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Full Length Research Paper

Southern Ethiopia Rift Valley lake fluctuations and climate

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This study describes hydrological fluctuations of Lake Abaya-Chamo via direct local measurements in relation to time-integrated climate anomalies. Reconstruction of an index involved compositing lake level and flow discharge station records in the period 1983-2009. Satellite and model interpolated rainfall, latent heat flux and run-off anomalies follow the hydrological records when summed over and lagged by one year. Correlation of ECMWF climate fields reveals that east Pacific and subtropical Atlantic surface temperature are influential on continuous flow discharge, while the Indian Ocean dipole has influence in September to November season. A predictive algorithm accounts for 44% of inter-annual fluctuations in the period 1983-2009. Rapid increases in lake level coincide with maturing El Niño and Indian Ocean dipole, bringing floods for example in October 1997. Early season dry spells prevail when the equatorial trough remains over Tanzania, and dry northerly winds accelerate evaporation such as in March 2000.

Key words: Ethiopia rift lakes, hydrological fluctuations.

INTRODUCTION

Ethiopia's Rift Valley contains many lakes and minor rivers, surrounded by > 2000 m mountains that induce seasonal rainfall, and lowlands prone to drought. Each year the rivers swell and the lakes rise ~ 0.5 m, but in some years the flow doubles while in others it is halved, stressing agrarian production. Much attention is given to the Nile River discharge from northwestern Ethiopia (Quinn, 1992; Eldaw et al., 2003; Potter et al., 2004) and its modulation by global climate (Camberlin, 1997; Camberlin et al., 2001; Segele et al., 2009; Jury, 2011) and local evapo-transpiration (Zeng and Eltahir, 1998; Zeng et al., 1999). Most of Ethiopia has a unimodal wet season (June-September), when surface winds arrive

from the Congo Basin and an upper easterly jet pulses every few weeks. Diurnal heating promotes late evening thunderstorms (Seleshi, 1991; Gebremariam, 2007) and run-off with an efficiency of 18% (Roskar, 2000; Dettinger and Diaz, 2000).

In contrast, the south-facing parts of the Ethiopian highlands that extend to the Kenya border (Figure 1a) experience a bi-modal wet season with peaks following equinox. One of the largest lake systems in the southern Rift Valley is Abaya-Chamo next to the urban center Arba Minch. The basin falls within the East African climate regime that is modulated by equatorial signals such as the Pacific El Niño and Indian Ocean dipole. Studies of

this lake system have found fluctuations in level attributable to land-use and climate (Servat et al., 1998; Awulachew, 2001, 2006; Schutt et al., 2002; Ayenew, 2002, 2009; Ayenew and Becht, 2007). The level of Lake Abaya-Chamo has been measured since 1970; abstraction and manipulation can be considered as limited. Its level has risen at the same time the local population has tripled (to 170/km²), contributing to deforestation and sedimentation (Belete, 2009). Lake Abaya spills southward into Lake Chamo via Kulfo (Schutt and Thiemann, 2006), forming a single basin with a water volume of 13 B m³ (Figure 1c). Lake Chamo, in turn, overflows via Woito to Lake Chew-Bahir in the arid Turkana Valley. Lake Abaya receives inflow from the north via the Bilate River and from smaller rivers (Gidabo, Gelana, Hare) draining mountains to the east and west, yielding a run-off of ~750 m³/year from rainfall that varies from 0.5 to 2 m/year over a basin of area 18,600 km² (Table 1). Gebremariam, (2007) reports peak rain rate of 23.7 mm/h in April with high suspended lake sediment (< 10 cm transparency depth). Potential evaporation losses are relatively steady and peak at 0.2 m/month in February-March when mean air temperatures and surface wind speeds reach 26°C and 2 m/s (Belete, 2009).

The objective of this paper is to reconstruct the fluctuations of Lake Abaya-Chamo using direct measurements supplemented with model estimates, to analyze seasonal to interannual variability and its global climate forcing, and to understand the meteorological processes underlying wet and dry spells, extending the work of Belete (2009) and the German Technical Cooperation with Arba Minch University.

MATERIALS AND METHODS

Ethiopia's southern rift valley lakes of Abaya and Chamo (5.6-6.9°N, 37.3-38.3°E) are the focus of this study (Table 1). Their daily levels and discharge are monitored by the Ministry of Water Resources: Lake Abaya has three level-recording stations, while Lake Chamo has one. Flow discharge is measured at nine stations, and three have complete records: two downstream (south) of Abaya, and one south of Chamo. Studies by Schutt et al. (2002) and Goerner et al. (2009) indicate that Lake Abaya-Chamo has risen (1.37 m) in recent decades due to a combination of tectonic uplift and sediment accumulation at the southern outlet. Following quality checks, averaging of overlapping data and linear interpolation of short gaps, complete time series were reconstructed from 1983 to 2009 to study hydrological fluctuations. Daily values were subsequently averaged to monthly (N=312) for analysis of seasonal to interannual variability.

In-situ hydrological records were supplemented with NASA satellite altimetry and gravity measurements (Tapley et al., 2004; Cretaux et al., 2011; Velpuri et al., 2012); and GPCCv6 gauge interpolated rainfall observations (Schneider et al., 2013) in the Abaya-Chamo basin (5.6-6.9°N, 37.3-38.3°E, Figure 1a,b). Area-averaged latent heat flux (LHF) and run-off estimated from the Global Land Data Assimilation System NOAHv2 model are employed (Rodell et al., 2004). Supporting datasets include: MODIS surface temperatures and enhanced vegetation index (EVI) (Huete et al., 2002), European Community reanalysis fields

Table 1. Lake Characteristics.

Parameter	Abaya	Chamo
Altitude (m)	1169	1110
Combined area (km ²)	18,600	
Mean length (km)	79	34
Mean width (km)	14	10
Max depth (m)	24	14
Volume (m ³)	9.8 x10 ⁹	3.2 x10 ⁹

(ECMWF) (Dee et al., 2011), NCEP-Coupled Forecast System (CFS) reanalysis fields (Saha et al., 2010) and National Climate Data Center (NCDC) v3 surface temperature, (Smith et al., 2008).

Cross-correlation of lake level and flow discharge with meteorological variables (Figure 3c) determined that a 1 year lagged running sum of GPCC6 rainfall, NOAH2 LHF and run-off anomalies best represents the hydrology. Hence their standardized departures were averaged with the reconstructed lake level and integrated flow discharge (Figure 2a, b) to create a continuous index with minimal trend (Figure 4a). This time series correlates above 50% with SST in the West Indian Ocean and tropical East Pacific.

To understand global climate influences on Abaya-Chamo hydrology, cross-correlation maps were computed at 6-month lead time. Candidate predictors were identified from the maps (Table 2, Figure 5a-c) and a multi-variate regression was calculated following the methods of Eldaw et al. (2003). Wet and dry spells were identified using streamflow records (Figure 2a) and their climate forcing was studied, with a focus on links to the Indian Ocean.

RESULTS

Basin characteristics

Ethiopia is blessed with ample water resources to due its mountainous topography and tropical latitude (Figure 1a). Yet in the Rift Valley, reduced orographic forcing of convection means that rainfall averages ~ 50 mm/month (Figure 1b). The Abaya-Chamo basin's mean satellite vegetation cover (EVI) and day-time surface temperature is illustrated in Figure 1c, d, while characteristics are listed in Table 1. The vegetation fraction varies from <0.2 in the northern valley where land surface temperatures exceed 35°C, to >0.6 in the cool (20°C) eastern mountains. The annual cycle (not shown) is warmest 40°C in early March and coolest 20°C in late July, with vegetation reaching a minimum 0.2 in early March and a maximum 0.4 in late June. Most years exhibit a uni-modal distribution. Lake surface temperatures are ~10°C cooler than the surrounding valley and consistent with nearby mountains, giving rise to local circulations and convective patterns embedded in a prevailing southeasterly wind (Figure 1b) with an upslope component.

Hydrology observations and annual cycle

The raw monthly *in-situ* records on flow discharge and

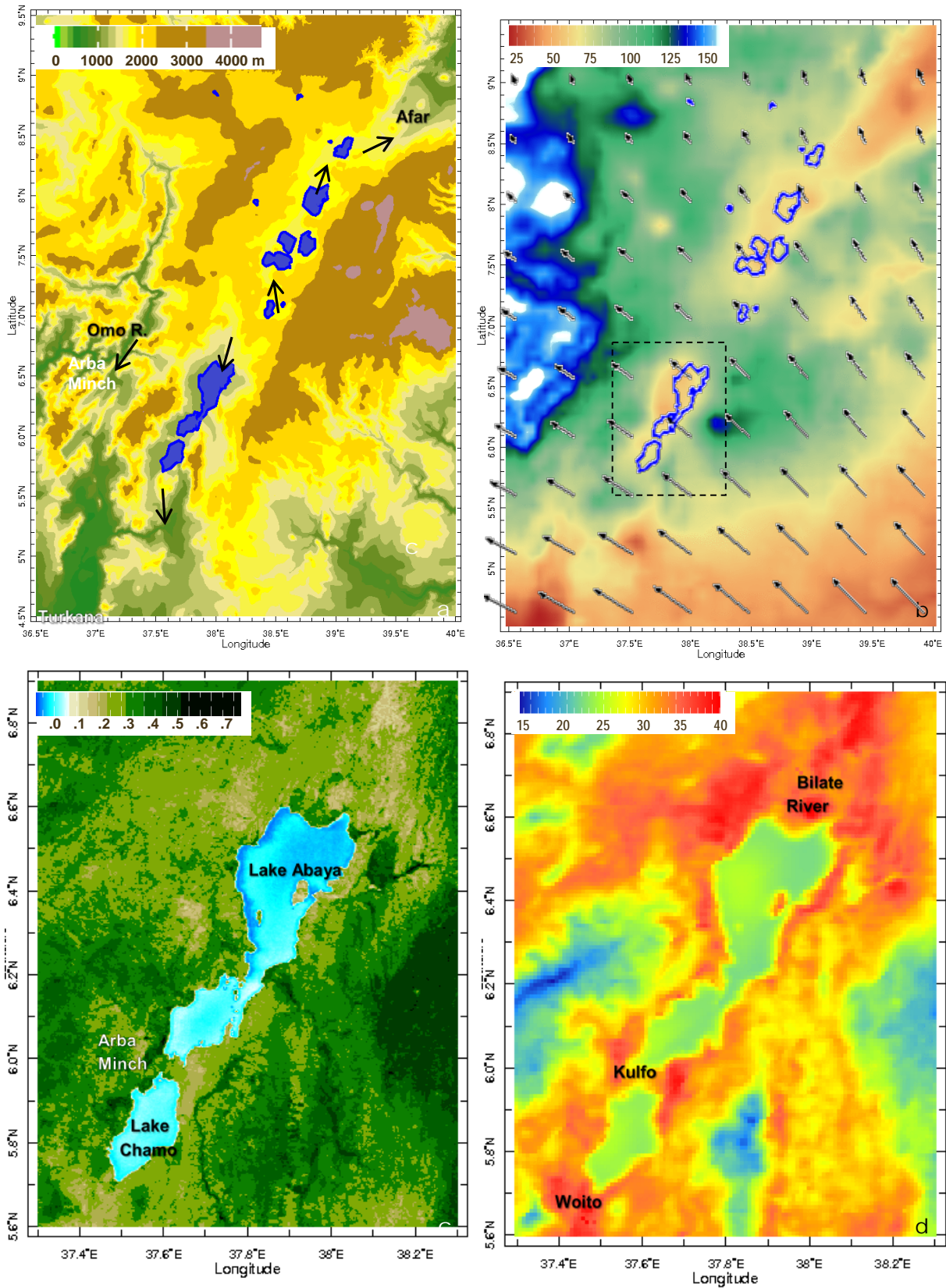


Figure 1. (a) Topography of Ethiopia's Rift Valley lakes and flows. (b) GPCPv6 mean rainfall (mm/month) and NCEP surface winds (max vector 5 m/s). (c) MODIS satellite 2000-2012 mean vegetation fraction and (d) day-time surface temperature of the Abaya-Chamo basin with stream gauges labelled. Dashed box in (b) is domain of (c,d).

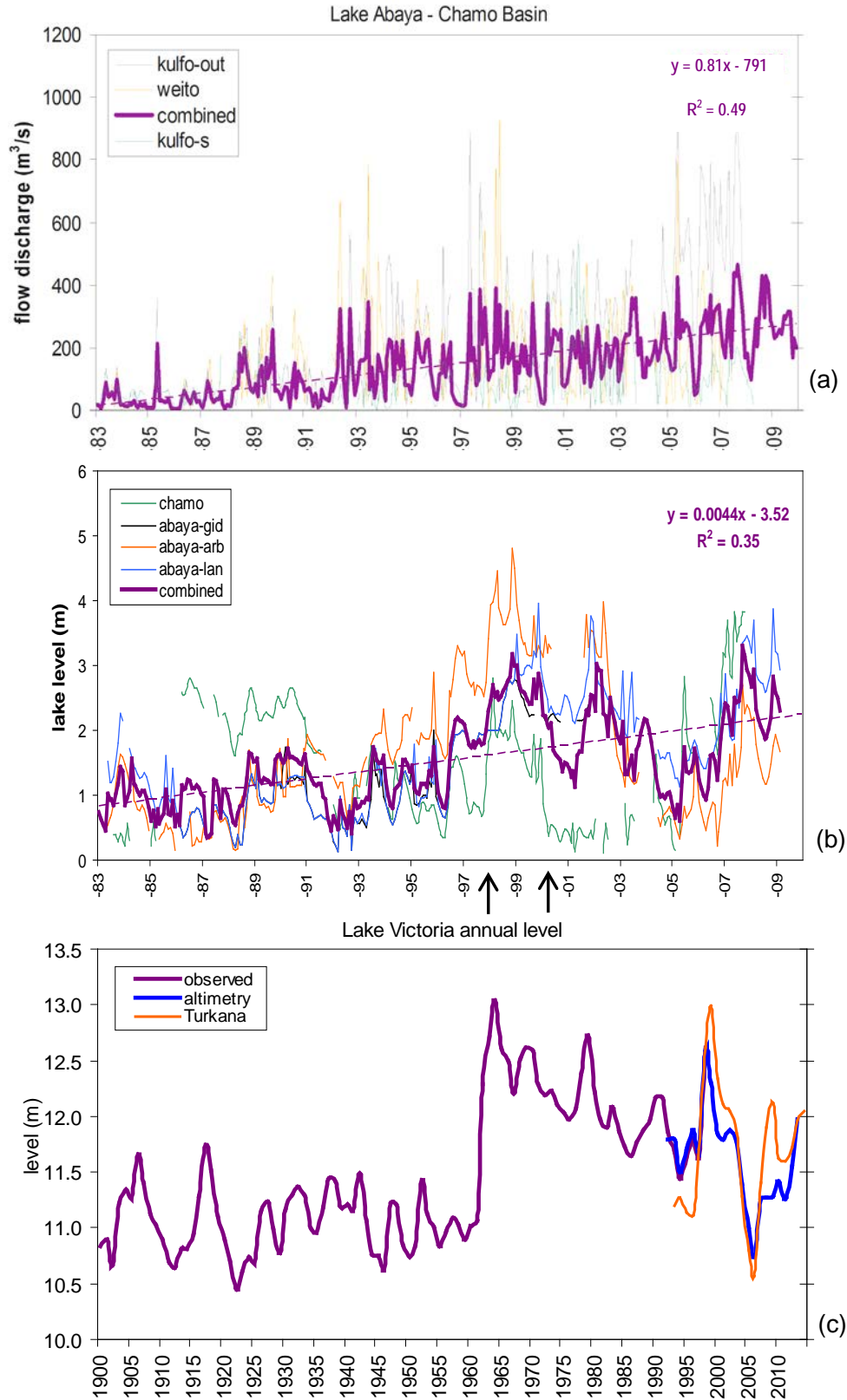


Figure 2. Raw monthly hydrological observations used to reconstruct the Abaya-Chamo: (a) flow discharge and (b) lake level. Thick purple line is average of stations in a,b; arrows point to case study wet and dry spells. (c) Lake Victoria levels since 1900 (observed purple, altimetry blue) and Lake Turkana altimetry levels since 1992 (orange line). Base levels (1133 m Victoria, 360 m Turkana) start at 10 m for comparison.

Table 2. Predictor characteristics.**Candidate predictors from the correlation maps**

Ts Pacific: 130W-70W, 20S-15N (surface temperature)

Ts Atlantic: 60W-10W, 0N-20N

Ts Europe: 15W-15E, 30N-50N

Ts w.Indian: 40E-70E, 20S-20N

U850 Indian: 50E-120E, 5S-5N (850 hPa zonal wind)

The 6-m lead multi-variate regression after elimination of weak predictors:

$$.86+(Ts\ Pac*0.208)+(Ts\ Eur*0.246)+(Ts\ Atl*0.327)$$

lake level at various stations are given in Figure 2. Both exhibit trends characterized by low and stable values up to 1993; a marked upward trend with increasing spread to 1998, and high and relatively stable values thereafter. The background up-trend is related to geological shifts and sedimentation (Goerner et al., 2009). The intermittent flow discharge at various stations and different reference levels for the lake are evident. Bewketu (2010) reviews the issues of data quality and notes uptrends of inflow to Lake Abaya and a 10% contraction of Lake Chamo shoreline since 1986. Here the data gaps were overcome by first establishing a base level then averaging all stations to a single time series. Further gaps at the end of the record in 2008 to 2009 were filled using proportionately scaled NASA satellite altimetry / gravity measurements, (eg. fitting overlapping records and projecting values forward).

Lake level data for nearby Lake Victoria and Lake Turkana provide a long-term context for our observations (Figure 2c). There is a huge 2 m rise in 1961 caused by a flood event similar to that described in short-term events (Figure 6); followed by a slow decline to present. Floods punctuate the Lake Victoria record in 1977 to 1978 and 1997 to 1998, and droughts in 1992 to 1993 and 2005 to 2006. Hence the uptrend in our Abaya-Chamo series (+.05 m/year) is at odds with lake levels 200 km to the south (-.02 m/year since 1965), making it advisable to partially detrend the record as described earlier.

