

Published in final edited form as:

Med Vet Entomol. 2010 June ; 24(2): 101–107. doi:10.1111/j.1365-2915.2010.00863.x.

Biological cost of tolerance to heavy metals in the mosquito

Anopheles gambiae

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Abstract

The global rate of heavy metal pollution is rapidly increasing in different habitats. *Anopheles* malaria vector species appear to tolerate many aquatic habitats with metal pollutants, despite their normal proclivity for 'clean' water (i.e., generally water free of organic matter). Investigations were conducted to establish whether there are biological costs for tolerance to heavy metals in *Anopheles gambiae* Giles *sensu stricto* (Diptera: Culicidae), and to assess the potential impact of heavy metal pollution on mosquito ecology. *Anopheles gambiae* s.s. were selected for cadmium, copper or lead tolerance through chronic exposure of immature stages to solutions of the metals for three successive generations. Biological costs were assessed in the fourth generation by horizontal life table analysis. Tolerance in larvae to cadmium (as cadmium chloride, CdCl₂), copper (as copper II nitrate hydrate, (Cu (NO₃)₂ · 2·5H₂O) and lead (as lead II nitrate, (Pb (NO₃)₂), monitored by changes in LC₅₀ concentrations of the metals, changed from, 6.07, 12.42 and 493.32 µg/L to 4.45, 25.02 and 516.69 µg/L, respectively, after 3 generations of exposure. The metal-selected strains had a significantly lower magnitude of egg viability, larval and pupal survivorship, adult emergence, fecundity and net reproductive rate than the control strain. The population doubling times were significantly longer and the instantaneous birth rates lower in most metal-selected strains relative to the control strain. Our results suggest that although *An. gambiae* s.s. displays the potential to develop tolerance to heavy metals, particularly copper, this may occur at a significant biological cost, which can adversely affect its ecological fitness.

Keywords

Anopheles gambiae; biological cost; cadmium; copper; heavy metals; lead; tolerance

Introduction

Urban settings represent potentially permanent hot spots for malaria vector production and malaria transmission in Africa (Matthys *et al.*, 2006; Afrane *et al.*, 2004; Robert *et al.*, 1998; Trape & Zoulani, 1987). Growing evidence suggests that *Anopheles gambiae* Giles *s.s.*, the most prolific African malaria vector, is expanding its ecological niche into polluted habitats. Recent studies found *An. gambiae* larvae thriving in a variety of anthropogenic urban water bodies, which contained pollution from domestic and/or industrial sewage (Djouaka *et al.*, 2007; Awolola *et al.*, 2007), including heavy metals in excess of natural loads (Mireji *et al.*, 2008). These mosquito larvae appear to have increased their tolerance, and possibly developed resistance, to the pollutants in their natural habitats.

Adaptation of this mosquito to the urban environment is a real threat that can seriously impact the health of the population. However, environmental changes and subsequent adaptation can have consequences on biological fitness of the mosquito (Reed *et al.*, 2003), especially if inherited resistance to selecting agents such as heavy metals develops (Orr, 1998). In the absence of compensatory secondary mutations (Levin *et al.*, 2000), the cost would be reflected in a decline of tolerant individuals in environments devoid of heavy metals; these individuals could be displaced by naïve populations with greater reproductive and growth rates (Agnew *et al.*, 2004).

The purpose of this study was to determine if *An. gambiae s.s.* populations exhibit a natural range of tolerances to the toxic effects of heavy metals, or if the degree of tolerance can be increased under selection pressure. We hypothesized that the tolerance to heavy metals we observed in wild *An. gambiae s.s.* populations (Mireji *et al.*, 2008) has an adverse effect on the biological fitness of the affected mosquito populations. Results could demonstrate the existence of a genetically controlled mechanism of resistance to heavy metals. A second aim was to define biological costs of tolerance to heavy metals in *An. gambiae s.s.* following generational selection by cadmium, copper or lead.

