

Mathematical Modelling of the Growth of *Cajanus cajan* in the Presence of Petroleum Oily Sludge Based on the Number of Shoots Produced

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ABSTRACT

The use of *Cajanus cajan* (pigeon pea) or Kacang dhal in the phytoremediation of petroleum sludge in this study is novel. One of the challenges in monitoring the efficiency or inhibition of remediation by this toxic substance is measuring growth indicator. The number of shoots produced under growth on this toxic substance can be used to monitor the efficiency or inhibition and the use of mathematical models in describing the growth curve based on this parameter can give tangible results that can be used in further modelling exercise. In this study we demonstrated on the feasibility of using mathematical models in obtaining growth parameters based on the number of shoots produced under various concentrations of toxicants. The popular modified Gompertz growth model again prove itself as the best models in describing plant growth. The coefficients obtained in this study will be very valuable in secondary modelling exercise. This is the first time that mathematically modelling of the growth rate of *C. cajan* measured as the increase in number of shoots is carried out to the best of our knowledge.

INTRODUCTION

The soil is a biological environment which receives pollutants and has the ability to exchange it with human and animals, through the horizontal transfer to groundwater and biological food chain [6]. Physicochemical methods used in the remediation of soils contaminated by oily sludge are costly and is environmental unfriendly methods [5]. The use of microorganisms can be efficient, but when dealing with oily sludge that limit aeration to the soil, this can led to a severe problem, leading to the discovery of phytoremediation [7]. Phytoremediation, a subgroup of bioremediation, utilizes special plants to remediate contaminated soils, sediments, surface and ground waters [8]. Legumes are recognized to present an edge on non-leguminous plants in phytoremediation

due to their capacity to fix nitrogen [9] and therefore, don't have to contend with microorganisms as well as other plants for restricted resources of accessible soil nitrogen at oil-contaminated sites. Typical appealing attributes of these plants are capacity to fix nitrogen; a significant constraining factor for successful degradation of pollutants) and it is also relatively drought tolerance [10].

Although there is a report on the use of *Cajanus cajan* to remediate hydrocarbon (diesel) pollution, the quantitative effect of the toxic property of hydrocarbon to the plant has received limited attention. Similar to microbial growth, plant growth rate is a valuable parameter that can reflect cellular mechanism [11]. One of the most important quantitative translation methods for measuring growth rate is mathematical growth modelling. The

olden ways of transforming growth of organisms over time by natural logarithm is fraught with inaccurate determination of growth rate and disruption of the error structure of the data. Primary mathematical models such as several available models such as Logistic [12,13], Gompertz [13–17], Richards [13,18], Schnute [13], Baranyi-Roberts [19], von Bertalanffy [20,21], Buchanan three-phase [22] and more recently Huang model [23] that can be used to accurately obtained growth rates that is valuable for secondary modelling exercise.

In this study, *Cajanus cajan* was utilized in remediating petroleum oily sludge amended soil. One of the parameters measured is the number of shoots. To date, the monitoring of plant growth rate affected by petroleum oily sludge through measuring the number of shoots present from affected seedlings has never been carried to the best of our knowledge and this is the first of such study.

MATERIALS AND METHODS

The experiment was carried out in the small garden of the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia in 2017. The petroleum oily sludge used in this study was obtained from Shell petrochemical company Malaysia [24]. The oily sludge was collected in clean jerry can and transported to the garden. An uncontaminated soil was collected from agricultural farm in Universiti Putra Malaysia. The seeds of *C. cajan* (pigeon pea) and plastic pots was obtained from a local grocery company.

About 3 kg of soil which was previously air dried and sieved was utilized in this study. The soil was previously sieved 4 mm before use. Aliquot of oily sludge was weighed to obtain 1%, 2%, 3%, 4%, and 5% (w/w) concentration and homogenously mixed by agitation to ensure homogeneity and uniform of soil to the oily sludge in a fume hood [25]. The pots were allowed to remain undisturbed for one week for the volatilization processes. The soil sample was taken for physicochemical analysis prior to treatment. After the one-week period, seeds of *C. cajan* was planted accordingly.

The viability of the seed of *Cajanus cajan* was tested using the floatation method and then surface sterilized with 10% Hydrogen peroxide. The seeds of was sown to a depth recommended for *C. cajan* with five seeds were planted in a hole. The pots were irrigated every day and emergence and quantity of shoot were observed [26].

