In Vitro Decontamination of Lead from Human Blood Plasma using *Syzygium aromaticum* Biosorbent.

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ABSTRACT

Adsorption of Pb (II) ions in human blood plasma onto *Syzygium aromaticum* biosorbent was investigated. The rate of adsorption was studied with time at optimum pH (7), optimum Pb (II) ion concentration (30.00 mgL^{-1}), optimum biosorbent dose (2 g) and at physiological temperature. Experimental data was fitted into Langmuir, Freundlich, and Temkin isotherm models. Data was found to follow the Langmuir model with correlation coefficient (R^2) value 0.5093, the maximum monolayer coverage capacity (q_m) 3.696×10^{-2} mgg^{-1}, Langmuir isotherm constant (K_L) 0.3258 Lmg^{-1}, separation factor (R_L) 9.282×10^{-2} indicating favourable isotherm. From Freundlich isotherm model, the sorption intensity (n) value obtained was 0.7130 which also indicated favorable sorption. The heat of sorption process was estimated from Temkin isotherm model to be 0.3752 $Jmol^{-1}$. Data was also fitted to pseudo first and pseudo second order kinetic models. Result followed the pseudo second order kinetics model with the experimental biosorption capacities of 0.3670 mgg^{-1}; its corresponding calculated biosorption capacities 0.3796 mgg^{-1} and (R^2) 1. The result obtained indicated chemisorption which involves the formation of chemical bonds between Pb (II) Ions and *Syzygium aromaticum*. Authors recommend *in vivo* study with experimental animals.

(Keywords: biosorption, lead, *Syzygium aromaticum,* isotherm, kinetics)

INTRODUCTION

Heavy metals in the environment are of major concern due to their toxicity to many life forms. Unlike organic pollutants, which are susceptible to biodegradation, metal ions do not degrade into any harmless end product [1] and tend to accumulate causing several diseases and health disorders in humans, and other living organisms [2]. Lead (Pb) is one of the heavy metals commonly found in the environment which serves no useful purpose in the body. The routes of exposure for inorganic Pb are inhalation and ingestion. Once absorbed, it is found in all tissues, but eventually 90% or more of the body burden is accumulated (redistributed) into bones with a biological half-life of years to decades [3]. High level of exposure affects the synthesis of haemoglobin, compromise the kidneys, gastrointestinal tract, joints and the reproductive system and result in an acute or chronic damage to the nervous system [4,5].

A range of methods has been used for heavy metal decontamination, and one of the most common is biosorption [6,7], which is the removal of metal or metalloid species, compounds and particulates from solution by biological materials [8]. The biosorption process involves several mechanisms that differ qualitatively and quantitatively, depending on the origin of the biomass, the adsorbate (species) involved and processing methods [9]. These mechanisms are generally based on physicochemical interactions between metal ions and functional groups present on the cell surface, which include ion exchange, complexation, electrostatic attraction and micro-precipitation [10].

Cloves are the dried flower buds of *Syzygium aromaticum*, a tree 10–20 m high which is indigenous to the Moluccas or Clove Island. They contain volatile oil (14 %-21%), tannins (10 %-13 %), phenol, sesquetrepene ester, and alcohol. The most important constituent of clove is the phenylpropene eugenol which gives this spice its pungent, distinctive aroma. Eugenol

makes up 70 % to 90 % of the essential oil and 15 % of the dry weight of clove buds. The traditional use indicates that Clove has several medicinal properties like antiviral, antimicrobial, antifungal, aphrodisiac, diuretic, odontalgic, stomachic, carminative and anaesthetic capacities [11, 12]. Figure 1 is *Syzygium aromaticum*, a tree and buds.

Figure 1: *Syzygium aromaticum* Plant and Buds.

Recent studies have shown that heavy metals can be removed from systems using plant materials such as neem leaf powder [13]; mango leaf biosorbent [9]; Ethiopian pepper [14] and sesame leaf [15]. To the knowledge of the authors no study has been conducted on Pb (II) ions decontamination from human blood plasma with *Syzygium aromaticum.* Therefore the present work seeks to investigate the biosorption potential of *Syzygium aromaticum* buds biosorbent in the decontamination of Pb (II) ions from human blood plasma solution. The optimum pH, biosorbent dosage, initial Pb (II) ion concentration would be determined and employed in the study of the rate of Pb (II) ions decontamination. Data obtained would be fitted into Langmuir, Freundlich and Tempkin isotherm models and kinetic studies carried out using pseudo first and second order models.

MATERIALS AND METHODS

Sample Collection and Preparation

Human blood was collected from the Department of Haematology, Ahmadu Bello University Teaching Hospital, Zaria, Kaduna, State, Nigeria. Plasma was extracted from the human blood by centrifugation and screened for Pb concentration. Dried *Syzygium aromaticum* buds (adsorbent) was bought from Sabo market, Zaria, Kaduna State, Nigeria, repeatedly washed with tap water and rewashed with deionized water to remove dust and solid impurities, dried in an oven for 24 hr at 60°C, ground to a constant weight, sieved with a 0.33 mm mesh size and stored in air tight plastic containers.