Materials and methods

Heavy metals

Cadmium, copper and lead were used in the following forms: cadmium chloride (CdCl_2) 99.99% pure, copper II nitrate hydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 2.5\text{H}_2\text{O}$) >99 % pure and lead II nitrate ($\text{Pb}(\text{NO}_3)_2$) 99.5% pure analytical salts, sourced from Fisher Scientific, Fair Lawn, NJ, U.S.A., Sigma-Aldrich, Laborchemikalien, GMBH, Germany and Prolabo, Fontenay, France, respectively.

Test insects

Anopheles gambiae s.s. mosquitoes were obtained from a colony kept by the International Center of Insect Physiology and Ecology (ICIPE), Nairobi Kenya. This colony was originally collected from Mbita field station (00.025°S, 34.013°E), South Nyanza province, Kenya in December, 2000, where *An. gambiae s.s.* is abundant in nature. At the time of this work, the colony was in the 35th filial generation post-field sampling. To our knowledge, the mosquitoes had not been exposed knowingly to heavy metals.

Mosquito rearing

Standard procedures for rearing *Anopheles* mosquitoes were followed (Ford & Green, 1972). All life stages were reared in an insectary under controlled environmental conditions ($28 \pm 2^\circ\text{C}$, 75 – 80 % RH and LD 12: 12 h photoperiod) in the Animal Rearing and Quarantine Unit (ARQU) of ICIPE, Nairobi, Kenya. From the day of emergence, adult mosquitoes were

provided with cotton wool soaked in a 10% sugar solution. Female mosquitoes were blood-fed on anaesthetized mice. Larvae were fed pulverized Tetramin fish food (Tetra GmbH, Melle, Germany). Approval for feeding mosquitoes on mice was obtained from the Kenya National Ethical Review Board (protocol number KEMRI/RES/7/3/1) with the Protocol reviewed by the KEMRI animal care and use committee (ACUC).

Generation of metal tolerant *An. gambiae* s.s. strain

Anopheles gambiae s.s. third-instar larvae were selected for heavy metal tolerance tests through f_1 - f_3 generations, in empirically determined, maximum acceptable toxicant concentrations (MATC) of cadmium, copper or lead. Eggs (1500 per replicate) and subsequent emergent immature stages (larvae and pupae) were exposed to the metal solutions, (separately for each metal) and in three replicates. The larvae were normally propagated in 1,500 mL of respective metal solutions in polypropylene cylindrical pans with a radius and height of 10.5 and 24.1 cm, respectively. The MATC were 0.36, 1.86 or 4.39 $\mu\text{g/L}$ for cadmium, copper and lead, respectively. Tolerance to cadmium, copper or lead in the first and third generations was monitored through LC_{50} acute toxicity testing (Chareonviriyaphap *et al.*, 2002). Toxicity range tests (24 h) of cadmium, copper or lead were conducted on the first and third generations using third-instar *An. gambiae* s.s. larvae. After determining the lower and upper toxicity ranges of each metal, 24 h acute toxicity tests were conducted. Lower and upper ranges were concentrations that caused more than 10 % or less than 96 % mortality, respectively. Three replicates ($n = 25$ larvae per replicate) were exposed to five lead, cadmium or copper concentrations within the established toxicity response ranges (Finney, 1971) in 400 mL of distilled water in the polypropylene cylindrical pans.

Larval mortalities were evaluated 24 h post exposure and LC_{50} determined by Probit Analysis. The larvae were not fed during the exposure period. The generation of LC_{50} values, and respective slopes for each metal selected strain, are presented in Table 1. A control colony was reared simultaneously in a separate room and handled in the same manner through all manipulations, but was not exposed to heavy metals. Emergent adult survivors from each treatment replicate and generation were propagated separately. There were a total of 12 colonies used for each of three generations: nine heavy metal tolerant treatment strains and three control strains.

All the concentrations were validated by direct quantitative determination of cadmium, copper or lead separately in each exposure concentration and replicate using Buck Scientific 210VGP flame atomic absorption spectrophotometer (BuckScientific, East Norwalk, CT, U.S.A.) according to the manufacturer's instructions. Quality control was achieved using certified reference sediment material for cadmium, copper and lead (IAEA 433) from the International Atomic Energy Agency (Wyse *et al.*, 2004).

