Ukrainian Journal of Nephrology and Dialysis
Scientific and Practical, Medical Journal
Founder:
- National Kidney Foundation of Ukraine

ISSN 2304-0238;
eISSN 2616-7352 **Journal homepage:** https://ukrjnd.com.ua

Research paper

Faten M. Abu Orabi[1], Mohammad Fawzi[1], Jamal Humaidi[2], Mohammed-Jamal A. Shammout[1], Rawand Saleh[1], Hedaya Hakawati[1], Ahmed Abu Rayyan[1]

doi: 10.31450/ukrjnd.3(83).2024.05

Heavy metal blood levels and their age-dependent changes in patients undergoing hemodialysis at Jordan Islamic Hospital

[1]Chemistry Department, Faculty of Science, Applied Science Private University, Amman, Jordan
[2]Department of Chemistry, College of Science, University of Hail, Hail, Kingdom of Saudi Arabia

Citation: Abu Orabi FM, Fawzi M, Humaidi J, Shammout M-JA, Saleh R, Hakawati H, et al. Heavy metal blood levels and their age-dependent changes in patients undergoing hemodialysis at Jordan Islamic Hospital. Ukr J Nephrol Dial. 2024;3(83):33-40. doi: 10.31450/ukrjnd.3(83).2024.05.

Abstract. *End-stage kidney disease (ESKD) is increasingly recognized as a major global health issue. Heavy metal exposure is a significant risk factor for ESKD. The present study aimed to assess the blood levels of cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn), and to investigate their variations with age among patients undergoing hemodialysis (HD).*

Methods. A cross-sectional study was conducted in Amman, Jordan, from January to December 2023. The study included 80 ESKD patients undergoing hemodialysis and 80 healthy controls, both groups aged 20-60 years, divided into four age ranges: 20-29, 30-39, 40-49, and 50-60 years. Blood samples were analyzed for Pb, Zn, Cd, and Cu using atomic absorption spectrometry. Data were analyzed using ANOVA, and p-values <0.05 were considered significant.

Results. The concentrations of Pb, Cd, and Cu were significantly higher in ESKD patients compared to healthy controls, while Zn levels were notably lower in ESKD patients. Specifically, Pb levels averaged 27.65 µg/dL in ESKD patients versus 1.06 µg/dL in controls (p = 0.006). Cd levels averaged 1.035 µg/dL in ESKD patients versus 0.0485 µg/dL in controls (p = 0.008). Cu levels averaged 270.1 µg/dL in ESKD patients versus 81.8 µg/dL in controls (p = 0.004). Zn levels were significantly reduced in ESKD patients, averaging 29.5 µg/dL compared to 82.45 µg/dL in controls (p = 0.035). Age-dependent variations showed that Pb (p = 0.01), Cd (p = 0.043), Cu (p = 0.01), and Zn (p = 0.037) levels increase with age in ESKD patients, suggesting age-related differences in metal accumulation and metabolism.

Conclusion: This study highlights significantly elevated levels of Pb, Cd, and Cu, and reduced Zn levels in ESKD patients undergoing hemodialysis compared to healthy controls. Age-dependent differences in metal concentrations suggest that the accumulation and metabolism of these metals may be influenced by age in ESKD patients. These findings underscore the need for further research into the impact of environmental toxins on kidney health.

Keywords: *end-stage kidney disease, heavy metals, lead, cadmium, copper, zinc, hemodialysis, environmental toxins.*

Conflict of interest. The author declares no conflict of interest.

Correspondence should be addressed to Mohammed-Jamal A. Shammout:
m_shammout@asu.edu.jo

Article history:
Received May 6, 2024, 2024
Received in revised form June 1, 2024
Accepted June 12, 2024

УДК: 616.61-085.38-073.27:[616.152+546.47/81](569.5)

Фатен М. Абу Орабі[1], Мохаммад Фавзі[1], Джамал Хумайді[2], Мохаммед-Джамаль А. Шаммут[1], Раванд Салех[1], Хедая Хакаваті[1], Ахмед Абу Райян[1]

Концентрація важких металів крові та їх вікові зміни у пацієнтів, які лікуються методом гемодіалізу в Ісламській лікарні Йорданії

[1]Хімічний факультет, відділ природничих наук, приватний університет прикладних наук,
Амман, Йорданія
[2]Хімічний факультет, Науковий коледж, Університет Хаіла, Хаіль, Королівство Саудівська Аравія

