

# Status of metals in serum and urine samples of chronic kidney disease patients in a rural area of Bangladesh: an observational study

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## Research Article

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## Abstract

The traditional causes of Chronic Kidney Damage (CKD) are Diabetes and Hypertension. However, recent studies reported the possible relations between metal exposure and CKD. This study aims to explore the status of metals in CKD patients compared to their healthy counterparts at Narayanganj, Bangladesh, through a cross-sectional study. In this study, 50 volunteers have involved; 30 CKD patients and 20 healthy controls. Five metals were measured from serum (Cu and Zn) and urine (Pb, Cd, and Cr) using Atomic Absorption Spectrophotometer (Varian AA240 FS). Compared to the controls and the CKD patients were exhibited a significantly higher level of Pb and Cr in their urine samples. As expected, it was observed that the cases had higher levels of Pb, Cd, and Cr in their urine. This signifies a potential association between heavy metal exposure and CKD. The serum levels of Cu were much higher than expected for CKD patients than controls, and the Zn values were in accordance with established literature. However, the level of Zn in blood was significantly lower in the CKD group compared to the control. This data suggests that the Cu imbalance in the serum of the CKD subjects might have been related to a myriad of reasons, one of which being exposed to large concentrations of the nephrotoxic metals such as Pb and Cr in this study. Large scale epidemiological study is warranted in Bangladesh to examine environmental health in relation to nephrology.

## Introduction

Bangladesh mainly has an agro-based economy with limited land to feed up a large population. Therefore, the application of pesticides and chemical fertilizers are obvious. A line of evidence suggests potential health and environmental hazards due to pesticide application in Bangladesh[1], and unregistered pesticide traces in environmental samples also indicate the weakness in the national pesticide's governance[2]. Moreover, it has been reported that toxic metals in fertilizers and pesticides might be attributed to Chronic Kidney Damage (CKD) [3]. In Bangladesh, CKD case has increased at an alarming rate over the past few decades [4]. CKD is a progressive disease that can be identified by a diminished estimated glomerular filtration (eGFR) rate ( $< 60$  mL/min/1.73m<sup>2</sup>) that persists for a period of a minimum of three months [5]. The major known causes of CKD are diabetes, hypertension, hyperlipidemia, structural diseases. Although some studies reveal a possible correlation of CKD with heavy metals, it has not been established for low-level environmental exposure[3]. Thus, the CKD of unknown etiology is termed as CKDu, which has been considered a matter of choice for environmental health research and nephrological research. It has been documented for some time, the deleterious effects of heavy metals such as Lead (Pb), Chromium (Cr), Cadmium (Cd), arsenic (As), mercury (Hg), etc. on the human body. However, the correlation of metals and CKD incidence still need to be explored as there is very limited research has been done and presented very mixed findings to explain the CKD etiology. For instance, Kim et al. (2015) reported in an epidemiological study that Pb, Hg, and Cd in blood were not associated with CKD, while Cd is associated in CKD cases having comorbidities such as hypertension or diabetes[6]. Whereas Chung et al. reported a significant association of blood Pb and Cd with renal dysfunction in Korean adults[7]. However, a systematic review by Moody et al. summarized with a mixed outcome based on epidemiological analysis regarding the association of As-CKD, Pb-CKD, and Cd-CKD. However, the descriptive studies were found elevated Cd levels in CKD populations[8].

Cd can cause osteoporosis by replacing calcium in the bone matrix. The principal organ targeted by Cd is the kidney. CKD has a significant association with elevated blood Cd levels, but less so with blood Pb and Hg [6]. Though Cd is one of the main factors for Chronic Renal Failure, according to Mohiuddin *et al.* in the context of Bangladesh, Cr and Pb is much above permissible in fertilizers than, Cd which is almost always below the detection limit[9]. And in the case of people who have a history of smoking (in stacked years), cadmium accumulation is found proportional with the higher number of years[10].

Zinc (Zn) and Copper (Cu) are essential metals. Alterations in Zinc and Copper metabolism have been frequently observed in patients with chronic kidney disease (CKD) and in those with diabetes. These elements have important roles in the biological systems, as components of proteins, enzymes, and antioxidants. Anorexia, low taste sensibility, hypogeusia glucose intolerance, healing difficulties, and anemia are common features of both CKD and diabetes that can be associated with copper and zinc abnormalities. Scarce information exists on the effects of the association between CKD and diabetes in the metabolism of Zn and Cu [11].

