ANALYSIS OF POTENTIAL ECOLOGICAL CHANGES ASSOCIATED WITH THE OUTBREAK OF BLACKFLIES (DIPTERA: *SIMULIIDAE*) IN ZOMBA, MALAWI.

MSc. (ENVIRONMENTAL SCIENCES) THESIS

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DECLARATION

I the undersigned hereby declare that this thesis/dissertation is my own original work which has not been submitted to any other institution for similar purposes. Where other people's work has been used acknowledgements have been made.

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CERTIFICATE OF APPROVAL

The undersigned certify that this thesis represents the student's own work and effort and has been submitted with our approval.

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To my Mum and late Dad, I always thank God for you.

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ABSTRACT

Blackflies are one of the insects of medical importance because of the parasites they transmit and the biting nuisance they cause. These flies have aquatic larvae and pupae that require fast flowing water for their development. Changes in the environment can make the blackflies reach pest proportions and cause outbreak. A study on the potential ecological changes associated with the outbreak of the blackflies was carried out. Changes in the physicochemical parameters of the water in Domasi river in one of the affected areas were analyzed. The test parameters included; total suspended solids, total hardness, total alkalinity, temperature, pH, electrical conductivity, dissolved oxygen, nitrates and phosphates. Recent water physicochemical data (2008) was collected and compared with historical physicochemical data (1985-2002) of the river to investigate the changes in the concentration in the parameters over the years. The data was later analyzed for the principle components (PC) that controlled the water quality for Domasi river over the years. The test parameters were reduced to 2 factors in September (2008), December (2008), and 3 previous years by principal component analysis (PCA). The spatial variation of the test physicochemical constituents was then plotted. The results of the factor scores were represented as contour diagrams to deduce their spatial variation. The data of the test parameters (for previous years and present) was further analyzed for significant changes over the years using students't-test. The test parameters that showed a significant change ($p<0.05$) over the years were tested for associations with blackfly larval densities.

Larval densities positively correlated with total hardness ($r=0.859$, $p<0.05$) and total suspended solids ($r = 0.755$, $p<0.02$). Furthermore, we examined changes in forest cover in the affected area. Land sat images of the years; 1984, 1994 and 2004 were used. The images were processed using Ilwis software and Arc GIS 9.2 was used to map the cover change. Results indicated that there has been significant ecological changes mainly in the concentrations plus spatial variation of the physicochemical constituents and forest cover in the affected area. It was evident that the outbreak of the blackflies in Zomba may have been mainly due to increases in total hardness and total suspended solids. The changes in the forest cover contributed to the changes in the water quality and the alteration of the microclimates in the sites where blackflies rest and breed, shortened their gonotrophic cycles and led to population outbreak.

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LIST OF COMMONLY USED ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
DOM	Dissolved Organic Matter
DO	Dissolved Oxygen
DM	Domasi
EC	Electrical conductivity
GPS	Global Positioning System
GIS	Geographical Information Systems
IC	Ion Chromatography
PCA	Principle Component Analysis
PC	Principle Component
pH	Hydrogen Ion concentration
RS	Remote Sensing
TDS	Total Dissolved Solids
TH	Total Hardness
TSS	Total Suspended Solids
N/A	Not Applicable
WNv	West Nile virus
NDOM	Natural Dissolved Organic Matter
PCR	Polymerase Chain Reaction

CHAPTER 1

INTRODUCTION

1.1 Background

Blackflies (Diptera: *Simuliidae*) are small bloodsucking flies of worldwide distribution, occurring in proximity of streams and rivers where their early stages develop (Bukacinski and Bukacinska, 2000). Only female flies suck blood which is a vital source of protein for egg development and maturation. Their bites cause extreme irritation their bird and mammalian hosts, including humans. Blackflies are also associated with reduction of livestock by affecting their productivity and health. They also transmit many human parasitic diseases; including onchorceciasis (river blindness). Onchocerciasis is caused by the filarial nematode *Onchorcerca volvulus (O.volvulus).*

The adult worms of *O. vovulus* lodge in nodules under the skin and release large numbers of microfilaria into surrounding tissues. When these worms die, they elicit an inflammatory reaction which produces a skin rash (onchordermal). If the condition is allowed to progress, lymphadenitis may occur and in the most extreme cases it results into squamous cell cancer (Enk, 2006). In most severe cases or advanced stage of the disease the optic nerve and cornea are attacked resulting in blindness. Other clinical manifestations include debilitating pruritis, fatigue, stigmatizing skin lesions such as de-pigmented "leopard skin," and the frequently super infected, hyper reactive sowda form (Enk, 2006).

An estimated 18-40 million people are currently affected worldwide, with about 270,000 having lost their sight (Courtright *et al.*,1994). Ninety-nine percent of the people living with this disease live in a broad band across the central parts of Africa (Centre for Disease Control (CDC), 2008). Although partially controlled by international mass treatment programs, onchocerciasis remains a major health problem and is endemic in more than 25 African nations, including Malawi.

The earliest modern study of the blackfly and its attendant health implications in Malawi was undertaken in Thyolo District in Shire Highlands in southern Malawi in the early 1980s. Skin snips from 23,373 people living in Thyolo highlands showed infection with *O. volvulus* to be unevenly distributed within the highlands area (Burnham, 1991). In the centre of the focus, most adults were infected, though the intensity of infection was light, not exceeding a geometric mean of 8 microfilaria per milligram of the skin. It was estimated that 327,000 persons lived in areas with prevalence of infection exceeding 10% and, 94,500 persons over the age of one year were infected with these filarial worms (Burnham,1991).

Until the above Rapid Epidemiological Assessments (REA), Thyolo District was the only district in Malawi recognized to have autochthonous transmission of *O. volvulus* (Courtright, 1994). However, subsequent field reports suggested that a new focus of onchocerciasis had developed in Mwanza, a district contiguous with Thyolo. A survey carried out in the northern half of Mwanza district, randomly selecting 62 villages for assessment, revealed that taking two iliac crest skin snips from 2,215 residents over the age of 15 years, one quarter had *O. volvulus* microfilaria. Autochthonous transmission had thus been demonstrated in Mwanza and illustrated the possibility of the spread of the disease to new foci in Malawi and the surrounding countries, due to historically recent shift in the distribution of populations of blackfly.

Major vectors of onchorceciasis in Africa are *Simulium neavei* and the *Simulium damnosum* complex. The same species were reported in Malawi (Pemba and Alezuyo 2006). The long-term presence of blackflies in various districts of Malawi is supported by oral tradition and the presence of blackflies in large numbers dates back to the days of John Buchanan (1885) when tea plantations were being established. Optimal environmental factors such as temperature, oxygen and food availability in the rivers may lead to an increase in fly numbers. As rural, subsistence farmers normally need also to be in close proximity to fresh water sources, the stage is set for blackfly attacks and microfilaria transmission.

1.2 Problem Statement

Although blackfly outbreaks have been a problem in Thyolo and Mwanza districts, this problem had not been reported elsewhere until 2006, when serious human attacks by blackflies were reported in Zomba District on the slopes of the Zomba-Malosa Plateau. *S.damnosum* complex occur naturally on this plateau (Pemba and Alezuyo, 2006). In response, rapid epidemiological surveys were conducted in the infested area of Zomba District and one person out of eleven tested positive for *O. volvulus* (Rapid Epidemiological Assessment (REA) WHO APOC, 2006). In Zomba, the detailed data on the biology and epidemiology of both the vector and the disease it transmits were collected.

These reports showed that the number of people infected was high, especially around Zomba-Malosa Mountain which is a major forest reserve in the district. This forest reserve has been under severe pressure from the nearby population and deforestation is very evident. Whether these ecological changes have led to the outbreak of blackfly populations in this district is unknown. This study therefore aims at investigating the relationship between environmental changes and blackfly outbreak in Malawi.

1.3 Significance of the study

The importance of understanding environmental variability and how this relates to the ecological conservation and human health cannot be over emphasized. This study will elucidate some of the factors that conditioned the increase in densities of the blackflies in Domasi river and its catchment area due to ecological changes. The study will contribute to the knowledge on the significance of ecological reserve determination for rivers and their catchments in Malawi for public health since rivers are important in determining distribution patterns and population dynamics of aquatic insects which include vector like the blackflies. The study will inform policies since sound decisions rely on scientific evidence.

1.4 Objectives of study

1.4.1 General Objective

The main objective of this study was to explore the relationship between ecological changes and the blackfly outbreak in Zomba.

1.4.2 Specific Objectives

(i) To determine the physicochemical changes of the water in Domasi river for possible causes of the outbreak of the blackflies.

(ii) To evaluate the changes in the forest cover of Domasi catchment due to land use change and possible relationships with the outbreak of blackflies.

CHAPTER 2

LITERATURE REVIEW

2.1 Blackfly and its life cycle.

Blackflies (sometimes called a buffalo gnat, turkey gnat or white socks) are a member of the family *Simuliidae* (Hutchinson, 2008). They are small insects (1 - 6mm), adults possessing a shiny somewhat humpbacked appearing thorax that ranges in color from black to various shades of gray or yellow. There are 166 known species of this family in the Afro tropical Region (Crosskey, 1980). In Malawi, 24 species have been reported (Roberts, 1988 and Dudley, 1997). Most of these species were collected in southern Malawi (Roberts, 1988). Sixteen species of *Simulium* are known to occur on the Zomba-Malosa Plateau (Pemba and Alezuyo, 2006).

Females deposit approximately 200 to 800 eggs throughout their life but the number of eggs and length of the life cycle vary between species (Palmer, 1997). The adult female life can be as little as six days and the development from egg to adult can be completed in less than two weeks (Robinson, 1997). Eggs are laid on vegetation under the water. Once eggs hatch, larvae emerge; attach themselves to aquatic or emergent vegetation as well as rocks. The larvae of *Simuliids* do not have a fixed number of instar stages; the majority has 6-9 instars. Larvae orient themselves into the current, using a cephalic structure with fine fans to filter feed on suspended organic matter (Ethan, 2002). They use tiny hooks which are located at the end of the abdomen to attach to the substrate.

The larvae often use silk to hold fast and lines to move, hold to their place and to facilitate feeding. The combination of the silk pad and abdominal hooks maintain the larvae in position on the substrate in the fast flowing water. The cephalic fans are held into the current and periodically contracted to bring food particles into its mouth. The filter feeding mechanism is passive with debris being filtered directly from the passing current. They pupate under water encased in a silken cocoon attached to vegetation or other objects in the stream and then emerge in a bubble of air as flying adults.

2.2 Ecology of blackflies

No insect population remains the same over time, their numbers vary and they are subject to fluctuations. The chief cause of these fluctuations is the instability of the environment and its subsequent influence on the insect's ecology (Clark *et al.*, 1970). Ecosystems face unprecedented crises in habitat fragmentation, destruction and ultimately extinction (Groves, 2003), Roux (2005) and coauthors indicated that rivers are the most endangered of all the varying ecosystems. The greatest threat to these systems is the loss of natural habitats and ecosystem processes, deforestation, introduction of alien species and climate change and these also affect blackfly populations (Vora, 2008; Driver *et al*., 2005).

In Malawi, the growing demand for food due to increase in human population has increased the alteration of natural ecosystems to agrarian ones causing changes in local microclimates. Since insects are cold-blooded (poikilothermic), they are extremely sensitive to temperature changes. They are more active at higher temperatures and less so when temperatures become low.

Increasing seasonal temperatures positively correlate to increases in insect feeding, faster growth rates, and rapid reproduction (Hart *et al.*, 2006). Blackflies are no exception to this, thus many physical and chemical parameters in the environment affect their population dynamics. Blackflies show preference for cold water which may have to do with much of the effects of temperature on oxygen availability on their reproduction rates. For example, eutrophication of water bodies due to input of nitrates, phosphates and organic particles, in fast flowing rivers favor the growth of the bacteria and algae (food for the blackflies) and create a favorable breeding condition for the blackflies.

Forest fragmentation, disturbance, and forest regeneration from abandoned land are some of the contributing factors to the high availability of debris/detritus in rivers. Availability of detritus material, especially the remains of dead plants together with the microorganisms that bring about their decay in rivers, provide a suitable environment for high blackfly breeding rates (Phulanya, 2006).

Oxygen is an important parameter for determining the amount of organic pollution in water and its presence is essential to maintain forms of biological life (Tebbut,1998). The availability of dissolved oxygen can be used as an index to determine the abundance of blackflies. Chemical oxygen demand can assess all the chemically oxidizable substances that can be directly related to the oxygen demand imposed by the organic pollutants in the breeding sites.

It is important to note that aquatic insects differ in their respiratory activity and therefore oxygen requirements. The species differences explain differences in species distribution and abundance observed in nature. Blackflies are sensitive to water pollution.

They prefer habitats with high organic matter (Giller and Malmqvist, 1998). Blackfly larvae are therefore known to be indicators of the physical and chemical changes in water bodies (Rubtsov, 1978).

2.3 Physical and chemical characteristics of water

Chemical and physical factors of water which most often are associated with blackfly larvae distribution and density include; dissolved oxygen, pH, conductivity, ionic position, alkalinity, water hardness, temperature, phosphates, nitrates, dissolved solids and suspended solids (Palmer,1997; Kim and Merrit, 1998). Tebbut (1998) outlined a detailed description of physical and chemical characteristics of water as follows:

Physical characteristics of water include temperature, solids, and electrical conductivity. Temperature is important for its effect on other water properties such as speeding up of chemical reactions and reduction of solubility of gases like oxygen. Solids are also one of the physical properties of water. The solids include the Total Suspended Solids (TSS) and the total dissolved solids (TDS). Thus, solids may be present in suspension or in solution. The total dissolved solids are due to soluble materials where as the total suspended solids are due to discrete particles which can be measured by filtering a water sample through a filter paper. Electrical conductivity (EC) depends on the quantity of salts in water sample. EC measurement provides a rapid indication of total dissolved solids in water.

As indicated by Tebbut (1998) chemical characteristics of water include: nitrates, pH, phosphates, alkalinity and water hardness. These chemical characteristics have different impacts on the biota in the aquatic environment including blackfly population dynamics. pH of water can be defined as the concentration of hydrogen ions present in water. It indicates the intensity of acidity or alkalinity of water sample when measured on a pH scale. Many chemical reactions are controlled by pH and biological activity is usually restricted to a fairly narrow pH range of 5-8.

Water hardness is another chemical characteristic of water. It is mainly due to metallic ions like calcium (Ca^{2+}) and magnesium (Mg). The mentioned metallic ions are usually associated with bicarbonates (HCO_3^-), sulphates (SO_4^{2-}), chlorides (Cl^-), and nitrates (NO_3^-). Hardness is usually expressed in terms of Calcium Carbonates ($CaCO_3$) and it is of two forms: Carbonate hardness and non Carbonate hardness. Carbonate hardness is due to metals associated with (HCO_3^-) and non carbonate hardness is due to metals associated with SO_4^{2-}, Cl^- and NO_3^-.

Dissolved Oxygen (DO) is another chemical characteristic of water. Its presence in water is essential to maintaining forms of biological life. Clean surface water or river water is normally saturated with DO but DO can be rapidly removed by the oxygen demand of organic substances (Tebbutt,1998).

Alkalinity is also worth mentioning as a chemical characteristic of water. It is mainly due to the presence of bicarbonates (HCO_3^-), carbonates (CO_3^{2-}) or hydroxide (OH^-). Most of the natural alkalinity in water is due to the bicarbonates (HCO_3^-).

Alkalinity provides a buffer to resist changes in pH. Alkalinity is normally divided into two: caustic alkalinity above pH 8.2 and total alkalinity above pH 4.5.

2.4 Population dynamics of blackflies

2.4.1 Predation

Blackfly larvae are vulnerable to predation (Malmqvist, 1994) and they are inferior competitors to net spinning Caddis flies e.g *Hydropsychidae* (Hemsphil and Cooper1983). De Moore (1992) listed a number of predator species of the blackflies and included caddis flies as one of the most important predators of the blackflies. Such predators have a very important influence in regulating populations of blackflies most of the times, although living in the fast flowing streams reduces this risk (Roberts, 1988; Palmer, 1997; Hart *et al*., 1996). Changes in water flows greatly influence blackfly larvae predation. Most of the blackflies' predators do not survive when the river velocity is high. Thus, blackflies reach pest proportions when the river levels are high because at this time they face little or no predation (Palmer, 1997). When competition is reduced recolonization after disturbance is rapid. During the times of usual events, local populations of the blackflies may be extinguished, but they may reintroduce themselves successfully from surviving populations when the conditions return to normal (Roberts, 1988).

2.4.2 Water Quality

High densities of small suspended solids, high turbidity, high total phosphorous and nitrogen concentrations, low cover of filament algae on the substratum and low altitude (Zhang *et al.,* 1998) affect population dynamics of blackflies. However, this varies from species to species. Bernotiene (2006) showed that the most important factor for the larvae of *S. rostratum* was water rich in nitrates, with a mean pH of at least 8 or higher, while larvae of *S. ornatum* preferred hard clean water with low pH.

Particle concentrations are positively related to blackfly species richness and abundance (Cummins and Klug, 1979). Wallace and Webster, (1996), revealed that suspended particles are the major source of organic carbon in stream water and they affect both the production dynamics and life history characteristics of suspension feeding benthos. Blackfly larvae capture suspended particles and dissolved organic matter non-selectively. Undigested particles which are released upstream by other invertebrates are compacted in the guts and released as fecal matter which is available to suspension feeders downstream (Wotton *et al.*, 1996). Thus, the blackfly larvae may play an important role in lotic food webs.

2.4.3 Suitability of site

A disturbance is defined as any discrete event with a frequency, intensity and severity outside a predictable range (Resh *et al.*, 1988). Malmquvist *et al.*, 1991, showed that the simuliids are very efficient and opportunistic colonizers. They prefer disturbed substrates (Downes and Lake, 1991) and are resistant to local disturbances (Rosser and Pearson, 1995). Sites with high species richness and abundance of blackflies are characterized by catchments with high proportion of forest cover, extended forest growth period, low frequencies of large water flow increments (Zhang *et al.,*1998).

The suitability as a site for immature stages of blackfly depends on water current velocity and food supply as well as the physical factors, chemical factors of the water (Roberts, 1988) as well as biotic factors. Biotic factors which influence the suitability of a site are competition and predation. Symbiosis may also play a role. Mccreadie *et al.*, (2004) showed a dependent symbiosis between blackflies and *trychomycete* fungi (*Harpellals: Legeriomyceteae*). They describe the relationship as mutualistic and the *trichomycetes* play a higher role in the survival of blackfly larvae.

In different sites, generally species distribution of the insects is of two types: macro-distribution and micro-distribution (Roberts, 1988). Several factors affect the macro-distribution. One of these is the selection by ovipositing females of sites where young stages develop. If the site chosen is not suitable, the young die and this in turn affects the population dynamics of the insects (Roberts, 1988). Although a river may be suitable for species of larval *Simuliids,* not all parts of the river are suitable. *Simuliid* species compositions and density vary at different sites in the river. The variations are due to particularities of a site such as low gradient causing a sluggish flow and altitude which can determine a species zonation by affecting climate or river temperature (Roberts, 1988).

2.4.4 Temperature

During their development aquatic invertebrates, including the blackflies must pass two critical thresholds for water and air temperature: a developmental threshold (which limits the development of larval stages) and a maturation threshold (which limits the development to pupal and adult stages) (Ward and Stanford, 1982). These temperature thresholds place limits on the duration of the aquatic stage, that is, the developmental time from egg through various larval instars to adult emergence (Rivers-Moore *et al.*, 2008a).

For *Simuliium chutteri,* the duration of the aquatic stage varies between 12 and 45 days, depending on water temperatures. A minimal daily water temperature of 18^0C was identified as a maturation threshold for *S. chutteri* by Palmer (1997), below which no pupation occurs. Therefore, no adults emerge when water temperatures are below 18^0C for prolonged periods of time (River-Moore *et al.*, 2008a). Water and air temperatures are therefore key determinants in the life cycle of the blackfly.

