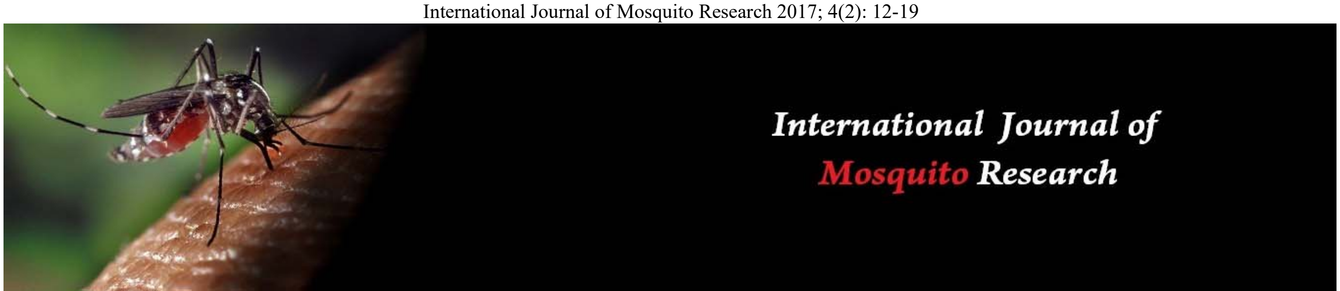

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Assessment of heavy metal concentration on *Aedes* mosquito breeding sites in urban area, Malaysia

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Abstract
The aim of the present study is to establish a baseline of the existing level of heavy metals concentration in positive *Aedes* mosquito larval habitat in the selected dengue hotspot area and non-hotspot area. *Aedes* survey was conducted in Subang Jaya Municipality areas to assess the concentration and distribution of heavy metals (Cd, Cr, Cu, Fe, Pb, Mn and Zn) in mosquito larval habitat. Water samples were (n = 141) collected and were analyzed using Atomic Absorption Spectrometer (AAS) and other standard laboratory protocols. Then, by using microscope, *Aedes* larvae species was determined and the weight of pupae and length of 3rd instars larvae was measured. The concentration of heavy metal in dengue hotspot area was found to be relatively higher than corresponding level on the non-hotspot area at all breeding site investigated. Consistent with other finding, the present study proves that the heavy metal concentration varies between container material and its concentration. Overall, the key dengue vectors are preferential adapted with the heavy metal concentration and thus may affect the development and its lifecycle.

Keywords: Dengue, *Aedes*, heavy metal, breeding sites, urban area, Malaysia

1. Introduction

Dengue continues to be one of the most important public health problems in Malaysia. The disease is transmitted by *Aedes* mosquitoes, which particularly in the tropics affecting humans and remains unique because its prevention depends entirely on vector control [1-4]. To date, the most widely used method to prevent dengue outbreaks is by attacking the mosquito's breeding sites, often called source reduction by eliminating the vector breeding sites if possible, and do a larviciding of breeding sites that cannot be eliminated. Although the control measures have been performed according to the plan, the epidemic pattern of the disease has shown variation in the number of cases reported weekly.

The abundance of mosquito species is influenced by availability of micro-habitat for breeding, physicochemical parameters of breeding sites and anthropogenic related factors [5]. Changes in the physicochemical and biotic characteristics of surface water habitats may create conditions either favorable or unfavorable to the breeding success of mosquitoes, depending on the ranges of tolerance of different species [4]. This can have implications for vector-borne diseases, because a habitat change that favors potential vector species can ultimately lead to increased rates of disease transmission [6].

Heavy metals have recently been reported in mosquito larvae breeding habitat [7]. Usually, heavy metals pollution can have a devastating effect on the ecological balance of aquatic environments, limiting the diversity of aquatic organism and plants. For instance, there are indications that the level of pollution in water bodies directly influences the diversity and abundances of larval stage mosquito species. According to the study conducted in Kisumu Kenya, copper was positively associated with the presence of *Ae. aegypti* and lead was associated with the presence of *An. Gambiae* and *Ae. aegypti* [8].

The presence of certain trace metals and nutrients in the water of breeding habitats produce a high numbers of mosquitoes such as the *Anopheline* and the *Culicines* in densely populated areas posing a risk of chronic malaria fatigue on the people in endemic communities [8]. Although there are many integrated approaches involving indoor residual spraying control measures within dwelling places of households, schools and markets in addition to larvaciding on the surface waters to control the various mosquitoes species have been seriously implemented, previous studies revealed that, all the control strategies could not yet avail a lasting solution to the problem [9, 10].