Scanning Electron Microscope (SEM) Analysis of Samples

Dry biosorbent, before and after Pb (II) ion (adsorbate) biosorption, were glued onto 10-mm diameter metal mounts and coated with gold under vacuum in an argon atmosphere. The coated samples were put in to the SEM unit and examined.

Determination of Lead Ion Concentration in Samples

All filtrates were digested with acid mixtures and the residual (unadsorbed) Pb (II) ion concentrations in the filtrates determined by Atomic Absorption Spectrometer (Varian AA240FS model).

Biosorption Data Evaluation: The percentage Pb (II) ion decontamination was determined using Equation 1:

$$\text{Biosorption (\%)} = \frac{C_0 - C_e}{C_e} \times 100 \qquad \textbf{(1)}$$

where C_0 and C_e (mgL^{-1}) are the initial and equilibrium Pb (II) ion concentration in the human blood plasma respectively. The amount of biosorbed Pb (II) ion (mg /g) was calculated from the decrease in the concentration of Pb (II) ions in the medium by considering the biosorption volume and used amount of the biosorbent as presented in Equation 2:

$$q_e = \frac{(C_i - C_e)\,V}{m} \qquad \textbf{(2)}$$

where q_e is the amount of Pb (II) ions biosorbed onto unit mass of the biosorbent (mgg^{-1}) at equilibrium, C_i and C_e are the initial and final (equilibrium) concentrations of the Pb (II) ions in the human blood plasma (mgL^{-1}), V is the

volume of the human blood plasma (dm^3) and m is the amount (g) of biosorbent used.

Optimization of Biosorption Parameters

The optimum pH of Pb (II) ion biosorption was studied by introducing 1.00 g of biosorbent into 60 cm^3 polypropylene containers containing 20 cm^3 of human blood plasma solution with 30 mgL^{-1} Pb (II) ion solution. The pH of composition was adjusted using 0.10 M NaOH and 0.10 M HCl solution to 2, 4, 6, 7, 8 and 10 respectively, agitated for 60 min while incubating in a 37^oC shaker (physiological temperature) and filtered using Whatman filter paper. The result showed optimum pH of Pb (II) ion biosorption to be 7.

The optimum biosorbent dosage study was conducted by introducing 0.50 g, 1.00 g, 1.50 g, 2.00 g, 2.50 g and 3.00 g of the biosorbent respectively into 20 cm^3 of human blood plasma solution containing 30.00 mgL^{-1} Pb (II) ions into 60 cm^3 polypropylene containers. The pH were adjusted using 0.10 M NaOH and 0.10 M HCl solution to pH 7 (optimum pH) and the composition was agitated for 60 min while incubating in a 37^oC shaker (physiological temperature) and filtered using Whatman filter paper. The result obtained showed that the optimum biosorbent dose is 2.00 g.

The optimum biosorbate dose was studied as follows: Concentrations of 10.00 mgL^{-1}, 20.00 mgL^{-1}, 30.00 mgL^{-1}, 40.00 mgL^{-1}, 50.00 mgL^{-1} and 60.00 mgL^{-1} of Pb (II) ions in 20.00 cm^3 human blood plasma solution were placed in 60 cm^3 polypropylene containers respectively. The optimum biosorbent dose (2.00 g) was added and the pH adjusted using 0.10 M NaOH and 0.10 M HCl solution to 7 (optimum pH) and the composition was agitated for 60 min while incubating in a 37^oC shaker (physiological temperature) and filtered using Whatman filter paper. The optimum concentration of Pb (II) ions in human blood plasma solution obtained was 30.00 mgL^{-1}.

Study of Rate of Pb (II) Ion Decontamination

The decontamination of Pb (II) Ions in human blood plasma solution was studied by monitoring the rate of Pb (II) Ion adsorption onto *Syzygium aromaticum* with time. 30.00 mgL^{-1} of Pb (II) ion in 20.00 cm^3 human blood plasma solution was placed in 60 cm^3 polypropylene containers respectively. The optimum biosorbent dose (2.00 g) was added and the pH adjusted using 0.10 M NaOH and 0.10 M HCl solution to 7 (optimum pH) and the composition was agitated for 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 min respectively while incubating in a 37^oC shaker (physiological temperature), filtered using Whatman filter paper and the amount of Pb (II) Ions adsorbed onto the adsorbent determined in each composition.

Statistical Data Analysis

Statistical data analysis was performed using the statistical functions of Microsoft Excel version Office XP (Microsoft Corporation, USA).

RESULTS AND DISCUSSION

Scanning Electron Microscope (SEM) Images

The SEM images of biosorbents before and after biosorption are presented in Figures 2 and 3 respectively for *Syzygium aromaticum.* From these images, it is clear that there is significant difference in the appearance of the biosorbent surfaces before and after biosorption. Images clearly highlight the action of biosorption on the surface morphology of the biosorbent. This implies that Pb (II) ion could adsorb on *Syzygium aromaticum.*

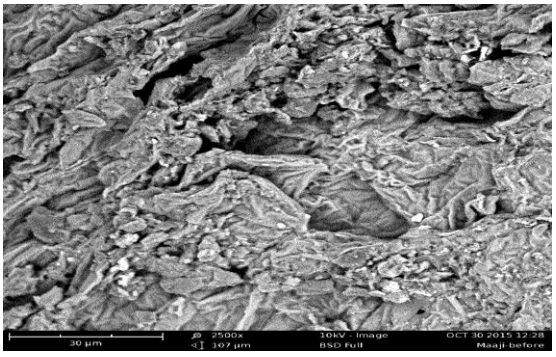

Figure 2: SEM of *Syzygium aromaticum* before Biosorption.

