

Urban mosquitoes and filamentous green algae: their biomonitoring role in heavy metal pollution in open wastewater channels in Nairobi, Kenya

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Abstract

Background

Levels of Mercury (Hg), Lead (Pb), Chromium (Cr), Cadmium (Cd), Thallium (Tl), and Nickel (Ni) in samples of wastewater, filamentous green algae (spirogyra) and urban mosquitoes obtained from open wastewater channels in Nairobi industrial area, Kenya, was established. Industrial wastewater may contain hazardous heavy metals upon exposure. Aquatic organisms in wastewater may accumulate the toxic elements with time. Therefore, human population living in informal settlements in Nairobi industrial area risk exposure to such toxic elements. Biomonitoring using aquatic organisms can be key in metal exposure assessment.

Results

Pb, Cr, & Ni levels ranged from 3.08 to 15.31 µg/L while Tl, Hg, & Cd levels ranged from 0.05 to 0.12 µg/L in wastewater. Pb, Cr, Ni, & Cd levels were above WHO, Kenya & US EPA limits for wastewater but Hg was not. Metals in tap water (control) which ranged from 0.01 to 0.2 µg/L was below WHO, US EPA, & Kenya standard limits. Pb, Cr, Tl, & Ni levels in assorted field mosquitoes were 1.3 to 2.4 times higher than in assorted laboratory-reared mosquitoes. Hg & Cd concentrations in laboratory-reared mosquitoes (0.26 mg/L & 1.8 mg/L respectively) was higher than in field mosquitoes (0.048 mg/L & 0.12 mg/L respectively). Pb, Cr, Ni, & Cd levels in green filamentous algae were 110.62, 29.75, 14.45, & 0.44 mg/L respectively and above WHO limits for vegetable plants. Hg level in algae samples (0.057 mg/L) was below WHO standard limits but above Kenya & US EPA limits in vegetables. Correlations for Pb & Hg ($r = 0.957$; $P < 0.05$); Cd & Cr ($r = 0.985$; $P < 0.05$) in algae samples were noted. The metal concentrations in the samples were in the order, wastewater < mosquitoes < filamentous green algae.

Conclusion

Samples of wastewater, mosquitoes and filamentous green algae from open wastewater channels and immediate vicinity, in Nairobi industrial area (Kenya) contained Hg, Pb, Cr, Cd, Tl, and Ni. Urban mosquitoes and filamentous green algae can play a role of metal biomonitoring in wastewater. The possibility of urban mosquitoes transferring the heavy metals to their hosts when sucking blood should be investigated.

Background

The wide application of heavy metals has raised concerns over their potential disadvantageous effects on human health and environmental modification [1]. Environmental pollution by heavy metals has been associated with mining, foundries, smelters, and other metal-based industrial operations [2]. Disadvantageous health effects associated with heavy metals in exposed humans and animals range

from cancer, systems disorders, developmental anomalies, neurologic and neuro-behavioral disorders, hematologic disorders, DNA damage, cellular and tissue damage, and gastrointestinal toxicity [3, 4, 5, 6, 7]. According to Tchounwou and his colleagues [1], heavy metals toxicity depends on their dose, route of exposure, chemical property as well as age, gender, genetics, and nutritional status of the exposed individuals.

Biological monitoring of water quality involves use of aquatic organisms to detect the pollutants [8]. For instance, heavy metals have previously been reported in mosquito larvae [9]. Biomonitoring of aquatic pollutants using *Culex* mosquito larvae is advantageous because their larvae are common in urban areas where pollution level is likely to be high, and secondly, the *Culex* larvae proliferate fast and have a sufficient developmental interval which gives time for heavy metal uptake [10, 11, 12].

According to Kitvatanachai and others [12], the routine collection of urban mosquitoes for medical research can also avail appropriate samples for monitoring environmental pollution by heavy metals. The uptake of pollutant metals by the mosquito larvae inhabiting contaminated water may occur through direct body absorption or indirectly through ingesting heavy metals contaminated materials. While adult mosquitoes suck nectar, honey and animal blood, their larvae filter algae and other plant materials from the water. However, the larvae of *Toxorhynchites* mosquitoes are predacious and feed on the larvae of other mosquito species, but in absence of a suitable prey they may feed on detritus or exhibit cannibalism [13]. According to Marten [14], abundance of algae usually provides favorable conditions for mosquito proliferation. Spirogyra filamentous algae which usually forms mats in the water serve as mosquito larvae food [15, 16]. Some species of algae however, including those in the order Chlorococcales and the blue green algae (Cyanobacteria) have larvicidal effect because they are indigestible and toxic to mosquito larvae respectively [14]. Hexane and chloroform extracts from marine Phaeophyta algae (*Padina gymnospora*) have been reported to display larvicidal activity against *Aedes aegypti* [17].

