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Modelling for Determination of Fe Uptake by *Brassica chinensis* Jusl var. *parachinensis* (Bailey) Tsen & Lee (Flowering White Cabbage)

(Pemodelan untuk Menentukan Penyerapan Fe oleh *Brassica chinensis* Jusl var. *parachinensis* (Bailey) Tsen & Lee (Kubis Bunga Putih))

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ABSTRACT

The study was conducted to determine the best model suitable for the determination of ferrum uptake in Brassica chinensis *(flowering white cabbage). A nonlinear regression model was selected to determine the amount of ferrum absorbed by each part of the* Brassica chinensis *plant namely the leaves, stems and roots. The Levenberg-Marquardt method was used to perform the nonlinear least square fit. This method employs information on the gradients and hence requires specification of the partial derivatives. A suitable model was obtained from the exponential regression model. The polynomial model was found to be appropriate for leaves, the mono-exponential model was suitable for stems and the simple exponential model for roots. The residual plots and the normal probability plots from each of the models indicated no substantial diagnostic problems, so it can be concluded that the polynomial and exponential regression models provide adequate fit to determine data on heavy metal uptake by the flowering white cabbage.*

Keywords: Brassica chinensis*; Fe*; *nonlinear regression; Levenberg-Marquardt method*

ABSTRAK

Kajian dijalankan untuk menentukan model yang sesuai dalam menentukan jumlah pengambilan Fe oleh Brassica chinensis *(sawi bunga). Model regresi tak linear dipilih untuk menentukan pengambilan logam berat oleh setiap bahagian sawi bunga iaitu daun, batang dan akar. Kaedah Levenberg-Marquardt digunakan untuk melakukan penyuaian melalui kaedah kuasa dua terkecil tak linear. Kaedah Levenberg-Marquardt menggunakan maklumat kecerunan dan memerlukan spesifikasi terbitan separa. Model yang sesuai diperoleh dengan menggunakan model regresi eksponen. Model polinomial sesuai untuk bahagian daun manakala model mono-eksponen sesuai untuk batang dan model eksponen ringkas untuk akar. Keputusan menunjukkan plot reja dan plot kebarangkalian normal daripada setiap model memenuhi andaian, maka dapat disimpulkan bahawa model regresi polinomial dan eksponen memadai bagi penentuan data pengambilan logam Fe oleh sawi bunga.*

Kata kunci: Brassica chinensi*; Fe*; *regresi tak linear; kaedah Levenberg-Marquardt*

INTRODUCTION

Trace elements such as ferrum (Fe), cuprum (Cu), Zn (Zn), Cobalt (Co), magnesium (Mg), manganese (Mn), boron (B) and natrium (Na) are known to be essential for the growth of plants (Berg 2008; Hopkins 1999). Fe, Cu, Zn and Mn are also heavy metals and important for agriculture and human health (Williams et al. 2000). Deficiency of these elements in the soil and plant may reduce agricultural productivity and affect human health (Alloway 2013).

Plants can accumulate trace elements, especially heavy metals, in or on their tissues due to their great ability to adapt to various chemical elements in the environment (Berg 2008). Thus plants are intermediate reservoirs through which trace elements from soil, water and air, move into the human body (Kabata-Pendias & Pendias 2001). Heavy metals can cause toxic effects to the plants and also to humans and animals that consume the plants if the concentration of the metals exceed the requirement of the plants. According to Berg (2008) and Hopkins (1999), Fe is one of the most important elements for plants nutrition and is required in large amounts. It plays a significant role in life-sustaining processes of the plant from respiration to photosynthesis, especially as the electron-transport chains (Kim & Guerinot 2007). Plants acquire Fe as ferric (Fe^{3+}) or ferrous (Fe^{2+}) ions although the Fe $^{2+}$ ion is more likely to be absorbed in much higher concentrations than the Fe^{3} ion (Roschzttardtz et al. 2013). Excess amounts of iron absorbed by the plant will be stored in the iron-storage protein, ferritin, in the roots of the plants (Connolly & Guerinot 2002; Hopkins 1999).

Several research studies have been carried out on Fe uptake by plants from various types of soil especially well drained and waterlogged soils. Nasser (2016) reported that Fe accumulated in high amounts in the roots of paddy plants at selected areas in MADA, Kedah, in amount ranging from 4990.0 to 20,584.89 mg/kg. Research by Gonnaskran et al. (2014) on paddy plant from IADA KETARA, Besut Terangganu showed that Fe accumulation was slightly lower than that from MADA averaging 2132.73 mg/kg. But paddy plants from Langkawi, Kedah indicated low Fe concentration in the roots between 0-32.59 mg/kg (Khairiah et al. 2013).

Studies were also conducted on Fe uptake by selected vegetables from Kelantan and Terengganu and it was shown that Fe accumulation in the roots of vegetables were very low compared to that in the root of the paddy plants which were in the range of 32.74-80.65 mg/kg (Khairiah et al. 2014). These findings show that Fe accumulation in plants is vary depending on the type of the soil and plants.

The variation among plants in their ability to absorb Fe is not consistent and is affected by the changing conditions of soil and stage of the plant growth. Soil conditions that influence the availability of heavy metals including Fe are pH, redox potential, organic matter, ion exchange capacity, clay content and Fe-Mn oxides in the soil (Helios Rybicka & Jędrzejezyk 1995; Rieuwerts et al. 1998). Tropical soils are known to contain high concentrations of Fe in the soil as Fe and Al oxide minerals (Habibah et al. 2014). For soils rich in the soluble Fe fractions, excessive Fe uptake can produce toxic effects on the plants. Symptoms of Fe toxicity are not specific and usually differ among plant species and the stages of their growth. The response of plants to Fe toxicity is highly variable among genotypes and plant species (Kabata-Pendias & Pendias 2001; Kim & Guerinot 2007; Morrissey & Guerinot 2009; Roschzttardtz et al. 2013).