Effect of heavy metal selection on immature *An. gambiae* s.s. survivorship

Egg hatchability—Samples of eggs ($n = 1500$) were collected separately from the third generation of selection for each of the strains (*i.e.* three heavy metals and one control * three replicates of each). Each sample of eggs was placed in 1,500 mL chlorine-free distilled water and the proportions of eggs that hatched were counted under a dissecting microscope (Leica WILD M3Z) 48 h post-exposure.

Survival rate of larvae to eclosion—All emergent larvae were normally reared in separate 1,500 mL containers, as described above (*i.e.* for each of the three heavy metals and one control* three replicates of each). The initial larvae density for each treatment and replicate was therefore determined by the number of eggs that had hatched in each sample of

eggs. Larvae pupating each day were noted and the pupae were placed into jars within emergence cages, separated by day, treatment and replicate.

The proportion of pupae that survived to produce adults, and the ratio of male: female adults—The numbers and sexes of adults emerging from each jar were recorded daily until the last adult emerged, constituting 12 readings of emergence rates (i.e. three heavy metal solutions plus one control * three replicates of each).

Effect of heavy metal selections on *An. gambiae* s.s. adult survivorship

A sample of emergent adults from each replicate was selected for adult life studies (Reisen & Mahmood, 1980). After males and females had been together in a cage long enough for mating to have occurred, samples of 40 males and 40 females (<12 h old) from the control and each of the metal-selected strains were placed in separate 4 L plastic containers in three replicates. Each sample of mosquitoes was supplied with cotton wool soaked in 10% sucrose and anaesthetized mice (female only) were provided daily as a blood-meal source. For oviposition, water in a plastic cup lined with filter paper (9 cm radius) was provided. Egg cups and sucrose cotton wool were changed daily. The eggs collected represented a contribution from 40 females. Mortality of both sexes was recorded daily until the last mosquito died. The three replicates of each metal and control treatment were reared separately throughout post-selection processes and the replicates had separate growing pans/cages for each line in all assessments.

Data Analysis

Mortality rates for testing the toxicity of each metal were corrected by Abbott's formula (Busvine, 1971) and then transformed to Probits (Finney, 1971) for linear regression analysis and the determination of 50 % lethal concentrations (LC₅₀); Probit analysis software was used (U. S. Environmental Protection Agency (EPA), Cincinnati, OH, U.S.A., Probit Program Version 1.5).

Horizontal life table analytical methods were applied to data for both immature and adult stages of various selection categories, since the cohorts were from distinct lines and were followed consistently through time from egg to adult (Reisen & Mahmood 1980).

Age-specific survivorship of adults (l_x) was determined as

$$l_x = y_x / y_0 \quad (1)$$

where y_x = the number of males or females alive on each day x and y_0 is the original number in the sample, so that the proportion is 1 on day 0.

The age-specific life expectancy (e_x) was computed as

$$e_x = T_x / l_x \quad (2)$$

where

$$T_x = \sum_x^w L_x \quad (3)$$

$$L_x = (l_x + l_{x+1})/2 \quad (4)$$

and, w = the day the last individual died (i.e. e_1 = the adult life expectancy at emergence in days).

In order to transmit *Plasmodium falciparum*, *P. malariae* or *P. vivax*, the anopheline vector must survive for ~ 8, 14 and 7 days, respectively, at temperatures and humidities similar to those applied in this study (Siddons, 1944). Assuming the infective meal is taken during the mosquito's second and third nights of adult life, the potential infective proportion of the population would consist of females not less than 10 days of age. Mean life expectancy at 10 days (e_{10}) was, therefore, computed for the female control and metal-selected strains. The net reproductive rate per cohort, or the total number of living females produced per female (R_0), was established as

$$R_0 = a \sum_{x=1}^w l_x m_x \quad (5)$$

where a = the mean proportion of females that survived from egg through adult emergence, and

$$m_x = E_x p. \quad (6)$$

where E_x is the mean number of larvae (i.e. hatched eggs) produced per female per age interval x , and p is the proportion of the offspring that were female.

