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#### **African Journal of Environmental Science and Technology**

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# African Journal of Environmental Science and Technology

Full Length Research Paper

# A new model of dynamic of plant biodiversity in changing farmlands: Implications for the management of plant biodiversity along differential environmental gradient in the Yellow River of Henan Province in the spring

#### **Bing-Hua Liao**

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The intermediate disturbance hypothesis (IDH) is often used as a model or investigating the linkages between disturbance intensity and biodiversity. However, the relationship between plant diversity and disturbance gradient makes it difficult to compare data from different researches and draw general models. It is importance to assess the relationship between disturbance gradients and herbaceous species diversity in the farmland center/farmland ridge/farmland boundary on varying spatial scales along the Yellow River watershed ecosystem of Henan Province in the arctic. Using community ecology techniques and quantitative measurements of disturbance, results show a linear relationship between weighted values of disturbance intensity and herbs species diversity (Shannon index), which were significantly, negatively correlated (P<0.01) in the differential ecosystem types (farmland center/farmlandridge/farmland boundary) along differential disturbance gradient. Thus, understanding a linear relationship between herbs diversity dynamics and disturbance gradient in the differential type's landscapes is important for further research of local ecosystem functions and the goal of agricultural sustainable development in the context of biodiversity conservation. These results indicated that a synthesis quantitative index of disturbance (e.g. weighting values of disturbance) is the most essential environmental factors affecting herbs species diversity in the ecosystem to building a model. Therefore, this model plays a vital part in conserving global biodiversity and maintaining global ecosystem function.

Key words: Model, herbs species diversity, disturbance gradient, significantly, the Yellow River.

#### INTRODUCTION

Ecological model of the relationship between species diversity and environment is important along environment

gradient (Boyd, 2012; Liao and Wang, 2010; Keeling et al., 2000). Ecosystems are composed of large numbers

**Table 1a.** The relationship between IDH and biodiversity from the different perspectives.

The relationship between IDH and biodiversity from the different perspectives	Author
Water level fluctuations, fire and grazing are essential for maintaining plant diversity.	Keddy (2005)
Increased diversity at intermediate disturbance was due primarily to increased evenness	. Aronson and Precht, (1995)
Diversity and soil properties were best at intermediate disturbance levels.	Zhang et al. (2010)
The IDH pattern was obtained for low frequency dependence and low immigration.	Cordonnier et al. (2006)
Generate a period-bubbling bifurcation structure and population dynamics that are most variable at intermediate disturbance frequencies.	Reluga (2004)

Table 1b. The physical geographic conditions of the Yellow River basin nature reserve.

Precipitation (mm)	Annual mean temperature(°C)	Latitude (°):	Longitude (°):	Elevation (m)†
800 - 1100	12.4 - 14.8	34.58 - 36.07	116.06 - 110.41	34 - 412

†Above sea level.

of plant species along environmental gradient, making species-centered studies of systemic processes and functions extremely difficult, if not out rightly impossible to carry out (Liao et al., 2011a, b). The intermediate disturbance hypothesis (IDH) suggests that species diversity will be maximal at intermediate levels of disturbance (Connell, 1978). Moreover, many experiments have assessed the relationship between plant species diversity and disturbance intensity from IDH perspective along different disturbance gradients in theoretical ecology (Leis et al., 2005). For example, Leis et al. (2005) found that disturbance up to intermediate levels can be used to maintain biodiversity by enriching the plant species pool. Further, Biswas and Mallik (2010) suggest that plant species richness and diversity, functional richness and diversity reached peaks at moderate disturbance intensity in riparian and upland plant communities.

Therefore, the relationship between plant species diversity and intermediate disturbance intensity has many real world applications in the field of ecological conservation and restoration (Table 1a).

Here, using plant community ecology techniques, this study investigated three key questions regarding the relationship between herbaceous plants species diversity and intermediate disturbance levels in the ecosystem types (e.g. farmland center, farmland ridge/farmland boundary, etc.). First, this study asked if the relationship between herbaceous species diversity dynamics and disturbance gradient from IDH perspective can be compressed into a general model that is universally applicable to ecosystem studies. Therefore, this study

shows how to link herbaceous species diversity and disturbance along disturbance gradient and can be applied to real world scenarios in order to prevent biodiversity loss and help in the management of degrading or degraded ecosystems.

#### **MATERIALS AND METHODS**

The dynamics of plant diversity in Henan Province section of the Yellow River basin are the results of the historical natural and anthropogenic activities (Table 1b, Figure 1).

Quantitative assessments mainly depended on disturbance of human-driven and season driven changes in herbaceous diversity and were based on a careful choice of landscape dynamics (Table 2).

Using community ecology techniques, we examined the influences of different disturbances on dynamics of herbaceous species diversity and disturbance interactions along the different environmental gradients in the Yellow River in the Henan Province from May 2010 to January 2011. Thirteen plots were established per 10 km length. A total of 30 plots were set. Each study plot, consisted of one 20 × 20 m tree layer plot, five (the center and four corners of the study plot) 2 × 2 m shrub layer quadrates and five 1 × 1 m herbaceous layer quadrates. Thus, these plots include 150 herbaceous layer quadrates in the farmland center, 150 herbaceous layer quadrates in the farmland boundary, and 150 herbaceous layer quadrates in the ridge of farmland from May 2010 to January 2011 (Table 3).

#### **Traditional indices**

These are the most widely used indices: Shannon index (Margalef, 1958); Shannon index of diversity is generally calculated as the

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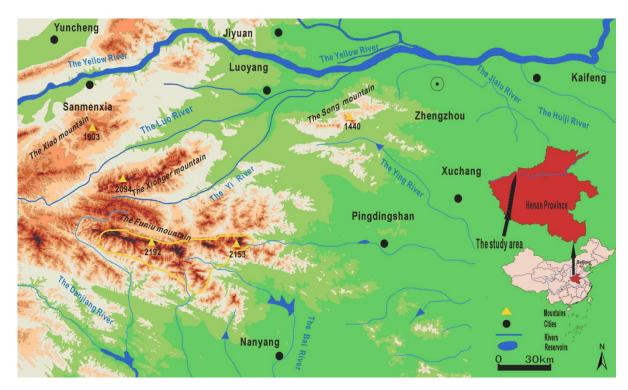


Figure 1. A digital cadastre map in the Yellow River of Henan Province.

**Table 2.** The selection of the weighting values of disturbance.

Quantitative measurements of disturbance to select and identify different disturbance	Differential agro-ecosystem types
Values of weighting of disturbance=Usage amount of herbicide	
+100/Return interval of using herbicide	Farmland center
+ 100/Return interval of tillage	Farmland ridge
+100/ The width of different types	Farmland boundary

**Table 3.** Investigation index along the elevation gradient variable.

Investigation	Layer	Community	Species	Height	Crow	Diameter
Community	Tree/shrub	Coverage	Species/ individual	Layer's	Crow height/	Basal
Investigation	/herbaceous	/community's age structure	number	Height	width	diameter

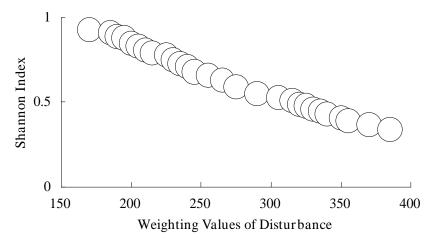
complement of H:  $H' = -\Sigma p_i \log(p_i)$ . Where i is the number of species and  $p_i$  is the proportion of the sample belonging to the i th species.

#### **RESULTS**

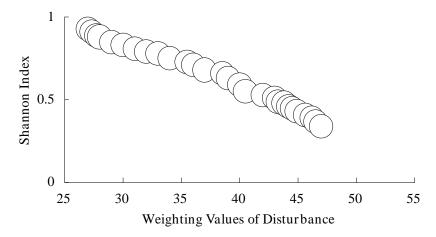
Disturbance is an important environmental factor affecting weed species diversity in anthropogenic activities in the farmland center/the farmland ridge areas/the farmland boundary areas. The relationship between values of

weighing disturbance and the weed species diversity investigated vary significantly in the herbaceous layer along the environmental gradient on varying disturbance scales in the different disturbance intensity farmland (Figures 2 to 4).

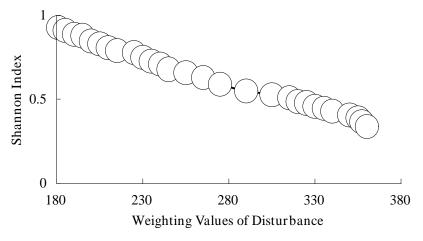
These results which shows that herb diversity (e.g. Shannon index) is expected to increase when weighting values of disturbance is reduced along disturbance gradients in the farmland areas of the Yellow River in the



**Figure 2.** The relationship between Shannon index and disturbance in the farmland center areas.



**Figure 3.** The relationship between Shannon index and disturbance in the farmland boundary areas.



**Figure 4.** The relationship between Shannon index and disturbance in the farmland ridge.

**Table 4.** The negative correlation between Shannon index and weighting values of disturbance.

Differential ecosystem types	Shannon index
Farmland center	-0.974**
Farmland ridge	-0.983**
Farmland boundary	-0.989**

Henan Province (Figures 2 to 4). Secondly, herbaceous diversity (e.g. Shannon index) is expected to decrease when weighting values of disturbance is increased along disturbance gradients in the farmland. Thus, our result implies that weak disturbance should lead to higher herbs diversity in the farmland.

These results suggest that herb species diversity (e.g. Shannon indicator) is expected to increase when weighting values of disturbance is reduced along disturbance gradients in the farmland boundary areas and the ridge of farmland of the Yellow River in the Henan Province (Figures 3 and 4). In addition, herb species diversity is expected to decrease when weighting values of disturbance is increased along disturbance gradients in the farmland boundary areas and the ridge of farmland (Figures 3 and 4). Therefore, this result implies that weak disturbance should lead to higher herbs species diversity in the farmland boundary areas. However, is there relationship between the species biodiversity and disturbance gradient in the Yellow River of Henan Province? To show this, the correlation between species biodiversity and the weighting values of disturbance was then analyzed (Table 4).

This results suggest a linear relationship between weighting values of disturbance and herb species diversity (Shannon index), which were significantly, negatively correlated (P<0.01). The results indicated that disturbance, largely determined by values of weighting of disturbance, are the most important environmental factors affecting herb species diversity (Table 4).

#### A general model of the links between herbs diversity and disturbance gradient from IDH perspective

The IDH is used as a framework for investigating the linkages between disturbance and species diversity. Unfortunately, the various IDH makes it difficult to compare data from different studies in order to draw general conclusions (Connell, 1978). It is therefore important to develop the model which is universally applicable across a range of agro-ecosystems so that the relationship between herbaceous species diversity and disturbance gradient can be more accurately quantified with quantitative measurements of disturbance intensity. Here, we proposed the use of model framework that

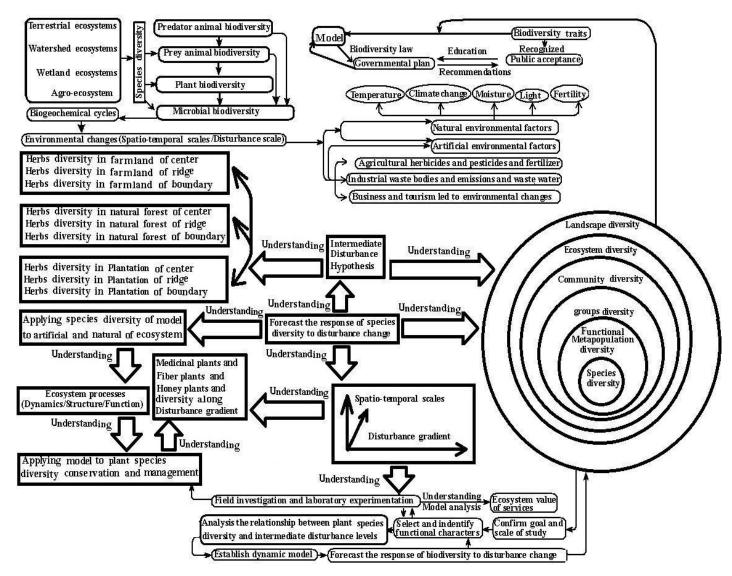
incorporates model (Figures 2 to 5) operating on varying spatial-temporal and disturbance scales for in-depth studies of the relationship between herbs species diversity and disturbance gradient (e.g. anthropogenic disturbance and natural disturbance) (Figures 5).

The model can help identify the relationship between herbaceous species diversity and disturbance intensity most relevant to tolerating environmental fluctuations or recovering from IDH perspective (Figure 5). The model is useful for ecosystem sustainable development in the context of biodiversity conservation. For example, Bartels and Chen (2010) explained that whether resource quantity or resource heterogeneity is the determinant of under story plant diversity in individual studies, was dependent on stand succession stage(s), presence or absence of intermediate disturbance, and forest biome within which the studies were conducted. Carvalheiro et al. (2011) suggest that presence of weeds allowed pollinators to persist within sunflower fields, maximizing the benefits of the remaining patches of natural habitat to productivity of this large-scale crop.

#### Applying general model to species conservation and ecosystem management

The model can use more theoretical ecology (e.g. diversity dynamics, species composition, food web structure, diversifying plant-microbial system, ecosystem stability/functioning, organic agriculture, biodiversity law, watershed ecosystem management, assessment of ecosystems and biodiversity, scale dependence) (Stutter et al., 2009; Shennan, 2008; Menalled et al., 2001, Bartels and Chen, 2010; Crowder et al., 2010; Bai et al., 2004; Davis et al., 2007; Bernez et al., 2002; Marris, 2010; Crawley and Harral, 2001). For instance, Menalled et al. (2001) suggested that agricultural management systems can have both immediate and long-term effects on weed species density, abundance and diversity. Shennan (2008) proposed that important elements for understanding biotic interactions include consideration of the effects of diversity, species composition and food web structure on ecosystem processes; the impacts of timing, frequency and intensity of disturbance; and the importance of multitrophic interactions in ecosystem. Stutter et al. (2009) proposed a mechanism whereby the establishment of vegetated buffer strips between cropland and watercourses on previous agricultural land causes a diversifying plant-microbial system. Bartels and Chen (2010) explained that whether resource quantity or resource heterogeneity is the determinant of under-story plant diversity in individual studies was dependent on stand succession stage(s), presence or absence of intermediate disturbance, and forest biome within which the studies were conducted.

