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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Full Length Research Paper

Bahir Dar tannery effluent characterization and its impact on the head of Blue Nile River

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A study was conducted to characterize Bahir Dar tannery effluent and determine its impact on the head of Blue Nile River using selected physicochemical parameters. Samples were taken from the direct effluent stream and four sampling sites (one upstream and three downstream) were selected along the river to determine its impact on the river. Samples were collected from October to March, 2010/11. Temperature, pH, conductivity and total dissolved solids (TDS) were measured *in situ* using a combined meter. The samples for the rest physicochemical parameters were collected from the sites using the appropriate method. In the laboratory, BOD_5 and COD were measured according to standard methods. Total nitrogen, total phosphorous, chloride and sulphide, were determined with Hach nutrient analysis kits and a Hach spectrophotometer. Total suspended solids were determined photometrically. The heavy metal, chromium (as Cr total), was determined using atomic absorption spectrophotometry according to standard methods. The results show that the impact of the effluent from biological oxygen demand (BOD), chemical oxygen demand (COD), NH_3-N, total nitrogen, chlorides, sulphides and chromium was significant with concentrations of 342±52.5, 850.75±96.2, 288±75.8, 462.5±130, 1408.13±405.3, 16.05±3.04 and 3.54±0.55, respectively and most of the effluent characteristics were beyond the provisional discharge limit set out by the Ethiopian Environmental Protection Authority. Analysis of variance indicated that all the physicochemical parameters except temperature, pH and total phosphorous significantly varied among sampling sites ($p<0.05$); the reference or upstream site having lower value than downstream sites. The result shows the pollution load of the effluent on the river and the urgent need for measures to be taken.

Key words: Tannery effluent, physicochemical parameters, Blue Nile River.

INTRODUCTION

Industrial waste is the most common source of water pollution in the present day (Ogedengbe and Akinbile, 2004) and it increases every year because most countries are getting industrialized. Worldwide, it is estimated that the industry is responsible for dumping 300-400 million tons of heavy metals, solvents, toxic sludge, and other wastes into waters each year (UNEP, 2010). Thus, the environment is under increasing

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pressure from wastes emanating from such industrial activities. As compared to other industries, leather tanning is one of the most polluting activities (Khan et al., 1999) as it consume huge amount of water in several stages, generating an enormous amount of liquid effluents (Farenzena et al., 2005) which are hazardous to the environment to which they are discharged.

Tannery wastewater is highly polluted in terms of biological oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), nitrogen, conductivity, sulphate, sulphide and chromium (Mondal et al., 2005) and in most developing countries tannery effluents are discharged directly into sewers or water bodies without treatment (Verheijen et al., 1996; Favazzi, 2002). The high BOD_5 content of the effluent will affect the survival of gill breathing animals of the receiving water body and high COD value indicate toxic state of the wastewater along with presence of biologically resistant organic substances. The high level of ammonia-N is toxic to aquatic organism and nitrogen may cause eutrophic condition. The high salinity and TDS of the effluent may result in physiologically stressful conditions for some species of aquatic organisms due to alterations in osmotic conditions. Studies show that increase in salinity causes shifts in biotic communities, limit biodiversity, exclude less tolerant species and cause acute or chronic effects at specific life stages. Changes in the ionic composition of water can also exclude some species while promoting population growth of others (Weber-Scannell and Duffy, 2007). The pollutants are poisonous to man and aquatic life resulting in food contamination.

In Ethiopia, although the use of leather and leather products goes back to prehistoric times, tanning hides and skins of animals into leather has been practiced as industrial activity since the last decades (EEPA, 2003). Presently, Ethiopia's leather industry is in the forefront of the leather sector development within the Eastern and Southern Africa region (UN, 2002). Currently, there are 20 operational tanneries that turnout wastes directly into the nearby water bodies like the other industrial activities. This makes industrial and chemical pollution to become the third major problem in the country and one of the great environmental concerns (Zinabu and Zerihun, 2002). This is becoming evident through the pollution of water bodies and human habitat in the major cities, rivers and lakes. Similarly, Bahir Dar Tannery is discharging its effluent into Blue Nile River. In view of the negative impact of this effluent on the environment, the present study aimed at determining the levels of physicochemical pollutants in effluent samples from the tannery and assessing its impact on Blue Nile River.

MATERIALS AND METHODS

Description of the study area

This study was conducted in Bahir Dar, the capital city of Amhara Region which is situated on the southern shore of Lake Tana, the source of Blue Nile (Abay) River. Bahir Dar Textile factory and Tannery are the most important industries in the city. Both the textile factory and the tannery discharged their effluent directly into the Blue Nile River. The downstream part of the river is used for domestic activities including drinking, irrigation and recreation (swimming and bathing). The use of the river in this way may lead to bioaccumulation of toxic pollutants like chromium which is hazardous to human beings as well as livestock.

Sampling

The study was conducted from October, 2010 to March, 2011. This time was selected to sample from both dry and wet periods so as to include possible seasonal effect. Direct physicochemical samples were taken from the effluent stream so as to compare with the country's discharge limit and four sampling sites (one upstream and three downstream) were established along the river length to assess the impact of the effluent on the river. One site directly below the source of pollution and two other sites at different intervals from the point of effluent discharge were chosen. The reference site was established directly above the effluent discharge where there is little disturbance. The sampling sites were designated as S_1 to S_4. The study area was mapped using Geographical Information System (GIS) using information obtained by Global Positioning System (GPS) (Figure 1).

Data collection

The physicochemical parameters include pH, temperature, BOD_5, COD, ammonia-nitrogen (NH_3-N), total nitrogen, total phosphorous, TDS, conductivity, total suspended solids (TSS), total chromium, chlorides and sulphides. These parameters were selected because they are considered to be deleterious on the receiving environment and they were included in the discharge limit. Temperature, pH, conductivity and TDS were measured *in situ* using combined pH/T°/TDS and conductivity meter. The sample for the rest physicochemical parameters were collected from the sites using a sampler which allows sampling from discrete depth and the sample was transferred into the storage bottle without agitation or aeration (Lind, 1979). Prior to sampling, the polyethylene bottle was cleaned with nitric acid and then washed and rinsed with distilled water. Then, the samples were taken to laboratory. In the laboratory, BOD_5 and COD were measured according to standard methods (APHA, 1998). Total nitrogen, total phosphorous, chloride, and sulphide, were determined with Hach nutrient analysis kits and a Hach spectrophotometer (DR010 Hach Co., Loveland, Colorado, USA). Total suspended solids were determined photometrically. The heavy metal chromium (as Cr total), was determined using atomic absorption spectrophotometer (Buck Scientific Model 210 VGP, USA) according to standard methods (APHA, 1998).

Data analysis

Descriptive statistics was used to analyze the physicochemical data. One-way ANOVA was used to compare the difference in physicochemical data among the sampling sites and means were separated using Tukey HSD.

RESULTS

The mean values for each parameter of the wastewater revealed that, most of them were beyond the standard provisional limit set by Ethiopian Environmental Protection Authority (EEPA, 2003) (Table 1).

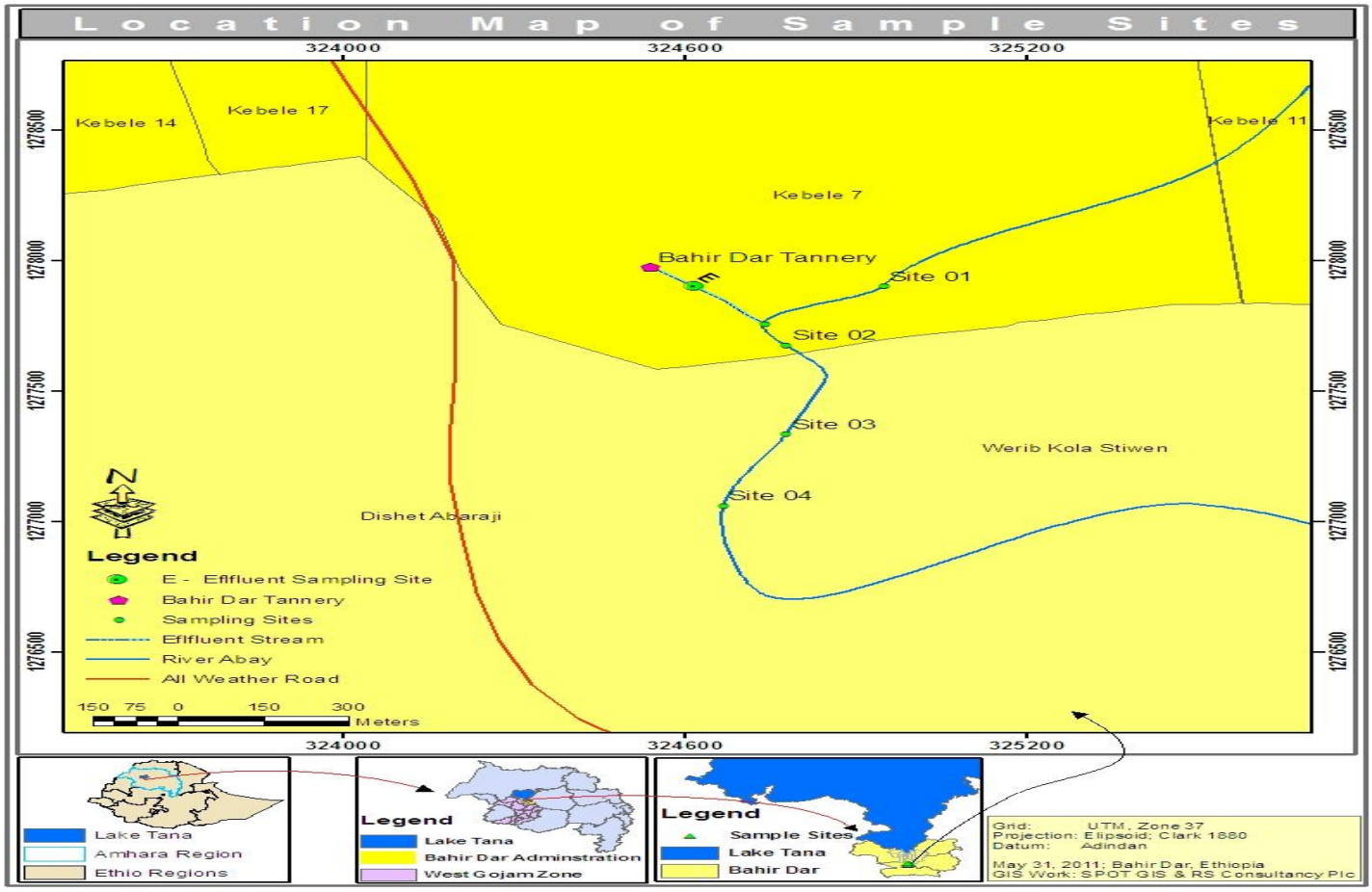

Figure 1. Map showing sampling sites on the effluent stream and along the Blue Nile River.

A detailed characterization of downstream water samples was also carried out to determine the pollution load of the effluent on Blue Nile River. The mean concentrations of pollutants and their variation along the sampling sites are presented in Table 2.

As shown in Table 2, the mean value of temperature and pH at downstream sites ranged from 20 to 24 and 7.13 to 7.15, respectively. Their value did not show significant variation among sampling sites ($F=2.22$, $p=0.16$, $R^2=0.69$ and $F=2.04$, $p=0.18$, $R^2=0.8$), respectively. The pollution profile for BOD_5 and COD along the downstream sites ranged from 34.9±9.05 to 73.4±13.2 mg/l and 107.3±26.7 to 206±32.6 mg/l, respectively. According to the ANOVA, the concentrations of these two parameters significantly varied among sampling sites ($F=20.1$, $P<0.0001$, $R^2=0.91$ and $F=26.23$, $P<0.001$, $R^2=0.93$), respectively. BOD_5 and COD values at the upstream site were significantly lower than the two downstream sites (S_2 and S_3). In addition, their concentrations at the last downstream site (S_4) were significantly lower than that of S_2, but there was no significant variation in their concentration between S_3 and S_4.

The levels of ammonia-N and total nitrogen in the water samples were in the range of 14.3±2.37 to 50±8.11 mg/l and 23±7.86 to 67.5±8.7 mg/l, respectively. Their values differed significantly among sampling sites ($F=26.81$, $P<0.0001$, $R^2=0.91$and $F=40.12$, $P<0.0001$, $R^2=0.94$), respectively. The upstream site had significantly lower concentration of ammonia-N than S_2 and S_3. But there was no significant difference in ammonia-N concentration between the last downstream site (S_4) and the upstream site.

Ammonia-N concentration at S_4 was also significantly lower than S_2, but there was no significant difference between S_3 and S_4. The concentration of total nitrogen significantly varied among all sampling sites; the upstream site having lower concentration (Table 2).

The level of phosphorous in the downstream water samples ranged from 5.3±0.98 to 6.5±1.1 mg/l while concentration of phosphorous at the reference site was 6.3±1.7 mg/l. This value did not show significant difference among sampling sites ($F=0.12$, $P=0.95$, $R^2=0.15$). Total dissolved solids, conductivity and TSS also vary in their values along the sites. Their values at

Table 1. Physicochemical characteristics of Bahir Dar Tannery effluent.

Physicochemical parameter	Mean ± SE	Range	Discharge Limit
Temperature (°C)	25.5 ± 2.2	20.3-30.5	40°C
pH	7.15 ± 0.09	7.13-7.16	6 - 9 pH units
BOD_5 (mg/l)	342 ± 52.5*	214-452	>90% Removal or 200 mg/l
COD (mg/l)	850.75 ± 96.2*	651-1023	500 mg/l
Total ammonia (as N) (mg/l)	228 ± 75.8*	96-420	30 mg/l
Total nitrogen (as N) (mg/l)	462.5 ± 130.8*	89-692	>80% Removal or 60 mg/l
Total phosphorus (as P) (mg/l)	11.5 ± 4.8*	1-24	>80% Removal or 10 mg/l
TDS (mg/l)	2003.25±74.5	1832-2193	-
Conductivity (µs/cm)	3953.25±150.3	3668-4374	-
TSS (mg/l)	339±68,6*	204-525	50 mg/l
Chromium (as total Cr) (mg/l)	3.535 ± 0.55*	1.98-4.51	2 mg/l
Chloride (as Cl) (mg/l)	1408.125 ± 405.3*	613.5-2517	1000 mg/l
Sulphide (as S) (mg/l)	16.05 ± 3.04	8.95-23.39	0.1 mg/l

*Means above the EEPA Discharge Limit.

Table 2. Variation in physicochemical characteristics at the sampling sites along the Blue Nile River (Temperature in °C, pH in pH units, conductivity in µs/cm and the rest in mg/l) in 2010/2011.

Site	pH	Temperature	BOD_5	COD	Ammonia-N	Total nitrogen
Upstream / Reference (S_1)	7.16^{a}	24.63^{a}	11.4^{c}	27.4^{c}	0.2^{c}	1.1^{d}
Just below the effluent discharge (S_2)	7.15^{a}	24.56^{a}	73.4^{a}	206.0^{a}	50.0^{a}	67.5^{a}
200 meters below S_1 (S_3)	7.13^{a}	20.93^{a}	55.0^{ab}	164.0^{ab}	32.0^{ab}	45.0^{b}
400 meters below S_1 (S_4)	7.14^{a}	23.18^{a}	34.9^{bc}	107.3^{b}	14.3^{bc}	23.0^{c}

Table 2. Contd.

Site	Total phosphorous	TDS	Conductivity	TSS	Chloride	Sulphide
Upstream / Reference (S_1)	6.3^{a}	150.2^{c}	307.0^{c}	41.0^{b}	34.0^{c}	0.03^{b}
Just below the effluent discharge (S_2)	6.0^{a}	1517.0^{a}	3051.0^{a}	222.0^{a}	299.6^{a}	0.55^{a}
200 m below S_1 (S_3)	5.3^{a}	1167.0^{ab}	2274.0b	157.5^{ab}	213.8^{b}	0.04^{b}
400 m below S_1 (S_4)	6.5^{a}	903.8^{b}	1793.7^{b}	108.8^{ab}	174.0^{b}	0.01^{b}

Means within a column followed by the same letter are not significantly different from each other according to Tukey HSD ($p<0.05$).

downstream sites ranged from 903.8±116.1 to 1517±196.8 mg/l, 1793.7±252.5 to 3051±397.2 µs/cm and 108.8±25.1 to 222±46 mg/l, respectively. The values of TDS significantly varied among sampling sites ($F=51.18$, $P<0.0001$, $R^2=0.95$). The upstream site had significantly lower TDS concentration than the three downstream sites. There was also significant difference between S_3 and S_4. Similarly, the values of conductivity showed significant difference among sampling sites ($F=54.3$, $P<0.0001$, $R^2=0.96$). The upstream site had significantly lower value than all downstream sites and its values at S_3 and S_4 were also significantly lower than S_2. Total suspended solids concentration showed significant variation among sampling sites ($F=6.09$, $P=0.02$, $R^2=0.7$); the upstream site having significantly lower value than the site just below the effluent discharge (Table 2).

The concentration of chromium at the two downstream sites was 0.165±0.11 (S_2) and 0.142±0.097 mg/l (S_3) while the concentration at the other downstream site (S_4) was not detected. Here, the concentration of Cr at the

Table 3. Literature on concentration of tannery effluents (pH in pH unit, Temperature in °C and the rest in mg/l).

Parameter	Present study	Literature	Reference	Discharge limit
Temperature	25.5	21.2±0.8	Seyoum et al., 2003	40
pH	7.15	4.74-8.66	Deepali et al., 2009	6-9
BOD_5	342	2982.5	Seyoum Leta et al., 2003	>90% or 200 mg/l
		840-1860	Haydar et al., 2007	
		585-639	Akan et al., 2009	
		1425-1500	Deepali et al., 2009	
COD	850.75	11123	Seyoum et al., 2003	500
		1320-54000	Haydar et al., 2007	
		2389-3784	Seyoum et al., 2003	
		1916-27810	Deepali et al., 2009	
NH_3-N	228	1330	Seyoum et al., 2003	30
Nitrogen	462.5	122.2	Seyoum et al., 2003	60 mg/l
Total phosphorous	11.5	16.65-19.93	Seyoum et al., 2003; Seyoum et al., 2003	10 mg/l
TDS	2003.25	42716.33	Deepali et al., 2009	-
TSS	339	3491.9-9485.33	Deepali et al., 2009	50
Total chromium	3.54	8-32.2	Seyoum et al., 2003; Akan et al., 2009	2 mg/l
Chlorides	1408.13	630.4-4313	Akan et al., 2009; Deepali et al., 2009	1000 mg/l
Sulphides	16.05	630.4	Seyoum et al., 2003	1 mg/l

reference site was not detected as that of S_4. Chloride and sulphide levels in the downstream samples varied between 174±66.46 to 299.6±98.13 mg/l and 0.008±0.002 to 0.55±0.23 mg/l, respectively. Chloride values at the downstream samples varied significantly among sampling sites ($F=45.85$, $P<0.0001$, $R^2=0.95$) with the reference site having significantly lower value than the downstream sites. Sulphides showed significant variation among sampling sites ($F=26.1$, $P<0.0001$, $R^2=0.9$). The site just below the effluent discharge had significantly higher value than the two downstream and the upstream sites (Table 2).

DISCUSSION

In this study, a detailed characterization of wastewater was carried out on Bahir Dar tannery effluent and the result showed that most of the parameters under investigation were high and well beyond the provisional standard limit set by EEPA (2003).

The values of temperature and pH in this study were within the discharge limit and below what Seyoum et al. (2003) and Deepali et al. (2009) reported (Table 3). The lower value of pH and temperature in this study might be due to variation in the sampling time of the day and the difference in production capacity of the tanneries. BOD_5 and COD mean values were above the discharge limit. But the values in this study were lower than what Seyoum et al. (2003), Haydar et al. (2007), Akan et al. (2009) and Deepali et al. (2009) reported (Table 3). These high levels of BOD_5 and COD values observed in the waste may be due to high amount of organic matter from various chemicals used during the processing of hides and skins. It has been reported that a significant part of chemicals used in the tanning process is not actually absorbed in the process and discharged into the environment (UNIDO, 1991), thereby increasing the levels of BOD_5 in the effluent. The high BOD_5 content of the effluent will affect the survival of gill breathing animals of the receiving water body and high COD value indicate toxic state of the wastewater along with presence of biologically resistant organic substances.

Ammonia-N and total nitrogen concentrations were above the discharge limit as shown in Seyoum et al. (2003) report. These high levels of ammonia-N and nitrogen might be attributed to several components in tannery effluent containing nitrogen as part of the chemical structure and the nitrogen contained in proteinaceous material of the skin (Bosnic et al., 2000). The high level of ammonia-N is toxic to aquatic organism and nitrogen may cause eutrophic condition. The mean phosphorous concentration of the effluent was somewhat similar to what Seyoum et al. (2003) and Akan et al. (2009) reported (Table 3) and showed little deviation from the discharge limit.

Total dissolved solids, conductivity and total suspended solids values in this study were found to be high. But Seyoum et al. (2003) and Deepali et al. (2009) reported values greater than the result of this study (Table 3). This deviation might be due to the variation in size and production capacity of the tanneries under investigation. The total dissolved solids may increase salinity of the water and thus may render it unfit for irrigation and other

purposes for the immediate downstream users while the suspended impurities cause turbidity in the receiving system.

Chromium level in this study is 36 times higher than the discharge limit set out by EEPA (2003). Similarly, sulphide and chloride values were above the discharge limit. These high values observed in the effluent may be due to the chemicals used by tanneries. Studies (Table 3) reported higher values of these pollutants and this might be also due to the variation in size and production capacity of the tanneries under investigation. Generally, the result showed that the effluent is rich in organic pollutants, but relatively poor in phosphorous content.

The mean concentration of pollutants along Blue Nile River indicated that the concentration of the various pollutants decrease downstream from the point where the effluent joins the river to the last downstream site (S_4). This is attributed to the dilution capacity of the river, uptake of pollutants by the vegetation along the river gradient and some reactions that can change the pollutants to harmless ones, may take place as the water flows downstream from the point pollution source.

Even though there was no significant variation among sites, the mean temperature value of the upstream site was slightly higher than the last downstream sites and the slight variation might be due to the difference in the sampling time. The pH in all sites was more or less neutral. The result in this study slightly deviates from the work of Seyoum et al. (2003) (Table 3) and this might be due to the size of the river and its dilution capacity.

The concentration of BOD_5 and COD at S_2 is very much reduced by 76-79% from the direct effluent owing to the dilution capacity of the river. The concentrations at downstream sites were well beyond the reference site indicating the organic pollution load of the effluent on the river. This increase in BOD_5 and COD increases rates of biological or chemical decomposition leading to oxygen depletion which produce both acute (mortality) and chronic (reduced growth, fecundity and disease resistance) impacts on aquatic biota (Allison, 1996).

The concentration of NH_3-N and nitrogen at S_2 showed 78-85% reduction from the direct effluent value. This rapid reduction might be due to the size of the river. Seyoum et al. (2003) reported total NH_3-N and nitrogen reduction of 92-95 and 69% respectively (Table 3). The standard provisional limits for NH_3-N and N of tannery wastewater are 30 and 60 mg/l respectively. All the values at downstream sites were beyond the limit and the value at the reference site. This indicated that the tannery wastewater was responsible for these pollutants. The nitrogen in ammonia form is toxic to aquatic organisms. In the environment ammonia-nitrogen is oxidized rapidly to nitrate, creating demand and low dissolved oxygen in the water. Moreover, the high nitrogen may cause eutrophication problem (USEPA, 2008). Eutrophication reduces dissolved oxygen in water and alters stream habitat available for macroinvertebrates, fish eggs and fish both of which are critical for fish and other aquatic life. This may disrupt the ecological cycle of the stream so that certain biological communities experience severe mortality.

The phosphorous content in downstream samples was within the standard provisional limit set by EEPA (2003). Its concentration did not show significant difference between the sites. The reference site had almost similar phosphorous content as that of the downstream sites showing that the tannery effluent was not responsible for this pollutant. This might be due to the addition of municipal wastewater into the river above the reference site. So in this study, phosphorous does not give concern as severe pollutant from the tannery and may not cause eutrophication.

Total dissolved solids, conductivity and total suspended solids at S_2 showed a 25-34% reduction than the direct effluent value. As compared to the other parameters, the values of the above parameters showed slight reduction at downstream sites. This might be due to their initial high concentration which made the dilution process slower. The values at the downstream sites also significantly differ from the reference site indicating the increasing impact of the tannery effluent on downstream water bodies. Studies by Seyoum et al. (2003) and Birnesh (2007) reported the same result (Table 3). High concentrations of total dissolved solids forms a layer on the bottom of water course and covers natural fauna on which aquatic life depends. This can lead to localized depletion of oxygen supplies in the bottom waters. It also reduces light penetration and thus photosynthesis in the water. On the other hand, high conductivity indicates salinity increment in the receiving water. This may result in adverse ecological effects on aquatic biota (Lefebvre and Moletta, 2006).

Chromium concentration at S_2 showed a 95% decrease from the effluent and it was not detected at S_4. The level at the reference site also was not detected indicating the pollution at downstream sites originated from the effluent. Similarly, chloride and sulphide contents at S_2 showed dramatic reduction with 96-98% from the direct discharge owing to the dilution effect of the river. Chloride concentrations at S_2 were significantly higher than the reference and the other downstream sites. But all the downstream sites have values beyond the standard provisional limit set out by EEPA (2003). The values indicated that most of the concentration of chlorides and sulphides at downstream sites were the contribution of the tannery effluent. The presence of these ions increases the salinity of the receiving water body. The discharge of high salinity and TDS effluents into receiving system may result in physiologically stressful conditions for some species of aquatic organisms due to alterations in osmotic conditions. Studies show that increase in salinity causes shifts in biotic communities, limit biodiversity, exclude less tolerant species and cause acute or chronic effects at specific life stages. Changes in

the ionic composition of water can also exclude some species while promoting population growth of others (Weber-Scannell and Duffy, 2007).

Conclusion

Even though tanning industries are very important for the country's economy and to improve standards of living of citizens, their waste is directly discharged into the nearby water body without treatment. Most of the physico-chemical parameters investigated in this study showed that almost all the effluent characteristics were above the provisional discharge limit set by the Environmental Protection Authority indicating the poor treatment mechanism employed by the tannery. Most of the physicochemical parameters along the river gradient were also high and beyond the discharge limit. This will create a problem for downstream users as they use it for domestic, agricultural and recreational value. So, this fact must regularly be brought to public awareness by using media or direct contact with the downstream users. The Environmental Protection Authority should also establish environmental protection laws which consider technical and financial capability of the industries so as to control industrial pollution. Not only establishment, the laws should also be enforced and environmental standards with their protocols should be followed with strict and continuous monitoring to safeguard the environment from heavy loads of pollutants and toxic substances.

Conflict of Interests

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

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Full Length Research Paper

Recovery and characterization of poly(3-Hydroxybutyric acid) synthesized in *Staphylococcus epidermidis*

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Polyhydroxyalkanoates (PHA) are biodegradable polyesters accumulated intracellularly as energy resources by bacterial species. In this study, fermentation process for production of PHA is carried out using sesame oil as carbon source. We studied recovery of poly(3-hydroxybutyric acid) (PHB) from *Staphylococcus epidermidis* by sodium hypochlorite digestion method. Recovered PHB sample was estimated by UV spectrophotometer. PHB from *S. epidermidis* was characterized and by these findings, we examined purified PHB by differential scanning calorimeter (DSC), a thermo gravimetric analyzer (TGA), thin layer chromatography (TLC) and infrared spectroscopy (IR). The results of our analysis of PHB while comparing with commercial source suggest that in DSC melting temperature of PHB was 173.36°C, TGA thermo grams of PHB sample was at 296.91°C, on TLC plate; Rf value was calculated as 0.71 and finally IR spectrum of the compounds showed characteristics bands for the groups CH, C=O and C-O, indicating the presence of PHB in the production medium.

Key words: Polyhydroxyalkanoates (PHA), poly(3-hydroxybutyric acid) (PHB), *Staphylococcus epidermidis.*

INTRODUCTION

Often, research and media attention on the renewable bioproduct industry is focused specifically on fuel alternatives. This is logical since diminishing fossil fuels supply nearly 80% of the global energy demands, and it is predicted that the current demand will increase by 56% by 2040 (U.S. Energy Information Administration, 2013). Currently, though, the renewable energy industry is one of the two fastest-growing industries globally, increasing at a rate of 2.5% per year (U.S. Energy Information Administration, 2013). By 2015, the demand for biodegradable plastics is estimated to reach 1.1 million tons (Metabolix, 2013a). To make environment free from plastics is one of the major interests to both decision makers and plastic industries (Chen, 2009). Poly(3-hydroxyalkanoic acid) (PHA) is a biodegradable polymer material that accumulates in numerous microorganisms under unbalanced growth conditions (Ribera et al., 2001). Poly β-hydroxybutyrate (PHB) is biopolymer that can be used as biodegradable plastics is the most common natural microbial PHA (Singh and Parmar, 2013). In terms of molecular weight, brittleness, stiffness, melting point and glass transition temperature, the PHB

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homopolymer is comparable to some of the more common petrochemical derived thermoplastics such as polypropylene (Sayed et al., 2009). Although PHB was found to be at advantage comparing to non-biodegradable plastics; its application is inevitably limited due to high production costs.

PHB is microbial polyester produced by many bacteria and stored in their cell in the form of granules, about 0.5 µm in diameter. β-hydroxybutyrate is connected by ester linkage and form PHB (Prasanna et al., 2011). PHB possesses only *R (alkyl group)* side chains (and lacks*S (Sulfur)* side chains) and hence reported as biodegradable materials (Anderson and Dawes, 1990; Saito et al., 1996; Jung et al., 2001); for example, vulcanized rubber. PHB is an intracellular product; the method applicable for its effective separation from other biomass component is complex and expensive.

Number of different methods for the recovery of PHB has been suggested. There are some of the known, effective methods for separation of PHB from bacterial cell: Physical method using a bead mill (Kunasundari and Sudesh, 2011), extraction method uses an organic solvent (Ibrahim and Steinbuchel, 2009), enzyme method (Kathiraser et al., 2007).

Finally, there must be a method which allows consistent recovery of the polymer with high purity. There may be a different requirement of purity of biopolymer which depends on its intended application and which ultimately designs the recovery method of PHB extraction.

In this work we studied PHB recovery from *Staphylococcus epidermidis* which possesses tendancy to utilize sesame oil and it has been reported previously that plant oils are desirable feed stocks for PHA production because they are also inexpensive in comparison with other carbon sources, such as sugar (Akiyama et al., 2003). Hence, here *S. epidermidis,* a known PHA-producing bacterium is utilized for study of PHB production and recovery of PHB from *S. epidermidis* worthy of investigation. PHB was recovered through a dispersion of a sodium hypochlorite solution and chloroform. In this paper we also described characterization and determination of native PHB like granules which recovered using various organic solvents.

MATERIALS AND METHODS

Cultivation of bacteria

S. epidermidis, isolated from edible oil contaminated sites and was used for PHB production (Marjadi and Dharaiya, 2011). *S. epidermidis* was grown and maintained in a modified mineral salts medium (MSM) (Marjadi and Dharaiya, 2011).

The production of PHB or copolymer was carried out by two-stage cultivation (Hartmann et al., 2010). First stage organisms were cultivated in the nutrient broth medium without any nutrient limitation, at 37°C and 150 rpm for 24 h. In second stage, after incubation, 2 ml of culture was taken to inoculate according to their dry cell weight (Marjadi and Dharaiya, 2011) the flask containing 200 ml of sterile production medium, and all the isolates were first grown for 72 h. at 37°C with shaking at 150 rpm in a carbon - rich MSB medium containing sesame oil (1% w/v) as a sole carbon source and cells accumulating PHB were cultivated in 250 ml of modified mineral salts basal medium (MMSB) as described by Marjadi and Dharaiya (2012).

Production and storage of PHB-containing biomass

To recover and characterizePHB produced in *S. epidermidis* after fermentation, the cell broth was concentrated bycentrifugation at 4,000 RPM for 15 min at 25°C, washed twice with distilled water,and then freeze dried. The resulting cell powder was stored at 4°C until they used further.

PHB recovery

After 96 h of incubation at 37°C, 10 ml of culture was taken into clean polypropylene centrifuge tubes which had been previously washed thoroughly with ethanol and hot chloroform to remove plasticizers. In each tube, 5 drops of formaldehyde were added in order to stop all biological activity and then centrifuged at 8000 rpm for 15 min. The supernatant was discarded and collected pellet was washed twice with 5 ml cold water and 2 ml cold hexane (Loba®) twice to remove hydrophobic residual oil (Kahar et al., 2004). The remaining pellet was dried in oven until obtaining the constant weight. The dried pellet was treated with the original volume of culture medium (10 ml) of 30 % (v/v) sodium hypochlororite (NaOCl) (Loba®) and the mixture was incubated at 37°C for 1 h. After incubation, the mixture containing the lipid granules was centrifuged at 6,000 rpm for 15 min and was washed with water, and then with 5 ml 96% cold acetone (Loba®) and followed by ethanol (Loba®) (1:1). The precipitates thus formed were allowed to dry to obtain PHB crystals.

PHB extraction

Extracted PHB crystals were re-dissolved in 5 mg in 5 ml (Sayyed et al., 2009) of chloroform in a test tube in water bath at 100°C for 20 min and filtered through Whatman No.1 filter paper and chloroform was evaporated by pouring the solution on sterile glass petri plate and then kept at 4°C in deep fridge. After some time, powder was collected from petri plates by slowly scratching for further analysis (Kuniko et al., 1989; Bowker, 1981; Ishizaki and Tanaka, 1991).

Estimation of PHB concentration

PHB concentration was estimated as suggested by Law and Slepecky (1961). Extracted PHB powder was transferred to clean test tube (Figure 1) and 10 ml of concentrated H_2SO_4 was added to the tube which was capped and heated for 20 min at 100°C in a water bath. PHB crystals were converted in to crotonic acid by dehydration (Aslim et al., 2002).The resultant brown colourcrotonic acid solution was cooled, and after thorough mixing, a sample was transferred to a quartz cuvette and the absorbance was measured at 235 nm in UV Spectrophotometer against a sulfuric acid blank. Standard curve of pure PHB (Sigma®, USA) was prepared by the modified method as suggested by Slepecky and Law (1960).

Characterization of PHB

The chemical structure and the thermal properties of PHB were

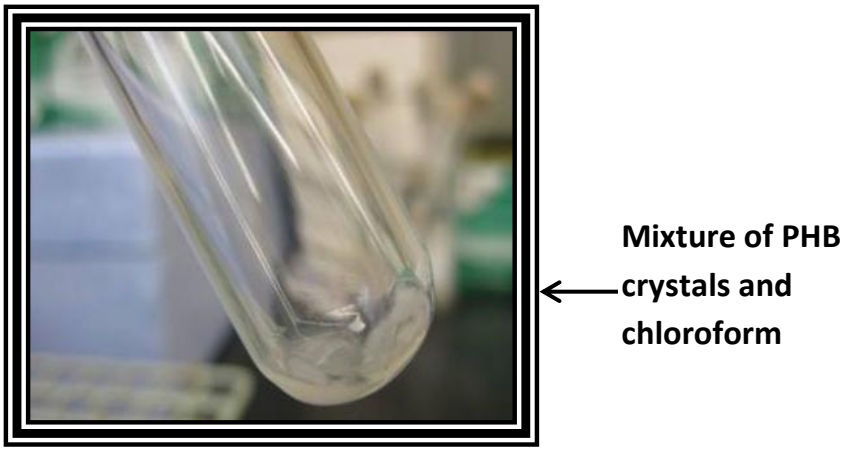


Figure 1. Test tube containing PHB crystals and Chloroform.

used as parameters for qualitative analysis of PHB. Characterization and determination of native PHB like granules involved precise measurements to analyze their physical properties and were characterized mainly by four methods in the present study: TLC, IR, DSC and TGA.

Thin layer chromatography (TLC)

TLC was carried out in a glass plate (10 × 5 cm^2) coated with silica (3 g/15 ml of chloroform), prepared using a spreader. About 50 µl of propanolysed organic phase which involve propanolysis of PHB in a tightly sealed vial (10 ml) to which 2 ml of dichloroethane and 2 ml of a solution of propanol-hydrochloric acid (4:1 [vol/vol]) (Panda et al., 2008) of sample was loaded on the TLC plate and allowed to run in the solvent system consisting of ethyl acetate and benzene (SRL®) (1:1) mixture for 40 min. The plate was left to dry after run and for staining 50 ml of iodine solution (Hi-media®) was vaporized in water bath at 80 to 100°C. TLC plate was kept over the beaker containing iodine solution for 5-10 min in order to get it saturated with iodine vapour. The Rf values of the spots were calculated using standard formula and compared with the standard chart (Rawte and Mavinkurve, 2002).

Infrared spectroscopy (IR)

IR analysis of PHB-like granules was performed using a commercial customer service, Aarti Industries, Tarapur, India. Briefly, extracted sample and standard PHB from Sigma® was separately made in to solid pellet by making an intimate mixture of a powder sample with potassium bromide for IR analysis. The relative intensity of transmitted light was measured against the wavelength of absorption on the region 800 to 4000 cm^{-1}using IR double beam spectrophotometer (Shimandzu®). IR spectra of samples were measured at ambient condition.

Differential scanning calorimetry (DSC)

DSC analysis of PHB-like granules was performed using a commercial customer service, Center of Excellence, Vapi, India. Briefly, differential scanning calorimetry was used to characterize the melting temperature (Tm) of samples which was done in a range of 30 to 450°C air at 10°C air /min. The melting temperature (Tm) and melting enthalpy (∆H) were determined from DSC endothermal peaks.

Thermo gravimetric analysis (TGA)

TGA analysis of PHB-like granules was performed using a commercial customer service, Center of Excellence, Vapi, India. Briefly, Thermo gravimetric analysis was used to determine the decomposition temperature (Tdecomp.) of PHB. Ten milligrams of PHB film were folded into a platinum tray and subjected to a heating rate of 20°C air/min from ambient to a final temperature of 500°C air.

RESULTS AND DISCUSSION

Lipid inclusion granules were stained black whereas the bacterial cytoplasm was stained pink in color confirming the presence of lipid inclusion granules inside the bacterial cell. PHB production was found to be influenced by the utilization of carbon from sesame oil.

PHB was isolated from the production medium by solvent extraction technique. The sodium hypochlorite digestion process enables in the digestion of cells and release of the PHB granules outside the cells for easy extraction of PHB. As present work involves utilization of edible oil as carbon source, extraction of PHB with a pre treatment of hexane helps in efficient removal of edible oil from fermentation broth.

After achieving constant weight of cell biomass at 105°C after 24 h in an oven, the biomass is treated with the hypochlorite solution, based on the fact that it can dissolve nearly all components of cell except PHB granules (Yu et al., 2006). The solvent extraction is widely used to recover PHB with high purity. Sodium hypochlorite breaks the cell wall of bacteria and facilitates elimination of Non-PHB Cellular Material (NPCM) resulted in the lysis of cells without affecting the PHB (Jacquel et al., 2008). The solvent system consisting of

Table 1. IR spectrum of sample and standard PHB.

Sample	Peak region	Comment
PHB Sample	1635	Carbonyl group (C=O)
	3097	Methine groups (CH)
	1089	Ester group (C-O)
	3578.55	Intramolecular H bond
	3415.06	H bond
PHB (Sigma)	1673	Carbonyl group (C=O)
	2928	Methine groups (CH)
	1076	Ester group (C-O)
	3330.13	Intramolecular H bond
	3417.70	H bond

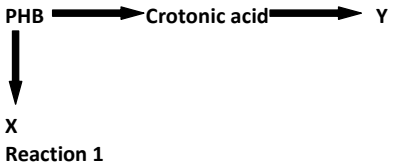
Table 2. Thermal properties of PHB

Sample	n (°C)	Δ*H* (J g-1)
PHB Sample	173.36	69.54
PHB (Sigma)	172.40	65.70

Tm: melting temperature, ΔH: melting enthalpy of the sample

1:1 mixture of ethanol and acetone washings serves to remove water which interferes with the extraction of the polymer into chloroform. It also proves to be specific and efficient for lysing the NPCM without affecting PHB. The present system also assists in extraction of cell lipid content and other molecules (except PHB) (Rawte and Mavinkurve, 2002).

The conversion to crotonic acid by hot concentrated sulfuric acid proved to be about one-third time more sensitive than alkaline hydrolysis of PHB extraction and analysis (Marjadi and Dharaiya, 2012). The protocol that was most efficient in determining PHB that is, β-elimination of crotonic acid; a brown colored compound affected by concentrated sulfuric acid was described by following reaction.



Reaction 1

Where, X refers to other degradation products of PHB and Y refers to degradation products of crotonic acid (Huang and Reusch; 1996).

The amount of PHB in the extracted samples was determined with UV spectrophotometer at 235 nm with reference to the standard graph of 3-hydroxy butyric acid (Data not shown here).

Thin layer chromatography (TLC)

As per the procedure described earlier, when the TLC plate sprayed with iodine vapour, PHB appeared as greenish-black spot surrounded by brown colour on white background. The comparison between standard and sample was performed using solvent system of ethyl acetate and benzene on TLC plate; Rf value (0.71) indicated the presence of PHB in the production medium by comparing with standard PHB.

Table 3. Initial and maximum decomposition temperatures evaluated from TGA.

Sample	Ti (°C)*	T max (°C)
PHB Sample	208.98	296.91
PHB (Sigma)	201.16	287.60

Ti: Initial thermal decomposition, *T max*: Maximum thermal decomposition

Infrared spectroscopy (IR)

IR spectrum of the compounds were recorded in the range of 800-4000 cm^{-1} and showed characteristics bands for the groups CH, C=O and C-O (Sindhu et al., 2011). The methine groups (CH) gave strong band in the range of 1360-1416 and 2914-3097. These frequency values were higher than the normal values because of polymerization.

The carbonyl group (C=O) gave strong band in the range of 1636-1673. These frequency values were lower than the normal value because of polymerization. The (C-O) group showed strong and broad absorption in the range of 1047-1089 (Table 1 and Figure 2).

Differential scanning calorimetry (DSC)

The thermal properties of PHB samples and commercial PHB were investigated by differential scanning calorimetry (DSC). The data are illustrated in Figure 3. Since the melting temperature of PHB is around 170-180°C (Matko et al., 2005), while that of the PHB sample is within the range (173.36°C), which is close to that of the commercial PHB (Table 2).

