

Study of Heavy Metal Levels in Nails and Hairs among Vegetable Farmers in Malaysia

N.Z.M. Saat, S.Y. Chow, A.R. Ghazali, Z.A. Hamid, S.H. Lubis, N. Mohamed, I. Ishak and H. Othman
Programme of Biomedical Science, School of Science Diagnostic and Applied Health,
Faculty of Health Science, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Abstract: The use of the pesticides and chemical fertilizers in agricultural sector releases plenty types of heavy metals to environment and brings adverse effects to human health. Arsenic (As), Cadmium (Cd), lead (Pb), Nickel (Ni) and Cobalt (Co) are toxic heavy metals to harm human health. Presence of keratin in hairs and nails allows the accumulation of metals elements in longer period. Thus, hairs and nails are suitable to be used as biological marker in the study of human exposure to heavy metal elements. The purpose of this study was to determine As, Cd, Pb, Ni and Co levels in hairs, fingernails and toenails of vegetable farmers. There were 90 Bangladesh farmers participated in this research by using universal sampling. Questionnaires have been carried out to collect the demographic data, working periods of the farmers and blood pressure. As, Cd, Pb, Ni and Co levels were analyzed by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Result showed that there was significant difference of As, Cd, Pb and Ni levels among hairs, fingernails and toenails, $p < 0.05$. The levels of As ($90.56 \pm 10.19 \mu\text{g L}^{-1}$) and Cd ($34.57 \pm 3.44 \mu\text{g L}^{-1}$) in toenails were found to be highest whereas the levels of Pb ($74.73 \pm 7.93 \mu\text{g L}^{-1}$) and Ni ($694.12 \pm 19.70 \mu\text{g L}^{-1}$) in fingernails were found to be highest with the mean level. ANOVA test showed that Co levels in toenails were significantly higher in farmers with hypertension ($22.51 \pm 3.61 \mu\text{g L}^{-1}$). Kruskal-Wallis test showed that As ($H = 10.64$, $p < 0.05$) and Cd ($H = 7.34$, $p < 0.05$) levels in toenails were significantly higher in farmers with hypertension. There was a significant weak negative relationship among As ($r = -0.44$, $p < 0.05$), Cd ($r = -0.43$, $p < 0.05$), Pb ($r = 0.32$, $p < 0.05$) and Ni ($r = -0.31$, $p < 0.05$) in fingernails together with Pb ($r = -0.29$, $p < 0.05$) levels in toenails with working periods of farmers shown by Spearman correlation test. Almost all farmers from different blood pressure groups shower after handling pesticides and fertilizers significantly ($p < 0.05$). The 66 famers have good knowledge from different working periods and there was a significant different between individual's knowledge and working periods ($p < 0.05$). As a conclusion, As, Cd, Pb, Ni, Co levels among vegetable farmers were lower than earlier studies and remained at a safety level.

Key words: Heavy metals, pesticides, fertilizers, toenails, fingernails, hairs

INTRODUCTION

Heavy metals are defined as metals or metalloid with density higher than 5 g/cm^3 or 5 times higher than water density (Hawkes, 1997). Mainly heavy metals exist naturally in the earth crust but those heavy metals can be released into environment through anthropogenic human activities such as mining operations, disposal of drainage and use of pesticides in agricultural sectors (Jing *et al.*, 2007). Heavy metals are persistent environmental contaminants since they cannot be degraded or destroyed (Duruibe *et al.*, 2007). Examples of heavy metals are lead (Pb), cadmium (Cd), zink (Zn), mercury (Hg), arsenic (As), chromium (Cr), copper (Co) and iron (Fe) (Duruibe *et al.*, 2007).

Some metals are extremely toxic to human (Duruibe *et al.*, 2007). These heavy metals are incorporated into the organism via different routes and can be stored in different tissues which lead to an internal bioconcentration that can induce different alterations and adverse effects. Human exposure to heavy metals occurs through food, air, industrial products and occupational exposure (Duruibe *et al.*, 2007). Heavy metals exposure occurs through three pathways (De Miguel *et al.*, 2007) such as oral ingestion, air ingestion and skin absorption due to dermal exposure (Luo *et al.*, 2012). The use of pesticides and chemical fertilizers in agricultural sectors will release the heavy metals causing accumulation of heavy metals in human body (Jing *et al.*, 2007).