The flowering white cabbage or 'choy sam' (*Brassica chinensis)* is an annual herb that is widely grown in Malaysia (Tindall 1986) and consumed daily as a vegetable. The leaves of the flowering white cabbage are either cooked and eaten as a vegetable or eaten raw in salads. The leafy cultivars have high nutritive value, whilst the heading forms have lower food value. The flowering white cabbage is tolerant to a wide range of soil conditions, including pH. This vegetable has the potential to absorb and accumulate high amounts of Fe thus posing a danger to humans as it is widely consumed daily. The aim of the present study was to determine the most appropriate model that can be used to estimate the amount of Fe uptake in different parts of the flowering white cabbage, namely the stems, leaves, and roots during the various growth stages until time of harvest.

Materials and Methods

A study on the flowering white cabbage (*Brassica chinensis* var. *parachinensis*) was carried out at Agrotek, Sepang, Selangor, located approximately 95 km from Kuala Lumpur, Malaysia. The Sepang soil in that area is mainly peat soil and the study area constituted one of the most important agricultural areas in Selangor. Three different plots were selected randomly and at each plot the vegetables (three replicates) were harvested once every three days for ten sampling days. The number of samples collected was 24.

The samples were taken to the laboratory, washed in running tap water followed by washing in three rounds of deionized-distilled water. The samples were swabbed with tissue before being separated into the root, stem and leaf. Samples were then oven dried at 70ºC until the weight was stable, then they were ground using a mortar and pestle (AOAC 1984).

Digestion was performed using HNO_3 and $HClO_4$ in the ratio (by volume) of 10:2, for three hours (AOAC 1984). The Fe concentration was determined by AAS (Perkin Elmer model 1100B). Data obtained was used to construct a model that can be used to determine the amount of Fe in the stems, roots and leaves. The detection limit for Fe and the recovery test are shown in Table 1. All glassware used for this experiment was acid soaked with 20% nitric acid for three days.

TABLE 1. Detection limit for Fe

Metal	Fe
Detection limit	5 µg/L
Recovery rate (%)	96.83%

Nonlinear Regression Model

The linear regression model provides a rich and flexible framework, however, it was not appropriate for all situations. There are many situations in science where the response variable and the predictor variables are related through a known nonlinear function. This would lead to a nonlinear regression model. Any model that is not linear for the unknown parameters is a nonlinear regression model.

In general, the nonlinear regression model can be written as $y = f(x, \boldsymbol{\theta}) + \varepsilon$ where $\boldsymbol{\theta}$ is a $p \times 1$ vector of unknown parameter and ε is an uncorrelated random error term with $E(\varepsilon) = 0$ and $Var(\varepsilon) = \sigma^2$. Also, another assumption is that the errors are normally distributed, as in linear regression. Since $E(y) = E[f(x, \boldsymbol{\theta}) + \varepsilon] = f(x, \boldsymbol{\theta})$, then $f(x, \boldsymbol{\theta})$ is called the expectation function for the nonlinear regression model (Myers et al. 2010). In a nonlinear regression model, at least one of the derivatives of the expectation function, with respect to the parameters, depends on at least one of the parameters. In a linear regression, these derivatives are not functions of the unknown parameters. Consider a linear regression model $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k + \varepsilon$ with the expectation function $f(x, \beta) = \beta_0 + \sum_{j=1}^{k} \beta_j x_j$. Then $\partial f(x, \beta) / \partial \beta_j = x_j$ for j=0, 1,..., k where $x_0 \equiv 1$. It should be noted that in the case of the linear model the derivatives are not functions of the β's (Piegorsch & Bailer 2005).

The nonlinear regression model should be used when a curvilinear relationship exists between the mean response and a predictor variable. Fortunately, many other functions can serve as viable regression models for environmental data. These functions cannot typically be transformed or linearized and are therefore truly nonlinear. In the present paper, the nonlinear regression model was fitted to the data from the flowering white cabbage leaves, stems and roots as the data showed a curvilinear relationship.

PARAMETER ESTIMATES

In nonlinear regression models, one must estimate the initial value of the parameter that should be used in the model. In the present study, the Levenberg-Marquardt fitting algorithm was used to estimate the initial value of the parameters. The Levenberg-Marquardt is a modified version of the Gauss-Newton method. Traditionally, the nonlinear least square is implemented via appeal to the Gauss Newton method. A method widely used in computer algorithms for nonlinear regression models is linearization of the nonlinear function followed by the Gauss-Newton iterative method of parameter estimation. Linearization is accomplished by the Taylor series expansion of $f(x_i, \boldsymbol{\theta})$. Suppose there is a sample of n observations on the response with the regressors, say y_i, x_{i1}, x_{i2}, …, x_{ik} for i=1,2,...,n. Consider the nonlinear regression model $y_i = f(x_i, \theta) + \varepsilon_i$ for i=1,2,...,n where $\underset{\sim}{x}_i = [1, x_{i1}, x_{i2}, \ldots, x_{ik}]$ for i=1,2,...,n. The least square function is $S(\boldsymbol{\theta}) = \sum_{i=1}^{n} [y_i - f(x_i, \boldsymbol{\theta})]^2$. To estimate the least-squares, it will be necessary to differentiate $S(\boldsymbol{\theta} = \sum_{i=1}^{n} [y_i - f(x_i, \boldsymbol{\theta})]^2$ with respect to each element of θ. This will provide a set of p normal equations for the nonlinear regression situation. The normal equations are $\sum_{i=1}^{n}[y_i - f(x_i, \theta][\partial f(x_i, \theta) / \partial\theta_j] = 0$ for j =1, 2,..., p. In a nonlinear regression model the derivatives in the large square brackets will be functions of the unknown parameters. Furthermore the expectation function will also be a nonlinear function (Piegorsch & Bailer 2005).