Intercomparisons of climate and hydrology observations are made for the annual cycle in Figure 3. The run-off and inflow is lowest in February and rise to a peak in May. Run-off stays above 0.7 mm/day from May to October then declines in the December to February dry season. The corresponding Lake Abaya-Chamo level (Figure 3a) is lowest in March and rises gradually to a peak by November. The lake level lags inflow from run-off, which in turn lags rainfall. Lake levels continually integrate the effects of climate, unlike inflows which reflect short-term events. The annual cycle of rainfall shows a major peak in April-May and a minor peak in September-October (Figure 3b). LHF remains high from April to November, while low values are found in December-February. Sensible heat flux (SHF), on the other hand, is greatest from January to March and is

consistent with latent heat flux the rest of the year. Precipitation exceeds evaporation only in April-May. Bewketu (2010) notes that Lake Abaya-Chamo level is better correlated with evaporation and run-off, than with local rainfall.

Hydrology response to climate

The hydrological response lag is determined by lag cross-correlation with monthly GPCC6 rainfall from 0-12 months. Observed lake level and outflow, and NOAA2 run-off all exhibit weak correlation at zero lag (Figure 3c). Hydrology variables cross-correlated with basin rainfall reach a plateau by 6-month lag and tail off gradually. Thus a 0-12 months lagged running sum (time integral) is considered optimal for merging meteorology variables (GPCC6 rainfall, NOAA2 LHF and run-off) with hydrology observations (lake inflow and level, Figure 2a,b). Together they form the Lake Abaya-Chamo index (Figure 4a). Its wavelet spectrum exhibits 2 to 5 years cycling with a weak harmonic at 8 to 9 years (Figure 4b). Subsequent analyses make use of this time-integrated index. Field correlation maps at 6-month lead time with respect to the Abaya-Chamo index are given in Figure 5a,b, with values < 90% confidence masked. For NCDC3 (detrended) surface temperature, positive correlations w.r.t. lake levels are noted in the east Pacific, subtropical Atlantic and western Europe. There is also a region of positive correlation in the tropical Indian Ocean. ECMWF 850 hPa zonal winds exhibit noteworthy correlations in the equatorial Pacific (+ westerly) and Indian Oceans (- easterly). Extracting five key predictors as detailed in Table 2, cross-correlations with the Abaya-Chamo index exhibit a 'crest' at 6-9 month lead time (Figure 5c). In multi-variate tests, west Indian Ocean predictors drop out because their influence is confined to August-December, while east Pacific and Atlantic predictors have continuous influence. After step-wise multi-variate regression, the predictive algorithm (Table 2, Figure 4d) achieves a 44% fit, with all inputs positive and the subtropical Atlantic surface temperature having largest coefficient. The algorithm follows the uptrend but over-predicts neutral cases. It forecasts 2 to 5 fluctuations and the 1997 to 1999 oscillation, but is too dry in 1993, 2008, and too wet in 1988, 2000. Thus Lake Abaya-Chamo rises during Pacific El Niño when the Indian Ocean dipole is active (Figure 5a,b). The 44% r^2 fit is statistically significant at 99% confidence, after deflating the degrees of freedom for target autocorrelation. The relationship between ENSO and the Indian Ocean dipole has been described by (Saji et al., 1999; Xie et al., 2001; Luo et al., 2010; Izumo et al., 2010). This feature is analogous to the thermocline see-saw of the Pacific, and is related to a sub-tropical ocean Rossby wave coupled with equatorial zonal winds (Jury and Huang, 2004; Jury, 2013). As the thermocline deepens in the west Indian Ocean with an incoming Rossby wave, SSTs increase > +1C and

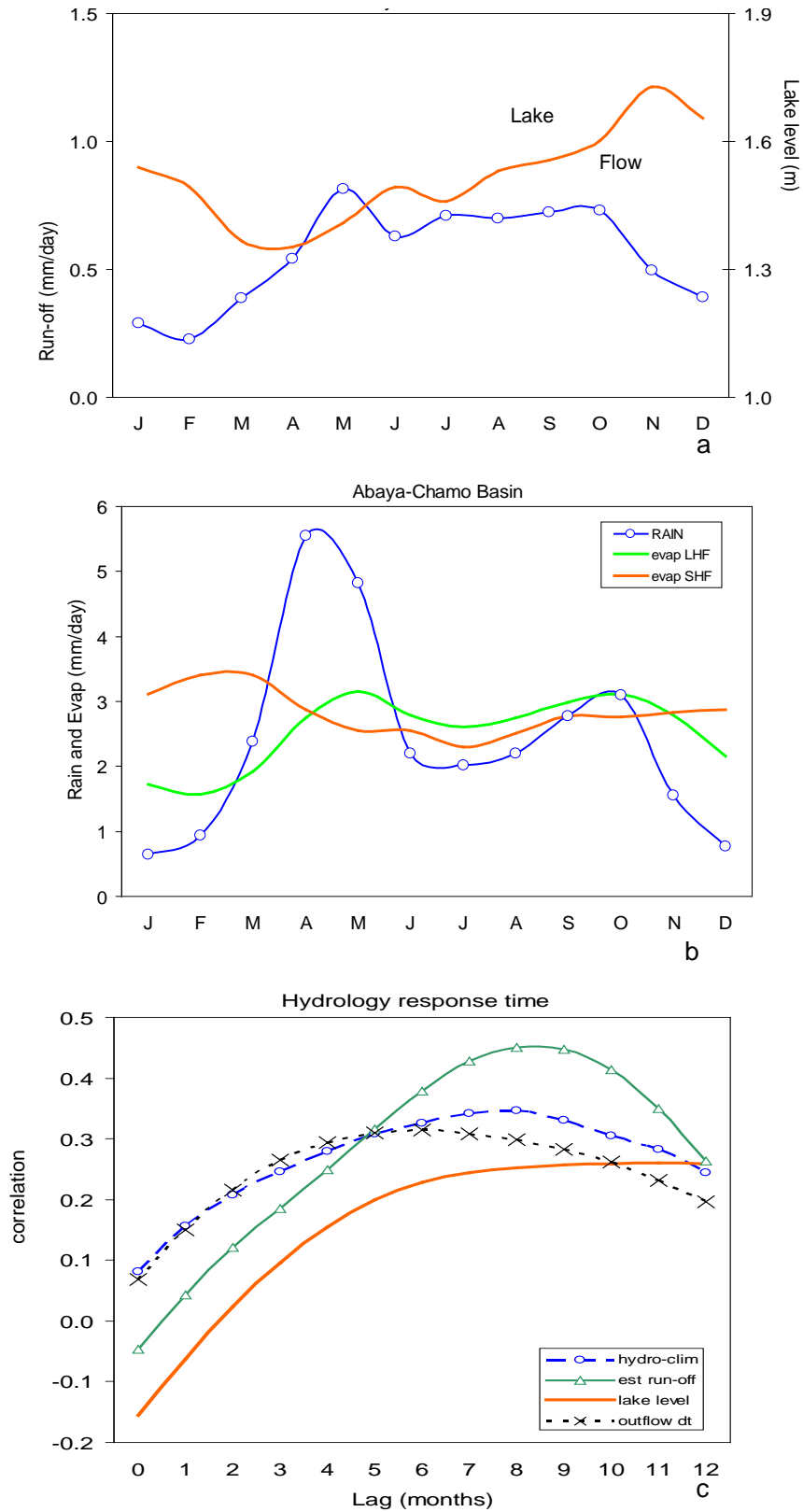


Figure 3. (a) Annual cycle of observed lake levels and flow discharge, (b) annual cycle of GPCP6 rainfall and NOAA2 latent and sensible heat flux (as proxy for evaporation), (c) correlation of monthly hydrological time series (from Figure 2a,b) with monthly GPCP6 rainfall.

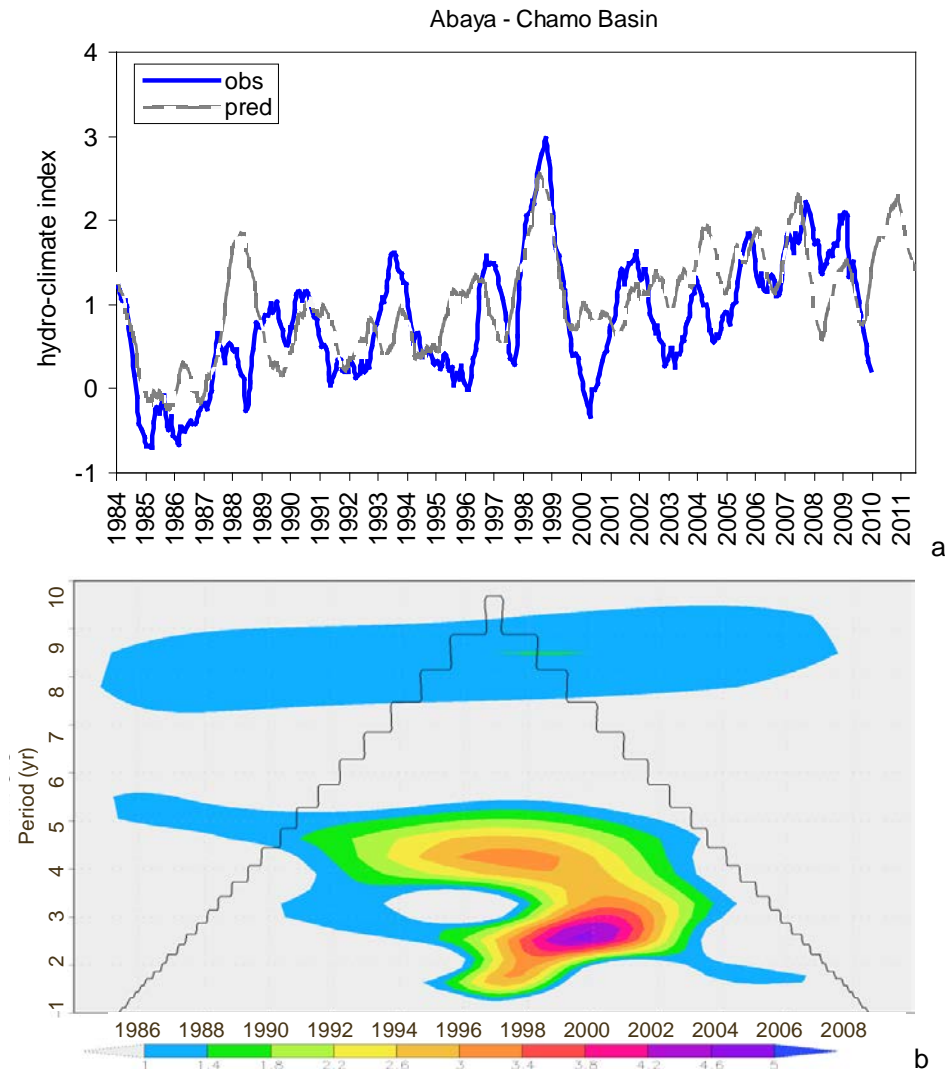


Figure 4. (a) Abaya-Chamo hydrology index (dark line), and (b) its wavelet spectral energy (period in years) shaded above 90% confidence. Subsequent correlation maps use this index. Faint line in (a) is prediction by the algorithm given in Table 2.

contribute to heavy rains over East Africa particularly after August (Behera et al., 2005; Yeshanew and Jury, 2007). The first event described below is one of those cases.

Short-term events

Events in the daily record of flow discharge are analyzed in this section, and two cases are selected for detailed study. The first case is the flood event of October to December 1997 (Figure 6) which has been well studied (Birkett et al., 1999; Goddard and Graham 1999; Mercier et al., 2002). Hydrological records indicate that Kulfo discharge reached $462 \text{ m}^3/\text{s}$ on 30 October, Lake Chamo rose 0.08 m on 20 November and Woito discharge set a record of $854 \text{ m}^3/\text{s}$ on 27 November 1997. A number of

pulses are seen in the time series (Figure 6a) which can be traced to westward moving convective waves arriving from the Indian Ocean (Morel et al., 2011). The rainfall distribution (Figure 6b) exhibited a narrow N-S wet zone along 38°E from Mombasa to Arba Minch. Winds over the Indian Ocean reflect a zonal overturning Walker circulation anomaly (Figure 6c) with rising motion over Ethiopia and descending motion west of Indonesia. The algorithm yields time series (Figure 4a) and scatterplot (Figure 5d).

Ethiopia and descending motion west of Indonesia. Sea surface temperature anomalies were $> +1.5^\circ\text{C}$ off Somalia and cool off Sumatra (Figure 6d), and easterly winds connected the two centers of action: the Indian Ocean dipole. This pattern was repeated in 1961 and 1977, adding $> 1 \text{ m}$ to Lake Victoria (Figure 2c).

Dry spells that diminish lake levels are related to high evaporation rates during heat waves. In Figure 7a the

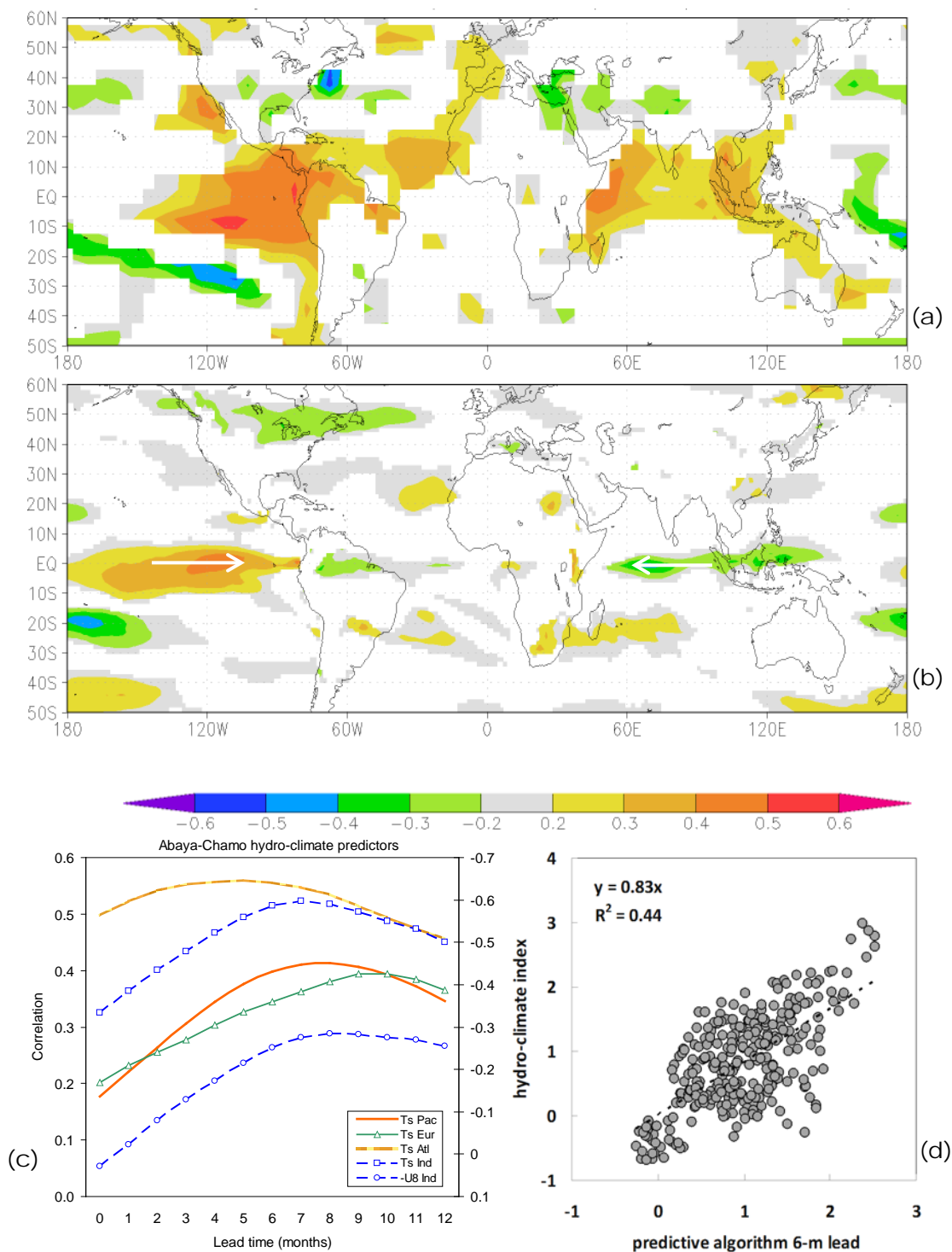


Figure 5. Global correlation map at 6-month lead time with respect to the continuous 1983-2009 Abaya-Chamo index (Figure 4a) for: (a) detrended NCDC3 surface temperature, and (b) ECMWF 850 mb zonal winds with arrows emphasizing key features. (c) Lag correlations for key predictors, and (d) scatterplot of predicted vs observed values with linear fit. Table 2 lists the details and Figure 4a gives the algorithm time series.