It has been reported that fertilizers contain significant levels of heavy metals with potential nephrotoxic properties [9], which may ultimately result in loss of kidney functions [6, 12, 13]. Jayatilake *et al.* have found evidence of greater inhibition of acetylcholinesterase among patients with chronic renal dysfunction in areas of a high prevalence of CKDu[14]. In their thorough study, they have shown higher numbers of CKDu affected people in the region with high levels of environmental Cadmium (Cd), Lead (Pb), aluminum (Al), and fluoride (F) [8]. The commonly used fertilizers in Bangladesh are urea, triple superphosphate (TSP), murate of potash (MoP), diammonium phosphate (DAP), gypsum, magnesium sulfate, etc. A study determined that heavy metal concentration in fertilizers collected from various parts of Bangladesh (Mymensingh, Chattagram, Rajshahi, and Bogra) showed Pb and Cr levels ranging 71.4–168.5 mg g<sup>-1</sup> and 260 – 302mg g<sup>-1</sup> respectively, in TSP. For DAP it was 21.43 – 371.4 mg g<sup>-1</sup> for Pb and 1210 – 1390 mg g<sup>-1</sup> for Cr. For MoP the content was 148.6 - 188.6 mg g<sup>-1</sup> for Pb and 296–310 mg g<sup>-1</sup> for Cr. For all the cases maximum allowable limit was 100 mg g<sup>-1</sup> for Pb, 500 mg g<sup>-1</sup> for Cr, and 10 mg g<sup>-1</sup> for Cadmium (Cd) [4]. Another study done in several districts of Bangladesh (Faridpur (Sadar), Saltha, Gazipur Sadar, Mymensingh (Sadar), Bagha, Nawabganj (Sadar), Pabna, Baghmara, Gomostapur, Charchat) showed that daily intake of heavy metal of an adult male from those areas is 19.7 µg of Cd, 74.1 µg of Pb, 423 µg of Cr on average [15].

Farmers, in general, belong to the low-income group and not well aware of their health risks from heavy metals or concerns for heavy metal or pesticide accumulation in the soil, their chief objective is the maximization of crop yield and consequently profit[1]. A World Bank survey found farmers usually sprayed their crops bare-footed (only 1% wore sandals), only 2% wore gloves, 3% wore protective eye-glasses, and 6% wore home-made cotton masks. Also, the concerning overuse of fertilizers is lent credibility by the following study conducted in the agrarian area of Tangail showed that 45% of farmers used 201-250 kg of urea per hector for cultivating Aus paddy, 45% of farmers used 201-250 kg of urea per hector for cultivating Aman paddy, 45% of farmers used 201-250 kg of urea per hector for cultivating Boro paddy. Where government standard amount was 141, 166 and 269 kg/hector for Aus, Aman, and Boro (varieties of paddy) respectively[16]. Therefore, the risk of toxic exposure is high among this occupational group in Bangladesh.

Heavy metals hamper homeostatic metabolic processes by binding to proteins or cation or sulfate/bicarbonate transporters or replacing metals in enzymes or by inducing reactive oxygen species (ROS). And it is to be noted that these heavy metals are ultimately transported to the kidney for excretion. It happens so that the heavy metals concentrations in the blood are not altered as significantly as in urine. Hence for the study of chronic renal failure due to low-level environmental exposure of heavy metals, it might be a matter of interest to study heavy metals (Pb, Cd, Cr) concentrations in the urine of CKD patients, and also by studying the levels of essential metals (Cu, Zn) in the blood serum to get an interesting clue about the kidney functions. Therefore, this research aimed to investigate the concentrations of Cd, Pb, Cr in urine and Cu and Zn in the blood serum samples of CKD affected people as well as healthy people. Besides, to examine the possible association between environmental heavy metal exposures and CKD for the first time in Bangladesh.

## Materials And Methods

### 2.1 Study Area and Population

A Cross-sectional study on a rural area, namely Sonargaon, Narayanganj, Dhaka, was performed because this area is abundant with industrial and agricultural activities (Banglapedia). Hence these two effects in tandem lead to large-scale contamination of the soil, groundwater, crops and affect the general population. Moreover, this area is at a communicable distance from Dhaka city, and there have been many research activities in this area. As a result, people are aware and are more inclined to participate in research. In Sonargaon, this study is the first of its kind.