GOODNESS OF FIT

Goodness of fit is used to check the model adequacy. For this purpose, the residual plot, normal plot, partial F-test and p-value were constructed for the data of the flowering white cabbage.

RESIDUAL PLOT

Graphical analysis of the residual plot is a very effective way to investigate the adequacy of fit of a regression model. These plots are generated by the SAS package. For any particular observed value of y, the corresponding residual is $e = y - \hat{y}$ which is the observed value of y minus the predicted value of y, where the predicted value of y or $\hat{y}$ is calculated using the 'least squares' prediction equation. In the present case y refers to the Fe uptake in the flowering white cabbage leaves, stems or roots. If the regression assumptions hold, the residuals should appear like they have been randomly and independently selected from a normally distributed population and their means should be equal to zero and with constant variance.

NORMAL PLOT

Another way of checking the normality assumption is to construct a normal probability plot of the residuals. This is a graph designed so that the cumulative normal distribution will be plotted as a straight line. To make a normal plot, firstly the residuals must be arranged in the order of the smallest to the largest. The ordered residuals will be denoted as $e_{(1)}$, $e_{(2)}$, …, $e_{(n)}$ where the ith residual in the ordered listing will be $e_{(i)}$. Then $e_{(i)}$ is plotted on the vertical axis against the normal percentiles on the horizontal axis. If the normality assumption holds, the normal probability plot should have the appearance of a straight line. A normal plot of the residual that does not appear as a straight line, indicates that the normality assumption is violated (Kutner et al. 2004; Montgomery et al. 2001).

PARTIAL F-TEST

A partial F-test allows the test of significance of a set of independent variables in a regression model. That is, the F-test can be used to test the significance of a portion of a regression model. Suppose the regression assumption holds and consider testing $H_0 : \beta_{j+1} = \beta_{j+2} = \ldots = \beta_{j+k} = 0$ versus H_1: at least one of $\beta_{j+1}, \beta_{j+2}, \ldots, \beta_{j+k}$ will not be equal to 0. The partial F-statistics is $F_{calc} = [\{SSE(RM) - SSE(FM)\}/\Delta_e]/[SSE(FM)/df_e(FM)]$ where $SSE(RM)$ refers to the error value of the sum of squares for the reduced model, and $SSE(FM)$ refers to the value of the error of the sum of squares for the full model. is the number of parameters constrained by H_0 and $df_e(RM)$ the degrees of freedom for the full model. Meanwhile $df_e(RM)$ refers to the degrees of freedom for the reduced model. H_0 will be rejected in favor of an alternative at the level of significance $\alpha = 0.05$ if F_{calc} is higher than $F^{\alpha}_{k,df_e(FM)}$.

THE p-VALUE

The p-value, or the probability value, is defined as the probability under H_0 of observing a test statistic as extreme as or more extreme than that actually tested. The p-value is related to the F_{calc} for the partial F-test as the area under the curve of the F_{calc} distribution to the right of F_{calc}. Then, H_0 can be rejected in favor of H_1 at the level of significance α if the p-value is less than α. Normally α is set to be equal to 0.05.

FITTING THE MODEL

LINEAR REGRESSION MODEL

As a starting point, it is necessary to develop a model relating to the amount of Fe uptake in the flowering white cabbage (y) to the days of growth (x), therefore a simple linear regression model is assumed. The least square fit is $\hat{y} = \hat{\beta}_0 + \hat{\beta}_1 x$ where $\hat{\beta}_0$ refers to the mean value of Fe uptake when the days of growth equal zero and $\hat{\beta}_1$ is the change in the mean value of Fe uptake associated with one-unit increase in days of growth. To visualize the data and begin the process of assessing the model fit, a scatterplot is constructed. From the scatterplot of y against x for each of the parts of the flowering white cabbage it is apparent from Figure 1, Figures 2 and 3 that a clear curvilinear relationship is indicated.

