Appraisal of Trace Elements in Scalp Hair of School Children in Kandal Province, Cambodia

A. Yavar, S. Sarmani, K. S. Khoo

Abstract—The analysis of trace elements in human hair provides crucial insights into an individual's nutritional status and environmental exposure. This research aimed to examine the levels of toxic and essential elements in the scalp hair of school children aged 12-17 from three villages (Anglong Romiot (AR), Svay Romiot (SR), and Kampong Kong (KK)) in Cambodia's Kandal province, a region where residents are especially vulnerable to toxic elements, notably arsenic (As), due to their dietary habits, lifestyle, and environmental conditions. The scalp hair samples were analyzed using the k0-Instrumental Neutron Activation method (k0-INAA), with a six-hour irradiation period in the Malaysian Nuclear Agency (MNA) research reactor followed by High Purity Germanium (HPGe) detector use to identify the gamma peaks of radionuclides. The analysis identified 31 elements in the human hair from the study area, including As, Au, Br, Ca, Ce, Co, Dy, Eu-152m, Hg-197, Hg-203, Ho, Ir, K, La, Lu, Mn, Na, Pa, Pt-195m, Pt-197, Sb, Sc-46, Sc-47, Sm, Sn-117m, W-181, W-187, Yb-169, Yb-175, Zn, and Zn-69m. The accuracy of the method was verified through the analysis of ERM-DB001-human hair as a Certified Reference Material (CRM), with the results demonstrating consistency with the certified values. Given the prevalent arsenic pollution in the research area, the study also examined the relationship between the concentration of As and other elements using Pearson's correlation test. The outcomes offer a comprehensive resource for future investigations into toxic and essential element presence in the region. In the main body of the paper, a more extensive discussion on the implications of arsenic pollution and the correlations observed is provided to enhance understanding and inform future research directions.

Keywords—Human scalp hair, toxic and essential elements, Kandal Province, Cambodia, k0-Instrumental Neutron Activation Method.

I. Introduction

HAIR serves as an efficient biomonitor for the distribution of essential and toxic elements due to several factors: (1) its collection is non-invasive; (2) it is stable and robust, with a composition that remains unchanged over time; (3) it requires no special storage or handling; (4) the sampling process is painless, straightforward, and does not require specialized skills, nor does it pose a risk of transmitting contagious diseases; (5) it uniquely captures the total body intake over an extended period, enabling a chronological mapping of changes based on hair length. Therefore, quantifying trace elements in hair is an effective strategy to assess long-term environmental

and occupational exposure to essential and toxic elements, as well as metabolic status regarding chronic nutritional deficiencies or diseases [1], [2].

Metal ions significantly influence numerous biochemical processes in the human body. Deficiencies or excesses of certain elements can affect health and potentially contribute to various diseases. These metallic elements can be divided into two primary groups: essential elements and toxic elements. The elements Al, B, Ca, Cl, Cr, Co, Cu, Ge, H, I, Fe, Mg, Mn, Mo, N, O, P, K, Se, Na, S, and Zn are deemed essential for optimal physiological functioning. However, excessive levels of Al, Co, Mn, Mo, and Se can be harmful. The elements Ag, Am, As, Ba, Be, Cd, Hg, Os, Pb, Po, Ra, Sb, Sr, Th, Tl, U, and V are recognized as toxic elements, with As, Cd, Hg, and Pb often classified as major toxic elements in biological samples [3], [4].

Metallic compounds, particularly heavy metals, are released into the environment through natural and anthropogenic pathways. These include the natural weathering of the Earth's crust, industrial discharge, mining operations, sewage effluents, soil erosion, and urban air pollution. Dietary habits, lifestyle, and environmental conditions influence trace element uptake levels in the human body. For most populations, the primary route of exposure to metallic elements is through diet (food and drinking water). Some individuals experience occupational exposure to these contaminants, and for urban dwellers, air pollution can be a more significant source than others. The contamination chain of heavy metals typically follows a cyclic order: industry, atmosphere, soil, water, food, and humans [5]-[7].