Heavy metals such as arsenic, cadmium and lead has no importance to human body yet toxic in vice versa although in small quantities (Duruibe *et al.*, 2007). Besides, heavy metals such as nickel and cobalt are needed in small quantities for human body but they can cause serious effect if exposure at high level. Nickel compounds are known as carcinogen to human body (Denkhaus and Salnikow, 2002) whereas cobalt can cause negative effects to respiratory system, hematopoietic tissue and thyroid gland (De Boeck *et al.*, 2003). Toxic effect of heavy metals is related to the release of reactive oxygen species which will lead to oxidative stress in human body (Ercal *et al.*, 2001).

Accumulation of heavy metals in human body normally occurs in the tissue which rich in keratin such as hair and nail. This is due to the presence of disulfide covalent bond between the keratin in hair and nail samples and positive metal ions. Thus, nail and hair samples are significant biomarker to determine the heavy metals levels besides blood and urine (Mehra and Juneja, 2005). Besides, collection of hair and nail samples is non-invasive (Slotnick and Nriagu, 2006). According to, human exposure to heavy metal at low level is a condition to cause poisoning and diseases due to non-biodegradable properties of heavy metals whereas accidental exposure at high level can cause serious effect immediately.

In this study, the levels of arsenic, cadmium, lead, nickel and cobalt in nail and hair samples from farmers were determined and those levels were then correlated with demographic factors, blood pressure and working periods. Knowledge, awareness and practices of pesticide use among farmers were determined with working periods and blood pressure.

MATERIALS AND METHODS

Method of collection and processing of samples was referred to Bass *et al.* (2001), Were *et al.* (2008), Rodushkin and Axelsson (2000), Mortan *et al.* (2002) and Mehra and Juneja (2005).

Study group: Subjects chosen for this study were vegetables farmers who were chosen via universal sampling from the list of registered farmer. A cross sectional study was conducted and a total of 90 male Bangladesh farmers took part in this study. Inclusion criteria of subjects for this study consisted of farmers had been working >1 year and have the age between 18-80 years old.

Questionnaire: Questionnaires were used to collect the information from farmers such as demographic data and

factors that will influence the level of heavy metals. Each subject was interviewed for the each section in the questionnaire which included personal background, disease history, knowledge of pesticides and fertilizers use, practice of pesticides and fertilizers use, attitude of pesticides and fertilizers use, lung function and medical examination.

Sampling: Samples were collected from the farmers who used pesticides and fertilizers. Nails were cut from 10 fingernails and toenails using stainless steel scissors whereas approximately 10 hairs were removed from the scalp. Both nails and hairs samples were then sealed in the plastic bags at room temperature.

Sample analysis: Samples collected were washed to remove the particles on them. Hair samples were cut into smaller pieces in the length 1 cm and were weighed into 0.0025 g. Samples in test tube were soaked in 10 mL of 0.5% Triton X-100 for 10 min and followed by 10 mL deionized water for 10 min and 8 mL acetone for 10 min. The samples were then rinsed three times with deionized water and one time with acetone. Nail samples were weighed between 0.010-0.013 g followed by soaking in 10 mL of Triton X-100 solution for 2 h and 8 mL of acetone for 1 h. Then, the samples were rinsed with deionized water five times. Subsequently, the samples were placed in incubator at 45°C overnight for drying process.

Both hair and nail samples were digested using nitric acid 65% and hydrogen peroxide 30% in a half closed opened digester. The 2 mL of nitric acid 65% was pipette into the each test tube contained sample and heated at 250°C to obtain a clear solution. Then, 0.2 mL hydrogen peroxide was pipette into the test tube till foam formation obtained. All samples were diluted with deionized water and filtered (6 µm) into centrifuge tube. The samples were stored in cold room at 4°C until the levels of heavy metals were conducted by using ICP-MS.

