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# Daily groundwater level fluctuation forecasting using soft computing technique

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**ABSTRACT:** The study presented here deals with forecasting daily groundwater level fluctuation (GLF) for monitoring of GLF pattern. The calculation model is based on the adaptive neuro-fuzzy inference system (ANFIS) and two algorithms of artificial neural networks (ANN) models, namely Levenberg-Marquardt (LM) and radial basis function (RBF). The objective in this study is to predict daily GLF for monitoring purposes. The first step was to investigate the effect of the number time lags as inputs for one-day-ahead prediction using the ANFIS algorithm. It was found that three input nodes containing three time-lag of well studied gave good prediction results. The second experiment was to predict the GLF one to seven steps ahead using the three input nodes. In this experiment, the three soft computing techniques were applied. The results indicate that the performances were decreasing by increasing the time step ahead, and in general there was no significant difference between the three techniques used. The best accuracy was for one-day-ahead prediction. The results obtained in this study suggest that GLF monitoring can be conducted by a forecasting model with considering time-lag as inputs. [Nature and Science. 2007;5(2):1-10].

**Keywords:** groundwater level fluctuation, forecasting, soft computing, artificial neural network, Levenberg-Marquardt, radial basis function, adaptive neuro-fuzzy inference system.

## INTRODUCTION

Groundwater is a highly valuable resource. Groundwater has agricultural, domestic, and industrial uses and is acquired by constructing and operating extraction wells. Measurement and analysis of groundwater level is needed for maintaining groundwater availability. Groundwater level modeling is important for environmental protection: maintaining the groundwater equilibrium system, controlling groundwater level fluctuation, and protecting against severe land subsidence. Groundwater management approaches based on a variety of simulation and prediction techniques and control measures have been proposed and adopted by researchers and relevant authorities to address the problem of providing long-term countermeasures against land subsidence and protection of groundwater resources. Recently, groundwater level fluctuation (GLF) analysis by means of forecasting or prediction has increased.

A common nonlinear method for groundwater problems is the artificial neural network (ANN). Many kinds of algorithms for training the network have been developed for GLF forecasting. A significant advantage of the ANN approach in system modeling is that one need not have a well-defined physical relationship for systematically converting an input to an output (Nayak et al., 2004). There have been various papers considering the application of ANN techniques in water resource problems. In surface hydrology, ANN applications have been used for runoff analysis (Gautam et al., 2000; Zhang & Govindaraju, 2003; Parida et al., 2006), rainfall forecasting (Luk et al., 2000; Toth et al., 2000; Trafalis et al., 2002; Ramirez et al., 2005), rainfall-runoff model (Sajikumar & Thandaveswar, 1999; Chiang et al., 2004; Lin & Chen, 2004; Rajurkar et al., 2004; Chen & Adam, 2006), and stream flow forecasting (Wang et al., 2006). In the groundwater domain, ANN has been used for groundwater reclamation (Ranjithan et al., 1993) and groundwater management (Coppola et al., 2003; Rao et al., 2003; Zaheer & Bai, 2003). Several papers have reported the use of ANN for groundwater level forecasting (Coulibaly et al., 2001; Daliakopoulos et al., 2005; Lallahem et al., 2005; Nayak et al., 2006). Gautam et al. (2004) reported that the groundwater table change before and after a bridge pier construction could be well analyzed by ANN. Many previous researchers have pointed out that ANN as non-linear model is a powerful tool to estimate a fluctuation of groundwater level with considering hydrological variables as inputs. A detailed theory and application of ANN in hydrology can be found in Govindaraju (2000a, b) and in Maier & Dandy (2000).

The application of a more promising soft computing technique, the fuzzy inference system (FIS), has recently been increasing in hydrology. Lu and Lo (2002) used self-organizing maps (SOM) and fuzzy theory for diagnosing reservoir water quality. Tayfur et al. (2003) developed fuzzy logic algorithms for

estimating sediment loads from bare soil surface. Wong et al. (2003) predicted volume of rainfall using SOM, BPNN (Backpropagation neural networks), and fuzzy rule systems. Alvisi et al. (2006) predicted water level using fuzzy logic and ANN.

The combination of ANN and FIS into the adaptive neuro-fuzzy inference system (ANFIS) has advantages in a computational framework. The learning capability of ANN can be used effectively for automatic fuzzy if-then rule generation and parameter optimization (Nayak et al., 2004). Several researchers have used ANFIS in hydrology. Ponnambalam et al. (2003) used ANFIS for minimizing variance of reservoir systems operation. Nayak et al. (2004) applied it to hydrologic time series modeling. Kisi (2005) investigated the ability of ANFIS and ANN to model the relationship between streamflow and suspended sediment. Chang and Chang (2006) used it to construct a water level forecasting system for flood periods. Tutmez et al. (2006) developed an ANFIS model for groundwater electrical conductivity, based on the concentration of positively charged ions in water.

Most of the previous researches analyzed monthly GLF. However, in this paper we propose to predict daily GLF. The daily GLF time series from two observation wells in Saitama City, Japan were used as a case study. The groundwater in the study is abstracted from a multilayer aquifer and the aquifer layer system cannot be clearly defined. The main objective of this paper is to develop a reliable groundwater level fluctuation forecasting system to generate trend forecasts. The forecasts, based on ANFIS and ANN techniques, are then compared to actual measurements recorded during a subsequent monitoring period. The ANN models were developed based on backpropagation with Levenberg-Marquardt algorithms (LM) and radial basis function (RBF). The daily GLF of the past two years and two months was used as dataset.

## MATERIAL AND METHODS

### 1. Artificial neural network

An artificial neural network (ANN) is different from a conventional system such as an analytical or statistical model. ANN is a network consisting of an arbitrary number of very simple elements called nodes. Each node is a simple processing element that responds to the weighted inputs it receives from other nodes (Lee et al., 2004). The arrangement of the nodes is referred to as the network architecture. There have been several types of ANN architecture and algorithm used successfully in groundwater level forecasting (Coulibaly, 2001; Daliakopoulos, 2005). The feed-forward neural network with Levenberg-Marquardt algorithm (LM) and radial basis function (RBF) will be presented. They are more reliable and faster to convergence.

#### *Feed-forward neural network*

A common type of feed-forward neural network (FFNN) consists of three layers: an input layer is connected to a hidden layer, which is connected to an output layer (Figure 1).

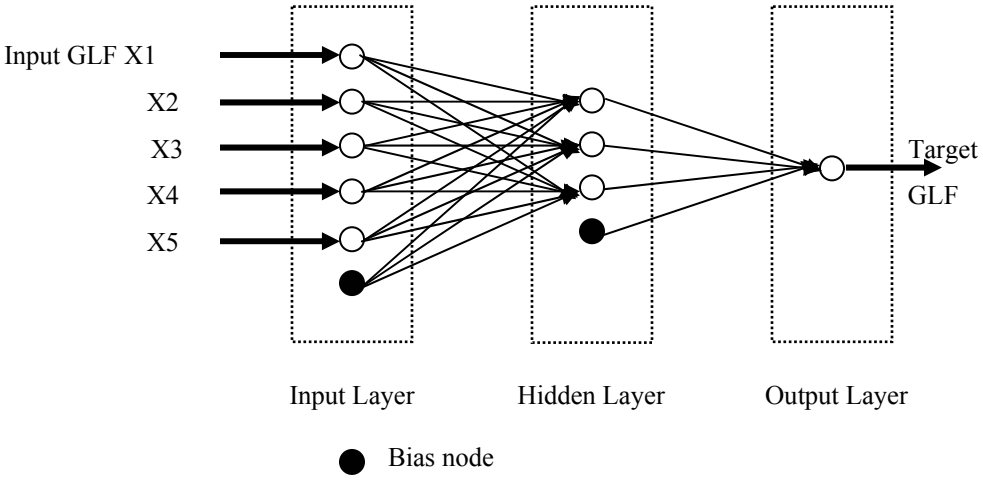


Figure 1. Topology of three-layer feed forward Artificial Neural Network

During this operation, each node *j* receives incoming signals from every node *i* in the previous layer. Each incoming signal ($y_i$) associates with a weight ($w_{ji}$). The net input, $x_j$, to node *j* is a sum of the incoming signal times the weight, as described in equation 1.

$$x_j = \sum_i y_i w_{ji} \qquad (1)$$

Note that this includes an extra node, called a *bias node*, which is assumed to have a value of 1 at all times. The weight on this extra node represents the bias as a threshold value.

The output signal $f(x_j)$ , which is a non-linear function, is produced by a transfer function of its summed input. The most commonly used transfer or activation function is the logistic sigmoid and hyperbolic tangent functions. In this study, the logistic sigmoid (eq. 2) transfer function is used as activation function between the input layer and hidden layer, and between the hidden layer and the output layer.

$$f(x_j) = \frac{1}{1+e^{x_j}} \qquad (2)$$

The nonlinear nature of this logistic transfer function plays an important role in the performance of the ANN. Other functions can be used as long as they are continuous and possess a derivative at all points.

The backward pass is concerned with error computation and weight update. The algorithm normally used in this operation is a backpropagation algorithm. Backpropagation neural networks (BPNN) were introduced by Rumelhart et al. (1986), and a good description of the BPNN in groundwater problems can be found in Ranjithan et al. (1993) and Govindaraju (2000a, 2000b), among others. In a backpropagation algorithm, the difference between the calculated output of the output layer and the desired output is backpropagated to the previous layer and the weights are adjusted. In this study, the weight and bias values update according to the Levenberg-Marquardt (LM) algorithm. This process continuously proceeds until the criterion achieved. The LM algorithm is widely applied to many different domains. It works extremely well in practice and is considered the most efficient algorithm. Like Quasi-Newton methods, the LM algorithm was designed to approach second-order training speed without having to compute Hessian matrix. To update weights and biases, the LM algorithm uses an approximation of the Hessian matrix.

$$W_{k+1} = W_k - \left[J^T J + \mu I\right]^{-1} J^T e \qquad (3)$$

The $W$ is weight and $J$ is the Jacobian matrix that contains first derivatives of the network errors with respect to the weights and biases, the e is a vector of network errors, and μ is a scalar that controls the learning.

***The Radial basis function network***

The radial basis function (RBF) network also consists of three layers, namely an input layer, a hidden layer or radial basis layer, and an output layer or linear layer. The input layer collects the input information. The hidden layer consists of a set of basis functions performing nonlinear transformations of the inputs. The most common transformation is Gauss function as the nonlinearity of the hidden nodes. The response of the *j*-th hidden node to $x_i$ is given by

$$h_{ij}(x) = \exp\left(-\alpha \left\| x_i - c_j \right\|^2\right) \qquad (4)$$

where $\|...\|$ is Euclidean norm, $c_j$ is the center of the basis function and α is a positive constant that determines the width of the symmetric response of the hidden node.

The output values of the network are computed as linear combination of these basis functions (hidden nodes),

$$\hat{y}_i = \sum_{j=1}^{K} w_j h_{ij}(x) \qquad (5)$$

where $w_j$ is the network connection weights and K is the number of hidden nodes. Assume that N samples of the signal are available for training. The center, $c_j$ , $1 \le j \le K$, can be selected from the network training input $x_i$ , $1 \le i \le N$. The weights can then be solved using the least squares method.

The RBF networks have been widely used for nonlinear system identification because of their simple topological structure and their ability to reveal in an explicit manner how the learning is proceeding (Lin & Chen, 2004). RBF networks have increasingly attracted interest for engineering applications due to their advantages over traditional multilayer perceptrons, namely faster convergence, smaller extrapolation errors, and higher reliability (Moradkhani et al., 2004). The architecture and training algorithms for radial basis function networks (RBF) are simple and clear.

## 2. ANFIS

Fuzzy logic, first introduced by Zadeh (1965), is about mapping an input space to an output space, and the primary mechanism of this mapping is a list of if-then statements called *rules*. Fuzzy rule-based modeling is a qualitative modeling scheme in which the system behavior is described using a natural language (Nayak et al., 2004). All rules are evaluated in parallel, rather than sequentially, and the order of the rules is unimportant. The rules refer to variables and the adjectives that describe those variables. The fuzzy components or steps are, in general: fuzzification of inputs, application of a fuzzy operator, application of an implication method, aggregation of all output, and defuzzification.

The fuzzy inference system (FIS) is based on the concept of fuzzy set theory and fuzzy reasoning. It is a method that interprets the values in the input vector and, based on some set of rules, assigns values to the output vector. There are two types of fuzzy rule system being widely used, and these two were proposed by Namdani (1974) and Takagi-Sugeno (1985). Takagi-Sugeno has been adapted for ANFIS used in this paper. The Takagi-Sugeno method uses a composite procedure for fuzzy inference and output defuzzification (Alvisi et al., 2006).

ANFIS was originally proposed by Jang (1993). ANFIS is a fuzzy system trained by an algorithm derived from neural network theory. The algorithm is a hybrid training algorithm based on backpropagation and the least squares approach. In this algorithm, the parameters defining the shape of the membership functions are identified by a backpropagation algorithm, while the consequent parameters are identified by the least squares method. An ANFIS can be viewed as a special three-layer feedforward neural network. The first layer represents input variables, the hidden layer represents fuzzy rules, and the third layer is an output.

## RESULT AND DISCUSSION

This paper investigates two schemes of soft computing technique, ANFIS and ANN models, for GLF forecasting. Records of daily GLFs were compiled from observation wells in the case study area for two years and two months (2003 to 2005). Two observation wells (Urawa1 and Urawa2) were analyzed. The daily GLFs that were missing for several days were interpolated using the cubic spline method. In order to ensure that all variables received equal attention during the calculation process, they were standardized (Maier & Dandy, 2000). By this consideration, the inputs and output desired were scaled in the range of 0.1 to 0.9 by normalizing with respect to minimum and maximum data before being fed into the calculation model. The training dataset of 730 daily records was used to train the network to obtain parameters. Another dataset consisting of 60 daily records was used as testing dataset.

The first set of analyses examined the impact of adding time lag by seven models (Model 1 to Model 7) on the ANFIS model for Urawa1 and Urawa2 data records. Model 1 contains two input nodes (time-lags $t$ and $t-1$) and Model 7 have eight input nodes (time-lags $t$, $t-1$,… $t-7$).The simulated outputs ($t + 1$) were evaluated by such goodness of fit statistics as root mean square error (RMSE), mean absolute error (MAE), mean relative error (MRE), and coefficient of determination ($R^2$). The RMSE statistic measures the residual variances that show global goodness of fit between calculated and observed GLF. The mean absolute error is the average of the absolute values of the residuals. The mean relative error measures the accuracy that less sensitive for the outlying values than the RMSE. The coefficient of determination (r-square) is the ratio of the explained variation to the total variation. It represents the percent of data (observed-predicted) that is the closest to the line of best fit.

Table 1 shows the performance results obtained in the training and testing period of the ANFIS approach. The experiment showed that the $R^2$ values became higher with additional time lag for the training period. However, the largest $R^2$ values occurred for Model 2 and Model 3 on Urawa2 and Urawa1 during the testing period, respectively. The RMSE values were outstanding during training and testing period for all models studied. The biggest RMSE was 0.165 m for Model 1 in the training period and 0.159 m for Model 7 in the testing period. It also can be seen from the calculated results that Model 2 and Model 3 have the highest $R^2$ in the testing period for Urawa2 and Urawa1, respectively. For further analysis, we decided

to use Model 2 to compare the performance of ANFIS with two ANN models (LM and RBF). The GLF predictions from one to seven ahead (daily) were performed.

Table 1. The performance results of ANFIS approach during training and testing period

| | Training | | Testing | |
|---|---|---|---|---|
| Model | RMSE | R2 | RMSE | R2 |
| | | *Urawa1* | | |
| 1 | 0.165 | 0.972 | 0.139 | 0.799 |
| 2 | 0.161 | 0.973 | 0.137 | 0.805 |
| 3 | 0.155 | 0.975 | 0.134 | 0.814 |
| 4 | 0.144 | 0.978 | 0.138 | 0.805 |
| 5 | 0.131 | 0.982 | 0.145 | 0.791 |
| 6 | 0.114 | 0.986 | 0.151 | 0.779 |
| 7 | 0.099 | 0.990 | 0.159 | 0.755 |
| | | *Urawa2* | | |
| 1 | 0.115 | 0.980 | 0.077 | 0.934 |
| 2 | 0.110 | 0.982 | 0.075 | 0.941 |
| 3 | 0.105 | 0.983 | 0.077 | 0.938 |
| 4 | 0.100 | 0.985 | 0.079 | 0.936 |
| 5 | 0.094 | 0.987 | 0.078 | 0.938 |
| 6 | 0.085 | 0.989 | 0.077 | 0.938 |
| 7 | 0.074 | 0.992 | 0.079 | 0.932 |

Figure 2 shows the comparison results of three soft computing models using input Model 2 in the testing period. The general tendency of predictions shows that the determination coefficient ($R^2$) values decrease when predicting time-step increase and the prediction of one day ahead has the biggest $R^2$ values. It can thus be suggested that the model is good to predict one-day-ahead. The statistics indices of one-day-ahead prediction are presented in Table 2. The maximum value of mean absolute error (MAE) was 0.275 meters using the LM algorithm for Urawa1 in the training period. The mean relative error (MRE) values did not exceed 1.75% in the testing period. The coefficients of determination ($R^2$) were also good. It is clear that the soft computing approach has high predictive power.

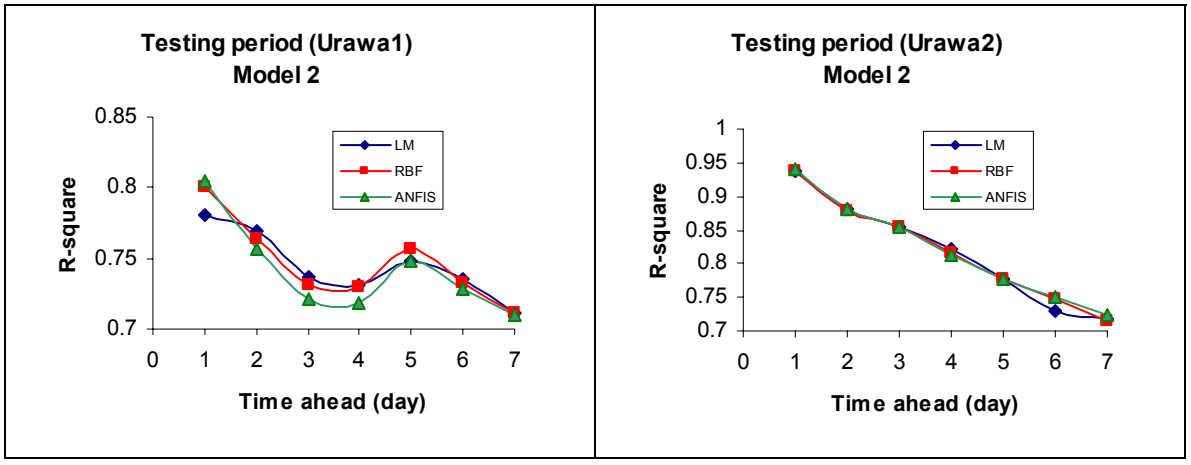


Figure 2. Comparison results of three different algorithms for lead time prediction using input Model 2.