In this study, the mean value of 'a' (proportions of females surviving to adult) for the control, cadmium-, copper- and lead- tolerant strains was 0.85, 0.16, 0.18 and 0.11, respectively, based on magnitude of female emergence and the observed sex ratio, and 'p' (proportion of offspring that were female) was 0.51, 0.49, 0.51, and 0.49, respectively. The p values are based on the observed sex ratio of the emerging adults from the non-selected control strain and metal-selected strains.

The age of mean cohort reproduction in days (T_0) was established as

$$T_0 = a \sum_x^w l_x m_x x / R_0 \quad (7)$$

starting at $x = 1$, the day of adult emergence.

The instantaneous rate of increase in females per female (r_m), was calculated using the Dobzhansky *et al.* (1964) modification of the original Euler-Lotka equation by the Newton Raphason iteration method where

$$1 = a \sum_{x=1}^w l_x m_x e^{-r_m(x+D)} \quad (8)$$

e is the base of natural logarithm and D is the duration in days from oviposition in the present generation to first oviposition in the offspring generation. D was considered to be the observed mean median emergence time for females plus the duration of the nulliparous period for that cohort. For non-selected control, cadmium-, copper- and lead-selected strains, D (in days) ranges were 14.66 – 17.33, 16.33 – 17.23, 16.56 – 19.07 and 14.23 – 14.85 days, respectively.

The mean generation time in days (G) was computed as

$$G = \ln R_0 / r_m \quad (9)$$

Since this value included D in the calculation, G was a realistic estimate of the time from mean oviposition in the present generation to mean oviposition of the offspring generation.

The instantaneous birth rate (b) was calculated as

$$b = \ln(1 + \beta) \quad (10)$$

and the instantaneous death rate (d) as

$$d = (b - r_m) \quad (11)$$

where

$$1/\beta = \sum_{x=1}^w l_x e^{-r_m(x+1)} \quad (\text{Birch, 1948}) \quad (12)$$

Population doubling time in days (T_d) was calculated as

$$T_d = \frac{\ln(2)}{r} \quad (\text{Elkinton, 1993}) \quad (13)$$

The effects of the metal selection on egg viability/hatchability, larval and pupal mortalities, and male and female emergence were evaluated by one-way analysis of variance (ANOVA) on the three replicate data sets, with the control and each of the metal treatments as factors. Means that were significantly different were identified using Tukey HSD post-hoc analysis.

Similarly, the effects of heavy metal selection on the sex ratios, fecundities, male and female mean life expectancies from emergence (e_1), net reproductive rates, mean life expectancies at 10 days (e_{10}), (R_0) mean cohort reproduction ages (T_0), instantaneous rates of increases (r_m), mean generation times (G), instantaneous birth rates (b), death rates (d), population doubling times (T_d), r_m/b and b/d among the treatments were also evaluated by one-way ANOVA on respective triplicate data sets, with the control, and each of the metal treatments as factors. Means that were significantly different were identified using Tukey HSD post-hoc analysis. ANOVA and Tukey HSD post-hoc analyses were conducted using SPSS statistical software (SPSS Corporation, Chicago, IL, U.S.A., Statistical Package version 11.5).

Results

Changes in tolerance with metal-selection

Cadmium was found to be the most toxic, followed by copper. The LC_{50} values of copper or lead selection generally increased with generation selection, while that for cadmium selection reduced for unknown reasons (Table 1). Overall, there was approximately a 1.36, 2.01 or 1.05 -fold change in the LC_{50} values following cadmium, copper or lead selection. Computed slopes of regression lines for each generation, an indicator of resistance vs tolerance development, indicate that the values for β did not vary significantly.

Effects of metal-selection on *An. gambiae* s.s. immature survivorship, adult emergence and fecundity

Effects of metal selections on various immature developmental attributes are presented in Table 2. The mean number of eggs that hatched was significantly higher in the controls (98.47 %) than in metal-selected strains (63.80–66.58%) ($F_{(3,11)} = 90.11$, $P < 0.001$). Similarly, the mean number of larvae that died was similar among the metals selected strains (68.86–79.00 %), and significantly higher than in control (10.13%) ($F_{(3,11)} = 121.23$, $P < 0.001$). Larval survivors were significantly higher in the controls (89.87%) than in metal-selected strains except in lead-selected strain, which had significantly less survivors (21.00%) ($F_{(3,11)} = 583.86$, $P < 0.001$). Pupal survivorship pattern was similar to that of the larvae with that exposed to cadmium (19.78%) being significantly higher than that to lead (14.12%) ($F_{(3,11)} = 5.23$, $P < 0.05$).