Temperatures place limits on the developmental time from egg through various larval instars to adult. Thus, maximum and minimum temperature thresholds are critical for timing insect development. Generally, the timing of insect development can be done using models which include; physiological, curvilinear and linear (degree-day) models (Byrd and Castner, 2001). All the mentioned approaches to measuring of insect development work, however, the physiological approach based on Sharp and Demichele model seem to have more theoretical validity and curvilinear model is relatively complicated to use in practice (Byrd and Castner, 2001). As such, linear (degree-day) is the mostly used approach to measuring insect development. The following formula is used to calculate degree-days;

$$DD = \frac{(Max\ daily\ temp + Min\ daily\ temp)}{2} - Min\ temp\ developmental\ threshold$$

Where;DD =Degree-Days *Equation* 1

2.4.5 Land use change

While dense populations of the blackflies are common in most of the rivers, studies scrutinizing ecological changes in a river catchment over a period of time and how these changes affect blackfly population dynamics are rare. Literature reveals increased populations of the blackflies due to human regulation of river flow and disturbance in the flow by channel modification, construction of engineering structures like weirs and energy dispators (Nyman 1995, Tebutt1998, Zhang *et al.,* 1998). Series of studies investigated by Meissner (2005) revealed that environmental stress explained the observed patterns in blackfly larval massive outbreaks and disappearance of some sensitive taxa and concluded that flow resistance is a key mechanism causing the outbreak of the blackflies.

However, long term studies by Bernotiene (2005) hypothesized the changes in blackfly abundance due to anthropogenic activity and further indicated other reasons for the abundance of blackflies as climatic and hydro chemical characteristics of the rivers. Zhang *et al*. (1998), explored on how flow regulation, suspended particles and other environmental factors affect abundance of blackfly larvae. The exploration of the mentioned factors was done on a regional scale where flow regulated and unregulated rivers were compared. Similar studies have been carried out on other dipterans especially Culicidae. Afrane *et al*, (2006) suggested that land use changes, deforestation in particular as one of the causes of mosquito outbreaks resulting into malaria outbreaks in Africa. They compared the survivorship and reproductive fitness of *Anopheles gambiae* in deforestated areas and forested areas and revealed an increased biting frequency of mosquitoes as a result of shortened gonotrophic cycles due to deforestation which inturn increase surface temperature. Studies by Jacob and co-authors (2009) explored the development spatial models for determining mosquito abundance and environmental factors that could aid in the risk prediction of West Nile virus (WNv) outbreaks. They indicate positive correlations between environmental variables and the abundance of *Culex pipiens* and further modeled spatial temporal variation of temperature and presence of West Nile virus (WNv) in *Culex pipiens*. Such studies are rare in diptera *Simuliidae* outbreaks. A growing body of literature reveals attempts on control of blackfly populations to relieve outbreaks. Not many studies have been done on the spatial-temporal analyses on the ecological changes associated with blackfly outbreaks.

CHAPTER 3

RESEARCH DESIGN, MATERIALS AND METHODS

3.1 Study area

Zomba district is one of the 12 districts in the southern region of Malawi. Total land area of the district is 2,580 square kilometers representing 3% of the total land area of Malawi (Government of Malawi (GOM), 2000). The highest point in the district is Zomba plateau which is 2,085 meters above sea level and the lowest point is lake Chilwa at 627meters above sea level (GOM, 2000). Zomba-Malosa Plateau, the most prominent geographical feature in this district, is the source of numerous rivers, one of such rivers is Domasi, which is one of the main foci of the recent blackfly population explosion and was chosen as the site of this study. The study area is located 15^0 17 South and 35^0 24 East (Figure 1).

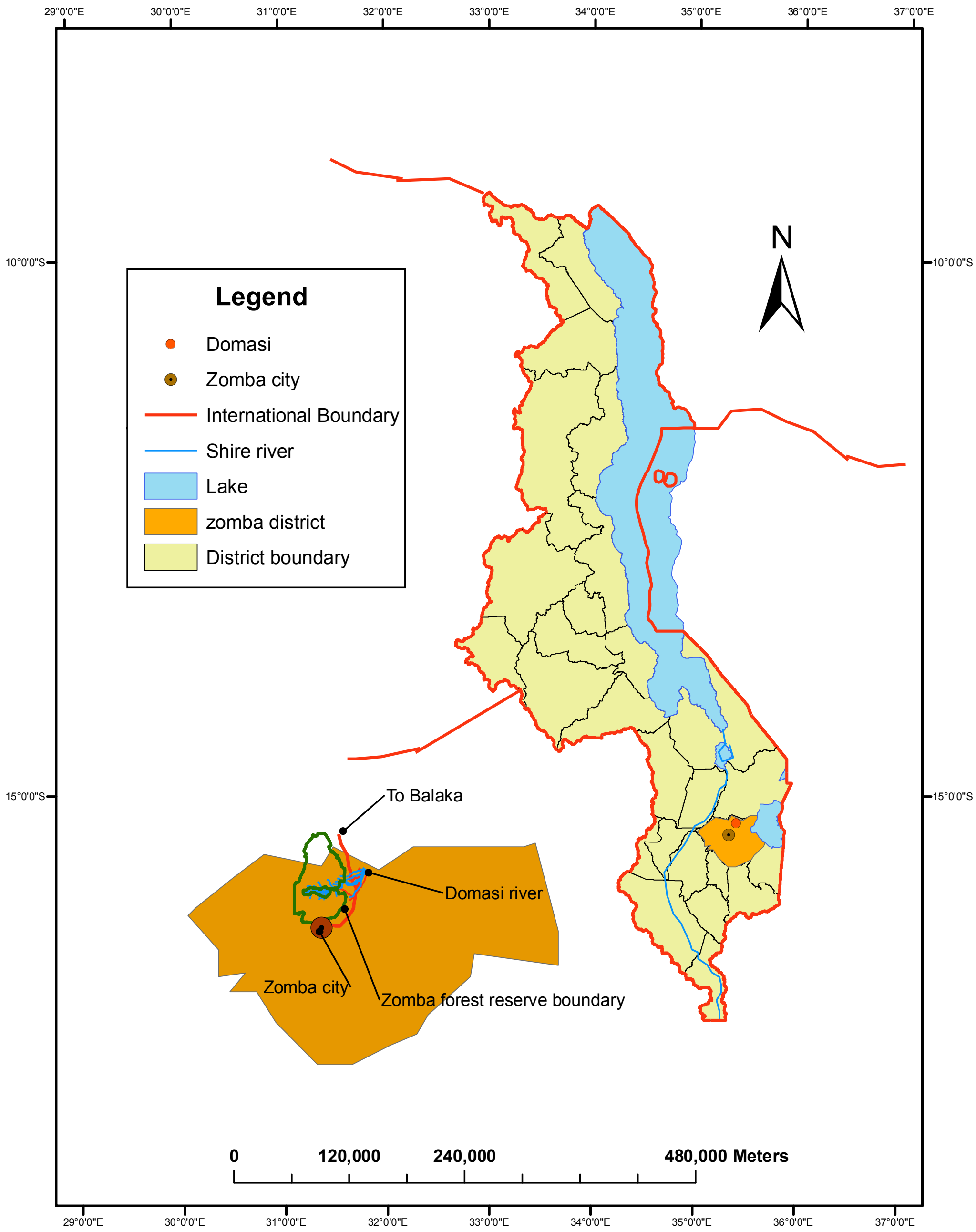


Figure 1. Map of Malawi showing the location of Zomba district

3.1.1 Domasi river

Domasi river flows through Domasi Valley and eventually into Lake Chilwa. The river includes a variety of water velocity, decreasing with altitude. Its catchment area has large parts which are deforested due to logging. The predominant species of blackflies in the area is *Simulium damnosum.* Rapid Epidemiological Assessments (REA) carried out in 2006 showed high levels of onchordermal conditions, warranting blackfly control WHO APOC (2006).

3.1.2 Sampling

Small portions of the river at systematically distributed points over the entire length of the river were selected. The sampling points in the Domasi river were determined by the flow stages of the river. The flow stages were determined with reference to the course of the river and for purposes of sampling three sections of the river were created arbitrary. The upper course which was highest sampling point was in the mountains of Zomba-Malosa and was characterized by natural vegetation (trees and grass). Water velocity was high in this section of the river. In the middle course of the river, the angle at which the river flows was less steep with most parts of the sides of the river covered with fields and less natural vegetation cover. The lower course of the river was almost flat with a gentle flow of the waters compared to the upper and middle course. Most parts of the river in the lower course are covered with reeds and bamboos. Most common activities in the lower course of the river were bathing and washing.

One of the primary goals of the water-quality investigation was to provide information that could be used to determine the composition of the whole volume of water that affect the population dynamics of the blackflies.

To determine adequately the instantaneous composition of the flowing stream, water samples were collected from sites which were representative of the entire flow at the sampling point at that instant. Furthermore, the sampling sites were chosen to represent adequately any changes that might occur. Changes occurring along the length of the stream were evaluated by adding more sampling points as Hem (1991) emphasized. Sampling points are shown in Figure 2 and brief description of the sampling sites is indicated in Tables 1 and 2.

Water samples were collected in two seasons: mid June to early September of 2008 and late December 2008. The sample sites were within the blackfly breeding sites.

The number of sample sites varied with season because of the varying number of breeding sites of blackflies. Water samples were collected and measured for physical parameters in two segments in the upstream section of the Domasi river, in the middle section and in the lower section in the dry season. In the wet season (December 2008) four segments were sampled in the upstream, two in the middle section and four in the lower section. Two sites were sampled in the middle section of the river because of accessibility problems in the wet season. Water samples were collected in triplicates in each of the segments for laboratory analysis of phosphates (PO_3), nitrates (NO_3), total suspended solids (TSS), alkalinity, total hardness and hydrogen ion concentration (pH).

Global positioning system (GPS) coordinates and photographs were taken at each sampling site.

Table1. Table showing sampling sites (coordinates) and site description (September, 2008)

Site Number	*Easting*	*Northing*	*Site Description*
DM 1	752573	8307764	Beyond H-parker mission
DM 2	753604	8307955	closer H-parker mission
DM 3	754199	8309259	Domasi water treatment plant
DM 4	754197	8309257	Domasi water treatment plant
DM 5	756642	8308747	M1 bridge
DM 6	757341	8309486	Institute bridge

Table 2. Summary table of sample sites coordinates and site description (December, 2008).

Site Number	*Easting*	*Northing*	*Site Description*
DM 1	752573	8307764	Beyond H parker Mission
DM 2	753604	8307955	Closer to H parker
DM 3	763597	8308115	At H parker mission Hospital
DM 4	757811	8309673	At Domasi secondary
DM 5	754197	8309257	Water treatment plant
DM 6	754199	8309259	Water treatment plant
DM 7	756642	8308747	At M1 Bridge
DM 8	754363	8309334	Domasi Prison
DM 9	757932	8309698	At Domasi market
DM 10	757341	8309486	At the institute bridge

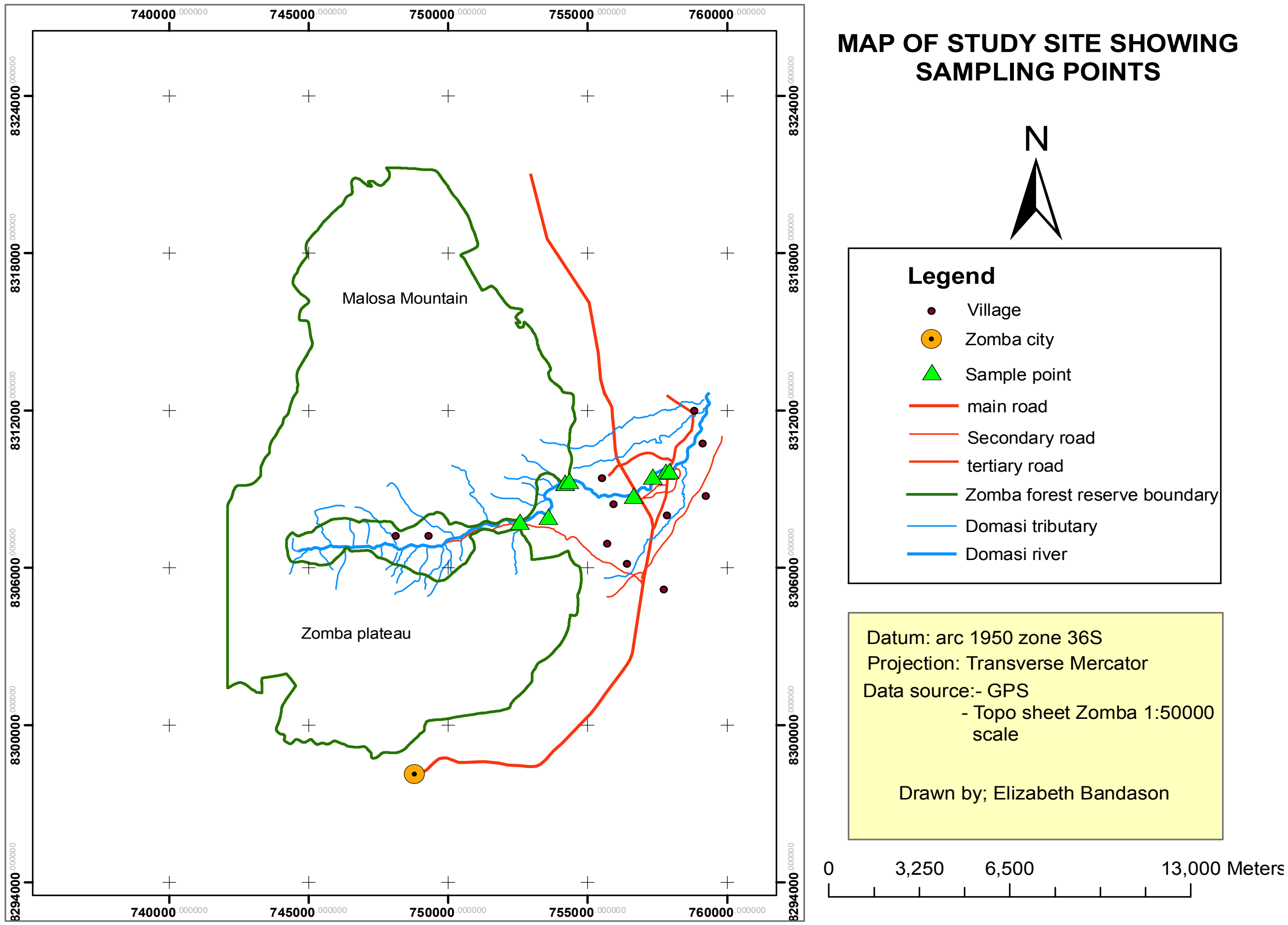


Figure 2. Map showing study site and sampling point

3.2 Establishment of hydro physicochemical data

3.2.1 Determination of electrical conductivity temperature and dissolved oxygen

The standard methods (Hem, 1991; APHA, 1992) were followed. In the determination of electrical conductivity (EC), temperature and dissolved oxygen (DO) of water samples were measured in the field immediately after the collection of samples using EC meter and DO meter (Cole Palmer, model 5946-75).

3.2.2. Determination of pH

Hydrogen ion concentration (pH) was determined using pH meter (Metrohm 827 pH lab). Before each measurement, the pH meter was calibrated with reference buffer solutions of pH = 4 and pH= 7. The values were then used to determine pH of 100ml of water samples as outlined in AOAC (1990) Determination of pH was made in unstirred samples to avoid loss of carbon dioxide and other volatile compounds.

3.2.3 Determination of alkalinity

Total alkalinity was determined using an acid-base titrimetric method. Water sample (25 ml) was pipetted into a 250 ml Erlenmeyer flask into which phenolphthalein (2-3 drops) were added to determine phenolphthalein alkalinity. Methyl orange indicator (2-3 drops) was added to the sample solution to determine total alkalinity. The mixture was then titrated with sulphuric acid (0.02 N) until the methyl orange end point, and then calculation done.

3.2.4 Determination of nitrates and phosphates

Water Samples were tested for phosphates and the nitrates. Ion Chromatography(IC) was used to test for nitrates and free phosphates. Samples were collected with addition of boric acid (2ml/1 litre sample) and neutralized with 5.0 N sodium hydroxide standard solutions.

3.2.5 Determination of Total Suspended Solids (TSS)

To determine Total Suspended Solids (TSS), evaporation method was used. Filter papers were pre-weighed. A water sample (10 ml), was filtered through a pre-weighed glass fibre filter funnel placed on a vacuum pump. The sample was washed with three 10 mls of distilled water on the filter allowing complete drainage between washings and with constant suctioning until filtration was complete (APHA, 1992). The filter funnel was thereafter removed from the vacuum pump to an aluminium planchet as a support. The filter funnel was then dried for one hour in an oven, and then cooled in a desiccator followed by weighing on an analytical balance. Suspended solids were calculated as follows:

$$mg/l\ SS = \frac{(A - B) \times 1000}{Sample(ml)} \qquad Equation\ 2$$

Where: *A*= weight of filter funnel + dried residue, mg; *B*= weight of the filter funnel, mg and *SS*=Suspended solids

3.2.6 Determination of Total Hardness (TH)

Ammonium chloride, (NH_4Cl) (16.9 g), was dissolved in concentrated ammonium hydroxide, (NH_4OH) (143 ml). Magnesium salt ethylene diamine tetraacetic acid (EDTA) (1.25 g) was added to the buffer solution which was later diluted to 250 ml with distilled water. The buffer solution was kept in a plastic container which was tightly stoppered to prevent loss of ammonium (NH_3) and pick up of carbondioxide (CO_2). The buffer solution was dispersed by means of a bulb operated pipette. Eriochrome Black T dye (0.5 g) (a sodium salt of 1-(1-hydroxy-2-napththylzo)-5-nitro-2-naphthol-4-sulfonic acid, No. 203 in the colour index.) was mixed together with NaCl (100 g) to prepare a dry powder mixture (the indicator). Analytical reagent grade disodium ethylenediamine tetra acetate dehydrate ($Na_2H_2C_{10}H_{12}O_8N_2.2H_2O$) (3.73 g), was weighed, dissolved in distilled water, and diluted to 1,000 ml. The titer was checked by standardizing against standard calcium solution to prepare EDTA titrant (0.01 M). Calcium (Ca) stock solution was prepared by weighing anhydrous calcium carbonate ($CaCO_3$), powder, Analytical Reagent grade (1.000 g) into a 500 ml Erlenmeyer flask. A funnel was placed in the neck of the flask and 1+1 HCl was added, slowly until all the $CaCO_3$ dissolved. Distilled water (20 ml) was added before boiling for a few minutes to get rid of CO_2. The mixture was cooled before adding a few drops of methyl orange indicator, and adjusted to the intermediate orange colour by adding NH_4OH (3 N) or 1+1 HCl, as required. The resultant solution was transferred quantitatively to a 1000 m1 volumetric flask and filled to the mark with distilled water. This standard solution was equivalent to 1.00 mg $CaCO_3$/1.00 ml. A sample volume that required less than 15 ml EDTA titrant was then selected without extending duration by 5 minutes, measured from the time of the buffer addition.

A water sample (25 ml) was diluted to with distilled water (50 ml) in a porcelain casserole before adding buffer solution (1ml) to give a pH of 10.0 to 10.1. The indicator solution (1 to 2 drops) was added. The EDTA standard titrant was then slowly added, with continuous stirring, until the last reddish tinge disappeared from the solution, adding the last few drops at 3-5 seconds intervals. At end point the solution was blue.

Total hardness (TH) was calculated using the following formula:

$$Total\ Hardness(EDTA) as\ mg/l\ CaCO_3 = \frac{A \times B \times 100}{ml\ Sample} \qquad Equation\ 3$$

Where *A*= ml titration for sample; *B* = mg $CaCO_3$ equivalent to 1.00 ml EDTA titrant

3.3.0 Historical physicochemical data

The physicochemical historical data for the sample sites was collected from central water lab in Lilongwe and Southern Region water office in Blantyre. Water temperature data for the past years was not available and in its absence, air temperature data was collected. The monthly air temperature data from 1991 to the year 2004 was obtained from the Meteological Department of Malawi in Lilongwe.

