Malaysian J Pathol 2015; 37(2) : 123 – 135

ORIGINAL ARTICLE

Effectiveness of bone cleaning process using chemical and entomology approaches: time and cost

LAI Poh Soon *BSc*, KHOO Lay See *MSc*, MOHD HILMI Saidin *BSc*, AHMAD HAFIZAM Hasmi *MPath*, MOHD SHAH Mahmood *MMedSc (Forensic Toxicology)*, NURLIZA Abdullah *MMedSc. (Forensic Anthropology)*, NAZNI Wasi Ahmad* *PhD*

*National Institute of Forensic Medicine, Hospital Kuala Lumpur (HKL) and *Institute of Medical Research (IMR), Kuala Lumpur*

Abstract

Skeletal examination is an important aspect of forensic pathology practice, requiring effective bone cleaning with minimal artefact. This study was conducted to compare between chemical and entomology methods of bone cleaning. Ten subjects between 20 and 40 years old who underwent uncomplicated medico-legal autopsies at the Institute of Forensic Medicine Malaysia were randomly chosen for this descriptive cross sectional study. The sternum bone was divided into 4 parts, each part subjected to a different cleaning method, being two chemical approaches i.e. laundry detergent and a combination of 6% hydrogen peroxide and powder sodium bicarbonate and two entomology approaches using 2nd instar maggots of *Chrysomyia rufifacies* and *Ophyra spinigera*. A scoring system for grading the outcome of cleaning was used. The effectiveness of the methods was evaluated based on average weight reduction per day and median number of days to achieve the average score of less than 1.5 within 12 days of the bone cleaning process. Using maggots was the most time-effective and cost-effective method, achieving an average weight reduction of 1.4 gm per day, a median of 11.3 days to achieve the desired score and an average cost of MYR 4.10 per case to reach the desired score within 12 days. This conclusion was supported by blind validation by forensic specialists achieving a 77.8% preference for maggots. Emission scanning electron microscopy evaluation also revealed that maggots especially *Chrysomyia rufifacies* preserved the original condition of the bones better allowing improved elucidation of bone injuries in future real cases.

Keywords: forensic anthropology; bone cleaning; chemical; maggots; emission scanning electron microscopy

INTRODUCTION

In forensic medicine, it is crucial to determine whether the injuries of a victim are inflicted during life or after death.[1] Examination of the bones without overlying soft tissue might provide evidence on type of weapons used and the nature of the injuries sustained. In addition, examination of the human skeleton enables the forensic pathologist and forensic anthropologist in determining the occupational and age-related osteological markers as well as the biological characteristics e.g. sex, race, stature and cultural affiliation.[2] Furthermore, traumatic and pathological details of the human skeleton can also help to determine cause of death and manner of death especially in a partially skeletonised or decomposed body. In order to have a better visualisation of skeletal remains during examination, it is important to process and clean up the bone effectively with minimal artefact. A processed clean bone must be suitable for archiving as court evidence and for education purposes. The effectiveness of cleaning bones and skeletal remains is also essential to the forensic odontologist in facial reconstruction, dental examination and bite mark analysis.

There are various methods available to process the bones. Maceration is a cleaning method that based on bacterial action on the skulls and bones in warm water. In the other ways, bones can also be chemically treated using detergents containing enzymes or undergo simmering and

Address for correspondence: Lai Poh Soon, Forensic Scientific Officer, National Institute of Forensic Medicine, Hospital Kuala Lumpur, Malaysia. Email: roysonn1@hotmail.com. Tel: +603 2690 6694; Fax: +603 2694 1422

boiling. There are also some suggestions to flesh out the skulls or skeletal remains using certain species of maggots and/or beetles. All of these have their strengths and weaknesses and any of them can be the best method at a particular time in a particular place for certain specimens.[3]

All bones are firmly attached to flesh which is mostly made of protein. Physically and mechanically removing flesh by using a scalpel, tweezers, scissors, probes (mounted needles), blunt table knife (scraper) and old toothbrushes have the potential of damaging delicate parts of the bones. As such, the remaining attached flesh could eventually be removed without damaging the bone either by artificially softening the protein or by using natural agents such as insects and bacteria. In certain cases, timely and slowly boiling within thirty minutes can effectively soften the flesh and remove muscles and ligaments from the bone surface depending on size of the bones. A drawback is that simmering and boiling fixes the fat in the bones and it will migrate to the surface, making the bone black and greasy a few years later. Alternatively, ammonium hydroxide and 3%–6% hydrogen peroxide are used to remove fats and oils but the bones will consequently decalcified over a long period of time. Thus the subtlest way to soften protein and remove fat was using enzymes either via maceration at 35°C or using washing powder.[4] Partly decomposed bodies found in a dried condition, with some mummified tissues attached, can usually be cleaned by soaking in detergent solution until the tissues soften. Since bone consists of protein as part of its structure, any protein denaturing process going too far will attack the bone resulting in the surface losing its ivory texture, then becoming chalky or eventually crumbly. Bleach also may remove the bone features and its natural colouring and subsequently made them brittle.

There are also the bone cleaning approaches using certain species of beetles or maggots as in "Bug Box".[5] One of the methods is dermestid beetles for small bones, while maceration is better for medium to large ones. Macerated skeletons are considered more thoroughly cleaned than bugged skeletons. Dermestids are much faster because of their larger size.[3] The larvae of dermestid beetles can delicately remove the flesh from a skeleton, leaving the bones well-articulated even in partially dried out carcasses found in dry summer. Maggots, on the other hand, preferred to work in a damp environment and can colonize anything dead, and quickly remove the flesh. The skeleton can be placed in a container or net bag to ensure retrieval of all the detached bones during this natural cleaning process.

