain CKDu hotspot regions are experiencing progressive trace metal accumulation that may become ecologically significant over longer temporal scales.

3.3.2. Bioaccumulation factors (BCF) of heavy metals/metalloids in rice plants

BCF analysis demonstrated substantial variability in the root uptake efficiencies of different metals (Table 4). Among all investigated elements, Cd exhibited the highest soil-to-root transfer efficiency, particularly in Dambethalawa (BCF = 0.740), indicating strong root accumulation potential. The elevated cadmium uptake observed in Dambethalawa despite relatively lower soil Cd concentrations suggests that factors other than total metal concentration, such as soil pH, redox potential, organic matter composition, and competitive ion interactions, strongly regulate Cd bioavailability within paddy ecosystems.

Table 4: Soil-to-root bioaccumulation factor (BCF) values of heavy metals/metalloids in rice plants cultivated in CKDu hotspot agroecosystems.

Site	**Soil-to-root bioaccumulation factor (BCF) values**						
	Cd	**Pb**	**As**	**Cr**	**Mn**	**Cu**	**Zn**
Ambagaswewa	0.526	0.200	0.054	0.030	0.300	0.250	0.350
Horowpothana	0.425	0.200	0.051	0.030	0.300	0.250	0.350
Girandurukotte	0.377	0.199	0.051	0.030	0.300	0.250	0.350
Dambethalawa	0.740	0.204	0.051	0.030	0.300	0.250	0.350

Therefore, As and Cr exhibited comparatively low BCF values (<0.06 and <0.03, respectively), indicating restricted uptake into root tissues. This may reflect stronger adsorption of these elements into soil minerals and iron oxides under flooded paddy conditions. Zn and Mn showed moderate accumulation capacities, consistent with their essential micronutrient functions within plant physiological processes. The relatively stable BCF values for Cu, Zn, and Mn across sites suggest more tightly regulated plant uptake mechanisms for essential trace elements. The findings collectively indicate that cadmium represents the most bioavailable and mobile toxic metal within the investigated agroecosystems.

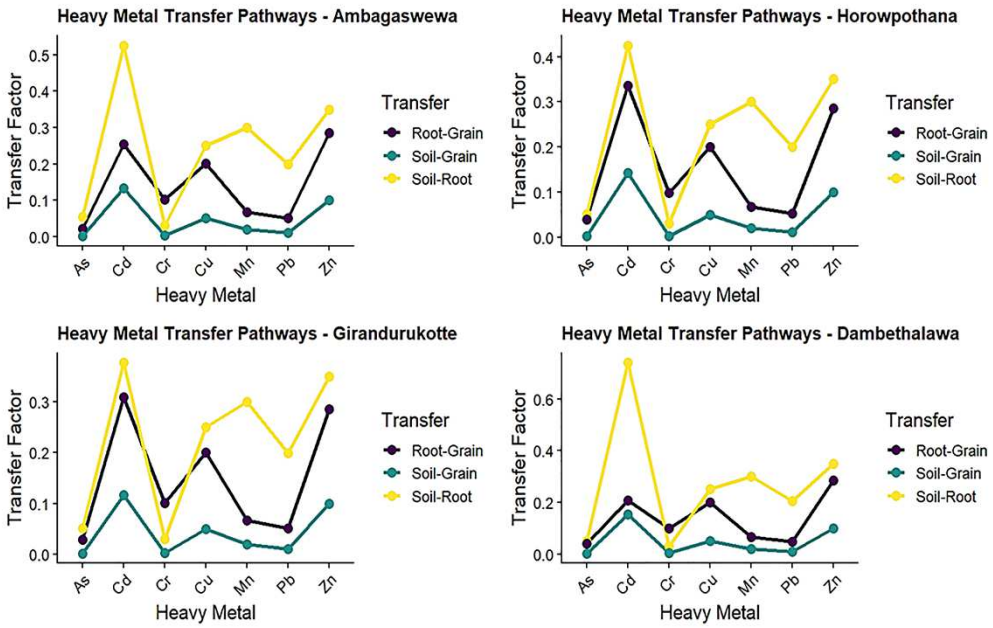


Fig 7: Soil-to-grain bioaccumulation factors (BCF) of heavy metals and the relationship between soil and grain Cd concentrations across the selected sampling locations.