Mathematical models for the effect of oil sludge on number of plant shoots

Fitting of the data

Fitting of the plant growth data measured as the number of shoot formed to the various growth models such as Logistic, modified Gompertz, modified Richards, modified Schnute, Baranyi-Roberts, von Bertalanffy, Buchanan three-phase and Huang models [27–29] was carried out using the Marquardt algorithm available from the CurveExpert Professional software (Version 1.6). The algorithm minimizes the sums of the square of the residuals, which is the differences between the predicted and observed values.

Statistical analysis

The quality of fit of the models to the experimental data was evaluated statistically using the Root-Mean-Square Error (RMSE) (Eqn. 1), adjusted coefficient of determination (R^2) (Eqn. 2), corrected AICc (Akaike Information Criterion) (Eqn.

3), bias factor (BF) (Eqn. 5) and accuracy factor (AF) (Eqn. 6) as carried out in previous works [30–34].

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (Pd_i - Ob_i)^2}{n - p}} \quad (\text{Eqn. 1})$$

$$Adjusted (R^2) = 1 - \frac{(1 - R^2)(n - 1)}{(n - p - 1)} \quad (\text{Eqn. 2})$$

$$AICc = 2p + n \ln\left(\frac{RSS}{n}\right) + 2(p + 1) + \frac{2(p + 1)(p + 2)}{n - p - 2} \quad (\text{Eqn. 3})$$

n and p represent the number of data points and the number of parameters, respectively. A model is more likely to be correct if it has the smallest AICc value compared to other models [35].

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^n \log \frac{(Pd_i / Ob_i)}{n}\right)} \quad (\text{Eqn. 5})$$

$$\text{Accuracy factor} = 10^{\left(\sum_{i=1}^n \log \frac{[(Pd_i / Ob_i)]}{n}\right)} \quad (\text{Eqn. 6})$$

RESULTS AND DISCUSSION

Cajanus cajan (pigeon pea) (Kacang dhal) in Malay is a very common legume crop that is frequently cultivated in tropical places, also it functions as a crucial supply of protein in our diet, it features a long root systems which usually fits under various soil condition and properties [36]. The synergistic strategy of legume-bacterial relationship by which legume plant provides rhizosphere bacteria with crucial organic nutrients and space, whilst the bacteria degrades hydrocarbons into less-toxic substances [37]. This synergy has allowed phytoremediation to proceed even in the presence of recalcitrant toxicant such as complex hydrocarbon containing significant heavy metals [26].

The number of shoots produced as a function of time from this plant was sigmoidal in shape with an observable log phases from three to five days indicating an inhibitory effect of petroleum oil sludge on the growth of the plant. Based on visual observation, the number of shoots appeared to be inhibited as well (Fig. 1). The number of shoots produced over time was then fitted to eight different models (Table 1). The resultant fitting shows visually acceptable fitting (Figs. 2-9). Based on all of the statistical tests, such as the lowest values for RMSE and AICc, and the highest value for the adjusted R^2 , the modified Gompertz model was chosen to be the best. Further statistical indicators such as the Accuracy Factor (AF) and Bias Factor (BF) values with their values closest to 1.0 (Table 2) also indicated that the modified Gompertz model was the best model for modelling the number of shoots produced by *Cajanus cajan*.

When a Bias Factor is equal to 1, this means that there exists an ideal match between the observed and predicted values. The values for the Accuracy Factor are usually ≥ 1 , with values higher than 1.0 indicates a model having a less precise property. The poorest performance was Von Bertalanffy with the lowest score for most of the statistics tests (Table 2).

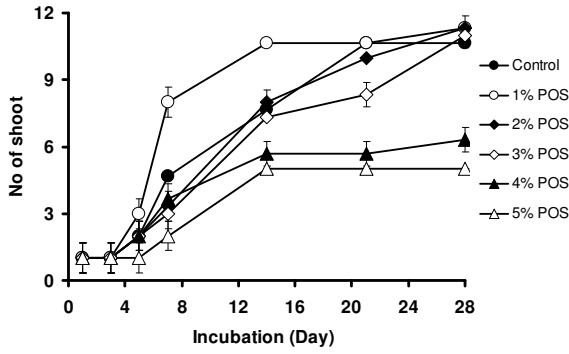


Fig. 1. The no of *Cajanus cajan* shoot produced as a function of time at various concentrations of petroleum oily sludge (POS). The error bars represent the mean \pm standard deviation of three replicates.

Table 1. Growth models used in this study.

