

## Full Length Research Paper

# A biocoagulant slow sand filtration for disinfection of *Toxoplasma gondii* oocysts from Mezam River in Bamenda, Cameroon

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An integrated low-tech biocoagulant-sand filter drum for disinfection of oocysts of *Toxoplasma gondii* targeted for developing countries was evaluated. Dirty and turbid water (130.3 NTU) from Mezam River and leachates from dump sites and stagnant water in Bamenda, Cameroon, was analyzed microscopically after centrifugation for oocyst of *T. gondii*. Leachates from dump sites and stagnant water in Bamenda city had a very high concentration of oocyst of *T. gondii* too numerous to count per 10 ml while the major Mezam River had 50 oocysts per 10 ml. Considering that is widely used for various domestic chores; filtration disinfection of *T. gondii* was considered. A bench scale disinfection of oocysts of *T. gondii* with 0.2 g of powdered *Moringa oleifera* seeds per 10 ml of contaminated water from Mezam River showed a reduction of 50 oocysts per 10 ml to 10 oocysts in 15 min retention time. To optimize this, a pilot scale up was carried out using 120 L (120,000 ml) of water from Mezam River pretreated with 2400 g of powdered *M. oleifera* seeds for 15 min retention time and filtered through a sand filter drum made of fine sand, coarse sand, charcoal and gravel for 1 h filtration time. The total mean values of oocysts counts for 120 L of water to be filtered were 600,000 per 120,000 ml. The oocysts counts reduced to 10,000 after pretreatment with 2400 g of powdered seeds of *M. oleifera* and after a final filtration through a sand filter drum, no oocysts of *T. gondii* was detected in the final treated water. The findings from this study suggests strongly that the application of natural coagulants and sand filtration systems could serve a simple low cost disinfection for oocysts of *T. gondii* from water systems in resource limited countries.

**Key words:** *Toxoplasma gondii*, oocysts, disinfection, water, biocoagulant, moringa, turbidity, sand filter, drum, Cameroon.

## INTRODUCTION

Toxoplasmosis is a widely distributed protozoan disease in Sub Saharan Africa, caused by *Toxoplasma gondii*. According to Dubey, the infective stages of *T. gondii* are capable of infecting a variety of vertebrates including humans (Dubey and Joes, 2008). Domestic and wild felids are capable of serving as definitive host and *T.*

*gondii* oocysts are excreted in their faeces (Ortis and Pinon, 2004). Toxoplasmosis is caused by ingesting *T. gondii* oocysts from contaminated water or foodstuff or by consuming *T. gondii* tissue cysts from infected hosts (Wallon et al., 1999). In Cameroon, and in most of Africa, many domestic animals are reared close to homes and