Kulfo discharge from Abaya to Chamo nearly stopped (< 2 m³/s) from 16-22 March 2000 coincidentally with basin-averaged maximum temperatures > 30°C. Lake Abaya-

Chamo level dropped from 2.72 m in November 1999 to 0.93 m by April 2001. The widespread nature of the March 2000 drought is evident in MODIS day-time land

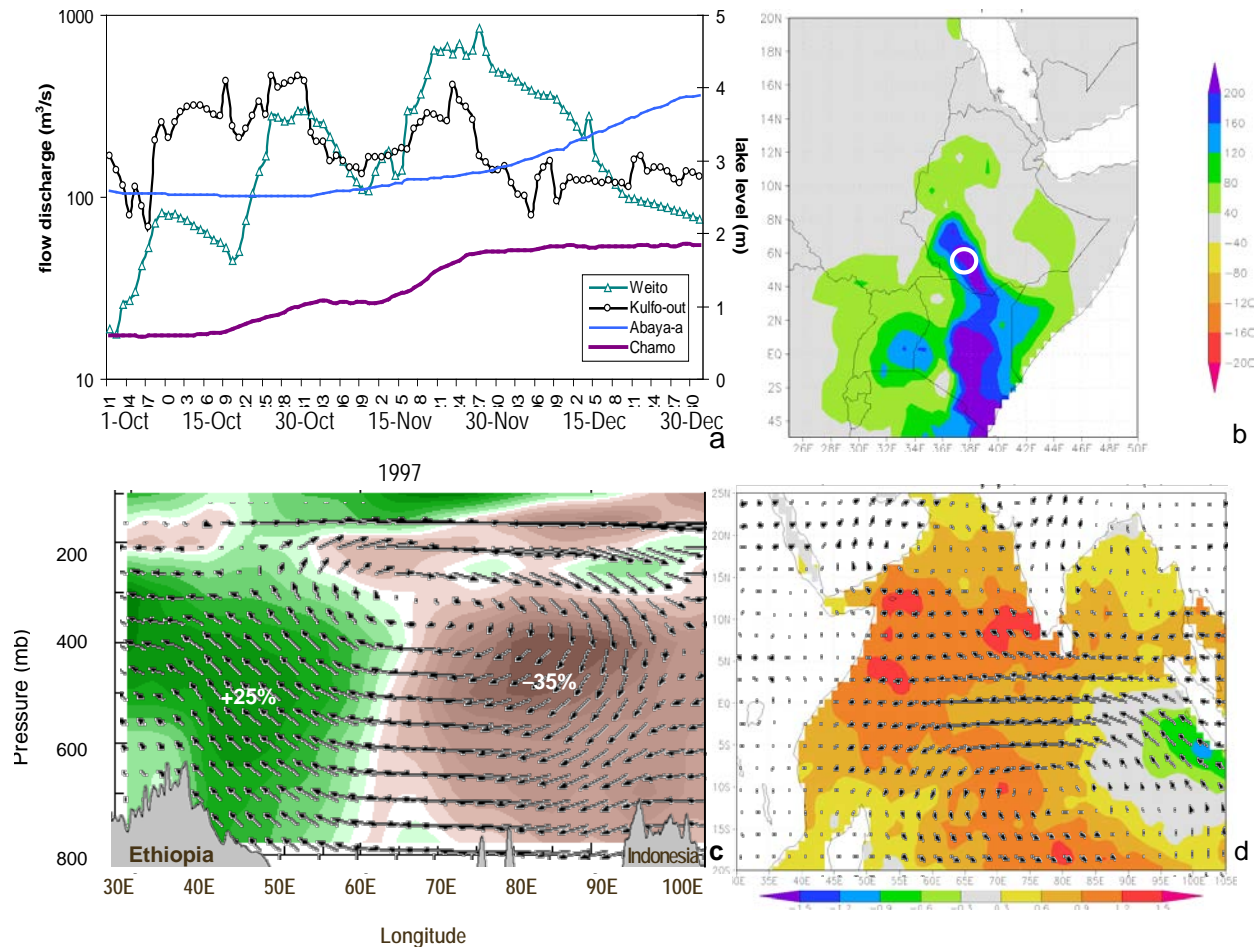


Figure 6. Wet spell (Oct-Dec 97): (a) hydrological observations, (b) rain anomalies (mm/month, circle is study area), Indian Ocean (c) zonal wind, vertical motion (vector, max 5 m/s) and humidity anomalies (shaded) in W-E section on 5N, and (d) sea surface temperature and 850 mb wind anomalies. The green and brown areas of (c) show a classic Indian Ocean dipole imprint.

surface temperatures $> 40^{\circ}\text{C}$ across Sudan, Ethiopia, Kenya and Somalia (Figure 7b). Satellite out-going longwave radiation (OLR) anomalies $> +20 \text{ W/m}^2$ formed an axis through southern Ethiopia in March 2000 (Figure 7d), while negative anomalies were evident south of the equator over Tanzania. A feature of this drought was the Hadley overturning circulation (Figure 7c), exhibiting sinking motions and northerly winds over Ethiopia (Figure 7d). The inter-tropical convergence zone remained south of its usual position (green shaded humidity, Figure 7c) and thus early season rains failed. Coincidentally, southern Africa experienced epic flooding (Jury and Lucio, 2004).

Conclusions

This study has described hydrological fluctuations of Lake Abaya-Chamo and its climate and weather forcing. While earlier studies found weak correlation between lake level and rainfall (Belete, 2009), here consideration of the

hydrological response lag yielded close relationships using time-integrated anomalies. The findings are summarized in the following points:

- (i) Direct measurements of large lakes are valuable as they integrate the effects of climate in space and time.
- (ii) Satellite and model interpolated data follow the hydrological record when summed over the preceding 12 months, especially GPCP6 rainfall, NOAA2 LHF and runoff, and ECMWF climate fields.
- (iii) The Pacific ENSO and subtropical Atlantic affects continuous flow discharge, while the Indian Ocean dipole has influence after August. A predictive algorithm accounts for 44% of variance at 6 month lead time.
- (iv) The Abaya-Chamo basin is on the edge of the East Africa climate regime shared with Kenya, wherein higher lake level coincides with a maturing El Niño.
- (v) Early season dry spells prevail when the equatorial trough remains over Tanzania, and the Hadley cell brings dry northerly winds that accelerate evaporation: March 2000.

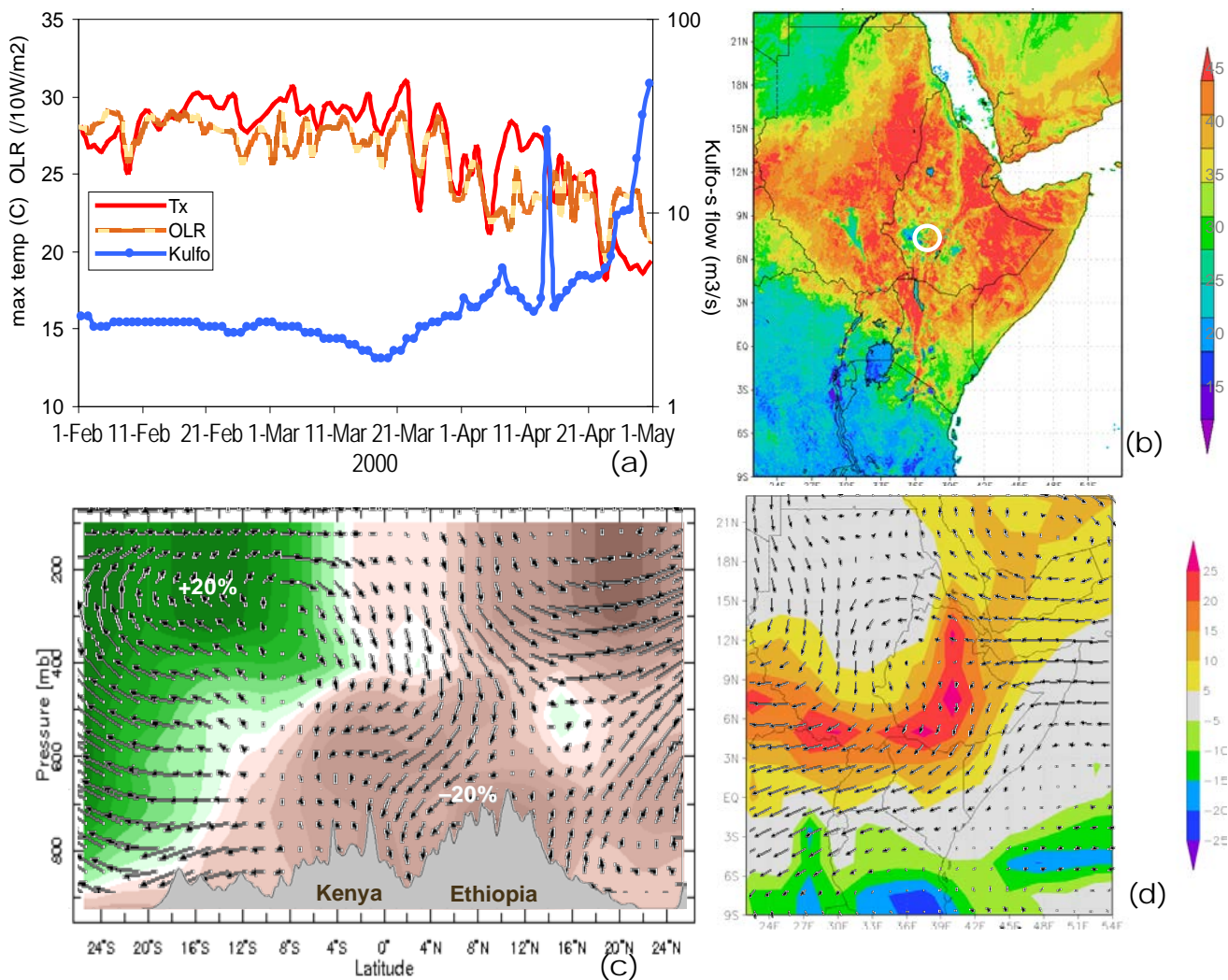


Figure 7. Dry spell (Feb-Apr 2000): (a) Time series of daily Arba Minch max temperature, satellite OLR anomalies and in-situ flow discharge, (b) MODIS day-time surface temperature (C) 5-21 March 2000 (study area circled). March 2000 (c) Meridional wind, vertical motion (vector) and humidity (shaded) anomalies in N-S section on 37E, and (d) NOAA satellite OLR and NCEP 700mb wind anomalies (W/m^2 , vector max 5 m/s).

(vi) Late season floods are related to easterly winds and a down-tilted thermocline (Jury and Huang, 2004, Yeshanew and Jury, 2007) that comprise the Indian Ocean dipole. In response, lake levels can rise 1m/month: October 1961, 1977, 1997.

Further research progress will depend on ready access to daily flow data, sustained local measurements timeously reported to international data centers, the calibration and operational use of mesoscale weather forecast models, and the maintenance of multi-disciplinary regional science networks. Although predictability was uncovered for Lake Abaya-Chamo, more than half of the fluctuations are unresolved. Thus coping mechanisms and strategies for resource switching in wet and dry years is needed, to keep the growing rural population on a sustainable socio-economic path.

Conflict of Interest

The authors have not declared any conflict of interest.

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Research, Melkasa.

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Full Length Research Paper

Effects of elevated temperature on seed germination and seedling growth on three cereal crops in Nigeria

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The effect of elevated temperature on seed germination and seedling growth was investigated on three Nigerian cereal crops: maize, rice and sorghum. Seeds were then allowed to germinate in controlled growth at 37 (control: room temperature), 40, 42, 45 and 50°C and monitored for germination, shoot length and root length for 24, 48, 72 and 96 h, respectively. Seeds were also sown in small poly bags in triplicates under the same temperature regimes and assayed for the physiological effects on the root, stem and leaf for 2 weeks after germination. Results showed a decrease in germination rate as temperature regimes increased. There was significant increase in the shoot length of maize at 37 and 40°C after 96 h of exposure to these temperature regimes but a drastic decrease at 42, 45 and 50°C, respectively. Root length of the maize plantlets also decreased when exposed to 45 and 50°C, respectively. Sorghum plantlets also showed shoot increase at 37, 40 and 42°C temperature regimes but decreased at 45 and 50°C, while root length decreased in length at 42, 45 and 50°C, respectively. Rice plantlets exposed to different temperature regimes showed an increase in shoot length at 37, 40, 42 and 45°C, respectively with a decrease only at 50°C and also a decrease in root length at 50°C. The results from the seedling experiments showed a drastic reduction in physiological parameters tested when compared with the control at $p < 0.05$. Root length of maize reduced from 13.10 ± 1.5 at 40°C to 3.80 ± 0.5 at 50°C, while length of stem of sorghum reduced from 5.00 ± 0.4 to 3.40 ± 0.5 . Rice had a reduction in leaf length of 6.70 ± 1.0 to 4.20 ± 1.5 . The results of the experiments also showed that sorghum was most affected by the increased temperature range among the three test crops. This study clearly indicates that the growth of maize, rice and sorghum seedlings showed signs of stagnation or decrease following rise in temperature. As temperature rise is part of the global climate change problem, the study clearly shows that climate change vis a vis increase in temperature will have strong negative implications on food crops in the nearest future especially in emerging economies like Nigeria.

Key words: Cereal crops, climate change, elevated temperature, seed germination, seedling growth.

INTRODUCTION

The International Panel on Climate Change (IPCC, 2007) has reported that by the end of the 21st century, the

earth's climate is predicted to warm by an average of 2 to 4°C. This according to Eitzinger et al. (2010) is due to

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anthropogenic and natural factors. Smith and Olesen (2010) described the emissions of greenhouse gases from agricultural systems as likely sources of global temperature increase. Extreme temperature has detrimental effect on plant growth and crop yield; this increase causes a reduction in growth and productivity (Southworth et al., 2000).

Wahid et al. (2007) reported that long exposure of high temperature during seed development triggers delaying in germination, seed vigor as well as dry mass reduction; while seed germination and seedling establishment stage play vital role for sustainable cropping the increase in temperature has exposed most of the world's crops to heat stress during some stages of their life cycle (Grass and Burris, 1995).

Again, the difficulty in the precise prediction of the projected agricultural impacts of climate change further adds to this problem (Watanabe and Kume, 2009). According to Gorai and Neffati (2007), the success of a plant to grow and establish is considerably dependent on optimum germination. As germination is a crucial stage in the life cycle of plants, any alter in this process will in general affect the plants growth and development.

Temperature is a major limiting factor affecting germination in the arid and semiarid areas (Huang et al., 2003; Tlig et al., 2008). Initial establishment of a plant species in high temperature area is related to germination response of seeds to temperature and early establishment usually determines if a population will survive to maturity (Huang et al., 2003; Song et al., 2005).

Liu et al. (2008) reported that temperature has a great influence on the distribution, growth, yield and quality in soybean; the study showed great sensitivity to temperature change. Gan et al. (2004) equally showed seed yields of canola to have decreased by 15% when high temperature stress was applied before flowering. The emergence of rice seeds during germination was delayed when temperature was increased to about 40°C as reported by Akman (2009). Morita et al. (2004) reported the reduction of grain formation of rice at an increased temperature of 34°C. In legumes, high temperature stress during reproductive development may physiologically affect flower abortion, sequent sink site and later pod abscission resulting a decreased number of seed per plant (Duthion and Pigeaire, 1991). Also, high temperature stress during reproductive development negatively affect cell expansion, cotylone cell number and thus seed filling rate, resulting in the lowered weight per seed (Munier-jolain and Ney, 1998). The effect of temperature on germination and seedling growth of Psyllium (*Plantago psyllium*), Marshmallow (*Althaea officinalis*) and Fennel flower (*Nigella sativa*) with regards to germination behavior and seedling growth characteristics is well documented in Saba et al. (2014), while Idikut (2013) has also reported the effects of light, temperature and salinity on seed germination of three maize forms.

Nigeria is said to be the 10th largest producer of maize

(*Zea mays*) in the world, and the largest maize producer in Africa, followed by South Africa (Cadoni and Angelucci, 2013a). While maize is grown in the entirety of the country (both yellow and white varieties), the North Central region is the main producing area. Seventy percent (70%) of farmers are smallholders, with an average 5 ha area of cultivated land accounting for 90% of total farm input. Gourichon (2013) reported Nigeria to be the largest sorghum (*Sorghum bicolor*) producer in West Africa, accounting for about 71% of the total regional sorghum output. Nigeria's sorghum production also accounted for 35% of the African production in 2007 (ATA, 2011) of which the country is the 3rd largest world producer after the United States and India. Rice (*Oryza sativa*) is grown approximately on 3.7 million ha of land in Nigeria, covering 10.6% of the 35 million ha of land under cultivation, out of a total arable land area of 70 million ha. 77% of the farmed area of rice is rain-fed, of which 47% is lowland and 30% upland. The range of grown varieties is diverse and includes both local (such as Dias, Santana, Ashawa, Yarsawaba, and Yarkuwa) and enhanced varieties of traditional African rice (such as NERICA) (Cadoni and Angelucci, 2013b).

As Nigeria is going through a transformation phase in the Agricultural sector to increase production in most of these commodity crops, there is a need to build into it the effects of climate change via increased in temperature on crops in other to ensure a successful transformation that will not only see an increase in food security but also crops that are able to adapt to these changes. With the effects of climate change via increased in temperature already has been experienced in the Northern parts of Nigeria (with an increase to about 42°C) in most cases.

This study is therefore aimed at studying the effects of elevated temperature on seed germination and seedling growth on three cereal crops: sorghum, maize and rice in Nigeria. In determining the physiological effects of increased temperature, the results of this study hopes to show how future temperature induced climatic change will have significant impacts on agriculture. The results of this study hopes to give scientists/policy makers insights worthy for consideration in development of the agricultural transformation agenda in Nigeria.

MATERIALS AND METHODS

Collections and sterilization of seeds

The test seeds of sorghum and maize were gotten from the open market in Gwagwalada, Abuja Nigeria, while the rice seeds were provided by Dr. S.A Afolabi's research group on rice transformation in the Biotechnology Advanced Laboratory, Sheda Science and Technology Complex, Abuja, Nigeria. This seeds were handpicked to make sure the best seeds were used for the experiments then subjected to some sterilization process. The method of Ali et al. (2013) was used for the sterilization process: this involved washing the fresh seeds with distilled water and then treated with 70% ethanol for 20 s followed by sterilizing with 10% sodium hypochlorite (commercial bleach) for 30 min. Seeds were rinsed five

times with autoclaved distilled water and then soaked in water for 24 h.

Experimental effects of elevated temperature

Experiment I

After the 24 h soaking, seeds were divided into five groups of 10 seeds in replicates. Seeds were then allowed to germinate on Petri plates with filter paper wetted with distilled water in controlled growth at 37°C (control: room temperature), 40, 42, 45 and 50°C. Seeds were then monitored for 24, 48, 72 and 96 h, respectively. When the radical emerged through the seed coat it was considered as germinated. The number of germinated seeds was recorded daily and in the last day (96 h), traits such as root length and shoot length were measured using five samples from each replicates in centimeters using a ruler. Germination percentage (GP) was calculated according to the methods of Saba et al. (2014) using the following formulas:

$$\text{Germination percentage (GP)} = S/T \times 100$$

S, Number of germinated seeds; T, total of number seeds.