Model	No of parameters	Equation
Modified Logistic	3	$y = \frac{A}{1 + \exp \left[\frac{4 \mu_m - (\lambda - t) + 2}{A} \right]}$
Modified Gompertz	3	$y = A \exp \left\{ - \exp \left[\frac{\mu_m e}{A} (\lambda - t) + 1 \right] \right\}$
Modified Richards	4	$y = A \left[1 + v \exp(1+v) \exp \left[\frac{\mu_m (1+v)}{A} \left(1 + \frac{1}{v} \right) (\lambda - t) \right] \right]^{\left(\frac{-1}{v} \right)}$
Modified Schnute	4	$y = \left(\mu_m \frac{(1-\beta)}{\alpha} \right) \left[\frac{1 - \beta \exp(\alpha \lambda + 1 - \beta - \alpha t)}{1 - \beta} \right]^{\frac{1}{\beta}}$
Baranyi-Roberts	4	$y = A + \mu_m x + \frac{1}{\mu_m} \ln \left(\frac{e^{-\mu_m x} + e^{-h_0}}{e^{-\mu_m x} - h_0} \right)$ $- \ln \left(1 + \frac{e^{\mu_m x} + \frac{1}{\mu_m} \ln \left(\frac{e^{-\mu_m x} + e^{-h_0}}{e^{-\mu_m x} - h_0} \right) - 1}{e^{(y_{max} - A)}} \right)$
Von Bertalanffy	3	$y = K \left[1 - \left[1 - \left(\frac{A}{K} \right)^3 \right] \exp \left(- \left(\mu_m x / 3K \right)^{\frac{1}{3}} \right) \right]^3$
Huang	4	$y = A + y_{max} - \ln \left(e^A + \left(e^{y_{max} - A} \right) e^{-\mu_m B(x)} \right)$ $B(x) = x + \frac{1}{\alpha} \ln \frac{1 + e^{-\alpha(x-\lambda)}}{1 + e^{-\alpha \lambda}}$
Buchanan Three-phase linear model	3	$Y = A, \text{ IF } X < \text{LAG}$ $Y = A + K(X-\lambda), \text{ IF } \lambda \leq X \leq X_{MAX}$ $Y = Y_{MAX}, \text{ IF } X \geq X_{MAX}$

Note:
 A= No of shoot lower asymptote;
 μ_m = maximum specific No of shootproduction rate;
 v= affects near which asymptote maximum no of shoot production.
 λ =lag time
 y_{max} = No of shoot upper asymptote;
 e = exponent (2.718281828)
 t = sampling time
 α, β, k = curve fitting parameters
 h_0 = a dimensionless parameter quantifying the initial physiological state of the reduction process. The lag time (h^{-1}) can be calculated as $h_0 = \mu_{max}$

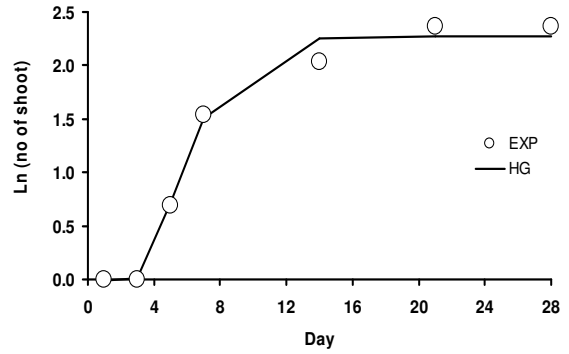


Fig. 2. The no of *Cajanus cajan* shoot produced as a function of time at 1% (w/w) petroleum oily sludge (POS) as modelled according to the Huang (HG) model.

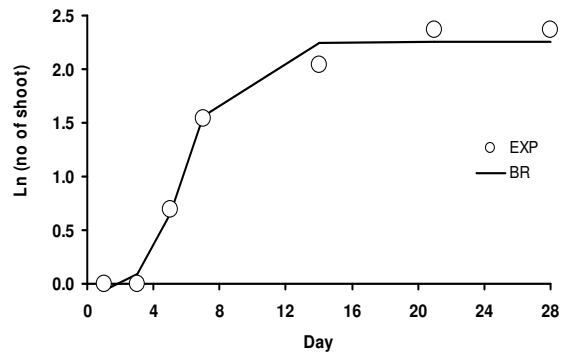


Fig. 3. The no of *Cajanus cajan* shoot produced as a function of time at 1% (w/w) petroleum oily sludge (POS) as modelled according to the Baranyi-Roberts (BR) model.