Among the analyzed heavy metals, Cd exhibited the highest bioaccumulation capacity across all sampling locations, indicating a greater tendency for Cd uptake and translocation into rice grains compared to the other metals. The highest cumulative Cd accumulation was observed in Dambethalawa and Ambagaswewa, suggesting enhanced mobility and bioavailability of Cd in these soils. Cr and As displayed the lowest BCF values across all locations, indicating limited transfer from soil to grain and comparatively lower bioavailability under the prevailing soil conditions.

The right panel illustrates the relationship between soil Cd concentration and rice grain Cd concentration using linear regression analysis (Fig 7). A positive linear trend was observed between soil and grain Cd concentrations, indicating that increased Cd levels in paddy soils contribute to elevated Cd accumulation in rice grains. The regression line with confidence intervals demonstrates a moderately strong positive association, suggesting that soil Cd content is an important predictor of Cd uptake by rice plants.

3.3.3. Transfer factor values of potentially toxic metals/metalloids in rice cultivated across four CKDu hotspot-associated paddy agroecosystems

The root-to-grain transfer factor (TF_{RG}) analysis demonstrated that Cd and Zn possessed comparatively higher translocation efficiencies into edible rice grains. Cadmium TF_{RG} values ranged from 0.207–0.336, with the highest transfer observed in Horowpothana, suggesting an increased dietary exposure risk through rice consumption. The relatively high Cd mobility may result from its chemical similarity to essential nutrients such as Zn and Ca, enabling Cd transport through plant nutrient pathways. In contrast, Pb exhibited extremely low TF_{RG} values (~0.05), indicating strong retention within root tissues and limited translocation to grains. Arsenic and Cr also showed relatively restricted grain transfer efficiencies.

Table 5: Soil-to-root transfer factor (TF_{SR}), root-to-grain transfer factor (TF_{RG}), and soil-to-grain transfer factor (TF_{SG}) values of heavy metals in rice plants cultivated within CKDu hotspot-associated paddy agroecosystems

Site	TF_{SR} values							TF_{RG} values							TF_{SG} values						
	Cd	**Pb**	**As**	**Cr**	**Mn**	**Cu**	**Zn**	**Cd**	**Pb**	**As**	**Cr**	**Mn**	**Cu**	**Zn**	**Cd**	**Pb**	**As**	**Cr**	**Mn**	**Cu**	**Zn**
Ambagaswewa	0.53	0.20	0.05	0.03	0.30	0.25	0.35	0.26	0.05	0.02	0.10	0.07	0.20	0.29	0.13	0.01	0.00	0.00	0.02	0.05	0.10
Horowpothana	0.43	0.20	0.05	0.03	0.30	0.25	0.35	0.34	0.05	0.04	0.10	0.07	0.20	0.29	0.14	0.01	0.00	0.00	0.02	0.05	0.10
Girandurukotte	0.38	0.20	0.05	0.03	0.30	0.25	0.35	0.31	0.05	0.03	0.10	0.07	0.20	0.29	0.12	0.01	0.00	0.00	0.02	0.05	0.10
Dambethalawa	0.74	0.20	0.05	0.03	0.30	0.25	0.35	0.21	0.05	0.04	0.10	0.07	0.20	0.29	0.15	0.01	0.00	0.00	0.02	0.05	0.10

The soil-to-grain transfer factor (TF_{SG}) analysis further confirmed Cd as the most efficiently transferred toxic metal from soil into edible rice grains, with values ranging from 0.116–0.153 (Table 5). Despite lower soil Cd concentrations in Dambethalawa, TF_{SG} values remained comparatively high, indicating that metal bioavailability and plant physiological mechanisms influence grain accumulation more strongly than total soil concentration alone. Pb, As, and Cr exhibited minimal soil-to-grain transfer, whereas Zn showed comparatively higher TF_{SG} values due to its essential biological function. Overall, the transfer factor analyses demonstrate that Cd represents the most significant metal in terms of bioaccumulation and potential dietary exposure risk within CKDu hotspot-associated paddy agroecosystems.