Thermo gravimetric analysis (TGA)

PHB sample and standard. The thermal degradation of Figure 4 and Table 3 shows the TGA thermo grams of extracted PHB proceeds by a one-step process with a maximum decomposition temperature at 296.91°C. This thermal degradation at maximum decomposition temperature of approximately 300°C is mainly associated with the ester cleavage of PHB component by β-elimination reaction (Choi et al., 2003). The temperature of 296.91°C was found to be the maximum decomposition temperature for biopolymer made with extracted PHB and it was almost similar with that of the standard PHB from

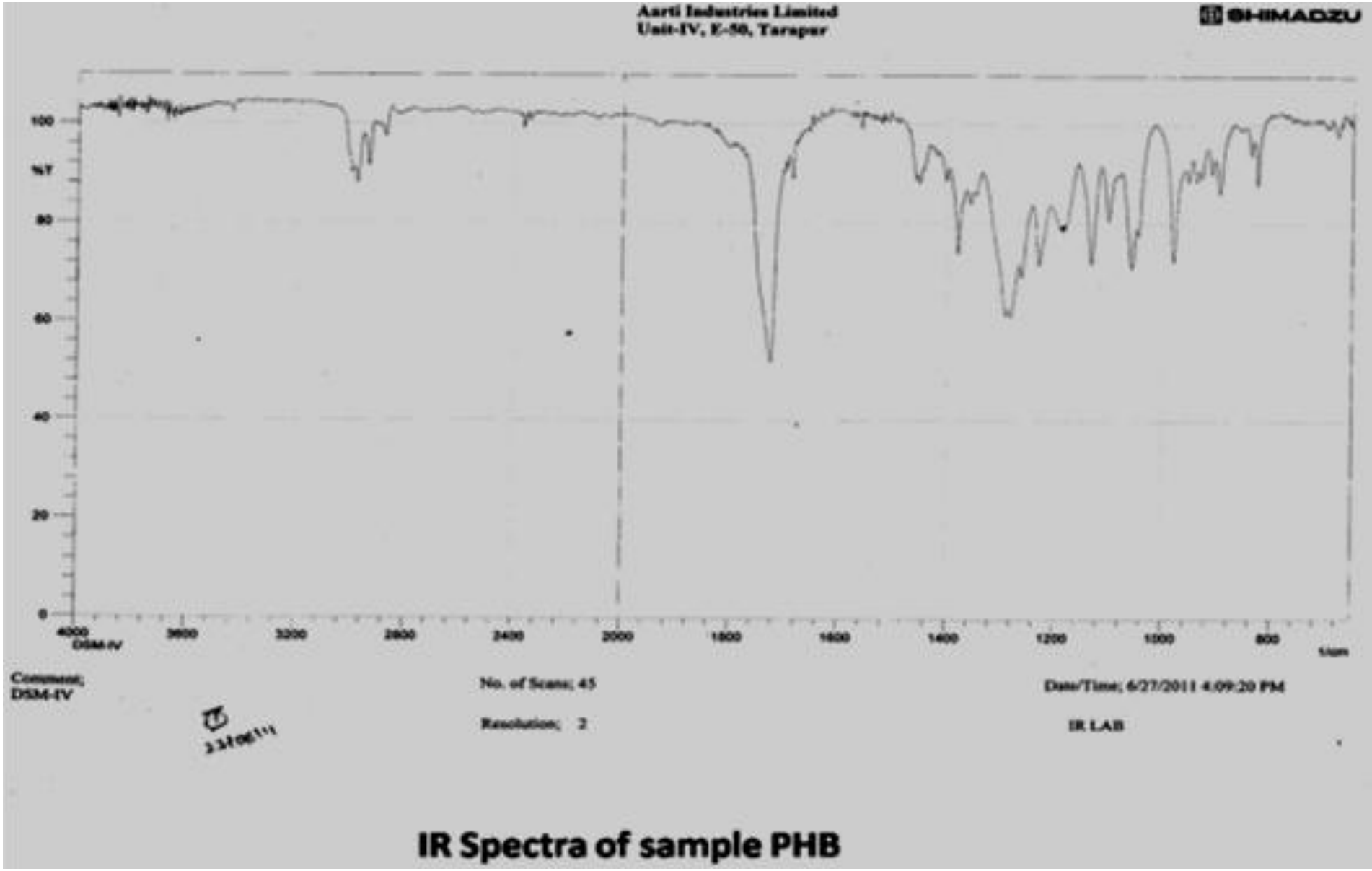


Figure 2. IR spectra comparison between sample and standard PHB.

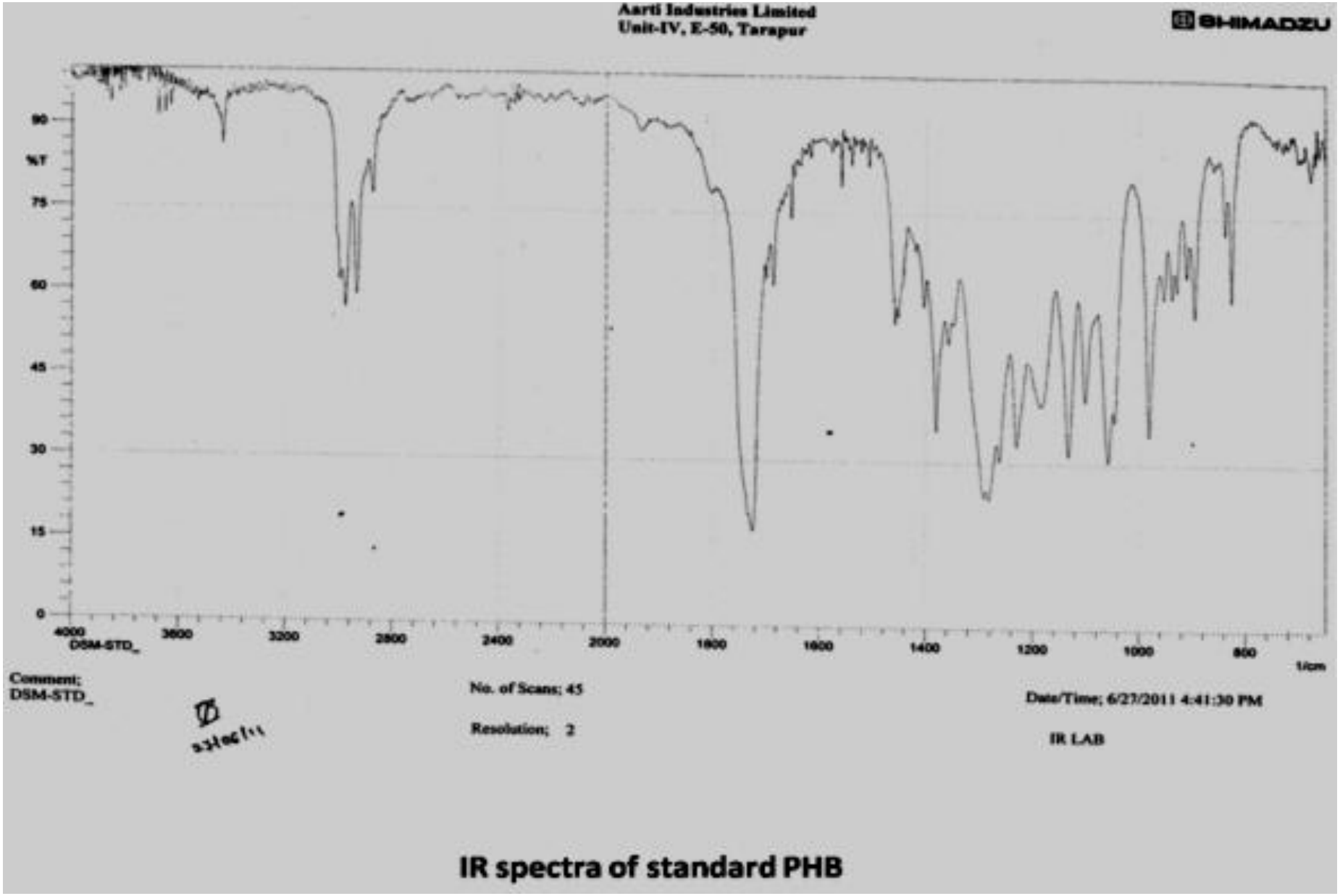
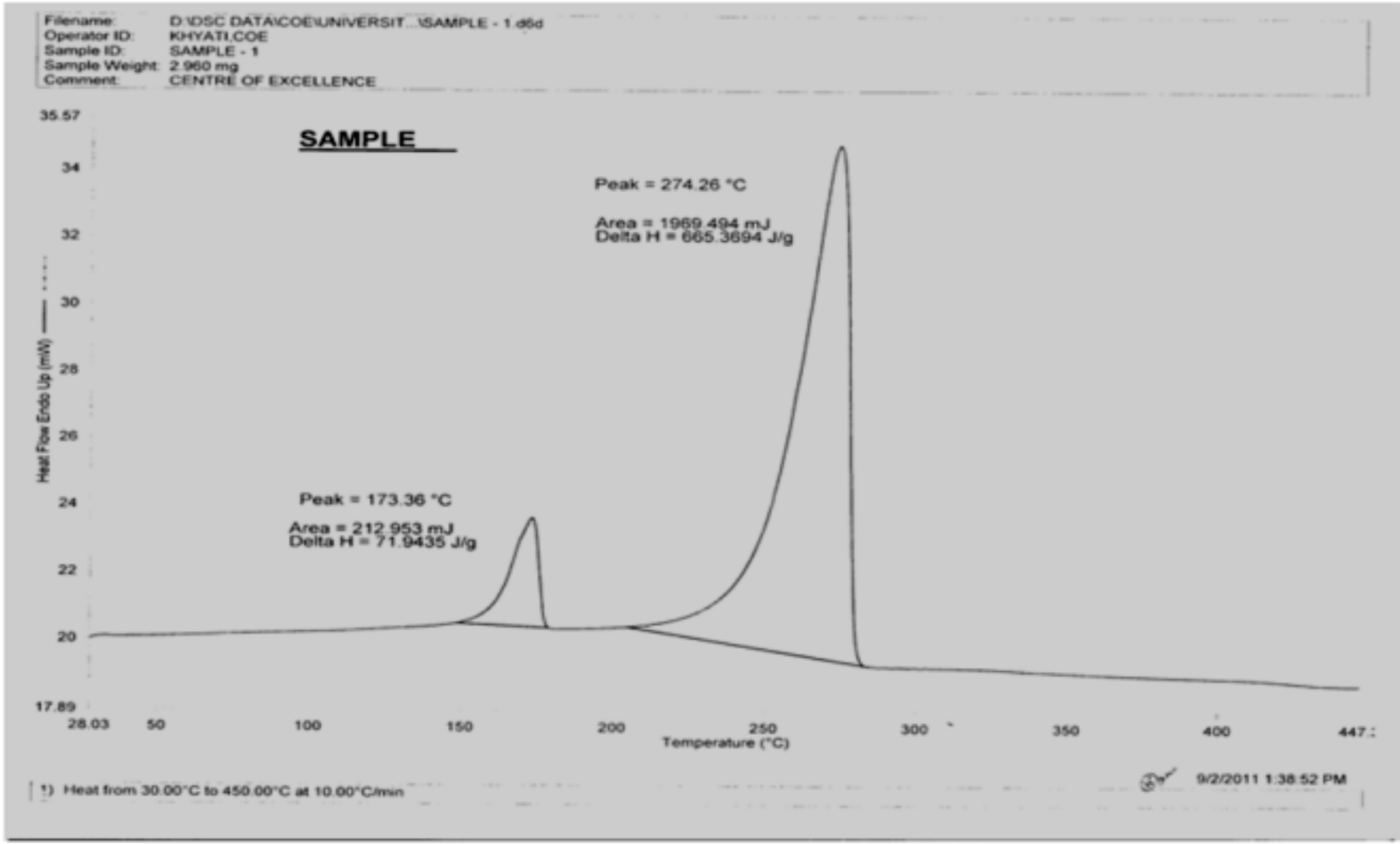
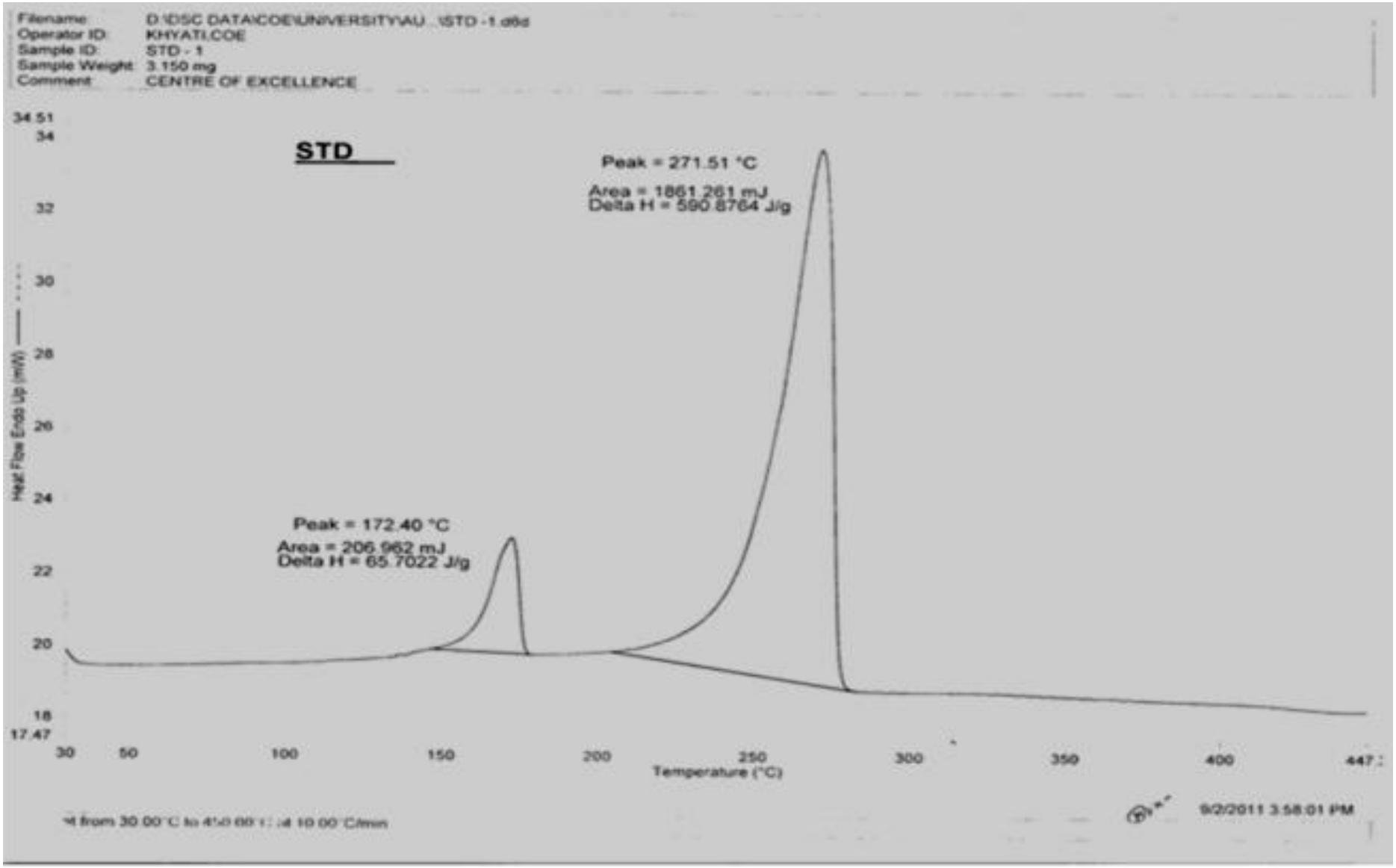


Figure 2. Contd.



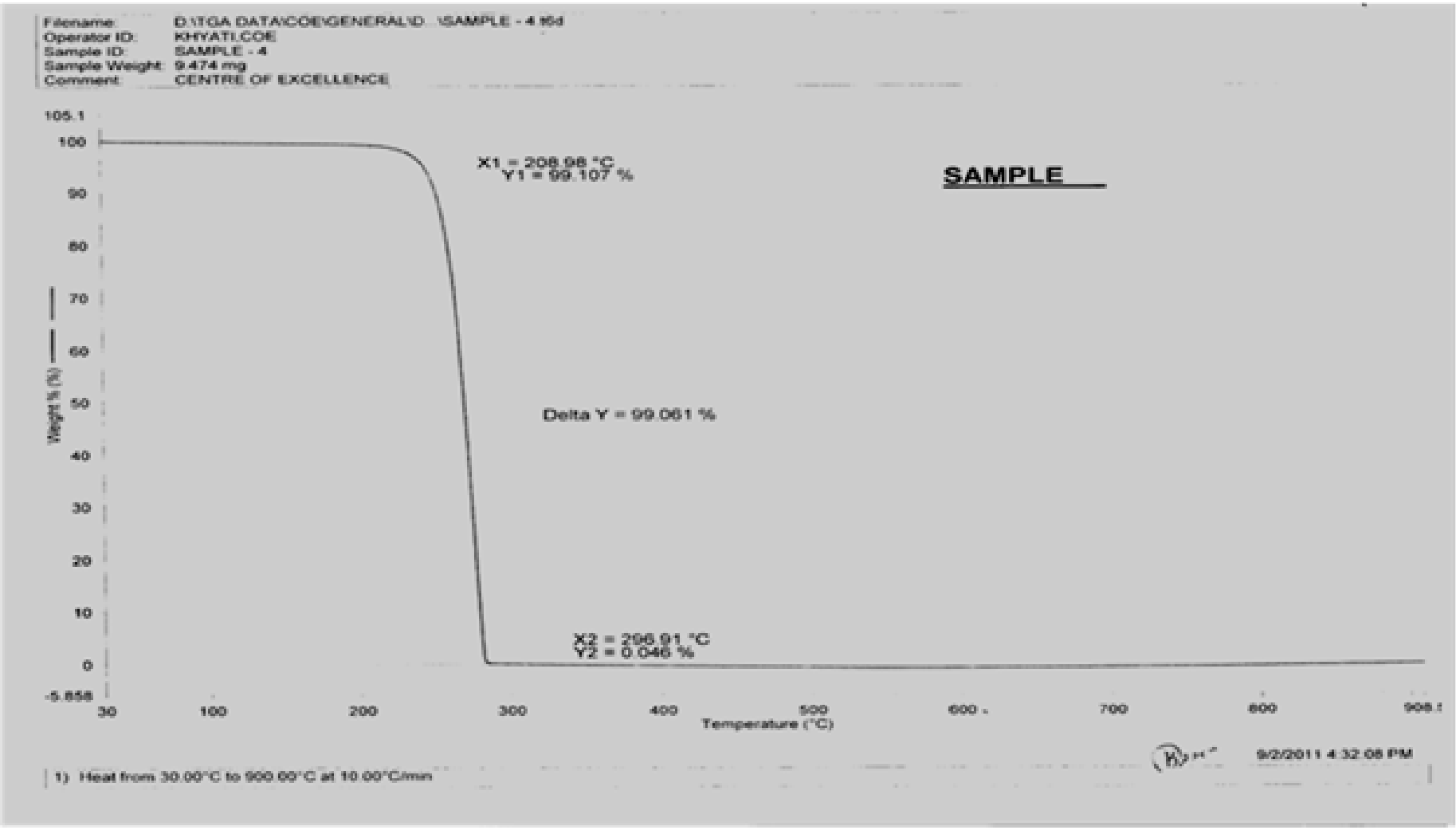
DSC thermo gram of sample PHB

Figure 3. DSC thermo gram comparison between standard and sample PHB.



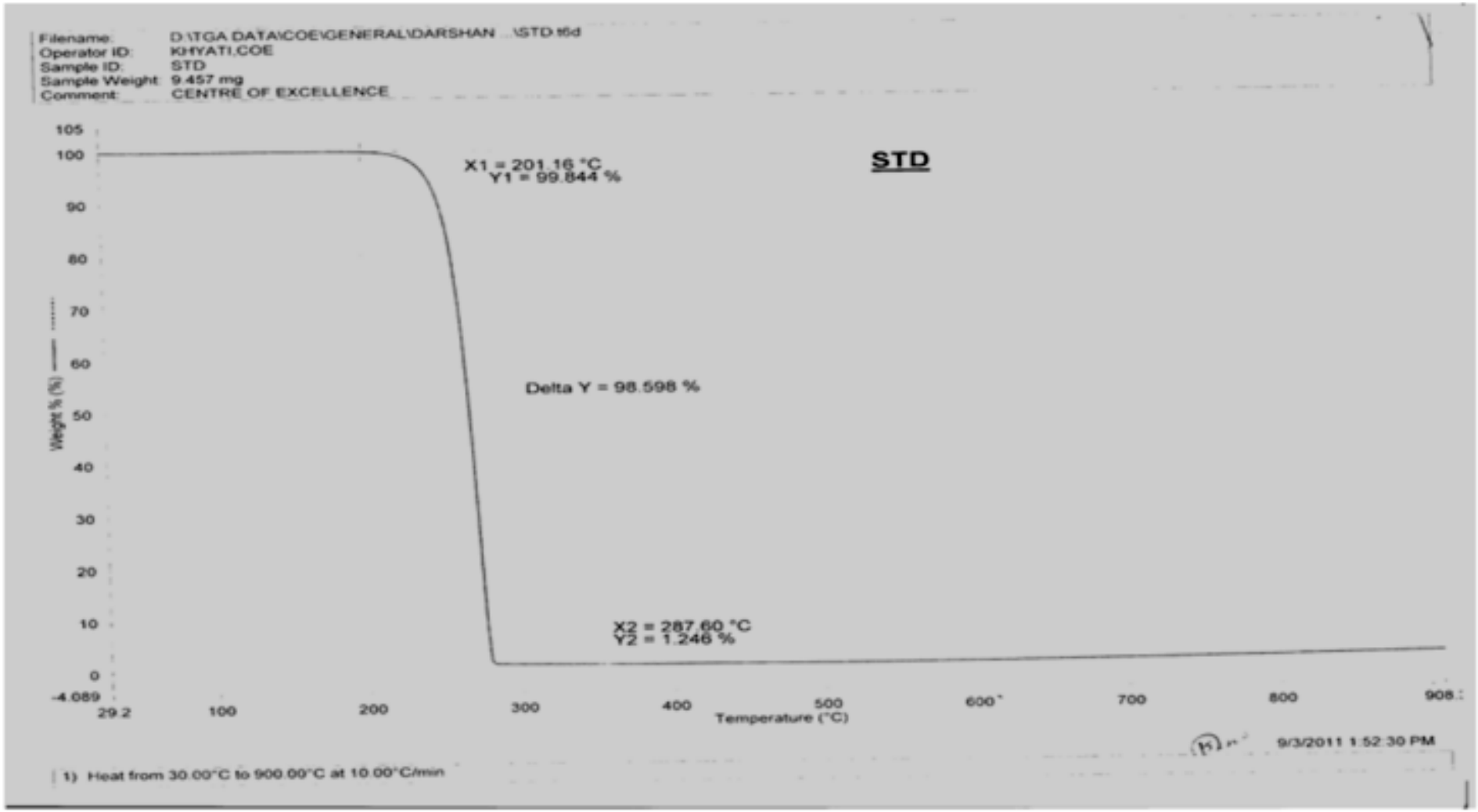
DSC thermo gram of standard PHB

Figure 3. Contd.



TGA thermo gram of sample PHB

Figure 4. TGA thermo gram comparison between sample and standard PHB.



TGA thermo gram of standard PHB

Figure 4. Contd.

Sigma (287.60°C).

Further, the characterization of PHB like granules by various methodologies and their comparison with standard PHB as described earlier shows that the extracted polymer from the microbial isolate possess almost similar properties and was finally confirmed to be PHB and of good quality.

Conclusion

Polyhydroxybutyrate (PHB) was successfully produced through biosynthesis in *S. epidermidis* and recovered appropriately. The method of PHB extraction also influences the quality of polymer. Therefore, bacterial cells were blended with chloroform using high speed homogenizer for a short time to cause lower damage of PHB the molecular weight. The identical PHB sample was verified to commercial PHB and other PHB data that reported in literatures. The obtained PHB has the same thermal properties as commercial PHB with higher molecular mass (approxinatly 3.9 × 106 Da) and lower degree of crystallinity.

Conflict of Interests

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

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Full Length Research Paper

Air quality assessment of carbon monoxide, nitrogen dioxide and sulfur dioxide levels in Blantyre, Malawi: a statistical approach to a stationary environmental monitoring station

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Air quality in urban areas is a cause of concern because of increased industrial activities that contribute to large quantities of emissions. The study assess levels and variations of carbon monoxide (CO), nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) in Blantyre, Malawi using a stationary environmental monitoring station (EMS). Results show that CO level (2.47 ± 1.23 mg m^{-3}) were below the Malawian limit value (10.31 mg m^{-3}). Although, NO_2 (4.02 ± 2.47 mg m^{-3}) and SO_2 (8.58 ± 2.88 mg m^{-3}) were significantly higher than allowable Malawian Standards (0.52 and 0.23 mg m^{-3}, respectively). Discernible variations in hourly, diurnal, monthly and seasonal CO, SO_2 and NO_2 were apparent. Independent t-test confirmed that day time values were higher than those at night ($p < 0.05$). Thus, variations in local weather affect the disparity in hourly and diurnal values. Analysis of variance (ANOVA) confirmed significant variations in monthly observations. Moreover, independent t-test showed that wet season CO (2.32 mg m^{-3}), SO_2 (5.10 mg m^{-3}) and NO_2 (9.41 mg m^{-3}) levels were higher than dry season values (CO = 2.32 mg m^{-3}; SO_2 = 3.42 mg m^{-3}; NO_2 = 8.13 mg m^{-3}). A hierarchical cluster analysis (HCA) divided the 10 months into three groups based on distribution of CO, SO_2 and NO_2, air temperature, wind speed and wind direction. Furthermore, factor analysis (FA) showed that air temperature had significant contribution to variations in mean values of CO, SO_2 and NO_2 for the entire study period. The study shows a need for constant urban air quality monitoring in Blantyre and all urban areas in Malawi. It is recommended that the experimental site widen the scope of the study by utilizing the flexibility of the EMS.

Key words: Air pollutants, principal component analysis, developing countries, environmental monitoring station, Kaiser normalization.

INTRODUCTION

Malawi, like many developing countries, has faced increased levels of urbanization and population growth over the last few years. Most cities in developing countries have population sizes more than twice that of 50 or so years ago (Baldasano et al., 2003). Urbanization and population growth have resulted in a corresponding

Table 1. Ambient air quality standards limits for Malawi (MSB, 2005).

Pollutant	Maximum concentration in ambient air	Average period
Suspended particulate matter	0.5	1 Year
- PM_{10}, µg/m^{-3}	25	1 Day
- $PM_{2.5}$, µg m^{-3}	8	1 Year
Carbon monoxide, mg m^{-3}	10.31	8 Hours
	40.10	1 Hour
Sulfur dioxide, mg m^{-3}	0.52	1 Hour
	0.21	1 Day
	0.05	1 Year
Nitrogen dioxide, mg m^{-3}	0.23	1 Hour
	0.06	1 Year
Ozone, mg m^{-3}	0.14	1 Hour
Lead, µg m^{-3}	0.50	1 Year
Photo-chemical oxidants (as ozone), mg m^{-3}	0.26	1 Hour
	0.08	4 Hours

increase in mobile and stationary fuel combustion emissions. Mobile sources e.g. motor vehicles, motor cycles and locomotives account for a large part of total emissions in major cities (Gerardo and Maricruz, 1997; Holloway et al., 2000; Makra et al., 2010). Of the mobile sources, diesel engines produce comparatively lower concentrations of CO and hydrocarbons (HC) than petrol engines (Bendelius, 1996). But, diesel engines emit large quantities of NO_x and SO_x as compared to petrol (Chan et al., 1997). These factors and challenges in waste management have contributed to the atmospheric deterioration such as acid rain, formation of smog and different ailments to people living in polluted air environment.

The atmospheric deterioration is deleterious to buildings, statues, devices, ecosystem integrity, causes visibility problems (Agrawal et al., 2003; Jalaludin et al., 2004; Lin et al., 2004; Kan et al., 2010; Fattore, et al., 2011) and many human health ailments (Cohen et al., 2004; Shah and Balkhair, 2011). Carbon monoxide and NO_2 are considered to be amongst other tropospheric O_3 precursors (Macdonald et al., 2011). Yet, tropospheric ozone is a threat to human health (WHO, 2003), has deleterious impact on vegetation (Fowler et al., 2009). During wet deposition, NO_2 and SO_2 react to produce acid rain which damages building structures and vegetation.

Human vulnerability to air pollutants depends on time and extent of sensitivity to particular air pollutants (Laumbach, 2010). The nature and significance of air quality issues depend on the many factors. Such factors include size of a city, physical and chemical industrial processes, meteorological processes, geographical features and social factors (Pires et al., 2008).

Urban air quality has received great attentions in recent years as attested by several research and documentation (Wolf, 2002; Agrawal et al., 2003; Vargas, 2003; Riga-Karadinos and Saitanis, 2005; Brajer et al., 2006; Oudinet et al., 2006). The growing concerns on air pollution have seen most developing countries introducing strict regulations (Bailey and Solomon, 2004; Mao and Zhang, 2003). Despite scientific investigations and abatement strategies, urban air pollution is still on the rise in many cities worldwide, or has experienced only small improvements (Makra et al., 2010). Besides, ambient air pollution serves as a major source of gaseous pollutants for indoor air quality (Freijer and Bolemen, 2000). In Malawi, we have the Malawi Standards (MS) that were developed and published by Malawi Standards Board (MSB, 2005). The MS stipulates threshold values for air quality (Table 1).

To improve air quality in cities, a need for air pollution control and prediction of trends is urgent. In addition, short-term forecasting of air quality is crucial since it assists in taking preventive and evasive action during episodes of elevated air pollution (Makra et al., 2010). Blantyre city is one of such cities where air pollution needs scientific evaluation and monitoring hence this study. But, information on CO, NO_2 and SO_2 pollution in Blantyre city is scarce and if any, the information is

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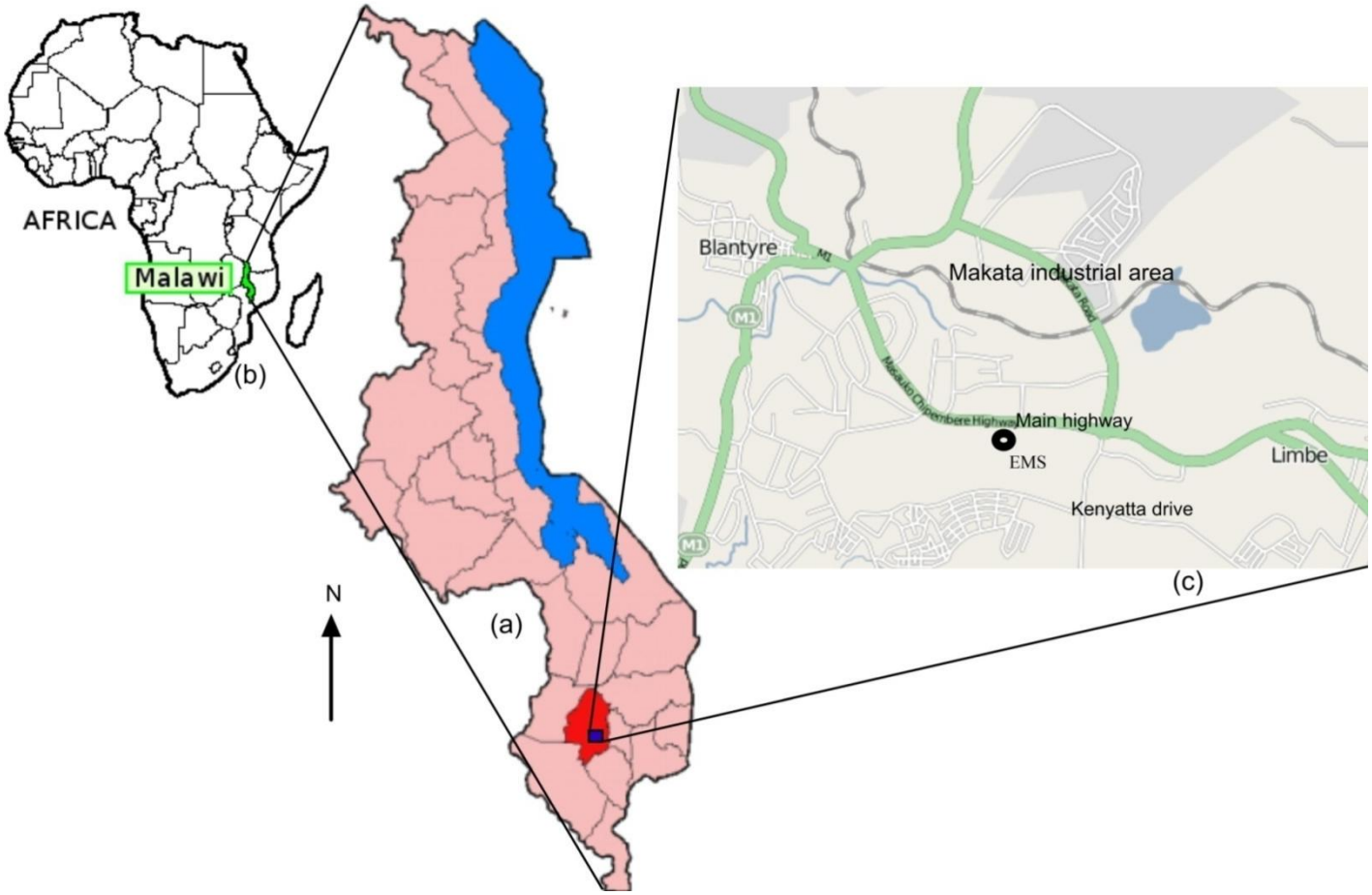

Figure 1. Location of Blantyre district in Malawi (a) found in southern Africa region (b). The blue rectangle is extruded to show the exact location of the study site.

unpublished (Mapoma et al., 2013; Mapoma and Xie, 2013).

The study evaluated diurnal, monthly and seasonal variations in CO, NO_2 and SO_2 levels. We envisaged high concentrations of CO, NO_2 and SO_2 during working hours (day time) as compared to non-working hours due to emissions from mobile and industrial activities. Furthermore, the study evaluated the effect of air temperature, wind speed and wind direction on levels of CO, NO_2 and SO_2. Data was collected between April 2011 and January 2012 using a fixed continuous active environmental monitoring station (EMS) located in Blantyre city along the main highway connecting Blantyre city and Limbe business district in Malawi (Figure 1).

MATERIALS AND METHODS

Experimental site

The study took place in Blantyre city, Malawi. Tables 2 and 3 summarize data on Blantyre City, elaborating on average climate condition, geographic data and commercial activities of the city. Furthermore, Figure 1 illustrates the geographical location of the city. Blantyre is a commercial city with high vehicle and motorcycle traffic in its main roads. Blantyre has designated industrial areas with Makata being the main industrial site (Figure 1). The experimental site is located near the main highway (Figure 1). The main highway (Chipembere) shown in Figure 1 connects Blantyre business district and Limbe business district. As such, the highway is one of the roads with higher vehicle traffic intensity during peak hours. Running parallel south of the main highway is another busy road (Kenyatta drive) that reduces congestion in the main highway when driving to Limbe (Figure 1). As such Kenyatta drive is one of the busiest roads in Blantyre.

Study design

A stationary EMS is located at 25 m above ground on top of the building at the experimental site in Blantyre city (Figure 1). The experimental site is at 1080 m mean altitude above sea level on coordinates 15°48'07"S, 035°01'37"E. The EMS (model MM900) is a dynamic and continuous data logger (http://www.nr.no/nb/projects/environmental-monitoring-station?

Table 2. Average climate data for Blantyre district[a].

Month	January	February	March	April	May	June	July	August	September	October	November	December	Year
Average high °C	28	28	28	28	26	24	24	26	29	32	31	29	27.8
Average low °C	20	19	19	18	15	13	13	14	17	19	21	20	17.3
Precipitation mm	208	206	170	43	8	3	3	3	5	20	86	132	887

[a]Source: http://www.weatherbase.com/weather/weather.php3?s=39676&cityname=Blantyre-Malawi.

Table 3. Summary of location and important productive activities in Blantyre.

Location / important productive activities	Description
District area	228 km^2
Population	1,895,973 (www.worldgazetteer.com, 2012)
Average Elevation	1,039 m above mean sea level
Coordinates	15°47'10"S; 35°0'21"E
Some commercial activities related to air quality issues	Manufacturing and production of paint, fertilizers, soft drinks, detergents, milk pasteurization, steel industries. Petroleum storage and distribution. Waste management Locomotive transportation Road transport network hub for the southern region

language=en). It is capable of monitoring a wide range of air quality parameters due to its flexibility. Up to 30 sensors can be attached to the equipment based on needs. At the moment of data collection, the EMS sensors available were for average air temperature (°C), wind speed (m s^{-1}), wind direction (degrees), SO_2 (parts per million (ppm)), NO_2 (ppm) and CO (ppm). Hourly recording of data for a continuous 24 h period was chosen for 10 months (1 April 2011 to 31 January, 2012). Choice of hourly data logging as opposed to half hourly recording was to lessen battery power loss. Thereafter, the units (ppm) for CO, SO_2 and NO_2 were converted to mg m^{-3} before data analysis (Formula 1). Then, hourly values were grouped into non-working and working h of the 24 h period. The non-working hours are between 7:00 pm and 7:00 am while the working hours are between 7:00 am and 7:00 pm.

$$x\,(\mathrm{mg\,m^{-3}}) = \frac{x\,(\mathrm{ppm}) \times \mathrm{Molar\ mass\ (g\,mol^{-1})} \times 1000\ \mathrm{mg\,g^{-1}}}{\mathrm{Molar\ volume\ (L\,mol^{-1})} \times 1000\ \mathrm{m^3\,L^{-1}}} \quad (1)$$

Statistical analysis

Data analysis used IBM® SPSS® statistics version 20 coupled with SigmaPlot 12.5 (http://www.sigmaplot.com/) for graphical illustrations. SPSS is a versatile statistical package used for statistical data analysis in many scientific and medical studies.

The SPSS base software includes descriptive statistics, parametric and non-parametric tests, linear regression and multivariate statistics (IBM, http://www-01.ibm.com/software/analytics/spss/). In this study, outliers and extreme values were removed using through box plot method. One sample t-test was used to compare CO, SO_2 and NO_2 mean values with national standards for air pollutants (MSB, 2005). Moreover, independent samples t-test compared diurnal and seasonal mean values while ANOVA assisted in understanding hourly and monthly variations amongst the dataset.

Multivariate statistics identified the underlying factors attributing to variations in CO, SO_2 and NO_2. The multivariate tools selected for this analysis were hierarchical cluster analysis (HCA) and factor analysis (FA). HCA and FA are quantitative and independent approaches used to classify months and making of correlations amongst variables, respectively. The goal of HCA was to examine and classify months based on distribution of the dataset. The Squared Euclidian distance

was used as a similarity/dissimilarity measure, while Ward's linkage method linked the clusters (Yidana, 2010; Hair et al., 2011). Raw data was standardized by converting each variable to standard scores by subtracting the mean and dividing by the standard deviation for each variable (Hair et al., 2011).

The FA technique was considered useful in this study to identify underlying variables that explain the pattern of correlations within the dataset. Principal component analysis (PCA) was the extraction method employed while the rotation method was Varimax with Kaiser Normalization (Hair et al., 2011).

RESULTS AND DISCUSSION

Levels of CO, NO_2 and SO_2

Table 4 presents results obtained between April 2011 and January 2012. The averages are direct computations from hourly recorded data over a 24 h continuous period. A one sample t-test compared the results with national air quality standards (MSB, 2005). With this test the mean value of CO (2.47 mg m^{-3}) was significantly lower than the threshold limit value (Table 4). Similar CO observations were made in an earlier performed in the main highway (Mapoma et al., 2013) near the experimental site (Figure 1). However, the current study's sampling and experimental design were different from that of Mapoma et al. (2013). The earlier study's sampling position was at 3 m elevation (ground surface reference) as compared to 25 m above ground. Conversely, the test results for SO_2 (4.02 mg m^{-3}) and NO_2 (8.58 mg m^{-3}) were significantly higher than standard limit values averaged over a 1 h period (Table 4). Such higher values are detrimental to infrastructure and a health hazard to human beings.

The recorded microclimatic variables show that November had the highest recorded air temperature over the experimental site while the lowest mean monthly air temperature was recorded in July (Table 4). On the contrary, the highest mean value for wind speed was in August and the lowest computed for June and December (Table 4).

The concentration of NO_2 and SO_2 were relatively highest in April 2011 and January 2012 (Table 4). The lowest concentrations for NO_2 and SO_2 were observed in August. Across the entire year, it shows that the month of August is the turning point since it is the month of the lowest recorded average NO_2 and SO_2 concentration.

Concentration of CO was highly variable with highest levels in April 2011 and January 2012 (Table 4). Thus, differences in local weather contributed to the observed trends with wind direction and air temperature as main factors (Figures 2 and 3, respectively).

The study scope concentrated on three pollutants due to lack of sensors to detect more pollutants. Besides, reliance on battery mains that requires constant checking and recharging has effect on continuity of data collection unless constant checking is employed.

Variances

Hourly and diurnal variations

The mean value of air temperature for the working hours was 21.7°C (standard deviation, SD = 4.05) while that of non-working hours was 18.4°C (SD = 3.51). This is an obvious characteristic considering daily sunlight energy routine. Even though, wind speed varied on hourly basis ($F = 4.077$, $p < 0.05$), the mean value for working hours (WS_{mean} = 4.88 mg m^{-3}; SD = 2.04) did not differ from that of non-working hours (WS_{mean} = 4.87 mg m^{-3}; SD = 2.10) as compared to when using independent t-test ($p < 0.05$).

The study noted remarkable hourly variations of CO, SO_2 and NO_2 characterized by high values during the day time as compared to night time (Figure 4). ANOVA results show significant variations in hourly values over the entire study period for CO ($F = 32.049$, $p < 0.05$), SO_2 ($F = 9.488$, $p < 0.05$) and NO_2 ($F = 12.709$, $p < 0.05$). Thus, indicating significant influence of human activities and variations in weather (Elminir, 2002). Independent samples t-test showed that working hours mean values of CO (2.81 mg m^{-3}), SO_2 (4.37 mg m^{-3}) and NO_2 (9.10 mg m^{-3}) were significantly higher ($p < 0.05$) than those of non-working hours mean values of CO (2.13 mg m^{-3}), SO_2 (3.66 mg m^{-3}) and NO_2 (8.06 mg m^{-3}). More so, the computed standard deviations (SD) for working hour values of CO (1.28), SO_2 (2.47) and NO_2 (2.86) were higher than those of non-working hours CO (1.08), SO_2 (2.42) and NO_2 (2.81) suggesting that working hour values varied more than non-working hour values (Figure 4). This suggests that these pollutants are a result of human activities. During working hours (day time) human activities are more than at night. Transportation in Blantyre city is more active during the day. The same can be said of heavy traffic in the main roads such as the Chipembere highway and industrial activities near the experimental site. Based on variations in air temperature (Figure 3) as compared to CO, SO_2 and NO_2 variations (Figure 4), much of the variations can be attributed to temperature changes and may be the persistent wind direction (Figure 2).

Monthly and seasonal variations

Across the entire study period, CO, SO_2 and NO_2 concentrations varied significantly amongst months ($F = 65.781$, 437.652 and 119.553 respectively; $p < 0.05$). Also, the variations in mean air temperature and wind speed across amongst months for the study period were significant ($F = 281.124$ and 53.977, respectively; $p < 0.05$). As mentioned earlier, the variation in weather for each month may contribute to the observed differences. A post hoc pair wise analysis implemented with least square deviations (LSD) showed some pairs of months

Table 4. Summary of results of the entire study period aggregated into minimum and maximum monthly values.