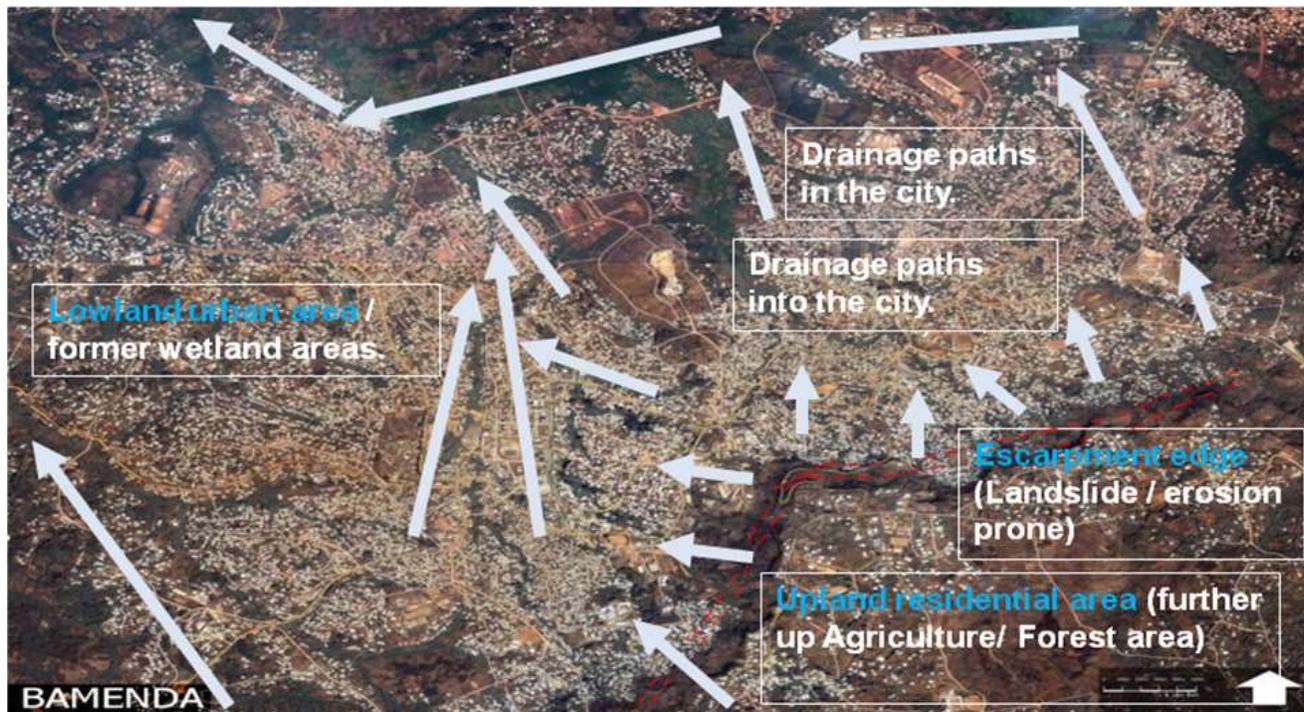


Figure 1. The map of Bamenda.

many cats stray in the wild, feed on and excrete on garbage as well as drink from surface water that are used by humans. Consequently, the impact of oocysts on toxoplasmosis epidemiology in Cameroon needs to be carefully studied because they are suspected to be associated with *T. gondii* seroprevalence in some emerging outbreaks of acute toxoplasmosis in humans and patients with compromised immune systems (De Moura et al., 2006; Yongabi, 2013). *T. gondii* oocysts are probably responsible for a significant part of infections in animals that are later consumed by humans (Sroka et al., 2006). The incidence of zoonotic toxoplasmosis in the Cameroonian population is high (Yongabi, 2013). Toxoplasma epidemiology in Cameroon has not been well reported. Less than 40% of the 22 million Cameroonians can afford clean and safe drinking water. Treated water still remains unaffordable for many Cameroonians. The treatment systems are expensive and not well implemented with poor plant management. The level of water and environmental pollution is increasing with increasing population amidst poor pollution management (Yongabi et al., 2011). The need to adopt simple sand filtration for water treatment is exigent. Slow sand filters have been observed as effective and lowcost (Yongabi et al., 2011). Furthermore, *Moringa oleifera*, a vegetable plant found across Africa

have been noted to possess coagulant activity and recommended for water purification (Yongabi et al., 2011). In routine microbial analyses of water in Sub Saharan Africa, the indicator organisms such as faecal coliforms have been applied. Such indicator organisms may not correlate well with the presence of oocysts in water bodies, thus rendering the reliance on this suspicious (WHO, 1984). Detection of *T. gondii* oocysts in environmental samples such as water and leachates in Cameroon and possible disinfection is crucial, as this coccidian parasite can be responsible for severe infections in humans and animals via ingestion of a single oocyst from contaminated water. In this paper, we report the potential use of *Moringa oleifera* and sand filter system to disinfect oocysts of *T. gondii* from surface water in Cameroon.

## MATERIAL AND METHODS

### Study site: Bamenda City Council

The study area was Bamenda metropolis and its environs in the North West Region of Cameroon. The map of the study area and description of the area is shown in Figure 1. Bamenda has three local councils under its municipal jurisdiction. Bamenda I, Bamenda II and Bamenda III. Approximately 85% of the inhabitants are

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**Figure 2.** River with garbage dumped into it.

subsistence farmers. The major development trends fall under, rehabilitating existing road network and creating new ones, reclaiming and developing wetlands and daily collection and disposal of garbage. Some of the greatest short-comings are the dilapidated state of roads and other networks; the poor state of habitations, especially in urban slums; the problem of waste management and drainage. Additionally, water and sanitation crisis, many homes do not have proper toilets. There is the haphazard digging of latrines and burials near habitations, with potentials to pollute the little underground water available. About 1,500 people are farming and grazing on the different watersheds of Age for and Bamendankwe and Akum, constituting the biggest threat to the forest, soil and water conservation in the area. The people at the foothills, plains and valleys (Mankon, Nkwen, Bamendankwe, Njah, Mbatu, Nsongwa, Chomba), estimated at more than 500,000, and the population of the Tubah Sub Division (Bambui, Kedjom, Sabga, Kedjom, Bamessing), estimated at more than 350,000, need the water from the watershed uphill. These areas constitute the slums of the city, with streams choked by forest and agricultural plants, and sanitary conditions are very poor, with malaria epidemics and periodic cases of cholera (Figures 2 and 3)

#### **Collection of water and leachate samples and processing**

Ten water sample from different points of the Mezam River, and ten leachate samples from waste dumps within the city of Bamenda were collected at different points located in the city. The samples were taken from places where cats, often excrete *T. gondii* oocysts. Samples were obtained according to the following procedure

described by Burns and Otterloo (1974); Ellis (1988); HACH (1990); APHA (1995) and Yongabi (2013a). A litre of the water and leachate were taken from the surface. Ten ml of each sample were placed in 10 ml test tubes for centrifugation.

