

Study of Heavy Metals in Hair and Nails of among Farmers in Kelantan, Malaysia

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Abstract: Heavy metals such as Arsenic (As), Cadmium (Cd) and Plumbum (Pb) in fertilizers and pesticides could cause health problems to human. The sources of exposure to these heavy metals can be ingestion, food and the use of pesticides and fertilizers in agricultural. A study was conducted in Kelantan among farmers. The purpose of this study was to determine levels of heavy metals according to gender, age group, blood pressure, smoking habits and period of time exposure to pesticide (years). There were 113 farmers participated in this study. The samples of hair and nails were collected from farmers. The levels of heavy metals such as As, Cd and Pb were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The results, showed that there was mean differences of Pb levels between age groups in hair samples ($p < 0.05$). Furthermore, using the pearson correlation, the results indicated that there was significant relationship between working experience and the level of Pb ($r = -0.020$, $p < 0.05$). However, there was no significant difference of Cd, Pb, As between gender, level of blood pressure (high, normal and low), smoking habit and residences in nails and hair sample. In conclusion, the levels of heavy metals was higher in nails compare to hair samples. The heavy metals was higher among male, older age group, hypertension and non-smoker.

Key words: Farmers, heavy metals, hair, nails, hair, pesticide

INTRODUCTION

Exposure to pesticide during working at workplace can occur through breathing, food chain and water that was polluted with heavy metals (Cang *et al.*, 2004). Arsenic (As), Cadmium (Cd), Mercury (Hg) and Plumbum (Pb) was among heavy metals that was found in pesticide and fertilizer (Llobet *et al.*, 2003). Exposure to heavy metals for a long period of time can cause Parkinson, indigestion, Alzheimer, heart disease, lung cancer and other (Ohsawa, 2009; Guzeva *et al.*, 2008; Mutter *et al.*, 2007). The use of pesticide and fertilizer especially among farmers can caused the release of toxic such as heavy metals to the air. The type of pesticide that was used by farmers mostly paraquat, alachlor, atrazine and simazine (WRI, 1998).

Heavy metals that were release to the environment were absorbed by human through ingestion and breathing. Heavy metals will part of the body as keratin on nails and hair. This is due to the cysteine and sulfide chain on keratin that combines the heavy metals to nails and hair. Therefore, nails and hair are suitable to be used in order analyze the exposure of human to the heavy metals (Hawkes, 1997). Heavy metals such as As, Cd and Pb do not have any benefit to human physiology and could caused toxicity to human even though in small

quantity. The toxicity of heavy metals was acute, chronic, neurotoxic, carcinogenic and mutagenic (Huang *et al.*, 2007). The toxicity effect of heavy metals in the human body has a relationship with the produce of Reactive Oxygen Stress (ROS) that can increase the oxidative stress in the human body (Jing *et al.*, 2007).

Earlier study on heavy metals was done in Vienna and Rome by Wolfsperger *et al.* (1994) using hair samples. The results indicates that male have higher As and low Cd compared to females. Furthermore, there was significance difference if Cd, cobalt and Chromium between smokers and non-smokers. Another study by Sukumar and Subramanian (2007) showed that the heavy metals levels of chromium, cuprum, nikel and zink difference according to residences.

Study has shown that As, Cd and Pb was found in fertilizer with the concentrations 19.4 until 271.0 mg kg⁻¹, 9.5 until 96.4 mg kg⁻¹ and 5.6 until 17.2 mg kg⁻¹ (Lin, 1996). The presence of heavy metals in pesticide and chemical fertilizer has been used widely in agriculture (Lone *et al.*, 2008).

The main goal in this study was too determine the heavy metal levels and find the the association of heavy metals with gender, age, working experience and blood pressure level among farmers in Kelantan.