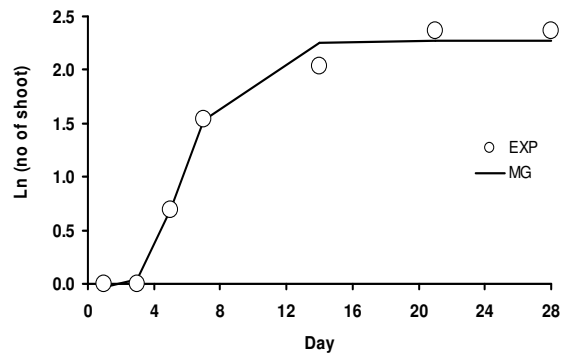


Fig. 4. The no of *Cajanus cajan* shoot produced as a function of time at 1% (w/w) petroleum oily sludge (POS) as modelled according to the modified Gompertz (MG) model.

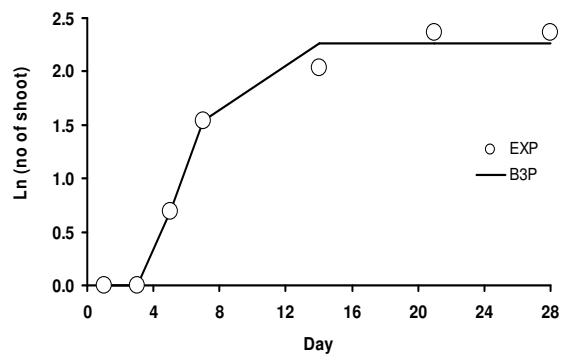


Fig. 5. The no of *Cajanus cajan* shoot produced as a function of time at 1% (w/w) petroleum oily sludge (POS) as modelled according to the Buchanan-3-phase (B3P) model.

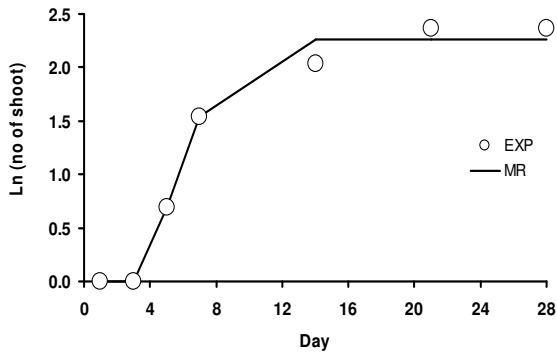


Fig. 6. The no of *Cajanus cajan* shoot produced as a function of time at 1% (w/w) petroleum oily sludge (POS) as modelled according to the modified Richards (MR) model.

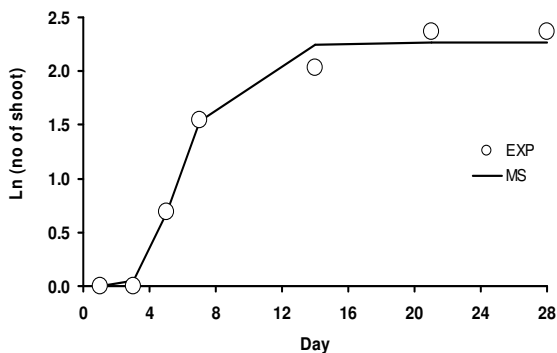


Fig. 7. The no of *Cajanus cajan* shoot produced as a function of time at 1% (w/w) petroleum oily sludge (POS) as modelled according to the modified Schnute (MS) model.

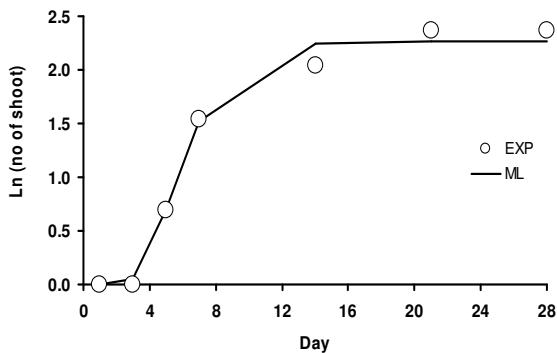


Fig. 8. The no of *Cajanus cajan* shoot produced as a function of time at 1% (w/w) petroleum oily sludge (POS) as modelled according to the modified Logistics (ML) model.

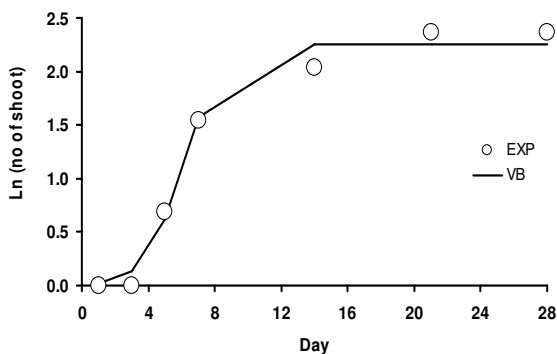


Fig. 9. The no of *Cajanus cajan* shoot produced as a function of time at 1% (w/w) petroleum oily sludge (POS) as modelled according to the Von Bertalanffy (VB) model.

