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Traced Metals and Nutrients in Mosquito Breeding Sites at the Obuasi Municipality of Ashanti Region in Ghana

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Abstract

A survey was conducted in the Obuasi Municipality to assess the impact of some trace metals and nutrients on mosquito breeding sites from 15 randomly selected communities. The water samples, collected fortnightly for eight months, were analyzed using spectrophotometry and other standard laboratory protocols at the AngloGold Ashanti Environmental Quality Assurance Laboratory. The results on trace metals, and nutrients did not reveal any significant pattern of attendant pollution that would influence the breeding patterns of mosquitoes. However, the trace metals ranged from 0.12±0.01mg/L - 13.42±0.01mg/L and 0.01±0.01mg/L - 0.30±0.01mg/L for Fe and Pb respectively in the waters whilst As and Zn were not detected. For the nutrients - nitrate values which were > 1 ranged from 2mg/L – > 40mg/L. Sulphate levels varied between < 20mg/L and > 200mg/L and were above the EPA Maximun Permissible Limit of 1.5 mg/L for polluted natural water bodies. There was no definite pattern in the concentrations of phosphates which varied between 0.1mg/L and 1.5mg/L in the waters. It is apparent that under rising temperature conditions of climate change, the mosquito's habitat may be highly favoured for adaptation and prolific breeding in the tropics and this further creates the opportunity for research partners to get actively involved in finding integrated control measures to counteract the life cycle of the pest.

Keywords: Anglogold Ashanti, Obuasi Municipality, Traced Metal Analysis, Mosquito Breeding Waters

1.0 Introduction

Mosquitoes exploit almost all types of lentic aquatic environments. Anopheles mosquito has been found to use fresh water habitats for breeding. Larvae of anopheles mosquitoes in clear water of suitable pH, temperature and nutrient conditions have been found to thrive in aquatic bodies such as fresh composition (Russel, 1999). However, high water or salt marshes, mangrove swamps, rice fields, current and flooding have been reported to lead to grassy

ditches, the edges of streams and rivers and anopheles species larval deaths due to reduction in small, temporary rain pools (CDC., 2007).

Diagnostic and scientific research has shown that many mosquito species prefer habitats without oxygen tension causing physical harm to the larvae with vegetation whilst some breed in open, sunlight pools (Okogun, 2005). Water of a near neutral pH of 6.8 – 7.2 is preferable for breeding of many species of mosquitoes whereas few species breed in tree holes or the leaf axils of some found to be most optimal for the weakening of the egg shells plants (CDC, 2004). Anopheline species are known to be for the first instar larvae stage to emerge, although large numbers are ground pools breeders (Okogun et al., 2003). Various chemical properties of the larval habitat observed in gutters, peri domestic runoff and domestic are related to vegetation and a wide range of heavy metal, nutrients and physicochemical characteristics of the water, ranging from pH, optimum temperature, concentration of ammonia, nitrate, phosphate, sulphate, iron, lead etc. have been found to affect larval development and survival (Mutero et al., 2004).

According to Okogun et al., 2003, mosquito eggs are white in color when first deposited but darken within 12 to 24 hours. Most species' eggs appear similar when seen by the naked eye, with the exception of the Anopheles spp., whose eggs have, floats attached to each side. When viewed with magnification, eggs of different species can be seen to vary from canoe-shaped to elongate or elongate-oval in shape. Some species lay eggs simply, and others glue them together to form rafts. The incubation period (elapsed time between oviposition and readiness to hatch) is dependent on environmental and genetic factors and varies considerably among different species (Gilvear & Bradley, 2000).

Permanent water and standing water species deposit their eggs directly on the water surface, and these may hatch in one to four days depending on temperature (Brandy and Holum, 1996). In addition, many floodwater and container-breeding species deposit their eggs on moist soil or other wet substrates. These eggs may hatch within a few days after being flooded, or the fully developed larvae may remain within the eggs for up to a year or more depending on immersion conditions. These quiescent eggs accumulate over time due to continued oviposition by blood-fed females. When temporarily flooded, they hatch, along with more recently deposited eggs. Populations can attain large numbers quickly this way. According to CDC, 2004, larvae (wigglers or wrigglers) of all mosquitoes live in the water. Near the last abdominal segment in most species is a siphon or air tube that serves as a respiratory apparatus when the larva suspends vertically below the water surface.