Therefore, it will nonetheless be a substantial challenge



**Figure 5.** The proposed framework of relationship between herbs species diversity and disturbance gradient that incorporates herbs species diversity dynamic from the intermediate disturbance level, and operating on varying spatial-temporal and disturbance scales. The model was based on some studies (Liao and Wang, 2010; Liao et al., 2011a, b).

to apply this model to specific real world policy problems (Gilbert, 2010; James and Vorhies, 2010; Marris, 2010).

#### Conclusion

Progress in three key areas will substantially increase efforts to gain a rigorous understanding on how the links between herbaceous species diversity (Shannon index) and disturbance intensity, and their interactions, influence the response of ecosystem properties to changing biodiversity. First, better understanding of the relationship between herbaceous species diversity and disturbance intensity is correlated, particularly with respect to the

predominant forces of human activities. Secondly, the model based on both field investigation of community ecology techniques of 450 herbs layer quadrates and scientific analysis, can use more and more theoretical ecology. Finally, the model can be useful in understanding the plant species diversity and disturbance intensity from IDH perspective. Understanding the model is essential for further research of local ecosystem functions and the goal of agricultural sustainable development in the context of herbs biodiversity conservation in natural or artificial disturbance ecosystem. Therefore, the model may help to ameliorate this situation of losses of plant species diversity (e.g. medicinal plants, honey plants, fiber plants, etc.).

#### **Future work**

The watershed ecosystems are results of the historical, natural and anthropogenic activities along differential environmental gradient in the Yellow River of Henan Province. However, there is an important question in the future: what are the change patterns for watershed landscape, habitat and biodiversity in the region.

#### **Conflict of Interests**

The author(s) have not declared any conflict of interests.

#### **ACKNOWLEDGEMENTS**

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## African Journal of Environmental Science and Technology

Full Length Research Paper

# Molecular characterization of intestinal protozoan parasites from children facing diarrheal disease and associated risk factors in Yamoussoukro, Côte d'Ivoire

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Diarrheal diseases are very common in children under 5 years and may lead to a delay of physical and mental development. Despite this knowledge, data on diarrheal diseases and socioeconomic determinants are still scarce in Côte d'Ivoire. This study is then conducted with the objective to fill part of this gap and specifically assess link between infant diarrhea occurrence and some major socioenvironmental factors. Stool samples were collected from children less than five suffering from diarrhea at Yamoussoukro Regional Hospital in central Côte d'Ivoire. Molecular species specific diagnosis was used to detect *Cryptosporidium parvum, Giardia intestinalis* and *Entamoeba histolytica*, three major protozoan parasites which cause diarrhea. Out of 306 stool samples examined, 62.75% were detected as positive at least for one of the protozoan parasite studied. Species specific prevalence was 36.93% for *C. parvum*, 20.92% for *G. intestinalis* and 22.55% for *E. histolytica*. Infection was more prevalent in children whose mothers were not educated although the difference was not statistically significant. No link was found between gender and infection while sanitation infrastructures, mother and children ages and water sources were found significantly associated with diarrhea occurrence. Awareness is then needed for women on lack of hygiene rules that could lead to diarrheal diseases burden.

**Key words:** Diarrheal diseases, children development, parasitic protozoan, molecular characterization, socio-environmental factors.

#### INTRODUCTION

Diarrhea is the second leading cause of under-five deaths worldwide, after pneumonia (UNICEF/WHO, 2009). Among those who survive diarrhea, the morbidity burden may affect their development depending on the

intensity of diarrhea and pathogen in question. Malnutrition, anemia, growth restrictions, cognitive delays, irritability, increased susceptibility to other infections and acute complications are some of the consequent morbidities.

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Intestinal parasitic infections have detrimental effects on the survival, appetite, growth and physical fitness (Sharma et al., 2004), school attendance (Nematian et al., 2008) and cognitive performance of school age children (WHO, 2005). In young children, diarrhea is particularly prevalent during the first 2 years of life and caused by parasitic diseases associated with impaired cognitive performance in later childhood (Niehaus et al., 2002; Walker et al., 2012). Infection early in life is, malnutrition and diarrhea, associated with poor cognitive function at school age (Berkman et al., 2002; Ijaz and Rubino, 2012).

Cryptosporidium parvum, Giardia intestinalis and Entamoeba histolytica are three major protozoan pathogens responsible for diarrhea in children under 5 years (Stark et al., 2009; Stark et al., 2011). C. parvum infection has been linked to chronic diarrhea, malabsorption and poor weight gain in children (Checkley et al., 1997: Adiei et al., 2004: Saneian et al., 2010: Rahi et al., 2013). Malabsorption in G. intestinalis infected children is probably due to several factors including increased epithelial permeability, bacterial overgrowth of the small intestine, loss of intestinal brush border surface area, villus flattening and inhibition of disaccharidase activities (Müller and von Allmen, 2005). E. histolytica is responsible for dysentery, anemia and could impact on infant growth (Ali et al., 2008; Hegazi et al., 2013).

The prevalence of intestinal parasitic infections is one of the most accurate indicators of socioeconomic conditions of a population and may be associated with several socio-cultural and economic determinant factors. It has been demonstrated that failure of children to fulfill their developmental potential and achieve satisfactory educational levels plays an important role in the intergenerational transmission of poverty (Grantham-McGregor et al., 2007). In countries with a large proportion of such children, national development is likely to be affected and, in turn, infectious diseases will become key factors influencing public health which could impaired Millennium Development Goal (MDG)4 (United Nations, 2010) regarding the under 5 mortality rate (U5MR) and infant mortality rate (IMR) (UNICEF/WHO, 2005; UNICEF/WHO, 2009).

Unlike microscopy of worm eggs, microscopic examination for the various intestinal protozoa is laborious, insensitive, and requires professional training. Sensitivity of microscopy for the different parasites does not exceed 60% even if concentration methods and skilful technical assistance are available (Hiatt et al., 1995). Nowadays a number of PCR-based assays have been developed for the identification of *G. intestinalis*, *E. histolytica* and *C. parvum* directly from human faeces. These assays have been proven to be considerably more sensitive than microscopy, highly specific and in contrast to microscopy are able to differentiate between the species and the subtype level. In addition, although a significant body of research exists on the relationship between diarrhea like-

likelihood and infrastructures, the impacts of water and sanitation infrastructures have been shown to vary by location, demanding country-specific analyses wherever diarrhea is a major cause of childhood morbidity and mortality.

Our objective is thus to investigate *G. intestinalis*, *E. histolytica* and *C. parvum* infection, based on molecular diagnosis, in children under five year suffering from diarrhea and attending Yamoussoukro CHR hospital in Central Côte d'Ivoire and examine link between infection and major socio-environmental factors. This could then contribute to appropriate policies aimed at controlling diarrheal diseases by improving mothers' hygiene practices when attending pediatric health care services.

#### **MATERIALS AND METHODS**

#### Study area and sampling

This study was conducted in the the pediatric unit of regional health care services (CHR) in Yamoussoukro District. The Autonomous District of Yamoussoukro, a city whose population was estimated to be 259,373 inhabitants in 2010 with population density of 104 inhabitants per km² is the political capital of Côte d'Ivoire. It is located at 240 km north of Abidjan, the economic and administrative capital of Côte d'Ivoire. The CHR is the greatest hospital in the district.

Mothers attending pediatric health care services from March to June 2013 with their children suffering from diarrhea cases were approached and a detailed explanation of the study was given by a local nurse in local languages or in French when possible. Following the explanation, if the mother is willing she may sign or thumb-print the informed consent form which was also translated into the local languages. A community health worker went to the mother's home and took a stool sample from the child in an interval of two hours after visit to the hospital. 200 mg of each stool were aliquot in cryotube label with code from each child and stored at -20°C before DNA extraction. A socio-economic questionnaire was administrated to investigate factors that could be associated with the occurrence of the diarrhea. The questions selected for analysis in this study referred to age (mother and children), shared or private toilet at home, and origin of water supply and education level of the mother. 306 stool samples were collected for analysis as well.

#### Molecular characterization

#### Extraction of genomic DNA

Genomic DNA was isolated from stool specimens by a cetyltrimethylammonium bromide (CTAB) extraction method as described by Khairnar and Parija (2007). Briefly, 50 mg of stool specimen, was dispersed in 250 μl of lysis buffer (0.25% sodium dodecyl sulfate in 0.1 M EDTA, pH 8.0), and 100 μg/ml of proteinase K was added. The lysate was incubated at 55°C for 2 h. Then 75 μl of 3.5 M NaCl followed by 42 μl of 10% CTAB/0.7 M NaCl (heated to 55°C) was added. After the components were mixed, the sample was incubated at 65°C for 30 min. This was followed by extractions with equal volumes of chloroform and then phenol-chloroform-isoamyl alcohol, and the DNA was precipitated with ice cold ethanol. The dried DNA pellet was dissolved in sterile distilled water and passed over a DNA clean-up spin column. The DNA was finally eluted from the spin column in 100 μl of Tris-EDTA (TE) buffer.

	;	Sex		
Protozoan	Male (N=153)	Female (N=153)	Χ²	P. value
	Number (%)	Number (%)	_	
C. parvum	54 (35.3)	59(38.56)	0.35	0.56
G. intestinalis	28(18.3)	36(23.53)	1.27	0.26
E. histolytica	30(19.61)	39(25.49)	1.52	0.22
Overall infection	92 (60.13)	100 (65.36)	0.9	0.34

**Table 1.** Sex related prevalence of protozoan species among diarrheal children.

**Table 2.** Frequency distribution of child age groups of the study children and their mothers.

Characteristics		Freq	X <sup>2</sup>	P. value		
		Positive (%)	Negative	Total	<b>X</b>	P. value
	0-24	128(69.19)	57	185		
Children age groups	25-36	56(53.84)	48	104	8.53	0.01
	>36	8(47.05)	9	17		
Total		192(62.75)	114	306		
	16-20	15(53.57)	13	28		
	21-25	31(50.82)	30	61		
Mothers age groups	26-30	41(64.06)	23	64	9.49	0.05
	31-35	79(72.48)	30	109		
	>35	26(59.09)	18	44		
Total		192(62.75)	114	306		

#### PCR amplification using species-specific oligonucleotides

The PCR method was applied to all stool sample DNA, which were stored at -20°C without preservatives using species-specific oligonucleotides. 2.5  $\mu l$  of DNA solution was used in the PCR reaction. PCR primer sequences used to amplify the genus Giardia and the species G. intestinalis are those reported by Mahbubani et al. (1991, 1992). The Cryptosporidium isolates DNA was amplified using polymerase chain reaction amplification and restriction fragment length polymorphism (PCR-RFLP) analyses of the TRAP-(Thrombospondin-Related Adhesive Protein of Cryptosporidium 2) as described in the literature (Peng et al., 1997; Sulaiman et al., 2002). In detail, nested PCR was used to amplify a fragment (266-366 bp) of the TRAP-C2 gene using two sets of oligonucleotide primers as described previously follow by restriction digestion for RFLP analysis enzymes BstEII, HaeIII (Nazemalhosseini et al., 2011).

Entamoeba genus was detected using genus specific PCR primers followed by nested multiplex PCR primers comprising *E. histolytica*, *E. dispar* specific primers (Khairnar and Parija, 2007).

#### Amplified products detection

Three microlitres of PCR-amplified DNA was detected by 2% agarose gel electrophoresis in TAE buffer (0.04 mM Tris-acetate, 0.001 mM EDTA, pH 8.0), stained in a solution of ethidium bromide (0.5 mgml<sup>-1</sup>) and visualized with a UV transilluminator.

#### Data analysis

Data recorded were analyzed using excel and STATA 9.0 software

(Stata, College Station, exas, United States). Chi-square ( $x^2$ ) tests were conducted to determine the relationship between parasites infection and major socio-environmental factors. Significance level was set at p<0.05 for all tests.

#### **RESULTS**

A total of 306 children with diarrheal diseases were included in this study out of the 405 children surveyed. One hundred and ninety two (192) of these children were infected with at least one of the three parasites detected by molecular markers, which make the overall prevalence 62.75%. Among infected children, 100 were female and 92 were male but no significant difference (p=0.34) between the two sexes was figured out (Table 1).

Children age groups were divided in three groups: birth to 24 months, 25-to 36 months and over 36 months. The group from birth to 24 months shows the highest prevalence (69.19%) and the difference was significant (p=0.01). Fewer infections (47.05%) were detected in the older children (>36 months) but this age group was less prevalent in the study (Table 2).

To see the mothers experience in caring for their children and influence on the occurrence of infection, the mothers' population was also divided in age groups with a 5-years interval between age groups. Protozoan infection was more prevalent in mothers' age group from

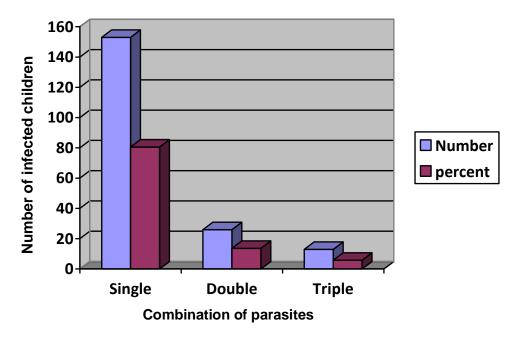


Figure 1. Single and mixed infections among children suffering from diarrheal.

	<b>Table 3.</b> Age related	prevalence of	protozoan	parasites among	diarrheal children.
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Parasites detected	Children age groups				<b>Y</b> <sup>2</sup>	P-value
raiasiles delected	0-24	25-36	> 36	Total (%)	_ ^	r-value
C. parvum	74	33	6	113(36.93)	1.99	0.37
G. intestinalis	42	18	4	64(20.92)	1.27	0.53
E.histolytica	46	20	3	69(22.55)	1.48	0.48
Overal infection	128	56	8	192(62.75)	8.53	0.01

31 to 35 years (72.48%) and lowest prevalence (50.82%) was found in group from 21 to 25 with a significant difference (p=0.05) between age groups (Table 2). Polyparasitism with double and triple infection was found in 26 and 13 children respectively out of the 192 positive cases but the majority was single infection (Figure 1). *C. parvum* was the most prevalent protozoan species found (36.93%) and *G. intestinalis* the less prevalent (22.55%). No significant difference was seen within each protozoan species among age groups but a significant different was detected among children age groups (p=0.01) when dealing with the overall infection (Table 3).

