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Exposome Wide Associations, DNA adduct and eGFR Dynamics: Evaluation of oxidative stress in CKDu pathogenesis

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Research Article

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Exposome Wide Associations, DNA adduct and eGFR Dynamics: Evaluation of oxidative stress in CKDu pathogenesis

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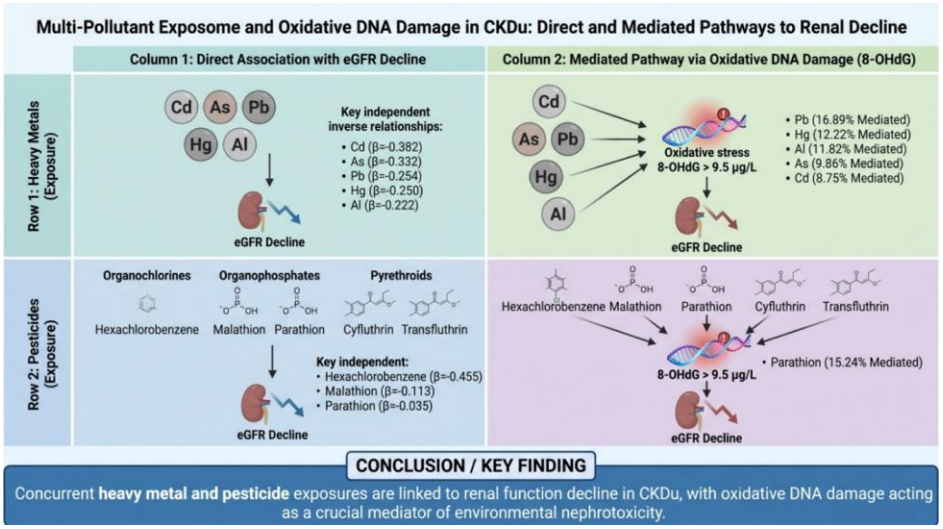
Graphical Abstract



Highlights

- Multi-pollutant exposomes profiling including heavy metals and multiclass pesticides
- CKDu cases had significantly higher serum levels of heavy metals (Cd, Hg, and Al) and agrochemicals (cyfluthrin, transfluthrin, and parathion) than the controls.
- We established a diagnostic threshold of 9.5 µg/L for urinary 8-OHdG (AUC=0.690, $p<0.001$), identifying individuals with significantly higher internal toxicant burdens.
- Causal Mediation Analysis confirmed that 8-OHdG mediates significant indirect eGFR decline for lead (16.89%), parathion (15.24%), mercury (12.22%), aluminum (11.82%), arsenic (9.86%), and cadmium (8.75%).

Abstract

Background: Chronic Kidney Disease of Unknown Etiology (CKDu) is a growing public health concern in agricultural regions, where environmental exposures are implicated as potential etiological agents. However, the combined nephrotoxic effects of complex heavy metal and pesticide mixtures remain poorly elucidated.

Methods: We conducted a population-based case-control study including 215 CKDu cases and 215 age- and sex-matched community controls without symptoms of CKDu. Serum concentrations of exposomes, including heavy metals (Al, Ni, As, Cd, Hg, and Pb) and pesticide residues (organochlorines, organophosphates, and pyrethroids), were measured using mass spectrometry, while oxidative DNA damage was estimated as urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG). A series of univariate, multivariate linear regression and mediation analyses were performed to explore independent exposure-outcome associations and identify underlying mechanistic pathways connecting environmental toxicants to estimated glomerular filtration rate (eGFR) decline.

Results: CKDu cases had significantly higher serum body burdens of heavy metals, most notably Al (32.32 vs. 13.43 μg/L, $p< 0.001$), Hg (16.17 vs. 7.55 μg/L, $p=0.001$), and Cd (3.17 vs. 0.10 μg/L, $p< 0.001$), as well as elevated levels of several pesticides, including cyfluthrin (11.33 μg/L vs <LLOQ, $p<0.001$) and transfluthrin (6.03 vs. 0.83 μg/L, $p=0.012$). In multivariate analysis adjusting for co-exposures, a significant inverse association was observed with eGFR for Cd ($\beta=-0.382$, As $\beta=-0.332$, Pb $\beta=-0.254$, Hg $\beta=-0.250$, and Al $\beta=-0.222$) with $p<0.05$. Using receiver operating characteristic (ROC) analysis, we identified a toxic threshold of 8-OHdG DNA adducts at 9.5 μg/L (AUC =0.690, $p<0.001$). Finally, mediation analysis revealed that oxidative DNA damage significantly mediated the effect of Pb (16.89%, parathion 15.24%, Hg 12.22%, Al 11.82%, As 9.86% and Cd 8.75% on eGFR decline($p<0.001$).

Conclusion: Our findings demonstrate that multi-pollutant exposome profiling can effectively capture complex interactions between environmental heavy metals and pesticides, which are critically involved in the pathogenesis of CKDu.

Keywords: CKDu, Heavy metals, Pesticides, DNA damage, 8-hydroxy-2'-deoxyguanosine

Introduction

Chronic kidney disease of unknown etiology (CKDu) has emerged as a major global public health crisis, disproportionately burdening rural agricultural communities across agricultural belts worldwide, including parts of South Asia and Latin America (Pillai & Murage et al., 2025). Unlike classical chronic kidney disease (CKD), which primarily stems from underlying metabolic disorders such as type 2 diabetes mellitus and severe hypertension, CKDu manifests as a progressive decline in estimated glomerular filtration rate (eGFR) in the absence of traditional clinical risk factors (KDIGO, 2024). Growing epidemiological evidence points toward environmental and occupational exposures—specifically, non-point-source exposure to complex chemical mixtures comprising heavy metals, persistent organic pollutants, and multi-class agricultural pesticides—as central environmental drivers of this tubulointerstitial pathology (Lebov et al., Wagoner & Lopez et al.). However, traditional single-pollutant paradigms fail to capture the cumulative and synergistic impacts of these real-world environmental co-exposures on renal health.

The conceptual framework of the exposome offers a paradigm shift by systematically assessing the total environmental exposure burden that an individual experiences over a lifetime and mapping its downstream physiological consequences. Agricultural workers and rural populations in Northern India face high multi-source environmental pressure via contaminated groundwater and intensive occupational pesticide application (Dhanorkar et al., 2023). While individual environmental toxicants—such as cadmium, lead, arsenic, and organophosphate or pyrethroid pesticides—have independently been linked to nephrotoxicity, real-world human exposures involve high-dimensional, highly correlated chemical mixtures (Verma et al., 2024, Verma et al., 2026). Evaluating the exposome-wide association study (EWAS) approach provides a comprehensive method to identify key toxicant drivers of renal function decline (quantified via eGFR) while accounting for high-dimensional co-exposure patterns (Yin & Zhao et al., 2024), Shi & Wang et al., 2024).