Резюме. *Термінальна стадія хронічної хвороби нирок (ХХН) є глобальною проблемою охорони здоров'я. Вплив важких металів є відомим фактором ризику розвитку та прогресування. Це дослідження мало на меті оцінити рівні кадмію (Cd), свинцю (Pb), міді (Cu) і цинку (Zn) у крові пацієнтів, які лікуються методом гемодіалізу (ГД) та дослідити їх вікові зміни.*

Методи. Це одномоментне перехресне дослідження було проведено в Аммані, Йорданія, з січня по грудень 2023 року. Дослідження включало 80 пацієнтів з ESKD, які проходили гемодіаліз, і 80 здорових контрольних груп, обидві групи віком 20-60 років, розділені на чотири вікові діапазони: 20-29, 30 -39, 40-49 і 50-60 років. Зразки крові аналізували на Pb, Zn, Cd та Cu методом атомно-абсорбційної спектрометрії. Дані аналізували за допомогою ANOVA, і p-значення <0,05 вважали значущими.

Результати. Концентрації Pb, Cd і Cu були значно вищими у ГД пацієнтів у порівнянні зі здоровими донорами, тоді як рівні Zn були статистично значущо нижчими. Зокрема, рівень Pb в середньому становив 27,65 мкг/дл у ГД пацієнтів проти 1,06 мкг/дл у контрольній групі (p = 0,006). Рівні Cd в середньому становили 1,035 мкг/дл у ГД пацієнтів проти 0,0485 мкг/дл у контрольній групі (p = 0,008). Рівень Cu становив 270,1 мкг/дл та 81,8 мкг/дл, відповідно (p = 0,004). Концентрація Zn була достовірно знижена у ГД пацієнтів та у середньому становила 29,5 мкг/дл порівняно з 82,45 мкг/дл у контрольній групі (p = 0,035). Аналіз вікових особливостей визначив, що концентрації Pb (p = 0.01), Cd (p = 0.043), Cu (p = 0.01), and Zn (p = 0.037) підвищуються з віком у ГД пацієнтів.

Висновки. Наше дослідження демонструє статистично значущо підвищені рівні Pb, Cd і Cu, а також знижені рівні Zn у ГД пацієнтів порівняно контрольною групою. На накопичення та метаболізм цих металів може впливати вік ГД пацієнтів. Ці висновки підкреслюють необхідність подальших досліджень впливу токсинів навколишнього середовища на здоров'я нирок.

Ключові слова: *термінальна стадія хронічної хвороби нирок, важкі метали, свинець, кадмій, мідь, цинк, гемодіаліз, екологічні токсини.*

Introduction. Over the past decade, end-stage kidney disease (ESKD) has become increasingly recognized as a global public health problem and an important cause of morbidity and mortality [1]. Although the burden of ESKD is well-defined in developed countries, there is growing evidence that the burden in developing countries may be even greater [1, 2]. Hemodialysis (HD), a life-saving treatment, involves filtering and purifying the blood using a machine to remove waste products and excess fluids in patients with ESKD. However, exposure to heavy metals, such as cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn), poses additional health risks for patients undergoing HD [3]. These metals, found in industrial processes and various environmental sources (water, air, food, cigarettes, gasoline, contaminated crops, seafood, etc.) can accumulate in the body over time, exacerbating kidney damage and complicating treatment outcomes [4, 5]. There are not less than 35 metals that are of great concern, because they are required for maintaining good health, but can become toxic in larger amounts [6]. Heavy metals are known environmental pollutants that have dramatically contributed to impaired kidney function. Metals used in industrial processes have been associated with contamination of drinking water, food, and soil, thereby increasing the risk of exposure among the general population [7]. Crommentuijn et al. showed that elevated metal levels in soil, lead to an increase in leaching losses of metals to groundwater and surface water, which will, after a considerable delay time, affect both drinking water quality and aquatic organisms [8]. Balkan endemic nephropathy (BEN) is a reported kidney illness in certain villages in Serbia, Bulgaria, Romania, Croatia, and Bosnia, that is caused by long-term exposure to polycyclic aromatic hydrocarbons (PAHs) or other toxic organic compounds [9]. Chronic exposure to low levels of environmental heavy metals such as Cd and Pb slowly accumulates in the body and causes

Mohammed-Jamal A. Shammout
m_shammout@asu.edu.jo

toxicological renal effects [5]. Choudhury et al. showed that, in Bangladesh, kidney failure cases have increased enormously over the past few decades, due to environmental hazards because of the application of toxic metals in fertilizers and pesticides [10].