Month		Air temperature (°C)	WS (m s^{-1})	CO (mg m^{-3})	SO_2 (mg m^{-3})	NO_2 (mg m^{-3})
April 11	Min	14.3	0.0	1.15	3.76	0.00
	Max	29.8	10.7	8.02	18.82	20.96
	Mean	20.4	4.5	3.04	7.91	11.26
	Std. Dev	2.7	1.9	1.12	3.32	5.07
May 11	Min	13.6	0.0	0.00	0.00	2.62
	Max	28.0	10.5	3.44	9.41	15.72
	Mean	20.2	4.4	2.32	3.73	8.49
	Std. Dev	2.6	1.7	0.64	1.15	1.37
June 11	Min	10.0	0.0	0.00	0.00	0.00
	Max	27.0	11.0	4.58	9.41	15.72
	Mean	18.0	4.1	1.95	3.83	8.67
	Std. Dev	3.6	2.6	0.92	1.69	1.76
July 11	Min	10.1	0.0	0.00	0.00	0.00
	Max	26.9	10.7	12.60	11.29	15.72
	Mean	17.0	4.7	2.81	3.18	7.77
	Std. Dev	3.7	2.2	2.15	1.45	1.75
August 11	Min	10.3	0.0	0.00	0.00	0.00
	Max	27.1	11.0	4.58	16.93	20.96
	Mean	17.0	5.8	2.02	2.89	7.67
	Std. Dev	4.0	2.3	1.07	1.63	1.93
September 11	Min	10.3	0.0	0.00	0.00	0.00
	Max	30.1	11.0	6.87	9.41	18.34
	Mean	20.9	5.2	2.32	3.28	7.80
	Std. Dev	3.2	1.8	0.75	1.28	1.88
October 11	Min	10.5	0.0	0.00	0.00	0.00
	Max	34.8	11.0	6.87	11.29	15.72
	Mean	22.5	5.4	2.52	3.61	8.41
	Std. Dev	5.0	2.1	0.99	1.84	2.32
November 11	Min	11.9	0.2	0.00	0.00	0.00
	Max	34.7	11.0	4.58	9.41	15.72
	Mean	23.5	5.5	2.66	3.31	8.68
	Std. Dev	3.9	2.3	0.91	1.60	2.56
December 11	Min	10.2	0.0	0.00	0.00	0.00
	Max	32.5	10.8	9.16	18.82	20.96
	Mean	21.5	4.4	2.39	3.59	8.03
	Std. Dev	3.1	1.7	0.85	1.53	2.32
January 12	Min	12.3	0.0	0.00	0.00	0.00
	Max	27.7	10.4	11.46	18.82	20.96
	Mean	20.7	4.6	3.10	6.60	10.18
	Std. Dev	2.5	2.4	2.28	4.52	5.27
	Overall mean	20.1	4.9	2.47	4.02	8.58
	MSB			10.31	0.21	0.23
	p-value			< 0.05	< 0.05	< 0.05

Min = Minimum, Max = maximum, Std. Dev = standard deviation, MSB = Malawi standards (MSB 2005), p-value = significance level for a one sample t-test.

that had similar results. The paired mean CO values that were not significantly different were for pairs of April/January, May/September, May/December, September/December and June/August ($p > 0.05$). The

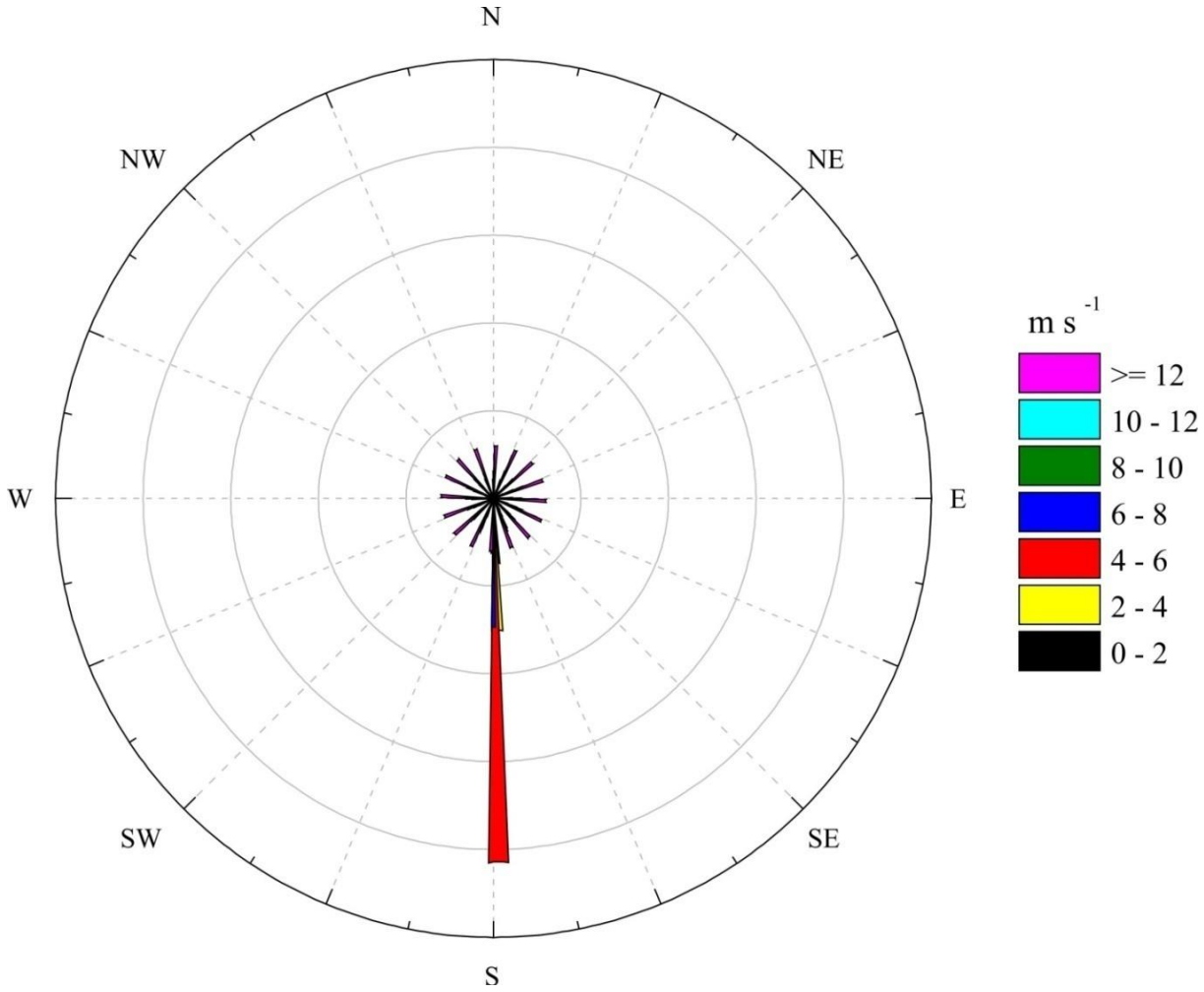

Figure 2. Wind direction in Blantyre illustrating the prevailing southerly winds consistent with a wind speed of 4-6 m s^{-1}.

months of May, June, October and December SO_2 mean values did not significantly differ ($p > 0.05$) as was the case for the months of July, September, November paired against each other. Similar observations were noted for NO_2 results for two groups (May, June, October, November) and (July, August, September, December) when paired against each other within the group ($p > 0.05$).

As indicated earlier, there were significant diurnal variations ($p < 0.05$) on a daily basis. As such, the same pattern showed up in seasonal diurnal variations except for wind speed (Figure 5). Furthermore, independent t-test showed that there were significant differences ($p < 0.05$) between wet season mean values (CO = 2.73 mg m^{-3}; SO_2 = 5.10 mg m^{-3}; NO_2 = 9.41 mg m^{-3}) and dry season mean values (CO = 2.32 mg m^{-3}; SO_2 = 3.42 mg m^{-3}; NO_2 = 8.13 mg m^{-3}). Similarly, there were significant differences between dry and wet season mean air temperature and wind speed ($p < 0.05$). Higher air temperature values were noted in wet season (21.7°C) as compared to dry season (19.3°C). But, higher wind speed values occurred in dry season (4.94 m m^{-1}) as compared to wet season (4.77 m m^{-1}) (Figure 3).

Temperature is a driving force in chemical reactions while lower wind speed may promote buildup of chemicals in the atmosphere since there will be less dispersive force to dilution effect. Thus, pollutants will readily react to form new compounds in ambient air such as (Bailey et al., 2005):

$NO_2 + O_2 \rightarrow NO_3$ (dry deposition) (2a)

$NO_3^- + H_2O \rightarrow HNO_3 + OH^-$ (wet deposition) (2b)

$CO + 2O_2 + h\nu \rightarrow CO_2 + O_3$ (fast process) (3)

$SO_2 + O_2 \rightarrow SO_3$ (slow process) (4a)

$SO_3 + H_2O \rightarrow H_2SO_4$ (4b)

During wet season, the humid atmosphere and high air temperature may promote photochemical reactions of

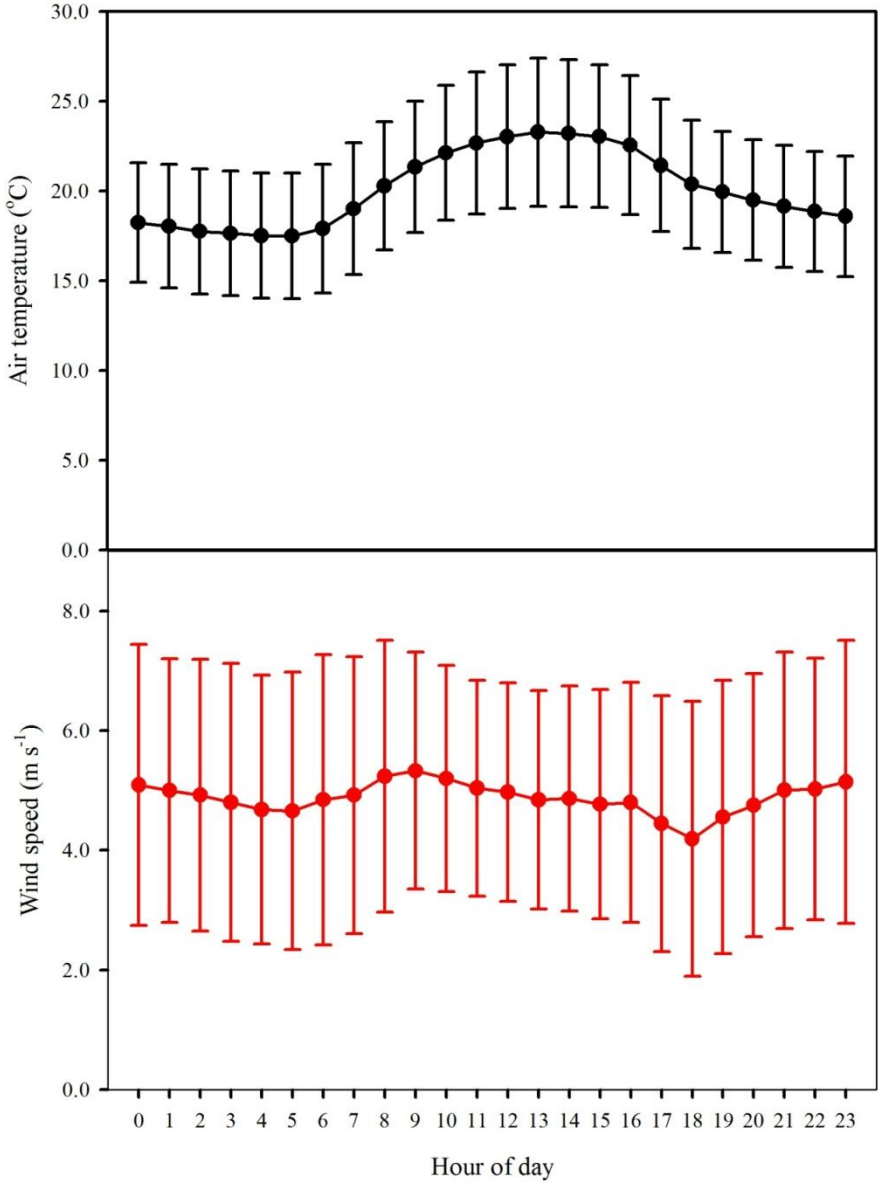

Figure 3. Hourly variations of air temperature and wind speed recorded using the EMS. The graphs indicate mean values where error bars represent standard deviation of mean.

NO_2 and SO_4 to form their acidic products leading to attenuation of the pollutant. For instance, in fog or cloud, SO_2 reacts with water to form sulfurous acid (H_2SO_3) followed by oxidation to form H_2SO_4, a similar outcomeof Equation 4 (Bailey et al., 2005). Yet, such products are caustic and may destroy natural and man-made infrastructure. However, low wind speed and maybe consistent wind direction lead to reduced air pollutant dilution. Also, maybe the fact that higher temperatures leads to air from lower level to rise, increasing pollutant concentration in air at the level of the EMS. As such, effective recording of emissions from vehicles and motor cycles by the instrument is increased. CO reacts in air to form CO_2 (Equation 3). Due to CO having a lower residence time than SO_2 and NO_2, the formation of CO_2 reduces the concentration in ambient air way below national threshold values in Blantyre.

Multivariate analysis

Hierarchical cluster analysis (HCA) revealed natural

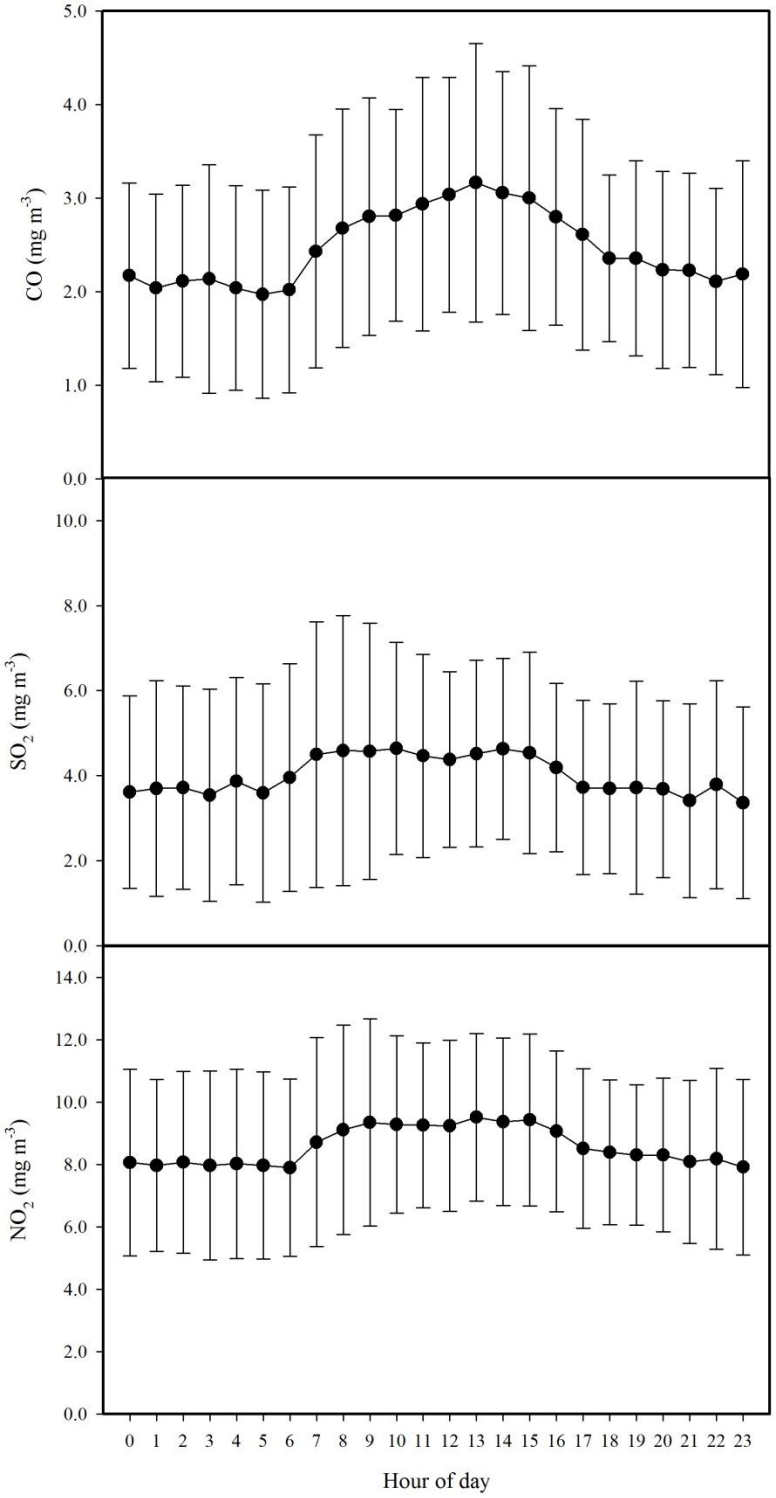

Figure 4. Hourly variations of CO, SO_2 and NO_2 recorded using the EMS. The graphs indicate mean values where error bars represent standard deviation of mean.

groupings of months within the study period. After drawing the phenon line at rescaled distance of 7.5, three clusters emerged. The choice of phenon line is based on semi-objective inspection of the dendrogram (Figure 6).

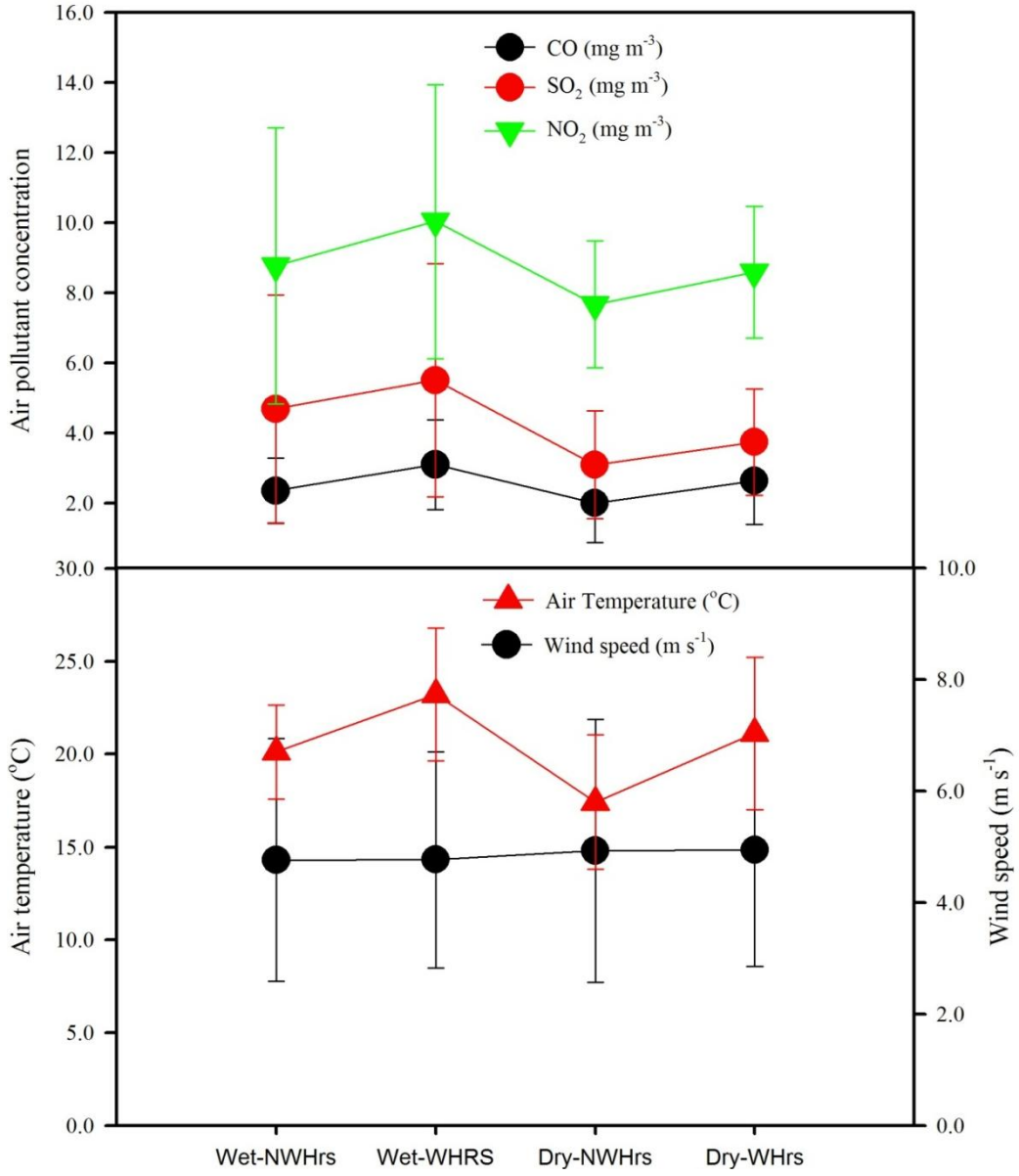

Figure 5. Diurnal and seasonal variations of air pollutants and microclimatic variables for the entire study period. The error bars represent standard deviations of mean. The labels in the x-axis represent wet season non-working hours, wet season working hours, dry season non-working hours and dry season working hours respectively.

Cluster I consists of August 2011, September 2011, October 2011 and November 2011. Cluster II is a group of May 2011, June 2011, July 2011 and December 2011 while Cluster III is a pair of April 2011 and January 2012.

The first cluster consists of months belonging to the dry warm season. The second cluster is dry cool and sometimes humid season except December which belongs to the warm wet season. The last cluster (April and January) is a pair of months in the wet season. Thus the first cluster describes months with lower air temperature, high wind speed and relatively lower CO, SO_2 and NO_2. The last cluster (April and January) describes months having the highest concentrations of CO, SO_2 and NO_2 (Table 4).

Furthermore, FA's principal component analysis (PCA) explained the impact of microclimatic variables on CO, SO_2 and NO_2. Based on significant Eigen values (Hair et al., 2011), PCA yielded two factors otherwise referred to as principal components (PCs) in this case. The two factors or PCs explained 52.3% of the total variance. The

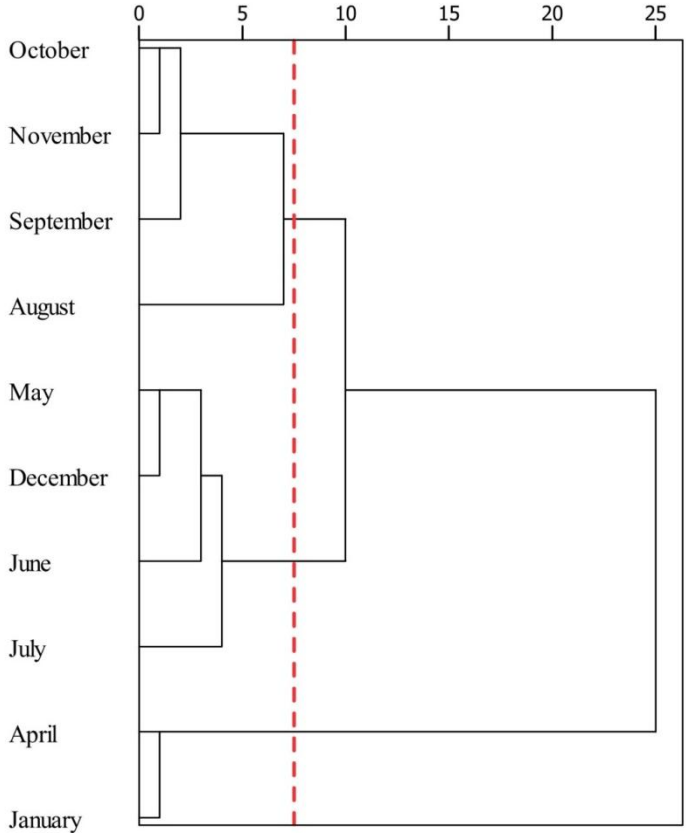

Figure 6. A dendogram produced from hierarchical cluster analysis. The phenon line (red) was drawn at a rescaled distance of 7.5 to identify monthly clusters based on distribution of CO, SO_2, NO_2, air temperature, wind speed and wind direction.

Table 5. Factor analysis' rotated component matrix showing significant factor loadings (principal components, PCs)[a].

Parameter	PC1	PC2
Air temperature		0.714
Wind direction		0.634
CO	0.669	-0.103
NO_2	0.933	
SO_2	0.940	
Wind speed		-0.498
Eigenvalue	2.039	1.099
Variance explained (%)	33.99	18.32

[a]Extraction method was principal component analysis and rotation method being Varimax with Kaiser normalization.

first factor accounted for 40.6% of the total variance. From the rotated component matrix (Table 5), PC1 explained the positive relationship amongst the three air pollutants (CO, SO_2 and NO_2) suggesting their co-occurrence in air in the vicinity of the experimental site.

The second PC identifies the positive high loading of air temperature, wind direction and negative loading of wind speed (Table 5). In this PC, air temperature has a negative relationship with wind speed. Furthermore, a significant positive loading of CO is explained in PC2. The negative influence of air temperature on CO is shown and so is the positive impact of wind speed on CO. The rotated component in space (Figure 7) illustrates the relationship amongst the variables where mostly air temperature shows a strong influence on CO, SO_2 and NO_2 as compared to wind speed and wind direction. From the correlation matrix (Table 6), there is a strong correlation between SO_2 and NO_2 ($p = 0.611$) which suggests similar sources of SO_2 and NO_2.

Besides vehicles, industrial activities closer to the experimental site such as milk production, fertilizer manufacturing, animal slaughter company and matches making that may involve burning of fossil fuels as well as sulfur and nitrogen ingredients or products are emission sources. Moreover, motor vehicles contribute large

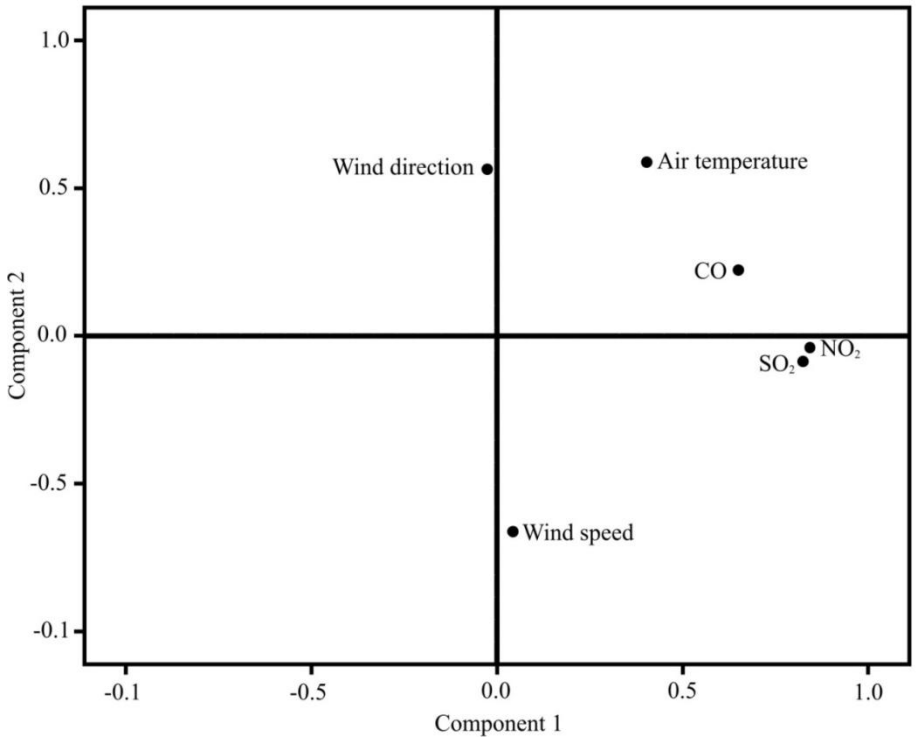

Figure 7. Rotated component graph showing relationship of variables in space.

Table 6. Correlation matrix of initial solutions showing coefficients and significance levels.

Parameter		Air temperature	WD	CO	NO_2	SO_2	WS
Correlation coefficients	Air temperature	1.000					
	WD	0.123	1.000				
	CO	-0.072	0.010	1.000			
	NO_2	0.006	0.022	**0.414**	1.000		
	SO_2	0.012	0.013	**0.438**	**0.900**	1.000	
	WS	-0.077	-0.047	0.011	-0.030	-0.012	1.000
P-value	Air temperature						
	WD	0.000					
	CO	0.000	0.236				
	NO_2	0.333	0.057	**0.000**			
	SO_2	0.192	0.171	**0.000**	**0.000**		
	WS	0.000	0.000	0.206	0.015	0.198	

WD = Wind speed, WS = wind direction.

quantities of atmospheric NO_2 pollutant in cities as compared to other sources (Makra et al., 2010). As such, most of the NO_2 detected may be from vehicular sources. Considering the low residence time of CO, we can conclude that the main source is vehicular emissions in the main highway (Mapoma et al., 2013) as opposed to industrial activities.

With the observed relationships between CO, SO_2 and NO_2 on one hand and micro climatic variables on the other, various remarks can be made from the results. Effect of wind speed and wet periods can be explained as: most of the time wind is calmer at night than day time, leading to a relatively stable atmosphere at night (Elminir, 2002). The stable atmosphere hinders mixing of air

leading to reduced concentrations of CO, SO_2 and NO_2 to rise to 25 m. Moreover, transport effects due to increased wind speed in dry season give an explanation for the dilution and clearing of the local air (Elminir, 2002). This explains in part the low concentrations of CO, SO_2 and NO_2 recorded at the experimental site at night and in dry season coupled with lower air temperature. Effect of changes in wind direction is significant on hourly variations in CO, SO_2 and NO_2 while consistent southerly winds over the entire study period (Figure 2) show that wind direction effect is not critical in explaining the seasonal variations.

Conclusions and recommendations

The observed CO level (2.47 ± 1.23 mg m^{-3}) fell below the Malawian limit value of 10.31 mg m^{-3}. But, NO_2 (4.02 ± 2.47 mg m^{-3}) and SO_2 (8.58 ± 2.88 mg m^{-3}) were significantly higher than allowable Malawian Standards (0.52 and 0.23 mg m^{-3}, respectively). Such higher values are detrimental to infrastructure and are a health hazard to human beings. The variations in hourly, diurnal, monthly and seasonal CO, SO_2 and NO_2 signify the important contributions of industrial and transportation activities in the city. Variations in vehicle traffic during the day (peak hour as compared to non peak hours), coincides with variations in emission levels. Independent t-test showed that wet season CO (2.32 mg m^{-3}), SO_2 (5.10 mg m^{-3}) and NO_2 (9.41 mg m^{-3}) levels were higher than dry season values (CO = 2.32 mg m^{-3}; SO_2 = 3.42 mg m^{-3}; NO_2 = 8.13 mg m^{-3}). Factor analysis' (FA) showed that air temperature had significant contribution to variations in mean values of CO, SO_2 and NO_2. Based on results, the study shows a need for constant urban air quality monitoring in Blantyre and urban cities in Malawi. It is recommended that the experimental site widen the scope of the study by utilizing the flexibility of the EMS.

Conflict of Interests

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

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Full Length Research Paper

Determination of optimum growth conditions and biodiesel production from filamentous algae

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Petroleum diesel combustion is a major source of greenhouse gas (GHG). It is also a major source of other air contaminants including NOx, SOx, CO and volatile organic compounds. Algae have emerged as one of the most promising sources for biodiesel production. In this study, a higher algae growth rate was observed in the experiments with excess Na_2SiO_4, trace metals, Na_2EDTA and excess vitamin solution, the increase was above 300%. It was also observed that the experiment that was supplied with CO_2 (without simultaneous sunlight exposure) for one hour, for 25 days and the beaker with excess NaH_2PO_4 solution, have shown a slower growth rate than the control. The results of the experiment on the effect of sunlight exposure for certain times daily for 25 days show that the growth rate is directly proportional to increase of sunlight exposure time (during the 90 min). The results of the experiment on the effect of simultaneous exposure to sunlight and CO_2 for certain times daily, for 25 days, show that the growth rate is directly proportional to the increase of sunlight and CO_2 exposure time.

Key words: Algal oil, biodiesel, transesterification, glycerine.

INTRODUCTION

The need of energy is increasing continuously due to the increase in population and industrialization. The continued use of petroleum sourced fuels is now widely recognized as unsustainable because of the depletion of supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment leading to increase of global warming. The combustion of fossil fuels is responsible for 73% of the CO_2 production (Narendra et al., 2010). With regards to global warming and as dependence on fossil fuels grows, the search for renewable energy sources that reduce CO_2 emissions becomes a matter of widespread attention (Ragauskas et al., 2006; Demirbas and Demirbas, 2007). In recent years, cultivation of microalgae has received renewed attention on account of their utility as a feasible CO_2 sequestration technology (Ono and Cuello, 2006; Hsueh et al., 2007; Jacob-Lopes et al., 2008).

In the last ten years, many studies have been conducted on biofuels for substituting fossil fuels and reduce the greenhouse gas emission (Bastianoni et al., 2008). Algae, especially microalgae, were found to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels (Chisti, 2007, 2008).

The idea of using algae as a source of fuel is not new (Chisti, 1980; Chisti 1981; Nagle and Lemke, 1990;

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Sawayama et al., 1995), but it is now being taken seriously because of the increasing price of petroleum and more significantly, the emerging concern about global warming that is associated with burning fossil fuels (Gavrilescu and Chisti, 2005). Microalgae can provide several types of renewable biofuels which include, methane, biodiesel (methyl esters) and biohydrogen (Gavrilescu and Chisti, 2005; Kapdan and Kargi, 2006; Spolaore et al., 2006). Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops (Shay, 1993).

Bioenergy is one of the most important components to mitigate greenhouse gas emissions and substitute of fossil fuels (Goldemberg, 2000). Petroleum diesel combustion is a major source of greenhouse gas (GHG). Apart from these emissions, petroleum diesel is also a major source of other air contaminants including NOx, SOx, CO, particulate matter and volatile organic compounds (Klass, 1998). Biomass is one of the better sources of energy (Kulkarni and Dalai, 2006). Large-scale introduction of biomass energy could contribute to sustainable development on several fronts, environmentally, socially and economically (Turkenburg, 2000; UNDP, 2008). Biodiesel is a nontoxic and biodegradable alternative fuel that is obtained from renewable sources. It is reported that algae were one of the best sources of biodiesel and are the highest yielding feedstock for biodiesel. It can produce up to 250 times the amount of oil per acre from soybeans (Hossain and Salleh, 2008). In fact, producing biodiesel from algae may be the only way to produce enough automotive fuel to replace current gasoline usage. The best algae for biodiesel would be microalgae. Microalgae have much more oil than macroalgae and it is much faster and easier to grow (Shay, 1993).

Algae contain anything between 2 and 40% of lipids/oils by weight (Wagner, 2007). Microalgae have much faster growth-rates than terrestrial crops. The per unit area yield of oil from algae is estimated to be between 18,927 and 75,708 L per acre, per year; this is 7 to 31 times greater than the next best crop, palm oil (around 2,404 L) (Wagner, 2007). In addition, the question “Food or Fuel?” is not raised.

Advantages of microalgal cultivation for biodiesel production over other oleaginous crops are: the former is not only easy to handle and more beneficial from economic point of view but also known for their bioremediation capabilities (Dayananda et al., 2005; Sazdanoff, 2006; Chisti, 2007; Huntley and Redalje, 2007; Li et al., 2008; Schenk et al., 2008; Tan et al., 2009). Some positive points of microalgal cultivation are: rapid growth rates, a high per-acre yield (7 to 31 times greater than the next best crop- palm oil), certain species of algae can be harvested daily, short life cycle (approximately 1 - 10 days), ability to synthesize and accumulate large quantities of lipids per dry weight biomass, algae biofuel contains no sulphur, algae biofuel is non-toxic, algae biofuel is highly bio-degradable, and algae consume carbon dioxide as they grow, so they could be used to capture CO_2 from power stations and other industrial plants that would otherwise go into the atmosphere, potential to grow in saline water and harsh conditions, less fertilizer and nutrient input requirements and it is the most promising non-food source of biodiesel. After extracting oil from microalgae, the remaining biomass portion can also be used as a high protein feed
for livestock (Schneider, 2006; Haag, 2007).

The algae used in this study is filamentous algae also known as "pond moss" or "pond scum" and these threadlike algae often occur in huge greenish masses floating upon the waters' surface. They can form dense mats in static water or long, rope-like strands in flowing water. Its filaments consist of series of cells being joined end to end giving a thread-like appearance.

The main aim of the research was to extract oil from a microalgae species and convert it to biodiesel and to determine the optimum growth conditions of the used algae species.

MATERIALS AND METHODS

Stock solutions preparation

The stock solutions were prepared according to the procedures of Guillard and Ryther (1962).

Filamentous algae cultivation

Cultivation of algae in a synthetic medium

The medium was prepared by adding 3.00 ml of $NaNO_3$ solution (150.008 g in 1 L distilled water), 3.00 ml of trace metal solution, 3.00 ml of $NaSiO_3.5H_2O$ solution (10 ml in 1 L distilled water), 3.00 ml of iron citrate solution (9.0008 g $FeCl_3$ and 9.00 g citric acid in 1 L distilled water), 3.00 ml of vitamin solution (Folic acid 0.015 g and 0.0156 g peptone bacteriological in 100 mL distilled water), 1.50 ml of NaH_2PO_4 (11.31 g in 1 L distilled water) and 1.50 ml of $Na_2EDTA.2H_2O$ solution, to a beaker containing 3000 ml of water and 19.62 g of filamentous algae was added.

Optimization of growth condition

The effects of different components concentration of the stock solution on the algae growth (Table 1)

One milliliter of $NaNO_3$ solution, 1.00 ml of trace metal solution, 1.00 ml of $NaSiO_3.5H_2O$ solution, 1.00 ml of iron citrate solution, 1.00 ml of vitamin solution, 0.50 ml of NaH_2PO_4 solution and 0.50 ml of $Na_2EDTA.2H_2O$ solution were added to each of nine beakers filled with 100.00 ml tap water. In the first beaker, 1.00 ml of $NaNO_3$ solution was added in excess, the second beaker, 1.00 ml of trace metal solution was added in excess, the third beaker, 1.00 ml of $NaSiO_3.5H_2O$ solution was added in excess, the fourth beaker 1.00 ml of iron citrate solution was added in excess, to the fifth beaker, 1.00 ml of vitamin solution was added in excess, the sixth beaker, 0.50 ml of NaH_2PO_4 solution was added in excess, the seventh beaker, 0.50 ml of $Na_2EDTA.2H_2O$ solution was added in excess, to

Table 1. The effect of different components of concentration of stock solution on the algae growth.

Stock solution in excess 1.00 ml	Initial mass of algae (g)	Mass of algae after 15 days (g)	Increase in mass (%)
$NaNO_3$	1.00	3.49	249 ↑
Trace metals	1.00	4.47	347 ↑↑
Na_2SiO_4	1.00	4.68	368 ↑↑
Iron citrate	1.00	3.74	274 ↑
Vitamin	1.00	4.04	304 ↑↑
NaH_2PO_4	1.0020	2.75	175 ↓
Na_2EDTA	1.00	4.29	329 ↑↑
CO_2	1.00	2.00	100 ↓
Control	1.00	2.86	186 –

Table 2. Results of the effect of sunlight exposure on growth after 25 days.

Sample	30 min sunlight daily	60 min sunlight daily	90 min sunlight daily
Initial mass (g)	1.00	1.00	1.00
Final mass (g)	2.75	2.87	3.01
Increase in mass (%)	175	187	201

Table 3. The effect of simultaneous CO_2 supplement and sunlight exposure after 25 days.

Experimental time	30 min		60 min		90 min	
	CO_2 and sunlight	Control (sunlight only)	CO_2 and sunlight	Control (sunlight only)	CO_2 and sunlight	Control (sunlight only)
Initial mass (g)	1.00	1.00	1.00	1.00	1.00	1.00
Final mass (g)	4.04	2.75	4.72	2.87	4.82	3.01
Increase in mass %	304	175	372	187	382	201

the eighth beaker, CO_2 was supplied for one hour daily, while the ninth was used as a control, no excess solution was added. One gram of algae was added to each beaker and CO_2 was supplied to each for 10 min.

The effects of sunlight exposure time on the algae growth (Table 2)

Three 250.00 ml beakers were filled with 200.00 ml of water and 1.00 ml of $NaNO_3$ solution, 1.00 ml of trace metal solution, 1.00 ml of $NaSiO_3.5H_2O$ solution, 1.00 ml of iron citrate solution, 1.00 ml ofvitamin solution, 0.50 ml of NaH_2PO_4 and 0.50 ml of $Na_2EDTA.2H_2O$ solutions were added, to each beaker. One gram of algae was added to each beaker and CO_2 was supplied to each for 10 min. The algae were exposed to sunlight at different times, for 30, 60 and 90 min, respectively. The growth rate in each beaker was observed.

The effect of simultaneous CO_2 supplement and sunlight exposure time (Table 3)

Three 250.00 ml beakers were filled with 200.00 ml of water and 1.00 ml of $NaNO_3$ solution, 1.00 ml of trace metal solution, 1.00 ml of $NaSiO_3.5H_2O$ solution, 1.00 ml of iron citrate solution, 1.00 ml of vitamin solutions and 0.50 ml of NaH_2PO_4 and $Na_2EDTA.2H_2O$ solution were added to each beaker.

One gram of algae was added to the beakers. The algae were supplied with CO_2 supplement and exposed to the sunlight simultaneously, for 30, 60 and 90 min, respectively. The growth rate in each beaker was observed.

The effect of available space on the growth rate

Filamentous algae, 34.33 g were collected from a pond and cultivated in two fish tanks. They were filled with 10.00 L of water, 20.00 ml of $NaNO_3$ solution, 20.00 ml of trace metal solution, 20.00 ml of $NaSiO_3.5H_2O$ solution, 20.00 ml of iron citrate solution, 20.00 ml of vitamin solution, 5.00 ml NaH_2PO_4 solution and 10.00 ml of $Na_2EDTA.2H_2O$ solution. To one tank, 22.88 g of algae were added and 11.45 g of algae was added to the second tank. The algae were exposed to sunlight and CO_2 supplement was bubbled into each tank for 90 min daily.

Extraction process

Harvesting

Ninety percent of the algae were collected using a fish net after 10 days of cultivation. The wet algae weighed 56.50 g and they were ground with a pestle in a mortar for 20 min. The ground algae were dried in an oven for 75 min at 80°C to release water. The dried algae weighed 26.85 g.