3.4.0 Larval density data

Larval density data was estimated using a larval density scale by Palmer (1997) from each of the sample sites.

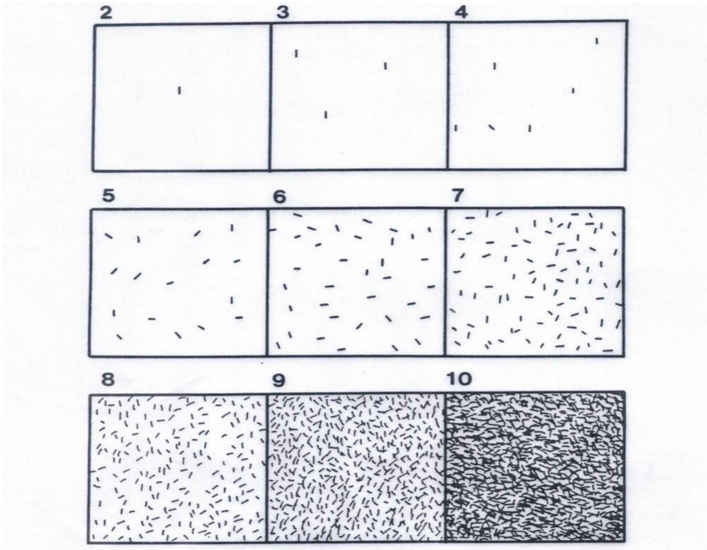


Figure 3. Larval Density Scale (Palmer, 1997)

3.5.0 Forest cover change

Land Sat satellite image for 2002 was collected from Geography and Earth Science Department of Chancellor College. The 1984 and 1994 Land Sat images were collected from Surveys' Spatial Data Analysis Department in Lilongwe. The images were processed using Ilwis 3.1 software and Arc GIS for georeferencing and digitizing. The satellite imagery data were not readily available; therefore, the analysis was only done for the available images regardless of seasons.

3.6.0 Statistical analysis of physicochemical data

Statistical analysis of the physicochemical data varies depending on the objectives of the study. In this project specific analyses were chosen to explain the variations of the hydro physicochemical constituents of Domasi river from the year 1985 to 2008 to the association with *Simulium* outbreak.

3.6.1. Principal component analysis (PCA)

Physical and chemical characteristics of water represent the chemical budget of water. Interpretation of the above mentioned a water physicochemical constituents is complex. Multivariate statistical analytical techniques were therefore used to characterize and interpret basic hydro-physicochemical data of Domasi river. Factor analysis offers a powerful means of identifying the similarities among the variables present in the physicochemical budget of water. It was therefore used to identify the likely principal factors that caused the variations in physicochemical compositions. A well known method of factor analysis is the Principal Component factor Analysis (PCA).

Factor analysis is useful because it brings out the relative significance of the combinations of variables that can be evaluated and the subsequent interpretation is simplified since these are statistical tools which reduce and categorize complex sets of data into groups with similar characteristics. The aim of the PCA of hydro physicochemical data was to explain the observed relationship in simple terms expressed as a new set of variables called factors through data reduction into an easily interpretable form. There are two modes of PCA; R-mode and Q-mode.

For hydro physicochemical data of water bodies, R-mode is often used. R-mode PCA provides several positive features that allow interpretation of data set more scientifically (Jayaprakash *et al*., 2007). R-mode PCA was done to identify a small number of factors that explain most of the indices observed in water quality and to assess changes in the hydrochemistry of Domasi river with combined factors. The following R-mode Principal Component Analysis model was used:

$$X_j = \sum_{r=1}^{p} a_{jr}\ f_r \varepsilon_j \qquad Equation\ 4$$

where the *rth* common factors, *p* was the specified number of factors, *'j'* was the random variation unique to the original hydro chemical variable X_j, a_{jr} was the loading of the *jth* variate on the *rth* factor. The model corresponded to the weights on principal components.

In this technique, eigen values or eigen vectors were extracted. A detailed explanation of the eigen values may be found in Anton (1984). In PCA technique, the group of variables which were extracted is called a factor or component (Jordan, 1995). These components accounted for a certain percentage of the total variation in the data.

In PCA, several factors may be extracted but most of them may account for a very small percentage of the total variation. Therefore, one of the most difficult parts of PCA technique was determining the number of meaningful components (Davis, 1973). In order to limit the number of factors extracted during PCA, a number of techniques are used to determine the number of meaningful factors to use in the analysis. One method is to select factors for which the eigen values represent at least ten percent of the total variance. Another method is to plot the eigen values in a scree plot and a third is to select components for which the eigen value is greater than one (Jordan,1995).

In this study, factors with eigen values greater than one were selected. The numbers of factors were determined by the total variance explained. A new factor was expressed as:

$$Factor = \sum (a_j \times I_i) \qquad Equation\ 5$$

Where a_j was the loading of index; I_i was the standardized monitoring data of I index. The numbers of hydro chemical variables retained in the factors were obtained by squaring the elements in the principal component matrix and summing the total within each hydro chemical variable. Scores that showed negative values denoted areas essentially unaffected by the said factors; most affected areas were denoted by extreme positive score (Dalton and Upchurch 1978).

3.6.2 Analysis of variance in physicochemical data

Environmental variables tend to change over a period of time. The change may be temporal or seasonal. PCA does not account for such changes. Therefore, to find out significant differences between means of the factors and variables analysis of variance (ANOVA) can be used. Student's t-test may also be useful when two sets of data are being compared. The Student's t-test is carried out on assumption that there is no difference in means of the variables which are being tested. A detailed description of Student's t-test is found in (Clarke and Cooke, 1998). In this study Student t-test was used to test for temporal and seasonal variation in the means of the test parameters. Student t-test was also used to test the variation of the concentration of the physicochemical constituents from site to site. This test was carried out considering the flow stages of the river.

3.6.3 Correlation studies:

A strong correlation between two variables can be exhibited by a high correlation coefficient (near +1 or -1) and its value around zero means no correlation between the variables. Variables showing correlation coefficient of >0.7 are considered to be strongly correlated where as those which exhibit a correlation coefficient between 0.5 and 0.7 show moderate correlation. Where variables are negatively correlated, it means there is an inverse relationship between them and where they are positively correlated it means that there is a direct relationship between them. Several methods are used to test for correlations between variables. These include partial least squares projection to latent squares regression (PLS) and multiple regression analysis.

PLS is a generalization of regression based on latent variables for finding the linear or polynomial relationships between a set of predictor variables (Zhang *et al*.1998). Both methods of testing for associations work and the choice of method vary with the objectives of the study.

Sometimes multiple comparisons may be a problem and need to be corrected using statistical tests such as Bonferroni correction which set significance cut-off at α/k where k are the number of parameters being tested. Thus, using the significance cut-off set using Bonferroni corrections, the null hypothesis is only rejected when p-value is less than α/k (McDonald, 2009). These corrections are important because they reduce the family wise error, when multiple tests are being carried out.

However, corrections for multiple tests may be costly because of the investable by- product of an increase in the false negatives which may lead to accepting of a null hypothesis where there is an effect which is statistically significant.

In this project multiple regression analysis was used to test for associations between the larval densities of the blackflies and the test parameters which showed a significant change over a period of time (1985-2002) to 2008 and multiple corrections were not done because they would be conservative and costly in case of this study

3.7.0 Spatial variation

Spatial distribution of the concentration of physicochemical parameters that may affect larval distribution, density and population dynamics of aquatic insects may also contribute to the suitability of a site for their larvae. The spatial distribution may be important for monitoring the concentration of the physicochemical constituents as well as location of sources of pollutants in the river. Geospatial analysis technologies such as Remote Sensing (RS) and Geographical Information Systems (GIS) provide tools for spatial analysis of data. Although technologies of similar nature are still being developed, they offer promise as tools for mitigating environmental health risks (Jacob *et al*., 2009)

In this study, spatial analysis of physicochemical data and the factor scores (principal components) which were obtained after PCA was done using Surfer software. The physicochemical constituent data, factor scores and GPS readings of the sample sites were mapped into grids. These grids were then used to create contour maps to show spatial variation. Thus, each physicochemical parameter and the principal components were represented as contour diagrams to show their spatial variation.

3.8.0 Limitations of the study

Larval density data for the previous years were not available and the physicochemical data for the previous years were limited. Satellite imagery data were not readily available in seasons and for the year 2008. It was beyond the scope of this study to investigate the changes in the microclimates of the blackflies in Domasi catchment. Such is the case because the change in the microclimates is due to several factors and not only deforestation. Thus, time and resources were limited to investigate all the environmental variables which have changed over the years to explain the outbreak of the blackflies.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Statistical summary of physicochemical constituents

Univariate results of chemical analyses and field measurements are listed in Table 3. The average water temperature was 20.1 ^{o}C in the wet season and an average of 19.9 ^{o}C was observed in the dry season showing a significant seasonal variation $p<0.002$. Conductivity showed a fairly consistent trend over the years $p >0.05$ with a mean of 30.992 µs in the past (1985-2002), 30.420 µs and 30.800 µs in 2008 dry and wet season respectively. Just like electrical conductivity, pH also showed a bit consistent trend with an insignificant variation over the years, $p>0.05$. However there is a clear trend of the waters becoming alkaline from a minimum pH of 5.600 in the past (1985-2002) to 6.180 and 6.250 in the dry and rainy season of 2008 respectively.

Results displayed an increasing trend of total hardness from (1985-2002) to 2008 $p<0.002$. It was that the water for Domasi river was softer in the past with a mean TH of 8.070 mg/l than in 2008; 46.540mg/l and 252.800mg/l in the dry and wet season respectively. Alkalinity of the water revealed a significant increasing trend $p<0.002$, with a mean of 8.362mg/l in (1985-2002) to 15.410mg/l and 57.500mg/l in 2008 respectively.

Results indicated an increasing trend of the nitrates over the years $p<0.002$. In 1985-2002, nitrate levels averaged to 0.030mg/l, while in 2008 nitrate levels increased to a mean of 0.050 mg/l and 0.300 mg/l in the dry and wet season respectively.

Total suspended solids also showed an increasing trend $p<0.002$ over the years. In 1985-2002, Domasi river had mean total suspended solids concentration of 3.170 mg/l, while in 2008, the total suspended solids showed an increased mean concentration of 22.670mg/l in the dry season a mean concentration of 3725 mg/l in the wet season.

A decrease in dissolved oxygen was observed seasonally, $p<0.001$. A mean concentration of 7.990mg/l and7.000 mg/l was observed in the dry and wet season respectively. Just like dissolved oxygen, phosphates showed a decreasing trend over the years $p<0.040$. A mean concentration of 0.579mg/l was observed in 1992-2002 data and a mean of 0.012mg/l, and 0.100mg/l, in the dry and wet season of the year 2008 respectively.

Table 3. Showing statistical summary of the test parameters for previous years (1985-2002), Dry season (2008) and Wet season (2008).

	Historical data (1985-2002)			Wet season (2008)			Dry season (2008)	
	Mean	Range	P-value	Mean	Range	P-value	Mean	range
Temperature °C	N/A	N/A	N/A	20.100	17.670-21.300	0.001	19.900	17.670-21.300
EC (µS)	30.992	20.000-41.950	0.314	30.800	25.470-43.170	0.625	30.420	25.470-43.170
pH	6.698	5.600-7.225	0.600	6.800	6.180-7.210	0.223	6.620	6.180-7.210
Nitrates(mg/l)	0.049	0.001-0.200	0.000	0.300	0.020-0.140	3.120E-14	0.050	0.020-0.140
Phosphates(mg/l)	0.674	0.039-0.910	0.030	0.100	0.001-0.040	1.310E-14	0.010	0.001-0.040
Alkalinity(mg/l)	8.362	0.340-10.650	0.000	57.500	21.530-9.570	1.560E-05	15.410	21.530-9.570
TH(mg/l)	8.070	0.002-24.000	0.000	252.800	43.540-49.860	2.140E-19	46.540	43.540-49.860
TSS (mg/l)	3.167	1.500-8.000	0.000	3725.000	17.330-28.000	6.000E-04	22.670	17.330-28.000
DO(mg/l)	N/A	N/A	N/A	7.023	7.400-8.570	1.000E-04	7.990	7.400-8.570

4.2 Temporal and Seasonal variation of the test parameters

4.2.1 Seasonal variation of physicochemical constituents in 2008

An increased concentration in the means of all the test parameters except for EC and hydrogen ion concentration (pH) was observed in the wet season (Figure 4a).

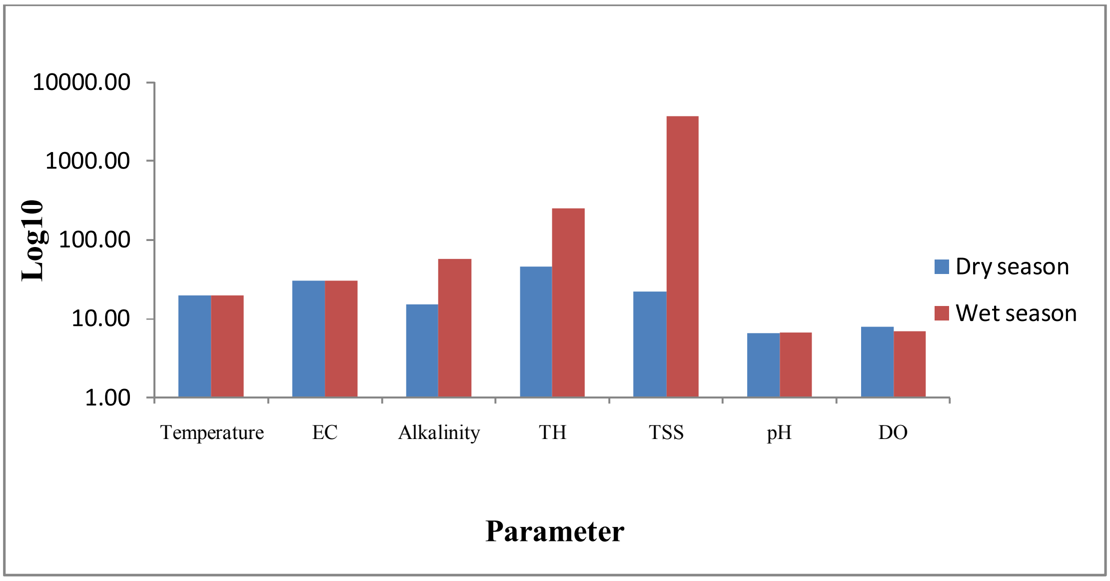


Figure 4a. Seasonal variation of temperature, EC, alkalinity, TH, pH and DO.

A significant increase in the total suspende d solids (TSS) was observed, seasonally $p < 0.001$. The increase of the total suspended solids in the wet season was attributed to increased runoff which carries with it solids into the Domasi river.

Further, a significant increase in total hardness in the wet season was evident $p < 0.001$. Total hardness is defined as the water that is rich in calcium /magnesium ions (Tebbutt, 1998) and these metals may be associated with nutrients like sulphates and nitrates. Thus, the increase in the total hardness in wet season would be due to the high loadings of the mentioned elements in the river due to excess water from the catchment.

Figure 4a indicates an increase in the total alkalinity (TA) in Domasi river in the wet season. In natural waters, alkalinity is due to dissolved carbon dioxide, bicarbonate and carbonate (Tebutt, 1998). The principal source of carbon dioxide species that produce alkalinity in surface waters is the CO_2 gas fraction of the atmosphere, or the atmospheric gases present in the soil or in the unsaturated zone lying between the surface of the land and the water table (Hem, 1991). It is important to note that CO_2 may be released by decay of dead plant material thus, a small part of the available CO_2 in soils species appears in runoff (Hem 1991). Thus, the major possible cause of increased alkalinity in the wet season in Domasi river could be the runoff.

Unlike the TSS, TH and TA, dissolved oxygen (DO) displayed a decreasing trend in the wet season, $p < 0.001$. The decrease in the concentration of DO in the wet season revealed an increase in the organic load in the river in rainy season. The DO concentration may be depleted by processes that consume dissolved, suspended, or precipitated organic matter (Hem, 1991). The sources of the organic load could be anthropogenic activities which include logging, and river bank cultivation which are evident along Domasi river course. These actions increase the amount of dissolved organic matter (DOM) and dissolved natural organic matter (NDOM). In addition to the oxygen required to stabilize carbonaceous matter, there is also a considerable amount of oxygen demand during the nitrification of nitrogenous compounds (Tebbutt, 1998) and this too leads to depletion of DO in water sources.

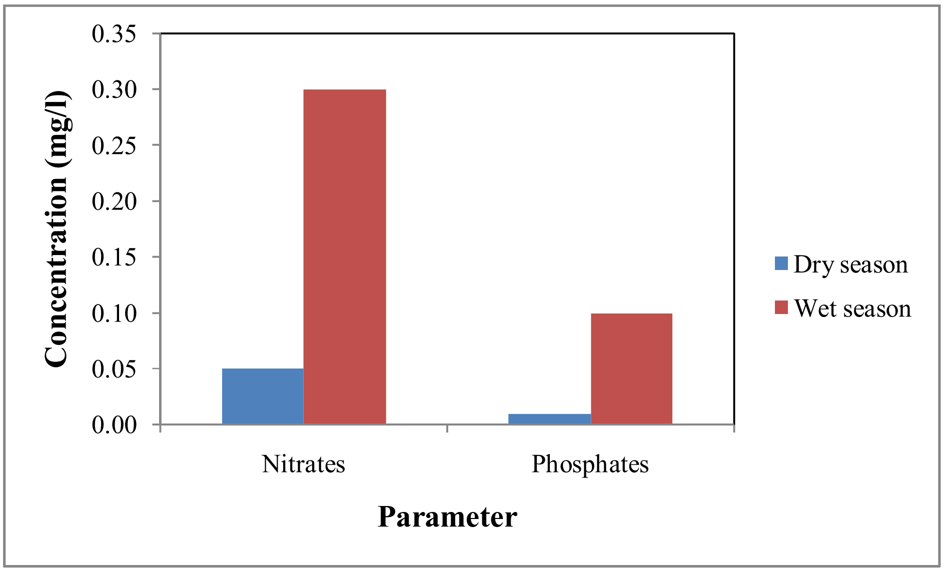


Figure 4b. Seasonal variation of Nitrates and phosphates in 2008

Nitrates occur naturally in surface waters (Tebutt, 1998). However, this study revealed a varying concentration of nitrates in Domasi river with seasons (Figure 4b). Results indicated an increased concentration of nitrates in the river, in the wet season. The increase could be due to intensive agricultural practices in the area which utilize nitrogenous fertilizers. Thus, in the wet season, due to rains there must have been an influx of nitrates into the river through leaching since they easily leach and easily change form due to their instability, hence increasing their concentrations in the wet season than in the dry season.

Just like the nitrates, phosphates are classified as one of the vital nutrients for plant growth. Thus, the use of phosphate fertilizers has a potential for increasing the phosphorus content of drainage or leaching of fertilized fields which are in close proximity with the river Domasi, and this may have contributed to the increase of phosphate concentrations in the wet season, $p<0.001$ (see Figure 4b).

However, leaching of the phosphates or the increase in phosphate content of drainage would be minor factor contributing to their increased concentrations in Domasi river as phosphates are not very mobile in soils. Soil erosion, therefore, may have added considerable amounts of the phosphates to Domasi river, as Hem (1991) indicates that considerable amounts of suspended phosphates may be added to streams due to soil erosion.