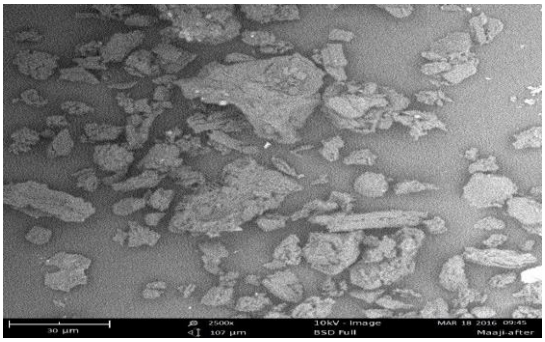

Figure 3: SEM of *Syzygium aromaticum* after Pb (II) Ion Biosorption.

Optimum pH

The optimization of pH on the biosorption of Pb (II) ions is presented in Figure 4. The percentage Pb (II) ions biosorbed increased from pH 2 (10.98%) to pH 7 (80.00%) then decreased to 18.53% at pH 10. The result suggests that optimum biosorption was obtained at pH 7. It has been documented that at low pH the biosorption capacity for most metal ions is low, because large quantity of hydrogen ions competes with metal ions at sorption sites. As the pH increases more negatively charged cell surface become available thus enhancing greater metal uptake [16]. At high pH, Pb (II) ions would precipitate out and this would hinder the biosorption process. Thus initial pH would play an important role in the decontamination of Pb from human blood plasma. Similar result was obtained in the biosorption of Pb (II) ions onto Bamboo Dust and Commercial Activated Carbons [17].

Optimum Biosorbent Dose

Biosorbent dose is a significant factor for effective biosorption. The percentage decontamination of Pb (II) Ions against various biosorbent dose is shown in Figure 5. The percentage of Pb (II) ions removed increased steadily with increase in biosorbent dose from

77.39% at 0.50 g of biosorbent to 82.99 % at 2.00 g. There was no significant rise in adsorption when the amount of biosorbent was increased to 2.50 g and 3.00 g respectively signifying that the optimum biosorbent dose is 2.00 g. This trend could be due to the formation of aggregates of the biosorbent at higher doses, which decreased the effective surface area for biosorption [18, 19]

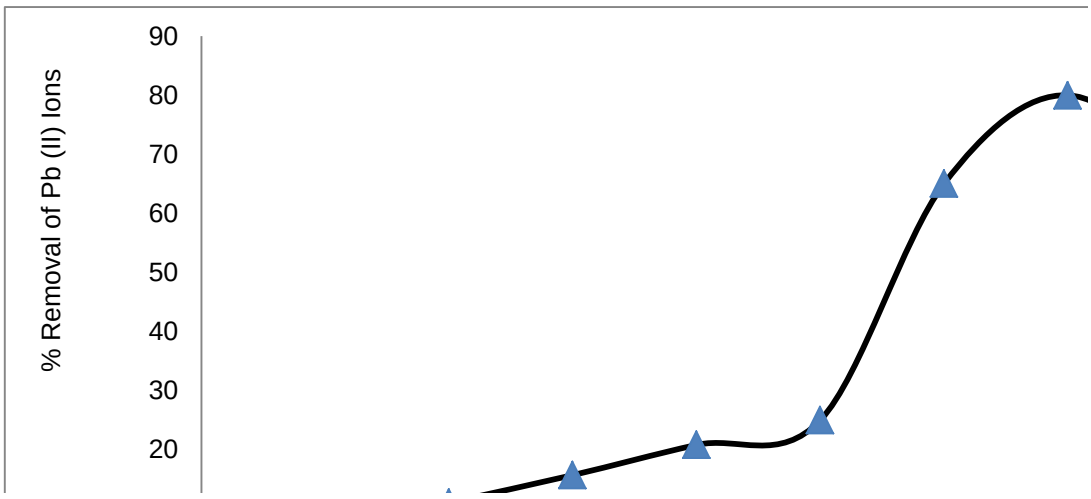

Figure 4: Percentage Removal of Pb(II) Ions against pH.