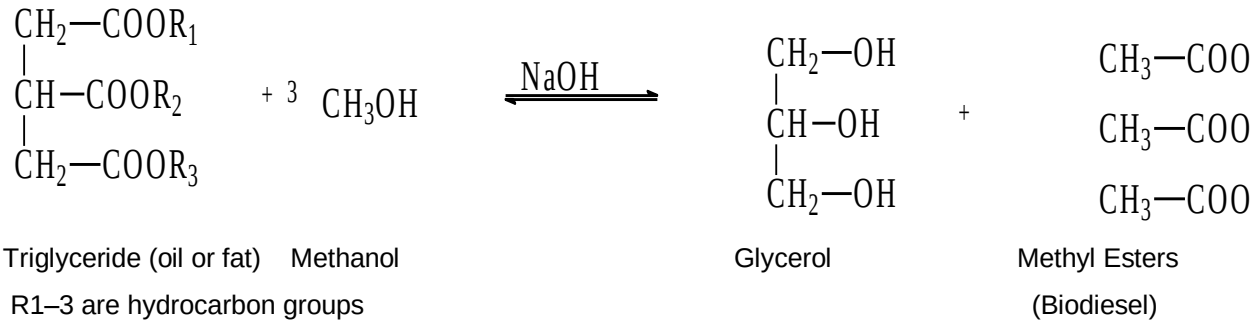

Figure 1. Transesterification reaction to produce biodiesel.

Drying time and temperature

Extraction was done several times on the filamentous algae at different drying times and temperatures in trials to find the best drying time and the optimum temperature. It was found that the best drying time was 75 min at 80°C.

Oil extraction

The two methods used are hexane solvent extraction and soxhlet extraction. The soxhlet extraction did not yield satisfactory results. The hexane solvent extraction used 20.00 ml of hexane and 20.00 ml diethyl ether which were mixed with the dried algae to extract the oil. The mixture was allowed to settle for 24 h. The biomass was collected by gravity filtration and weighed 25.58 g. The extracted oil was collected after filtration. The hexane and the diethyl ether were evaporated using a rotary evaporator.

Transesterification (Figure 1)

Algal oil (0.80 ml) was mixed with sodium methoxide which was prepared by dissolving 0.0122 g of NaOH in 1.20 ml of methanol. The mixture was shaken for one hour, and thereafter transferred to a separatory funnel and allowed to settle for 12 h.

In the separatory funnel, the layers were clearly formed; biodiesel on top and glycerol at the bottom. The bottom layer was drained into a vial and stored. The biodiesel was washed three times with water. The obtained biodiesel was heated at 54°C for 20 min to evaporate all the residual water. Approximately 0.20 ml of biodiesel was obtained and it was then stored in a vial. The extraction and the transesterification processes were repeated several times using the method described by Hossain and Salleh (2008).

RESULTS

Cultivation

The effects of different components of stock solution concentration on the algae growth

A higher growth rate was observed in the beakers with excess Na_2SiO_4, trace metals, Na_2EDTA and excess vitamin solution, the increase was above 300%. It was also observed that the beaker that was supplied with CO_2 (without simultaneous sunlight exposure) for one hour and the beaker with excess NaH_2PO_4 solution showed a slower growth rate than the control.

The effect of the sunlight exposure time, for 25 days, on algae growth rate

The results of the experiment studying the effect of sunlight exposure for certain times daily for 25 days, showed that the growth rate is directly proportional to increase of sunlight exposure time (for 90 min).

The effect of CO_2 supplement and sunlight exposure simultaneously on the growth after 25 days

The results of the experiment on the effect of simultaneous exposure to sunlight and CO_2 for certain times daily, for 25 days, showed that the growth rate is directly proportional to the increase of sunlight and CO_2 exposure time.

The effect of available space on the growth rate

The growth rate in the tank which containing 11.45 g algae was 2.9% after 10 days, while the growth rate in the tank which contained 22.88 g algae was 2.6% after 10 days and under the same experimental conditions. This is in agreement with a different study done by Campbell (2008).

Extraction

Filamentous algae extraction and transesterification results

Extraction was done several times on the filamentous algae at different drying times and temperatures in trials to find the best drying time and the optimum temperature. Although, the dry mass was not constant but based on the ratio between the dry mass and the amount of oil produced it was found that the best drying time, in our experiments, was 75 min at 80°C. Algal oil was obtained as shown in Table 4. With the transesterification process, only some of the algal oil was converted to biodiesel.

Table 4. Extraction and transesterification results of filamentous algae oil.

Dry mass (g)	Biomass (g)	Drying time (min)	Amount of algal oil extracted (ml)	Biodiesel produced (ml)
1.23	0.78	60 min at 80°C	Approximately 0.20	No biodiesel produced
26.85	25.58	85 min at 80°C	0.80	Approximately 0.20 ml of biodiesel produced
13.97	12.70	75 min at 80°C	1.30	Approximately 0.5ml of oil produced
31.01	29.93	180 min at 85°C	1.00	No biodiesel produced
18.83	18.52	170 min at 60°C	0.70	No biodiesel produced
21.42	18.94	30 min at 80°C	0.50	No biodiesel extracted
14.77	13.21	20 min at 80°C	0.70	No biodiesel extracted
25.98	21.35	20 min at 80°C	0.50	0.2 ml biodiesel extracted

Some of the oil solidified during the process. Results in Table 4 shows that the best drying time, in our experiments, was 75 min at 80°C.

DISCUSSION

In the experiment investigating the effect of the sunlight exposure time only and the effect of simultaneous CO_2 supplement and sunlight exposure in 25 days, it was observed that the growth rate is directly proportional to the length of sunlight exposure time and CO_2 supplement simultaneously. This may be attributed to enhanced photosynthesis which in turn increase the growth rate. Space plays a role in the growth of algae, the results of the experiment on the effect of available space indicated that the algae that were sparsely populated in the growth tank had a higher growth rate. In the experiment on the effect of different components' concentrations of media solution on the algae growth, it was found that excess of trace metal and silicate solutions have shown the highest growth rates. This is because they play a structural role in the chloroplast membrane, maintains the green colour and assist in the breakdown of purine, which is in accordance with report of Salisbury and Ross (1992).

Conclusion

The experiment on the effect of different nutrients on the growth of algae showed a higher growth rate in beakers with excess Na_2SiO_4, trace metals, Na_2EDTA and excess vitamin solution, the increase was above 300%. It was also observed that in the experiment which was supplied with CO_2 (without simultaneous sunlight exposure) for one hour and the experiment with excess NaH_2PO_4 solution, there was a slower growth rate than that of the control.

The results of the experiment on the effect of sunlight exposure for certain times daily, for 25 days, showed that the growth rate is directly proportional to the increase in sunlight exposure time (within 90 min).

The results of the experiment on the effect of simultaneous exposure to sunlight and CO_2 for certain times daily, for 25 days, showed that the growth rate is directly proportional to the increase of simultaneous sunlight and CO_2 exposure time (within 90 min).

The results of the experiment on the optimum drying time and temperature, showed that the best drying time was 75 min at 80°C. Algal oil was extracted using hexane and diethyl ether in 1:1 ratio. Through transesterification reaction, some of the algal oil was converted to biodiesel.

Conflict of Interests

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

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Review

Marine biotoxins and its detection

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The incidences of intoxication due to the consumption of marine foods have been increasing in recent years. This is due to the presence of biotoxins in foods of marine origin. The biotoxins will be accumulated in the marine foods due to the consumption of toxic biota of marine origin. When this contaminated food is taken by the humans or animals, those toxins will be transferred to them causing intoxication and lethality. Among these intoxications, most of them are caused by the harmful algal blooms (HAB). In order to avoid the harmful effects from marine biotoxins, it is necessary to have the proper knowledge. In this manuscript, the different types of biotoxins, source of intoxication, characteristics of toxins, detection and control measures are discussed in detail.

Key words: Harmful algal blooms, harmful algal blooms (HAB), ciguatara fish poisoning (CFP), paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP) blooming, detection.

INTRODUCTION

Microscopic planktonic algae of the world's oceans are critical food for filter-feeding bivalve shellfish (oysters, mussels, scallops and clams) as well as for the larvae of commercially important crustaceans and fin fish. Over the last several decades, countries throughout the world have experienced an escalating trend in the incidence of "harmful algal blooms" (HABs) (Anderson, 1989; Hallegraeff, 1993). HAB events are characterized by the proliferation and occasional dominance of particular species of toxic or harmful algae. When toxic algae are filtered from the water as food by shellfish, their toxins accumulate in those shellfish to levels that can be lethal to humans or other consumers. Another type of HAB impact occurs when marine fauna are killed by algal species that release toxins and other compounds into the water. HABs include species of microscopic, usually single celled eukaryotic plants that live in estuarine and marine waters. A "bloom" occurs when algae grow very quickly or "bloom" and accumulate into dense visible patches near the surface of the water (National Office for Marine Biotoxins and Harmful Algal Blooms, 1999)

During the past two decades, the frequency, intensity and geographic distribution of harmful algal blooms has increased, along with the number of toxic compounds found in the marine food chain. Different explanations for this trend have been given such as increased scientific awareness of toxic algal species, increased utilization of coastal waters for aquaculture, transfer of shellfish stocks

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from one area to another, cultural eutrophication from domestic, industrial and agricultural wastes, increased mobility of humic substances and trace metals from soil due to deforestation and/or by acid precipitation (acid rain), and unusual climatic conditions (Hallegraeff et al., 1995).

A poorly defined but potentially significant concern relates to sublethal, chronic impacts from toxic HABs that can affect the structure and function of ecosystems. Adult fish can be killed by the millions in a single outbreak, with long- and short-term ecosystem impacts (Okaichi et al., 1989; Kim et al., 1999). Likewise, larval or juvenile stages of fish or other commercially important species can experience mortalities from algal toxins (White et al., 1989). HABs also cause mortalities of wild fish, seabirds, whales, dolphins and other marine animals. Non-toxic blooms of algae can cause harm, often due to the high biomass that some blooms achieve, and the deposition and decay of that biomass, leading to anoxia. Chronic toxin exposure may have long-term consequences that are critical with respect to the sustainability or recovery of natural populations at higher trophic levels (Ramsdell et al., 2005). Only a few of the many thousands of species of algae are associated regularly with toxic or harmful algal blooms (National Office for Marine Biotoxins and Harmful Algal Blooms, 1999). Shellfish poisoning syndromes include Ciguatara fish poisoning (CFP) and paralytic (PSP), diarrhetic (DSP), ciguatara (CFP), neurotoxic (NSP) and amnesic (ASP) shellfish poisoning based on human symptoms.

TOXINS

Ciguatara fish poisoning (CFP)

Ciguatera poisoning in humans and domestic animals is caused by potent neurotoxins produced by benthic dinoflagellates including *Gambierdiscus toxicu*, *Prorocentrum concavum*, *Prorocentrum hoffmannianum*, *Prorocentrum lima*, *Ostreopsis lenticularis*, *Ostreopsis siamensis*, *Coolia monotis*, *Thecadinium* and *Amphidinium carterae.* In the tropics and subtropics toxic dinoflagellates living on coral reefs are eaten by small herbivorous fish grazing on coral which in turn are eaten by larger carnivores. The poisons move up the food chain into the organs of larger top-order predators such as coral trout, red bass, chinaman fish, mackerels and moray eels and cause ciguatera fish poisoning, CFP, in people who eat these fish (Kim, 1999; Klöpper et al., 2003; Leikin, and Paloucek, 1998).

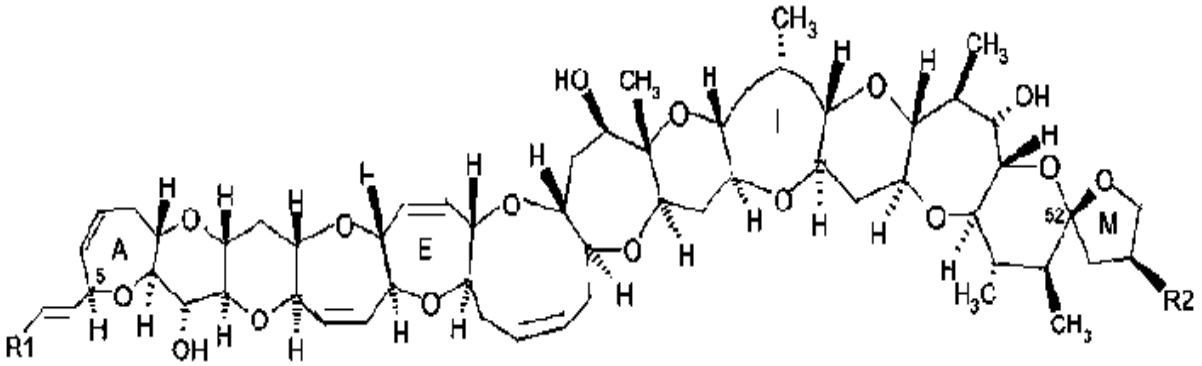
Toxins produced: Ciguatoxin, Maitotoxin

CFP produces gastrointestinal, neurological and cardiovascular symptoms. Generally, diarrhea, vomiting and abdominal pain occur initially, followed by neurological dysfunction including reversal of temperature sensation, muscular aches, dizziness, anxiety, sweating and numbness and tingling of the mouth and digits. Paralysis and death have been documented, but symptoms are usually less severe although debilitating. Recovery time is variable, and may take weeks, months, or years. Rapid treatment (within 24 h) with mannitol reported to relieve some symptoms. There is no antidote, supportive therapy is the rule, and survivors recover. Absolute prevention of intoxication depends upon complete abstinence from eating any tropical reef fish, since there is currently no easy way to measure routinely ciguatoxin or maitotoxin in any seafood product prior to consumption, (Nielsen and Tonseth, 1991; Partensky and Sournia, 1986; Partensky et al., 1988; Partensky et al., 1991; Passow, 1991; Perez et al., 2001; Rafuse et al., 2004; Schnorf et al., 2002; Tangen, 1977; Taylor et al., 1995; Tillmann, 2004).

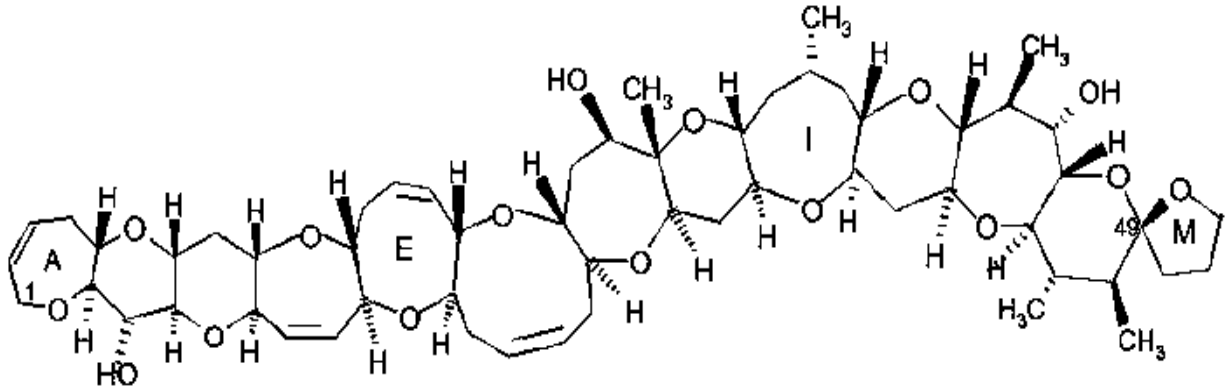
Chemical properties

Ciguatoxins are lipid-soluble polyether compounds consisting of 13 to 14 rings fused by ether linkages into a most rigid ladder-like structure (Figure 1). They are relatively heat-stable molecules that remain toxic after cooking and exposure to mild acidic and basic conditions. Ciguatoxins arise from biotransformation in the fish of precursor gambier toxins (Lehane and Lewis, 2000; Lehane, 2000).

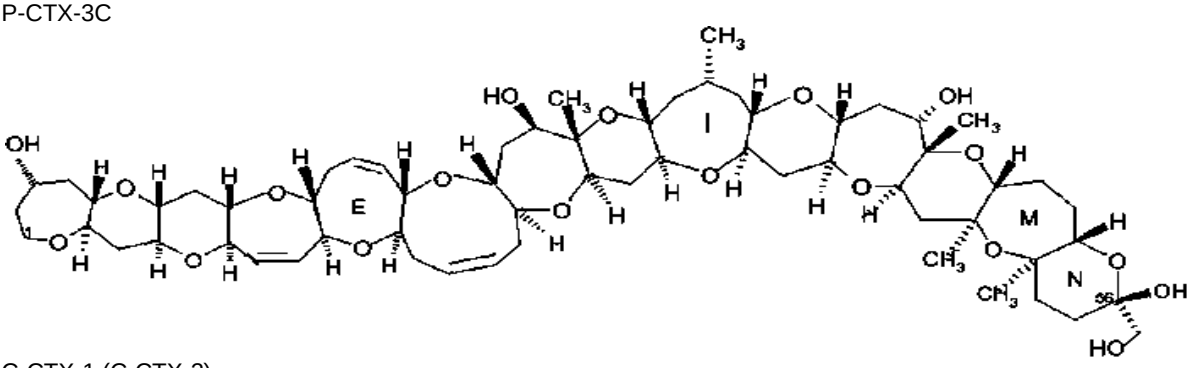
In areas in the Pacific, the principal and most potent ciguatoxin is Pacific ciguatoxin-1 (P-CTX-1, mol. wt. 1112). Its likely precursor is gambiertoxin-4B (GTX-4B). The main ciguatoxins in the Pacific, P-CTX-1, P-CTX-2 and P-CTX-3, are present in fish in different relative amounts (Lehane and Lewis, 2000; Lehane, 2000). Caribbean (and Indian Ocean) ciguatoxins differ from Pacific ciguatoxins. Caribbean CTX-1 (C-CTX-1) is less polar than P-CTX-1. Structures of two Caribbean ciguatoxins (C-CTX-1 and C-CTX-2) were elucidated in 1998. The structures of more than 20 congeners of ciguatoxin were elucidated. Structural modifications were mainly seen in the both termini of the toxin molecules and mostly by oxidation (Naoki et al., 2001; Yasumoto et al., 2000). Multiple forms of ciguatoxin with minor molecular differences and pathogenicity were described. CTX-1 is the major toxin found in carnivorous fish and poses a human health risk at levels above 0.1 µg/kg fish (De Fouw et al., 1999). The energetically less favored epimers, P-CTX-2 (52-epi P-CTX-3), P-CTX-4A (52-epi P-CTX-4B) and C-CTX-2 (56-epi C-CTX-1) are indicated in parenthesis. 2, 3-Dihydroxy P-CTX-3C and 51-hydroxy P-CTX-3C have also been isolated from Pacific fish (Lewis, 2001). Various species of parrotfish have previously been reported to contain a toxin less polar than CTX-1, named scaritoxin. Judging from the reported



	R1	R2
P-CTX-1:	$^{1}CH_2OHCHOH$	OH
P-CTX-3 (P-CTX-2):	$^{1}CH_2OHCHOH$	H
P-CTX-4B (P-CTX-4A);	$^{1}CH_2CH$	H



P-CTX-3C



C-CTX-1 (C-CTX-2)

Figure 1. Structure of Pacific (P) and Caribbean (C) ciguatoxins (CTXs). Source: Yasumoto et al., 2000 and Lewis, 2001.

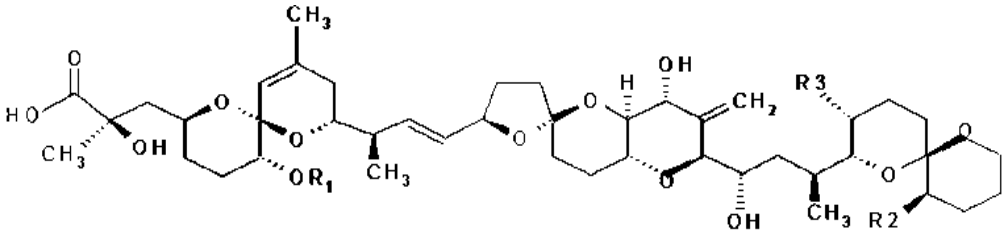
chromatographic properties, scaritoxin seems to correspond to a mixture of CTX-4A and CTX-4B (De Fouw et al., 1999).

Diarrhetic shellfish poisoning (DSP)

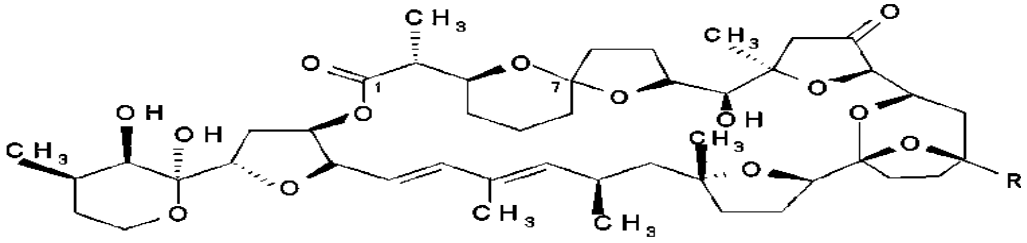
Diarrhetic shellfish poisoning (DSP) is produced by dinoflagellates in the genera *Dinophysis* and *Prorocentrum* like *Dinophysis*, *Prorocentrum*, *Dinophysis fortii*, *Dinophysis acuminata*, *Dinophysis norvegica*, *Dinophysis acuta* (Murakami et al., 1982; Lee et al., 1989; Jackson et al., 1993).

Toxin produced: Okadaic acid

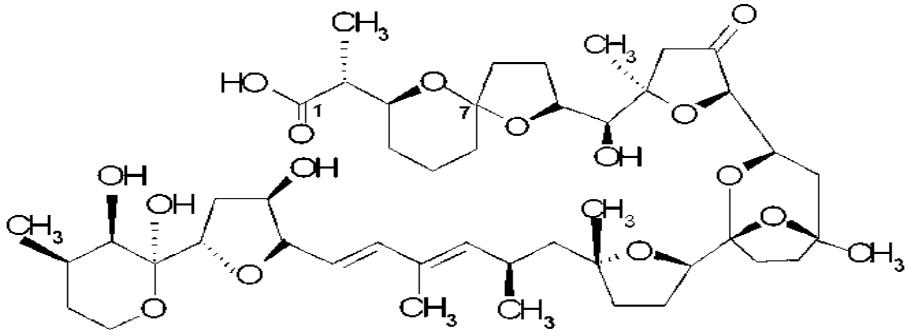
DSP produces gastrointestinal symptoms, usually beginning within 30 min to a few hours after consumption of toxic shellfish. The illness, which is not fatal, is



	R1	R2	R3
Okadaic acid (OA)	H	H	CH_3
Dinophysistoxin-1 (DTX1)	H	CH_3	CH_3
Dinophysistoxin-2 (DTX2)	H	CH_3	H
Dinophysistoxin-3 (DTX3)	acyl	CH_3	CH_3



		R	C-7
Pectenotoxin-1	(PTX1)	CH_2OH	*R*
Pectenotoxin-2	(PTX2)	CH_3	*R*
Pectenotoxin-3	(PTX3)	CHO	*R*
Pectenotoxin-4	(PTX4)	CH_2OH	*S*
Pectenotoxin-6	(PTX6)	COOH	*R*
Pectenotoxin-7	(PTX7)	COOH	*S*



	C-7
Pectenotoxin-2 seco acid (PTX2SA)	R
7-epi-PTX2SA	S

Figure 2. Chemical structures of okadaic acid, dinophysistoxins and pectenotoxins. Source: Yasumoto et al., 2001.

characterized by incapacitating diarrhea, nausea, vomiting, abdominal cramps and chills. Recovery occurs within three days, with or without medical treatment, (Climent and Lembeye, 1993; Climent et al., 2001; Clement, 1999; Cohen, 1974; Cosper et al., 1989; Currie et al., 2000).

Chemical properties

The DSP toxins are all heat-stable polyether and lipophilic compounds isolated from various species of shellfish and dinoflagellates (Draisci et al., 1996a) (Figures 2 and 3). Although diarrhea is the most characteristic symptom of intoxication, several other effects may be of relevance and some of the toxins in the DSP complex (PTXs and YTXs) do not yield diarrhea at all (Van Egmond et al., 1993). Re-evaluation of their toxicity will probably lead to these toxins being removed from their classification as DSP toxins (Quilliam, 1998a). The different chemical types of toxins associated with the DSP syndrome comprise:

a) The first group, acidic toxins, includes okadaic acid

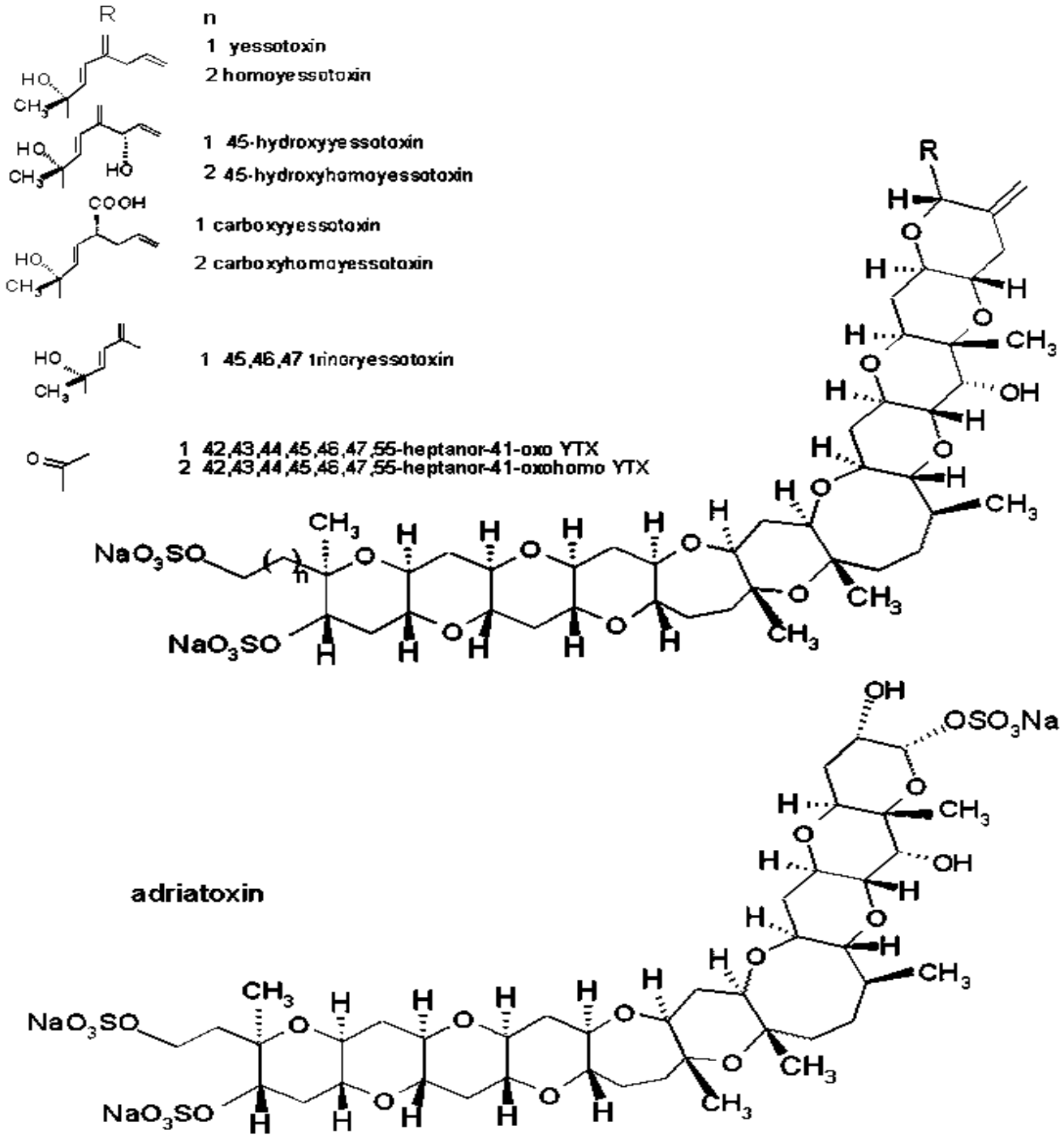


Figure 3. Chemical structures of yessotoxins and adriatoxin. Source: Ciminiello et al., 1998; 2002 and Yasumoto et al., 2001.

(OA) and its derivatives named dinophysistoxins (DTXs). Okadaic acid and its derivatives (DTX1, DTX2 and DTX3) are lipophilic and accumulate in the fatty tissue of shellfish. These compounds are potent phosphates inhibitors and this property is linked to inflammation of the intestinal tract and diarrhea in humans (Van Apeldoorn et al., 1998; Hallegraeff et al., 1995). OA and DTX1 are also tumor promoters in animal test systems (Draisci et al., 1996a; Van Egmond et al., 1993). DTX1 was first detected in *Dinophysis fortii* in Japan; DTX2 was identified in shellfish in Ireland during a DSP episode (Van Egmond et al., 1993). DTX2 was isolated also from a marine phytoplankton biomass mainly consisting of *Dinophysis acuta* (James et al., 1999). A new isomer

of DTX2, named DTX2B, was isolated and identified in Irish mussel extracts (James et al., 1997). DTX3 originally described a group of DSP toxin derivatives in which saturated or unsaturated fatty acyl groups are attached to the 7-OH group of DTX1. More recently it has been shown that any of the parent toxins, OA, DTX1 and DTX2, can be acylated with a range of saturated and unsaturated fatty acids from C_{14} to C_{18} (Hallegraeff et al., 1995; Wright, 1995). In a report of an EU meeting, it was stated that chain length of the fatty acid can vary from $C_{14}$to C_{22} and that the number of unsaturation varying from 0 to 6. The most predominantly saturated fatty acid in DTX3 was palmitoyl acid (EU/SANCO, 2001). These acylated compounds also possess toxic activity. Since these compounds have only been detected in the digestive gland of contaminated shellfish, it has been suggested that they are probably metabolic products and not *de novo* products of toxin producing micro algae (Wright, 1995). Suzuki et al. (1998) demonstrated the transformation of DTX1 to 7-*O*-acyl-DTX1 (DTX3) in the scallop *Patinopecten yessoensis*. The ester bond in the acylated compounds can be hydrolyzed by heating in 0.5 M NaOH/90 percent methanol solution at 75°C for 40 min. The ester bond in DTX3 was also easily hydrolyzed by lipase and cholesterol esterase (EU/SANCO, 2001).

Two naturally occurring ester derivatives called diol esters were isolated from some *Prorocentrum* species. These diol esters did not inhibit phosphatase *in vitro*. However, it should be noted that these allylic diol esters may be somewhat labile and could be hydrolysed to yield the active parent DSP toxin (Hallegraeff et al., 1995). Draisci et al. (1998) reported the detection of another OA isomer and called it DTX2C. The structure of DTX2C is not yet elucidated. The compound was isolated from *Dinophysis acuta* collected in Irish waters.

b) The second group, neutral toxins, consists of polyether-lactones of the pectenotoxin group (PTXs). Ten (10) PTXs have been isolated until now and six out of these have been chemically identified; PTX1, -2, -3, -4, -6 and -7. Since PTX2 (PTX2,CH_3) is found in phytoplankton only (*Dinophysis fortii* in Japan and Europe) and never in shellfish, it is suggested that an oxidation occurs in the hepatopancreas of shellfish producing other PTXs (PTX1, CH_2OH; PTX3, CHO; PTX6, COOH) (Draisci et al., 1996a; Yasumoto et al., 2001; Van Apeldoorn et al., 1998). Sasaki et al. (1998) identified PTX4 and PTX7 as spiroketal isomers of PTX1 and PTX6, namely *epi*-PTX1 and *epi*-PTX6, respectively. Suzuki et al. (1998) demonstrated oxidation of PTX2 to PTX6 in scallops (*Patinopecten yessoensis*). Two new artifacts, PTX8 and PTX9, were also isolated but their structures are not yet elucidated. Daiguji et al. (1998) isolated two new pecteno toxins from the green shell mussel *Perna canalicus* from New Zealand and from *Dinophysis acuta* from Ireland and elucidated the structures as pectenotoxin-2-seco acid (PTX2SA) and 7-*epi*-pectenotoxin-2 seco acid (7-*epi*-PTX2SA), respectively.

c) The third group includes a sulphated compound called yessotoxin (YTX), a brevetoxin-type polyether, and its derivative 45-hydroxyyessotoxin (45-OH-YTX) (Draisci et al., 1996a; Van Egmond et al., 1993). Yessotoxin was first isolated from the digestive organs from scallops (*Patinopecten yessoensis*) in Japan (Ciminiello et al., 1999) and is believed to be produced by microalgae. The yessotoxins do not cause diarrhoea. Yessotoxin attacks the cardiac muscle in mice after intra peritoneal injection, while desulphated yessotoxin damages the liver (Van Egmond et al., 1993). In the digestive gland of Adriatic mussels (*M. galloprovincialis*) besides yessotoxin, two new analogues of yessotoxin, homoyessotoxin and 45-hydroxyhomoyessotoxin were identified by Ciminiello et al. (1997, 1999). Tubaro et al. (1998) also detected homoyessotoxin in *M. galloprovincialis* from the Adriatic Sea during a bloom of *Gonyaulax polyhedra* (*Lingulodinium polyedrum*). Satake et al. (1997) and Satake et al. (1999) isolated YTX and 45, 46, 47-trinoryessotoxin from cultured cells of the marine dinoflagellate *Protoceratium reticulatum*. The production of yessotoxins by *P. reticulatum* differed from strain to strain. Ciminiello et al. (1998) detected again a new analogue of YTX, adriatoxin (ATX), in the digestive glands of DSP infested Adriatic mussels collected in 1997 along the Italian coast (Emilia Romagna). In addition, four further analogues of yessotoxin, carboxyyessotoxin (COOH group on C_{44} of YTX instead of double bond), Carboxyhomoyessotoxin (COOH group on C_{44} of homoYTX instead of double bond) (Ciminiello et al., 2000a, b), 42,43,44,45,46,47,55-heptanor-41-oxo YTX and 42,43,44,45,46,47,55-heptanor-41-oxohomo YTX (Ciminiello et al., 2001, 2002) in Adriatic mussels (*Mytilus galloprovincialis*) were identified.

d) Unexplained human intoxication, with DSP symptoms, following the consumption of mussels from Killary, Ireland in 1995 was resolved by the isolation of a new toxin ($C_{47}H_{71}NO_{12}$), tentatively named Killary Toxin-3 or KT3 (Satake et al., 1998a).

NEUROTOXIC SHELLFISH POISONING (NSP)

Neurotoxic shellfish poisoning (NSP) is caused by toxins produced predominantly by *Gymnodinium* species like *Gymnodinium breve*, *Karenia brevis*. Several species of phytoplankton in New Zealand have been found to produce NSP toxins. These include *Gymnodinium c.f. breve, Gymnodinium c.f. mikimotoi* (which may include three separate species), *G. galatheanum* and a species of *Heterosigma* (Mackenzie et al., 1995a; Haywood, 1998). The identity of the causative agent in the 1993 NSP event in Northland is uncertain: both *Gymnodinium c.f. breve* and *Gymnodinium c.f. mikimotoi* were present in elevated numbers at the time (Chang, 1996; Mackenzie et al., 1995b).

Toxins produced: Brevetoxins

NSP produces an intoxication syndrome nearly identical to that of ciguatera. In this case, gastrointestinal and neurological symptoms predominate. In addition, formation of toxic aerosols by wave action can produce respiratory asthma-like symptoms. No death has been reported and the syndrome is less severe than ciguatera, but nevertheless debilitating. Unlike ciguatera, recovery is generally complete in a few days.

Monitoring programs (based on *Karenia brevis* cell counts) generally suffice for preventing human intoxication, except when officials are caught off-guard in previously unaffected areas (Passow, 1991; Perez et al., 2001; Rafuse et al., 2004; Schnorf et al., 2002; Tangen, 1977; Taylor et al., 1995).

Chemical properties

The NSP toxins, called brevetoxins, are tasteless, odorless, heat and acid stabile, lipid-soluble, cyclic polyether neurotoxins produced by the marine dinoflagellate such as *Gymnodinium breve* (or *Ptychodiscus brevis*). The molecular structure of the brevetoxins consists of 10 to 11 transfused rings; their molecular weights are around 900. Ten brevetoxins have been isolated and identified from field blooms and *G. breve* cultures (Benson et al., 1999) (Figure 4). These brevetoxins show specific binding to site-5 of voltage-sensitive Na^+ channels leading to channel activation at normal resting potential. This property of the brevetoxins causes the toxic effects (Cembella et al., 1995). PbTx-2 is the major toxin isolated from *G. breve*.

Four brevetoxin analogues (Figures 5 and 6) were isolated from contaminated shellfish. The brevetoxin analogues were analysed in cockles (*Austrovenus stutchburyi*) (BTX-B1) (Ishida et al., 1995) and Green shell mussels (*Perna canaliculus*) (BTX-B2, BTX-B3 and BTX-B4) (Morohashi et al., 1995, 1999; Murata et al., 1998) and differed from brevetoxins isolated from dinoflagellate cultures. Apparently BTX-B1, BTX-B2, BTX-B3 and BTX-B4 are metabolites formed by the shellfish itself as they were not found in field blooms or *G. breve* cultures. The presence of BTX-B2, BTX-B3 and BTX-B4 in *Perna canaliculus* does suggest that metabolic pathways in this species are more complicated than those in cockles (*A. stutchburyi*). However, the major toxins in shellfish were left unelucidated because of the extreme difficulty in isolation (Morohashi et al., 1999).

In addition to brevetoxins, some phosphorus containing ichthyotoxic compounds resembling anti cholinesterases, have also been isolated from *G. breve* (Figure 7). One example is an acyclic phosphorus compound with an oximino group in addition to a thiophosphate moiety, namely O,O-dipropyl(*E*)-2-(1-methyl-2-oxopropylidene) phosphorohydrazidothioate-(*E*) oxime (Van Apeldoorn et al., 2001).

Paralytic shellfish poisoning (PSP)

Paralytic shellfish poisoning (PSP) toxins are present in some genera of dinoflagellates and one species of cynobacteri. Several species of the genus *Alexandrium* (formerly named *Gonyaulax* or *Protogonyaulax*) are identified as contaminators in shellfish. These are *Alexandrium tamarensis*, *Alexandrium minutum* (syn. *Alexandrium excavata*), *Alexandrium catenella*, Alexandrium fraterculus, *Alexandrium fundyense* and Alexandrium cohorticula. Other clearly distinct dinoflagellates have also been recognised as sources of the STXs.

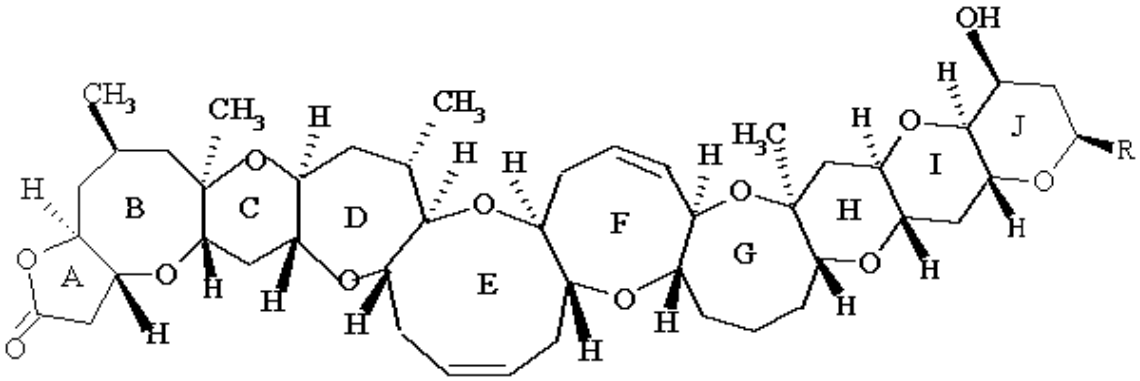
These are *Pyrodinium bahamense* and *Gymnodinium catenatum* (Mons et al., 1998). The toxicity of the dinoflagellates is due to a mixture of STX derivatives of which the composition differs per producing species and/or per region of occurrence.

Toxins produced: Saxitoxins

PSP, like ASP, is a life threatening syndrome. Symptoms are purely neurological and their onset is rapid. Duration of effects is a few days in non-lethal cases. Symptoms include tingling, numbness, and burning of the perioral region, ataxia, giddiness, drowsiness, fever, rash, and staggering.

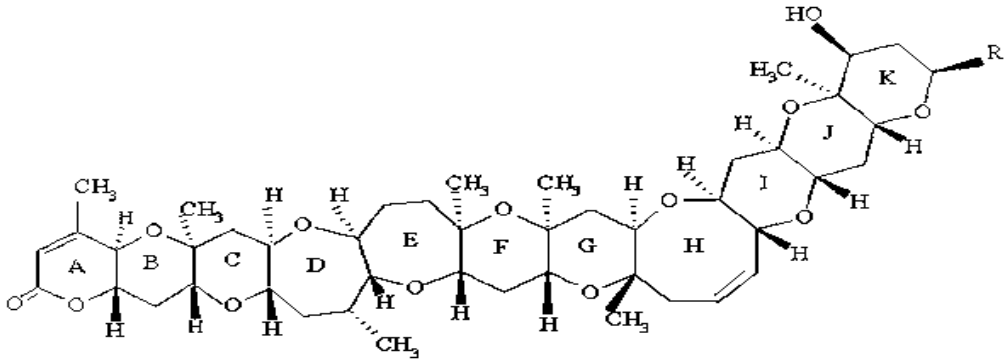
The most severe cases result in respiratory arrest within 24 h of consumption of the toxic shellfish. If the patient is not breathing or if a pulse is not detected, artificial respiration and CPR may be needed as first aid. There is no antidote, supportive therapy is the rule and survivors recover fully. PSP is prevented by large-scale proactive monitoring programs (assessing toxin levels in mussels, oysters, scallops, clams) and rapid closures to harvest of suspect or demonstrated toxic areas. Paralytic shellfish poisoning (PSP) has been reported to occur after eating puffer fish, filter feeding shellfish and molluscs.

If ingested by humans, PSP produces neurologic symptoms such as tingling and burning of the mouth and tongue, numbness, drowsiness and incoherent speech. These symptoms occur within 30 min to two hours after ingestion and in severe cases cause ataxia, muscle weakness, respiratory paralysis and death. The Toxic Exposure Surveillance System (TESS) of the American Association of Poison Control Centres has identified 10 illnesses of presumed puffer fish poisoning due to exposure from PSP after eating puffer fish from the area of Titusville, Florida, (Klöpper et al., 2003; Leikin and Paloucek, 1998; Lembeye et al., 1993; Lembeye, 1981; Luckas et al., 2005; MacKenzie et al., 1996; Mahoney et al., 1990).



Type 1 (A) brevetoxins:

PbTx-1,	R = $CH_2C(=CH_2)CHO$
PbTx-7,	R=$CH_2C(=CH_2)CH_2OH$
PbTx-10,	R=$CH_2CH(CH_3)CH_2OH$



Type 2 (B) brevetoxins:

PbTx-2	R = $CH_2C(=CH_2)CHO$
oxidized PbTx-2	R=$CH_2C(=CH_2)COOH$
PbTx-3	R=$CH_2C(=CH_2)CH2OH$
PbTx-8	R=CH_2COCH_2Cl
PbTx-9	R=$CH_2CH(CH_3)CH2OH$
PbTx-5	the K-ring acetate of PbTx-2
PbTx-6	the H-ring epoxide of PbTx-2

Figure 4. Chemical structures of type A and B brevetoxins (Hua et al., 1996).