4.2.2 Temporal variation of the test physicochemical parameters (1985-2002) and 2008

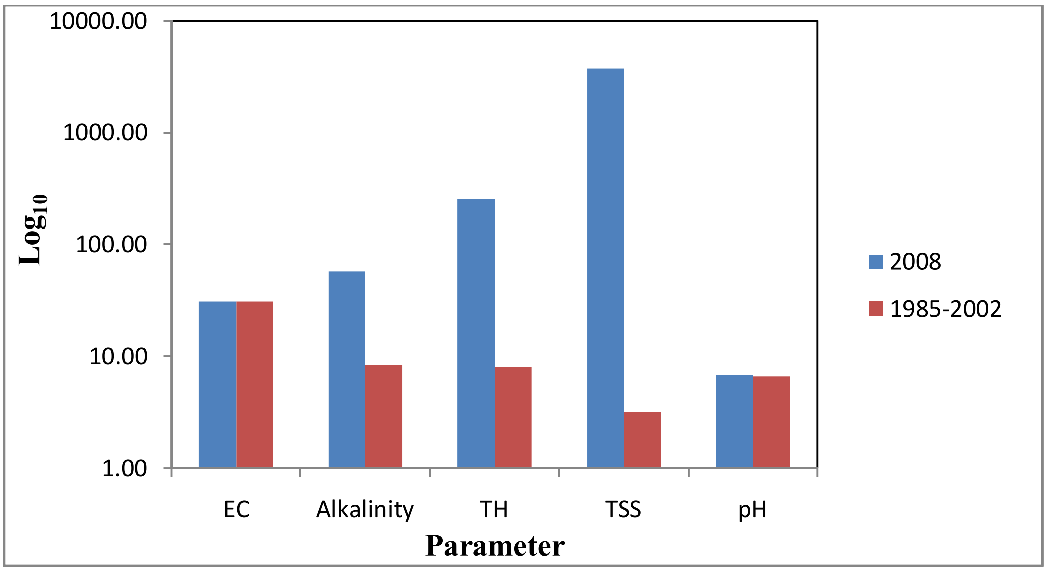


Figure 5a. Temporal variation in EC, Alkalinity, TH, TSS and pH.

From Previous (1985-2002) to recent years (2008), Total Hardness (TH) is higher than total alkalinity (TA) this indicates non carbonate hardness (Chow, 1964). Non carbonate hardness is dominated by nutrients and these include the nitrates. Thus, the increase in the TH over the years $p<0.002$ indicated an increase in nutrient load in Domasi river.

As in the wet season, results showed a significant increase of TSS over the years $p<0.001$. The increase in TSS was mainly due to accumulated debris/detritus material from the catchment of the river as a result of logging and changes in the land use activities.

Figure 5a indicates an increase in alkalinity over the years $p<0.001$. Subsequent export of carbonate alkalinity (HCO_3^- + CO_3^{-2}) from soils in the catchment to Domasi river may account for the dramatic increase of alkalinity from previous years (1985-2002) to recent years (2008). Studies by Raymond and Cole (2003) revealed dramatic increase in alkalinity in a river due to increased rainfall and flow in the river basin and further linked the increased alkalinity to amount and type of land cover. Thus, the increased alkalinity in Domasi river over the years can be attributed to changes in land use activities which altered the amount and type of land cover for Domasi catchment and triggered the export of the carbonates and bicarbonates from soils in the catchment into the river. No significant variations were observed in electrical conductivity (EC) and pH.

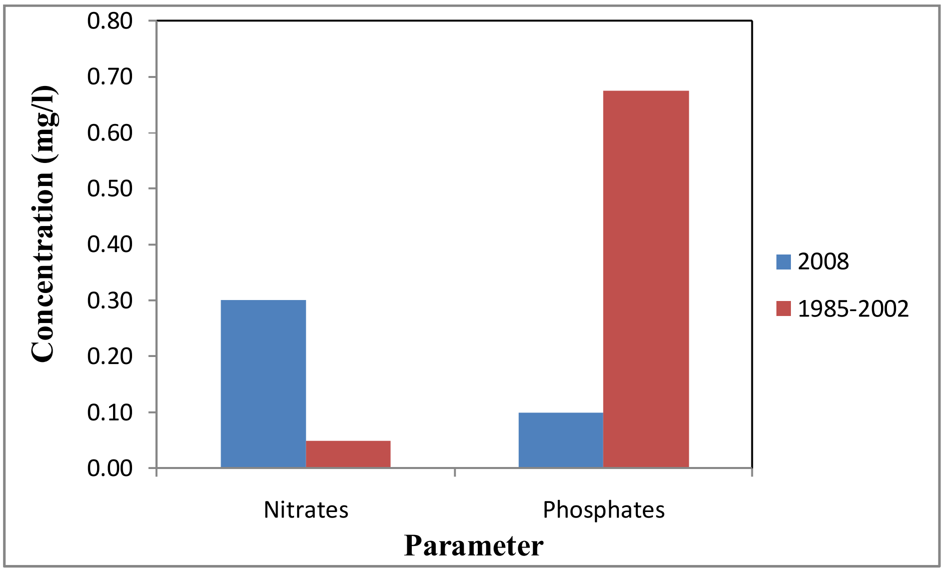


Figure 5b. Temporal variation of Nitrates and Phosphates over the years

There was a significant increase in the concentration of nitrates (NO_3), $p<0.001$. This could be due to anthropogenic activities and changes in land use practices over the years. The gradual alteration of Domasi catchment from the natural ecosystem to agrarian one in which application of nitrogenous fertilizer in the agricultural land which is in proximity with the river Domasi could be a possible source of the nitrates in the river.

On the contrary, the phosphates showed a decreasing concentration over the years $p<0.000$. The availability of the phosphates in natural waters is dependent on several factors and these include; co precipitation and adsorption as well as uptake by biota, the use of phosphates by aquatic vegetation as well as increase in the metal oxides which increase the adsorption of phosphates and reduce the availability of phosphate ions in water (Hem 1991). This study did not attempt to tease out the causes of the decrease in the phosphates in Domasi river over the years.

4.3.0 Comparison of physicochemical parameters from site to site

The overviews of the variation of the physicochemical parameters from upstream, middle stream and lower stream are shown in Tables 4, 5, 6 and 7. The comparison of the physicochemical parameters was done taking into consideration different flow stages of the river. In the previous years (1985-2002) there was no significant difference in EC upstream to lower stream $p>0.05$. However, Students' t-test showed a significant increase in electrical conductivity along Domasi river from upstream to downstream (upstream and middle, $p < 0.005$; upstream and downstream, $p< 0.004$; middle and downstream, $p<0.003$, in July, 2008 and in September, 2008, middle and lower, $p<0.030$, upper and lower, $p<0.010$. In December, 2008 (upstream and middle, $p<0.002$, middle and downstream, $p<0.001$, upstream and downstream $p<0.001$).

The amount of dissolved oxygen decreased downstream (upper and lower, $p<0.001$, middle stream and lower $p<0.005$ in July, 2008), (upper and middle, $p<0.020$ in September, 2008) and in December, 2008 (upper and middle stream $p=0.005$, middle stream and downstream $p<0.005$). This trend revealed the increase of organic load in Domasi river from upper stream to lower stream.

On the other hand, water temperature increased downstream (upper and middle, $p < 0.003$; upper and lower stream, $p < 0.010$; middle and lower stream, $p < 0.020$) in July, 2008. In September, (upper and lower stream; $p < 0.001$, upper and middle stream $p< 0.004$, middle and lower stream $p< 0.001$) and in December, 2008, (upper and middle $p< 0.002$, upper and lower $p< 0.001$, middle and lower $p< 0.010$).

In common with the majority of other organisms,body temperature of insects is determined by temperature of their surroundings. Within the range of ambient temperatures to which insects are naturally exposed, the rates of their physiological processes vary directly with temperature. This might explain why not all parts of Domasi river are affected by the blackflies because of the variations in temperature of the water and other physicochemical constituents.

It is known that water temperature is affected by air temperature. Thus a change in the air temperature leads to a change in the water temperature. Thus, in the absence of water temperature data, air temperatures may be used as a proxy measure to simulate water temperature. Air temperature data for Domasi catchment was therefore collected. Student t-test of the air temperature showed a significant increase of the temperatures from the year 1991 to 2008 ($p<0.05$). Air temperature change over the years is shown in Figure 19.

Table 4. Summary table of P-values of physical and chemical parameters between sites (July, 2008).

	Upper and Middle	Upper and Lower	Middle and Lower
EC	0.004	0.003	0.002
Temperature	0.002	0.007	0.016
pH	0.397	0.001	0.523
Dissolved Oxygen	0.164	0.000	0.004
Alkalinity	0.005	0.080	0.999
TSS	1.000	0.772	0.589

Note: $H_0 = \mu_1 = \mu_2$

Table 5. Summary table of P-values of physical and chemical parameters between sites (September, 2008).

	Upper and Middle	Upper and Lower	Middle and Lower
EC	0.095	0.009	0.023
Temperature	0.003	0.000	0.000
pH	0.775	0.414	0.369
Dissolved Oxygen	0.014	0.074	1.000
Total hardness	0.324	0.009	0.017
Nitrates	0.016	0.250	0.186
Phosphates	0.010	0.461	0.011

Note: $H_0 = \mu_1 = \mu_2$

Table 6. Summary table of P-values of physical and chemical parameters between sites (December, 2008).

	Upper and Middle	Upper and Lower	Middle and Lower
EC	0.005	0.002	0.001
Temperature	0.001	0.000	0.004
pH	0.005	0.002	0.001
DO	0.005	0.108	0.004
Total hardness	0.038	0.023	0.004
Nitrates	0.270	0.136	0.297
Phosphates	0.738	0.446	0.654
TSS	0.193	0.050	0.055
Alkalinity	0.423	0.118	0.129

Note: $H_0 = \mu_1 = \mu_2$

Table 7. Summary table of P-values of physical and chemical parameters between sites in the previous years (1985-2002).

	Upper and Middle	Upper and Lower	Middle and Lower
EC	0.427	0.433	0.095
Temperature	N/A	N/A	N/A
pH	0.550	0.841	0.515
DO	N/A	N/A	N/A
Total hardness	0.171	0.033	0.054
Nitrates	0.188	0.225	0.181
Phosphates	0.433	0.010	0.220
TSS	0.226	0.022	0.066
Alkalinity	0.949	0.000	0.142

Note: $H_0 = \mu_1 = \mu_2$

4.4.0 Spatial variation of the test parameters

The concentration of the tested physicochemical constituents was distinguished by different colours. Green indicated areas with low concentration, the blue colour denoted areas with moderate concentration and red indicated the areas with high concentration of the physicochemical constituents. For the principal components (PCs) green indicated areas where a particular factor had very low impact and with a negative score, blue indicated areas affected by the said principal component to an average degree and red areas most affected with extreme positive scores. The arrow indicates the direction of flow of the river from upstream towards the lower stream (downstream). Plots for the spatial variation of the physicochemical constituents are shown in figure 6 to figure 14.

4.4.1Hydrogen ion concentration (pH)

From the plots, there has been a significant shift in the spatial variation of the pH of Domasi river from high to low in the upper section, low to moderately high in the middle section and from low to moderately high in the lower section.

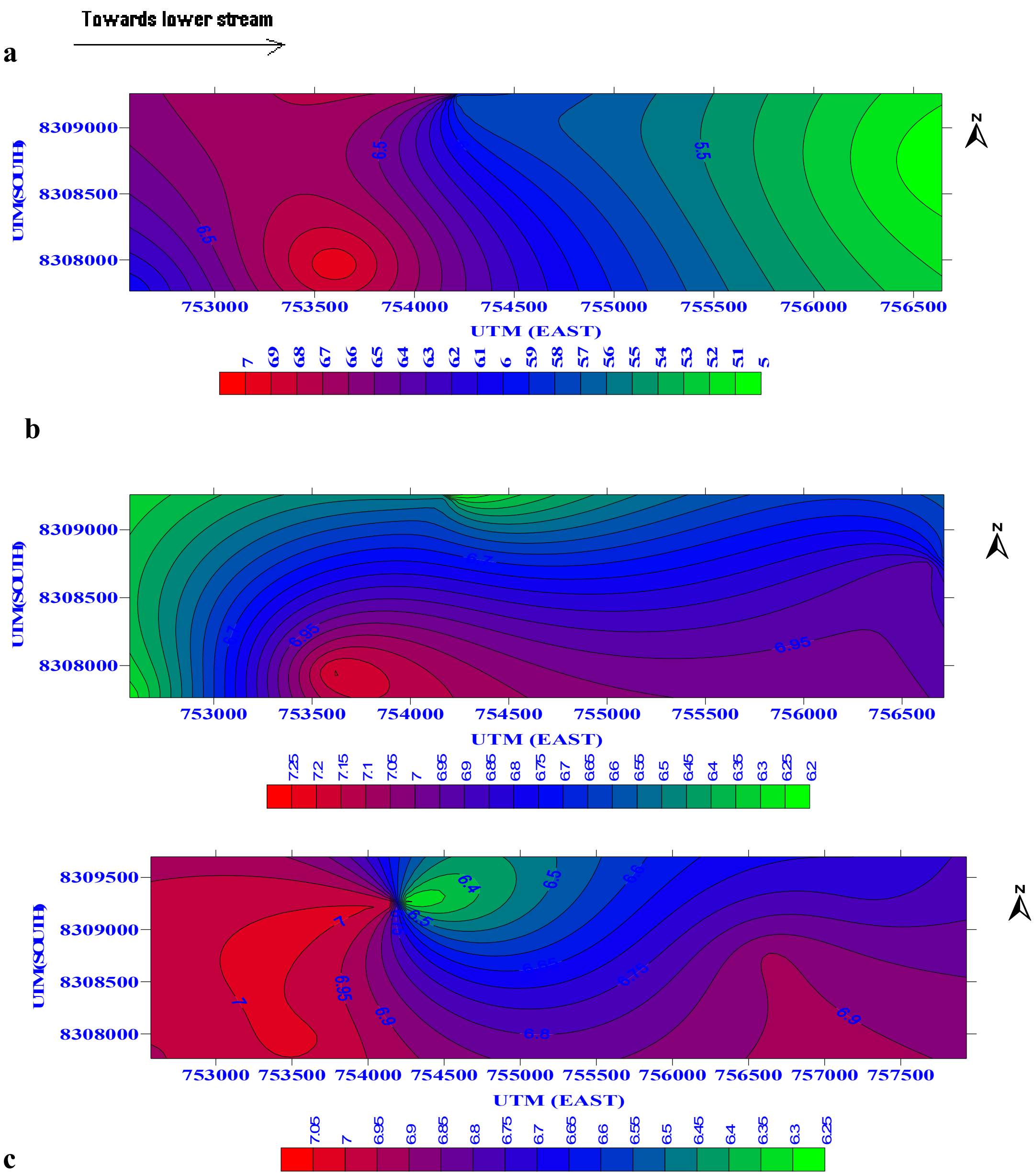


Figure 6. Spatial variation of pH (hydrogen ion concentration). **a** previous years, **b** September, 2008, **c** December,2008.

4.4.2 Alkalinity

The spatial distribution of alkalinity in the previous years showed a decrease in concentration with decrease in the altitude of the river. Over the years the spatial variation of alkalinity has changed from high to low concentration from the upper to lower section of the river in both wet and dry season in 2008.

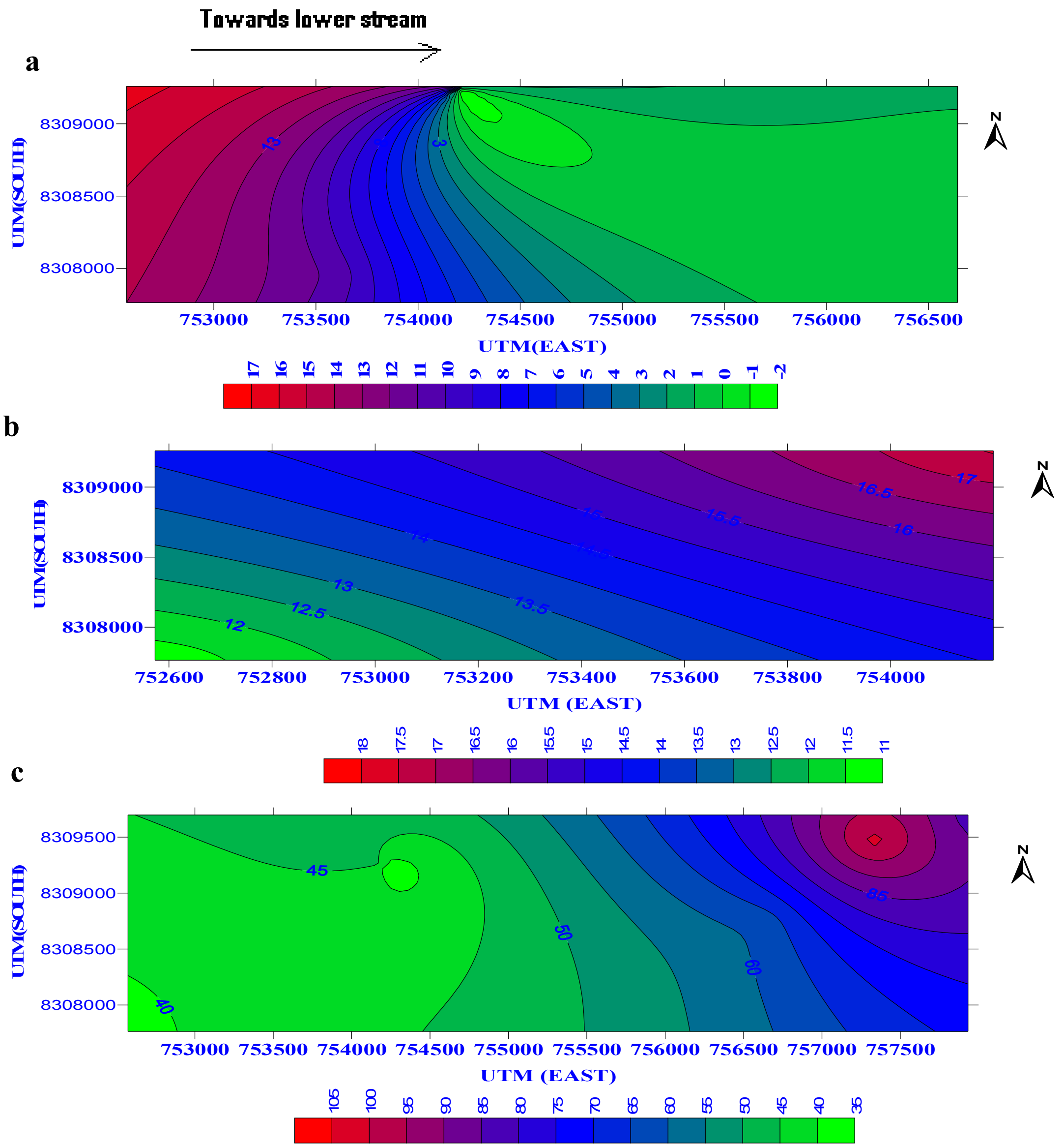


Figure7. Spatial variation of alkalinity: **a** previous years, **b** June,2008 **c** December,2008

4.4.3 Electrical conductivity (EC)

In the previous years (1985-2002) electrical conductivity decreased from upstream to downstream. Plot of recent data show a change in the spatial variation of EC from low in the upstream to high downstream in September, 2008 to moderately high in the upstream except for some areas which registered low EC to moderate in the lower stream.