Statistical analysis: All data obtained analyzed by using Program Statistical for Social Science (SPSS) Version 20. The levels of each heavy metal were shown in mean (µg L⁻¹) together with Standard Error of Mean (±SEM). This study employed Kruskal Wallis test and one-way ANOVA to compare the mean level of heavy metals among hairs, fingernails and toenails and to compare the mean level of heavy metals with blood pressure. To find out the correlation of level of heavy metals with working period, Spearman correlation and Pearson correlation tests were carried out. On the other hand to determine the correlation of knowledge, attitude and practice of pesticides and fertilizers use with working period and blood pressure, Chi-square correlation were used in this study.

RESULTS AND DISCUSSION

In the findings, the mean value of each heavy metal level among hair, fingernail and toenail samples from farmers are presented in Table 1. There were a significant difference shown in arsenic ($H = 17.95$, $p < 0.05$), cadmium ($H = 63.56$, $p < 0.05$), lead ($H = 25.87$, $p < 0.05$) and nickel ($H = 157.01$, $p < 0.05$) among hair, fingernail and toenail samples. Table 2 shows comparison of heavy metals levels between two each samples. There were a significant difference shown in cadmium, lead and nickel levels between hair and fingernail between hair and toenail and fingernail and toenail. Arsenic level is only shown significant in between hair and fingernail samples ($U = 1768.00$, $p < 0.05$).

Table 1: Comparison of means of heavy metals levels among hair, fingernail and toenail samples

Parameters	Mean±SEM		H-value	p-value
	Sample size (n)	Heavy metals level (µg L ⁻¹ (ppb))		
Arsenic				
Hair	83	75.27±9.350	17.95	0.00*
Fingernail	80	1.76±0.230		
Toenail	80	90.56±10.19		
Cadmium				
Hair	83	23.21±2.960	63.56	0.00*
Fingernail	80	1.29±0.380		
Toenail	80	34.57±3.440		
Lead				
Hair	83	37.59±1.520	25.87	0.00*
Fingernail	80	74.73±7.930		
Toenail	80	66.74±17.63		
Nickel				
Hair	83	36.21±3.230	157.01	0.00*
Fingernail	80	694.12±19.70		
Toenail	80	95.21±10.66		
Cobalt				
Hair	83	12.11±1.580	4.61	0.10
Fingernail	80	17.62±0.380		
Toenail	80	14.91±1.640		

Table 2: Comparison of mean of heavy metals levels between two each samples

Parameters	U-value	p-value
Arsenic		
Hair with fingernail	1768.00	0.00*
Hair with toenail	3241.50	0.79*
Fingernail with toenail	2673.00	0.06*
Cadmium		
Hair with fingernail	1633.50	0.00*
Hair with toenail	2629.00	0.02*
Fingernail with toenail	1137.00	0.00*
Lead		
Hair with fingernail	1842.50	0.00*
Hair with toenail	2438.50	0.00*
Fingernail with toenail	2478.50	0.01*
Nickel		
Hair with fingernail	161.000	0.00*
Hair with toenail	1731.00	0.00*
Fingernail with toenail	174.000	0.00*

*Significant ($p < 0.05$)

Table 3 shows comparison of heavy metals levels in samples of farmers with the blood pressure groups. There were significant difference shown in the arsenic ($H = 10.64$, $p < 0.05$), cadmium ($H = 7.34$, $p < 0.05$) and cobalt ($F = 3.33$, $p < 0.05$) levels in toenail among the blood pressure groups. From the Table 4, arsenic and cadmium levels in toenail were significant difference in between normal with pre-hypertension group and normal with

Table 3: Comparison of heavy metals levels in hairs and nails of farmers among the blood pressure group