Chemical properties

The PSP toxins form a group of closely related tetra hydropurine compounds that make up four subgroups: i) Carbamate (STX, neo STX and Gonyautoxins (GNTX1-4); ii) N-sulfo-carbamoyl (GNTX5-6, C1-4); iii) Decarbamoyl (dc-) (dcSTX, dcneoSTX, dcGNTX1-4); and iv) deoxydecarbamoyl (do-) (doSTX, doneoSTX and doGNTX1) components. At least 21 PSP toxins (Figure 8) mainly from marine dinoflagellates and shellfish that feed on toxic algae have been identified. Attempts to isolate PSP toxins began more than one hundred years

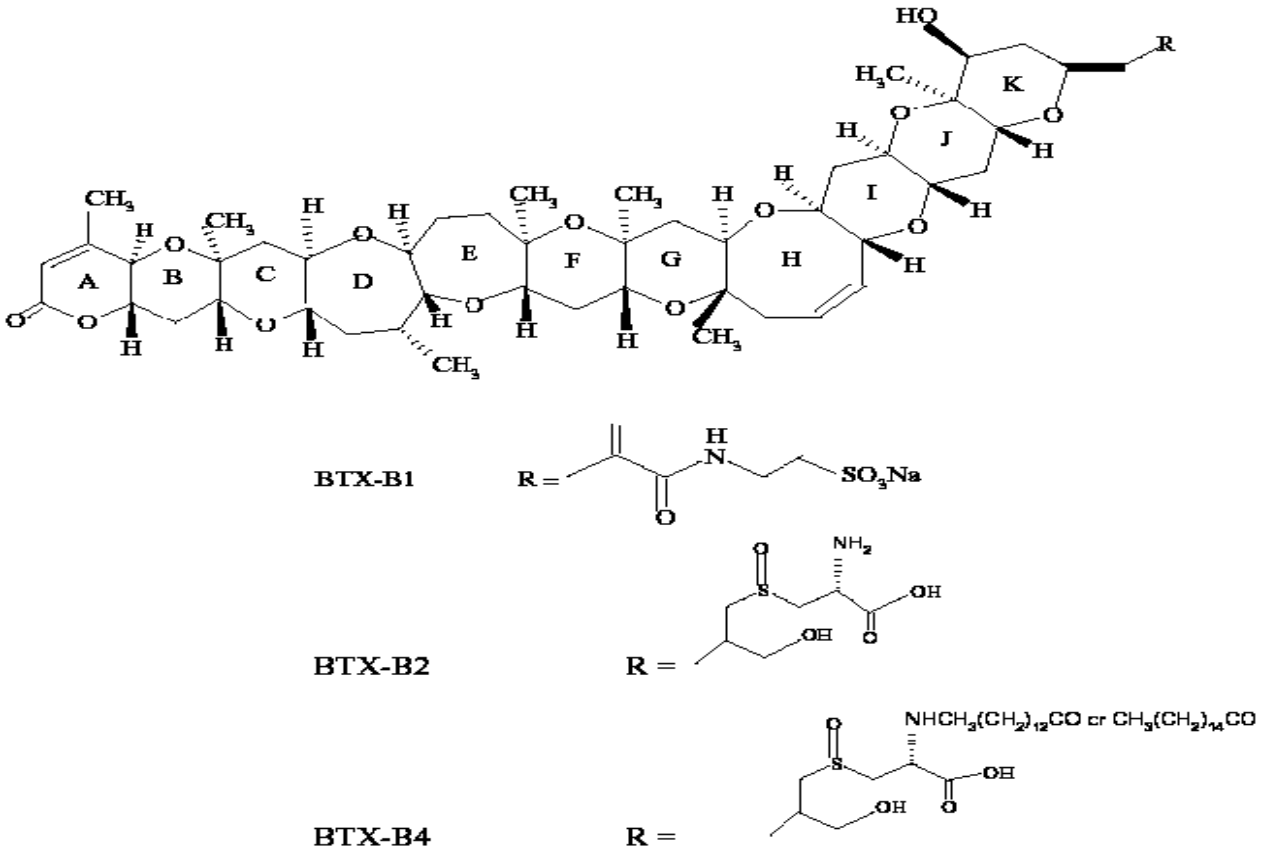
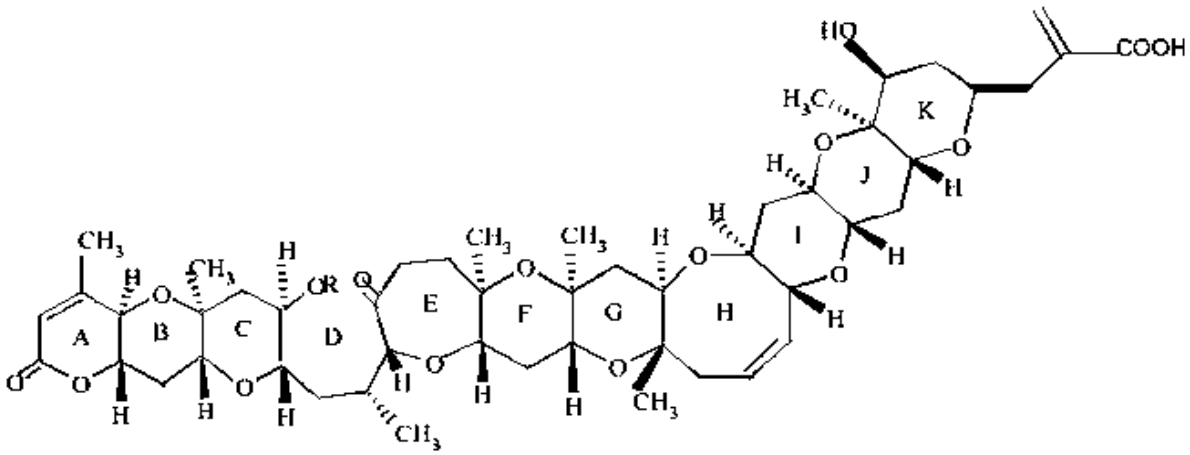


Figure 5. Chemical structures of brevetoxin analogues BTX-B1, -B2 and -B4 isolated from contaminated shellfish. Source: Yasumoto et al., 2001.



BTX-B3
R = $CH_3(CH_2)_{12}CO$ or $CH_3(CH_2)_{14}CO$

Figure 6. Chemical structure of brevetoxin analogue BTX-B3 isolated from contaminated shellfish. Source: Yasumoto et al., 2001.

ago but their occurrence as mixtures of compounds with different ionizable functionalities complicated isolation procedures and early progress was slow. The development of ion-exchange chromatography, guided by mouse bioassays, eventually led to the isolation of a water-soluble basic toxin from the Alaska butter clam (*Saxidomus giganteus*). This compound was later given the trivial name saxitoxin (STX).

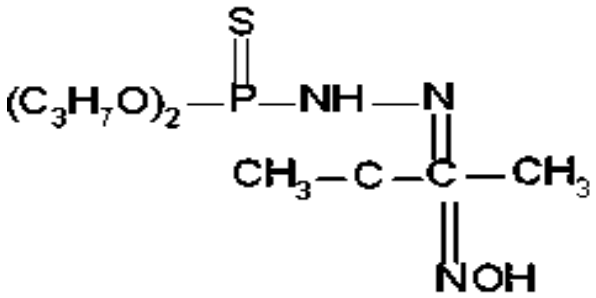


Figure 7. Phosphorus containing ichthyotoxic toxin isolated from *G. breve*. Source: Van Apeldoorn et al., 2001.

In 1975, the first crystalline derivative of STX was synthesized and the structure was studied (Bower et al., 1981). By means of X-ray crystallographic and nuclear magnetic resonance (NMR) spectroscopic studies the structure of STX was elucidated (Figure 8 for the chemical structures of STX and other PSP toxins). The dihydroxy or hydrated ketone group on the five rings is essential for its poisonous activity. Catalytic reduction of this group with hydrogen to a monohydroxy group eliminates the activity. Removal of the carbamoyl group side-chain on the six-membered ring, leaving a hydroxyl group in its place, produces a molecule with about 60% of the original toxic activity. The presence of this active hydroxyl group establishes a means for the preparation of various derivatives of STX (Mons et al., 1998). The PSP toxins are heat stable at acidic pH (with the exception of the N-sulfo-carbamoyl components) but unstable and easily oxidized under alkaline conditions (Mons et al., 1998).

Amnesic shellfish poisoning (ASP)

Amnesic shellfish poisoning (ASP) is caused by Domoic acid (Wright et al., 1989). Species within the genus *Pseudo nitzschia* produce Domoic acid. This toxin is unusual in being produced by diatoms rather than dinoflagellates and some causative organisms like *Pseudo nitzschia australis*, *Pseudonitzschia pungens*.

Toxins produced: Domoic acid

ASP can be a life-threatening syndrome. It is characterized by both gastrointestinal and neurological disorders. Gastroenteritis usually develops within 24 h of the consumption of toxic shellfish; symptoms include nausea, vomiting, abdominal cramps and diarrhea. In severe cases, neurological symptoms also appear, usually within 48 h of toxic shellfish consumption. These symptoms include dizziness, headache, seizures, disorientation, short-term memory loss, respiratory difficulty, and coma.

In 1987, four victims died after consuming toxic mussels from Prince Edward Island, Canada. Since that time, Canadian authorities have monitored both the water column for the presence of the causative diatom, and shellfish for the presence of the toxin, domoic acid. Shellfish beds are closed to harvesting when the domoic acid concentration reaches 20 µg/g shellfish meat. Fish and crab viscera can also contain domoic acid, so the risk to human consumers and animals in the marine food chain is more significant than previously believed (Rafuse et al., 2004; Schnorf et al., 2002).

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Chemical properties

Discrimination between some species of *Pseudo-nitzschia* is virtually impossible under a light microscope because of morphological similarity between species-some species differ in details that can only be detected under an electron microscope. However, whole cell DNA probes have been developed to distinguish between species (Rhodes et al., 1998), and these are utilised by industry in risk management when deciding whether to implement voluntary closures to harvesting, pending the results of shellfish toxicity testing.

Amnesic shellfish poisoning (ASP) is caused by domoic acid (DA) (Figure 9), a naturally occurring compound belonging to the kainoid class of compounds that have been isolated from a variety of marine sources including macro- and microalgae (Wright and Quilliam, 1995). DA is a crystalline water-soluble acidic amino acid. It can be purified by a variety of chromatographic methods and contains a strong chromophore that facilitates detection by UV spectroscopy.

DA was originally discovered as a product of a red macroalgae *Chondria armata* and was later isolated from several other red macroalgae. However, these seaweeds were not the source of DA in the first reported ASP incident on Prince Edward Island in Canada in 1987. The source of DA in this outbreak of ASP was found to be the diatom Pseudo- *nitzschia* (formerly *Nitzschia*) pungens forma multi series. DA is a potent neurotoxin and the kainoid class of compounds to which DA belongs, is a class of excitatory neurotransmitters that bind to specific receptor proteins in neuronal cells causing continual

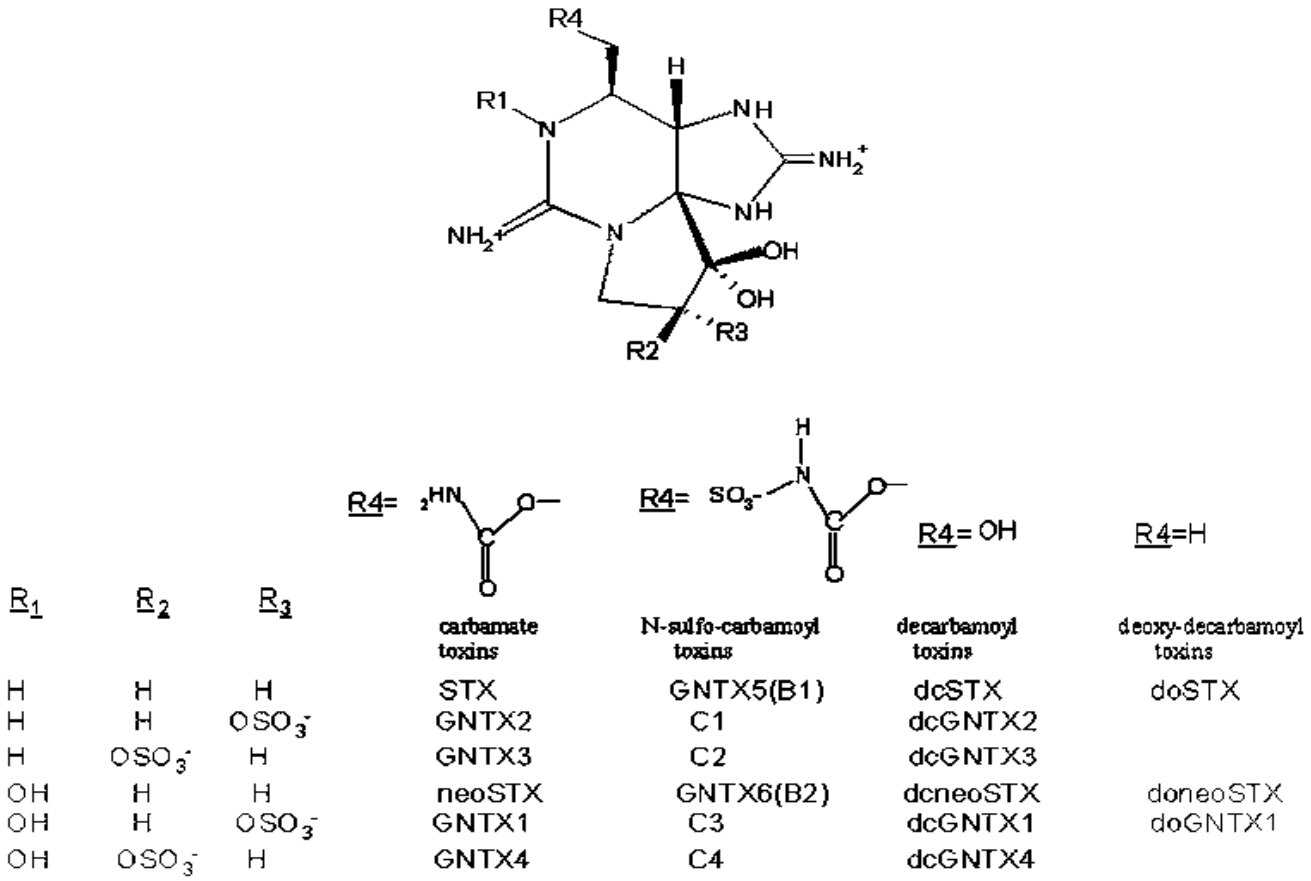

Figure 8. Chemical structures of PSP toxins. Source: Mons et al., 1998; Quilliam et al., 2001.

depolarization of the neuronal cell until cell rupture occurs (Wright, 1995).

Investigation of the kainoids present in *Chondria armata* resulted in the discovery, in minor amounts, of the geometrical isomers isodomoic acid A, B and C (Figure 9) as well as domoilactones. None of these isomers, found in seaweed, were detected in extracts of plankton or shellfish tissue. However, three other geometrical isomers (isodomoic acids D, E and F) and the C5' diastereomer (Figure 8) were isolated from both plankton cells and shellfish tissue (Wright and Quilliam, 1995; Ravn, 1995). The geometrical isomers can be prepared in the laboratory by brief exposure of dilute solutions of DA to UV light, and are therefore not considered to be *de novo* products of the plankton. Pharmacological studies indicate that these photoisomers bind less strongly to the kainate receptor proteins than DA itself suggesting that they are not as toxic as the parent amino acid. Formation of the C5' diastereomer is accelerated with warming. This C5' diastereomer shows almost the same binding efficacy to the kainate receptor as DA itself (Wright and Quilliam, 1995). Zaman et al. (1997b) reported the isolation of two new isomers of DA from the red alga *Chondria armata*, i.e. isodomoic acid G and H (Figure 9).

DETECTION OF MARINE TOXIC

In all cases, the marine HAB toxins that cause the human poisoning syndromes consist of families or groups of structurally related compounds, with individual derivatives exhibiting potencies that can significantly differ from other congeners (Van Dolah, 2001). During food web transfer, HAB toxins can also be metabolized or bio-transformed into structurally different compounds. The broad chemical and structural diversity of algal toxins and their derivatives and metabolites, coupled with differences in their potency account for many of the challenges associated with their detection in ocean observatory programs. Traditionally, biotoxin monitoring programs have relied on measurements of toxins in shellfish samples collected weekly or bi-weekly from key locations in areas affected by HABs (Shumway et al. 1988).

Toxin measurement methods can be grouped into three main types: chemical, *in vitro*, and *in vivo* assays (Hallegraeff et al., 2003). The latter (bioassays) have had a long history in HAB toxin detection, but are obviously not amenable to automation and high-throughput analysis in ocean observatories, so the only options in that context are measurements of toxin in seawater using either

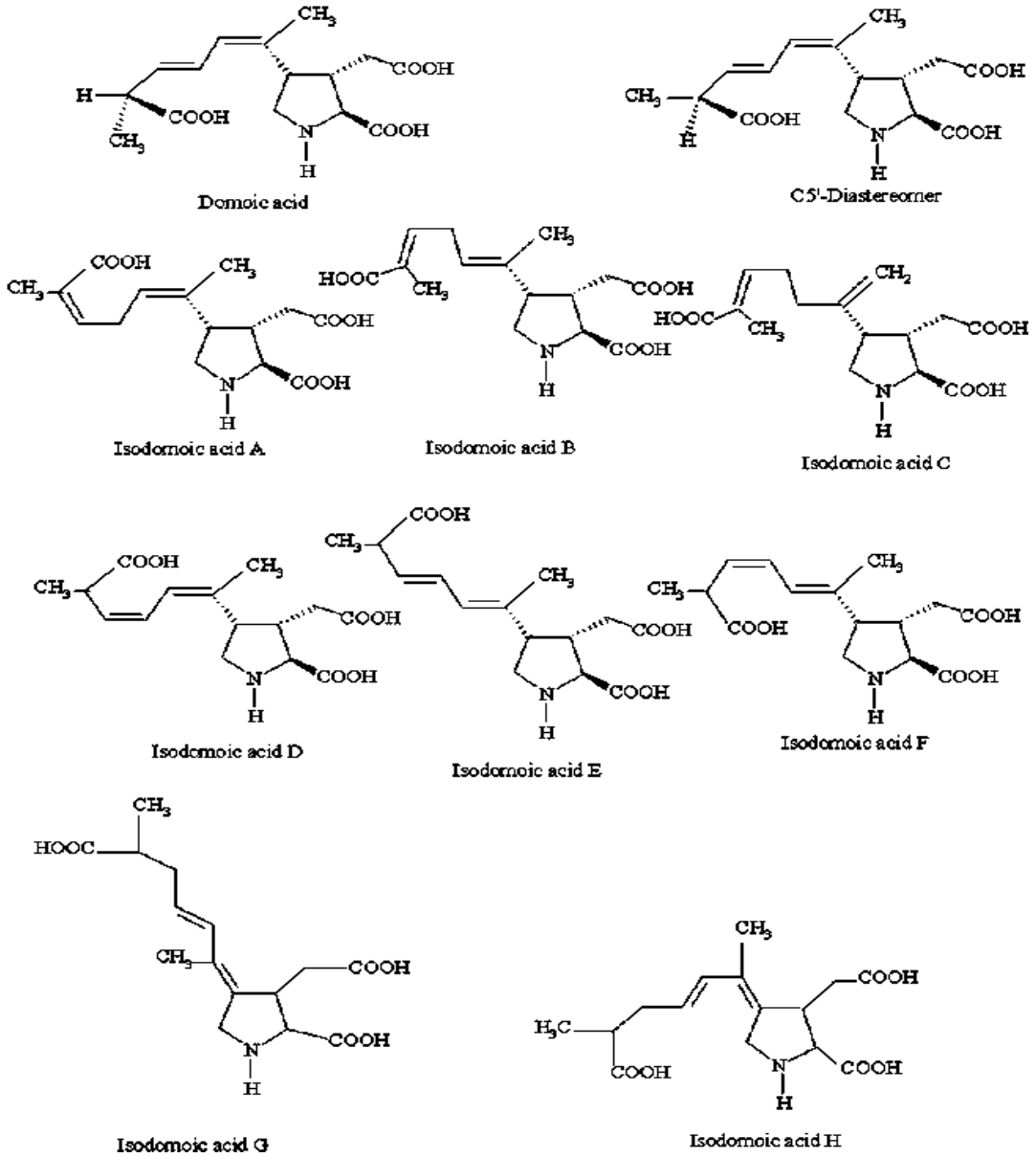

Figure 9. Chemical structures of domoic acid and its isomers. Source: Wright and Quilliam, 1995, Zaman et al. (1997b).

chemical analyses or *in vitro* assays. This immediately introduces some concerns, as considerable work will be needed to relate measurements of toxins dissolved in seawater, or in particulate form in that water, to the risk to human consumers of shellfish or fish.

Chemical methods for toxin analysis include high performance liquid chromatography (HPLC), and mass spectrometry coupled to liquid chromatographic separation (Quilliam 1996). Of these two alternatives, only mass spectrometry shows the potential for use in ocean observatories, and the challenges remain significant due to the diversity, size, and solubility of the toxins, as well as the matrices in which they occur (for example, particulate versus dissolved). Another constraint is the need to perform spectrometry in a vacuum and underwater, which poses significant engineering challenges. Progress has been good, however. For example, a small, modular mass spectrometer has been developed and mounted in an Autonomous Underwater Vehicles (AUV) (Wenner et al., 2004). That system consists of an *in situ* membrane-introduction linear-quadrupole mass spectrometer capable of detecting dissolved gases and volatile organic compounds at sub parts-per-billion concentrations. This instrument is still under development and has not been configured for HAB toxins, but future designs may permit the analysis of HAB toxins that occur dissolved in seawater

(for example, brevetoxins, domoic acid, okadaic acid).

Analysis of toxins in particulate form will require a different approach, such as laser desorption mass spectrometry (LDMS), which is widely employed in analytical laboratories due to its simplicity of operation and rapid analysis times. One benefit of LDMS is that many different types of materials can be vaporized and ionized by a tightly focused laser beam (Cotter 1997). This can avoid sample purification or preparative techniques, which is critical to deployment of such technologies in a moored or mobile configuration in an Ocean Observing System (OOS), as it will greatly reduce sampling and handling requirements, and thus power drain, space needs, and reagent needs as well. LDMS has been used for the detection of bacterial spores, vegetative cells, viruses, and toxins in aerosol environments (Fenselau and Demirev, 2001), and efforts are underway to apply this method to HAB cells and dissolved toxins in seawater.

Another important and rapidly developing group of HAB toxin detection methods comprises the *in vitro* assays. One subgroup the functional assays, relies on detection of a toxin's biochemical activity while the other structural assays depends on recognition of chemical structure at the molecular level (Cembella et al., 2003; Van Dolah and Ramsdell, 2001). A variety of functional assays have been developed for the detection of HAB toxins, including cyto toxicity assays (Manger et al., 1995), enzyme inhibition assays (Della Loggia et al., 1999), and receptor binding assays (Van Dolah et al., 1994). Nevertheless, retention of the biological activity of a cell line or a receptor preparation outside the laboratory remains a significant, and thus far, insurmountable obstacle to *in situ* use of these assays (Sellner et al., 2003).

In contrast, structural assays show considerable promise for automated deployment in an observatory system. These assays rely on the structural or conformational interaction of a toxin with a recognition factor such as an antibody. Antibody-based assays have been developed for a variety of HAB toxins and many of these tests are now commercially available (Laycock et al., 2001; Cembella et al., 2003). One novel immunoassay utilizes surface plasmon resonance (SPR) in a portable system developed for rapid field quantification of toxin levels in both shellfish and seawater (Stevens et al., 2007).

The SPR assay had a limit of detection of 3 ppb domoic acid and a quantifiable range from 4 to 60 ppb. Comparison of analyses with standard HPLC protocols gave an excellent correlation. This same technology should also function for detection of domoic acid (and other algal toxins for which antibodies are available) in concentrated algal extracts or high dissolved levels in seawater. With refinement of the extraction protocols and generation of higher affinity monoclonal antibodies, detection of much lower levels of toxin should be possible, leading to eventual application of automated SPR biosensors on moorings. Another novel and potentially useful approach for *in situ* observations is a competitive immunoassay using screen printed electrodes (SPEs; Kreuzer et al., 2002; Micheli et al., 2004). Excellent sensitivity and accuracy has been achieved with HAB toxins such as okadaic acid, brevetoxin and domoic acid and for all toxins investigated, results compared favourably with other toxin analysis techniques. The advantages of speed of analysis, simplicity of design, *in situ* measurement capability, stability (storage up to four weeks prior to use), and disposability make screen printed electrodes (SPE) immune sensors good candidates for observatory instrumentation. Adaptation of this and other immunoassay technologies to robotic systems and deployment in remote locations is thus possible, but will require further development effort.

CONCLUSION

Only a small number of HAB species can be detected using optical measurements, either *in situ* or remotely from space, and therefore instruments that can detect the vast majority of HAB species need to have capabilities for sample collection, concentration, and manipulation. The chemistries and procedures for cell identification and enumeration using molecular probe assays of various types are well established.

Conflict of Interests

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

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Full Length Research Paper

A precision nutrient variability study of an experimental plot in Mukono Agricultural Research and Development Institute, Mukono, Uganda

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The spatial soil fertility status of a 2.5 ha experimental plot was generated by mapping the soil nutrient concentration and fertility status using GIS kriging technique. The research was conducted in Mukono Zonal Agricultural Research and Development Institute, Mukono, Uganda in October 2013. Soil samples across the experimental plot were randomly taken for laboratory analysis of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and the organic matter content. The mean values of N, P, K, Ca, Mg and the organic matter content were 0.16%, 13.7 ppm, 0.44 cmol/kg, 5.35 cmol/kg, 4.83 cmol/kg and 2.78% respectively. The spatial concentration of each element and the organic matter was carried out by the interpolation technique using the 3D Analyst/Raster Interpolation/Kriging Tools while the overlay operations to generate the soil fertility map was carried out using the 3D Analyst/Raster Math Tools in ArcMap. The autocorrelation analysis was carried out using the Spatial Statistics/Spatial Autocorrelation Tools. The autocorrelation analysis indicated N, Ca, Mg and organic matter to be somewhat clustered each with the Moran's 1 Index of 0.37, P was clustered with Moran's 1 Index of 0.5 while potassium pattern was neither clustered nor dispersed. The spatial soil fertility pattern reflected the distribution of nutrient concentration.

Key words: Nutrient variability mapping, Kriging technique, fertility mapping.

INTRODUCTION

The mapping of nutrient distribution in soils had been reported in previous investigations (Jobbagy and

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Jackson, 2001; She and Shao, 2009; Craine and Dybzinski, 2013; Laiho et al., 2004; Salehi et al., 2013). The five major factors influencing soil formation and nutrient distribution were climate, organisms, relief, parent materials and time (Gebrelibanos and Assen, 2013). The effects of climate and organisms were explained in Jobbagy and Jackson (2000) on the distribution of soil organic carbon in the soil profile where it was observed that at a given climate, the percentage soil organic carbon in the soil was deepest in the shrublands, intermediate in grassland and shallowest in forests. The effects of parent rock and parent materials on soil chemical properties and nutrient distribution was discussed in the relationship among the six main rock types identified in south west Nigeria and the corresponding soil associations (Smyth and Montgomery, 1962; Periaswamy and Ashaye, 1982). In the report, the soils of Iwo soil association, were related to the coarse grained granite and gneisses, Ondo soil association related to the medium grained granites and gneisses, Egbeda soil association related to the fine grained biotite gneisses and schists, Itagunmodi soil association related to the amphibolites, Okemessi soil association related to quartz gneisses and schists, Mamu soil association related to sericite schists.

The spatial distribution pattern of soil nutrients and their relationships with topographic factors were reported in a research conducted by Song et al. (2011) in the Huangshui River drainage basin, China where slope curvature was observed to have significant effects on spatial distribution of nutrients and the generated digital mapping of the soil nutrients provided data support for the precise management of soil resources in the study area.

The use of remotely sensed imagery in assessing soil nutrient concentration was demonstrated by Chen et al. (2000) in the determination of soil organic carbon concentration using aerial photograph of a bare soil of a 115-ha field, located in Crisp County, Georgia. The use of geostatistical technique in mapping soil nutrient content was also demonstrated in the research by Ismail and Junusi (2009) in a Durian Orchard at Beudang, Malaysia using the Geostatistic Plus (GS++) tool to quantify the spatial nutrient content to predict nutrient values at unsampled location.

The spatial variability of soil nutrients could be conducted by either the grid-cell or grid point methods. The grid-cell method involved dividing fields into square cells and composting soil cores to give one sample per cell while the grid point method involved soil sampling at grid intersection points spaced on a square grid. Soil nutrient maps could then be generated by such methods as Delaunay triangulation, polynomial trend surface, inverse distance squared gridding, point kriging and block kriging (Wollenhaupt et al., 2013).

The spatial distribution of nutrient which employed interpolation methods were demonstrated in the spatial interpolation of soil pH across the Loess Plateau, China using the inverse distance weighted (IDW), splines, ordinary kriging and cokriging methods (Liu et al., 2013). The several other investigations conducted to assess spatial variability of nutrients had been previously reported (Sadeghi et al., 2006; Shah et al., 2013).

The objective of the research was to use the GIS kriging technique to produce precision soil nutrient concentration and fertility maps of a 2.5-ha experimental land in Mukono Agricultural Research and Development Institute Mukono, Uganda.

MATERIALS AND METHODS

Soil sampling and laboratory analysis

Soil samples to a depth of 25 cm from randomly selected locations across the 2.5 hectare experimental area were taken for laboratory analysis. Soil samples were air-dried and sieved through a 2 mm sieve and analysed for N, P, K, Ca, Mg and organic matter content following the laboratory procedures described by Carter (1993). Organic carbon was determined by oxidising soil sample with dichromate solution and later titrated with ferrous sulphate solution. The total nitrogen was determined using micro-kjeldahl method and the available P determined by the Bray P-1 method. The exchangeable cations of K, Ca and Mg were extracted by leaching 5 g of soil with 100 ml ammonium acetate at pH 7 and the potassium in the leachate determined with a flame spectrophotometer while Ca and Mg were determined with atomic absorption spectrophotometer.

Interpolation technique for spatial distribution of nitrogen, phosphorus, potassium and the organic matter content and overlay operations for production of soil fertility map

The values of the nutrients input in Microsoft Excel and saved in coma delimited (csv) file format was added as a layer on the map in ArcMap in the Projected Coordinate Systems WGS 1984 UTM zone 31N. It was added as a layer and exported to Shape file through the Data/Export Data pathway. The spatial distribution of N, P, K, Ca, Mg concentration and the organic matter content was carried out separately for each element with the 3D Analyst/Raster Interpolation/Kriging Tools while the overlay operations to generate the soil fertility map was carried out using the 3D Analyst/Raster Math/Plus Tools in ArcMap. The autocorrelation analysis was carried out using the Spatial Statistics/Analyzing Patterns/Spatial Autocorrelation Tools.

RESULTS

Table 1 shows the coordinates of the perimeter and the

Table 1. Coordinates of the perimeter and the soil sampling locations.

Coordinates of the experimental land perimeter		Coordinates of the soil sampling locations	
Easting	**Northing**	**Easting**	**Northing**
470299	42304	470320	42292
470435	42326	470363	42301
470481	42242	470420	42312
470316	42214	470324	42267
470299	42304	470329	42228
		470354	42241
		470390	42238
		470381	42259
		470439	42252
		470431	42284
		470362	42271
		470339	42280
		470400	42285
		470410	42262

soil sampling locations of the 2.5 ha experimental plot while Figure 1 shows the spatial representation of soil sampling locations. The sampling locations were randomly selected across the experimental land with each representing an area within 10 m radius. On the map part of the administrative block and the major road from the main gate are shown.

Table 2 shows the values of nutrient elements with the mean values and the organic matter content as 0.16%, 13.7 ppm, 0.44 cmol/kg, 5.35 cmol/kg, 4.83 cmol/kg and 2.78%, respectively.

Figures 2, 3, 4, 5, 6 and 7 show the spatial concentration of nitrogen, phosphorus, potassium, calcium, magnesium and the organic matter content, respectively, while Figure 8 showed the soil fertility map. The autocorrelation analysis indicated N, Ca, Mg and organic matter to be somewhat clustered with the Moran's 1 Index of 0.37, phosphorus was clustered with Moran's 1 Index of 0.5 while potassium was neither clustered nor dispersed with Moran's 1Index of 0.28. The soil fertility classes I, II and III on the soil fertility map indicated low, medium and high fertility status respectively.

DISCUSSION

The attribute data which indicated the coordinates of the perimeter and soil sampling locations in Table 1 was used to generate the spatial data in Figure 1. This corroborated the previous study by Murray and Shyy (2000) on the integration of attribute and spatial data for identifying patterns in spatial information. The previous investigation by Andrienko and Andrienko (2001) also reported on the technique of analysis of numerical data associated with area geographical objects. The attribute data had been described to contain information about the features on a map that was linked to the map and the linkage achieved by specifying variables in the attribute data set and composite association in the spatial definition that had the same values (GISTUTOR, 2001).

The spatial concentration of each of N, P, K, Ca, Mg and the organic matter content in Figures 2 to 7 was generated through interpolation method of kriging. The interpolation technique enabled predicted values to be assigned to all other locations to create continuous surface representation of the nutrient concentration. This corroborated with the research findings of Oliver and Webster (1999) in the stochastic models of spatial variation in the mapping of soil salinity in the Jordan Valley of Israel and also the herbaceous cover in semi-arid Botswana. The geostatistical interpolation technique of kriging was also used to prepare the landslide susceptibility analysis map of Kota Kinabalu in Malaysia to locate areas prone to landslides (Roslee et al., 2012). The degree of accuracy of kriging technique in the prediction of soil properties was explained in the report of Omran (2012) in the descriptive tools of semivariograms to characterize the spatial patterns of continuous and categorical soil attributes. The application of kriging technique had been premised on the principle that soil properties closer together would tend to be more alike

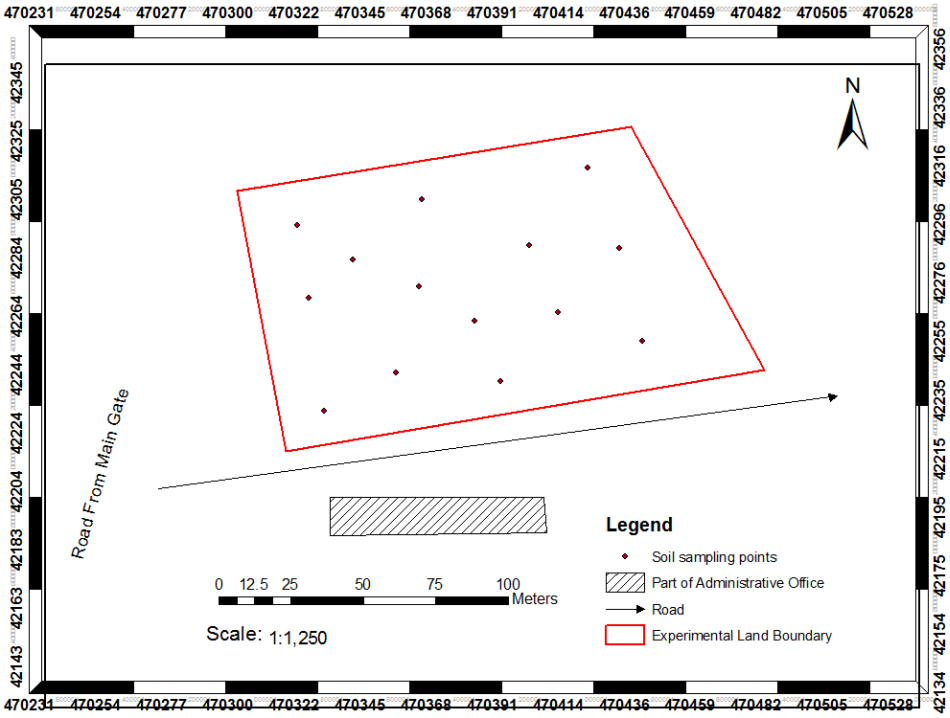

Figure 1. Experimental site with soil sampling locations.

Table 2. The values of N, P, K, Ca, Mg and the organic matter content at each sample location.

Easting	Northing	Nitrogen (%)	Phosphorus (ppm)	Potassium (cmol/kg)	Calcium (cmol/kg)	Magnesium (cmol/kg)	Organic matter (%)
470320	42292	0.16	13.4	0.36	4.48	4.88	2.78
470363	42301	0.12	11.8	0.28	3.68	4.22	2.12
470420	42312	0.14	11.2	0.21	3.24	3.86	2.12
470324	42267	0.19	15.7	0.70	8.17	5.56	3.60
470329	42228	0.18	14.8	0.65	7.49	5.48	3.46
470354	42241	0.19	15.6	0.54	6.56	5.42	3.24
470390	42238	0.18	14.9	0.71	6.52	5.52	3.42
470381	42259	0.14	13.9	0.43	5.52	4.86	2.86
470439	42252	0.13	13.2	0.34	3.86	4.14	2.26
470431	42284	0.14	11.6	0.24	4.42	4.64	2.88
470362	42271	0.15	13.8	0.42	5.58	5.24	2.88
470339	42280	0.17	14.1	0.51	6.51	5.38	2.86
470400	42285	0.15	13.6	0.32	4.40	4.12	2.14
470410	42262	0.14	13.6	0.41	4.42	4.32	2.24

than the distant points and the interpolation was a prediction made within the spatial extent of the measured locations.

The spatial concentration of the nutrient elements from the autocorrelation analysis which indicated N, Ca, Mg and organic matter to be somewhat clustered each with

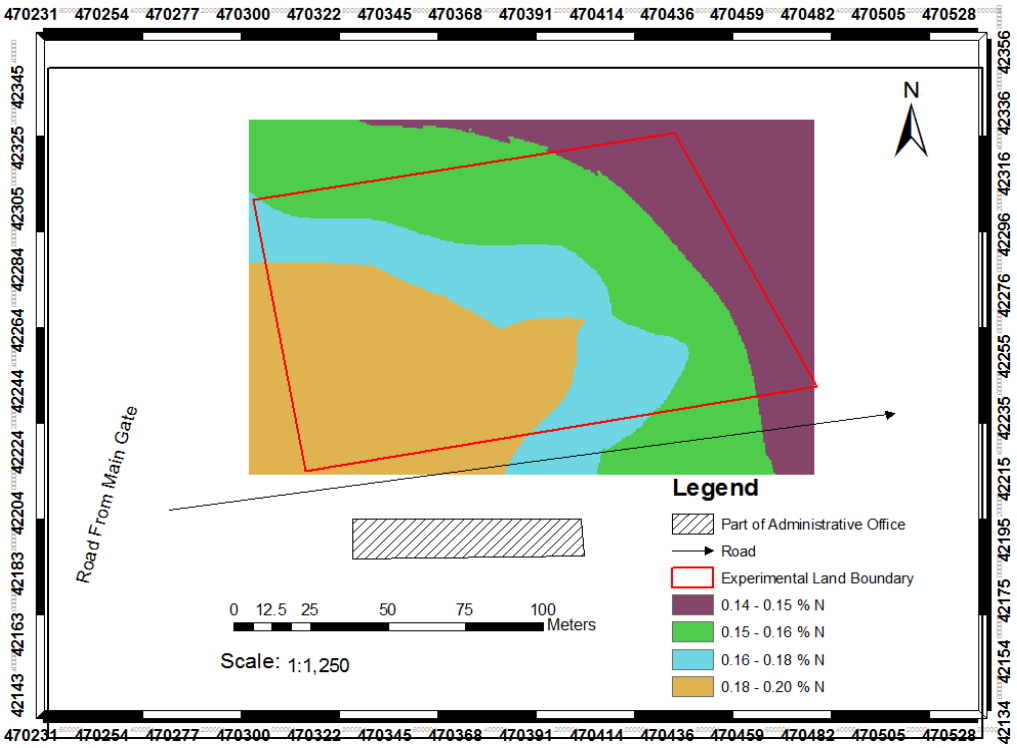

Figure 2. Nitrogen spatial concentration.

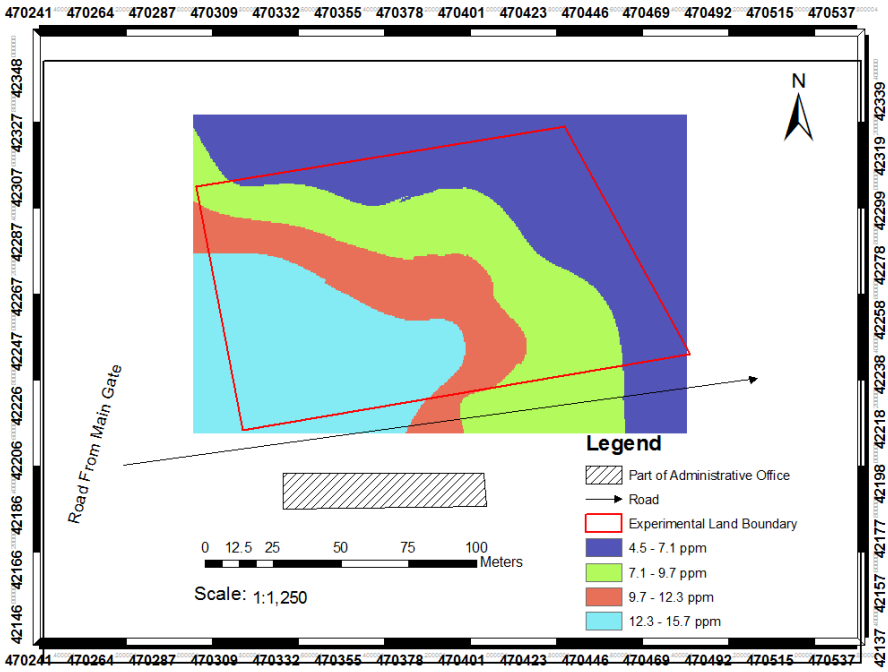

Figure 3. Phosphorus spatial concentration.