Elevated arsenic levels in groundwater have been identified as a major problem in at least ten provinces of Cambodia, with Kandal being one of the most severely affected. Estimates, based on groundwater quality and population data for Kandal Province, suggest that over 100,000 people are at high risk of chronic As exposure [9]. These areas are typically associated with low-lying regions along the Mekong and Bassac Rivers. Several studies have explored the chemical, biological, and physical processes contributing to the spatial distribution and heterogeneity of As in Cambodian groundwater [8]-[12]. Despite this, there is a dearth of information on baseline element concentrations in the study region. Therefore, the present work aims to ascertain the concentrations of toxic and

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essential elements in human hair from three villages in Kandal province, namely Anglong Romiot (AR), Svay Romiot (SR), and Kampong Kong (KK). In addition, correlations between As levels and other elements in human hair samples from our study region will be determined using Pearson's correlation test. A one-way ANOVA test will be performed to gather information about the content of elements in human hair in relation to age and gender within the study region.

II.EXPERIMENTAL PROCEDURE

A. Sample Preparation and k₀-INAA of Samples

Human hair samples were collected from healthy school children aged between 12 and 17 years (with a hair length of 2 to 3 cm) in three villages of Kampong Kong (KK), Svay Romiot (SR), and Anglong Romiot (AR) in Kandal province, Cambodia. In each village, 10 hair samples were obtained using stainless steel scissors and cut to a length of 2 mm before being stored at room temperature in plastic bags.

For an accurate assessment of heavy metal content, hair samples were first rinsed with ultra-pure water, followed by three washes with acetone, and again rinsed with ultra-pure water three times. The samples were then oven-dried at 90 °C for 2 hours. Hair samples, weighing between 70 and 90 mg, were subsequently packed into vials of 1 cm diameter and length. Gold (Au) and zirconium (Zr) monitors were utilized to determine the k0-INAA parameters. The monitors consisted of Al-0.1%Au alloy wire (IRMM-527a, diameter 1 mm, length 10 mm) and Zr foils (IRMM, 99.9%, 125 µm thick), respectively. These monitors were cut and carefully weighed; the Au monitors ranged from 17.8 to 26.0 mg and the Zr monitors ranged from 12.5 to 27.5 mg. The monitors were heat sealed inside polyethylene vials and packed in heat-resistant plastic, with each vial containing one Au monitor and one Zr monitor.

To evaluate the results, three ERM-DB001-human hair CRMs were prepared. The CRMs were dehumidified in an oven at 90 °C for 2 hours, then carefully weighed (in the range of 100 to 110 mg) and heat sealed into separate vials. Four vials, each containing one monitor vial, one hair sample vial, one CRM vial, and one blank vial (to offset background radiation) were packed together in two layers of heat-resistant plastic. This package was prepared for irradiation at three RR channels, while 22 other RR channels were used to irradiate similar packs without CRM vials. All packs were irradiated for 6 hours in 30 RR irradiation channels of the MNA research reactor.

To detect radionuclides of 198Au and 97Zr/97mNb, both monitors were counted for about 5 minutes after a one-day decay period, while irradiated zirconium was counted for the measurement of 95Zr after 3 days of cooling, with a 15-minute counting period. The hair samples and CRMs were counted after 1 day, 7 days, and 21 days cooling periods to determine the short, medium, and long half-life radionuclides, at 15.8 cm, 7.8 cm, and 3.8 cm distances in the HPGe detector, respectively [13], [14]. The Høgdahl convention based on the k₀-INAA method was used to determine elemental concentrations in hair samples and CRMs.

B. Result Assessment

The analysis process and precision of measurements were evaluated using the CRM ERM-DB001-human hair (European Reference Materials – Belgium). Additionally, the accuracy of the analytical measurements was assessed using the z-score, as:

$$z = \frac{\left| C_i - C_{ref,i} \right|}{\sqrt{\sigma_i^2 + \sigma_{ref,i}^2}} \tag{1}$$

where C_i is the concentration of element i in the sample; $C_{ref,i}$ is the concentration of the certified value for element i; σ_i is uncertainty of the concentration of element i in the sample; $\sigma_{ref,i}$ is uncertainty of the certified respective consensus value for element i. When using z-score, if z < 2, the result is classified as anticipated; if 2 < z < 3, the quality of measurement is "alarming"; and z > 3, the results are "out of control" [14].