Experiment II

Five (5) seeds each of sorghum, maize and rice were sown in small poly bags in triplicates under the same temperature regimes of 37°C (control: room temperature), 40, 42, 45 and 50°C for 2 weeks. The seedlings were watered every 2 days with equal amount of water (100 ml) After the 2 weeks of growth the seedlings were assayed for the physiological effects on the root, stem and leaf, 2 weeks after germination. The SPSS software was used to show the analysis of variance (ANOVA) at 0.5 level of significance.

RESULTS

Figure 1 shows the percentage rate and germination rate of the three cereal crops as it is affected by different temperature regimes. It was noticed that as temperature increased from 37 to 50°C over a 96 h period, the percentage of germination reduced from 100% to about 60% in rice and 40% in maize and sorghum, respectively. Shoot length of maize plantlets is represented in Figure 2. Results showed a significant increase in shoot length at 37 and 40°C after 96 h of exposure to these temperature regimes but a drastic decrease in shoot length on plantlets grown on temperature regimes of 42, 45 and 50°C, respectively. Root length of the maize plantlets also decreased when exposed to 45 and 50°C, respectively (Figure 3). Figure 4 shows the shoot length of sorghum plantlets after 96 h of exposure to different temperature regimes. It was noticed that the plantlet shoots increased after 96 h when exposed to 37, 40 and 42°C temperature regimes but decreased at 45 and 50°C. Root length of sorghum plantlets after 96 h of exposure to different temperature regimes showed a decrease in length at 42, 45 and 50°C, respectively (Figure 5). Rice plantlets exposed to different temperature regimes showed an increase in shoot length

at 37, 40, 42 and 45°C, respectively with a decrease only at 50°C and also a decrease in root length at 50°C (Figures 6 and 7). The effects of elevated temperature on root length, leaf length, width of leaves as well as the length of stem of the maize seedlings after 2 weeks is represented in Table 1. The results showed a drastic reduction in physiological parameters tested when compared with the control (37°C) at $p > 0.05$. Root length of maize reduced from 13.1 ± 1.5 at 40°C to 3.80 ± 0.5 at 50°C. Leaf length of maize seedlings also reduced from 15.40 ± 3.6 at 40°C to 5.80 ± 1.1 at 50°C. The length of stem also significantly reduced at $p < 0.05$ from 6.90 ± 0.8 to 4.00 ± 0.7 , respectively. Table 2 represents the effects of elevated temperature on sorghum seedlings. The root length of the seedlings reduced from 6.30 ± 1.3 at 40°C to 1.50 ± 0.1 at 50°C. Length of stem of sorghum seedlings reduced from 5.00 ± 0.4 to 3.40 ± 0.5 . The effects of elevated temperature on rice seedlings are also shown in Table 3. Leaf length was significantly reduced from 6.70 ± 1.0 to 4.20 ± 1.5 between 40 and 50°C. There was also a significant reduction in stem length from 5.90 ± 0.5 at 40°C to 3.00 ± 0.1 at 50°C.

DISCUSSION

During the life cycle of a plant, seeds have the highest resistance to adverse environmental conditions, whereas seedlings are often more sensitive, and this is especially true for most plant species (Gutterman, 2002). Therefore, successful establishment of a plant species is dependent to adaptive mechanisms of seed germination and of seedling growth (Mijani et al., 2013). This study was able to show that temperature played an important role in germination as seen in the decrease in the percentage germination rate with increase in the temperature regimes. It was noticed that seed germination was reduced from 42 to 50°C when compared with the control 37 and 40°C, respectively. Essemine et al. (2010) have reported that inhibition of seed germination with increase in temperature often occurs through induction of ascorbic acid (ABA). Seeds exposed to very high temperatures in this study must have produced more ABA thus inhibiting germination (Toh et al., 2008). In this study, it was noticed that germinated seedlings showed seedling vigor, reduced radicle as well as plume growth. Kumar et al. (2011) as well as Piramila et al. (2012) have documented these physiological stress caused by increase in temperature on mungbean (*Phaseolus aureus* Roxb) and the black gram plant. At very high heat stress (45°C) the rate of germination of wheat was strictly prohibited and caused cell death and embryos for which seedling establishment rate was also reduced (Cheng et al., 2009). Plant height, number of tillers and total biomass were reduced in rice cultivar in response to high temperature regimes (Mitra and Bhatia, 2008). Flores and Briones (2001) studied germination responses of six desert species to elevated temperatures and observed that along with increasing

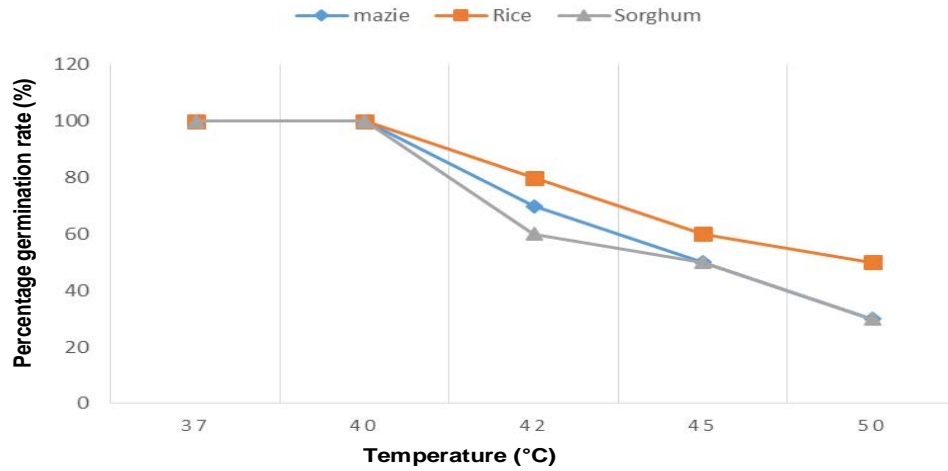


Figure 1. Percentage germination rate of the three cereal crops.

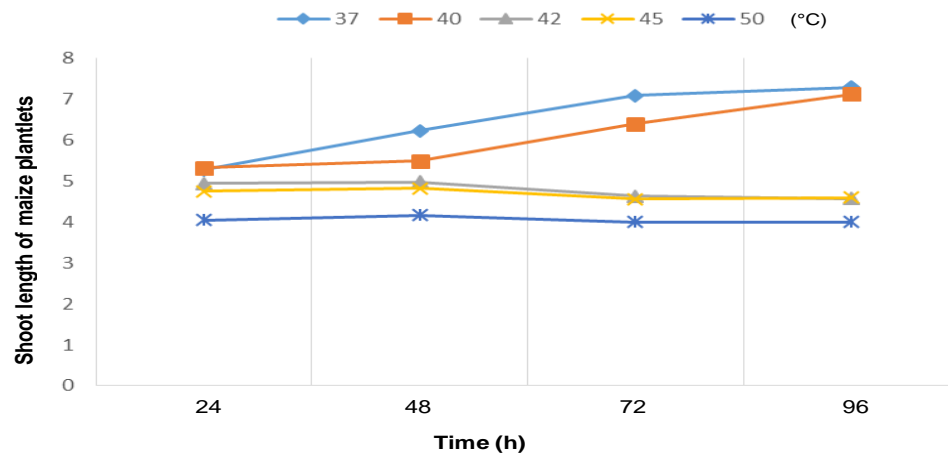


Figure 2. Shoot length of maize plantlets after 96 h of exposure to different temperature regimes.

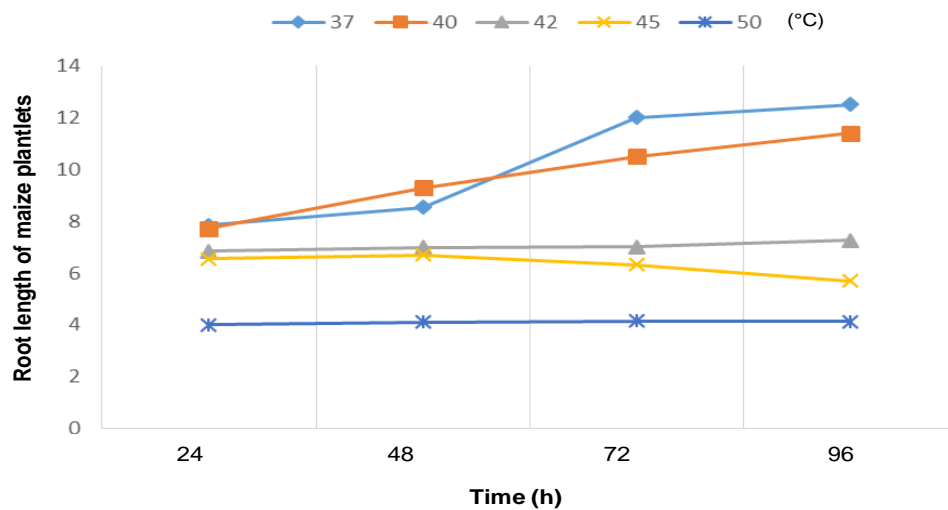


Figure 3. Root length of maize plantlets after 96 h of exposure to different temperature regimes.

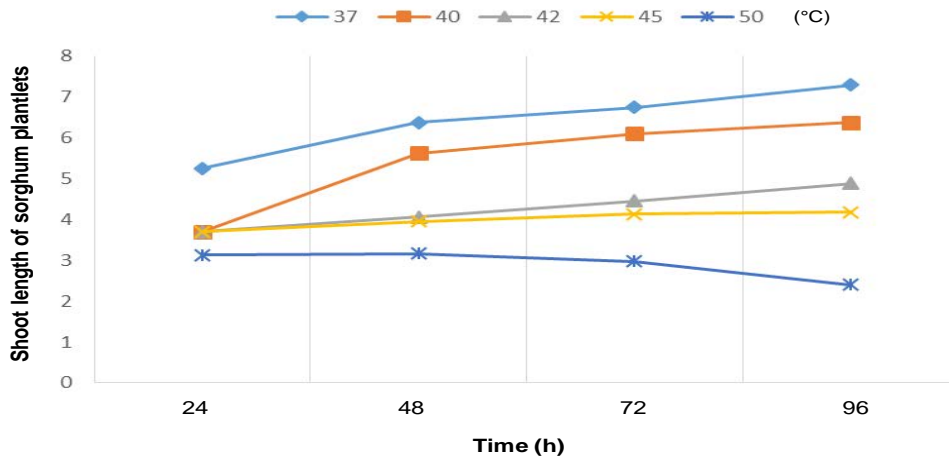


Figure 4. Shoot length of sorghum plantlets after 96 h of exposure to different temperature regimes.

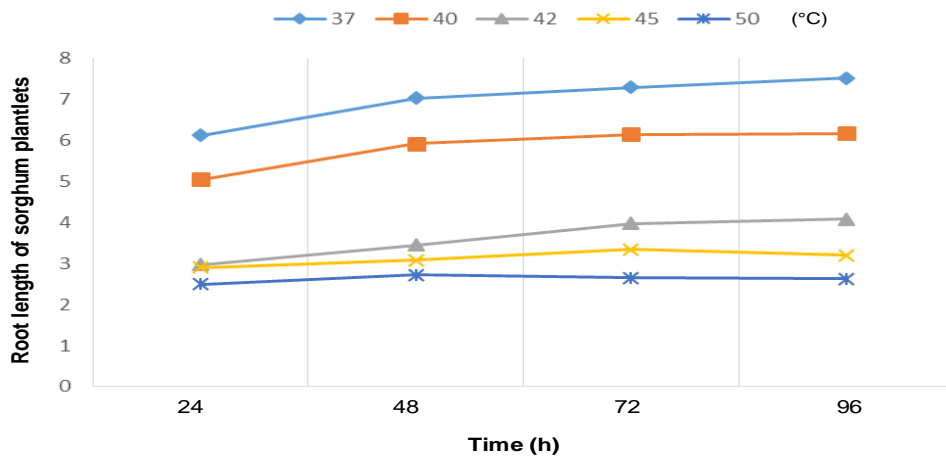


Figure 5. Root length of sorghum plantlets after 96 h of exposure to different temperature regimes.

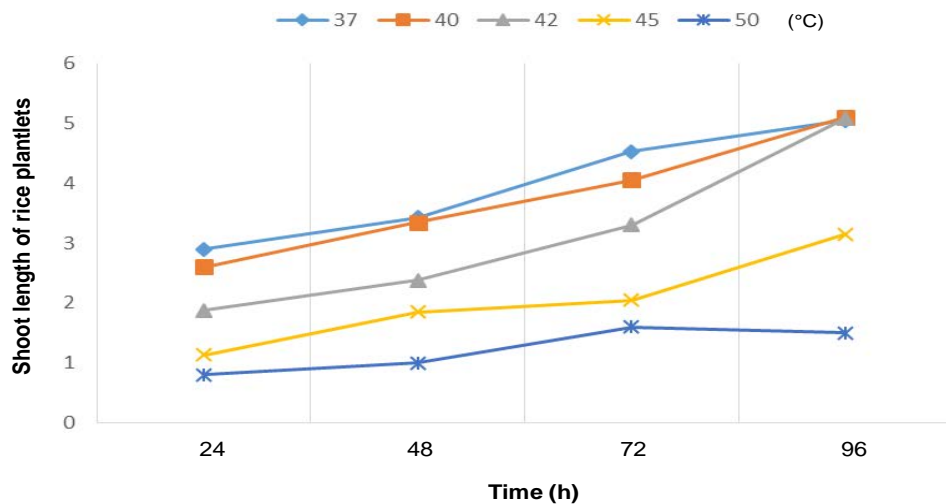


Figure 6. Shoot length of rice plantlets after 96 h of exposure to different temperature regimes.

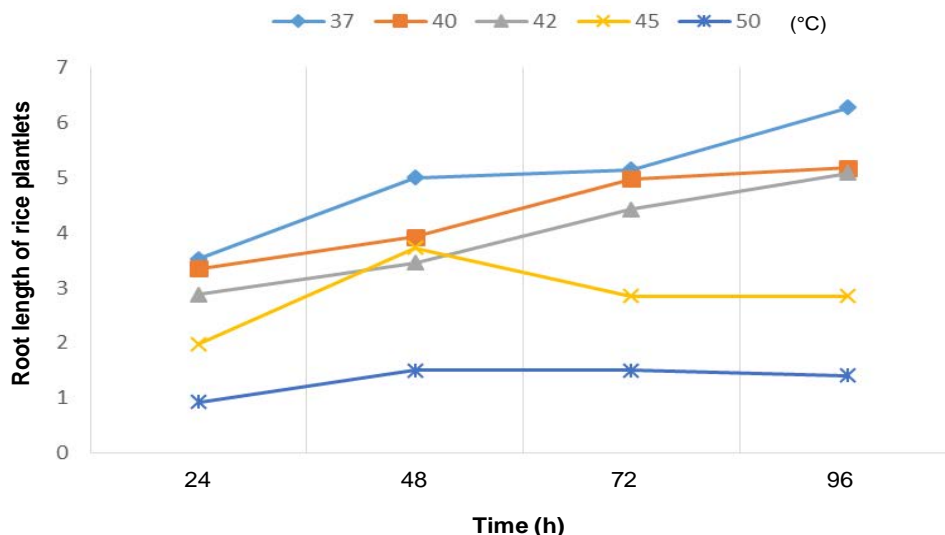


Figure 7. Root length of rice plantlets after 96 h of exposure to different temperature regimes.

Table 1. The effects of elevated temperature on maize after 2 weeks of elevated temperature exposure.

Physiological parameter (cm)	MCO	MEI	MEII	MEIII	MEIV
Length of root	16.30 ± 1.0	13.10 ± 1.5	11.60 ± 2.9	8.50 ± 1.5	3.80 ± 0.5
Length of leaf	19.90 ± 1.6	15.40 ± 3.6	11.60 ± 1.7	9.70 ± 3.2	5.80 ± 1.1
Width of leaf	1.30 ± 0.1	1.20 ± 0.1	1.00 ± 0.1	0.90 ± 0.1	0.50 ± 0.1
Length of stem	7.80 ± 0.3	6.90 ± 0.8	5.30 ± 0.8	4.00 ± 0.4	4.00 ± 0.7

MCO, Control experiment at 37°C; MEI, experiment at 40°C heat Stress; MEII, experiment at 42°C heat stress; MEIII, experiment at 45°C heat stress; MEIV, experiment at 50°C heat stress.

Table 2. The effects of elevated temperature on sorghum after 2 weeks of elevated temperature exposure.

Physiological parameter (cm)	SCO	SEI	SEII	SEIII	SEIV
Length of root	8.90 ± 2.3	6.30 ± 1.3	6.10 ± 1.9	5.50 ± 1.6	1.50 ± 0.1
Length of leaf	11.10 ± 0.6	8.80 ± 2.2	7.70 ± 0.4	6.90 ± 2.5	5.80 ± 1.1
Width of leaf	0.50 ± 0.1	0.20 ± 0.0	0.03 ± 0.1	0.30 ± 0.1	0.10 ± 0.1
Length of stem	8.60 ± 2.2	5.00 ± 0.4	4.20 ± 0.9	4.00 ± 2.3	3.40 ± 0.5

SCO, Control experiment at 37°C; SEI, experiment at 40°C heat stress; SEII, experiment at 42°C heat stress; SEIII, experiment at 45°C heat stress; SEIV, experiment at 50°C heat stress.

Table 3. The effects of elevated temperature on rice after 2 weeks of elevated temperature exposure.