Socio-environmental factors such as mothers' education level, sanitation infrastructure used (shared or family private) and water sources were assessed (Table 4). The difference in the prevalence of protozoan infection in children was insignificant (p=0.06) with regards to the mother education level although infection in children from not educated mother (NE) was the highest (Table 4).

Infection was less common in family with private sani-

tation as compared to community sanitation and the difference was very significant (p<0.0001). Regarding sources of population water supply, tank and well users were more susceptible to infection than tap users with a significant difference (p<0.0001).

#### **DISCUSSION**

E. histolytica, G. intestinalis and C. parvum are known to be the most important diarrhea-causing protozoa (Stark et al., 2011) and in addition in September 2004, Giardia and Cryptosporidium were included in the "Neglected Diseases Initiative" (Savioli et al., 2006). To overcome the lack of sensitivity of the microscopic observation of intestinal protozoa (Stensvold and Nielsen, 2012), in this study, we decided to use sensitive and specific molecular markers to investigate protozoan parasites E. histolytica, G. intestinalis and C. parvum which are three of the most common intestinal protozoan parasites infecting humans worldwide.

Ob and stanistic		F	$\chi^2$			
Characteristic		Positive (%)	Negative (%)	Total	X	P. value
	HE	31 (57.41)	23	54		
Mothers education level*	NE	65 (72.22)	25	90		
mothers education level*	PE	44(63.77)	25	69	6.11	0.1
	SE	52(55.91)	41	93		
Total		192(62.,75)	114	306		
Sanitation	Private	61(38.61)	97	158		
Sanitation	Shared	131(88.51)	17	148	87.80	< 0.0001
Total		192(62.75)	114	306		
	Tank	51(94.44)	3	54		
Water sources	Тар	91(84.26)	108	199	83.45	< 0.0001
	Well	50(94.33)	3	53		

**Table 4.** Major socio-environmental factors related to prevalence of protozoan parasites among diarrheal children.

Mothers education level\*: HE (High education), NE (No education), PE (Primary education), SE (Secondary education).

114

306

192(62.75)

A prevalence of 62.75% was observed on 306 diarrheal stools collected from children under the age of 5. The remaining 37.25% could be due to other pathogens causing diarrheal disease such as enteropathogens, bacteria (*Enterotoxigenic Escherichia coli* and other obligate pathogenic *E. coli, Campylobacter jejuni, Vibrio cholera, Yersinia species, Shigella* species, *Salmonellae*, cytotoxigenic *Clostridium difficile*), viruses (*Rotavirus, Noro* viruses and *Enteric* adenoviruses) and parasites such as the helminths (*Strongyloides stercoralis* and *Ascaris lumbricoides*) and protozoa (e.g. *Blastocystis hominis* and *Isospora*).

Total

Stools of diarrhea infection are different in infant mushy stool subjected to breastfeeding which was clearly distinguished with watchful eye of physician (Messou et al., 1996). No association was found between gender and overall occurrence of parasitic infection in contrast with other studies conducted in other town in Côte d'Ivoire where authors demonstrated an association between gender and the occurrence of G. intestinalis and E. histolytica infection (Ouattara et al., 2010). Our results were nevertheless consistent with theirs in terms of a significant association between the prevalence of infection and age groups of children and their mothers. The highest prevalence of infection in children from 0 to 2 years is consistent with that reported in the literature (Guerrant et al., 1992, Walker et al., 2012). Indeed, diarrhea diseases leading to childhood malnutrition, morbidity and mortality cause 1.8 million deaths annually worldwide among children less than five years, 80% occurring in the first two years of life (Walker et al., 2012).

At species specific level, *C. parvum* species presented the highest prevalence (36.93%). *C. parvum* infection is common worldwide, and its frequency may be related to

the inadequacy of sanitation. In general, the infections are more common in developing countries and an infection rate of 33.83% is reported for children aged less than 5 years with diarrhea in Iraq (Rahi et al., 2013).

C. parvum is unfortunately scarcely studied in Côte d'Ivoire unlike E. histolytica and G. intestinalis (Ouattara et al., 2010; Schmidlin et al., 2013) despite its importance as a major pathogen that can cause diarrhea and even death in young children. The most recent study in Ivorian area reported prevalence of 15.0 and 14.4% of G. intestinalis and E. histolytica/E. dispar, respectively (Schmidlin et al., 2013). These rates are less than what we reported here and could be due to the fact that molecular techniques are more sensitive in addition to advantages that permit differentiation of E. histolytica from E. dispar, and also because we collected stools from children alleged as suffering from diarrheal diseases.

Literature reported that the prevalence of intestinal parasitic infections is one of the most accurate indicators of socioeconomic and environmental conditions of a population (Astal, 2004) and may be associated with several determinant factors, such as adequate sanitation, fecal pollution of water and foods, as well as host age and type of infecting parasite (Quinn, 2009). Our results confirmed that of others where sanitation and water sources influence occurrence of diarrheal diseases (Godana and Mengiste, 2013) but we found no significance influence of mother education level on pathogen transmission, contrary to some literature report (Cochrane et al., 1982; Grosse et al., 1989) despite the fact that protozoan parasites were more common in children from mother with no education level.

37.25% of children suffering from diarrhea were not in-

fected with one of the three protozoa parasite studied but could be infected with other pathogens causing diarrhea as feces were recognized as diarrheal infection stool by pediatricians. The apparent polyparasitism shows that other pathogens than those detected could co-exist in these infections.

#### Conclusion

Limited published data are available from Ivorian body of research on protozoan pathogens associated with children diarrhea. This first study with specific genetic markers on diarrheal children shed light on some factors that contribute to the occurrence of protozoan diarrhea.

Although the molecular diagnosis of parasites used in this study cannot be used routinely in hospitals due to its cost, it gives the true prevalence with respect to its sensitivity and specificity and confirms microscopic misdiagnosis.

Our recommendations are consistent with research that could lead to the development of rapid diagnostic test at the hospital level. Advice on washing hands after using the toilet and the care of the drinking water for children could avoid a lot of inconvenience as a result of diarrheal diseases.

#### **Conflict of Interests**

The author(s) have not declared any conflict of interests.

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# African Journal of Environmental Science and Technology

Full Length Research Paper

# Prospects and challenges of vermiculture practices in southwest Nigeria

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The prospect of vermiculture in the south-western coast of Nigeria was studied. One hundred and two respondents were interviewed in the three coastal towns of Badagry, Epe and Igbokoda. The results show that 94% of respondents made use of earthworms as fish bait, 37% buy their worms and 57% collected by themselves. The respondents, who buy, however, noted that their suppliers do not breed the worms but search for them from marshy areas. The price value placed on the worms ranged between 0.35 and \$3.10 per kg; while the most quoted prices were 0.35 and \$0.80 (16.7% each). The average quoted price was \$0.70 per kg. Forty-six percent (46%) of respondents were willing to buy earthworms if supplied to them; 49% were willing to serve as sales agents if contacted and 66.7% opined that earthworm was their choicest fishing bait. The results indicate positive expectation for vermiculture as a business venture. Vermiculture should, however, put into consideration the production of Alma millsoni and Libyodrilus violaceus, the species quoted as the most preferred and effective for fishing. Vermiculture will go a long way in solving the problem of earthworm scarcity among these people, reduce the stress put on natural populations earthworms, thus conserving the worms and also reduce damage done to the soil environment in the course of excavating for worms.

Key words: Vermiculture, earthworm, prospects.

#### INTRODUCTION

Earthworms, today, are being considered world over in the degradation of waste and as an alternative to inorganic fertilizers. There role in improving soil fertility is well known (Edwards and Lofty, 1977; Owa et al., 2003; Owa et al., 2004a, 2004b). Earthworms have also been suggested by Deolalikar and Mitra (1996) as good substitute or feed supplement for fishmeal in aqua culture.

Deolalikar and Mitra (1996) have used vermicompost

prepared from paper mill solid waste for fertilizing aquacultural tanks and found an increase in net primary productivity from 32.08 to 220.83 MgC/m/h. Vermicompost application also facilitates better growth of rohu fish (*Labeo rohita*) when compared with other commercially available organic manures (Deolalikar and Mitra, 1997). There is an increasing demand for protein-rich raw materials in fish and other animal feed industry. Fishmeal is the main protein component of fish feed.

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Earthworm species used	Frequency	Percent (%)
Alma millsoni only	33	37.5
A. millsoni and Libyodrilus violaceus	34	38.6
A. millsoni, L. violaceus and Eudrilus eugeniae	1	1.1
L. violaceus only	13	14.8
Unspecified choice	7	8.0
Total	88	100.0

**Table 1.** Species of earthworms used for fishing.

Earthworm is generally used as bait by the anglers. However, large-scale vermiculture has the potential of supplying earthworm meal as a substitute of fishmeal. The earthworm meal contains all the essential amino acids required in fish feed (Dedeke et al., 2010). The methionine and lysine availability are higher than that of fishmeal.

Vermicomposting and vermiculture technology is applicable to the rural as well as the urban society. It not only helps in commercial aqua-farming but also acts as a convenient source of earthworm for growing ornamental fishes in aquarium. Thus, Vermicomposting can be included as a component of sustainable life-style. Application of vermiculture and vermicomposting in aquaculture is eco-friendly and bio-ethically acceptable (Deolalikar and Mitra, 1997).

Vermiculture, which involves the breeding of earthworms under controlled conditions, has been carried out on species in temperate regions and a few studies have been done in India. Here in Africa, Nigeria in particular, the prospects of vermiculture has not been duly explored, particularly as regarding indigenous species. The present study was therefore carried out to establish a baseline for future works on the prospects of vermiculture in Nigeria, using the coastal areas of south-western states as case study. The research also put into consideration the economic value of the earthworm, which is much needed in the study of the economics of the belowground biodiversity.

#### **METHODOLOGY**

The study was carried out in the coastal towns of Badagry, Epe and Igbokoda, southwest Nigeria. This area is characterized by heavy rainfall spanning more than six months of the year and reaching 1200 mm in the months of June and July. Temperature here ranges between 29 and 32°C.

One hundred and two respondents were interviewed, using a structured questionnaire having both multiple choice and openended questions. The questionnaire was divided into three (3) sections, with section A addressing the socio-economic characteristics of the respondents. Sections B and C addressed the issues related to knowledge of earthworm types and uses and the culturing and sourcing of earthworms, respectively.

The questionnaires were administered to fishermen along the coastal line of the sampling locations. These areas are well known

fishing spots in the study area and are mainly visited by fishermen who are involved in commercial fishing, thus their need for and use of earthworms is believed should be regular.

The data obtained was analysed for frequencies and simple percentage of the earthworm species used by respondents, cost of purchasing earthworms and alternative baits, aside from earthworm, used mostly for fishing by the respondents.

#### **RESULTS**

The questionnaires were administered to 46, 25 and 31 individuals at Igbokoda, Epe and Badagry respectively. Almost all the respondents practiced fishing either as fulltime or as part-time occupation. Ninety-four percent (94%) of them make use of earthworms as fish bait and the most commonly used species were Alma millsoni and Libyodrilus violaceus (Table 1). Most of the respondents collected earthworms by self-sourcing (60%), while some 37% both buy and collect earthworms directly. Most collect earthworms on their own because suppliers were scarce and there might be the need to cover long distances in search of suppliers. However, 3% of the respondents do not collect worms themselves but go in search of suppliers. These along with those who both buy and collect noted that they travel long distances in search of suppliers. For instance, some of the respondents in Epe (one of the sampling location) claimed that they go as far as Ibadan, a city which is about 250 km away, in search of suppliers. The respondents, however, noted that none of their suppliers bred earthworms.

Some respondents (46%) showed willingness to buy earthworms if suppliers were available, while some 49% indicated readiness to serve as earthworm sales agent if contacted.

The price range for one kilogram (1 kg) of earthworms was put at between 0.35 and ₦3.10 by the respondents (Table 2). The highest prices quoted were 0.35 and ₦0.80 (16.7% of respondents for each price). The average price was ₦0.70/kg. The most frequent rate of purchase of earthworms by the respondents was twice per week (Table 3). Seventy-three per cent (73%) of respondents picked earthworm as their choicest fishing bait (Table 4) while other baits used for fishing include fish organs, insect larvae, crayfish, small snail, bath soap, etc (Table 5).

**Table 2.** Prices at which respondents valued 1 kg of earthworms.

Price (₦)	Frequency	Percent (%)
0.01 to 0.35	23	29.7
0.36 to 0.70	17	21.8
0.71 to 1.40	35	44.9
1.41 to 2.11	1	1.3
Above 2.11	2	2.6
Total	78	100.3

Table 4. Choicest bait.

Choicest bait	Frequency	Percent (%)
Anything available	19	20.7
Earthworms	68	73.9
Fries	1	1.1
Crab	1	1.1
Crayfish	3	3.3
Total	92	100.0

#### DISCUSSION

The results show that there is the need to develop the breeding of limicolous earthworm species, particularly A. millsoni and L. violaceus. There are no literatures indicating any efforts so far on breeding these species of worms. Most commonly used earthworm for culturing is Eisenia fetida that has wide range of temperature tolerance, has very high reproductive potential and is less sensitive to density pressure (Watanabe and Tsukamoto, 1976; Tsukamoto and Watanabe, 1977; Hartenstein et al., 1979; Hartenstein, 1981; Reinecke and Kriel, 1981). Eudrtilus eugeniae, which is commonly called 'African night crawler', has also been reported as an efficient earthworm to maintain as culture (Kale, 1994). Other species that have been successfully cultured are Perionyx excavatus and P. sansibaricus (Kale, 1998, 2002). However, these species, except E. eugeniae are not found in the West African sub-region, where the present study was carried out. The possibility, though of adapting such species to the tropical climate of this region has not been tested.

Since vermiculture is not expected to serve the fishing industry alone, there might also be the need to ascertain the usability of these species in other areas of need of vermiculture such as waste degradation and vermicomposting. There is, however, the possibility that the reason why these species are widely used in fishing in this area is their availability within the marshy terrains of these communities. Introducing other species of earthworms may not easily attract patronage as locals do not give in to change easily.