Certain species of algae have been reported to uptake heavy metals from contaminated water through biosorption and bioaccumulation [18]. Such species can therefore be used as indicators of the extent of water pollution and in removing pollutants from the wastewater, a process known as phytoremediation. Phytoremediation has emerged as a desirable technology which uses plants for removal of environmental pollution [19]. Both micro and macro algae have been shown to uptake heavy metals from contaminated water naturally and from experimental solutions in the laboratory [20, 21]. Aquatic organisms in the lower trophic levels are better tools for natural biomonitoring of metal since they are among the first in the food chain to be exposed to the pollutants [22]. The heavy metals taken up by the aquatic producers flow into the consumers in a food web through the various aquatic food chains.

The current study was therefore designed to establish the levels of heavy metals in samples of wastewater, filamentous algae (Order Zygnamatales: Genus Spirogyra) and mosquitoes (Order Diptera: Family Culicidae) both larvae and adults, that were obtained from open wastewater channels and the

immediate vicinity in Nairobi industrial area, Kenya. The metallic elements studied were chromium (Cr), cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni), and thallium (Tl).

Results

Physico-chemical parameters of wastewater samples:

The mean range for pH, temperature, total dissolved solids (TDS) and electrical conductivity (EC) of the wastewater samples were 7.28 to 8.78, 16.75 to 26.05° C, 160.33 to 544.67 ppm, and 336.67 to 1134.33 µS/cm respectively (Table 1). All the wastewater samples obtained from the study area were alkaline, with those from Chief's camp (B-1), Kartasi industries (F) and Sinai (G) sites being more alkaline at pH 8.13, 8.59 and 8.78, respectively. Samples of wastewater from open, shallow, and exposed channel at Sinai (G) site had a temperature of 26.05° C compared to samples from shaded channels and with a vegetation cover at Davis & Shirtliff sampling site (E) that had a temperature of 16.75°C. Increased TDS corresponded to increased EC and vice versa. The TDS (ppm) of wastewater samples at sampling sites, Railways lower (C), Railways upper (D), and Sinai (G) were 562.00, 575.33 and 544,67 ppm; while the EC (µS/cm) of the wastewater samples in the same sites were 1134.33, 1072.33, and 1074.33 µS/cm respectively. Both TDS and EC recorded were above the recommended limits by WHO (Table 1). The physico-chemical parameters observed and recorded in the current study from the eight sampling sites differed significantly (F-test, $P < 0.05$).

Levels of heavy metals in samples of wastewater and tap water:

The Pb levels were highest ranging from 13.62 to 15.31 ppb, followed by Ni (4.96 to 6.91 ppb) and the lowest was Tl at 0.05 ppb. The mean concentrations of the heavy metals in acid digested wastewater samples followed an ascending order of $Tl < Hg < Cd < Cr < Ni < Pb$ (Table 2). Mean concentration of Cr (7.49 ± 2.12 ppb) in wastewater samples that were not digested with acids was significantly higher than for the other elements studied (Table 2). The mean concentrations of Pb and Cr in acidified wastewater samples were above the limits set by WHO, US EPA and Kenya. The levels of Hg, Cd, and Ni in acidified wastewater samples were below the limits set by WHO and Kenya. The level of Hg in wastewater samples was above the US EPA limit which is set at 0.00003 ppm (0.03 ppb). The mean concentration of thallium in wastewater was 0.04 ppb but standard limits for WHO, Kenya and US EPA were missing in the literature accessed. The mean concentrations of Hg, Pb, Cr, Cd, Tl and Ni in samples of tap water ranged between 0.01 to 0.2 ppb which were far below the standard limits set by WHO, US EPA, and Kenya (Table 2).

Levels of the selected heavy metals in filamentous green algae

Filamentous green algae were sampled from 4 out of 8 (50 %) sampling sites (Table 3). The mean concentration of heavy metals in the samples of wastewater and in filamentous green algae collected from the same site differed significantly ($P > 0.05$). The average heavy metal concentrations in filamentous green algae samples were between 500 to 5000 times more than the mean concentration of the same metals in wastewater samples in the same sampling site (Tables 2 and 3). The mean

concentrations of heavy metal in filamentous green algae followed an ascending order of Hg < Tl < Cd < Ni < Cr < Pb and ranged from 0.057 to 110.62 ppm (Table 3). The algae samples obtained from Railways Lower (D) and Davis & Shirtliff (E) sampling sites had significantly higher levels of heavy metals ($P < 0.05$) compared to those collected from Kartasi sampling sites (F1a & F1b) as shown in Table 3. Concentrations of Hg, Pb, Cr, Cd and Tl were 1.93 to 2.75 times higher in room temperature dried algae samples (that is before metal analysis) than in lyophilized algae samples (Table 3). The Ni level was however higher in lyophilized algae samples than in the room temperature dried algae samples (Table 3). The mean concentration of heavy metals in algae samples obtained from open wastewater channels were above the limits set for plants (vegetables) by WHO, Kenya and US-EPA except for thallium where the standard limits were missing in the literature accessed (Table 3).