Even in cases where environmental toxins have been associated with impaired renal clearance, the internal biological mechanisms that translate multi-pollutant exposure into nephron injury

remain insufficiently characterized. Oxidative stress—specifically, oxidative DNA damage—is hypothesized to serve as a critical pathological nexus. Toxic heavy metals and active pesticide metabolites disrupt mitochondrial respiratory chains, induce reactive oxygen species (ROS) overproduction, and deplete endogenous antioxidant defenses within renal tubular epithelial cells (Ho et al., 2022; Gobe et al., 2010). Environmental toxicants—including heavy metals (Cd, As, Hg, Pb, and Al) and active residues of organophosphate, organochlorine, and pyrethroid pesticides—induce severe oxidative stress through the overproduction of reactive oxygen species (ROS), such as superoxide anions ($O2^{\cdot-}$), hydrogen peroxide (H2O2), and highly reactive hydroxyl radicals ($^{\cdot}OH$) (Jomova et al., 2025). The proximal tubular epithelial cells of the kidney are exceptionally vulnerable to this oxidative burden because of their high metabolic rate, rich mitochondrial density, and primary role in concentrating xenobiotics during filtration. When radicals directly attack the C-8 position of guanine nucleobases in nuclear and mitochondrial DNA, 8-hydroxy-2'deoxyguanosine (8-OHdG) is generated as a primary oxidative adduct. 8-OHdG, a major oxidative DNA adduct, serves as a sensitive, quantitative internal biomarker of systemic and renal genomic oxidative damage (Valavanidis et al., 2009; Ayna et al., 2025). High-sensitivity liquid chromatography-tandem mass spectrometry (LC-MS/MS) quantification of 8-OHdG allows for the precise evaluation of whether oxidative DNA injury acts as a direct biological mediator bridging complex environmental toxicant burdens to renal functional decline (Blahova et al., 2023; Guo et al., 2024 & Chen et al., 2024).

To address these gaps, we utilized single- and multi-pollutant multivariate modelling and regression analysis to identify independent chemical predictors of eGFR decline. This study deployed advanced targeted mass spectrometry platforms—specifically, inductively coupled plasma mass spectrometry (ICP-MS) for heavy metal profiling, gas chromatography-tandem mass spectrometry (GC-MS/MS) for multi-class pesticide quantification, and LC-MS/MS for 8-OHdG analysis to explore its potential mediation in the causation of CKD.

Methodology

Study Population

This case-control study was conducted in the northern region of Uttar Pradesh, India, at the Nephrology OPD of the Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, between August 2022 and March 2024. This study was conducted in collaboration with the Department of Biochemistry. Individuals with chronic kidney disease were included based on the Kidney Disease Improving Global Outcomes (KDIGO) guidelines, specifically having an estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m^2 for at least three months (KDIGO, 2012). The study focused on patients diagnosed with chronic kidney disease of unknown etiology (CKDu), a diagnosis made by the treating nephrologist after ruling out other known causes of CKD, such as diabetes mellitus, hypertension requiring multiple medications, polycystic kidney disease, congenital kidney disease, and chronic glomerulonephritis. Patients with a history of acute kidney injury or those who required renal replacement therapy were excluded. Controls were selected from other OPDs visiting for routine checkups with proper kidney health (eGFR $\geq$ 90 mL/min/1.73 m^2) and no prior hospital diagnosis of kidney disease after matching for age, sex, and geolocalization. All baseline histories and details were obtained from the study participants after providing written informed consent and undergoing blood and urine sample collection.

A total of 215 patients with unexplained chronic kidney disease (fulfilling the CKDu criteria) and 215 age- and sex-matched normal controls were enrolled in the present study.

This prospective case-control study was approved by the Institutional Ethical Committee (IEC 21/24), and all study procedures adhered to the principles of the Declaration of Helsinki revised version (2013) and institutional ethics guidelines (Cantin et.al, 2014).

Exposure: Quantification of pesticides and metals

All pesticides were quantified using a Shimadzu Nexis 2030 GC–MS/MS, and heavy metal levels were quantified using a NexION 1000 Perkin Elmer ICP–MS system. All serum and urine samples were prepared according to our previous study (Verma et al., 2024; Verma et al.,2026). The intra-day and inter-day coefficients of variation (CV) for all the exposomes ranged from 1.39% to 3.45% and 1.26–3.61%, respectively. Calibration curves showed excellent linearity (R^2 $\geq$ 0.998). The method detection limits (MDLs) are listed in Table 3.

Mediator: Quantification of oxidative stress marker 8-OHdG

For the quantification of 8-OHdG, a Qtrap 5500+ LC-MS/MS system was employed. The working parameters for the quantification of 8-OHdG are listed in Table S1 and Fig.1. The average spiked recovery rate of 8-OHdG was 112.4%, with intra-day and inter-day analytical precision, expressed as the CV, 4.71% and 3.12%, respectively. Calibration curves showed excellent linearity ($R^2 \geq 0.998$). The MDL of 8-OHdG was 0.19 ng/mL.

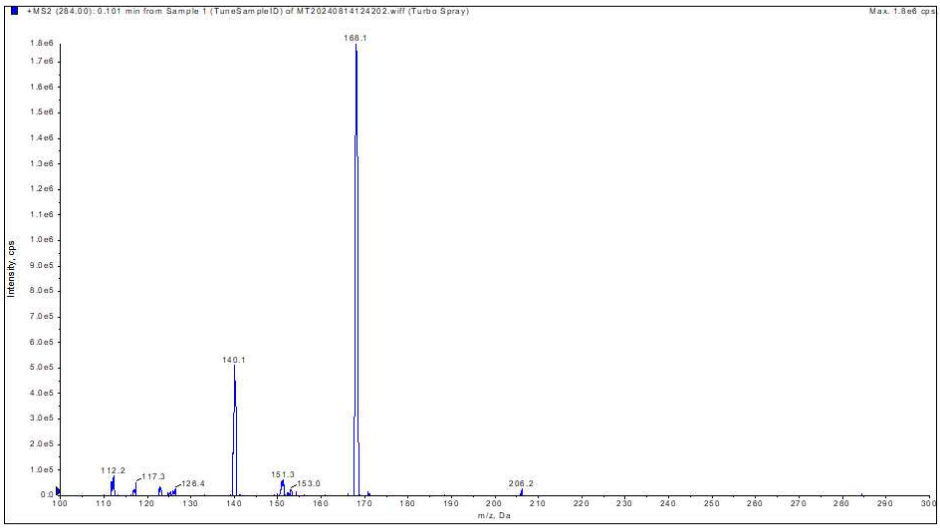


Fig.1: Mass Spectrum of 8-OHdG

Outcome: Estimated Glomerular Filtration Rate (eGFR)

Participants were diagnosed and categorized according to the KDIGO guidelines (KDIGO, 2012). The estimated GFR was calculated using the CKD-EPI creatinine equation given by Stevens and Levin (Levey & Stevens et.al, 2009). Participants were assigned to CKD progression according to eGFR levels as follows: stage I (>89 mL/min/1.73 m^2), stage II (89–60 mL/min/1.73 m^2), stage III (59–30 mL/min/1.73 m^2), stage IV (29–15

mL/min/1.73 m^2), and stage V (<15 mL/min/1.73 m^2). Blood and urine samples were collected from stage V patients before dialysis.