The physicochemical characteristics of the breeding habitats have been related to the behavior of mosquito species [8]. By doing so, it might be possible to predict whether the population of mosquitoes has the potential to further rise due to unpredicted changes that could be associated with the changing climatic conditions such as heavy rainfall and unexpected flooding, urbanization, mass consumption and mass waste generation and consequential pollution of the environment [9]. In addition, creation of slums and improper disposal of waste water from various channels may directly or indirectly constitute mosquito breeding sites [10].

Most studies focus on the adult stage of dengue vectors, and surprisingly few studies are directed to understanding the biology and ecology of the immature stages [11-15]. Yet, these stages determine the abundance, dynamics and fitness of the adults, and as a consequence, dengue transmission. In order to overcome this shortcoming, this study was designed to understand element of the aquatic immature stages.

Through this study, a baseline of existing levels of heavy metal in potential mosquito larval habitat was established. On the other hand, the relationship between concentration of heavy metals and the presence of mosquito larvae in water bodies will provide a fundamental knowledge of the biology and ecology of mosquitoes. Mosquito breeding generally occurs in a wide range of habitats with different types of waters. These are known to be specific for many mosquito species, although the factors involved are not clearly known. The physical and chemical nature of the water probably determines the selection of breeding sites.

2. Materials and methods

This study was carried out in Subang Jaya, where a total of 17 ecological sampling sites were determined from the 4 sub-district (Puchong, Subang, Kinrara and Seri Kembangan) based on the the calculated frequency index, duration index and intensity index from reported dengue cases between 2006 and 2008 (Table 1).

The methodology of this project is divided into two parts which is (i) *Aedes* survey and (ii) heavy metals analysis. *Aedes* survey involved the process of collection, identification and determination of larvae and pupae in each breeding site while heavy metals analysis consisted of collection, preparation, preservation and analysis of water samples using Atomic Absorption Spectrophotometer (AAS).

A survey was conducted to inspect for the presence and density of larvae in all potential mosquitoes breeding sites in two types of breeding site known as permanent or temporal breeding sites. Permanent breeding sites refers to drainage canal, watering point, erosion pit, retention ponds, and lakes, while examples for temporal habitat of larval mosquito are flower vases, uncovered barrels, buckets, and discarded tires. Temporary breeding sites can appear during the rainy season in tires, step tracks, puddles, ditches and garbage cans, or in debris on construction sites [17]. The types of breeding sites and container material of breeding site were also recorded and was categorized into eleven groups [(1) aluminium, (2) cement, (3) ceramic, (4) fiber, (5) glass, (6) metal, (7) paper, (8) plastic, (9) polystyrene, (10) PVC, and (11) rubber].

All larvae and pupae collected from positive breeding sites were placed into bottles, preserved with alcohol and brought back to the laboratory. The number of larvae and pupae were calculated to determine the density of larvae in each breeding site. Lastly the 3rd instars of larvae and pupae were separated to measure the individual length and weight respectively.

Water samples were randomly collected in plastic bottles using dipper technique. The water was immediately acidified (pH less than 2.0), by adding 1 ml of analytical grade concentrated HNO_3. This preservation was done to prevent heavy metal adsorption onto the container walls as well as to inhibit the activity of micro-organisms, which might cause changes in trace metal levels in the water samples.

In-situ parameters measured include pH, dissolved oxygen, conductivity, and turbidity. Water samples were analyzed for cadmium, chromium, copper, iron, lead, manganese and zinc by Atomic Absorption Spectrophotometer (AAS).

Table 1: Detailed description with geographic information of the study populations

Zone	Location	Category	No of sample taken
Puchong	Taman Puchong Perdana	Hotspot	4
Puchong	Taman Puchong Indah	Hotspot	4
Subang	PJS 9	Hotspot	7
Subang	PJS 7	Hotspot	8
Seri Kembangan	Taman Universiti	Hotspot	9
Seri Kembangan	Taman Serdang Jaya	Hotspot	6
Subang	USJ 6	Hotspot	7
Subang	USJ 11	Hotspot	3
Seri Kembangan	Taman Sri Serdang	Hotspot	10
Puchong	Taman Kinrara	Hotspot	5
Puchong	Bandar Puchong Jaya	Hotspot	4
Puchong	Bandar Puteri	Hotspot	1
Puchong	Kampung Batu 3	Hotspot	11
Seri Kembangan	Taman Batu 13	Hotspot	9
Seri Kembangan	Taman Sg. Besi Indah	Hotspot	7
Seri Kembangan	Serdang Jaya	Hotspot	8

