



## Evaluated the adverse effects of cadmium and aluminum via drinking water to kidney disease patients: Application of a novel solid phase microextraction method



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### ABSTRACT

In present study aluminum (Al) and cadmium (Cd) were determined in ground water samples and assesses human health risks associated with elevated concentrations of toxic metals in dissolved form, using a novel solid phase microextraction (SP $\mu$ E). Ground water sample (n = 200) and biological sample (blood) of patients having chronic kidney disorders (CKD) along with healthy control subjects of same area (southern part of Pakistan) were collected. A simple system, including the micropipette tip packed with modified ionic liquid-activated carbon cloth (IL-ACC) coated with 8-hydroxyquinilone (8-HQ) attached to syringe. The analytes in water and acid digested blood samples were manually drawn for 2–10 cycles (drawing/discharging) at different pH range. The analytes sorbed on coated ACC were then desorbed with 2.0 mol L<sup>-1</sup> HNO<sub>3</sub> in ethanol by drawing/discharging cycles for 1–5 times. The concentration of extracted analytes was determined by electrothermal atomic absorption spectrometer. The influence of different variables on the extraction efficiency of Cd and Al, were optimized. The Al and Cd concentrations in groundwater were found to be elevated than recommended limits by the World Health Organization. The urinary N-acetyl-h-glucosaminidase values were significantly higher in CKD patients as compared to referent subjects (p < 0.001). The significant variation in levels of Cd and Al were observed in blood samples of CKD patients than referents subjects (p < 0.01). The strong positive correlation among Al and Cd levels in groundwater versus blood samples of CKD patients (r = 0.82–0.85) p < 0.01) was observed than those values calculated for referent subjects (r = 0.425–0.536).

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### 1. Introduction

To evaluate the environmental pollution due to different toxic metals is enormously important; especially in developing countries of south Asia, wherever the elevated exposure to biological life including human beings is severe, most probably due to lack of information and low socioeconomic status (Malik et al., 2010). The epidemiological studies in recent years have revealed adverse impacts of toxic metals in water used for drinking and other domestic purposes create different physiological disorder in human

beings (Flaten, 2001; Reddy and Gunasekar, 2013). The high exposure of Cd adversely effects the normal physiological functions of kidney and interfere with the renal handling of plasma derived proteins (Garçon et al., 2007; Järup and Åkesson, 2009; Satarug et al., 2011; 2003). The adverse effect attributed to Cd exposure is also attributed to tubular proteinuria levels, as it can creates irreversible damage to renal functions (Garçon et al., 2007). It is also associated with age-related decline in glomerular filtration rate and reduce in the filtration reserve capacity (Satarug et al., 2003; Wang et al., 2010). Thus, precise finding of Cd in drinking water has been very important (Shrivastava and Patel, 2010).

The humans are exposed to non-essential element, aluminum (Al) frequently from different sources including drinking water. Generally, it is not frequently soluble in water at neutral pH (Flaten, 2001). During recent times, a great quantity of Al has been dis-

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charged into the environment by waste release, low pH of water and soils extract from acidic rain (Khan et al., 2009).

The  $200 \mu\text{gL}^{-1}$  of Al is maximum permissible level in drinking water by WHO (World Health Organization, 2004). Recently, a profound attention has been increased by the adverse impact of Al toxicity in human life (Venturini-Soriano and Berthon, 1998). It was reported that high exposure of Al might be stored in the brain through diverse ways (diet, waters and different medication), which might creates adverse impacts on the nervous system (Flaten, 2001; Sińczuk-Walczak et al., 2004). The Al causes severe health related problems in humans and it might be one of the factors causes renal osteodystrophy, Parkinson and Alzheimer's disease (Sang et al., 2008). The most important pathological alteration associated with toxicity of Al in Alzheimer's disease, is its presence in neuro-fibrillary tangles in different parts of the brain. The high level of Al interferes through and variations in calcium metabolism in numerous organs such as brain (Klaassen, 2001). The poisoning of Al in end stage of CKD who have long-term dialysis is might creates adverse impacts on health such as dialysis encephalopathy, osteomalacia and anemia (Jeffery et al., 1996). It is well recognized that continuing ingestion of Al via different route, can create harmful effects on human health (Jeffery et al., 1996). The very low levels Al determination has been very substantial in clinical and environmental samples, because due to its adverse function in the normal physiology of human body (Bishop et al., 1997). Consequently, there is a very strong requirement to assess Al in water of natural resources (Becaria et al., 2006). It is usually difficult to determine very small quantity of Cd and Al in biological and environmental samples directly due to interference of matrix, or the analyte concentration is below the limit of detection of the instrument. Therefore, separation and preconcentration methods are quiet essential.