Significantly more males (87.78%) ($F_{(3,11)} = 719.26$, $P < 0.001$) and females (84.67%) ($F_{(3,11)} = 758.91$, $P < 0.001$) also emerged from control than from any of the metal selected strains (11.24–18.00 %). The male: female sex ratios were also similar ($F_{(3,11)} = 3.09$, $P > 0.05$) among the strains. Fecundity (i.e. the mean number of eggs per female per group) was significantly higher ($F_{(3,11)} = 52.24$, $P < 0.001$) in control than metal-selected strains by 2.4, 1.7 and 2.1-fold in cadmium-, copper- and lead-selected strains, respectively. Overall, general survivorship was significantly higher in control than in metal selected populations.

Effect of metal selection on *An. gambiae* s.s. adult survivorship and fitness

The effects of metal tolerance on the biological fitness of *An. gambiae* s.s. adults are presented in Table 3. The copper-selected strain had lower mean life expectancy (e_1) than cadmium or lead-selected strains. Female life expectancies at 10 days (e_{10}) were similar ($F_{(3,11)} = 3.45$, $P > 0.05$) between all treatments. Net reproductive rates (R_0) were significantly higher ($F_{(3,11)} = 8.17$, $P < 0.01$) in controls by 6-, 20- and 21-fold than in the cadmium-, copper- and lead-selected strains, respectively. Metal-selected strains had significantly lower natural rates of increase (r_m) ($F_{(3,11)} = 5.53$, $P < 0.05$) but longer population doubling times (T_d) ($F_{(3,11)} = 7.81$, $P < 0.01$) than the respective control strains. Additionally, instantaneous birth rates (b) were significantly lower ($F_{(3,11)} = 5.72$, $P < 0.05$) in

copper- or lead-selected strains than in the respective control strains. R_m/b ($F_{(3, 11)} = 12.61$, $P < 0.01$) and b/d ratios ($F_{(3, 11)} = 8.28$, $P < 0.01$) were significantly higher in control than in metal selected strains.

Discussion

This study demonstrates the potential of *An. gambiae* s.s. mosquitoes to withstand chronic exposure to heavy metals in the range occurring in metal-polluted natural habitats (Mireji *et al.*, 2008). Our findings are in harmony with recent reports (Awolola *et al.*, 2007; Djouaka *et al.*, 2007) that indicate the inherent capacity of this vector to adapt genetically to chemically altered habitats encountered in urban environments. This phenomenon may account for recent evidence of rapid increases in population sizes of some disease vectors in urban cities (reviewed by Robert *et al.*, 2003). The present results indicate 23-fold lower tolerance to the metals within our colony relative to the conditions in nature (Mireji *et al.*, 2008), which may be attributed to the relatively shorter selection period (three generations) compared to the natural populations in polluted habitats. The present study also indicates that tolerance occurs at significant fitness costs to the species, which is reflected in reduced survivorship and fecundity, as previously suggested (Orr, 1998; Reed *et al.*, 2003). How this would affect the ecological performance of the mosquito remains unknown and detailed observations of affected field populations over a longer time scale may be necessary to determine the actual ecological fitness status in nature. In our previous study in polluted habitats (Mireji *et al.*, 2008), we found *An. gambiae* s.s. thriving in significantly higher concentrations of heavy metals than used in the present study, indicating that following survival under initial exposures, intensive selection must have occurred and that the acquired fitness was passed over to subsequent generations.