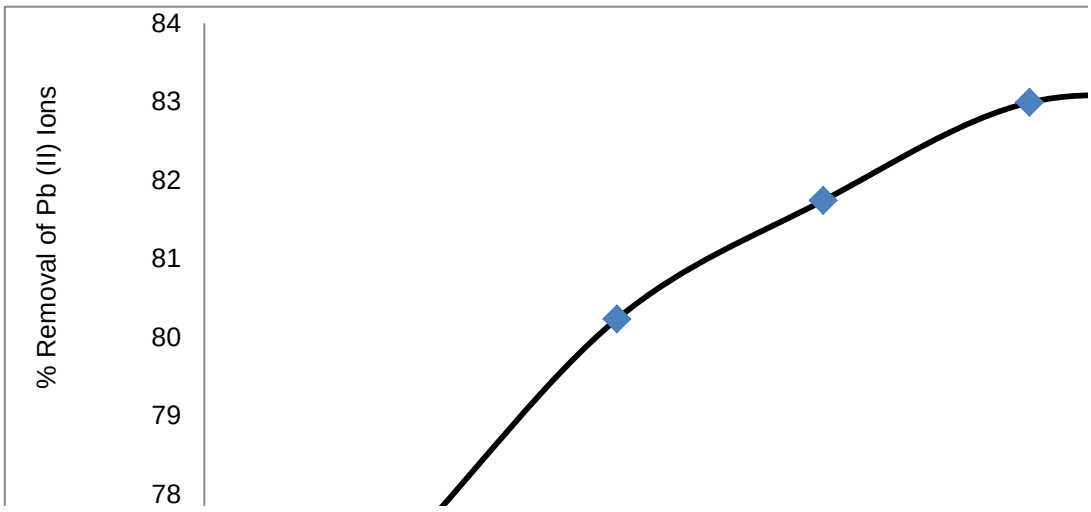

Figure 5: Percentage Removal of Pb(II) Ions against Biosorbent Dose.

Optimum Pb (II) Ion Concentration

Figure 6 is the plot of percentage decontamination of Pb (II) Ions by the adsorbent at various concentrations of Pb (II) Ions. The percentage Pb (II) ions removed increased with a corresponding increase in the initial concentrations of Pb (II) ions from 30.03% at 10.00 mgL^{-1} to 93.50% at 30.00 mgL^{-1} and no further percentage increase in adsorption occurred at increased Pb (II) ion concentrations. This trend suggests that increase in Pb (II) ion concentration resulted in increase in the number of available molecules per binding site of the biosorbent thus indicating a higher probability of binding of Pb

(II) ions to the biosorbent. Therefore, the probability of chemical interaction between the biosorbent and the Pb (II) ions is enhanced by reason of the high availability of Pb (II) ions in solution. At higher concentrations there was no increase in the percentage recontamination this could be due to the diminishing or saturation of Pb (II) ions binding sites on the biosorbent at concentrations higher than 30.00 mgL^{-1}. Similar trend has been reported [20].

Rate of Pb (II) Ion Decontamination

Figure 7 shows the rate of percentage decontamination of Pb (II) ions with contact time onto the biosorbent. As the contact time increased, there was increase in the percentage decontamination of Pb (II) ions. The percentage increased from 87.61% at 10 min to 94.01% at 90 min (equilibrium adsorption) and no further decontamination with time. The result showed that the decontamination of Pb (II) ions was rapid in the first 30 min then increased gradually till the equilibrium at 90 min and the biosorption became almost constant thereafter with increase in time indicating saturation of the binding sites

Biosorption Isotherms

The biosorption isotherm is the relationship between equilibrium concentrations of solute (adsorbate) in the solution and the biosorbent at constant temperature that is how the biosorption molecules distribute between the liquid phase and the solid phase when the biosorption process reaches an equilibrium state [21]. The data obtained from the rate of percentage decontamination of Pb (II) ions with contact time onto the biosorbent was fitted with Langmuir, Freundlich and Tempkin isotherms, respectively.

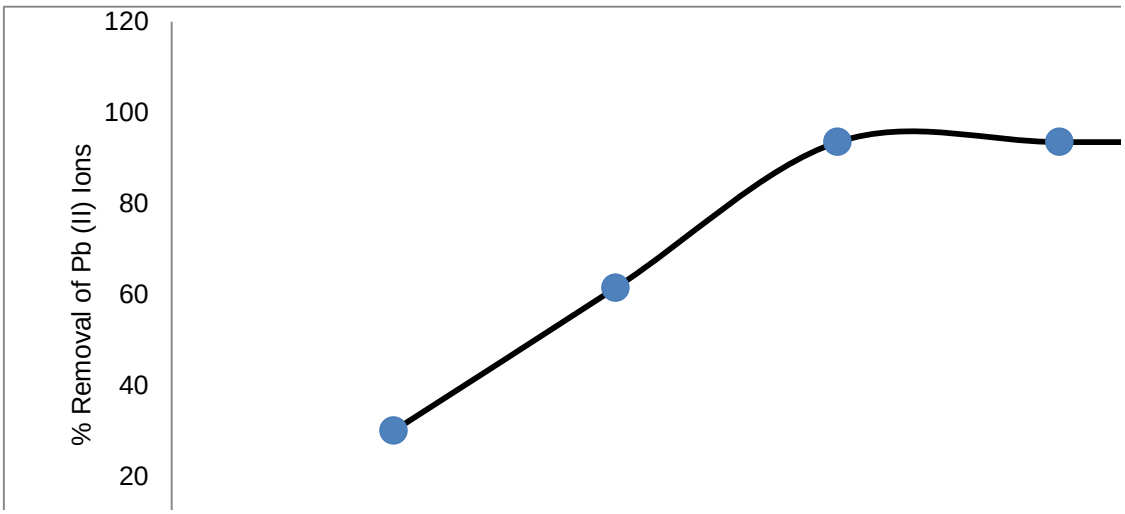

Figure 6: Percentage Removal of Pb(II) Ions against Concentration.