**Table 3.** How frequently respondents buy earthworms.

Time of purchase	Frequency	Percent (%)
Daily	7	18.9
Once a week	11	29.7
Twice a week	12	32.4
Every fortnight	2	5.4
Once a month	1	2.7
Thrice a week	4	10.8
Total	37	100

Those who travel long distances in search of suppliers claimed the reason is the cumbersome process involved in digging for the worms, while some who collected themselves complained of the cost of transportation if they have to go in search of suppliers. Vermiculture would go a long way in solving these problems, particularly if the vermiculture farmer either supplies the anglers at the harbour or set up the tanks near the fishing harbour. The vermiculture farm also have the benefit of helping to check the problem of habitat destruction done during the process of excavating for earthworms, by both anglers and their earthworm suppliers. This will also go a long way in helping to conserve the natural populations of earthworm, hence the productivity of the soil environment as relating to fertility and water movement.

#### Conclusion

The science of vermiculture in Nigeria has not been explored because little is known about the biology of our indigenous species of earthworms, particularly regarding reproductive timing and hatching rates. The present research serves as a baseline for intending earthworm breeders. Vermiculture has a viable prospect among the people of south-western Nigeria.

Vermiculture, however, still needs to be explored here by scientists, for information on the biology of the local species, vermicomposting potentials, and the protein content of the individual species need to be known to guide in specifying species to use in feed formation. Scientist equally needs to explore the fields of biotechnology and genetics as a way of developing better varieties of worms taking into consideration:

- 1. Age at maturity;
- 2. Number of cocoons produced; and
- 3. Size of worms.

#### Conflict of Interests

The author(s) have not declared any conflict of interests.

Table 5. Alternative baits to earthworms.

Bait	Frequency	Percentage (%)
Crayfish	3	3.1
Insect larvae, and adults	6	6.1
Small fishes and fish organs	11	11.2
Amphibian larvae	1	1.0
Bivalve and small fishes	1	1.0
Insect larvae and eba	1	1.0
Amphibian and insect larvae	1	1.0
Insects, small snail and crabs	1	1.0
Snail and small fish	3	3.1
Insects, crabs and amphibian larvae	1	1.0
Small fishes, snails and amphibian larvae	1	1.0
Fish, insects and eba	1	1.0
Insects, eba, amphibian larvae and diatoms	3	3.1
Fish, insects and snails	3	3.1
Fish and eba	2	2.0
Insects, snails and diatoms	11	11.2
Fish and insects	12	12.2
Insects and diatoms	4	4.1
Fish, insect and amphibian larvae	2	2.0
Fish, insect, eba and amphibian larvae	1	1.0
Fish, and amphibian larvae	1	1.0
Fish, insect, eba and snail	1	1.0
Fish, insect, bivalve, amphibian larvae, diatoms and snail	1	1.0
Fish, insect, snail and amphibian larvae	1	1.0
Insects and crabs	1	1.0
Fish and crayfish	6	6.1
Fish, crayfish and crab	3	3.1
Fish and crab	1	1.0
Fish, insect, crayfish, toilet soap and crab	1	1.0
Crayfish and snail	1	1.0
Fish, bread, eba and toilet soap	1	1.0
Fish, eba and toilet soap	1	1.0
Fish, insect, and crayfish	1	1.0
Fish, crayfish and snail	4	4.1
Fish, eba and crayfish	3	3.1
Fish, insect, eba, crab and crayfish	1	1.0
Insect, eba, crab and crayfish	1	1.0
Total	98	100

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## African Journal of Environmental Science and Technology

#### Review

# Basement and alluvial aquifers of Malawi: An overview of groundwater quality and policies

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This paper highlights the quality of groundwater in basement and alluvial aquifers of Malawi through literature assessment. Groundwater in these aquifers serves about 60% of Malawian population. Alluvial aquifers yield high groundwater in excess of 10 L/s and more mineralized than basement aquifers. The values from literature are presented as ranges. The geochemical quality of both aquifer types are classified as good. However, in some cases values higher than maximum permissible limits (MPL) are worrisomely apparent. Significant levels for some elements have been demonstrated. Although groundwater policies and instruments are available, more groundwater research, monitoring, data archiving is needed.

Key words: Alluvial aquifer, basement complex, groundwater quality, borehole water, shallow wells, Malawi.

#### INTRODUCTION

Potable water is a core to sustainable development. Most of the surface water in use for domestic and industrial purposes is highly contaminated, thus requiring a great deal of treatment before reaching the end user. In some regions, even this surface water is scarce owing to the great distances covered just to get to the nearest water point. Besides, a great deal of treatment required to make surface water fit for use makes it a difficult task for communities, especially in developing countries. The alternative is groundwater, which is probably the cheapest and safest potable water source (Foster, 1986). Groundwater is perceived to be cleaner in terms of

contaminants. However, depending on various factors, groundwater may be contaminated as well. Its physicchemical characteristics may exceed recommended quality standards. This may arise from either geogenic control or anthropogenic interference. Various quality parameters in groundwater have been assessed (Adekunle et al., 2007; Rowland et al., 2008; Muneer et al., 2010). In some instances, dangerous high quantities of health threatening elements have been documented (Smedley and Kinniburgh, 2002; Xie et al., 2013; Wen et al., 2013) Correlations and impacts of groundwater contaminants human health have been

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highlighted (Lyman et al., 1985; Hall et al., 2002). To safeguard human health, policies and standards regarding the use of groundwater for domestic and commercial purposes have been formulated in various countries. Most countries use the WHO standards (WHO, 2008) or have modified them to suit local conditions.

Most Malawians in rural areas depend on groundwater for domestic purposes (Government of Malawi (GOM), 2011). Open shallow wells have been in use since time immemorial. Recently, a lot of boreholes have been sunk through government initiatives, non-governmental organizations and international agencies (Stoupy and Sugden, 2003; Grimason et al., 2013). Borehole wells offer contamination freer water and guarantee safety to the drawer. Open wells on the other hand are prone to deliberate contamination and present physical dangers of falling in. Urban areas in Malawi depend much on surface water for potable water use, however this water flow originate from base flow in hills and mountains (Ngongondo, 2006). As such, variations in aquifer water quantity and quality has a significant contribution to variations in river water volume and quality.

Various policy documents have been formulated in Malawi as regards water resources use and degradation (GOM, 2011). The scientific community has been keen on quantity and quality of groundwater in Malawi as well as the SADC region. However, research work is still fragmented and data is not easy to come-by. This paper seeks to identify literature on Malawi's groundwater and use the same to summarize physico-chemical and biological values and related policies. At the same time, it will highlight various strides undertaken in Malawi as regards groundwater research and present areas where most study results have been published. The goal is to bring together values in one paper that will act as a starting point when identifying references about groundwater literature in Malawi.

#### **SOURCES OF INFORMATION**

A desk review approach was employed in this study. Google scholar, PubMed and Elsevier Scorpus were used to search for journal articles. General information about Malawi and policy documents were sourced from various government websites and published materials related to groundwater. Besides, some information came from theses available on university websites. The use rights were considered.

#### **GEOLOGY OF MALAWI**

#### Geography, general relief and climate

Malawi is a sub-Saharan African country south of the

equator situated between latitudes 9° 22′ and 17° 7′ south and between longitudes 32° 40′ and 35° 55′ east. It is bordered by Mozambique to the south east and south west, Zambia to the west and north-west and Tanzania to the north east and north (Figure 1A). It has a geographical area of 118480 km². According to 2012 world bank records, Malawi has a population of 15.91 million (World Bank, http://data.worldbank.org/indicator/SP.POP.TOTL).

Mkandawire (2004) explained the geologic and climate characteristics of Malawi as follows. Malawi has an array of relief that strongly influences climate, hydrology and groundwater occurrence. The topography consists of (1) Plateaus that are gently undulating surfaces with broad valleys and large level areas on the interfluves generally located at 900-1,300 m above mean sea level (msl). They are extensively covered by a thick weathered material: (2) Mountainous areas that rises abruptly from the plateau, located 2,000-3,000 m above msl covered by a more erosion resistant underlying strata; (3) Escarpment that fall steeply from the plateau areas underlain by poor and fractured aquifer where erosion significantly strips away the weathering products; and, (4) The Rift valley floor (hosting the alluvial plain) located below 600 m above msl, is gently sloping and of very low relief (Figure 1B). There is considerable potential for groundwater in these areas. Based on topography, the country is divided into Water Resource units (Figure 2).

In terms of climate, Malawi's seasons are influenced by the migration of the Inter-Tropical Convergence Zone (ITCZ). However, climatic conditions are complex due to the wide topographic variation and the influence of Lake Malawi. Much higher rainfall is experienced in uplands as compared to low lands. The mean annual temperature is 22–23°C with less latitudinal effect.

Rainfall variability has a significant impact on variations in both water availability and total potential discharge in a particular catchment (McCarthy et al., 2001; Bloomfield, 2002; Ngongondo, 2006). However, processes other than rainfall play a significant role in the hydrologic processes of any catchment (Ngongondo, 2006). The numerous rivers, lakes, dams, lagoons and marshes existing in Malawi (GOM, 2011) connect with groundwater in either recharge zones or mainly in discharge zones.

#### Aguifer geology and characteristics

The geological setting of Malawi is mainly characterized by crystalline metamorphic and igneous rocks of Precambrian to lower Palaeozoic age (Carter and Bennett, 1973; Chilton and Smith-Carington, 1984). This is referred to as the basement complex that covers approximately 70% of Malawi's landscape and supplying water to about 60% of the population (National Statistics Office, 2002). The major lithological units of the basement aquifer complex are syenitic granites, charconoctic and ultra-basic

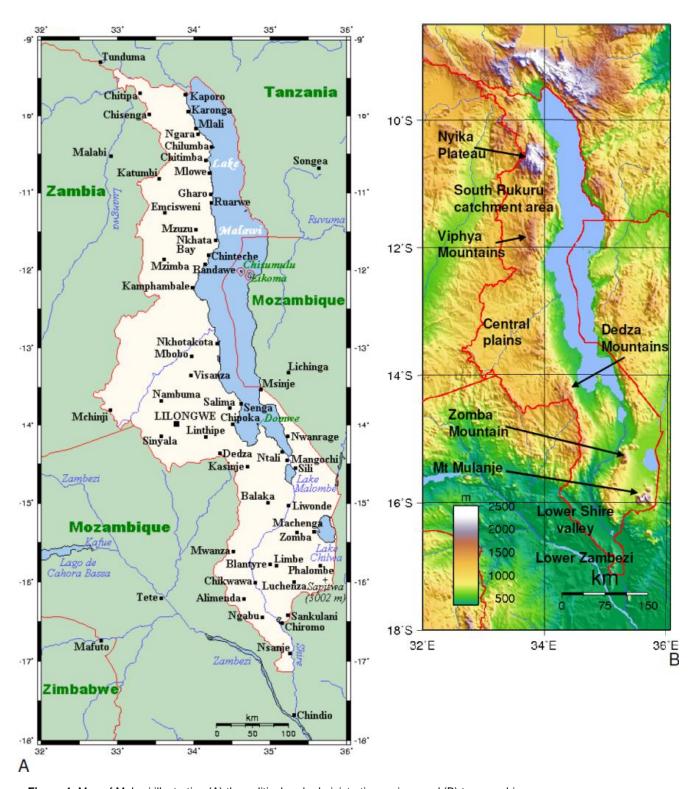
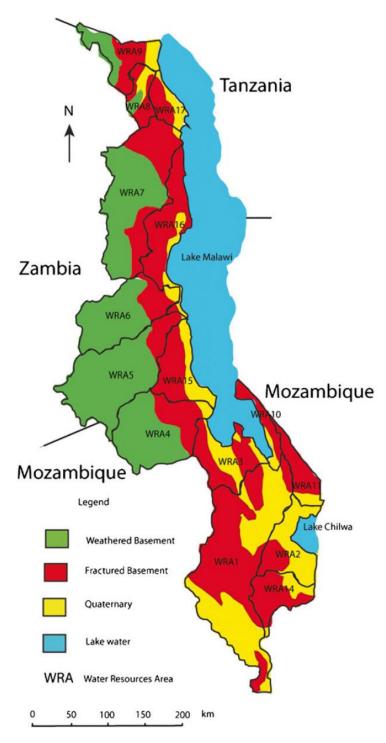


Figure 1. Map of Malawi illustrating (A) the political and administrative regions and (B) topographic zones.

gneisses, schistis, granulalite and quartzites (Mkandawire, 2004; Monjerezi and Ngongondo, 2012). Large inselberg of these rocks rises above the plateau as a result of

epeirogenic events (Chilton and Smith-Carington, 1984). The lift valley has modified and interrupted this land-scape. Groundwater is of variable qualities and quanti-



**Figure 2.** Outline of the geology of Malawi and Water Resource Areas (WRA) (Robins et al., 2013).

ties, unevenly distributed in time and space and is subjected to poor conservation and management (GOM, 2007). Aquifer recharges from broad interfluves and groundwater discharge in surface depressions. In terms of chemistry, the shallow component is less mineralized than the deeper component (McFarlane and Bowden, 1992). This basement is divided into weathered and fractured basement aquifers (Robins et al., 2013) (Figure 2). Besides basement complex, the quaternary alluvial aquifer exists in Malawi (Chimphamba et al., 2009; Robins et

al., 2013) located mainly along the Lake Malawi shores and Lower Shire valley within the Great Lift Valley floor (Figure 2).

According to Makandawire (2004), transmissivity of the weathered basement aquifers are generally in the range of 5 to 35 m²/day with estimated hydraulic conductivities ranging from 0.01 to 1 m/d. The storage coefficient, on the other hand, has been assumed to range from 0.01 to 0.001 (Mkandawire 2004). It is suggested that the groundwater is under unconfined to confined conditions. The water table is closer to the surface in the valley region and deeper in higher grounds (Mkandawire, 2004).

Borehole yields are generally highest where the saturated thickness of the weathered zone is greatest and the parent bedrock coarsest (Chilton and Smith-Carington, 1984). In general, however, the weathered basement aquifer produces low borehole yields (GOM, 2007; Chilton and Smith-Carington, 1984). Despite this, domestic water use (either shallow wells or hand-pump boreholes) can be sufficiently obtained (Chilton and Smith-Carington, 1984). On the other hand, alluvial aguifers are high yielding with recorded yields in excess of 10 L/s (Chimphamba et al., 2009; GOM, 2007). McFarlane et al. (1992) observed and concluded that very flat land at least one kilometer from the berg is generally likely to be high yielding than sites close to the berg in the plains of central region of Malawi. This was done through statistical analysis using data from about 1500 boreholes in this region.

Despite the relative expanse of the aquifer system, significant physical and chemical characteristics are observed on a local scale due to differences in mineralogy and structure (Chilton and Smith-Carington, 1984). The heterogeneity in terms of hydraulic characteristics and lithology is due to fractures, weathered zones and intrusions that control the occurrence of aquifers (Chimphamba et al., 2009; Pritchard et al., 2007).