Figure 3 shows the results of one-day-ahead prediction and observed GLF in the testing period using Model 2. The most striking result to emerge from the calculation is that there are no significantly different results among the three algorithms used. All predicted values tend to slightly underpredict groundwater level. (*Underpredicted* means that predicted values were shallower in depth than what was observed; *overpredicted* means they were deeper than the observed values.) The maximum positive deviation was 0.3746 meters for Urawa1 using the RBF algorithm and the maximum negative was 0.2938 meters for Urawa1 using the LM algorithm. It was observed that the prediction errors are falling around ± 0.2 meters. It can be seen from Figure 4 that of the predicted results, 85% are satisfactory. Based on these results, we can predict or monitor daily groundwater fluctuation with considering some time lag as inputs of the model.

Table 2. The performance of LM, RBF and ANFIS models during training and testing period

| | Training | | | | Testing | | | |
|---|---|---|---|---|---|---|---|---|
| Algorithms | MAE | RMSE | MRE | R2 | MAE | RMSE | MRE | R2 |
| | | | | *Urawa1* | | | | |
| LM | 0.275 | 0.517 | 3.523 | 0.897 | 0.117 | 0.141 | 1.748 | 0.781 |
| RBF | 0.132 | 0.164 | 1.865 | 0.972 | 0.112 | 0.138 | 1.671 | 0.801 |
| ANFIS | 0.130 | 0.161 | 1.832 | 0.973 | 0.112 | 0.137 | 1.663 | 0.805 |
| | | | | *Urawa2* | | | | |
| LM | 0.088 | 0.110 | 0.913 | 0.982 | 0.059 | 0.073 | 0.622 | 0.941 |
| RBF | 0.089 | 0.112 | 0.930 | 0.981 | 0.060 | 0.075 | 0.637 | 0.938 |
| ANFIS | 0.088 | 0.110 | 0.916 | 0.982 | 0.060 | 0.075 | 0.632 | 0.941 |

**CONCLUSION**

This study has demonstrated the predictive value of soft computing techniques for groundwater level estimation. The groundwater level prediction for this study was done under two scenarios. First stage was one-day-ahead prediction with time lag input models using an ANFIS algorithm. It was found that Model 2, the (*lag t, t-1, t-2*) input model, gave satisfactory prediction results. The advanced study was designed to test predictions of groundwater level fluctuation for seven time steps ahead using input Model 2. In this second stage, we compared three soft computing techniques: the ANFIS, LM, and RBF algorithms. It was found that the three algorithms produced no significant differences in prediction results. In general, the results showed that the prediction accuracy was decaying by increasing time step ahead, with the best accuracy being for one day ahead. It was found that predicted values were close to the observed ones and 85% fell in the range of ± 0.2 meters. Overall, these results suggest that all three soft computing algorithms can predict daily groundwater level with high accuracy using time lag as inputs networks. Further studies are recommended, especially using a much larger number of observation wells, to cover regional variations and to allow more comprehensive analyses. Furthermore, the larger number of data samples is needed to assess the long term tendency of groundwater level.

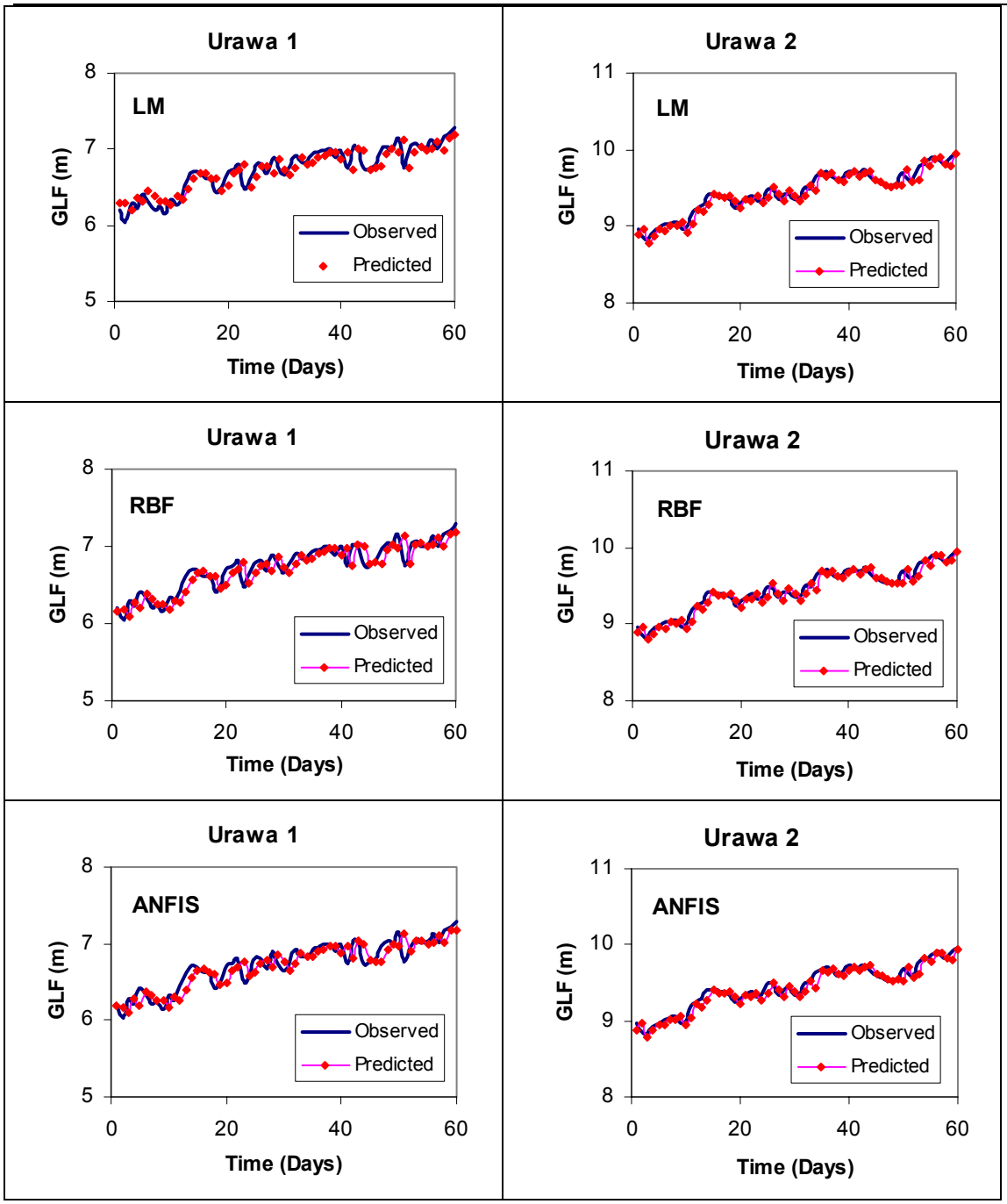


Figure 3. Comparison between observed and predicted GLF using ANFIS during testing period for input Model 2.

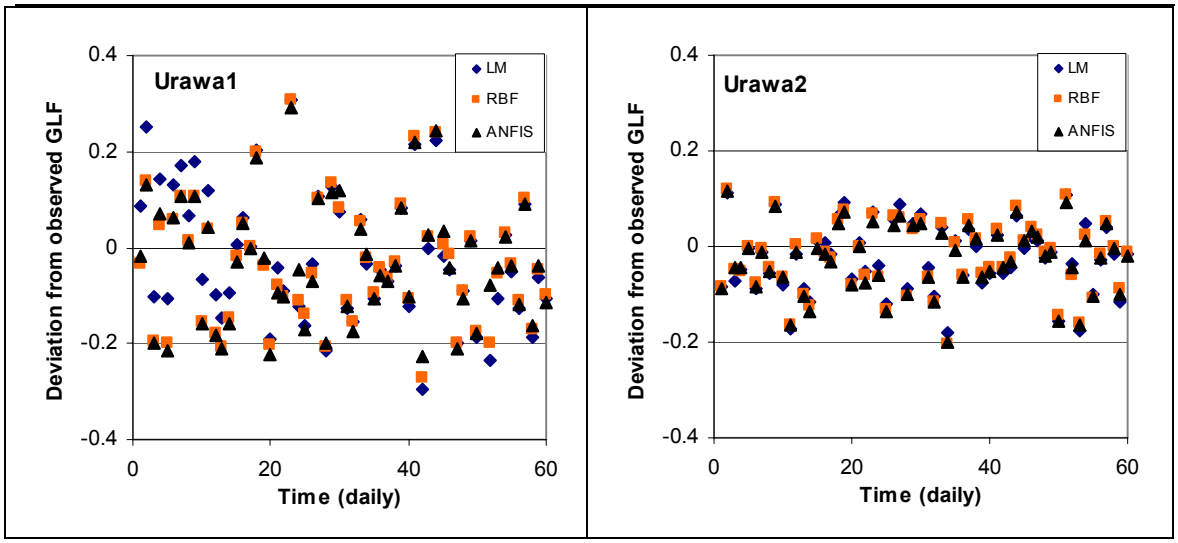


Figure 4. One day ahead prediction deviation from observed GLF during testing period

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**REFERENCES:**

1. Alvisi, S., Mascellani, G., Franchini, M. And Bardossy, A. (2006) Water level forecasting through fuzzy logic and artificial neural network approaches. Hydrology and Earth System Sciences 10: 1-17.
2. Chang, F.-J. And Chang, Y.-T. (2006) Adaptive neuro-fuzzy inference system for prediction of water level in reservoir. Advances in Water Resources 29: 1-10.
3. Chen, J. and Adam B.J. (2006) Integration of artificial neural networks with conceptual models in rainfall-runoff modeling. Journal of Hydrology 318: 232-249.
4. Chiang, Y.-M., Chang, L.-C. and Chang F.-J. (2004) Comparison of static-feedforward and dynamic-feedback neural networks for rainfall-runoff modeling. Journal of Hydrology 290: 297-311.
5. Coppola, E., Poulton, M., Charles, E., Dustman, J. and Szidarovszky, F. (2003) Application of artificial neural networks to complex groundwater management problem. Natural Resources Research 12: 303-320.
6. Coulibaly, P., Anctil, F., Aravena, R. and Bobée, B. (2001) Artificial neural network modeling of water table depth fluctuations. Water Resources Research 37(4): 885-896.
7. Daliakopoulos, L.N., Coulibalya, P., Tsanis, I.K. (2005) Groundwater level forecasting using artificial neural networks. Journal of Hydrology 309: 229-240.
8. Gautam, M.R., Watanabe, K. and Saegusa, H. (2000) Runoff analysis in humid forest catchment with artificial neural network. Journal of Hydrology 235: 117-136.

9. Gautam, M.R., Watanabe, K. and Ohno, H. (2004) Effect of bridge construction on floodplain hydrology-assessment by using monitored data and artificial neural networks models. Journal of Hydrology 292: 182-197.
10. Govindaraju, R.S. (2000a) Artificial neural networks in hydrology. I: Preliminary concepts. By the ASCE task Committee on application of artificial neural networks in hydrology. Journal of Hydrologic Engineering 5: 115–123.
11. Govindaraju, R.S. (2000b) Artificial neural networks in hydrology. II: Hydrologic application. By the ASCE task Committee on application of artificial neural networks in hydrology, Journal of Hydrologic Engineering 5: 124-137.
12. Jang, J.S.R. (1993) ANFIS: Adaptive network based fuzzy inference system, IEEE Trans. On System, Man, and Cybernetics 23(3): 665-685.
13. Kisi, O. (2005) Suspended sediment estimation using neuro-fuzzy and neural network approaches. Hydrological Sciences Journal 50(4): 683-696.
14. Lallahem, S., Mania, J., Hani, A. and Najjar, Y. (2005) On the use of neural networks to evaluate groundwater levels in fractured media. Journal of Hydrology 307: 92-111.
15. Lee, S., Ryu, J.H., Won, J.S. & Park, H.J. (2004) Determination and application of the weights for landslide susceptibility mapping using an artificial neural network. Engineering Geology 71: 289-302.
16. Lin, G.-F. and Chen, L.-H. (2004) A non-linear rainfall-runoff model using radial basis function network. Journal of Hydrology 289: 1-8.
17. Lu, R.-S. and Lo, S.-L. (2002) Diagnosing reservoir water quality using self-organizing maps and fuzzy theory. Water Research 36: 2265-2274.
18. Luk, K.C., Ball, J.E. and Sharma, A. (2000) A study of optimal model lag and spatial inputs to artificial neural network for rainfall forecasting. Journal of Hydrology 227: 56-65.
19. Maier H.R. and Dandy G.C. (2000) Neural networks for the prediction and forecasting of water resources variables: a review of modelling issues and applications, Environmental Modelling and Software 15(1): 101-124.
20. Moradkhani, H., Hsu, K.-L., Gupta, H.V. and Sorroshian, S. (2004) Improved streamflow forecasting using self-organizing radial basis function artificial neural networks. Journal of hydrology 295: 246-262.
21. Namdani, E.H. (1974) Appplication of fuzzy algorithm for control of simple dynamic plant, Proc. IEEE 121:1585-1889.
22. Nayak, P.C., Sudheer, K.P., Rangan, D.M. and Ramasatri, K.S. (2004) A neuro-fuzzy computing technique for modeling hydrological time series, Journal of Hydrology 291: 52-66.
23. Nayak, P.C., Rao, Y.R.S., and Sudheer, K.P. (2006) Groundwater level forecasting in a shallow aquifer using artificial neural network approach. Water Resources Management 20: 77-90.
24. Parida, B.P, Moalafhi, D.B. and Kenabatho, P.K. (2006) Forecasting runoff coefficients using ANN for water resources management: The case of Notwane Catchment in Eastern Botswana. Physics and Chemistry of the Earth 31: 928-934.
25. Ponnambalam, K., Karray, F. and Mousavi, S.J. (2003) Minimizing variance of reservoir systems operations benefits using soft computing tools. Fuzzy sets and systems 139: 451-461.
26. Rajurkar, M.P., Kothyari, U.C. and Chaube, U.C. (2004) Modeling of the daily rainfall-runoff relationship with artificial neural network. Journal of Hydrology 285: 96-113.
27. Ramelhart, D.E., Hinton, G.E. & Williams, R.J. (1986) Learning representations by back-propagation error. Nature 323: 533-536.
28. Ramirez, M.C.V., Velho, H.F.de C. and Ferreira, N.J. (2005) Artificial neural network technique for rainfall forecasting applied to the Sao Paulo region. Journal of Hydrology 301: 146-162.
29. Ranjithan, S., Eheart, J.W. & Garrett, J.H. (1993) A neural network-based screening for groundwater reclamation under uncertainty, Water Resources Research 29: 563-574.
30. Rao, S.V.N., Thandaveswara, B.S., Bhallamudi, M.S. and Srinivasulu, V. (2003) Optimal groundwater management in deltaic regions using simulated annealing and neural networks. Water Resources Management 17: 409-428.
31. Sajikumar, N. and Thandaveswara, B.S. (1999) A non-linear rainfall–runoff model using an artificial neural network. Journal of Hydrology 216: 32–55.
32. Takagi, T. and Sugeno M. (1985) Fuzzy identification of systems its application to modeling and control, IEEE Trans. On System, Man, and Cybernetics 15(1): 116-132.

33. Tayfur, G., Ozdemir, S. and Singh, V.P. (2003) Fuzzy logic algorithms for ruoff-induced sediment transport from bare soil surfaces. Advances in Water Resources 26: 1249-1256.
34. Toth, E., Brat, A. and Montanari, A. (2000) Comparison of short-term rainfall prediction models for real-time flood forecasting. Journal of Hydrology 239: 132-147.
35. Trafalis, T.B., Richman, M.B., White, A. and Santosa, B. (2002) Data mining techniques for improved WSR-88D rainfall estimation. Computers & Industrial Engineering 43: 775-786.
36. Tutmez, B., Hatipoglu, Z. And Kaymak, U. (2006) Modelling electrical conductivity of groundwater using an adaptive neuro-fuzzy inference system. Computers & Geosciences 32: 421-433.
37. Wang, W., Van Gelder, P.H.A.J.M., Vrijling, J.K. and Ma, J. (2006) Forecasting daily streamflow using hybrid ANN models. Journal of Hydrology 324: 383 – 399.
38. Wong, K.W., Wong, P.M., Gedeon, T.D. and Fung, C.C. (2003) Rainfall prediction model using soft computing technique. Soft Computing 7: 434-438.
39. Zadeh, L.A., Fuzzy sets. Information and control 8(3): 338-353.
40. Zaheer, I. and Bai, C.-G. (2003) Application of artificial neural network for water quality management. Lowland Technology International 5(2): 10-15.
41. Zhang, B. and Govindaraju, R.S. (2003) Geomorphology-base artificial neural networks (GANNs) for estimation of direct runoff over watersheds. Journal of Hydrology 273: 18-34.

# Genetic Variability for Biological Nitrogen Fixation Traits in Tropical Soybeans (*Glycine max* (L) Merr)

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**ABSTRACT:** Twenty five soybean (*Glycine max* (L) Merrill) genotypes were sown at the Teaching and Research Farm of UNAAB in Nigeria during the late planting season in year 2004 to obtain data for grain yield and seven Biological Nitrogen Fixation (BNF) traits. The data were subjected to analysis of variance (ANOVA) and were later used in the computation of heritability estimates for grain weight, days to flower, nodulation rating, days to maturity, number of nodules, nodule weight, primary root length and number of secondary roots. Varieties were observed to be genotypically diverse with respect to the traits evaluated. Large genotypic and phenotypic variations were also observed for the characters. Nodule number and nodule weight that had positive and significant association with nodulation rating, with relatively high broad-sense heritability estimates were adjudged as possible selection criteria for genetic improvement for BNF. Genotype TGx 1921-2F was identified as the only genotype that has the potentials for genetic manipulation of host plant for the improvement of soil nitrogen among the genotypes that were evaluated. [Nature and Science. 2007;5(2):11-15].

**Keywords:** Native rhizobia, Nodulation, Organic biological nitrogen fixation, tropical soil

## Introduction

The initial breeding approach of the International Institute of Tropical Agriculture (IITA) soybean scientists in the improvement of biological nitrogen (N) fixation (BNF) to meet the demand for Nitrogen in soybean in the early 1980s was to breed for promiscuous varieties that would nodulate freely with indigenous soil rhizobia to make inoculation unnecessary (Pulver *et al*., 1982; Kueneman *et al*., 1984). Promiscuous soybean varieties are those that are compatible with and could form effective symbiotic association with a large majority of native rhizobial strains (IITA, 1983) thus, making the use of synthetic fertilizers unnecessary. Compatible lines are lines that have high number of nodules with high biomass production. Shoot dry matter yield (biomass production) in particular has been reported to have strong and positive association with soybean grain yield (Okogun and Sanginga, 2003).

Reports in the last decade (Sanginga *et al*., 1997; Okogun and Sanginga, 2003) have shown that promiscuous soybean varieties derived about 85 kg N from BNF. Positive response of soybean to inoculation (yield increase of 179%) has also been reported to occur in situations where indigenous bradyrhizobial cells were fewer than 10 cells per grain of soil or where the rhizobial populations were not effective (RENEASA, 1996). Sanginga (2003), Okogun and Sanginga (2003) have confirmed that tropical soil rhizobial strains were not effective in fixing enough biological nitrogen to sustain soybean growth and, thus, the need for a starter dose of nitrogen fertilizer or a search for more effective rhizobial strains.