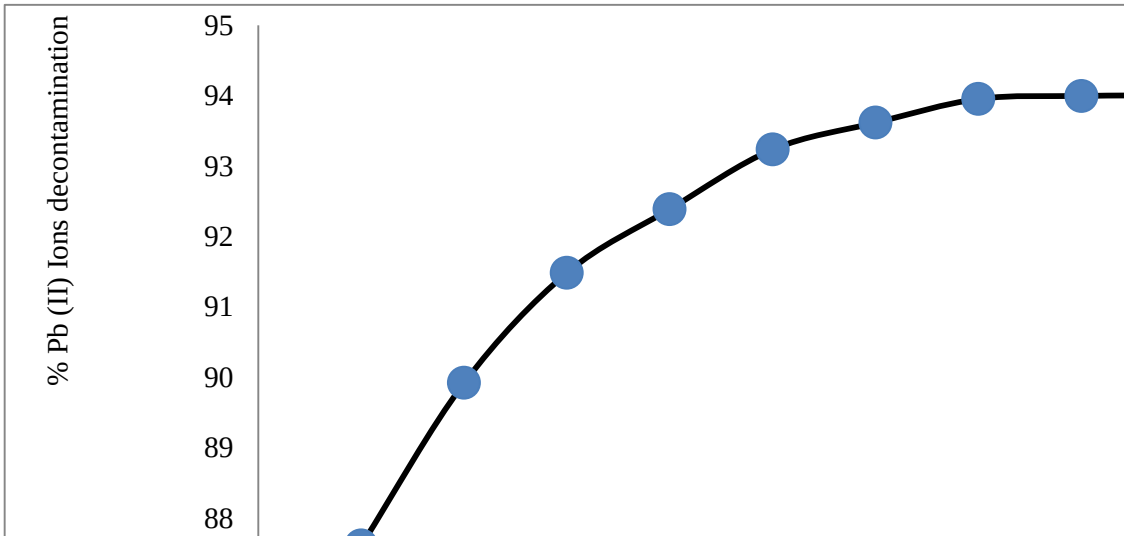

Figure 7: Percentage Removal of Pb(II) Ions with Time.

Langmuir Isotherm: The Langmuir equation assumes that solid surface presents a finite number of identical sites which have uniform energy; there is no interaction between biosorbed species meaning that the amount biosorbed has no influence on the rate of biosorption and a monolayer is formed when the solid surface reaches saturation. The Langmuir equation is as presented in Equation 3:

$$q_e = \frac{K_L q_m C_e}{1 + K_L C_e} \quad (3)$$

where q_e and q_m are the equilibrium and monolayer biosorption capacities of the biosorbent ($molg^{-1}$) respectively. C_e is the metal ion concentration at equilibrium in the solution ($molL^{-1}$) and K_L is the Langmuir constant ($Lmol^{-1}$) related to the free energy of biosorption. Equation 3 is usually linearized to obtain Equation 4:

$$\frac{C_e}{q_e} = \frac{1}{K_L q_m} + \frac{1}{q_m} C_e \quad (4)$$

By plotting C_e/q_e versus C_e' Langmuir constant (K_L), monolayer biosorption capacities (q_m) could be obtained from the straight line graph. The Langmuir constant (K_L), is employed to determine the suitability of the biosorbent to sorbate by using Hall separation factor (R_L), dimensionless [22] and R_L can be calculated with Equation 5:

$$R_L = \frac{1}{1 + K_L C_0} \quad \textbf{(5)}$$

where C_0 is the initial concentration of adsorbate in the solution (mgL^{-1}), K_L is the constant related to the energy of adsorption (Langmuir Constant). R_L value indicates the adsorption nature to be either unfavourable if R_L>1), linear if R_L =1, favourable if 0< R_L<1 and irreversible if R_L=0.

The Langmuir isotherm model (Figure 8) shows that the correlation coefficient (R^2) is 0.5093 and from the data calculated in Table 1, the maximum monolayer coverage capacity (q_m) was determined to be 3.696×10^{-2} mgg^{-1}, Langmuir isotherm constant (K_L) is 0.3258 Lmg^{-1}, the separation factor (R_L) is 9.282×10^{-2} which is greater than 0 but less than 1 indicating that Langmuir isotherm is favorable [23].

Freundlich Isotherm: The Freundlich isotherm is an empirical expression based on biosorption on a heterogeneous surface. The Freundlich model is represented in Equation 6:

$$\ln q_e = \ln K_F + \left(\frac{1}{n}\right) \ln C_e \quad \textbf{(6)}$$

where K_F (Lg^{-1}) and n are Freundlich isotherm constants being indicative of the extent of the biosorption and the degree of non-linearity between solution concentration and biosorption, respectively.