The popular techniques currently used in Malaysia are laundry detergents and chemicals such as a combination of hydrogen peroxide and sodium bicarbonate. Research into new approaches with validated effectiveness is generally important to be applied within the setting of the work of the National Institute of Forensic Medicine Malaysia at Hospital Kuala Lumpur (HKL). This study was conducted specifically to compare between chemical and entomology methods, aimed at enabling both forensic pathologist and forensic anthropologist to choose the most cost effective and time effective method in order to improve the forensic service.

MATERIALS AND METHODS

This was a cross-sectional and descriptive study performed at the Institute of Forensic Medicine Malaysia, HKL during a three-month period. Subjects were randomly chosen from dead bodies of ages between 20 and 40 years old, who underwent medico-legal autopsies from 16th December 2013 to 15th March 2014.

The power of study was 80%, fixed with the 95% confidence interval (CI) whilst the level of precision was ± 5% (e value = 0.05). The assumed maximum value of variability in this research was 50% and therefore the p value was 0.5 and q value was $1 - p = 0.5$. The calculated sample size as derived from the formulas was approximately 30 samples collected within a three-month period in order to represent the population sample.

Only 10 samples were collected due to constraints in obtaining samples. Sample unit was the number of normal intact sternum bones extracted from fresh bodies, which were then cut into four equal parts using an autopsy saw, each part to undergo different bone cleaning methods There were four methods of bone cleaning conducted in this research, two being based on a chemical approach and two methods based on an entomology approach.[6] All cases were handled by adopting biohazard procedures when cleaning bones. The procedures of the four methods are outlined below:

(1) *Laundry detergent with normal tap water (chemical approach).*

1. The sternal bone was weighed using an

electronic balance.

2. The bone was totally immersed in a solution of approximately 0.1 litres of detergent (Kuat Harimau-Oxibleach, obtained commercially) made up to 0.5 litres with tap water.
3. The bone and solution were sealed in a container and left at room temperature. The solution was replaced with new detergents at the Day 6 if the bone was not cleaned to a score of 1 or less.

(2) *6% hydrogen peroxide and powder sodium bicarbonate with normal tap water (Chemical approach).*[7]

1. The sternal bone was weighed using the electronic balance.
2. The bone was totally immersed in a solution of approximately 0.05 kilograms of sodium bicarbonate to approximately 0.1 litres of 30% hydrogen peroxide made up to 0.5 litres with tap water.
3. The bone and solution were sealed in a container and left at room temperature.
4. If the change of score was not beyond 1 compared to the previous observation i.e. at Day 0 or Day 6, it would be replaced with new chemical solution 3 days later i.e. at Day 3 or Day 9 respectively.

(3) *2nd instar maggots of Chrysomyia rufifacies (Entomology approach)* (Figure 1).

1. The size of the bone was measured in cm^3 by displacement of tap water (1 ml = 1 cm^3).
2. The sternum bone was weighed using an electronic balance.
3. The bones and sufficient quantities of *Chrysomyia rufifacies (C.r.)* 2nd instar maggots (5 maggots per cm^3 of bone) were placed into a container and sealed with piece of cloth or gauze with tiny holes to hinder maggots from crawling out.
4. An open lid was used to cover container.
5. The 3rd instar maggots of *C.r.* were changed with new 2nd instar maggots of *C.r.* at Day 6, only if the bone was not completely cleaned to a score of 1 or less.

(4) *2nd instar maggots of Ophyra spinigera (Entomology approach)* (Figure 2).

1. The size of the bone was measured in cm^3 by displacement of tap water (1 ml = 1 cm^3).
2. The sternal bone was weighed using an electronic balance.
3. The bones and sufficient quantities of *Ophyra spinigera (O.s.)* 2nd instar maggots (5 maggots per cm^3 of bone) were placed into a container and sealed with a piece of cloth or gauze with tiny holes to hinder the maggots from crawling out.
4. An open lid was used to cover the container.
5. The 3rd instar maggots of *O.s.* were changed with new 2nd instar maggots of *O.s.* at Day 6, only if the bone was not completely cleaned to a score of 1 or less.

Evaluation of effectiveness of bone cleaning

All pre- and post-processed sternal bones were photographed at Days 0, 3, 6, 9 and 12. All weights of the sternal bones were recorded accordingly to dates and duration of the bone cleaning process after clearing off detachable soft tissues using autopsy forceps at Days 3, 6, 9 and 12 and compared to Day 0.

To reduce subjectivity, a scoring system for grading the outcome of cleaning was used (Table 1). Scoring was performed by three different HKL forensic scientists at Days 3, 6, 9 and 12.

FIG. 1: 2nd instar maggots of *Chrysonmyia rufifacies* (entomology approach)

FIG. 2: 2nd instar maggots of *Ophyra spinigera* (entomology approach)

TABLE 1: Scoring system for bone cleanliness

SCORE	Criteria
0	No flesh and bone looks clean and whitish with no smell
1	Little flesh attached but bone appears white with little smell
2	Little flesh attached but bone is yellowish dirty with little smell
3	Soft tissues still attached with decomposed body smell ("*Sickening sweet smell*")
4	Abundant soft tissue attached and no progress seen, with decomposed body smell ("*Sickening sweet smell*")

The effectiveness of the bone cleaning methods was evaluated based on average weight reduction per day and median number of days to achieve the average score of less than 1.5 within 12 days of the bone cleaning process. The lowest median number of days to achieve the average score of less than 1.5 and highest weight reduction per day would be considered the most effective method.

In addition, three (3) sets of the post-treated bones which had completed the 12-day cleaning period were randomly chosen amongst the preserved bone (dried and not mouldy) and given to three different HKL forensic specialists to evaluate the best cleaned bones without knowing the actual method used. Forensic specialists evaluated the cleaned bones by direct observation on the defleshing status, colour, smell and the morphology of the bones. The best method was the one with the highest percentage derived from the total of the method preference validated by the forensic specialists over the total number of validated sets of bones.