Cd significantly damages the lungs, it is a nephrotoxic environmental pollutant; that can cause serious kidney disease [11]. In studies where animals were given cadmium in food or water, the animals developed high blood pressure, anemia, liver disease, and nerve or brain damage [12]. The United States Environmental Protection Agency (USEPA) lists kidney damage as the major effect of cadmium [13]. The WHO has set a drinking water quality guideline of 3 µg/L for cadmium, and levels in drinking water are usually less than 1 µg/L [14]. Allowable blood Cd concentration is in the range of 0.5 to 2.0 ng/mL (0.05- 0.2 µg/dL), and acute toxicity will be observed when levels exceed (5 µg/dL) or 50 ng/mL [15]. Cd has a worrying half-life of over 7.4 years, making it very difficult to eliminate. The main sources of cadmium exposure are smoking, contaminated food and water, pigments, plastics, coatings, and battery manufacturing [5]. According to the Agency for Toxic Substances and Disease Registry (ATSDR) [12], food and tobacco are the primary sources of exposure in the United States. As soon as cadmium enters the body, most of it binds to metallothionium. These compounds are eliminated through the glomerulus but are reabsorbed and stalled in the renal tubules. The slowly degrading of Metallothionium constantly releases highly toxic free cadmium. It is then passively excreted in the urine, but also causes oxidative stress in the renal tubules. As the kidneys' function declines, they not only lose their ability to eliminate toxins, but they also become less able to perform other functions [4].

Pb is another environmental pollutant, it is a useful element in the industry but has severe health problems in the human body, which leads to serious damage to the kidneys. Pb in blood circulation is either excreted by the kidneys or accumulates in bone. The half-life of Pb in the blood is around 35 days, and up to 30 years in bones. It has been reported, that chronic low Pb exposure (blood Pb level < 5-10 µg/dL) can potentially contribute to the development of ESKD, and chronic high Pb exposure (blood Pb level > 60 µg/dL) can cause Pb nephropathy, which is characterized by glomerular sclerosis, tubular atrophy, tubulointerstitial fibrosis, and finally reduced glomerular filtration rate (GFR). The common sources of exposure to Pb are gasoline, batteries, ammunition, coatings, food, and water contaminated by Pb pipes [7].

Cu, due to its role as a catalytic cofactor or structural component for many enzymatic reactions, is considered one of the most essential trace elements found in all living organisms. The main sources of Cu exposure are ambient air, potable water, seeds, nuts, beans, and grains [16]. Allowable blood Cu concentration ranges from 70 to 140 µg/dL [17, 18]. Cu can be absorbed into the human body through ingestion, inhalation, and even dermal contact. Excess levels of cellular Cu can be cytotoxic, which results in the production of highly reactive oxygen species (ROS), responsible for lipid peroxidation in membranes [16].

Zn is one of the most abundant trace metals in humans and the second essential element, after Iron needed by the body. Zn in small amounts is relatively harmless. Only high doses have toxic effects [19]. Drinking water seldom contains zinc at concentrations above 0.1 µg/L [14]. Arora et al. showed that normal blood Zn concentration ranges from 70 to 180 µg/dL [20]. Despite the extensive documentation of heavy metal exposure and its impact on kidney function, there is limited research specifically addressing the variations in blood levels of Cd, Pb, Cu, and Zn among patients undergoing hemodialysis across different age groups.

The present study aimed to assess the blood levels of Cd, Pb, Cu, and Zn and to investigate their variations with age among patients undergoing HD.

Patients and methods. The study design and participants. A cross-sectional study was conducted in the urban area of Amman from January to December 2023 due to its high potential for heavy metal contamination from industrial and geographical sources. The study includes patients with kidney failure from one of the most famous renal dialysis centers in Jordan (Islamic Hospital/Amman) which falls into the jurisdictions of Amman City the capital of the Hashemite Kingdom of Jordan [21]. Approval was obtained from the Ethics Committee of the Jordan Islamic Hospital (approval number 645/2022/1) and the Deanship of Higher Education and Scientific Research in Applied Science Private University (approval number 354/9/2/10). A written informed consent was obtained from all of the participants.

Eighty male patients with kidney failure undergoing hemodialysis were recruited. Exclusion criteria included diabetes, high blood pressure, hepatitis B and C, and HIV. Patients were aged 20-60 years and treated at the Renal Dialysis Unit at the Islamic Hospital. A control group of 80 healthy, non-smoker males aged 20-60 years was also included to avoid physiological differences between genders. To achieve the study goal, the participants of both groups were subdivided into the following age groups: A (20–29 years), B (30–39 years), C (40–49 years), and D (50–60 years).

