African Journal of Pure and Applied Chemistry

Academic
Journals

ABOUT AJPAC

The **African Journal of Pure and Applied Chemistry (AJPAC)** *is an open access journal that publishes research analysis and inquiry into issues of importance to the science community. Articles in AJPAC examine emerging trends and concerns in the areas of theoretical chemistry (quantum chemistry), supramolecular and macromolecular chemistry, relationships between chemistry and environment, and chemicals and medicine, organometallic compounds and complexes, chemical synthesis and properties, chemicals and biological matters, polymer synthesis and properties, nanomaterials and nanosystems, electrochemistry and biosensors, chemistry and industry, chemistry and biomaterials, advances in chemical analysis, instrumentation, speciation, bioavailability. The goal of AJPAC is to broaden the knowledge of scientists and academicians by promoting free access and provide valuable insight to chemistry-related information, research and ideas. AJPAC is a bimonthly publication and all articles are peer-reviewed.*

African Journal of Pure and Applied Chemistry (AJPAC) *is published twice a month (one volume per year) by Academic Journals.*

Contact Us

Editors

African Journal of Pure and Applied Chemistry

Table of Contents: Volume 8   Number 8   August, 2014

ARTICLES

Research Articles

Full Length Research Paper

Kinetic and equilibrium studies of the heavy metal remediation potential of *Helix pomentia*

Adeoye, Moriam Dasola[1]*, Azeez, Lukman Adeyemi[1], Lawal, AbdulAzeez Tunbosun[2], Olayiwola, Olajumoke Abidemi[3] and Shitta, Olanrewaju Razaq[1]

[1]Industrial and Environmental Chemistry Unit, Chemical Sciences Department, Fountain University, Osogbo Osun State, Nigeria.
[2]Industrial and Environmental Chemistry Unit, Chemical Sciences Department, Al-Hikmah University, Ilorin Kwara state, Nigeria.
[3]Chemical Sciences Department. Oduduwa University, Ipetumodu, Ile-Ife, Osun State, Nigeria.

Heavy metals are one of the major pollutants in water and are very toxic. However, chitosan – a waste biomass of agricultural products possess the ability to chelate heavy metals in water. In this study, the application of snail (*Helix pomatia*) shell based chitosan for remediating heavy metals contaminated Osun River (OSR), Odo-ebu stream (OBS) and Aso well water (AWW), all in Osogbo community, Nigeria; and the binding efficiency of chitosan for heavy metals in these water sources were evaluated. The result shows the prevalence of Iron (Fe), Cadmium (Cd), Zinc (Zn), Copper (Cu), Chromium (Cr), and Lead (Pb) ions in the three water samples with OBS and OSR having higher metal concentrations in the order: Pb > Cd > Fe > Zn > Cr > Cu compared with AWW which follows the order: Fe > Zn > Pb > Cr > Cd > Cu. The concentration range of heavy metals in OBS and OSR were (0.0011 -2.831) mg/L and (0.0012 - 1.687) mg/L respectively, while for AWW it was (0.0004-0.0237) mg/L and below the Standard Organization of Nigeria (SON) 2007 and World Health Organization (WHO) 2004; maximum permissible level of metal ions in drinking water. The prepared chitosan is a selective candidate for remediating four heavy metals ions from these water samples, with the maximum sorption capacities for the metal ions in the sequence: Pb > Cd > Fe > Cr. The sorption behaviour followed a pseudo-second-order model, while the equilibrium data correlated well with the Langmuir isotherm models with the R_L values in the range $0 < R_L < 1$.

Key words: Heavy metals, *Helix pomatia*, chitosan, dumpsite, equilibrium studies.

INTRODUCTION

Heavy metals are of great concern due to their contrasting toxicities, long-term mobilities, bio-availabilities and their non-degradable nature in the environment. They enter the environment through a

*Corresponding author. E-mail: dasoladeoye@yahoo.com, Tel: 08028944246.

Figure 1. Map of Osogbo showing sampling area.

variety of anthropogenic and industrial activities, poor waste disposal mechanism; and pose serious threats to plants and human health (Kanamadi et al., 2003; Jude and Augustin, 2007). Heavy metals such as copper, zinc, nickel, chromium, cadmium and iron present in most Nigerian rivers have been found in concentrations well above acceptable and permissible levels (Eniola et al., 2010). Although, degradation of water quality has been claimed to be most severe in the four states that contain 80% of the nations industries that is, Lagos, Rivers, Kano and Kaduna state but this has extended to other regions. Thus, the importance of environmental quality of Osogbo (an urban settlement) in Osun state (south-western Nigeria) has attracted a great deal of interest due to the increasing population of the inhabitant, increasing industrialization, poor land use system, agricultural activities, industrialization and anthropogenic impact which has made the water and land being polluted (Olajire and Imeokparia, 2001). This is of great concern due to the great threat on health and economic development (Mustapha, 2008).

Bioremediation, the use of waste-derived materials or microbes to detoxify and degrade environmental contaminants provide safe and economic alternatives for the removal of toxic metal ions from wastewater or any aquatic environment and soil (Volesky, 2001). It also converts wastes into useful materials for soil and water decontamination. Chitosan, a poly-b (1-4)-2-amino-2-deoxy-D-glucosed derived from chitin - a natural biopolymer found in the outer shell of crustaceans such as crabs, shrimps and prawns has been found to have sorption capacity for several metal ions (Adewuyi et al., 2009; Yildiz et al., 2010). The metal sorption capacity of chitosan varies with crystallinity, affinity for water, deacetylation degree and amino group content. Kinetic studies have demonstrated that the rate of metallic ion sorption onto chitosan differs depending on the raw material (snail, shrimp, crab or lobster shells), preparation method, chemical modification, and chitosan particle shape (Martha, 2008).

This study presents the kinetic and uptake performance of the binding potential and selectivity of prepared biopolymers- chitosan for heavy metal ions (Cr, Pb, Cd, Fe) in the Osun River, Odo Ebu and Aso Hotel well water which serve as the major sources of water for the Isale-Osun community. The importance of this study lies on the direct evaluation of the metal sorption capacity of chitosan from real contaminated water samples from Isale-Osun community.

MATERIALS AND METHODS

Chitosan preparation

Helix pomatia (snail) shell procurement, deproteination, demineralization and N-deacetylation of the chitin were carried out using the methods of Adewuyi et al. (2009) and Taboada et al. (2003). All the chemicals used were of analytical grade products of British Drug House (BDH), Poole, England. De-ionized-distilled water was also used throughout the whole processes. The resulting chitosan was collected, washed and oven dried at 60°C for 4 h. The degree of deacetylation of the prepared chitosan was carried out using literature method of Tolaimate et al. (2002). Ultra Violet-Visible spectra analysis (using JENWAY spectrophotometer) of the prepared chitosan sample, covering the wavelength range of 190 to 800 nm with quartz cells (with a thickness of 0.2 cm) before and after treatment with the different water samples to determine its wavelength of adsorption.

Sampling and metal analysis

Surfaces water samples from Aso Hotel well water, Ebu stream (Odo Ebu) and Osun River water, all in the vicinity of dumpsite located at Isale Osun axis of Asubiaro area (Lat 07,44° N Long 04.74°E) in Osogbo, Osun state were collected (Figure 1). The pH and temperature of these water samples was determined using

Jenway 3505 pH- portable meter. The water samples were digested using the method of Sallau et al. (2011). 50 cm^3 of each sample was treated with 5 cm^3 of conc. HNO_3 and heated on a hot plate with gradual addition of conc. HNO_3 as necessary until the solution boils. It was then evaporated to about 20 cm^3; 5 cm^3 of conc. HNO_3 was finally added, covered and allowed to cool and then filtered. The pH of these solutions was initially fixed at a pH higher than that for the chitosan solubilization threshold (pH around 6.2). The filtrate was poured into a 50cm^3 standard volumetric flask and made up to the mark with distilled water. Metal analyses of the digested samples were determined with Solaar AAS series 711047v1.22 atomic absorption spectrometer. Detection limits were estimated from digested blank (deionized water) which was run during the analysis. Triplicate digestions and analyses were run and average values were reported. Same procedure was repeated after the samples had been treated with chitosan.

Equilibrium studies

Equilibrium studies were carried out using the method of Adeogun et al. (2010). Adsorption was performed in a set of 100 ml flasks wherein the water samples (100 ml of each sample) were placed. Equal mass of 0.2 g of the prepared chitosan of particle size 40 mesh was added to the samples and kept on an isothermal shaker (orbital shaker) at 25 ± 1°C for 48 h for equilibrium to be reached between the solid-solution mixture. Similar procedure was followed for another set of 100 mls flasks containing the water samples without adsorbate (to be used as a control). The pH was adjusted to 4.8 - 6.0 by adding few drops of diluted HCl or NaOH (0.1 $moldm^{-3}$). The flasks were then removed from the shaker and the final concentrations of heavy metals in the solutions were determined by Atomic Absorption Spectroscopy (AAS). Each experiment was duplicated under identical conditions. The amount of adsorption at equilibrium, $q_e^/$ (mg/g), was calculated by:

$$q_e^/ = \frac{(C_0 - C_e)v}{w} \tag{1}$$

Where: C_o and C_e (mg/ml) are the concentrations of metal ion at initial and equilibrium stage respectively, V is the volume of the solution (ml), and W is the mass of dry adsorbent used (g).

Adsorption kinetics

The kinetic experiments were identical to those of equilibrium tests except that the aqueous samples were taken at different time intervals from the solid-solution mixture, and the concentrations of heavy metals in the solutions were similarly measured (Adeogun et al., 2010). The amount of adsorption q_t (mg/g), at time t, was calculated by:

$$q_t = \frac{(c_0 - c_t)v}{w} \tag{2}$$

C_o and C_t (mg/ml) are the concentrations of the metal ions in the samples at initial and any time t, respectively, V is the volume of the solution (ml) and W is the mass of dry adsorbent used (g). An ideal sorbent for metal decontamination should not only have a large sorbate capacity but also a fast sorption rate (Crini and Badot, 2008). According to Sud et al. (2008), predicting the rate at which sorption takes place and the binding mechanism are essential to determine the efficiency of a sorption process. These may be controlled by: (i) solute transfer from the bulk solution to the boundary film that surrounds the sorbent's surface, (ii) solute transport from the boundary film to the sorbent's surface, (iii) solute transfer from the sorbent's surface to the active intraparticle sites, and (iv) interaction(s) between solute and binding sites of the sorbent (intra-particle diffusion). The pseudo- first order and pseudo-second order kinetic and intra-particle diffusion (chemical binding reaction) kinetic models were employed to determine the rate constant and the controlling mechanism of the sorption process. These were done to further confirm the best fit kinetic model (s) for the sorption process. Although, several metal sorption studies have shown that most sorption kinetics usually followed the pseudo-second-order kinetic model. The pseudo-first-order and intra-particle diffusion equations were generally acclaimed to be applicable over the initial stage of the adsorption process (Mohan et al., 2006; Pan et al., 2009). The linear forms of pseudo- first order, pseudo- second order the intra-particle diffusion equations are as expressed in Equations 3, 4 and 5 respectively (Lagergren, 1898; Kamari, 2011; Ho and McKay, 2000).

$$\ln(q_e^/ - q_t) = \ln(q_e) - k_1 t \tag{3}$$

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \left(\frac{1}{q_e}\right) t \tag{4}$$

$$q_t = K_{id} t^{0.5} \tag{5}$$

Where: $q_e^/$ and q_t (mg/g) are the amount of metal ions sorbed (mg/g) at equilibrium and at time t (min) respectively while k_1 (g/mg/min), k_2 (g/mg/min) and k_{id} (mg/g/$min^{0.5}$) are the rate constants of the pseudo-first –order, pseudo-second-order and intra-particle diffusion equations for the bio-sorption processes respectively (Kamari et al., 2011).

Adsorption isotherm

Adsorption isotherm study was carried out using the Langmuir adsorption isotherm (Langmuir, 1916). Langmuir isotherm assumes monolayer adsorption onto a surface containing a finite number of adsorption sites of uniform strategies of adsorption with no transmigration of adsorbate in the plane of surface (Fytianos et al., 2003). The energy term in Langmuir equation varies as a function of the surface coverage (Fytianos et al., 2003). The applicability of the isotherm equation was judged by the correlation coefficients, R^2. The linear form of Langmuir's isotherm model is given by the equation:

$$\frac{C_e}{q_e^/} = \frac{C_e}{q_{max}} + \frac{1}{q_{max} \cdot b} \tag{6}$$

C_e is the equilibrium concentration of the adsorbate (heavy metal ion) (mg/L); q_e, the amount of adsorbate adsorbed per unit mass of adsorbent (mg/g); q_{max} and b are Langmuir constants which are related to monolayer adsorption capacity and affinity of adsorbent towards adsorbate respectively. The important characteristic of the Langmuir isotherm is expressed in terms of a dimensionless equilibrium constant separation parameter (R_L) (Saifuddin and Kumaran, 2005), which is defined as:

$$R_L = \frac{1}{(1 + b \, c_0)} \tag{7}$$

Where: b = the Langmuir constant, C_o = the highest metal ion concentration (mg/L).

Table 1. Weight of bio-sorbent obtained during production.

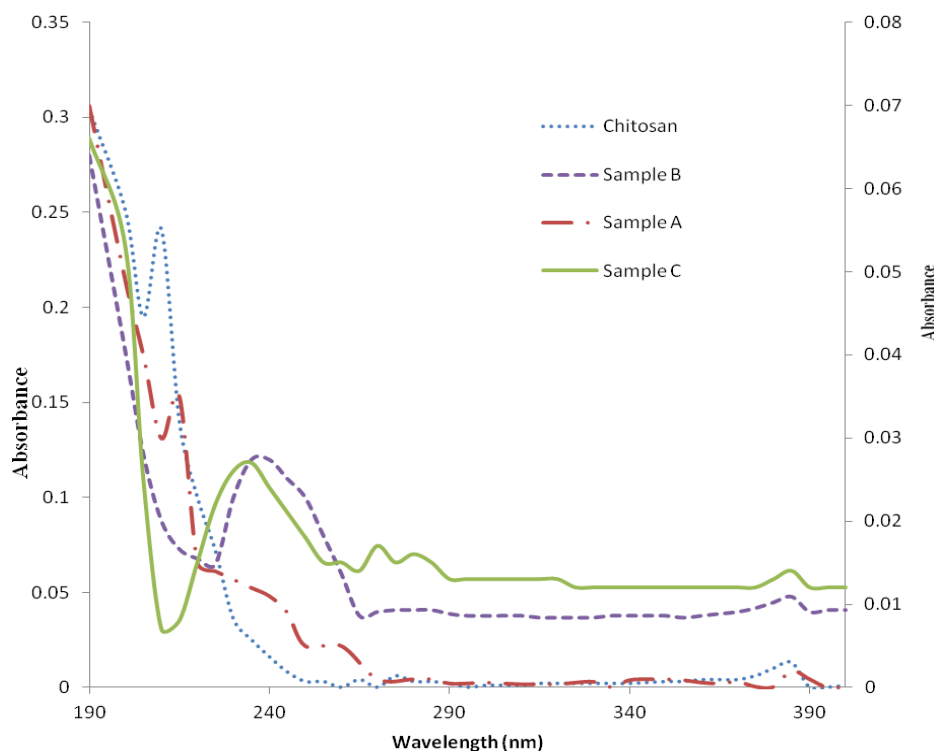| Stages of production	Dry weight (g)
Ground Shell	200.00
Demineralized Shell	56.23
Deproteinized Shell (Chitin)	50.26
Deacetyled Shell (Chitosan)	40.50

Figure 2. Electronic absorption spectra showing variation of the absorbance maximum as a function of metal ion concentration in solution. Sample A: Aso well water;   Sample B: Ebu Stream water; Sample C: Osun river water. The curves of Samples A and C are plotted against the secondary axis.