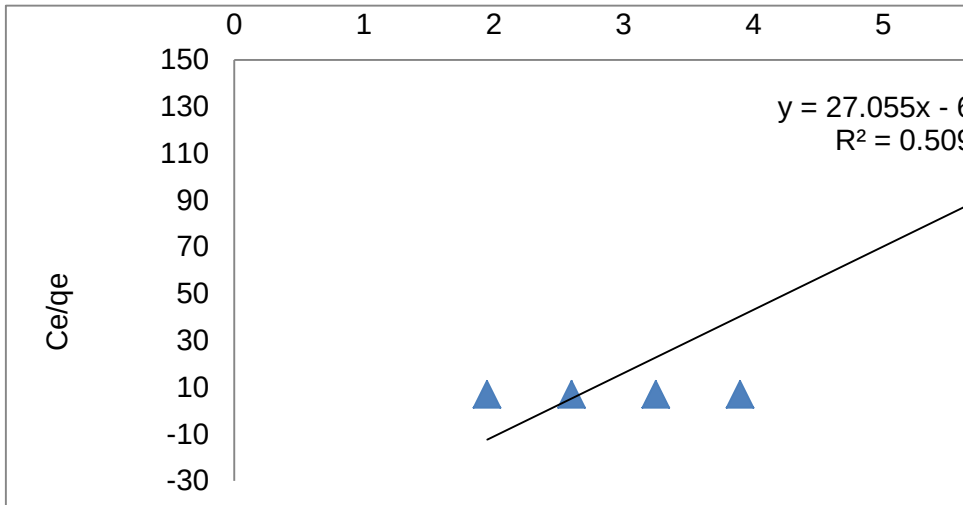

Figure 8: Lagmuir Isotherm for the Biosorption of Pb(II) Ions.

Table 1: Isotherms of Pb (II) Ions Biosorption onto *Syzygium aromaticum* at 37°C.

Langmuir isotherm				Freundlich isotherm			Temkin isotherm		
q_m	K_L	R_L	R^2	n	K_F	R^2	A	B	R^2
0.03696	0.3258	0.09282	0.5093	0.7130	1.4515	0.4795	15.6943	0.2193	0.3462

A plot of ln q_e versus ln C_e gives a straight line which is the Freundlich isotherm model of Pb (II) ion biosorption (Figure 9), which was employed to generate K_F and n from the intercept and slope values. K_F is a useful index to determine the biosorption capacity of the biosorbent. The n value indicates the degree of non-linearity between solution concentration and adsorption as follows: if n=1, then adsorption is linear, if n<1, then adsorption is chemical process; if n>1, the adsorption is physical process [24]. As presented in Table 1 the obtained n value was 0.7130 which is less than 1 indicating chemisorption process.

Tempkin Isotherm: Temkin isotherm takes into account the interactions between adsorbents and metal ions to be adsorbed and is based on the assumption that the free energy of sorption is a function of the surface coverage [25]. The equation is presented in Equation 7:

$$q_e = RT/b_T In(AC_e) \quad \textbf{(7)}$$

The linearized Temkin isotherm is presented in Equation 8:

$$q_e = BInA + BInC_e \quad \textbf{(8)}$$

where RT/bT = B, T(0K) is the temperature, R is the ideal gas constant 8.314Jmol-1K-1), *A*(L/g) is the equilibrium binding constant corresponding to the maximum binding energy, *B*(J/mol) is a constant related to heat of sorption and b_T is the Temkin isotherm constant. The linear plot of qe versus lnCe is the Temkin isotherm for the biosorption data which is presented in Figure 10 and enables the determination of constants A and B.

From the result obtained presented in Table 1, the obtained correlation coefficient (R^2) is 0.6462, the value of A is 10.3377 Lg^{-1}, B_T= 0.3752 $Jmol^{-1}$**.**

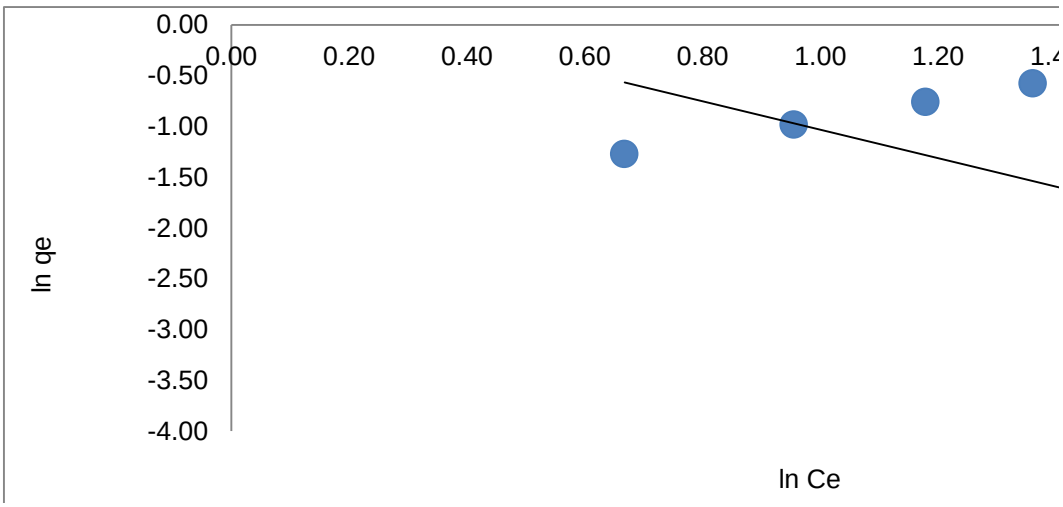

Figure 9: Freundlich Isotherm for Biosorption of Pb(II) Ions.