Physiological parameter (cm)	RCO	REI	REII	REIII	REIV
Length of root	8.30 ± 1.0	7.10 ± 1.5	7.60 ± 2.9	6.50 ± 1.5	5.80 ± 0.1
Length of leaf	8.20 ± 0.1	6.70 ± 1.0	5.80 ± 0.6	4.90 ± 3.2	4.20 ± 1.5
Width of leaf	0.70 ± 0.1	0.50 ± 0.1	0.50 ± 0.1	0.20 ± 0.1	0.20 ± 0.1
Length of stem	6.80 ± 0.3	5.90 ± 0.5	5.30 ± 0.5	4.00 ± 0.5	3.00 ± 0.1

RCO, Control experiment at 37°C; REI, experiment at 40°C heat Stress; REII, experiment at 42°C heat stress; REIII, experiment at 45°C heat stress; REIV, Experiment at 50°C heat stress.

temperature mean germination time was reduced. Plant growth and development, particularly reproductive

processes are affected by temperature more than any other environmental factor when water is not a limiting

factor (Thuzar et al., 2010). There was significant decrease ($P < 0.05$) in the physiological parameters (Root length; leaf length, width of leaf and length of stem) in the seedling. Growth decrease due to high temperature could be due to the effects on the reproduction at both organ and process levels (Huan et al., 2000). Kakani et al. (2002) equally reports a possibility of reduction in process levels due to increased temperature regimes.

It was noticed that the plants at 45 and 50°C had a change in texture and color; this has also been reported by Apple et al. (2000). Elevated temperature directly and negatively affected the morphology of the seedlings. Seedlings grown under elevated temperatures had greater numbers of malformed buds, and abnormal needle primordial tissue compared with seedlings in the ambient temperature treatments as reported by Apple et al. (2000). In addition, the seedlings grown under the higher temperatures were shorter and more "bush-like" in morphology, thus hindering their ability to gain height as noticed in this study when temperature was elevated from 45 and 50°C (Olszyk et al., 1998a, b). Note that the present study did not span over the entire reproductive stages. However, Prasad et al. (2003) have reported that despite an increase in photosynthesis, the detrimental effect of high temperature on seed yield was not alleviated by elevated carbon dioxide concentrations but rather by the increase in temperature regimes.

High temperature stress has become a major concern for crop production worldwide because it greatly affects the growth, development, and productivity of plants. However, the extent to which this occurs in specific climatic zones depends on the probability and period of the increased temperature and on the diurnal timing of it too (Meehl et al., 2007). Results from this study clearly indicate that the growth of seedlings of maize, rice and sorghum showed signs of stagnation or decrease following rise in different temperature regimes. As the present rate of emission of greenhouse gases gradual increase in the world's ambient temperature, resulting in global warming vis a vis climate change, plant responses and adaptation to elevated temperatures, and the mechanisms underlying the development of heat-tolerance, need to be better understood for important agricultural crops and this is worth considering in the Nigerian Agricultural Transformation Agenda.

Conflict of Interests

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

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Full Length Research Paper

Enhancing container transportation traceability based on intelligent product and web-services: Model driven approach

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Containers transportation involves various intermediate supply-chain actors. These containers are subject to myriad risks that can occur at any moment. In addition, the absence of a specialized stakeholder for securing containers flow and the lack of communication among actors impedes identifying responsibilities when an adverse event arises. For this purpose, we advocate the use of traceability system to enhance containers transportation security. This objective is achieved through enhancing systems interoperability to collect necessary information for identifying risks. Thus, we opted for urbanizing a traceability system to meet the new requirements related to risk management. In this paper, we propose the use of web-services to overcome the problems related to the heterogeneity of systems used by supply-chain stakeholders. Thus, we proposed a development process based on model driven architecture (MDA) to automate the code generation of these web-services. Moreover, we used the concept of intelligent product (IP) to propose an intelligent container able to communicate with external systems and to manage information related to its traceability. This solution is validated by a real case study.

Key words: Intelligent product (IP), model driven architecture (MDA), service oriented architecture (SOA), business process (BP), traceability.

INTRODUCTION

Supply-chain activities are often subject to undesirable events, particularly in case of containers transportation. Establishing a risk management processes for securing containers flow is a tedious task, due to the heterogeneity and the distribution of actors involved in the supply-chain processes. This implies the participation of several actors

who have different priorities concerning the establishment of risk management process (Erkut et al., 2007). This situation is compounded by the absence of an actor who has the visibility over the entire supply-chain activities. We have the conviction that traceability systems have the potential for improving containers transportation security

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(Boukachour et al., 2011). These systems allow the establishment of a process for collecting necessary information to identify risk events. Furthermore, information related to containers traceability is the basis for investigating the responsibilities of supply-chain actors. To achieve this goal, the traceability system should consider the supply-chain specificity.

The reticular organization of supply-chain actors and their interventions in various networks hinder the cooperation for enhancing traceability. On the one side, the involved actors are reluctant to share information that describes their internal operations due to the risk of information disclosure to competitors. On the other side, there is a lack of interoperability among the traceability system and the heterogeneous systems used by these actors. However, despite these constraints, new regulations impose the adoption of a traceability system, particularly in case of hazardous materials transportation. Thus, adopting traceability should be considered as a competitiveness factor for supply-chain actors.

This work is motivated by supporting the implementation of the Container Safety Initiative (CSI) (Yang, 2011). This initiative aims to remedy the emergence of fraudulent activities, exploiting the vulnerabilities of multi-modal containers transportation. For this purpose, CSI imposes suspicious containers inspection in foreign seaports before goods shipping. However, inspection activities cost and the limited resources allocated for this mission impose the establishment of a decisional process for targeting high risks containers. Consequently, we envisage exploiting traceability information to improve the relevance of suspicious containers targeting.

The first objective is to propose a Service oriented architecture (SOA) for Geo-location Optimization and Secure Transport (GOST) traceability system, and the use of Intelligent Product (IP) concept to develop an intelligent container. The second objective seeks to ease systems adaptation for sharing traceability information based on the Model Driven Architecture (MDA). The MDA automates generation of the web-services foreseen to be interfaced with the GOST system.

RELATED WORKS

Traceability

According to ISO8402, traceability is associated with the ability to trace the history, the use and the location of an entity, based on recorded identifications (Aung and Chang, 2014). Accordingly, product traceability focuses on the identification of its origin, its location and the operations that altered product's state based on the data provided by the information system of each supply-chain actor involved in the product's life cycle.

Traceability systems are based on a set of basic

functionalities. According to Karlsen et al. (2012), traceability system must be able to trace both products and activities, by determining traceable unit and elementary activities. Regattieri et al. (2007) focused on the ability of the traceability system to describe and to communicate product characteristics, taking into consideration confidentiality constraints.

Traceability systems are used in various fields such as food industry (Jin and Zhou, 2014), quality control of products (Aung and Chang, 2014), improving supply chain visibility (Woo et al., 2009) and enhancing risk management in case of hazardous goods transportation (Planas et al., 2008). Bechini et al. (2008) have proposed a generic traceability model for controlling products quality during transportation in food industry. This model allows tracing both of the physical product and the activities executed by the actors involved in the food industry. Thus, it represents a solid basis for developing an efficient traceability system that minimizes recovery cost in case of food contamination. However, this model was not experimented in a real case study. Woo et al. (2009) have proposed a traceability system that enables products monitoring, taking into consideration the granularity of the traceable unit (container, pallet and product). In the MITRA project, traceability system was also used for monitoring the intervention for incidents related to transportation of hazardous materials in Europe (Planas et al., 2008). This project aims to improve the intervention of civil security centers in case of accidents related to hazardous goods transport using new technologies such as satellite navigation systems, geographic information systems and risk knowledge databases.

The aforementioned application proves that traceability systems are often used to enhance risk management in different domains. In this context, GOST traceability system was developed as part of an industrial partnership to enhance security and safety of containers transportation. This system is axed on real-time monitoring of containers carrying hazardous materials in a multi-modal transportation network, and taking into consideration the risk management issues (Boukachour et al., 2011). GOST functioning is axed on containers geo-location. Furthermore, it monitors transport conditions using a set of captors embedded on containers, and generates alerts when predefined rules are transgressed. For example, convoys of dangerous goods have the obligation to go through specific roads far from urban areas. Thus, the traceability system will verify if the convoys have passed through the predefined itinerary, or they broke this rule. Research on traceability field is more focused on technological issues and geo-location. However, only few works have tackled problems related to the interoperability of traceability systems with external systems, and how traceability information can be gathered in a collaborative way. The establishment of such solution will overcome the problems associated with

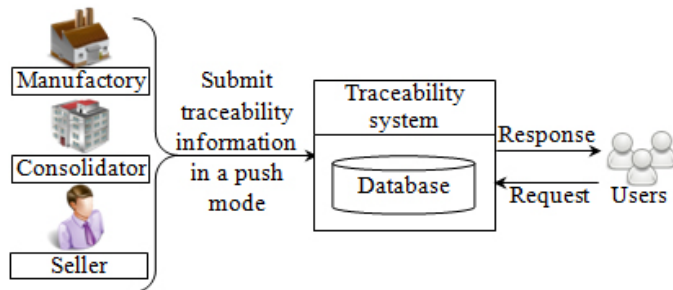


Figure 1. Current functioning mode of GOST.

the mass data of traceability and palliate the problems related to the confidentiality. Furthermore, process for developing a traceability system must lead to an agile and scalable solution, easy to align with the supply-chain's dynamic. This will facilitate the frequent adaptation of the traceability system to meet the new requirements of risk management, the changes of the involved stakeholders and their roles.

In this we will study the case of GOST traceability system. This system is based on centralized architecture and a push strategy for collecting traceability information. This information is archived in GOST database to respond to external requests. Figure 1 describes the current functioning mode of GOST traceability system.

The shortcomings related to this functioning mode manifest in a massive collection of information likely to be useless without an external request that requires the analysis of traceability information. Furthermore, the centralized architecture brings into focus the risk of information disclosure and the reliability of the entity that ensure this task.

Intelligent product

The development of technologies for products self-identification has emerged the concept of IP. The aim of this concept is to bridge the gap between products' physical and informational representation. According to McFarlane et al. (2003), an IP is characterized by an identifier, communication with the environment, information gathering, the ability to communicate its features and decision making. The IP's definition proposed by Kärkkäinen et al. (2003), is focused on the ability of a product to take decisions. Olli (2007) have focused on the ability of an IP to perceive its environment and how it responds to changes.

Meyer et al. (2009) have proposed an IPs classification according to three criteria; intelligence level, intelligence location, intelligence aggregation. The first criterion distinguishes the product that manages its information, the product that notifies problems occurrence and the product that analysis information to take a decision. The

second criterion distinguishes the product with an embedded intelligence and the case of external intelligence through network. The third criterion distinguishes the case of simple IP considered as an atomic entity, and the case of composed IP that manages information related to its components. Wong et al. (2002) have proposed a simplified classification of IP axed on the intelligence level. Consequently, IPs are classified into products characterized by a communicative model or decisional model. The first model includes products that are able to gather information about themselves and the context where they are located. Moreover, these products are able to establish connectivity with external systems in order to communicate information. The second model encompasses products endowed with reasoning processes that exploits the gathered information to take decision concerning their destiny.

The implementation of IP concept is not limited to a single standard. The works we reviewed tackle the problems related to IP implementation from two points of view. The first one is axed on a theoretical approach and aims to propose a conceptual model of IP. The second one is axed on the experimentation of technologies for the realization of this concept.

From a theoretical point of view, Framling et al. (2003) have used the ID@URI concept to link the physical product to a software agent, which manages information related to its life-cycle. An IP model based on the combination of Holons and software agents was proposed by Valckenaers et al. (2009). The Holon concept was coined by Fischer et al. (2003) and covers both of the notions "whole" and "part". Accordingly, this concept consists of a fractal structure that is stable, coherent and that consists of several Holons as sub-structures and is itself a part of a greater whole. The software agent represents a program located in an environment and it is able to perceive and act on its environment. The agents display an amount of autonomy and are able to act in dynamic and uncertain environment (Fischer et al., 2003).

The IP model presented by Xiaoyu et al. (2009) is based on intelligence through network model. It is based on embedding data unit into product to be connected to services deployed by external servers. From an experimental point of view, Kiritsis (2011) has exploited this concept to enrich gathering information related to a product life-cycle, to ease the diagnosis and maintenance of gaming consoles. An application of IP was realized by Ngai et al. (2005) for tracking containers and forklifts inside warehouses.

IP concept presents a significant potential for ameliorating traceability systems functioning. It allows building an intelligent container able to perceive its environment, to manage its traceability information and to communicate with external systems. This goal can be achieved based on the technological solutions deployed by GOST system.

Model driven architecture

The frequent need to align the IT solutions used by an organization to its new business needs must be supported by a new approach to simplify this task. In addition, it is wise to consider the software maintenance phase in its development cycle (Liang and Des, 2009). To overcome this problem, the MDA approach has been proposed by the Object management group (OMG) to reduce the cost of development and adaptation of systems by automating the code generation from a system modeling.

The MDA approach is based on a software engineering idea which calls for the separation of the functional specification of a system and details related to the implementation platforms. This approach aims to separate the organizational and functional aspects of a system from the technological implementation decisions. MDA covers different phases of software development and promotes the automatic generation of a large part of the code. Moreover, the main objectives of this approach are reflected in the portability, the interoperability and the reusability, by the separation of architectural concerns (Estefan, 2008).

MDA is a modeling framework composed of multilevel models of abstraction. This allows capturing the business logic of the system and its functional and non-functional needs, along with a sufficient level of details needed for the automated code generation.

According to José-Luis and Pablo (2013), the key feature of this approach is its ability to define transformations for the passage from abstract models to more specific models. This is the mapping that establishes the link among the basic concepts of different models. Therefore, the modification of a model is reflected in the other models by a series of automated systemic changes which limit the system adaptation costs.

Business process (BP)

According to Muehlen and Indulska (2010), a BP is a collection of actions that address a set of input values to provide a result. Based on these definitions, we equate the BP to a structured set of activities performed by one or more actors and satisfying a set of constraints to achieve the goal set by its designer.

In a changing environment, BPs management and adaptation are key factors for organizations survival. Thus, the importance of processes improvement and understanding of their structures by unspecialized actors, have emerged the need for new modeling techniques. Vergidis et al. (2008) classified modeling techniques in three sets. The first technique is communicative and based on the use of diagrams modeling. The second technique is based on mathematical models to provide a precise specification of BPs. The third technique gives a

linguistic description and simplifies the automation of the processes implementation.

There are several modeling standards for BPs such as Xml Process Definition Language (XPDL), Business Process Modeling Language (BPML), and Business Process Execution Language (BPEL). These are intended to simplify the implementation of BP by exploiting XML notation. However, these aforementioned standards do not have a graphical notation for BP modeling. Therefore, only a small number of specialized actors can operate the generated models. To overcome this problem, the OMG proposed the Business Process Modeling Notation (BPMN) which is a schematic standard simplifying the understanding of BPs. BPMN provides a visual and intuitive description of a BP (Vergidis et al., 2008). Chinosi and Trombetta, (2011) proposed a comprehensive study of this modeling notation, specification and relationship with other BP modeling tools.

BPMN allows BP modeling at several levels of details. This standard is suitable for modeling of internal and external organizational processes. For internal processes, it takes account of the internal activities of organizations and makes abstraction of interactions with external actors. Regarding the modeling of external BPs, it considers the collaborative aspect of process modeling activities that ensure the interaction between the organization and its environment. This aligns with the needs of activities modeling in a supply chain.

MATERIALS AND METHODS

Satisfying the inherent need for containers traceability in supply-chain's context is a tedious task. Accordingly, we adopt an approach structured into four steps to alleviate the complexity of this study. This approach is based on the use of these aforementioned concepts (MDA, Web-Service, and IP); in addition, it details the steps to follow from problem analysis to the solution implementation. Figure 2 illustrates the main steps of this approach.

The first step - objectives description - aims to present how we exploit the IP concept to propose an intelligent container able to manage the information related to its life cycle. In the second step, we propose a new distributed architecture for GOST based on the integration of the intelligent container and web-services to overcome the problems related to the centralization of information and traceability information sharing.

The second step - domain analysis - consists mainly of identifying the domain needs, specifying the characteristics of the system to implement and understanding its use context. This analysis simplifies the identification of actors involved in the process of containers transportation and details the role of each stakeholder. At first, we adopt a case oriented approach relying on the Use case diagram (UCD) of Unified modeling language (UML) to achieve a static view of system actors and functions. In a second step, we use the boundary, entity, and control (BEC) diagram (2011)]. This diagram uses the functional requirements determined by the UCD to specify the classes to be implemented. To achieve a dynamic view of the system, we use the BPMN to model the execution sequence of the functions identified by the UCD.

The third step - web-service generation - deals with problems related to adaptation of the systems used by the supply-chain actors to enable traceability information sharing. For this purpose,

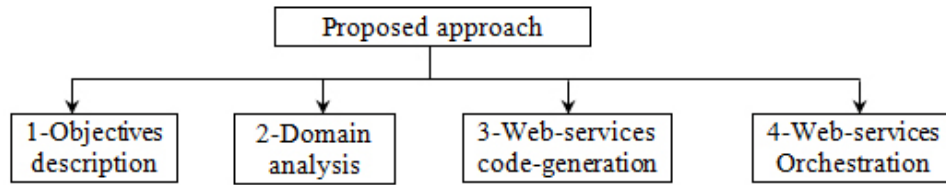


Figure 2. Principal steps of the proposed approach.

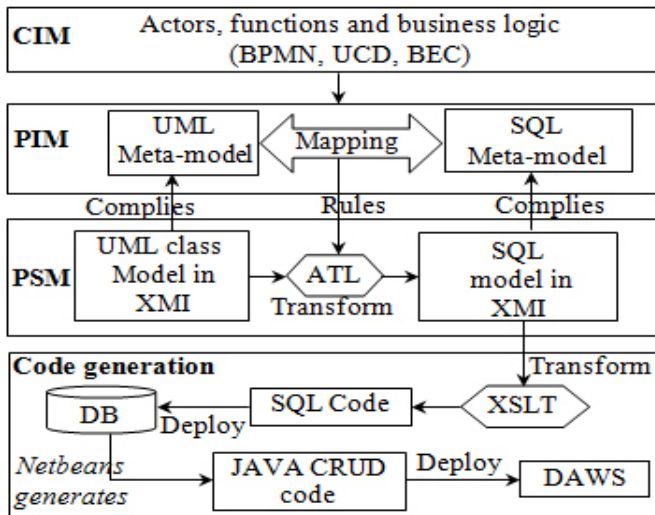


Figure 3. MDA process for generating web services.

we opted for web-services to collect information. This choice is supported by the ease of web-services invocation by the intelligent containers through internet. The aim of this step is to generate automatically web-services based on a MDA process. Figure 3 gives an overview of this process.