The value of R_L indicates the shape or type of the isotherm to be either unfavourable ($R_L > 1$), linear ($R_L = 1$), favourable ($0 < R_L < 1$) or irreversible ($R_L = 0$) (Saifuddin and Kumaran, 2005).

RESULTS AND DISCUSSION

Chitosan yield and characterisation

Yield of chitosan

The yield after each process is as presented in Table 1. The snail shell yielded approximately 20% of chitosan. This implies that snail shells (*Helix pomatia*) can be considered as a better source of chitosan compared to freshwater crab (*Potamon potamios*) whose yield is about 4.65% (Bolat et al., 2010), however, the yield can be improved if loss of mass due to washing can be reduced to the minimum possible. A contributing factor to this significant amount of yield is the non-edibility of the snail shell itself.

Characterisation of the prepared chitosan

The degree of deacetylation (%DD) of this snail based as calculated using potentiometric titration following Tolaimate et al. (2002) was found to be 65%. The electronic absorption spectra of pure and used chitosan sample depicting the effect of chelation on the spectra properties of chitosan is as shown in Figure 2. The

Table 2. The pH of the water samples and the initial concentration of heavy metal ions in the samples.

Sample	Concentration (mg/L)							
	pH	T(^{0}C)	Fe	Cd	Zn	Cu	Cr	Pb
A	6.94	27.7	0.0237	0.0007	0.0077	0.0004	0.0016	0.0019
B	8.90	29.4	0.0702	0.288	0.0275	0.0011	0.0036	2.831
C	8.33	28.7	0.0710	0.151	0.0139	0.0012	0.0040	1.687

Sample A: Aso well water; Sample B: Ebu Stream water; Sample C: Osun river water.

Table 3. Conc. of heavy metals ion in samples after treatment with chitosan.

Sample	Concentration (mg/L)					
	Fe	Cd	Zn	Cu	Cr	Pb
A	nd*	nd*	nd*	nd*	0.0001	0.0002
B	0.0163	0.0008	nd*	nd*	0.0001	0.0133
C	0.0153	0.0001	nd*	nd*	0.0004	0.0135

Sample A: Aso well water;   Sample B: Ebu Stream water; Sample C: Osun river water, nd*: not detected.

Table 4. Maximum permissible level of metal ions in drinking water.

Metals	SON (mg/L)	WHO (mg/L)
Pb	0.01	0.01
Cd	0.003	0.003
Cr	0.05	0.05
Cu	2	1
Zn	-	3
Fe	-	0.3

(NIS, 2007; WHO, 2004).

prepared chitosan absorbs at a wavelength of about 210 nm which is close to the characteristic bands (208 nm) reported for highly deacetylated chitosan (Tolaimate et al., 2002). There is shift in the wavelength (λ_{max}) of absorption of the treated chitosan with each water sample (215, 235 and 236 nm for Aso well, Ebu stream and Osun river water treated chitosan respectively). This bathochromic shift indicated the binding of metal ions with chitosan which is one of its potential applications.

Evaluation of heavy metals in the sample

The concentration of heavy metals in the prepared chitosan-treated and untreated water samples as reported in Tables 2 and 3 indicated the prevalence of six heavy metals (Fe, Cd, Zn, Cu, Cr, and Pb) in all the three samples with Odo-ebu stream (OBS) and Osun River (OSR), having higher metal concentrations compared with AWW water. The pH and temperatures are also functions of these metal concentration accumulation. The level of contamination by heavy metal in Ebu stream and Osun River follow the order: Pb > Cd > Fe > Zn > Cr > Cu while for Aso well water, it was Fe > Zn > Pb > Cr > Cd > Cu. OSR and OBS samples whose sources are not too far from dumpsite contain high concentrations of Pb ions, followed by Cd, thus making both samples the most contaminated of the three water samples. Other metal ions concentration in OBS and OSR samples are also very high when compared with AWW sample. Although, sample from AWW can be said to be unpolluted since the concentrations of most of the analyzed heavy metals are below the allowable standards of SON and WHO limits for drinking water (Table 4), but it may not be declared as being totally safe for drinking due to the risk of bioaccumulation in the human body (Majolagbe et al., 2013; Sekabira, 2010). The high concentrations of these metals can be attributed to leaching from dumpsite which is about 50 m to Ebun stream, and 100 m to Osun River. The high Pb concentration could be coming from exhaust from heavy vehicles (trucks) that ply the routes and burning of refuse at the dumpsite. The activity of
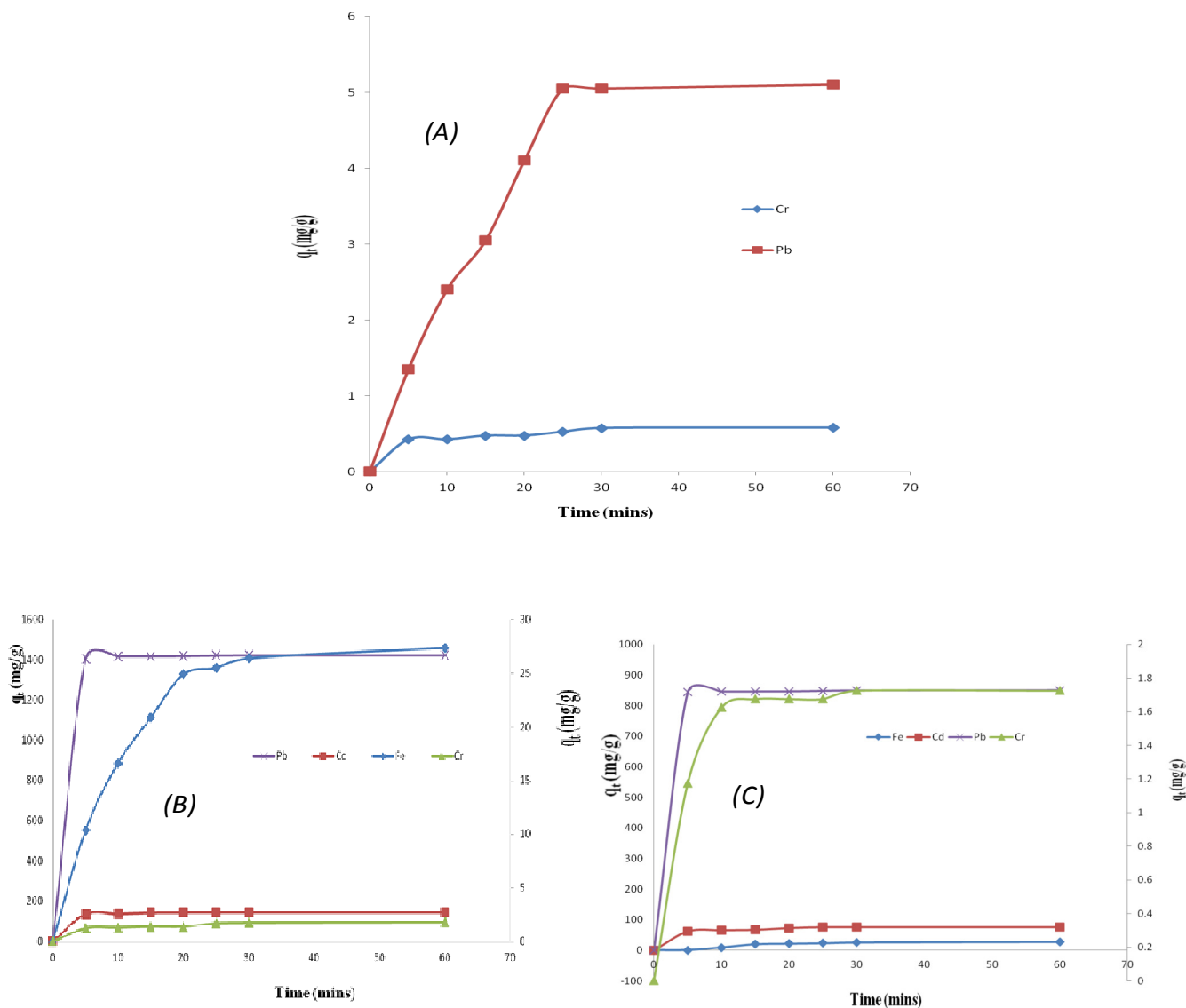
Figure 3. (a) Variation in Adsorption Capacity of chitosan with time for   Sample A (Aso well water), **(b)** Variation in Adsorption Capacity of chitosan with time for   Sample B (Ebu stream), **(C)** Variation in adsorption capacity of chitosan with time for sample C (Osun river water). The absorption of Cd and Fe were not considered in sample A as the initial concentration of these metals in the sample was significantly low (Table 2). In Figure 3b, Fe and Cr were plotted via the secondary axis ditto Cr in Figure 3c.

inhabitant e.g. washing of clothes and vehicles along the bank of Osun River could also be responsible for high concentration of the other metals. Some of these heavy metals have been reported to cause vomiting, dizziness, mortality, morbidity, pulmonary disorder and haematological disorder and cancer (Majolagbe et al., 2013).

Effect of contact time and adsorption capacity of chitosan

The time profiles of metal ions sorption by chitosan

carried out on the three water samples at 25°C are presented in Figure 3. The amount of metal ions sorbed increased with contact time before plateauing, beyond which no more ions were removed from the samples (the concentrations of heavy metal ions left in the sample after treatment with chitosan are presented in Table 3). At this point, equilibrium has been reached between the amount of the heavy metal ions desorbed from the chitosan and heavy metal ions sorbed onto it.   The selectivity of chitosan for the heavy metals ions (Pb, Cd, Cr, Fe) vary while Cu and Zn were below the detection limit. This can be explained on the basis of these metal ions ionic radii, hydration energies and their concentration in solution
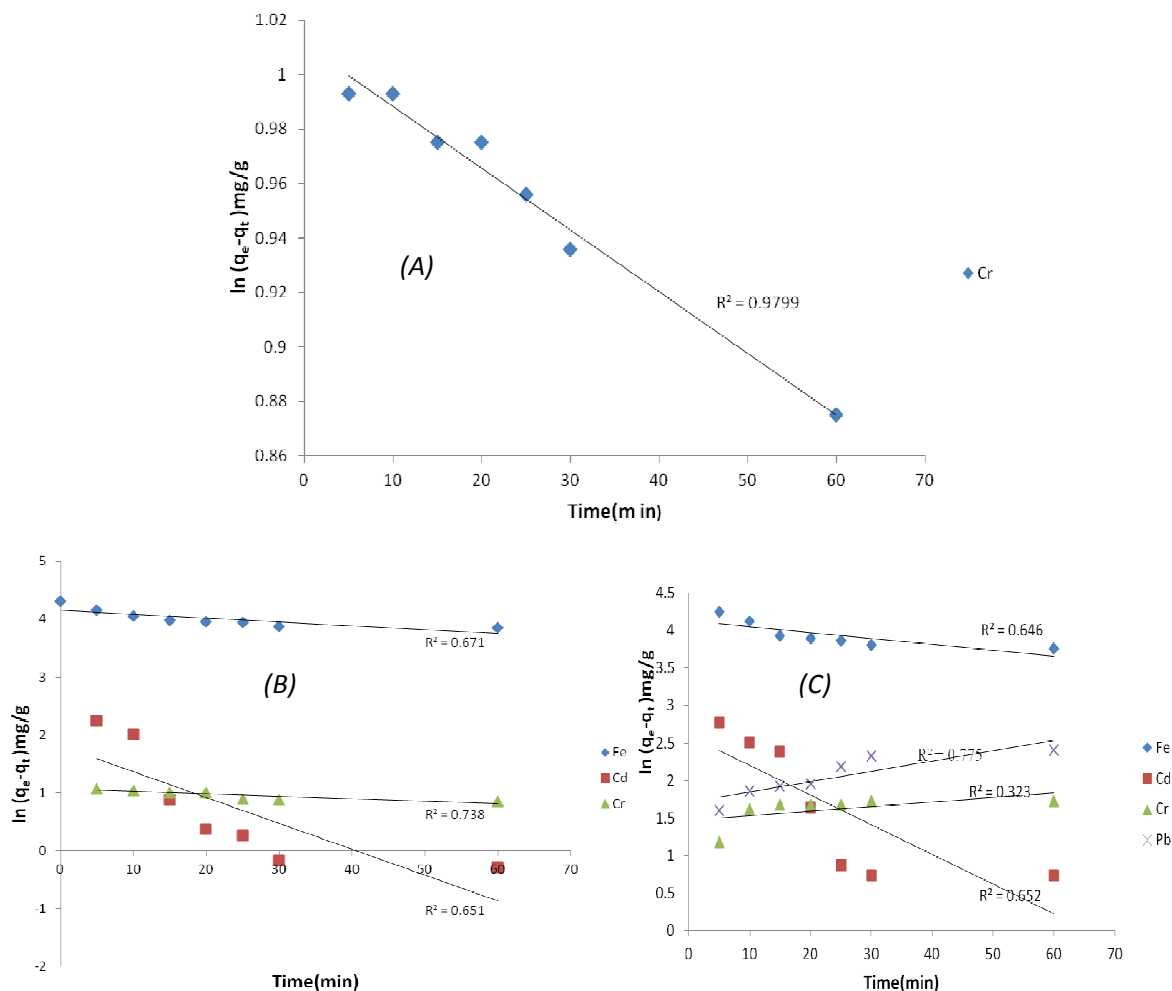
Figure 4. (a) Pseudo-first order adsorption kinetic plot for Sample A (Aso well), **(b)** Pseudo-first order adsorption kinetic plot for Sample B (Ebu stream), **(c)** Pseudo-first order adsorption kinetic plot for Sample C.