Scanning electron microscopy

Emission scanning electron microscope was performed on one randomly selected set of processed bone (the set having 4 samples of the same bone, each sample being cleaned by a different process). A small fragment, less than 3 cm square, was cut with an electric saw from the selected bone, coated with platinum of 40nm thickness and mounted on an aluminium SEM stub for imaging using a ESEM PHILIPS scanning electron microscope. The SEM analysis included the features at the floor and the walls of the bones, the presence of debris or remaining flesh attached. A scoring system (Table 2) was used by the 3 of HKL forensic scientists to evaluate the SEM images obtained. The lowest average score was regarded the best result.

Cost-effectiveness

The cost effectiveness of each method was calculated based on the average cost per case used in each method to reach a score of less than 1.0. Cost of materials was based on purchase price per unit and the quantity of materials used up to the specific day for the desired score to be achieved.

Ethics Committee approval

This study was registered with the Malaysian National Medical Research Register (ID: NMRR-13-1607-18439) and exempted from full board review by the Malaysian Ministry of Health Medical Research and Ethics Committee.

RESULTS

The lowest mean score against time was achieved when detergent and chemicals were used (Figure 3). However, considering the average weight reduction per day within 12 days, bone cleaning with maggots *C.r.* was the highest being 1.4 gm per day, while the least effective was the chemical method whereby only 0.5 gm on average was cleared per day (Tables 3-6;

TABLE 2: SEM scoring criteria

SEM Score	Criteria
4	0-20% area free from debris
3	21-40% area free from debris
2	41-60% area free from debris
1	61-80% area free from debris
0	81-100% area free from debris

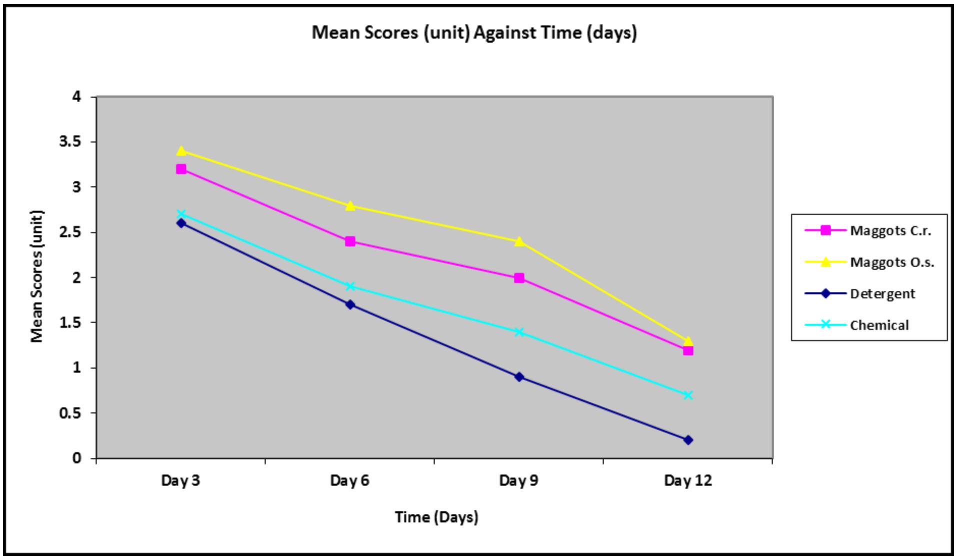

FIG. 3: Mean scores achieved by the 4 methods against time (days). The scoring system is as explained in Table 1.

Figure 4). Across time, weight reduction rates of all methods was highest during the first 3 days and then flattened after day 6 or Day 9. The exception was the detergent method, where the weight reduction decreased from Day 0 until Day 6, then accelerated but flattened from Day 9 onwards.

Based on the median number of days to achieve a score of less than 1.5 within 12 days, the detergents method was the shortest, being 7.5 days followed by chemical and maggots *C.r.* being 9.8 and 11.3 days respectively (Tables 3-6; Figure 5). The least effective was the maggots *O.s.* method, which required almost 12 days to clear off the flesh. Median number of days was considered instead of the average number of days because some of the bones did not clean properly within the 12-day period and in such cases an estimation of time to reach the desired score was calculated through projection. Hence, median was chosen as a more suitable measure of central tendency to exclude extreme outliers.

Blinded validation by forensic specialists were conducted on 3 sets of the preserved bones. Forensic specialists validated the preserved bones by direct observation of the bones. The

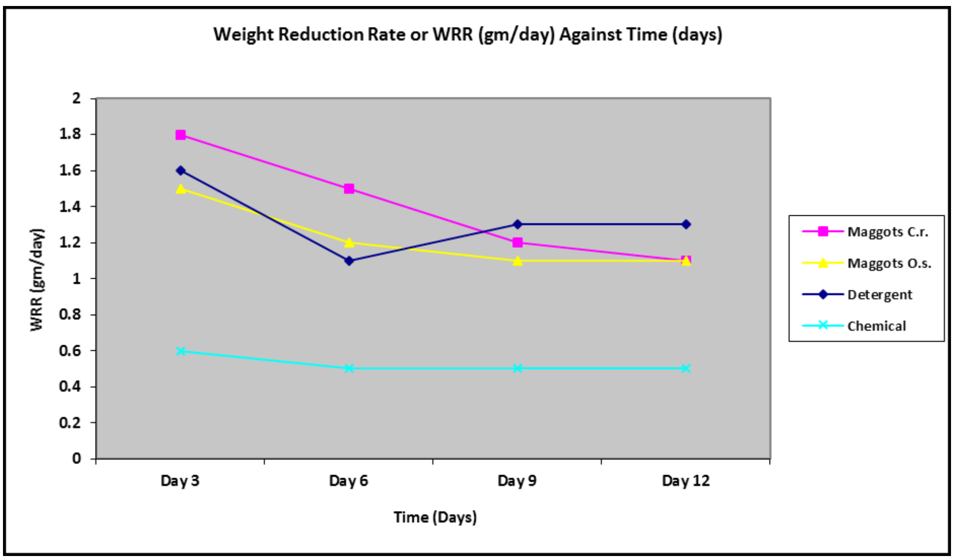

FIG. 4: Weight reduction rate or WRR (gm/day) achieved by the 4 methods against time (days). WRR is calculated against day 0.