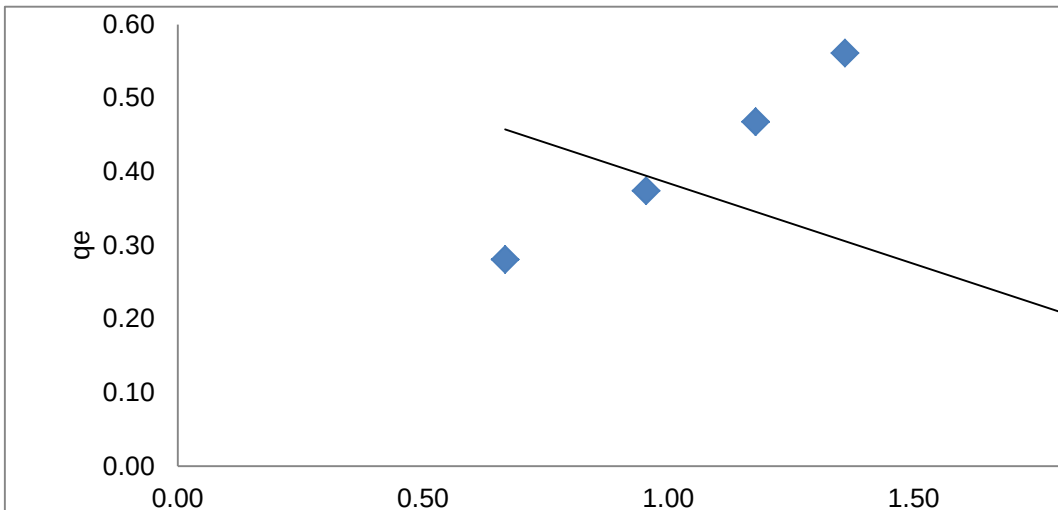

Figure 10: Temkin Isotherm for the Biosorption of Pb(II) Ions.

Biosorption Kinetic Studies

The data obtained from the rate of percentage decontamination of Pb (II) ions with contact time onto the biosorbent was studied with pseudo first and second order kinetics.

Pseudo First Order Kinetic Model: The integrated form of pseudo first order kinetic model is as expressed in Equation 9:

$$Log(q_e - q_t) = Log\ q_e - \frac{K_1}{2.303}\ t \qquad \textbf{(9)}$$

where q_e (mmolg^{-1}) and q_t (mmolg^{-1}) are the mass of metal biosorbed at equilibrium and at time (t) respectively and K_1 (min^{-1}) is the first order rate constant of biosorption. Figure 11 is a plot of log (q_e-q_t) versus t which gives a straight line and K_1 is evaluated from the slope of the graph while qe was determined from the intercept. As presented in Table 2, the experimental biosorption capacities of Pb (II) ion is 0.3670 mgg^{-1}; its corresponding calculated biosorption capacities using the 0.1221and the correlation coefficient (R^2) is 0.9077

Pseudo Second Order Kinetic Model: The pseudo second order kinetic model is expressed in Equation 10 [26]:

$$\frac{t}{q_t} = \frac{1}{K_2 \times q_e^2} + \frac{t}{q_e} \qquad \textbf{(10)}$$

where q_e (mmolg^{-1}) and q (mmolg^{-1}) are the mass of metal biosorbed at equilibrium and at time (t) respectively, K_2 (min^{-1}) is the second order rate constant of biosorption. Figure 12 is a linear plot of t/q_t against t. As presented in Table 2, the experimental biosorption capacities of Pb (II) ion is 0.3670 mgg^{-1}; its corresponding calculated biosorption capacities is 0.3796 mgg^{-1} and the correlation coefficient (R^2) is 1.

Therefore the pseudo second order biosorption model could be accepted as more suitable to describe the biosorption kinetics of Pb (II) ions on *Syzygium aromaticum* and this relies on the assumption that the rate limiting step is most likely to involve chemical interactions involving binding of the ions to the surface by bonding as strong as covalent bond [27].

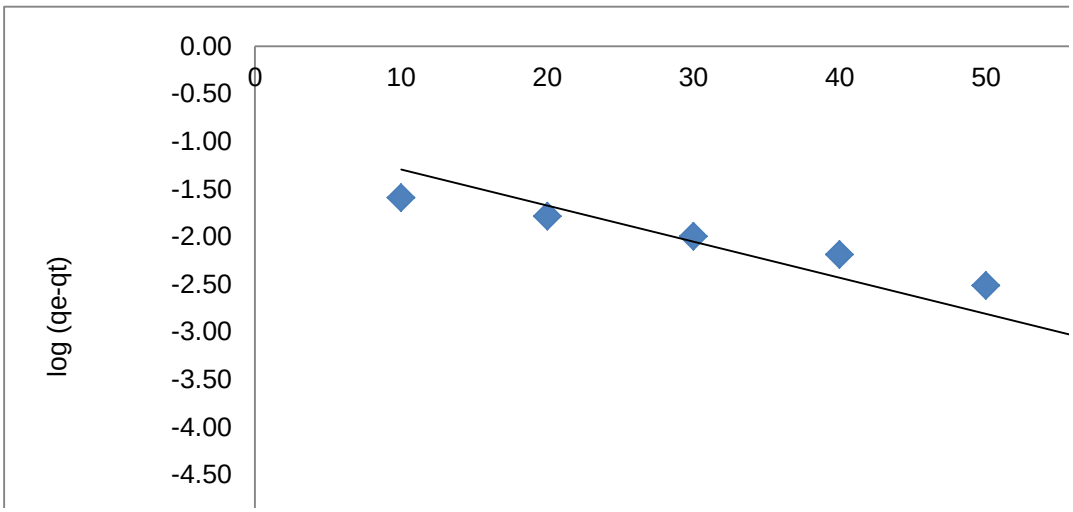

Figure 11: Pseudo First Order Kinetics of Pb(II) Ions.