The sampling population selected were adults who were full-time or part-time involved in agricultural activities. A voter list of the 47 villages of Sonargaon was collected, and certain people were requested to appear at the Research sites as volunteers for this study. The selected people were invited to the research center and briefed about the cause of the invitation and were assigned IDs. Researchers along with physicians filled up datasheets, which recorded the healthy and CKD, affected people of the selected area age, profession, habits, occupation, education, monthly income, food habits, etc. The healthy and CKD affected people of the selected area were told to come early in the morning on a different day when their blood and urine were collected. The biological samples (Blood and Urine) of 50 representative people from 16 of the 47 villages were collected in metal-free containers. The controls and CKD affected people of the selected area have been divided into two groups, namely 'exposed' and 'controls'. The patients of CKD were confirmed by examining parameters like eGFR, serum creatinine, and ACR over 3 months. Only the confirmed cases of CKD, who did not have High Blood Pressure or Diabetes but had abnormal levels of eGFR and ACR were considered for our study. The controls are the people who have not been diagnosed with CKD.

Fig. 1. Schematic diagram of our research

This study was approved by the ethics committee of the collaborating hospital and the Institutional Review Board (IRB), Atomic Energy Centre, Bangladesh Atomic Energy Commission, Dhaka, and written informed consent was obtained from all of the volunteers, including patients and healthy controls.

### 2.2 Chemicals and Reagents

In our study, all chemical reagents were of analytical grade or of supra pure quality (E. Merck, Germany). High purity deionized water (Milli-Q System, Millipore, Thermo scientific, MA USA) with the resistivity  $18.2\text{M}\Omega\text{ cm}^{-1}$  was used for the preparation of all solutions. Nitric acid ( $\text{HNO}_3$ ) (70%, purified by redistillation,  $\geq 99.999\%$  trace metals basis) was obtained from Sigma Aldrich (St. Louis, MO, USA). Single element standard reference metal solution of Pb, Cd, Cr, Cu, Zn ( $1000\text{ }\mu\text{g/L}$ ; Spectropure, USA) from Agilent Technologies (Santa Clara, CA, USA) was procured. Quality control standard was prepared from multielement standard solution ( $500\text{ }\mu\text{g/L}$ ; Agiland, Santa Clara, USA).

As the tubes and pipette tips (Eppendorf type; Brinkmann Instruments, Inc., Westbury, NY 11590) are unused, they were directly soaked in 5% nitric acid for 24 hours, followed by washing in deionized water for another 24 hours, and then dried and kept in cleaned capped plastic containers.

### 2.3 Sample Preparation and Analysis

The urine was transported from the sample collection site in iceboxes and was finally preserved at  $-80\text{ }^\circ\text{C}$ . Blood was collected in Polypropylene tubes and stored in an icebox. Later it was subjected to centrifugation at 3000 rpm for 15 minutes at room temperature. Thus, serum was obtained, and the serum was preserved at  $-80\text{ }^\circ\text{C}$  in metal-free polypropylene tubes.

After receiving the serum and urine samples in the Analytical Chemistry Laboratory, dilution of samples was rendered to 10 mL using ultrapure deionized water if required. Analytical calibration standards, internal quality control, and spiked samples with matrix from known standards were prepared from the standard stock solution. A calibration curve for each metal of  $0.1\text{--}10\text{ }\mu\text{g/L}$  was constructed. The Pb, Cr, and Cd in urine and Cu, Zn in serum were determined by Atomic Absorption Spectrophotometer (Varian AA240 FS) equipped with hollow cathode lamp. The wavelength and lamp current of Pb, Cd, Cr, Cu, Zn are 217.0 nm and 10mA, 228.8 nm and 4mA, 357.9 nm and 7mA, 324.8 nm and 4mA, 213.9 nm and 3mA, respectively. The type of flame was Air/Acetylene, the flow rate of Air and Acetylene was 13.50 L/min and 2.90 L/min, respectively. Moreover, the purity of Acetylene gas was 99.99% pure used for the flame. Replicate samples/standards, quality control standard, spike recovery, and method blanks were used to monitor the performance of the instrument and the quality of the data. The recoveries of spiked samples were from 88 to 99%.

### 2.4 Statistical Analysis

For statistical analysis SPSS Statistics 22.0 (IBM Corp, Armonk, NY, USA) was used, including analysis of variance, correlation analysis, and principal component analysis. Origin 2019 (OriginLab Corp, Northampton, MA, USA) was used for distribution tests and charting. The Monto Carlo simulation was performed using Crystal Ball Software (11.1 Oracle Inc., Oracle, CA, USA).