TABLE 3: Data from maggots (*Chrysomyia rufifacies – C.r.*) method

		Maggots (*Chrysomyia rufifacies - C.r.*)										
		Day 0 / Time		Day 3 / Time		Day 6 / Time		Day 9 / Time		Day 12 / Time		Score ≤ 1.5 (total days)
Case No.	Start Date	Wt (gm)	Score	Wt Δ (gm)	Score	Wt Δ (gm)	Score	Wt Δ (gm)	Score	Wt Δ (gm)	Score	
1	26/12/2013	20.0	4.0	5.8	3.0	6.4	2.7	7.9	2.7	8.6	2.0	*15.0
2	8/1/2014	26.2	4.0	2.2	3.3	11.8	2.7	14.8	2.3	20.6	1.3	12.0
3	8/1/2014	13.6	4.0	1.6	3.0	4.8	2.3	7.3	1.7	8.7	0.7	9.8
4	8/1/2014	15.6	4.0	1.8	3.3	5.8	2.3	7.1	2.0	9.0	1.0	10.5
5	19/2/2014	15.1	4.0	3.9	3.0	6.2	2.7	6.2	1.7	7.2	1.0	10.5
6	19/2/2014	27.6	4.0	9.8	3.0	13.3	2.3	14.4	1.7	15.3	1.0	10.5
7	19/2/2014	25.8	4.0	7.5	3.3	9.8	2.3	10.9	2.3	12.9	1.3	12.0
8	19/2/2014	26.1	4.0	8.7	3.7	12.1	2.3	12.3	2.0	14.7	1.3	11.3
9	11/4/2014	36.3	4.0	6.4	3.0	12.0	2.3	16.7	2.0	17.5	1.3	11.3
10	23/4/2014											
	Average	22.9	4.0	5.3	3.2	9.1	2.4	10.8	2.0	12.7	1.2	11.4
	Per Day	1.4		1.8		1.5		1.2		1.1	**Median**	11.3

*calculated via projection rate

bones which had undergone maggot's treatment were evaluated to be the best preserved bones. *C.r.* (55.6%) and *O.s.* (22.2%) together made up to 77.8% of the preference while detergent scored 22.2% (Table 7). The average score given by the forensic specialists was independent or regardless on the best methods chosen by them (Table 8) but mostly depending on macroscopic observation on the surface architecture of the cleaned bones.

Emission scanning electron microscope (Figure 6) showed the least and almost no

TABLE 4: Data from maggots (*Ophyra spinigera – O.s.*) method

		Maggots (*Ophyra spinigera – O.s.*)										
		Day 0 / Time		Day 3 / Time		Day 6 / Time		Day 9 / Time		Day 12 / Time		Score ≤ 1.5 (total days)
Case No.	Start Date	Wt (gm)	Score	Wt Δ (gm)	Score	Wt Δ (gm)	Score	Wt Δ (gm)	Score	Wt Δ (gm)	Score	
1	26/12/2013	13.4	4.0	0.9	4.0	3.5	2.7	6.6	2.3	7.9	1.7	*13.0
2	8/1/2014	28.6	4.0	4.4	3.7	7.7	3.0	10.1	3.0	16.8	2.0	*15.0
3	8/1/2014	16.7	4.0	1.5	3.3	2.8	3.0	4.8	3.0	8.7	1.3	12.0
4	8/1/2014	15.3	4.0	1.3	3.7	3.5	3.0	5.0	2.7	7.3	1.0	11.3
5	19/2/2014											
6	19/2/2014	20.3	4.0	7.5	3.0	7.8	2.7	9.0	1.7	9.2	1.0	9.8
7	19/2/2014	28.1	4.0	5.9	3.3	7.9	2.7	8.8	2.7	13.2	1.3	12.0
8	19/2/2014	38.6	4.0	5.8	3.0	8.3	2.7	9.6	2.3	17.7	1.7	*13.0
9	11/4/2014	31.9	4.0	5.5	3.3	9.4	2.7	16.6	2.0	18.6	1.3	11.3
10	23/4/2014	33.5	4.0	6.8	3.7	12.5	3.0	15.9	1.7	19.2	0.7	9.8
	Average	25.2	4.0	4.4	3.4	7.0	2.8	9.6	2.4	13.2	1.3	11.9
	Per Day	1.2		1.5		1.2		1.1		1.1	**Median**	12.0

* calculated via projection rate

TABLE 5: Data from detergent (Harimau Kuat : Oxi-Bleach) method

		Detergent (Harimau Kuat: Oxi-Bleach)										
		Day 0 / Time		Day 3 / Time		Day 6 / Time		Day 9 / Time		Day 12 / Time		Score ≤ 1.5 (total days)
Case No.	Start Date	Wt (gm)	Score	Wt Δ (gm)	Score	Wt Δ (gm)	Score	Wt Δ (gm)	Score	Wt Δ (gm)	Score	
1	26/12/2013	13.5	4.0	5.7	2.3	5.7	1.7	9.3	0.3	13.5	0.0	6.8
2	8/1/2014	25.2	4.0	7.8	2.7	7.1	2.0	15.1	1.3	25.2	0.0	8.3
3	8/1/2014	17.2	4.0	4.9	3.0	5.0	2.0	10.3	1.0	17.2	0.0	7.5
4	8/1/2014	17.4	4.0	4.4	2.7	4.6	1.7	8.9	1.3	17.4	0.0	7.5
5	19/2/2014	9.8	4.0	1.5	2.3	3.1	1.3	5.6	0.7	9.8	0.0	6.0
6	19/2/2014	19.4	4.0	2.8	2.3	5.6	2.0	11.3	1.0			7.5
7	19/2/2014	33.5	4.0	5.3	2.7	10.7	2.0	16.2	1.0	17.5	0.3	7.5
8	19/2/2014	29.3	4.0	3.4	2.7	6.8	1.7	12.0	1.3	12.4	0.7	7.5
9	11/4/2014	25.4	4.0	7.2	2.3	7.8	1.3	11.3	0.3			6.0
10	23/4/2014	24.5	4.0	6.3	3.0	7.5	1.7	12.7	1.0	12.8	0.7	7.5
	Average	21.5	4.0	4.9	2.6	6.4	1.7	11.3	0.9	15.7	0.2	7.2
	Per Day	1.3		1.6		1.1		1.3		1.3	**Median**	7.5