#### **GROUNDWATER QUALITY IN MALAWI**

#### **Introductory remarks**

As mentioned earlier, spatial variation in chemical and physical quality is attributed to the heterogeneity of the aquifer system, groundwater flow regime and weathering processes (Chilton and Foster, 1995). Some shallow wells dry up in dry season forcing people to use surface water (e.g. rivers) which are grossly contaminated. Contamination of shallow wells is due to sanitation facilities being close to the wells (Dzwairo et al., 2006). The Water Resources Act of Malawi stipulates that water points be upstream of sanitation facilities. Fourie and van Ryneveld (1995) wrote that pollution from on-site is influenced by (1) varying subsurface conditions especially

saturated versus unsaturated zones, (2) varying contaminant characteristics mainly mobility and persistence, and (3) varying mechanism of movement through materials.

Determination of quality of groundwater is very vital. For instance, determination of nitrates is considered significant as an indicator for water quality due to its association with blue baby syndrome effect, possible carcinogen and crude fecal pollution indication (Fourie and van Ryneveld, 1995). In general, determination of physical and chemical quality serves to provide a guide on the quality of groundwater for all purposes. In the case of poor quality, the results help in identifying and prescribing the water borne health implications in an area.

#### Aquifer quality characteristics

Various studies on groundwater quality have been carried out in several districts in Malawi. Table 1 summarizes the range of biological, physical and chemical quality parameters observed in various districts. The values in Table 1 are quoted from various papers published in refereed journals, conference proceedings and reports. However, due to limited papers found so far on Malawian groundwater, the values presented were all found from the search engines. The ranges are presented as they are found in the cited papers. Some large values where rounded off to remove decimal points. Some significantly higher range values are explained by the various authors themselves. However, this paper will try to highlight some of the issues already explained by the various authors on the values. The summarized results are for both boreholes and shallow wells. Shallow wells can be open unprotected or closed protected wells (Pritchard et al., 2007, 2008, 2009; Mkandawire, 2008). As such, high variations in range of values are expected if protected shallow wells are sampled together with unprotected open wells. Thus, the combined range of values will definitely result in a higher range. Most of the highest values for shallow wells are expected for open unprotected wells. Makandawire (2008) and Pritchard et al. (2008, 2009) observed higher values for fecal coliforms (FC) in unprotected open shallow wells both during dry and wet seasons. Similar observations were made for other parameters such as sulfates (SO<sub>4</sub>), turbidity, pH and electric conductivity (EC). Some parameters improve during wet season while others worsen (Prichard et al., 2007, 2008, 2009). The difference in range values for the same district is an indication of the complexity of the aguifer. This depends on sampling location within the district even though the aquifer is either basement complex or alluvial. A synopsis of the results is summarized in Table 1a and

**Table 1a.** Range of expected Physical, Chemical and Biological quality of groundwater in Balaka, Blantyre, Chikhwawa, Chiradzulu, Karonga and areas in Central plains of Malawi.

Parameter	Balaka <sup>a</sup>	Blantyre	Chikhwawa	Chiradzulu	Dedza <sup>k</sup>	Karonga <sup>l</sup>	Central Plains <sup>m</sup>
		6.38 - 7.85 <sup>b</sup>	6.6 - 8.00 <sup>f</sup>	6.14 – 7.91 <sup>c</sup>			
pH	6.7 - 8.1	6.42 –10.3 <sup>c</sup>	$5.0 - 9.0^{h}$	$6.25 - 7.47^{e}$	6.4 - 7.7	6.3 - 8.1	6.4 - 7.0
		6.56 – 8.81 <sup>d</sup>	$6.9 - 7.7^{j}$				
TDS, mg/L	152 - 686	176 – 342 <sup>d;e</sup>	713- 2250 <sup>a</sup>	74 – 179 <sup>c</sup>	99 – 2120	50 – 950	N/A
100, mg/L	102 000	172 – 338 <sup>c</sup>	16 – 2653 <sub>.</sub> 9 <sup>9</sup>	71 – 162 <sup>e</sup>	00 2120	00 000	14// (
		1.3 – 114 <sup>b</sup>	0 – 209 <sup>i</sup>	$0.0 - 812^{c}$			
Turbidity, NTU	0.0 - 5.1	$0.0 - 62^{e}$	0 - 5.6 <sup>j</sup>	$1.9 - 30.3^{e}$	1.5 - 12.5	N/A	N/A
F0 0/	054 4400	$0.0 - 86^{c}$	4.50 0000f	474 000 <sup>0</sup>	105 1150	400 4705	N1/A
EC, μS/cm	254 – 1428	180 – 383 <sup>b</sup>	$1450 - 2800^{\circ}$	174 – 299 <sup>c</sup>	195 – 4156	120 - 1735	N/A
		287 – 577 <sup>c</sup>	254 – 15540 <sup>g</sup>	106 – 290 <sup>e</sup>			
		294 – 570 <sup>e</sup>	221 – 6574 <sup>j</sup>	0.0.0.400	0.5.040	4 7 000	0 40
<b>-</b>		13.6 – 35 <sup>b</sup>	3.8 –11767 <sup>i</sup>	$0.0 - 0.18^{c}$	8.5 – 248	4.7 – 990	2 – 19
Chloride, mg/L	N/A	0 - 0.22 <sup>c</sup>	8.1 –426 <sup>j</sup>	$0.0 - 0.09^{e}$			
		0 - 0.14 <sup>e</sup>					
Fluoride, mg/L	0.4 - 10.0	0.37 - 0.87 <sup>b</sup>	0.59 - 1.93 <sup>f</sup>		0.2 - 1.1	N/A	$0.5 - 7.02^{n}$
Traditad, mg/L	0.1 10.0		$0.1 - 4.8^{j}$		0.2 1.1	IN/A	0.0 7.02
		0 - 3.2 <sup>c</sup>		$0.0 - 4.8^{\circ}$			
NO <sub>3</sub> , (mg/L	N/A	0 - 0.61 <sup>d</sup>	0.0 –178 <sup>j</sup>	$0.0 - 0.03^{e}$	0.0 - 4.7	0.0 - 12.3	N/A
		0 - 1.92 <sup>e</sup>					
		$0 - 83^{c}$	1.6 – 985 <sup>h</sup>	$0.0 - 113^{c}$	5.1 – 887	0.0 - 505	114 – 2490
SO <sub>4</sub> , mg/L	0.0 - 33	$0 - 79^{d}$	$1.1 - 2600^{i}$	$0.0 - 11.0^{e}$			
		0 - 18.5 <sup>e</sup>					
			143 –1314 <sup>h</sup>				
HCO <sub>3</sub> , mg/L	N/A	N/A	65 – 3110 <sup>i</sup>	N/A	96 – 728	7.9 – 666	N/A
CO <sub>3</sub> , mg/L	N/A	N/A	$0.0 - 569^{g}$	N/A	0.0 - 49	0.0 - 72	N/A
			15.0 – 1178 <sup>j</sup>				
Sodium, mg/L	N/A	15.3 – 24.3 <sup>b</sup>	20.2 – 2050 <sup>h</sup>	N/A	11.5 – 314	2.4 – 166	28 – 163
Oddiam, mg/L	14// (	10.0 24.0	$10 - 8320^{i}$	14/71	11.5 514	2.4 100	20 100
			$0.5 - 24.3^{j}$				
Potassium, mg/L	N/A	N/A	0.3 - 24.3 $0.4 - 13.8^{h}$	N/A	0.3 - 21.9	1.2 – 11.6	0.8 - 1.4
			$9.9 - 309.8^{h}$				
0.1.1	<b>N</b> 1/A	<b>N</b> 1/A		<b>N</b> 1/A	45.0 054	0.0 440	00 555
Calcium, mg/L	N/A	N/A	6 – 876 <sup>i</sup>	N/A	15.8 – 351	3.6 – 412	62 – 555
			20.3 – 121 <sup>j</sup>				
			5.9 – 410.4 <sup>h</sup>				
Magnesium, mg/L	N/A	N/A	1.0 – 1302 <sup>i</sup>	N/A	7.7 - 50.1	1.2 – 117	16 – 343
			12.8 – 54.3 <sup>j</sup>				
Iron , mg/L	N/A	$0.1 - 0.8^{b}$	$0.0 - 0.6^{j}$	N/A	0.0 - 1.1	N/A	0.02 - 0.23
Hardness,	108 - 1080	$47 - 325^{\circ}$	$6.0 - 1370^{i}$	3 – 165 <sup>c</sup>	71 – 1036	N/A	N/A
mg CaCO₃/L		47 – 210 <sup>d</sup>		5.0 – 120 <sup>e</sup>			
my CaCOyL		$56 - 220^{e}$		5.0 - 120			
		$0 - 11000^{b}$		0.00000			
Fecal coliforms, cfu/100 mL	0 - 4230	0 - 28450 <sup>c</sup>	0 – 9250 <sup>i</sup>	0 – 29600°	N/A	N/A	N/A
,		0 – 17175 <sup>d</sup>		13 – 4550 <sup>e</sup>			

Superscript denotes the main source of data for the district except where noted within the table: <sup>a</sup>Pritchard et al., 2008; <sup>b</sup>Palamuleni, 2002; <sup>c</sup>Pritchard et al., 2007; <sup>d</sup>Mkandawire, 2008; <sup>e</sup>Pritchard et al., 2009; <sup>f</sup>Sajidu et al., 2008; <sup>g</sup>Monjerezi et al., 2012a; <sup>h</sup>Monjerezi et al., 2012b; <sup>i</sup>Monjerezi and Ngongondo, 2012; <sup>j</sup>Grimason et al., 2013; <sup>k</sup>Kushe, 2009; <sup>h</sup>Wanda et al., 2013; <sup>m</sup>McFarlane and Bowden, 1992; <sup>n</sup>Msonda, 2003.

**Table 1b.** Range of expected Physical, Chemical and Biological quality of groundwater in Machinga, Mchinji, Mulanje, Mzimba, Nkhatabay, Rumphi and Zomba-Phalombe plains of Malawi.

Parameter	Machinga°	Mchinji <sup>p</sup>	Mulanje <sup>c</sup>	Mzimba <sup>q</sup>	Nkhatabay <sup>r</sup>	Rumphi <sup>l</sup>	Zomba-Phalombe
pН	6.70 - 9.37	6.4 - 7.4	5.22 - 6.93	6.0 - 8.7	5.8 - 7.1	6.5 - 7.8	$5.5 - 7.0^{a}$
	$7.05 - 9.50^{f}$						6.30-7.15 <sup>f</sup>
							$6.5 - 7.2^{t}$
TDS, mg/L	43 – 1772	39 - 215	27 – 161	29 - 1896	501 - 108	24 - 810	29 – 185 <sup>a</sup>
Turbidity, NTU	Trace - 4.00	1 - 11	0.32 - 502	Bdl - 740	N/A	N/A	$2.0 - 147^{a}$
EC, μS/cm	1050 - 31000	76 - 416	28.4 - 268	50 - 316	71 – 168	40 - 1630	129-805 <sup>f</sup>
	55 – 1676 <sup>f</sup>						126 - 1806 <sup>t</sup>
Chloride, mg/L	0.003 - 0.59	<0.01-17	0.0 - 0.96	4.7 - 990	3.5 - 12.3	6.0 - 399	0.004-0.59 <sup>s</sup>
			0.01 - 0.17 <sup>e</sup>				$0.0 - 40.5^{t}$
Fluoride, mg/L	0.35 - 10.3	0.01-1.21	N/A	Bdl - 1.7	0.40 - 1.33	N/A	0.26-6.51 <sup>f</sup>
	0.24-7.51 <sup>f</sup>						$0.0 - 4.5^{t}$
NO <sub>3</sub> , mg/L	Trace - 58.3	0.01-0.39	0.0 - 2.0	Bdl - 12.3	0 - 0.90	0.0 - 3.7	$0.003 - 1.36^{a}$
			$0.0 - 0.01^{e}$				$0.0 - 48^{t}$
SO <sub>4</sub> , mg/L	2.9 - 110	4.2-58.5	0.0 - 7	Bdl - 505	0 - 0.91	0.0 - 53	2.9-110 <sup>s</sup>
			$0.0 - 5.0^{e}$				$0.0 - 10^{t}$
HCO <sub>3</sub> , mg/L	44.4 – 497	38.6-164	N/A	7.9 - 666	8.5 - 148	25 - 551	N/A
CO <sub>3</sub> , mg/L	0.00 - 26.7	0	N/A	Bdl - 72.0	-	0.0 - 31	N/A
Sodium, mg/L	N/A	6 - 13	N/A	2.4 - 166	1.39 – 11.2	3.1 - 40	$4 - 53^{t}$
Potassium, mg/L	N/A	0.7 - 6.8	N/A	1.0 – 11.6	0.84 - 6.16	1.0 - 12	N/A
Calcium, mg/L	N/A	9 - 45.4	N/A	3.6 - 412	6.94 - 43.9	7.4 - 142	$4 - 47^{t}$
Magnesium, mg/L	N/A	2.7 - 12.6	N/A	0.97 – 117	1.23 - 4.32	1.0 - 45	N/A
Iron, mg/L	N/A	0.01 - 3.5	N/A	Bdl – 2.7	0.15 - 1.98	N/A	N/A
Hardness	3.49 – 199	25 - 161	0.0 – 325	14 - 1489	N/A	N/A	$3.0 - 220^{t}$
mg CaCO₃/L	J.49 - 199	20 - 101	0.0 – 325	14 - 1409	IN/A	IN/ A	3.0 – 220
Fecal Coliforms, cfu/100 mL	N/A	0 - 43	0 – 4020	N/A	0 - 0.5	N/A	0 – 6550 <sup>a</sup>

Superscript denotes the main source of data for the district except where noted within the table: <sup>a</sup>Pritchard et al., 2008; <sup>c</sup>Pritchard et al., 2008; <sup>b</sup>Vanda et al., 2013; <sup>c</sup>Sajidu et al., 2007; <sup>p</sup>Mleta, 2010; <sup>d</sup>Vanda et al., 2011; <sup>c</sup>Kanyerere et al., 2012; <sup>c</sup>Chimphamba et al., 2009; <sup>c</sup>Von Hellens, 2013.