Sample preparation and Laboratory Analysis

The analysis of DNA adducts was performed in urine. First, 2 mL of urine was centrifuged at 10000 rpm for 5 min at 4^0C. Then, 1 mL of urine from the supernatant was separated in 2 mL centrifuge tubes, followed by the addition of 1 mL acidified acetonitrile. The mixture was vortexed properly and left in -20^0C for 5 min for protein precipitation. The mixture was again centrifuged at 10000 rpm for 5 min at 4^0C. The supernatant was removed and completely evaporated using a nitrogen evaporator (Guo Z et al., 2024). The residue was redissolved in 1 mL of nucleus-free water and injected into LC-MS/MS. All urinary 8-OHdG were adjusted with urinary creatinine (Fig. S1).

Covariates: Face-to-face interviews were conducted by specially trained interviewers to collect sociodemographic information, including age, sex, maternal education, and annual household income. Lifestyle habits, including water sources, use of cookware, and food habits, and working in agriculture were also noted. Medical history was also considered, including history of hypertension, use of any painkillers, frequent fainting, or any kind of dizziness.

Statistical analysis

Continuous data are expressed as mean ± SD or median (interquartile range) based on normality testing via the Shapiro-Wilk test. Categorical parameters are presented as counts and percentages (n, %) and evaluated using the chi-square test. Non-parametric continuous variables between cases and controls were compared using the Mann-Whitney U test.

Univariate linear regression models were used to evaluate the associations between individual serum toxicants and eGFR or 8-OHdG levels. Multivariate linear regression models incorporated co-pollutants and key potential confounders to isolate independent effect estimates (β-coefficients). Receiver Operating Characteristic (ROC) curve analysis was used to establish the optimal clinical cutoff threshold for 8-OHdG in predicting CKDu risk.

CMA was conducted to quantify the proportion of toxicant-induced eGFR reduction mediated through 8-OHdG-driven oxidative DNA damage, reporting estimated direct effects, indirect effects, and percentage mediation proportions. All analyses were conducted using SPSS version 22.0 (IBM, USA), with statistical significance set at $p < 0.05$.

Result

Baseline Demographics and Clinical Characteristics

A total of 430 participants—comprising 215 patients with chronic kidney disease of unknown etiology (CKDu) and 215 age- and sex-matched healthy controls—were included in the study, with both groups displaying well-balanced baseline sociodemographic profiles (Table 1). The mean age was 46.4±15.37 years in the CKDu group and 44.3±11.64 years in the control group (p=0.113), while male participants constituted 49.3% (n=106) of the cases and 47.9% (n=103) of the controls (p=0.846). Most participants resided in rural areas (71.2% cases vs. 68.8% controls; p=0.598) and engaged in agricultural occupations (54.8% cases vs. 49.3% controls; p=0.246), although a significantly higher proportion of CKDu patients relied on untreated groundwater than the controls (78.1% vs. 68.8%; p=0.028). Clinical evaluations confirmed severe renal impairment in the CKDu cohort, marked by a significantly lower median estimated glomerular filtration rate (eGFR) compared to the controls (18.00 (11.00–32.00) vs. 118.50 (106.00–128.00) mL/min/1.73 m^2; p<0.0001), alongside substantial elevations in serum urea (82.00 vs. 22.17 mg/dL; p<0.0001) and serum creatinine (3.48 vs. 0.68 mg/dL; p<0.0001) (Table 2).

Table 1: Demographic profile and exposure determinants in CKDu cases vs. controls

Variable	CKDu (n=215) n (%)	Controls (n=215) n (%)	p-value
Age (years) Mean ± SD	46.4 ± 15.37	44.3 ± 11.64	0.113
Gender Distribution M F	 106 (49.3) 109 (50.7)	 103 (47.9) 112 (52.1)	0.846

Residence Rural Urban	153 (71.2) 62 (28.8)	148 (68.8) 67 (31.2)	0.598
Occupation Agriculture Non-Agricultural	118 (54.8) 97 (45.2)	106 (49.3) 109 (50.7)	0.246
Socio-economic Status Lower Middle	125(58.1) 90(41.9)	110(51.2) 105(48.8)	0.146
Water habits Groundwater Purified water	168(78.1) 47(21.9)	148(68.8) 67(31.2)	0.028*

*The chi-square test was used to calculate the p-value. p<0.05 is considered as significant was mentioned in *. Abbreviations: CKDu, chronic kidney disease of unknown etiology; eGFR, estimated glomerular filtration rate. M:Male, F:Female*

Table 2: Clinical baseline biochemical profiles of study participants

Biochemical Parameters	**CKDu (n=215) Median (Q1-Q3)**	**Controls (n=215) Median (Q1-Q3)**	**p-value**
eGFR (mL/min)	22.00 (15.00-32.00)	118.50 (106.00-128.00)	<0.0001*
Urea (mg/dL)	82.00 (57.54-125.53)	22.17 (18.62-27.64)	<0.0001*
Creatinine (mg/dL)	3.48 (2.13-5.35)	0.68 (0.52-0.83)	<0.0001*
Sodium (mmol/L)	138.00 (136.00-140.00)	137.00 (122.00-140.00)	<0.0001*
Potassium (mmol/L)	4.80 (4.21-5.33)	4.21 (3.95-4.55)	<0.0001*
SGOT (U/L)	24.60 (20.00 - 32.50)	29.13(21.96-38.52)	0.001*
SGPT (U/L)	20.74 (14.45-28.70)	29.27(23.30-39.20)	<0.0001*

*The Mann-Whitney test was used to calculate the p-value. P <0.05 was considered significant and is indicated by *. Abbreviations: eGFR: Estimated Glomerular Filtration Rate, SGOT: Serum*

Glutamic-Oxaloacetic Transaminase, SGPT: serum glutamic pyruvic transaminase*, DBilirubin: Direct Bilirubin, TBilirubin: Total Bilirubin, ALP: Alkaline phosphatase*

Exposome Distribution and Body Burden Profiles

As shown in Table 3, serum toxicant profiling revealed significantly elevated environmental toxicant burdens among CKDu cases compared to controls ($p<0.001$) across nearly all measured species, except 2-phenylphenol ($p = 0.004$), with overall detection rates reaching up to 95.3% for serum Cd. Among the heavy metals, serum Al and Hg exhibited the highest absolute concentrations in cases, with median levels reaching 32.32 µg/L (IQR: 24.22–97.25) vs. 13.43 µg/L in controls for Al ($p<0.001$), and 16.17 µg/L (IQR: 11.11–26.66) vs. 7.55 µg/L in controls for Hg ($p<0.001$), alongside significant enrichments in serum As, Cd, Ni, and Pb ($p<0.001$). Synthetic pyrethroids and organophosphates also showed pronounced elevations in CKDu patients, highlighted by median Cyfluthrin1 concentrations of 11.33 µg/L in cases versus levels below the method detection limit (<MDL) in controls ($p<0.001$), accompanied by significant increases in transfluthrin (6.03 vs. 0.83 µg/L; ($p<0.001$), parathion (2.92 vs. 2.79 µg/L; ($p<0.001$), and hexachlorobenzene (0.22 µg/L vs. <MDL; ($p<0.001$).