3. Results

A total of 103 positive samples were collected from dengue hotspots area in Subang Jaya (Puchong, Subang, Kinrara, and Seri Kembangan); where 85 samples were identified as *Ae. albopictus,* 14 samples are *Ae. aegypti* and the remaining 4 samples are culex species. *Ae. albopictus* was identified the most dominant species across the study site. The distribution density of immature *Aedes* varied widely among the container material. Figure 1 indicate the portion pattern of breeding site by container material. The largest portion of the chart is plastic with 38% of the total samples collected followed by metal (21%), and rubber (12%), ceramic (10%), PVC (9%), aluminum (3%), polystyrene (2%) and glass (2%) respectively. The smallest portion was dominated by paper, cement and fiber containers contributing 1% each as the material for breeding site.

Based on the container materials, the *Aedes* larvae and pupae were collected and count for identification. The details of typology of *Aedes* production sites based on different container material are shown in Table 2. The productivity of the different container material were calculated in order to evaluate the density of *Aedes* mosquitoes. The highest productivity was found in breeding sites with ceramic material (ρ:48.4) while the lowest was in paper material (ρ:3).

Table 2: Detailed description with geographic information of the study populations

Container Material	No. Positive	Total of *Aedes* immatures	Productivity (ρ)	Mean Length of 3rd instar larvae (cm)	Mean Wet weight of pupae (g)
1. Aluminium	4	74	18.5	0.687±0.304	2.590±1.668
2. Concrete	2	29	14.5	0.670±0.137	2.720±0.000
3. Ceramic	16	775	48.4	0.592±0.081	2.396±1.055
4. Fiber	2	76	38	0.670±0.059	1.625±0.637
5. Glass	3	26	8.7	0.670±0.059	2.070±0.529
6. Metal	20	753	37.7	0.659±0.051	1.908±0.636
7. Paper	1	3	3	0.330±0.000	0.000±0.000*
8. Plastic	54	2011	37.2	0.614±0.042	2.347±0.442
9. Polystyrene	3	90	30	0.637±0.142	2.363±1.299
10. PVC	12	373	31.1	0.691±0.049	2.290±0.992
11. Rubber	18	710	39.4	0.671±0.039	2.040±0.686
Total	135	4920	306.5	0.635±0.024	2.223±0.264

*not detected

Productivity = Total Aedes larvae / No. positive

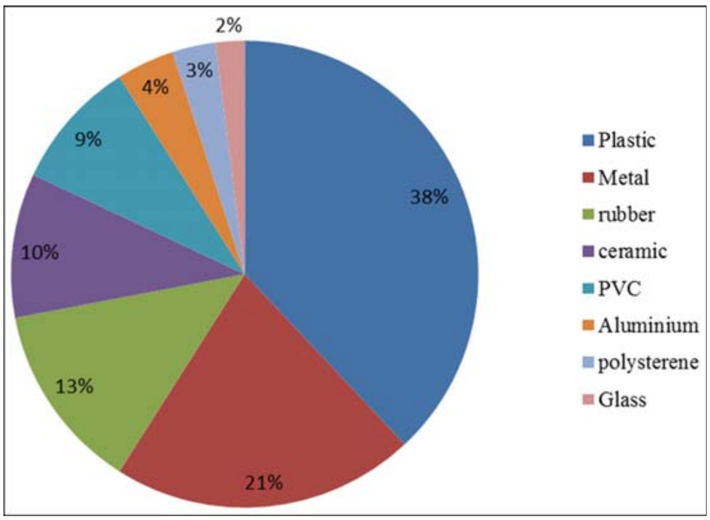
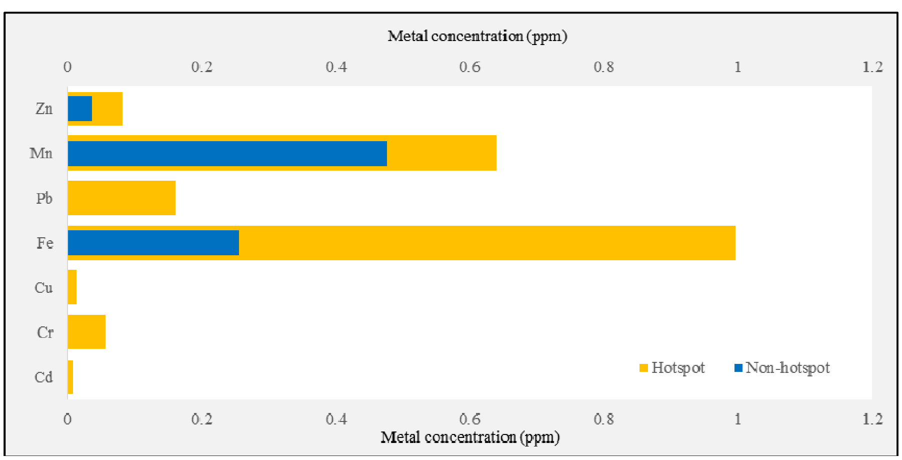