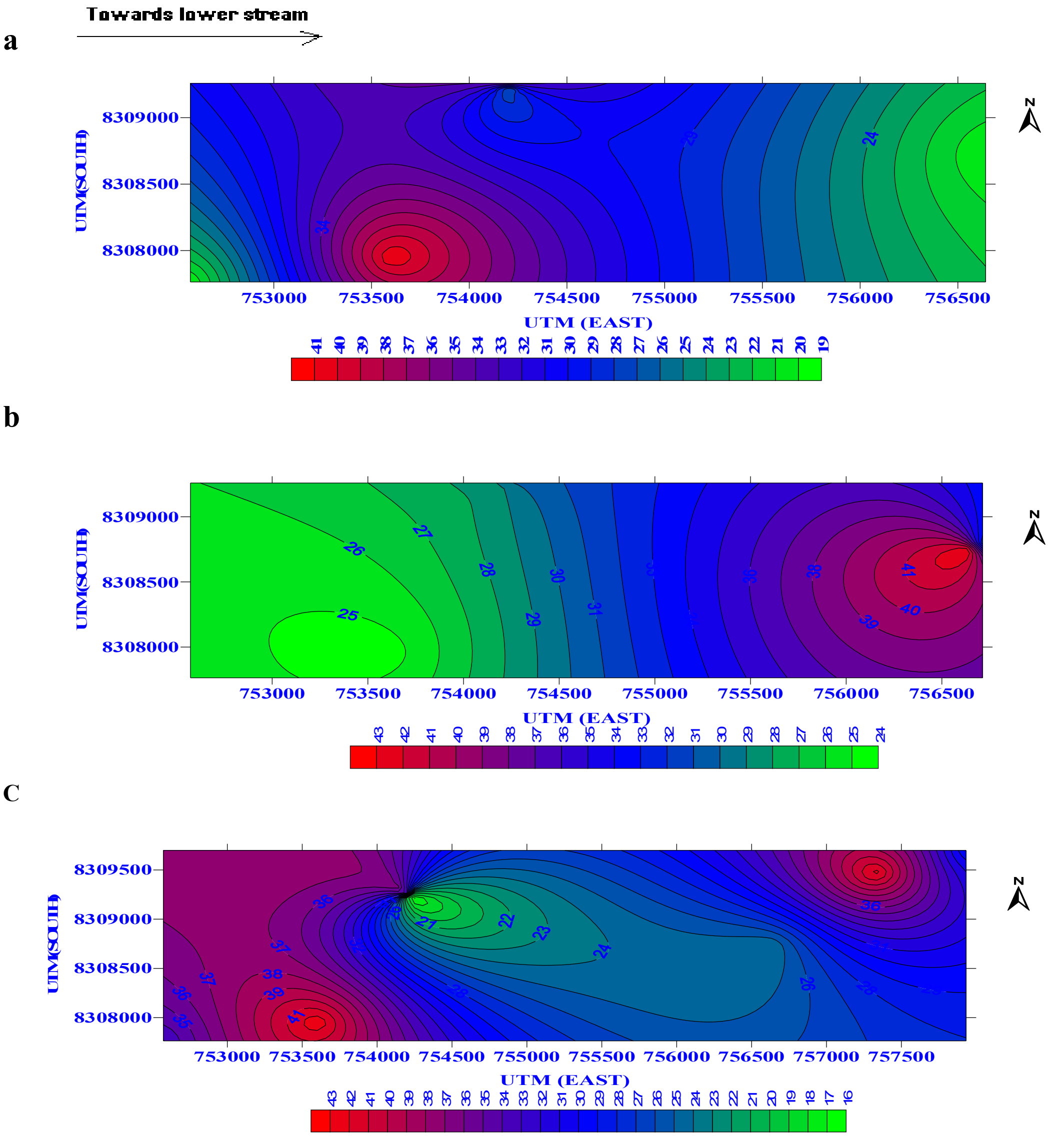


Figure 8. Spatial variation of electrical conductivity (EC); **a** previous years, **b** September, 2008, **c** December, 2008.

4.4.4 Nitrates

The spatial variation of the nitrates showed the same pattern from previous years to September 2008. The nitrates showed an increasing concentration from upstream to downstream. However, the concentration of the nitrates changed in December, 2008 from moderate in the upstream to moderate-high in the downstream.

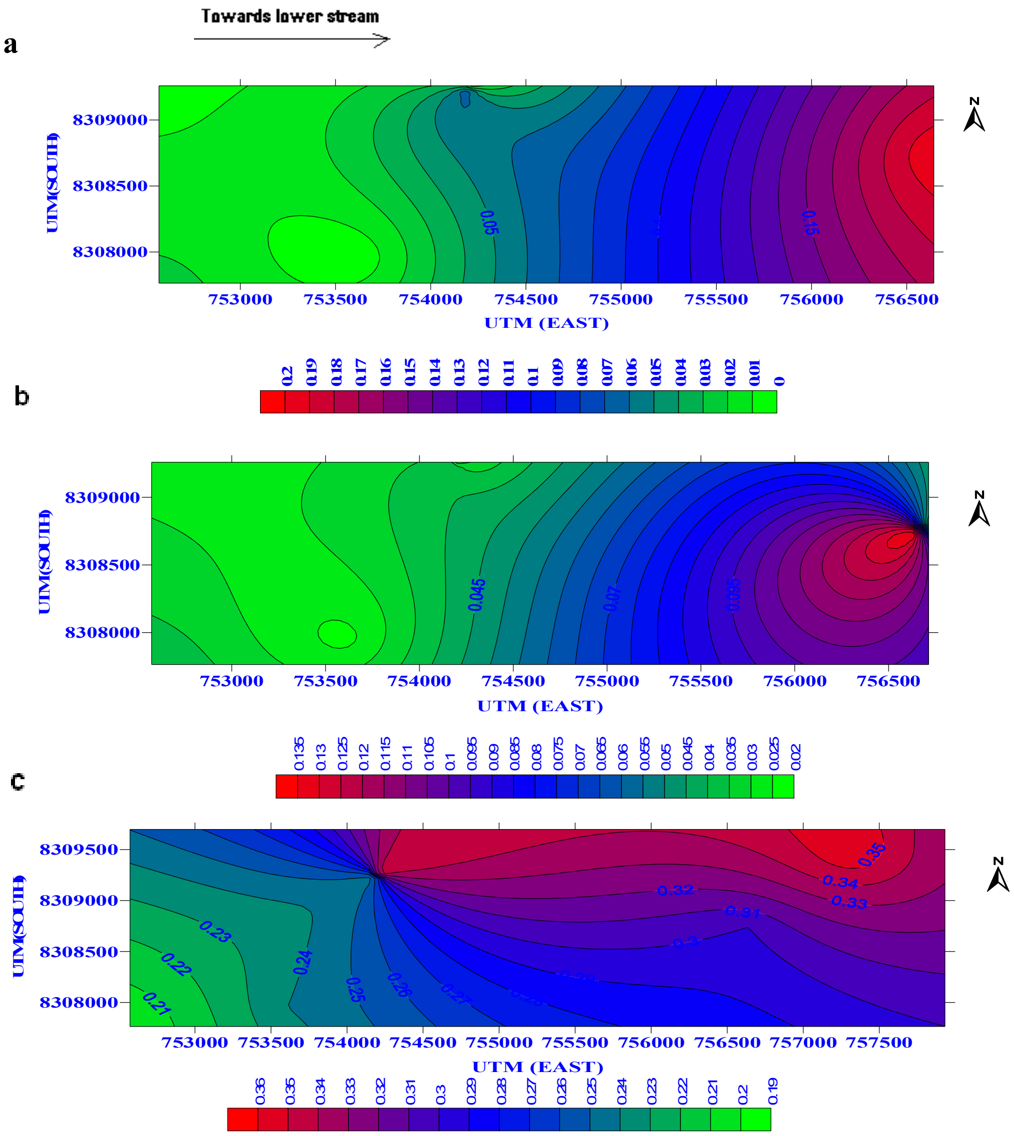
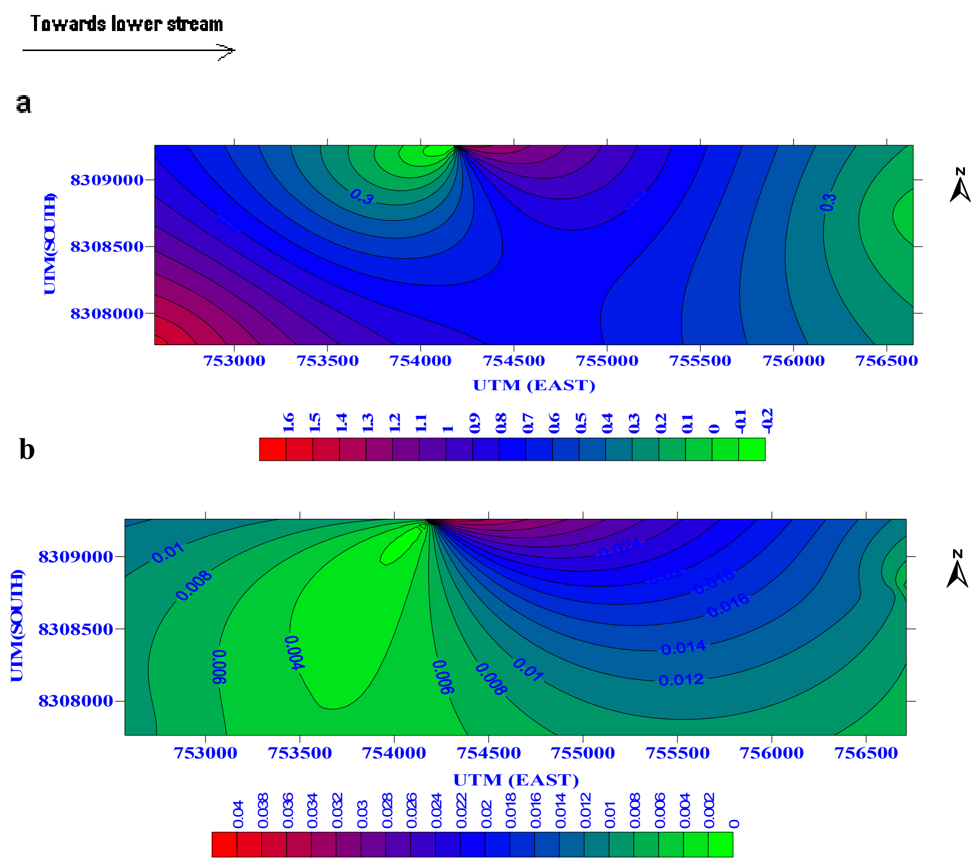


Figure 9. Spatial variation of nitrates (NO_3^-); **a** previous years, **b** September,2008, **c** December,2008

4.4.5 Phosphates

The plot for the previous year's concentration showed a higher concentration of the phosphates in the upper course and a moderate concentration towards the middle course of the river. In the dry season (September 2008) the plot showed a low concentration of the phosphates from the upper course of the stream to a moderate concentration in the middle course and high concentration in the lower course. In the rainy season the concentration of the phosphates showed moderate concentration in the middle course concentration in the lower course of the river.



c

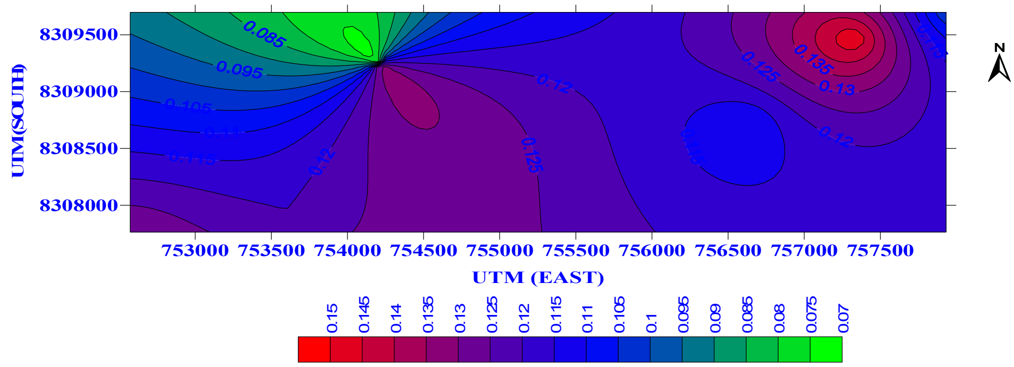


Figure10. Spatial variation of phosphates (PO_4); **a** previous years, **b** September, 2008, **c** December, 2008

4.4.6 Total Hardness (TH)

The concentration of total hardness of Domasi river was higher in the upstream and decreased with decrease in the altitude in (1985-2002). Recent(2008) plots of the spatial distribution of total hardness showed an increase in the TH concentration from upstream to downstream in both dry and wet season.

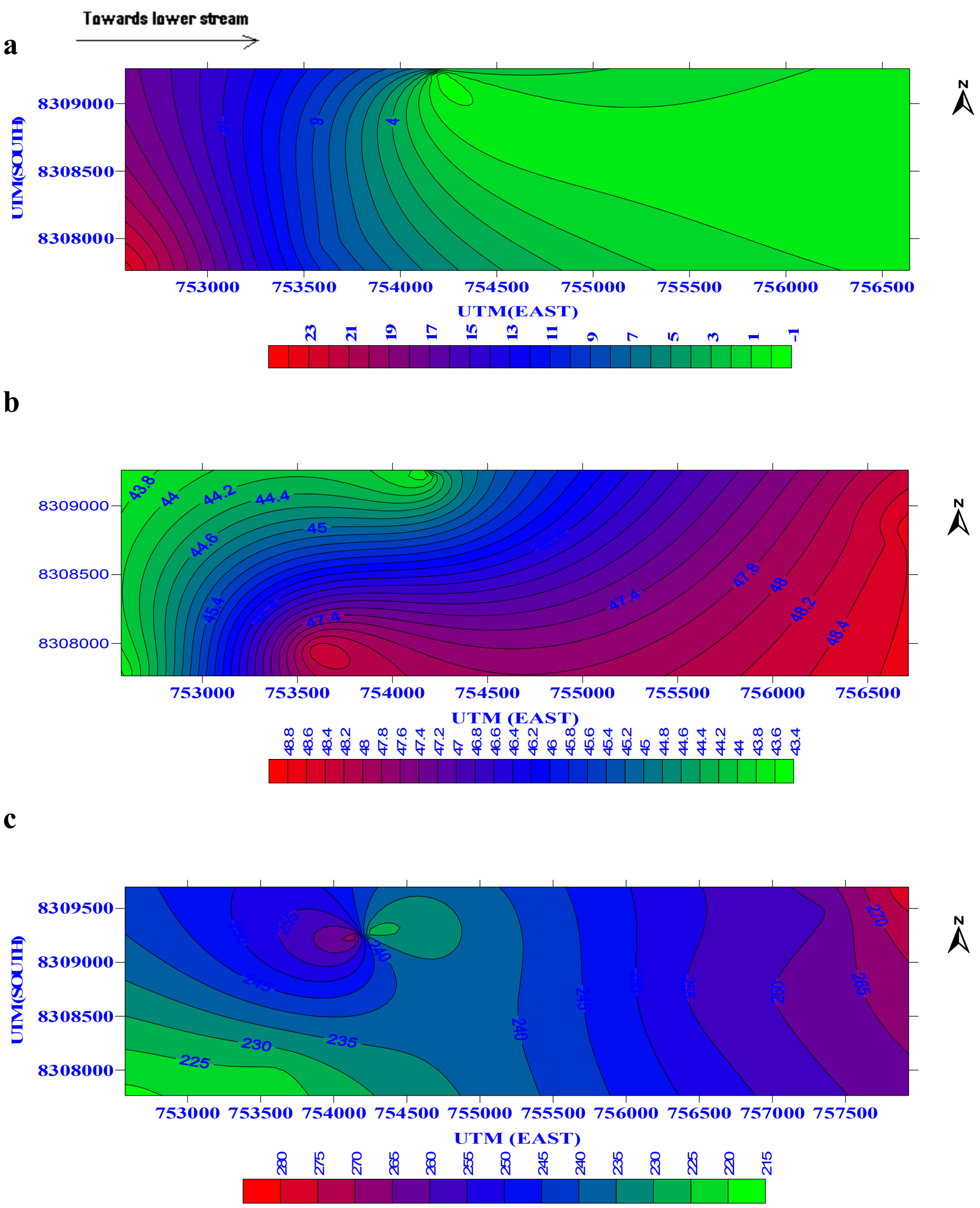


Figure11.Spatial variation of total hardness (TH); **a** previous years, **b** September,2008, **c** December,2008.

4.4.7 Dissolved oxygen(DO)

In both seasons, dissolved oxygen increased from upstream to lower in the rainy season (December,2008). Lower and middle parts of the river showed a higher concentration of DO than the upstream.The contrary was noted in the dry season(September,2008). The DO in the dry season showed a decrease from high to moderate along the course of the river. However, some parts of the upstream showed a higher depletion of DO denoting higher organic pollution.

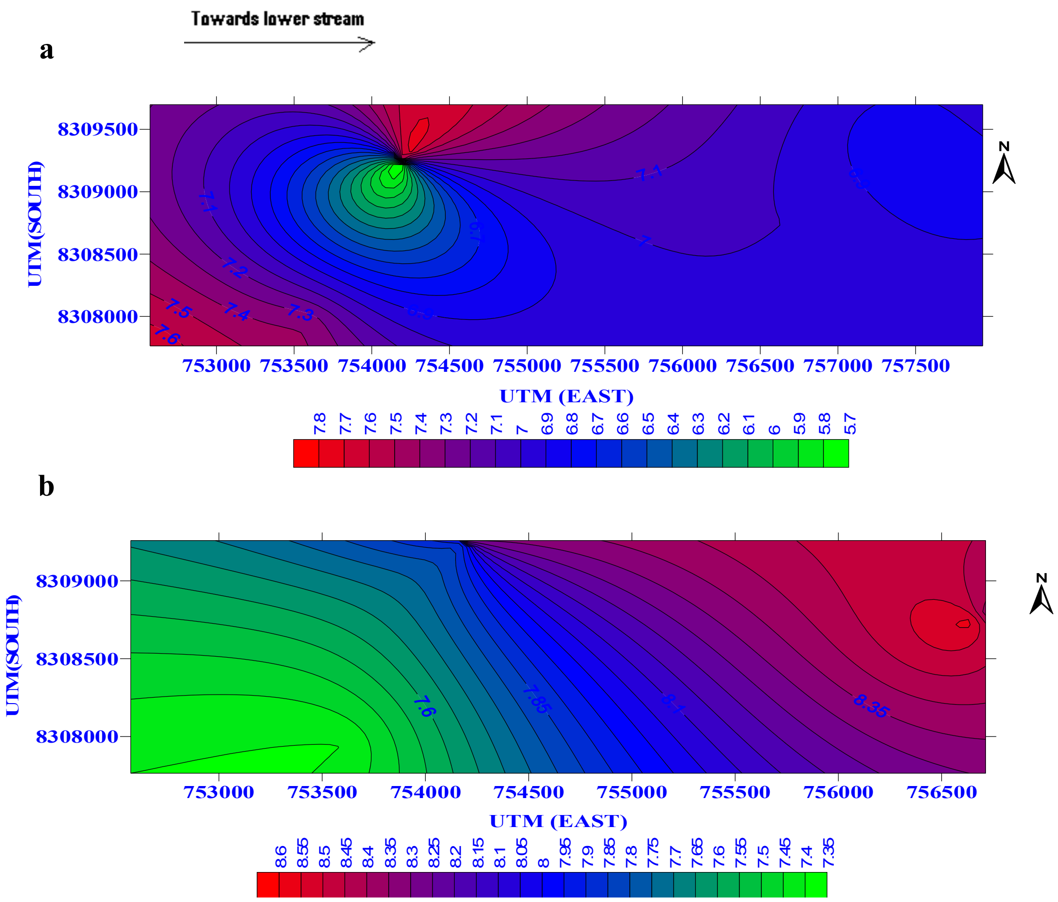
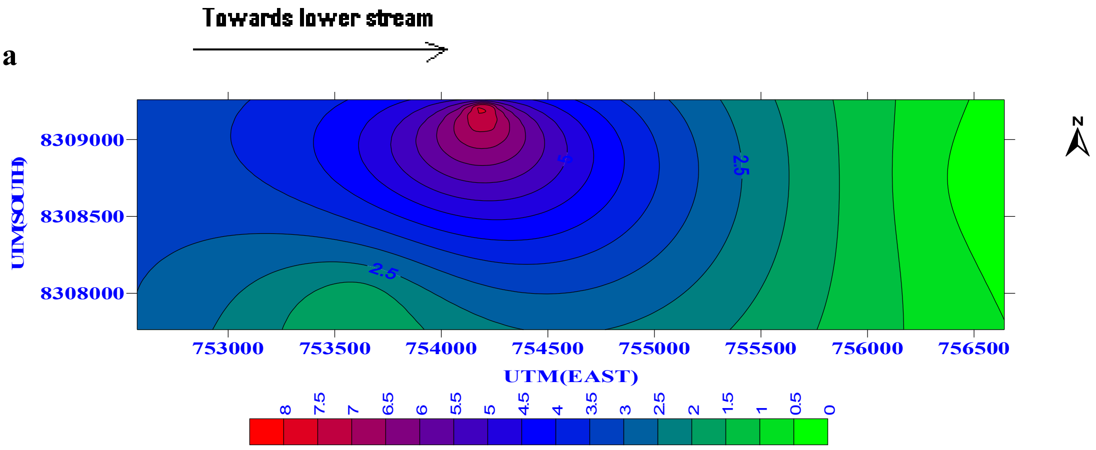