MATERIALS AND METHODS

Background of the subjects: This study was conducted in Dec. 2010 until April 2011. The objective of the study to determine the levels of heavy metals in hair and nails of farmers that was exposed to pesticide. Heavy metals that was measured Pb, Cd and As. This study was done in Bachok and Pasir Putih, Kelantan, Malaysia. About 113 farmers involves in this study. Most of subjects was vegetables and tobacco farmers. The farmers that was selected was those who fulfilled the acceptance criteria. The rejection criteria was farmers who have chronic disease such as heart failure, diabetes, pregnant and didn't give consent. Samples of nail and hairs was taken and was disinfected with ethanol.

In the present study, toenails and hair sample were taken from 113 farmers by using universal sampling. The inclusion criteria of the farmers were had been a farmer for at least 1 years and didn't have a chronic disease such as heart failure, diabetes and a hypertension. The questionnaire was used consist of the background of the farmers, number of years working as farmers, safety equipment that is used during the used of pesticide, diseases history (diabetes, hypertension, cholesterol and chemical fertilizer. The samples of toenails were taken using nail clippers. Mean while the samples of hair were taken up to 10 hairs using a scissors.

Sampling: All the samples that were taken were kept at room temperature. The method of taking samples and sample process were using methods from earlier researchers on heavy metals (Mehra and Juneja, 2005; Bass *et al.*, 2001; Were *et al.*, 2008; Rodushkin and Axelsson, 2000; Mortan *et al.*, 2002). The samples of hair from 113 farmers were cut into 1 cm and then were weight using the electronic weighing machine in order to get the hair samples 0.0025 g. The hair samples were soaked in Triton X-100, deionize water and acetone. Meanwhile, the toenails were scraped to remove all the dirt at the toenails. Then the toenail was weighed to get the weight of 0.01 g. Then the toenails were soak in the Triton X-100 for 2 h and followed by acetone. Next, both of the samples were process following the method of digestion and desiccation (Mehra and Juneja, 2005; Bass *et al.*, 2001; Were *et al.*, 2008; Rodushkin and Axelsson, 2000; Mortan *et al.*, 2002). The Inductively Coupled Plasma-Mass Spectrometry (ICPMS) were used to measure the levels of heavy metals such as As, Cd and Pb.

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Statistical analysis: All the data were analyzed using Statistical Package of Social Science (SPSS) Version 17. The statistical analysis that was used are Mann-Whitney, Kruskal Wallis, one way analysis of variance and Pearson correlation. The level of significance is 0.05.

RESULTS AND DISCUSSION

This study involves of 113 farmers, age range from 21 until 80 years old. The number of years working as farmers was 1 until >40 years. The results showed that most (55.7%) of the farmers aged >50 years old. In this study, the 28 farmers (40%) indicated that they have <21 years of experience working as farmers. A total of 16 farmers (22.9%) has experience as farmers >40 years (Table 1). Further more there are 92 male and 21 female farmers. According to age group age 25 until 41 years, 42 until 58 and >58 years was 15 (13%), 54 (48%) and 43 (38%), respectively. Meanwhile, according to blood pressure group <120, 120-139 and >140 mmHg was 25 (23%), 36 (32%) and 50 (45%), respectively. According to working experience 1-17 years, 18-34 and >34 years was 34 (31%), 45 (41%) and 31 (28%).

Table 1: Demographic data

Characteristics	n	Percentage
Gender		
Male	92	81.4
Female	21	18.6
Age		
25-41	15	13.4
42-58	54	47.2
>58	43	38.4
Working experience		
1-20 years	34	30.9
21-40 years	45	40.9
≥41 years	31	28.2
Smoking habits		
Smokers	46	41.1
Non-smokers	66	58.9
Blood pressure group		
Normal	25	22.5
Prehypertension	36	32.4
Hypertension	50	45.0

Table 2, showed that the Pb and As among male was higher compared to female in nails (0.642 ± 0.510) and (0.012 ± 0.008), respectively. Meanwhile samples in hair indicates that the Pb, As and Cd was higher among female farmers (0.517 ± 0.252) and male (0.013 ± 0.012). However, there was no median difference using Mann-Whitney test ($p > 0.05$). Table 3 indicates the comparison of heavy metals between age group. In nail samples, the Pb levels was higher among older farmers with age 59-75 which is 0.819 ± 0.688 . Mean while for Cd, it was higher among younger age group which is 1.242 ± 1.098 . Using the one way Analysis of Variance (ANOVA) the results indicated that was significance mean difference of Pb between age group in hair samples ($F = 3.59$, $df = 2$, $p < 0.05$). Using the Post-Hoc test there was mean difference of Pb between age group 25-41 years and 42 until 58 years with age group > 58 years ($p < 0.05$).