Table 3: Serum exposome concentrations and detection rates among study participants

Family	Exposomes	MDL	DR (%)	Median IQR Cases	Median IQR Control	p-value
Trace elements	Al	0.117	78.8	32.32 (24.22-97.25)	13.43 (<MDL-21.22)	<0.001*
	Ni	0.099	81	5.04 (3.60-6.90)	1.26 (0.35-2.52)	<0.001*
	As	0.004	75.41	4.04 (2.49-6.73)	0.79 (.42-1.42)	<0.001*
	Cd	0.015	95.3	3.17 (1.45-7.28)	0.10 (<MDL-0.48)	<0.001*
	Hg	0.002	56.9	16.17 (11.11-26.66)	7.55 (<MDL-13.69)	<0.001*
	Pb	0.024	22.35	1.58 (0.71-4.86)	0.24 (<MDL-0.55)	<0.001*
Pesticides	Chlorneb	0.02	47.29	1.37 (1.23-	0.80 (0.58-	<0.001*

				1.59)	1.05)	
	HCB	0.12	63.52	0.22 (0.15-0.29)	<MDL (<MDL, <MDL)	<0.001*
	BetaHCH	0.04	72.9	0.88 (0.33-2.48)	0.68 (0.33-1.19)	<0.001*
	ppDDE	0.22	56	1.08 (0.59-1.66)	0.62 (0.30-1.40)	<0.001*
	Phenylphenol	0.09	49.4	0.16 (0.12-0.19)	0.15 (0.12-0.18)	0.024*
	Malathion	0.01	32.9	0.57 (0.39-1.60)	0.20 (0.03-0.39)	<0.001*
	Parathion	0.03	77.65	2.92 (1.56-5.54)	2.79 (0.08-3.25)	<0.001*
	Carbophenothion	0.11	54.58	0.69 (0.46-0.85)	0.47 (0.31-0.51)	<0.001*
	Azinphosmethyl	0.08	49.41	0.62 (0.41-3.45)	0.37 (0.22-0.49)	<0.001*
	Transfluthrin	0.34	80.35	6.03 (1.51-10.19)	0.83 (0.55-1.50)	<0.001*
	Cyfluthrin1	0.02	58.82	11.33 (7.69-15.56)	<MDL (<MDL, <MDL)	<0.001*
	Flucythrinate1	0.81	81.88	2.51 (1.55-3.07)	1.93 (1.15-2.63)	<0.001*
	Flucythrinate2	10.22	58.82	26.10 (12.08-54.39)	19.66 (12.91-36.09)	<0.001*

*Abbreviations: MDL, method detection limit; DR, detection rate; IQR, interquartile range; Al, aluminum; As, arsenic; Ni, nickel; Cd, cadmium; Hg, mercury; Pb, lead; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; ppDDE, para-para Dichlorodiphenyldichloroethylene. The Mann-Whitney test was used to calculate the p-value. $p<0.05$ was considered significant, as indicated by *.*

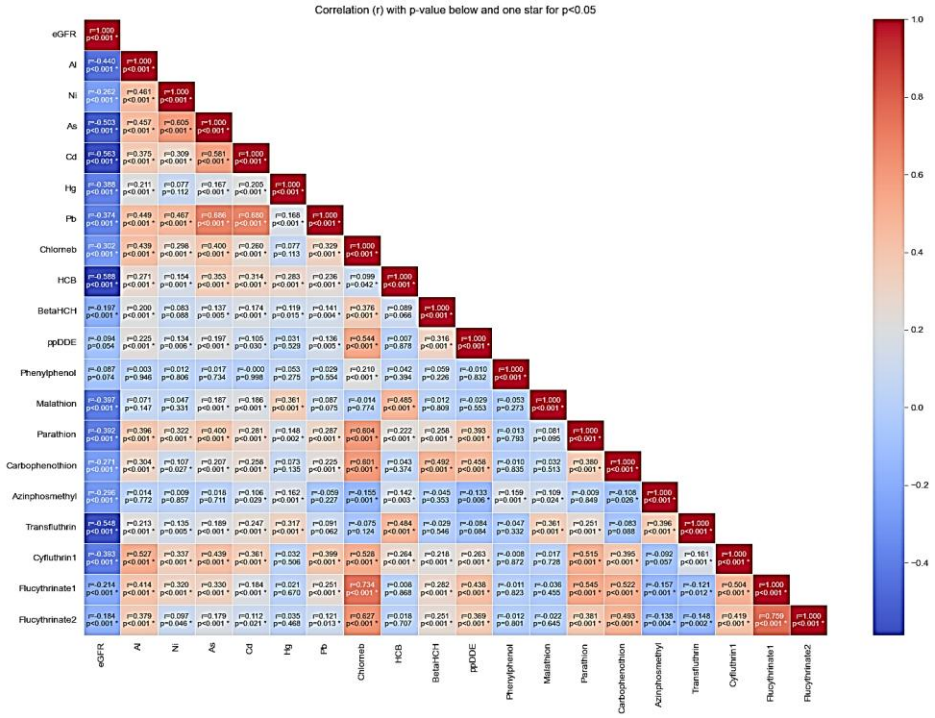


Fig 2. Correlation matrix of eGFR and exposomes

The correlation matrix in Fig.2 evaluates pairwise relationships among estimated GFR, heavy metals, and multi-class pesticide residues, revealing consistent, statistically significant inverse correlations ($p<0.001$) between eGFR and almost all measured toxicants—most strongly with HCB ($r=-0.588$), Cd ($r=-0.563$), transfluthrin ($r=-0.548$), As ($r=-0.503$), and Al ($r=-0.440$). Pronounced intra-class co-exposures are evident among heavy metals, highlighted by strong positive correlations between Pb and As ($r=0.686$, $p<0.001$), Pb and Cd ($r=0.680$, $p<0.001$), and As and Ni ($r=0.605$, $p<0.001$), as well as within pesticide classes, such as Flucythrinate1 and Flucythrinate2 ($r=0.759$, $p<0.001$) and Chlorneb with Parathion ($r=0.604$, $p<0.001$). Additionally, key cross-class correlations between metals and pesticides—including Al with Cyfluthrin1 ($r=0.527$, $p<0.001$) and Chlorneb ($r=0.439$, $p<0.001$)—demonstrate a complex multi-pollutant exposure pattern in the target population.

Exposome-Wide Linear Regression Models with eGFR Decline

Univariate linear regression models demonstrated that all evaluated heavy metals and the majority of organochlorine, organophosphate, and pyrethroid pesticides had strong inverse associations with eGFR ($p<0.001$). In multipollutant multivariate regression models adjusted for

co-exposures and confounders, Cd (β=-0.434, p<0.001) and As (β=-0.325, p<0.001) retained the strongest independent inverse relationships with eGFR among heavy metals, followed by Pb (β=-0.251), Hg (β=-0.245, p<0.001), and Al (β=-0.223, p<0.001). All pesticides lost statistical significance after multivariate adjustment (Table 4).