#### **Concentration technique and centrifugation**

Concentration technique was used to detect *T. gondii* oocysts. This procedure included the concentration of 50 to 1000 oocysts per litre by flocculation or filtration, purification and detection of oocysts as described by Dubey and Jones (1988) and Pekzar et al, (1993). 10 ml of the water and leachate samples each were centrifuged at 300 rpm for 5 min; the supernatant were discarded and the deposit were examined directly under the microscope at x10 and x 40 magnifications after which stained films with mythelene blue were also examined microscopically (Villena et al., 2004)

#### **Microscopic detection**

The light microscopy was used. For the detection of unsporulated and sporulated oocysts, epifluorescence technique with UV light was used (excitation filter 330 to 385 nm, dichroic mirror 400 nm, barrier filter 420 nm). This facilitated the detection because both unsporulated and sporulated oocysts exhibit typical blue auto fluorescence (Yongabi, 2013; Yongabi, 2013b). The materials used in the construction of the sand filter were locally gotten at a river bed and included; 150 L carrying capacity drum (plastic), 1½ yards of hose, four clips, three nipples, strainer or sieve, sharp river sand



**Figure 3.** Subsurbs of Bamenda, a foot bridge with stray pig feeding close to a water fetching point.

(coarse and fine), charcoal and gravel. All these materials (sand, gravel and charcoal) were carefully washed and rinsed repeatedly in clean water (Yongabi, 2010). The laying of the materials in the drum was done in the order: laying of perforated hose connected to the collector tank, then a layer of gravel, followed by a layer of charcoal, then coarse sand (2 mm in size) and two layers of fine sand (0.15 to 0.30 mm size) on top. Ten litres of each of these materials were filled into the filtration drum. A test trial was carried out by flushing the set up repeated with clean water (Yongabi et al., 2011). The moringa pretreated water was then passed through the system. A hundred (100) gram of Moringa powder was sprinkled into 100 L of turbid water at a residence time of 25 min filtered using sack muslin cloths before pouring the filtrate into the sand packed drum. The final filtered water was collected in drum 2 and samples taken for analyses (Figure 4). Three water samples from Mezam River and leachates were collected and subjected to all these treatments. Mature seeds of *M. oleifera* were obtained from Maroua in the far north region of Cameroon. A stock of the powder was prepared and kept for use. A 2000 seeds were deshelled and pulverized in clean mortar using a pestle. The powder (from 2400 g) was sprinkled onto 120 L of the dirty pond water in a 150 L capacity drum (see picture) and stirred using a clean wood stirrer and the set up allowed to sit for 15 min retention time. It was then filtered off using a muslin sack cloth and the filtered water was then passed through a sand filter drum.

#### pH

The pH of the water samples before and after treatment with biocoagulants and sand filter was measured using a pH meter

model pH 1 to 125. The electrodes of the pH meter were standardized by calibrating in acidic and basic buffers raised on distilled water. The pH was taken by inserting the electrodes into test tubes containing wastewater samples and pH read off from the meter screen. The values obtained were consistent with values from HACH DR 2000.

## RESULTS AND DISCUSSION

The result of the initial oocysts of *T. gondii* counts from Mezam River per 10 ml was 50 (Table 1). These counts per 10 ml suggest that *T. gondii* oocysts are very prevalent in environmental samples in Cameroon. It was also observed that the leachates from wastes dumped in and around Bamenda had very high oocysts counts per 10 ml, much more than in water sample from Mezam River. In previous studies, Dubey and Jones (2008) reported the presence of *T. gondii* oocyst from environmental samples in the United States. In this study, *T. gondii* oocysts were detected in Mezam River. Sroka et al. (2006) reported the occurrence of *T. gondii* in water from wells located on farms. In a related study in 2004, Ortis and Pinon also detected oocysts of *T. gondii* in water samples. Waste management in Cameroon and in Bamenda in particular is poorly managed with many domestic animals that stray around on the major street.