(Rhazi et al., 2002). The time required to attain this state of equilibrium is termed the equilibrium time, and the amount of heavy metal ions adsorbed at the equilibrium time reflects the maximum adsorption capacity of the adsorbent under those operating conditions. It was found that 96.04% of Pb, 99.83% of Cd, 94.47% of Cr and 77.6% of Fe were bound to chitosan within 15 min. The initial high amount of metal ions sorbed indicates instantaneous sorption, which can be attributed to the availability of binding sites on the sorbents. However, as these sites progressively react, the sorption of metal ions slowed before attaining equilibrium. The ability of chitosan to bind a large amount of metal ions within 15 min suggests that they are effective sorbents. Furthermore, the rapid kinetics has significant practical importance as it will facilitate the application to smaller reactor volumes ensuring efficiency and economy (Kamari et al., 2011).

The maximum adsorption capacities of each heavy metal based o$_n$ the samples are presented in the Table 1.

This further shows the selectivity of chitosan for adsorbing particular heavy metals ions when more than one heavy metal ions is present in solution. Chitosan showed the highest maximum adsorption capacity for Pb amongst the four heavy metal ions detected after metals sorption in OBS and OSR water samples. The selectivity of the snail shell based chitosan in these samples for the metal ions follow the sequence: Pb > Cd > Fe > Cr.

Adsorption kinetics

The kinetic parameters and their values obtained for the heavy metals sorption processes of the prepared chitosan on the three water samples are presented in Figures 4(a – c), 5(a - c) and Table 5. The experimental equilibrium sorption capacities (q$_e$ experimental) determined from the contact time study were in good agreement with the theoretical equilibrium sorption
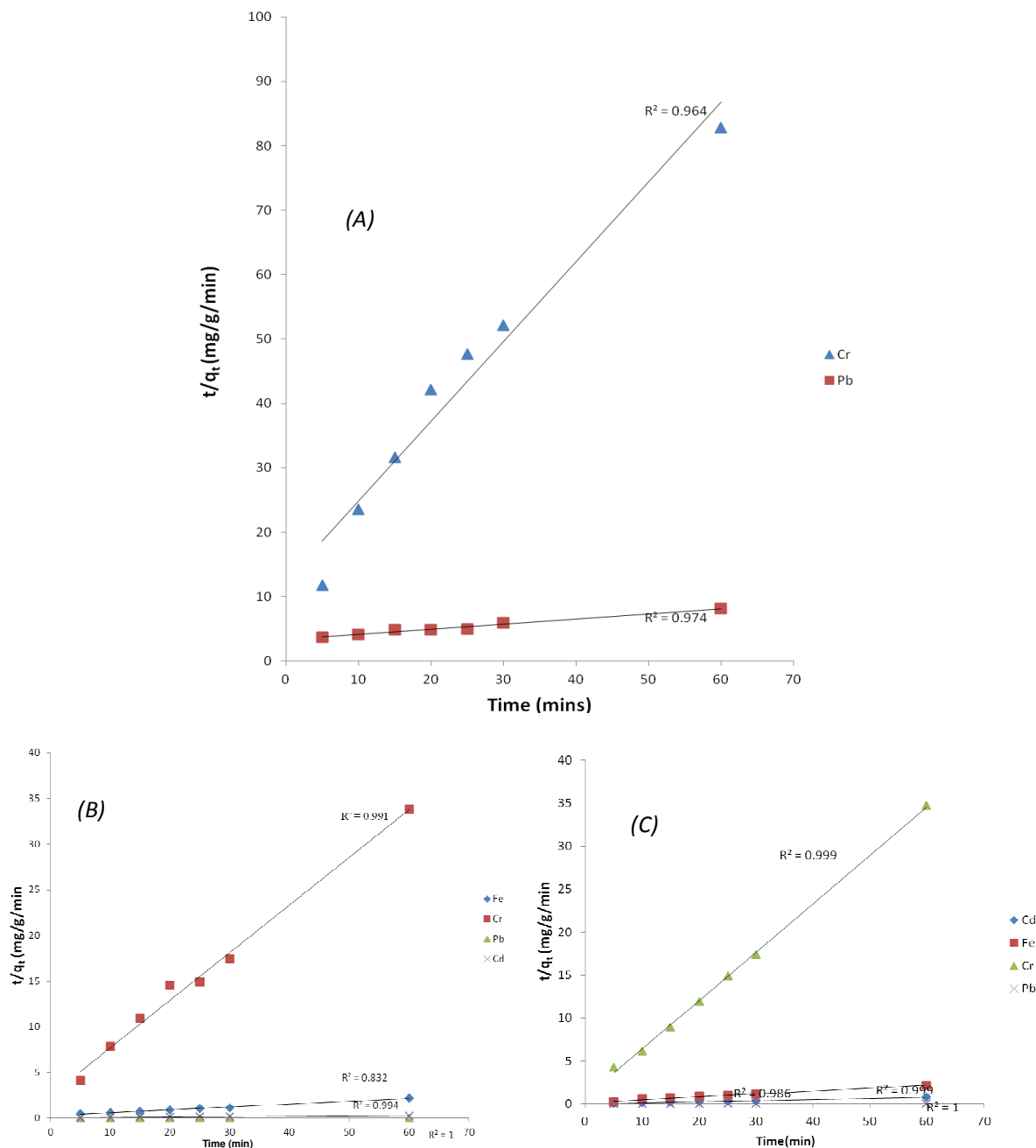
Figure 5. (a) Pseudo-second order adsorption kinetics plot for Cr and Pb in sample A, (b) Pseudo-second-order adsorption kinetic plot for Sample B, (C) Pseudo-second order adsorption kinetic plot for Sample C.

capacities (q_e theoretical) calculated from the pseudo-second-order kinetic model than for the pseudo-first-order. Moreover, experimental sorption data correlated well to the pseudo-second-order kinetic model (R^2 values being greater than 0.95 in most cases except for Cd metal sorption and range from 0.984 to 0.999). Lagergren's pseudo-first order and Intra-particle diffusion equations did not fit well for those metals they can be

plotted, the R^2 values are relatively small and in some cases, having negative values. This had earlier been reported by Kamari et al. (2011) and Mohan et al. (2006) who concluded that pseudo-first-order equations did not fit well to the whole range of contact time for heavy metal sorption processes of chitosan but were only applicable over the initial stages of the processes of adsorption The results suggest that the binding of metal ions studied onto

Table 5. Comparison of the Intra-particle diffusion, pseudo first- and pseudo second-order adsorption rate constants, calculated and experimental q_e, values for the heavy metals ions in the water samples.

Metal	$q_{e\ exp.}$ (mg/g)	Intra-particle diffusion		Pseudo-first order model			Pseudo-second order model		
		k_{id} (mg/g/min $^{0.5}$)	R^2	$q_{e\ cal.}$ (mg/g)	K_1 (10^{-03}) (g/mg/min)	R^2	$q_{e\ cal.}$ (mg/g)	K_2 (10^{-03}) (g/mg/min)	R^2
				Sample A					
Pb	5.05	0.805	0.67	-	-	-	12.59	1.87	0.97
Cr	0.58	0.106	0.61	2.75	2.03	0.98	0.81	123	0.96
				Sample B					
Pb	1.42×10^3	274.8	0.092	12.86	-14.00	0.37	1.0×10^3	25.03	0.99
Cr	1.73	0.365	-4.81	2.90	4.00	0.74	1.92	1110	0.99
Cd	1.44×10^2	27.61	0.17	6.11	44.00	0.65	333.3	0.173	0.83
Fe	2.64×10^1	4.554	0.52	63.50	6.03	0.67	32.26	3.44	0.99
				Sample C					
Pb	846.33	164.2	0.089	5.55	-13.22	0.775	733	0.15	0.99
Cr	1.73	0.320	0.43	4.34	-6.01	0.323	1.78	395	1.00
Cd	75.63	13.96	0.40	13.49	39.04	0.652	83.30	7.23	0.99
Fe	25.68	4.089	0.53	62.18	7.05	0.646	30.30	5.45	0.99

Table 6. Langmuir Isotherm constant for heavy metal analysed in the samples.

Metal	b(X 10^3)	q_{max}	R_L(X10^{-7})
	Sample B		
Pb	7.00	1428.57	1.00
Cd	18.5	135.14	4.00
Cr	26.7	1.25	30.0
	Sample C		
Pb	40	833.33	0.30
Cd	3.18	62.89	50.00
Cr	4.50	1.11	2,000.00

chitosans was best described by the pseudo-second order kinetic model and that the chemical binding reaction was the rate-limiting step as discussed by Ho and McKay (2000).

Adsorption isotherm

The adsorption isotherm indicates how the adsorbed molecules distribute themselves between the liquid phase and the solid phase when the adsorption process reaches an equilibrium state. The analysis of equilibrium adsorption data by fitting them to an isotherm models is an important step to show that the Langmuir model can be used for design purpose (Haghseresht and Lu, 1998). When C_e/q_e was plotted against C_e, a straight line with slope, $1/q_{max}$ was obtained, and are presented in Figure 6(a – c). The Langmuir constants 'b' and 'q_{max}' were calculated from this isotherm and their values are presented in Tables 6. The large values of b signify that the chitosan has a very high affinity for the heavy metals coupled with a high binding energy. The conformation of the experimental data, q_{max}, to that obtained using Langmuir isotherm model indicated the homogeneous nature of snail shell based chitosan surface, i.e., each metal ion molecule/chitosan biosorption has equal adsorption activation energy. The linear plot obtained further buttress the monolayer form of adsorption. Also from Table 4(a and b), values of R_L were found to be less than 1 but greater than zero in all cases. This confirmed that the biosorbent prepared from the snail shell is favourable for biosorption of Pb, Cd, and Cr ions
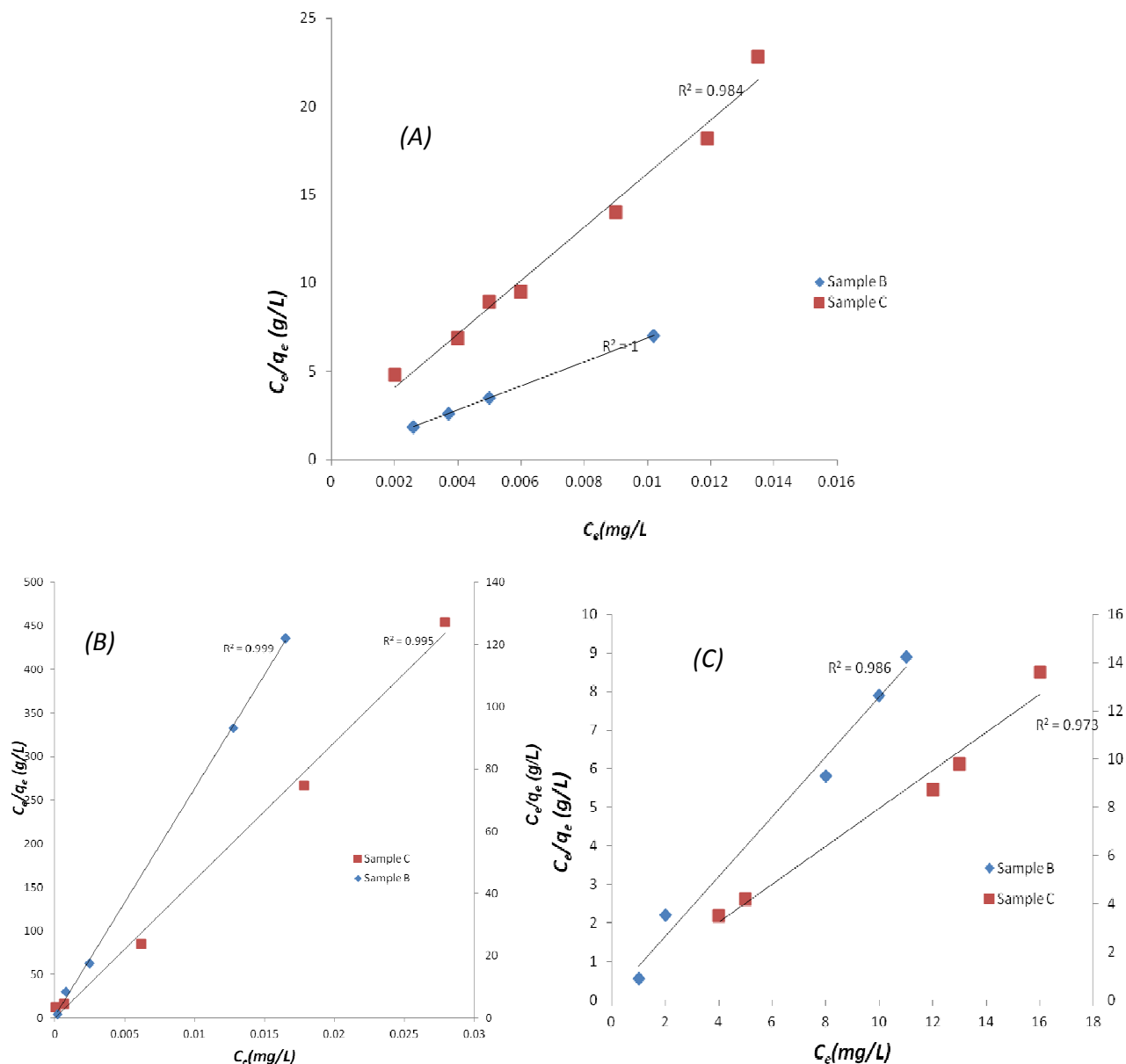
Figure 6 (a) Langmuir adsorption isotherm for the biosorption of lead ions, (b) Langmuir adsorption isotherm for the bio-sorption of Cadmium ions. sample B is plotted against the secondary axis, (c) Langmuir adsorption isotherm for the bio-sorption of chromium ions at 25 °C. Sample C is plotted against the secondary.

under the conditions used in this study.

Conclusion

The findings from this study have shown that the Ebu stream and Osun river water samples were highly polluted with heavy metals and chitosan has great affinity for these metal ions in the analyzed samples in the following order: Pb > Cd > Fe > Cr. The adsorption kinetics follows pseudo-second order and the

experimental equilibrium sorption capacities determined from the contact time study were in good agreement with the theoretical equilibrium sorption capacities calculated from the kinetic model. The Langmuir model fitted the isotherm equilibrium data with $R^2 \geq 0.985$. The values of maximum sorption capacities also correlated well with those determined theoretically and experimentally. R_L values obtained also confirmed that the prepared biosorbent is favorable for biosorption of heavy metals. Similar results were reported by Kamari et al. (2011) for crab shell based chitosan and Adeogun et al. (2010)

using adsorbent obtained from plumb shell.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Adeogun AI, Bello OS, Adeoye MD (2010). Biosorption of lead ions on biosorbent prepared from plumb shells (*Spondias mombin*): Kinetics and equilibrium studies. Pak. J. Sci. Ind. Res. 53(5):246-251.

Adewuyi S, Adeoye MD, Sanyaolu NO (2009). Equillibruim removal efficiency of Nickel metal ion by chitosan. Proc. of the 33rd ann. Inter. Conf. Chem. Soc. Nig. (CSN), Abeokuta, Nigeria. pp. 403-405.