The MDA process starts by the specification of the Computation independent model (CIM) (Elfazziki et al., 2012). It deals with the conduct of a domain analysis to understand the mission of the studied system. The second stage of this process focuses on the specification of the Platform independent model (PIM) of the source model and the target model. It aims to define the UML class diagram meta-model and the Server query language (SQL) meta-model, to perform the mapping between their basic concepts as well as to ensure validity of the model to transform and the model to generate. The third step in this process is based on the specification of transformation rules from the mapping of the two meta-models using Object constraints language (OCL). The transformations are performed using the Atlas transformation language (ATL) (Elfazziki et al., 2012).

The ATL transforms the source Platform specific model (PSM) representing the class diagram expressed in XML Metadata Interchange (XMI), to a target PSM compliant with SQL expressed in XMI. The resulting PSM is transformed into SQL statements using the eXtensible stylesheet language transformations (XSLT) (Ming-zhe, 2013) to deploy a Database (DB). The last step of this process is based on the use of Netbeans development environment to generate the Create, Read, Update, and Delete (CRUD), JAVA code required for managing the DB. The JAVA classes generated for tables' consultation are deployed as Data access web-services (DAWS).

The last step of the proposed approach aims to orchestrate the generated DAWS according to the BP logic. This allows us to generate a new composite web-service that provides the information related to a container traceability based on the DAWS deployed by the supply-chain actors. To achieve this goal, we exploit the BPMN diagram expressed in the CIM. The BPMN is supported by the BPEL that provides a description of the interactions between the BP and the web-services. Thus, the implementation of the sequence of BP activities defines the order of granular web-services invocation to achieve the BP goal. Therefore, the execution of this process is equivalent to the invocation of a new composite web-service, based on the orchestration of granular web-services interfaced with the BP.

APPLICATION AND RESULTS

This section describes the implementation of each step of the proposed approach.

Objectives description

To improve the functioning of the GOST traceability system we propose a distributed architecture of this system based on web-services. This new architecture delegates traceability information gathering to the intelligent container. This latter is capable to collect and communicate information related to its life-cycle. This new solution allows container to get only its information at the appropriate time. Thus, it is based on a pull mode for collecting traceability information, and a decentralized architecture where each supply-chain actor ensures the storage of traceability information. Figure 4 gives an overview of the new functioning mode of GOST.

The proposed architecture imposes to supply-chain stockholders to deploy DAWS to share traceability information related to containers. These web-services are exploited by the intelligent container to generate a new composite web-service that provides the intervention description of each supply-chain actor involved in the container life-cycle.

The new functioning mode of GOST needs an IP model that overcomes the simple connection between physical product and its informational representation for a model able to interact with heterogeneous systems. For this aim, we propose an enriched model of IP to build an intelligent container able to exploit the deployed DAWSs.

The proposed model of the intelligent container is

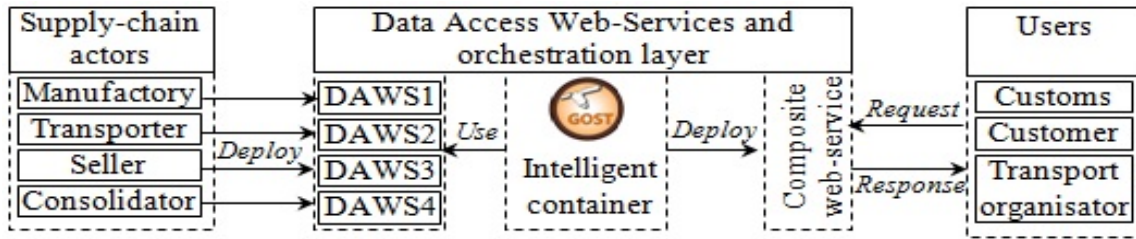


Figure 4. The proposed new functioning mode for GOST.

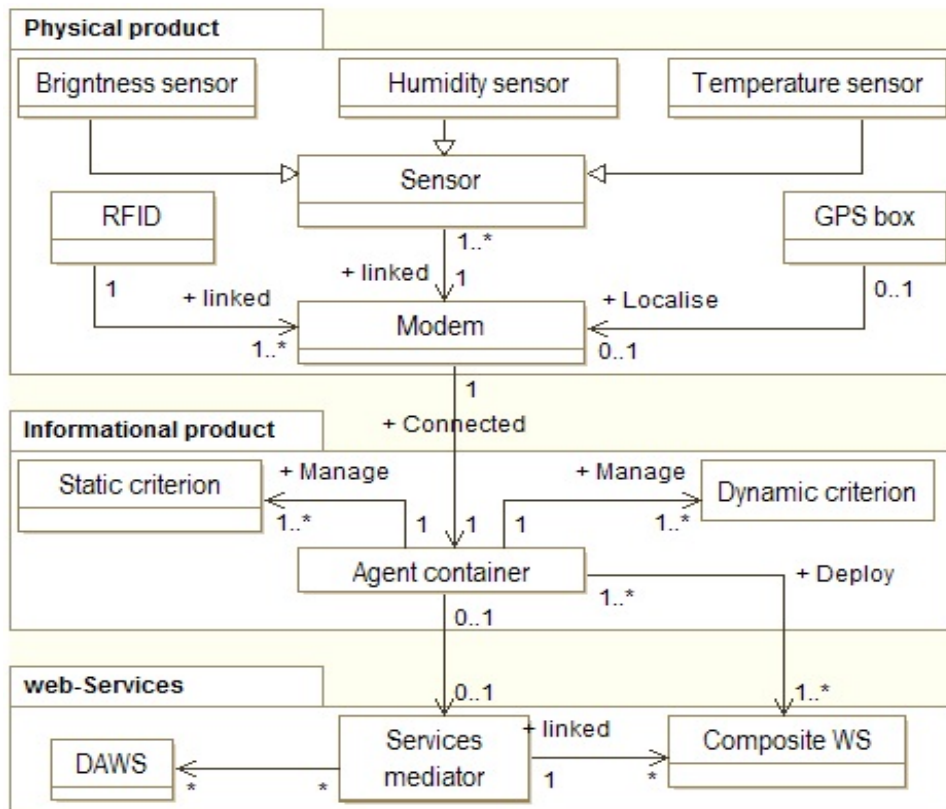


Figure 5. Enriched intelligent container model.

structured into three packages. The first package represents physical products and it consists of Radio frequency identification (RFID) tag for identification, GPS box for geo-location, a set of sensors to monitor container's internal state and a modem to communicate through network. The second package represents the informational product. It is composed of static information describing the products properties, dynamic information such as temperature evolution during the transportation and a container agent that manages this information. The third package represents the services layer. It is comprised of granular DAWS and a web-service mediator needed by container agent to deploy composite web-services. The composite web-service corresponds to the

orchestration of the granular DAWS deployed by the supply chain actors that have intervened during container transportation. The container exploit this new web-service to gather information related to its life cycle. For example, Figure 5 shows the intelligent container model.

Domain analysis

The studied case treats the process of preparation and delivery of a Less-than-container-load (LCL). This container is based on the consolidation of parcels sent by different clients to the same destination. Each sender prepares his parcel and sends it to the consolidator,

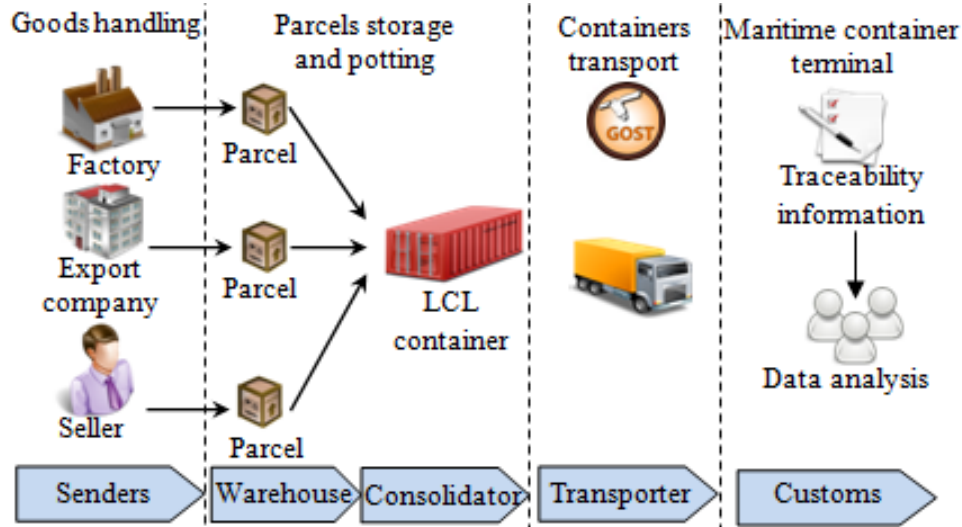


Figure 6. Case study: Less-than-container-load preparation and transport process

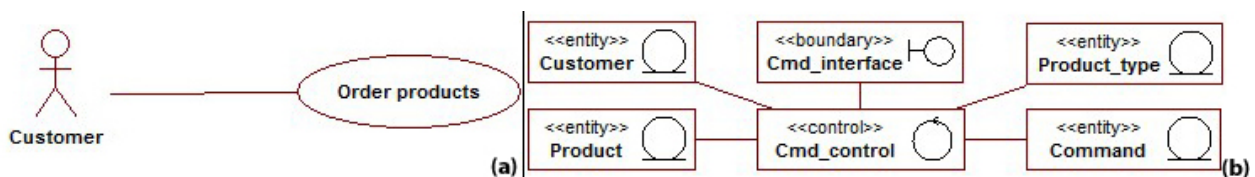


Figure 7. (a) order product use case diagram, (b) BEC diagram.

which provides storage of these parcels during the required time to receive enough parcels to fill the container. These parcels are packed in a container that will be transported to the maritime container terminal by a hauler. The consolidator provides information describing the nature and origin of goods. This information is used by customs to allow the container access to the terminal; Figure 6 illustrates this process.

The establishment of a traceability process must be preceded by an analysis to determine the information to be collected. To do this, the BEC diagram identifies persistent entities of the information systems used by the stakeholders. These entities carry information about the supply-chain actors' activities. We present a simple example of a seller making an order. BEC diagram (Figure 7) shows that the system used by the seller consists of interface and control classes, which represent the user interface and the entity that supervises operations performed by the seller. We focus on persistent entities of the system and we determine which entities are holding traceability information about the order preparation. Therefore, we must take account of persistent classes determined by the BEC diagram (Figure 7), which are command, customer, product and product type.

For a dynamic view of the preparation and delivery process of a LCL container to the seaport, we opted for a model based on BPMN. The modeling process is performed following the description level defined by Silver (2009). Thus, the following model provides a macroscopic view focusing only on the treatment of parcels sent by the seller, without detailed internal sub-processes of each stakeholder (Figure 8).

Web-services code generation

The process of generating web-services using the MDA approach starts with the specification of the CIM for capturing business requirements. This was detailed in the previous section - domain analysis - by specifying a static view of the main features of the system and a dynamic view by modeling the studied BPs.

The web-services generation is based firstly on the description of the traceability data shared by the supply chain actors, and secondly on the creation of new databases that can be accessed by DAWS. To do this, the actors give a detailed description of the traceability data. Then, these data will be refined to specify an UML class diagram. This class diagram is used for SQL code

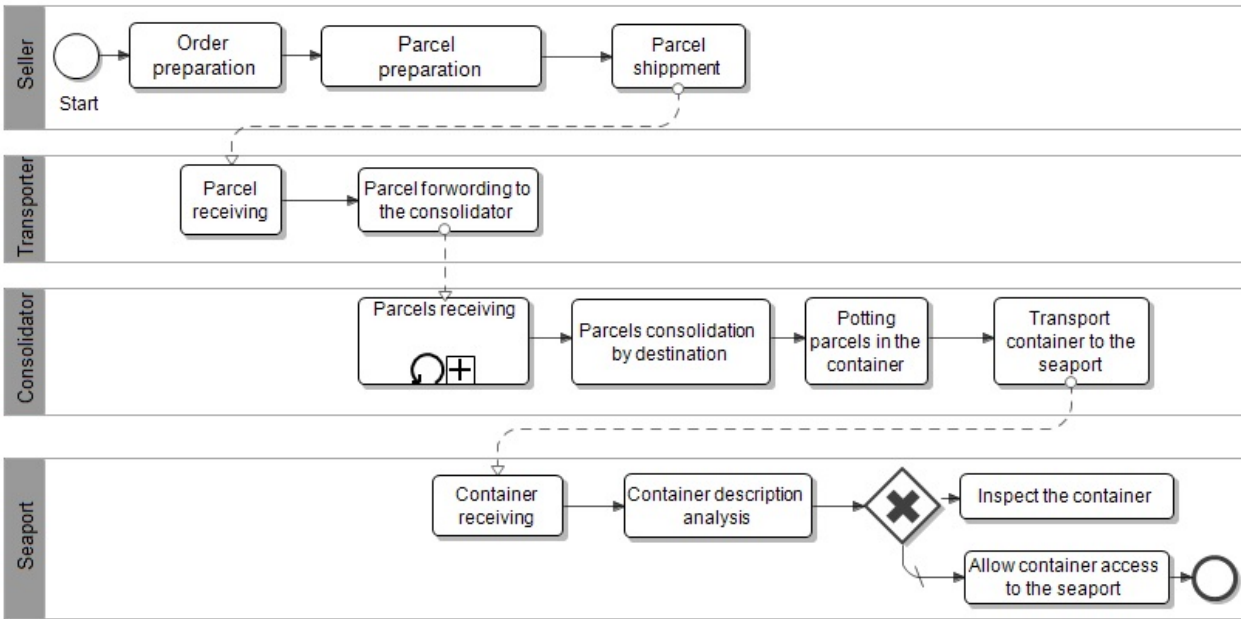


Figure 8. Case study: Less-than-container-load preparation and transport process.

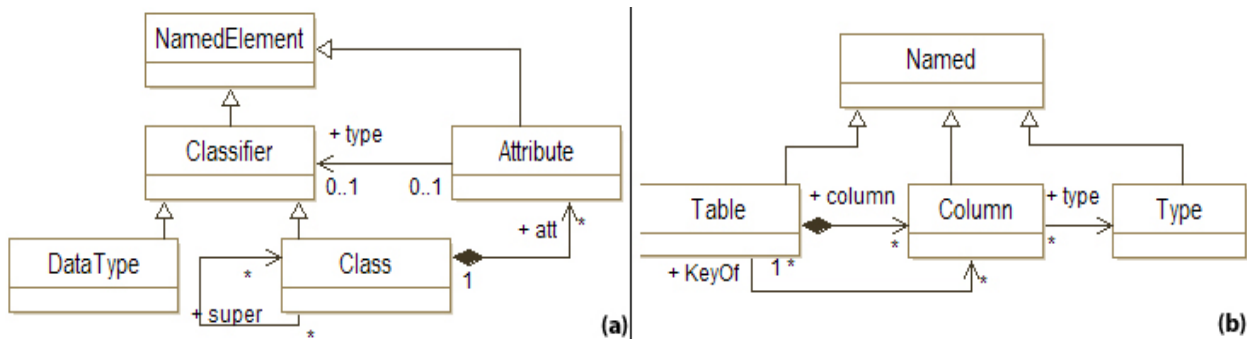


Figure 9. (a) UML class diagram meta-model (b) SQL meta-model.

generation, necessary to create the new database. This brings us to use in a first stage a class diagram meta-model to ensure compliance of the input model with the specifics of an UML class diagram, and in a second stage, a SQL meta-model that will guarantee compliance of the generated model to the specifics of the SQL implementation platform. Figure 9 shows a simple version of the meta-models used specifically for the case study.

The transition from a class diagram to a SQL model is based on transformation rules between the two models. These transformation rules are derived from the mapping which determines the correspondence among the elements of these aforementioned meta-models. These correspondences are detailed as follows:

Named Element to Named: Establishes a link between the two models parent classes; we use parent classes in

order to get main node during models specification using XML. Thus, all the classes composing a meta-model extend one main class.

DataType to Type: Binds attribute types of a class to columns types of a table, for example it binds the String to Varchar type; this guarantee that the parameters used by the deployed web-services correspond to au columns' type in databases.

Class to Table: Transforms the model classes into SQL table. Thus the generated SQL tables are used to deploy the database that will be interrogated by the web services.

Attribute to column: Allows the transformation of the attributes of a class in columns in a SQL table; this is the

<pre> <?xml version="1.0" encoding="ASCII"?> <xmi:XMI xml:version="2.0" xmlns:xmi="http://www.omg.org/XMI" xmlns="Class"> <Class name="Consolidator"> <attr name="idCriterion" multiValued="false" type="/1"/> <attr name="idContainer" multiValued="false" type="/1"/> <attr name="value" multiValued="false" type="/1"/> </Class> </xmi:XMI> </pre>	<pre> <?xml version="1.0" encoding="ISO-8859-1"?> <xmi:XMI xml:version="2.0" xmlns:xmi="http://www.omg.org/XMI" xmlns="Relational"> <Table name="Consolidator" Key="/1/@col.0"> <col name="objectId" KeyOf="/1"/> <col name="idCriterionId"/> <col name="idContainerId"/> <col name="valueId"/> </Table> </xmi:XMI> </pre>
(a)	(b)

Figure 10. (a) Input model class diagram (b) Output SQL model expressed with XMI.

```

CREATE TABLE Consolidator ( idContainer varchar(255), c1
varchar(255), c2 varchar(255), c5 varchar(255), c6
varchar(255), c9 varchar(255), c10 varchar(255), c18
varchar(255) );
ALTER TABLE Consolidator ADD CONSTRAINT
pk_Consolidator PRIMARY KEY (idContainer);

```

Figure 11. Generated SQL code: consolidator table.

basis for creating the tables for recording traceability data.

Association Column+: Transforms the connection between a class and its attributes to a table and its columns; this transformation rule brings together the generated columns relatively to the table that includes them.

Association KeyOf: Determines the primary key of the generated table. The key is used for identifying the databases records in unique manner.