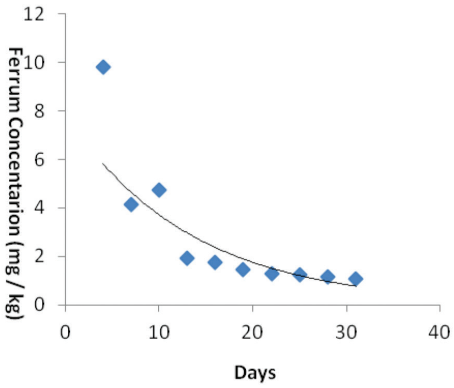

FIGURE 1. Scatterplot for Fe uptake in leaves of flowering white cabbage

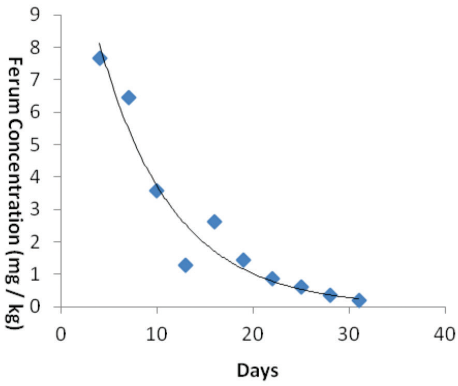

FIGURE 2. Scatterplot of Fe uptake in stem of flowering white cabbage

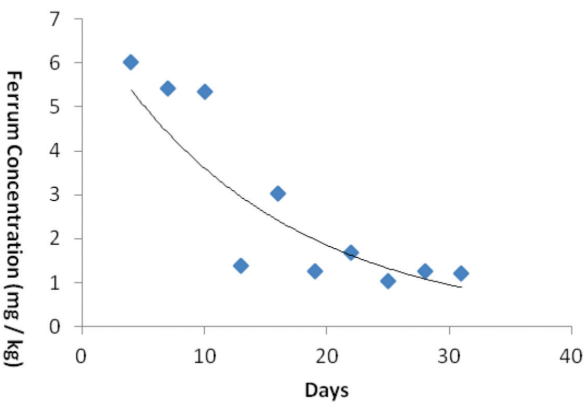

FIGURE 3. Scatterplot of Fe uptake in root of flowering white cabbage

It may be possible to match the observed behavior of the plot to one of the curves and use the linearized function to represent the data. However, initially the linear regression model will be fitted to the data. A summary of the SAS output of the linear regression model for the flowering white cabbage leaves, stems and roots are shown in Table 2.

TABLE 2. The parameter estimates and R^2 for linear regression models for Fe uptake in flowering white cabbage leaves, stems and roots

Parameter estimates and R^2	Leaves	Stems	Roots
Intercept	7.081	7.008	6.117
Days of growth	-0.242	-0.257	-0.191
R^2	0.628	0.788	0.729

Hence, the regression model for each of the parts of the flowering white cabbage is:

Model for leaves, $\hat{y} = 7.081 - 0.242x$

Model for stems, $\hat{y} = 7.008 - 0.257x$

Model for roots, $\hat{y} = 6.117 - 0.191x$

where $\hat{y}$ is the fitted amount of Fe uptake in the flowering white cabbage and x is the number of days of growth. The F-test was used to test the significance of the linear regression model. The p-values for the model F-statistics of the flowering white cabbage leaves, stems and roots were 0.0063, 0.0006 and 0.0017, respectively. As all of these p-values for the model F-statistics of the flowering white cabbage were less than 0.05, there is strong evidence that the linear regression model relating to Fe uptake in the three parts of the flowering mustard plant to the days of growth were significant.

Another useful measure is the value of R^2, the simple coefficient of determination. R^2 is a measure of the variability in amount of Fe uptake in the flowering white cabbage (y) that can be explained by the days of growth (x) of a linear regression model. It can take a value between 0 and 1. The nearer the value of R^2 is to 1, the larger the proportion of the total variation explained by the model, the greater the utility of the model in predicting y. From Table 2, the values of R^2 for the linear regression model of the flowering white cabbage leaves, stems and roots were 0.628, 0.788 and 0.729, respectively. As all of these values were not very close to 1, the linear regression model is not the best model for explaining the proportion of the total variation in the data of the flowering white cabbage.

The plot of residual versus the predicted values ($\hat{y}$) for each part of the flowering white cabbage plant are shown in Figures 4, 5 and 6. The residuals plots showed a distinct pattern, that is, they moved systematically from positive to negative and back to positive again as the days of growth increased. This indicated model inadequacy and

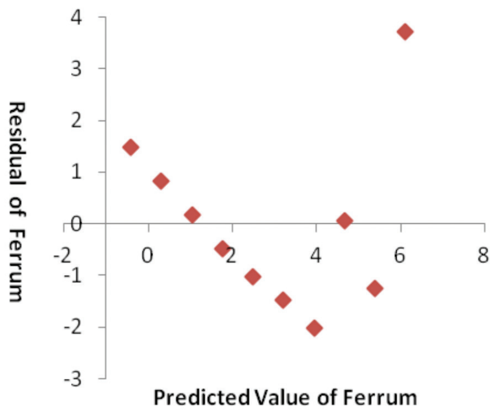

FIGURE 4. Residual plot after fitting linear regression model to Fe uptake in white cabbage leaves

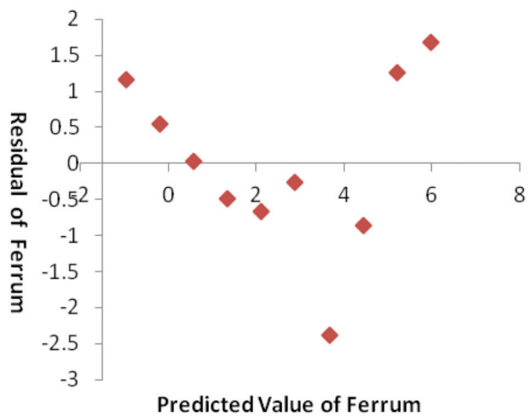

FIGURE 5. Residual plot after fitting linear regression model to Fe uptake in white cabbage stems

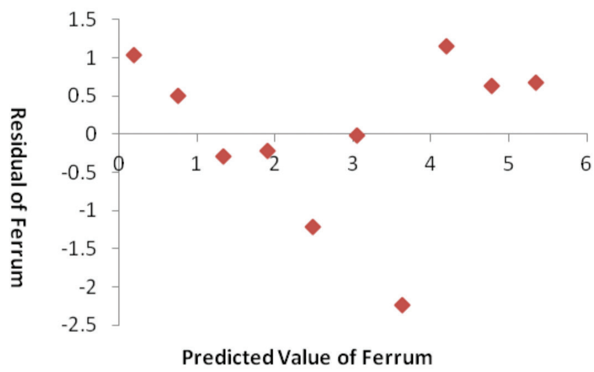

FIGURE 6. Residual plot after fitting linear regression model to Fe uptake in white cabbage roots

implied that the linear relationship had not captured all the information in the 'days of the growth' variable. It should be noted that there was apparent curvature in the scatterplot in Figures 1, 2 and 3. It is apparent that some other model form should be considered. Therefore a nonlinear model needed to be considered for use in the analyses.