Overall, soil-to-root transfer factors were consistently higher than soil-to-grain transfer factors for all metals and locations, indicating that rice roots function as the primary accumulation compartment for heavy metals. Comparatively lower soil-to-grain transfer factors demonstrate that only a limited proportion of absorbed metals is ultimately translocated into edible rice grains. Root-to-grain transfer factors showed intermediate values, reflecting the internal movement of metals from roots toward reproductive tissues.

Among the investigated metals, cadmium (Cd) exhibited the highest transfer efficiencies across most pathways and sampling locations. Particularly elevated soil-to-root and root-to-grain transfer factors were observed for Cd in Dambethalawa and Horowpothana, indicating enhanced Cd mobility and translocation potential. This suggests a greater risk of Cd accumulation in edible rice grains and associated dietary exposure. Zinc (Zn) also showed relatively high transfer efficiencies, especially in the soil-to-root and root-to-grain pathways, likely due to its essential physiological role in plant metabolism and nutrient transport.

In contrast, lead (Pb), arsenic (As), and chromium (Cr) exhibited comparatively lower transfer factors, particularly in the soil-to-grain pathway, indicating restricted movement into edible tissues. Pb showed moderate root accumulation but limited grain translocation due to strong adsorption to soil particles and low mobility within plants. Copper (Cu) and manganese (Mn) demonstrated moderate transfer behavior, with greater accumulation in roots than grains. Spatial variability among sampling locations was also evident, suggesting that soil physicochemical properties, irrigation quality, and agricultural management practices influence heavy metal bioavailability and plant uptake. The results identify Cd as the most mobile and environmentally significant toxic metal within the rice cultivation systems studied.

3.3.4. Ecological risk factor (Er) and potential ecological risk index (PERI) values for heavy metals in paddy soils

The ecological risk assessment results presented in Table 6 indicate substantial spatial variation in the contribution of individual heavy metals to the overall environmental risk within the studied paddy agroecosystems. Among the investigated metals, cadmium (Cd) consistently exhibited the highest ecological risk factor (Er) values across all sampling locations, ranging from 5.544 in Dambethalawa to 17.684 in Girandurukotte. This elevated Cd-associated risk is primarily attributed to its high toxic response factor and strong environmental persistence despite relatively low measured concentrations. In contrast, manganese (Mn), chromium (Cr), and zinc (Zn) exhibited comparatively low Er values in all locations, indicating minimal ecological threat from these metals under the present contamination levels.

Girandurukotte recorded the highest cumulative Potential Ecological Risk Index (PERI) value (30.704), followed by Ambagaswewa (26.793) and Horowpothana (24.067), whereas Dambethalawa exhibited the lowest PERI value (13.011). The comparatively elevated PERI values observed in Girandurukotte reflect the combined influence of higher concentrations of Cd, Pb, As, Cu, and Zn in this agroecosystem, suggesting stronger anthropogenic contamination and prolonged agricultural metal accumulation. Although Pb and Cu displayed moderate contributions to the overall ecological risk, their Er values remained substantially lower than those of Cd, confirming that Cd is the dominant ecological risk contributor among the investigated metals.

Table 6. Ecological risk factor (Er) values and cumulative potential ecological risk index (PERI) for heavy metals in paddy soils collected from CKDu hotspot-associated agroecosystems in Sri Lanka.

Site	Er-Cd	Er-Pb	Er-As	Er-Cr	Er-Mn	Er-Cu	Er-Zn	PERI
Ambagaswewa	14.984	2.117	3.524	1.079	0.633	4.027	0.430	26.793
Horowpothana	12.940	1.892	3.231	1.051	0.620	3.914	0.420	24.067
Girandurukotte	17.684	2.632	3.887	1.072	0.663	4.323	0.444	30.704
Dambethalawa	5.544	0.876	2.006	0.942	0.526	2.797	0.319	13.011

According to Hakanson's ecological risk classification, all investigated sites fall within the "low ecological risk" category (PERI < 150), indicating that the present contamination status does not yet represent severe ecological

impairment. Nevertheless, the consistently elevated contribution of Cd across all sites is environmentally important because long-term agricultural intensification, continuous fertilizer application, and metal accumulation may progressively increase ecological risk over time. The relatively low PERI values observed in Dambethalawa further support the interpretation that this site experiences comparatively lower anthropogenic metal loading and reduced contamination pressure.