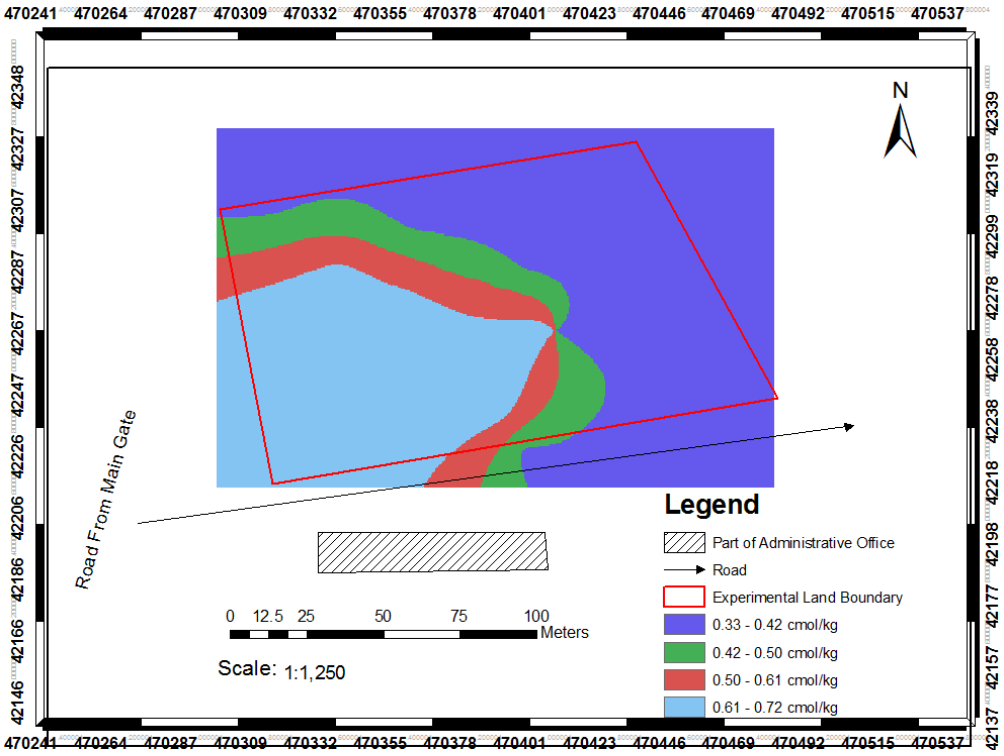

Figure 4. Potassium spatial concentration.

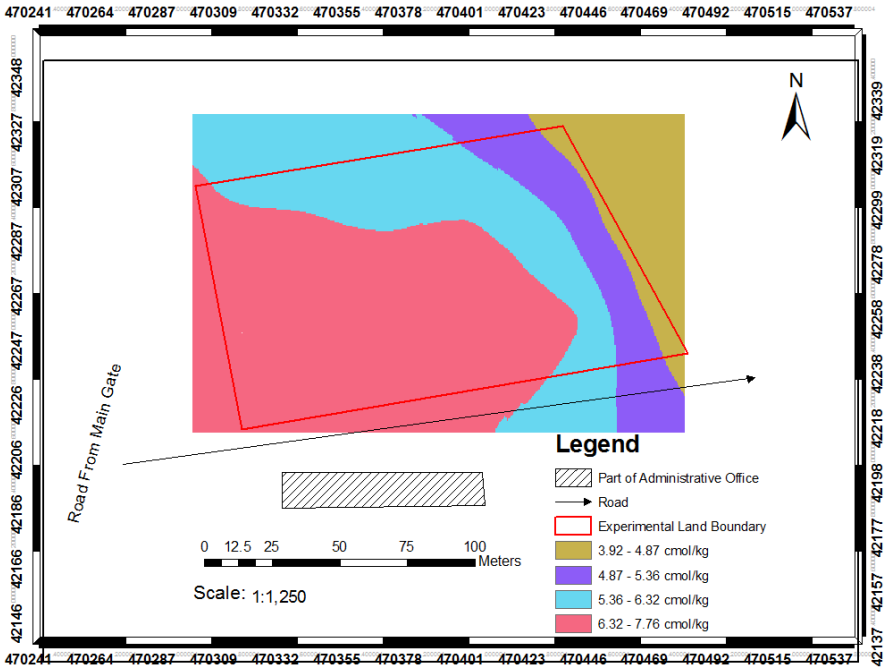

Figure 5. Calcium spatial concentration.

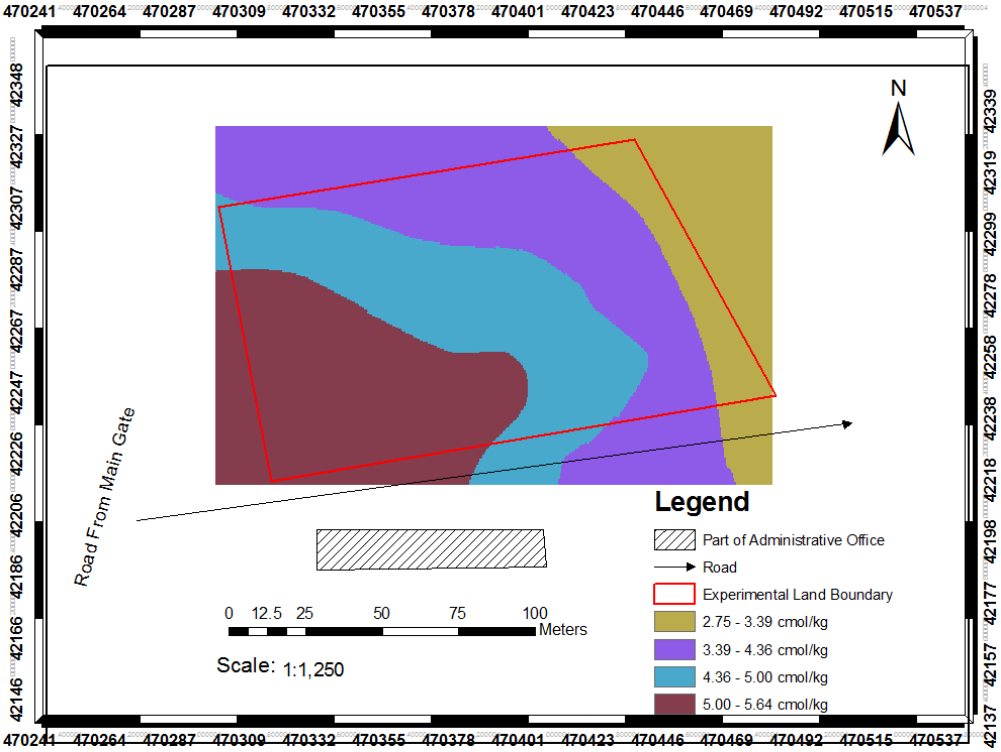

Figure 6. Magnessium spatial concentration.

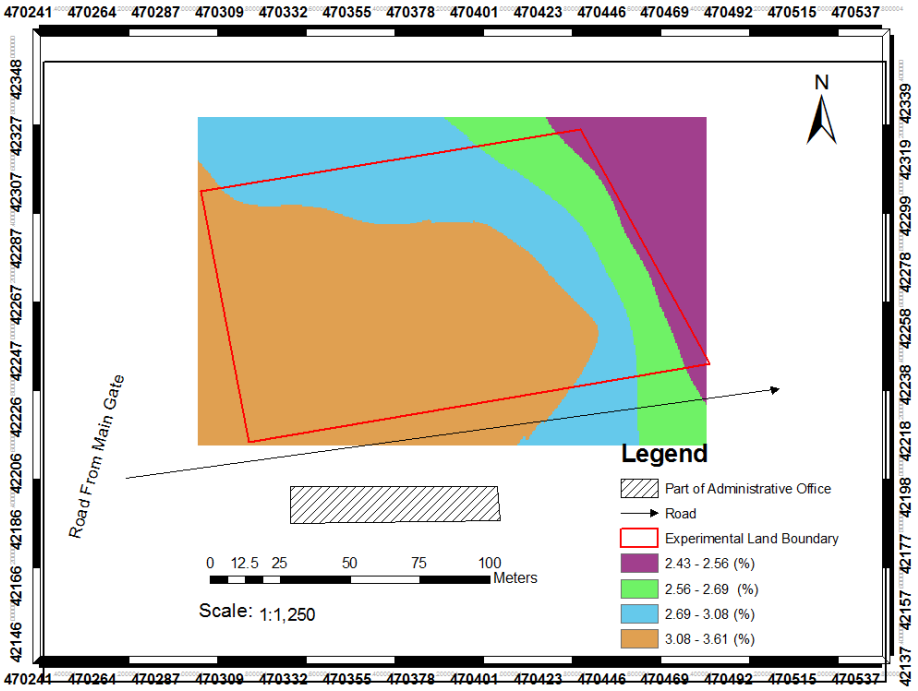

Figure 7. Organic matter spatial distribution.

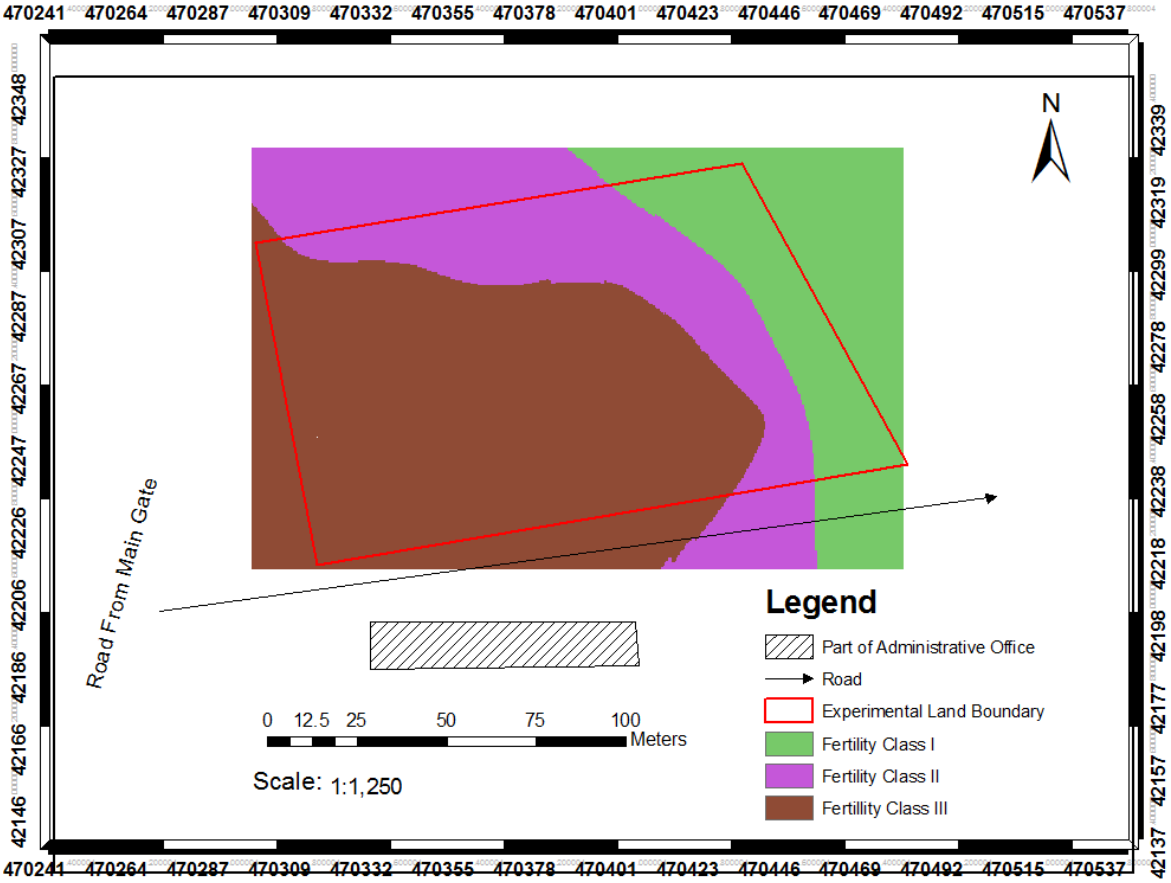

Figure 8. Soil fertility map.

the Moran's 1 Index of 0.37, P with clustered pattern with Moran's Index of 0.5 and K which was neither clustered nor dispersed with Moran's Index of 0.28 corroborated earlier observation of Huo et al. (2010, 2011) on the improvement of spatial interpolation accuracy of heavy metals concentration in soils and also in the autocorrelation analysis of soil pollution data on soils in Taiwan (Chu and Chang, 2011).

The spatial nutrient concentration maps integrated in an overlay operation to generate the soil fertility map corroborated the overlay procedure adopted by Onunkwo-Akunne et al. (2012) for the production of industrial, residential and waste disposal maps that were further superimposed to produce a composite land use map useful for regional and urban planning.

The spatial distribution of the soil fertility status of the experimental land as shown in Figure 8 reflected the spatial concentration of nutrients in Figures 2 to 7 which corroborated the previous observation of Salehi et al. (2013) on the determination of soil fertility status from the nutrient concentration.

Conclusion

The somewhat clustered nutrient concentration pattern observed could be adduced to past fertilizer application and cropping pattern on the experimental land. The high mean values of 0.44 and 5.35 cmol/kg for K and Ca, respectively could be attributed to past continuous application of CAN and NPK. A situation map of nutrient distribution was generated with the use of GIS Kriging technique.

Conflict of Interests

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

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Full Length Research Paper

Effect of heat build-up on carbon emissions in chimato compost piles

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A study was conducted to determine impacts of heat build-up of chimato compost piles TD0, TD20, TD40, TD50, TD60, TD80 and TD100, made by blending maize stalks with 0, 20, 40, 50, 60, 80 and 100% *Tithonia diversifolia*, respectively, on carbon losses and emissions during composting. Compost piles temperatures that determined heat built-up were obtained from previous studies. Organic carbon and total carbon of chimato composts were determined using Kjeldahl method. Relatively, greater carbon reductions were observed in compost piles TD0, TD20, and TD40 (that experienced prolonged high thermophilic temperatures (temperature >60°C)) than compost piles TD50 and TD60 (that experienced a short-lived thermophilic temperatures (45≤60°C)). The prolonged high temperatures increased kinetic energy of chemical species CO_2 and CH_4 that became more volatile and probably escaped from the compost piles in large quantities resulting in significant carbon emissions. Relatively, short-lived heat built-up in TD50 and TD60 resulted in minimal carbon reduction, hence minimal carbon emissions. Therefore, chimato composts TD50 and TD60 significantly reduce compost pile carbon emissions (p=0.05, α=0.001) and should be recommended to mitigate effects of climate change.

Key words: *Tithonia diversifolia*, heat built-up, carbon emissions, carbon reduction, prolonged thermophilic temperatures.

INTRODUCTION

Agriculture sector plays vital role in sustaining growth and reducing poverty in many countries in Africa including Malawi. However, depletion of soil fertility as well as organic matter has been reported to continuously and negatively affect sustainable agricultural production in Malawi (Elias et al., 2013). Application of composts in agricultural filed has been identified as one of the potential initiative to replenish soil fertility and organic matter. It is reported that application of well matured and stable composts into agricultural fields significantly increases soil carbon content by diverting inorganic atmospheric carbon compounds such as dioxide (CO_2) and methane (CH_4) into soil organic carbon compounds (Biala, 2011; Gill et al., 2012; Biddlestone and Gray, 1987). Maturity and stability of compost is partly dependent on type of feedstock that influence compost pile moisture content, aeration and heat build up. Well matured and stable composts gradually decompose

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under aerobic conditions (Biddlestone and Gray, 1987; Christensen and Peacock, 1998).Compost piles that mature into well matured and stable composts usually meet certain minimum blending composition of nitrogen rich ingredients and carbon rich ingredients (WSU, 2010; Mlangeni et al., 2013). Such requirements make ingredients to be digested very fast by microbes and the compost pile stays in high thermophilic stage for relatively short time (Mlangeni, 2013).

On the contrary, immature and unstable compost is usually produced when fewer quantities of nitrogen rich have been blended with disproportional large quantities of carbon rich feedstock. Such ingredient composition limits microbial activities. Microbes use limited available nitrogen to slowly digest ingredients in prolonged high thermophilic temperatures. The high temperatures ensure availability of microorganisms that multiply rapidly at specific temperature range (WSU, 2010). Therefore, compost piles that stay in the high thermophilic phases for a long time are symptomatic to prolonged exothermic oxidation reactions that built-up large amount of heat (WSU, 2010). Large generation of prolonged heat is attributed to high active microbial activities taking place in the compost piles due to quantities of high carbon content cellulosic materials (lignin) whose oxidation and digestion generate large amounts of heat (Agromisa**, 1990**). The compost piles stay longer in the thermophilic stage because of greater quantities of hard to digest materials in the feedstock that prolongs maturity period. Thus, the compost piles' prolonged stay in the thermophilic stage may have a negative impact on kinetic energy of chemical species available in the compost pile, which may also be symptomatic to volatilization and consequently to loss of such chemical species.

Smallholder farmers in Malawi are encouraged to make and use chimato compost in their fields. However, such farmers use low quality ingredients such as grass and maize stalks. Use of nitrogen rich ingredients such as *Tithonia diversifolia* biomass is recommended (Mlangeni et al., 2013). Temperature progression of chimato compost piles made using 80 and 100% of *T. diversifolia* was reported to rapidly increase to high thermophilic peak temperature (54.5 and 55°C respectively) before rapidly dropping down to mesophilic phase (35.0°C) (Mlangeni, 2013). Temperature progression of chimato compost piles made using 60 and 50% of *T. diversifolia* was reported to experience prolonged stay in the high thermophilic phase peak temperature (65.0 and 70.0°C), respectively, symptomatic to well matured compost (Biddlestone and Gray, 1987; Biddlestone and Gray, 1987; Darlington, 2010). The blending is reported to generate a balanced heat build-up conducive to initiate a self-accelerated process of decomposing the feedstock (Mlangeni et al., 2013). Chimato compost piles' heat build-up determines both nitrogen and carbon content of the chimato composts. Too high temperatures deactivate mesophilic microbes whereas a longer duration of high temperatures accelerates losses of methane (CH4), carbon dioxide (CO_2) and nitrogen loss as ammonia NH_3 and nitrogen oxide through volatilization (Carr, 1998; CAW, 2012). Heat built-up in compost pile is dependent on quality of ingredients used in constructing the compost piles (Mlangeni, 2013), whereas quantity of CO_2, methane and other nitrides diverted is dependent on type, stability and quality of composts. High quality ingredients such as *T. diversifolia* enhances rapid and active microbial activity that break down chemical bonds of compounds and transforms chemical energy (Biddlestone and Gray, 1987; WSU, 2010) contained in the bonds to heat, which raises the temperature of the compost pile (Mlangeni, 2013). Blending maize stalks with *T. diversifolia* of greater than 40% ensures availability of both carbon and nitrogen in sufficient amounts to enhance optimal active and rapid microbial activities. It is suggested that optimal heat build-up may have significant impacts on residual compost nitrogen and carbon. This study was conducted to determine impacts of compost piles heat build-up of chimato compost produced by blending maize stalks with different quantities of *T. diversifolia* on carbon losses and carbon emissions during composting.

MATERIALS AND METHODS

Study site

The study was carried out at Natural Resource College farm (130 85' S 330 38, E) in Lilongwe, Malawi. Natural Resource College farm is about 13 km south west of Lilongwe town and 3.5 km off Lilongwe-Mchinji road. It lies on an altitude of 1146 m above sea level. Natural Resource College experiences mean annual temperature of 20°C and mean annual relative humidity of 68%. It receives an annual mean rainfall of 892 mm of which 85% falls between November and March (DARETS, 2002).

Experimental design and treatments

Six standard chimato composting treatments Td0, Td20, Td40, Td50, Td60, Td80 and Td100 were made. In each case, *T. diversifolia* biomass was blended with maize stalks in the ratio (TD/MS) 0:100, 20:80, 40:60, 50:50, 60:0, 80:20 and 100:0 respectively by mass. Tender green *T. diversifolia* shrubs of about 8 weeks old (counted from the date the *T. diversifolia* shrubs were slashed for regeneration) were used. Both maize stocks and the *T. diversifolia* materials were cut into pieces ranging from 5.0 to 10.0 cm. The small sizes were chosen to increase surface area onto which microbes would act on, to enhance efficient diffusion of air throughout the entire pile and to enhance active and rapid aerobic decomposition (Nalivata, 2008; Michel et al, 2010). Each compost pile was adequately watered to optimize compost pile moisture content. Initial diameter of each compost pile was 1.5 m wide, 1.5 m high and conical shape. The piles were then plastered on the outside with mud as shown in Figure 1. Air vents (Figure 1c) were made at the bottom and middle of the piles to allow air circulate through the biomass to enhance aerobic degradation (Nalivata, 2008).

Figure 1. a) Un-plastered compost pile; **b)** freshly plastered conical piles; **c)** matured chimato compost piles.

Analysis of chimato composts

Compost samples were purposely collected from different locations of the matured chimato compost pile. Samples from each treatment were homogeneously mixed to get a composite representative sample. The samples were analyzed for total Kjeldahl carbon and organic carbon using Kjeldahl method (Jeffery et al., 1989).

Data analysis

Data was analyzed using SPSS 17.5 version. Level of variation of organic carbon and total Kjeldahl carbon were determined using analysis of various (ANOVA).

RESULTS AND DISCUSSION

Effect of prolonged stay in high thermophilic phase on carbon losses

Study results indicates that amounts of carbon losses in chimato compost TD0 and TD20 were significantly different from carbon losses of chimato composts TD50, TD60, TD80 and TD100 ($p=0.018$; $\alpha=0.05$; Wilcoxon). Carbon losses in TD0 was not significantly different from carbon losses in treatment TD20 and TD40 ($p=0.207$; $\alpha=0.05$; Wilcoxon). However, greatest carbon losses were observed in chimato compost TD0 followed by chimato compost TD20 then chimato compost TD40 (Figure 2). Carbon losses in TD0, TD20 and TD40 highly correlated with chimato compost piles' prolonged stay in the high thermophilic stage, implying that prolonged stay in the high thermophilic stage (prolonged heat built-up) was responsible for the observed carbon losses (Carr, 1998; CAW, 2012). The heat increased kinetic energy of the chemical species which probably became more volatile and escaped the compost pile. High temperatures in compost piles are symptomatic to rapid active microbial activities likely to generate large heat (WERL, 2005; WSU, 2010). Thus, the observed carbon losses could be attributed to prolonged exothermic decomposition reaction of carbon atoms that induced the observed high-prolonged temperatures (heat built-up) and that released significant quantities of carbon dioxide (Biala, 2011; Biddlestone and Gray, 1987).

Since prolonged stay in the thermophilic stages suggests prolonged active microbial activities, production of excessive methane and carbon dioxides in compost piles TD0 and TD20 was inevitable. TD0 and TD20 relatively contained large quantities of large quantities of hard to digest carbon containing polymers, such carbon atoms were digested for a longer time thereby releasing enormous heat which raised the temperature of the compost piles. In this process, microbes used and recycled limited quantities of nitrogen used as source of building materials of microbe structure and carbon atoms as source of energy (Biala, 2011; Gill et al., 2012).

Secondly, prolonged stay in high thermophilic phase is symptomatic to prolonged generation of large amounts of heat (WERL, 2005; Mlangeni, 2013) likely to increase kinetic energy of chemical species in the compost piles. The prolonged stay in the thermophilic phase cause chemical species such as carbon dioxide (CO_2), methane (CH_4), ammonia (NH_3) and nitrogen oxides (NO_x) acquire kinetic energy that significantly increases chemical species' random motions in the compost piles. The rapid random motion would either trigger faster rates of reaction with other chemical species in the compost pile or accelerates volatilization from the compost piles. Methane (CH_4) species, which has about 300 times impact on climate change (EPA, 2008; Bernal et al., 1998), could readily react with oxygen under aerobic conditions to produce carbon dioxide, which is of less impact thereby significantly reducing carbon emission of more dangerous greenhouse gas. In addition, methanogenesis would likely occur under conditions of high temperatures as well as low supply of ammonia (Biala, 2011; Gill et al., 2012). Ammonia would be readily available in compost piles with greater quantities of nitrogenous feedstock. Volatilization and escape of chemical species especially carbon dioxide (CO_2) and

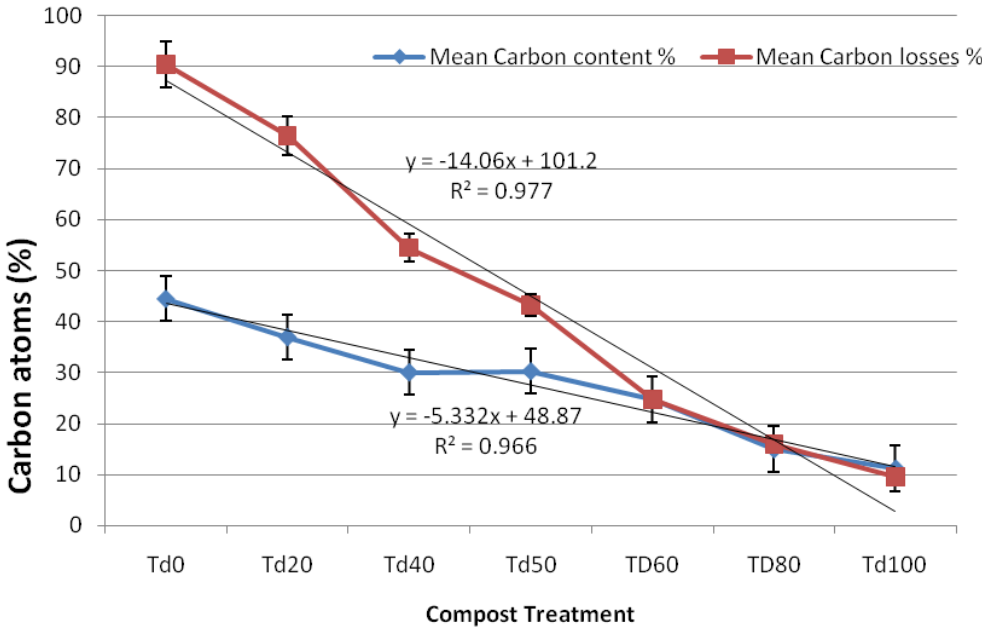

Figure 2. Carbon retainance and losses in various compost treatments.

methane (CH_4) could be enhanced by cracks of compost piles that quickly develops due to high heat built up of compost piles (WERL, 2005; Gill et al., 2012; Biddlestone and Gray, 1987). Thus, carbon dioxide (CO_2) and methane (CH_4) escaped from the piles at fast rate greatly increasing carbon losses of the resultant chimato composts. Therefore, the significant carbon reduction observed in TD0 could be attributable to described carbon losses which would increase carbon emissions. Secondly, microbes had probably plenty nitrogen atoms used to digest available little carbon atoms, which was accomplished with greater speed and significantly short-lived heat build-up of compost piles.

Further analysis has shown that quantity of *T. diversifolia* is negatively correlated with carbon losses in the compost pile up to 60% of *T. diversifolia* content in the compost piles. A linear equation model was developed as follows:

$$Y = -17-46x + 106.63 \quad (1)$$

Where, y=quantities of *T. diversifolia* and x = carbon lost in the compost piles.

A linear model revealed that *T. diversifolia* is inversely related to carbon losses suggesting that as quantities of *T. diversifolia* increase amounts of carbon lost decreases. Carbon is lost asbeyond 60% of *T. diversifolia* content in the compost piles, carbon losses increased. Carbon could be lost into the surrounding environment as carbonates leachate hence not contributing to carbon emission.

Effect of shorter stay in lower thermophilic stage on carbon emission

As shown in Figure 2, lowest carbon losses were observed in compost TD60 followed by TD50 whose compost piles experienced moderate stay in the high thermophilic stage. The observation implies that compost piles' moderate stay in the high thermophilic phase was due to a balanced blending proportion of carboceous and nitrogenous feedstock. A balanced blending proportion of carboceous and nitrogenous provided an optimum microbial activities that generated an optimum heat build-up in compost piles (Mlangeni and Chiotha, 2013); EPA, 2008; Bernal et al., 1998) that greatly limited production of excessive methane and carbon dioxides. Td50 and Td60 did not experience prolonged heat build-up that forced microbes to reuse the available nitrogen to build up their structures thereby causing significant nitrogen losses nor did they experience short-lived heat build-up which was wet and induced favorable conditions for anaerobic decomposition and significant nitrates losses.

Effect of longer stay in mesophilic stage on carbon emission

As shown in Table 1, TD100 and TD80 experienced a short lived stay in the thermophilic phase and relatively longer stay in the mesophilic phase. The short lived stay in thermophilic phase and longer stay in mesophilic phase resulted from rapid active microbial activities that quickly degraded the ingredients in relatively short time. Thus, less heat was generated in the compost piles

Table 1. Effect of *T. diversifolia* on carbon and nitrogen losses.

Treatment	Initial N (%)	Percentage of N lost (%)	Total C (%)	C lost	Std Error
Td0	1.2	56.67	44.5	90.5	5.0
Td20	1.4	53.57	37.0	76.5	5.0
TD25	1.57	56.05	29.4	67.5	5.0
Td40	1.7	51.76	30.1	44.5	3.5
Td50	2.3	47.83	30.3	33.3	2.0
Td60	2.5	44.80	24.7	24.8	2.5

implying minimal occurrence of decomposition of carboceous materials. The feedstock provided little quantities of hard to digest macromolecules such as cellulose and polyphenols which were easily digested thereby releasing less heat than those with lower composition *T. diversifolia* in the feedstock. Since ammonia volatilization occurs in compost piles with high nitrogen content, Td80 and Td100 were certain to experience reductions of both total Kjeldahl nitrogen and nitrate-N. Nitrates were leached out of the compost piles as leachate or as slurry (Manahan, 2008) collected under the piles. Hence, the short-lived heat build-up in Td80 and Td100 is associated with decreased recorded amounts of total Kjeldahl nitrogen and nitrate-N while the less prolonged were recorded in Td80 and Td100 (Mlangeni, 2013).

Conclusion and recommendations

The results have shown that compost piles that experienced moderate heat build-up experienced minimum carbon losses. Either the heat build-up or the microbial activities or both were responsible for the low carbon losses. However, balanced blending proportion of carboceous and nitrogenous materials in feedstock of compost piles TD50 and TD0 played a significant role in limiting rapid microbial activities which subsequently limited generation of heat in the compost piles. Subsequently, the limited generated heat also limited kinetic energy of chemical species such as NH_4, CO_2 and CH_4 and became more volatile and escaped from the compost piles in large quantities hence the observed greater carbon reduction which increased carbon emission. Making and using chimato composts produced by blending *T. diversifolia* biomass with maize in the ratio of 50:50≤Td/MS≤60:40 greatly reduce compost pile carbon emissions and should be recommended to mitigate effects of climate change.

Conflict of Interests

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

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Full Length Research Paper

Assessment of environmental degradation of soil and groundwater: A case study of waste disposal in Benin West Moat - Ekenwan gully Benin City, Edo State, Nigeria

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The ancient Benin moat of 2.00 to 5.00 m width, and 10 to 30 m depth almost concentrically dug round the City, is supposed to be an important socio-cultural artifact if well preserved. It has served its good purpose of defense and protection of the ancient Bini civilization. Now, modern urbanization pressure has defaced and is degrading the moat and its environs through house development, soil quarrying, gullying, flood catchment, and waste disposal. Because of its extent, it is pertinent to understand its pedo-geological and hydrogeological setting in order to assess the impact of the waste disposal on the soil and groundwater systems. Random; 11 soil (pool) samples at 0.0 to 0.5 m depth, and eight groundwater samples from pumping boreholes, 0.5 to 10.0 m on both sides of the moat were drawn. Samples were analyzed for their chemical, heavy metal constituents (including microbial in water). Comparisons of means with pristine locations in previous studies were conducted using one way analysis of variance (ANOVA) at 5% α level of significance. Results reveal contamination of soil with Al, Cd, Fe, Pb and THC at $P<0.05$. The groundwater also is polluted with presumptive coliform of 1.0×10^2 to 1.5×10^3 cfu/ml^{-1} and heavy metals; Al, Cd, and Pb at $P<0.01$ indicating high significant difference. This indicates that the once protective moat is now a likely area of phytotoxicity and general environmental toxicity to man if chronic exposure is allowed by continual waste disposal.

Key words: Wastes disposal soil and groundwater contamination in Benin West-Moat, case of environmental toxicity.

INTRODUCTION

Urban degradation is drawing concern in Nigeria. In Edo state, it has been estimated that land loss due to gully erosion, sheet - rill erosions constitute about 5% degraded land (Ehiorobo and Izonyon, 2011). One of the most basic but non-renewable resource is soil and once lost, it is difficult and costly to replace within near future.

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A visible form of environmental degradation is waste disposed through open surface landfills or open dumps. These can be cleared up, the land cleaned up but a most visible and potentially dangerous environment degradation includes soil erosion and gully sites used as waste dumping points. Both soil and groundwater qualities can be costly and challenging to remediate or recover (Akujieze, 2006; Akujieze and Ezomo, 2010).

Gully erosion gnaws away massive earth system of an area, apart from geomorphological distortion, it opens up the geology and renders the groundwater systems highly vulnerable to contamination and pollution (Akujieze, 2004), especially when such open sites are used for waste disposal.

Typical example is the Benin West moat - Ekenwan gully that terminates at the headwater of Ogba River from where deep gully of about 1 km long had developed along the river valley. The situation in Benin City is that gully sites and the ancient Benin Moat often (10 to 30 m) deep and 2.00 to 5.00 m wide had been converted to flood channels and receptors to all types of wastes that vary from domestic, agricultural to industrial wastes. For decades, the moat has suffered erosion, gullying, sand quarrying and waste filling due to the pressure of urbanization. Some of the moat segments are perennially waterlogged with waste dumps with residential homes abounding along and around the moats. Some of the residential houses source their domestic water supply through boreholes not sufficiently distant from the moats.

The degradation effects of the use of Benin Moat - Ekenwan gully as waste disposal sites need to be investigated with particular reference to the soil and groundwater system to ensure safety of health and human life in the Urban City of Benin.

This study was aimed at determining the level of contamination of soil and groundwater by the waste disposal into moats/gully sites and environs. This would provide data for further environmental management, through effective waste disposal and groundwater protection for the area.

MATERIALS AND METHODS

The Benin West moat is a partially concentric open trench system dug in ancient times as defensive perimeters round the capital city-seat of power of the ancient Bini Kingdom. It consists of the North, South, East and West sectors. The one under study is the Western sector here referred to as Benin West Moat.

Focus of data acquisition was on map of the surface outline, geology of the Benin West Moat-Ekenwan gully area, the soil chemistry and the groundwater setting including the groundwater quality.

In determining the surface outline, geomorphology, geology and groundwater disposition of sub-basin of the Benin West moat: topographic map of Benin City sheet 258 on scale 1:50,000 was digitalized and gridded on mesh size of 500 m (Figures 1 and 2). Mapping was conducted for more precise spatial location and tracing of moat outline, roads, streets, soil and geological features. Horizontal and vertical height, measurements of positions were made using tapes and geographic positioning systems (GPS - Gemini model). Moat top surface, wall sides and bottom heights dimensions were recorded. Soil and rock horizons with depth were correlated to obtain a stratigraphic setting of the moat-gully system. Water table depths were determined to understand how close the water table is to the floor/bottom of the gully/moat (Figure 3).

The West Moat Ekenwan-Ogba River gully system lie within latitudes (6° 23′ 00″ - 6° 19′ 00″) N and longitudes (5° 34′ 00″ - 5° 36′ 00″) E in Egor-Oredo local government area of Benin City, covering an estimated area of 64.0×10^6 m (Figure 3). The Benin Westmoat traces the western sector of ancient Bini moat. It is traced from an area around Eghosa Grammar School, Okhoro - New Lagos Road Junction, then 800 m westerly across Ebo Street, then southerly besides Iyoha Road for about 1400 m to Textile Mill Road besides which it stretches westerly for about 1500 m into Aruosaghe Road. From there the moat continues southwesterly for another 2000 m across Siluko Road, Erhumwense, 2nd Cemetery Road in Owina Street and is routed for another 1800 m across Ekenwan-Geli-Geli-Sea port Road. The Ekenwan-Ogba gully system intensifies threatening to cut the road, at Ogba River/Spring head area and routes southerly for a distance of about 1005 m along the Ogba River valley. The Ogba River drains in a southerly direction (Figures 1, 2 and 3).

Random soil/sediment sampling was conducted by taking samples from the top 0.0m -0.5m moat/gully and >0.5 to 4.0 m at moat bottom/floor using hand auger, trowel and shovels. Disturbed soil samples were taken into polythene bags, sealed labeled and sent to Macgill Engineering for physicochemical and heavy mineral analysis whose mean results were compared with mean values of control samples of Anoliefo et al. (2001) and Akujieze (2004) to assess soil if contamination had occurred as a result of waste disposal into the moat and gully environs. These mean values were subjected to one-way statistical analysis of variance (ANOVA) at α of 5% significant level. Eight (8) groundwater samples were randomly drawn from residential pumping wells 0.5 to 10.0 m at both sides of the moat. Samples were drawn after two volume of well water was wasted. Groundwater samples for microbial tests were filled into sterile glass bottles and immediately sealed, while samples for dissolved metals were filled into 2 l PVC bottles, previously soaked in 10% nitric acid, rinsed with dilute ionized water and sealed. All the water samples were labeled and stored in iced coolers (0 to 4°C) and taken to the laboratory for analysis. Concentrations of Cr, Fe, Cu, Al, Cd, Zn, Pb and Mn were determined calorimetrically using 2D spectrophotometer. In determining the quality of groundwater, World Health Organization WHO (2011) and NIS (2007) permissible limits were used for comparison of chemical parameters. To understand groundwater contamination level, a one-way statistical comparison of means of heavy metal concentration through analysis of variance (ANOVA) at α of 5% significant level was conducted on the mean values of the present work and those of Akujieze (2004) and Akujieze and Oteze (2007), some four distant boreholes taken randomly far away from the moat - gully environment.

General geology

The study area is underlain by sedimentary Benin Formation which has been described severally in Short and Stubble (1967), Akujieze (2004), Akujieze and Oteze (2006).

Drifts and soil-cover characterized the formation over lateritized reddish brown clayey sand capping highly porous friable white sands, pebbly sands and clay stringers with basal indurated ferruginous pebbly - coarse grained sandstone. The Benin Formation is poorly bedded and occasionally cross - bedded at greater depths. Detailed geology and lithostratigraphy of Benin Formation are illustrated in Tables 1 and 2 and Figures 1, 2 and 3.

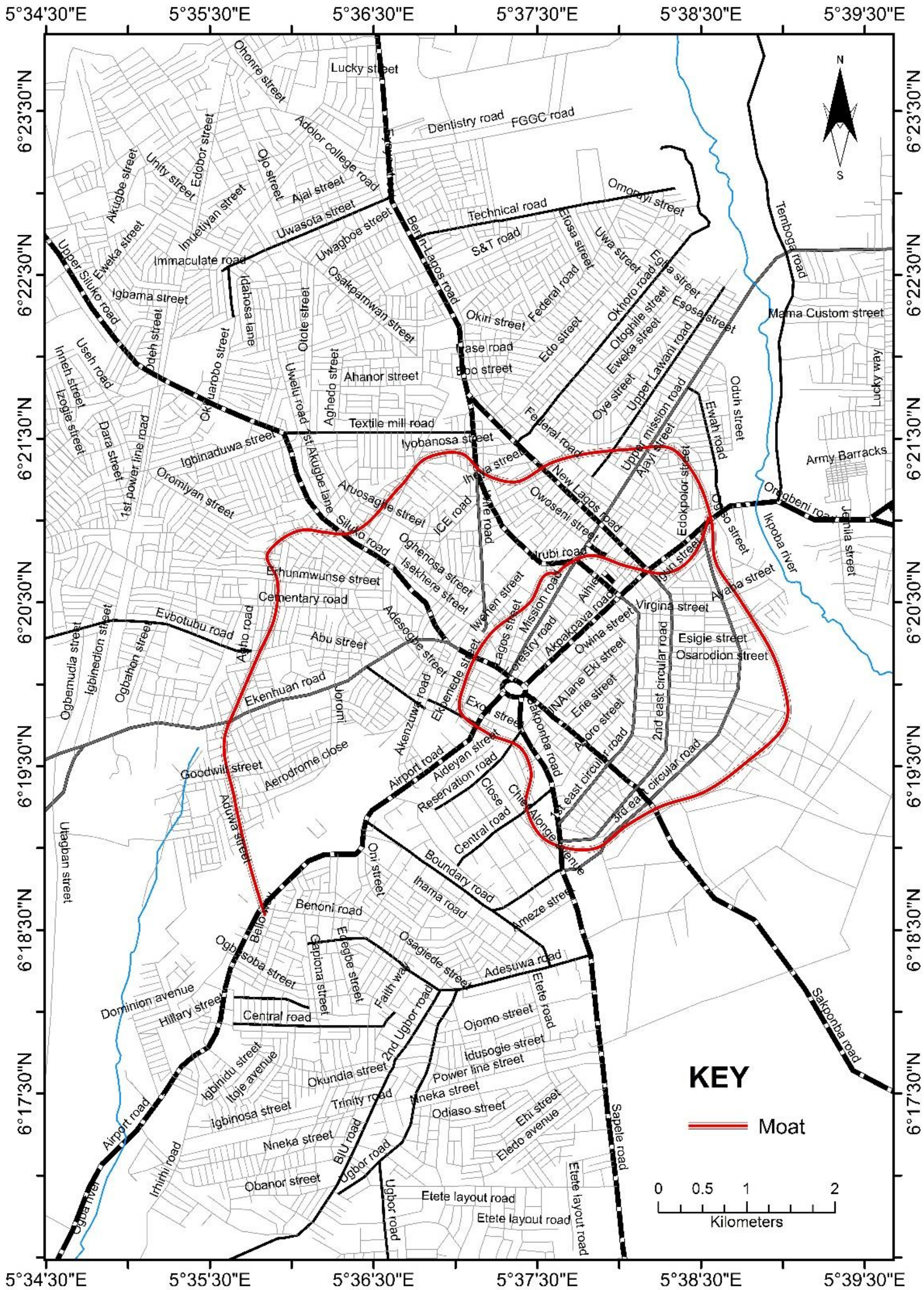


Figure 1. Map of Benin City town showing moat system.

RESULTS AND DISCUSSION

The Ekenwan gully geology is illustrated in Figure 3. The sedimentary units as shown in borehole lithology logs belong to the Benin Formation. It is remarkable that the logs revealed unconfined aquifer system are devoid of clayey stringers, which is significant in giving way to free downward percolation of water bearing contaminants (of heavy metals) into and across the water table.