Levels of heavy metals in mosquito samples

There were no adult mosquitoes trapped at site C; and similarly, no mosquito larvae were available for sampling at sites B, F and H. However, the mean concentrations of Hg, Pb, Cr, Cd, and Ni in adult *Culex* mosquitoes' samples collected from Donholm site (H) was significantly higher than the means for the same elements at Kartasi site (F) as shown in Table 4. Similarly, the mean concentrations of Hg, Pb, Cr, Cd, Tl, and Ni in mosquito larvae samples collected from Sinai site (G) were significantly high (Table 4). The mean concentration of heavy metals in field mosquitoes' samples followed an ascending order of Tl < Hg < Cd < Ni < Pb < Cr while that for the laboratory reared mosquito samples was Tl < Hg < Cd & Ni < Cr < Pb (Table 4). The mean concentration of Pb, Cr, Tl, and Ni in assorted field mosquito samples were 1.3 to 2.4 times more than the mean concentration in the assorted laboratory reared mosquito samples. The mean concentrations for Hg (0.26 mg/L) and Cd (1.8 mg/L) in assorted laboratory-reared field mosquitoes were 4.4 and 20 times more respectively than in assorted field mosquitoes which were 0.059 mg/L (Hg) and 0.09 mg/L (Cd) as shown in Figure 1 and Table 4. The mean concentration of Pb, Cr, Cd, Tl, and Ni at Kartasi sampling site, in filamentous algae samples was 3 to 29 times higher than in assorted field mosquito samples (Tables 3 and 4). The level of Tl was below the method detectable level which was set at 0.02 ppm in both the assorted field and laboratory reared mosquito samples (Figure 4 and Table 4).

The mean concentration of Pb, Cr, and Ni in both assorted field and assorted laboratory-reared mosquitoes' samples ranged from 2.33 to 10.53 ppm, which was above the WHO permissible limits (0.5 to 2.0 ppm) for freshwater fish (Table 4). The level of Hg in both assorted field and assorted laboratory-reared mosquitoes' samples ranged from 0.06 to 0.26 ppm and was below the WHO permissible Hg levels (0.5 ppm) for freshwater fish. Similarly, the mean concentration of Cd in assorted laboratory-reared mosquito samples was 1.8 ppm and was above the WHO permissible Cd limits (1.0 ppm) for freshwater fish while the mean Cd level in assorted field mosquito samples was 0.09 ppm (Table 4). WHO permissible limits for thallium in freshwater fish were missing in the literature accessed, and therefore no comparisons were made.

Correlation of the heavy metal levels in wastewater, algae, and mosquito samples

Pairs of heavy metal concentrations such as Pb & Hg; Cd & Cr; Tl & Hg in algae samples correlated strongly, positively, and significantly (Table 5), where an increase in one element corresponded to an increase in the partner element in the pair of metals analyzed. Similarly, concentration of Tl & Cd in wastewater samples correlated positively (Table 5). Strong positive correlations indicated a close association of the elements in samples of wastewater and filamentous algae. A few strong negative correlations between Pb (wastewater) & Hg (algae) where $r = -0.921$, at significance level of 0.079, and between Pb (algae) & Pb (wastewater) where $r = -0.974$, at significance level of 0.026, were observed (Table 5).

Table 6 shows inter-elemental correlation of the mean concentration of pairs of heavy metals in wastewater samples obtained from Sinai site (G). Pairs of elements such as Cd & Pb; and Ni & Pb correlated positively and significantly ($P < 0.05$). Similarly, Cd & Pb; and Tl & Pb in samples of mosquito larvae trapped from wastewater at Sinai showed strong positive significant and negative significant correlations respectively ($P < 0.01$) as shown in Table 6.

Discussion

According to Azam and others [39], insects are the dominant invertebrate faunal group that has been used in biomonitoring and bio assessment studies. This is because insects have a strong relationship with ecology [40]. Insects are abundant and they possess diverse morphologies and functions which enable them to display unique biochemical and genetical responses after their exposure to environmental changes including pollutions. In the current study, field urban mosquitoes were evaluated for their possible role of bio-indication for heavy metal pollution in wastewater since they frequently encounter such water in open channels when accomplishing their life processes including feeding and reproducing. The results showed that the field urban mosquito samples, majority of which belonged to *Culex* species as previously reported [41], had high levels of Pb and Cr compared to the laboratory-reared *Anopheles* and *Aedes* control mosquitoes that were reared in KEMRI – Nairobi, Kenya. This implied that the mosquitoes that breed in contaminated wastewater channels may have absorbed and accumulated Pb and Cr into their body tissues. This observation was supported further by establishing that the wastewater samples from the open channels from which the mosquito larvae were obtained had higher levels of the heavy metals when compared to tap water (Table 2). Dechlorinated tap water is used for rearing of mosquitoes at KEMRI, Nairobi. Previous studies have shown that *Culex* mosquito larvae can be tools for natural biomonitoring of heavy metals since they are among the first in the food chain to be exposed to the heavy metal pollutants [22, 42]. It was also observed in the current study that the laboratory-reared mosquito samples had a slightly higher level of metals including Hg, Cd & Ni (Table 4). This could have been probably attributed to the rearing processes, equipment used, insectary, insect feed, and routine procedures in the rooms adjacent to the insectary where the rearing of mosquitoes took place. According to van der Fels-Klerx and colleagues [43], insects can become exposed to chemical hazards from the substrate used to grow them. Some of the heavy metals are known to escape into the air as tiny particulates [44] which would then easily contaminate the insectary and the mosquitoes being reared. Laboratories have been associated with increased concentration of specific pollutants depending on the