There are other potential implications of our results for the ecological performance of mosquitoes. First, because of the relatively high net reproductive rate (>1) of mosquitoes, metal-selected strains may be highly successful at colonizing habitats (Elkinton, 1993), although depressed egg viability and reduced fecundity of the adult population might lead to substantially reduced population sizes in successive generations. *Anopheles gambiae* s.s. is known to proliferate in different types of habitat in different seasons (Mbogo *et al.*, 1995), some of which have been found recently to be contaminated with heavy metals (Mireji *et al.*, 2008). It may thus be anticipated that the dominance of metal-selected versus naïve populations would alternate seasonally in different habitats, depending on levels of heavy metals. Development of effective molecular markers for the two types of populations; complementary field and controlled laboratory studies may help shed some light on these questions.

The differences in the patterns of tolerance to different metal selections may also suggest different underlying biological regulatory processes for each metal, including their uptake (Buchwalter & Luom, 2005). The metal uptake could have been through larval permeable body surfaces (Rainbow, 2007), the gut (Wang, 2002) or both. Additionally, significant variation between the effects of the metals on survivorship and fecundity indicate major differences in their effective toxicities to mosquitoes (Hare, 1992); the rate of detoxification of the respective metals could be an important determinant (Rainbow, 2002; Marsden & Rainbow, 2004). The variation may also be due to differences in 'carry over' or transmission of some of the metal molecules from mother to offspring and/or other indirect maternal effects. Detailed comparative studies on molecular and physiological processes associated with tolerance and fitness, and particularly those that underlie sequestration of the metals and their bio-availability and bio-magnifications, are needed to elucidate reasons for these differences.

In conclusion, although *An. gambiae* s.s. displays the potential to develop tolerance to increasing levels of heavy metal, it occurs at a significant biological cost, which can adversely affect the mosquito's ecological performance and fitness. Our study provides a starting point for further detailed complementary field and controlled laboratory studies to shed some light on the long-term ecological implications of our findings.

Acknowledgments

We thank Mr. Salim Mwatsahu of the Department of Chemistry, Kenyatta University, Nairobi, Kenya, for his technical assistance in Atomic absorption spectroscopy analysis of the samples and Ms. Milkah Gitau (ICIPE, Nairobi) for her technical assistance with mosquito rearing. This study was funded by National Institutes of Health (NIH) Grant no. NIH ICIDR U19 A145511 and NIH Fogarty ABC Grant no. D43 TWO1142.

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Concentrations ($\mu\text{g/mL}$) that impose 50% mortality (LC_{50}) of third instar *An. gambiae* s.s. larvae in naïve population, and following three successive generational exposures to heavy metals

Table 1

Strain	Generation	LC_{50} ($\mu\text{g/L}$)	95% CI	Slope ($\beta \pm \text{SE}$)	χ^2
Cadmium	F ₀	6.07	4.80 – 7.77	2.81 \pm 0.41	0.17
	F ₃	4.45	3.29 – 5.89	2.65 \pm 0.49	0.34
Copper	F ₀	12.42	8.34 – 18.45	1.76 \pm 0.29	1.52
	F ₃	25.02	18.20 – 35.59	2.12 \pm 0.33	0.31
Lead	F ₀	493.32	245.22 – 1007.77	1.03 \pm 0.18	2.60
	F ₃	516.69	272.44 – 1072.20	1.00 \pm 0.16	1.55

Figures indicated in brackets are 95% confidence intervals about respective median value.

Table 2
Mean (\pm SE) and percentage developmental attributes of metal-selected and non-selected control *An. gambiae* s.s. strains