## 2.5 Monte Carlo Simulation

The probabilistic risk assessment of heavy metals was performed using a simulation of Monte Carlo. Weak correlation or independence between the input variables was assumed in this simulation. Input variables (Pb, Cd, Cr, Cu) were modeled as specific probability distribution functions (Table 4). To ensure the reliability of the results, 20,000 random iterations of each input variable were carried out in each simulation. The input variables were randomly extracted from the defined probability distributions (Table 4). In this study, the 5th, 25th, 50th, 75th, and 95th percentiles for each heavy metal were extracted from the probability distribution (Fig.4).

## Results

### 3.1 Metals concentration levels in the urine samples

The concentrations of metals in the urine samples of CKD patients and control subjects with their descriptive statistics are presented in table 1. Pb levels in urine samples from CKD patients are ranging from 30 µg/L to 1040 µg/L with a mean value of 594.86 µg/L. In the control group, the mean value of Pb was 38.48 µg/L (ranging from 10 to 90 µg/L) (Table 1). In the case of Cr in the urine sample, the mean concentration of 77.38 µg/L was found for CKD having a range from 2.75 to 116 µg/L. On the other hand, in the control group, the mean concentration of Cr was 18.00 µg/L with a range of 5.6 to 29.09 µg/L (Table 1). The mean concentration of Cd in the urine samples of CKD cases was 19.69 µg/L with a range from 1.56 to 45 µg/L. Whereas, mean Cd concentration of 17.50 µg/L was found in the control group with a range from 8 to 30 µg/L in the urine samples (Table 1). Comparative status of Pb, Cr, and Cd in urine samples from CKD cases and healthy counterpart is presented in Fig 2. For Pb and Cr, a significantly elevated concentration was found in the urine samples of CKD patients compared to healthy people. However, in the case of Cd, the difference in concentration level was not significant between CKD and healthy people (Fig. 2).

### 3.2 Metals concentration levels in the blood samples

In the blood samples of CKD patients and their healthy counterparts, 2 metals were studied, i.e., Cu and Zn. The descriptive statistics of the data was given in table 1. The mean concentration of Cu in blood samples was 737.08 µg/L in the CKD group ranging from 30 µg/L to 1185.31 µg/L. In the case of Zn, the mean concentration in blood samples of CKD cases was 519.78 µg/L having a range from 29 to 1042.31 µg/L (Table 1). On the other hand, for the healthy control group mean Cu concentration in blood samples was 511.58 µg/L with a range of 2014.45 to 690.89 µg/L. Whereas, Zn in blood samples of the control group was ranging from 636.78 µg/L to 1054 µg/L with a mean of 771.8 µg/L (Table 1). Statistical comparison was conducted to examine the differences between these 2 metals in the blood of both study subjects and results are presented in Fig. 3. For both of the metals, a significant difference was found. Cu is significantly increased in the blood samples of CKD cases compared to their healthy counterpart (Fig. 3). On the other hand, Zn was found significantly lower in the CKD group compared to their healthy control group (Fig. 3).

### 3.3 Pearsons` correlation and PCA of the metals in the study groups

Pearsons

*c* or *relation was perfect or med* →  $f \in \text{daposib} \leq \text{association amongd} \Leftrightarrow \text{erentvariab} \leq s(\text{Tab} \leq 2)$ . Assign if *icantpositivec* or *rela* urine sample and urine level Cr and blood level Zn in CKD group at  $p < 0.01$ , and with urine level Cd, blood level Cu at  $p < 0.05$  (Table 2). Urine level of Cr in the CKD group was also showed a significant association with urine level Pb, blood level Cu, and Zn in CKD patients. However, urine level Cd was only significantly associated with the blood level of Zn in CKD cases at  $p < 0.05$  (Table 2). In addition, the Blood level of Cu in CKD cases is significantly associated with the blood level of Zn in the same group. Moreover, the Pb in urine level was significantly associated with the urine level Cd in the control group. Four principal components were identified with significant association PC1 comprised with a strong influence of urine level Pb and Cr, and Cd in CKD group followed by PC2 formed with urine level Pb and Cd in the control group; PC3 comprised of blood level Cu and Zn in CKD, and negative association of blood level Cu in the control group; and PC4 made of urine level Cr and blood level Zn in the control group (Table 3).