Therefore, the ecological risk assessment demonstrates that although the studied paddy agroecosystems currently exhibit low cumulative ecological risk, cadmium remains the most critical contaminant due to its toxicity and dominant contribution to PERI values. These findings emphasize the importance of continuous monitoring of Cd-containing agricultural inputs, particularly phosphate fertilizers, in CKDu-endemic agricultural regions to prevent future environmental deterioration and associated human health risks.Ecological risk factor (Er) values and cumulative potential ecological risk index (PERI) for heavy metals in paddy soils of CKDu hotspot agroecosystems.

The cumulative potential ecological risk index (PERI) values ranged from 13.01 to 30.70 (Fig 8**),** indicating an overall low ecological risk category for all study regions. Nevertheless, Girandurukotte displayed the highest cumulative PERI value, suggesting that this agroecosystem represents the most environmentally vulnerable location among the investigated CKDu hotspots. Although the PERI values remain below internationally recognized high-risk thresholds, the dominance of Cd within the ecological risk profile is environmentally important because even moderate soil Cd accumulation can lead to long-term food-chain transfer and chronic human exposure through rice consumption.

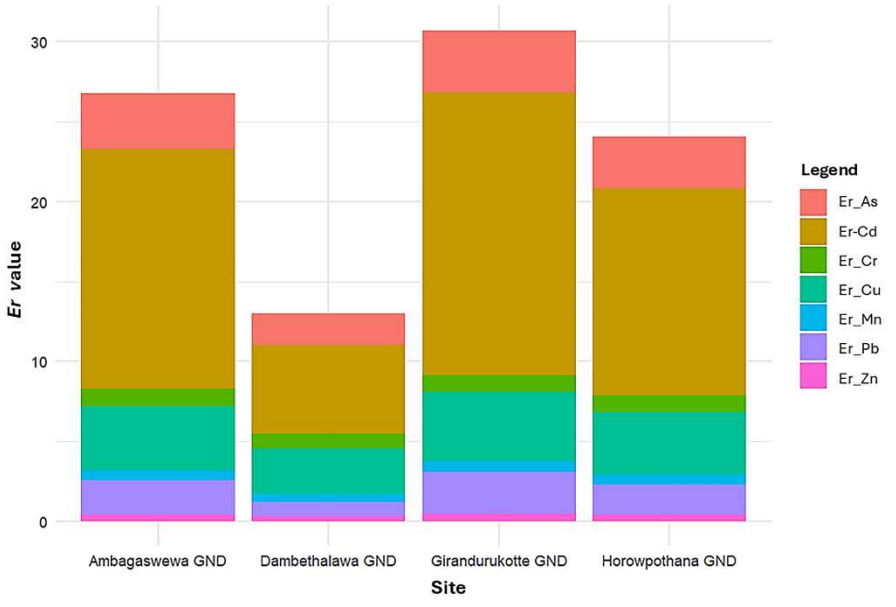


Fig 8: Cumulative Ecological Risk Factor (Er) values across CKDu hotspot-associated paddy agroecosystems in Sri Lanka

The Potential Ecological Risk Index (PERI) analysis demonstrated clear spatial variability in the cumulative ecological risk associated with heavy metal contamination among the investigated paddy agroecosystems. Among

the four study locations, Girandurukotte exhibited the highest PERI value (approximately 31), indicating the greatest overall ecological burden of heavy metals within the study region. This elevated ecological risk likely reflects intensified anthropogenic inputs, prolonged agrochemical application, irrigation-mediated contaminant accumulation, and enhanced geochemical retention of toxic metals within the paddy soil system.