Chemicals and reagents. Nitric acid (HNO_3) and Hydrochloric acid (HCl) of analytical reagent grade were purchased from E. Merck Germany. A certified standard solution reagent for atomic absorption containing Zn (LABCHEM, USA), Cd and Pb (super-Sigma-Aldrich, Germany), and Cu (CARLO ERBA, Germany) was used for preparing stock and working standard solutions. A 5% HNO_3 solution in distilled water was used to dilute these standard solutions. The preparation and handling of blank solutions were the same as that of standard solutions. All standard solutions contained deionized water (Local supplier), hydrogen peroxide (H_2O_2), and nitric acid (HNO_3) (perk grade 65%) [22, 23].

Apparatus and instruments. All glassware and equipment were cleaned with nitric acid and distilled water. Blood sample analysis was performed using the Atomic Absorption Spectrometer (AAS) model Nova 800F, which operates in the wavelength range of 200-700 nm, using a single beam optic mode for absorbance and concentration data processing [24, 25].

Sample and Standard Solution Preparation. Working standards for each heavy metal were prepared from their standard solutions using 5% HNO3 as a diluent to construct calibration curves. Blood samples (3-5 ml) were collected from the antecubital vein of ESKD patients and controls using disposable pyrogen-free needles and transferred into heparinized tubes containing lithium heparin.

Preservation and Digestion of Blood Samples. Blood samples were wet digested and stored at -20°C until analysis. Wet digestion using nitric acid involved heating 5 ml of blood with 10 ml of nitric acid at 160°C for two hours. The digested samples were filtered, cooled, treated with hydrogen peroxide, diluted to 50 ml, and filtered again [23, 25-27].

Statistical analysis. The Kologrimov-Smirnov was used to assess data distribution. Data are presented as mean (M) and standard deviation (SD) using EXCEL 2019. The results were analyzed using analysis of variance (ANOVA), with a p-value of <0.05 considered significant.

Results. The characteristics of the study participants are summarized in Table 1.

Table 1

Characteristic of the study participants

Samples	Age groups (year)	Hb (g/dL)	Serum creatinine (g/dL)	Smoking habits	Sex	Total No.
Healthy Controls	A 20-29	15.18 ± 0.73	0.68 ± 0.069	Non-smoker	Males	80
	B 30-39	15.4 ± 0.52	0.68 ± 0.58			
	C 40-49	15.2 ± 0.59	0.73 ± 0.094			
	D 50-60	15.03 ± 1.056	0.68 ± 0.0.76			
Patients undergoing HD	A 20-29	9.03 ± 0.56	7.4 ± 0.85	Non-smoker	Males	80
	B 30-39	9.8 ± 1.14	7.5 ± 1.4			
	C 40-49	10.2 ± 0.51	7.1 ± 1.3			
	D 50-60	9.4 ± 0.78	7.5±1.2			

Abbreviation: Hb, hemoglobin.

Hemoglobin and serum creatinine levels were similar across different age groups in both the control and ESKD groups. However, significant differences were found in the blood levels of Cd, Pb, Cu, and Zn between ESKD and control groups as shown in Table 2.

Table 2

ANOVA test of normal ranges and average concentrations of heavy metals in blood samples of patients with ESKD

Elements	Allowable blood level (μg/dL)	Healthy group (μg/dL)	ESKD group after dialysis (μg/dL)	p-value
Pb	≤ 5 [34]	1.06 ± 0.23	27.65 ± 6.95	0.006
Zn	70 to 180 [24]	82.45 ± 4.5	29.5 ± 2.3	0.035
Cd	0.05 to 0.2 [17]	0.0485 ± 0.0268	1.035 ± 0.39	0.008
Cu	70 to 140 [20,21]	81.8 ± 2.9	270.1 ± 5.05	0.004

Abbreviations: Cd, cadmium; Cu, copper; Pb, lead; Zn, zinc.

As you can see in Table 2, Pb, Cd, and Cu concentrations measured after dialysis were significantly higher in ESKD patients compared to healthy controls. In contrast, Zn concentrations were significantly lower in ESKD patients compared to healthy controls.

For further analysis, both groups were subdivided into age ranges: A (20-29), B (30-39), C (40-49), and D (50-60), as shown in Tables 3 and 4.