#### Physical characteristics

Average values of pH in most cases indicate near-neutral groundwater compositions with a range of 6 - 8. The permissible levels of pH in groundwater for Malawi is 6 – 9 (Malawi Standards Board (MSB), 2005a). According to Table 1a and b, groundwater pH values lower than 6 for some places in Chikhwawa, Mulanje, Nkhatabay and Zomba can be encountered as well as those beyond pH 9 for some places in Machinga. Msilimba and Wanda (2013) reported a range of pH for groundwater in Mzuzu as 4.9 – 6.3 an indication of acidic waters in this area. Most of the groundwater recorded so far have pH range within the acceptable levels.

Total dissolved solids (TDS) were quoted as low, 24 mg/L with most of the higher values below the MPL value of 1000 mg/L (MSB, 2005a). However values higher than 1000 mg/L are reported in some areas such as those found in Chikhwawa, Dedza, Karonga, Machinga and

Nkhatabay. These are same places where higher electrical conductivities were also observed. Chilton and Smith-Carington (1984) observed values of total dissolved solids up to around 2500 mg/L in Livulezi (central) and Dowa West (south-central). On the other hand, Bath (1980) concluded that total dissolved solids up to 2900 mg/L can be expected in Nkhotakota and surrounding lake shore areas in colluviums and weathered basement.

Higher turbidity range (Table 1) were observed for studies done in Blantyre (Palamuleni, 2002; Pritchard et al., 2007; Mkandawire, 2008; Pritchard et al., 2009), Chikhwawa (Monjerezi and Ngongondo 2012), Chiradzulu (Pritchard et al., 2007), Mulanje (Pritchard et al., 2007; Pritchard et al., 2009), Mzimba (Wanda et al., 2011) and Zomba-Phalombe plain (Pritchard et al., 2008). As mentioned earlier some of these observations were made for open unprotected wells (Pritchard et al., 2007, 2008, 2009; Mkandawire, 2008). The maximum

permissible level for groundwater in Malawi is 25 NTU (MSB, 2005a) as such some places in the studied areas recorded in Table 1 had values higher than the MPL value. However, the average values indicated by authors themselves indicate that groundwater has values within acceptable levels in terms of turbidity.

The electrical conductivity (EC) values are in the range of  $35-2800~\mu\text{S/cm}$  (Table 1). However, in some instances values higher than the maximum permissible level (MPL) of  $3500~\mu\text{S/cm}$  (MSB, 2005a) can be expected for some places in Chikhwawa, Dedza and Machinga. Bath (1980) reported that electric conductivity as high as 7700  $\mu\text{S/cm}$  can be observed in some areas in Malawi. Chilton and Smith-Carington (1984) also found mostly low-conductivity groundwater in basement aquifers from the Livulezi (central) and Dowa West (south-central) areas. In the Nkhotakota area on the western shores of Lake Malawi, Bath (1980) reported electrical conductivity values of  $180-4600~\mu\text{S/cm}$ .

#### Chemical quality

The chloride (CI) MPL for borehole and shallow well drinking water in Malawi is 750 mg/L (MSB, 2005a). However, chloride concentrations up to 4000 mg/L have been recorded in Shire Valley and lower than 60 mg/L in Nkhotakota area (Bath, 1980). BGS (2004) observed concentrations of chlorides up to 2100 mg/L in groundwater from alluvial deposits close to the edge of the Karoo sediments. In South Rukuru catchment area, chloride concentrations of 4 - 2000 mg/L were reported by Bath (1980). Except for Chikhwawa (Monjerezi et al., 2012b; Monjerezi and Gongondo, 2012), Karonga (Wanda et al., 2013), Mzimba (Wanda et al., 2011), and Rumphi (Wanda et al., 2013), all the areas in Table 1 show values below the MPL of 750 mg/L. Nevertheless, the high values recorded in these areas are not rampant, an indication that the average majority of sampled groundwater had values less than 750 mg/L with very few exceptions. This is what is making the range to be so highly spread.

It is expected that areas located in Rift Valley floor will have high fluoride (F) levels (BGS, 2004). As such, Malawian groundwater in the alluvial plains, are likely to be most affected. Sporadically, some groundwater in the weathered basement may also have high concentrations greater than Malawi standards of 6 mg/L (MSB, 2005a). Groundwater fluoride levels less than 1 mg/L in areas of the basement complex in Malawi and concentrations between 2 mg/L and 10 mg/L from the alluvial regions have been reported by UN (1989). Bath (1980) reported a median concentration of fluoride <1 mg/L in basement aquifers of the Nkhotakota area. However, one of the samples recorded a value higher than 1 mg/L that resulted in an inflated range of <1 – 7 mg/L. Elsewhere,

concentrations in the basement aquifer of South Rukuru are reported in the range <0.1-3.3 mg/L, nonetheless most of the results were below 0.6 mg/L (Bath, 1980). Much higher fluoride concentrations, up to 15 mg/L in some samples, have been reported by Bath (1980) in the lower Shire valley. At Ulongwe in Machinga District, fluoride levels of 8.6 mg/L have been shown to exist (Sibale et al., 1998). Carter and Bennett (1973) also reported existence of fluorides in Karonga (8.0 mg/L), Mwanza (3.4 mg/L), Mazengera in Lilongwe (7.0 mg/L), Nathenje in LiLongwe (7.0 mg/L), Nkhotakota (9.6 mg/L) and Nsanje (5.8 mg/L). Spatial and temporal analyses of fluorides levels in Nathenje in Lilongwe (part of central plains) indicate higher values for central part of the area and during dry season, respectively (Msonda et al., 2007). From Table 1, higher values of fluoride concentrations are expected in some places in Balaka, Central plains, Machinga, Nsanje and Zomba-Phalombe plains. There is evidence of fluorosis problem in these areas (Msonda et al., 2007, Sajidu et al., 2007). Values of fluorides higher than 6 mg/L are worrisome in Malawi. However the problem of fluoride is sporadic in the basement aquifer and not widely spread in the alluvial aquifer (UN, 1989).

The nitrates (NO<sub>3</sub>) MPL for groundwater used for drinking in Malawi is 45 mg/L (MSB, 2005a). Bath (1980) summarized fluoride data showing fluoride concentrations much less than the MPL value in Nkhotakota, Bua, South Rukuru catchement area and Lower Shire valley. Sporadically high concentrations of nitrates in groundwater from the lower Shire valley (as high as 18.5 mg/L) have been observed (Bath, 1980). Nevertheless, the study concluded that nitrate concentrations were mostly less than 5 mg/L and many were below 0.7 mg/L. Also, UN (1989) reported groundwater nitrate values less than 1 mg/L from both the basement and alluvial aquifers in Malawi. Observations from Table 1 indicate that nitrates higher than 45 mg/L are expected in some places in Chikhwawa (Grimason et al., 2013), Machinga (Sajidu et al., 2007) and Zomba-Phalombe plain (von Hellens 2013).

In terms of sulfates, Chilton and Smith-Carington (1984) reported concentrations higher than 2000 mg/L in Dowa west of central plains of Malawi. Sulfate up to 2400 mg/L were recorded in groundwater from alluvial deposits close to the edge of the Karoo sediments (BGS, 2004). The MPL for sulfates in borehole and shallow wells drinking water for Malawi is 800 mg/L (MSB, 2005a). According to Table 1a, higher values were observed in some places in Chikhwawa (Monjerezi et al., 2012b; Monjerezi and Gongondo, 2012), Dedza (Kushe, 2009) and Central plains of Malawi (McFarlene and Bowden, 1992). In general, sulfate levels in weathered basement and alluvial aquifers are mainly below the MPL value.

Chimphamba et al. (2009) indicate that the expected bicarbonate (HCO<sub>3</sub>) concentrations in weathered base-

ment aquifer are 100 – 500 mg/L while for alluvial aquifers across the country it is expected that the values fall within 200 – 1000 mg/L. Most values for the districts recorded in this study are below 500 mg/L in the weathered basement except for some samples in Dedza (Kushe, 2009), Karonga (Wanda et al., 2011) and Mzimba (Wanda et al., 2013). In Chikhwawa (Lower Shire valley floor), the values in some places were above the expected maximum value of 1000 in alluvial aquifer. Carbonate (CO<sub>3</sub>) concentrations were as high as 569 mg/L (Table 1) in places recorded in this review so far.

The nationwide expected value for sodium (Na) is in the range 5 - 70 mg/L in weathered basement and 20 -1500 mg/L in alluvial aquifer. On the other hand, expected values for potassium (K) are in the range of 1 - 6 mg/L for weathered basement aguifer and alluvial aguifer (Chimphamba et al., 2009). The MPL for sodium in Malawian groundwater used for drinking is 500 mg/L (MSB, 2005a). However, that of potassium is not stipulated. Nevertheless, potassium MPL for drinking water (tap water) is stipulated as 50 mg/L (MSB, 2005b). Bath (1980) reported sodium concentrations for Nkhotakota (0.4 - 720 mg/L) and Lower Shire (14 - 3550 mg/L). From Table 1, sodium concentrations falling outside nationwide expected range are reported in various places within the recorded districts in both weathered basement and alluvial aguifers such as Chikhwawa (Alluvial aguifer mainly), Dedza, Karonga, Central plains and Mzimba. In terms of drinking water quality for sodium, some places in Chikhwawa (Lower Shire) and Nkhotakota area are expected to have sodium values higher than MPL value of 500 mg/L (Bath, 1980; Monjerezi et al., 2012b; Monjerezi and Gongondo, 2012; Grimason et al., 2013). Similarly, some places in Chikhwawa, Dedza, Karonga, Mzimba and Rumphi exhibit high concentrations of potassium (Table 1) than the nationwide expectation of 1 - 6 mg/L but below 50 mg/L MPL (MSB, 2005b) stipulated for tap water. This indicates that in most places in these areas, the values will be below the acceptable maximum normal drinking water standard for potassium.

The MPL for calcium (Ca) and magnesium (Mg) in groundwater used for drinking in Malawi is 250 and 200 mg/L, respectively (MSB, 2005a). In Nkhotakota region, the groundwater is mainly of calcium-(magnesium)-bicarbonate type. In South Rukuru catchment area, concentrations of calcium up to 500 mg/L and magnesium up to 280 mg/L have been reported (Bath, 1980). According to Chimphamba et al. (2009), the nationwide expected value for calcium is in the range of 10 – 100 mg/L in weathered basement and 50 - 150 mg/L in alluvial aguifer. On the other hand, expected value for magnesium is in the range of 5 – 50 mg/L for weathered basement aguifer and 20 - 100 mg/L in alluvial aguifer (Chimpahmba et al., 2009). Based on the range indicated in Table 1, some places both in basement and alluvial aguifers had calcium values outside the expected nationwide range and

in some instances higher than the recommended maximum permissible value of 250 mg/L, e.g. in some places found in Chikhwawa (Monjerezi et al., 2012b; Monjerezi and Gongondo, 2012), Dedza (Kushe, 2009), Karonga (Wanda et al., 2013), Central plains (McFarlene and Bowden, 1992), Mzimba (Wanda et al., 2011) and Rumphi (Wanda et al., 2013). Similarly, magnesium values in some places had values outside the expected nationwide range and in some instances higher than the recommended MPL of 200 mg/L such as in Chikhwawa and Central plains (Table 1).

The MPL for total iron (Fe) in groundwater for Malawian aguifers is 3 mg/L (MSB, 2005a). The nationwide expected range is 1 - 5 mg/L in both weathered basement and alluvial aquifers (Chimphamba et al., 2009). According to UN (1989), the range for total iron in weathered basement rocks and alluvial sediments is 1 – 5 mg/L. In Lower Shire Valley, a range of <0.1 – 84 mg/L have been reported and 0.8 - 82 mg/L in the weathered basement aquifers of Nkhotakota area by Bath (1980). Also, Bath (1980) recorded a range of total iron of <0.1 – 59 mg/L in Bua area of Central Malawi and <0.2 - 65 mg/L in South Rukuru catchment area. From Table 1, the highest value is 3.53 mg/L for Mchinji which falls below the maximum permissible level and within the expected range of 1-5mg/L. Chilton and Smith-Carington (1984) explains the high values observed by Bath (1980) as possibly emanating from sampling methodology employed at that time that might have included particulate iron in the sample and likelihood of contamination from the pump material.

The MPL of total hardness (TH) in groundwater used for drinking is 800 mg CaCO<sub>3</sub>/I (MSB, 2005a). The results in Table 1 indicate most values less than the MPL. However in some places the values recorded by cited authors in Table 1 show values above 800 mg/L in some places like Balaka, Chikhwawa, Dedza and Mzimba. It is therefore expected that some places in these areas will produce groundwater exhibiting characteristics of high hardness and unsuitable for drinking.

Manganese (Mn) concentrations of 0.1-0.4 mg/L have been reported in Central plains (McFarlane and Bowden, 1992), <0.001 mg/L in Mchinji (Mleta, 2010), <0.4 mg/L in Mzimba (Wanda et al., 2011) and 0-0.29 mg/L in Nkhatabay (Kanyerere et al., 2012). The values are below the MPL of 1.5 mg/L (MSB, 2005a).

Trace elements such as copper, lead, strontium, cadmium, boron, barium, beryllium, chromium, mercury have been analyzed in Nkhatabay and Lower Shire (McFarlene and Bowden, 1992; Kanyerere et al., 2012; Monjerezi et al., 2012; Grimason et al., 2013). However, no significant values were identified in these areas.

Phosphates of 0.16-1.16 were recorded in Nkhatabay (Kanyerere et al., 2012) while Sajdu et al. (2007) indicated that phosphates are not an issue in Machinga. Ammonia (0.0-0.5 mg/L) and nitrite (0.0-0.06 mg/L) were examined in samples in Blantyre (Mkandawire,

Parameter	Good	Fair	Moderate	Poor
EC, μS/cm	0-750	750-1500	1500-3000	3000-6000
Na, mg/L	0-115	115-230	230-460	460-920
Mg, mg/L	0-30	30-60	60-120	60-120
Hardness, mg CaCO <sub>3</sub> /L	0-250	250-500	500-1000	1000-2000
CI, mg/L	0-180	180-360	360-710	710-1150
SO <sub>4</sub> , mg/L	0-145	145-290	290-580	580-1150

**Table 2.** Standards for drinking water in arid regions adopted in Malawi (Government of Malawi-UNDP, 1986).

2008) and were found to be lower than the MPL set by WHO (2008) of 1.5 and 3 mg/L, respectively. Arsenic is not a problem currently based on spot checks carried out by the ministry as well as studies done by various researchers in Malawi (Pritchard et al., 2007, 2008; Mkandawire, 2008; Kanyerere, 2012). All the arsenic values reported are below the MPL of 0.5 mg/L (MSB, 2005a).