In the studied case, ATL provides the transition from a class diagram to a SQL database model. Therefore, the implementation of the defined specifications mapping covers the transformation rules in Object constraints language (OCL). For example, "DataType to Type" is the transformation rule that determines the relationship between an UML class attribute type and a SQL table column type. It is implemented as following:

```

rule DataType2Type {
  from dt : Class !DataType
  to out : Relational !Type( name<- dt.name )
}

```

ATL requires the specification in Ecore of the two meta-models, SQL and class diagram. To do this, we used Eclipse modeling framework (EMF), which provides tools to generate Ecore meta-models from the diagrams shown in Figure 10. In addition, the ATL input model

must be specified in XMI (XML Metadata Interchange). This is achieved using the EMF tools, which enables the generation of XMI code corresponding to a class diagram, along with ensuring the validity of the generated code with its meta-model. The Figure 10 shows the XMI input (a) and output (b) models.

The changes made by the ATL allow the passage from an input model to an output model -expressed in XMI- that meets the specifics of the implementation platform. The output model is not directly exploitable and requires a second transformation type "ModelToText" to generate SQL code for deploying the new DB. This step is completed by using the eXtensible Stylesheet Language Transformations (XSLT). It is a XML transformation language proposed by the W3C to convert XML documents into other languages. Thus, the transition from the generated XMI model to SQL is done by specifying a XSLT script that determines the transformation rules. These rules are executed using a XSLT processor. The following script shows the implementation code of the generated DB table that corresponds to the consolidator with the criteria that describe his interventions (Figure 11).

The second phase of the process of generating DAWs covers the use of the Netbeans IDE to generate the CRUD classes in Java, needed for the DB operation. We focus on classes of tables' consultation to meet the need of collecting traceability information. The Netbeans IDE provides tools for identifying entity classes from the deployed DBs. In this step, the generated classes are deployed as WS. The following script shows an example


```

<?xml version="1.0" encoding='UTF-8'?> <operation name="findAll">
<definitions xmlns:tns=           <input wsam:Action=
"http://seller.ws/" xmlns=       "http://seller.ws/sellerWS/findAllRequest"
"http://schemas.xmlsoap.org/wsdl/" message="tns:findAll"/>
targetNamespace="http://seller.ws/" <output wsam:Action=
name="sellerWS">                 "http://seller.ws/sellerWS/findAllResponse"
<types>                          message="tns:findAllResponse"/>
<xsd:schema>                      </operation>
</types>                          </portType>
<message name="findAll">          <binding name="sellerWSPortBinding"
<message name="findAllResponse"> type="tns:sellerWS">
<portType name="seller">         <service name="sellerWS">

```

Figure 12. WSDL script describing the generated web-service.

of the WSDL file describing the deployed DAWS to retrieve information about a seller (Figure 12).

Web-services orchestration

In this step, we propose a solution to use DAWS for the deployment of a new composite web service. This latter is invoked by the intelligent container to meet the traceability requests. Thus, the solution is based on the use of BP logic to orchestrate the granular DAWSSs.

To exploit the BPs modeling for orchestrating DAWS, we opted for the BPEL Designer proposed by Eclipse IDE. It allows modeling the invocation order of DAWS along with the implementation of activities that represent the BP shown in Figure 9. The BPEL Designer simplifies the generation of BPEL code that will execute the BP. As a result, it enables automating the execution of the BPs by linking its activities to DAWS. This allows the generation of composite WS based on the orchestration of DAWS. The intelligent container will respond to external requests by operating the composite web-service. Figure 13 shows a model for the BPEL invocation of three DAWSSs.

DISCUSSION

The objective of this work is to urbanise the GOST traceability system to meet the evolving data needs to improve the risk management during containers transport. For this purpose, we specified a new distributed architecture of GOST system based on web-services and the concept of intelligent container. Furthermore, a development process based on the MDA was proposed to automate the code-generation of web-services to facilitate the adaptation of systems used by supply-chain actors to share traceability information.

Our first contribution is the proposal of an enriched model of intelligent container through the specification of web-service layer. The aim of this contribution is to

enhance the connectivity of the container with the external system and to delegate gathering the traceability data to each intelligent container. This solution allows us to avoid confidentiality problems related to the risk of data divulgation. Thus we differentiate from existing IP models axed on data gathering.

Our second contribution manifests in the proposal of a development based on MDA for web-service generation. The web-services were chosen because they allow to build more flexible architecture and to speed the integration of existing applications. Moreover, the proposed process minimizes the costs of adapting the heterogeneous systems to communicate with the intelligent container. The main advantage of this process is reflected in the separation of the system aspects in several models and automating the transition from one model to another using transformation rules as earlier specified. It was pointed out that this process is structured into many steps likely to be laborious; however it is based on open sources solutions and it is founded on well know standards. Thus, this approach contributes to minimize development times and can be adopted to accelerate software development cycle with other technologies.

As a perspective of this work, it is assumed that this solution can be improved by the use of Enterprise service web (ESB) (Ming-zhe, 2013). Thus, the connectivity among intelligent containers and external systems can be ensured by the configuration of an ESB. Furthermore, we can avoid the generation of the DAWS by accessing the databases directly using the adapters proposed the ESB; however this solution required a special configuration for each database.

CONCLUSION AND PERSPECTIVES

This paper was aimed to enhance traceability system functioning to meet the needs of securing containers transportation. For this, a decentralized structure of GOST traceability system based on the exploitation of the

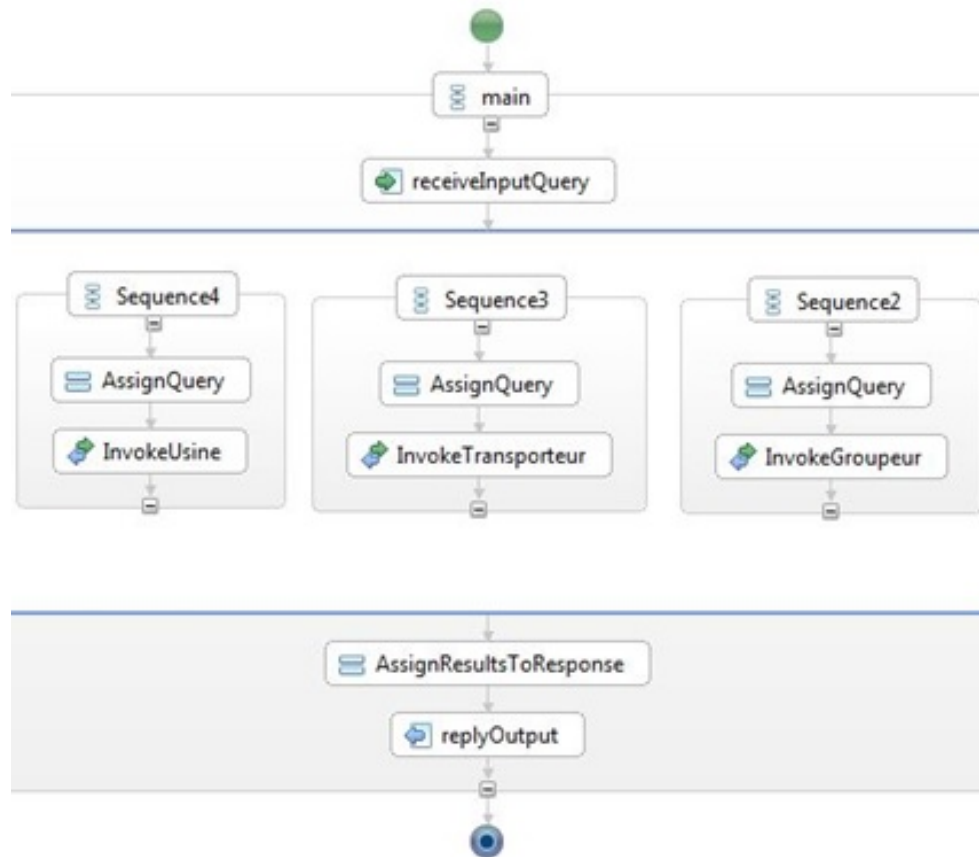


Figure 13. BPEL diagram for orchestrating the generated web services.

IP concept and web-services was proposed. The main contribution of this work is reflected in the proposal of a model-driven architecture approach and web-services to simplify the collection of traceability information. To this end, the ATL was used to automate the generation of web-services based on an UML class diagram. In addition, the BPMN diagram was used to orchestrate the generated web-services according to the business logic. Work is currently underway for the proposal of a new solution to ease the collection of traceability information based on the configuration of an enterprise service bus. The main contribution of this alternative manifests in the availability of open source adapters for interfacing legacy systems with GOST traceability system.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGMENTS

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Full Length Research Paper

Antioxidant and antibacterial activities of saponin fractions of *Erythropheleum suaveolens* (Guill. and Perri.) stem bark extract

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***Erythropheleum suaveolens* stem bark saponins fractions were investigated for antioxidant and antibacterial activities. *In vitro* systems such as, 1, 1- diphenyl-2-picrylhydrazyl (DPPH) radical, reductive potential and inhibition of lipid peroxidation were carried out to determine the antioxidant activities. The antimicrobial activity was determined using the hole-in-plate agar diffusion technique and the minimum inhibitory concentration (MIC) of each fraction was determined using the agar dilution method. All the fractions scavenged DPPH radicals but not comparable with ascorbic acid. These fractions also inhibited lipid peroxidation induced with highest percentage inhibition exhibited by 70:30 fraction which compare favourably with tannic acid. The reducing potential decreased with increasing concentration for all the fractions. The result of antibacterial studies revealed that 70:30 fraction inhibited the tested bacteria to a greater extent than the other fractions while the determination of the MIC of each fraction against the organisms showed that 70:30 fraction possessed a broader spectrum of activity against gram positive cocci of medical importance and also inhibited all the gram negative organisms tested. The study indicates saponin fractions of *E. suaveolens* stem bark to be potential candidates as antioxidant and antimicrobial agents with the 70:30 saponin fractions being the most promising and that these two activities augment each other.**

Key words: *Erythropheleum suaveolens*, saponins, 1, 1- diphenyl-2-picrylhydrazyl (DPPH), lipid peroxidation, reductive potential and antimicrobial.

INTRODUCTION

Saponins are surface-active, high molecular-weight and chemically complex group of compounds that occur naturally in plants, to a lesser extent in lower marine organisms and some bacteria (Riguera, 1997; Yoshiki et

al., 1998; Price et al., 1987). Saponins generally are composed of two major parts, one or more sugar or oligosaccharide moieties glycosidically linked to a triterpenic or steroidal liposoluble structure (aglycone or

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sapogenin). The aglycone may contain one or more unsaturated C–C bonds. This combination of polar and non-polar structural elements in their molecules explains their soap-like behaviour in aqueous solutions (Tsukamoto et al., 1995; Oleszek and Stochmal, 2002). Reported pharmacological properties elicited by some isolated saponins include hypocholesterolaemic, anticarcinogenic, immunostimulant, hypoglycemic, molluscicidal, antiprotozoa, antioxidant etc (Hostettmann and Marston, 1995). Such findings raise the possibilities of exploiting plant extracts rich in these metabolites as pharmacological and veterinary products, and herbal medicines (Attele et al., 1999; Oakenfull, 1981). Saponins have found wide applications in beverages and confectionery, as well as in cosmetics (Price et al., 1987; Petit et al., 1995; Uematsu et al., 2000).

Erythrophleum suaveolens is an example of plant rich in saponins. It is a perennial tree of about 30 m in height, bole seldom straight, slightly buttressed, often low-branching and producing a dense spreading crown. It is referred to by various names by natives. It is referred to in English as sassy, sasswood, red water tree and ordeal tree. The species are of ethnological interest as ordeal poison as indicated in many of their Europeans and African names. The English 'red water tree' is derived from the capacity of the stem bark decoction or infused in water to turn the water red (Burkill, 1985).

The stem bark is the most interesting part of the tree being the source of most notorious ordeal poison in Africa to trial criminals accused of serious misdemeanors, or person suspected or accused of witchcraft.

Ngounou et al. (2005) in their studies on the antimicrobial activity of diterpenoid alkaloids (amide norcassaide and norerythrosua-veolide) from *E. suaveolens* reported that these compounds showed potent antimicrobial activities against bacteria and yeasts.

The antibacterial potentials of *E. suaveolens* aqueous and chloroform fractions against some selected bacterial isolates were investigated by Aiyegoro et al. (2007). The two fractions were reported to compare favourably with the standard antibiotics, streptomycin and ampicillin at concentration of 1 mg/ml and 10 µg/ml respectively.

Antioxidants are the body's natural defense mechanisms against the damaging effects of "free radicals" and oxidation reactions that damage cells and cause disease. The main function of antioxidants is to prevent oxidation in various ways. It has been known for some time that antioxidants play a very important biological role in the body by preventing against oxidative damage (particularly oxidative DNA damage), thus preventing cardiovascular, neurological and carcinogenic diseases and delaying chronic health problems like cataracts (Williamson et al., 2000; Morton et al., 2000).

Usually, polyphenols and carotenoid pigments being the major nutritional antioxidants in food attract most of the research in this area. Some saponins have also been found to have anti oxidative or reductive activity (Francis

et al., 2002). From our recent investigations (Akinpelu et al., 2012) saponin fractions from the stem bark of *E. suaveolens* protected red blood cells to some degree and one of the fractions (70:30) compete favourably with standard drug used in protecting the cell membrane integrity. This study is to investigate further the antioxidant and antibacterial activities of *E. suaveolens* fractions. This is a view to discover and explain possible mechanisms through which saponin exert its antioxidant and antibacterial activities.

EXPERIMENTAL

Plant materials

Dried stem-barks of *E. suaveolens* were obtained from the Central Local Market (Oja Tuntun) in Ile-Ife, Osun State, Nigeria. The plant was identified and authenticated by Dr. H .C. Illoh, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The voucher specimen copy has been deposited at the IFE Herbarium Obafemi Awolowo University, Ile-Ife, Nigeria.

Sources of microorganisms

The test organisms employed for screening antimicrobial activities of the extracts were gram positive organisms (*Bacillus subtilis*, *Clostridium sporogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus capitis*, and *Staphylococcus xylosus*), gram negative organisms (*Escherichia coli*, *Shigella* spp., *Proteus* spp., *Klebsiella* spp., and *Pseudomonas aeruginosa*) and yeasts (*Candida albicans* and *Candida pseudotropicalis*). All organisms were obtained from the Department of Pharmaceutics Microbiology Laboratory of Obafemi Awolowo University, Ile-Ife, Nigeria. The bacteria and yeasts were sub-cultured into fresh nutrient agar plates and sarbouraud dextrose agar plates respectively 24 h before use for antimicrobial test.

Preparation of alcoholic extract

Ethanol extract of the stem bark of *E. suaveolens* was prepared according to the procedure as earlier described by Oyedapo and Amos (1997). Powdered (900 g) dried stem bark was suspended in 4.2 L of 80% (v/v) ethanol for 72 h at room temperature. The suspension was filtered through two layers of cheese-cloth. The extraction process was repeated four times until the extract became clear. The filtrates were combined and concentrated under reduced pressure on Edwards High Vacuum Pump rotatory evaporator (Edward Vacuum Co-operation, Crawley, England) at 35°C to give a coffee brown residue. The total crude ethanolic extract weighed 500 g which was 55.56% of the starting material and was stored in the dessicator until required for further processing.

Isolation of saponins from crude ethanolic extract of *E. suaveolens*

Isolation of crude saponin was carried out according to a procedure that was based on the methods described by Abdel-Gawad et al. (1999) and Wagner et al. (1984). The ethanolic extract (10 g) was washed twice with chloroform (50 ml x 2) and, also twice with ethylacetate (50 ml x 2). The residue was allowed to dry and then dissolved in 50% (v/v) methanol. The water-methanol solution was extracted three times (100 x 3) with butanol. On evaporation, a

syrupe residue was obtained which was taken up in methanol. The residue was dissolved in 50 ml 50% (v/v) methanol followed by the addition of diethylether (100 ml) to precipitate crude saponins. The upper diethylether layer was carefully removed; the residue was dissolved in a little amount (10 ml) of methanol and was poured into diethylether (200 ml). The upper diethylether was again removed and the residue was dissolved as described earlier and precipitated by the addition of diethylether. The precipitate was further purified by repeated dissolution in methanol and precipitation with diethylether until a cream light brown precipitate was obtained. The procedure was carried out until 350 g of crude ethanolic extract was analysed. A total yield of 55.35 g of light brown precipitate termed crude saponins was obtained.

Fractionation of crude saponin

The crude saponin was fractionated on silica gel (Kiesel gel 60; 60-200 mesh) column chromatography using gradient elution mixture of dichloromethane with methanol (DCM: MeOH) according to the modified method of Lin et al. (2008). A clean dry glass column (4 x 40 cm) was carefully packed with silica gel (Kiesel gel 60) to the required height of 30 cm. The silica gel was over layered with alumina powder (aluminium oxide) to a height of 5 cm. The column was washed and equilibrated with dichloromethane (200 ml x 2). Crude saponin (4 g) was thoroughly mixed with dry silica gel powder in a mortar with pestle. The sample was then layered carefully on top of alumina powder and a small amount of clean silica gel was carefully poured on top of packed gel to a height of 2 cm. Elution was carried out with the solvent mixtures (DCM:MeOH; 100:0 – 0:100). Fractions were collected for each of the solvent mixtures and evaporated to dryness at 40°C on rotatory evaporator to give various fractions for each solvent system. Each fraction was evaporated to dryness and kept for further analyses.

Tests for saponin

Saponin was tested for according to a procedure that was based on those earlier reported by Evans (2002) and Sofowora (2008) with slight modifications.

Frothing test

Saponin (5 mg) in a test tube was dissolved in distilled water (5 ml). The mixture was shaken vigorously until frothing. The production of honeycomb-like frothing which persisted after warming at 50°C for up to 15 min was indicative of saponin.

Lieberman-Burchard reaction test

Saponin (0.2 g) was dissolved in 1.0 ml acetic anhydride in a test tube, and then concentrated sulphuric acid (2 ml) was added carefully through the side of a test tube by means of a Pasteur pipette. The occurrence of a bluish-green colour in the upper liquid layer on a reddish-brown ring at the interface of the two liquid layers was taken as a positive test for the presence of steroidal nucleus.