Aspect	Attribute	Control		Cadmium		Copper	
		\bar{x}	%	\bar{x}	%	\bar{x}	%
Egg	Viability*	1477.00 \pm 8.50 ^a	98.5	997.33 \pm 34.72 ^b	66.5	998.67 \pm 18.98 ^b	66.6
	Mortality	149.67 \pm 2.67 ^a	10.1	687.00 \pm 19.47 ^b	68.9	687.67 \pm 20.67 ^b	68.9
Larvae	Survivorship	1327.33 \pm 6.06 ^a	89.9	310.33 \pm 27.42 ^b	31.1	311.00 \pm 29.72 ^b	31.1
	Mortality	33.33 \pm 2.03 ^{ab}	2.5	61.33 \pm 9.17 ^a	19.8	46.00 \pm 8.50 ^{ab}	14.8
Pupae	Survivorship	1293.33 \pm 4.33 ^a	97.5	248.67 \pm 18.22 ^b	80.2	264.33 \pm 21.94 ^b	85.2
	Emergence*	658.33 \pm 11.46 ^a	87.8	126.33 \pm 8.29 ^b	16.8	128.67 \pm 12.47 ^b	17.2
Adult female	Emergence*	635.00 \pm 11.14 ^a	84.7	121.67 \pm 10.04 ^b	16.2	135.00 \pm 9.54 ^b	18.0
	Fecundity	129.48 \pm 8.11 ^a	-	54.88 \pm 0.76 ^{bc}	-	47.18 \pm 6.27 ^c	-
Sex ratio	(Male/total)	0.51 \pm 0.01 ^a	-	0.51 \pm 0.00 ^a	-	0.49 \pm 0.01 ^a	-

Different letters (superscripts) in the same row (treatments) denote mean differences that are significant at the 0.05 level of *P* by Tukey HSD multiple comparisons; Viability = No of eggs hatching from 1500 eggs exposed; Mortality = No of larvae or pupae that died in larval or pupae stage respectively; Survivorship = No of larvae or pupae that developed into the next stage of the life cycle; Emergence = No of male or female adult mosquitoes that emerged from the 1500 eggs exposed; Fecundity = Mean number of eggs laid per female per group;

* = % in relation to total number of eggs exposed, and assuming a 1:1 sex ratio

Table 3

Adult life table characteristics of control and heavy metal selected *An. gambiae* s.s. strains

Attribute	Control	Cadmium	Copper	Lead
e ₁	8.05 ± 0.98 ^a	5.84 ± 0.53 ^a	5.95 ± 0.94 ^a	6.15 ± 0.79 ^a
Female	6.99 ± 0.89 ^a	5.45 ± 0.35 ^a	4.66 ± 0.27 ^b	5.02 ± 0.48 ^a
e ₁₀	5.43 ± 0.60 ^a	3.42 ± 0.27 ^a	3.20 ± 0.91 ^a	3.61 ± 0.51 ^a
R ₀	26.75 ± 8.01 ^a	4.62 ± 3.07 ^b	1.36 ± 0.23 ^b	1.29 ± 0.06 ^b
T ₀	7.72 ± 0.46 ^a	8.00 ± 0.49 ^a	6.51 ± 0.39 ^a	6.99 ± 0.27 ^a
r _m	0.19 ± 0.06 ^a	0.05 ± 0.03 ^b	0.04 ± 0.02 ^b	0.01 ± 0.00 ^b
G	21.58 ± 0.29 ^a	23.79 ± 1.24 ^a	18.53 ± 5.42 ^a	23.10 ± 0.43 ^a
b	0.24 ± 0.01 ^a	0.16 ± 0.04 ^a	0.15 ± 0.01 ^b	0.12 ± 0.01 ^b
d	0.10 ± 0.02 ^a	0.11 ± 0.01 ^a	0.13 ± 0.01 ^a	0.11 ± 0.01 ^a
T _d	4.90 ± 0.66 ^a	28.83 ± 11.61 ^b	36.80 ± 6.53 ^b	67.52 ± 12.81 ^b
r _m /b	0.60 ± 0.08 ^a	0.25 ± 0.10 ^b	0.09 ± 0.04 ^b	0.09 ± 0.02 ^b
b/d	2.68 ± 0.47 ^a	1.40 ± 0.22 ^b	1.10 ± 0.05 ^b	1.10 ± 0.02 ^b

Different letters (superscripts) denote mean differences that are significant at the 0.05 level of *P* by Tukey HSD multiple comparisons. e₁ = mean life expectancy from emergence in days; e₁₀ = mean life expectancy in days at 10 days post emergence. R₀ = net reproductive rate in living female progeny per female per generation; T₀ = age in days at mean cohort reproduction; r_m = instantaneous rate of increase in living female per female; G = mean generation time in days; b = instantaneous birth; d = death rate, assuming stable age distribution and T_d = population doubling time in days