Table 4 indicates the comparison of heavy metals according to blood pressure group. Farmers that has hypertension showed have higher Pb in nails (0.876 ± 0.628). However, for As famers at prehypertension group have higher As levels which is 0.014 ± 0.009 . Meanwhile for hair samples farmers in prehypertension group have the higher Pb levels which is 0.488 ± 0.225 . There was no statistically significance difference of heavy metals among blood pressure group. Table 5 indicates that non-smoker have higher Pb and Cd levels which is 0.791 ± 0.734 and $0.400 \pm 0.101 \mu\text{g g}^{-1}$. The heavy metals especially Pb and Cd in nails and hair was higher among non-smokers.

Table 2: Mean and SEM of heavy metals according to gender

Sample (Nails)	Sample size	Heavy metals		
		Pb	As	Cd
Male	11	0.604 ± 0.458	0.006 ± 0.005	0.0522 ± 0.038
Female	36	0.403 ± 0.405	0.012 ± 0.010	1.562 ± 0.943
Test statistic		-0.514	-0.064	-1.181
Male	11	0.418 ± 0.181	0.003 ± 0.001	0.009 ± 0.008
Female	36	0.517 ± 0.252	0.004 ± 0.002	0.013 ± 0.012
Test statistic		-1.905	-0.911	-1.200

Table 3: Mean and SEM of heavy metals according to age group

Samples	Sample size	Heavy metals		
		Pb	As	Cd
Nails				
25-41	11	0.604 ± 0.458	0.006 ± 0.005	1.242 ± 1.098
42-58	36	0.403 ± 0.405	0.012 ± 0.010	0.009 ± 0.005
> 58	37	0.819 ± 0.688	0.011 ± 0.005	0.110 ± 0.002
Test statistic		4.586	1.407	2.103
Hairs				
25-41	14	0.502 ± 0.159	0.004 ± 0.003	1.242 ± 1.212
42-58	42	0.473 ± 0.242	0.003 ± 0.001	0.009 ± 0.006
> 58	21	0.345 ± 0.102	0.003 ± 0.002	0.110 ± 0.052
Test statistic		3.590*	0.166	0.446

* $p < 0.05$

Table 6 showed the relationship between working period and heavy metals levels. The results showed that there was negative correlation between Pb levels and working period ($r = -0.02$, $p < 0.05$). The working experience as farmers was associated with exposure to heavy metals. The results shows that the longer period of working experience the higher level of Pb.

The use of pesticide has increased the health problems among farmers indirectly. The results from this study indicates that the range of heavy metals was in the similar range with earlier study. The As levels in nails in the present study was lower compared to study by Hashem and Othman (2001), Mielke *et al.* (2005) and Steinkellner *et al.* (2003) maybe due to the water and soil pollution of As. Meanwhile, for the hair sample the heavy metals was also lower compared to earlier study by Nowak and Chmielnicka (2000) and Meyer *et al.* (1999). Earlier study on the water samples shown that the As, Cd, mercury, mangan and lead was higher than the water quality standard. The samples was taken from the underground water resources and river in Kelantan (Mustapha and Anuar, 2011). Therefore, there is a possibilities that heavy metals in Kelantan may be due to the water pollution of As and Cd.