nature of experiments that are being conducted [45]. In a study on indoor air quality in research laboratories, Valavanidis and Vatissta [46] established that respirable suspended particulates (RSP) reached $700 \mu\text{g}/\text{m}^3$ in spring and summer period. Similarly, Rumchev and colleagues [47] in their study on indoor air quality in 15 university laboratories established that the particulate matter ($\text{PM}_{2.5}$ and PM_{10}) were significantly high in Chemistry, Engineering and Biology laboratories. Suspended particulates in the air may include black carbon, heavy metals, spores, dust, pollen grains, liquid aerosols among others, and they tend to be in large quantities in heavily polluted areas and premises.

The mean concentration of heavy metals was highest in filamentous green algae, followed by field mosquito larvae or adult mosquito samples and lowest in wastewater, giving an ascending sequence of wastewater < mosquitoes < filamentous algae. At Kartasi sampling site, the mean concentration of Pb, Cr, Cd, Tl, and Ni in filamentous algae samples was 3 to 29 times higher than in assorted field mosquito samples. This observation was in line with a previous study carried out by Kitvatanachai and others [12] which showed that the levels of Pb was higher in *Cx quinquefasciatus* than in wastewater from the factories and the areas close to the factories. Aquatic insects accumulate heavy metals in their bodies from contaminated aquatic ecosystems because they become exposed during their vital developmental stages and processes including embryogenesis, larval development, and pupation [48, 49]. The emerging and surviving imagoes of aquatic insects are therefore likely to have elevated levels of heavy metal in their bodies as well. In our current studies, field assorted mosquitoes had high concentration of Pb and Cr when compared to the assorted laboratory-reared mosquitoes. Previous studies though, have shown that the process of metamorphosis can be a survival challenge for aquatic insects in metal contaminated aquatic ecosystems because the larvae become exposed to extra stress that enhance the mortality of the imagoes [50]. The urban mosquitoes, majority of which are *Culex pipiens* [41] can breed in wastewater, although when exposed to increased specific heavy metal concentration, their breeding potential is reduced [51]. According to Dom and colleagues [52], the *Aedes* mosquitoes, the key dengue vectors appear to develop adaptations to cope with increased heavy metal concentration in polluted waters. The urban mosquitoes that can breed and survive in polluted waters especially in crowded areas are therefore a health hazard because they can serve as vectors of infectious diseases as well as pollutants contaminated blood sucking insects. It was also shown in our study that the mean levels of Pb, Cr, and Ni in assorted field mosquito samples, which comprised of adults and their larvae, were above the WHO permissible levels for freshwater fish. This agreed with a previous study that reported an increased Pb levels in *Cx. quinquefasciatus* mosquito larvae that were obtained from Pb-contaminated wastewater [12]. Both the assorted field and assorted laboratory-reared mosquito samples had mean Hg levels that were below the WHO permissible levels for freshwater fish. This was in line with a study carried out in North America in which the methylmercury in mosquitoes was a little less than that typically found in fish [53].

From previous studies, algae belonging to the genus *spirogyra* have a significant potential absorbent for heavy metal from contaminated water [54, 55]. Our current study established that the mean concentration of heavy metals in filamentous green algae was 500 to 5000 times more than the mean concentration of

the same metals in the wastewater samples collected from the same site. According to Sunish and Reuben [56], filamentous algae in the mosquito breeding water have nutritive value necessary for mosquito development and adult emergence. Therefore, when the mosquito larvae feed on heavy metal contaminated filamentous algae, the heavy metals may get transferred into their tissues. Feeding process is one of the main pathways through which aquatic invertebrates obtain metals from their surroundings [53, 57]. Our study clearly illustrates occurrence of bioaccumulation of heavy metals in the mosquitoes and aquatic filamentous algae inhabiting contaminated open wastewater channels in Nairobi industrial area, Kenya. This was in line with previous studies which established that contaminated wastewater could lead to a build-up of heavy metals in soils, food crops and macrophytes [58, 59]. According to Gokce [60], use of algae for environmental biomonitoring can be advantageous and suitable because algae is spatially dense, easy to sample where available and store. Similarly, mosquitoes can breed rapidly in stagnant water and are easy to sample, especially the larvae.