Parameters	Blood pressure (mmHg)	Mean±SEM		
		Heavy metals level (µg L ⁻¹ (ppb))	F/H value	p-value
Hair				
Arsenic	Normal	59.31±16.99	1.28	0.28 ^a
	Pre-hypertension	74.84±12.29		
	Hypertension	107.58±26.11		
Cadmium	Normal	17.48±5.420	1.27	0.28 ^a
	Pre-hypertension	23.58±3.900		
	Hypertension	32.70±8.110		
Lead	Normal	33.29±1.960	4.40	0.11 ^b
	Pre-hypertension	40.13±2.250		
	Hypertension	35.69±3.460		
Nickel	Normal	31.22±5.650	0.72	0.32 ^a
	Pre-hypertension	36.74±4.290		
	Hypertension	43.69±9.560		
Cobalt	Normal	9.49±2.900	1.16	0.32 ^a
	Pre-hypertension	12.08±2.070		
	Hypertension	17.25±4.340		
Fingernail				
Arsenic	Normal	1.46±0.310	1.13	0.57 ^b
	Pre-hypertension	2.00±0.360		
	Hypertension	1.41±0.350		
Cadmium	Normal	1.28±0.870	0.98	0.61 ^b
	Pre-hypertension	1.18±0.440		
	Hypertension	1.87±1.060		
Lead	Normal	67.35±15.89	1.55	0.46 ^b
	Pre-hypertension	78.87±10.71		
	Hypertension	72.46±16.74		
Nickel	Normal	679.79±36.95	0.25	0.88 ^b
	Pre-hypertension	698.41±27.99		
	Hypertension	706.32±34.68		
Cobalt	Normal	17.76±0.910	1.92	0.38 ^b
	Pre-hypertension	17.37±0.460		
	Hypertension	18.35±0.730		
Toenail				
Arsenic	Normal	64.87±18.72	10.64	0.00 ^{b*}
	Pre-hypertension	88.63±13.66		
	Hypertension	140.69±22.26		
Cadmium	Normal	24.73±5.810	7.34	0.03 ^{b*}
	Pre-hypertension	35.45±4.870		
	Hypertension	48.15±7.130		
Lead	Normal	42.27±3.560	3.28	0.19 ^b
	Pre-hypertension	77.81±30.89		
	Hypertension	69.79±17.93		
Nickel	Normal	81.27±13.33	0.33	0.85 ^b
	Pre-hypertension	105.32±16.88		
	Hypertension	83.84±19.70		
Cobalt	Normal	9.68±2.760	3.33	0.04 ^{a*}
	Pre-hypertension	15.27±2.270		
	Hypertension	22.51±3.610		

^aOne-way ANOVA; Normal = <120/80 mmHg, ^bKruskal Wallis test; Pre-hypertensi = 120/80-139/89 mmHg, *Significant ($p < 0.05$); Hipertensi = 140/90 mmHg

hypertension group. Post hoc Bonferroni test shows cobalt level in toenail was significant difference in between normal and hypertension group (Table 5).

Table 6 shows the relationship between working periods as farmers and the level of heavy metals in hair and nails samples. There were a weak negative

Table 4: Comparison of arsenic and cadmium levels in toenail between two each group of blood pressure

Parameters	U-value	p-value
Arsenic in toenail		
Normal with pre-hypertension	397.00	0.16 ^a
Normal with hypertension	52.00	0.00 ^{a*}
Pre-hypertension with hypertension	169.00	0.02 ^{a*}
Cadmium in toenail		
Normal with pre-hypertension	400.00	0.20 ^a
Normal with hypertension	65.50	0.01 ^{a*}
Pre-hypertension with hypertension	188.00	0.05 ^{a*}

^aMann-Whitney test; ^{*}Significant (p<0.05)

Table 5: Comparison of cobalt level in toenail between two each group of blood pressure

Parameters	Mean±SEM	p-value
Cobalt in toenail		
Normal with pre-hypertension	-5.59±3.72	0.29 ^a
Normal with hypertension	-12.83±5.00	0.03 ^{a*}
Pre-hypertension with hypertension	-7.24±4.50	0.24 ^a

^{*}Post Hoc Bonferroni test; ^{*}Significant (p<0.05)

relationship between arsenic (r = -0.44), cadmium (r = -0.43), lead (r = -0.32) and nickel (r = -0.31) levels in fingernail and also lead level (r = -0.29) in toenail with working periods as farmer (p<0.05).