Larvae of Anopheles, however, breathe through a cluster of small abdominal plates, which causes them to lie flat close to the underside of the water surface when not diving. Larvae of some species are predaceous (e.g. Toxorhynchites rutilis and Psorophora ciliate) and prey on other invertebrates, including mosquito larvae. Most larvae are filter feeders, ingesting anything smaller than about 10 microns by vibrating their mouth brushes and sweeping in particulate matter and small organisms from the surrounding water (CDC, 2004).

This study examined under field and laboratory conditions some of the trace metal and nutrient characteristics of the breeding sites for the development and fecundity in Anopheline and Culicine mosquitoes. Prodigious numbers of mosquitoes can hatch simultaneously under the proper conditions. In rapidly developing broods, survival of the immature stages can be quite high, but estimates for many species indicates that immature survival is normally less than 5 percent. But 5 percent of millions represents a sizable number. Irrespective of population densities, if they transmit disease or preferentially feed on humans, which many species do, they become appropriate targets for control activities (Okogun et al., 2003).

1.1 Problem Statement and Justification for the Study

The people of Obuasi Municipality have been complaining of malaria issues over the past decades due to the poor sanitary effects on the environment that is surrounded by many water bodies, and mining as a result of shallow excavations have been a source of breeding sites for mosquitoes as vectors which develop the parasite for malaria transmission. Due to the release of certain trace metals and nutrients into the water, breeding of the various mosquitoes such as the *Anopheline* and the *Culicines* can produce numbers that are densely populated posing so much chronic malaria fatigue on the people in endemic communities (Bradley & Kutz, 2006). Despite the fact that a number of integrated approaches involving indoor residual spraying control measures experiments within dwelling

places of household, schools and markets in addition to larvaeciding on the surface waters to control the various mosquito species have been seriously implemented by the AngloGold Malaria Centre in Ghana just as in Kenya and other tropical countries, previous studies conducted revealed that, all the control strategies could not yet avail a lasting solution to the problem (CDC, 2004). Trying to use the integrated approach by adopting a research principle to investigate what goes on in terms of adaptability of the mosquitoes at their breeding sites to varying conditions of trace metal and nutrient concentration could therefore, help determine appropriate malaria control strategies through sound environmental management principles (Bradley & Kutz, 2006).

The rationale was to relate the trace metal and nutrient characteristics of the breeding habitat with the behavior of mosquito species of public health significance. By so doing, 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 in the Obuasi Municipality and its adjoining peri-urban communities. Additionally, creation of slums and improper disposal of waste water from various channels may directly or indirectly constitute mosquito breeding sites. These amongst other factors, are apparent reasons why it was absolutely necessary to conduct a research to assess the water quality in relation to the species of mosquito larval development by purposively sampling point sources of fresh and stagnant waters in Ghana using the Obuasi Municipality as a case for the study. Suitability of the mosquito breeding sites and the level of water quality in terms of its heavy metal and nutrient characteristics could determine the type of mosquito larval development in a particular water body (Brandy and Holum, 1996).

1.2 Research Objective

The general objective was to assess the nutrient some trace metal and characteristics of water from various mosquito breeding sites within the Obuasi Municipality and other adjoining pri-urban communities.

1.2.1 The Specific Objectives were to:

The specific objectives were to:

1. Survey stagnant water bodies for mosquito larvae at breeding sites.
2. Collect water samples from located breeding sites to the laboratory for analysis.
3. Determine the nutrient and trace metal concentrations of the water samples in which mosquitoes breed.

1.3. Methodology

This work was done under surveillance on experimental bases. Here, some preliminary field survey and mapping of sampling points was done using the Obuasi Municipal Map for identification of various communities. This surveillance for stagnant water bodies for mosquito larvae was done within the sampling duration of eight months in 2010/2011 within fifteen sampling communities. Moreover, water samples were collected from located mosquito breeding sites to the laboratory for analyses using various standard methods. Spectrophotometer was used in determining the nutrients. High electron spectrophotometer was used for general analysis of some traced metals (Fe, Pb, As and Zn) in the water samples.