C. Statistical Analysis

Data was subjected to statistical analysis using IBM SPSS software, version 22. The normality test was evaluated using Skewness and Kurtosis z-values (results should fall within the range of -1.96 to +1.96) and the Shapiro-Wilk test (results should exceed a p-value of 0.05). One-way ANOVA, with the significance level set at 95%, was employed to investigate potential statistical differences in elemental concentrations between different genders and age groups. Additionally, possible correlations between Arsenic and other metal elements' distribution in the hair samples within each population group were assessed using Pearson's correlation analysis [15].

III.RESULTS AND DISCUSSION

The results of the study, including mean, standard deviation, minimum and maximum values, and p-value of the one-way ANOVA test for 31 essential and toxic elements in the scalp hair of participants from the three villages of AR, SR, and KK in the Kandal province of Cambodia are summarized in Table I. Among the essential elements, the highest concentrations in the hair samples of the KK village were observed for Ca (13935 ± 1545 mg/kg), and K (89.69 ± 29.46 mg/kg), whereas Mn (101 \pm 99 mg/kg), and Na (230 \pm 27 mg/kg) were highest in the AR village, and Zn $(388 \pm 189 \text{ mg/kg})$ was highest in the SR village. Additionally, Co $(0.74 \pm 0.43 \text{ mg/kg})$ was only detected in the SR village. Among the toxic elements, As (2.81 ± 1.36) , Hg (4.26 ± 2.90) , and Sb (0.14 ± 0.03) presented the highest levels in the KK, SR, and AR villages, respectively (Table I). A oneway ANOVA test revealed statistically significant differences in elemental concentrations between the three villages (p < 0.05) (Table I).

For optimal body functioning, maintaining both an appropriate level of bio-elements and a balanced ratio among these elements is crucial. Therefore, the relationship between different elements is of great importance. Increasing levels of toxic elements in the environment can affect the balance of

other elements [2], [3], [16]. Given the importance of arsenic in Cambodia, studying the correlation between As and other elements in the study area is beneficial. Figs. 1 and 2 display the correlation between As concentration and other elemental concentrations in the hair samples from the KK and SR villages, respectively (As was not detected in the AR village), using

Pearson's correlation test. Strong statistical correlations were found between As concentration and Br, Ce, Dy, Hg-197, Hg-203, Ho, Ir, Lu, Mn, Pa, Pt-197, Sc-46, Sc-47, Sm, Yb-169, Yb-175, Zn, and Zn-69m in the KK village (R > 0.3). However, only weak correlations were found for Br, K, La, and Sc in the SR village (R < 0.3).

TABLE I

COMPARISON OF ELEMENTAL CONCENTRATIONS IN SCALP HAIR SAMPLES OF CHILDREN AGED 12 TO 17 FROM THREE VILLAGES IN KANDAL PROVINCE OF