spontaneous scattering of flesh or particles on the surface of the bone cleaned maggots compared to those by chemicals and detergent. The average SEM score for maggots *C.r.* and *O.s.* were 1.56 and 1.78 respectively (Table 7; Table 9), with the best being 61 - 80% area free from debris. Bones treated with chemicals were generally 60% free from debris compared to detergent with less than 60% area free from debris.

Cleaning using maggots *C.r.* was the most cost-effective, incurring an average cost of MYR 4.11 per case (Table 10; Figure 5) followed closely by laundry detergent (MYR 4.20) and maggots *O.s.* (MYR 4.33). The chemical method was the most expensive at MYR 94.80 per case (Table 11).

TABLE 6: Data from chemicals (hydrogen peroxide + sodium bicarbonate) method

		Detergent (Harimau Kuat: Oxi-Bleach)										
		Day 0 / Time		Day 3 / Time		Day 6 / Time		Day 9 / Time		Day 12 / Time		Score ≤ 1.5 (total days)
Case No.	Start Date	Wt (gm)	Score	Wt Δ (gm)	Score	Wt Δ (gm)	Score	Wt Δ (gm)	Score	Wt Δ (gm)	Score	
1	26/12/2013	11.2	4.0	2.4	2.0	3.3	1.3	5.1	0.7	5.7	0.3	5.3
2	8/1/2014	25.1	4.0	2.6	3.0	5.3	2.0	6.7	1.7	9.8	1.0	10.5
3	8/1/2014	14.7	4.0	0.7	2.7	1.5	2.0	2.3	1.7	3.7	1.0	10.5
4	8/1/2014	13.0	4.0	1.3	2.7	2.7	2.0	4.2	1.7	5.5	0.7	9.8
5	19/2/2014											
6	19/2/2014	22.4	4.0	2.1	2.7	3.0	1.7	4.1	1.3	5.5	0.7	7.5
7	19/2/2014	33.3	4.0	3.1	2.7	4.8	1.7	6.3	1.7	10.7	1.0	10.5
8	19/2/2014	26.7	4.0	1.0	2.7	1.4	1.7	1.7	0.7	3.4	0.7	6.8
9	11/4/2014	26.6	4.0	1.9	2.7	3.9	2.0	4.6	1.7	5.0	0.7	9.8
10	23/4/2014	24.9	4.0	1.2	3.0	1.7	2.3	4.7	1.7	5.1	0.7	9.8
	Average	22.0	4.0	1.8	2.7	3.1	1.9	4.4	1.4	6.0	0.7	8.9
	Per Day	0.5		0.6		0.5		0.5		0.5	**Median**	9.8

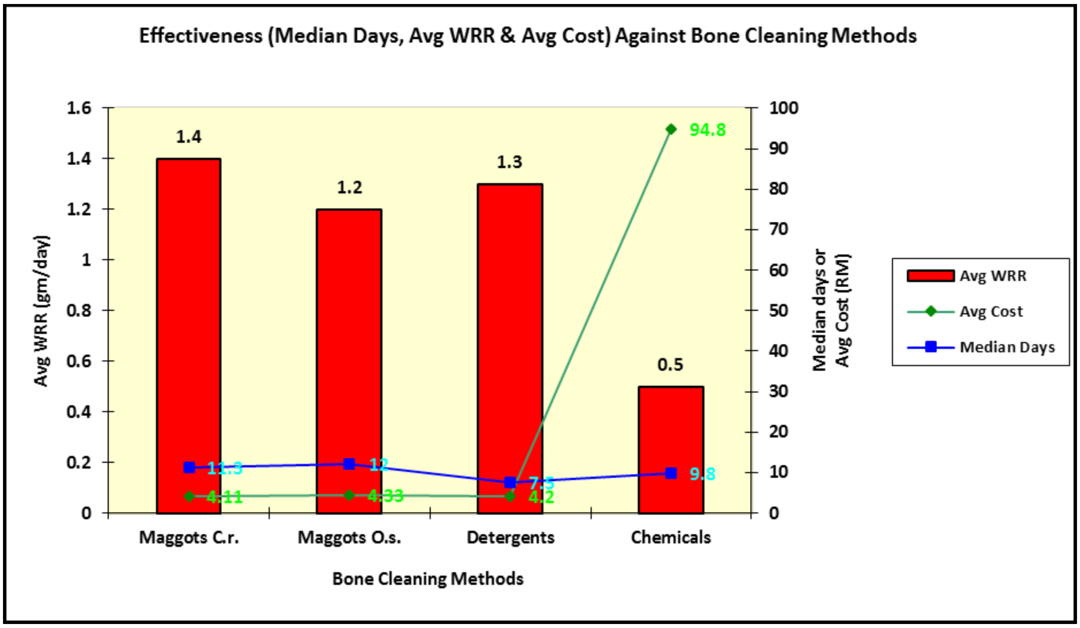

FIG. 5: Effectiveness (median days, average WRR and average cost) against bone cleaning methods. Median days based on achieving score of 1.5 or less within 12 days. Avg = Average