NONLINEAR REGRESSION MODEL

Leaves Polynomial models are widely used in situations where the response is curvilinear, as even complex nonlinear relationships can be adequately modeled by polynomials over a reasonably small range of the $x_i's$. This model with an extension of the simple linear relationship is the addition of the higher order polynomial terms. A general form of the p-th order polynomial regression model is $Y_i = \beta_0 = \beta_1(x - \bar{x}) + \beta_2(x - \bar{x})^2 + \ldots + \beta_p(x - \bar{x})^p + \varepsilon_i$ where $i = 1, 2, \ldots, n$.

With the usual homogeneous-variance, the normal-error assumption; that is ε_i is independent, identically distributed as $N(0, \sigma^2)$ for $i = 1, 2, \ldots, n$. By assuming $p < n - 1$, there should be at least $p + 1$ distinguishable values among the $x_i's$. This model is used to represent a simple curvilinear relationship between $E[Y_i]$ and x_i (Ahmad Mahir et al. 2007). Parameter estimates and statistical inferences for this model would follow the normal linear regression procedures. A scatterplot of the flowering white cabbage leaves is shown in Figure 1. This display and knowledge of the production process suggest that a quadratic model may adequately describe the relationship between the amount of Fe uptake in the leaves $g(x; \beta)$ to the days of growth (x). Thus, the polynomial model shows best fit to the data as shown in Table 3. The model of Fe uptake in the leaves is as follows:

$$g(x; \beta) = 1.368 - 0.242x + 0.020x^2 \tag{1}$$

From the model, $\hat{\beta}_1 = -0.242$ is the estimate of the linear effect parameter and $\hat{\beta}_2 = 0.020$ is the estimate of the quadratic effect parameter. The $\hat{\beta}_0 = 1.368$ is the estimate of the mean of $g(x; \beta)$ when $x = 0$ if the range of the data includes $x = 0$. Otherwise this parameter has no adequate interpretation.

STEMS

One possible model to account for the curvilinear effect a mono-exponential decay model for the stem of the flowering white cabbage, that is

$$g(x; \beta) = \beta_0 + \beta_1 e^{-\beta_2 x} \tag{2}$$

From model (2), x refers to days of growth, $g(x; \beta)$ is the Fe uptake, dependent on days, β_0 the maximum Fe uptake and β_1 the difference between β_0 and the value of Fe uptake when days of growth equal 0 and β_2 is a scaling term. Here the Levenberg-Marquardt iterative fitting algorithm was used. For the partial derivatives, $\partial g / \partial \beta_0 = 1$, $\partial g / \partial \beta_1 = e^{-\beta_1 x} = e^{-\beta_2 x}$ and $\partial g / \partial \beta_2 = -x\beta_1 e^{-\beta_2 x}$.

The initial values to start with in the Levenberg-Marquardt iterative procedure, included (2). If $Y_i = g(x; \beta) = \beta_0 + \beta_1 e^{-\beta_2 x_i}$, then $E[Y_i] = \beta_0 + \beta_1 e^{-\beta_2 x}$. As $x \to \infty$ then $E[Y_i] \to \beta_0$ if $\beta_0 > 0$. Hence the initial value of β_0 or β_{00} can be set equal to the observed response at the highest value of x. In the case stated, it will be the lowest of the Y_i values or $Y_1 = \min_{i \in \{1,2,\ldots,n\}} \{Y_i\}$. Therefore, selection of $\beta_{00} = Y_1 =$

TABLE 3. The fitted model, F_{calc} and p-values for the data of flowering white cabbage leaves, stems and roots

	Leaves	Stems	Roots
Model	polynomial	Mono-exponential	simple exponential
Fitted	$g(x, \beta) = 1.368 - 0.242x + 0.020x^2$	$g(x, \beta) = 0.022 + 13.114e^{0.123x}$	$g(x; \beta) = 8.770e^{-0.079x}$
F_{calc}	24.52	57.51	39.10
p-value	<0.0007	<0.0001	<0.0001

0.193 was carried out since it was the lowest value of the response variable. As for the initial values of β_1 and β_2; since, the, by taking the natural logarithm on both sides, $ln(Y_i - \beta_0) = ln\beta_1 e^{-\beta_2 x_i})$ therefore $ln(Y_i - \beta_0) = ln(\beta 1) - \beta_2 x_i$.

If $U_i = ln(Yi - \beta_0)$ and $z_i = -x_i$, by regressing $U_i = ln(Y_i - \beta_{00})$ on z_i the intercept for this regression line will be $\beta_{10} = e^{\ln \beta_2}$ and the estimated slope $\beta_{20} = \beta_2$. From the above results, the estimated $\beta_{10} = e2.73314$ and $\beta_{20} = 0.14945$ can be obtained. Then the nonlinear model will be fitted with the initial value; $\beta_{00} = 0.193, \beta_{10} = 15.38111, \beta_{20} = 0.14945$. After the initial value of the parameters are estimated, the next step will be to model the data of Fe uptake in the flowering white cabbage stems using the mono-exponential model. The results showed that the value of β_0, β_1 and β_2 converged to -0.022, 13.114 and 0.123 respectively after four iterations. Thus from Table 3, the fitted model for the flowering white cabbage stem is $g(x; \beta) = -0.022 + 13.114e^{0.123x}$.