CAMBODIA USING ONE-WAY ANOVA TEST

Villages						O ANOVA				
Radionuclide	AR			SR			KK			One-way ANOVA (p-value)
	Average	Min	Max	Average	Min	Max	Average	Min	Max	(p-value)
As-76	-	-	-	1.88 ± 0.88	1.00	2.76	2.81 ± 1.36	1.69	4.32	0.378
Au-198	0.03 ± 0.01	0.02	0.05	0.41 ± 0.79	0.02	1.82	0.31 ± 0.42	0.02	1.05	0.310
Br-82	3.37 ± 1.03	2.21	4.93	2.48 ± 0.75	0.90	3.33	3.02 ± 0.97	2.11	5.61	0.122
Ca-47	12983 ± 3201	10170	16467	-	-	-	13935 ± 1545	12390	15481	0.667
Ce-141	11.99 ± 10.56	2.06	24.66	3.88 ± 2.00	1.88	5.88	3.31 ± 2.97	1.56	6.74	0.260
Co-60	-	-	-	0.74 ± 0.43	0.42	1.22	-	-	-	-
Dy-159	-	-	-	-	-	-	4186 ± 2657	1529	6844	-
Eu-152m	0.03 ± 0.01	0.02	0.03	0.03 ± 0.01	0.01	0.04	0.03 ± 0.01	0.02	0.04	0.825
Hg-197	2.85 ± 1.62	0.51	5.59	-	-	-	4.52 ± 1.45	2.79	7.40	0.065
Hg-203	3.52 ± 2.45	1.90	8.93	4.26 ± 2.90	1.96	11.00	3.37 ± 1.20	1.40	5.19	0.715
Ho-166	0.72 ± 0.05	0.67	0.77	-	-	-	1.86 ± 1.21	0.80	3.53	0.175
Ir-194	0.92 ± 0.90	0.20	2.08	0.84 ± 1.06	0.28	2.43	0.29 ± 0.19	0.14	0.50	0.611
K-42	82.94 ± 32.80	51.51	141	76.00 ± 20.35	48.47	102	89.69 ± 29.46	61.11	151	0.616
La-140	1.58 ± 2.11	0.58	6.36	1.42 ± 0.51	0.98	2.28	1.03 ± 0.45	0.59	2.11	0.625
Lu-177	0.39 ± 0.38	0.15	0.83	2.08 ± 1.26	0.81	3.33	2.72 ± 2.20	0.52	4.92	0.219
Mn-56	101 ± 99	13.17	254	61.39 ± 31.39	39.79	97.40	46.99 ± 1.78	45.21	48.76	0.566
Na-24	230 ± 27	186	276	195 ± 42	103	262	223 ± 36	181	290	0.099
Pa-233	2.35 ± 2.27	0.43	5.31	1.68 ± 1.59	0.21	3.46	1.03 ± 1.17	0.19	2.80	0.545
Pt-195m	-	-	-	-	-	-	754 ± 680	74	1434	-
Pt-197	3265 ± 2859	418	9636	-	-	-	3843 ± 1309	2307	6442	0.632
Sb-122	0.14 ± 0.03	0.11	0.17	-	-	-	-	-	-	-
Sc-46	-	-	-	0.06 ± 0.02	0.05	0.08	0.06 ± 0.02	0.04	0.08	0.842
Sc-47	21241 ± 7769	12814	36391	-	-	-	25907 ± 13468	12870	50169	0.388
Sm-153	0.82 ± 0.30	0.49	1.25				0.95 ± 0.75	0.36	2.55	0.707
Sn-117m	-	-	-	283 ± 21	262	305	-	-	-	-
W-181	-	-	-	116155 ± 8549	107606	124704	-	-	-	-
W-187	0.64 ± 0.03	0.61	0.67	-	-	-	-	-	-	-
Yb-169	-	-	-	-	-	-	6.00 ± 5.10	0.90	11.10	-
Yb-175	1.61 ± 1.55	0.06	3.16	4.07 ± 3.49	0.58	7.55	8.52 ± 6.86	1.66	15.38	0.245
Zn-65	346 ± 162	164	703	388 ± 189	145.60	709	358 ± 235	128	866	0.893
Zn-69m	425 ± 192	159	861	502 ± 227	209	875	444 ± 280	81	1018	0.764