Table 4: Mean and SEM of heavy metals between blood pressure group

Samples	Sample size	Heavy metals		
		Pb	As	Cd
Nails				
Normal	20	0.479 ± 0.394	0.004 ± 0.001	0.002 ± 0.003
Prehypertension	24	0.352 ± 0.233	0.012 ± 0.010	0.008 ± 0.002
Hypertension	39	0.876 ± 0.628	0.011 ± 0.005	0.457 ± 0.229
Test statistic		1.146	1.763	9.436
Hairs				
Normal	22	0.460 ± 0.220	0.003 ± 0.001	0.009 ± 0.006
Prehypertension	23	0.003 ± 0.001	0.003 ± 0.002	0.011 ± 0.010
Hypertension	32	0.399 ± 0.178	0.004 ± 0.002	0.011 ± 0.010
Test statistic		1.362	0.330	0.422

Table 5: Mean and SEM of heavy metals according to smoking habits

Samples	Sample size	Heavy metals		
		Pb	As	Cd
Nails				
Smokers	40	0.440 ± 0.322	0.013 ± 0.010	0.011 ± 0.010
Non-smokers	44	0.791 ± 0.734	0.010 ± 0.006	0.400 ± 0.101
Test statistic		-1.191	-1.066	-0.259
Hairs				
Smokers	28	0.460 ± 0.220	0.003 ± 0.001	0.009 ± 0.006
Non-smokers	49	0.003 ± 0.001	0.003 ± 0.002	0.011 ± 0.010
Test statistic		-1.426	-0.579	-1.160

Table 6: Correlation coefficient between heavy metals and working experience

Heavy metals	Working experience	
	Nails	Hairs
Pb	0.107	-0.020*
As	-0.117	-0.016
Cd	-0.028	-0.079

* $p < 0.05$

The heavy metals levels was higher in nails compared to hair samples. This is because the nails directly exposed to the pesticide. Furthermore, the accumulation of heavy metals in nails was difference compare to hair samples because the growth of nails and hair was differed (Sukumar, 2002; Were *et al.*, 2008). In nails, the growth was 3.47 mm per month and hair was (0.601.4 cm) per month. In this study, there was no significant mean difference of heavy metals according to gender, similar to other study by Chojnacka (2010). This is because the farmers male and female use the safety equipment such as gloves, hat and boots during spraying pesticide to the vegetables.

This study shown that there was no difference of heavy metals levels according to age group. This was similar with study by Alkan (2008) and Komarnicki (2000). The higher levels of heavy metals among age group >59 years old was solely working as farmers. In this study, the heavy metals was higher among male farmers. This is similar to earlier study by Sukumar (2002). In this study showed that there was no difference of heavy metals levels according to hypertension category. This findings was contrast with previous study by Eum *et al.* (2008) who found that there was relationship between hypertension and heavy metals. In this present study, the higher age group showed higher Pb levels compared to middle age group. This is due to the exposure of heavy metals when the farmers used pesticide without using safety equipment. This is similar with earlier study indicated that there was relationship between heavy metals and age (Alkan, 2008).

This study shown that As, Cd and Pb shown no significance difference between smokers and non smokers. This finding was similar with finding by Sukumar and Subramanian (2007) who indicates that heavy metals were not influence by smoking habits. This study shown the heavy metals Pb has significant relationship with the period of time working as farmers. This study contrast with study by Gil *et al.* (2011) who indicates that there was relationship between working experience as farmers and heavy metals. The longer working experience was believe to have relationship with the heavy metals levels. There was significant relationship of Pb levels and working experience (years) in hair samples. This is due to the longer period of time expose to pesticide among farmers.

CONCLUSION

The heavy metals levels especially Pb showed higher level among farmers. According to demographic factors the heavy metals was higher among male, 59-75 years old, hypertension, non-smokers and Pasir Puteh and longer

working experience (years). However, with the safety measures that have been taken by the farmers was effective and therefore the results showed that the heavy metals were low and there were no reported cases of heavy metals poisoning effects among farmers. Therefore, the usage of pesticide among farmers was still below the levels that can cause poisoning and toxic among farmers.

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