Bolat YŞ, Bilgin A, Günlü L, Izci SB, Koca SÇ, Koca HU (2010). Chitin Chitosan yield of freshwater crab (*Potamon potamios*, Olivier 1804) shell. Pak. Vet. J. 30(4):227-231.

Crini G, Badot PM (2008). Application of chitosan, a natural polysaccharide, for dye removal from aqueous solutions by adsorption process using batch studies: a review of recent literature. Prog. Poly. Sci. 33:399-447. http://dx.doi.org/10.1016/j.progpolymsci.2007.11.001

Eniola EB, Chukwu LO, Olaide BS (2010). Hydro-chemistry, macro-invertebrate fauna and fish production of acdja fishing sites in a Tropical Lagoonal Ecosystem. J. Am. Sci. 6(1):22-28.

Fytianos K, Voudrias E, Kokkalis, E (2003). Sorption-desorption behaviour of 2,4-dichlorophenol by marine sediments. Chemosphere 40:3-6. http://dx.doi.org/10.1016/S0045-6535(99)00214-3

Haghseresht F, Lu GQ (1998). Adsorption characteristics of phenolic compounds onto coal-reject-derived adsorbents. Ener. Fuels. 12:1100-1107. http://dx.doi.org/10.1021/ef9801165

Ho YS, McKay G (2000). The kinetics of sorption of divalent metal ions onto sphagnum moss peat. Water Res. 34:735-742. http://dx.doi.org/10.1016/S0043-1354(99)00232-8

Jude CI, Augustin AA (2007). Equilibrium sorption isotherm studies of Cd(II), Pb(II) and Zn(II) ions detoxification from waste water using unmodified and EDTA-modified maize husk. Elect. J. Biotech. 10(4): ISSN: 0717-3458. DOI: 10.2225/vol10-issue4-fulltext-15. http://dx.doi.org/10.2225/vol10-issue4-fulltext-15

Kamari A, Pulford ID, Hargreaves JSJ (2011). Binding of heavy metal contaminants onto chitosans: An evaluation for remediation of metal contaminated soil and water. J. Env. Mgt. 92:2675-2682. http://dx.doi.org/10.1016/j.jenvman.2011.06.005

Kanamadi RD, Ahalya N, Ramachandra TV (2003). Review Paper: Biosorption of Heavy Metals. Res. J. Chem. Environ. 7(4):71-79.

Lagergren S (1898). Zur theorie der sogenannten adsorption gelöster stoffe. Kungliga Svenska Vetenskapsakademiens. *Handlinga*r 24:1-39.

Langmuir I (1916). The constitution and fundamental properties of solids and liquids. J. Am. Chem. Soc. 38:2221-2295. http://dx.doi.org/10.1021/ja02268a002

Majolagbe TA, Azeez L, Salawu HO, Adeoye MD, Lawal AT, Agbaogun BKO, Tijani KO (2013). Assessment of heavy metal pollution in soils and wells around municipal dumpsites in Osogbo metropolis. Environ. Sci.-An Ind. J. 8(2):56-61.

Martha B (2008). Adsorption of Metallic Ions onto Chitosan: Equilibrium and Kinetic Studies Royal Institute of Technology. Department of Chemical Engineering and Technology. Division of Transport Phenomena, Stockholm, Sweden. Licentiate Thesis pp. 3-4.

Mohan D, Pittman Jr CU, Steele PH (2006). Single, binary and multi-component adsorption of copper and cadmium from aqueous solutions on Kraft lignin-a biosorbent. J. Colloid. Interf. Sci. 297:489-504. http://dx.doi.org/10.1016/j.jcis.2005.11.023

Mustapha MK (2008). Assessment of the water Quality of Oyun Reservoir, Offa, Nigeria, Using Selected Physico-Chemical Parameters. Tur. J. Fish. Aq. Sci. 8:309-319.

Olajire AA, Imeokparia FE (2001). Water quality assessment of osun river: studies on inorganic nutrients. Environ. Monit. Assess. 69:17-28. http://dx.doi.org/10.1023/A:1010796410829

Pan X, Wang J, Zhang D (2009). Sorption of cobalt to bone char: kinetics, competitive sorption and mechanism. Desal. 249:609-614. http://dx.doi.org/10.1016/j.desal.2009.01.027

Rhazi M, Desbrieres J, Tolaimate A, Rinaudo M, Vottero P, Alagui A, El Meray M (2002). Influence of the nature of the metal ions on the complexation with chitosan. Application to the treatment of liquid waste. Euro. Poly. J. 38:1523-1530. *www.elsevier.com/locate/europolj.*

Saifuddin MN, Kumaran P (2005). Removal of heavy metal from industrial wastewater using chitosan coated oil palm shell charcoal. Elec. J. Biotech. 8(1):22-25 ISSN: 0717-3458 DOI: 10.2225.

Sallau AA, Ekanem EO, Abubakar AB (2011). The 34th Annual International Conference of the Chemical Society of Nigeria (CSN) at Unilorin, EN001- EN007 Sattelite map of osogbo. <a href=http://www.maplandia.com/nigeria/osun/osogbo/.

Sekabira K , Origa HO, Basamba TA , Mutumba, G, Kakudidi E (2010). Int. J. Environ. Sci. Tech. 7(3):435-446. http://dx.doi.org/10.1007/BF03326153

Standard Organization of Nigeria (2007). Nigerian Standard for Drinking Water Quality. pp.1-30.

Sud D, Mahajan G, Kaur MP (2008). Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions - A review. Bioresour. Technol. 99:6017-6027. http://dx.doi.org/10.1016/j.biortech.2007.11.064

Taboada S, Carbrera G, Cardenas G (2003). Retention capacity of chitosan for copper and Mercury ions. J. Chilian Chem. Soc. 48(1):10.

Tolaimate A, Rhazi M, Desbrieres J, Rinaudo M, Vottero P, Alagui A, El-Meray M (2002). Influence of the nature of the metal ions on the complexation with chitosan. Application to the treatment of liquid waste. Eur. Polymer J. 38:1523-1530. http://dx.doi.org/10.1016/S0014-3057(02)00026-5

Volesky B (2001). Detoxification of metal-bearing effluents: biosorption for the next century. Hydromet. 59:203-216. http://dx.doi.org/10.1016/S0304-386X(00)00160-2

World Health Organization (WHO) (2004). The Guidelines for Drinking Water Quality, Third edition. *www.who.int/water_sanitation_health/dwq/gdwq3rev*

Yildiz B, Şengül B, Ali G, Levent I, Seval BK, Soner Ç, Habil UK (2010). Chitin-Chitosan Yield of Freshwater Crab (*Potamon potamios*, Olivier 1804) Shell. *Pak. Vet. J*. ISSN: 0253-8318 (PRINT) 2074-7764 (ONLINE). Accessible at: www.pvj.com.pk. 675-2682

Full Length Research Paper

Effect of solvent type on the yields and mineral compositions of the leaf extracts of *Moringa oleifera* L.

Adeoye, M. Dasola[1], Lawal, A.Tunbosun[2]*, Azeez, L. Adeyemi[1] and Olayiwola, O. Abidemi[3]

[1]Industrial and Environmental Chemistry Unit, Chemical Sciences Department, Fountain University, Osogbo Osun State, Nigeria.
[2]Industrial and Environmental Chemistry Unit, Chemical Sciences Department, Al-Hikmah University, Ilorin Kwara State, Nigeria.
[3] Chemical Sciences Department. Oduduwa University,Ipetumodu, Ile-Ife, Osun State, Nigeria.

The present study was conducted for the leaves extract of *Moringa oleifera* L (*Moringeaeae*) in different solvents: Fermented maize marsh (Omidun /FMM), hot water (HW), cold water (CW), palm-wine (PW), ethanol, dichloromethane (DCM) and n-hexane to determine the effects of solvent properties on the extraction yields and the compositions of dietary minerals (k, Ca, Mg, Zn, Cu, and Fe) for *M. oleifera*. The observed yields follow the trend: PW > CW > FMM > ethanol >HW > DCM> n-hexane. Fermented maize marsh extracts recorded the highest mean compositions for Ca and Mg (560.50 ± 34.5 and 485.75 ± 67.32); palm-wine extracts recorded the highest compositions of Fe and Cu (7.42 ± 1.22 and 0.50 ± 0.01), while cold water and hot water extracts recorded the highest compositions of K (2045.14 ± 265.02) and Zn (2.16 ± 0.11) respectively. The lowest concentrations of these trace metals which range from 0.06 ± 0.01 to 6.47 ± 0.35 was recorded in n-hexane extracts. Statistical analysis of the mineral constituents using two sample t-tests studies at $p < 0.05$ also revealed great significant differences between the means of the mineral elements in the extracts except in few cases. The high yields of these extracts in PW, CW and FMM indicate that these solvents have great affinity and similar solubility properties for the different compositions of *M. oleifera*.

Key words: *Moringa oleifera,* fermented maize marsh, solvatochromic, extraction, toxicological.

INTRODUCTION

Green plants provide most of the world's molecular oxygen and are the basis of a good number of the earth's ecologies, especially on land. Plants enrich our lives as flowers and ornaments, and generally contain high amounts of essential nutrients, vitamins, minerals and fatty acids. These are important in animal feed and human nutrient for osmotic adjustment and to activate enzymes, hormones and other organic molecules that enhance the growth, function and maintenance of life processes by living organisms (Aslam et al., 2005). Minerals cannot be synthesized by animals and must be provided from plants or mineral-rich water (Mosha et al., 1995; Al-Kharusi et al., 2009).

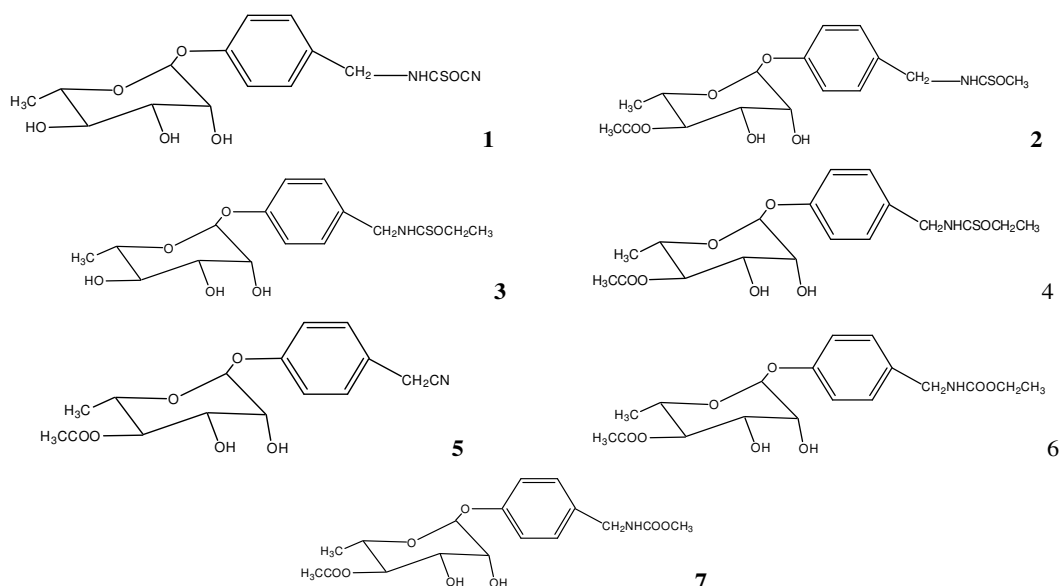*Moringa oleifera* Lam [syn. *M. ptreygosperma* Gaertn.]

Figure 1. Structures of some selected phytochemicals from moringa (1) niazidin (2) niazicin (3) niazimcin (4) niaziminin (5), niazirinin (6) niazinin (7) niazicinin (Reubena, 1995).

plants, widely distributed and naturalized species of a monogeneric family Moringaceae is commonly called ben oil tree but locally known as *Zogeli* among the Hausa speaking people of Nigeria (Toba et al., 2010). It has been reported to be a rich source of protein, vitamins, β - carotene, aminoacids, various phenolics and essential minerals like calcium (Ca) and potassium (K) (Toba et al., 2010; Makkar and Becker 1996). It has also been reported to be a good source of natural antioxidants such as ascorbic acid, flavonoids, phenolics and carotenoids which enhance the shelf-life of fat containing foods. Its oil and micronutrients have also been acclaimed to contain antitumour, antiepileptic, antidiuretic, anti-inflammatory and venomous bite characters (Hsu, 2006), while its aqueous leaf extracts has been reported to regulate thyroid hormone and can be used to treat hyperthyroidism since it exhibits an antioxidant effect (Kumar and Pari, 2003). The biodiesel derived from *M. oleifera* oil by alkali-catalyzed trans-esterification with methanol is reported to be an acceptable substitute for petrol and diesel. Its cetane number was found to be the highest reported for a biodiesel fuel with much better oxidative stability (Mahajan et al., 2007). The seeds also serve as one of the cheap and best coagulants for water treatment (Kalogo et al., 2000).

The minerals and nutritional values of *M. oleifera* and their bioavailability have been a subject of great studies and there have been reported considerable variations among these constituents in both the leaves and seeds of this plant. These depend on factors like genetic background, location, environment and cultivation methods (Aslam et al., 2005; Brisibe et al., 2009; Toba et al., 2010).

Cold water, hot water, palm wine or fermented maize mash (omidun) has been used traditionally as solvent for herbal extraction in Nigeria. The leaves, barks or roots of some herbal plants are either cooked or soaked in these solvents to treat certain diseases. However, there are limited reports on the influence of these solvents on the mineral and bioactive compositions of these herbal extract. Most of the bioactive components contained in plant matrices are medium-sized molecules which due to the presence of aromatic delocalized π-electrons in it, the

molecules are highly polarizable. Their high polarizability makes the molecules liable to a variety of specific interactions with various solvents, e.g., protonation, hydrogen bonding, and specific solvation (Andri et al., 2009). Thus, for toxicological reasons, drug and medicine manufacturers are increasingly required to minimize the number of solvents employed in pharmaceutical processes (Kolar et al., 2002). The presence of a solvent in the extract may also affect the kinetics of crystallization and the morphology of the crystals, which is an important factor in determining the product quality (Andri et al., 2009).

To date, twenty three active compounds had been isolated and elucidated from *M. Oleifera* leaves and these had been claimed to depend on their species, sources and ages (Rubeena, 1995; Toba et al., 2010). These compounds are novel glucosides with hypothensive properties; some of these are niaziminin, niazinin, niazicinin, niazimicin, niazirin, niazirinin, niazicin and niazimin in Figure 1 (Rubeena, 1995). It is therefore pertinent in this work to confirm the presence/ retention of these compounds in the extracts of *Moringa* leaves

Corn

↓

Clean for 2 to 3 days

↓

Wet mill

↓

Sieve and discard pomace

↓

Ferment filtrate and allow to sediment

↓

for 1-3 days

↓

OGI

Figure 2. Flow diagram for the preparation of ogi /omidun (FAO, 1999).

ecotype in Osogbo, a South Western Nigeria town through their functional groups and to study the effect of solvent on its mineral compositions.