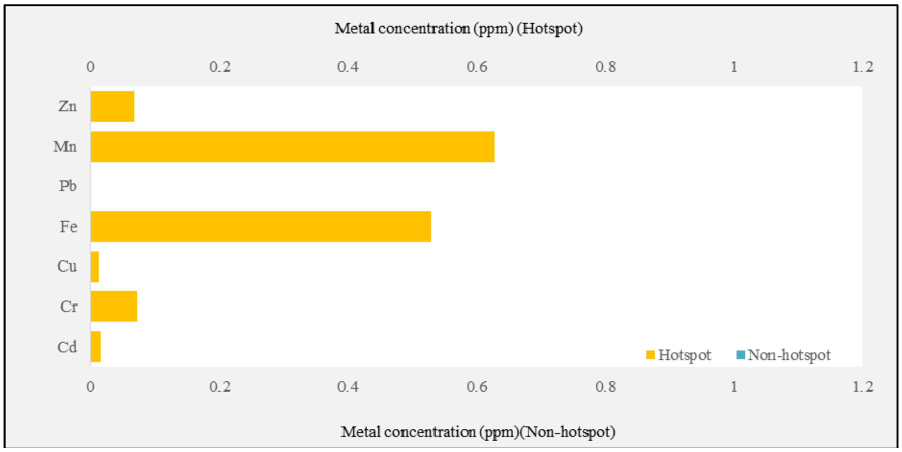
Fig 1: Pattern of breeding site by container material

3.1 Determination of heavy metal concentration in positive mosquito habitat.

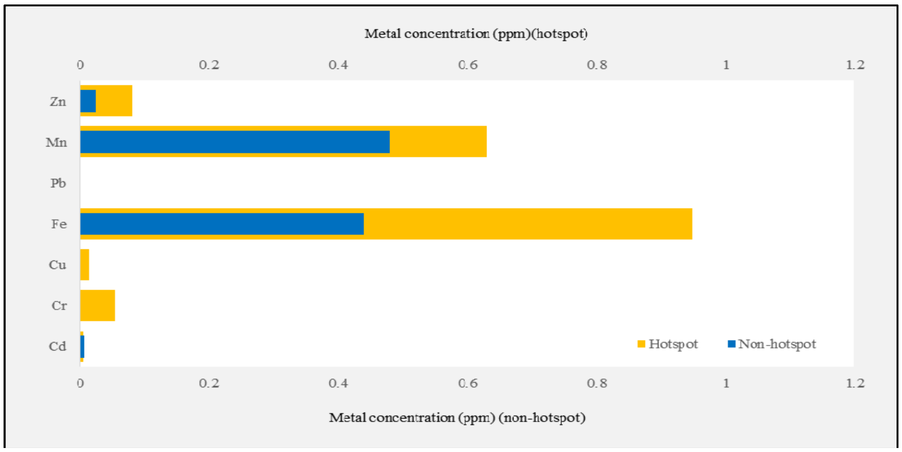
In the comparison of metal concentrations, the concentration of iron was found to be highest in a hotspot area at 0.996 ppm while in the non-hotspot area manganese was the highest at 0.477 ppm. Chromium, copper and lead were only detected in samples from hotspot areas. The least concentration of heavy metal is cadmium at 0.008 ppm at hotspot area and for the non-hotspot area the least heavy metal concentration is zinc at 0.036 ppm. Concentrations of all heavy metals were consistently higher in water from hotspot area compared to non-hotspot area. The biggest difference involved Iron, where more than four-fold difference was observed. The complete result of each heavy metals are shown in Figure 2a.