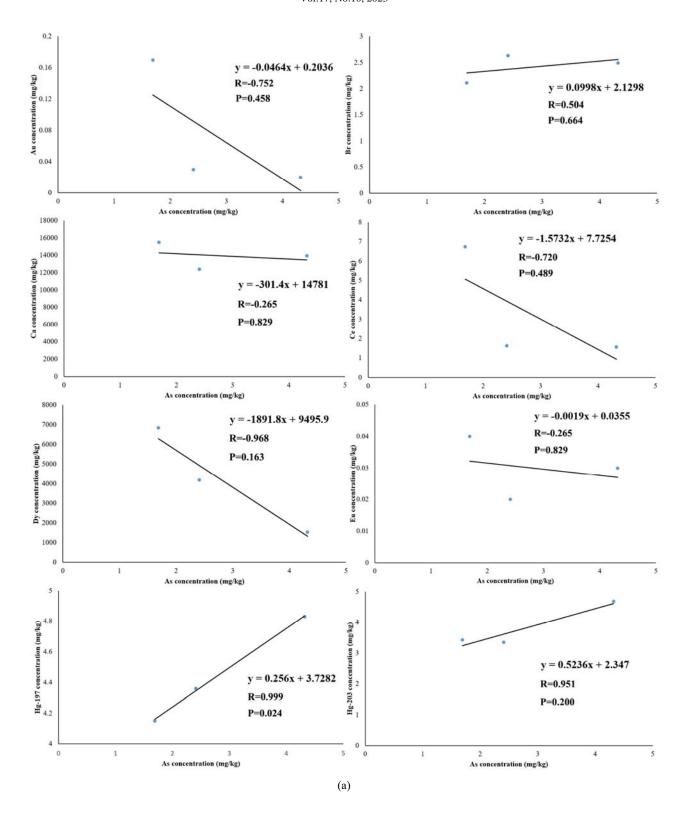
Min: minimum; Max: maximum

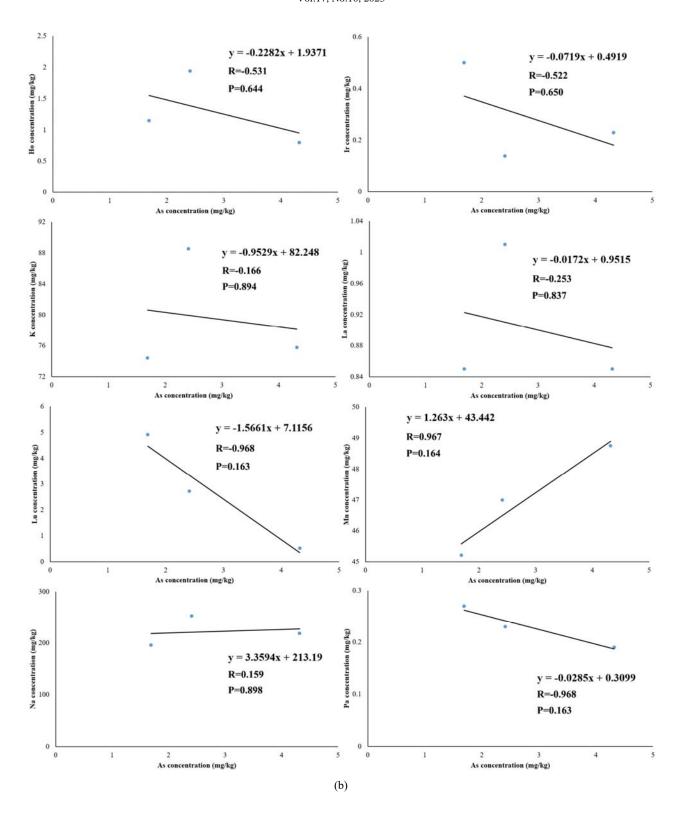
The gender and age-based disparities, and the associated statistical data are presented in Table II. A one-way ANOVA test was conducted to evaluate statistical differences in elemental concentrations between males and females as well as different age groups. The p-value of the ANOVA test was below 0.05 for Au, Zn, and Zn-69m in gender-based comparisons, suggesting statistically significant differences. The highest levels of toxic elements were observed in the scalp hair of 17-year-old participants (Table II). Statistically significant differences were found between all participant age groups except Sc-46, Sm, and W-181 (with p-values of 0.00, 0.01, and 0.00, respectively).