MATERIALS AND METHODS

Solvents

Fermented maize mash (*omidun*), prepared by soaking and fermentation of maize grains in cold water for 28 to 72 h, wet milled, sieved and left to sediment for 48 h (Figure 2) were collected from traditional processors of ogi in Osogbo, Osun State, Nigeria. This solvent was from ogi prepared from white maize grains (FAO, 1999; Abdulrahaman and Kolawole, 2006). The *omidun* samples were collected aseptically in sterile screw cap bottles. Fresh palm-wine was bought in undiluted form from the local palm-wine taper while other reagents/chemicals (dichloromethane, ethanol, n- hexane) used were analytical grade products of British Drug House (BDH). The reagents were redistilled twice and their boiling points were used as index of purities. The standards used were prepared using distilled water.

Collections and identification of plant materials

Fresh leaves of *Moringa* were collected from *M. oleifera* plants located in Fountain University compound, Osogbo in Osun State southern/western Nigeria between January and February, 2013. The plant materials had earlier been taxonomically identified by Dr. Awodoyin, R.O. of Botany Herbarium, University of Ibadan. The leaves were air- dried until a constant weight was obtained (one week). They were pulverized to powdery form using a well-cleaned 240 v 4 L blender machine. The powdered samples were then stored in airtight containers, properly kept and labeled for further analysis. Soil samples of the land on which *M. oleifera* is grown were also taken within 10 cm depth. And, its pH determination was done at ratio of 1:1 in distilled water (Anjorin et al., 2010).

Determination of moisture and ash contents

2 g fresh samples of *M. oleifera* leaves (in duplicate) were weighed. The moisture contents in the samples were determined using the method of the Association of Official Analytical Chemists (AOAC, 1990). This was done by weighing the leaves in the crucible and drying in oven at 105°C until a constant weight was obtained. Determination of the ash content was done by ashing at 550°C for about 3 hrs (Ogbe and John, 2011). The ash resulted from the combustion was then allowed to cool to lowest temperature and weighed.

Preparation of *M. oleifera* extracts

The extracts of *M. oleifera* in different solvents were prepared by soaking 8 g of dried powdered samples each in 100 ml of distilled coldwater, distilled hot water, omidun, palmwine, dichloromethane, n- hexane, ethanol respectively for 24 h in an airtight reagent bottles. These were then filtered. The filtrates were concentrated in rotary vacuum evaporator to yield an oily golden yellowish mass. The concentrates of all extracts mentioned above were finally air dried to constant weight and the total yield of each sample were determined. 2 g of each of the concentrates were treated with 20 ml of concentrated Trioxonitrate (V) acid (HNO_3) heated to reflux on an electric hot plate at 70 to 90°C until digestion was completed (Anjorin et al., 2010). Side by side, 20 ml of HNO_3 was also added to empty beakers which served as blank. The contents of the beaker were allowed to cool, filtered through whatman No. 2 filter paper into volumetric flasks and made up to volume of 100 ml with distilled water before the Atomic Absorption Spectroscopy analysis was done. These were carried out in triplicates.

Determination of the compositions of dietary minerals in the *M. oleifera* extracts

Chemical analysis

Macro elements (K, Ca, Mg) and trace elements (Zn, Cu, Fe) concentrations in the *M. oleifera* extracts in different solvents were estimated on Atomic Absorption Spectrophotometer (AAS) (Buck Scientific Modelling 2010/2011 V.G.T made in England) at the Agronomy Department of the University of Ibadan. Infra-red analysis of the functional groups in the *M. oleifera* extract and UV- Visible spectroscopy analysis of the concentrates in the wavelength range of 190 to 800 nm in different solvents were also determined.

Statistical analyses

The analyses in this study were carried out in triplicates. For each, a mean value and standard deviation were calculated. Statistical analysis of the results obtained involved the use of Aspin-Welch unequal variance two sample t-tests.

RESULTS AND DISCUSSION

Characterization of the extracts of *M. oleifera* leaves

The extracts of *M. Oleifera* leaves were obtained in amorphous form as golden to dark brown colour in different solvents. The moisture contents, the extracts yields, the mineral composition and the results of the spectra analysis (UV and IR) showing the effect of

Table 1. Yields of *M. oleifera* extracts in different solvents.

Solvent	Yield (g)
Hot water	1.13 ± 0.02
Cold water	2.0 ± 0.01
n-hexane	0.2 ± 0.02
D.C.M	1.0 ± 0.11
Ethanol	1.2 ± 0.03
F.M.M	1.9 ± 0. 01
Palm-wine	6.77 ± 0.12

F.M.M., Fermented maize mash; DCM, dichloromethane.

Table 2. Mineral composition of *M. oleifera* leaves extracts in different solvents.

Extracts	Ca (mg/L)	Mg (mg/L)	K (mg/L)	Fe(mg/L)	Cu (mg/L)	Zn (mg/L)
Cold water	484.33±75.77[a]	292.82±16.09[a]	2045.14±265.02[a]	1.38±0.04[a]	0.08±0.01[a]	0.64±0.12[a]
Hot water	512.18±17.18[b]	197.25±26.37[b]	1775.99±98.66[b]	2.78±0.17[a]	0.06±0.01[b]	2.16±0.11[b]
F.M.M	560.50±34.5[b]	485.75±67.32[c]	1875.15±183.53[b]	3.47±0.07[b]	0.11±0.02[a]	1.14±0.05[c]
D.C.M	20.40±1.43[c]	12.71±3.02[d]	33.93±3.77[c]	6.63±0.33[c]	0.09±0.02[a]	0.29±0.02[d]
n-hexane	4.22±0.28[d]	1.56±0.09[e]	6.47±0.35[d]	0.70±0.01[d]	0.06±0.01[b]	0.14±0.03[e]
Ethanol	5.19±0.04[d]	54.75±4.12[f]	625.21±28.27[e]	5.89±1.38[c]	0.13±0.05[a]	0.33±0.02[d]
Palm-wine	152.50±24.47[e]	212.50±35.85[b]	216.50±12.56[f]	7.42±1.22[c]	0.50±0.01[b]	0.44±0.03[f]

D.C.M, 1, 2-dichloromethane, F.M.M, fermented maize mash (Omidun). Data are expressed as Mean ±SD. Means with different superscripts in a column are significantly different at $p < 0.05$.

Table 3. Summary of the Infra-red (IR) analysis showing the different functional groups in the extracts of *M. oleifera* leaves.

Functional groups	(υ_{max}) cm^{-1}						
	Cold water	Hot water	F.M.M	Palm- wine	Ethanol	D.C.M	n-hexane
υ O-Hstr	3396	3425	3430	3404s	3457.82	3453	3445.71
υ OH/ N-Hstr	-	-	-	3373	-	-	-
υ C-H str in CH$_2$	2976.95	2980.2	-	2978.34	-	-	2927.75
υ C=C str (aromatic ring)	1648.66	1643.09	1637.86	1643.94	1639.21	1638.36	1638.54
υ C=O str (carbamate)	-	-	-	-	-	-	1726.42
υ -C=O amide	-	-	-	-	-	-	1589.63
υ C-O thioamide	1400	1398		1399.50	-	-	1374.23
υ C-OH stretch/def	1047	1048.30	1090	1048.86s	1048.57	-	-
υ Subst bzc	662.26	668.33	609.32	662.26	601.00	-	

F.M.M, Fermented maize mash; D.C.M, dichloromethane

different functional groups and the effect of extractive solvents in the spectra shifts of *M. oleifera* leaves extract**s** are as presented in Tables 1 to 4 and in Figures 3 to 15. Moisture contents: 10.22% ± 0.11. Ash values: 7. 93% ± 0.32

pH Values and soil samples

The soil on which the plants grow is loamy soil while its pH was slightly acidic (pH 6.55). This was in line with the findings of Anjorin et al. (2010). The moisture contents (10.22% ± 0.11) and total ash values (7. 93% ± 0.32) for *M. oleifera* leaves obtained in this study were slightly higher than those reported by Moyo et al. (2011) and Ogbe and John (2011) for *M. oleifera* leaves. The variations in the reported values of these parameters may be due to differences in the ages of the trees or their different stages of maturity and possibly due to difference in agroclimatic conditions (Ogbe and John, 2011).

Table 4. UV-Visible Absorption Bands of different solvents extracts of *M. oleifera*.

Extracts	Band I		Band II		Band III		Band IV	
	(λ_{max})nm	Absorbance	(λ_{max})nm	Absorbance	(λ_{max})nm	Absorbance	(λ_{max})nm	Absorbance
Cold water	300	2.304	-	-	-	-	-	-
Hot water	295	1.565	-	-	-	-	-	-
F.M.M	300	2.081	-	-	-	-	-	-
Palm wine	300	2.018	-	-	-	-	-	-
Ethanol	295	1.553	410	0.452	-	-	665	0.116
D.C.M	295	1.331	410	0.724	-	-	665	0.250
n-Hexane	-	-	415	0.234	435	0.126	470	0.143

F.M.M., Fermented maize mash; D.C.M., dichloromethane.
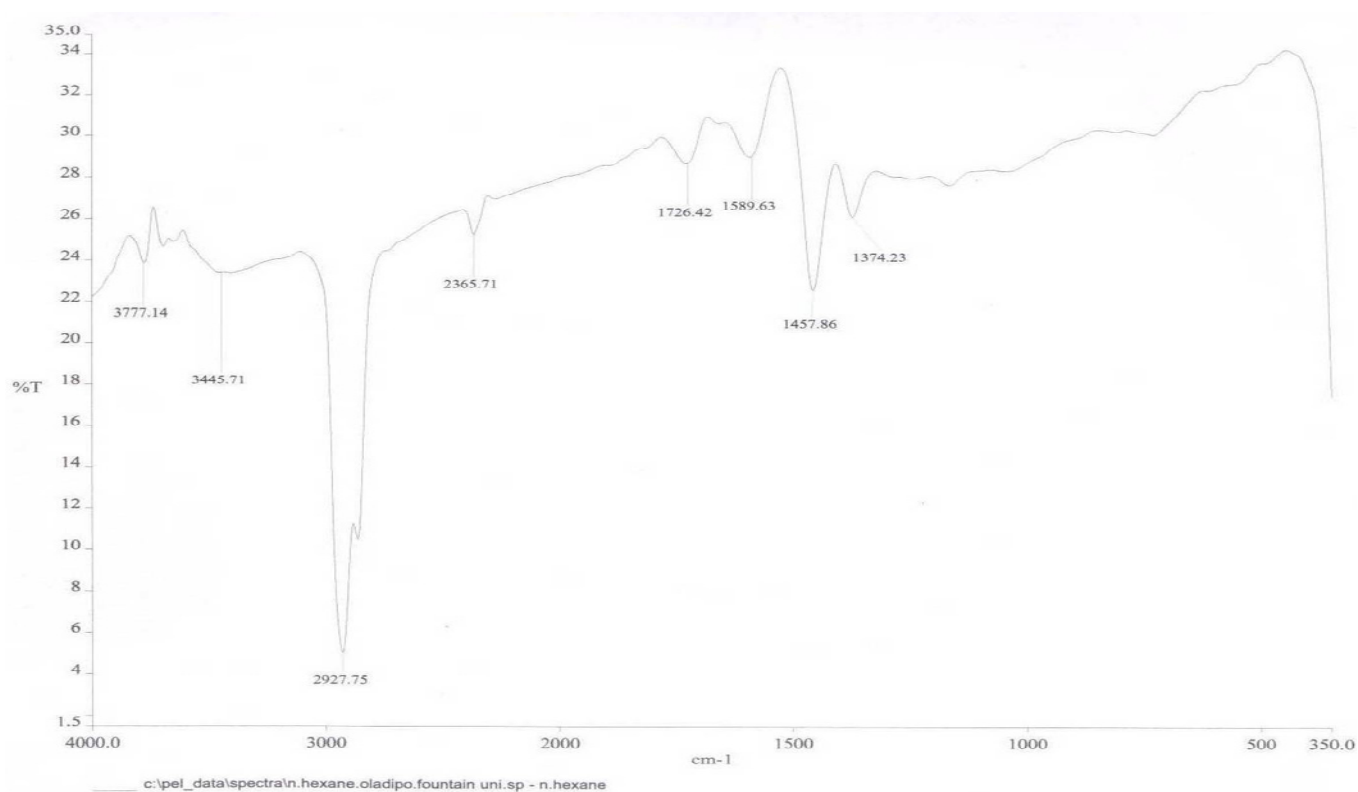
Figure 3. IR Spectrum of n-hexane extract of *M. oleifera*.

Extracts yields

Plant matrices including leaf, stem and root contain various solute molecules with more than one functional group. Therefore it is difficult to predict the solubility and interactions of the solute in a particular solvent (Andri et al., 2009). Polarity was defined by Snyder as the relative ability of a molecule to engage in strong interactions of all kinds and is the sum of all possible interactions (Andri et al., 2009). Thus, the concept of polarity can be used as alternative way of considering solubility.