In this finding, awareness and practice of pesticides and fertilizers use among farmers by blood pressure groups shown in Table 7. Almost all farmers from different

Table 6: Correlation of heavy metals levels in nail and hair with working periods of farmers

Parameters	Correlation coefficient	p-value
Hair		
Cadmium	-0.20	0.08 ^a
Lead	-0.01	0.96 ^b
Nickel	-0.16	0.15 ^a
Fingernail		
Arsenic	-0.44	0.00 ^{b*}
Cadmium	-0.43	0.00 ^{b*}
Lead	-0.32	0.00 ^{b*}
Nickel	-0.31	0.00 ^{b*}
Cobalt	0.18	0.11 ^b
Toenail		
Arsenic	-0.04	0.76 ^a
Cadmium	0.02	0.89 ^a
Lead	-0.29	0.01 ^{b*}
Nickel	0.17	0.15 ^b
Cobalt	-0.02	0.85 ^a

^aPearson correlation; ^bSpearman correlation; ^{*}Significant (p<0.05)

Table 7: Awareness and practice of pesticides and fertilizers use by blood pressure group

Questions	Answer	Blood pressure group			χ^2 -value	p-value
		Normal	Pre-hypertension	Hypertension		
Have you been involve in talk about the proper way to use Personal Protective Equipment (PPE)?	Yes	23	35	10	1.68	0.46
	No	3	11	3		
Do you aware about Personal Protective Equipment (PPE)?	Yes	23	37	11	0.79	0.79
	No	3	9	2		
Wear hats while spraying pesticides and handling fertilizers	Yes	16	33	9	1.23	0.57
	No	11	13	4		
Had longed-sleeved clothes while spraying pesticides and handling fertilizers	Yes	21	42	12	3.15	0.22
	No	6	4	1		
Had longed-sleeved pant while spraying and handling fertilizers	Yes	23	42	12	0.81	0.64
	No	4	4	1		
Wear goggle while handling pesticides and fertilizers	Yes	14	31	8	1.74	0.41
	No	13	15	5		
Wear mask while handling pesticides and fertilizers	Yes	21	33	12	2.43	0.28
	No	6	13	1		
Wear cotton glove when handling pesticides and fertilizers	Yes	3	5	3	1.40	0.55
	No	24	40	10		
Wear rubber glove when handling pesticides and fertilizers	Yes	17	26	7	0.41	0.84
	No	10	20	6		
Wear long boots when handling pesticides and fertilizers	Yes	24	41	11	0.63	0.73
	No	2	6	2		
Drink while handling pesticides and fertilizers	Yes	1	1	0	0.63	1.00
	No	21	42	11		
Eat while handling pesticides and fertilizers	Yes	1	1	0	0.63	1.00
	No	21	42	11		
Smoking while handling pesticides and fertilizers	Yes	0	3	0	2.40	0.28
	No	22	40	11		
Change cloths after handling pesticides and fertilizers	Yes	15	32	10	2.08	0.34
	No	7	10	1		
Wash hand and face after handling pesticides and fertilizers	Yes	19	35	11	2.46	0.33
	No	3	8	0		
Shower after handling pesticides and fertilizers	Yes	12	33	10	7.86	0.02 [*]
	No	10	10	0		

^{*}Significant (p<0.05)

Table 8: Awareness and practices of pesticide and fertilizers use by working period as farmer