1.3.1 Methods of Analyses for Heavy (Traced) Metals in Mosquito Breeding Waters

1.3.2 Iron

Iron level of the water was analyzed using the FerroVer Method. In principle, the FerroVer Iron Reagent converts all soluble iron and most insoluble forms of iron in the sample to soluble ferrous iron. The ferrous iron reacts with the 1.10 phenanthroline indicator in the reagent to form an orange colour in proportion to the iron concentration. The initial concentration of iron was determined by selecting Program 265 Iron, FerroVer from the Hach Programs. A clean, round sample cell was filled with a known volume of the water sample diluted to 10mL and the content of one FerroVer Iron Reagent Powder Pillow was then added to it. The sample cell was swirled to mix the contents and the timer icon pressed to begin a three-minute reaction period. Another sample cell was filled with 10mL distilled water (the blank) and placed in the cell holder of the spectrophotometer after thoroughly wiping it. The ‘Zero’ button was

pressed and a 0.00 mg/L Fe concentration was displayed. After the three-minute reaction period, the prepared sample was also placed in the cell holder and 'Read' button pressed. The concentration of iron was displayed in mg/L Fe.

1.3.3 Lead (Pb)

The reagents used include; Potassium sodium titrate, Ammonium Solution - 25% (0.91), EBT, and 0.1M titriplex solution. About 50ml of the raw water sample was measured into the conical flask whilst 2.0g of potassium sodium titrate was added and this preceded the addition of one drop of EBT. Depending on the pH of the water, 2ml ammonium solution was added to the mixture and then heated to about 40°C and titrated quickly with 0.1 M titriplex solution until colour of the solution changed from red to green (microburet). The concentration of lead was then calculated using the formula:

1ml of 0.1M Titriplex =20.721mg of Pb.

1.3.4 Zinc, (Zn)

About 25ml of solution containing Zn salt solution was measured into a 100ml volumetric flask. It was then diluted to 100ml, and finally transferred into a 250ml conical flask where it was coarsely neutralized with 10% NaOH (since the solution was acidic) and a one spoonful of EBT was added. Similarly, 1ml Ammonium solution was finally added and the mixture then titrated with 0.10ml Titriplex III solution until the colour changed to green.

Calculation: 1ml of 0.10m Titriplex iii solution =6.538/ml of Zn.

1.3.5 Arsenic

50ml of the water sample was measured into a 100ml volumetric flask. 1g of ammonium chloride was then added. Afterwards, 5ml of 25ml ammonium solution and 0.1M magnesium sulphate were further added respectively up to the mark with distilled water and then shook vigorously. The solution was allowed to stand for about 15 minutes followed by repeated shaking. After being settled, the mixture was filtered and the first 10-20 filtrate discarded. About 50ml of the remaining filtrate was titrated against 0.1M Titriplex III solutions after adding 1drop of EBT indicator until colour of the solution changed to green.

Calculation:

1ml of 0.1M Titriplex III solution= 1ml 0.1M magnesium sulphate =7.49mg of As = C

As (mg/L) = Cx50x100/100000 = C/200

NB: C value is dependent on volume of water used for the titration.

1.3.6 Sulphate

A test kit was used for the determination of sulphate ions in surfaces water and sewage. The sample tube was rinsed several times with the test sample and filled up to 20 ml mark. The bottle containing the SO_4^{-1} was held vertically and about 10 drops of SO_4^{-1} was added and mixed. One level measuring spoon of SO_4 was added and dissolve by swirling. The test mixture becomes more or less turbid. After 1 minute the liquid from the sample tube was poured into the measuring tube until the black cross on the bottom of the measuring tube was no longer visible (as observed directly from above). The sulphate concentration could be read off directly from the graduation on the measuring tube (bottom of the meniscus curve).