Effectiveness of soil rhizobial strains is influenced by prevailing environmental conditions such as moisture (Nantakorn and Weaver, 1982) and competition for nodule sites (Harold and Fudi, 1992). Excess soil moisture of more than 10% (Osa-Afiana and Alexander, 1979) probably, affects the rhizobial population and, thus, their symbiotic activities (Okogun and Sanginga, 2003).

Efforts have been made to incorporate the promiscuous gene into elite soybean varieties developed between 1991 and the present (FAO, 1999; Ojo, 2002) and selection for promiscuity and nodulation as reflected by the number of nodules and biomass production has been attempted (Sanginga *et al*., 1997; Sanginga *et al*., 2000).

It has been reported earlier that inheritance of promiscuity in tropical soybean was conditioned by a few major genes (IITA, 1980) suggesting that the trait is qualitatively inherited.

Genetic evaluation of BNF traits, particularly, the estimation of the degree of genetic determination of those traits that have significant association with grain yield among selected tropical soybean lines could provide a fast and cost effective means of knowing the traits that can be used as good predictors of yield in this crop. This is because heritability estimates indicate how easy or difficulty it will be to produce a change in a given trait by applying selection (Graham and Welch, 1996) and the amount of grain anticipated from such a selection is best given by heritability (Borojevic, 1990). The exercise could result

in an intelligent choice of the best lines and the right breeding procedure needed for their genetic improvement for effective nitrogen fixation in tropical soils without the use of synthetic fertilizers. Sanginga *et al*. (1997) have observed that the choice of breeding lines could influence the potential contribution of fixed nitrogen to farming system.

This research was intended to (1) examine genetic variability for BNF traits in selected soybean lines (2) examine possible association that exist between grain yield and BNF traits and (3) identify which of the traits that can be used as selection criteria for BNF and thus, grain yield in tropical soybeans.

**Materials And Methods**

Twenty five tropical soybean genotypes obtained from IITA, Ibadan, Nigeria were sown in the Teaching and Research Farm of the University of Agriculture, Abeokuta, (UNAAB), Nigeria in August 2004.

After land preparation that involved ploughing and harrowing, seeds of each genotype were sown by drilling in four-row plots in randomized complete block design with three replications. Rows were 75 cm apart. On emergence, seedlings were thinned to a plant-to-plant spacing of 5 cm leaving a population of 266, 667 plants per hectare.

Hand weeding was employed as required. Data were collected on number of days to flowering, number of nodules at flowering, weight of fresh nodules, length of primary root, number of secondary roots and nodulating rating. At maturity data were collected for days to maturity and grain yield per plot.

**Days to flowering**: - this was determined as the period from date of planting to the date when 50% of the plant in the plot was at full bloom stage.

**Days to maturity**: - was determined as the period between date of planting and the date when plants in the plot had matured physiologically and the pods were brown in color.

**Nodulating rating**: - was a rating from 1 to 5, root having very few nodules was rated 1, few nodules rated 2, moderate nodule rated 3, plenty nodules rated 4 while very plenty nodules was rated 5.

**Number of nodules**: - roots of ten plants were carefully dug up, packed in polythene bags together with the nodules that had become detached during digging and were transported to the laboratory where they were washed and number of nodules was counted. The mean value was then recorded.

**Weight of fresh nodules**: - after counting the nodules, it was weighted with a sensitive scale.

**Grain yield**: - grain yield in kg/plot was determined on clean dry grains of plants harvested from two middle rows of each plot.

**Length of primary root**: - ten plants were randomly picked from boarder rows and tap roots were measured per plant for length of primary root (cm) using a thread and meter rule. The mean length was then determined.

**The number of secondary roots** was also recorded as the mean number of roots of ten plants selected randomly from the boarder row.

The plot means of each character were subjected to analysis of variance (ANOVA). Simple correlation coefficients were obtained between all possible combinations of traits using Pearson correlation coefficient analysis. Also, estimates of broad-sense heritability for each character were obtained for possible use as selection criteria.

**Result And Discussion**

Table 1 presents the mean values of the twenty five soybean genotypes that were evaluated for eight BNF traits. Mean grain yield per plot ranged from 160 kg/plot for TGx 1923-4F to 433 kg/plot for TGx 1903-8F. Genotype TGx 1921-2F with highest nodulation rating of 3.4 matured in 87 days just as genotypes TGx 1921-1F, 1921-23F and TGx 1924-1F. Incidentally, genotype TGx 1921-2F recorded the highest number of nodules (21.0), biggest nodule weight (0.3 g/plant), very long primary root (13 cm) as well as highest number of secondary roots (10.0).

Table 1. Mean value of 25 soybean genotypes evaluated for grain weight and biological nitrogen fixation characters

| S/N | Variety | GWK g/plot | DF | NR | DM | NN | NW g/plt | RL | SN |
|---|---|---|---|---|---|---|---|---|---|
| 1 | $TG_X$ 1903-8F | 433 | 40 | 1.7 | 83 | 7.0 | 0.1 | 9.8 | 10.0 |
| 2 | $TG_X$ 1908-6F | 413 | 42 | 3.0 | 83 | 9.7 | 0.2 | 9.6 | 8.0 |
| 3 | $TG_X$ 1921-7F | 353 | 41 | 3.3 | 81 | 14.0 | 0.2 | 11.0 | 8.0 |
| 4 | $TG_X$ 1903-4F | 347 | 41 | 3.0 | 84 | 8.0 | 0.1 | 11.0 | 5.7 |
| 5 | $TG_X$ 1920-1F | 347 | 42 | 2.0 | 84 | 2.0 | 0.02 | 12.0 | 6.3 |
| 6 | $TG_X$ 1924-1F | 340 | 47 | 3.0 | 87 | 12.0 | 0.2 | 10.0 | 9.3 |
| 7 | $TG_X$ 1923-3F | 327 | 41 | 3.0 | 84 | 16.0 | 0.2 | 13.0 | 10.0 |
| 8 | $TG_X$ 1924-4F | 323 | 46 | 2.3 | 91 | 4.3 | 0.1 | 9.5 | 6.0 |
| 9 | $TG_X$ 1909-3F | 317 | 42 | 3.0 | 81 | 10.0 | 0.1 | 13.0 | 8.0 |
| 10 | $TG_X$ 1903-7F | 307 | 41 | 2.0 | 81 | 3.7 | 0.05 | 10.0 | 8.3 |
| 11 | $TG_X$ 1921-6 | 307 | 42 | 3.3 | 80 | 15.0 | 0.3 | 12.0 | 7.7 |
| 12 | $TG_X$ 1919-1F | 293 | 42 | 2.3 | 81 | 4.7 | 0.1 | 11.0 | 7.3 |
| 13 | $TG_X$ 1922-1F | 260 | 41 | 2.3 | 81 | 7.7 | 0.2 | 11.0 | 8.3 |
| 14 | $TG_X$ 1925-1F | 259 | 46 | 2.0 | 87 | 4.7 | 0.0 | 10.0 | 4.7 |
| 15 | $TG_X$ 1909-2F | 253 | 42 | 3.3 | 81 | 7.0 | 0.2 | 13.0 | 9.0 |
| 16 | $TG_X$ 1985-1D | 240 | 40 | 2.3 | 83 | 6.0 | 0.1 | 11.0 | 6.0 |
| 17 | $TG_X$ 1921-13F | 240 | 42 | 2.3 | 81 | 9.7 | 0.1 | 11.0 | 9.0 |
| 18 | $TG_X$ 1904-2F | 220 | 42 | 2.0 | 84 | 4.0 | 0.1 | 14.0 | 6.0 |
| 19 | $TG_X$ 1921-1F | 220 | 41 | 2.7 | 87 | 11.0 | 0.1 | 11.0 | 8.7 |
| 20 | $TG_X$ 1921-2F | 220 | 42 | 3.4 | 87 | 21.0 | 0.3 | 13.0 | 10.0 |
| 21 | $TG_X$ 1904-4F | 220 | 42 | 3.0 | 81 | 5.0 | 0.1 | 12.0 | 6.7 |
| 22 | $TG_X$ 1921-23F | 200 | 41 | 2.7 | 81 | 8.0 | 0.1 | 11.0 | 7.0 |
| 23 | $TG_X$ 1930-20E | 173 | 42 | 1.3 | 81 | 1.3 | 0.03 | 12.0 | 11.0 |
| 24 | $TG_X$ 1921-20F | 173 | 44 | 2.0 | 81 | 14.0 | 0.2 | 13.0 | 7.0 |
| 25 | $TG_X$ 1923-4F | 160 | 41 | 3.3 | 87 | 8.0 | 0.1 | 13.0 | 6.3 |
| | Mean | 227.3 | 42.0 | 2.6 | 83.6 | 8.6 | 0.1 | 11.5 | 7.8 |
| | LSD | 140.2 | 1.00 | 1.44 | 1.41 | 7.36 | 0.11 | 3.33 | 3.36 |

GW = Grain weight, DF = Days to flower, NR = Nodulation rating, DM = Days to maturity, NN = Nodule number, RL = Primary root length, SN = Secondary root number

Mean squares from analysis of variance for BNF traits (Table 2) showed that the twenty five soybean genotypes were highly variable with respect to grain yield, days to flowering, days to maturity, number of nodules, nodule weight and number of secondary roots to confirm the observed variations stated above.

Table 2. Mean squares from analysis of variance of biological nitrogen fixation (BNF) characters in twenty five varieties of soybean

| S O V | df | GW | DF | NR | DM | NN | NW | RL | SN |
|---|---|---|---|---|---|---|---|---|---|
| Replication | 2 | 47225.65** | 0.37 | 1.29 | 0.36 | 7.72 | 0.01 | 7.34 | 3.21 |
| Variety | 24 | 16305.72** | 8.52** | 1.04 | 25.02** | 70.61** | 0.02** | 4.46 | 8.42* |
| Error | 48 | 7292.32 | 0.37 | 0.77 | 0.74 | 20.08 | 0.01 | 3.84 | 4.19 |

*Significant at 5% probability level, ** Significant at 1% probability level.
SOV= source of variation, df=degree of freedom, GW= Grain weight, DF= Days to flower, NR= Nodulation rating, DM= Day to maturity, NN= Nodule number, NW= Nodule weight, RL= Root length, SN= Secondary Root Number

Observed correlation coefficients between grain yield and BNF traits as presented in Table 3 revealed that only the primary root length recorded a significant but negative correlation with grain yield. Days to flowering and days to maturity recorded non-significant and negative correlation with grain yield. However, days to flowering and days to maturity were positively and significantly correlated (0.472). Nodulation rating was equally positively and significantly associated with nodule weight (0.674) and number of nodules (0.633). Significant and positive association between two characters suggests that these characters can be improved simultaneously in a selection programme (Hayes *et al*., 1955). This is because a significant positive association shows a mutual relationship as selection for one trait would lead to selection and consequent improvement for the other traits.

Genetic manipulation of selected soybean genotypes for number of nodules, nodule weight and number of secondary roots would be an effective means of rating tropical soybean genotypes for BNF characteristics according to the current study. However, the length of primary root would not have any meaningful contribution to grain yield because both were negatively and significantly correlated.

Table 3. Correlation coefficients between Grain weight and Biological Nitrogen-Fixation characters

| | GW | DF | NR | DM | NN | NW | RL | SN |
|---|---|---|---|---|---|---|---|---|
| GW | 1.000 | -0.046 | 0.077 | -0.044 | 0.054 | 0.073 | -0.561** | 0.119 |
| DF | | 1.000 | -0.047 | 0.472* | 0.012 | 0.038 | -0.186 | -0.239 |
| NR | | | 1.000 | 0.093 | 0.633** | 0.674** | 0.191 | 0.017 |
| DM | | | | 1.000 | 0.063 | 0.040 | -0.263 | -0.245 |
| NN | | | | | 1.000 | 0.856** | 0.208 | 0.392* |
| NW | | | | | | 1.000 | 0.212 | 0.325 |
| RL | | | | | | | 1.000 | 0.002 |
| SN | | | | | | | | 1.000 |

*Significant at 5% probability level ** Significant at 1% probability level.
GW= Grain weight, DF= Days to flower, NR= Nodulation rating, DM= Day to maturity
NN= Nodule number, NW= Nodule weight, RL= Root length, SN= Secondary Root Number

Table 4 presents the phenotypic and genotypic variances and estimates of broad-sense heritability ($H_B$) of eight traits that were evaluated. Days to flowering and days to maturity had the largest estimates of 88 and 92%, respectively and thus were least affected by the effects of the environment. Nodule number and nodule weight recorded average estimates of 46 and 47% respectively, whereas nodulation rating, primary root length and number of secondary roots had $H_B$ estimates that were ridiculously low (5 – 25%).

Table 4. Phenotypic and genotypic variance and heritability of eight traits that were evaluated

| S/N | Traits | Phenotypic variance | Genotypic variance | Heritability ($H_B$) |
|---|---|---|---|---|
| 1 | Grain weight | 10296.77 | 3004.47 | 0.29 |
| 2 | Days to flowering | 3.09 | 2.72 | 0.88 |
| 3 | Nodulation rating | 0.86 | 0.09 | 0.11 |
| 4 | Days to maturity | 8.83 | 8.10 | 0.92 |
| 5 | Nodule number | 36.92 | 16.84 | 0.46 |
| 6 | Nodule weight | 0.0088 | 0.0041 | 0.47 |
| 7 | Primary root length | 4.05 | 0.21 | 0.05 |
| 8 | Secondary root number | 5.60 | 1.41 | 0.25 |

Very low estimates of $H_B$ suggest large effects of the environment and consequently larger phenotypic variances. High soil moisture (Nantakorn and Weaver, 1982), competition among local strains of Rhizobia for nodule sites (Harold and Fudi, 1992) and few number of native Bradyrhizobial cells in a unit grain of soil (Okogun and Sanginga, 2003) have been reported as some of the factors that prevent effective nodulation of soybean lines.

This study shows that number of nodules and weight of nodules were positively associated with nodulation rating. Thus, the two traits could be used as selection criteria for BNF traits among tropical

soybean genotypes. As genetic manipulation of host plants offers the greatest potential for the improvement of nitrogen levels in soybean (Sanginga *et al*., 1997) genotype TGx 1921-2F with highest nodulation rating, highest number of nodules, highest nodule weight as well as highest number of secondary roots could be selected for a future genetic improvement of biological nitrogen fixation.

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**References**

1. Borojevic, S. 1990. Principles and Methods of Plant Breeding. Slavko Borojevic (ed) pp. 72 – 74 (Development in Crop Science) Vol. 17. Elsevier Science Publications, Amsterdam.
2. F. A. O. Food and Agriculture of the United Nations. Manual for Training in Seed Technology and Seed Production. R. K. Triveds, L. A. Usman and J. C. Umeh (Eds) 1999 166p.
3. Graham, R. D. and Welch, R. M. 1996. Genetic control of characteristics pg. 76 – 78. In: Breeding for staple food corps with high micronutrient density. International Fool Policy Res. Institute. Washington DC.
4. Harold, H. K. and Fudi, L. L.. Potential for increasing biological nitrogen fixation in soybeans, *Mant. Soil* 1982 141: 119-135.
5. Hayes, H. K., Forrest, R. I. and Smith, D. C.. Methods of plant breeding, correlation and regression in relation to plant breeding McGraw Hill company Inc. 2nd edition pp. 1955 439-451.
6. IITA, 1980. International Institute of Tropical Agriculture. Annual Report for 1979. Ibadan, Nigeria.
7. IITA, 1983. International Institute of Tropical Agriculture. Soybean lines for the lowland tropics. Research Highlights, 1983: 86-88.
8. Kueneman, E. A., Root, W. R., Dashiell, K. E. And Hohenberg, J.. Breeding soybean for the tropics capable of nodulating effectively with indigenous *Rhizobium* spp. *Plant Soil*, 1984 82: 387-396.
9. Nantakorn, B. and Weaver, R. W.. Survival of cowpea rhizobia as affected by soil temperature and moisture. In: Graham, P. H., Harris Sc (eds) paper presented at a workshop 9 – 13 March, 1981. CIAT, Cali Colombia. 1992
10. Ojo, D. K. 2002. Food legume for Health and Wealth. Achievements and challenges in soybean breeding. Presentation at the 5th Annual Conference of the Alumni association, University of Agriculture, Abeokuta, Nigeria, 12th January, 2002.
11. Okogun, J. A. and Sanginga N.. Can introduced and indigenous rhizobial strains compete for nodule formation by promiscuous soybean in the moist savanna agroecological zone of Nigeria? *Bio Ferti. Soils* 2003 38: 26-31.
12. Osa-Afiana, L. O. and Alexander, M.. Effects of Moisture on the survival of Rhizobium in soil. *Soil Sci. Am J*. 1979 43: 925-930.
13. Pulver, E. L., Kveneman, E. A. and Rao, V. A.. Identification of promiscuous nodulating soybeans efficiency in Nitrogen fixation. *Crop Sci*. 1982 25: 660-663.
14. RENEASA,. Rhizobium Ecology Network of East and Southern Africa. Legume Inoculating response and farmer perceptions of Nitrogen fixation and Legume inoculants. Technical report of Phase II activities. 1996
15. Sanginga, N.. Role of biological nitrogen fixation in legume based cropping systems; a case study of West Africa farming systems. *Plant and Soil* 2003 252: 25-39.
16. Sanginga, N. Dashiell, K., Okogun, J. A. and Thottapilly, G.. Nitrogen fixation and Nitrogen contribution in promiscuous soybeans in Southern Guinea Savanna of Nigeria. *Plant Soil* 1997 195: 257-266.
17. Sanginga, N., Thottapilly, G. and Dashiell, K.,. Effectiveness of rhizobia nodulating recent promiscuous soybean selection in the moist savanna of Nigeria. *Soil Bio. Biochem*. 2000 32: 127-133.

# Morphometric Studies in *Eudrilus Eugeniae* Populations from Different Locations in Lagos, Nigeria

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**Abstract:** Morphological variation was studied within earthworm species – *Eudrilus eugeniae* populations collected from 14 different locations within Lagos State in Nigeria. Mature earthworms were identified by the presence of the clitellum and position of clitellum assisted in specie identification. Morphometric data collected on the earthworms include body weight, length of clitellum, body size diameter, total number of segments and body volume. Results from the statistical analysis showed that the earthworms though of same species were separated into 3 distinct groups based on the morphometirc parameters and not soil type. These 3 distinct groups may represent different lineages within the earthworm - *Eudrilus eugeniae.* [Nature and Science. 2007;5(2):16-21].

**Keywords:** Earthworms, *Eudrilus eugeniae.* variation, clitellum

## Introduction

The earthworm species, *Eudrilus eugeniae* commonly referred to as the West African night crawler, occurs all over the world but mostly in West African regions (Shagoti 1985, Segun 1998). It is largely found in West Africa from Ghana to Nigeria to West Cameroun and Gabon. It grows well at a temperature of more than $25^0$C but best at $30^0$C (Viljoen and Reinecke 1992), attaining maximum weight, length and number of segments in about 15 to 20 weeks (Rodriguez and Lapiere 1992). It ranges in size from about 10cm in length to huge specimens of over 12cm and size may depend on habitat (Segun 1998). The total number of segments in *Eudrilus eugeniae* varies from about 80 to over 100 with the location of a thick cylindrical collar – the clitellum between segments 13 – 20 (Pirrone 1985). It has a purple sheen and the posterior segments are evenly tapered to a point (Blackburn 1989).