(a) Mean concentrations of heavy metal (ppm) in water of larval in hotspot and non-hotspot area (Overall)



(b) Mean concentration of heavy metal for *Ae. aegypti* in hotspot and non-hotspot area



(c) Mean concentration of heavy metal for *Ae. albopictus* in hotspot and non-hotspot area

Fig 2: The mean concentration of heavy metal detected in positive breeding sites for hotspot (yellow histogram) and non-hotspot (blue histogram): (a) Overall mean concentration of dengue vectors; (b) Concentration of heavy metal for *Ae. aegypti* breeding sites and (c) Concentration of heavy metal for *Ae. albopictus* breeding sites

The concentration of heavy metals was intensively analysed based on the presence of *Aedes* species which is *Ae. albopictus* and *Ae. aegypti*. Through this, comparison on concentration of heavy metals for both mosquitoes species habitat was made. Figure 2b shows the mean concentration of heavy metals at hotspot and non-hotspot area for *Ae. aegypti* species. There are obvious difference of heavy metals concentration between hotspot and non-hotspot for *Ae. aegypti* species. From all collected sample at hotspot area, 14 of the samples are *Ae. aegypti* while there is none of *Ae. aegypti* species found at non-hotspot area. However, the highest concentration of heavy metals at hotspot area is manganese which is 0.628 ppm while the lowest is copper with 0.011 ppm.

For *Ae. albopictus* species (Figure 2c), the total numbers of samples in hotspot area are 85 and 35 for the non-hotspot area. With the exception of cadmium, concentrations of all other metals were consistently higher in water from hotspot area than from non-hotspot area. The highest concentration of heavy metals for hotspot area is iron which is 0.949 ppm, while the highest concentration of heavy metals in the non-hotspot area is manganese which is 0.479 ppm. Lead is not detected at both hotspot and non-hotspot area. The lowest heavy metal concentration at both hotspot area and non-hotspot area is cadmium at 0.006 ppm and 0.007 ppm, respectively.

3.2 Distribution of heavy metal in relation to container material

Figure 3 shows the concentration of heavy metals which was classified accordingly by container material. Cadmium is highly present in Aluminium container (Cd: 0.06 ppm), while chromium and copper is higher in glass container (Cr: 0.074 ppm; Cu: 0.033 ppm). Iron was highly detected in Aluminium container with 3.218 ppm.

Moreover, manganese was highly detected on ceramic container and zinc was found highly in rubber container. Iron was found in all container types and was much higher in concentration compared to other metals. Then, concrete and polystyrene container shows high concentration of manganese with 0.348 ppm and 0.507 ppm respectively. Breeding habitat material from paper had the presence of the least amount of heavy metals, testing positive for only chromium at 0.057 ppm.