Calculations:

$MgSO4^{2-}/l = mgSO_4^{2-} \times 1000/$ mL sample

1.3.7 Phosphate

For Phosphate analysis, the Stannous chloride method was used. About 0.05ml of phenolphthalein indicator was added to 100ml of sample that was free from colour and turbidity. An acid was added drop wise to neutralize the sample that turned pink. Then, 4.0ml of molybdate reagent 1 and 0.5ml stannous chloride reagent 1 were added with thorough mixing. About 10ml of the prepared sample was taken and the absorbance at wavelength 690mm was read after zeroing with distilled water. The corresponding phosphate concentration was taken on the calibrated curve and calculated using the formula:

Mg/L P = mg P in 100ml x 1000

Sample Volume

1.3.8 Nitrate-Nitrogen (NO_3-N)

In principle Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with gentisic acid to form an amber coloured solution. The concentration of Nitrate-nitrogen was determined by selecting Program 353 N, Nitrate MR from the Hach Programs. A clean, round sample cell was filled with a known sample volume to 10mL and the contents of one NitraVer 5 Nitrate Reagent Powder Pillow was added to it. The sample cell was shaken vigorously to mix the contents and the timer icon pressed to begin a one-minute reaction period. The timer icon was pressed again after the one-minute reaction for a five-minute reaction period to begin. Another sample cell was filled with 10mL distilled water (the blank) and placed in the cell holder of the spectrophotometer after thoroughly wiping it. The 'Zero' button was pressed and a 0.00 mg/L NO_3^--N concentration was displayed. After the five-minute reaction period, the prepared sample was also placed in the cell holder after wiping the sample cell and the 'Read' button was pressed. The concentration of Nitrate-nitrogen was displayed in mg/L NO_3^-N.

1.3.9 Method of Data Analyses

Multivariate analysis was conducted on all nutrients and heavy metal data using Statistical Package for Social Scientists (SPSS) Version 18.0 at $P<0.05$ level of significance.

1.4 Results

1.4.1 Heavy Metal Concentration in Mosquito Breeding Water

Table 1. Mean concentration of heavy metals from mosquito breeding water sampling sites as (homogenous subsets) from October, 2010 – November, 2010.

Communities	Iron (Fe)	Lead (Pb)
1.Gausu	9.67±0.01	0.30±0.01
2.Bedieso	0.31±0.01	0.30±0.01
3.Mensakrom	13.42±0.01	0.30±0.01
4.Kunka New Town	2.71±0.01	0.02±0.01
5.Nyameso	0.23±0.01	0.01±0.01
6.Sanso	1.36±0.01	0.04±0.01
7.Nyiraeso	1.72±0.01	0.02±0.01
8.Bediem	0.45±0.01	0.04±0.01
9.Bongobiri	0.18±0.01	0.05±0.01
10.North Nyamebekyere	0.82±0.01	Nil
11.Binsere	3.90±0.01	Nil
12.Abompe Extension	0.12±0.01	0.08±0.01
13.Nyameso: Control B	0.31±0.01	0.02±0.01
14.Akaporiso	1.60±0.01	Nil
15.Akaporiso : Control A.	1.50±0.01	0.01±0.01
EPA MPL	n/a	0.1

The mean difference is significant at the 0.05 level. n/a= not at all

From Table 1 above, the mean Fe concentration in water samples from the mosquito breeding sites from all the

fifteen communities ranged from 0.12±0.01mg/L - 13.42±0.01mg/L. The lowest value was recorded at the Abompe Extension site where as the highest value at Mensakrom. The next higher Fe values of 9.67±0.01mg/L, 3.90±0.01mg/L and 2.71±0.01mg/L were also recorded at Gausu, Binsere and Kunka New Town respectively. A site-by-site multivariate comparison of means for Fe between the Akapori control site A and the rest of the sites revealed that there was significant differences between them (P<0.000). A similar comparison between control site B at Nyameso and the rest of the sites revealed significant differences between them (P<0.000).