Inter-elemental analysis of the metals in the different samples collected from the channels revealed several strong, positive, and significant correlations. Such pairs included Pb & Hg; Cd & Cr; Tl & Hg in algae samples. These correlations suggested that the pairs of the metals may have had a common source, most likely the industries whose wastes were draining into the open channels in the study area. Such industries probably were releasing specific wastes that were rich in certain elements, hence a positive correlation of such elements. This explanation was in line with previous studies carried out in Nigeria and Pakistan [61, 62]. The significant correlation coefficients between pairs of metals in samples of wastewater, filamentous algae and mosquito larvae strongly suggested that the sources of the heavy metal pollution in the study area was mainly anthropogenic.

Our current study raises a few public health implications such as, people can easily become exposed to heavy metal pollutants when clearing and unblocking the wastewater channels when they clog. Prolonged heavy metal exposure can lead to serious toxicity and exposure to potential carcinogenic agents in humans [63]. The heavy metal contaminated wastewater pollutes the surface runoffs after the rains, which then spread the pollutants into the residential areas, soils, crops and public places. Contaminated wastewater may overflow from the channels onto the highways during the heavy rains hence exposing the road users to the pollutants. The mosquitoes that breed successfully from contaminated wastewater channels may accumulate heavy metals in their bodies with time through direct diffusion of such metals into their bodies or by ingesting heavy metal contaminated plant materials that includes algae. Such mosquitoes may therefore serve as both disease vectors as well as pollutants contaminated piercing and blood sucking insects. Studies to verify whether mosquitoes with elevated heavy metals in their tissues can spread such elements through their bites are however lacking. Such a study can involve comparing the levels of heavy metals in salivary glands of mosquitoes exposed and those not exposed. In India, wastewater has been used for microalgae cultivation for biofuel production [64]. The current study has shown that algae present in contaminated wastewater absorbs and accumulates the pollutants, in this case heavy metals. The levels of heavy metals in the algae were higher than in wastewater due to bioaccumulation in the current study. Therefore, harvesting microalgae grown in contaminated wastewater can be a health hazard especially where the level of ignorance and

poverty levels are high and inadequate use of safety measures when handling such algae. In Kenya, commercial cultivation of microalgae for biofuel production is still facing many challenges [65] and even when this economic activity picks, use of untreated wastewater to cultivate the microalgae should be highly discouraged.

Conclusion And Recommendation

The heavy metal concentration in the samples analysed followed an ascending sequence of wastewater < mosquitoes < filamentous algae. The mean concentration of Pb, Cr, and Ni were relatively higher than those of Tl, Hg, and Cd in wastewater, filamentous algae, and field mosquito samples. The mean concentration of the heavy metals in field mosquito samples followed an ascending order of Tl < Hg < Cd < Ni < Pb < Cr. The levels of Pb, Cr, Tl, and Ni in assorted field mosquito samples was significantly higher than in assorted laboratory-reared mosquito samples. The assorted laboratory-reared mosquito however had slightly higher levels of Hg and Cd when compared to the assorted field mosquitoes. The levels of Pb, Cr, and Ni in both the field and laboratory reared mosquitoes were above the WHO permissible limits for freshwater fish. The concentration of Pb, Cr, Ni, and Cd in wastewater were above the limits set by WHO, Kenya and US EPA for wastewater (effluents) however, the level of Hg in wastewater was within the limits set by WHO, Kenya and US EPA. The levels of Pb, Cr, and Cd in algae samples were above the limits set for plants (vegetables) by WHO, Kenya and US EPA while Ni concentration in algae were above the WHO limit in plants. The level of Hg in algae samples was above the limit set by Kenya & US EPA for plants. Strong significant positive correlation ($P < 0.05$) for Pb & Hg and Cd & Cr in algae samples were noted. This study established that the filamentous algae and mosquitoes obtained from open wastewater channels at Nairobi industrial area, Kenya, had higher levels of Pb, Cr, Ni and Cd. Both filamentous algae and urban mosquitoes growing and breeding respectively in contaminated wastewater in open drainage channels can bio-accumulate the heavy metals and therefore have the potential of being used for heavy metal pollution biomonitoring. There is need for efficient wastewater treatment in urban areas for reuse to reduce significant exposure of the vulnerable population to the hazardous contaminants in the wastes. Strict environmental and public health policies should be formulated and adopted by the county and national governments to control environmental pollution by industrial effluents. Public awareness on the toxic nature and health risks of untreated wastewater should be done in addition to utilizing safety precautionary measures by municipal workers and youth groups when unclogging and cleaning up the open waste channels in urban areas in Kenya. The possibility of urban mosquitoes transferring the heavy metals when sucking blood should be investigated. Growing of microalgae for biofuel production, using untreated wastewater should be discouraged.