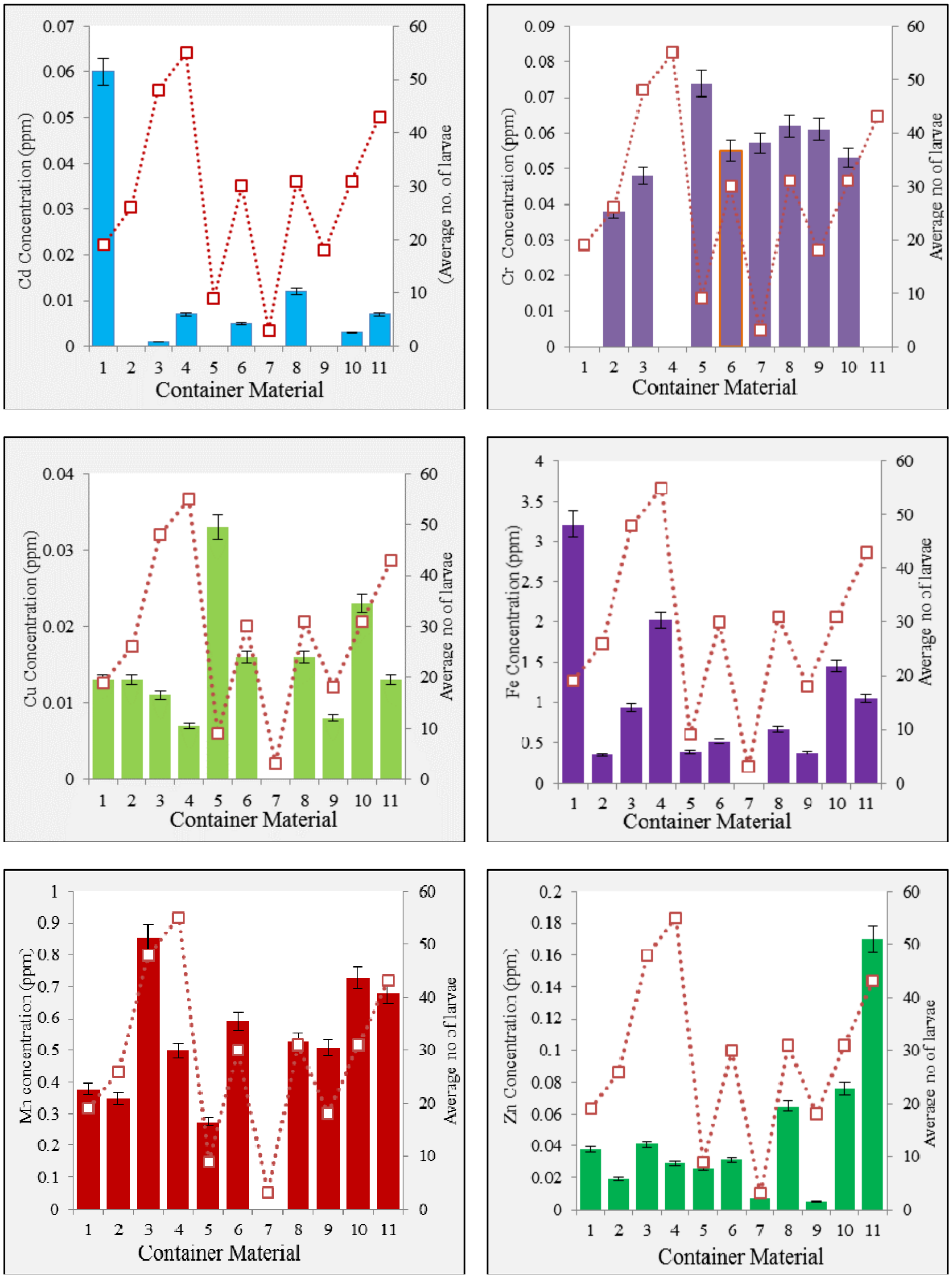


Fig 3: Distribution of each heavy metal concentration by container material (1) Aluminium, (2) Concrete, (3) Ceramic, (4) Fiber, (5) Glass, (6) Metal, (7) Paper, (8) Plastic, (9) Polystyrene, (10) PVC, (11) Rubber.

The concentration of heavy metal in hotspot and non-hotspot breeding sites depicted by different container materials as shown in Table 3. In non-hotspot areas, chromium, copper and lead were not detected in any samples, while cadmium was detected in only plastic container material. No metal were detected in container material made from aluminum, concrete, fiber, glass or paper. In hotspot areas, lead was the least detected metal and only detected in PVC container material. All container material tested positive for at least one metal. Iron was the metal detected in the most number of samples (91%) and in the highest concentration.

Table 3: Heavy Metal concentration by container material: (a) Hotspot area (b) Non hotspot

(a) Hotspot area

Container Material	Metal Concentration (mg/L)						
	Cd	Cr	Cu	Fe	Pb	Mn	Zn
Aluminium	-	0.015	0.006	3.218	-	0.378	0.038
Concrete	-	0.038	-	0.011	-	0.396	0.005
Ceramic	-	0.012	0.010	0.037	-	0.777	0.010
Fiber	-	-	0.007	3.942	-	0.565	0.050
Glass	-	0.049	0.011	0.261	-	0.274	0.026
Metal	0.001	0.007	0.010	0.418	-	0.456	0.034
Paper	-	0.057	-	-	-	-	-
Plastic	0.003	0.018	0.010	0.763	-	0.511	0.079
Polystyrene	-	0.031	0.004	0.492	-	-	0.017
PVC	0.001	0.013	0.013	1.051	0.013	0.539	0.075
Rubber	0.001	-	0.013	1.100	-	0.533	0.203