Lead concentration in mosquito breeding water on the other hand, ranged from 0.01±0.01mg/L - 0.30±0.01mg/L. The lowest value was recorded at Akaporiso Control sit A and Nyameso whilst the highest value was recorded at Gausu, Bedieso, and Mensakrom. However, no lead traces were found in water samples from the North Nyamebekyere and Binsere sampling sites. A site-by-site multivariate comparison of means for Pb between the Akaporiso control site A and the rest of the sites revealed that there were significant differences between only it, the Abompe Extension, Akaporiso and Bongobiri (P<0.000 and P<0.002) respectively. A similar comparison between control site B at Nyameso and the rest of the sites for Pb revealed much significant differences between it and the Abompe extension (P<0.000). No arsenic and lead traces were detected in the raw water samples from all the sites throughout the period of analyses.

i. Nutrients in Mosquito Breeding Waters

Table 2 Mean concentration of nutrients in mosquito breeding water sites in the Obuasi Municipality

Communities	**Nitrate Range (1-40mg/L)**	**Sulphate Range (20-200mg/L)**	**Phosphate range (0.1-1.5mg/L)**
1.Gausu	6	36	1.2
2.Bedieso	2	56	< 0.1
3.Mensakrom	3	25	1.5
4.Kunka New Town	< 1	< 20	< 0.8
5.Nyameso	< 1	< 20	0.1
6.Sanso	1	< 20	> 1.5
7.Nyiraeso	34	29	1.4
8.Bediem	> 40	96	0.1
9.Bongobiri	8	52	1.2
10.North Nyamebekyere	< 1	46	0.9
11.Binsere	23	> 200	0.1
12.Abompe extension	20	30	0.2
13.Nyameso: control B	< 1	142	0.2
14.Akaporiso	17	136	0.5
15. Akaporiso: control A.	< 1	< 20	< 0.1
EPAMPL	50	1.5	2

From table 2 above, the nitrate level in water samples from the various mosquito breeding sites varied and its concentrations were relatively lower. It was generally < 1mg/L at the Kunka New Town, Nyameso and Sanso. This pattern was similar to the values recorded at both control sites A and B (> 1mg/L) at Akaporiso and Nyameso respectively.

The nitrate values which were > 1 in the mosquito breeding waters ranged from 2mg/L – > 40mg/L. The lowest value was recorded at Bedieso and the highest at Bediem. The mid ranging values of nitrate from the descending order of highest in the mosquito breeding waters were (34, 23, 20, 17, 8, 6 and 3) mg/L from the Nyiraso, Binsire,

Abompe Extension, Akaporiso, Bongobiri, Gausu and Mensakrom sites respectively. At all the sites except for Bediem, the concentrations were relatively below the EPA Maximum Permissible Limit of 50 mg/L for nitrates in fresh natural and waste water bodies.

Additionally, there was no definite pattern in the concentrations of sulphates which varied between < 20mg/L and > 200mg/L in the mosquito breeding waters. It was < 20mg/L at four water sources namely; Kunka New Site, Nyameso, Nanso and Akapori Control Site A respectively and > 200mg/L at the Binsere site. The mid ranging values from the descending order of next highest from the mosquito breeding waters were (142, 136, 96, 56, 52, 46, 36, 30, 29 and 20) mg/L for Nyiraso control site B, Akaporiso, Bediem, Medieso, Bongobiri, Noth Nyamebekyere, Gausu, Abompe Extension, Nyireso and Mensakrom sites respectively (Table 2). From the entire mosquito breeding water sources, the concentration of sulphur was above the EPA Maximun Permissible Limit of 1.5 mg/L in natural water bodies.

Similarly, there was no definite pattern in the concentrations of phosphates which varied between < 0.1mg/L and > 1.5mg/L in the mosquito breeding waters. It was < 0.1mg/L at the Bedieso and Akaporiso Control Site A respectively and > 1.5mg/L at the Nanso site. The mid ranging values from the next descending order highest were (1.5, 1.4, 1.2, 0.9, 0.5, 0.2, 0.1 and < 0.8) mg/L from Mensakrom, Nyiraeso, Bongobiri, Gausu, North Nyamebekyere, Akaporiso, Abompe extension, Nyameso control site B, Binsere, Bediem, Nyameso and Kunka New Town respectively. In all the mosquito breeding water sites except for Sanso, the levels of phosphate were below the EPA Maximum Permissible Limit of 2mg/L in natural aquatic environments. 1.5 Discussion

According to Brandy & Holum (1996), the presence of heavy metals and nutrients in aquatic environments serve as direct sources of water pollution which affects the potential survival of different organisms whose lifecycle largely depend on water. However, the results shown above did not reveal any significant pattern of attendant heavy metal or nutrients pollution or contamination of the fresh waters that probably served as the direct breeding grounds of the various mosquito species as closely monitored in this study.