Several analytical techniques, atomic absorption spectrometry (Kazi et al., 2009a), inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Liang et al., 2003) and spectrofluorimetry are used for the determination of trace levels of Al in environmental and biological samples (Abdolmohammad-Zadeh and Sadeghi, 2010). However, all of the techniques are required enrichment methods for the determination of trace amounts of Al (Panhwar et al., 2014; Khan et al., 2009). The generally used techniques for the separation and preconcentration of Al consists of liquid-liquid extraction (Gharehbaghi et al., 2009), solid-phase extraction (SPE) (Arain et al., 2014a), and cloud point extraction (Khan et al., 2009; Arain et al., 2014b).

The aims of present work were to develop a novel strategy "solid phase microextraction (SP $\mu$ E)" and to assess the relative distribution and apportionment of certain toxic metals in drinking water (groundwater) and biological sample (blood) of CKD patients. For comparative purposes healthy subjects of same age group and residential areas was also selected as referents. It is assessed that the work would assist to recognize the association of Cd and Al in drinking water with the CKD patients and their possible role in the complication of kidney disorders.

## 2. Materials and methods

### 2.1. Study population

Fifty five CKD male patients aged 40–60 years, living in the rural areas near by Hyderabad city, while fifty nine male subjects having same age, dietary habit and socioeconomic status (mostly the relative of patients) as control/referent group. The CKD patients attend the urology ward as outdoor patient department (OPD), of the Liaquat Medical University Hospital (LUMHS) Jamshoro, Pakistan, during 2013–2014, were selected. The information about the toxic

effects of Al and Cd, was provided to local population by leaflets and loudspeakers. The rural health center workers were inquired to provide a screening project to those persons who were unaware of the health message. The local population of the selected area consumed generally groundwater for drinking and other domestic purposes, due to lack of municipal treated water. All CKD patients and healthy control were provided verbal consent in local language (because most of the study subjects were illiterate), while confirming that they have accepted the conditions of providing blood samples and other information. To all referents and patients a questionnaire was provided to obtain details of their physical health, dietary habits, ethnic origin, and age. The physical examinations of selected population were achieved at local health center and LUMHS Hyderabad, Pakistan. The height, weight, blood pressure of the patients and healthy referents were measured at once. The different biochemical data was obtained from the pathological laboratory of LUMHS

### 2.2. Instrumentation

A double beam Perkin-Elmer atomic absorption spectrometer model A Analyst 700 (Norwalk, Connecticut, USA) equipped with a graphite furnace HGA-400, pyrocoated graphite tubes, an auto sampler AS-800. The instrumental parameters for the measurement of studied metals (Al and Cd) were followed as suggested by the manufacturer. The blood samples were acid digested in a domestic microwave oven (Pel, PMO23, Japan).

### 2.3. Reagents and glassware

The ultra-pure water obtained from ELGA Labwater System (Bucks, UK) was used throughout the experimental work. Hydrogen peroxide and nitric acid from Merck (Darmstadt, Germany) were of analytical reagent-grade. The certified standard solution of Al and Cd (1000 ppm) purchased from Fluka Kamica (Buchs, Switzerland), were further diluted to prepare working standard solutions. Furthermore, matrix modifiers were engaged to analyse Cd and Al obtained from Sigma Aldrich (Milwaukee, Wisconsin, USA). To check the accuracy of the analytical method, the certified matrix matched samples for water and blood samples, SRM 1643e and Clincheck control-lyophilized human whole blood were obtained from National Institute of Standards and Technology (Gaithersburg, MD, USA) and (Recipe, Munich Germany) respectively. All the plastic materials and glasswares used were formerly treated for a 24 h in 5 M nitric acid and washed with double distilled water and then by ultra-pure water.

### 2.4. Sampling and pretreatment

#### 2.4.1. Water sampling

The forty seven groundwater samples were taken from depth of greater than 40 ft from rural areas of district Hyderabad, Sindh province. The samples of groundwater were collected from motor and hand pumps directly into the sampling bottles. For comparative purpose, water samples of domestically treated (N = 50), supplied to urban population of Hyderabad city. The pH of groundwater samples were measured as defined in earlier study (Kazi et al., 2009b). The water samples were preserved at 4 °C for further treatment.

#### 2.5. Blood sampling

The 10 mL of venous blood samples were collected from each studied subject in tubes containing  $>1.5 \text{ mg K}_2\text{EDTA/mL}$  (metal-free) obtained from Becton Dickinson, Rutherford, USA. The blood samples of each CKD patient and control were kept at  $-4 \text{ }^\circ\text{C}$ , till further analysis.

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