ROOTS

Scatter plots of Fe uptake in the flowering white cabbage roots indicate a curvilinear relationship between Fe uptake and days. Hence, the simple exponential model is the best model to fit this data is the simple exponential model :

$$g(x; \beta) = \beta_0 e^{-\beta_1 x} \quad (3)$$

From model (3), the parameter β_0 was the initial value of Fe uptake when days of growth were 0 and the parameter β_1 was the rate of exponential decay. For this purpose, the Levenberg-Marquardt iterative fitting algorithm was used for partial derivatives of the model (3). The two derivatives were $\partial g / \partial\beta_0 = e^{-\beta_1 x}$ and $\partial g / \partial\beta_1 = \beta_0 x e^{-\beta_1 x}$. The pseudo-variable can be created by taking the logarithm $ln\ g(x; \beta) = ln\ \beta_0 - \beta_1 x$ which will be obtained. This fact can be used to regress the pseudo-variable.

From the results, the value for parameter estimates of the intercept and slope were 1.95218 and 0.06686, respectively. Therefore, the initial estimates were $\beta_{00} = 7.04403$ and $\beta_{10} = 0.06686$. After the initial value of the parameters were estimated, the next step was to model the data of Fe uptake in the flowering white cabbage roots using the simple exponential model. The results showed that the value of β_0 and β_1 converge to 8.7703 and 0.0789, respectively after three iterations. Thus from Table 3, the fitted model for the flowering white cabbage roots is $g(x; \beta) = 8.7703^{-0.0789x}$.

RESULTS AND DISCUSSION

LEAVES

The residual and normal probability plots for the polynomial regression model are given in Figures 7 and 8. The residual plot did not show any serious model inadequacy. The normal probability plot of the residuals indicated that the error distribution was approximately normal. The observed p-value < 0.0007, indicated that the polynomial model was significant. Investigation on the contribution of the parameters to the model is needed. That is, $H_0 : \beta_1 = \beta_2 = 0$, needs to be tested. From the SAS output, $SSE(RM) = 69.07930$, $SSE(FM) = 8.62822$, $df_e(FM) = 7$, $\Delta e = 2$. By using the partial F-test, the value of $F_{calc} = 24.52$ will be obtained and this value can be compared to $F^{0.05}_{(2,7)} = 4.737$=. As F_{calc} clearly exceeded the critical point, it can be concluded that both the linear and the quadratic terms contributed significantly to the model. The other summary statistics for this model were R^2=0.8751. As the value of R^2 was close to 1, the model can be used to explain a large proportion of the total variation and will have great use in predicting Fe uptake in the flowering white cabbage leaves. Therefore the fitted model for Fe uptake in the leaves of the flowering white cabbage is $g(x; \beta) = 1.368 - 0.242x + 0.020x^2$.

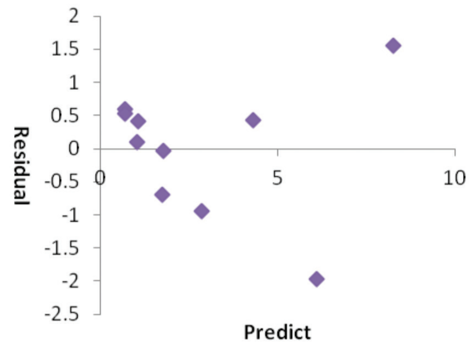

FIGURE 7. Residual plot after fitting polynomial model to Fe uptake in white cabbage leaves

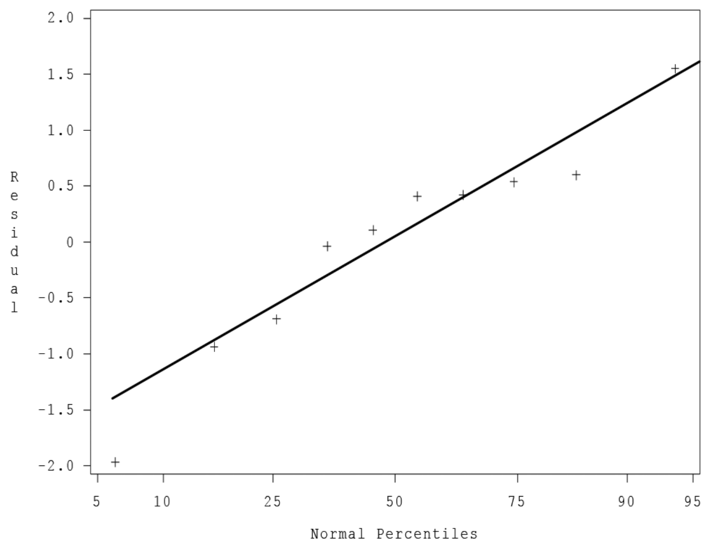

FIGURE 8. Normal probability plot of residuals after fitting polynomial model to Fe uptake in white cabbage leaves