Questions	Answer	Working period As farmer (years)			χ^2 -value	p-value
		Short (<2)	Average (2-7)	Long (≥ 8)		
Have you been involve in talk about the proper way to use Personal Protective Equipment (PPE)?	Yes	18	28	20	1.79	0.44
	No	2	9	6		
Do you aware about Personal Protective Equipment (PPE)?	Yes	17	30	22	0.20	0.87
	No	3	7	4		
Wear hats while spraying pesticides and handling fertilizers	Yes	16	22	19	1.54	0.49
	No	7	14	6		
Had longed-sleeved clothes while spraying pesticides and handling fertilizers	Yes	21	30	22	0.82	0.72
	No	2	6	3		
Had longed-sleeved pant while spraying and handling fertilizers	Yes	22	31	22	1.40	0.60
	No	1	5	3		
Wear goggle while handling pesticides and fertilizers	Yes	14	21	17	0.60	0.78
	No	9	15	8		
Wear mask while handling pesticides and fertilizers	Yes	18	27	19	0.08	1.00
	No	5	9	6		
Wear cotton glove when handling pesticides and fertilizers	Yes	4	4	2	0.96	0.69
	No	19	32	22		
Wear rubber glove when handling pesticides and fertilizers	Yes	9	24	17	5.68	0.06
	No	14	13	7		
Wear long boots when handling pesticides and fertilizers	Yes	21	32	21	0.64	0.77
	No	2	4	4		
Drink while handling pesticides and fertilizers	Yes	0	2	0	2.76	0.34
	No	19	30	24		
Eat while handling pesticides and fertilizers	Yes	0	2	0	2.76	0.34
	No	19	30	24		
Smoking while handling pesticides and fertilizers	Yes	1	1	1	0.14	1.00
	No	18	31	23		
Change cloths after handling pesticides and fertilizers	Yes	15	24	17	0.88	0.70
	No	3	8	7		
Wash hand and face after handling pesticides and fertilizers	Yes	17	27	20	0.36	0.84
	No	2	5	4		
Shower after handling pesticides and fertilizers	Yes	15	23	16	1.48	0.54
	No	3	9	8		

Table 9: Individual's knowledge on pesticides handling by blood pressure group

Category/Score	Blood pressure			χ^2 -value	p-value
	Normal	Pre-hypertension	Hypertension		
Low (<10)	8	12	3	2.10	0.72
Medium (10-11)	12	19	7		
High (>11)	7	19	3		

blood pressure groups shower after handling pesticides and fertilizers significantly ($p < 0.05$). Most of the farmers aware about the Personal Protection Equipments (PPE) and possess good awareness and practice.

Table 8 shows awareness and practice of pesticides and fertilizers use among farmers by working period as farmer. Almost all farmers from different working periods have good awareness and practice against pesticides and fertilizers use. Most of them wore hat, mask, long-sleeve shirt and pant, wash hands and faces and shower after handling pesticides and fertilizers and did not smoke, drink and eat while handling pesticides and fertilizers.

Individual's knowledge on pesticides handling by blood pressure group is shown in Table 9. The 67 farmers posses high score in understanding the use of pesticides and personal protection equipments (≥ 10 scores). Table 10 shows individual's knowledge on pesticides

Table 10: Individual's knowledge on pesticides handling by working period as farmer

Category/Score	Working period as farmer			χ^2 -value	p-value
	Short	Average	Long		
Low (<10)	6	13	3	11.58	0.02*
Medium (10-11)	14	10	13		
High (>11)	3	16	10		

*Significant ($p < 0.05$)

Table 11: Individual's attitude on pesticides handling by blood pressure group

Category/Score	Blood pressure			χ^2 -value	p-value
	Normal	Pre-hypertension	Hypertension		
Not good (<24)	3	12	3	5.35	0.25
Good (24-26)	16	17	5		
Very good (>26)	4	13	2		

handling by working period as farmer. The 66 famers have good knowledge from different working periods and there was a significant different between individual's knowledge and working periods ($p < 0.05$).

Table 11 shows individual's attitude on pesticides handling by blood pressure groups. The 57 farmers have good attitude in protecting themselves from the exposure of pesticides although, there was no significant difference

Table 12: Individual's attitude on pesticides handling by working period as farmer

Category/Score	Working period as farmer			χ^2 -value	p-value
	Short	Average	Long		
Not good (<24)	5	9	4	4.15	0.38
Good (24-26)	10	17	11		
Very good (>26)	12	7	9		

shown. The 66 farmers from different working periods have good attitude on pesticides handling shown in Table 12.

The influence of environmental pollution on human health can be determined by metabolically inactive tissues such as nails and hairs due to easy of sample collection, transportation and preparation for analysis. Analysis of heavy metals normally can use alternative biomarkers such as blood and urine but these samples are only meant for short-term exposure (Van Dael *et al.*, 2001). In this study, nails and hairs are used to determine the heavy metals levels in human body because evaluation of the biological markers can be used to monitor the elements accumulated over a period such as from a few weeks to a few months (Rodushkin and Axelsson, 2000).