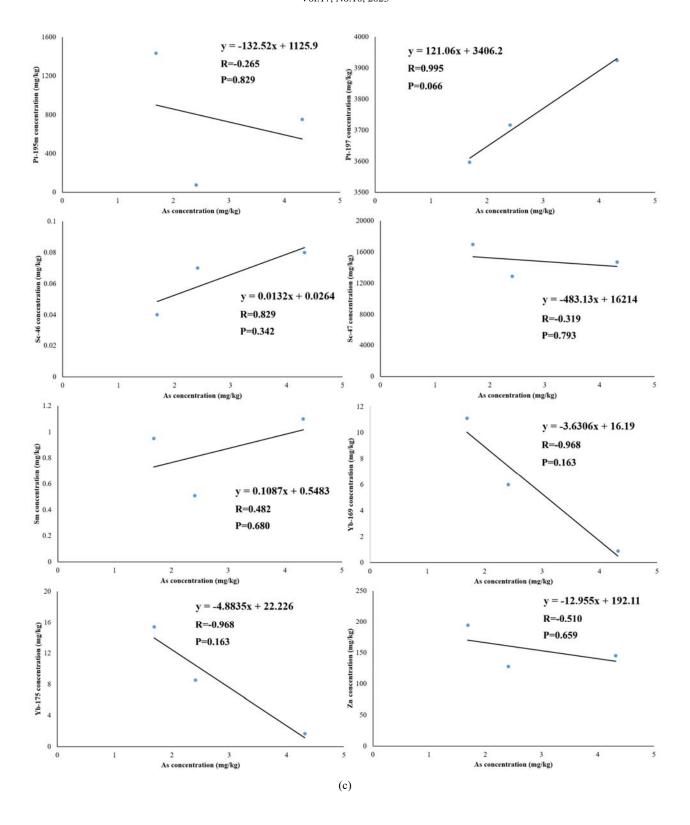
Table III presents the average concentrations of Cu, Se, and

Zn in CRM ERM-DB001-human hair, along with z-score measurements that validate our results. The z-scores of this study were below 2, indicating satisfactory results. This suggests a good agreement between our measured values and the certified ones.

Given that the elemental concentrations in hair samples can vary significantly with geographical location, nutritional status, and environmental conditions [3], [4], [16], the data obtained in this study are of sufficient quality to be used as reference values for toxic and essential elements in the hair of teenage individuals in our study area. The results, including As and Hg, are presented as a comprehensive database in this manuscript.







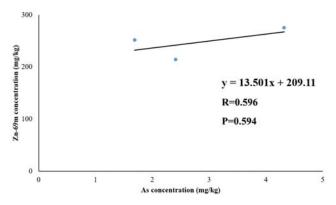
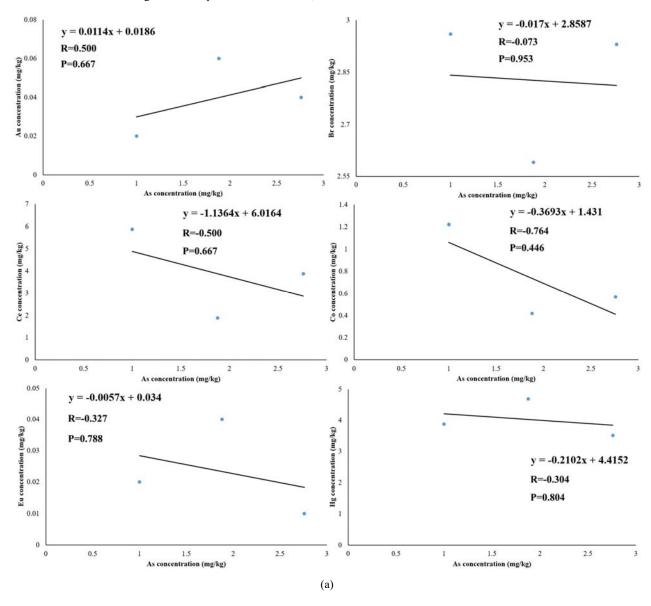
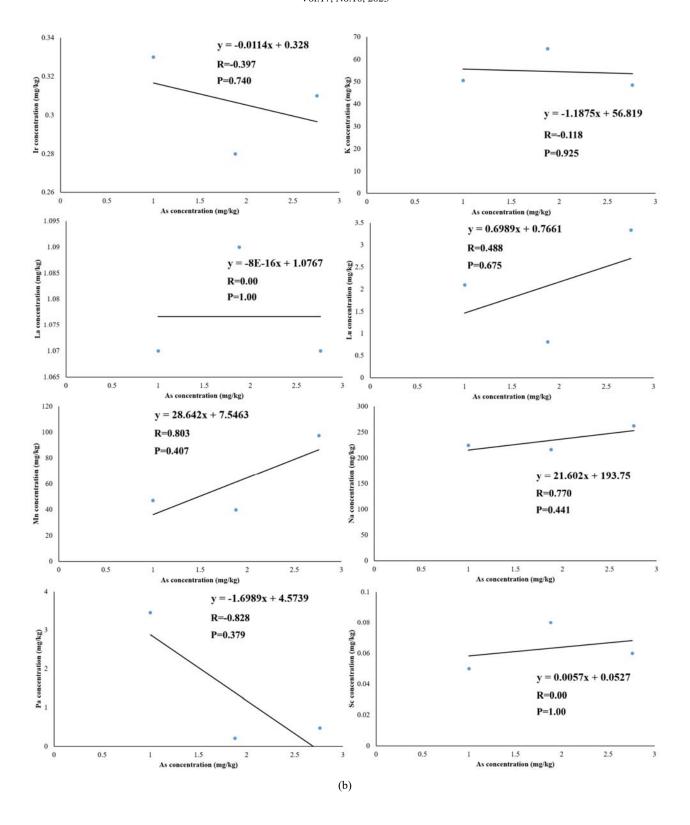