Table 4: Univariate and multivariate linear regression models evaluating eGFR as the dependent variable

Exposomes	Univariate		Multivariate	
	beta coefficient	p-value	beta coefficient	p-value
Al	-0.440	<0.001	-0.223	<0.001
As	-0.503	<0.001	-0.325	<0.001
Ni	-0.262	<0.001		
Cd	-0.563	<0.001	-0.434	<0.001
Hg	-0.388	<0.001	-0.245	<0.001
Pb	-0.374	<0.001	-0.251	<0.001
Chloroneb	-0.532	<0.001*		
HCB	-0.131	0.103		
Beta HCH	-0.187	0.045		
pp DDE	-0.004	0.583		
Phenylphenol	-0.11	0.108		
Malathion	-0.438	<0.001*		
Parathion	-0.317	<0.001*		
Carbophenothion	-0.299	0.004*		
Azinphos-methyl	-0.273	<0.001*		
Transfluthrin	-0.471	<0.001*		
Flucythrinate 1	-0.215	0.004*		
Flucythrinate 2	-0.143	0.059		
Cyfluthrin	-0.225	0.049*		

Abbreviations: *eGFR as a dependent variable after adjusting confounding variables (age, gender, and water habits), Al: Aluminum, As: Arsenic, Ni: Nickel, Cd: Cadmium, Hg: Mercury, Pb: Lead, HCB: Hexachlorobenzene, Beta HCH: Beta Hexachlorocyclohexane, pp DDE: para-para Dichlorodiphenyldichloroethylene p- value. p<0.05 is considered as significant was mentioned in *.*

Exposome-Wide Linear Regression Models with 8-OHdG

Multivariate linear regression analysis evaluating 8-OHdG accumulation identified serum Cd as the primary independent driver of oxidative DNA damage (β=0.279, p<0.001), with Hg (β=0.120, p=0.010) and Al (β=0.104, p=0.044). Among the pesticides, only parathion (β=0.463, p=0.026) showed a significant association in multivariate analysis (Table 5).

Table 5: Univariate and multivariate linear regression models evaluating 8-OHdG as the dependent variable

Exposomes	Univariate		Multivariate	
	beta coefficient	p-value	beta coefficient	p-value
Al	0.246	<0.001*	0.104	0.044*
As	0.256	<0.001*		
Cd	0.359	<0.001*	0.279	<0.001*
Hg	0.204	<0.001*	0.120	0.010*
Pb	0.320	<0.001		
Ni	0.086	0.078		
Chloroneb	0.058	0.231		
Hexachlorobenzene	0.123	0.011*		
Beta HCH	0.006	0.900		
pp DDE	0.042	0.394		
Phenylphenol	0.015	0.766		
Malathion	0.041	0.458		
Parathion	0.104	0.047*	0.463	0.026*
Carbophenothion	0.097	0.046*		
Azinphos-methyl	0.016	0.744		
Transfluthrin	0.106	0.029*		
Flucythrinate 1	0.092	0.058		
Flucythrinate 2	0.061	0.211		
Cyfluthrin	0.191	<0.001*		

Abbreviations: *8-OHdG as a dependent variable after adjusting confounding variables (age, gender, and water habits), Al: Aluminum, As: Arsenic, Ni: Nickel, Cd: Cadmium, Hg: Mercury, Pb: Lead, HCB: Hexachlorobenzene, Beta HCH: Beta Hexachlorocyclohexane, pp DDE: para-para Dichlorodiphenyldichloroethylene p- value. p<0.05 is considered as significant was mentioned in *.*

Performance measurement of DNA adduct (8-OHdG)

The levels of 8-OHdG were distributed among CKDu and controls, as shown in Fig.3a. Receiver Operating Characteristic (ROC) curve analysis in Fig.3b established a diagnostic toxicity threshold for 8-OHdG at 9.5 µg/L (AUC =0.690, 95% CI: 0.640-0.740, p<0.001). Dichotomizing the cohort using this cut-off revealed that individuals above the threshold possessed significantly higher toxicant levels, including serum Al (34.18 vs. 21.48 µg/L; p<0.001), As (5.11 vs. 1.63 µg/L; p<0.001), Cd (3.26 vs. 0.66 µg/L; p<0.001), Hg (16.72 vs. 12.00 µg/L; p=0.004), and parathion (1.34 vs. 1.07 µg/L; p=0.004) (Table 6).

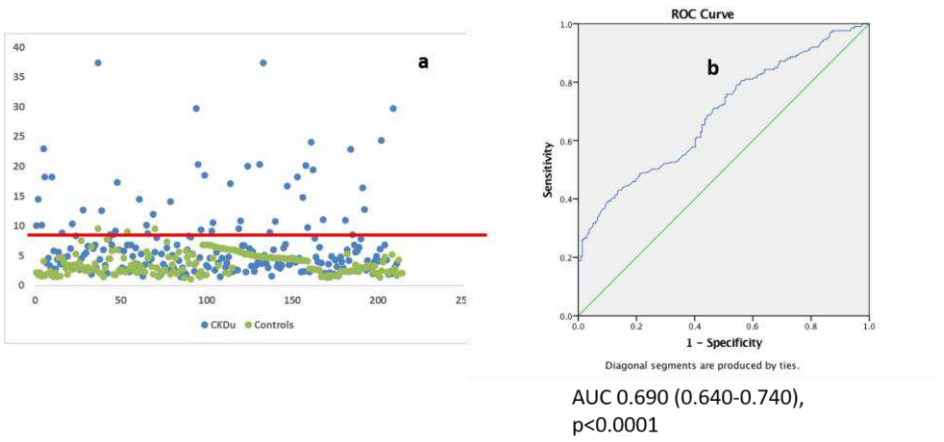


Fig. 3 Diagnostic performance and concentration distribution of 8-OHdG in CKDu (a) Scatter plot showing individual concentrations of 8-OHdG in patients with CKDu of unknown etiology (CKDu; blue dots) compared to healthy controls (green dots). (b) Receiver Operating Characteristic (ROC) curve demonstrating the ability of 8-OHdG to discriminate between CKDu cases and controls. The marker shows moderate diagnostic accuracy with an area under the curve (AUC) of 0.690 (95% CI: 0.640–0.740, p < 0.0001). The green diagonal line represents a non-discriminatory reference model.