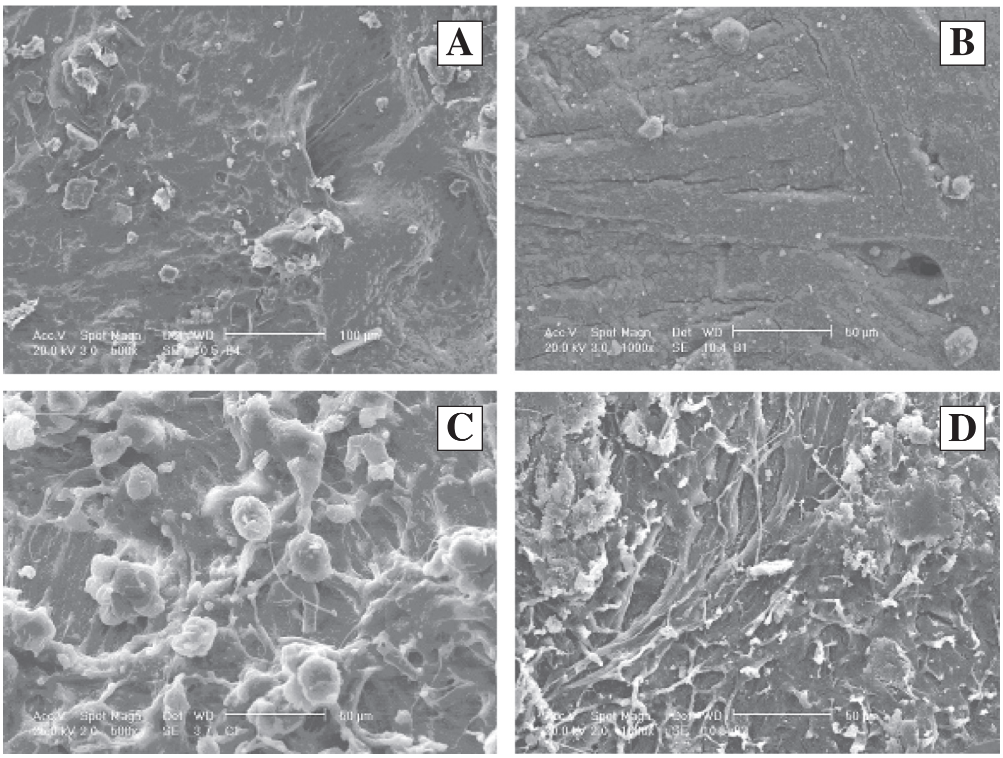

FIG. 6: Emission scanning electron micrographs of bone cleaned by (A) maggots *Ophyra spinigera* with average score 1.78 (~65% free from debris), (B) maggots *Chrysomyia rufifacies* with average score 1.56 (~70% free from debris), (C) detergent with average score 2.44 (~51% free from debris) and (D) chemicals with average score 2.00 (~60% free from debris).

TABLE 7: Summary of blind validations

No.	Method	Preferred choice of forensic pathologists		Average SEM score by forensic scientists
		Number	Percentage	
1	Detergent	2	22.2%	2.44
2	Chemicals	0	0.0%	2.00
3	*C.r.*	5	**55.6%**	**1.56**
4	*O.s.*	2	**22.2%**	**1.78**
	Total	9	100.0%	

TABLE 8: Details of blind validation scores by forensic pathologists

Specialist	Set	Methods	Flesh	Color	Smell	Average score	*Method chosen
Specialist 1	A	A1	3	2	2	2.33	4
		A2	1	1	1	1.00	
		A3	1	0	1	0.67	
		A4	2	2	2	2.00	
	B	B1	0	1	1	0.67	4
		B2	2	1	1	1.33	
		B3	3	3	2	2.67	
		B4	2	2	2	2.00	
	C	C1	1	2	2	1.67	3
		C2	1	1	1	1.00	
		C3	2	2	2	2.00	
		C4	3	2	2	2.33	
Specialist 2	A	A1	1	2	0	1.00	3
		A2	1	1	0	0.67	
		A3	0	2	0	0.67	
		A4	1	2	0	1.00	
	B	B1	0	2	0	0.67	1
		B2	0	1	0	0.33	
		B3	0	3	0	1.00	
		B4	1	3	0	1.33	
	C	C1	1	2	0	1.00	3
		C2	0	1	0	0.33	
		C3	1	2	0	1.00	
		C4	1	3	0	1.33	
Specialist 3	A	A1	1	3	1	1.67	1
		A2	0	0	1	0.33	
		A3	0	2	1	1.00	
		A4	0	2	1	1.00	
	B	B1	0	1	0	0.33	3
		B2	0	1	0	0.33	
		B3	0	3	1	1.33	
		B4	0	3	1	1.33	
	C	C1	0	2	1	1.00	3
		C2	0	1	1	0.67	
		C3	0	3	1	1.33	
		C4	1	2	1	1.33	

Note: *Method chosen was based on validation form filled up by forensic pathologists regardless of the average score given.

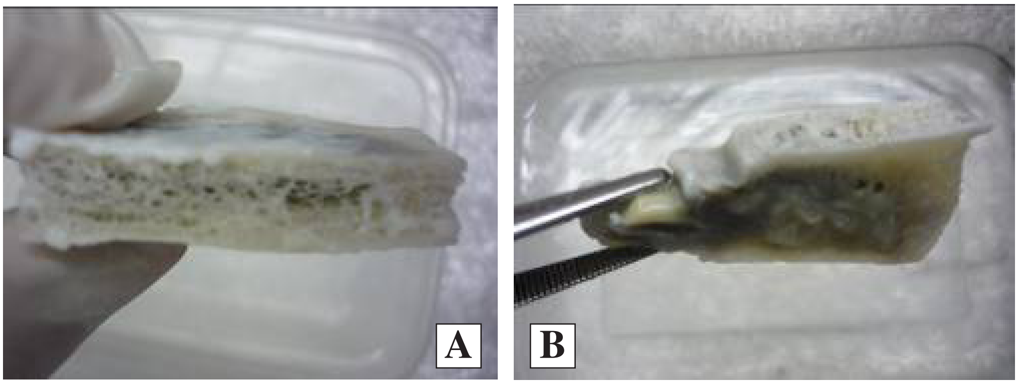

FIG. 7: Bones treated with chemicals (A) shows brittle bone that is easily broken, while with detergent (B) shows porous bone surface due to protein digestion.