Table 2: Kinetic Parameters for Biosorption of Pb (II) Ions onto *Syzygium aromaticum.*

	Pseudo First Order			**Pseudo Second Order**		
q_e (Experimental)	q_e(cal.) mg/g	K_1 (min^{-1})	R^2	q_e(cal.)mg/g	K_2(min^{-1})	R^2
0.3760	0.1221	0.08728	0.9077	0.3796	2.7902	1

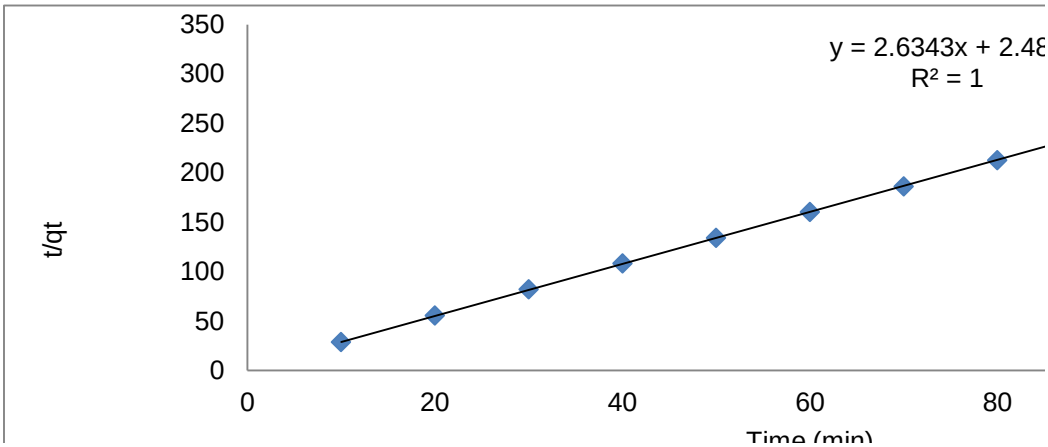

Figure 12: Pseudo Second Order Kinetics of the Biosorption of Pb(II) Ions.

Intraparticle Diffusion Model

This model is employed in the identification of the diffusion mechanism and rate controlling steps that affects the adsorption process [28]. The theory is mathematically presented in Equation 11:

$$qt = K_{id}t^{1/2} + C \qquad (11)$$

where K_{id} is the intraparticle diffusion rate constant (mg/gmin$^{1/2}$), C is the intercept (mg /g).

A plot of percentage decontamination (qt) versus quare root of time ($t^{1/2}$) gives a linear relationship (Figure 13). From this plot k_{id} value was determined to be 0.473 from the slope and R^2 = 0.6885 while the intercept (89.614) reflects the boundary layer effect [29].

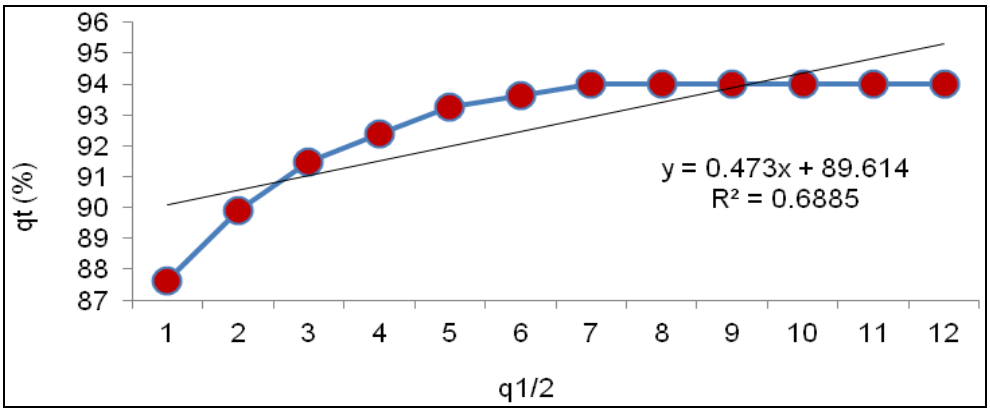

Figure 13: Percentage Removal versus Square Root of Time.

If the regression of the plot is linear and passes through the origin, then intraparticle diffusion is the sole rate determining step [30]. However, the linear plots of the percentage decontamination of Pb did not pass through the origin. This indicated that the intraparticle diffusion was not the rate determining step [31].

CONCLUSION

Syzygium aromaticum was evaluated as possible biosorbent for removal of Pb (II) ions from Human Blood Plasma. Result showed that Pb (II) ions could be effectively removed from Human Blood Plasma by the biosorbent. The biosorption was dependent on pH, concentration of Pb (II) ions and amount of adsorbent. The biosorption followed the Langmuir model and the kinetics of biosorption was well represented by Pseudo second order kinetic model.

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