Table 6: Median toxicant concentrations stratified by 8-OHdG status

Exposomes	**Positive (Median Q1-Q3)**	**Negative (Median Q1-Q3)**	**p-value**
Al (µg/L)	34.18 (24.76-97.84)	21.48 (4.46-37.22)	<0.001*
As (µg/L)	5.11 (3.03-7.91)	1.63 (0.71-3.42)	<0.001*

Cd (µg/L)	3.26 (1.48-10.25)	0.66 (0.09-2.67)	<0.001*
Hg (µg/L)	16.72 (9.00-31.50)	12.00 (6.12-18.98)	0.004*
Parathion (µg/L)	3.51 (2.45-6.04)	2.75 (0.14-3.41)	<0.001*
Chlorneb (µg/L)	1.34 (1.25-1.44)	1.07 (0.71-1.40)	0.004*

Abbreviations: *Al, aluminum; As, arsenic; Cd, cadmium; Hg, mercury. Mann–Mann-Whitney test was used to calculate p-values. Statistical significance was set at $p < 0.05$.*

Causal Mediation Analysis of 8-OHdG on Renal Dysfunction

Formal causal mediation modelling in Fig.4 confirmed that systemic 8-OHdG oxidative DNA damage functions as a statistically significant biological mediator connecting heavy metal and pesticide exposures to eGFR decline. Among the heavy metals, Pb displayed the largest indirect effect mediated via 8-OHdG (Indirect Effect β=-0.076, p<0.001), accounting for 16.89% of its total effect on renal function, followed by Hg (12.22% mediated; β=-0.054, p<0.001), Al (11.82% mediated; β=-0.059, p<0.001), As (9.86% mediated; β=-0.055, p<0.001), and Cd (8.75% mediated; β=-0.054, p<0.001). Among pesticide exposures, 8-OHdG likewise mediated substantial proportions of eGFR decline for flucythrinate1 (19.78% mediated; β=-0.053, p=0.019), flucythrinate2 (16.37% mediated), and parathion (15.24% mediated; β=-0.057, p<0.001) and transfluthrin (6.36% mediated; β =-0.032, p<0.001) (Table S2).



Fig 4: Causal mediation analysis evaluating 8-OHdG as an intermediary between environmental exposomes and eGFR decline

Discussion

Chronic Kidney Disease of Unknown Etiology (CKDu) represents a major global public health concern, particularly in tropical and agricultural belts (Wan et al., 2021). Unlike classic chronic kidney disease (CKD) secondary to metabolic conditions such as diabetes or hypertension, CKDu is primarily characterized by tubulointerstitial nephritis and rapid renal functional loss among young agricultural workers (Jacobson et al., 2021). While environmental pollutants have long been hypothesized as central drivers, establishing specific etiologic pathways has been challenging due to the multi-pollutant nature of environmental exposures.

By integrating multi-platform serum biomonitoring, this study provides a comprehensive characterization of the most prevalent exposomes in a rural population from Northern India, linking complex co-exposures of heavy metals and pesticides to chronic kidney disease of unknown etiology (CKDu) through targeted mass spectrometry platforms. This study demonstrates that individuals with CKDu possess significantly higher internal body burdens of multi-class environmental pollutants—including heavy metals (Cd, As, Pb, Hg, and Al) and synthetic agrochemicals—compared to matched controls from the same endemic regions. Our statistical modelling isolates independent toxicant contributions to renal functional impairment and establishes 8-OHdG-mediated oxidative DNA damage as a mechanistic pathway linking chemical exposomes to progressive nephron damage.

Our demographic analysis revealed that the reliance on untreated groundwater was significantly higher among patients with CKDu (78.1%) than among matched controls (68.8%, $p=0.028$). Untreated groundwater in intensive agricultural zones serves as a primary sink for both geogenic bedrock constituents and anthropogenic runoffs. Intensive agrochemical application combined with regional hydrogeochemical leaching facilitates the transport of heavy metals and agrochemicals into drinking-water aquifers (Ahmad et al., 2019; Khan et al., 2020). These epidemiological patterns mirror regional reports from agricultural belts across India and Central America, where drinking untreated surface or groundwater has been independently linked to elevated CKDu prevalence (Chen et al., 2024; CPCB 2007). In rural settings, where untreated groundwater serves as the main source of drinking and cooking, daily oral ingestion delivers a chronic, low-dose toxicant mixture that maintains sustained internal tissue burdens.

In real-world settings, human exposure involves complex multi-pollutant mixtures rather than isolated toxicants. Our multivariate regression analyses revealed that heavy metals retained powerful inverse relationships with eGFR, even after mutual adjustment for concurrent chemical exposures. Cd ($\beta=-0.382$) and As ($\beta=-0.332$) emerged as the strongest independent continuous drivers of eGFR loss. Proximal tubular epithelial cells internalize Cd via receptor-mediated endocytosis of complexes. Once inside S1 and S2 tubule segments, free triggers mitochondrial disruption, disrupts intercellular junctions, and induces tubular apoptosis (Rho et al., 2025; Johri et al., 2010). This aligns with global biomonitoring data showing that chronic Cd accumulation

drives tubular dysfunction and eGFR loss (Rho et al., 2025). As impairs cellular bioenergetics by uncoupling ATP synthesis and inactivating pyruvate dehydrogenase complexes, while Pb promotes oxidative damage and microvascular sclerosis in renal tissue. Epidemiologic studies confirm that elevated blood Pb and As levels are strongly correlated with reduced eGFR and elevated clinical risk of CKD progression (Rho et al., 2025; Turkington et al., 2025). Elevated serum Al (in cases vs. in controls) impairs renal clearance and accelerates intracellular iron overload, which drives iron-dependent lipid peroxidation (ferroptosis) in proximal renal tubules (Liang et al., 2025; Guangji et al., 2025).

The persistent detection of HCB retained a strong independent inverse association with eGFR (β=-0.455, $p<0.001$). Resistant to metabolic degradation, HCB accumulates in lipid membranes and induces chronic endoplasmic reticulum (ER) stress in the renal parenchyma. Parathion (β=-0.035) and malathion (β=-0.113) maintained significant inverse associations with eGFR. Beyond acetylcholinesterase inhibition, organophosphates induce interstitial inflammation and pro-inflammatory signaling pathways (such as activation) within tubular cells (Wan et al., 2021; Jacobson et al., 2021). Cyfluthrin1 exhibited substantial serum accumulation in cases (11.33 µg/L vs. <MDL in controls). Synthetic pyrethroids disrupt voltage-gated ion channels, promote membrane lipid peroxidation, and decrease mitochondrial membrane potential, exacerbating metal-induced nephrotoxicity (Costa et al., 2015; Reddam et al., 2022). The high body burdens of toxic heavy metals and multi-class pesticide residues observed among CKDu cases align with emerging global paradigms that frame CKDu as an environmentally driven tubulointerstitial disease.