Earthworms are becoming increasingly important as a common assay organism for soil fertility tests especially since they represent a major group in the terrestrial environments (Weeks and Svendsen 1996). They are known to contribute to soil processes through faecal excretion in form of casts, burrows, feeding and digestion (Tian *et al.* 2000). In the tropics, they are known to help in plant residue decomposition (Tian *et al.* 1995) and also convert plant residue into soil organic matter (Lavelle 1988).

There is a great taxonomic diversity among tropical earthworms with the dominant ones represented by the families Almidae, Glossoscolecidae, Megascolecidae, Ocnerodrilidae and Eudrilidae – to which *Eudrilus eugeniae* belongs (Segun 1998). Variability in terms of morphological measurements has been reported among different species of earthworms (Gregory and Herbert 2002, Heethoff *et al.* 2003, Sims and Gerard 1985). Variation in total body length as measured from the first to last segment was reported by Vitturi *et al.* 2000.

The aim of this study is to test for phenotypic differences within an earthworm species - *Eudrilus eugeniae* using quantitative measurements. The earthworms were sampled in 14 different locations with a view to using the traits measured to determine if different groups/lineages exist within the *Eudrilus eugeniae* population

## Materials and Methods

Sexually mature earthworms as determined by the presence of the clitellum were collected from 14 different localities in the Lagos state of Nigeria (Figure 1). Fifteen specimens were collected from each study site. Most of the earthworms were sampled from under leaf litter and small logs in the early hours of the day (usually by 8am) by digging and handsorting. Collected specimens were identified according to Segun (1998). Samples were taken immediately with good quantity of soil from its habitat to the laboratory for further analysis.

Characteristics determined at the point of sampling include the colour of the earthworm and position of the clitellum. Other measurements determined in the laboratory using five worms per location include body weight, length of clitellum, diameter of posterior and anterior ends; total body length, body size diameter and total number of segments. Body volume was calculated according to Gregory and Herbert (2002) as follows: $\prod L\ (½d)^2$

Where L = total body length
d = body size diameter

Length measurements were taken with a metre rule to the nearest millimeter and body weight was measured to the nearest 0.01gram.

Test of means were performed by the least significant difference method using a pooled error variance. The integer values were combined with continuous data and analyzed by cluster analysis using Pearson correlations as distance and the average linkage method (Swofford and Olsen 1990) to represent the collection in a dendogram.

**Results**

Colour and position of clitellum and soil types from which the earthworms were collected are as presented in Table 1. It was observed that earthworms from University of Lagos (Unilag), Maryland, Ajah, Lekki, Suru-lere, Abule-Egba and Alausa appeared very dark in colour at the anterior (head) end and lighter at the posterior (tail) end. These earthworms were collected from soils which were clay/loam. Earthworms from Ikorodu, Badagry, Ogba and Mushin which were from loam/humus soil were purple in colour, while those from Ketu, Owode/Ajegunle and Bariga were collected from clay soil and appeared purple-grey in colour. There was also a consistency in the position of the clitellum for the earthworms in all locations.

Table 2 shows the results of body measurements across all locations sampled. Earthworms from Badagry and Mushin had the highest body size diameter of 5.22mm and 5.10mm respectively with body volume of 3091.28 $mm^3$ and 2861.10 $mm^3$. However, earthworms from Badagry and Ogba had the highest values for body weight. The earthworm with the highest number of segments was those from Mushin while those from Ajah and Bariga had the lowest number of 88 segments.

Results from the cluster analysis as presented in the dendogram in Figure 2 shows that earthworms were divided into three major cluster groups. Earthworms from Unilag (1) and Badagry(3) in addition to Ikorodu (2) were isolated and joined at the lowest cluster level which was close to coefficient of similarity of 0.0 for Unilag and Badagry and a value of 0.01 for Ikorodu. The earthworms from the other eleven locations clustered at level 3 indicating some similarity in the earthworms based on the characters used in the assessment. It was further observed that earthworms from Ajah (6) and Owode/Ajegunle (9) clustered together at the highest level of 0.44. Earthworms at the cluster level 3 had 3 sub-clusters with earthworms in the group designated "b" exhibiting high values in body sizes as measured by body volume and body length. The earthworms from Surulere (8) were in a distinct group "c", while those from other locations were in the group "a" and had low to medium values for body weight and length.

Table 1. Collection locality, soil type, position of clitellum and earthworm colour

| Locality of collection and location number | Soil type | Colour | Clitellum position |
|---|---|---|---|
| Unilag (1) | Clay/Loam | Dark head/light tail | 13 - 20 |
| Ikorodu (2) | Loam/Humus | Purple | 13 - 20 |
| Badagry (3) | Loam/Humus | Purple | 13 - 20 |
| Maryland (4) | Clay/Loam | Dark head/light tail | 13 - 20 |
| Ketu (5) | Clay | Purple-Grey | 13 - 20 |
| Ajah (6) | Clay/Loam | Dark head/light tail | 13 - 20 |
| Lekki (7) | Clay/Loam | Dark head/light tail | 13 - 20 |
| Suru-lere (8) | Clay/Loam | Dark head/light tail | 13 - 20 |
| Owode/Ajegunle (9) | Clay | Purple-grey | 13 - 20 |
| Abule-Egba (10) | Clay/Loam | Dark head/light tail | 13 - 20 |
| Ogba (11) | Loam/Humus | Purple | 13 - 20 |
| Alausa(12) | Clay/Loam | Dark head/light tail | 13 - 20 |
| Bariga (13) | Clay | Purple-grey | 13 - 20 |
| Mushin (14 | Loam/Humus | Purple | 13 - 20 |

Table 2. Body size measurements in *E. eugeniae*

| Location | Average body weight(g) | Length of clitellum (mm) | Average body size diameter (mm) | Body length (mm) | Avarege body volume ($mm^3$) | Average no. of sements | Diameter at anterior end (mm) | Diameter at posterior end (mm) |
|---|---|---|---|---|---|---|---|---|
| Unilag | 0.53 | 3.26 | 4.12 | 129.0 | 1710.43 | 91 | 2.5 | 1.2 |
| Ikorodu | 0.93 | 5.02 | 5.00 | 115.0 | 2258.92 | 95 | 3.2 | 3.0 |
| Badagry | 1.07 | 6.18 | 5.22 | 144.0 | 3091.28 | 94 | 5.0 | 4.0 |
| Maryland | 0.90 | 3.92 | 4.10 | 127.0 | 1624.62 | 92 | 3.0 | 1.5 |
| Ketu | 0.87 | 2.10 | 4.10 | 130.0 | 1717.00 | 92 | 2.0 | 1.5 |
| Ajah | 0.75 | 5.00 | 4.00 | 130.0 | 1634.30 | 88 | 2.0 | 1.0 |
| Lekki | 0.84 | 4.50 | 4.00 | 125.0 | 785.70 | 92 | 3.0 | 1.5 |
| Suru-lere | 0.74 | 4.90 | 4.10 | 121.0 | 1598.30 | 89 | 2.1 | 1.1 |
| Owode/Ajegunle | 0.83 | 4.00 | 4.00 | 120.0 | 1508.60 | 92 | 2.0 | 1.0 |
| Abule-Egba | 0.79 | 5.10 | 4.25 | 120.0 | 1703.10 | 92 | 3.2 | 1.1 |
| Ogba | 1.26 | 3.20 | 4.40 | 145.0 | 2205.66 | 93 | 2.4 | 1.6 |
| Alausa | 0.45 | 2.10 | 4.00 | 125.0 | 1571.71 | 92 | 2.8 | 1.5 |
| Bariga | 0.95 | 4.90 | 4.40 | 150.0 | 2281.71 | 88 | 2.0 | 1.0 |
| Mushin | 0.96 | 4.00 | 5.10 | 140.0 | 2861.10 | 96 | 2.5 | 1.9 |

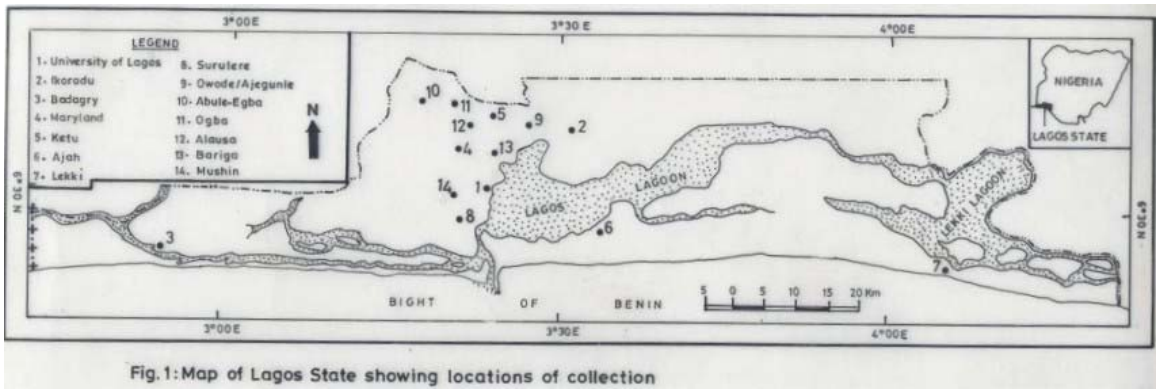

Fig. 1: Map of Lagos State showing locations of collection

Figure 1. Map of Lagos State showing locations of collection

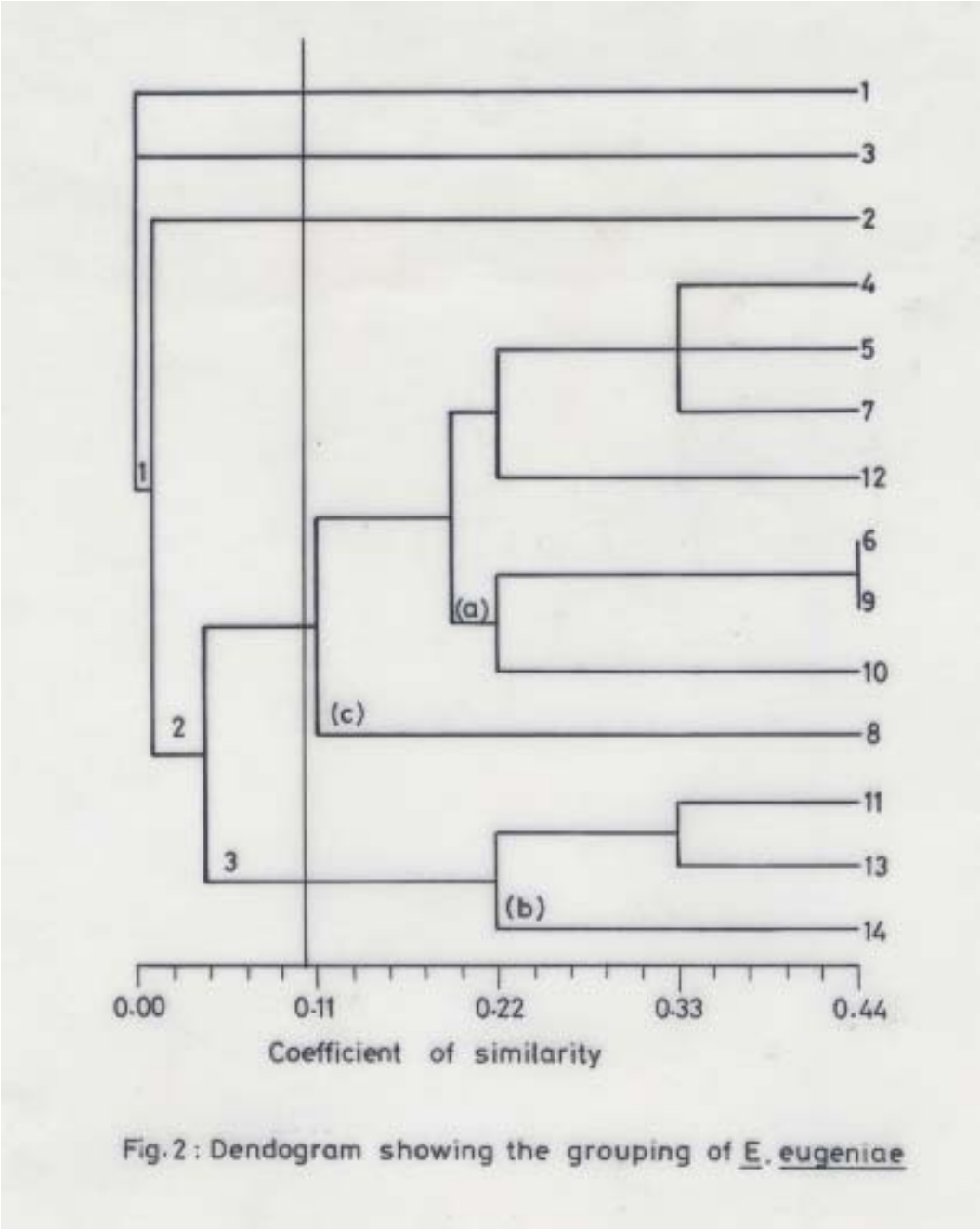


Figure 2. Dendogram showing the grouping of E. eugeniae

**Discussions**

One of the most important results from this study of the earthworm species is the separation of the collections into 3 major groups. The collections from Unilag and Badagry though in the same cluster were distinct from each other based on their coefficient of similarity. The collection in cluster 2 which was from Ikorodu was also isolated from every other cluster while the collections from the other eleven locations

were in cluster 3. Collections were studied based on body size characters, thus differences in clustering could be attributed to the body size measurements across locations. Sims and Gerard (1985) suggested that the environments inhabited by most earthworms were quite stable and these determined the time it takes to mature in some species. Also Satchel (1980) classified lumbricid earthworms into 2 distinct categories – the 'r' and 'k' selected species based on their body sizes and lifestyles. The 'r' selected species usually had small body sizes, shorter incubation and maturation times and inhabited stable surface environments. The k-selected species however had large body sizes, and inhabited stable environments. Even though the age of these earthworms were not determined in the study, they were all sexually mature at the time of collection as shown by the presence of the clitellum. Collections from Maryland, Ketu, Ajah, Lekki, Alausa and Owode/Ajegunle had small body sizes and were from a clay and/ loamy soil, thus having some relationship with r-selected species. Furthermore earthworms from Unilag and Bariga though they were collected from the clay/loam soil type they were quite varied in their body weights. Earthworms from Badagry and Ikorodu were from the loam/humus soil type which usually contains large amounts of decaying plant and animal materials which when used as food by the earthworms may have contributed to their large body sizes. Thus the soil type may not be a strict determinant of body size as collections from Bariga had high body size and it was from a clay soil.

From this study, it was further observed that soil type and body weight may not be the only factors in the grouping of the earthworms but in addition average body volume, body length and number of segments may have also helped in discriminating amongst earthworms from different locations. This is in line with extensive studies on the parthenogenic earthworm *Octolasion tyrtaeum* in different localities in Europe that have shown that it consists of distinct morphological and/or genetic lineages. Studies in Germany, on adult *O. tyrtaeum* showed that in some habitat specimens had large sizes, in some small and in some both sizes co-existed (Meinhardt 1974 as cited by Heethoff *et al.* 2003). This may have been the case with collections from Unilag, Ikorodu, Badagry and Suru-lere which tend to be distinct from each other and all the others. However, studies in Finland by Terhivuo and Saura (1993) showed that in a total of 238 *O. tyrtaeum* specimens from 8 locations using izozyme electrophoresis with 3 loci, a total of 24 clones were observed. However there was no correlation between body size and clonal affiliation. Thus it was concluded that variation was due to local environment rather than genetics. In another study by Heethoff *et al.* 2003 using adult *O. tyrtaeum* worms from Germany and Canada genetic distance was measured using mitochondrial cytochrome oxidase II (C011) sequences. Results showed that *O. tyrataeum* consisted of two morphologically and genetically distinct lineages.

This present study though preliminary and not on *O. tyrtaeum* but *E. eugeniae* using simple statistics on morphological measurements has shown that three different lineages which are morphologically distinct exist in the Lagos area of Southwestern Nigeria. Further study using larger sample sizes, wider sample area and other genetic parameters for a more effective discrimination of specimens is suggested.

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**References**

1. Blackburn, J.C. 1989. External anatomy of earthworms. *Sci.* **207**: 2572 – 2577.
2. Gregory, T.R. and P.D.N. Herbert. 2002. Genome size estimates for some oligochaete annelids. *Can. J. Zool.* **80**: 1485 – 1489.
3. Heethoff, M., K. Etzold and S. Scheu. 2003. Mitochondrial C011 sequences indicate that the parthenogenetic earthworms *Octolasion tyrtaeum* (Savigny 1826) constitutes of two lineages differing in body size and genotype. *Pedobiologia.* **47**: 9 – 13.
4. Lavelle, P. 1988. Earthworm activities and the soil system. *Biol. Fert. Soils.* **6**: 237 – 251.
5. Pirrone, S. 1985. The earthworm baits market in North America. *Heredity.* **59**: 1019 – 1029.

6. Rodriguez, A.C. and I.R. Lapeire. 1992. Increase in weight, length and number of segments of *Eudrilus eugeniae* (Oligochaeta: Eudrilidae) at $24^0$C. *Rev. Biol.* **6**: 215 – 221.
7. Segun, A.O. 1998. Tropical Zoology 2nd edition. University Press, Ibadan. 283pp.
8. Shagoti, U.M. 1985. Analysis of developmental rates and body size in earthworms. *Heredity.* **80**: 29 – 40.
9. Sims, R.W. and R. Gerard. 1985. A review of the economic importance of earthworms. *Science.* **29**: 152 – 160.
10. Swofford, D.L. and G.J. Olsen. 1990. Phylogeny Reconstruction P411-501D.M. in Hills and C. Moritz (eds) Molecular Systematic. Sineaur Associates, Sunderland, MA.
11. Terhivuo, J. and A. Saura. 1993. Clonal and morphological variation in marginal populations of the parthenogenetic earthworms *Octolasion tyrtaeum* and *O. cyaneum* (Oligochaeta Lumbricidae) from eastern Fennoscandia. *Boll. Zool.* **60**: 87 – 96.
12. Tian, G., L. Brussard and B.T. Kang. 1995. Breakdown of plant residues with contrasting chemical compositions: Effect of earthworms and millipedes. *Soil Biol. Biochem.* **27**: 277 – 280.
13. Tian, G., J.A. Olimah, G.O. Adeoye and B.T. Kangi. 2000. Regeneration of earthworm populations in a degraded soil by natural and planted fallows under humid tropical conditions. *Soil. Sci. Soc. of America. J.* **64**: 222 – 228.
14. Viljoen, S.A. and A.J. Reinecke. 1992. The temperature requirements of epigeic earthworm species *Eudrilus eugeniae* (Oligochaete); a laboratory study. *Soil. Biol. Biochem.* **24**: 1345 – 1350.
15. Vitturi, R., M. S. Colomba, A. Pirrone and A. Libertini. 2000. Physical mapping of Rdna genes (TTAGGG)n telomeric sequence and other karyological features intwo earthworm of the family Lumbricidae(Annelida: Oligochaeta). Heredity 85: 203-207.
16. Weeks, J.M. and C. Svendsen. 1996. Neutral red retention by lysosomes from earthworm (*Lumricus rubellus*) coelomocytes: A simple biomarker of exposure to soil copper. *Env. Toxicol. Chem.* **15**: 1801 – 1805.