Generally, it has been observed that alluvial aquifers probably have high salinity values particularly in lower Shire valley, eastern Bwanje valley and around Lake Chilwa (Bath, 1980; Monjerezi et al., 2011). In contrast, low salinity values for groundwater from weathered basement in the Bua catchment of western Malawi are on record (Bath, 1980). Elsewhere, in colluviums and basement aquifer, a very variable salinity has been observed in South Rukuru catchment area and in some instances high salinity values were recorded around Emcisweni in Mzimba District (Bath, 1980).

#### Fecal coliforms

Aguifer microbial contamination is from pit latrines, septic tanks, cesspool, leaky sewers and landfill leachate (Pedley and Howard, 1997). Major variations in fecal coliforms are observed between wet and dry seasons. Wet seasons are expected to produce shallow well waters with high fecal coliforms, especially if open and unprotected. However boreholes exhibit lower and below MPL for fecal coliform. Fecal coliform values of 129 – 920 colony forming units (cfu)/100 mL were observed in shallow wells in Mzuzu (Msilimba and Wanda, 2013). The authors concluded that the wells are contaminated since the values exceed the maximum recommended value of 50 cfu/100 ml (MSB, 2005a). Similar situations were observed by authors in Balaka, Blantyre, Chikhwawa, Chiradzulu, Mulanje and Zomba-Phalombe plain (Table 1).

#### Overall quality of groundwater

In general, high contents of cations, anions and total dissolved solids (TDS) are limiting factors to the use of groundwater (BGS, 2004; Kundell, 2008). However,

based on Table 2, groundwater quality in both basement and alluvial aquifer in Malawi can be classified on average as good in terms of major cations, total hardness and sulfates. But, there are sporadic exceptions in few cases in some places within the studied districts where the groundwater quality in some parameters ranges from moderate to poor as can be observed from Table 1 when compared with Table 2.

#### **POLICIES ON GROUNDWATER IN MALAWI**

GOM (2008) identified challenges facing the water sector as (1) unharmonized policies and laws, (2) inadequate stakeholder coordination, (3) poor catchment management, (4) lack of capacity and (5) inadequate water supply and sanitation. Policies and legislation related to water resources, on the other hand, have been formulated such as the National Environmental Policy (NEP), National Strategy for Sustainable Development (NSSD), Environmental Management Act (EMA), National Environmental Action Plan (NEAP), District Environmental Action Plans (DEAPs), National Water Policy (NWP), Water Resources Act (WRA), Water Works Act (WWA) and other sectoral policies which also focus on the water resource such as the Agriculture Policy and the Forestry Policy (GOM, 2011). Standards on groundwater are available and the Malawi Standards Board is responsible for their formulation and updates. However, there are still coordination, enforcement and monitoring challenges that reduce the impact of these instruments (GOM, 2011).

Malawi is a member of the Southern Africa Development Community (SADC) that has a regional ground-water vulnerability initiative (Robins et al., 2007). The SADC established a water sector in 1996 (Molapo and Puyo, 2004). Besides, SADC has a regional strategic action plan for integrated water resource development and management that establishes rules and procedures to implement joint management of water resources between countries. There is also the Zambezi river basin commission within SADC for which Malawi is party to (Molapo and Puyo, 2004). The regional grouping established a subcommittee for hydrogeology that serves as supervisor or steering group for implementation of projects

of regional magnitude (Molapo and Puyo, 2004). SADC protocol on shared watercourse systems of 1995 (and revised in 2000) places great emphasis on governance of shared water (Ramoeli, 2002; Pietersen 2005; Molapo and Puyo, 2004). It should be noted that the aquifers in Malawi are shared among adjacent states (Mkandawire, 2004; Turton et al., 2004). However, the geographic extent of the transboundary aquifers is not known which leads to management problems (Turton et al., 2004). Above all, Government of Malawi is trying its best to achieve the targets set in the millennium development goals (MDGs) and other policies. It is conclusively clear that Government of Malawi is striving at best in ground-water resources management despite the existence of various challenges.

## SUMMARY OF INADEQUACIES AND POSITIVE STRIDES ON GROUNDWATER IN MALAWI

There have been some studies on groundwater in Malawi as shown in the preceding sections. Besides quality studies, some cited examples include (1) investigation of the weathered basement aquifers (Jones, 1985; Acworth, 1987; Chilton and Foster, 1995), (2) major village water supply programme featuring the introduction of village level operation and maintenance of hand pumps (Smith-Carington and Chilton, 1983), (3) Dambo research (McFarlane and Whitlow, 1990; McFarlane and Bowden, 1992) and (4) improving community based management of boreholes (DeGabriele, 2002).

However, no time series hydrologic data of any type are being collected and archived except for data collected during the drilling of boreholes (Robins et al., 2006). There is no regular groundwater monitoring done indicating a deficiency in sustainability interventions of the resource (Mleta, 2010; GOM, 2011). On a positive note, percentage of rural population with improved water source including boreholes, protected dug wells, is 82% as at 2011 as compared to 75% in 2008 (World Bank, http://data.worldbank.org/indicator/SH.H2O.SAFE.RU.Z). The responsibility of monitoring groundwater quality is vested in the hands of Department of Water through its regional and district offices.

Some significant studies on improvement of ground water quality in Malawi have been carried out such as defluoridation using clays (Msonda, 2003), gypsum (Masamba et al., 2005) and bauxite (Sajidu et al., 2008 and 2012). Pritchard et al. (2009) studied the potential of using indigenous methods of purifying water by utilizing plant extracts. These studies have yielded remarkable results. Various groundwater modeling application studies have been done in some areas (Monjerezi et al., 2012b; Wanda et al., 2013). Besides, a comprehensive groundwater resource assessment has not been examined for Malawi yet (Mkandawire, 2004).

#### **CONCLUSIONS**

Groundwater quality in Malawi can be classified as good except for some sporadic zones where some elements exceed recommended drinking water guideline values; Chimphamba et al. (2009) quoted standards adopted by Government of Malawi for drinking water in arid regions. Alluvial aguifers have high values of fluorides in some places, a situation that leads to fluorosis problems. Traces of similar incidences have been observed in weathered basement aguifers. High content of cations, anions and total dissolved solids have led to well abandonment. Saline groundwater conditions have been observed in some places like eastern Bwanje valley, Lake Chilwa and Lower Shire. However serious incidences of groundwater contamination are not imminent as indicated by fecal coliform, nitrate and sulfate observations, except in shallow wells. The amount of chemical elements found in these aguifers can be attributed mainly to the geological setting of the aquifer complex itself. The paper has shown that most of the studies are concentrated in southern region especially the Shire Valley. Thus, the conclusions are based on district level studies. More flow modeling and reactive groundwater modeling are needed to understand the hydrochemistry of the aguifer as well as mobilization and speciation of chemical species within. Transboundary groundwater data sharing, archiving and monitoring need to be in place for a better management of the shared aguifer. Multi-country research on the basement aguifer is necessary to promote sharing of knowledge between research establishments and water management institutions.

#### **Conflict of Interests**

The author(s) have not declared any conflict of interests.

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# African Journal of Environmental Science and Technology

Full Length Research Paper

# Histopathological and biochemical disrupting effects of Escravos crude oil on the liver and heart in Chinchilla rabbits

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The benefit of exploration and exploitation of crude oil to the Nigerian economy is not without its negative consequences. Apart from the indirect exposure to crude oil due to spillage, the consumption of this crude oil by the rural populace living in oil rich regions as traditional medicine for illnesses have evoked local and international concerns. The aim of this study was to investigate the histological and biochemical disrupting effects of Escravos crude on the liver and heart in Chinchilla rabbits. A total of thirty Chinchilla rabbits aged twelve to fourteen weeks and weighing 1.2 to 1.45 kg were used. Crude oil was orally given at the doses of 15, 20, 25 and 30 mg/kg body weight, corresponding to groups B, C, D and E, respectively for 28 days while group A (control) received distilled water. The result show a dose dependent significant increase in the serum concentrations of total cholesterol, creatine kinase, C-reactive protein, alanine transaminase and aspartate transaminase (p<0.05). The histological findings include: lymphocytic infiltration, cirrhosis, fibrosis, hemosiderin, oedema, mild tissue scaring and tissue necrosis. Thus, this result suggests that Escravos crude oil is a potential biochemical disruptor and can also affect the micro-architecture of liver and heart.

Key words: Escravos crude oil, liver, heart, biochemical parameters, histology, Chinchilla rabbits.

#### INTRODUCTION

The over dependence on the monetary benefit of crude oil exploration and exploitation and neglect of its environmental consequences has made the problem of crude oil pollution a recurrent issue. The impact of crude oil spillage and discharge on the ecosystem as a result of oil exploration activities is an obvious problem of environmental concern (Otitoju and Onwurah, 2007; Ovuru and Ekweozor, 2004). The largest contributor to the oil spill

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in total, besides corrosion of pipes and tanks, is the rupturing or leaking of production infrastructures that are described as, "very old and lack regular inspection and maintenance" (Nwilo and Badejo, 2001).

According to Dede et al. (2002), cases of misuse of this substance by individuals have been reported, as it is known to be liberally used by some of the indigenes who believe that it can repel witches when applied either topically or given orally to afflicted individuals, while other countries such as Kenya, Tanzania, Zimbabwe, Ghana and Tunisia depend on crude oil for unorthodox treatment of ailments such as stomach ache, diarrhoea, respiratory distress and convulsion.

Generally, various studies on crude oil have revealed that it has serious deleterious effects on soils (Jeroh et al., 2011; Mary and Dolor, 2007), plants (Baek et al., 2004; Agbogidi et al., 2007), aquatic life (Ndimele et al., 2010; Daka and Ekweozor, 2004) and even organisms such as the macrobenthic invertebrates (Arimoro and Adamu, 2008). However, humans and other animals are also adversely affected. The constituents of crude oil can irritate the skin and mucous membrane on contact. Irritant effects can range from slight reddening to burning, swelling (oedema), pain and permanent skin damage. Commonly reported effects of acute exposure to crude oil through inhalation or ingestion include: difficulty in breathing, headaches, nausea, confusion and other central nervous system effects (Akpofure et al., 2000). The aim of this study was to investigate the structural and biochemical disrupting effects of Escravos crude oil on the liver and heart in Chinchilla rabbits.

#### **MATERIALS AND METHODS**

#### Test sample

The Escravos blend crude oil (with reference number 863) used in this study was provided by Warri Refining and Petrochemical Company Effurun, Delta State. The crude oil was exposed to sunlight in shallow pans (25 x 25 x 5 cm) for 24 h at the site of the study to allow the extremely light and volatile fractions to evaporate leaving behind the stable components. This product simulates the naturally occurring condition following spillage (Neff et al., 2000).

#### Animals/experimental design

A total 30 Chinchilla rabbits aged 12 to 14 weeks and weighing 1.2 to 1.45 kg were obtained from the Faculty of Agriculture, Ebonyi State University Abakaliki (EBSU). The animals were examined, treated for ectoparasites using Lymectin (Hebei New Century Pharmaceutical C0. Ltd) by a veterinarian and allowed to acclimatize for two weeks at the Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus. The animals were randomly divided into five groups, containing 6 rabbits each (3 males and 3 females).

The research plan consisted of five groups designated Groups A (control), B, C, D and E. Group B to E were orally given a sub-lethal dose of 15, 20, 25 and 30 mg/kg body weight of the Escravos crude oil, respectively, with due consideration of their body weight (those with greater body weights have their dose divided into two; one in

the morning one at night). The different doses of the liquid Escravos crude oil were measured in weight on an electronic weighing balance and given orally (oral gavage) for 28 days.

#### **Animal treatment**

The experiment was conducted in accordance with the Guidelines of the U.S. National Institute of Health (NIH) on the care and use of laboratory animals. The animals were kept under standard and good laboratory conditions (12 h light and 12 h darkness, temperature (30  $\pm$  4.5°C), humidity and ventilation). Overnight, prior to exposure, the animals (rabbits) were starved of solid food and their body weights were taken weekly and for the duration of the study to check for weight loss or gain which is associated with toxicity. The rabbits were fed grower pallets (from Vital feed Ltd, Jos, Plateau State, Nigeria) and water  $ad\ libitum\ for\ 28\ days.$ 

#### Sample collection, organ harvest and microscopy

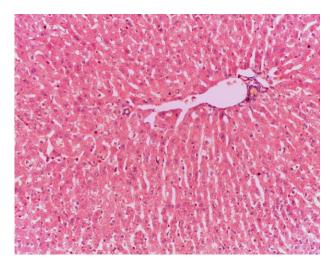
On the 29<sup>th</sup> day (morning), the animals were anaesthetized using cotton wool damped in chloroform with due consideration of their body weights. The blood samples, obtained by marginal ear vein puncture, were drawn into tubes using 22 gauge sterile needles. For biochemical analyses, blood samples collected into plain test tubes were centrifuged (Rotofix 32®-Hettich) at 3000 g for 10 min; the serum was collected and kept at -20°C until analysis. Animals were sacrificed; the heart and liver excised, blotted dry to remove traces of blood and weighed using an electronic weighing balance (using 210/0.1 mg digital balance ESJ-210-4). The excised heart and liver were fixed in 10% formal saline, processed through paraffin wax, sectioned and slices of 3 µm thickness were stained using Haematoxylin and Eosin (H&E), Van Gieson and the Gordon and Sweet's staining Techniques (Avwioro, 2002). Photomicrograph of the stained tissue sections were taken for documentation. The processing of the heart and liver were made at Histopathology Unit in the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

#### Biochemical analysis

The serum liver enzyme concentrations were estimated using the colourimetric method (Sood, 2009). The modified- IFCC (UV) method was adopted for the estimation of serum creatine kinase (CK-MB) as described by Stein (1985). Enzyme linked immunosorbent quantitative method as described by Powell et al. (1979) was used to determine the concentration of C-reactive protein while enzymatic end point method was used to estimate the serum total cholesterol concentration (Sood, 2009). Kits from Randox Laboratories, United Kingdom and Diagnostic Automation Inc., Calabasas were used. The concentrations of the biochemical parameters were measured using ELISA machine (MR 96 USA) and spectrophotometer. The biochemical analyses were carried out using the facilities of Reene Laboratories Onitsha.