### 3.4 Probabilistic Health Risk Assessment

Considering the variation of concentrations of heavy metals in urine and serum samples, risks were simulated using the Monte Carlo method. The 5th, 25th, 50th, 75th, and 95th percentile values of the distributions of heavy metals are shown in Fig 4. The Pb, Cu were higher than Cr, Cd one even at the 5th percentile, suggesting that most of the residents were exposed to a significant health risk for Pb, Cu. The order of the 95th percentile risk value of metals studied in the control and CKD group showed the pattern as follows:  $\text{Cu} > \text{Pb} > \text{Cr} > \text{Cd}$  (Fig.4). A similar trend was aobserved in case of 75th, 50th and 5th percentile.

## Discussion

In this study, it was found that the concentration of serum Copper lies within 214.45 – 1185.31 µg/L and serum Zinc within 319.45 – 1042.31 µg/L. DiDonato et al.[17] reported that serum Copper for healthy human beings must lie between 100 and 150 µg/L and Barsoum et al. [18] reported that serum Zinc must lie between 660-1100 µg/L. It appears that the mean values obtained for serum Copper are much more than their recommended values, while the mean of the serum Zinc levels are within the recommended limit. However, the mean for Copper is less than the Zinc values as per the recommendations of DiDonato and Barsoum. The mean for serum Copper for case and control are 816.99 µg/L and 511.49 µg/L, respectively. The increase in the copper levels in CKD affected people are expected [19]. The mean serum Zinc level for the case and control are 578.35 µg/L and 771.80 µg/L, respectively. The decrease in levels of zinc found in this investigation is also expected as per Shih *et al.* [19]. The mean serum Copper and Zinc for cases were higher and lower, respectively than that of controls, which is in agreement with the literature. However, it must be noted that the mean for copper concentration in serum found is about 4 times higher

than 150 µg/L (the recommended value). It might be concluded that this has resulted from a copper-rich diet of the people of Sonargaon or long term exposure to heavy metals. Also, the standard deviations were quite large indicating that both serum Copper and Zinc data varied greatly from man to man.

Out of 50 urine samples, 30 of them are having a very high concentration of Pb (250 – 1040 µg/L), and only 20 samples are having Lead concentration levels (10 – 90 µg/L), which are comparatively lower. According to Yaman *et al.* [20], the normal levels of Lead in the urine may be assumed to center about a mean of 35 µg/L. For unexposed human urine, the Lead and Cadmium concentration must be below 80 µg/L and 1 µg/L, respectively. The mean urinary Lead for cases and controls are 656.50 µg/L and 38.48 µg/L. It is evident that the CKD patients have much higher levels of Lead in their urine in contrast to controls. The highest urinary Lead excretion mean values are obtained for individuals from Panam Gabtoli, Uttar Khansardi, and Hariya Chowdhury para, which are 1040, 926.67, and 910 µg/L, respectively. And the lowest values are obtained for individuals from Bhabanipur, Khamargaon, Dighi Chandpur, Dakarband, and H. Khaser Kanda, which are 10, 20, 30, 35, and 55 µg/L, respectively. The urinary excretion of Lead is very high in people of 10 of the 16 villages, which is much more above the normal range. It might be possible that the former areas are much more contaminated with Lead than the latter due to heavy exposure of industrial effluents containing Lead or by overuse of fertilizers containing Lead.

The mean urinary Cadmium for case and control was 20.67 µg/L and 17.51 µg/L, respectively. The CKD patients had higher levels of Cadmium in their urine in contrast to controls. This agrees with the reported trend [13] but to a limited extent. The highest mean values for urinary Cadmium excretion were found for individuals from locality Uttar Khansardi, Panchabati, Hariganj, Basundardi, H, Khaser Kanda, Bhabanipur, Dhanpur, Panam Gabtoli and Haria Chowdhury para which was 45.00, 30.00, 23.00, 22.00 and 21.00 µg/L respectively. The lowest values were obtained for individuals from Dighi Chandpur, Khamargaon, Haria Khaser Kanda, and Paschim Damodari, which were 8.00, 8.00, 8.00, 9.90 µg/L, respectively. It might be possible that the former areas are much more contaminated with Cadmium than later. However, no area is under the safe limit of healthy levels of urinary excretion of Cadmium. This finding is very alarming as we know Cadmium is a known carcinogen and a toxic metal.

The mean urinary Chromium, for exposed and control, are 85.35 µg/L and 18.00 µg/L. Kiilunen *et al.*[21] have found the average value for urinary excretion of Cr for normal or unexposed human beings is 0.125 µg/L and recommended that the values must be below 0.57 µg/L. It is evident that the CKD patients had higher levels of Chromium in their urine in contrast to controls. All members from the villages had urinary chromium excretion values that were very high, except individuals from Dakarband, Vhabanipur, and Dakarband, which had means of 8.00, 5.60, and 12.00 µg/L, respectively. It might be possible that all the areas under study are contaminated with Chromium. However, no area was under the safe limit of healthy urinary excretion of Chromium.