The total yields of *M. oleifera* in the solvents of choice (dichloromethane, n-hexane, Fermented Maize Mash, Ethanol and water) as shown in Table 2 indicated that palm-wine and non polar (n- hexane) extracts had the highest and lowest yields respectively. All the extracts obtained from the solvents used were observed as dark brown or golden brown semi-solids. The high yields of these extracts in palm-wine and fermented maize marsh (Omidun) indicated that these solvents has high affinity and similar solubility properties for the different compositions of *M. oleifera* because they contain hydroxyl group which is hydrophyllic showing that the molecules in these extracts dissolves better in these
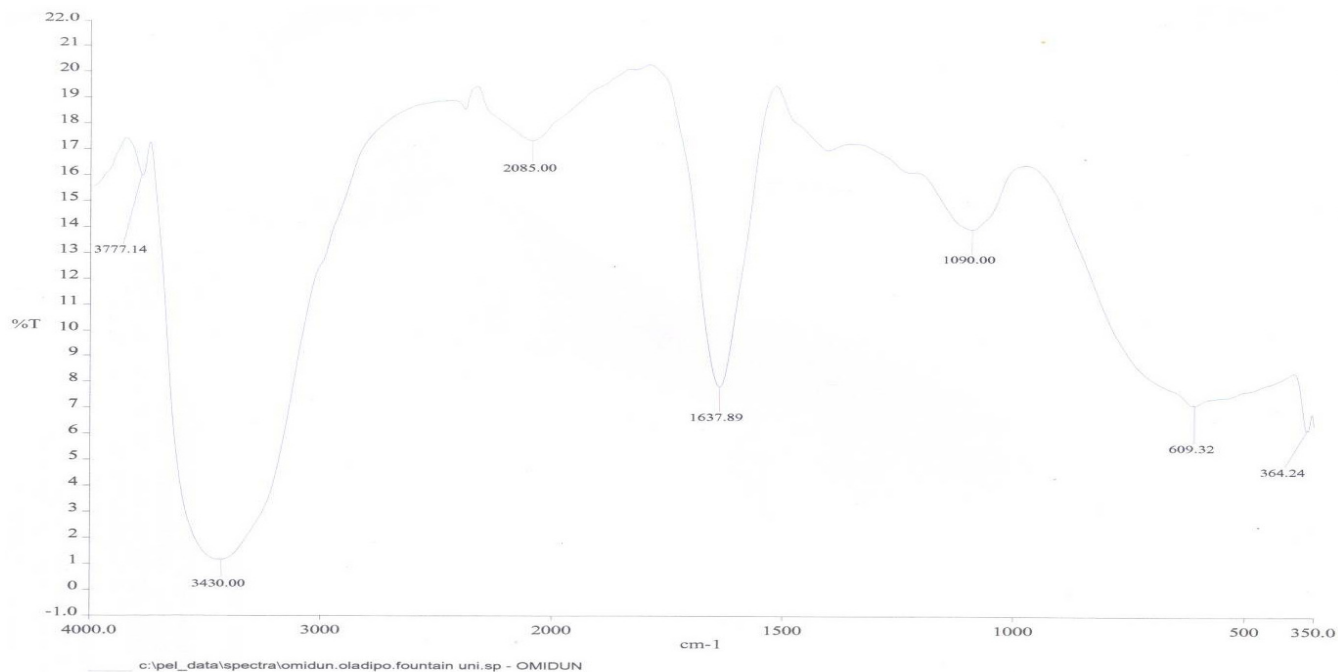
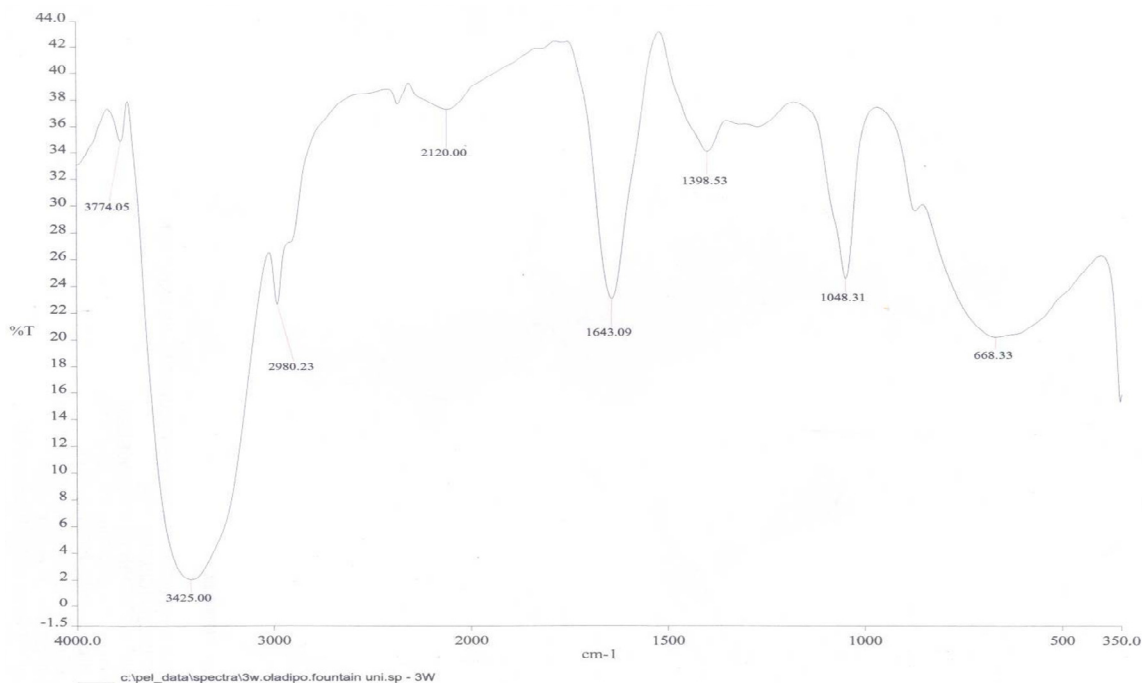**Figure 4.** IR Spectrum of Fermented maize mash extracts of *M. oleifera.*

Figure 5. IR Spectrum of Hot water extracts of *M. oleifera.*

solvents. The extract yields of the plants decreased with decreasing solvent polarity. Low polar solvents such as dichloromethane and n-hexane were not able to significantly extract the *M. oleifera*. However, there is also requirement from environment considerations and toxicological implication to reduce the quantity of organic solvents used in particular chlorinated solvents (including dichloromethane) in the extraction of consumable items
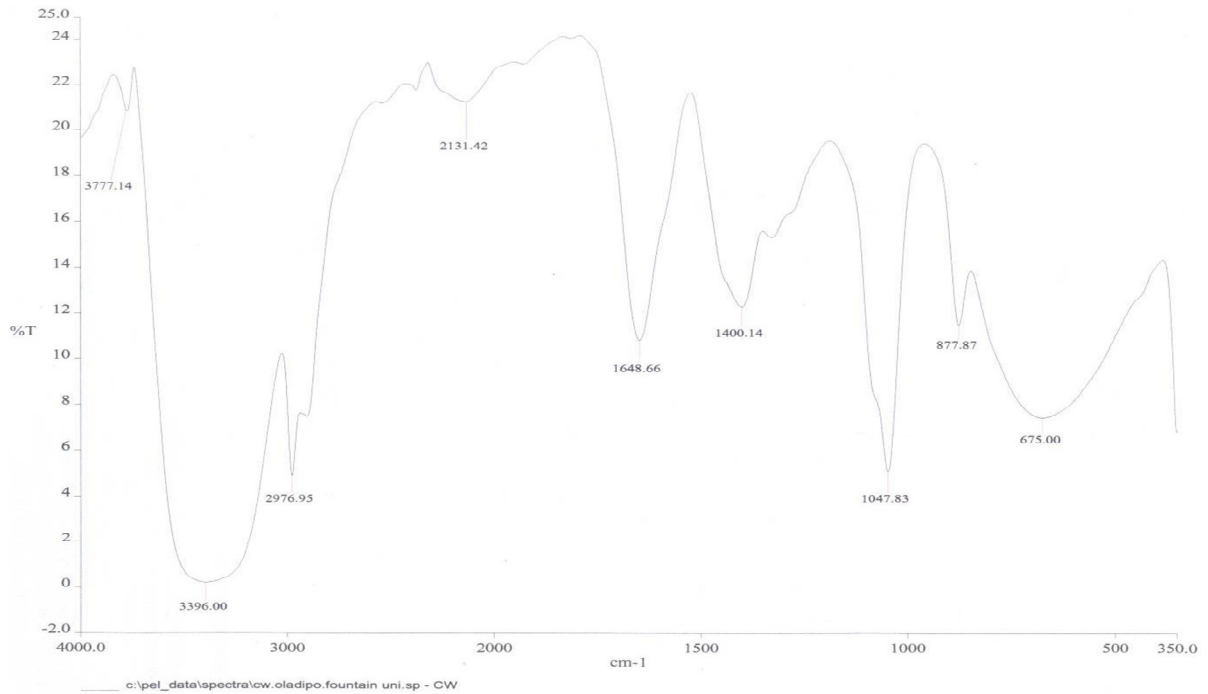
Figure 6. IR Spectrum of cold water extracts of *M. oleifera*.
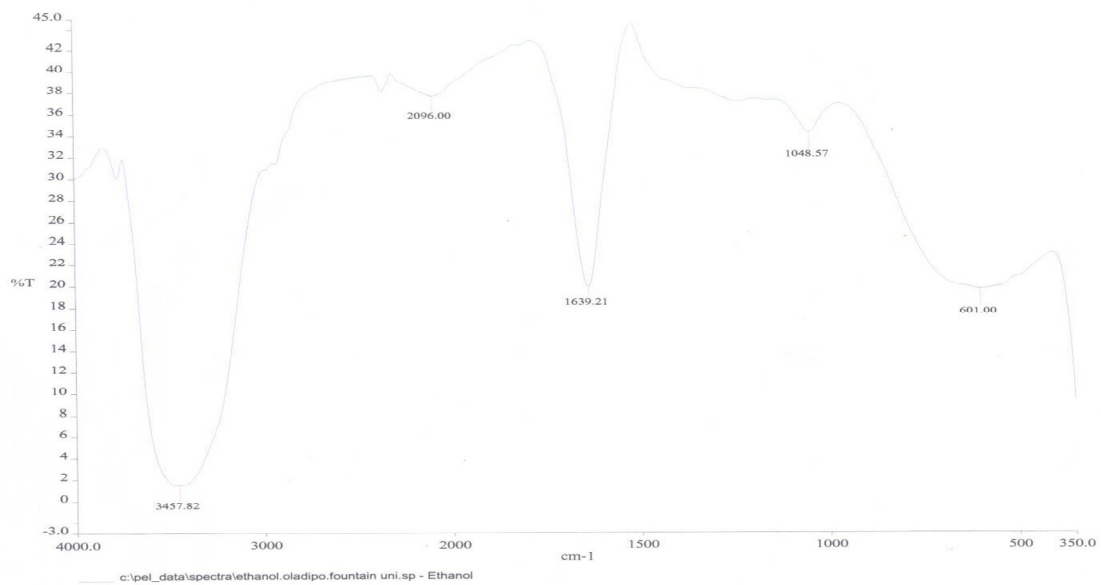
Figure 7. IR Spectrum of ethanol extracts of *M. oleifera*.

(Barwick, 1997).

Distribution of minerals in *M. oleifera* extracts

The level of the important dietary mineral constituents (k, Ca, Mg, Zn, Cu and Fe) that are determined in this study

in different solvent extracts of *M. oleifera* are as summarized in Table 2. In all the extracts and for the macronutrients analysed, concentrations of K was the highest while that of Mg was the lowest. For the micronutrients analysed, Fe concentrations was the highest while Cu was the least. This report supported the previous findings of Moyo et al. (2011) that *Moringa*
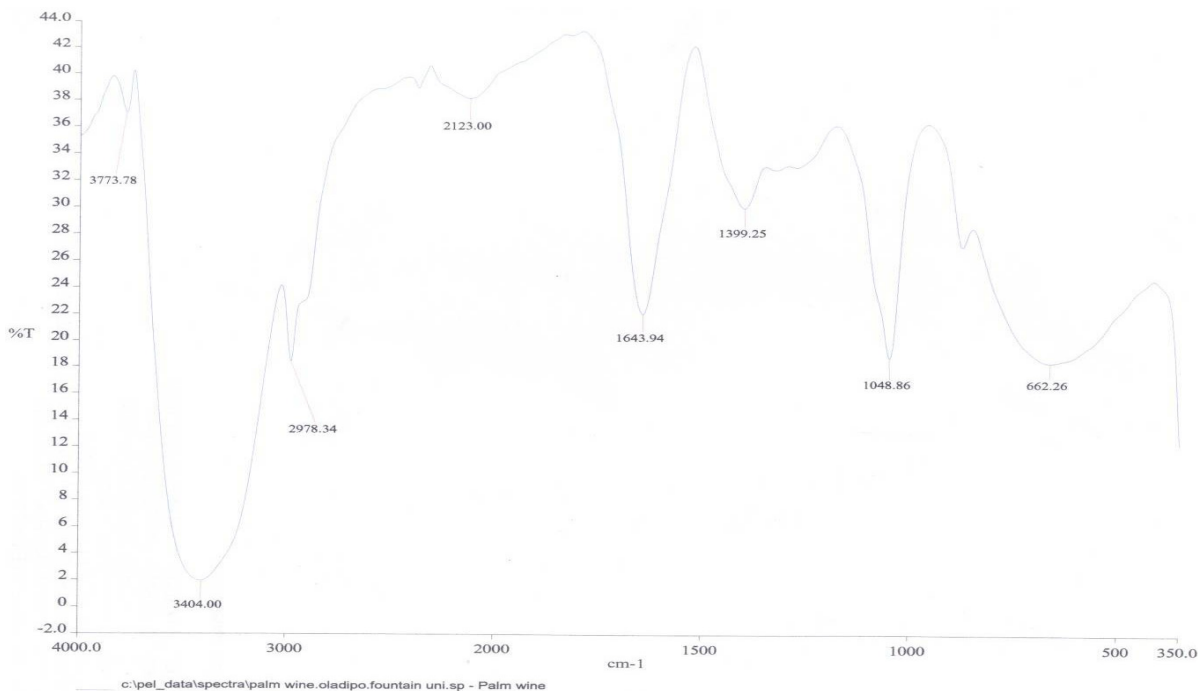
Figure 8. IR Spectrum of alm wine extracts of *M. oleifera*.

seeds and leaves could be a potential source of Fe supplement for humans and livestock.

Fermented maize marsh extracts recorded the highest mean compositions for Ca and Mg (560.50 ± 34.5 and 485.75 ± 67.32); palm-wine extracts recorded the highest compositions of Fe and Cu (7.42 ± 1.22 and 0.50 ± 0.01) while cold water and hot water extracts recorded the highest compositions of K (2045.14 ± 265.02) and Zn (2.16 ± 0.11) respectively. The lowest concentrations of these trace metals which range from 0.06 ± 0.01 to 6.47 ± 0.35 was recorded in n-hexane extracts. Statistical analysis of the mineral constituents using two sample t-tests studies at $p < 0.05$ also revealed great significant differences between the means of the mineral element in the extracts except for Ca contents in hot water and FMM extracts, as well as, in n-hexane and ethanol extracts pair; K concentrations in hot water and FMM extracts; Fe concentrations in cold water and hot water, in addition to, dichloromethane and ethanol extracts pair; Cu in cold water, FMM and dichloromethane; and Zn concentrations in ethanol and dichloromethane. This indicates that solvents polarity and types has great influence on the mineral and bioactive compositions of *M. oleifera* extracts.

The high values of Ca and K in Omidun extracts may be as a result of the high nutritional and therapeutic values of *ogi*. Earlier reports have also shown the presence of microorganisms and their attendant minerals, vitamins and soluble nitrogenous compounds present in ogi which cannot also be ruled out in omidun. This might be an added advantage for the highest yields of these

elements in the omidun extracts. This indicated that the extraction process is a function of the solvents compositions.

The similarity in some of the mineral contents of the extracts obtained with cold water, hot water and omidun also attests to the high affinity and similar solubility properties of these polar solvents for *M. oleifera*.

