CHAPTER FIVE

GENERAL DISCUSSION

5.0 The Effect of Water Quality Parameters on the Breeding and Survival of *A. aegypti*

In line with the research objectives enlisted earlier, the main purpose of this study was firstly to determine water quality parameters such as pH, weight of suspended solids, rate of filtration and the concentrations of As, Co, Cr, Mn, Fe, Zn, Mg, Cd, Cu, Pb and Sn of the 16 freshwater samples that were collected from the environment. Secondly, it was to determine the significant effect of these parameters on the breeding and survival of *A. aegypti*.

From Table 4.1, it could be shown that all the collected water samples had a slightly acidic pH with the exception of water sampled at the construction site. The acidity in these water samples could have contributed to the accumulation of heavy and trace metals. Nordberg *et al.* (1985) noted that acid precipitation increases metallic ion concentrations. Acid precipitation is thought to be the main cause of mobilization of lead in soils and deterioration of surface materials containing lead and Cd. Decreasing pH increases corrosiveness of water enhancing the mobilization of metal salts from soil and metallic compounds.

From the data recorded in Table 4. 4 of the survival of *A. aegypti* in the 16 different water samples a few interesting observations can be made. The larvae bred in the Cheras rainwater sample showed a higher mortality at the pupal stage compared to all other water samples. From Figure 4.3 it can be shown that the rainwater sample collected from Cheras had a comparatively higher Cd content. The locality from which this rainwater sample was collected has heavy traffic congestion and the smoke emitted from these vehicles can cause severe pollution to the rain water. A review by Horner (1996) revealed that Cd, Pb and Zn were extracted from car tyres using simulated acid-rain solutions (pH 2.5). The respective mean concentrations of Cd, Pb and Zn that leached into the soil were 22, 1160 and 1235 ppm respectively. Dannecker (1991) found that in a heavily congested area with heavy traffic, during a rainfall, the content of noxious substances such as Cd in the collected rainwater reached a level that was risky if the surface waters served as drainage ditches. This may explain the high Cd concentration in the Cheras rainwater sample. Larval survival in this sample preceding the pupal stage was on the average of 99%. However, a drastic drop to about 45% survival was observed from the pupa to the adult stage (as shown in Figure 4.6). As the *Aedes* mosquito is known to survive and proliferate rapidly in rainwater, the reason for pupal mortality in this particular rainwater sample warrants further investigation.

A. aegypti survived in Sungai Keroh (Point E) water sample but a steady decrease in survival was shown in all 3 stages of egg, larva and pupa (Figure 4.2.2). Only an average of 78% of eggs hatched and 79% of the hatched larva survived to the point of pupa. Only 73% of the pupae survived to the adult stage. From the scatterplot in Figure 4.2, it is observed that Sungai Keroh (Point E) depicts a heavily polluted river carrying in its content effluents from nearby factories and organic pollutants discharged from the residents at its banks. A comparatively higher concentration of As and Mn was clearly evident in this water sample. Kalbitz and Wennrich (1998) found that the concentration of As in water samples are positively correlated with dissolved organic matter. The behavior of As in the soil and aquatic systems is also dependent on various processes the most important ones being oxidation-reduction, precipitation, adsorption-desorption, ligand formation and biomethylation. The distribution of As(III)/As(V) mainly depends on the redox potential and the pH. The redox reactions involved are catalyzed by manganese oxides. Therefore a high content of As in an aquatic system is often associated with a high concentration of Mn in it. Further investigation has to be carried out to verify the effect of varying concentrations of As and Mn on the breeding and survival of *A. aegypti*.

A. aegypti is known to be a container breeder and oviposition sites are often in stagnant water bodies. The ordination of the sources of these water samples (Figure 4.1) shows that stagnant waters from an abandoned land (Point I), swamp water (Point II), pools from a construction site (Point III), rusty container (Point IV), drain near an industry (Point V) and pond were (Point VI) seen to have a high concentration of Co and Cr. It is known that Co is adsorbed onto organic carbon (Sanchez *et al.*,1988) where the peat soil samples rich in organic carbon has a higher sorption for Co compared to soil samples with less organic carbon content. This suggests the importance of organic complexes for the retention of Co at low pH where hydrolysis of metals and sorption is expected to be low. An investigation by Kalbitz and Wenrich (1988) on the mobilization of heavy metals in polluted wetland soils and its dependence on dissolved organic matter suggests that the concentration of Cr in the soil is positively correlated with dissolved organic matter causing a higher concentration of these elements in these spots. The high organic content in the soil surrounding the stagnant water bodies could have retained elements such as Co and Cr which leached from the soil into the water that stagnated after a rainfall.

An interesting deduction can be derived from the survival of *A. aegypti* in stagnant waters as seen from Figure 4.8. Although the hatching rate was on an average of 80%, the survival of larva to the emergence of adult mosquitoes was only on an average of 60%. It can be deduced that the survival of *A. aegypti* to the adult stage is considerably low. Further study has to be carried out to determine the parameters responsible for hampering the survival of *A. aegypti* in stagnant water bodies.