Table 3

Heavy metal concentrations in ESKD patients' blood samples (Mean ± SD)

Age Group	Pb (µg/dL)	Zn (µg/dL)	Cd (µg/dL)	Cu (µg/dL)
A (20-29)	13.8 ± 8.0	26.2 ± 2.3	1.02 ± 0.36	251.4 ± 5.5
B (30-39)	28.3 ± 6.1	27.9 ± 2.4	0.93 ± 0.41	267.2 ± 4.8
C (40-49)	33.67 ± 6.6	29.8 ± 2.2	1.06 ± 0.37	279.7 ± 5.2
D (50-60)	34.82 ± 7.1	34.1 ± 3.1	1.13 ± 0.42	286.1 ± 4.7
p-value	0.01	0.037	0.043	0.01

Abbreviations: Cd, cadmium; Cu, copper; Pb, lead; Zn, zinc.
p-value of <0.05 is considered significant.

Table 4

Heavy metal concentrations in healthy control group blood samples (Mean ± SD)

Age Group	Pb (µg/dL)	Zn (µg/dL)	Cd (µg/dL)	Cu (µg/dL)
A (20-29)	0.8 ± 0.2	78.7 ± 4.2	0.032 ± 0.01	78.1 ± 2.3
B (30-39)	0.94 ± 0.23	92.3 ± 5.1	0.046 ± 0.024	79.3 ± 2.4
C (40-49)	1.1 ± 0.15	81.6 ± 4.7	0.057 ± 0.031	83.6 ± 3.2
D (50-60)	1.4 ± 0.32	77.2 ± 4.1	0.059 ± 0.042	86.2 ± 3.8
p-value	0.021	0.042	0.033	0.023

Abbreviations: Cd, cadmium; Cu, copper; Pb, lead; Zn, zinc.
p-value of <0.05 is considered significant.

Discussion. According to the Centers for Disease Control and Prevention (CDC) [28], the average Pb level in the blood of 5 µg/dL or 0.24 µmol/L or above is considered elevated in adults. We observed a significantly higher ($p = 0.006$) concentration of Pb in patients with ESKD compared to the healthy control group and reference (allowable) blood levels. Our findings align with previous studies [29, 30], which reported that patients with kidney failure who smoke had significantly higher levels of Pb in their blood samples compared to non-smoker healthy controls. This is consistent with the study by Kim et al. [5], which demonstrated that chronic exposure to low levels of environmental heavy metals, such as Pb, slowly accumulates in the body and causes toxicological renal effects. Additionally, Humudat et. Al. found elevated Pb concentrations in dialysis fluid [31].

According to Arora et al., the allowable blood Zn concentration ranges from 70 to 180 µg/dL [20]. We observed a significantly low ($p = 0.035$) concentration of Zn in patients with ESKD compared to the healthy control group and reference (allowable) blood levels. These findings were consistent with previous reports [3, 32, 33] which showed Zn deficiency in patients undergoing HD due to its removal during hemodialysis However, the results published by Ogabiela et al. were not consistent with our findings, as they indicated significant levels of whole blood Zn in the studied population in Dareta Village, Nigeria [34].

One probable reason for the low Zn levels in blood samples of ESKD patients is that exposure to Pb and Cd via environmental pollution can replace Ca and Zn, respectively, by competing for binding sites in biological systems. It is also known that these elements and their compounds can be toxic if their concentrations exceed certain limits [35, 36]. Arredondo et al. mentioned that alterations in Zn and Cu metabolism have been noticed in patients with ESKD [37]. Orr and Bridges showed that in previous studies, low doses of Zn supplement have been shown to reduce renal toxicity induced by Cd [32].

According to the CDC, in adults, the allowable Cd level in the blood is 0.05-0.2 µg/dL, and levels above 5 µg/dL are considered elevated [28]. We observed a significantly higher ($p = 0.008$) concentration of Cd in patients with ESKD compared to the healthy control group and reference (allowable) blood levels. Our findings align with a 2015 study [5] demonstrating that chronic exposure to low levels of environmental heavy metals like Cd gradually accumulates in the body, causing toxic renal effects. Additionally, our results are consistent with a 2008 study [29] that reported significantly higher Cd levels in the blood samples of patients with kidney failure compared to non-smoker controls.

According to LaGow et al. and Heitland et al., the allowable blood copper Cu level concentration in males is 70 to 140 µg/dL [17, 18]. We observed a significantly higher ($p = 0.004$) concentration of Cd in whole blood compared to the healthy control group and reference blood levels. Our findings were consistent with a study conducted by Niu et. al. [38], which showed that the levels of intracellular Cu were significantly elevated in the case of kidney fibrosis. Moreover, our findings also match with the results shown by Ahmad et al. [36] who reported that raised blood copper levels have been associated with chronic kidney disease.