Antioxidant assay

DPPH radical assay

The antioxidant activity of the extracts, on the basis of their scavenging activity of the stable 1, 1 – diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by

Braca et al. (2001). Aqueous extract (0.1 ml) was added to 3 ml of 0.004% methanol solution of DPPH. Absorbance at 517 nm was taken after 30 min and the percent inhibition activity was calculated using the expression below:

$$\text{Percentage Inhibition Activity} = (A_0 - A_e / A_0) \times 100$$

where A_0 = Absorbance without extract and A_e = Absorbance with extract.

Lipid peroxidation and thiobarbituric acid reactions

A modified thiobarbituric acid reactive species (TBARS) assay as described by Ohkawa et al. (1979) was used to measure the lipid peroxide formed using egg yolk homogenate lipid rich media. Egg homogenate [0.5 ml of 10% (v/v)] and 0.1 ml of extract were added to a test tube and made up to 1ml with distilled water; 0.05 ml of copper sulphate (0.07 M) was added to induce lipid peroxidation and the mixture was incubated for 30 min. Then 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8 % (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulphate were added and the resulting mixture was vortexed and then heated at 95°C for 60 min. After cooling, 5.0 ml of butanol were added to each tube and centrifuged for 10 mins. The absorbance of the organic layer was measured at 523 nm. Percentage inhibition of lipid peroxidation by the extract was calculated using the expression $[(1-E)/C] \times 100$, where C is the absorbance value for fully oxidized control and E is the absorbance in the presence of extract.

Reducing power

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Different concentrations of extract (100 – 1000 µg) in distilled water was mixed with phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 20 min. To the mixture was added 10% (w/v) trichloroacetic acid, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.1% $FeCl_3$ (0.5 ml) and the absorbance was measured at 700 nm, increased absorbance of the reaction mixture indicated reducing power. Ascorbic acid was used as a standard to compare the reducing power of the extracts.

Antimicrobial assay

Colonies of 20 h old cultures of all test organisms were suspended in sterile normal saline and the density adjusted to 0.5 MacFarland Standard. This was swabbed on fresh Mueller-Hinton agar for the bacteria and sarbouraud dextrose agar for the yeast with the aid of sterile cotton tipped applicator. The water soluble extracts were reconstituted with sterile water to give the different concentrations tested. The different extracts were then introduced into holes bored in the agar plates. Sterile distilled water was used as negative control while cefuroxime and ketoconazole was used as positive control antibiotics for the bacteria and yeast respectively. All plates were left in the fridge for 1 h to allow diffusion of test extracts before incubating at 37°C for the bacteria and 25°C for the yeasts. Zones of inhibition were measured in millimeter (mm) after 24 h of incubation.

Minimal inhibitory concentration (MIC)

The MIC of the extract was determined for each of the test organisms using the agar dilution susceptibility tests. Duplicate

Table 1. DPPH radical scavenging activities of saponin fractions of *E. suaveolens* stem bark and ascorbic acid.

Sample	Concentration ($\mu\text{g/ml}$)	% Inhibition \pm SD (n = 3)	Regression equation	IC ₅₀ ($\mu\text{g/ml}$)
90:10 Sap Fraction	250	28.40	Y=-0.060x +41.52 (r ² = 0.8741)	141.33
	330	25.50		
	500	11.40		
80:20 Sap Fraction	250	25.20	Y=-0.061x +43.23 (r ² = 0.8481)	110.98
	330	26.60		
	500	11.10		
70:30 Sap Fraction	250	26.60	Y=-0.069x +45.18 (r ² = 0.8136)	69.86
	330	27.10		
	500	9.00		
60:40 Sap Fraction	250	24.60	Y=-0.070x +44.93 (r ² = 0.8547)	72.43
	330	26.00		
	500	8.60		
Ascorbic acid	2	13.01	Y=7.60x +2.34 r ² =0.9996	6.89
	4	27.41		
	6	43.81		
	8	73.40		

IC₅₀ – half maximal inhibitory concentration; Sap – Saponin

oven-dried Mueller-Hinton agar plates containing different concentration of test extracts in agar were inoculated with the test bacteria using a multi-inoculator and incubated at 37°C for up to 72 h. The yeasts were tested in duplicate Sabouraud dextrose agar containing the different concentrations of the extracts and incubated at 25°C. The presence or absence of growth of each test organism was noted on each plate. The minimum concentration inhibiting the growth of a test organism was recorded as the MIC against the organism.

RESULTS AND DISCUSSION

DPPH-Radical scavenging activity

DPPH free radical scavenging method has been widely applied for evaluating antioxidant activity in a number of studies (Brad-Williams et al., 1995). The principle of DPPH method is based on the reduction of DPPH in the presence of a hydrogen donating antioxidant. Quite a number of naturally occurring antioxidants have been demonstrated to play prominent roles in inhibiting both free radicals and oxidative chain reactions within tissues and membranes (Nsimba et al., 2008).

In Table 1 is the result of the DPPH scavenging activity of *E. suaveolens* stem bark saponins. It was observed that *E. suaveolens* stem bark saponins inhibited DPPH free radical scavenging activity better at lower concentrations, that is, 250 and 330 $\mu\text{g/ml}$. Although the DPPH radical scavenging abilities of the saponin fractions were less than that of ascorbic acid but the result showed that *E. suaveolens* saponin fractions has the proton-donating ability and could serve as free radical

inhibitors or scavenger, acting possibly as primary antioxidant.

Reducing power

The ability of *E. suaveolens* stem bark saponin fractions and ascorbic acid (AA) to reduce Fe³⁺ / ferricyanide complex to ferrous form is represented in Figure 1. Low values indicate high antioxidant activities. The reductive potential of saponin fractions decreased with increase in fraction concentrations. Fraction 60:40 showed highest reductive potential at 200 $\mu\text{g/ml}$ used followed by 70:30, 80:20 and 90:10 fractions respectively, the more polar the fraction the higher the reductive activity. The reductive potential of AA was higher than that of saponin fractions at all concentrations, however it should be noted that the reductive potential of saponin fractions were still appreciable. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995).

Inhibition of lipid peroxidation

Peroxidation of lipids has been shown to be cumulative effect of reactive oxygen species, which disturb the assembly of the membrane causing changes in fluidity and permeability, alterations of ion transport and inhibition of metabolic processes (Nigam and Schewe, 2000). The extract exhibited strong lipid peroxidation inhibition at 1000 $\mu\text{g/ml}$ in the order 70:30>80:20>60:10>80:20 and compared favourably with

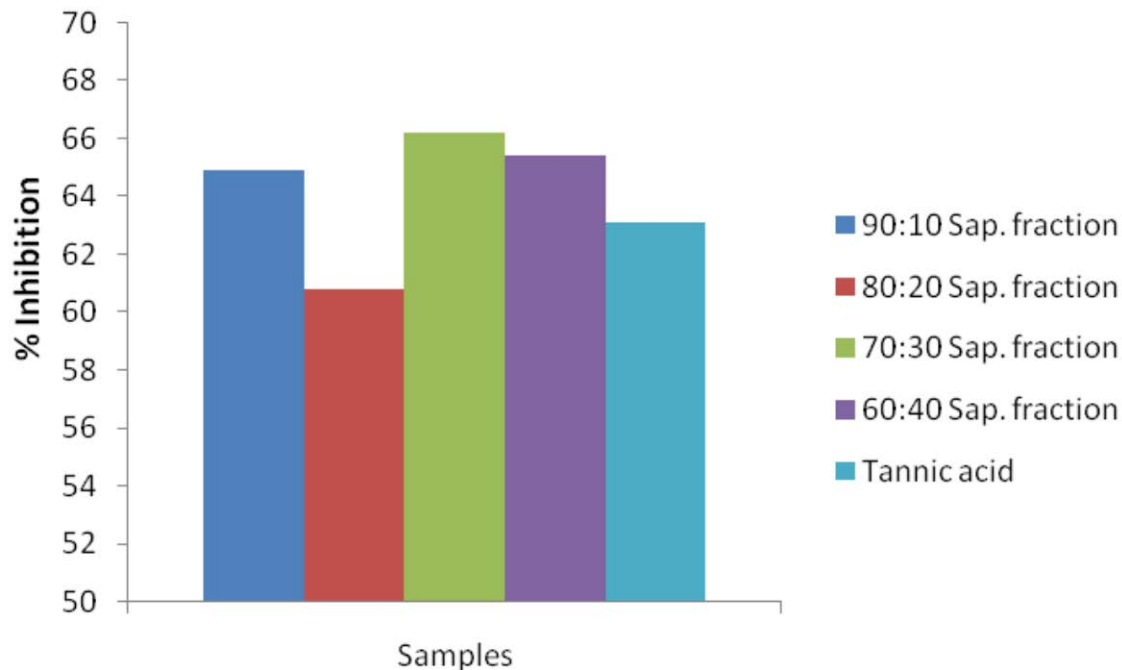


Figure 1. Lipid peroxidation inhibition of saponin fractions of *E. suaveolens* stem bark and tannic acid, Sap – Saponin.

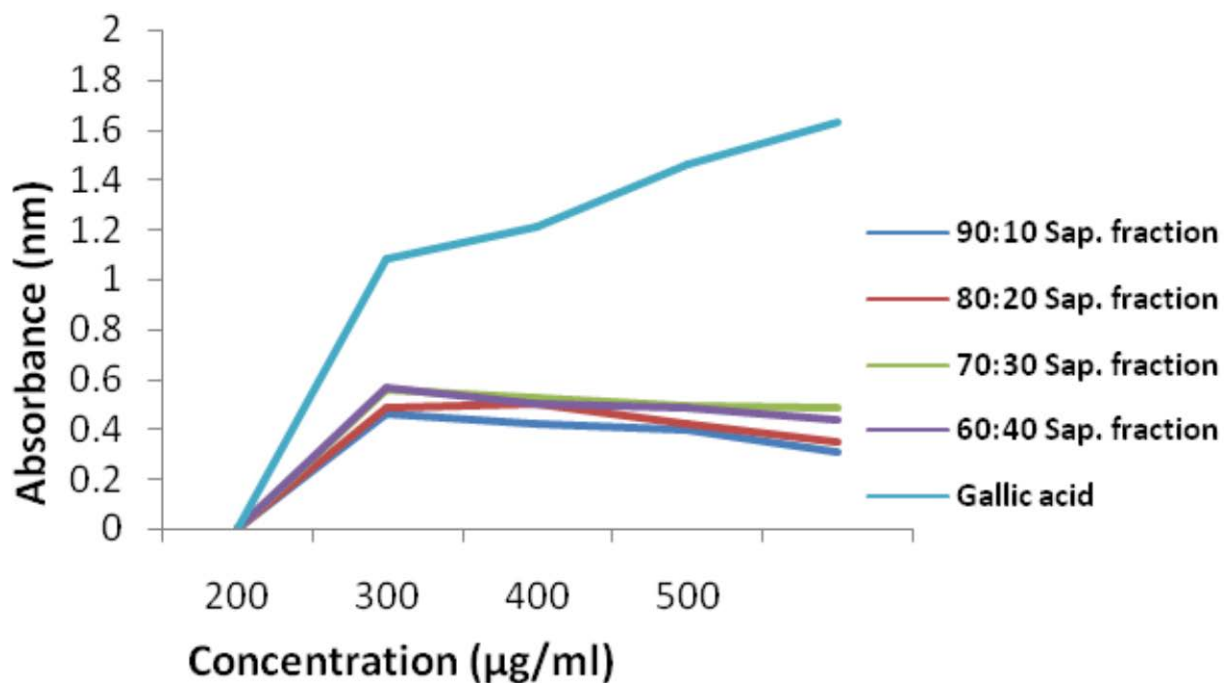


Figure 2. Reducing power of saponin fractions of *E. suaveolens* stem bark and gallic acid, Sap – Saponin.

tannic acid at the same concentration (Figure 2). This result suggests that *E. suaveolen* saponins could play a role in protecting the physicochemical properties of membrane bilayer from free radical induced severe

cellular dysfunction. The fractions inhibit the oxygen free radicals formation and this may be one of the possible mechanisms of membrane stability activities these fractions as reported by Akinpelu et al. (2012).

Table 2. Zones of inhibition of the different saponin fractions of *E. suaveolens* stem bark.

Fraction / concentration	Organism / Zones of inhibition (mm)					
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>C. pseudotropicalis</i>
100 mg/ml						
A	12	12	0	0	0	10
B	10	8	0	0	0	0
C	18	14	11	0	12	14
D	11	10	0	0	11	14
50 mg/ml						
A	10	10	0	0	0	8
B	8	7	0	0	0	0
C	15	12	10	0	10	12
D	9	8	0	0	10	12
25 mg/ml						
A	8	8	0	0	0	7
B	0	0	0	0	0	0
C	12	9	8	0	8	10
D	7	0	0	0	7	10

A: 90:10 fraction; B: 80:20 fraction; C: 70:30 fraction; D: 60:40 fraction.

Antimicrobial studies

The zones of inhibition of the different saponin fractions of *E. suaveolens* stem bark to *B. subtilis*, *S. aureus*, *E. coli*, *Ps. Aeruginosa*, *C. albicans* and *C. pseudotropicalis* are presented in Table 2. The results of the antimicrobial studies of the saponin fractions of the bark of *E. suaveolens* revealed that it had antimicrobial activity which was concentration dependent. The determination of the zones of inhibition of same concentration of each fraction showed that all the fractions were more active against the gram positive bacteria *B. subtilis* and *S. aureus* than the gram negative organisms. All the fractions were also found to be active against *C. pseudotropicalis* with the exception of the 80:20 fraction while *C. albicans* was inhibited by the 70:30 and the 60:40 fractions. This shows that the saponin fractions of *E. suaveolens* had more activity against the gram positive organisms than the gram negative bacteria. This is expected and it suggests that the mechanism of action of the saponins in the tested fractions of the plant is related more to cell wall integrity and functions since the difference in the two groups of bacteria is in the cell wall. The cell wall of gram-positive bacteria is less chemically complex than that of the gram negative bacteria (Lamikanra, 2010) and gram-negative bacteria are known to be resistant to the action of many antimicrobial agents including antimicrobial plant extracts (Kambezi and Afolayan, 2008; El-Mahmood, 2009). The anti-staphylococcal activity of this saponin fraction is significant due to the fact that the spectrum of activity

included the methicillin-resistant *S. aureus* (MRSA) strains which are important causes of many hospital and community acquired infections (CDC, 2012; Taiwo et al., 2004).

The results of the study also showed that the 70:30 fraction inhibited the tested bacterial to a greater extent than the other fractions (Table 3). The determination of the minimum inhibitory concentration of each fraction against a wide range of organisms which included both the gram negative and gram positive bacteria as well as *Candida* species showed that not only was the 70:30 fraction more active, it also possessed a broader spectrum of activity against gram positive cocci of medical importance and also inhibited all the gram negative organisms tested. This included the notorious multi-resistant organism *Pseudomonas aeruginosa* (Bousseimi et al., 2005; Olayinka et al., 2004; Bradford, 2001). This activity was not observed with any of the other fractions. This result suggests that the antimicrobial principles in these fractions are likely to be more than one and the 70:30 fraction probably contains the most potent principles than any of the other fractions.

Conclusion

Hence it could be surmised that the *E. suaveolens* saponins could be used to prevent damage caused by free radicals and infections caused by pathogenic bacteria. In addition, 70:30 *E. suaveolens* saponin fraction stem bark could be a useful starting point if the

Table 3. Minimum inhibitory concentration (MIC) of saponin fractions of *E. suaveolens* stem bark.

Organism	Source	No. of strains tested	MIC of fraction (mg/ml)			
			A	B	C	D
<i>B. subtilis</i>	Reference NCTC	1	>8	20	<4	8
<i>B. subtilis</i>	Reference NCIB	1	<2	20	<4	2
<i>B. cereus</i>	Reference NCIB	1	>8	<20	8	>8
<i>S. aureus</i>	Reference NCTC	1	4	20	8	2
<i>S. aureus</i>	Reference NCIB	1	>8	20	8	2
MRSA	Clinical	11	< 2 (1); 4 (1); 8 (1); >8 (8)	> 10 (4); > 20 (7)	<4 (2); 8 (9)	2 (3); >8 (8)
<i>S. epidermidis</i>	Clinical	3	8 (1); >8 (2)	>10 (2); >20 (1)	<4 (2); 8 (1)	2 (2); >8 (1)
<i>S. capitis</i>	Clinical	2	8	>10	<4	-
<i>S. xylosus</i>	Clinical	1	-	-	8	-
<i>Cl. Sporogenes</i>	Reference NCIB	1	-	-	8	-
<i>E. coli</i>	Reference ATTC	1	-	-	8	-
<i>E. coli</i>	Commensal	1	-	-	8	-
<i>Shigella spp</i>	Reference NCIB	1	-	-	8	-
<i>Shigella spp</i>	Clinical	1	-	-	8	-
<i>Proteus spp</i>	Reference NCIB	1	-	-	8	-
<i>Proteus spp</i>	Clinical	1	-	-	8	-
<i>Klebsiella spp</i>	Reference NCIB	1	-	-	8	-
<i>Klebsiella spp</i>	Clinical	1	-	-	8	-
<i>Pseudomonas fluorescense</i>	Reference NCIB	1	-	-	8	-
<i>Pseudomonas aeruginosa</i>	Reference ATTC	1	-	-	8	-
<i>C. albicans</i>	NCYC	1	-	-	>8	4
<i>C. pseudotropicalis</i>	NCYC	1	8	-	8	4

A: 90:10 fraction; B: 80:20 fraction; C: 70:30 fraction; D: 60:40 fraction.

active saponin principles are to be exploited for development into antimicrobial chemotherapeutic agents in line with the ongoing search for substances to replace the antibiotics in current clinical use which because of the emergence and spread of resistant organisms are less useful than before (Iwata, 1992; Chopra et al., 1997).

Conflict of Interest

The authors have not declared any conflict of interest.

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