The Ogba spring head is the target area for groundwater source in Ekenwan area of Benin. The area host a nest of boreholes serving Benin City Public water works. The water table there lies at 0.3 - 3.0 m below the ground surface with prolific yield of about 228 m^3h^{-1} and

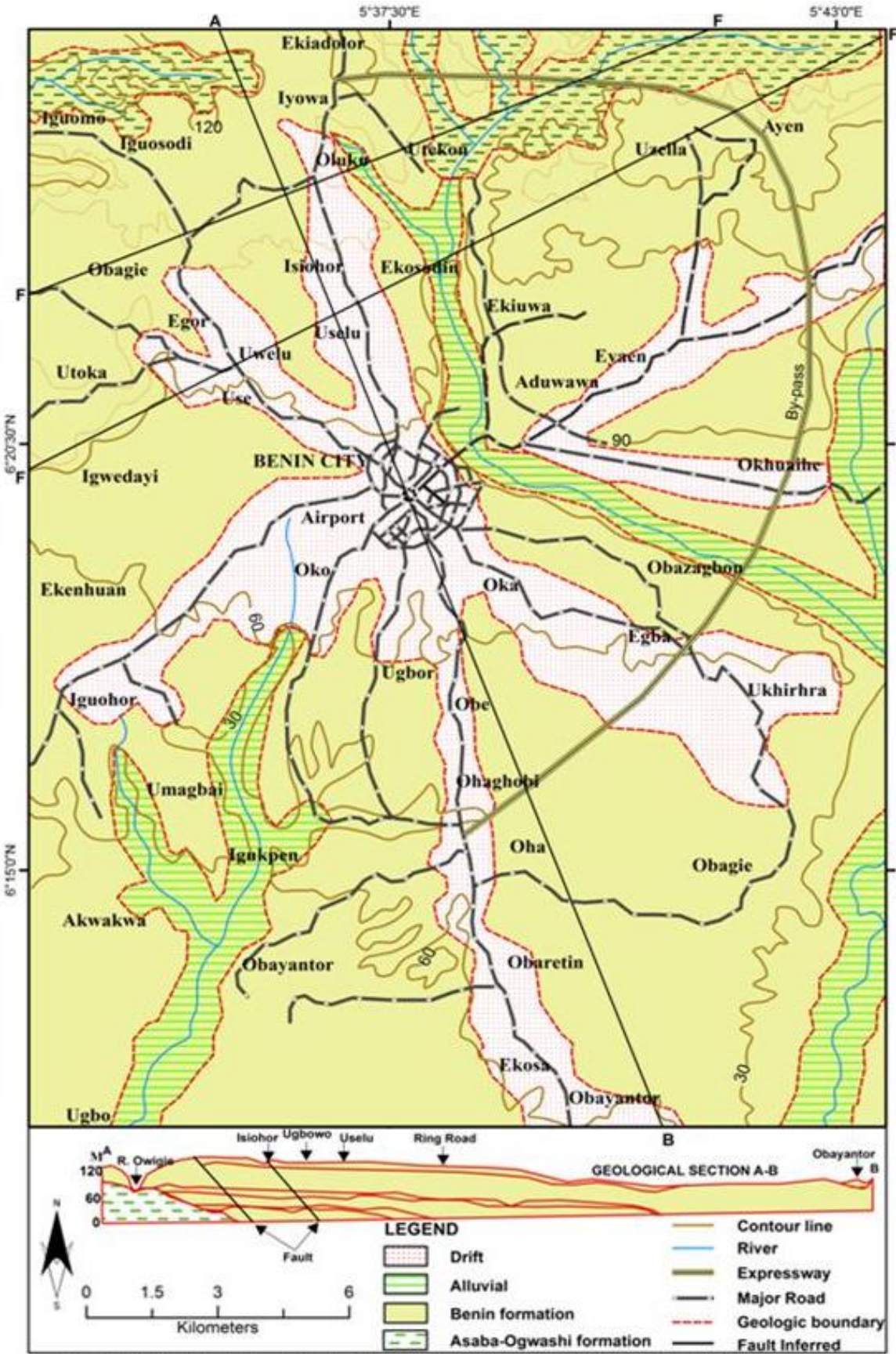


Figure 2. Geological map of Benin City and environs (Akujieze, 2004).

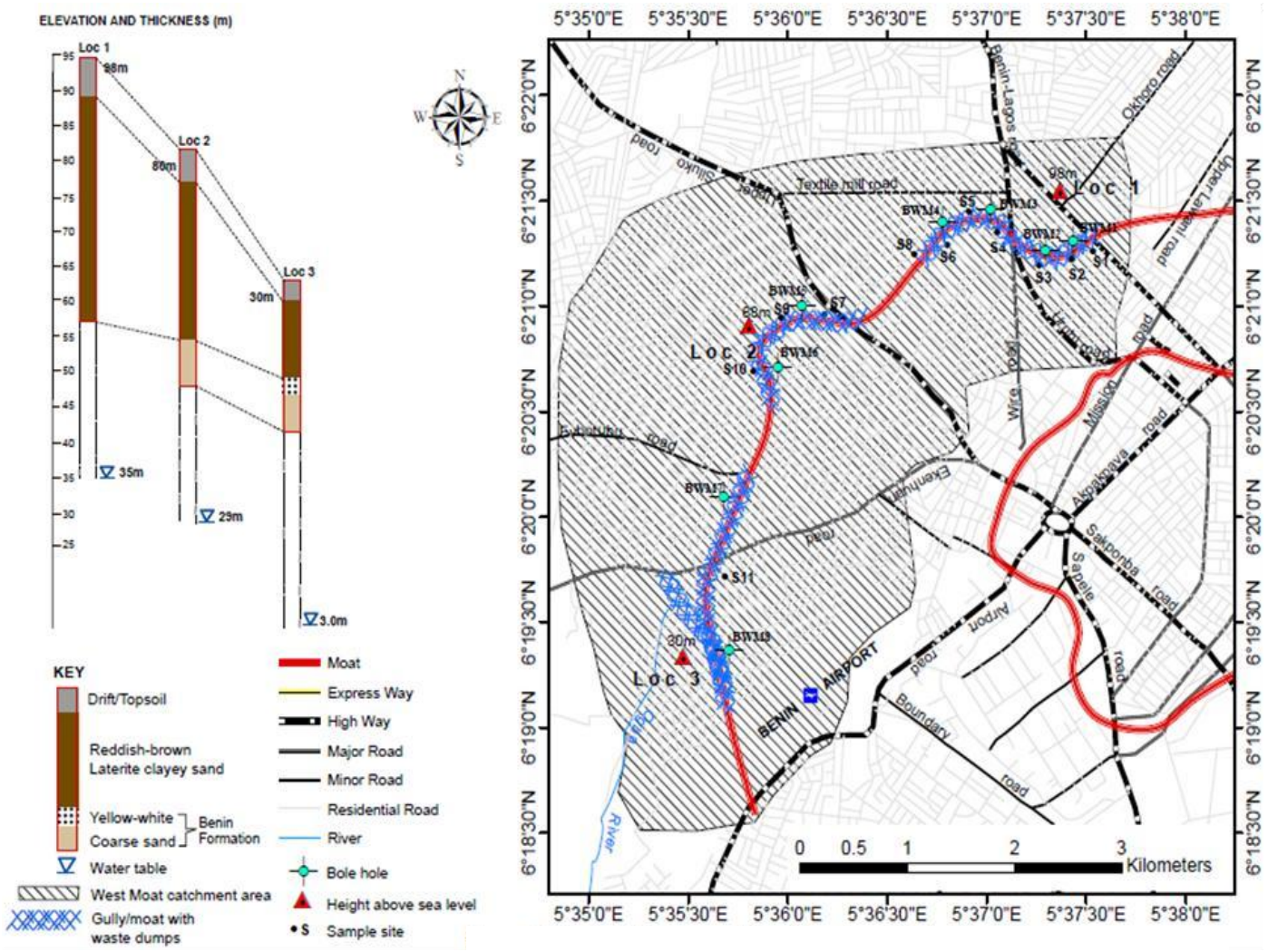


Figure 3. Geological section and stratigraphy of Benin west moat.

Table 1. Geology and Hydrostratography of Benin West Moat - Ekenwan Gully.

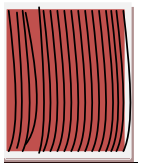
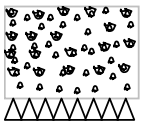
Sedimentary unit	Lithological description
Drift	Loose light gray-dirty white sands, silt, mudflows
Alluvium (Only at River Banks)	Light gray - brown - dirty white sands, silt, clays gravels and pebbles.
Benin Formation	Top reddish brown clay sand, capping thick sequences of poorly bedded friable - loose sand gravelly - pebbly sand and pinkish - white clay stringers.
Asaba-Ogwashi (Azagba-Ogwashi Formation)	Dark gray-woody clay, alternating with dark clay and lignite.

draw-down of 6.7 m (Akujieze and Oteze, 2006).

Soil quality

Tables 3 and 4 illustrate the soil chemical and heavy metals constituents of Benin West moat and Ekenwan Gully waste dumpsites. The background soil quality is taken from Anoliefo et al. (2001) while the control for the heavy metal constituents is taken from Akujieze (2004). The justification for these backgrounds is because of the following reasons: (1) They are prestine un-impacted locations within Benin City urban, (2) they are within the same tropical climatic conditions in terms of rainfall and temperature, (3) they are from the same Oxisol soil type;

Table 2. Typical Benin West Moat stratigraphy.

Age	Formation	Ground elevation and thickness (m)	Section	Unit		Rock description
Recent	Alleviation of top soil	95.00	~ ~ ~ ~ ~~ ~ ~ ~ ~~~	4	Drifts	Brown gray silty sand
		94.50		3	Top Soil	Sandy loam gray
Pleistocene-oligocene	Benin formation	92.00 82.00		2	Laterite	Top reddish brown clayey sand with mud crack in some places
		60.00 55.50 50.00 Not to scale		1	Sand	Yellow iron stained – white friable loose sand

the reddish brown lateritic tropical soil, (4) the soil type is underlain by the same parental Benin Formation rock type. (5) Time variant monitoring advantage can be obtained to understand levels of contaminant changes. Table 3 shows that the general trend in centimoles per kilogram of soil (c mol kg^{-1}) in the control area is Ca>Mg>k>Na that is, mean value of Ca (0.98)>Mg (0.40)>K (0.147)>Na (0.00) but this generally accepted trend (Ellis and Mellor, 1995) is not the same in the Benin West Moat-Ekenwan gully waste dump sites where the mean concentration is as follows; Na (2.70) > Ca (2.29) > K(1.22) > Mg (0.49) indicating that Na is more prominent. This upset suggests an impact. ANOVA results show that Na mean concentration occurs at P<0.001 indicating very high significant difference against control mean. While Ca, Mg, K, occur at P>0.05 indicating no significant difference. Total hydrocarbons (THC) occur in soil samples B_{WS} (7, 8, 9, 10 and 11) ranging from 0.11 to 0.24 mg/kg with an average concentration of 0.166 mg/kg which is significant. It indicates that the moat-gully site receives automobile waste products used auto oil and grease and other waste petroleum product. There is no significant difference (P>0.05) in the mean concentration of C (0.82%) in moat-Ekenwan gully dumpsites against (0.80%) in the control site, also (phosphorus) P (6.84 mg/kg) in moat against P (12.37 mg/kg) control mean value.

However, site-specific variation of phosphorus (P) indicates higher concentration (18.29 $mgkg^{-1}$) at shallower 0.0 to 10 cm depth of control site, than in deeper levels (Anoliefo et al., 2001). There is generally lower concentrations of C and P in soil, probably due to absence of or low organic wastes that generate C and P or due to leaching effect due to high rainfall in a predominantly sandy (791 to 94.1%) and porous soil. The Benin West moat-Ekenwan gully waste dumpsite soil has effective cation exchangeable capacity (ECEC) mean value of 3.77 (c $molkg^{-1}$) higher than control soil mean value of 2.42 c $molkg^{-1}$. Consequently, there is availability of more exchangeable H^+ ion of mean 0.33 c $molkg^{-1}$ above control level at P<0.05 indicating a significant difference.

There is higher concentration of Al^{3+}0.87 c $molkg^{-1}$ in the moat-gully waste dumpsites than in control soil site with mean of 0.45 c $molkg^{-1}$. This may have ensured higher acidity in the waste dumpsites of the moat-Ekenwan-gully erosion sites. According to Ellis and Mellor (1995), where exchangeable (H^+) ion is available in an already acidic soil, additional Al^{3+} ions play important role in generating more soil acidity. The hydrogen ion concentration pH in the control soil ranging between 5.3 to 5.9 with a mean value of 5.6 is higher than the moat-gully soil sites between 3.80 to 5.5 with mean of 4.57 which is more acidic than the control at P>0.05 indicating a significant difference which may have been dictated by waste disposal into the environment.

Table 4 shows that there is heavy metal build-up in soil of the Moat-Ekenwan gully waste dumpsites. Fe occurs with mean concentration of 729.65 mg/kg as against 0.075 mg/kg mean in control at P<0.001 very high significant difference. This compares favorably well with Fe in dumpsite in Imeokparia et al. (2009b). Mn with average value of 285.11 mg/kg against control average of 0.005 mg/kg at (P<0.001) indicating very high significant difference. As also Ni with mean of 0.84 mg/kg; both comparing fairly well with similar work in Benin City by (Ukpebor et al., in Imeokparia et al., 2009a) as illustrated in Table 5. Cr with mean concentration of 0.28 mg/kg

Table 3. Chemical properties of the soils and sediments from Benin West moat - gully Sites in comparison with means of Anoliefo et al., at α 5% significant value in one way ANOVA.

Sample Code	Depth (m)	pH	C	N	P	c molkg^{-1}						Clay	Silt	Sand	THC
			(%)	(%)	(mg/kg)	Ca	Mg	Na	K	H	Al	ECEC	(%)	(%)	(%)	
Bw_{s1}	0.0 - 0.5	4.10	0.22	0.011	6.79	1.92	0.56	0.28	0.14	0.40	0.56	3.30	19.9	1.0	79.1	ND
Bw_{s2}	0.0 - 0.1	5.50	0.74	0.058	26.96	5.04	1.12	0.27	0.15	0.30	0.55	6.88	9.9	2.5	87.6	ND
Bw_{s3}	0.0 - 0.5	4.30	0.65	0.047	3.11	0.72	0.40	0.26	0.19	0.20	1.20	2.97	19.9	1.0	79.1	ND
Bw_{s4}	0.0 - 0.5	4.10	0.51	0.039	2.54	0.64	0.32	0.25	0.11	0.90	0.40	2.62	20.9	0.5	78.6	ND
Bw_{s5}	0.0 - 0.5	3.80	0.35	0.022	2.77	0.80	0.64	0.28	0.13	0.60	0.50	2.95	17.9	1.0	81.1	ND
Bw_{s6}	0.0 - 0.5	4.10	0.54	0.038	2.69	0.72	0.48	0.26	0.07	0.30	0.90	2.73	19.9	1.0	79.1	ND
Bw_{s7}	0.0 - 0.5	5.00	0.86	0.067	17.73	4.40	0.32	0.27	0.22	0.10	0.96	5.31	13.9	2.5	83.6	0.11
Bw_{s8}	0.0 - 0.5	4.70	1.02	0.085	4.69	2.48	0.24	0.27	0.06	0.30	0.89	3.35	8.9	2.0	89.1	0.24
Bw_{s9}	0.0 - 0.5	4.90	1.15	0.093	3.51	3.20	0.16	0.28	0.07	0.10	1.58	3.81	4.4	1.5	94.1	0.18
Bw_{s10}	0.0 - 0.5	4.80	1.24	0.034	6.81	1.90	0.67	0.35	0.16	0.41	1.21	3.32	19.9	1.0	79.1	0.13
Bw_{s11}	0.0 - 0.5	5.00	1.75	0.042	3.68	3.40	0.58	0.29	0.18	0.35	0.89	4.30	20.9	0.5	78.6	0.17
Mean		4.572727	0.820909	0.04873	6.8436	2.2927	0.4991	2.70818	1.1245	0.33273	0.876364	3.776364	32.4	1.318182	82.645	0.166
SD	0.161245	0.523624	0.445252	0.02512	8.0725	1.558	0.2648	8.05673	3.2757	0.24017	0.359757	1.290041	55.84308	0.716684	5.3266	0.0503
K_a	0-10 cm	5.90	1.40	0.16	18.29	1.10	0.30	ND	0.19	ND	0.40	2.38	0.0	0.0	0.0	0.0
K_b	10-20 cm	5.30	0.20	0.18	6.50	0.87	0.51	ND	0.09	ND	0.50	2.46	0.0	0.0	0.0	0.0
Mean		5.60	0.8	0.17	12.37	0.985	0.405	ND	0.14	ND	0.45	2.42	0.0	0.0	0.0	0
KS-D		0.424264	0.848528	0.01414	8.3014	0.1626	0.1485	ND	0.0707	ND	0	0.056569	0.0	0.0	0.0	0
P. values		P<0.05	P>0.05	P<0.001	P>0.05	P>0.05	P>0.05	P<0.001	P>0.05	P<0.05	P>0.05	P>0.05				

Control (ka, kb) of Anoliefo et al. (2001), ND = not done, P>0.05 = no significant difference, P<0.05 = least significant difference, P<0.01 = high significant difference, P<0.001 = Very high significant difference.

against control value of 0.02 mg/kg and Cu 2.2 mg/kg against control value of 0.15 mg/kg both occurring at P<0.01 indicating high significant difference respectively. Pb average of 0.92 mg/kg against control concentration of 0.22 mg/kg and Cd mean value of 0.97 mg/kg against background of 0.47 mg/kg occur at P>0.05 indicating no significant difference. There is generally low value of Pb, Cd, Cu, Cr and Ni in the waste dump site. This could be explained by the tendency of increasing acidity associated with pH changes with attendant redox activity and probable metal speciation. According to Akujieze et al. (2012) bioavailability of bounding heavy metals can be altered by changes in pH, organic matter content, and the redox status of contaminated soils.

Chemical properties of groundwater

From the groundwater chemical constituents illustrated in Table 6, it could be observed that the hydrogen ion concentration pH varies from 6.40 to 7.06 with an average of 6.65 and may be described as slightly acidic on the average. This may be a reflection of an interaction between surface acidic soil environment and sub-surface groundwater. All the physico-chemical parameters and most chemical constituents except phosphorus and heavy metals like Fe, Pb, Cd and Al are within World Health Organization acceptance limit for potable water. Phosphorus occurs above European Economic Community (EC, 1980) permissible limit. There is presumptive coliform and Cd in all the groundwater samples, suggesting an impact from the disposal of waste into the Benin West Moat-Ekenwan Gully.

The heavy metals (Table 7) shows that Fe occurs above (WHO, 2011) permissible limit in boreholes 1, 2, 3, 4, 5 and 6 but at P>0.05 that is, no significant difference when compared with the Fe mean in Akujieze (2004). This implies that although Fe is increasing above WHO (2011) limit, its increase is generally not significant. While the mean of Cu in this work (0.0475 < 0.11 mg/l mean in Akujieze 2004) at P<0.01 indicating high significant difference and greater than mean of 0.03mg/l of Akujieze and Oteze (2007) both of which are below 2.0mg/l of WHO (2011) and so suggesting that Cu is not posing any threat of

Table 4. Heavy metals concentration (mg/kg) in the soils and sediment from Benin west moat-Ekenwan gully sites in comparison with means of (Akujieze, 2004) at α of 5% significant level in one way ANOVA.

S/N	Location and Depth(m) (pool samples)		Fe	Mn	Zn	Cu	Cd	Pb	Cr	Ni	Al
1	BW_{s1}	0.0 - 0.5	1121.45	413.52	248.71	3.50	1.89	1.25	0.44	6.13	0.0
2	BW_{s2}	0.0 - 0.5	403.50	217.80	125.60	2.27	0.0	0.94	0.32	5.18	0.52
3	BW_{s3}	0.0 - 0.5	948.70	335.90	278.20	3.15	0.75	0.26	0.41	5.45	0.31
4	BW_{s4}	0.0 - 0.5	621.50	294.20	180.50	1.84	0.49	0.39	0.19	7.40	0.11
5	BW_{s5}	0.0 - 0.5	723.60	258.40	145.00	1.20	0.72	0.30	0.15	6.48	0.23
6	BW_{s6}	0.0 - 0.5	745.70	286.20	175.80	1.12	1.65	0.75	0.14	4.87	0.40
7	BW_{s7}	0.0 - 0.5	801.20	293.00	195.80	0.97	0.77	0.81	0.30	6.18	0.29
8	BW_{s8}	0.0 - 0.5	723.50	216.80	140.20	1.36	0.92	1.74	0.15	4.31	0.13
9	BW_{s9}	0.0 - 0.5	620.70	189.30	120.00	2.52	0.81	1.35	0.30	6.72	0.27
10	BW_{s10}	0.0 - 0.5	680.50	250.50	205.50	3.80	1.81	1.55	0.41	6.8	0.35
11	BW_{s11}	0.0 - 0.5	635.80	380.60	188.60	3.20	0.95	0.83	0.30	4.8	0.28
Mean			729.65	285.1109	182.1736	2.266364	0.978182	0.924545	0.282727	5.847273	0.262727
SD			186.5758	69.72868	49.67139	1.035657	0.580031	0.501326	0.111184	0.984348	0.143881
K_{OT}		0.0 - 0.2	0.13	0.01	0.33	0.13	0.04	0.19	0.01	0.0	ND
K_{OB}		0.2 - 0.5	0.02	0.0	0.33	0.18	0.90	0.25	0.03	0.0	ND
Control Mean			0.075	0.005	0.33	0.155	0.47	0.22	0.02	0	ND
SD			0.055	0.005	0	0.025	0.43	0.03	0.01	0	ND
P. value			P<0.001	P<0.001	P<0.001	P<0.01	P>0.05	P>0.05	P<0.01	P<0.001	P<0.05

K_{OT}, K_{OB} = Control values of Akujieze (2004), ND = not done, P>0.05 = no significant difference, P<0.05 = least significant difference, P<0.01 = high significant difference, P<0.001 = very high significant difference.

Table 5. Concentration of Heavy Metals Mg/kg in Top Soil Samples in Refuse Dumps in Benin City. Adapted from; Imeokparia et al. (2009).

Dump site location	Distance from dump site (m)	Metal concentration mg/kg							
		Zn	Ni	Cu	Pb	Cr	Cd	Mn	Fe
Iyaro	0.00	-	130±3.3	30±1.0	159.54±14.22	120±3.30	10.0±1.20	294.5	-
	50.0	-	11.4±7.0	13.25±1.02	26.4±1.98	11.15±1.3	10.0±1.14	40±1.58	-
Siluko	0.00	-	708.0±17	16.7±0.64	63.90±2	24.0±2.3	29±0.98	344±15	-
	50.0	-	62.0±2.0	11.05±1.22	4.8±0.09	9.2±0.94	6.9±0.82	211±11.46	-
West Circular	0.00	-	54.0±1.74	30.0±2.36	80±3.22	35.0±3.0	7.30±0.99	228±4.4	-
	50.0	-	15.11±1.10	5.9±0.64	18.0±1.30	6.15±0.8	5.00±0.78	54.0±2.27	-

Table 6. Physiochemical parameters of groundwater along Benin West moat-Ekenwan gully sites Benin City.

S/N	Sample location	Colour Hazen	Turbidity(UTC)	Conductivity μScm^{-1}	Total solid mg/l	pH	Alkalinity (mg/l)	Total hardness (mg/l)	Chloride (Cl) mg/l	Nitrate (NO_3) mg/l	Sulphate (SO_4) mg/l	Phosphate (P_2O_4) mg/l	Sodium (Na) mg/l	Potasium (K) mg/l	Calcium (Ca) mg/l	Magnesium (Mg) mg/l	Iron (Fe) mg/l	Chromium (Cr) mg/l	Copper (Cu) mg/l	Zinc (Zn) mg/l	Lead (Pb) mg/l	Cadmium (Cd) mg/l	Aluminium (Al) mg/l	Presumptive coliform cfu/ml	E.coli
Bwm_1	Ebo Street	3.0	0.0	47.60	38.10	6.50	18.30	13.00	36.21	0.30	1.80	1.20	1.18	5.80	2.81	1.46	0.11^+	0.030	0.05	0.06	0.01	0.01	0.55*	$1.2\times10^{3+}$	0.0
Bwm_2	Eyobo area	3.0	0.0	49.90	40.90	6.60	24.40	24.40	26.63	0.25	1.70	1.90	3.04	6.70	8.42	0.73	0.15^+	0.021	0.04	0.04	0.02*	0.02	0.01	$1.0\times10^{2+}$	0.0
Bwm_3	Idugboe area	1.0	0.0	165.60	132.50	6.60	73.20	13.00	39.76	0.40	1.20	1.45	6.24	5.90	2.49	0.24	0.03	0.022	0.03	1.82	0.02*	0.01	0.65*	$1.3\times10^{3+}$	0.0
Bwm_4	Ehaikpen	0.0	0.0	96.10	77.10	6.50	24.40	23.00	35.86	0.20	1.30	1.80	1.88	4.33	5.61	2.19	0.25^+	0.036	0.03	1.34	0.01	0.01	0.48*	$1.4\times10^{3+}$	0.0
Bwm_5	Owigie	2.5	3.2	150	128	6.40	70.5	23.8	28.5	0.45	1.65	1.66	1.88	6.85	8.30	0.89	0.16^+	0.035	0.05	1.36	0.01	0.01	0.46*	$1.4\times10^{3+}$	0.0
Bwm_6	Owina	2.5	2.8	160	130	6.60	30.2	23.5	29.8	0.35	1.70	1.59	2.65	5.66	7.20	1.56	0.18^+	0.036	0.06	0.04	0.01	0.02	0.30*	$1.3\times10^{3+}$	0.0
Bwm_7	Erhumse	3.0	3.75	70	0.0	7.00	40.14	0.0	16.00	0.66	0.9	0.60	9.27	8.60	10.00	14.60	0.03	0.030	0.06	0.70	0.04*	0.02	0.48*	$1.5\times10^{3+}$	0.0
Bwm_8	Evbotobu	3.0	2.00	90	0.0	7.06	36.76	0.0	20.00	0.50	0.6	0.99	4.00	4.20	5.00	4.60	0.04	0.04	0.06	0.65	0.03*	0.02	0.55*	$1.5\times10^{3+}$	0.0
Mean		2.25	1.47	103.65	68.33	6.658	39.74	15.09	29.10	0.389	1.357	1.399	3.77	6.01	6.23	3.28	0.118^+	0.31	0.05	0.69	0.02	0.15	0.44	1212.5	0.0
WHO 2011		15.0	5.0	1,200	500	6.15-9.5	250.0	100.0	200.0	25.00	200.00	0.35^E	200.0	12.00	75.00	30.00	0.1	0.05	2.00	3.00	0.01	0.003	0.20	10.00	0.0
NIS 2007		3.0	5.0	1,000	1,500	6.0-8.5	100	50.0	250.0	50.0	100.0	-	200.0	10.0	75.0	20.0	0.3	0.05	1.00	5.00	0.01	-	0.50		0.0

$^+$Above WHO (2011) permissible limit for potable water E.E.C. (1980) Standard Limit European Economic Community. *Above WHO (2011) and NIS (2007) permissible limit for potable water.

Table 7. Heavy metals concentration (mg/l) and their mean values in groundwater along Benin west moat-Ekenwan Gully Sites in comparison with values from borehole sources far away from the moat in Benin City.

S/N	Location	Fe (mg/l)	Cu (mg/l)	Zn (mg/l)	Pb (mg/l)	Cd (mg/l)	Cr (mg/l)	Al (mg/l)	Mn
Bw_{H1}	Ebo Street	0.11	0.05	0.06	0.01	0.01^+	0.030	0.55^+	ND
Bw_{H2}	Eyoba	0.15	0.04	0.04	0.02^+	0.02^+	0.021	0.01	ND
Bw_{H3}	Idugboe	0.03	0.03	1.32	0.02^+	0.01^+	0.022	0.65^+	ND
Bw_{H4}	Ehaikpen	0.25	0.03	1.34	0.01	0.01^+	0.036	0.48^+	ND
Bw_{H5}	Owigie	0.16	0.05	1.36	0.01	0.01^+	0.035	0.46^+	ND
Bw_{H6}	Owina	0.18	0.06	0.04	0.01	0.02^+	0.036	0.30^+	ND
Bw_{H7}	Erhumse	0.03	0.06	0.70	0.04^+	0.02^+	0.030	0.48^+	ND
Bw_{H8}	Evbotobu	0.04	0.06	0.65	0.03^+	0.02^+	0.040	0.55^+	ND
$Mean_1$		0.12	0.05	0.69	0.02^+	0.02^+	0.03	0.44^+	ND
STD		0.08	0.01	0.60	0.01	0.01	0.01	0.20	0.0
$*B_{H30}$	Ugbiyoko	0.05	0.11	1.63	0.00	ND	ND	ND	0.01
$*B_{H31}$	Gapiona/Ugbor	0.03	0.05	1.31	0.01	ND	0.01	ND	0.01
$*B_{H35}$	Egor	0.03	0.12	0.32	0.01	ND	ND	ND	0.01
$*B_{H39}$	GRA/Ugbor	0.02	0.16	0.98	0.01	ND	ND	ND	0.03
$*Mean_2$		0.0325	0.11	1.06	0.0075	ND	0.0025	0.00	0.015
P. value		P>0.05	P<0.01	P>0.05	P>0.05	P>0.05	P<0.001	P<0.001	P<0.001
$Mean_3^{\#}$		0.08	0.03	0.40	0.04	0.00	0.00	0.00	0.00
WHO 2011		0.1	2.0	3.0	0.01	0.003	0.05	0.20	0.

*Akujieze (2004), $^{\#}$Akujieze and Oteze (2007). $^+$Above WHO (2011) permissible limits for potable water. ND = Not done, P>0.05 = No significant difference, P<0.05 = least significant difference, P<0.01 = high significant difference, P<0.001 = very high significant difference.

contamination. The same with Zn which occurs at P>0.05; no significant difference in the mean concentration of 0.688 mg/l against 1.06 mg/l of Akujieze (2004) and 0.40mg/l (Akujieze and Oteze, 2007) all < 3.0 mg/l of WHO (2011). Pb occurs at the threshold of 0.1 mg/l in almost all the groundwater samples, and groundwater from boreholes 2, 3, 7 and 8 contain Pb concentrations above (WHO, 2011) permissible limits. With the mean occurring at P>0.05, no significant difference; it implies that the aquifer is receiving a uniform influx of Pb, signaling potential Pb pollution with time if the source(s) of input (probably diffused) remain(s) unchecked. Cd occurred above (WHO 2011) 0.003 mg/l in all the eight groundwater samples at P<0.001 indicating very high significant difference. Al occurred at above WHO (2011) permissible limit of 0.2 mg/l in groundwater samples from boreholes 1, 3, 4, 5, 6, 7 and 8.

Conclusion

It is evident that waste disposal into the Benin West Moat - Ekenwan gully sites impacted soil and groundwater in the environment; by acidifying the soil pH and contaminating the soil with Fe, Pb, Cd and Al. The presence of total hydrocarbon THC (0.166 mg/l) can be critical if allowed to continue because of dangers of possible chlorination under the humid environment. Al in the waste dumps at P<0.05 when compared with other control means is significant and was reported by Anoliefo et al. (2001) in another location in Benin City, where high accumulating of Al in soil caused high phytotoxicity inhibiting root growth and plant uptake of water and nutrients. Al, Pb, Cd, are not only high in soil but also in groundwater of this study area where Al and Cd occur at P<0.001 in ANOVA comparison of means with reference means from other works in Benin City at α of 5% indicating very high significant difference. Apart from the phytotoxicity, long exposure of Al, Pb, Cd, Cu, Cr and Mn to man can pose serious health hazards. According to Erah et al. (2002), chronic exposure of man to Pb can cause Pb-poisoning, interference in red-blood cell chemistry, nervous system damage, mental defects, and IQ depressions in children. Cd in man and animals causes kidney and liver dysfunction, hypertension and heart diseases. Solutions include: (1) greater public awareness against indiscriminate waste disposal into the moats, (2) Clearing the moat of waste, (3) soil recovery and remediation, and (4) groundwater treatment and aquifer remediation. The handicap in this study includes lack of facilities for greater understanding of the speciation processes of the heavy metals in the waste soil. Therefore, there is need for provision of facilities and funds for more studies to facilitate soil/groundwater interaction for better groundwater quality monitoring to understand metal mobility and contamination toward better control and environmental management for soil and groundwater conservation.

Conflict of Interests

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

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Impacts of climate change on invasive *Lantana camara* L. distribution in South Africa

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Climate change and invasive species are now seen as two major contributors to global biodiversity change. The combined effects of these two factors have serious implications for biodiversity and agriculture. *Lantana camara* L. (*sensu lato*) (lantana) is a woody shrub that is highly invasive in many countries of the world including South Africa where it has a profound impact on biodiversity, water resources and agriculture. Strategies to manage and control this highly noxious weed will benefit from information on its likely potential distribution under current and future climate. CLIMEX, a species distribution modelling software, was used to develop a process-oriented niche model to estimate its potential distribution under current and future climate scenarios. Model calibration was carried out with phenological observations and geographic distribution records of lantana. The potential distribution of lantana under current climate showed a good match to its current distribution in South Africa. Under future scenarios, the climatically suitable areas for lantana were projected to contract in the northern provinces of Limpopo and Mpumalanga as well as coastal areas of Western Cape Province. However, lantana's potential distribution may expand further inland into new areas in KwaZulu-Natal and Eastern Cape provinces. The results suggest that lantana management initiatives in areas where climatic suitability is likely to decline should focus on controlling the density of invasion rather than curbing range expansion. On the other hand, areas where climatic suitability is projected to increase will require ongoing monitoring to prevent further range expansions.

Key words: CLIMEX, niche models, species distribution models, biotic invasions, weeds, climate change.

INTRODUCTION

The major reason for many deliberate introductions of non-native species throughout the world has been for the provision of benefits to human societies. Food, shelter and aesthetic enjoyment are included among these benefits. However, many of these introduced species have become invasive in natural as well as agricultural ecosystems (Groves et al., 2001). An invasive species is broadly defined as an introduced species that becomes established and spreads outside its native range (Jeschke and Strayer, 2005). Biological invasions have been the focus of much attention and research because it has led to increasing biotic homogenization of the Earth's

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flora and fauna (Hobbs, 2000; Mooney and Hobbs, 2000). The main impacts of invasive species are a global loss of biodiversity (Czech and Krausman, 1997; Dirzo and Raven, 2003) and alteration of ecosystem structure and function (Binggeli, 1996; Vitousek et al., 1997; Sutherst, 2000). Plant invaders, in particular, can impact native ecosystems through changes in fire regime, nutrient cycling, hydrology and energy budgets, thus causing a reduction in the abundance or survival of native species (Mack et al., 2000). The economic costs associated with biological invasions are also substantial due to lost yields and control efforts, particularly in agriculture (Vitousek et al., 1997). Furthermore, based on the overwhelming evidence for rapid climate change effects (IPCC, 2007), it is essential to consider the influence of climate change on the rate and extent of biological invasions (Walther et al., 2009). The immediate effect of climate change on invasive species will most likely be shifts in their distributions facilitated by changes in temperature and rainfall patterns that define their range boundaries. Climate change may favour species that can tolerate a wide range of climatic conditions and thus such species may have greater competitive success than most native species (Walther et al., 2009; Sutherst et al., 2007a).

Lantana camara L. (*sensu lato*) (lantana) is an invasive species that has had substantial negative impacts in many tropical and subtropical countries outside its native range of central and northern South America and the Caribbean. Sixty countries or island groups between 35°N and 35°S are included in its global distribution (Day et al., 2003). It has been ranked among the world's worst 100 invasive alien species (Lowe et al., 2000) while Sharma et al. (2005) considered it as one of the world's ten worst weeds. Its major impacts include a reduction in native species diversity, local extinctions, decline in soil fertility and allelopathic alteration of soil properties as well as an alteration of ecosystem processes (Day et al., 2003). It was introduced into South Africa in the mid 1800s for horticultural purposes (Richardson et al., 1997; Day and Neser, 1999) and due to its ability to hybridize easily with other entities, there are now reportedly up to 40 varieties in this country (Graaf, 1986). Lantana has been classified as a widespread species that has invaded many biomes including forest, savannah, fynbos, Indian Ocean coastal belt and grassland with the species being particularly prominent in the savannah and Indian Ocean coastal belt biomes (Vardien et al., 2012). Various studies have identified lantana as a major invader in South Africa (Richardson et al., 1997; Robertson et al., 2003; Nel et al., 2004), with over 2 million hectares invaded by this species (Le Maitre et al., 2000). Lantana is also listed as a category one weed (prohibited weeds that must be controlled in all situations) in the Conservation of Agricultural Resources Act. Furthermore, in an assessment of the potential impacts of plant invaders on biodiversity, water resources and rangeland productivity in South Africa, lantana scored the highest in terms of its impacts on biodiversity (Le Maitre et al., 2004). It also has a large impact on water resources by using up to 97.14 m^3 of the surface water resources (Le Maitre et al., 2000). This species has also been the subject of the most intensive biological control programme in South Africa (Richardson et al., 1997; Urban et al., 2011). In the last 23 years, 30 possible biological control agents have been evaluated and seven were found suitable for release into South Africa (Urban et al., 2011). However, only five of these have been established but they do not provide adequate control since they neither kill the lantana plants, nor stop the weed population increase (Day and Neser, 1999; Urban et al., 2011). The limited knowledge on the taxonomy of this species has made it difficult to select effective biological control agents and thus lantana is still not under adequate control and remains a problem in many areas of South Africa (Richardson et al., 1997; Urban et al., 2011).

Information on the expected potential distribution and relative abundance of this species under current and future climate scenarios is necessary for risk assessment and formulation of effective management strategies by biosecurity agencies in South Africa. Ecological niche models are useful tools in such instances (Peterson et al., 2011).

In simple terms, occurrence records of the target species in one region are used to calibrate the model and then projected onto other regions where the species may or may not currently be invasive (Peterson et al., 2011). Thus, the species' 'environmental envelope' or its preferred climate is inferred from its occurrence data (Barry and Elith, 2006). One major assumption that underlies such models is that climate is the primary factor defining the potential range of plants and other poikilotherms (Woodward, 1987). A range of software is now available which can be used to model species' current and future distributions (Kriticos and Randall, 2001; Hirzel and LeLay, 2008) one of which, CLIMEX, has been widely used to assess invasion risks from invasive alien species (Kriticos et al., 2011a; Chejara et al., 2010; Kriticos and Leriche, 2010; Taylor et al., 2012a, b). It is a mechanistic model (Hijmans and Graham, 2006) which is well suited for applications that involve transferability or projections of species distribution into novel environments (Randin et al., 2006), such as investigating the impacts of climate change on species' potential ranges (Kriticos et al., 2011a).

So, the objectives of this study were (i) to use the CLIMEX modelling package to develop a model of the climate responses of lantana, and (ii) use this model to assess the impacts of climate change on its potential distribution in South Africa using two global climate models (GCM), CSIRO-Mk3.0 and MIROC-H based on the A1B and A2 SRES (Special Report on Emissions Scenarios) emission scenarios for 2030, 2070 and 2100.

MATERIALS AND METHODS

CLIMEX software

CLIMEX for Windows Version 3 (Hearne Scientific Software, 2007; Sutherst et al., 2007a) was employed in model development of the potential distribution of lantana under current and future climate scenarios. The basis of this software is an eco-physiological growth model which assumes that a population experiences a favourable season with positive growth and an unfavourable season with negative population growth. Geographic distribution data and phenological observations are used to infer parameters that describe a species' response to climate (Sutherst et al., 2007b). The parameters can then be applied to novel climates so that the potential distribution of a species in new regions or under climate change scenarios can be deduced (Kriticos et al., 2011a). The temperature (temperature index) and moisture (moisture index) requirements of a species are used to determine the potential for population growth during favourable climate conditions, termed the annual growth index (GI_A). The likelihood of survival during unfavourable conditions is described by four stress indices (cold, wet, hot and dry) and up to four interaction stresses (hot-dry, hot-wet, cold-dry and cold-wet). Weekly calculations of the growth and stress indices are combined into an overall annual index of climatic suitability, the ecoclimatic index (EI), which is theoretically scaled from 0 to 100. An EI value of zero indicates that the species will not be able to survive at that location, 1-10 indicate marginal habitats, 10-20 can support substantial populations while EI values >20 are highly favourable (Sutherst et al., 2007b). A detailed description of parameters is provided in Sutherst and Maywald (1985). The methodology described in Sutherst and Maywald (1985), Kriticos et al. (2011a) and Shabani et al. (2012) was used to fit the CLIMEX parameters for lantana.

Meteorological data and climate change scenarios

The CliMond 10′ gridded climate dataset (Kriticos et al., 2011b) was employed to carry out the modelling component of the study. In this dataset, historical climate (averaging period 1950-2000) is represented by five variables, average minimum monthly temperature (*T*min), average maximum monthly temperature (*T*max), average monthly precipitation (*P*total) and relative humidity at 09:00 h (*RH*09:00) and 15:00 h (*RH*15:00). Potential future climate in 2030, 2070 and 2100 is represented by the same five variables, based on the CSIRO-Mk3.0 and MIROC-H Global Climate Models (GCM) (Gordon et al., 2002) with the A1B and A2 SRES scenarios (IPCC, 2000). The A1B scenario depicts a balanced use of fossil and non-fossil resources in the future whereas the A2 scenario depicts a varied world with high population growth coupled with slow economic development and technological change. The major reason for not including the B family of scenarios in this study was based on the findings that recent global temperature increases were much higher than the hottest IPCC scenarios (Rahmstorf et al., 2007).

CLIMEX parameters

The Global Biodiversity Information Facility (GBIF) is a database of natural history collections around the world for various species and it is available for download. A total of 4126 records on the global distribution of lantana were downloaded from GBIF. However, only 1740 of these were used in parameter fitting and the others were discarded since many records did not have geolocations or were repetitions. Of these, 1139 were native and 601 were exotic records. Data on the alien distribution of lantana were also used for fitting stress parameters (SAPIA, 2006; Press et al., 2000; Chen and Gilbert, 1994; Thakur, 1992; Jafri, 1974; Biswas, 1934). Inclusion of native and alien distribution data in model parameterization ensured that the complete range of environmental conditions in which lantana may occur was covered. Seasonal phenology data from the southern states of Brazil (Winder, 1980, 1982) was used to fit growth parameters. Australia has extensive distribution data on lantana and this was reserved for model validation and thus not used in parameter fitting. Iterative adjustment of each parameter was conducted until a satisfactory match was obtained between the potential and known distribution of lantana in these areas, that is, to ensure that the maximum number of occurrence points fell within the modeled distribution. The aim was to achieve maximum EI values near known large and healthy populations and to minimize EI values outside the recorded distribution of lantana.

Stress parameters

The southern limits of lantana distribution in Argentina and northern limits in Nepal, Pakistan and China were defined by applying two cold stress mechanisms. The cold stress temperature threshold (TTCS) was set at 5°C with the frost stress accumulation rate (THCS) at -0.004/week based on the observation that lantana seldom occurs where temperatures frequently fall below 5°C (Cilliers, 1983). Furthermore, the cold-stress degree-day threshold (DTCS) was set at 15°C days, with the stress accumulation rate (DHCS) set at -0.0022/week so that the potential distribution was restricted to the known southern limits in Buenos Aires and northern limits in India, Nepal and China. The heat stress parameter (TTHS) was set at the same level as the limiting high temperature (DV3), 33°C, with a stress accumulation rate (THHS) of 0.001/week. This setting allowed lantana to persist along the Western Ghats (Murali and Sidappa Setty, 2001) as well as in Bengal and Assam in India where it is reportedly common (Biswas, 1934). The dry stress parameter was set at the same level (0.1) as the lower soil moisture threshold (SM0) with the stress accumulation rate set at -0.01/week. This excluded the species from the drier western parts of South Africa where it survives only as an ornamental plant (Cilliers and Neser, 1991). The wet stress threshold (SMWS) was set to 1.6 and the accumulation rate (HWS) set at 0.01/week. These were fitted based on the observations that lantana can tolerate up to 3000 mm of rainfall per year as long as the soil is not waterlogged for prolonged periods (Day et al., 2003; Thaman, 1974). These settings allowed the species to grow well in Indonesia and the Philippines (Holm et al., 1991) as well as in central Burma, but excluded it from the wetter coastal areas (Biswas, 1934).