Methods And Materials

Sampling sites:

Samples were collected from 8 different sampling sites that were coded A to H, at Nairobi industrial area, Kenya. The sites included Tetrapak (A); Chief's Camp at Land Mawe (B); Two sites at Railways near

Enterprise/ Lunga Lunga roads junction (C & D); Davis & Shirtliff along Dondori road (E); Kartasi Industries (F); Rok Industries near Sinai urban slums (G); and Donholm Swamp/Kenya Power & Lighting Station (H) as shown in Figure 2. Samples of wastewater, filamentous green algae, and mosquito larvae were collected from the open wastewater channels. Adult mosquitoes were trapped at night from the factory premises (for the security of the traps) near the open wastewater channels.

Collection and preparation of wastewater samples:

A standard 350 mL dipper was used to collect the wastewater samples from the open channels and placed into clean reagent plastic bottles. The samples were collected in triplicates in equal portions. Two separate portions were separately digested with concentrated hydrochloric (HCL) acid and concentrated nitric (HNO_3) acid respectively by adding three drops of the respective acid per 100ml of wastewater sample. Acidification was meant to inhibit adsorption of dissolved elements onto the interior walls of the plastic bottles as well as preventing microbial reactions [66]. A third portion of wastewater was not acidified to act as a control. Similarly control triplicate samples of tap water were also collected from selected sites in the study area, two portions were acidified while one portion was not. All the samples were labeled appropriately, packaged, and stored in low temperature.

Measuring the physico-chemical parameters of water samples:

The physico-chemical parameters of the wastewater samples including temperature, pH, electrical conductivity (EC), and total dissolved solids (TDS) were measured immediately after collection of wastewater samples at the site using a digital electronic device (HANNA Instruments, H1991300, Romania) and recorded appropriately.

Collecting samples of filamentous green algae:

Filamentous green algae (Figure 3) were collected in triplicates from the open wastewater channels using a large plastic strainer and packaged in well labeled brown paper bags. The strainer was then rinsed in deionized water before being used again. All the samples collected were transferred to Kenyatta University Biochemistry laboratory for further processing.

Preparation of green algae samples for heavy metal analysis:

The filamentous green algae samples were divided into two parts. One part was air dried at room temperature for several days while the remaining part was lyophilized (freeze dried). Both air dried and lyophilized algae samples were ground and sieved to obtain a fine powder as described by Ngure & Kinuthia [67]. The powder was then weighed and packaged in well labeled brown small envelopes to await metal analysis. Briefly, lyophilization involved extracting the algae samples using de-ionized water for 36 hours on an electrical shaker, followed by filtering the extract obtained using clean muslin cloth on a water pump. About 200 ml of the filtrate was then put on clean stainless-steel tray and placed in the deep

freezer for 24 hours at negative 45°C. The samples were then retrieved and placed in a freeze-drier for a further 24 hours at negative 50°C to complete lyophilization.

Outdoor trapping of adult mosquitoes:

Adult mosquitoes were trapped using surveillance standard Centers for Disease Control and prevention (CDC) light traps as described by Mweya and colleagues [68] using carbonated dry ice as the bait. The traps were set in potential breeding sites and amidst the vegetation where applicable (Figure 4) within the factory premise. The trapping commenced from 6:00 PM to 6:00 AM each day. The average number of CDC traps set per sampling site per night was seven depending on the size of the compound. The mosquito trapping activity was carried out daily for two weeks. The field mosquitoes were trapped near the sites where wastewater and algae samples had been collected from.

Collection of mosquito larvae from wastewater:

Mosquito larvae were collected during the day preferably midmorning, from open wastewater channels. Three dips (triplicate) were taken to obtain the larvae from the wastewater, using the standard 350 mL dipper. If less than ten mosquito larvae were captured in the first three attempts, additional two dips were done to obtain a sizable number. The dipper contents were then transferred onto a white plastic tray. The mosquito larvae were sorted, counted and their number per dip per site recorded. The larvae were then placed in plastic Whirl-Pak[®] bags (Bio Quip, Rancho Dominguez, CA) which were approximately half full of the same wastewater from which the larvae were collected. The Whirl-Pak bags containing the larvae were then tightly closed to retain air before transporting to the laboratory as described by Rueda and others [69], where they were identified and preserved.

Preservation of adult mosquitoes and mosquito larvae in the field:

The trapped mosquitoes were processed as described by Tchouassi and colleagues [70]. The trapped mosquitoes were anaesthetized and killed using triethylamine while still in the trap. The mosquitoes were then sorted, counted, and put in Nunc tubes. The adult mosquitoes were then preserved in liquid nitrogen until when they were required for identification at Kenya Medical Research Institute (KEMRI), before processing them further for metal analysis. Similarly, the mosquito larvae were preserved as described by James-Pirri and others [71]. Briefly, the mosquito larvae were retrieved from the Whirl-Pak bags and placed in hot water at a temperature of 87°C for 50 seconds after which they were removed using a strainer. The larvae were then preserved in Dietrich's solution and later transferred into 75% ethanol for further preservation until when they were required for identification and processing for metal analysis.