In this study, heavy metals arsenic, cadmium, lead and nickel were significantly higher in nails compared to hairs. Nails contain more α -keratin (22% cysteine) compared to hairs (14% cysteine) (Mandal *et al.*, 2003). Heavy metals levels are different in hair, fingernail and toenail are because of the differences between the growth of samples. Fingernail can grow approximately 3.47 mm per month, toenails grow 1.62 mm per month and hairs grow 1.4 cm per month (Pragst and Balikova, 2006). Arsenic and cadmium levels are higher in toenails because toenail is less exposure and contaminate to exogenous compounds (Barbosa *et al.*, 2005). Lead and nickel levels were higher in fingernails because fingernails are more exposed to environment (Sukumar, 2006).

Exposure of heavy metals can injure vascular system causing many types of diseases such as high blood pressure, edema and atherosclerosis (Prozialeck *et al.*, 2008). Arsenic, cadmium and cobalt in toenails were significantly higher compared to fingernails and hairs supporting the statement that toenail is a better biological marker. Exposure to arsenic can cause dysfunction of vascular endothelium and inactivate the nitric oxide synthase enzyme (Balakumar and Kaur, 2009). Besides, exposure of cadmium at high level will lead to renal dysfunction leading tubular proteinuria (Jarup *et al.*, 1998). High exposure of cobalt can cause myocardial dysfunction and cardiovascular disease too.

In this finding, heavy metals arsenic, cadmium, lead and nickel in fingernails and lead levels in toenails were

shown weak negative relationship with working period as farmer. This may be due to most of the farmers possess good attitude and practice on pesticides use to prevent the exposure of heavy metals. This will help to reduce the accumulation of heavy metals inside body. Besides, fertilizers used in are organic fertilizers such as SUKU BUMI fertilizer preventing the release of organic wastes to soil.

From the result of awareness and practices on pesticides use by blood pressure groups and working period most of the farmers have good awareness on the techniques to use pesticides and personal protective equipments when working. This can help them to prevent harm including heavy metals material from entering the body. Besides, most of the farmers have medium scores (10-11 score) in knowledge on pesticides handling. This shows that the farmers are lacking about knowledge in pesticides handling that can lead to careless on the sustainability of pesticide use and ignore the cause of pesticides pollution. However, almost all of the farmers have good attitude on pesticides handling and Personal Protective Equipments (PPE). Protective equipments are a tool to reduce exposure of heavy metals from environment.

CONCLUSION

There were significantly higher of heavy metals levels in nail compared to hair among farmers. Arsenic, cadmium and cobalt levels in toenail were significantly higher in farmers who have hypertension. Other factors such as nutrition, socioeconomic status and lifestyle have to be considered for a realistic approach. Based on the results of knowledge, attitude and practice on pesticides handling, most of the farmers possess good behavior and responsible in dealing the pesticides use to reduce the exposure of heavy metals. As a conclusion, the levels of heavy metals in nail and hair samples among vegetable farmers are lower compared to previous studies. However, the control of environment levels and human exposure to these metals is still important and other alternatives to control the pesticides and fertilizers use have to be implemented.

ACKNOWLEDGEMENTS

Special thanks to Malaysian Agricultural Research and Development Institute for the permission to conduct this study on farmers and all the laboratory assistants from departments of Biomedical Science. Researchers wished to thank Ministry of Higher Education Malaysia and Universiti Kebangsaan Malaysia for research grant ERGS/1/2011/SKK/UKM/03/12.