From the correlation analysis carried out, a few observations could be made as shown in the correlation matrix in Table 4.9. The rate of mortality at the embryonic phase was positively correlated with the concentration of As and rate of filtration. From the multiple regression analysis in Table 4.10, the final selected independent variables used to predict mortality at the egg to larval phase were As, Cd, Pb and rate of filtration. In a study to determine the biological effects of heavy metals on the development of *A. aegypti*, Rayms-Keller *et al.* (1998) found that mosquito eggs immersed in 32ppm Cu or 5ppm Cd did not hatch. The arrest of hatching was in part reversible by the removal of heavy metals. However, the concentration of the Cu or Cd in the water samples in this investigation were lower than these concentrations and therefore the effect on egg survival need not have to be the same. Further investigation by immersing the *A. aegypti* eggs in different concentrations of Cd and Cu is needed to verify the actual concentration of these elements needed to induce *A. aegypti* embryonic mortality.

Della Torre *et al.* (1993) found that copper contained larvicidal properties against *A. albopictus* (a close relative of *A. aegypti*) The results show that the strongest lethal effect was on the first stage larva. Bellini *et al.* (1998) confirmed that metallic copper could be used as a practical method for preventing *A. albopictus* development in small containers such as flower saucers found in urban areas. In this study, multiwire electric cable was used as a source of metallic copper in flower saucers colonized by *A. albopictus*. A linear regression coefficient of 0.68 was obtained between Cu concentration in the water during larval development and the relative production of adults. Larval mortality was higher in earlier instars with less evident effect on third instar larva and pupa. The strongest larvicidal action is presumed to partially explain the effect of metallic Cu on *A. albopictus* larva.

In another investigation, O'Meara *et al.* (1992) conducted a study on reduced mosquito production in cemetery vases with copper liners. Water-holding stone vases were sampled in four Central Florida cemeteries to compare the prevalence of mosquitoes in containers with and without metallic liners. Overall, immature mosquito forms were found in more than 60% of the vases lacking liners and in more than 50% of the vases with Al liners. Significantly, fewer vases with copper liners were positive for mosquitoes. High mortality and a lack of development were observed in a field test involving the introduction of *A. aegypti* larvae into stone vases with copper liners.

The investigations made so far on the effect of metallic copper on the mortality of *A. albopictus* and *A. aegypti* did not take into account the concentration of dissolved Cu in aquatic sources. The reason behind the lack of data from this project to support the positive correlation between the concentration of Cu and mortality at larva phase could be due to the low concentration of Cu in the water samples collected from the environment that was not sufficient to evoke a response.

At the pupa phase, however, it can be deduced from the correlation matrix in Table 4.9 that the rate of mortality was correlated with the concentrations of Co, Cr and Cd. Rayms-Keller *et al.* (1998) found that the mortality rate of third instar larvae exposed to heavy metals for 24 hours was dependent on the concentration of metals and their doses. The 50% lethal concentration (LC50) endpoints were 3.1ppm for Hg, 16.5ppm for Cd and 33ppm for Cu. Braeckman *et al.* (1997) evaluated the toxicity of $CdCl_2$, $HgCl_2$ and MeHgCl on the C6/36 cell line of *A. albopictus*. This cell line proved to be a suitable tool for studying heavy metal toxicity in insect cells. Since data on heavy metal toxicity in invertebrate cell cultures are almost nonexistent, the results obtained were based on *in vivo* and *in vitro* invertebrate studies. Viability and proliferation were assessed by dye exclusion and DNA quantification respectively. These viability tests were carried out with and without fetal calf serum in the media. The 3 metal species decreased viability to different extents with MeHgCl concentration decreasing viability to a greater extent than $HgCl_2$ and $CdCl_2$ concentration. Fetal calf serum had a positive effect. In serum deprived cultures, LD50 values were 140.2, 2.51 and 2.08 mumol/L for $CdCl_2$, $HgCl_2$ and MeHgCl respectively. The viability curve for $CdCl_2$ under serum free conditions suggests the induction of a cell defense system. The 3 metal species also inhibited cell proliferation with MeHgCl exerting the greatest inhibition followed by the concentration of $CdCl_2$ and finally the concentration of $HgCl_2$. In summary, low MeHgCl concentration caused both cell death and inhibited cell proliferation. $HgCl_2$ disrupted the plasma membrane and $CdCl_2$ inhibited cell proliferation.

The findings of the present study reaffirm the negative correlation that exists between the survival of immature *A. aegypti* and the concentration of Cd in the water source. Rayms-Keller *et al.* (1998) found that the mortality rate of third instar larva exposed to heavy metals for 24 hours was metal and dose-dependent. The 50% lethal concentration (LC50) endpoints were 3.1ppm for Hg, 16.5ppm for Cd and 33ppm for Cu. A proportion of third instar larva exposed to Cu or Cd for 24 hours failed to produce a dissectable peritrophic matrix. However, further study needs to be conducted specifically on different concentrations of Cd in relation to pupal mortality as it can be noted that Cheras rainwater which had a comparatively higher concentration of Cd and also recorded a higher pupal mortality rate compared to the rest of the water samples. However, no specific study has been carried out on the mortality of *A. aegypti* pupa in relation to the concentration of Co and Cr and the findings in this study warrants further investigation.