Figure 12. Spatial variation of dissolved oxygen (DO); **a** September, 2008, **b** December, 2008

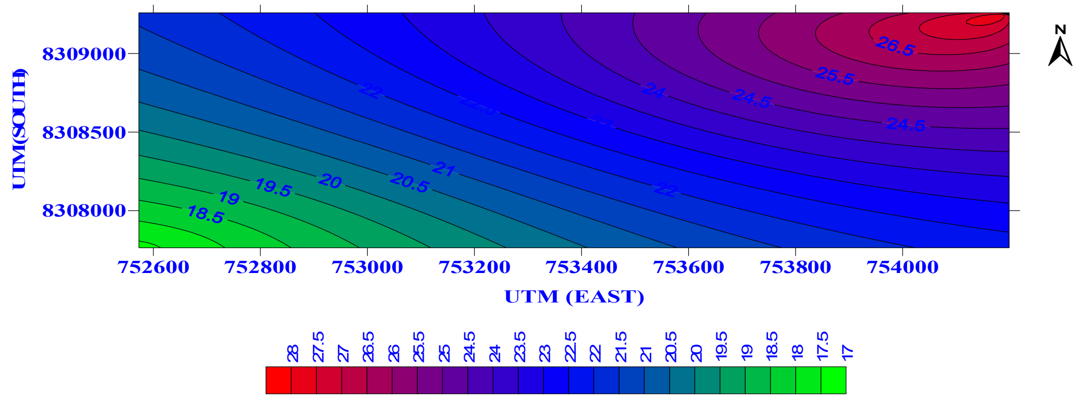
4.4.8 Total suspended solids (TSS)

Domasi river had low particle concentration in the upstream and the concentration increased downstream in the previous years. In the recent years, (2008) in the dry season the particle concentration registered low to moderate concentration in the upstream, moderate in the middle and moderately high in the lower section in the dry season. In the rainy season when the concentration of TSS was high in the upstream low in the middle and moderately high in the lower section.

a



b



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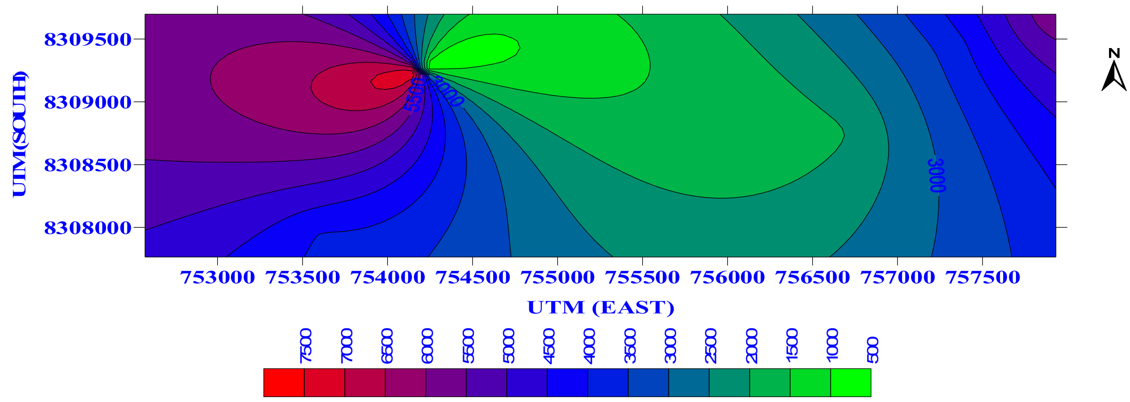


Figure 13. Spatial variation of total suspended solids (TSS); **a** previous years, **b** June 2008 **c** December, 2008

4.4.9 Temperature

In the dry season water temperatures were moderately high in the upstream and increased downstream. In the rainy season much of the upstream registered low temperatures but increased downstream.

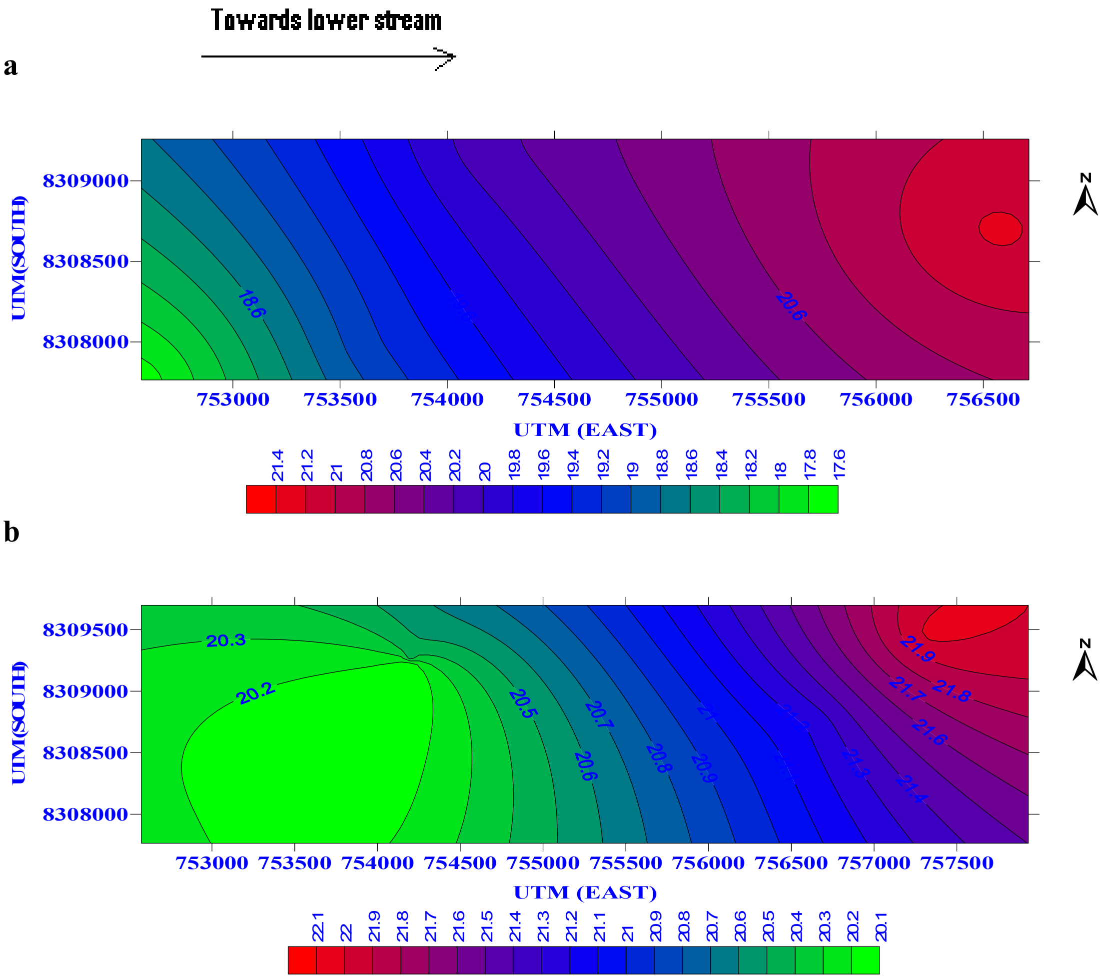


Figure 14. Spatial variation of temperature **a** September 2008 **b** December 2008

4.5.0 Principal component analysis (PCA) and spatial variation of the Factor scores and larval densities.

Test parameters were reduced to three factors in previous years (1985-2002), 2 PCs in September and December 2008, using Statistical Package for Social Scientists (SPSS) Version 16.0. The pattern of factor loadings is shown in table 8. Only factors with a score greater than 0.5 are shown and used.

Table 8. Factor loadings of the physicochemical data

Parameter	Previous years			Dry season		Wet season	
Factor	1	2	3	1	2	1	2
Temperature				0.900		0.887	
pH	0.970				0.749		-0.725
DO				0.823			0.585
EC	0.925			0.937		0.976	
Total hardness		0.904				0.884	
Nitrates	-0.795			0.766		0.719	
Phosphates		0.860			-0.863		
Alkalinity	0.686					0.801	
TSS			0.945				-0.933
Eigen values	3.547	1.610	1.137	3.280	1.620	4.160	2.160
Variance (%)	50.667	23.004	16.236	46.240	23.910	44.320	26.040
Cumulative (%)	50.667	73.672	89.908	46.240	70.150	44.320	70.370

Factor 1 of the previous years which explained 50.67% of the variance had high loadings in pH, electrical conductivity, nitrates and alkalinity. This factor controlled the upper section towards the middle and not much of the lower section of the river (figure 15a). Factor 2 of the previous years consisted of total hardness, and phosphates. It accounted for 23.00% of the total variance. This factor controlled much of the upper section of Domasi river (Figure 15b). The third factor of the previous years comprised the total suspended solids. This factor accounted for 16.24% of the total variance. It controlled the middle section (Figure 15c).

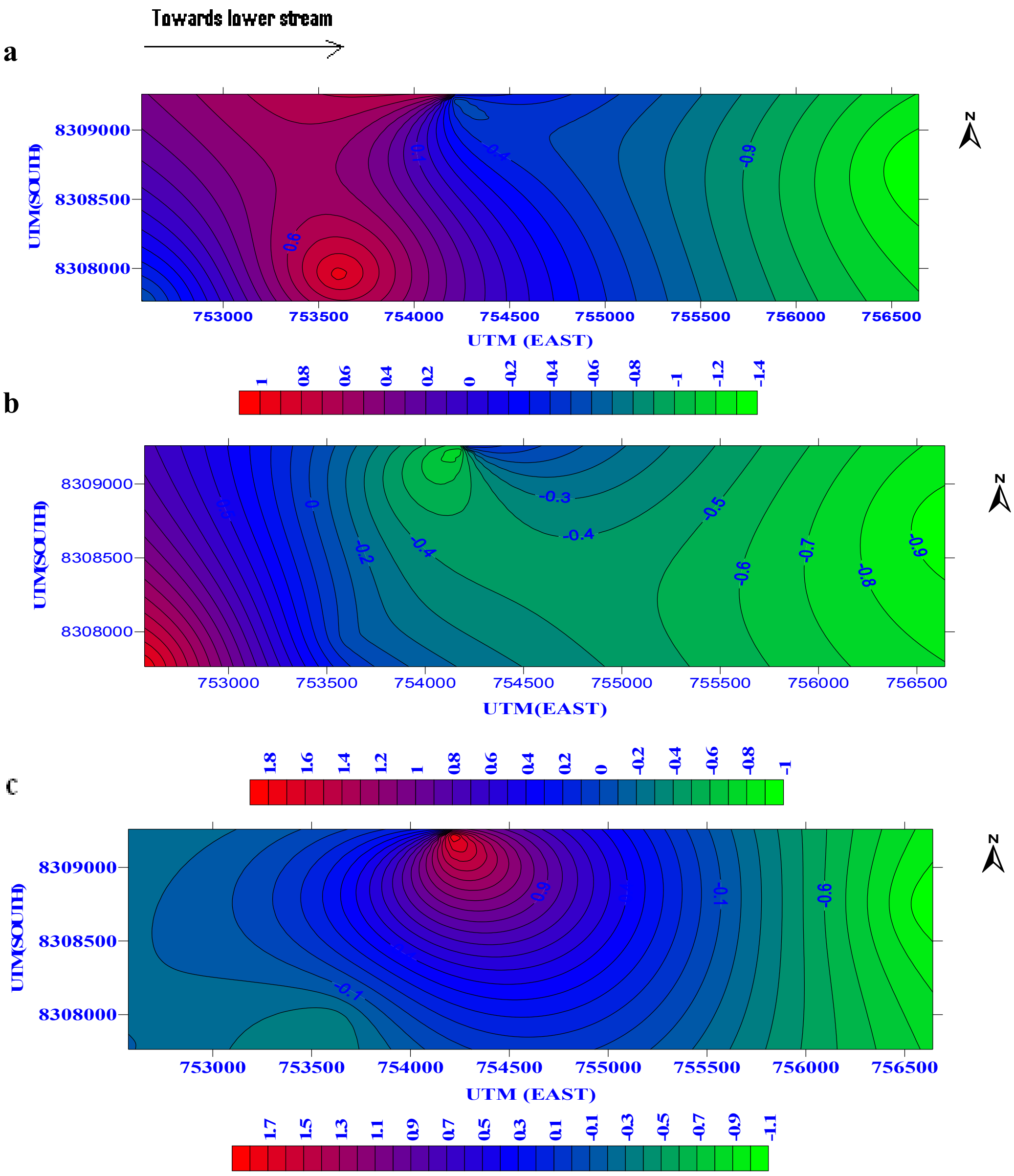
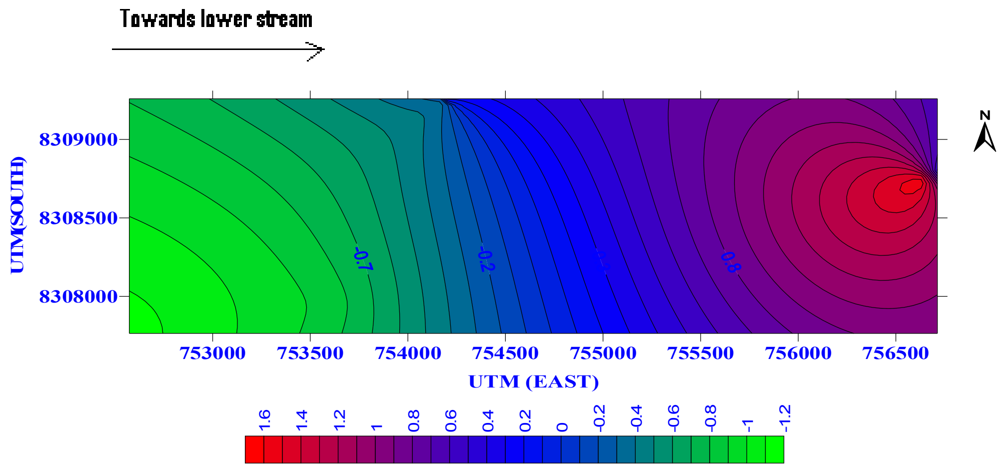


Figure 15. Spatial variation of the factor loadings previous years; **a** Factor 1, **b** Factor 2 **c** Factor 3

In (September, 2008), two factors were extracted. Factors 1 had high loadings in nitrates, temperature, electrical conductivity, and dissolved oxygen representing agricultural and other anthropogenic sources. It controlled 46% of the total variations. This factor affected the water quality in the lower section and this had a positive impact on the larval densities (see figure 16a and 18a). Factor 2 controlled 23 % of the total variations. This factor registered high loadings in phosphates and pH. This controlled the upper and the middle section (figure 16b).

a



b

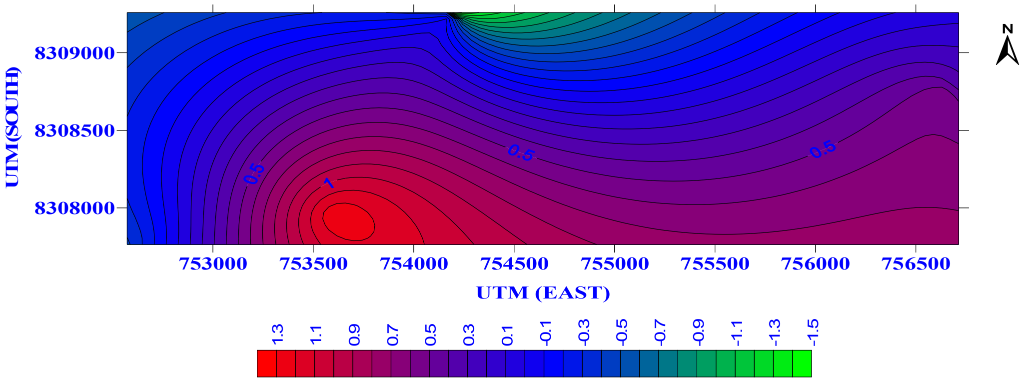


Figure 16. Spatial variation of the factor loadings; **a** Factor 1 September,2008 **b** Factor 2 September,2008

In wet season (December, 2008), factor 1 controlled 44.32% of the total variations. It showed high loadings in temperature, electrical conductivity, Nitrates, total hardness and alkalinity and negatively affected the upper section of Domasi river and positively affected the lower section (Figure 17a). Factor 2 of the wet season controlled 26 % of the total variations and showed high loadings in total suspended solids, dissolved oxygen and pH. This factor controlled the upper section and the middle section with positive loadings (Figure 17b). This factor had low impact in the uppermost part of the river and a higher impact in the upper section towards the middle section of the river and showed a moderate impact in the lower section.