Ultra violet/visible spectroscopy (UV/Visible) analysis

From the ultra violet/ visible spectral (Figures 9 to 15) and the summary of the ultra violet/ visible spectra analysis, that is, the wavelength maximum absorption (λ_{max}) and their corresponding absorbance for the leaves extract of *M. oleifera* in different solvents media at room temperature in Table 4, two prominent bands were observed for the extracts in the organic solvents used: Polar protic (ethanol), polar aprotic (dichloromethane), and non polar (n-hexane) while three bands were observed in the aqueous solvents (cold water, boiled water, FMM and palm-wine). The highest absorption bands of these extracts appear at 295 nm/300 nm while the lowest were obtained at 210 to 285 nm in all the solvents except in non polar n-hexane. This non appearance of band 1 in the spectra of the extracts in organic solvent could be due the non transparency of this band to these solvent.

Band 1(295 nm) in cold water is shifted to longer wavelength, in both palm-wine and FMM and the intensities as observed also increased in this solvent.
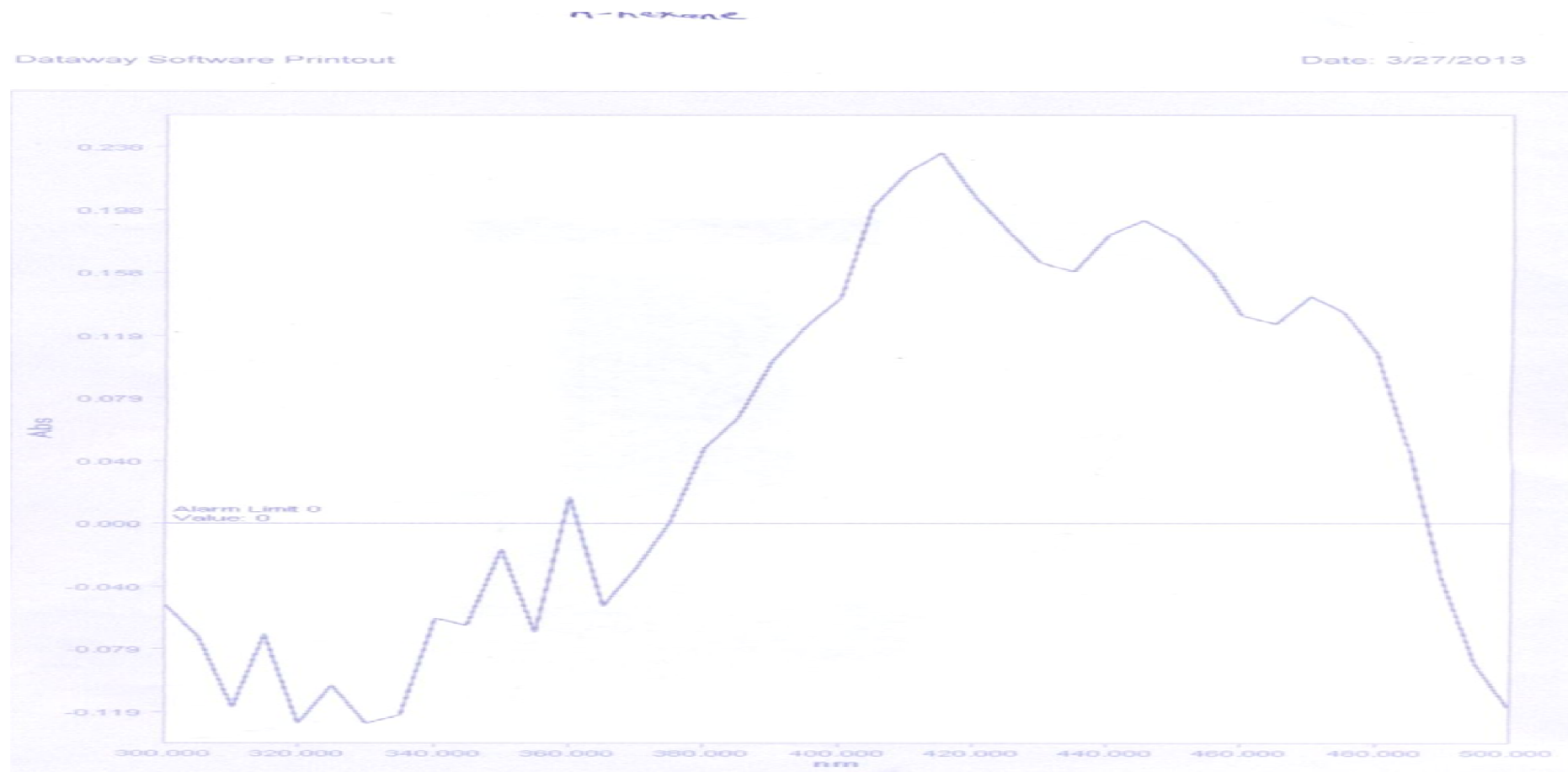
Figure 9. UV-Visible spectrum of n-hexane extracts of *M. oleifera.*

This shows that solvents have great influence (through interaction e.g., protonation, hydrogen bonding, and specific solvation effect) on the molecules of the compounds (Kolar et al., 2002). The absorbance at 295 nm was retained for the extracts in dichloromethane (DCM) and ethanol but with lower intensity (hypochromic shifts), Band 2 (410 nm) was also retained in ethanol but with increase in intensity of absorption. Increase in solvents polarity has little or no effects on the absorption maximum (λ_{max}). The band which occurred at 295 nm in DCM, boiled   water and ethanol were shifted to longer wavelength, that is, 300 nm (Batochromic shift) in Omidun and cold water.

Infrared spectroscopy (IR)

Two or more compounds are one if their spectra agree   in   position   and   relative   intensities
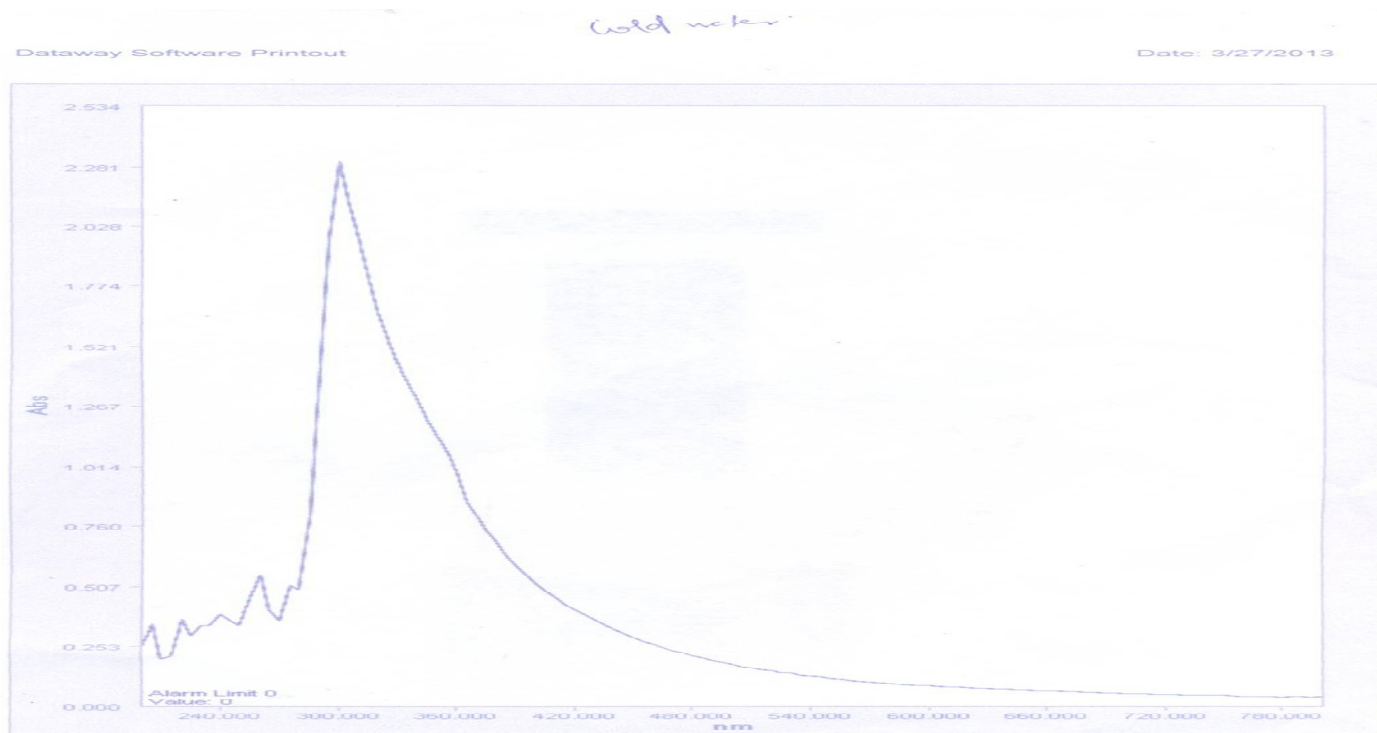
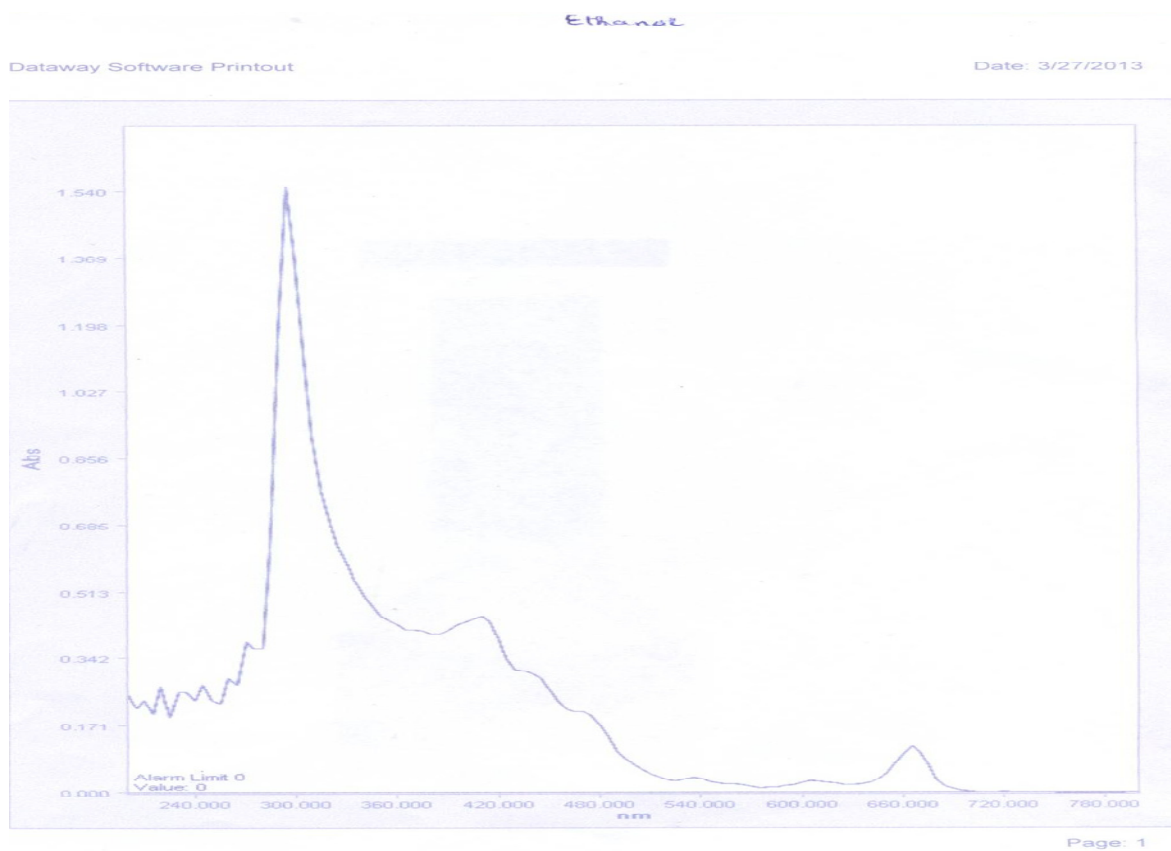**Figure 10.** UV-Visible spectrum of cold water extracts of *M. oleifera*.

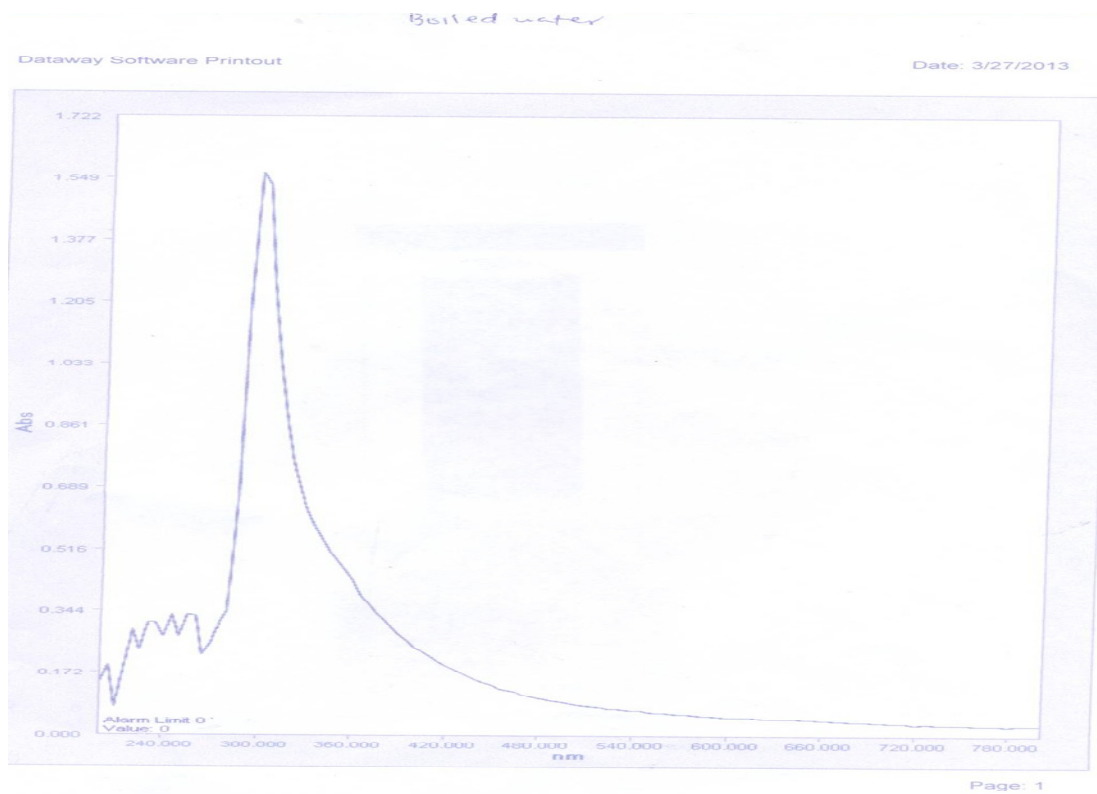**Figure 11.** UV-Visible spectrum of ethanol extracts of *M. oleifera*.