In effect, analysis of the heavy metal concentration generally revealed much lower levels detected in the mosquito breeding surface waters when compared with the EPA Maximum Permissible Limits. Generally, it was ranging from 0.12±0.01mg/L - 13.42±0.01mg/L and 0.01±0.01mg/L - 0.30±0.01mg/L for Fe and Pb respectively in the water sources whilst As and Zn traces were not detected from the water

Similarly, the nutrients (nitrate, sulphate and phosphate) concentrations in the mosquito breeding waters were not found to follow any definite trend in this study. The absolute nitrate values which were > 1mg/L ranged from 2mg/L – > 40mg/L was within the EPA. At all the sites except for Bediem, the concentrations were relatively below the EPA Maximum Permissible Limit of 50 mg/L for nitrates in fresh natural and waste water bodies. It was additionally found that sulphates which varied between < 20mg/L and > 200mg/L in the mosquito breeding waters was above the EPA Maximun Permissible Limit of 1.5 mg/L for polluted natural water bodies. However, there was no definite pattern in the concentrations of phosphates which varied between < 0.1mg/L and > 1.5mg/L in the entire mosquito breeding waters and the concentration were generally below the EPA Maximum Permissible Limit of 2mg/L for polluted natural water bodies except for the Sanso site where it fluctuated above 1.5mg/L.

Mosquitoes, especially the Anopheles species are commonly found to be breeding in fresh waters (WHO, 2006). A close comparison of the surface water quality clearly reveals that the concentrations of the trace metals and nutrients were generally non detective or minimal and could not negatively affect the freshness of the mosquito breeding waters. It therefore, confirms the fact that the waters were generally good mosquito breeding grounds once other physico-chemical conditions such as temperature, pH, dissolved oxygen content electrical conductivity etc. were assessed to be suitable based on a similar study conducted on these waters within the same period by the authors.

Scientific studies in the past have revealed that some adult mosquitoes are terrestrial and capable of flight with piercing and sucking mouthparts; the females feed mostly on animal blood and plant nectar, but, males ‘antennae is required (Bradley & Kutz, 2006). The adults of some species remain within a few hundred feet in fresh waters of where they spent the larval stage, whereas others may migrate up to 50 miles or more (Bradley & Kutz, 2006) . Eggs develop a few days after females take a blood meal. Females oviposit on the water, in crevices in the soil, or on other favored substrates or special niches that are or will subsequently be flooded, such as natural and artificial containers

or tree holes, and the cycle repeats itself particularly in fresh waters (Curtis, 1996). An observation of the presence of mosquito larval species in the fresh waters closely monitored throughout the study confirm the fact that the concentration of heavy metals and nutrients were really low and could not negatively alter the water quality to an extent that would significantly affect the adaptability of the breeding mosquitoes. Otherwise, the mosquitoes might have developed some resistance to the existing heavy metal and nutrient conditions (Russel, 1999).

Conclusion

This study revealed that more opportunities are available to research and improve upon current malaria control programmes if additional knowledge is sought and utilized in relation to the predisposing factors in the environment such as trace metal and nutrients concentrations that directly or indirectly affect the breeding pattern and natural adaptation of the various mosquito types which have unique and much varying developmental characteristics. So, professional science researchers must get involved immediately because our goal to save life through developmental research that aims at controlling the malaria vector is an important preamble which is similar to maintaining the scope of our health status as individuals.

Recommendations

It is worth to implement a number of recommended practices in order to improve upon these developmental research findings. Regular survey within some monitoring mosquito breeding sites such as, Akaporiso, Bongobiri, and North Nyamebekyere for more larviciding for at least four times a week is worth it. There is the urgent need to fill all the stagnant ponds permanently from breeding of 3rd and 4th instars, besides draining them through digging channels. In this regard, the waste rocks from the mining catchment area can be brought with the trucks to fill those particular areas to avoid oxbow lakes formation any time the rains set in.

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