Table 9: Details of blind validation scores by forensic scientists (SEM)

Scientists	Methods	Images	Score	Average
Scientist 1	A	1	3	2.00
		2	2	
		3	1	
	B	4	2	2.33
		5	1	
		6	4	
	C	7	1	*1.00
		8	0	
		9	2	
	D	10	2	1.67
		11	2	
		12	1	
Scientist 2	A	1	4	2.33
		2	2	
		3	1	
	B	4	3	2.67
		5	1	
		6	4	
	C	7	3	*2.00
		8	1	
		9	2	
	D	10	3	*2.00
		11	2	
		12	1	
Scientist 3	A	1	3	*1.67
		2	2	
		3	0	
	B	4	2	2.33
		5	1	
		6	4	
	C	7	3	*1.67
		8	0	
		9	2	
	D	10	2	*1.67
		11	2	
		12	1	

*Lowest score amongst the 4 methods in 1 set of bones.

TABLE 10: Cost analysis (entomology approach)

		Maggots (*C.r.*)						Maggots (*O.s.*)					
No.	**Start date**	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Total MYR**	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Total**
1	26/12/2013	4.00	0.00	1.00	0.00	0.00	5.00	4.00	0.00	0.00	0.00	0.00	4.00
2	8/1/2014	4.00	0.00	0.00	0.00	0.00	4.00	4.00	0.00	1.00	0.00	0.00	5.00
3	8/1/2014	4.00	0.00	0.00	0.00	0.00	4.00	4.00	0.00	1.00	0.00	0.00	5.00
4	8/1/2014	4.00	0.00	0.00	0.00	0.00	4.00	4.00	0.00	1.00	0.00	0.00	5.00
5	19/2/2014	4.00	0.00	0.00	0.00	0.00	4.00						
6	19/2/2014	4.00	0.00	0.00	0.00	0.00	4.00	4.00	0.00	0.00	0.00	0.00	4.00
7	19/2/2014	4.00	0.00	0.00	0.00	0.00	4.00	4.00	0.00	0.00	0.00	0.00	4.00
8	19/2/2014	4.00	0.00	0.00	0.00	0.00	4.00	4.00	0.00	0.00	0.00	0.00	4.00
9	11/4/2014	4.00	0.00	0.00	0.00	0.00	4.00	4.00	0.00	0.00	0.00	0.00	4.00
10	23/4/2014							4.00	0.00	0.00	0.00	0.00	4.00
Average							**4.11**						**4.33**

Units are in Malaysian ringgit (MYR)

DISCUSSION

Total weight reduction itself is not good enough to reflect the effectiveness of cleaning because it would be affected by the amount of flesh attached to the bone prior to the cleaning process. Hence, the use of the weight reduction rate could eliminate this factor. The average weight reduction per day within 12 days for the detergent method was most probably unreliable and unusual as the trending rose again at day 6 up to 1.3 gm per day from 1.1 gm per day. This was most probably due to bone decalcification from the effects of bleaching agents contained in the laundry detergents. Hence, its actual weight reduction per day within 12 days would be lower than achieved by maggots during the bone cleaning process. Maggots was the best in this aspect of evaluation because they fed on the flesh to grow from the early larva stage until the final 3rd instar and some of them had already turned into pupae within the 12-day period.

The lowest median number of days to achieve an average score of less than 1.5 within 12 days for the detergent methods was impressive possibly due to the effects of enzymes in the laundry detergents quickly digesting the protein and fat attached to the bones. However, the use of maggots was totally dependent on the

TABLE 11: Cost analysis (chemical approach)

		Detergent (Oxi-Bleach)						Chemicals					
No.	**Start date**	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Total MYR**	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Total MYR**
1	26/12/2013	3.10	0.00	1.10	0.00	0.00	4.20	48.40	0.00	46.40	0.00	0.00	94.80
2	8/1/2014	3.10	0.00	1.10	0.00	0.00	4.20	48.40	0.00	46.40	0.00	0.00	94.80
3	8/1/2014	3.10	0.00	1.10	0.00	0.00	4.20	48.40	0.00	46.40	0.00	0.00	94.80
4	8/1/2014	3.10	0.00	1.10	0.00	0.00	4.20	48.40	0.00	46.40	0.00	0.00	94.80
5	19/2/2014	3.10	0.00	1.10	0.00	0.00	4.20						
6	19/2/2014	3.10	0.00	1.10	0.00	0.00	4.20	48.40	0.00	46.40	0.00	0.00	94.80
7	19/2/2014	3.10	0.00	1.10	0.00	0.00	4.20	48.40	0.00	46.40	0.00	0.00	94.80
8	19/2/2014	3.10	0.00	1.10	0.00	0.00	4.20	48.40	0.00	46.40	0.00	0.00	94.80
9	11/4/2014	3.10	0.00	1.10	0.00	0.00	4.20	48.40	0.00	46.40	0.00	0.00	94.80
10	23/4/2014	3.10	0.00	1.10	0.00	0.00	4.20	48.40	0.00	46.40	0.00	0.00	94.80
Average							**4.20**						**94.80**

Units are in Malaysian ringgit (MYR)

number of maggots used for bone cleaning. For this study, only 5 maggots per cm^3 of bone were used and the median number of days was slightly higher than detergents. If the number of maggots was doubled, definitely it would shorten the time taken, possibly as fast as less than 6 days depending on the amount of flesh attached to the bones. Hence, this factor could be solved by using more maggots when a larger portion of bone needs to be cleaned. Furthermore, the feeding habits of maggots was affected by the environment and the condition of the flesh attached to it. For instance, warm but damp surroundings enhanced feeding provided the flesh was not extremely dry.