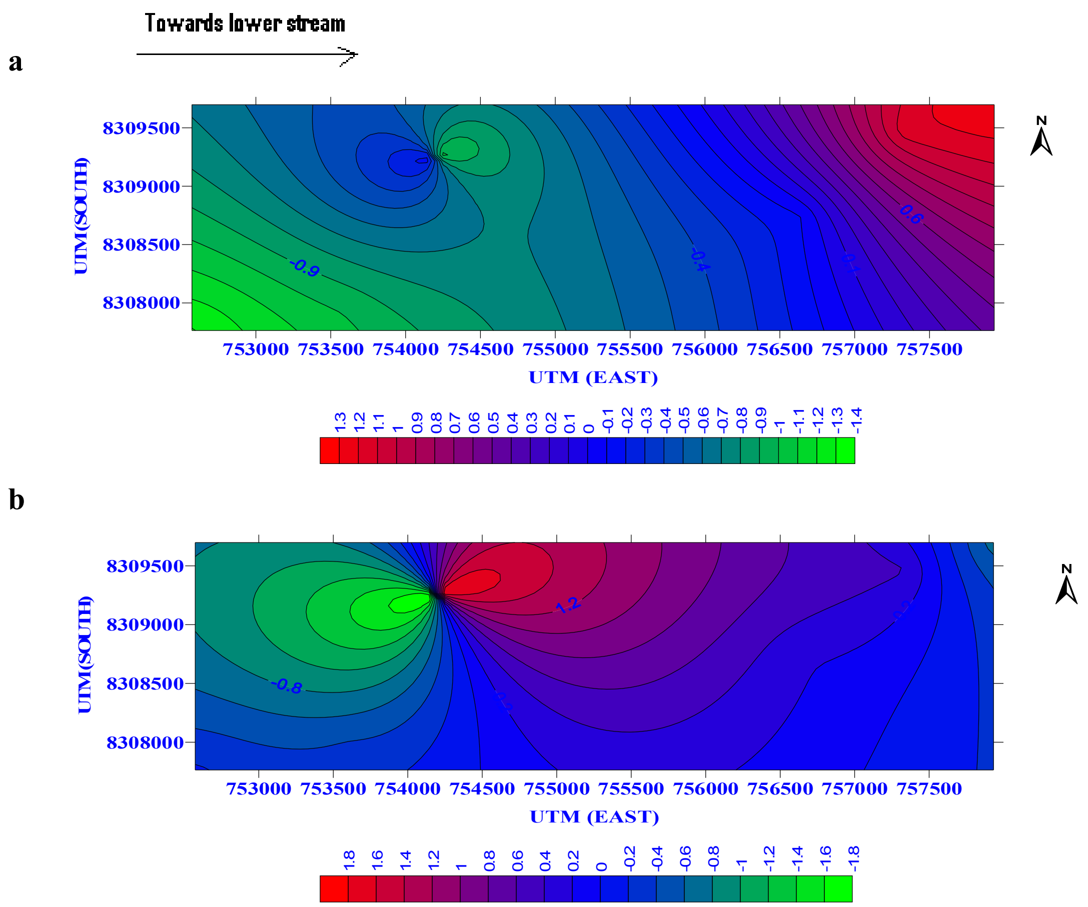


Figure 17. Spatial variation of the factor loadings; **a** Factor 1 December, 2008, **b** Factor 2 December, 2008

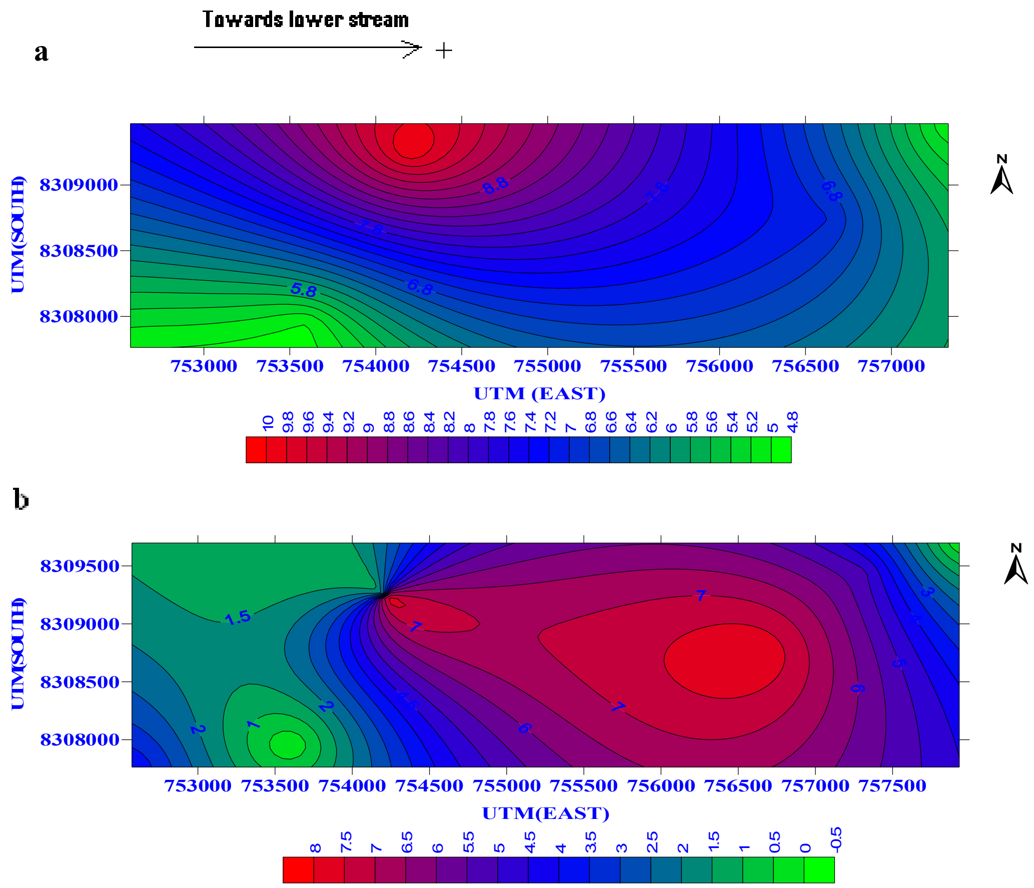


Figure 18. Spatial variation of the larval densities: **a** Larval density (dry season) **b** Larval density (wet season).

4.6.0 Correlation studies of physicochemical constituents and larval densities

In this study, the correlation between various elements and larval densities has been presented in table 9 and 10. The correlation matrices show high levels of positive and negative correlations among different physicochemical constituents and also the blackfly larval densities. Not all physicochemical constituents showed important implications on blackflies. Only electrical conductivity, temperature, Phosphates, total suspended solids, total hardness and dissolved oxygen were associated with blackfly larvae density.

4.6.1 Impact of the Total Suspended Solids (TSS) on the blackfly larvae

The composition and concentration of TSS strongly correlated with larval density ($r=0.755$, $p<0.050$). Suspended particles are crucial to blackfly larvae because they are of higher nutritional quality (Zhang *et al.*, 1998). Studies by Wallace *et al.,* 1982, revealed the presence of adenosine triphosphate in small suspended particles (solids). Kondratief and Simmons, 1985 indicated a more dense bacterial population on the suspended particles which were small in size and concluded that there is an increased quality for microbial habitation associated with suspended particles which are small in size. The bacteria are important food source for blackfly larvae (Zhang et al, 1998). Literature has revealed the fast and abundant growth of the blackfly larvae following floods because of the increased total suspended solids (Vora, 2008). Total suspended solids have 95% organic content (Parmer, 1997) and the blackflies feed on the organic matter non selectively (Zang *et al*., 1998). The upsurge of the blackflies can therefore be attributed to the increased suspended solids since the blackflies are suspension feeders.

4.6.2 Impact of Total Hardness on the blackfly larvae

Water hardness correlated well with the larval densities (r =0.859, p<0.020). Total hardness also showed a significant correlation with the nitrates (r =0.950, p<0.003). This study revealed increased non carbonate hardness (associated with nutrients) concentration in Domasi river. Thus, the strong positive correlation between total hardness and the larval blackflies indicates high preference of the blackflies to sites with high nutrient load since they are suspension feeders and feed on the nutrients in the water non selectively as it flows by.

4.6.3 Impact of dissolved oxygen on the blackfly larvae

A strong negative correlation between the larval densities and dissolved oxygen was observed (r = -.732, p =0.050). The decrease in dissolved oxygen suggested an increase in oxygen demand by microbes in the processes of biodegradation of the organic load in the rivers as clean surface waters are normally saturated with dissolved oxygen as suggested by Tebbutt (1998). Thus, the negative correlation between larval blackflies and dissolved oxygen denoted preference of the larval blackflies for sites with high organic matter (NDOM and DOM).

4.6.4 Impact of electrical conductivity on the blackfly larvae

Multiple regression analysis of the study showed a significant positive correlation of EC with nitrates (r =0.935 p<0.004 and r =0.917 p<0.006) in dry and wet season respectively (Table 8 and 9). Electrical conductivity is a measure of the water's ability to conduct current in relation to the amount of dissolved salts in the water. The increase in electrical conductivity can be attributed to anthropogenic activities.

Along Domasi river, river bank cultivation, bathing and washing are very common downstream. The mentioned anthropogenic activities lead to high loadings of natural dissolved organic matter (NDOM) which the larval blackflies feed on non-selectively.

4.6.5 Impact of water temperature on the blackfly larvae

The blackfly larval densities correlated positively with water temperature ($r = 0.819$ $p<0.030$). Water temperature thresholds place limits on the duration of the aquatic stage, developmental time from egg through various larval instars to adult emergence from the pupal stage of the blackfly. Survival of the blackflies is determined by the water temperature. Thus, an increase in water temperature would cause an increase in larval densities of the blackflies.

Table 9. A correlation matrix showing the physicochemical parameters association with the larval *Simuliids* in September 2008. R is the multiple correlation coefficient and P the probability associated with the contribution of the test parameters to the larval densities**.**

		Temp	pH	DO	EC	TH	Nitrat	Phosph	Larva
Correlation	Temp	—							
Coefficient (r)	pH	-.373	—						
	DO	.821	-.570	—					
	EC	.749	-.646	.586	—				
	TH	.652	-.085	.488	.738	—			
	Nitrat	.488	-.518	.220	.917	.623	—		
	Phosphat	-.046	-.372	.336	.003	-.234	-.081	—	
	Larva	.819	-.413	.709	.815	.859	.593	-.296	—
P-Values	Temp	—							
	pH	.233	—						
	DO	.023	.119	—					
	EC	.043	.083	.111	—				
	TH	.080	.436	.163	.047	—			
	Nitrat	.163	.146	.337	.005	.093	—		
	Phosphat	.466	.234	.258	.497	.328	.440	—	
	Larva	.466	.208	.057	.024	.014	.107	.284	—

Table 10. A correlation matrix showing the physicochemical parameters association with the larval *Simuliids* in December 2008. R is the correlation coefficient and P the probability associated with the contribution of the physicochemical parameters to the larval densities.

		pH	DO	Temp	EC	Alkalini	TH	TSS	Nitrate	Phosph	larva
Correlation Coefficient (r)	pH	—									
	DO	.429	—								
	Temp	-.977	-.416	—							
	EC	-.988	-.345	.985	—						
	Alkalini	-.625	-.323	.655	.659	—					
	TH	-.900	-.506	.925	.894	.365	—				
	TSS	-.588	-.368	-.656	-.680	-.293	-.585	—			
	Nitrate	-.937	-.312	.925	.935	.425	.950	-.717	—		
	Phosph	.915	-.718	-.268	-.268	-.073	-.141	.837	-.379	—	
	Larva	.273	-.732	-.245	-.245	-.193	-.058	.755	-.281	.813	—
P-Values	pH	—									
	DO	.198	—								
	Temp	.000	.206	—							
	EC	.000	.251	.000	—						
	Alkalin	.092	.266	.079	.077	—					
	TH	.007	.153	.004	.008	.239	—				
	TSS	.110	.236	.079	.068	.286	.111	—			
	Nitrate	.003	.273	.001	.003	.201	.002	.054	—		
	Phospha	.356	.054	.304	.295	.446	.395	.019	.229	—	
	Larva	.300	.049	.320	.246	.357	.456	.041	.295	.024	—

4.7.0 Zomba-Malosa Plateau deforestation

Historical satellite data indicated the presence of a very thick forest in Domasi river catchment. The satellite image for 1984 showed that trees were abundant in the Domasi river catchment. Almost the whole Zomba-forest reserve aproximately, 1901.800 hactares had thick forest cover (see figure 20). Satellite image of the same river catchment in 1994 showed a good proportion of an area approximately 8228.916 hactares with a change in the forest cover from thick to sparsely covered, leaving 10889.822 hactares of the forest reserve with thick forest cover (Figure 21). A recent satellite image in 2002 for the same catchment showed a near absence of natural trees along the Domasi river and its catchment. Only 8929.084 hactares had a thick forest cover while 6970.6123hactares was sparecely covered and 3118.405 hactares was thinly covered (Figure 22).

4.7.1 Effects of Deforestation on Domasi catchment.

Large scale conversion of forest land to clear land warms the surface temperature by 0.8 degrees year round in the subtropics and the tropics (Bounoua, *et al.*2002). In southwestern Uganda, Lindblade *et al*. (2000) found that changes in the land cover in the areas which surrounded cultivated swamps led to an increase in minimum and maximum temperature by 0.8–0.9°C. A plot of air temperature data for Domasi catchment as a function of time showed a linear relationship between air temperatures and time $R^2 = 0.871$, $p<0.05$. Figure 19 shows a graph of air temperature as function of time (years) from 1990 to 2008. The land cover changes contributed to the changes in the temperatures for the catchment by affecting the energy balance of the land surface (Afrane, 2005). Morphologic changes in vegetation changes the albedo and physiologic changes in vegetation alter heat flux (Afrane, 2006).

Land cover changes in Domasi catchment did not only affect the energy balance of the land surface but also the health of Domasi river as Wallace *et al.*1997, emphasized that there is an existing ecological link between the forest streams to their surrounding terrestrial catchments. In addition, a growing body of literature highlights the link between anthropogenic disturbances and river health (see for example, Allan *et al*., 1997; Rivers-moor *et al.*, 2008b). Thus, the alterations to the land cover of Domasi catchment contributed to gradual changes in the water quality of Domasi river over the years as (Growns and David 1991; Fortino *et al.,*2004) highlights that alterations to a terrestrial catchment of a river have significant influences on water quality. Therefore, a change in forest cover due to landuse in Domasi catchment represents a change in runoff over the years. This affected aquatic environment and the biota (Rivers-moor *et al*., 2008b) including the blackflies.

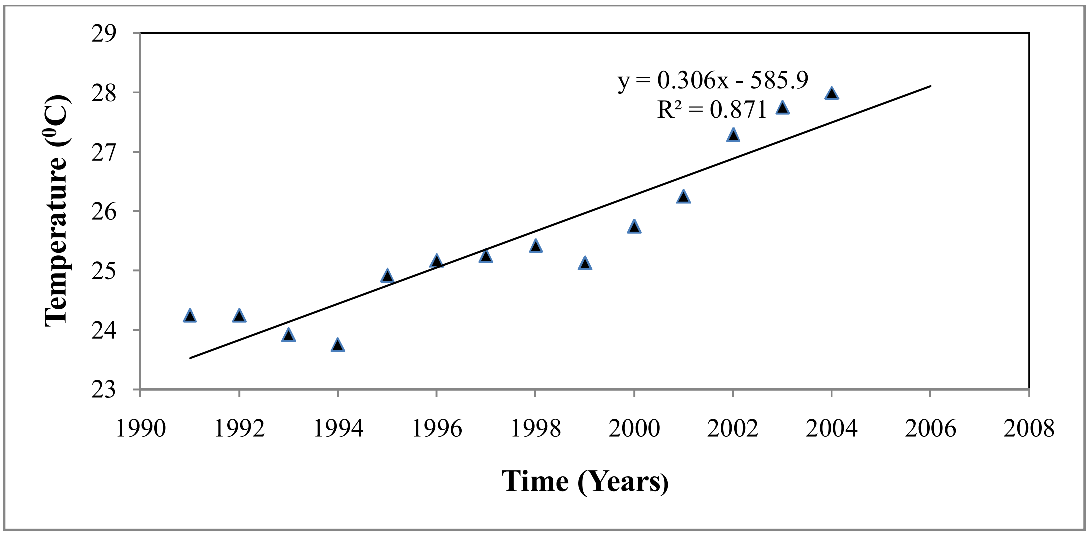


Figure 19 showing a change in air temperatures for Domasi catchment as a function of time in years