A central insight from this investigation is the role of 8-OHdG as a functional mediator along the exposure-nephrotoxicity axis. Proximal tubular epithelial cells (PTECs) are metabolically active and highly susceptible to xenobiotic-induced reactive oxygen species (ROS) production (George et al., 2017). When persistent environmental toxicants induce mitochondrial membrane disruption and excessive ROS generation, the resulting oxidative attack on DNA guanine nucleobases generates 8-OHdG. Unrepaired 8-OHdG lesions promote genomic instability, tubular cell apoptosis, and subsequent tubulointerstitial fibrosis (Valavanidis et al., 2009; Ayna et al., 2025). A high urinary 8-OHdG concentration is associated with CKD. On careful ROC

analysis, the precise cut-off threshold of 8-OHdG to predict CKD was 9.5µg/L with 99.1% sensitivity, above which participant profiles displayed significantly elevated internal concentrations of Al, As, Cd, Hg, and parathion. Our causal mediation analysis formally quantified this pathway, demonstrating that 8-OHdG-mediated oxidative DNA damage accounted for significant proportions of total eGFR decline across multiple toxicants, most notably Pb (16.89%), Hg (12.22%), Al (11.82%), and parathion (15.24%). These causal mediation findings align with recent environmental toxicological studies, such as Chen et al. (2024), who reported that 8-OHdG significantly mediates renal functional decline under multiple pollutant exposures. Persistent oxidative DNA damage impairs transcriptional integrity, triggering premature cellular senescence and apoptosis. Because human proximal tubule cells possess limited regenerative capacity under chronic toxic stress, ongoing oxidative damage ultimately leads to tubulointerstitial fibrosis and progressive nephron loss (Peng et al., 2023; Chevalier et al., 2016). Direct, non-mediated pathways accounted for the remaining of eGFR decline, operating through complementary pathways such as direct membrane disruption, trace mineral displacement, mitochondrial pore transition, and pro-inflammatory cytokine cascades (Ebert et al., 2021; Ahmed et al., 2025).

These findings highlight the essential priorities for environmental management and regulatory policies in agricultural zones. Regulatory protocols typically evaluate chemical safety on a single compound basis. Public health agencies (such as the EPA and WHO) should incorporate mixture toxicity frameworks, such as cumulative hazard indices and Bayesian mixture modelling, to account for multi-pollutant interactions.

Furthermore, the strong association between untreated groundwater ingestion and disease risk and the expansion of community-level drinking water infrastructure with reverse osmosis and activated carbon filtration to remove heavy metals and pesticide residues are critical. The limitation of this study is secondary accumulation due to reduced eGFR (reverse causation) as it cannot be fully ruled out in case-control designs, requiring future longitudinal cohort validation. Although our previous hospital-based studies demonstrated a high exposome burden across Lucknow, Barabanki, Gonda, Lakhimpur Kheri, Bahraich, and Gorakhpur, the tertiary care sampling limits the generalizability to the broader community and precludes localized mapping

of exposure hotspots. Furthermore, systemic oxidative stress in extra-renal organs was not evaluated. Although 8-OhdG is a telltale of oxidative stress, its increase signifies the role of oxidative stress in the causation of CKDu among our studied exposomes. Longitudinal cohort studies incorporating prospective environmental water/soil sampling and biobank tracking are recommended to validate these findings. Unmeasured environmental variables, such as occupational heat stress or dietary patterns, could also contribute as co-factors in disease progression.

Targeted interventions aimed at mitigating groundwater contamination using filtered water and providing targeted antioxidant or cellular protection strategies may offer viable pathways to alleviate the heavy burden of CKDu in vulnerable agricultural communities.

Conclusion

Our study showed that exposure to Al, As, Cd, Hg, and Pb among the studied exposomes was associated with CKDu. Oxidative DNA damage is a key mechanistic link between environmental exposure and renal dysfunction, offering novel etiological insights and potential avenues for environmental intervention in high-risk occupational populations.

Statements and Disclosure

Declaration of Conflicting Interests: The authors declare no conflicts of interest regarding the publication of this manuscript.

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References:

Ahmad I, Chaurasia S (2019) Study on heavy metal pollution in Ganga River at Kanpur (UP). Journal of Emerging Technologies and Innovative Research 6(6):391–398 26.

Ahmed AE, Madkhali MH, Alhashim HA, Damanhouri LM, Bagasher AM, Alharbi AM, Almohammadi AF, Alomrani NA, Alghaithi AM, Hibili NH. Nephrotoxic Effects of Endocrine-Disrupting Chemicals: A Systematic Review. Cureus. 2025 Dec 19;17(12).

Ayna A, Caglayan C, Taysi S. Cellular and molecular mechanisms of oxidative DNA damage and repair. Medicina. 2025 Nov 11;61(11):2013.

Bláhová L, Janoš T, Mustieles V, Rodriguez-Carrillo A, Fernandez MF, Bláha L. Rapid extraction and analysis of oxidative stress and DNA damage biomarker 8-hydroxy-2′-deoxyguanosine (8-OHdG) in urine: Application to a study with pregnant women. International Journal of Hygiene and Environmental Health. 2023 May 1;250:114175.

Cantín M (2014) World medical association declaration of Hel sinki: ethical principles for medical research involving human subjects. Reviewing the latest version. Int J Med Surg Sci 1(4):339346. https:// doi. org/ 10. 32457/ ijmss. 2014. 042

Central Pollution Control Board (2007) Status of groundwater quality in India. Part - I. Groundwater Quality Series: GWQS/09/2006. New Delhi: Ministry of Environment and For ests, Government of India. https:// kupdf. net/ downl oad/ status- of- groun dwater- quali ty-in- india- part-i_ 5b00f d55e2 b6f57 e1d86 96ca_ pdf

Chen Q, Deng Q, Liao Q, Liu Y, Zhang Z, Wu D, Lv Y, Qin J, Liu Q, Li S, Long Z. 8-OHdG mediates the association of co-exposure to fifty-five typical endocrine-disrupting chemicals with renal function: a cross-section investigation in Southern Chinese adults. Environmental Science and Pollution Research. 2024 May;31(21):30779-92.https://doi.org/10.1007/s11356-024-33266-1

Chen Q, Deng Q, Liao Q, Liu Y, Zhang Z, Wu D, Lv Y, Qin J, Liu Q, Li S, Long Z. 8-OHdG mediates the association of co-exposure to fifty-five typical endocrine-disrupting chemicals with renal

function: a cross-section investigation in Southern Chinese adults. Environmental Science and Pollution Research. 2024 May;31(21):30779-92.

Chevalier RL. The proximal tubule is the primary target of injury and progression of kidney disease: the role of the glomerulotubular junction. American Journal of Physiology-Renal Physiology. 2016 Jul 1;311(1):F145-61.

Costa, L.G. The Neurotoxicity of Organochlorine and Pyrethroid Pesticides. In *Handbook of Clinical Neurology*; Elsevier: Amsterdam, The Netherlands, 2015; Volume 131, pp. 135–148. ISBN 978-0-444-62627-1.

Dhanorkar MA, Verma J, Kulshreshtha MR, Chandra A, Rao NS. Environmental risk factors and mechanisms of injury in chronic kidney disease of unknown etiology–fitting the pieces of the puzzle. Indian Journal of Kidney Diseases. 2023 Jul 1;2(3):67-77.

Ebert T, Neytchev O, Witasp A, Kublickiene K, Stenvinkel P, Shiels PG. Inflammation and oxidative stress in chronic kidney disease and dialysis patients. Antioxidants & redox signaling. 2021 Dec;35(17):1426-48.

George B, You D, Joy MS, Aleksunes LM. Xenobiotic transporters and kidney injury. Advanced drug delivery reviews. 2017 Jul 1;116:73-91.

Gobe G, Crane D. Mitochondria, reactive oxygen species and cadmium toxicity in the kidney. Toxicology letters. 2010 Sep 15;198(1):49-55.