Though the urinary excretion of Cadmium and Chromium are lower in the 16 villages, they are well above the recommended range for almost all of the villages. The urinary excretion of all three metals is highest for Panam Gabtoli and Haria Baidyer Para, while the lowest values can be attributed to Bhabanipur, Khamargaon, Dighi Chandpur, and Dakarband.

The above paragraphs depict that the urinary excretion of Lead, Cadmium, and Chromium are higher for the cases than the controls. One of our research parameters is that the CKD patients who did not have hypertension or diabetes were specifically chosen. It proves a positive correlation between exposure to nephrotoxic heavy metals and CKD. Also, the exposure might cause an imbalance of Copper concentration in their blood serum, which might affect their kidneys and other internal organs in the long term. This findings are also support the out of the probalistic health risk assessment of this study (Fig.4). Highest risk was observed due to high exposure of Cu followed by the Pb. Accumulation of Cu in the serum of CKD indicates the lower urinary excretion of Cu might be associated with the high probalistic risk factor for CKD patients in this study. This may also be concluded that CKD patient might be affected by low serum Zn level which triggers the low profile of antioxidants in the patient might be stimulated by the high level of Cu. However, this findings warrants further large scale study in this issue to clarify the exact mechanism.

## Conclusion

In comparison with other lethal ailments, CKD is comparatively obscure in Bangladesh. This collaborative study being the first of its kind in Bangladesh, found that most residents of the area had elevated levels of heavy metals in their urine. Moreover, residents with the highest values of Pb, Cd, Cr in their urine suffered from CKD and an imbalance of Cu concentrations in their blood serum. Although in our study, a positive correlation was established. Further research into the correlation between nephrotoxic metals and CKD is necessary to comprehend CKD and its treatment.

## Declarations

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### Compliance with Ethical Standards

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Data availability:** All data generated or analysed during this study are included in this published article.

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## Tables

	N	Minimum	Maximum	Mean	Std. Deviation	Variance	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
CKD urine Pb	35	30	1040	594.8603	256.0849	65579.45	-0.395	0.398	-0.057	0.778
CKD urine Cr	35	2.75	116	77.3883	26.64065	709.724	-1.402	0.398	1.745	0.778
CKD urine Cd	35	1.56	45	19.6937	9.10494	82.9	0.895	0.398	1.532	0.778
CKD blood Cu	35	30	1185.31	737.0878	298.1705	88905.65	-0.779	0.398	0.422	0.778
CKD blood Zn	35	29	1042.31	519.7827	222.1036	49330.01	-0.048	0.398	0.867	0.778
Control urine Pb	20	10	90	38.4825	22.463	504.587	1.14	0.512	1.045	0.992
Control urine Cr	20	5.6	29.09	18.0025	7.87408	62.001	0.065	0.512	-1.51	0.992
Control urine Cd	20	8	30	17.507	7.3631	54.215	0.108	0.512	-1.18	0.992
Control blood Cu	20	214.45	690.89	511.5831	127.9366	16367.79	-0.694	0.512	-0.04	0.992
Control blood Zn	20	636.78	1054	771.8009	113.3127	12839.76	0.968	0.512	0.458	0.992

Table 1: Descriptive statistics for the metal's concentrations in urine and blood samples of CKD cases (n=35) and control cases (n=20).

Table 2: Pearson correlation for among different variables in the study.

	P Pb U	P Cr U	P Cd U	P Cu B	P Zn B	C Pb U	C Cr U	C Cd U	C Cu B	C Zn B
P_Pb_U	1									
P_Cr_U	.776**	1								
P_Cd_U	.401*	.396*	1							
P_Cu_B	.405*	.675**	0.168	1						
P_Zn_B	.551**	.542**	.368*	.614**	1					
C_Pb_U	-0.041	0.001	-0.301	0.013	-0.219	1				
C_Cr_U	0.239	0.016	-0.124	0.013	-0.058	0.365	1			
C_Cd_U	-0.092	-0.047	-0.251	0.148	-0.342	.462*	0.079	1		
C_Cu_B	-0.151	-0.223	-0.282	-0.172	-0.436	-0.009	-0.02	0.158	1	
C_Zn_B	-0.095	-0.296	-0.385	-0.01	-0.209	0.042	0.204	0.057	0.108	1