Limitations. First, the relatively small sample size of 80 ESKD patients and 80 healthy controls may limit the generalizability of the findings. Additionally, conducting the study at a single hospital in Amman may not represent other regions or populations. Second, the cross-sectional design captures data at one point in time and does not account for temporal variations in heavy metal levels or kidney function. Longitudinal studies are needed to explore the long-term effects of heavy metal exposure. Third, while some variables were controlled, other potential confounders, such as dietary habits, occupational exposure, and broader environmental pollution, were not comprehensively assessed. Fourth, the study measured heavy metal concentrations but did not assess sources or duration of exposure. Including detailed exposure assessments would provide a clearer understanding of heavy metal sources. Fifth, variations in hemodialysis protocols, including methods and frequency, were not considered, which could affect heavy metal levels. Sixth, the study focused on specific age ranges (20-29, 30-39, 40-49, 50-60 years), potentially limiting the applicability of results to other age groups. Lastly, the study only analyzed four heavy metals (Cd, Pb, Cu, Zn). Including other metals or toxins might provide a more comprehensive view of the heavy metal burden in ESKD patients.

Conclusions. Our study reveals that ESKD patients undergoing HD have significantly higher blood concentrations of Pb, Cd, and Cu compared to healthy controls, while Zn levels are significantly lower. Age-dependent variations show that Pb, Cu, Cd, and Zn levels increase significantly with age in ESKD patients, suggesting age-related differences in metal accumulation and metabolism. Regular monitoring of heavy metal concentrations in patients with ESKD is recommended to manage and mitigate potential toxic effects. Future research should explore the mechanisms of heavy metal accumulation and investigate potential therapeutic strategies (e.g. Zn supplementations) to counteract toxic effects. Additionally, identifying the sources of heavy metal exposure could inform public health initiatives aimed at reducing environmental contamination.

Conflict of Interest. The authors declare that they have no conflicts of interest.

Funding Sources. This study project [DRGS-2020-2021-12] was funded by the Deanship of Scientific Research in Applied Science Private University, Amman, Jordan.

Data Availability. The data supporting the study findings is available from the corresponding author upon request.

The authors' contributions:

Faten M. Abu Orabi: Supplying financial resources, and writing methodology;

Mohammad Fawzi: Conceptualization, methodology, writing-original draft, writing, reviewing and editing, and approving the final version of the manuscript;

Jamal Humaidi: Formal analysis, equipment, and reagent preparation;

Mohammed-Jamal A. Shammout: The manuscript preparation, organizing, reviewing and editing the manuscript;

Rawand Saleh and Hedaya Hakawati: Data curation and formal analysis, software;

Ahmed Abu Rayyan: Idea for the research and article/hypothesis generation, methodology, supervision, resources, project administration,

Acknowledgment. The authors would like to thank the administration of the Applied Science Private University and the Deanship of Scientific Research for their assistance and support in completing this project.

References:

1. *Francis A, Harhay MN, Ong ACM, Tummalapalli SL, Ortiz A, et al;* American Society of Nephrology; European Renal Association; International Society of Nephrology. Chronic kidney disease and the global public health agenda: an international consensus. Nat Rev Nephrol. 2024;20(7):473-485. doi: 10.1038/s41581-024-00820-6.
2. *Kolesnyk M, Stepanova N, Dudar I, Bagdasarova I, Fomina S.* Nephrology in Ukraine. In: Moura-Neto, J.A., Divino-Filho, J.C., Ronco, C. (eds) Nephrology Worldwide. Springer, Cham; 2021. p. 675–685. doi: 10.1007/978-3-030-56890-0_50.
3. *Wakino S.* Trace Elements and Their Management in Dialysis Patients—Pathophysiology and Clinical Manifestations. Kidney and Dialysis. 2023; 3(3):274-296. doi: 10.3390/kidneydial3030025.
4. *Pizzorno J.* The Kidney Dysfunction Epidemic, Part 1: Causes. Integr Med (Encinitas). 2015;14(6):8-13.
5. *Kim NH, Hyun YY, Lee KB, Chang Y, Rhu S, Oh KH,et. al.* Environmental heavy metal exposure and chronic kidney disease in the general population. J Korean Med Sci. 2015;30(3):272-277. doi:10.3346/jkms.2015.30.3.272.
6. *Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN.* Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol. 2014;7(2):60-72. doi: 10.2478/intox-2014-0009.
7. *Tsai HJ, Wu PY, Huang JC, Chen SC.* Environmental pollution and chronic kidney disease. Int J Med Sci. 2021;18(5):1121-1129. doi: 10.7150/ijms.51594.
8. *Crommentuijn T, Polder MD, van de Plassche EJ.* Maximum permissible concentrations and negligible concentrations for metals, taking background concentrations into account. RIVM Report 601501 001. National Institute of Public Health and the Environment. [Internet].1997, Bilthoven,

The Netherlands. Available from: https://www.rivm.nl/bibliotheek/rapporten/601501001.html.