Morphological identification of the trapped field mosquitoes:

Both mosquito larvae and adults were identified using morphological features up to species level under a stereomicroscope. Appropriate mosquito taxonomic keys for the Sub-Sahara Africa and the East African region [72, 73, 74] were used.

Laboratory rearing of mosquitoes:

Anopheles gambiae s.s., Kisumu strain and *Aedes aegypti*, Mombasa strain laboratory colonised mosquitoes were reared in the laboratory at KEMRI, following the protocol described by Das and colleagues [75]. Mosquito rearing was carried out in the insectary that was maintained at a temperature ranging from 27 to 28°C and approximately 80 % humidity on a 12h/12h light and darkness cycle. Optimal larval concentrations were maintained to avoid possible effects of competition. Mosquito larvae were fed on finely ground *Sera Vipar staple diet*TM (Sera, Germany) while adults were offered a fresh 10% (w/v) glucose solution meal daily and fed on hamster (*Mesocricetus auratus*) as a source of blood meals for egg production. Mosquito larvae were reared in de-chlorinated tap water. De-chlorination of the tap water was achieved by allowing the tap water in a bucket to stand in the insectary chamber for at least 24 hours. These laboratory-reared mosquitoes obtained served as a control in the current study to enable us to compare the levels of heavy metals in field trapped and laboratory mosquitoes.

Preparation of the mosquito samples for metal analysis:

Both the field and laboratory-reared mosquitoes were separately dried from an open room on brown papers, ground and then sieved to obtain a fine powder. The mosquito powder was then weighed, packaged in small new brown envelopes and labeled appropriately for metal analysis.

Analysis of heavy metals for the different samples:

The analysis of heavy metals was carried out at Mineral Laboratories, Bureau Veritas Commodities Ltd, Vancouver, Canada. The protocols included *aqua regia* digestion ultra-trace inductively coupled plasma mass spectroscopy (ICP-MS) for algae and mosquito samples; and ICP-MS (solutions > 0.1% total dissolved solids (TDS)) for water samples as described by the American Herbal Products Association [76]. The digest solution was nebulized, and sample aerosols transferred to argon plasma. The high temperature plasma then produced ions, which were then introduced into the mass spectrometer. The mass spectrometer then sorted out the ions according to their mass-to-charge ration and finally, the ions were quantified with an electron multiplier detector. Certificates of analysis and quality control reports for all the samples analyzed were awarded by the Bureau Veritas, Canada.

Data Analysis:

The statistical package for the social sciences (SPSS) version 20 for Windows at 5% level of significance was used for data analysis. Descriptive statistics involved computing mean, standard error (SE), and standard deviation (SD) for the different variables measured in wastewater, algae, and mosquito samples. One-way analysis of variance (ANOVA) was used to establish whether the differences within and between groups were significant or not. Tukeys and Games-Howell *Post hoc* tests were carried out to establish the pairs of variables that were significantly different. Correlation analysis was carried out to establish the nature of relationship, level of significance between concentrations of heavy metals in

different samples. Pairwise correlation coefficients for the levels of selected heavy metals in wastewater, algae, and mosquito samples were also computed.

Limitations

We acknowledge the limitations of the current study which included: limited samples of filamentous green algae from the sampling sites and the challenge faced in obtaining adequate powdered mosquito samples for adults and larvae separately for metal analysis, hence forcing us to prepare assorted (mixed) mosquito samples for metal analysis.

Abbreviations

ANOVA: Analysis of Variance

AHPA: The American Herbal Products Association

CDC: Centers for Disease Control and prevention

CPCB: Central Pollution Control Board

EMC Environmental Management and Coordination (water quality) regulation

IARC: International Agency for Research on Cancer

ICP-MS: Inductively Coupled Plasma Mass Spectroscopy

KEBS: Kenya Bureau of Standards

KEMRI Kenya Medical Research Institute

NEMA: National Environmental Management Authority

MDL: Method Detection Limit

OKWQS: Oklahoma Water Quality Standards

SWQS: Surface Water Quality Standards

SPSS: Statistical Package for the Social Sciences

TDS Total Dissolved Solids

US EPA: United States Environmental Protection Agency

WHO: World Health Organization

Declarations

Ethical approval and consent to participate

Not applicable

Consent of publication

Not applicable

Availability of supporting data

All the datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Conflict of interests

The authors declare that there are no conflicts of interests, both financial and non-financial competing interests, associated with this manuscript.