# Chemical Factors in Erosion-Induced Soil Degradation in Owner-Managed Farms in Central Southeastern Nigeria

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**Abstract:** Guided mainly by a reconnaissance survey and geological map of the study area 18 owner-managed farms were identified and used for a study in 2004 which investigated the principal chemical factors in erosion-induced soil degradation. Field sampling along a transect resulted in the collection of 3 soil samples per farm. These samples were prepared for analyses and results showed varying rates of degradation using land degradation index (LDI) among soil parameters and locations. Least LDI values were found in total sulphur. Soil pH (PRIN I), organic carbon (PRIN 2) and total nitrogen (PRIN 3) explained a greater proportion of the total variance in erosion-induced degradation among soils. Some farms clustered, indicating similarity in management. Further research should include physical, biological, and other soil related factors associated with erosion-induced degradation. [Nature and Science. 2007;5(2):22-29].

**Keywords:** Chemistry, degradation, erosion, farms, principal component analysis, low input farming, tropical soil.

## Introduction

As populations grow, farmers are forced to cultivate smaller plots where the soil eventually becomes depleted, or expand into fragile hillsides (Scherr and Yadav, 1996). This scenario is common in the sub-Saharan humid tropics and this could be one of the reasons for low productivity (Eswaran *et al,*. 1997). The situation worsens as farmers may not adopt modern technologies (Reich *et al.,* 2001) amidst inappropriate management practices such as continuous cropping, burning, deforestation (Mbagwu and Obi, 2003). They noted that 85% of the cause of land degradation world wide is due to soil erosion, which according to Brady and Weil (1999) starts from slight to medium to severe to extreme severe soil and vegetative degradation. This is brought about by a prolonged interface between human –induced and natural factors (Di Falco *et al.,* 2006).

In central Eastern Nigeria, the major causes of soil degradation are soil erosion due to high rainfall deforestation, fragile nature of the soil and farming activities (Igwe 2003). As a consequence of soil erosion by the agency of water, soil nutrients are depleted leading to decline in crop production (Henao and Baanante, 2001). Changes in soil properties due to erosion may be accountable for the vulncability and reduction in the cherished qualities of soils. Oti (2002) reported a decline in water stable aggregates and this makes soils unstable to raindrop impact thereby enhancing disintegration and slaking. Increased soil erosion promotes soil compaction (Mainville et al., 2006) and loss of weakened top layers of soil (Farella *et al.,* 2001) especially in agroecosystems characterized by high rate of deforestation (Sierra, 2002). About 300 tons of soil loss per year were recorded in Honduras due to soil crosion (Thurow et al., 2002)

Chemistry of soil is also altered as a result of soil erosion on farmlands. It results in net decrease in soil carbon and nitrogen (Mainville *et al.,* 2006) due to leaching, burning and volatilization (Roulet et al., 1999). Loss of organic matter results in soil structural instability since it is a major binding agent (Mikha and Rice, 2004). Mbagwu and Auerswald (1999) reported low exchangeable sodium percentage due to soil erosion. Increase in aluminium saturation and reduced calcium magnesium ratios were also reported with heightened severity of soil erosion (Oti, 2002). In addition to the above, removal of epipedal layers may lead to subsoil exposure, and consequently heavy metals pollution (Mainville *et al.,* 2006). There show that eroded soils are sick (IIRR, 2005), hence a major limitation in most farmlands in Africa (Lal, 1995) due to declining agricultural productivity and farmers income (Hans *et al.,* 2006). The main aim of this investigation was to identify specific soil chemical properties altered by soil erosion that would serve as reliable predictors of status of soil degradation.

## Materials and Methods

**Study area:** The study site (Abia and Imo State in central southeastern Nigeria lies between latitudes $4^{0}40^{1}$ and $8^{0}15^{1}$N and $6^{0}40^{1}$ and $8^{0}15^{1}$ E. Predominant parent materials underlying soils of the site include Alluvium Coastal Plain Sands, Shale, Lower Coal measures, Upper Coal Measures, Falsebedded Sandstones. This guided field sampling as follows: Akwette, Oguta and Oweninta (Alluvium), Okeikpe, Owerri and Umuahia (Coastal Plain Sands) Arondizuogu, Bende and Nkporo (Shale) Arochukwu, Ohafia and Uturu (Lower Coal Measures), Abiriba, Item and Ihube (Upper Coal Measures and Ezere, Okigwe and Umulolo (Falsebedded Sandstones). The study site is generally a lowland area, with few scarpy landscapes in the northeast orientation. It has a humid tropical climate, with marked wet and dry seasons of nine and three months, respectively. Mean annual rainfall ranges from 2000-2500 mm while annual temperatures range from 26-29$^{o}$C. It has a typical rainforest vegetation which has been drastically altered by anthropogenic activities. Farming is a major socio-economic activity in the area and is mainly practiced at subsistence level. Soil fertility regeneration is by use of bush fallow, whose length has been drastically reduced due to demographic pressure on land (Onweremadu, 1994). Mixed cropping is popular in the area.

**Field studies:** After a reconnaissance survey of the area and guided by the geology map of the region, field sampling using the transect technique was conducted in 18 owner-managed farms in the study area in 2004. In each geological zone, 3 owner-managed farms were selected and this activity was done on 6 geological zones, giving a total of 18 farms. On each farm, surface soil samples were collected representing a bulked sample, sample from eroded portion and sample from non-eroded portion of the farm. This gives a total of 54 soil samples used for the study. These soil samples were collected based on differences in erosion-related morphological properties, such as thickness of A-horizon, incidence of rills, topography, sandiness and soil colour. The farms were under similar management practices and of 2-year fallow length. Soil samples were air-dried, crushed and sieved using 2-mm sieve in readiness for various laboratory analyses on selected chemical prosperities. The 18 bulked soil samples were used for characterizing surface soil of the studies farmlands while samples from eroded and non-eroded portions were used in investigating status of soil degradation.

**Laboratory analyses:** Particle size distribution was determined by hydrometer method according to the procedure of Gee and Or (2002). Soil pH water was estimated electrometrically in soil liquid ratio of 1:2.5 as described by Hendershot *et al*. (1993). Soil organic carbon was estimated by wet digestion (Nelson and Sommers, 1996). Total nitrogen (TN) was determined by microkjeldahl method (Bremner, 1996). Total sulphur content of soils was estimated by potassium nitrate/nitric acid digestion method (Blauchar, 1986) while available phosphorus was obtained by Bray 2 method (Olsen and Sommers, 1982). Exchangeable cations were estimated by inductively coupled plasma atomic emission spectrometer (ICP-AES) (Integra XMP, GBC, Arlington Heights, IL).

**Soil degradation determinations:** Status of soil degradation was computed from results of laboratory analyses of samples from eroded (E) and non eroded (NE) portions of the farmland, using the land degradation index as described by Barrow (1992). The LDI is given as follows:

$$LDI = \left( \frac{D}{ND} \times 100\ \% \right) - 100$$

Where LDI = Land degradation index
D = Value of soil parameter in the degraded tract
ND = Value of soil parameter in the non –degraded tract
100% = Percentage grade
100 = Constant representing ideal soil state

**Statistical analyses:** Principal component analysis was performed on the values of soil chemical properties measured in the on – farm studies with the aid of SAS computer package after values have been subjected to linear correlation analysis to produce correlation matrix. Cluster analysis was used to group the 18 farmers fields according to similarity in certain response patterns.

**Results and Discussion**

**Soil properties:** With the exception of shale-derived soils, other soils were very sandy (Table 1) and this is attributable to the nature of parent materials. Climate promotes physical weathering, which according to Esser *et al*.(1992) exerts a great influence on the distribution of silt and clay fractions. Soils were strongly to moderately acidic, possibly due to differences in parent materials and land use since all the sampled points are within similar agroecology. Exchangeable basic cations were low, suggesting that they have been leached away and/or excessively used for crop production without replenishment as it is a low-input low- output agriculture. Organic fractions were low, and this is consistent with the findings of Igwe and Stahr (2004) in their study of soil of southeastern Nigeria. The low organic fractions content could be attributed to young age of fallow coupled with influences of high temperature and associated rapid mineralization (Esu *et al.,* 1991). Low values of available phosphorus (Av. P) were found in all soils studied irrespective of lithological origin. Although not investigated, P- availability is governed by one or a combination of chemical and mineralogical properties, such as clay type, clay content, sesquioxides, organic carbon, pH and calcium carbonate content (Burt *et al.,* 2002). Again, there are many mechanisms through which it can leave the soil through uptake, runoff and leaching (Giesler et al., 2005). Sulphur content is relatively high and Isirimah *et al.* (2003) attributed this to strong to moderate acidity of soils. Results of soil chemical properties in eroded (E) and non eroded tracts of farmands are shown in Table 2, indicating lower values of studies parameters in E parts of the farm in all the locations. These results reflect on the negative values of land Degradation Index (LDI) as presented on Table 3. The negative values indicate losses and this differed with location and chemical parameters assessed. Highest percentage degradation in pH in the study (- 14.5%) was recorded on very sandy soils of Owerri (Coastal Plain Sands), Oguta (lacustrine Alluvium) and Abiriba (Upper Coal Measures). The study area is of rainforest agroecology with extreme sandiness which suggests excessive leaching of basic cations. But soils derived from Shale also showed spectacular degradation, implying that some other factors contribute to soil acidity

**Soil degradation:** High LDI values on OC were recorded mainly on soils Lower Coal Measures Abiriba and Item), Falsebedded sandstones (Umulolo and Ezere) and fluvial Alluvium (Owerrinta). With the exception of Owerrinta other locations are within the drier part of the region, characterized by sparsely vegetated rainforest. The vegetal form, climate and land use may have interactively reduced OC content of epipedal layers studies. These factors also affected basic cations content and availability of phosphorus and total nitrogen. Least LDI were recorded in total sulphur content of soil, suggesting greater sufficiency of this macronutrient in soils of central southeastern Nigeria. Higher sulphur sufficiency status is possible if organic matter is sustained the latter being a reservoir if the former (Isirimah *et al.,* 2003). Nonetheless, elemental sulphur is stable and predominates in highly oxidized to slightly reducing acidic conditions.

The main chemical factors in E and NE portions soils of the farms studied are shown in Tables 4 and 5, respectively. In eroded portions of farmlands, soil (pH PRIN 1) explained 51.8 of the total variance, followed by OC (PRIN 2), explaining 20.4% and total nitrogen (PRIN 3) , contributing 13.6% of the total variance. Similar trend was observed in NE where pH, OC and TN explained 38.7, 24.8 and 14.5% of the total variance. respectively. These results show that soil reaction controlled greatly to the distribution, abundance and activity of other chemical soil properties. Lower pH does not favour basic cations which significantly influenced soil –water relations (Dontsova *et al.,* 2004). The results suggest the relevance of pH and organic fractions in the sustainable management of soils of southeastern Nigeria. Erosion of epipedal layers which account for high soil organic storage in the study area (Onweremadu, 2007).

Cluster analysis resulted to an ordination plot of 18 farmers' fields based on similarity of LDI values (Fig. 1), indicating that all farms in the same quadrant can be managed alike. It implies that similar farm-input model can be made for farms on each quadrant and this is a hallmark in precision farming. However, there is need for inclusion of other non-chemical parameters since soil productivity is a result of interactions among soil and soil-related factors. This reduce bias and errors thus increasing predictiveness of models generated from the soil data.

Table 1. Properties of soils of the study site

| Location | Clay (g $kg^{-1}$) | Silt (g $kg^{-1}$) | Sand (g $kg^{-1}$) | pH (Water) | Ca | Mg | K | Na | OC | TN | Avp | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 890 | | ← Cmo/$kg^{-1}$ ← | | | | (g $kg^{-1}$) | | (mg $kg^{-1}$) | |
| Akwette | 90 | 20 | 870 | 5.5 | 0.9 | 1.0 | 0.3 | 1.0 | 2.14 | 0.196 | 38.0 | 140 |
| Oguta | 11.0 | 20 | 880 | 5.2 | 0.7 | 0.8 | 0.2 | 0.8 | 1.89 | 0.192 | 6.0 | 135 |
| Owerrinta | 100 | 20 | 880 | 5.1 | 0.8 | 0.9 | 0.2 | 0.6 | 1.55 | 0.98 | 7.0 | 131 |
| Okeikpe | 110 | 10 | 850 | 4.9 | 1.0 | 0.4 | 0.4 | 0.2 | 2.13 | 0.101 | 38.0 | 127 |
| Owerri | 130 | 20 | 860 | 4.9 | 0.9 | 0.4 | 0.3 | 0.2 | 2.32 | 0.212 | 38. | 132 |
| Umuahia | 120 | 20 | 700 | 5.0 | 0.6 | 0.5 | 9.2 | 0.2 | 1.92 | 0.112 | 34.0 | 127 |
| Arondkwgu | 280 | 80 | 550 | 5.1 | 2.1 | 0.6 | 0.5 | 0.2 | 2.10 | 0.146 | 5.0 | 125 |
| Ende | 350 | 100 | 600 | 5.3 | 2.5 | 1.1 | 0.3 | 0.3 | 2.25 | 0.223 | 9.0 | 129 |
| Nkporo | 300 | 100 | 750 | 5.3 | 2.6 | 0.9 | 0.4 | 0.2 | 2.28 | 0.250 | 8.0 | 123 |
| Arochukwu | 200 | 150 | 770 | 4.8 | 1.7 | 0.4 | 0.5 | 0.2 | 2.06 | 0.129 | 9.0 | 125 |
| Ohafia | 180 | 50 | 750 | 4.9 | 1.2 | 1.1 | 0.6 | 0.1 | 2.86 | 0.106 | 6.0 | 122 |
| Uturu | 170 | 80 | 920 | 4.9 | 0.8 | 0.9 | 0.5 | 0.1 | 1.51 | 0.11 | 4.0 | 123 |
| Aburiba | 60 | 20 | 890 | 4.4 | 0.6 | 0.5 | 0.2 | 0.2 | 1.22 | 0.098 | 5.0 | 120 |
| Item | 80 | 30 | 900 | 4.3 | 0.4 | 0.3 | 0.3 | 0.1 | 1.36 | 0.073 | 3.0 | 118 |
| Ihube | 70 | 30 | 900 | 4.1 | 0.8 | 0.2 | 0.2 | 0.1 | 1.13 | 0.92 | 5.0 | 117 |
| Ezere | 80 | 20 | 900 | 4.4 | 0.5 | 0.5 | 0.3 | 0.3 | 1.28 | 0.068 | 3.0 | 116 |
| Okigwe | 90 | 20 | 890 | 4.5 | 1.4 | 0.4 | 0.3 | 0.2 | 1.33 | 0.104 | 6.0 | 121 |
| Umulolo | 95 | 20 | 905 | 4.3 | 1.2 | 0.5 | 0.4 | 0.1 | 1.16 | 0.112 | 6.0 | 119 |

Table 2. Soil Chemical Properties of Eroded (E) And Non-Eroded portons of surveyed farms

| S/ No | Location | **pH Water Eroded** | OC | **TN** | **Avail P.** | Ca | **Mg** Non-eroded | **K** | Na | **S** | pH Water | **OC** | **T N** | **Avail P.** | **Ca** | **Mg** | **K** | Na | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Akwette | 5.3 | 2.03 | 0.192 | 36 | 0.70 | 0.82 | 0.18 | 0.88 | 138 | 5.9 | 2.41 | 0.256 | 44 | 1.01 | 0.98 | 0.28 | 0.92 | 148 |
| 2 | Oguta | 4.7 | 1.88 | 0.188 | 4 | 0.62 | 0.66 | 0.06 | 0.69 | 135 | 5.5 | 2.12 | 0.243 | 12 | 0.76 | 0.82 | 0.10 | 0.77 | 152 |
| 3 | Owerrinta | 4.7 | 0.86 | 0.083 | 4 | 0.68 | 0.69 | 0.14 | 0.51 | 128 | 5.3 | 1.55 | 0.116 | 14 | 0.98 | 0.86 | 0.20 | 0.63 | 144 |
| 4 | Okeikpe | 4.6 | 1.98 | 0.099 | 34 | 0.82 | 0.20 | 0.28 | 0.10 | 125 | 5.2 | 2.50 | 0.212 | 48 | 1.21 | 0.50 | 0.44 | 0.16 | 140 |
| 5 | Owerri | 4.7 | 2.02 | 0.196 | 35 | 0.71 | 0.25 | 0.16 | 0.05 | 128 | 5.5 | 2.46 | 0.251 | 50 | 0.90 | 0.40 | 0.24 | 0.06 | 136 |
| 6 | Umuahia | 4.2 | 1.61 | 0.099 | 32 | 0.40 | 0.15 | 0.06 | 0.03 | 124 | 5.4 | 1.96 | 0.242 | 70 | 0.70 | 0.40 | 0.14 | 0.06 | 156 |
| 7 | Arondizuogu | 4.8 | 1.80 | 0.141 | 3 | 1.88 | 0.90 | 0.28 | 0.09 | 122 | 5.6 | 2.55 | 0.218 | 12 | 3.00 | 1.40 | 0.36 | 0.15 | 135 |
| 8 | Bende | 5.1 | 1.96 | 0.193 | 7 | 2.06 | 0.70 | 0.18 | 0.04 | 125 | 5.9 | 2.40 | 0.222 | 15 | 3.86 | 1.10 | 0.24 | 0.05 | 136 |
| 9 | Nkporo | 5.2 | 1.98 | 0.197 | 6 | 3.20 | 0.20 | 0.26 | 0.08 | 120 | 6.0 | 2.46 | 0.231 | 13 | 3.70.2. | 0.50 | 0.30 | 0.10 | 125 |
| 10 | Arochukwu | 4.4 | 1.66 | 0.118 | 3 | 1.28 | 0.82 | 0.26 | 0.10 | 122 | 4.8 | 2.21 | 0.196 | 9 | 20 | 1.44 | 0.34 | 0.16 | 127 |
| 11 | Ohafia | 4.4 | 1.40 | 0.099 | 2 | 0.86 | 0.66 | 0.40 | 0.05 | 120 | 5.0 | 1.84 | 0.124 | 7 | 1.46 | 0.90 | 0.56 | 0.09 | 127 |
| 12 | Uturu | 4.5 | 1.01 | 0.069 | 4 | 0.50 | 0.42 | 0.09 | 0.06 | 122 | 4.8 | 2.16 | 0.198 | 10 | 1.22 | 1.40 | 0.18 | 0.15 | 128 |
| 13 | Abiriba | 4.1 | 1.03 | 0.072 | 2 | 0.42 | 0.20 | 0.06 | 0.02 | 116 | 4.8 | 2.12 | 0.194 | 8 | 0.90 | 0.90 | 0.14 | 0.06 | 124 |
| 14 | Item | 4.0 | 0.92 | 0.066 | 1 | 0.10 | 0.10 | 0.08 | 0.03 | 114 | 4.6 | 1.85 | 0.133 | 7 | 0.40 | 1.60 | 0.22 | 0.08 | 123 |
| 15 | Ihube | 3.9 | 1.12 | 0.081 | 2 | 0.50.0. | 0.18 | 0.08 | 0.03 | 112 | 4.4 | 1.94 | 0.125 | 8 | 0.90 | 0.99 | 0.18 | 0.05 | 127 |
| 16 | Ezere | 3.9 | 0.86 | 0.056 | 1 | 20 | 0.20 | 0.06 | 0.06 | 114 | 4.4 | 1.67 | 0.111 | 4 | 0.60 | 0.90 | 0.14 | 0.10 | 124 |
| 17 | Isikwoato | 4.0 | 1.10 | 0.099 | 4 | 0.90 | 0.30 | 0.16 | 0.03 | 118 | 4.5 | 1.93 | 0.127 | 9 | 1.96 | 0.80 | 0.28 | 0.05 | 130 |
| 18 | Okigwe Umulolo | 4.0 | 0.98 | 0.094 | 5 | 0.88 | 0.25 | 0.18 | 0.07 | 114 | 4.6 | 2.18 | 0.129 | 11 | 1.98 | 0.96 | 0.26 | 0.09 | 131 |

**ID:** OC (organic carbon) and T.N. (Total nitrogen) are in g $kg^{-1}$.
Ca, Mg, k and Na are in Cmol/Kg
Available phosphorus (Bray II method: 1:25) and Total sulphur are in mg $kg^{-1}$.