9. *Voice TC, McElmurry SP, Long DT, Dimitrov P, Ganev VS, Peptropoulos EA.* Evaluation of the hypothesis that Balkan endemic nephropathy is caused by drinking water exposure to contaminants leaching from Pliocene coal deposits. J Expo Sci Environ Epidemiol. 2006;16(6):515-524. doi:10.1038/sj.jes.7500489.
10. *Choudhury TR, Zaman SZ, Chowdhury TI, Begum BA, Islam MA, Rahman MM.* Status of metals in serum and urine samples of chronic kidney disease patients in a rural area of Bangladesh: An observational study. Heliyon. 2021;7(11):e08382. doi: 10.1016/j.heliyon.2021.e08382.
11. *Fatima G, Raza AM, Hadi N, Nigam N, Mahdi AA.* Cadmium in Human Diseases: It's More than Just a Mere Metal. Indian J Clin Biochem. 2019;34(4):371-378. doi: 10.1007/s12291-019-00839-8.
12. ATSDR. Agency for Toxic Substances and Disease Registry. Draft toxicological profile for cadmium. Atlanta, GA: US Department of Health and Human Services, Public Health Service. [Internet].2012. Available from: https://www.atsdr.cdc.gov/ToxProfiles/tp5-p.pdf.
13. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) on Cadmium. National Center for Environmental Assessment, Office of Research and Development, Washington, DC. [Internet]. 1999. Available from: https://www.epa.gov/sites/default/files/2016-09/documents/cadmium-compounds.pdf.
14. WHO, Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda. [Internet]. 2022 World Health Organization. Geneva. Available from: https://pubmed.ncbi.nlm.nih.gov/35417116/.
15. *Moreau T, Orssaud G, Lellouch J, Claude JR, Juguet B, Festy B.* Blood cadmium levels in a general male population with special reference to smoking. Arch Environ Health. 1983;38(3):163-7. doi: 10.1080/00039896.1983.10543998.
16. *Tapiero H, Townsend DM, Tew KD.* Trace elements in human physiology and pathology. Copper. Biomed Pharmacother. 2003;57(9):386-98. doi: 10.1016/s0753-3322(03)00012-x.
17. *LaGow B, et al.* PDR Lab Advisor. A Comprehensive Point-of-Care Guide for Over 600 Lab Tests. First ed. Montvale, NJ: Thomson PDR. [Internet].2007:675. Available from: https://books.google.com.ua/books/about/PDR_Lab_Advisor.html?id=YEFDNAAACAAJ&redir_esc=y.
18. *Heitland P, Köster HD.* Biomonitoring of 37 trace elements in blood samples from inhabitants of northern Germany by ICP-MS. J Trace Elem Med Biol. 2006;20(4):253-62. doi: 10.1016/j.jtemb.2006.08.001.
19. *Paun S, Tudosie M, Petris R, Macovei R.* The effects of Zinc on human body, including on renal failure and renal transplantation. J Med Life. [Internet]. 2012;5(Spec Issue):137-140. Available from: https://pubmed.ncbi.nlm.nih.gov/31803301/.
20. *Arora PN, Dhillon KS, Rajan SR, Sayal SK, Das AL.* Serum Zinc Levels in Cutaneous Disorders. Med J Armed Forces India. 2002;58(4):304-6. doi: 10.1016/S0377-1237(02)80083-1.
21. *Jaber S.M.* Landsat-based vegetation abundance and surface temperature for surface urban heat island studies: the tale of Greater Amman Municipality. Annals of GIS.2018; 24(3): 195-208. doi: 10.1080/19475683.2018.1471519.
22. *Li W, Li X, Su J, Chen H, Zhao P, Qian H, et. al.* Associations of blood metals with liver function: Analysis of NHANES from 2011 to 2018. Chemosphere. 2023; 317:137854. doi: 10.1016/j.chemosphere.2023.137854.
23. *Batool M, Ahmad K, Zahidqureshi, Mahboob N, Nimra.* Determination of heavy metal toxicity in blood and health effect by AAS (detection of heavy metals and its toxicity in human blood. Arch. nanomed. 2018;1(2), 22-8, doi: 10.32474/ANOAJ.2018.01.000107.
24. *Sun H-F, Li Y-H, Ji Y-F, Yang L-S, Wang W-Y, Li H-R.* Environmental contamination and health hazard of lead and cadmium around Chatian mercury mining deposit in western Hunan Province, China. Trans. Nonferrous Metals Soc. China. 2010;20(2):308-314. doi: 10.1016/S1003-6326(09)60139-4.
25. *Ashish B, Neeti K, Himanshu K.* Copper Toxicity: A Comprehensive Study. Res J Recent Sci.2013;2(ISC-2012):58-67.
26. *Xiaoyu Li, Lijuan Liu, Yugang Wang, Geping Luo, Xi Chen, Xiaoliang Yang, et. al.* Heavy metal contamination of urban soil in an old industrial city (Shenyang) in Northeast China. Geoderma.2013;192: 50-58. doi: 10.1016/j.geoderma.2012.08.011.
27. *Morais S, e Costa FG, de Lourdes Pereir M.* Heavy Metals and Human Health. Environmental Health - Emerging Issues and Practice. InTech. 2012: 227-246. doi: 10.5772/29869.
28. The Center for Disease Control and Prevention (CDC). National Institute of Occupational Safety and Health (NIOSH). Adult Blood Lead Epidemiology and Surveillance (ABLES). [Internet]. 2022. Available from: https://www.cdc.gov/niosh/lead/programs/index.html.
29. *Kazi TG, Jalbani N, Kazi N, Jamali MK, Arain MB, Afridi HI, et. al.* Evaluation of Toxic Metals in Blood and Urine Samples of Chronic Renal Failure Patients, before and after Dialysis. Ren Fail. 2008; 30(7):737-745, doi: 10.1080/08860220802212999.