Authors Contributions

GK – designed the proposal, sourced for funding, collected samples from the field and was involved in data analysis; VN – oversaw heavy metals analysis of the samples, collection of samples from the field and their preparation for metal analysis; and assisted in data analysis; LK - reviewed the proposal; oversaw rearing of the Anopheles and Aedes mosquitoes in the laboratory for control experiments; and advised on data analysis. All the authors reviewed and corrected the manuscript before it was submitted for publication.

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Tables

Due to technical limitations, table 1, 2, 3, 4, 5, 6 is only available as a download in the Supplemental Files section.

Figures

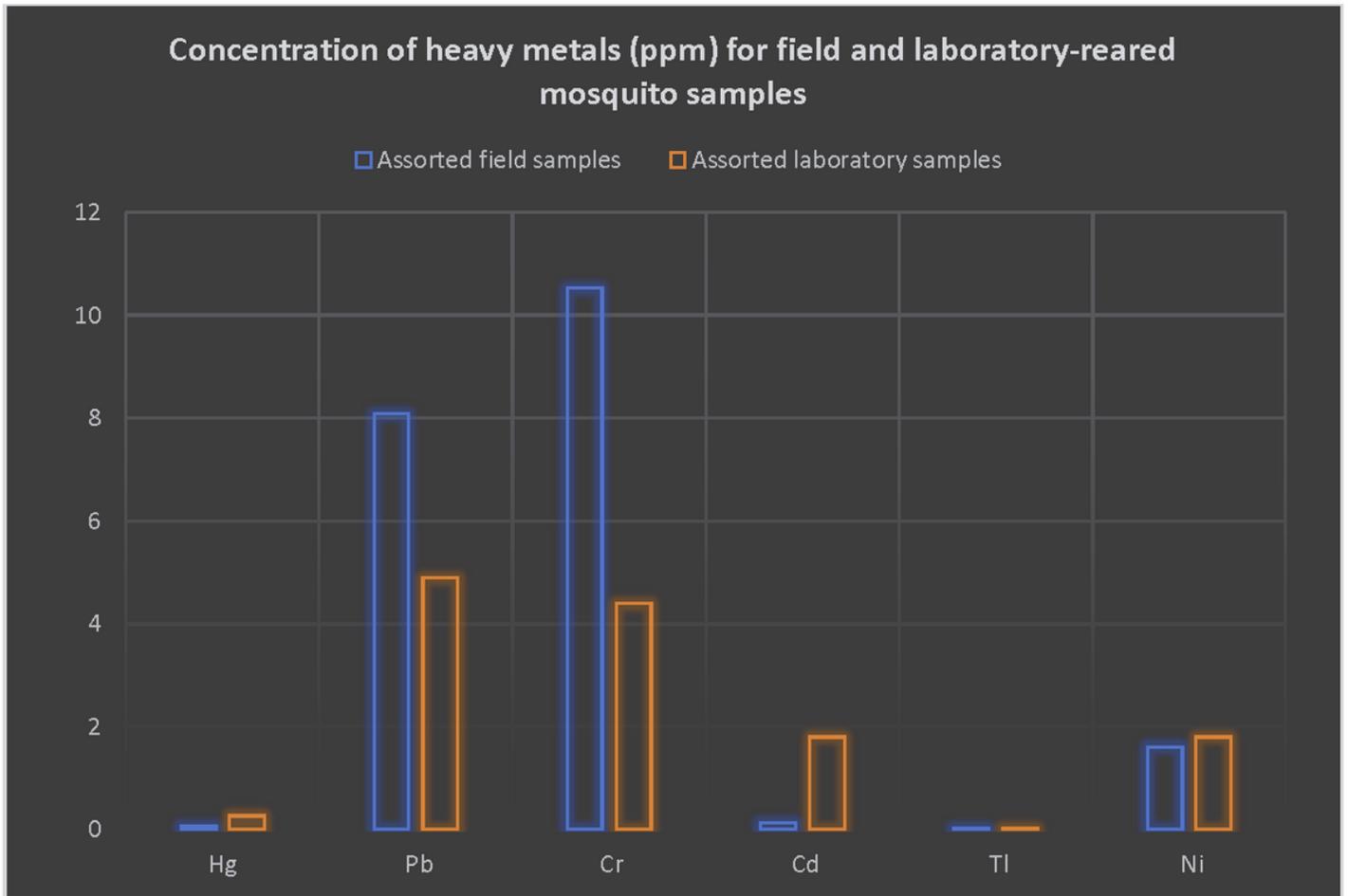


Figure 1

Showing comparison of heavy metal concentration (ppm) in assorted field and assorted laboratory-reared mosquito samples.

Showing the filamentous green algae collected from open wastewater channels near Kartasi industries, Nairobi.



Figure 4

Showing CDC mosquito trap on a tree branch in the premise of Kartasi Industries, Nairobi.

Supplementary Files

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