STEMS

The result for the stems of the flowering white cabbage indicated rapid convergence with p-value < 0.0001 indicating that the model was very significant. To test H_0 : $\beta_1 = \beta_2 = 0$, it was necessary to appeal to the discrepancy measure approach and fit a simple exponential model under H_0. The values of $SSE(RM) = 62.39362$ on 9 degree of freedom with $SSE(FM) = 3.5795$ on 7 degree of freedom were obtained. The F-statistics were then calculated and compared to $F^{0.05}_{(2,7)} = 4.737$. Since $F_{calc} = 57.5079$ and this value clearly exceeded the critical point, it can be concluded that the parameters differed from zero. This was confirmed by the 95% confidence interval for β_1 and β_2 where $8.751 < \beta_1 < 17.4758$ and $0.0460 < \beta_2 < 0.2005$ did not contain zero. For model adequacy assessment, Figure 9 gives a plot of the residual from the mono-exponential fit. It can be seen that generally there was random spread. The plot did not reveal any serious problem with inequality variance. The normal probability plot of the residual plot in Figure 10 showed that there was no indication of serious model inadequacies. Therefore the fitted model for Fe uptake in the stems of the flowering white cabbage is $g(x; \beta) = -0.022 + 13.114e^{0.123x}$.

ROOTS

The p-value for the simple exponential model was less than 0.0001, which means that the data exhibited significant Fe uptake in the roots of the flowering white cabbage. The parameters β_0 and β_1 were significant as the 95% confident interval, $5.5653 < \beta_0 < 11.9744$ and $0.0436 < \beta1 < 0.1142$ did not include zero. To test for the model adequacy, the hypothesis testing used was $H_0 : \beta_1 = \beta_2 = 0$ versus $H_1 : \beta_1 \neq 0$. From the results, the value of $SSE(RM) = 37.30851$,

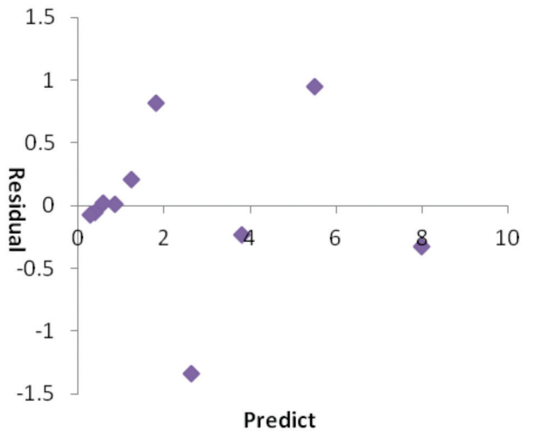

FIGURE 9. Residual plot after fitting mono-exponential model to Fe uptake in white cabbage stems

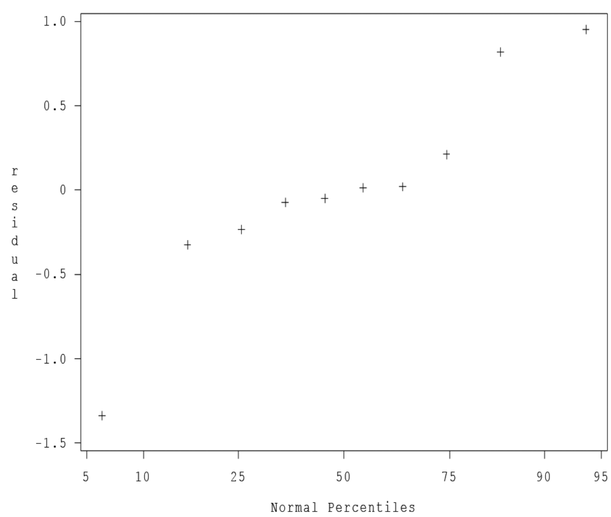

FIGURE 10. Normal probability plot monoexponential model for data on Fe in stems

$SSE(FM) = 6.3375$, $df_e(FM) = 8$, $\Delta_e = 1$. Appealing this value to the test statistical equation gave $F_{calc} = 39.095$. By comparing this value to $F^{0.05}_{(1,8)} = 5.138$, F_{calc} was higher than $F^{0.05}_{(1,8)}$, so β_1 in the simple exponential model was significant. The residual plot in Figure 11 appeared generally reasonable, although there was a slight hint that it increased and decreased after one point. It is reasonable to presume that the constant variance assumption for the model was not violated. The normal plot in Figure 12 of this model indicated that the residual spread was almost a straight line. Thus the normality assumption for the residual holds. It is clear that the simple exponential model is suitable to model the data for the flowering white cabbage roots and the fitted model is $g(x; \beta) = 8.770e^{-0.0788x}$.

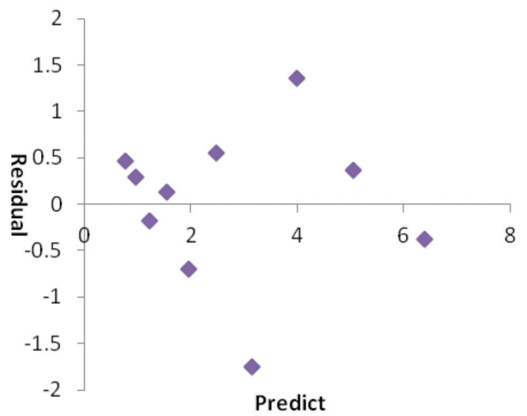

FIGURE 11. Residual plot from fit of simple exponential the residual model for data on Fe uptake in roots