*(-): not detected

(b) Non-hotspot area

Container Material	Metal Concentration (mg/L)						
	Cd	Cr	Cu	Fe	Pb	Mn	Zn
Aluminium	-	-	-	-	-	-	-
Concrete	-	-	-	-	-	-	-
Ceramic	-	-	-	-	-	0.653	0.026
Fiber	-	-	-	-	-	-	-
Glass	-	-	-	-	-	-	-
Metal	-	-	-	0.789	-	0.655	0.005
Paper	-	-	-	-	-	-	-
Plastic	0.007	-	-	0.298	-	0.413	0.041
Polystyrene	-	-	-	0.143	-	0.507	0.168
PVC	-	-	-	0.529	-	0.089	0.015
Rubber	-	-	-	0.346	-	0.503	0.005

*(-)not detected

4. Discussion

The assessment of the heavy metal in potential urban hotspot and non-hotspot area was attempted by examining the relative concentration of heavy metal in both areas. Result revealed that, the heavy metal concentration is relatively higher in hotspot area compared to non-hotspot area. These results are consistent with a study conducted by Paul *et al*., (2008) on distribution of heavy metals in urban and rural areas. The factors underlying this difference may be complex and detailed comparative studies are needed on the sources of pollutants and particularly their physical and chemical characteristics, precipitation patterns, and composition of flora growing in the areas. There is a study reported that elevated levels of the heavy metals observed in human made, as compared to natural habitats, underscores the anthropogenic nature of heavy metal pollution in the cities (Paul *et al*. 2008). Khandekar *et al*., (1987) mentioned that the concentration of heavy metal such as lead (Pb), cadmium (Cd), zinc (Zn) and copper (Cu) in air, water, surface soil and food are greater in high traffic zones [20].

Aedes mosquitoes serve as vectors to transmit the dengue virus to human. These mosquitoes do not demonstrate a marked seasonal variation and is available throughout the year. They breed in artificial and natural containers and receptacles which hold clean and clear water. Some of the preferential breeding sites are containers such as ant traps, earthen jars, flower pots, drums, concrete tank, coconut shells and discarded tires [21]. The heavy metal concentration varies between container types indicating it serves as a receptacle for domestic and industrial pollutant exposure and act as preferential breeding sites.

The present data have shown that there is no significant pattern on the existing level of heavy metal detected with the abundance of larvae as closely monitored in this study. *Aedes* mosquitoes are normally known to proliferate almost exclusively in relatively unpolluted environment [19]. Concerning the effect of heavy metal on the development of the mosquitoes at larval stage, the Spearman correlation coefficient was carried out. A complex but non-significant association was found between heavy metal concentration and larval abundance of *Aedes* mosquitoes except for copper which show a significant correlation. The results indicate that the elevated levels of heavy metal in different breeding habitats have been adapted by the *Aedes* larval mosquitoes. Thus, this condition (presence of heavy metal in mosquitoes breeding habitat) may affect its survival and their lifecycles.

With regards to environmental contamination, research on heavy metal effects on mosquito biochemistry has started only recently. Only scattered information on effects of heavy metal stress on metabolism is available. In contrast, the biological impact of heavy metals on aquatic insects has been extensively studied in nature and in the laboratory [22]. In addition to mortality, exposure of heavy metals can result in changes in terms of their fecundity and fertility of mosquitoes [22]. However, some mosquitoes can survive in polluted wastewater as mentioned by Kitvatanachai *et al*., (2011). Moreover, mosquitoes are also sensitive to bio-reporters of heavy metals contamination because the exposure occurs during critical stage of insect development such as embryogenesis, larval development and pupation. The toxicity of some heavy metals against these mosquitoes is not yet known.

Aquatics insects accumulate heavy metals and have long been subjugated as indicator species of environmental pollution [23]. They are sensitive bio-indicators of heavy metal contamination because exposure takes place during vital stages of insect development such as embryogenesis, larval development and pupation [24]. In addition to mortality, exposure of aquatic insects to heavy metals can result in locomotion, behavior, oviposition and mating changes. Basically, aquatics organisms can absorb and accumulate heavy metals in their tissues that can induce expression of metal-responsive genes capable of encoding heat shock, metallothionein and metal-binding proteins in invertebrates. This is associated with the development of pesticide resistance among mosquito. Frequently a pest becomes resistant to a pesticide because it evolves physiological changes that protect it from the chemical.

4. Conclusion

In conclusion, concentration of most heavy metals in mosquito larval habitat in urban hotspot area is relatively high due domestic and industrial exposure. Most mosquito species examined, appear to perform well in these habitats, and further studies are needed to elucidate the underlying mechanisms.

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