#### Statistical analysis

Mean values ( $\pm$ SD) of the biochemical parameter and organ weights were taken for analysis. The data was tested for homogeneity of variance and significantly different results were established by one-way ANOVA using the SPSS software application (version 16). Pair-wise comparisons were made using the Post hoc test. The accepted level of significance was set at p<0.05. The Pearson's correlation was made to compare the blood levels of (i) Aspartate transaminase and C-reactive protein, (ii)



**Figure 1.** Group A: Photomicrograph of the liver tissue with normal micro-architecture. Stained by Haematoxylin and Eosin stain (H&E), 200x.

Table 1. Mean ±SD and pair-wise comparison of the biochemical parameters between the control group and the treated groups.

D	Group						
Parameter	A (control)	B (15 mg/kg)	C (20 mg/kg)	D (25 mg/kg)	E (30 mg/kg)	P-value	
Aspartate transaminase	17.00±3.16	19.40 ± 2.07 (0.854)	45.60 ± 29.06 (0.037)	65.20 ± 31.00 (0.001)	41.40 ± 10.52 (0.072)	0.008	
Alanine transaminase	27.83±2.71	35.60 ± 4.93 (0.061)	40.20 ± 8.93 (0.005)	45.00 ± 9.75 (0.000)	40.00 ± 3.54 (0.005)	0.003	
Creatine kinase	9.42±4.36	13.69 ± 4.57 (0.412)	16.23 ± 3.27 (0.196)	30.08 ± 17.38 (0.001)	20.15 ± 3.98 (0.048)	0.008	
C-reactive protein	0.24±0.11	$0.38 \pm 0.12 (0.147)$	$0.51 \pm 0.25 (0.008)$	$0.57 \pm 0.19 (0.002)$	$0.46 \pm 0.07 (0.032)$	0.019	
Cholesterol	1.44±0.18	$1.91 \pm 0.33 (0.007)$	$2.20 \pm 0.19 (0.002)$	$2.29 \pm 0.24 (0.000)$	2.92± 0.23 (0.000)	0.000	

P-value is significant at p < 0.05; significant increases in the serum concentration of the biochemical parameters when the control group was compared with the treated groups (p < 0.05). One-way ANOVA and Post Hoc test.

aspartate transaminase and creatine kinase, and (iii) creatine kinase and C-reactive protein, with the accepted level of significance set at 0.01.

#### **RESULTS**

#### **Behavioural effect**

After two days of the crude oil administration, the animals in the treated groups D and E became restless. The latter was followed by loss of appetite and decreased locomotion. Soon after the tenth day, they regained their appetite.

#### **DISCUSSION**

Crude oil extracted from different wells and locations have different chemical compositions, which may finally determine their toxicity (Neff et al., 2000). The result of this study elucidates the potency of Escravos crude oil to induce organ toxicity and disrupt normal metabolic processes in relation to serum concentration of C-

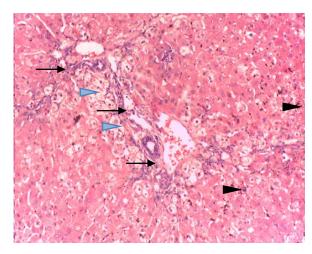
reactive protein (CRP), aspartate transaminase (AST), alanine transaminase (ALT), creatine kinase (CK-MB), cholesterol, weights of the liver and heart.

Significant increases in the weight of the liver and heart were observed when the control group were compared with the treated group (Table 2 Figure 1), which could be attributed to the different histopathological findings observed in the tissues. The significant decrease in the mean change in body weight per week (Table 2) could be linked to the reduction in appetite observed in the treated groups. Cholesterol is an unsaturated steroid alcohol. It is used by the liver, testes and adrenal gland as a major metabolic precursor for the biosynthesis of bile acids, and steroid hormones which include male and female sex steroids (androgens and oestrogens) and adrenal steroid hormones (Morrissey, 2006). In this study, a significant increase in serum cholesterol concentration was observed in the treated group of both sexes (Table 1). The result is in conformity with the findings of Afonne et al. (2013). The observed cholesterol increase may be an indication of tissue fibrosis which culminated to cirrhosis (Figures 4, 5 6 and 7) and or renal retention disease resulting in diminished removal of lipoprotein from the

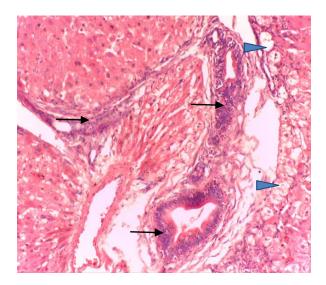
Table 2. Mean ± SD change in body weight of animals (kilogram) and weight of liver and heart (kilograms) in the test and control groups.

Moight	Group					
Weight	A (control)	B (15 mg/kg)	C (20 mg/kg)	D (25 mg/kg)	E (30 mg/kg)	P-value
Mean change in weight (kg)	$0.012 \pm 0.004$	$0.09 \pm 0.003$	$0.02 \pm 0.001$	$0.08 \pm 0.001$	$0.08 \pm 0.004$	0.019
Weight of Liver (kg)	$0.068 \pm 0.05$	$0.077 \pm 0.08$	$0.080 \pm 0.05$	$0.091 \pm 0.06$	$0.087 \pm 0.08$	0.000
Weight of heart (kg)	$0.041 \pm 0.005$	$0.045 \pm 0.005$	$0.047 \pm 0.003$	$0.053 \pm 0.005$	$0.052 \pm 0.003$	0.006

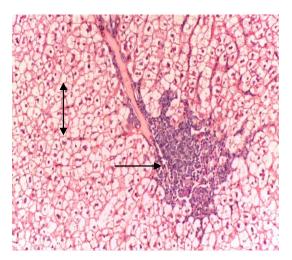
P-value is significant at p<0.05; significant decrease in the mean change in body weight per week and significant increases in the weight of the liver and heart (P<0.05). One-way ANOVA.



**Figure 2.** Group B: Photomicrograph section of the liver with evidence of stromal proliferation, hydropic hepathocytes (marked by blue arrow heads), lymphocytic infiltration (marked by arrows) and hemosiderin (coarse, dark brown and refractile granules-marked by black arrow heads). Stained by H&E technique, 200x.



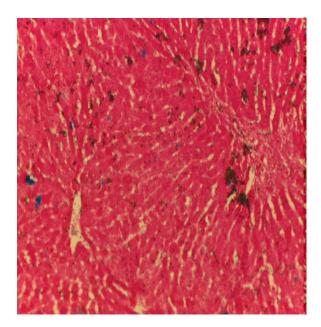
**Figure 3.** Group C: Photomicrograph of the liver tissue with dilatation of the portal triad, dense stroma, hydropic cells (marked by blue arrows) and lymphocytic infiltration (marked by blue arrow heads) within and around the portal triad. Stained by H&E technique, 200x.



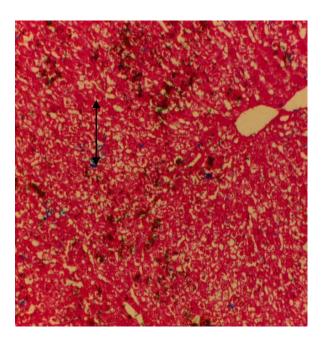
**Figure 4.** Group E: Photomicrograph of the liver tissue with fat deposits clogging the blood supply to the hepatocytes, marked hydropic cells (ballooning degeneration-marked by the double headed arrow), karyorhexis, cirrhosis and fibrosis (marked by arrow). Stained by H&E technique, 200x.

plasma, thus causing the concentration of cholesterol to increase markedly.

C-reactive protein (CRP) is an acute phase protein synthesized by the liver and is normally present as a trace constituent of serum or plasma at levels less than 0.3 mg/dl (Kushner et al., 1994; Macy et al., 1997). Its physiological roles are numerous and varied, but with several functions similar to those of immunoglobulins, CRP appears to function in host defense (Schultz and Arnold, 1990). As elevated CRP values are always associated with pathological changes, the CRP assav provides useful information for the diagnosis, therapy and monitoring of inflammatory processes and associated disease (Shine et al., 1981; Dixon, 1984; Hind and Pepys, 1984; Kushner, 1991). The result of this study showed a significant increase in CRP concentration (Table 1) in the treated animals. The Inflammatory process marked by lymphocytic infiltration (Figures 2 and 3) proves a vivid connection between the assayed CRP and the structural changes evident in the section of the liver. Additionally, measurement of CRP by high-sensi-tivity CRP assays



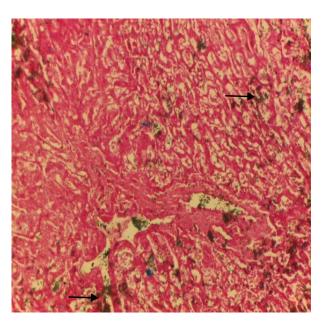
**Figure 5.** Group A (control): Photomicrographed section of a normal liver. Stained by Gordon and Sweet's technique. X200.



**Figure 6.** Group D: Photomicrograph of the liver tissue with mild onset of cirrhosis (marked by areas of distorted liver stroma). Stained by Gordon and Sweet's technique, 200x.

may add to the predictive value of other cardiac markers (myoglobin, creatine-kinase-MB, Troponin I and T), which are used to assess the risk of cardiovascular and peripheral vascular disease (Ridker et al., 1997, 1998).

Creatine kinase (CK-MB) is primarily found in striated muscle, brain and heart tissues. Ck-MB activity is useful



**Figure 7.** Group E: photomicrograph of the liver tissue with hydropic cells and cirrhosis (demonstrated by poorly marked darkly stained reticulin fibres). Stained by Gordon and Sweet's technique, 200x.

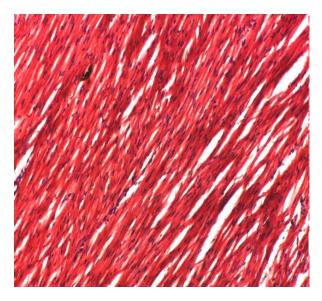
in diagnosis of myocardial infarction, re-infarction and the sizing of infarction (Braunwald et al., 2000; Apple and Murakami, 2005). In this study, a significant increase in CK-MB was observed (Table 1). The result from the Pearson's correlation shows a strong correlation between the AST, CK-MB and CRP levels in the serum of the treated animals (Table 3). Tissue necrosis and mild muscle scarring were evident in the histological sections of the heart tissue from group E (treated animals) used in this study (Figure 9 and 11). When compared with the control group (Figure 8). The micro-architectural changes found in the heart could be attributed to the increased cholesterol observed in the treated groups which may have caused arteriosclerosis, ischemia and finally necrosis in the heart tissue. The latter, is in consonance with the reports of Milinkovitch et al. (2013), who found similar effects on juvenile golden grey mullet (Liza aurata).

AST is an enzyme found mostly in the heart muscle, liver cells, skeletal muscles and kidneys. Injury to these tissue result in the release of the enzyme in the blood stream. Elevated levels are found in myocardial infarction, cirrhosis and hepatitis (Sood, 2009). The result from this study showed significant increases in the levels of AST and ALT (Table 1). This result is in accordance with the reports of Sese et al. (2013) who reported similar increase after administering Bonny light crude oil to male Chinchilla rabbits. Cirrhosis is a result of advanced liver disease, characterized by replacement of liver tissue by fibrosis (scar tissue), regenerative nodules; lumps that occur due to attempted repair of damaged tissue, elevated

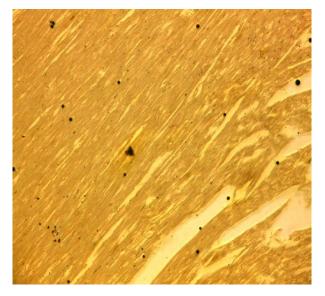
Table 3. Correlation between aspartate transaminase, C-reactive protein (CRP) and creatine kinase (CK).

Variables	R-value	P-value	Remark
Aspartate transaminase correlated with CRP	0.518**	0.007	Positive correlation
Aspartate transaminase correlated with CK	0.622**	0.001	Positive correlation
Creatine kinase correlated with CRP	0.574**	0.002	Positive correlation

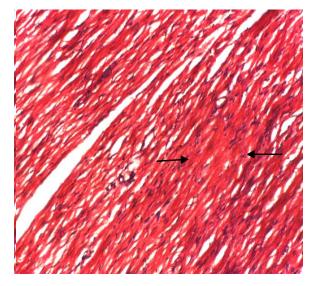
<sup>\*\*</sup>Correlation is significant at 0.01 level (2-tailed); significant strong positive correlations between the serum concentration of the biochemical parameters at 0.05 and 0.01 (\*\*) confidence interval. Pearson's correlation.



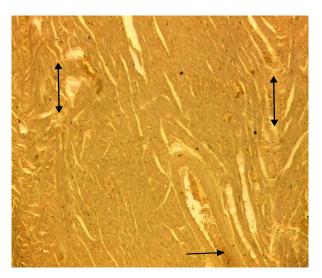
**Figure 8.** Group A (control): Photomicrograph of the heart tissue with normal architecture. Stained by Van Gieson technique, 200x.



**Figure 10.** Group A (control): Photomicrograph of the heart tissue with normal architecture. Stained by Van Gieson technique, 200x.



**Figure 9.** Group E: Photomicrograph of a heart section with mild distortion (and discontinuation) of the muscle alignment. There is also an area of mild tissue necrosis (marked by arrows). Stained by H&E technique, 200x.



**Figure 11.** Group E: Photomicrograph of a section of the heart with evidence of increased vascularization, oedema (marked by arrow) and mild muscle scaring (degeneration of collagen fibres-marked by double headed arrows). Stained by Van Gieson's technique, 200x.

cholesterol and hemochromatosis among others. According to the reports by Udeme and Etim (2012), the Nigerian crude oil blends have been observed to contain some trace metals such as Pb, Cd, Cr, Mn, Zn, Cu, and Co at a low concentration but with high values of Ni, V and Fe. The Fe in the crude oil may have caused the iron deposits (hemosiderin) found on the liver tissues of the treated groups (Figures 2 and 4) when compared with the control group (Figure 1).

The biochemical and the histological findings evident in the photomicrographs elucidated the deleterious effect of crude oil on the liver and heart, whether by indirect contact due to oil spillage or by ingestion as a traditional medicine. This result suggests that Escravos crude oil has the potential to cause biochemical toxicity and can affect the micro-architecture of the liver and heart.

#### Conflict of interests

The author(s) have not declared any conflict of interests.

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