Fig. 1 Relationship between As concentration and Au, Br, Ca, Ce, Dy, Eu, Hg-197, Hg-203, Ho, Ir, K, La, Lu, Mn, Na, Pa, Pt-195m, Pt-197, Sc-46, Sc-47, Sm, Yb-169, Yb-175, Zn, and Zn-69m concentration scalp hair samples of 12-17 school children from Kampong Kong (KK) village in Kandal province of Cambodia; R and P are Pearson's correlation coefficients





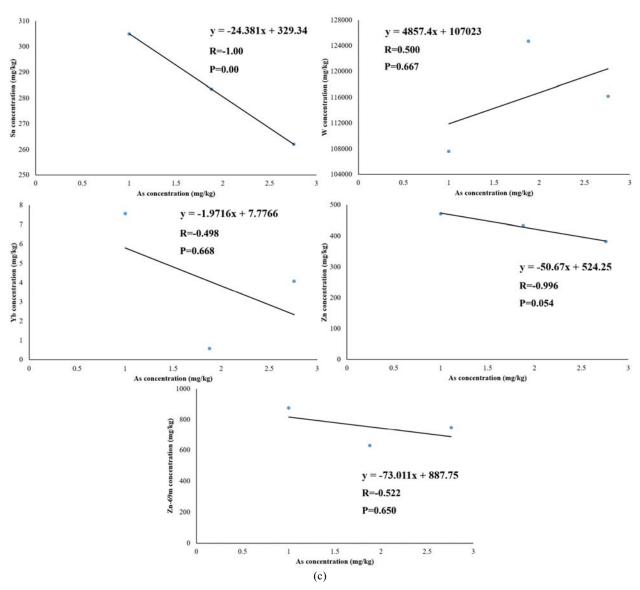


Fig. 2 Relationship between As concentration and Au, Br, Ce, Co, Eu, Hg, Ir, K, La, Lu, Mn, Na, Pa, Sc, Sn, W, Yb, Zn, and Zn-69m concentrations in scalp hair samples of 12-17 school children from Svay Romiot (SR) village in Kandal province of Cambodia; R and P are Pearson's correlation coefficients

IV.CONCLUSION

This study assessed the contents of 31 elements in the scalp hair of school children from three villages in the Kandal province of Cambodia. Among the essential elements, the highest levels of Ca and K were found in the hair samples from the KK village; Mn and Na were highest in the AR village; and Co and Zn were highest in the SR village. Additionally, among the toxic elements, the highest values of As (2.81 ± 1.36) , Hg (4.26 ± 2.90) , and Sb (0.14 ± 0.03) were found in the KK, SR, and AR villages, respectively. A comparison of elemental concentrations across all samples was performed using a one-way ANOVA test, revealing statistically significant differences (p < 0.05).