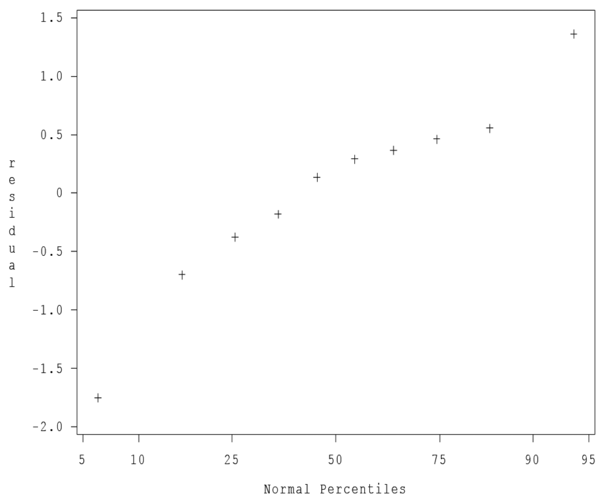

FIGURE 12. Normal probability plot of simple exponential model for data on Fe uptake in roots

CONCLUSION

This study showed that models used fulfill the normality and constant variance assumption while the goodness of fit test indicate that all the models are significant in indicating the plant activity Therefore the polynomial, mono-exponential and simple exponential regression models can be used to model the data of the flowering white cabbage leaves, stems and roots, respectively.

Analyses through the scatterplot of all the models for leaves, stems and roots of the flowering white cabbage showed that Fe uptake was high during the first ten days. After that it decreased gradually until the 30th day. It can be concluded that Fe uptake is high in the leaves, stems and roots of the flowering white cabbage for the first ten days from sowing. It is therefore best to harvest the flowering white cabbage after the 10th day.

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REFERENCES

Ahmad Mahir Razali, Wan Rozita Wan Din & Khairiah Jusoh. 2007. Fitting of nonlinear regression model to heavy metal uptake by Spinach leaves. *Proceedings of the 3rd IMT-GT Regional Conference on Mathematics, Statistics and Applications*. Universiti Sains Malaysia.

Alloway, B.J. 2013. Introduction. In *Heavy Metals in Soils*. 3rd ed., edited by Alloway, B.J. New York: Dordrecht Heidelberg: Springer.

AOAC. 1984. *Official Method of Analysis*. 14th ed. Virgina: Sidney Williams, Association of Official Chemist, Inc.

Berg, L. 2008. *Introductory Botany: Plants, People, and the Environment*. Australia: Thomson.

Connolly, E.L. & Guerinot, M.L. 2002. Iron stress in plants. *Genome Biology* 3(8): 1024.1-1024.4.

Goonasakran, R.R., Khairiah, J., Ismail, B.S., Mohamed Kaberi Ghani & Shafea Ab. Wahab. 2014. The bioaccumulation of heavy metals in paddy plant. *The International Journal of Environmental Sustainability* 93: 11-14.

Habibah, J., Khairiah, J., Ismail, B.S. & Kadderi, M.D. 2014. Iron speciation in selected agricultural soils of Peninsular Malaysia. *Journal of Environmental Science and Technology* 7(3): 154-165.

Helios Rybicka, E. & Jędrzejczyk, B. 1995. *Applied Clay Science* 10: 259-268.

Hopkins, W.G. 1999. *Introduction to Plant Physiology*. 2nd ed. New York: John Wiley.

Kabata-Pendias, A. & Pendias, H. 2001. *Trace Elements in Soils and Plants*. 3rd ed. Boca Raton: CRC Press.

Kim, S.A. & Guerinot, M.L. 2007. Mining iron: Iron uptake and transport in plants. *Federation of the European Biochemical Societies* 581: 2273-2280.

Khairiah, J., Buthina S. Saad, Habibah, J., Naseer Salem, Alias Bin Semail & Ismail, B.S. 2014. Heavy metals content in soils and vegetables grown in an inland valley of Terengganu and a river Delta of Kelantan, Malaysia. *Research Journal of Environmental and Earth Sciences* 6(6): 307-312.

Khairiah, J., Azie Rahimah Ramlee, Habibah, J., Zulkifle Ismail & Ismail, B.S. 2013. Heavy metal content of paddy plant in Langkawi, Kedah, Malaysia. *Australian Journal of Basic and Applied Sciences* 7(2): 123-127.

Kutner, M.H., Nachsheim, C.J. & Neter, J. 2004. *Applied Linear Regression Models*. 4th ed. New York: McGraw-Hill/Irwin.

Montgomery, D.C., Peck, E.A. & Vining, G.G. 2001. *Introduction to Linear Regression Analysis*. 3rd ed. New York: John Wiley & Sons.

Morrissey, J. & Guerinot, M.L. 2009. Iron uptake and transport in plants: The good, the bad and the inonome. *Chemical Review* 109(10): 4553-4567.

Myers, R.H., Montgomery, D.C., Vinning, G.G. & Robinson, T.J. 2010. *Generalized Linear Models: With Applications in Engineering and the Sciences*. 2nd ed. Hoboken, New Jersey: John Wiley & Sons.

Piegorsch, W.W. & Bailer, A.J. 2005. *Analyzing Environmental Data*. Chichester: John Wiley and Sons.

Rieuwerts, J.S., Thornton, I., Farago, M.E. & Ashmore, M.R. 1988. Factors influencing metal bioavailability in soils: Preliminary investigations for the development of a critical loads approach for metals. *Chemical Speciation & Bioavailability* 10(2): 61-75.

Roschzttardz, H., Coéjéro, G., Divol, F., Alcon, C., Verdeil, J-L., Curie, C. & Mari, S. 2013. New insights into Fe localization in plant tissues. *Plant Science* 4: 1-11.

Tindall, H.D. 1986. *Vegetables in the Tropics*. Basingstoke: Macmillan Press.

Williams, L.E., Pittman, J.K. & Hall, J.L. 2000. Emerging mechanisms for heavy metals transport in plants. *Biochimia et Biophysica Acta* 1465: 104-126.

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