\*\* Correlation is significant at the 0.01 level (2-tailed).  
\* Correlation is significant at the 0.05 level (2-tailed).  
P: CKD; C: Control; U: Urine; B: Blood

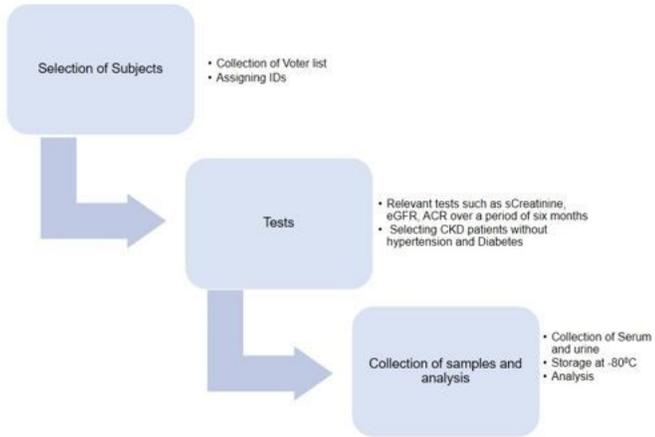
Table 3: Rotated Component Matrix for PCA analysis

	PC1	PC2	PC3	PC4
P_Pb_U	<b>0.88</b>	-0.103	0.069	0.197
P_Cr_U	<b>0.86</b>	0.113	0.011	-0.155
P_Cd_U	<b>0.578</b>	-0.311	0.283	-0.36
P_Cu_B	-0.34	0.353	<b>0.55</b>	-0.196
P_Zn_B	0.124	-0.473	<b>0.658</b>	0.01
C_Pb_U	0.045	<b>0.752</b>	0.039	0.336
C_Cr_U	0.2	0.256	0.127	<b>0.77</b>
C_Cd_U	-0.069	<b>0.811</b>	-0.139	-0.051
C_Cu_B	-0.184	0.061	<b>-0.806</b>	-0.029
C_Zn_B	-0.349	-0.089	-0.188	<b>0.679</b>
Eigenvalues	2.181	1.77	1.541	1.402
% of Variance	21.812	17.698	15.411	14.015
Cumulative %	21.812	39.51	54.921	68.936

Table 4: The probability risk distribution of heavy metals in patient

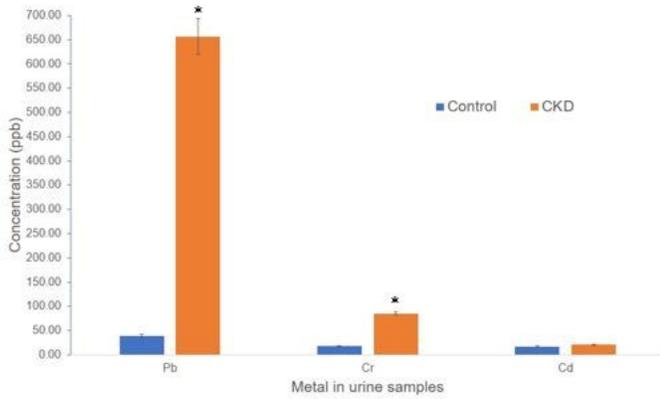
Probability	Pb	Cd	Cr	Cu	Zn
5%	1830.10	73.35	286.65	2580.04	1922.46
50%	2234.07	87.14	316.81	3005.15	2242.86
75%	2420.60	93.25	329.96	3210.46	2388.39
95%	2689.79	101.73	348.66	3520.09	2604.84