30. *Makarov OO, Pisarev EO, Sheiman BS, Kulizkiy MV, Peretyatko DV, Vihor VO, et al.* Changes of Nonessential Trace Elements Levels in Whole Blood Of End Stage Renal Disease Adult Patients. Ukr J Nephrol Dialys. 2013;2(38):114-125. doi: 10.31450/ukrjnd.2(38).2013.09.
31. *Humudat YR, Al-Naseri SK.* Heavy Metals in Dialysis Fluid and Blood Samples from Hemodialysis Patients in Dialysis Centers in Baghdad, Iraq. J Health Pollut. 2019; 10(27):200901. doi: 10.5696/2156-9614-10.27.200901.
32. *Orr SE, Bridges CC.* Chronic Kidney Disease and Exposure to Nephrotoxic Metals. Int J Mol Sci. 2017;18(5):1039. doi: 10.3390/ijms18051039.
33. *Damianaki K, Lourenco JM, Braconnier P, Ghobril JP, Devuyst O, Burnier M, et al.* Handling of zinc in chronic kidney disease patients and the role of circulating zinc levels in renal function decline. Nephrol Dial Transplant. 2020;35(7):1163-1170. doi: 10.1093/ndt/gfz065.
34. *Ogabiela EE, Okonkwo EM, Agbaji AS, Udiba UU, Hammuel C, Ade-Ajayi AF, et. al.* Trace Metal Level of Human Blood from Dareta Village, Anka, Nigeria. Global Journal of Pure and Applied Sciences. [Internet]. 2011;17(2): 183-188. Available from: https://www.ajol.info/index.php/gjpas/article/view/78786.
35. *Mueller PW, Price RG, Finn WF.* New approaches for detecting thresholds of human nephrotoxicity using cadmium as an example. Environ Health Perspect. 1998;106(5):227-30. doi: 10.1289/ehp.98106227.
36. *Ahmad S, Ärnlöv J, Larsson SC.* Genetically predicted circulating copper and risk of chronic kidney disease: A mendelian randomization study. Nutrients. 2022;14(3):509. doi: 10.3390/nu14030509.
37. *Arredondo M, Martínez R, Núñez MT, Ruz M, Olivares M.* Inhibition of iron and copper uptake by iron, copper and zinc. Biol Res. 2006;39(1):95-102. doi: 10.4067/s0716-97602006000100011.
38. *Niu YY, Zhang YY, Zhu Z, Zhang XQ, Liu X, Zhu SY, et. al.* Elevated intracellular copper contributes a unique role to kidney fibrosis by lysyl oxidase mediated matrix crosslinking. Cell Death Dis. 2020 Mar 31;11(3):211. doi: 10.1038/s41419-020-2404-5