REFERENCES

- Balakumar, P. and J. Kaur, 2009. Arsenic exposure and cardiovascular disorders: An overview. *Cardiovasc. Toxicol.*, 9: 169-176.
- Barbosa, F., Jr., J.E. Tanus-Santos, R.F. Gerlach and P.J. Parsons, 2005. A critical review of biomarkers used for monitoring human exposure to lead: Advantages. Limitations Future Needs, 113: 1669-1674.
- Bass, D.A., D. Hickock, D. Quig and K. Urek, 2001. Trace element analysis in hair: Factors determining accuracy, precision and reliability. *Alt. Med. Rev.*, 6: 472-481.
- De Boeck, M., M. Kirsch-Volders and D. Lison, 2003. Cobalt and antimony: Genotoxicity and carcinogenicity. *Mutation Res.*, 533: 135-152.
- De Miguel, E., I. Irribaren, E. Chacon, A. Ordonez and S. Charlesworth, 2007. Risk-based evaluation of the exposure of children to trace elements in playgrounds in Madrid (Spain). *Chemosphere*, 66: 505-513.
- Denkhaus, E. and K. Salnikow, 2002. Nickel essentiality, toxicity and carcinogenicity. *Crit. Rev. Oncol. Hematol.*, 42: 35-56.
- Duruibe, J.O., M.O.C. Ogwuegbu and J.N. Ekwurugwu, 2007. Heavy metal pollution and human biotoxic effects. *Int. J. Phys. Sci.*, 2: 112-118.
- Ercal, N., H. Gurer-Orhan and N. Aykin-Burns, 2001. Toxic metals and oxidative stress. Part 1. Mechanisms involved in metal-induced oxidative damage. *Curr. Top. Med. Chem.*, 1: 529-539.
- Hawkes, S.J., 1997. What is a heavy metals?. *J. Chem. Educ.*, 74: 1374-1374.
- Jarup, L., M. Berglund, C.G. Elinder, G. Nordberg and M. Vahter, 1998. Health effects of cadmium exposure: A review of the literature and risk estimate. *Scand. J. Work, Environ. Health*, 24: 1-51.
- Jing, Y.D., Z.L. He and X.E. Yang, 2007. Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. *J. Zhejiang Univ. Sci. B*, 8: 192-207.
- Luo, X.S., J. Ding, B. Xu, Y.J. Wang, H.B. Li and S. Yu, 2012. Incorporating bioaccessibility into human health risk assessments of heavy metals in urban park soils. *Sci. Total Environ.*, 424: 88-96.
- Mandal, B.K., Y. Ogra and K.T. Suzuki, 2003. Speciation of arsenic in human nail and hair from arsenic-affected area by HPLC-inductively coupled argon plasma mass spectrometry. *Toxicol. Applied Pharmacol.*, 189: 73-83.
- Mehra, R. and M. Juneja, 2005. Elements in scalp hair and nails indication metal body burden in polluted environment. *J. Sci. Ind. Res.*, 64: 119-124.
- Mortan, J., V.A. Carolan and P.H.E. Gardiner, 2002. Removal of exogenously bound elements from human hair by various washing procedures and determination by Inductively Coupled Plasma Mass Spectrometry. *Anal. Chimica Acta*, 455: 23-34.
- Pragst, F. and M.A. Balikova, 2006. State of the art in hair analysis for detection of drug and alcohol abuse. *Clin. Chimica Acta*, 370: 17-49.
- Prozialeck, W.C., J.R. Edwards, D.W. Nebert, J.M. Woods, A. Barchowsky and W.D. Atchison, 2008. The vascular system as a target of metal toxicity. *Toxicol. Sci.*, 102: 207-218.
- Rodushkin, I. and M.D. Axelsson, 2000. Application of double focusing sector field ICP-MS for multielement characterization of human hair and nails. Part I. Analytical methodology. *Sci. Total Environ.*, 250: 83-100.
- Slotnick, M.J. and J.O. Nriagu, 2006. Validity of human nails as a biomarker of arsenic and selenium exposure: A review. *Environ. Res.*, 102: 125-139.
- Sukumar, A., 2006. Human Nails as a Biomarker of Element Exposure. In: *Reviews of Environmental Contamination and Toxicology*, Ware, G.W., H.N. Nigg and D.R. Doerge (Eds.). Springer, USA., pp: 141-177.
- Van Dael, P., L. Davidsson, R. Munoz-Box, L.B. Fay and D. Barclay, 2001. Selenium absorption and retention from a selenite- or selenate-fortified milk-based formula in men measured by a stable-isotope technique. *Br. J. Nutr.*, 85: 157-163.
- Were, F.H., W. Njue, J. Murungi and R. Wanjau, 2008. Use of human nails as bio-indicators of heavy metals environmental exposure among school age children in Kenya. *Sci. Total Environ.*, 393: 376-384.