## Figures



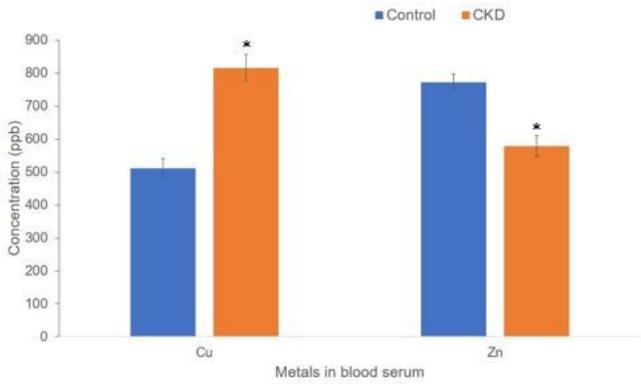
**Figure 1**

Schematic diagram of our research

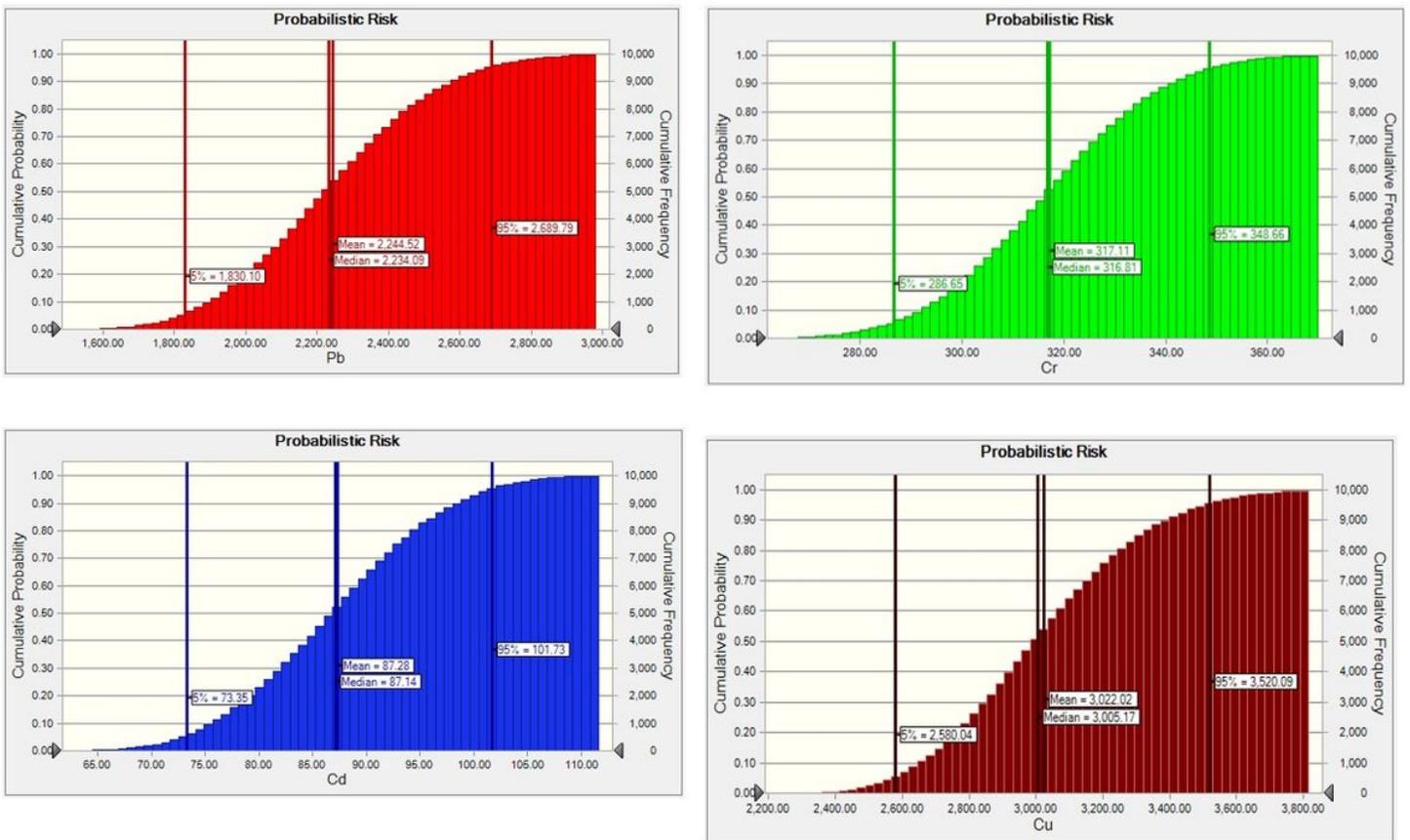


**Figure 2**

Comparative average concentrations of metals ( $\pm$  SE, n=35) in the urine of CKD patients and controls. Asterisk (\*) denotes a significant difference at  $p > 0.01$  compared to the control.



**Figure 3**  
Comparative average concentrations of metals ( $\pm$  SE, n=20) in the blood serum of CKD patients and controls. Asterisk (\*) denotes a significant difference at  $p < 0.05$  compared to the control.



**Figure 4**  
Cumulative distribution of Pb, Cd, Cr, Cu for probabilistic risk of CKD patients and controls in study area