Table 3. Land Degradation Index (Ldi) on Farm Research (After Barrow, 1992) Parameters S

| S/No | Location | pH Water | OC. | T.N | Avail P. | Ca | Mg | K | Na | S |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Akwette | -10.1 | -15.8 | -25.0 | -18.1 | -30.6 | -16.4 | -35.8 | -4.3 | -6.7 |
| 2 | Oguta | -14.5 | -11.3 | -22.6 | -66.6 | -18.4 | -19.5 | -40.0 | -10.3 | -11.1 |
| 3 | Owerrinta | -11.3 | -44.5 | -28.4 | -71.4 | -30.6 | -19.7 | -30.0 | -19.0 | -11.1 |
| 4 | Okeikpe | -11.5 | -20.8 | -53.3 | -29.1 | -32.2 | -60.0 | -36.3 | -37.5 | -10.7 |
| 5 | Owerri | -14.5 | -17.8 | -21.9 | -30.0 | -21.1 | -50.0 | -33.3 | -16.6 | -5.8 |
| 6 | Umuahia | -22.2 | -17.8 | -59.0 | -54.2 | -42.8 | -62.5 | -57.1 | -50.0 | -20.5 |
| 7 | Arondizuogu | -14.2 | -29.4 | -35.3 | -75.0 | -37.3 | -35.7 | -22.2 | -40.0 | -9.6 |
| 8 | Bende | -13.5 | -18.3 | -13.0 | -53.3 | -46.6 | -36.3 | -25.0 | -20.0 | -8.0 |
| 9 | Nkporo | -13.3 | -19.5 | -14.7 | -46.1 | -13.5 | -60.0 | -13.3 | -20.0 | -4.0 |
| 10 | Arochukwu | -8.3 | -24.8 | -39.7 | -66.6 | -41.8 | -43.0 | -23.5 | -37.5 | -3.9 |
| 11 | Ohafia | -12.0 | -23.9 | -20.1 | -71.4 | -41.0 | -26.6 | -28.5 | -44.4 | -5.5 |
| 12 | Uturu | -6.2 | -53.2 | -65.1 | -60.0 | -59.0 | -70.0 | -50.0 | -60.0 | -4.6 |
| 13 | Abiriba | -14.5 | -51.4 | -62.8 | -75.0 | -53.3 | -77.7 | -57.1 | -66.6 | -6.4 |
| 14 | Item | -13.0 | -50.2 | -50.3 | -85.7 | -75.0 | -83.3 | -63.6 | -62.5 | -7.3 |
| 15 | Ihube | -11.3 | -42.2 | -35.2 | -75.0 | -44.4 | -81.8 | -55.5 | -40.0 | -11.8 |
| 16 | Ezere Isikwuato | -11.3 | -48.5 | -49.5 | -75.0 | -66.6 | -77.7 | -57.1 | -40.0. | -8.0 |
| 17 | Okigwe | -11.1 | -43.0 | -22.0 | -55.5 | -54.0 | -62.5 | -42.8 | -40.0 | -9.2 |
| 18 | Umulolo | -13.0 | -55.0 | -27.1 | -54.5 | -55.5 | -73.9 | -30.76 | -22.2 | -12.9 |

Table 4. Principal component analysis of soil properties in eroded portion of the farmers' fields.

| | Prin 1 | Prin 2 | Prin 3 |
|---|---|---|---|
| $pHH_2O$ | 0.433 | 0.069 | 0.031 |
| OC | 0.408 | 0.078 | -0.330 |
| TN | 0.413 | 0.058 | -0.144 |
| P | 0.226 | -0.334 | -0.588 |
| Ca | 0.250 | 0.556 | -0.059 |
| Mg | 0.292 | 0.064 | 0.623 |
| K | 0.203 | 0.482 | 0.044 |
| Na | 0.283 | -0.454 | 0.347 |
| S | 0.394 | -0.350 | 0.099 |
| Eigenvalue | 4.66 | 1.84 | 1.22 |
| % Var. | 51.8 | 20.4 | 13.6 |
| Cum. Var.% | 51.8 | 72.3 | |

Table 5. Principal Component Analysis of soil properties in the Non-Eroded portion of the farmers' fields

| | Prin 1 | Prin 2 | Prin 3 |
|---|---|---|---|
| $pH_{H2O}$ | 0.470 | 0.164 | 0.119 |
| OC | 0.356 | 0.406 | -0.082 |
| TN | 0.485 | 0.098 | 0.022 |
| P | 0.404 | -0.231 | -0.381 |
| Ca | 0.099 | 0.583 | 0.064 |
| Mg | -0.161 | 0.304 | 0.614 |
| K | 0.015 | 0.378 | -0.235 |
| Na | 0.234 | -0.259 | 0.607 |
| S | 0.404 | -0.320 | 0.172 |
| Eigenvalue | 3.48 | 2.24 | 1.31 |
| Var. % | 38.7 | 24.8 | 14.5 |
| Cum. Var.% | 38.7 | 63.5 | 78.1 |

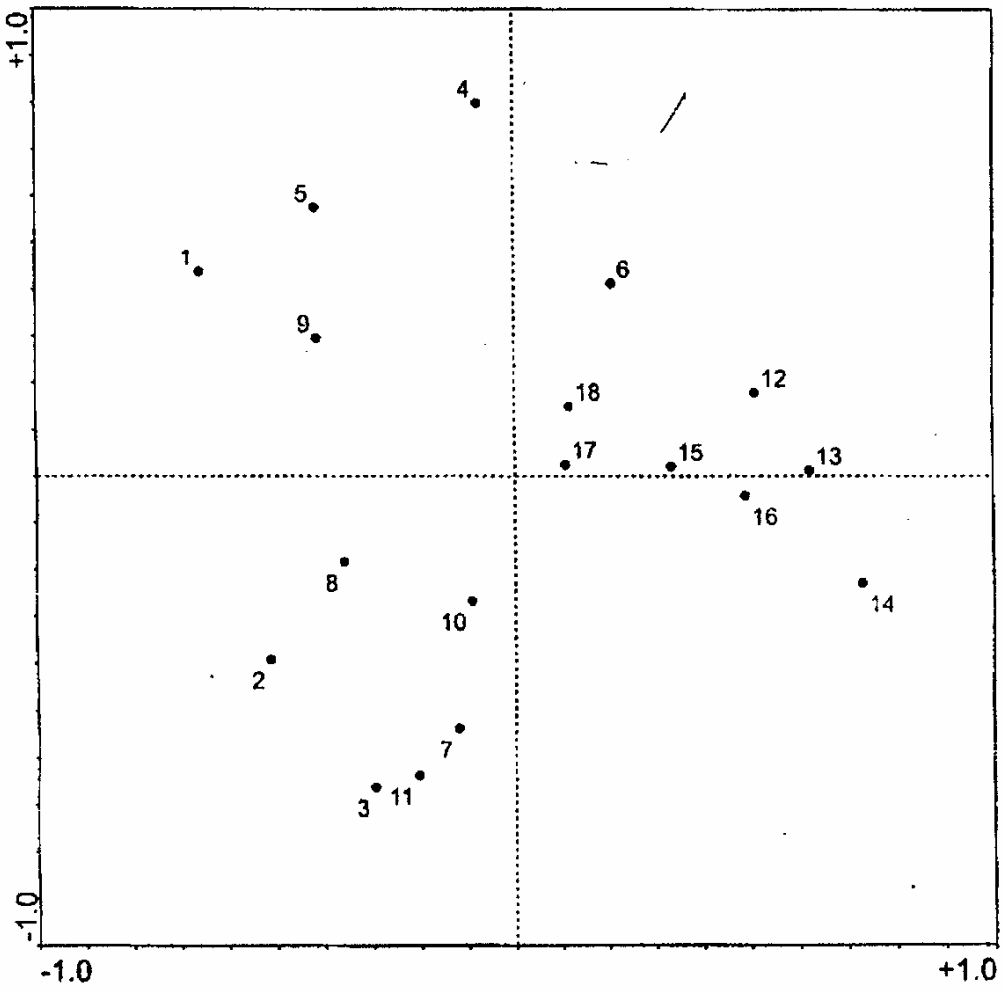


Figure 1. Ordination plot of 18 farmers fields based on similarity in land degradation index values.

| ID: | | | |
|---|---|---|---|
| | 1 = Akwette | 7 = Arondizuogu | 13 = Abiriba |
| | 2 = Oguta | 8 = Bende | 14 = Item |
| | 3 = Owerrinta | 9 = Nkporo | 15 = Ihube |
| | 4 = Okeikpe | 10 = Arochukwu | 16 = Isikwuato |
| | 5 = Owerri | 11 = Ohafia | 17 = Okigwe |
| | 6 = Umuahia | 12= Uturu | 18 = Umulolo |

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**References**


1. Akamigbo, F.O.R. 2001. Survey classification and land use in wetland soils in Nigeria. An invited paper presented at the 27th Annual conference of soil Science Society of Nigeria Nov. 5-9 2001. University of Calabar, Calabar Nigeria.
2. Barrow, C.J. 1992. Land degradation Cambridge University Press, New York.
3. Blauchar, R.W. 1986 measurement of sulphur in agriculture Agron Mongraph, ASA, CSSA and SSSA, Madison, WI. Pp. 27
4. Brady, N.C. and R.R. Weil 1999. The nature and properties of soils. 12th edition. Prentice Hall, NJ,
5. Bremner, J.M. 1996. Nitrogen total. In Sparks, D.L. (ed) Methods of soil analysis. Part 3 chemical methods. 2nd edition. ASA and SSA Book Series No 5 Madison, WI. Pp. 1085-1121.
6. Burt, R., M.D. Mays, E.C. Benhamand and J.F. Ma. 2002. Phosphorus characterization and correlation with soil properties of selected benchmark soil of the United states. Commun. Soil Sci. Plant Anal., 33: 117-141.
7. Di Falco, S., J. Chavas and M. Smale, 2006. Farmer management of production risk on degraded lands: the role of wheat genetic diversity in Tigray region, Ethiopia. Discussion paper 153, Int Foods Policy Res. Ins Washington DC pp. 30
8. Dontsova, K.M., L.D. Norton, C.T. Johnston and J.M. Bigham. 2004. Influence of exchangeable cations on water adsorption by soil clays. Soil Sci Soc Am., J., 68:1218-1227.
9. Esser, K.B., J.G. Bockhain and P.A Helmke 1992. Mineral distributions in soils formed in the Indiana dunes, USA. Geoderma, 54: 91-105
10. Esu, I.E., A. C. Odunze and J.P. Mobera 1991. Morphological, physicochemical and mineralogical properties of soil in the Talata-Mafara area of Sokoto State Samaru J. Agric. Res., 8: 41-56.
11. Eswaran, H., R. Almaraz, E. Van Den Berg and P.F. Reich 1997. An assessment of soil resources of Africa in relation to soil productivity. Geoderma, 77: 1-18.
12. Farella, N., M Cucotte, P. Louchouarn and M. Roulet. 2001 Deforestation modifying terrestrial organic transport in the Pio Tapajos, Brazilian Amazon. Org. Gecohem., 32: 1443-1458.
13. Gee, G. W and D. Or 2002. Particle size analysis. In: Dane J.H. and G.C. Topps (ed). Methods of soil analysis, part 4. physical methods. Soil Sci. Soc. Am Book series No. 5 ASA and SSSA, Madision, WI. Pp 255-293.
14. Giesler, R., T. Petersson, L. Lovgren and P. Persson. 2005 Phosphorus sorption in aluminium – and non –rich humus soils soil Sci. Soc Am. J., 67:77-86.
15. Hans, G.I. P. Jansen, J. Pender, A. Damon and R. Schipper. 2006. Rural development policies and sustainable land use in the hillside areas of Houduras: A quantities livelihoods approach. Research Report 147, Int. food Policy Res. Ins., pp 1-8.
16. Henao, J. and C. Baanante. 2001. Nutrient depletion in the agricultural soils of Africa. In: Pinstrup – Andersen, P. and R. Pandya–Lorch (eds) The unfinished agenda: Perspectives in over coming hunger, poverty and environmental degradation Int. Food policy Res. Inst., Washington, DC., pp. 159-164
17. Hendershot, W.H., H. Lalande and M. Duquette, 1993. Soil reaction and exchangeable acidity. In: carter, M.R. (ed). Soil sampling and methods of analysis. Can. Soc. Soil Sci Lewis Publishers London, pp. 141-145.
18. Igwe, C.A, and K. Stahr 2004. Water –stable aggregates of flooded of flooded inceptions from southeastern Nigeria in relation to mineralogy and chemical properties. Austr. J. Soil Res., 42:171-179.
19. Igwe, C.A. 2003. Soil degradation response to soil factors in central Eastern Nigeria. proceedings of the 28th Annual Conference of Soil Science Society of Nigeria at National Root Crops Research Institute, Umudike Umuahia, Nigeria, 4-7 Nov 2003. pp. 228-234
20. IIRR (International Institute of Rural Reconstruction). 2005. Conservation agriculture. A manual for farmers and extension workers in Africa IIRR and Act Nairabi. Kenya.
21. Isirimah, N.O., A.A. Dickson and C. Igwe. 2003. Introductory soil chemistry and biology for agriculture and biotechnology OSIA INTL Publishers Ltd, Port Harcourt, Nigeria pp. 36-97.
22. Lal, R. 1995. Erosion crop productivity relationship for soils of Africa. Soil Sci Soc Am J., 59: 661-667.
23. Maineville, N., J. Webb, M. Lucotte, R. Davidson, O. Betancourt, E. Cueva and D. Mergler. 2006 Decrease in soil fertility and release of mercury following deforestation in the Andean Amazon, Napo River valley, Ecyador. Sci. Total Environ., 368:88-98.

24. Mbagwu, J.S.C. and K. Auerswald. 1999. Relationship of percolation stability of soil aggregates to land use, selected structural indices and stimulated rainfall erosion. Soil Till Res., 50:197-206.
25. Mbagwu, J.S.C. and M.E. Obi 2003. Land degradation agricultural productivity and rural poverty. Environmental implications. Proceedings of the 28th annual conference of soil Science society of Nigeria National pool crop Research institute, Umudike Umuahia, Nigeria 407 Nov 2003 pp 1-11.
26. Mikha, M.M. and C.W. 2004. Tillage and manure effects on soil and aggregate –associated carbon and nitrogen. Soil Sci. Soc. Am, J., 68:609-816
27. Nelson D.W. and L.E. Sommers. 1996. Total carbon, organic carbon and organic matter. In: Sparks, D.L. (ed). Methods of soil analysis part, 3 changeable 2nd edition. ASA and SSSA Book sense N0 5 Madison, WI. Pp 960-1010
28. Olsen, S.R. and L.E. Sommers 1982 Phosphorus. In: Page, A.L., R.H. Miller, and D.R. keeney (eds). Methods of soil analysis, Part 2. chemical methods, ASA, Madison , WI pp 403-430.
29. Onweremadu, E.U. 1994 Investigation of soil and other related constraints to sustained agricultural prodyctivity of soils of Owerri zone in Imo State, Nigeria. M.Sc. Thesis accepted by the University of Nigeria, Nsukka, Nigeria.
30. Onweremadu, E.U. 2007. Spatial variability of soil carbon in floodplain soils of southeastern Nigeria. Int. J. Agric Res, Academic Journals USA (In Press).
31. Oti, N.N. 2002. Discriminant functions for classifying erosion degraded lands at Otamiri, Southeastern Nigeria. Agro-Sci 3 (1): 34-40.
32. Reich, P.F., S.T. Numbem, R. A Almaraz and H. Eswaran. 2001. land resource stresses and desertification in Africa Agro-Sci., 2 (2) : 1-10
33. Roulet, M., M. Lucotte, N Farella, G. Serigue, H. Coeiho and C.J. Sousci Passos. 1999. Effects of recent human colonization of the presence of mercury in Amazonian ecosystems. Water Air Soil Pollut., 112:297-313.
34. Scherr, S.J. and S. Yadav. 1996. Land degradation in the developing World: Implications for food, agriculture, and the environment to 2020. Discussion paper 14, Int. Food policy Res. Ins. Washington DC. Pp. 36.
35. Sierra, R. 2000. Dynamics and patterns of deforestation in the Western Amazon: The Napo deforestation front, 1986-1996. Appl. Geogr., 20: 1-16.
36. Thurow, T.L., A.P. Thurow, X Wu, and H Perotto-Baldivioso. 2002. Targeting soil conservation investments in handuras. Choices (Summer) pp. 20-25.

# Chromium (III) in Relation to Soil Properties and Bioaccessibility in Soils Polluted by Animal Wastes

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**Abstract**: Research on the concentration of chromium (111) (Cr 111)) was conducted in soils polluted by livestock wastes before the onset of the rainy seasons in 2006. Soil samples were randomly collected from surface soil horizons of 5 locations near university livestock farm. Soil samples were air-dried, crushed and sieved before laboratory analysis for some soil properties and chromium (Cr) analysis. Results showed that bioaccessibility of Cr (111) was constantly higher for I day aging than in 100 days aging. The amount of Cr (111) oxidized to Cr (V1) was less than 10 gm $kg^{-1}$ in the studied soils. Soil properties influenced both Cr (111) sorption and bioaccessibility, with clay, total organic carbon (TOC), total inorganic carbon (TIC), double deionized pH (pH DD1), and cation exchange pH capacity (CEC) giving a high $r^2$ value of 0.8. In all the models, variance inflation factor (VIF) was less than 4.0, suggesting insignificant collinearity and high reliability. The model can therefore be used as a good decision support tool in remediation of soils in the area. [Nature and Science. 2007;5(2):30-36] (ISSN: 1545-0740).