Given that arsenic is a particularly significant toxic element in Cambodia, correlations between As and other elements in the studied area were determined using Pearson's correlation test. Strong statistical correlations were found between As concentration and Br, Ce, Dy, Hg197, Hg203, Ho, Ir, Lu, Mn, Pa, Pt197, Sc46, Sc47, Sm, Yb169, Yb175, Zn, and Zn69m concentrations in the KK village (R > 0.3). In contrast, weak statistical correlations were identified only for Br, K, La, and Sc in the SR village (R < 0.3).

The gender and age differences in elemental distributions were also analyzed using a one-way ANOVA test, which revealed statistically significant differences for most elements. The accuracy of the method was verified by analyzing ERM-DB001-human hair as a CRM, with z-score results indicating satisfactory performance. Given that elemental concentrations in hair samples can vary significantly with geographical location, nutritional status, and environmental features, the results of this study could serve as a valuable reference for toxic and essential elements in the hair of teenagers in our studied area.

TABLE II
DISTRIBUTION OF ELEMENTAL CONCENTRATIONS IN HUMAN HAIR SAMPLES
OF THE STUDY POPULATION BY GENDER AND AGE

	Gender Age						
Element	M F			Max	-		
2101110111	(mg/kg)	г (mg/kg)	p- value	(mg/kg)	Age of max	p- value	
As	2.81	1.88	0.378	4.32	17	0.063	
Au	0.04	0.47	0.044	0.73	14	0.293	
Br	2.95	2.93	0.960	3.48	15	0.718	
Ca	13364	12540	0.705	13935	14	0.983	
Ce	7.74	3.75	0.542	9.35	17	0.907	
Co	0.92	0.74	0.745	1.22	14	0.398	
Dy	3579	6473	0.374	6473	14	0.854	
Eu152m	0.026	0.028	0.760	0.03	16	0.428	
Hg197	3.42	5.22	0.097	4.51	12	0.885	
Hg203	3.29	4.53	0.211	4.34	17	0.974	
Но	1.54	0.93	0.541	2.06	13	0.636	
Ir	0.77	0.22	0.55	1.18	16	0.651	
K	85.81	75.02	0.414	97.22	12	0.962	
La	1.43	1.09	0.497	2.26	14	0.406	
Lu	1.56	2.08	0.684	3.33	17	0.884	
Mn	68.25	102.77	0.432	159	13	0.382	
Na	224	197	0.082	243	17	0.562	
Pa	1.85	1.16	0.510	2.88	14	0.430	
Pt195m	687	2578	0.059	1532	14	0.630	
Pt197	3444	4388	0.480	5849	14	0.717	
Sc46	0.060	0.065	0.777	0.08	12	0.000	
Sc47	22790	26455	0.608	32727	17	0.654	
Sm	0.93	0.36	0.357	2.55	13	0.010	
Sn117m	273	305	0.334	305	15	0.334	
W181	124704	111880	0.333	124704	16	0.000	
W187	0.76	0.62	0.277	0.85	17	0.114	
Yb169	5.23	6.23	0.854	6.23	14	0.0979	
Yb175	3.78	8.03	0.313	8.48	13	0.695	
Zn	272.30	569.08	0.000	471	12	0.441	
Zn69m	347	696	0.000	556	12	0.547	

M: elemental concentration in male hair; F: elemental concentration in female hair; P-value: p-value of one-way ANOVA test; Max: maximum value of elemental concentration versus candidate's age; Age of Max: age of maximum value of elemental concentration.

 $TABLE\ III$ Comparison of Obtained Cu, Se, and Zn Concentrations by $\kappa_0\text{-}INAA$ with the Certified Value in ERM-DB001-Human Hair

-					
	Element	This work (mg/kg)	Certified value (mg/kg)	z-score	
	Cu	42.59 ± 4.67	33 ± 4	1.56	
	Se	3.50 ± 1.15	3.24 ± 0.24	0.22	
	Zn	202.53 ± 5.04	209 ± 12	0.50	

ACKNOWLEDGMENTS

This study received partial financial assistance through project funding from FRGS/1/2013/SG02/UKM/02/2 and DPP-2013-021.

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