Table 2: Statistical analysis of the various fitted models.

Model	p	RMSE	R^2	adR^2	AF	BF	AICc
Huang	4	0.15	0.99	0.97	1.03	1.00	45.32
Baranyi-Roberts	4	0.17	0.99	0.96	1.04	0.99	46.90
modified Gompertz	3	0.04	1.00	1.00	1.03	1.00	-15.32
Buchanan-3-phase	3	0.13	0.99	0.98	1.03	1.00	2.03
modified Richards	4	0.15	0.99	0.97	1.03	1.00	45.64
modified Schnute	3	0.06	1.00	1.00	1.03	1.00	31.54
modified Logistics	3	0.16	0.98	0.97	1.05	0.99	4.00
von Bertalanffy	4	0.14	0.99	0.97	1.05	1.00	2.74

Note:

- p no of parameters
- adR^2 Adjusted Coefficient of determination
- BF Bias factor
- AF Accuracy factor

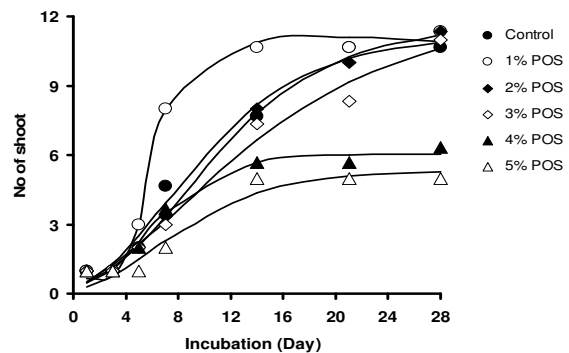


Fig. 10. Natural logarithm transformation of the no of *Cajanus cajan* shoot produced as a function of time at various concentrations of petroleum oily sludge (POS).

The coefficients for the modified Gompertz model for the number of shoots produced under various concentrations of petroleum oily sludge (POS) are shown in Table 3. It was observed that the lag period increased as the concentration of POS was increased from 1.335 d for the control to 5.2 d for the production of shoot of *C. cajan* in the presence of 5% (w/w) POS. In addition, the rate of shoot production also appears to be inhibited as the concentration of POS was increased from 0.717 d^{-1} in the control to 0.409 d^{-1} for the shoot production of *C. cajan* in the presence of 5% (w/w) POS (Table 3) indicating that POS caused an inhibition to the growth of *C. cajan*. The effect inhibitory effect of hydrocarbon on *C. cajan* has been reported before [26,38]. *C. cajan* seeds immersed in spent engine oil for several hours exhibit poor to almost no germination [39].

Table 3. Number of shoot production coefficients at various petroleum oily sludge (POS) concentrations as modelled using the modified Gompertz model.

	Control	1% POS	2% POS	3% POS	4% POS	5% POS
Lag (d)	1.335 ± 0.365	1.9 ± 0.314	2.411 ± 1.078	1.8 ± 0.46	2.294 ± 1.252	5.208 ± 1.122
Y_{max}	11.18 ± 0.854	10.9 ± 0.283	11.64 ± 0.501	12.04 ± 1.998	6.032 ± 0.29	5.056 ± 0.28
μ_{max}	0.717 ± 0.128	0.674 ± 0.049	0.698 ± 0.063	0.521 ± 0.089	0.544 ± 0.111	0.409 ± 0.12

CONCLUSION

The use of *Cajanus cajan* (Pigeon pea) or Kacang dhal in the phytoremediation of petroleum sludge in this study is novel. One of the challenges in monitoring the efficiency or inhibition of remediation by this toxic substance is measuring growth indicator. The number of shoots produced under growth on this toxic substance can be used to monitor the efficiency or inhibition and the use of mathematical models in describing the growth curve based on this parameter can give tangible results that can be used in further modelling exercise. In this study we demonstrated on the feasibility of using mathematical models in obtaining growth parameters based on the number of shoots produced under various concentrations of toxicants. The popular modified Gompertz growth model again prove itself as the best models in describing plant growth. The coefficients obtained in this study will be very valuable in secondary modelling exercise. This is the first time that mathematically modelling of the growth rate of *C. cajan* measured as the increase in number of shoots is carried out to the best of our knowledge.

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