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**Keywords:** Bioavailability, collinearity, edaphic properties, heavy metal, modeling, soil pollution, and sorption

## Introduction

Heavy metals, such as chromium, lead, calcium and mercury, have not been shown to be essential for life. (Isirimah et al., 2003) but they are continuously introduced into the environment by anthropogenic activities. The principal sources of heavy metals pollution in the environment are domestic sewage sludge, industrial processing of ores, waste dump s, animal and human excrete, and these heavy metals are released in assorted and unjustifiable amounts (Nweke and Ekpete, 2003). Chromium (Cr) is one of such biotoxic heavy metals whose presence in the environment is widespread due to usage in many agro- industrial processes.

Chromium is thermodynamically stable in two oxidative states, namely chromium (111), which is cationic, and chromium (IV), which is anionic

It has been reported that cationic chromium is more stable and less problematic while the reverse is the case for the latter (Patterson et al., 1997). Isirimah et al., (2003) observed that Cr (VI) moves in well-aerated soils of moderate to high pH. These two major forms of Cr pose health risks when ingested (Skoworonski et al., 2001) but Cr (V1) is considered most harmful being carcinogenic and mutagenic at low concentration (Levis and Bianchi, 1992). However, the presence of Cr (111) in an environment is not healthful due to its potential to oxidize to hexavalent forming (Fendorf et al., 1992) thus become more bioaccessible (Rodriguez et al., 1998; Skoworonski et al., 2001). Decreased mobility of Cr (111) form in soils has been attributed to strong adsorption to the charged soil surface, formation of iron and aluminum oxides and hydroxides as well as formation of complex molecules with organic compounds in the soil. These organo metallic derivatives are often volatile and may concentrate in fatty tissue and cause chromosomal damage (Nweke and Ekpete, 2003).The main aim of this study was to assess the distribution of chromium Cr (111), relating it to some properties of polluted soil proximal to a livestock farm in Owerri municipality southeastern Nigeria.Specific objective was to relate the findings in the main objective to bioaccessibility.

## Materials and Methods

**Study area**: The study was conducted before the onset of dry seasons in 2006 at Owerri southeastern Nigeria, lying on latitude $5^0$ 43'14", 623 and longitude $7^0$ 39' 34".490 (Handheld Global Positioning System) Receiver (Garmin LTD, Kansas, USA). Coastal plains sands (Benin Formation) are the

predominating geological material in the area (Orajaka, 1975). The area is dominated by lowland geomorphology (Ofomata, 1975) and the site is specifically 55 meters above sea level (Readings of Handled Global Positioning System) Receiver (Garmin, LTD, Kansas, USA). Owerri has mean annual rainfall ranging from 2250 to 2500 mm and mean annual temperature range of 27-28$^0$C (Federal Department of Agricultural Land Resources, 1985). Three months of dryness and bimodal rainfall peak characterize the study area.

The predominant vegetation is evergreen rainforest whose density has been reduced by farming and deforestation. Small/ holder livestock production is common with over 80% of rural families keeping West Africa Dwarf ruminants and mixed breeds of local and exotic chicken (Ejiogu, 1990). But, urbanization has led to the establishment of commercial farms, especially poultry and piggery while facilities of Agriculture in five tertiary institutions located in the town resulted in the mounting of livestock demonstration farms, including the Teaching and Research Farm of the Federal University of Technology Owerri, Nigeria. Consequent upon, dump sites are found near livestock farms. The livestock farm influences about. 10,000m$^2$ of arable farmland: Random surface soil sampling field studies was done at the dumpsite Six sampling sites were identified during a reconnaissance visit to the area, and 5 soil samples were collected from each sampling site. Soil samples were triplicates giving a total of 90 soil samples for the study. Soil samples were in air-dried, crushed and sieved using 2-mm sieve. Sieved soils were further disaggregated by gently grinding them in a mortar using a pestle, and this was re-sieved to provide a particle size fraction less than 250 mm (Rodriguez et al, 1999).

**Laboratory analysis:** Silt and clay fractions of soil samples were determined by hydrometer method (Gee and Or, 2002). Soil pH was estimated using double deionized (DDI) water in soil/solution ratio of 1:2. The pH of the clear supernatural was measured with a microprocessor ionalyzer/901 9orion Research, Beverly, MA) using a combination of glass and calomel electrode (Beckman Fullerton, CA). Total organic carbon (TOC) and total inorganic carbon (TIC) were determined by combustion method using Perkin-Elmer 2400 Series 11 CHNS/O analyzer. Before the determination of TOC, soil samples were pre-treated to remove TIC, which involved near boiling, 3 M HCL extraction method on agitated samples. Soil TIC was calculated as a deference between total soil carbon, which involved no pretreatment, and TOC. Extractable iron oxide (FeO) and Manganese oxide (MnO) were estimated using dithionite – citrate-bicarbonate (DCB) methods (Mehra and Jackson, 1960). Total chromium (Cr) in soils was estimated using a modified EPA method 3052. In this method, the soil was digested in a CEM microwave; model MDS-8ID, with hydro fluoric and nitric acid. Earlier, boric acid was added before sample analysis to facilitate the removal fluoric from solution through the formation of fluoroboric acid.

These soil samples were stored and analyzed for total $Cr_T$ using inductively coupled plasma (ICP).

**Bioaccessibility:** Ten grams of sieved soil was weighed and placed in 200-ml glass centrifuge vessel along with 100 ml of 500 mg kg $^{-1}$ Cr (111) as Cr CL at pH 4.The slurry was agitated on a reciprocal shaker for 48 hours, centrifuged and the supernatant decanted for analysis. This was done 4 times and after the fourth addition of Cr, soils were washed 3 times with DDI water and air-dried. After air-drying, soils were crushed, homogenized and wetted with DDI water to achieve a 25% moisture content. Thereafter, soils were kept in a container out of direct light and 25% moisture content was maintained. This was followed by the inclusion of soils for 100 days (duration of study).In vitro bioaccessibility was attempted in which a physiologically based extraction test (PBET) was adopted from Ruby et al. (1999). Triplicate 0.4 g moist samples were placed in 50 ml polyethylene tubes to which 30ml 0.4 m glycerine at pH 1.5 and temperature of 37$^0$ agitated at 30+_ 2 revolutions per minute (rpm) for 60 minutes. The supernatant was separated from the solid through centrifugation. The pH of the supernatant was measured to ensure that the pH was within +_0.5 units of the initial pH. Then bioaccesibility was computed as follows:

$$\textbf{\%}\ \text{Bioaccessibility} = \frac{(\text{Cr in PBET supernatant (Ug mL}^{-1}) \times 30\ \text{mL}/0.3\text{g dry soil})}{(\text{Cr in soil surface (mg kg} -1)}$$

After this, Cr analysis was performed. The PBET supernatant, soil spiking solution and equilibrium solution were measured for Cr and equilibrium solution were measured for Cr (VI) and $Cr_T$. The value of Cr(VI) was estimated using a modified s-diphenyl- carbohydrazide colourimetric method (Bartlett and James, 1979) using a UV-VIS Spectrophotometer at a wavelength of 540 Nm (HP model

8453(VI)immediately to prevent reduction of Cr (VI) to Cr(III), Palo Alto, CA). Rapidly cooled PBET solutions were used to perform analysis of Cr (III) by glycine decoding to Jardine *et al., (*1999). Total chromium was estimated using Perkin Elmer Analyst 800 atomic absorption spectrophotometer (Wesley P.A.). Value of Cr(III) was computed as a difference between $Cr_T$ and Cr (Vi).

**Data analysis:** Statistical analyses were conducted in SAS version 8.0 (SAS Institute, 2001). Logarithmic functions were used to minimize soil variables, which typically spanned several orders of magnitude. Pearson correlation analysis with uncorrected probabilities was used to examine relationships between bioaccessibility of Cr (III) and selected soil properties, Soil parameters with P- values < 0.10 were considered in a stepwise multiple linear regression analysis to develop models predicting bioaccessibility of Cr (III) from soil properties

**RESULTS**

**Soil properties**: Results of selected soil properties in the studied soils are shown in Table 1. Soils were generally acidic and of low cation exchange capacity. Soil pH values were slightly higher than those reported by Mbagwu *et al*. (2001) in the same region. Silt and clay contents were low and these results are consistent with the findings of Igwe et al. (1995). In all soil locations, total organic carbon (TOC) dominated total inorganic carbon (TIC). The same trend was exhibited by extractable iron oxide (FeO) over extractable manganese oxide (MnO). Earlier studies in the region (Igwe and Stalr, 2004) reported least values of MnO when compared with oxides of iron, aluminum and potassium.

**Soil properties and chromium sorption**

Chromium sorption (Cr III) in soils are related according to the data on Table 2. Soil pH influenced Cr adsorption and desorption, and consequently surface precipitation. The Cr (III) was increasingly precipitated as pH rose above 5.0 while it was complexed at lower pH value. Similarly, TIC increased Cr(III) sorption but there was no correlation between soil pH and TIC, suggesting a local effect of soil pH in Cr(III) sorption. The model also suggested that cation exchange capacity (CEC) promoted Cr (III) sorption, that is the higher the (CEC), the greater the Cr (III) sorption. Among the particle sizes determined, clay content had a positive correlation with Cr (III) adsorption and this could be attributed to the predominantly negatively charged sites of clay minerals. This condition is created by isomorphic substitution taking place at the charged sites. (Klein and Hurlbut, 1993). Negatively charged sites attract $Cr^{3+}$ leading to the formation of an electrostatic bond. Relating this to CEC, it implies that greater availability of negatively charged sites increases CEC and as a greater propensity for $Cr^{3+}$ to sorb. Again, clay sized particles have largest specific surface suggesting greater ability to attract and accommodate $Cr^{3+}$ . In a similar study, Kaiser *et al*. (1996) observed increased sorption of dissolved organic carbon with increased surface area. By these results, the independent variables of soil pH, Clay content, CEC and soil carbon component can be used to predict Cr (III) sorption as dependent variable. Value of variance Inflation factor (VIF) was slightly larger than no multicollinearity among tested variables. The relationship describing Cr (III) sorption was:

Cr (III) (g $kg^{-1}$ on soil) = -186.3 + (82.9 x clay) +113.3x CEC) +(180 X TIC) +(184.4 pHDDI)…..(1)

The Cr (III) sorption was strongly correlated with soil properties ($r^2$ =0.6).

**Aging, soil properties and chromium bioaccessibility**: Aging influenced the amount of Cr (III) that is soluble and available in soils. Bioaccessibility values were consistently higher for 1 day aging compared with 100 days (Table 3), with values ranging from 12.2 to 21.4 g $kg^{-1}$ and 5.1 to 8.6 g $kg^{-1}$ for 1day and 100 days, respectively. As obtained from the PBET extractment, a value of Cr (VI) following the oxidation of Cr (III) was less than 10 g $kg^{-1}$ (that is less than 1%).Bioaccessibility of Cr (III) was influenced by clay content, TIC, TOC, pH, $pH_{DDI}$ (DDI) and CEC (Table 4). The model attributes show that Cr (III) bioaccessibility decreases as TIC increases and clay content decreases. In other words, as the clay content increased, the quantity that is bioaccessible also increased. Step-wise multiple regression analysis also suggested that Cr (III bioaccessibility was significantly correlated with, clay (P<_0.001) and TOC (P<_ 0.001) of soils (Table 4). The relationships explaining Cr (III) bioaccessibility is given as follows:

Cr (III bioaccessible =17.6 +(0.5 x clay
- (4.1 x TOC)……..(2)
Cr (III) bioaccessible = 17.6 +(6.3 X pH) + (7.1 X CEC
- (8.2 x TIC) ……(3)

With an $r^2$ value of 0.8, the P value was less than 0.01, implying that parameter attributes were statistically rigorous at 99% confidence, with VIF values approximately 1.000(Table 4)

Table 1. Selected properties of soils studied (mean Values)

| Location | TOC G $kg^{-1}$ | TIC G $kg^{-1}$ | FeO G $kg^{-1}$ | MnO g $kg^{-1}$ | Silt G $kg^{-1}$ | Clay G $kg^{-1}$ | CEC cmol $kg^{-1}$ | pH DD1 |
|---|---|---|---|---|---|---|---|---|
| A1 | 1.6 | 0.6 | 5.1 | 1.0 | 20 | 50 | 6.1 | 5.6 |
| A2 | 0.9 | 0.2 | 4.8 | 0.4 | 18 | 80 | 7.7 | 5.8 |
| A3 | 1.7 | 0.5 | 2.8 | 0.3 | 19 | 87 | 78 | 5.6 |
| A4 | 2.0 | 0.4 | 5.2 | 1.0 | 21 | 92 | 9.2 | 5.7 |
| A5 | 1.1 | 0.6 | 2.2 | O.2 | 20 | 82 | 6.2 | 4.9 |
| B1 | 1.2 | 0.4 | 4.6 | 0.9 | 22 | 79 | 6.3 | 4.8 |
| B2 | 0.9 | 0.4 | 4.0 | 0.7 | 18 | 68 | 5.8 | 4.9 |
| B3 | 2.7 | 0.5 | 3.6 | 0.6 | 19 | 70 | 8.8 | 5.3 |
| B4 | 2.1 | 0.3 | 1.9 | 0.2 | 16 | 68 | 8.2 | 5.6 |
| B5 | 0.8 | 0.5 | 2.4 | 0.3 | 14 | 81 | 4.8 | 5.8 |
| C1 | 2.2 | 0.3 | 1.9 | 0.2 | 18 | 70 | 8.3 | 5.8 |
| C2 | 1.6 | 0.5 | 1.6 | 0.1 | 25 | 74 | 6.0 | 4.8 |
| C3 | 1.1 | 0.5 | 1.9 | 0.3 | 2.0 | 68 | 5.8 | 4.9 |
| C4 | 1.6 | 0.4 | 4.8 | 0.8 | 21 | 82 | 5.9 | 4.9 |
| C5 | 1.9 | 0.5 | 5.2 | 1.0 | 18 | 79 | 6.0 | 5.0 |
| D1 | 1.2 | 0.3 | 2.3 | 0.3 | 30 | 90 | 10.6 | 5.4 |
| D2 | 0.8 | 0.4 | 2.6 | 0.5 | 22 | 6.8 | 4.8 | 4.8 |
| D3 | 0.9 | 0.4 | 2.1 | 0.2 | 28 | 72 | 4.8 | 5.0 |
| D4 | 2.6 | 0.5 | 2.6 | 0.4 | 24 | 68 | 8.8 | 5.8 |
| D5 | 1.8 | 0.5 | 4.9 | 0.8 | 21 | 71 | 6.0 | 4.8 |
| E1 | 0.9 | 0.6 | 5.1 | 0.9 | 20 | 72 | 4.6 | 4.7 |
| E2 | 1.2 | 0.4 | 4.2 | 0.6 | 21 | 73 | 4.8 | 4.7 |
| E3 | 1.5 | 0.3 | 6.1 | 1.1 | 18 | 80 | 8.6 | 5.8 |
| E4 | 2.8 | 0.5 | 2.9 | 0.4 | 22 | 69 | 2.8 | 5.3 |
| E5 | 2.1 | 0.4 | 2.2 | 0.3 | 26 | 72 | 7.6 | 5.7 |
| F1 | 1.6 | 0.5 | 3.1 | 0.6 | 20 | 83 | 8.9 | 5.8 |
| F2 | 1.9 | O.4 | 2.2 | 0.3 | 18 | 82 | 9.6 | 5.6 |
| F3 | 2.1 | 0.5 | 1.8 | 0.2 | 19 | 78 | 8.6 | 5.5 |
| F4 | 2.0 | | 2.0 | 0.3 | 22 | 82 | 10.0 | 5.3 |
| F5 | 1.8 | | 2.6 | 0.3 | 18 | 81 | 9.6 | 5.2 |

TOC = total organic carbon, TIC =total inorganic carbon, CEC=cation exchange capacity, DDl = double deionized, FeO = extractable Fe oxide, MnO = extractable Mn oxide.

Table 2. Parameter estimates, standard errors and other statistics got from a multiple regression analysis relating Cr(III) sorption to some soil properties

| Parameter | Value | SE | P | V/F |
|---|---|---|---|---|
| Intercept | -186.4 | 64.1 | <0.001 | - |
| Clay (g $kg^{-1}$) | 82.9 | 17.8 | <0.001 | 1.016 |
| CEC (cmol $kg^{-1}$) | 113.3 | 23.2 | 0.015 | 1.080 |
| TIC (g $kg^{-1}$) | 180.2 | 56.6 | 0.042 | 1.524 |
| pH (DDl) | 184.4 | 32.4 | <0.001 | 1.801 |
| $r^2$ | 0.6 | | <0.001 | |

Table 3. Solid phase concentrations of Cr (III) in soils with their corresponding bioaccessibility after 1 and 100 days incubation (mean –values)

| Location | Cr- in soil (g $kg^{-1}$) | Cr (III) bioaccessibile 1 day. (g $kg^{-1}$) | Cr(III) 100 days (g $kg^{-1}$) | Boiaccessible Value of Oxidized Cr (VI) ( g $kg^{-1}$) |
|---|---|---|---|---|
| A | 38.3 | 16.4 | 7.6 | 4.2 |
| B | 42.1 | 19.6 | 8.2 | 4.5 |
| C | 53.6 | 21.4 | 8.6 | 4.7 |
| D | 36.8 | 15.3 | 6.6 | 3.8 |
| E | 33.2 | 12.2 | 5.1 | 2.9 |
| F | 44.6 | 20.8 | 8.3 | 4.1 |

Table 4. Parameter estimates, standard errors and other statistics obtained from a multiple linear regression analysis that related some soil properties to Cr (III) bioaccessibility.

| Parameters | Value | SE | P | VIF |
|---|---|---|---|---|
| Intercept | 17.6 | 2.1 | <0.001 | - |
| Clay content (g $kg^{-1}$) | 0.5 | 0.8 | <0.001 | 1.001 |
| TOC (g $kg^{-1}$) | -4.1 | 0.7 | <0.001 | 1.001 |
| TIC (g $kg^{-1}$) | -8.2 | 0.9 | <0.001 | 1.002 |
| $PH_{DDI}$ | 6.3 | 1.2 | <0.001 | 1.002 |
| CEC (Cmo1$kg^{-1}$) | 7.1 | 0.8 | <0.001 | 1.002 |
| $r^2$ | 0.8 | | <0.001 | |

SE = Standard error, P = probability, VIF = Variance inflation factor, TOC = Total organic carbon, TIC = Total inorganic carbon.

**Discussions**

Generally, soils of the study site have high potentials for sorption activities, Presence of organic deposits from livestock pens coupled with presence of FeO, suggest high sorption of Cr (III). Gerke and Hermann (1992) showed that the phosphate sorption in Fe-humic substance mixture was six to seven times larger than amorphous iron oxide, due to the formation of ternary complex between humic substances and phosphate. Similarly, Fe- humic substances create negative charges, which may attract $Cr^{3+}$ in soils. On the other, the more mobile Cr(VI) anions may be attracted to the positively charged ferric substances in the highly oxidized soils of the study area. The presence of MnO in soils although low has great influence on

sorption behaviour of soils of the site. Negra *et al*.(2005) noted that the chemical influence of Mno in the soil system may be much greater than that suggested by its relatively low abundance. Manganese oxides are powerful oxidizers known to oxidize Cr (III) to Cr (VI) (Kozuh *et al.,* 2000; Kim *et al*. 2002). The values of FeO and MnO may have influenced sorption of Cr (III0 )although they were not used as independent variables in the study

The determined soil properties had significant effect on Cr (III) sorption (P<_0.05) (Table 2). The values of VIF suggested that collinearity between the independent variables was not significant (Table 2) and it can be inferred from this result that the parameter estimates are reliable. Because, VIF values were less than 4.0, collinearitly does not pose a problem in this model

Bioaccessibility of Cr (III) decreased towards 100 days, suggesting that the aging effect is related to the enhanced stability of Cr (III) on the soil surface with time. Karthein et al. (1991) attributed this sampling with time to structural re-orientation of Cr surface, bonds and slow precipitation reaction . It is also possible that after 100 days, aging effects on bioaccessibility decreases to insignificance. Bioaccessibility of Cr (III) in soils did not show any distinct trend in response to location, and this could be attributed to low intensive field sampling of soils used in the determination.