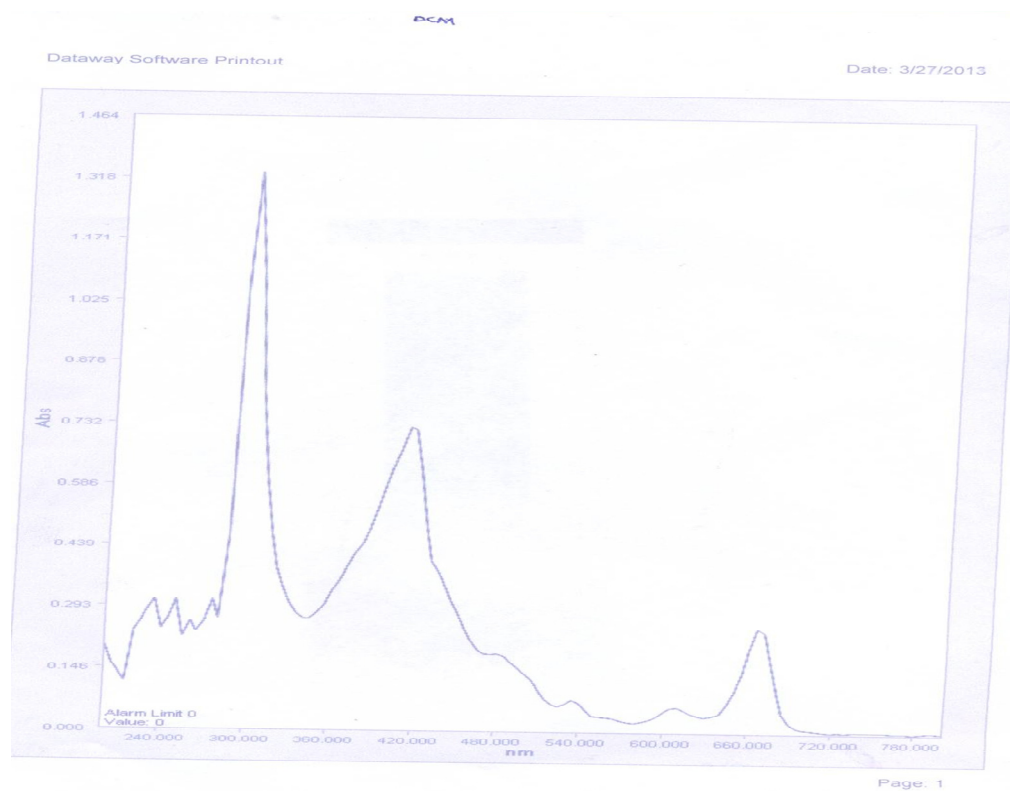**Figure 12.** UV-Visible spectrum of Hot water extracts of *M. oleifera*.

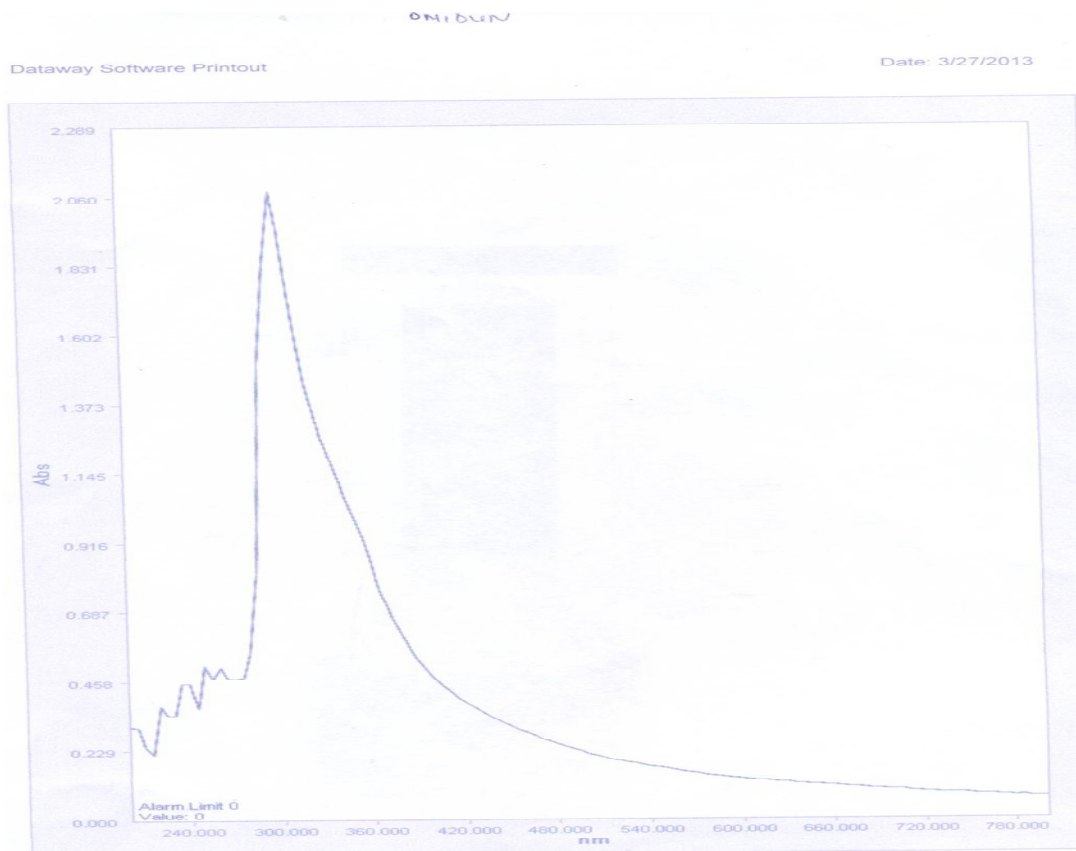**Figure 13.** UV-Visible spectrum of Dichloromethane extracts of *M. oleifera*.

ONIDUN

Dataway Software Printout

Date: 3/27/2013

Figure 14. UV-visible spectrum of fermented maize marsh extracts of *M. oleifera*.

Palm wine

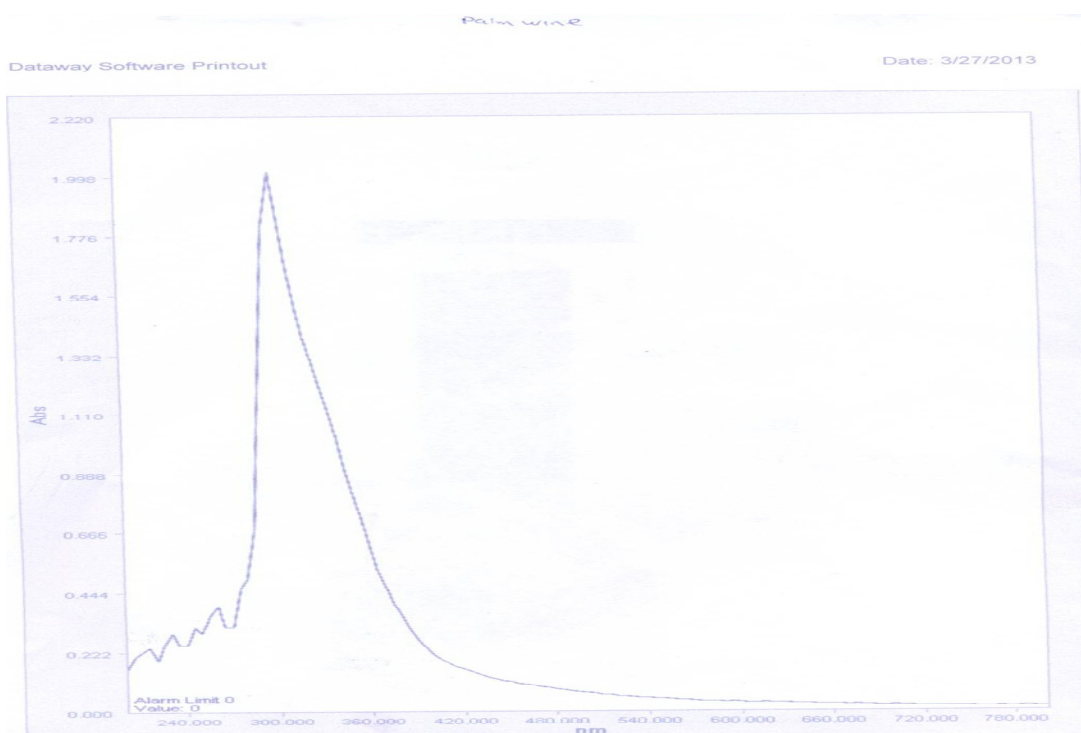Dataway Software Printout

Date: 3/27/2013

Figure 15. UV-Visible spectrum of palm-wine extracts of *M. oleifera*.

of the bands. Thus, the functional groups of the active compounds (Figure 1) in the studied *M. oleifera* extracts and the effect of different solvents on them were confirmed by (IR) analysis. Figures 3 to 8 and Table 3 show the characteristic absorption bands exhibited by the IR spectrum of the studied leave extracts in different solvents. These observed functional groups are comparable with those obtained from the proposed structures of the isolated compounds in Figure 1 with slight variations due to changes in solvent (Rubeena, 1995).

Conclusion

This study shows that solvents polarities and types have great influence on the various chemical compositions of *M. oleifera* plant extracts due to its various interactions with them. Palm wine gave the highest yields of the extracts and highest compositions of micronutrients (Fe and Cu). Fermented maize marsh gave highest composition of the macronutrients (Ca and Mg) and micronutrients (Zn) components. Statistical analysis of the mineral constituents using two sample t-tests studies at $p<0.05$ generally reveals great significant differences in the attendants studied mineral compositions in the extracts except in few cases. The Moringa from this source was found to be a good source of important minerals and this further confirmed that this plant is a viable food supplement feeds additives for dietary minerals in animal and human beings.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Abdulrahaman AA, Kolawole OM (2006). Traditional preparations and uses of maize in Nigeria. Ethnobot. Leaflets 10:219-227.
Al-Kharusi LM, Elmardi MO, Ali A, Al-Said FAJ, Abdelbasit KM, Al-Rawahi S (2009). Effect of mineral and organic fertilizers on the chemical characteristics and quality of date fruits. Int. J. Agric. Biol. 11:290–296.
Andri CK, Masitah H, Harcharan S (2009). Effects of solvent properties on the Soxhlet extraction of diterpenoid lactones from Andrographis paniculata leaves Sc. Asia. 35:306-309.
Anjorin TS, Ikokoh P, Okolo S (2010). Mineral composition of Moringa oleifera leaves, pods and seeds from two regions in Abuja, Nigeria. Int. J. Agric. Biol. 12:431-434.
AOAC (1990). Official methods of analysis, Association of Official Analytical Chemists, Washington, D.C., USA. 15th Edition. pp. 807-928.
Aslam M, Anwar F, Nadeem R, Rashid U, Kazi TG, Nadeem M (2005). Mineral composition of *Moringa oleifera* leaves and pods from different regions of Punjab, Pakistan. Asian J. Plant Sci. 4:417-421. http://dx.doi.org/10.3923/ajps.2005.417.421

Barwick VJ (1997). Strategies for solvent selection - a literature review. Trends Anal. Chem. 16:293-309. http://dx.doi.org/10.1016/S0165-9936(97)00039-3
Brisibe EA, Umoren UE, Brisibe F, Magalhaes PM, Ferreira JFS, Luthria D, Wu X, Prior RL (2009). Nutritional characterization and antioxidant capacity of different tissues of Artemisia annua L. Food Chem. 115:1240-1246. http://dx.doi.org/10.1016/j.foodchem.2009.01.033
FAO corporate document repository (1999). Fermented cereals. A global perspective. Chapter 2: Cereal fermentations in African countries. pp. 1-2.
Hsu R (2006). Moringa Oleifera Medicinal and Economic Uses. International course on Economic Botany. National herbarium, Leiden, The Netherlands.
Kalogo Y, Rosillon F, Hammes F, Verstraete W (2000). Effect of a water extract of Moringa oleifera seeds on the hydrolytic microbial species diversity of a UASB reactor treating domestic wastewater. Lett. Appl. Microbiol. 31:259-264. http://dx.doi.org/10.1046/j.1365-2672.2000.00814.x
Kolar P, Shen JW, Tsuboi A, Ishikawa T (2002). Solvent selection for pharmaceuticals. Fluid Phase Equil. 194(7):771-82. http://dx.doi.org/10.1016/S0378-3812(01)00716-6
Kumar A, Pari L (2003). Antioxidant action of Moringa oleifera Lam. (drumstick) against antitubercular drugs induced lipid peroxidation in rats. J. Med. Food. 6:255-259. http://dx.doi.org/10.1089/10966200360716670
Mahajan SG, Mali RG, Mehta AA (2007). Effect of Moringa oleifera Lam. seed extract on toluene diisocyanate –induced immune-mediated inflammatory responses in rats. J. Immunotoxicol. 4:85-96. http://dx.doi.org/10.1080/15476910701337472
Makkar HPS, Becker K (1996). Nutritional value and anti-nutritional components of whole and ethanol extracted Moringa oleifera leaves. Animal Feed Sci. Tech. 63:211-228. http://dx.doi.org/10.1016/S0377-8401(96)01023-1
Mosha TC, Pace RD, Adeyeye S, Mtebe K, Laswai H (1995). Proximate composition and mineral contents of selected Tanzanian vegetables and effect of traditional processing on the retention of ascorbic acid, riboflavin in and thiamine. Plant Food Hum. Nutri. 48:235–245. http://dx.doi.org/10.1007/BF01088445
Moyo B, Patrick J, Masika AH, Voster M (2011). Nutritional characterization of Moringa (Moringa oleifera Lam.) leaves Afri. J. Biotech. 10(60):12925-12933.
Ogbe AO, John PA (2011). Proximate study, Mineral and Anti-nutrient composition of Moringa oleifera leaves harvested from Lafia, Nigeria: Potential benefits in poultry nutrition and health. J. Microbiol. Biotech. Food Sci. 12:1(3):296-308.
Rubeena S (1995). Studies in the chemical constituents of M. Oleifera L and preparation of potential biologically significant derivatives of 8-Hydroxyquinoline. H.E.J. Research Institute of Chemistry University of Karachi, Pakistan Ph.D thesis.
Toba SA, Pius I, Simon O (2010). Mineral Composition of Moringa oleifera Leaves, Pods and Seeds from Two Regions in Abuja, Nigeria. Int. J. Agric. Biol. ISSN Print: 1560–8530; ISSN Online:1814–959609–398/SBC/2010/12–3–431–434 http://www.fspublishers.org

African Journal of Pure and Applied Chemistry

Related Journals Published by Academic Journals

- African Journal of Mathematics and Computer Science Research
- International Journal of the Physical Sciences
- Journal of Geology and Mining Research Technology
- Journal of Environmental Chemistry and Ecotoxicology
- Journal of Internet and Information Systems
- Journal of Oceanography and Marine Science
- Journal of Petroleum Technology and Alternative Fuels