Growth parameters

The limiting low temperature (DV0) was set at 10°C based on Winder (1980) observation that 'cold winter temperatures caused cessation of growth with a substantial loss in leaves and side-branches'. This was based on seasonal phenology data from Iguazu (25°33′S, 54°34′W) in Brazil where winter temperatures can get as low as 8°C and also Stirton (1977) observation that in South Africa, lantana is found in areas with a mean annual surface temperature greater than 12.5°C. The 10°C value was chosen as a compromise between the South African distribution data and the phenology data from Iguazu. The limiting high temperature DV3 was set at 33°C based on summer temperatures in Iguazu which rarely exceed 33°C and where lantana grows rapidly during summer (Winder, 1980). The lower (DV1) and upper (DV2) optimal temperatures were set at 25 and 30°C, respectively, based on seasonal phenology at Iguazu, and these provided a good fit to the observed South American, Asian and South African distribution. The lower moisture threshold (SM0) was set at 0.1 which excluded

Table 1. The CLIMEX parameter values that were used for *Lantana camara* L; taken from Taylor et al. (2012a).

Parameter	Mnemonic	Value
Limiting low temperature	DV0	10°C
Lower optimal temperature	DV1	25°C
Upper optimal temperature	DV2	30°C
Limiting high temperature	DV3	33°C
Limiting low soil moisture	SM0	0.1
Lower optimal soil moisture	SM1	0.5
Upper optimal soil moisture	SM2	1.2
Limiting high soil moisture	SM3	1.6
Cold stress temperature threshold	TTCS	5°C
Cold stress temperature rate	THCS	-0.004 /week
Minimum degree-day cold stress threshold	DTCS	15°C days
Degree-day cold stress rate	DHCS	-0.0022 /week
Heat stress temperature threshold	TTHS	33°C
Heat stress temperature rate	THHS	0.001 /week
Dry stress threshold	SMDS	0.1
Dry stress rate	HDS	-0.01 /week
Wet stress threshold	SMWS	1.6
Wet stress rate	HWS	0.01 /week

lantana from the drier western parts of South Africa where it survives only as an ornamental (Cilliers and Neser, 1991). Lantana grows well during the months of January to March in Iguazu (Winder, 1980) and thus the lower (SM1) and upper (SM2) optimum moisture thresholds were set at 0.5 and 1.2, respectively, to improve species growth during these months. The upper soil moisture threshold (SM3) was set at 1.6 to permit growth in the Philippines and Indonesia where it has been reported as a troublesome weed (Holm et al., 1991). The parameters were checked to ensure that they were biologically rational (Table 1). They were then used to model potential lantana distribution in South Africa under the reference climate (averaging period 1950-2000) as well as climate change scenarios. For a more detailed explanation of the parameter-fitting procedure, refer to Taylor et al. (2012a, 2012b) and Taylor and Kumar (2012).

RESULTS

Historical climate

There is a good match between the current global distribution of lantana and the modelled global climatic suitability (Figure 1). The CLIMEX modelling shows that large parts of the tropics and subtropics have suitable climatic conditions for lantana. Most of the central, eastern and parts of western Africa as well as Madagascar show climatic suitability for this species. The southern states of USA, large parts of South and Central America and Asia are also projected to be climatically suitable. Although there is a good match between the current global distribution and the modelled global climate suitability, this does not account for the occurrence records from Mediterranean Europe because lantana is mostly grown as an ornamental plant in this region (Garibaldi et al., 2008). Furthermore, parts of Africa that have been projected as climatically suitable do not show many occurrence records because there is a chronic lack of data across much of Africa, where lantana is certainly present. Model validation was conducted using the distribution data for Australia as these records were not used for parameter fitting. The occurrence records match well with the modelled climate suitability for the continent (Figure 2), and the present Australian distribution is consistent with the Ecoclimatic Index. Approximately 87% of the occurrence records fall within the suitable and highly suitable categories.

The occurrence records (Figure 3) agree well with the modelled climate suitability (Figure 4) and the present South African distribution is consistent with the Ecoclimatic Index. The model suggests coastal areas of KwaZulu-Natal, Eastern Cape and Western Cape provinces to be climatically suitable. Climatic suitability is also suggested for parts of Northern provinces such as Mpumalanga, Limpopo and the North West. Central and western provinces such as the Free State and Northern Cape are projected as being unsuitable, primarily due to dry or cold stress.

Future climate

The potential distribution of lantana under future climate scenarios show a substantial contraction in climatically suitable areas in the northern provinces of Limpopo and Mpumalanga as well as coastal areas of Western Cape

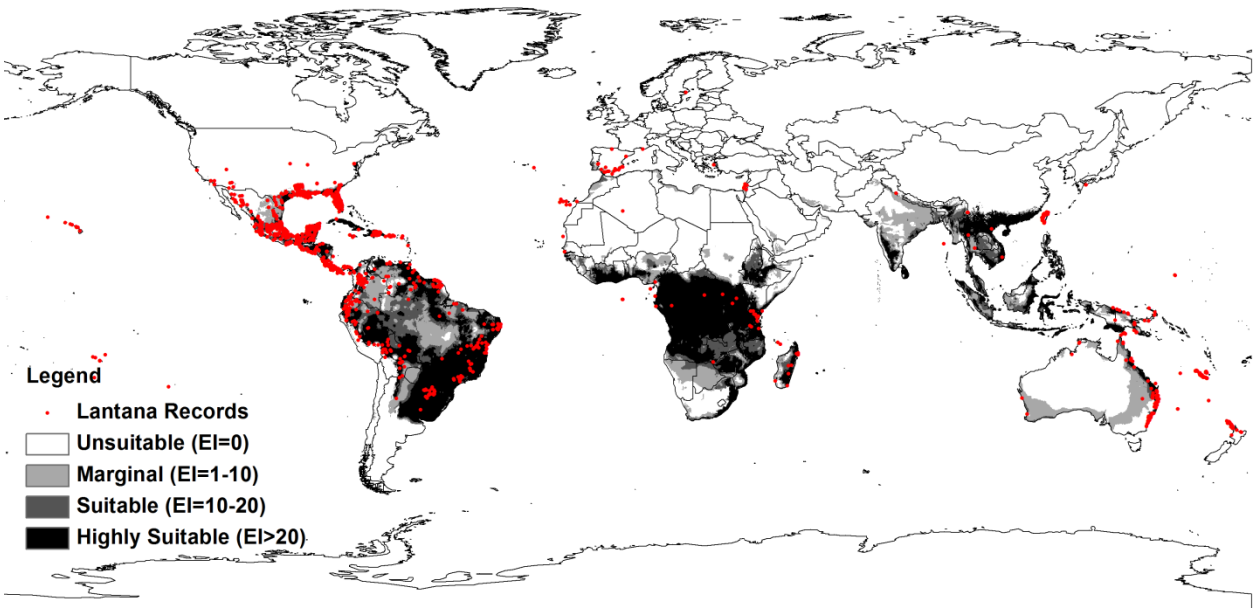

Figure 1. The current global distribution of lantana based on records taken from the Global Biodiversity Information Facility 2007 together with the current potential global distribution of lantana modelled by CLIMEX for reference climate (averaging period 1950-2000).

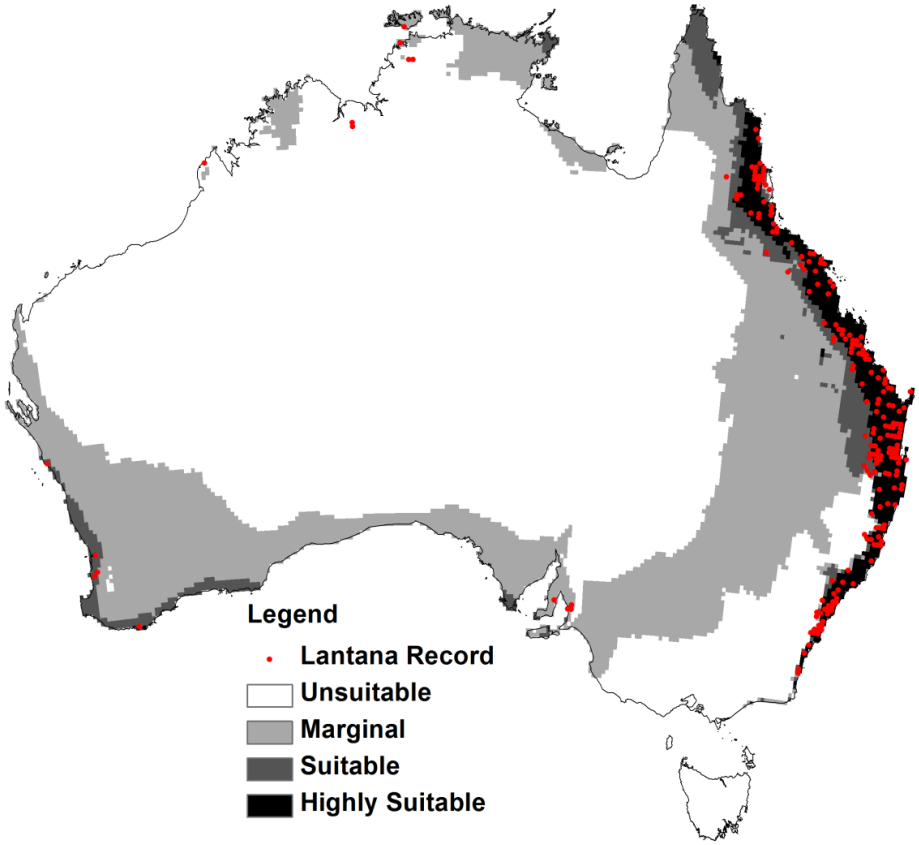

Figure 2. Current Australian distribution of lantana based on records from Australia's Virtual Herbarium together with the current potential distribution of lantana modeled by CLIMEX for reference climate (averaging period 1950-2000).

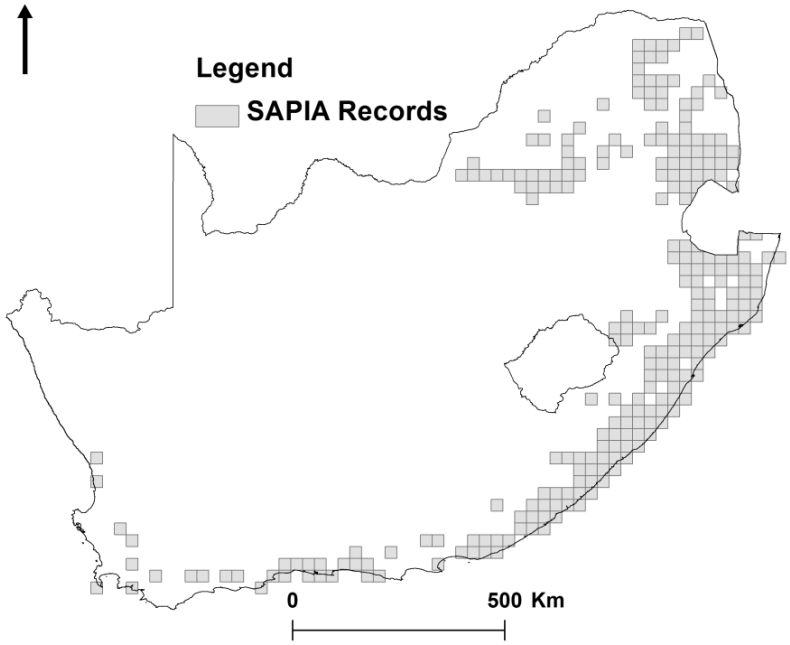

Figure 3. Distribution and relative abundance of lantana in Southern Africa (Southern African Plant Invaders Atlas Database, 2006).

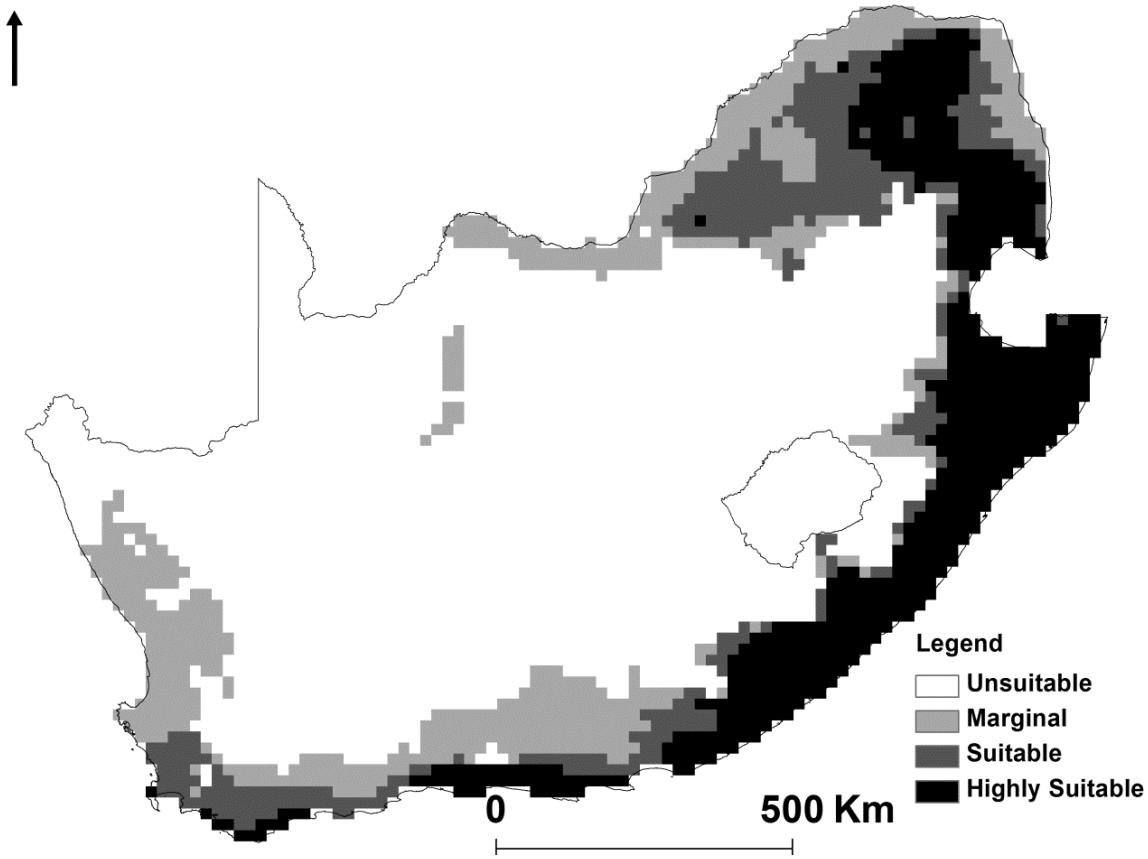

Figure 4. The climate (EI) for lantana in South Africa based on CLIMEX under historical climate (averaging period 1950-2000).

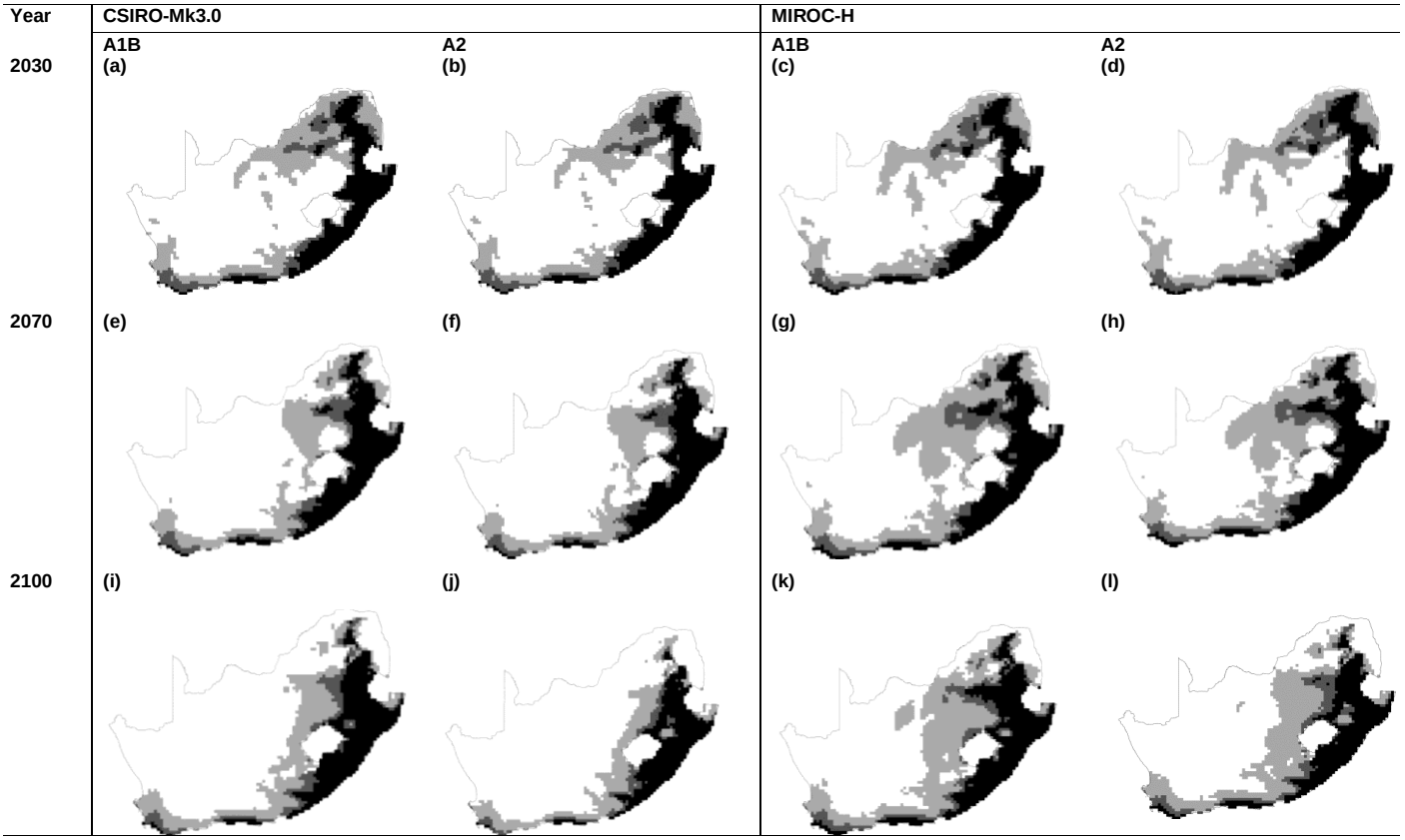

Figure 5. The climate (EI) for lantana in three time periods projected using CLIMEX under the CSIRO-Mk3.0 and MIROC-H GCM running the SRES A1B and A2 scenarios.

province and this trend was exacerbated by 2100 (Figure 5). The contraction in climatically suitable areas was more pronounced in the results shown by the CSIRO-Mk3.0 GCM as compared to the MIROC-H GCM. However, very little variation was seen between the two emission scenarios. The results also show that, in the future, lantana's potential distribution may expand further inland into new areas in KwaZulu-Natal and Eastern Cape provinces. This projection was consistent for both the GCMs.

DISCUSSION

The validation using Australian distribution data showed a good match with the modelled climate suitability for the continent. A good fit was also observed between model output and the current global distribution records as well as the current South African distribution. Under current climate, the model projects coastal areas of KwaZulu-Natal, Eastern Cape and Western Cape provinces and parts of Northern provinces such as Mpumalanga, Limpopo and the North West to be climatically suitable. A previous study (Rouget et al., 2004) that utilized climatic envelope models (CEMs) to assess the potential distribution of invaders (lantana was one of the species that was assessed) in South Africa found that under current climate, some of the worst perceived invaders in the country had less potential to increase in range as compared to other species (Le Maitre et al., 2000, 2004). Lantana was identified as one of such species. Our assessment of potential lantana distribution under current climate agrees with this assessment. Based on a comparison of Figures 3 and 4, lantana appears to have spread to occupy its potential range. However, it could continue to invade new habitats and increase its density within this range. Furthermore, the results of the climate change modelling show the potential for substantial range expansion in KwaZulu-Natal and Eastern Cape provinces, an assessment also shown in a study conducted by Vardien et al. (2012). Therefore, it would be prudent to formulate management strategies that would prevent lantana from expanding its range in the Eastern Cape and northern KwaZulu-Natal. These could include the formation of strategic containment lines or quarantine barriers. Land managers in these regions need to be alerted to the long term threat and undertake on-going monitoring of the identified areas. In such instances, the

maps resulting from this study are useful tools for informing individuals and organizations involved in invasive species management. Furthermore, in the short term (2030), provinces such as Limpopo, Mpumalanga and Western Cape remain at risk of invasion consistently under both GCMs although their climatic suitability becomes diminished by 2100. These areas would benefit from a concerted effort of weed control measures in the short term. This would be an effective strategy in terms of reducing impacts on natural resources since climatic suitability is projected to contract for these areas by 2100.

Two factors may affect the accuracy of the results presented here based on the assumptions underlying the modelling process. First, CLIMEX does not explicitly incorporate the effects of non-climatic factors that affect species' distributions such as dispersal potential, biotic interactions, topography, land-use and disturbance activities. The main assumption underlying this modelling environment is that climate is a major determinant of species' distributions although other factors can be considered in a stepwise fashion after the climate modelling has been completed. Second, the results are only indicative of the direction and magnitude of change that may be expected in the future due to the uncertainties associated with the state of climate modelling and uncertainty in future global greenhouse gas emission patterns (Kriticos et al., 2006). The maps show areas of climatic suitability for lantana and are not predicted future distributions. The dispersal capability of lantana and efforts on the part of land managers to curb its proliferation may cause the actual range of the species to fall below the potential.

Lantana has had a profound negative impact on biodiversity, water resources and agriculture in South Africa (Le Maitre et al., 2000, 2004), and, thus, the potential distribution maps presented here can be used to develop broad strategic control plans so that the management of this noxious weed can be adapted to the challenges of climate change. In particular, they can inform decisions concerning the effective allocation of resources for weed management in the short term and also the long term. An additional impact of climate change will be on biocontrol agents that are being used for biological control of lantana since the distribution of such agents will also likely alter with climate change (Kriticos et al., 2009). This is particularly pertinent given the considerable amount of resources that have been used in the biological control of lantana in South Africa. Ongoing monitoring of current lantana biological control programmes will be essential so that changes may be detected early and appropriate action taken. Moreover, disturbance plays a key role in the spread of lantana (Day et al., 2003, Stock et al., 2009), and, therefore, it would be practical to focus management strategies on reducing disturbance. This is particularly true for protected areas such as national parks and nature reserves as these contain environmental assets of high conservation value. For example, roads and rivers may provide channels for disturbance through propagule dispersal or creation of open spaces (Alston and Richardson, 2006). Therefore, eradication of existing infestations around such disturbances together with on-going monitoring to avoid re-infestation should form part of the management strategy to reduce the chances of further spread through protected areas. There will always be a need to control lantana in areas containing biodiversity of high conservation value because of its characteristics as a highly competitive weed. In such cases, the results from this study can inform targeted management actions, particularly where climatic suitability has been projected to increase in conservation areas under climate change. However, a recent study has shown that, despite intensive management of lantana in Australia, India and South Africa, little evidence exists for success. An adaptive management approach has been suggested which focuses on the positive qualities of this species rather than the negative qualities (Bhagwat et al., 2012). The results presented here can also be useful under such a scenario by identifying areas where such innovative management approaches can be tested without further endangering biodiversity.

Conflict of Interests

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

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Full Length Research Paper

Culture-dependent characterization of hydrocarbon utilizing bacteria in selected crude oil-impacted sites in Bodo, Ogoniland, Nigeria

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This investigation was carried out to characterize microbial communities in selected crude oil polluted sites in Bodo community, Gokana Local Government Area of Rivers State, Nigeria. Total heterotrophic bacterial counts ranged from 0.7 to 1.37x10^7 cfu/g and 0.2 to 5.9x10^6 cfu/ml while counts of hydrocarbon utilizing bacteria ranged from 0.1 to 8.0 x 10^6 and 0.2 to 7.5 x 10^5 cfu/ml for soil, sediment and water, respectively. Physiochemical parameters of all samples were determined. The ranges obtained were temperature 31-33°C, pH 7.5-8.2, conductivity 1134 - 7680 µs/cm, total nitrogen 792.4 - 886.3 mg/kg, nitrate 36.55 - 42.70 mg/kg, total organic carbon 2.06 - 2.18%, total petroleum hydrocarbon 1007 - 1104 mg/kg, vanadium 0.001 - 0.007 mg/kg, iron 3.772 - 4.889 mg/kg, chromium 52.40 - 66.20 mg/kg, nickel 40.02 - 41.62 mg/kg, lead 17.30 - 19.40 mg/kg and zinc 35.10 - 39.50 mg/kg for soil and sediments while water had total nitrogen 868 mg/l, nitrate 40.6 mg/l, total organic carbon 3.1 mg/l, total petroleum hydrocarbon 768 mg/l, nickel 39.2 mg/l, lead 17.3 mg/l and turbidity 250 NTU. Bacteria isolates characterized belonged to these genera *Bacillus, Proteus, Pseudomonas, Flavobacterium, Corynebacterium, Serratia, Micrococcus, Klebsiella, Enterobacter* and *Azotobacter.* The findings reveal that there is a high population of active indigenous hydrocarbon utilizing bacteria which can be monitored and enhanced to bring about bioremediation in the study area.

Key words: Hydrocarbon pollution, soil, water, sediments, hydrocarbon utilizing bacteria, Bodo, Ogoniland.

INTRODUCTION

Petroleum is at present, Nigeria's and indeed the world's most important derived energy source (Moffat and Linden, 2005). However the growth and activities of petroleum and petroleum associated industries in Nigeria and in other parts of the world has lead to increased oil pollution in our environment. Crude oil, because of its characteristics is one of the most significant pollutants in the environment as it is capable of causing serious damages to humans and the ecosystem (Okpokwasili, 1996).

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Oil producing areas of Nigeria especially the Niger-Delta area have experienced the devastating consequences of crude oil spills to both terrestrial and aquatic environments in the past 50 years of crude oil exploration and production (Adati, 2012). One of the major reasons for prolonged negative impact of oil spill on the environment could probably be absence of adequate and qualitative scientific baseline data which is required to provide informed and quick response to emergent environmental challenges (Akinde et al., 2012). The Niger Delta is among the ten most significant wetland and marine ecosystems in the world but unsustainable oil exploration activities has rendered the Niger Delta region one of the five most severely petroleum damaged ecosystems in the world (FME, 2006).

Ogoniland is located in the Niger Delta and oil exploration and production activities have been ongoing in this area since the 1950s. Ogoniland is now characterized by oil fields and installations that have remained dormant for several decades. Past spills, lack of maintenance, oil trapping and damage to oil infrastructures have been a common sight in this region and the environment has been without remediation or partially remediated over the years. UNEP report (2011) concluded that pollution of soil by petroleum hydrocarbons in Ogoniland is extensive in land areas, sediments and swampland. The investigation also showed that the surface water throughout the creeks contains massive hydrocarbons.

Hydrocarbons interact with the environment and micro-organisms determining the fate of the contaminant relative to their chemical nature and microbial degra-dative capabilities respectively. Provided the polluted has requisite values for environmental factors that influence microbial activities and there are no inhibitors of meta-bolism, there is a good chance that there will be a viable and active population of hydrocarbon utilizing micro-organisms in the environment (Chikere et al., 2011, 2012b). Considering the large quantity of oil going into the Niger Delta environment especially farmlands and rivers, the need to cleanup crude oil contaminated sites has become a key environmental issue (Vincent et al., 2011).

Conventional methods such as physical removal are the first response option. It is worthy to note that they do not achieve a complete cleanup of the oil spills. Current mechanical methods typically recovers not more than 10-15% of crude after a major spill and almost always leaves the receiving body in worse conditions (Abu and Dike, 2008).

Due to the abilities of certain microbes to mine-ralize hydrocarbon components into environmentally friendly substances such as carbon dioxide and water, the ability of bacteria in breaking down hydrocarbons has gained growing attention in modern day research (Wackett and Hershberger, 2001; Kadali et al., 2012). Biodegradation by microbes is the key removal process of hydrocarbons which is controlled by hydrocarbon physicochemistry, environmental conditions, bioavailability and the presence of catabolically active microbes (Stroud et al., 2007).

This study was conducted to ascertain the microbial diversity associated with the chronically oil-inundated Bodo community in Ogoniland and to some extent ascertain their natural propensity to utilize petroleum hydrocarbons.

MATERIALS AND METHODS

Sampling

One sample each of crude oil polluted water, soil and sediment were collected under aseptic conditions from Bodo community in Ogoniland, Rivers State in the Niger Delta using appropriate equipments. Soil was collected at 0-15 cm soil auger into sterile polyethylene bags. Sediment was collected with an Eckman grab while water was collected into sterile bottles. Samples were collected at different parts of each site, bulked for homogeneity and thereafter transported to the laboratory at 4°C in ice pack.

Determination of physicochemical parameters of samples

Physicochemical parameters such as pH, moisture content, nitrate, phosphate, total organic carbon (TOC), turbidity, salinity, temperature, conductivity and heavy metals were determined using methods from APHA (2008).

Chromatographic analysis

Residual total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) were extracted from the samples and quantified using gas chromatograph-flame ionization detector (GC-FID).

Enumeration of total heterotrophic bacteria

Total heterotrophic bacterial (THB) counts were determined using spread plate method on plate count agar (PCA). From each sample 1 g or 1 ml was homogenized in 9 ml of 0.85% normal saline using Heindolph vortexing machine. Decimal dilutions (10-fold) of the suspensions were plated out on agar medium and incubated at 30°C for 24 h. The colony forming units were afterwards enumerated.

Enumeration of hydrocarbon utilizing bacteria

Hydrocarbon utilizing bacteria (HUB) were enumerated by a method adopted from Hamamura et al. (2006) which involved the dilutions of appropriate sample suspensions and plating out on Bushnell-Haas agar (Sigma-Aldrich, USA). Hydrocarbons were supplied through the vapour phase to putative hydrocarbon utilizers by placing sterile Whatman No.1 filter papers impregnated with 5 ml Okono crude oil on the lids of the inverted plates and incubated for 14 days at 30°C.

Purification and characterization of hydrocarbon utilizing bacteria

Discreet colonies of different HUB were randomly picked using a sterile inoculating wire loop and sub cultured for purification by

Table 1. Physicochemical parameters.

Parameter	Sediment	Soil	Water
pH	7.86	5.40	7.66
Electrical conductivity (µS/cm)	7240	1134	3159
Total nitrogen (mg/kg)	886.25	867.20	672.2
Total phosphorous (mg/kg)	11.2	60.4	4.92
Total organic carbon (%)	2.18	5.3	3.1
Nickel (mg/kg)	41.62	41.5	39.2
Lead (mg/kg)	19.40	17.3	17.3
Zinc (mg/kg)	39.5	36.5	32.4
Salinity (PPT)	NA	NA	19.66
Turbidity (NTU)	NA	NA	250
TPH (mg/kg)	1104	1007	768
PAH (mg/kg)	92.6	53.25	85.56

Total 2. Heterotrophic and hydrocarbon utilizing bacterial counts.

Sample	Mean values of THB	Mean values of HUB
Soil (cfu/g)	$1.0x10^7$	$0.5x10^6$
Water (cfu/ml)	$3.1x10^6$	$3.9x10^5$
Sediment (cfu/g)	$2.7x10^6$	$1.0x10^6$

streaking on nutrient agar plates and incubated at 30°C for 24 h. Individual colonies were predominantly identified using biochemical tests as described in Bergy's Manual for Determinative Bacteriology (Holt et al., 1994).

Degradation screening

Representative HUB isolates were further screened for oil degradation capability under aerobic conditions by inoculating a calibrated loop full of 18 h old culture of each hydrocarbon utilizing bacterium into Bushnell Haas Broth containing 1 ml of Okono medium crude oil. Biodegradation was scored by turbidity and emulsification of oil-in-mineral broth medium after 14 days incubation at 30°C (Kostka et al., 2011).

RESULTS AND DISCUSSION

Soil, sediment and water physicochemical parameters are shown in Table 1. The parameters determined indicated that the samples had been exposed to hydrocarbon contamination with traces of other organic and inorganic contaminants (Chikere, 2010, 2012a).

These pollutants cause damages to humans and the ecosystem if not effectively remediated. The contamination may have resulted in the low pH of 5.40 observed in soil as compared to pristine soil while pH in sediments and water were observed to be 7.86 and 7.66, respectively which is neutral to slightly alkaline. Previous studies have demonstrated that the pH range optimal for biodegradation of hydrocarbons is 6-7 (Eweis et al., 1998; Aparna et al., 2010). Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants especially nitrogen, phosphorus and in some cases iron. Addition of nutrients is necessary to enhance biodegradation of crude oil pollutants.

The presence of microbial activity was determined by the enumeration of culturable total heterotrophic bacteria and total hydrocarbon utilizing bacteria as presented in Table 2 and soil sample recorded highest cfu count for THB with a mean value of 1.0×10^7 cfu/g and sediment highest in HUB count with a mean value of $1.0 \times 10^{6.}$ A similar observation was reported by Ibiene et al. (2011) and Eze and Okpokwasili (2011).

The high counts recorded in soil sample could be attributed to the myriad of nutrients, high organic matter concentration and other ecological factors that influence the survival of soil microorganisms that play important roles in the decomposition and recycling of nutrients. Continuous input of petroleum-based pollutants usually results in an enriched microbial community capable of surviving toxic contamination. The difference between THB and HUB counts was observed to be minimal/-insignificant which suggests that most of the micro-organisms present in the various sample sites are hydrocarbon degraders that can withstand the concentration of crude oil and also use them as source of carbon.

Totally, 47 pure cultures were able to grow on mineral salt medium (Bushnell Haas Agar) with crude oil as carbon source and were identified using phenotypic and biochemical tests. The population of culturable hydrocarbon degraders from soil, water and sediment samples investigated showed that majority of the bacteria were Gram negative belonging to *Gamma proteobacteria* group, this corroborates with the findings of Kaplan and Kitts (2004) although some Gram positive isolates were also observed. A total of 30 isolates had the ability to utilize crude oil and they belonged to the genera *Bacillus, Proteus, Klebsiella, Pseudomonas, Micrococcus, Serratia, Enterobacter, Flavobacterium, Corynebacterium* and *Azotobacter* as presented in Table 3. The flora represents the normal heterotrophic bacteria present in the various samples.

Majority of these organisms isolated were *Proteus*, *Enterobacter* and *Pseudomonas*. These predominant genera isolated have been shown to contain high numbers of oil degrading species from oil polluted sites, and this gives evidence that these species are probably the active degraders in this environment. These isolates have also been demonstrated by other researchers to be hydrocarbon degraders (Sarma and Sarma, 2010; Ebrahimi et al., 2012). Watanabe (2001) isolated *Micrococcus*, *Pseudomonas* and *Bacillus* from sediments as marine petroleum hydrocarbon degraders. Chikere et al. (2009) reported the prevalence of *Flavobacterium*, *Enterobacter, Norcardia* and *Acinetobacter* in a hydrocarbon

Table 3. Characterization of bacterial isolates.

Isolate code	Gram reaction		Tentative identity	Degradative screening
HUBSED1	+	R	*Corynebacterium* sp.	Y
HUBSED2	+	R	*Corynebacterium* sp.	Y
HUBSED3	-	R	*Serratia* sp.	Y
HUBSED4	-	R	*Pseudomonas* sp.	Y
HUBSED5	-	R	*Proteus sp.*	Y
HUBSED6	-	R	*Flavobacterium* sp.	Y
HUBSED7	-	R	*Proteus* sp.	Y
HUBSO1	-	R	*Proteus* sp.	Y
HUBSO2	-	R	*Enterobacter* sp.	Y
HUBSO3	-	R	*Enterobacter* sp.	Y
HUBSO4	-	R	*Flavobacterium* sp.	Y
HUBSO5	-	R	*Pseudomonas* sp.	Y
HUBSO6	+	R	*Bacillus* sp.	Y
HUBW1	+	C	*Micrococcus* sp.	Y
HUBW2	+	R	*Bacillus* sp.	Y
HUBW3	-	R	*Klebsiella* sp.	Y
HUBW4	-	R	*Enterobacter* sp.	Y
HUBW5	-	R	*Serratia* sp.	Y
HUBW6	-	R	*Proteus* sp.	Y
HUBW7	-	R	*Azotobacter* sp.	Y
HUBW8	+	C	*Micrococcus* sp.	Y

Y = Yes, R = rod, C = cocci.

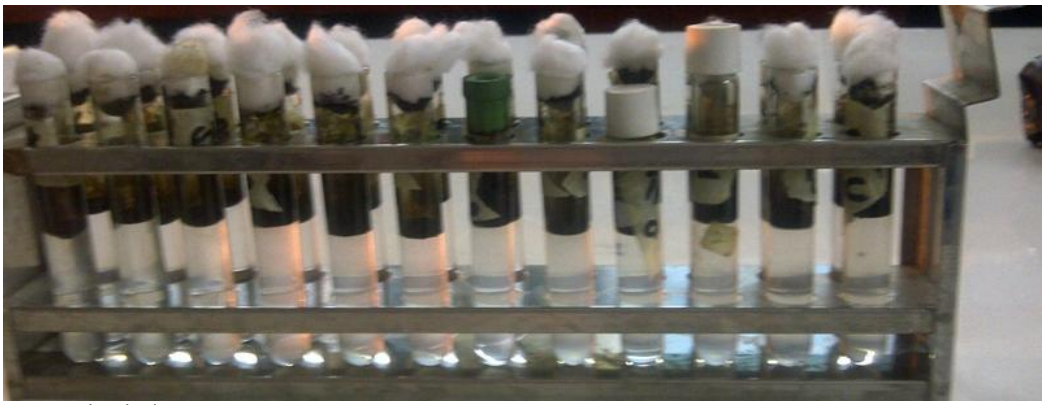
polluted marine sediment undergoing bioremediation. In a related study conducted by Ibiene et al. (2011) at Aluu and Mogho communities in Port Harcourt, Rivers State, Nigeria, *Micrococcus, Bacillus, Corynebacterium, Vibrio, Pseudomonas* and *Flavobacterium,* were isolated from a contaminated soil undergoing bioremediation by natural attenuation. Obire and Nwanbeta (2002) also reported the isolation of *Serratia, Pseudomonas, Proteus, Klebsiella*, *Micrococcus* and *Staphylococcus* species from samples collected from petroleum hydrocarbon contaminated soil in Port Harcourt while Eze and Okpokwasili (2010) isolated *Flavobacterium, Proteus, Bacillus, Klebsiella, Lactobacillus* among other bacteria from Okpoka-Woji river sediment serving as a sink for industrial effluents. Table 3 also indicates that the crude oil degradative ability of the individual bacterial isolates was significant as evidenced by turbidity and emulsification of 1 ml of crude oil in 10 ml of Bushnell-Haas broth after 14 days incubation when compared with the test isolates on day zero incubation as presented in Figure 1. Bacteria that are capable of utilizing hydrocarbons as energy and carbon sources in broth culture have been shown to produce bioemulsifiers or bio-surfactants that assist in the transport of hydrocarbons into the cell via efficient uptake systems (Atlas and Philip, 2005; Olga et al., 2008; Satpute et al., 2010; Cho et al., 2011). Previous studies have demonstrated that the bacterial genera characterized in the present investigation contain known hydrocarbon utilizing species (Chaillan et al., 2004; Brito et al., 2006; Olga et al., 2008; Kadali et al., 2012).

Conclusion

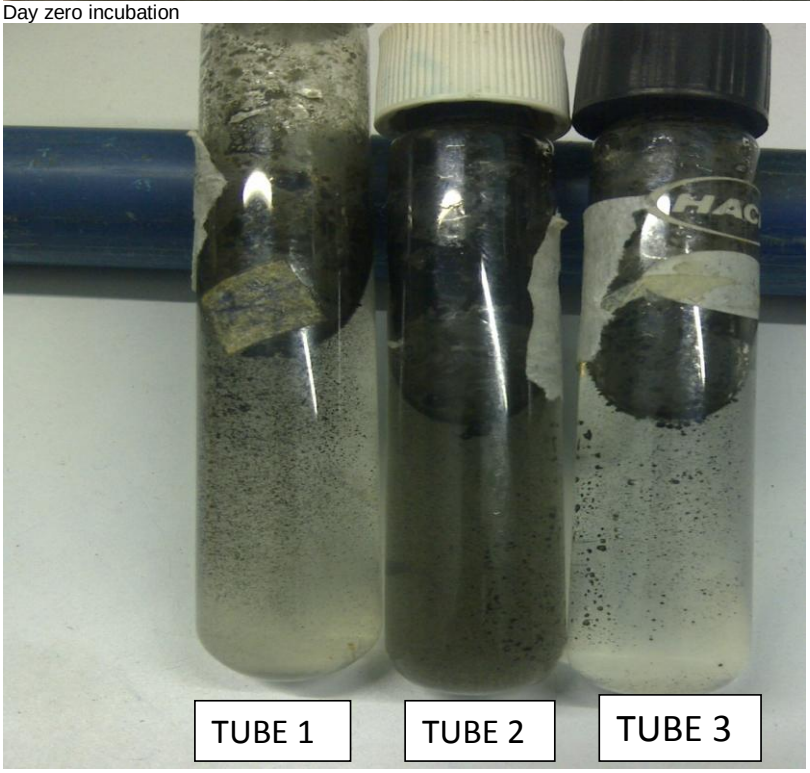
One of the major reasons for prolonged negative impact of oil spill on the environment is probably the absence of adequate and qualitative scientific baseline data. These findings have revealed that there is an appreciable population of indigenous hydrocarbon utilizing bacteria in oil-polluted sites in Bodo which can be monitored and enhanced to increase the bioremediation rate in this chronically oil-impacted area. it is pertinent to study the community dynamics of hydrocarbon degrading bacteria in oil-polluted ecosystems using cultivation-independent 16S rRNA-gene-based and functional-gene-based methods in order to fully undertsand the biochemical reactions that underpin hydrocarbon degradation during bioremediation projects (Chikere, 2013).

Conflict of Interests

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



Day zero incubation



Day 14 incubation

Figure 1. Biodegradation screening of hydrocarbon utilizing bacteria. Day zero incubation of isolates in test tubes in rack above. Day 14 incubation showing crude oil emulsification by specific isolates. Tube 1: Crude oil degradation by HUBSED2 (*Corynebacterium* sp.); Tube 2: Crude oil degradation by HUBSED4 (*Pseudomonas* sp.); Tube 3: crude oil degradation by HUBSO4 (*Flavobacterium* sp.).

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