**Figure 4.** Sand filter drum. Drum 1 (left), sand filter media; drum 2 (right), collection /storage of filtered water.

**Table 1.** Physico-chemical and detection of Oocysts of *T. gondii* before filtration.

Parameter	Mezam River sample 1	Mezam River sample 2	Mezam River samples 3	Mean values (X)
Temperature (O°C)	27.0	26.0	27.1	25.0
PH	7.6	7.5	7.6	7.1
Turbidity (NTU)	130.3	130.1	130.3	130.2
Total solids mg/dm <sup>3</sup>	466.0	466.0	466.0	466.0
<i>Toxoplasma gondii</i> , Oocyst counts (cfu/10 ml)	50	50	50	50

TNTC – Too Numerous To Count, \* Water source, people use the water for other domestic chore, - Stray animals, cats, dogs and pigs drink from the source sporadic, - Analysis was done in the peak of rainy season (July to August, 2013 and 2014)

Cats in Cameroon are hardly confined and very common to find cat faeces as well as other faces of other animals on wastes dumped on the major streets. In Cameroon, rivers and streams are generally used for recreation as well as water fetched for household chores. The detection of *T. gondii* oocysts in Mezam River suggests that surface water must be checked for *T. gondii*. This pathogen poses a serious risk to pregnant women and

potential fetal transmission (Wallon et al., 1999; Cook et al., 2002). Slow sand filtration has been reported to be more than 99.99% efficient in removing pathogens from water. However, certain groups of organisms may still leached through the filter bad especially when poorly constructed (Yongabi et al., 2011). The efficacy of the slow sand filter systems on removal of *T. gondii* oocysts has not been previously reported.

**Table 2.** Physico-chemical and detection of *Toxoplasma gondii* oocyst after pretreatment with 0.2 g of *Moringa oleifera* (Lam) seed powder.

Parameter	Mezam River sample 1	Mezam River sample 2	Mezam River sample 3	Mean values (X)
Temperature	27.0	27.0	27.0	27.0
PH	7.0	7.0	7.0	7.0
Turbidity (NTU)	29.5	29.5	29.5	29.5
Total solids mg/ml	350	350	350.1	352.025
<i>Toxoplasma gondii</i> , Oocyst counts (cfu/10 ml)	10	10	10	10

**Table 3.** Physico-chemical and detection of *T. gondii* after purification with sand filter drum.

Parameter	Mezam River sample 1	Mezam River sample 2	Mezam River sample 3	Mean values (X)
Temperature (0°C)	25.1	25.1	25.1	25.1
PH	7.4	7.4	7.4	7.4
Turbidity (NTU)	12	13	13	12.5
<i>Toxoplasma gondii</i> , Oocyst counts (cfu/120,000 ml)120 L 600,000 Oocysts detected	nil	nil	nil	nil

The results from this study also showed conclusively that combining *M. oleifera* seeds powder on to slow sand filter system potentially reduced drastically oocysts of *T. gondii* from the contaminated water (Tables 2 and 3). Yongabi et al. (2011) reported the beneficial effects of *M. oleifera* extracts in disinfection of bacterial contamination of water. The toxic effects of plant extracts such as *M. oleifera* on *T. gondii* have not been previously reported. The efficacy of *M. oleifera* and slow sand filter systems in the removal of other pathogens has been observed (Eilert, 1978; Pollard et al., 1995; Yongabi, 2010). With the sand filter system, very dirty water for consumption can be recovered. Although, coliforms may be absent after treatment, a few oocyst of *T. gondii* may be present. The combined effects of a *M. oleifera* hybrid sand filter drum demonstrated 100% disinfection of oocyst of *T. gondii*. This observation is very important in that the materials are usually low cost and readily available in Africa. Additionally, information available on the prevalence of *T. gondii* oocysts in the environment in most African countries are lacking. This observation has been reported elsewhere. Very little information is available on the presence of *T. gondii* oocysts in naturally contaminated water (Villena et al., 2004; De Moura et al., 2006; Sroka et al., 2006; Villena et al., 2004; Sroka et al., 2006; Vaudaux et al., 2011). However, soil is also an environmental source of Toxoplasmosis in humans (Teutsch et al., 1979; Stagno et al., 1980; Weigel et al., 1999). It was indicated that pregnant women mostly get infection from soil (Cook et al., 2002). The conclusion made is that *T. gondii* infection can be prevented in Cameroon and in Africa at large through appropriate filtration methods using low cost materials.

### Conflict of interests

The authors did not declare any conflict of interest.

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