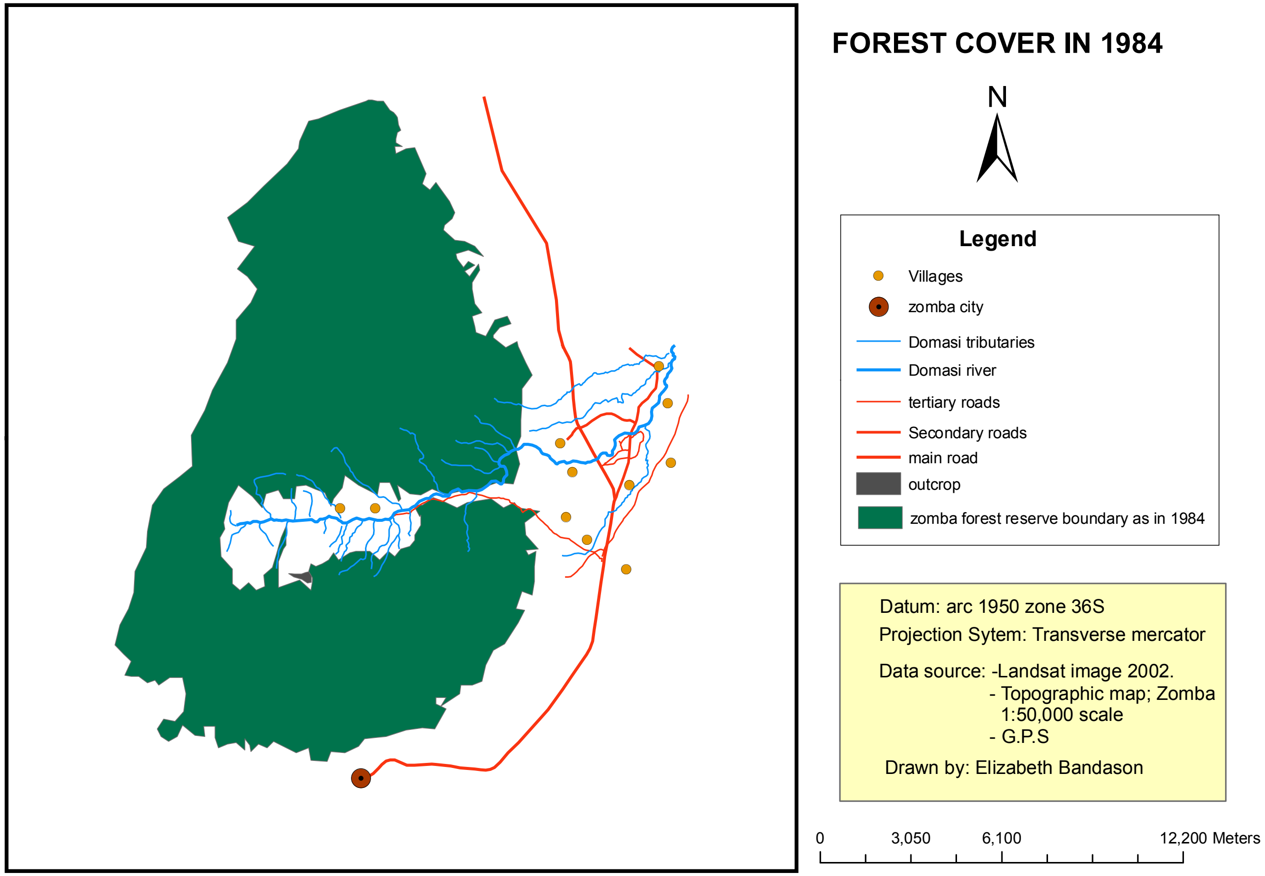


Figure 20. Showing a thick forest cover of Domasi Catchment in 1984

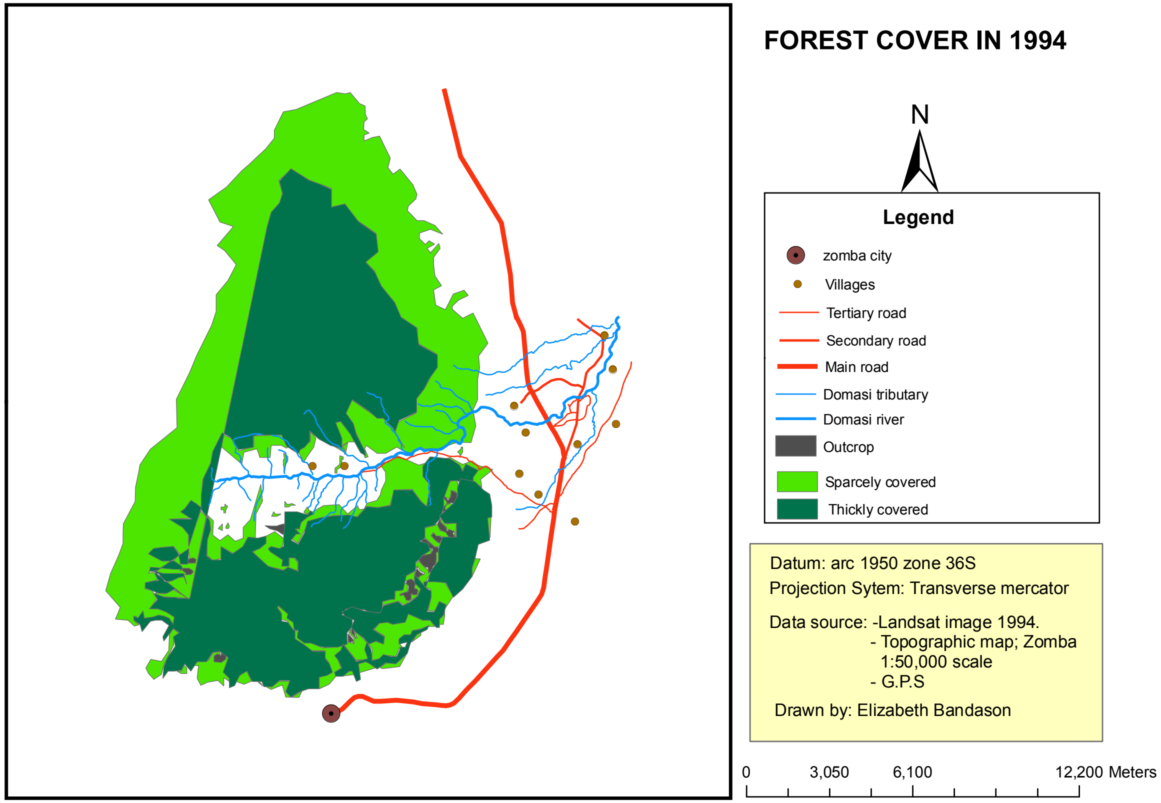


Figure 21. Showing a reduced thick Forest cover of Domasi Catchment in 1994

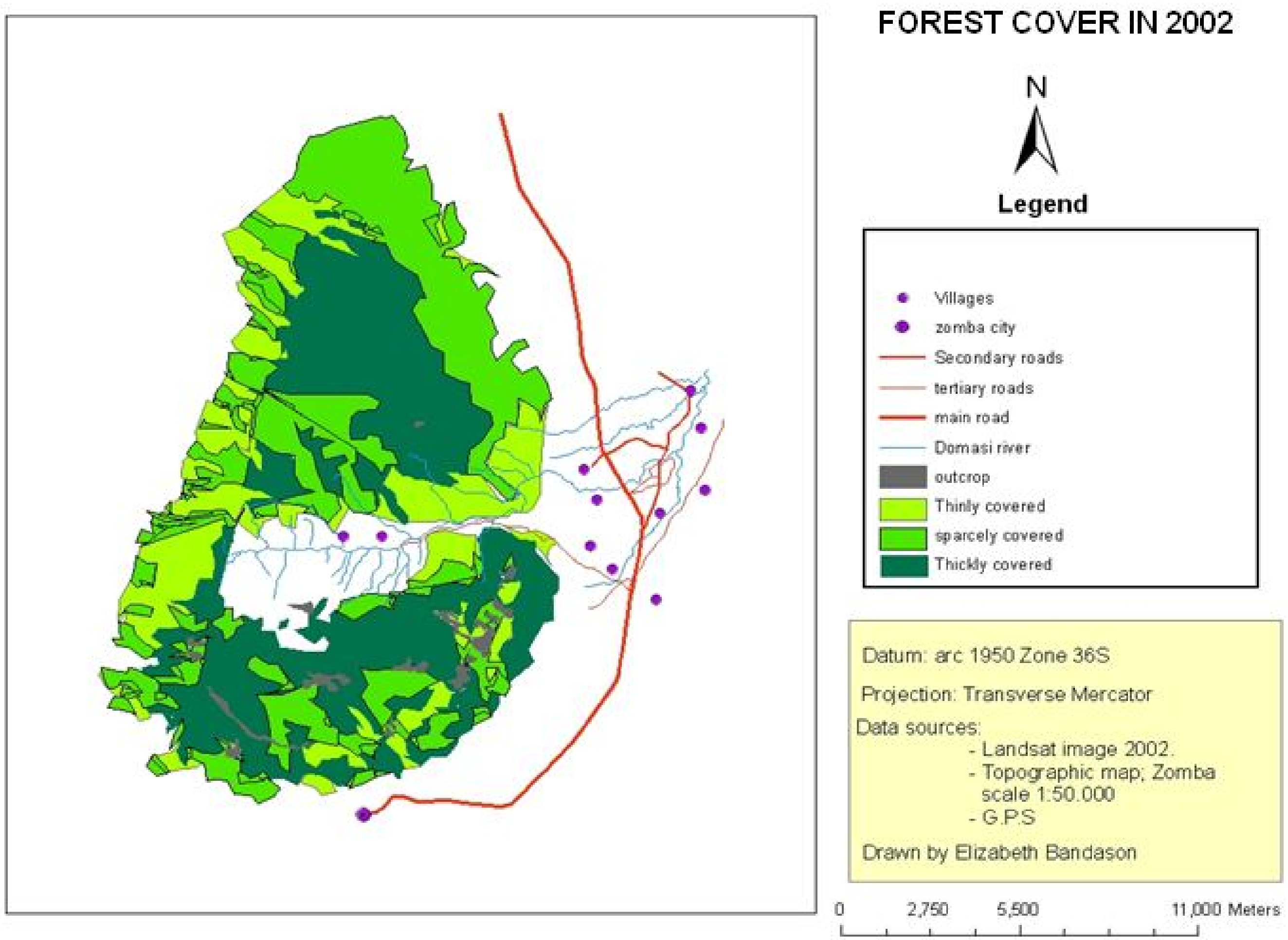


Figure 22. Showing extensively reduced thick Forest cover for Domasi Catchment in 2002

4.7.2 Effects of Deforestation on Blackflies (Diptera: Simuliidae)

The change in the land cover of Domasi catchment over the years as shown in figures 20, 21 and 22 could have modified the microclimate of the blackflies which might have had an effect on the blackfly survivorship and fitness. This affected the history traits of the blackflies. Vora (2008) showed an increase in the distribution of savannah blackflies after deforestation. Deforestation brought a whole scale ecosystem reconstitution. It contributed to creation of ecological niches favoring proliferation of vectors like the blackflies. Deforestation which contributed to the increase in the surface temperature in the Domasi catchment (see Figure 19 and 21), contributed to the higher feeding rates of the blackflies there by shortening the gonotrophic cycles. Since blackfly survivorship and fecundity are important determinants of their growth dynamics, the altered microclimates of the blackflies due to increased temperatures which caused the adult blackflies to feed more frequently also made them lay more eggs, more frequently. In the 1980's, the forestry cover was thick. This maintained low surface temperatures and decreased blackfly feeding rates and made the blackfly life cycles longer.

Land use change especially deforestation in Domasi river catchment might have significantly altered water temperatures of Domasi river. In rivers such as Domasi, riparian deforestation is associated with changes (usually increases) in water temperature and temperature variability (Steedman *et al.*1998). Thus, the blackfly development process especially larval stage was hastened with increases in the water temperatures. As indicated by (Regier *et al.* 1990), water temperature is a key to the physiology and ecology of aquatic biota. It is a limiting factor that affect distribution, behavior and survival of aquatic biota (Steedman *et al.*1998) including the blackflies. The temperatures of water bodies support the breeding of *S .damnosum* (Wilson et al, 2005). In West Africa, Ocran *et al.*,1982 found that there was variation in the distribution of members of *Simulium damnosum* depending on ecological zones and seasons.

Apart from causing changes in the water temperature, deforestation in Domasi catchment meant increased runoff which carried with it some organic debris increasing the total suspended solids in the river. The increase in the total suspended solids increased food quality and quantity for the blackflies over the years, hence their outbreak.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.0 Conclusions

This study was designed to investigate ecological changes that potentially led to the outbreak of blackflies (Diptera *Simuliidae*) in Zomba. Statistical and geospatial analyses which were applied to hydrochemical data set and land sat imagery data were used to provide insight into the factors and processes which were associated with the outbreak of the blackflies in Zomba.

In conducting studies like this, it would have been easier to compare directly climatic and water quality data with biological data. This would have involved collecting or accessing Black fly abundance data over time and compare to changes in environmental factors in order to show causality. Lack of direct comparison was one of the limitations in this study.

Principal component analysis (PCA) offered an effective means of manipulating, interpreting and presenting data concerning temporal and seasonal variability of the hydrochemical variables associated with the outbreak of blackflies in the study area.

The study, through spatial distribution of the factor scores delineated changes in the test physicochemical variables over the years. Areas of high scores of factor 1 in both dry and wet seasons in recent years (2008) are located in the lower section of the stream indicating anthropogenic pollution of the stream over the years. While areas of high scores of factor 3 in the previous years represented a small section of the river in the affected by total suspended solids, areas affected by high scores of factor 2 in the 2008 represented a large section of the upper stream affected by total suspended solids. This indicated changes in the land use activities in the Domasi catchment which increased total suspended solids in the river over the years. Thus, the source of total suspended solids was delineated as part of the catchment of the upper stream which constitutes major forest reserve in the area.

Hydrochemical results on the temporal variation obtained from students't-test of the concentrations of the test parameters showed significant increases in the total hardness (TH) (non-carbonate type), total suspended solids, alkalinity and nitrates. The increase in the TH was attributed to increase in the nutrient load in the river over the years while the increase in alkalinity was attributed to large exports of carbonates and carbonates from the soils as a result of runoff in the catchment area.

Results of correlation studies between hydrochemical parameters and the larval densities revealed positive correlations of larval densities with total suspended solids, water temperature and total hardness. Negative correlation of larval densities with dissolved oxygen revealed preference of larval blackflies for places with high organic matter. Thus, increase in the total suspended solids, total hardness and organic matter in the river over the years, represented an increase in food quality and quantity of the blackflies.

Forest cover change analysis using GIS and remote sensing technologies provided further information about ecological changes in the catchment over the years. Landsat imagery data indicated a decrease in the forest cover for the catchment from 1984. This indicated changes in energy balance of the study area as the increase in temperatures of the study area was evident over the years. This increase in temperatures contributed to changes in the microclimates of blackflies in the places they breed and rest and caused high blood ingestion rate in the adult blackflies, decreased their gonotrophic cycles and contributed to their increased populations.

This study has revealed changes in the blackfly populations as a consequence of ecological change. Information obtained on the ecological changes therefore represents a base for future ecological work which will help in planning, protection and decision making regarding ecological reserve determination of catchments and rivers to safeguard public health.

5.1 Recommendations

- Insect ecology has a predictive potential and it is necessary that ecological studies of vectors be enhanced to relieve insect outbreaks. Many of the major vector problems are associated with man made changes in the environment. This study, therefore recommends restoration of disturbed sites in Domasi catchment to relieve blackfly outbreaks.

- Further studies using remote sensing techniques and statistical modeling should be done to come up with a model which can be used to predict the outbreak periods of the blackflies in Zomba, Malawi.

- This study did not thoroughly investigate the effect of deforestation on microclimates. Further studies are required to investigate the changes in the microclimates in an ecosystem due to land use change.

- Detailed studies on the effects of microclimatic changes caused by deforestation on the survivorship and reproductive fitness of *Simuliium damnosum* in affected areas may contribute to the knowledge on the blackfly outbreaks and control.

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APPENDICES

Appendix I: Summary of results.

Table a. Summary table of all the test parameters at each site in the previous years (1985-2007).

Site	1	2	3	4	5	6
Conductivity (mS)	20.500	41.950	36.500	-	64.300	20.000
pH	6.300	7.230	6.860	7.210	6.670	5.600
Alkalinity (mg/L)	14.000	10.650	4.970	12.240	0.560	0.340
Hardness (mg/L)	23.500	8.740	5.050	7.420	0.013	0.002
TSS (mg/L)	6.000	5.500	3.500	3.000	5.800	-
Nitrate (mg/L)	0.025	0.001	0.001	0.001	0.024	0.200
phosphates	1.550	0.910	1.495	0.046	0.230	0.039

Table b. Summary table of all the test parameters at each site in July, 2008.

Site	1	2	3
Water Temperature (°C)	15.400	17.300	16.300
DO (mg/L)	8.800	9.400	7.700
Conductivity (mS)	20.800	24.800	27.200
pH	6.110	6.200	6.270
Alkalinity (mg/L)	11.160	17.540	17.540
Hardness (mg/L)	-	-	-
TSS (mg/L)	17.330	23.000	28.000

Table c. Summary table of all the test parameters at each site in September, 2008.

Site	1	2	3	4	5	6
Temp (°C)	17.670	19.100	20.130	20.170	21.000	21.300
DO (mg/L)	7.400	7.400	8.370	7.870	8.370	8.570
EC (μs)	25.500	25.470	27.900	28.600	31.930	43.170
pH	6.260	7.210	6.180	6.500	6.620	6.940
TA (mg/L)	-	-	-	-	-	-
TH(mg/L)	43.540	48.400	44.580	44.250	48.600	49.860
TSS (mg/L)	-	-	-	-	-	-
Nitrate (mg/L)	0.040	0.023	0.026	0.040	0.035	0.137
phosphates	0.009	0.004	0.043	0.001	0.005	0.011

d Summary table of all the test parameters at each site in December, 2008.

site	1	2	3	4	5	6	7	8	9	10
Temp (°C)	20.300	20.100	20.370	20.400	20.100	21.230	21.730	22.000	22.000	22.030
DO (mg/L)	7.670	7.370	7.570	7.130	5.500	7.000	7.330	6.900	6.930	6.830
EC (µs)	21.770	21.960	24.170	24.170	28.770	28.900	32.930	42.860	42.530	39.970
pH	6.930	7.000	6.250	6.940	6.840	6.910	6.860	6.700	6.750	6.710
TA (mg/L)	38.130	47.470	38.140	47.660	47.670	57.200	57.210	57.210	82.630	101.690
TH (mg/L)	218.530	223.300	225.210	260.380	263.370	258.630	252.400	288.190	279.120	259.080
TSS(mg/L)	4830	3980	340	5560	6970	1910	3170	740	5970	3780
NO_3(mg/L)	0.203	0.244	0.349	0.29	0.252	0.301	0.302	0.442	0.328	0.355
PO_4^-	0.128	0.117	0.108	0.061	0.117	0.11	0.112	0.147	0.095	0.151

e. Summary table for larval densities in September 2008

Site	Larval density
DM1	2
DM2	2
DM3	9
DM4	8
DM5	-
DM6	5

f. Summary table for larval densities in December 2008

Site	Larval density
DM1	5
DM2	5
DM3	-
DM4	-
DM5	10
DM6	7
DM7	-
DM8	3
DM9	9
DM10	5

Appendix II: Plots for principle components

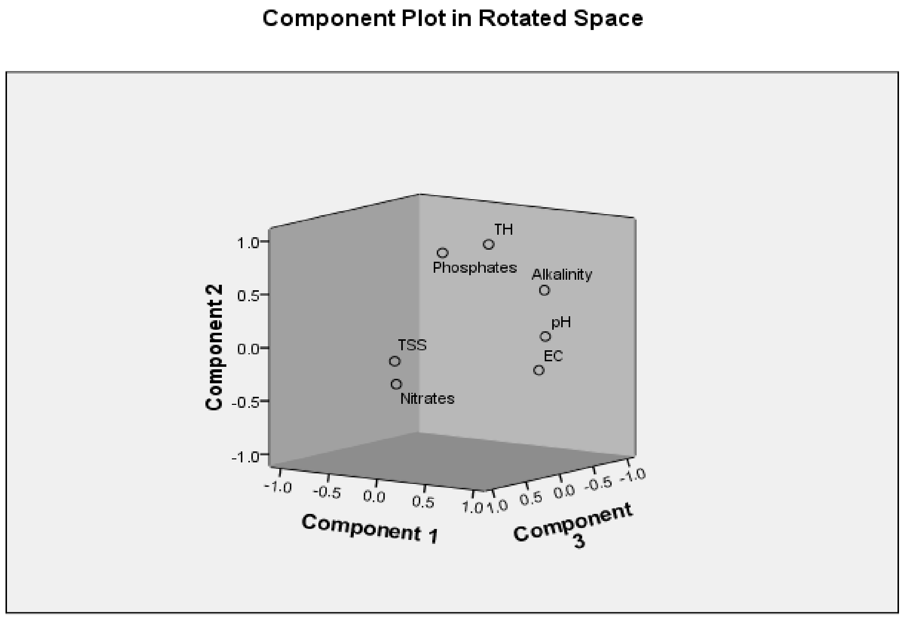

Figure I. Component loading plot for previous years (1985-2002)

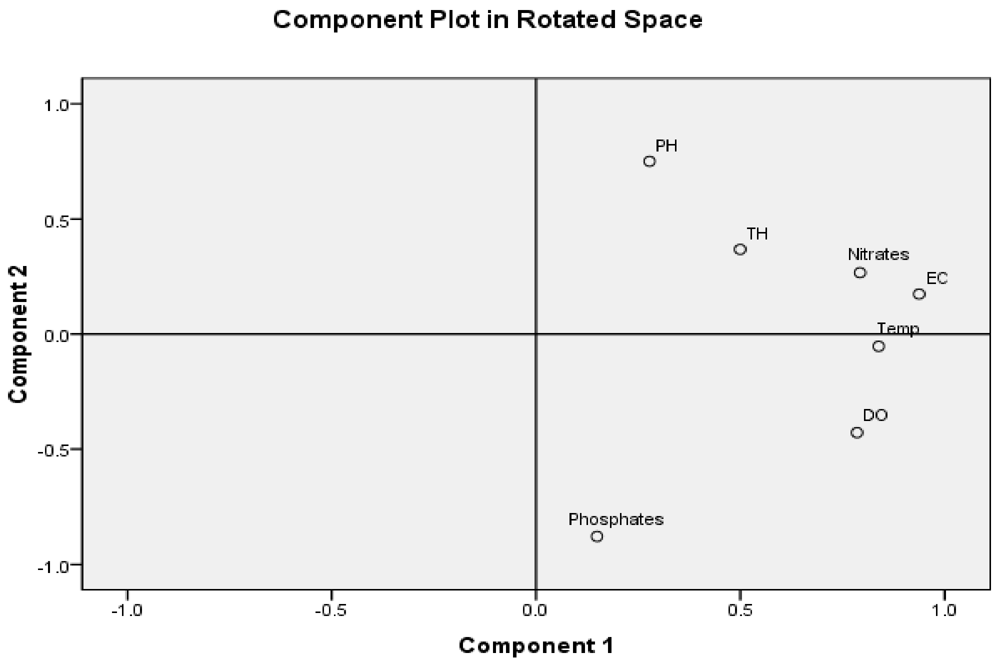

Figure II. Component loading plot for September, 2008

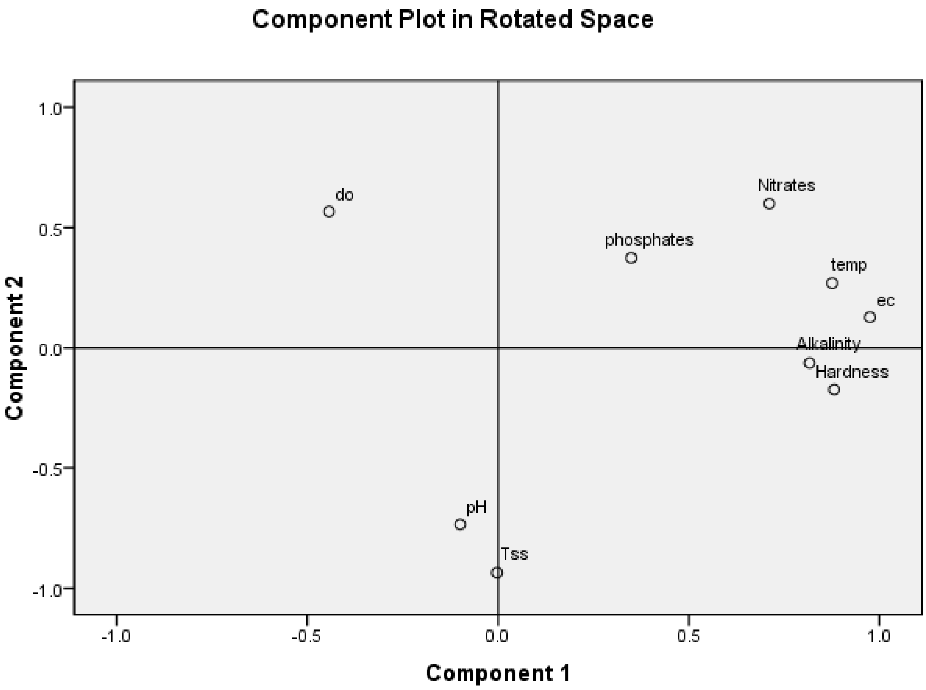

Figure III. Component loading plot for December 2008