Guangji W, Huixin P, Hongfei H, Hai L, Yang F, Shihua L, Yanxin H, Huixiong Y, Wencheng C. Aluminum exposure induces ferroptosis in spermatogenic cells of mice through iron overload and lipid peroxidation. Free Radical Biology and Medicine. 2025 Sep 23.

Guo Z, Ren X, Liu P, Deng Y, Bian J, Ge Y, Xu B, Tang X, Li X, Huang H, Liu J. Co-exposure to parabens, bisphenol A, and triclosan and the associations with dyslipidemia in Chinese older adults: The mediation effect of oxidative stress. Environmental Research. 2024 Dec 1;262:119835.

Guo Z, Ren X, Liu P, Deng Y, Bian J, Ge Y, Xu B, Tang X, Li X, Huang H, Liu J. Co-exposure to parabens, bisphenol A, and triclosan and the associations with dyslipidemia in Chinese older adults: The mediation effect of oxidative stress. Environmental Research. 2024 Dec 1;262:119835.

Ho HJ, Shirakawa H. Oxidative stress and mitochondrial dysfunction in chronic kidney disease. Cells. 2022 Dec 25;12(1):88.

https://kdigo.org/wp-content/uploads/2024/03/KDIGO-2024-CKD-Guideline.pdf

Jacobson MH, Wu Y, Liu M, Kannan K, Li AJ, Robinson M, Warady BA, Furth S, Trachtman H, Trasande L. Organophosphate pesticides and progression of chronic kidney disease among children: A prospective cohort study. Environment international. 2021 Oct 1;155:106597.https://doi.org/10.1016/j.envint.2021.106597

Johri N, Jacquillet G, Unwin R. Heavy metal poisoning: the effects of cadmium on the kidney. Biometals. 2010 Oct;23(5):783-92.

Jomova K, Alomar SY, Nepovimova E, Kuca K, Valko M. Heavy metals: toxicity and human health effects. Archives of toxicology. 2025 Jan;99(1):153-209.

Khan R, Saxena A, Shukla S (2020) Evaluation of heavy metal pollution for River Gomti, in parts of Ganga Alluvial Plain, India. SN Appl Sci 2:1451. https:// doi. org/ 10. 1007/ s42452- 020-03233-9

Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO (2012) Clinical practice guidelines for the evaluation and management of chronic kidney disease. Kidney Int Suppl 2013(3):1–150

Lauria G, Genchi G, Curcio R. Mitochondrial Toxicology of Heavy Metals and Pesticides: Transport Systems, Mitochondrial Dysfunction and Permeability Transition. International Journal of Molecular Sciences. 2026 Jul 17;27(14):6347.

Lebov JF, Engel LS, Richardson D, Hogan SL, Sandler DP, Hoppin JA. Pesticide exposure and end-stage renal disease risk among wives of pesticide applicators in the Agricultural Health Study. Environmental research. 2015 Nov 1;143:198-210.

Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feld man HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J (2009) CKD-EPI (Chronic Kidney Disease Epidemiology Col laboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 150(9):604–612. https:// doi. org/ 10. 7326/ 0003- 4819- 150-9- 20090 5050- 00006

Liang L-D, Zhao X-Y, Li L, Liang S, Zhang J-M, Lv J-N, Deng F-L, Lu C-L, Li Q, Huang Q-W, Huang M-J, and Peng H-X (2025) AlCl3 exposure induces nephrotoxicity in mice by mediating ferroptosis through the NRF2 signaling pathway. Front. Toxicol. 7:1692410. doi: 10.3389/ftox.2025.1692410

Peng Z, Wang H, Zheng J, Wang J, Xiang Y, Liu C, Ji M, Liu H, Pan L, Qin X, Qu X. Is the proximal tubule the focus of tubulointerstitial fibrosis?. Heliyon. 2023 Feb 1;9(2).

Pillai A, Murage P, Haque F. Understanding the relationship between climate change-related meteorological factors and chronic kidney disease (CKD) and CKD of unknown origin (CKDu) in low-and middle-income countries: A systematic review & meta-analysis. Environmental research. 2025 Sep 22:122928.

Reddam, A.; McLarnan, S.; Kupsco, A. Environmental Chemical Exposures and Mitochondrial Dysfunction: A Review of Recent Literature. *Curr. Environ. Health Rep.* **2022**, *9*, 631–649.

Rho JH, Lee S, Kwon JY, Hong YS. A comparative study on the paradoxical relationship between heavy metal exposure and kidney function. Diagnostics. 2025 Jan 2;15(1):86. https://doi.org/10.3390/diagnostics15010086

Shi X, Wang X, Zhang J, Dang Y, Ouyang C, Pan J, Yang A, Hu X. Associations of mixed metal exposure with chronic kidney disease from NHANES 2011–2018. Scientific Reports. 2024 Jun 6;14(1):13062.

Singh S, Verma J, Gupta N, Pathak AK, Rajput MS, Tiwari V, Kulshrestha MR. Association of Arsenic and Nickel with Markers of Insulin Resistance and Beta Cell Dysfunction: A Case–Control Study in Indo Gangetic Plain: S. Singh et al. Biological Trace Element Research. 2025 Oct;203(10):5108-26.

Turkington RE, Hukriede NA, Ho J, Jayasundara N, Sanders AP. Metal mechanisms of mitochondrial toxicity: recent review of arsenic, cadmium, and lead-induced nephrotoxicity. Environmental Science and Pollution Research. 2025 May;32(24):14439-51.

Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2′-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. Journal of environmental science and health Part C. 2009 May 7;27(2):120-39.

Verma J, Rao N, Kulshrestha MR, Singh S, Singh S, Tiwari V, Sharma MT. Exploring the Association Between Potentially Toxic Elements and Chronic Kidney Disease of Unknown Etiology in Eastern Uttar Pradesh, India. Biological Trace Element Research. 2026 Feb;204(2):1084-98.

Verma J, Rao N, Kulshrestha MR, Tiwari V, Sharma MT, Chan dra A (2024) Chronic kidney disease from agricultural commu nities—association and accumulation of hexachlorobenzene, malathion, and parathion pesticides. Toxicol Environ Health Sci 16(3):299–309. https:// doi. org/ 10. 1007/ s13530- 024- 00222-y

Wagoner RS, López-Gálvez NI, Casarez IJ, Canales RA, Beamer PI, Farland LV, von Hippel FA, de Zapien JG, Rosales CB, Furlong MA. Longitudinal associations and interactions of heat and metal (loid) exposure with kidney outcomes in Mexican agricultural workers. Environmental research. 2025 Aug 5:122533.

Wan ET, Darssan D, Karatela S, Reid SA, Osborne NJ. Association of pesticides and kidney function among adults in the US population 2001–2010. International Journal of Environmental Research and Public Health. 2021 Sep 29;18(19):10249. https://doi.org/10.3390/ijerph181910249

Yin G, Zhao S, Zhao M, Xu J, Ge X, Wu J, Zhou Y, Liu X, Wei L, Xu Q. Complex interplay of heavy metals and renal injury: New perspectives from longitudinal epidemiological evidence. Ecotoxicology and Environmental Safety. 2024 Jun 15;278:116424.

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