Ambagaswewa and Horowpothana displayed intermediate PERI values, suggesting moderate ecological pressure relative to Girandurukotte. The similarity in PERI levels between these two agroecosystems indicates comparable contamination dynamics and environmental conditions governing metal accumulation and mobility. The observed ecological risk in these regions may be associated with long-term fertilizer usage, sediment-associated transport processes, and repeated cultivation cycles characteristic of intensively managed paddy ecosystems.

In contrast, Dambethalawa exhibited the lowest PERI value (approximately 13), indicating comparatively lower ecological risk and reduced heavy metal enrichment within this agroecosystem. The relatively low ecological burden observed in Dambethalawa suggests limited anthropogenic contamination inputs and potentially more favorable soil geochemical conditions that restrict heavy metal accumulation.

Although all PERI values remained below internationally recognized thresholds for moderate ecological risk, the results are environmentally significant because they demonstrate progressive trace metal enrichment within CKDu hotspot agroecosystems. Importantly, the relatively higher ecological risk observed in Girandurukotte corresponds closely with the elevated contamination, and hierarchical clustering patterns identified in the study, collectively confirming the presence of localized contamination hotspots. The PERI framework additionally highlighted the dominant contribution of cadmium to the cumulative ecological risk profile. Despite relatively low absolute concentrations, cadmium substantially influenced the overall PERI values due to its high toxic-response coefficient and strong ecological toxicity. This finding is particularly important because cadmium exhibits high mobility in flooded paddy soils and possesses strong bioaccumulation potential within rice-based food chains. Therefore, the PERI analysis demonstrates that while the investigated agroecosystems remain within the low ecological risk category, continuous agricultural intensification and long-term agrochemical inputs may progressively elevate ecological vulnerability in CKDu-endemic paddy landscapes. These findings emphasize the importance of implementing sustainable fertilizer management strategies, continuous environmental monitoring, and targeted mitigation measures to minimize future ecological deterioration and associated human health risks.

4. Conclusion

This integrated, multi-matrix assessment provides the most comprehensive evidence base to date linking fertilizer-derived PTE inputs to soil contamination, bioaccumulation, and dietary exposure risk in CKDu-endemic paddy farming communities of Sri Lanka. Triple Superphosphate emerged as the dominant anthropogenic PTE vector, carrying critically elevated Cd (6.74 mg/kg), Pb (124.34 mg/kg), and Cr (186.36 mg/kg) concentrations that accumulate progressively in paddy soils under repeated application and slow-turnover flooded conditions.

Cadmium distinguished itself across all environmental matrices as the most ecologically and toxicologically critical element, exhibiting the highest soil-to-root bioconcentration factors (up to 0.740), root-to-grain transfer factors (0.207–0.336), and dominant contribution to cumulative PERI values at every site despite comparatively

low absolute soil concentrations. Girandurukotte consistently emerged as the most contaminated and ecologically vulnerable agroecosystem, with the highest soil PTE burdens, most pronounced IDW-interpolated hotspots, and highest PERI value (30.70). The geo-epidemiological correspondence between these spatial contamination patterns and documented CKDu prevalence zones provides quantitative empirical support for chronic, low-dose dietary cadmium exposure as a significant aetiological contributor to renal tubular injury in Sri Lanka's dry zone.

Although all sites currently fall within the low ecological risk classification, the trajectory of progressive Cd enrichment under continued TSP application demands urgent policy intervention. The positive linear association between soil and grain Cd concentrations identifies fertilizer input management as the most actionable lever for reducing dietary exposure. Priority measures should include mandatory PTE quality certification for imported phosphate fertilizers, regulatory Cd limits in TSP aligned with international frameworks, and incentivisation of low-Cd phosphate alternatives. Longitudinal studies incorporating redox-sensitive speciation analysis, irrigation water quality assessment, and rice genotype characterisation are essential to fully resolve the temporal dynamics of Cd accumulation and its aetiological contribution to CKDu.

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Data Availability The data used to support the results of this research are shown in the manuscript and available from the corresponding author upon request.

Declarations

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