Forensic specialists had chosen the application of maggots as the best probable cleaning method via blind validation. The use of maggots successfully preserved the original colour, bone surface architecture and morphology with fairly good defleshing of soft tissues or minimal soft tissues present. Chemical was the least preferred. This is probably due to less efficacy of morphological preservation of the bones especially when it caused bones to become more brittle and easily broken off. Detergents which contain a lot of enzymes also digested off the protein of the bones in addition to the protein in the flesh causing a porous bone surface. The chemical method was not preferred due to flesh still being attached and chemical action leading to a brittle appearance of the bones as shown in Figure 7.

Emission scanning electron microscope is a high-tech instrument that provides high-resolution three-dimensional surface images and an increased field of depth.[8] The routine use of macroscopical observation in forensic anthropology is common whereas microscopical studies are rarely reported in the literature. However, there are a few reports on SEM analysis of forensic bone trauma[9] and soft tissue wounds.[10] It has also been used for analysis of surfaces of teeth and in forensic anthropology for analysis of burned bones or burned teeth. In this study, particles or flesh remaining could be seen under SEM microscopically to reveal the cleanliness and morphological features of the bones. The application of chemicals and detergent had clearly caused deterioration of bone morphological features due to decalcification and bleaching effects. Hence, maggots which purely focus on the flesh understandably did not cause any disturbance to the bone morphology and retain it at an original condition to facilitate the trauma analysis (if required).

The use of maggots *C.r.* was the most cost-effective method, excluding maintenance cost. The IMR is a readily available source as it provides the services for colonization of flies and its maggots on a daily basis to the other governmental and non-governmental agencies. Although detergent was also comparatively cost-effective, its cost would increase when applied to increasing size of bones. This would not happen to the cost of the maggots as the cost is relatively maintained or only slightly raised up with increasing bone size to be cleaned since the rearing costs of the flies would be almost similar regardless of number of maggots requested at any particular time.

CONCLUSION

Using maggots was the most time-effective and cost-effective bone cleaning method, achieving an average weight reduction of 1.4 gm per day, a median of 11.3 days to achieve desired score and an average cost of MYR 4.10 per case to reach the desired score within 12 days. Even though laundry detergent achieved a slightly faster median number of days (7.5 days), its decalcification effect made the bone brittle and gave misleading weight reduction. The number of maggots used can be increased to achieve a faster cleaning process without costing much more compared with detergent and chemicals. This conclusion was also supported and validated by the Forensic Specialists using blind validation with 77.8% preference for maggots. Furthermore, Emission scanning electron microscope evaluation also revealed that bone cleaning using maggots especially *Chrysomyia rufifacies* preserved the original condition of the bones better allowing elucidation of bone injuries (if any) consistent with the results of this study. However this is only a small scale study on bone cleaning methods. A bigger scale project using whole body skeletons in an enclosed damp area for the bone cleaning using maggots should be considered to test whether these conclusions can be extended. The main outcome of such a study can lend support for setting up an enclosed area for bone cleaning using maggots under controlled conditions.

ACKNOWLEDGEMENT

The authors are grateful to colleagues of the Institute of Forensic Medicine in HKL for assistance throughout the study as well as the cooperation from Madam Heah Sock Kiang and Mr Hazmizam Hamzah of IMR for the colonization of maggots. Special appreciation is due to Mr Santhana Raj of the IMR for the SEM analysis for validation of this study.

REFERENCES

1. Ohshima T. Forensic wound examination. Forensic Sci Int. 2000; 113(1-3): 153–64.
2. Robert P, David B. The use of Forensic Anthropology. 2nd ed. 2009.
3. Hinshaw SH. Use of dermestid beetles for skeleton preparation [Internet]. [cited 2013 Nov 8]. Available from: http://deepwater.org/bioteacher/11-Ecology/dermestids/colony-maintenance.htm.
4. Nawrocki SP. Cleaning bones [Internet]. 1997 [cited 2013 Nov 9]. Available from: http://archlab.uindy.edu/documents/CleaningBones.pdf.
5. Sullivan LM, Romney CP. Cleaning and preserving animal skulls [Internet]. 1914 [cited 2013 Nov 9]. Available from: http://extension.arizona.edu/sites/extension.arizona.edu/files/pubs/az1144.pdf.
6. Chin HC, Ahmad NW, Lim LH, *et al*. Predation on pupa of *Chrysomya rufifacies* (Marquart) (Diptera: Calliphoridae) by parasitoid, *Exoristobia philippinensis* Ashmead (Hymenoptera: Encyrtidae) and *Ophyra spinigera* larva (Diptera: Muscidae). Trop Biomed; 2009; 26(3): 369–72.
7. Forensic Medicine Department. Bone-cleaning guidelines adopted from Hospital Tengku Ampuan Afzan (Kuantan) and Hospital Queen Elizabeth (Kota Kinabalu); 2010.
8. Alunni-Perret V, Muller-Bolla M, Laugier JP, *et al*. Scanning electron microscopy analysis of experimental bone hacking trauma. J Forensic Sci. 2005; 50(4): 796-801.
9. Humphrey JH, Hutchinson DL. Macroscopic characteristics of hacking trauma. J Forensic Sci. 2001; 46(2): 228–33.
10. Rawson RB, Starich GH, Rawson RD. Scanning electron microscopic analysis of skin resolution as an aid in identifying trauma in forensic investigations. J Forensic Sci. 2000; 45(5): 1023–7.