Low values of oxidized Cr (III) (that is, Cr (III) to Cr (VI) values, tend to suggest minimal oxidation reactions due to low MnO content of soils. It is also possible that oxidized products of anionic chromium (Cr) were tightly held by the soil exchange complex. As surface soil samples were used in the study, organic matter may have reduced oxidized Cr (Eary and Rai, 1991. Fendorf and Li, 19960).

Model attributes (Table 4) indicate that the parameter had good association; ($r^2$ – 0.8), hence reliable predictions of Cr (III) sorption in the site. It implies that 80% of the variability in Cr (III) bioaccessibility was explained. Low values of VIF (nearly 1.000) shows that there was no redundant information in soil properties and there was insignificant co- linearity between independent variables. Low standards error (SE) on the estimated values (Table 4) are further indicators of the reliability of parameter estimates.

Based on the results of the step- wise multiple regressions (Table 4), Cr (III) had an inverse relationship with the TIC implying that as TIC, increases bioaccessibility of Cr (III) decreases. Total inorganic carbon promotes the formation of solid phase Cr (III) hydroxides, which are sparingly soluble even undersevere acidic soil milieu. The precipitation of these hydroxides cause them in cover the soil surfaces thereby making Cr (III0) difficulty bioaccessible. Also the relationship between bioaccessibility and TOC and Clay content was smaller to that relating it to TIC that relating increased TOC resulted to decreased bioaccessibility of Cr (III). Organic matter is a major sorbent for heavy metal cations (Sparks, 1995) since it substantially contributes to the generation of negative charges in the soil system. These heavy metal cations when complexed are not easily relased since carboxylic bonds hold them. The implication of these complexation reactions between heavy metal cation and TOC is that groundwater is sparingly contaminated.

## Conclusions

Soil properties influenced Cr (III) sorption characteristics in the studied soils. The amount of bioaccessible Cr (III) also depended on the soil properties and decreased with time. Bioaccessiblity of Cr (III) can be predicted with high degree of reliability using clay content, TIC, TOC, pH and CEC, having an $r^2$ value of 0.8.

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## References

1. Bartlett, R. J., and Kimble, J.M. Behaviour of chromium in soils: Trivalent Forms. J.Environ.Qual; 1979 53:379-383

2. Eary, L. E. Rai, D. Chromate reduction by surface under acidic conditions. Soil Sci. Soc. Am. J 1991., 55:676-683.
3. Fedorf, S.E. Li,. G. Kinetics of chromate reduction by ferrous iron. Environmental. Science. Technolology 1996, 30:1614-1617.
4. Gee, G.W. Or, D. Particle size analysis. In: Dane. J.H. and Topp. G.C. (eds). Methods of soil analysis, Part 4. Physical methods. Soil Sci. Soc. Am Book series No.5 ASA and SSSA, Madison, WI 2002, 255-293
5. Gerke, J., Harmann, R. Adsorption of orthophosphate to humic- Fe complexes and to amorphous
6. Fe-oxide. Z. Pflanzenernachr. Bodenkd, 1992 155:233_236.
7. Isirimah, N.O., Dickson, A.A, Igwe, C.A. 2003. Introductory oil chemistry and biology for agriculture and biotechnology. OSIA Int'L Publishers LTD, Port Harcourt, Nigeria 2003
8. Jardiine, P. M., Fendorf, S.E; Mayes, M.A., Brooks, S.C., and Bailey, W.B. Fate and transport of hexavent chromium in undisturbed heterogeneous soil. Environ.Sci. Technol., 33:2939-2944.
9. Kaise, L, Guggernberger, G Zech. W. Sorption of DOM and DOM fractions in Forest soils. Geoderma,1996 74:281-303
10. Klein, C Hurbut, C. (1993). Manual of mineralogy.21st edition. Wiky, New York.
11. Kim, J.G., Dixon, J. B. Chusuei, C.C., Deng, I. Oxidation of chromium (III) to (VI) by manganese oxides. Soil. Sci. Am. J.,2002 66:306-315
12. Kozuh, N., Stipar, J. Goene, B.Reduction and oxidation processes of chromium in soils. Environmental. Science. Technology 2000, 34:112
13. Levis, A.G. Bianchi, V. Mutagenic and cytogenic excess of chromium compounds. In Langjards, S. (ed) Biological and environmental aspects of chromium. Elsevier Biomedical Press, New York 1982, 171-208
14. Mehra, O.P Jackson, M.L Iron oxide removed from soils and clays by a dittronite- citrate buffered with sodium incaclonate. Clays Miner 1960 7:317-327.
15. Negra, C.C., Ross, D.S, Lanzirotti, A. Oxidizing behaviour of soil manganese: Interactions among abundance, oxidation state, and pH. Soil Sci. Soc. Am.2005, 69:87-95
16. Nweke, S.O, Ekpete, O.A. Qualitative determination of Hg, As and Cd in Ntanwo-Ogba Creek Water Africa. J.Environ. Stud 2003. 4:73-75
17. Patterson, R..R., Fendorf, S.and Fendorf, M. Reduction of hexavalent chromium by amorphous non-sulphide. Environmental. Science Technology 1999 31:2039-2044.
18. Rodriguez, R.R.. Basta, N.T., Casted, S.W, and Pace, L.W. (1999). Ai in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid and solid media. Enviro. Sci. Technology. 33:642-640.
19. Ruby, M.V., School, R., Brat TIN, w., GOLDADE, m., post, Post, G., Harnois, M., Mosby, D.E., Casted, S.W, Berti.,W., Carpenter, M., Edwards, D., Cragin, D., Chappel, W. Advances in evaluating the oral bioaccessibility of in organics in soils for use in human risk assessment. Environmental. Science Technology. 1999, 33:3697-3705.
20. SAS Institute. (2001). SAS version 8.0 cary, N.C. Igwe, C.A Stalir, K. Water-stable aggregates of flooded inceptisols from southeastern Nigeria I relation to mineralogy and chemical properties and yield of maize and Bambara groundnutAustralian Journal of Soil Research. 42: 171-179.
21. Showoronski, G., Seide, M, Abdel-Rahman, M.S. Oral biaccessibility of trivalent and hexavelent chromium in soil by simulated gastric fluid. . J. Toxicol. Environ. Health, Health 2001, 63:351-362.
22. Sparks, D.L. Environmental soil chemistry. Academic Press, New York, 1995.

# Micropropagation of Cassava (*Manihot esculantum* Crantz) Using Different Concentrations of Benzyaminiopurine (BAP)

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**Abstract:** An investigation conducted on the effect of Benzylaminopurine (BAP) on cassava plantlets cultures. Murashige and skoog medium containing different concentrations of BAP was used to subculture two varieties of cassava plantlets; TMS 98/0379 and TMS 98/0581, which were thereafter incubated in a culture room at 28°C ± 2 and exposed to artificial illumination of 2000 -2500 lux for sixteen hours daily. Results showed that BAP application had some inhibitory effects on the two cassava varieties as only the control treatment (no BAP application) recorded the best growth in most of the growth parameters observed (height, leaves, nodes), and thus differing significantly ($P<0.05$) from other BAP levels. Between the experimental units, TMS 98/0379 appeared to grow better in terms of height, leaf and number of nodes while TMS 98/0581 recorded the greatest fresh weight.[ Nature and Science. 2007;5(2):37-40] (ISSN:1545-0740).

## Introduction

Cassava improvement for increased production necessitates addressing various factors that beset its production, which include pests and diseases, requirement of large quantities of planting material for its propagation and low multiplication ratio (Dahniya and kellon, 1983; Okigbo, 1986). Although plant breeding and genetics has contributed immensely in the improvement of crop plants, it has been observed more recently that micropropagation has become an irreplaceable tool in the improvement and genetic manipulation of plants especially vegetatively propagated crops (Onwubiko and Mbanaso, 2006). And fortunately cassava is ranked fourth in order of importance after rice, wheat and maize in crops for micropropagation in Africa (Johan *et al*, 1998).

The establishment of culture media and adjustment of their concentrations are the keys to success in micropropagation. Generally, cassava researchers have used specific treatment to obtain good development (Guohua, 1998; Joseph *et al*, 1999; Peng *et al*; 2001; Matand *et al*, 2004). Also studies on other crops have reported controlling some Morphogenic developmental stages using a single treatment (Triagiano and Gray, 1996; Matand *et al*, 2004). This work therefore is an investigation on the effect of various levels of Benzylaminopurine (BAP) composition of culture media interacted with two cassava varieties for regeneration of cassava plants from stem explants.

## Materials and Method

This study was carried out in the tissue culture laboratory of National Root Crop Research Institute (NRCRI) Umudike, Abia State Nigeria. Umudike is located at latitude 5° 25[1]N, longitude 7° 35[1] and at 122m above sea level. The two cassava plantlets (TMS 98/0379 and TMS 98/0581) used for this study were collected from the tissue room in the tissue culture laboratory of NRCRI Umudike and the nutrient media (BAP) contained inorganic and organic constituents. Six different levels of BAP concentrations (0, 0.25, 0.5, 0. 75, 1.0 and 1.25 $Mgl^{-1}$) were used for the study.

The two cassava varieties used were five months old plantlets cultured on a basal medium with height above 5cm. The Plantlets were brought out on Petri dishes using sterile forcepts and the tissues subdivided into one-node length sterile scalpel. The node cuttings were transferred into fresh media of different BAP concentrations. The necks of the culture vessels were flamed before replacing the cover. All these operations were carried out under strict aseptic conditions in the laminar airflow cabinet. The culture vessels were carefully labeled and incubated in the culture room at 28°C ±2 where they received artificial illumination of 2000-2500 Lux for 16 hours daily.

The experimental design used for this study was a 2x6 factorial in a completely randomized design (CRD) with 5 replications.

Data collection on plant height, number of leaves, number of developed nodes were collected on 2 weeks interval for 6 weeks after subculture (WAS). With electronic weighing balance fresh weight of the

plantlets was determined after removal of plantlets from culture vessels on the 6th week. The statistical analysis used in analyzing the result collected was genstat.

**Results**

At 2,4 and 6 WAS, BAP application did not influence plant height in the two cassava varieties, rather in the control there was significant difference($p<0.05$) in plant height as the greatest mean (3.88) was recorded (Table 1) Again at BAP level 1.25 $mgt^{-1}$ TMS 98/0581 recorded a high percentage of callus formation showing *in vitro* recalcitrance.

The result on number of cassava leaves at 2,4 and 6 WAS was almost similar to that of plant height. Increase or decrease in BAP concentration was not consistent with leaf formation in the two cassava varieties. However at 0.75 mg $l^{-1}$ BAP, TMS 98/0581 recorded the highest number of leaves at 6 WAS (Table 2) although this was not significantly ($P<0.05$) greater than the leaf formation in the control.

A significant difference in the number of nodes was however observed in 2,4 and 6 WAS between the two cassava varieties (Table 3) Apparently, TMS 98/0379 had the highest number of nodes. Generally, the highest number of nodes was observed at 4 and 6 WAS at 0.00 Mg $l^{-1}$ BAP concentration, although this was not significantly different from other hormonal levels.

Similar to the result observed in the number of cassava leaf, the flesh weight of the two cassava varieties of constants increase or decrease in BAP concentration (Table 4). The highest fresh weight was recorded for the two cassava varieties at 1.0 Mg $l^{-1}$ BAP concentration although it was not significantly different ($P>005$) with the result from the control and other levels of BAP. Comparatively TMS 98/0581 recorded a greater fresh weight.

Table 1. Effect of different concentration of BAP on plant height at 2, 4 and 6 WAS

| Treatments | Weeks after Subculture | | |
|---|---|---|---|
| Cassava varieties | 2 | 4 | 6 |
| TMS 98/0379($c_1$) | 1.30 | 1.72 | 2.12 |
| TMS 98/03819 ($c_2$) | 1.04 | 1.72 | 1.12 |
| LSD (0.05) | 0.28 | 1.19 | 1.47 |
| LSD (0.05) | | | |
| BAP Mgl $^{-1}$ | | | |
| 0.00 ($B_0$) | 1.78 | 2.58 | 3.88 |
| 0.25 ($B_1$) | 1.26 | 1.47 | 1.53 |
| 0.50 ($B_2$) | 1.24 | 1.46 | 1.72 |
| 0.75 ($B_3$) | 1.10 | 1.49 | 1.67 |
| 1.00 ($B_4$) | 1.79 | 0.80 | 0.99 |
| LSD 0.05 | 0.48 | 0.57 | 0.64 |

Table 2. Effect of different concentration of BAP on number of leaves at 2,4and 6 WAS

| Treatments | Weeks after Subculture | | |
|---|---|---|---|
| Cassava varieties | 2 | 4 | 6 |
| TMS 98/0379($c_1$) | 1.65 | 2.84 | 2.87 |
| TMS 98/03819 ($c_2$) | 0.95 | 2.26 | 2.78 |
| LSD (0.05) | 0.46 | 0.80 | 0.91 |
| LSD (0.05) | | | |
| BAP Mgl $^{-1}$ | | | |
| 0.00 ($B_0$) | 2.20 | 3.70 | 4.40 |
| 0.25 ($B_1$) | 1.32 | 2.47 | 2.14 |
| 0.50 ($B_2$) | 1.02 | 2.95 | 3.43 |
| 0.75 ($B_3$) | 1.18 | 2.82 | 3.43 |
| 1.00 ($B_4$) | 1.00 | 2.00 | 2.00 |
| LSD 0.05 | 1.07 | 1.35 | 1.54 |
| LSD (0.05) | 0.80 | 1.39 | 1.58 |

Table 3. Effect of different concentration of BAP on number of nodes at 2,4and 6 WAS

| Treatments | Weeks after Subculture | | |
|---|---|---|---|
| Cassava varieties | 2 | 4 | 6 |
| TMS 98/0379($c_1$) | 3.04 | 3.75 | 4.74 |
| TMS 98/03819 ($c_2$) | 1..60 | 2.64 | 3.75 |
| LSD (0.05) | 0.47 | 0.73 | 1.00 |
| LSD (0.05) | | | |
| BAP $Mgl^{-1}$ | 2.50 | 4.50 | 6.10 |
| 0.00 ($B_0$) | 2.88 | 3.36 | 4.22 |
| 0.25 ($B_1$) | 2.48 | 3.61 | 4.71 |
| 0.50 ($B_2$) | 2.34 | 3.46 | 4.33 |
| 0.75 ($B_3$) | 2.10 | 2.30 | 3.30 |
| 1.00 ($B_4$) | 1.63 | 1.94 | 2.82 |
| LSD 0.05 | 0.81 | 1.27 | 1.74 |

Table 4. Effect of different concentration of BAP on number of nodes at 2, 4 and 6 WAS

| Treatments | 6 Weeks after Subculture |
|---|---|
| Cassava varieties | |
| TMS 98/0379($c_1$) | 0.18 |
| TMS 98/03819 ($c_2$) | 1.23 |
| LSD (0.05) | 2.06 |
| LSD (0.05) | |
| BAP $Mgl^{-1}$ | 0.25 |
| 0.00 ($B_0$) | 0.14 |
| 0.25 ($B_1$) | 0.19 |
| 0.50 ($B_2$) | 0.11 |
| 0.75 ($B_3$) | 0.42 |
| 1.00 ($B_4$) | 0.13 |
| LSD 0.05 | 3.56 |

**Discussion**

The *in vitro* application of different concentration of synthetic cytokinins (BAP) considerably inhibited the performance of the two cassava varieties used in the study. The fact that all the parameters evaluated were better in the control treatment (no BAP application) shows that the endogenous levels of BAP in these cassava varieties were adequate. Hence, exogenous application of BAP led to supra – optimal amounts which induced some inhibitory effects. This observation is consistent with the physiological behaviour of hormones which have two concentration maxima for promotive and inhibitory effects. Berrie (1984) reported that synthetic cytokinins are inhibitory at high concentration. Again, the effect of any particular exogenously applied growth hormone is influenced by a variety of other factors in the internal environment of the plant, especially other hormones in the plant (Curtis *et al*, 1985; Preece, 1987).

Comparatively, TMS 98/0379 appeared to have performed better in terms of height, leaves, and nodes. This is in agreement with the findings of Curtis *et al.* (1985), who reported that the response to a particular hormonal message does not only depend on its content but also upon how it is "read" by its recipient. The *in vitro* recalcitrance observed in TMS 98/0581 at BAP concentration 1.25 $Mgl^{-1}$ is also in agreement with the findings on *in vitro* propagation of cassava; that above 1.0 µm BAP, shoot lengths were decreased which made subculture of nodes more difficult (Smith *et al*, 1986). Also, Anura (2006) observed that higher levels of kinetin (Cytokinin) induced meristem cultures to form callus.

**Conclusion and Recommendation**

Apparently, from the forgoing, the use of plant growth regulators like BAP has great potential and can offer meaningful and useful results. However, cassava cultures using basal media (no hormone) appears to be the most practical and effective method for maximum *in vitro* cassava performance.

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**References**

1. Anura, H. (2006). Tissue culture and meristem culture in sweet Potato M. Sc. Thesis Unpublished. pp. 1-10.
2. Berrie, A.M. (1984) Germination and growth regulators. In: Advance Plant Physiology. Malcom. W.B. ed. English Language Book Society/Longman England. 987 pp.
3. Cutis, H. and Barnes, N. (1985) Invitation to Biology, Worth Publishers. New York 4th edition. pp 373.
4. Dahniya, N.T. and Kallon, S.N. (1983) Rapid Multiplication of Cassava by direct Planting. In: Tropical Root Crops Production and Uses in Africa. Terry, E.R., Doku, E.V., Arene, O.B. and Mahnungu, N.M. (eds) Proceedings of the second Trienniel symposium of the International Society of Tropical Root Crops – Africa Branch held in Douala Cameroun, 14-19 August, 1983.
5. Guohua, M. (1998). Effects of Cytokinins and auxins on cassava shoot organogenesis and somatic embrryogenesis from somatic embryo explants.I. Plant Cell Tissue and organo cultures 54 (1): 1-7.
6. Johan, A.B., Barbara, R.W. and Edgar J.D.(1998). Plant Biotechnology: a tool for development in Africa. Electronic Journal of Biotechnology Vol.1.N0 3.
7. Joseph, T., Yeoh, H.H. and Loh, C.S. (1999) cynogenesis in somatic embryos and plantlets of cassava ( *Minohot esculentum* Crantz) J. sci food Agric 79:1071-1074.
8. Matand, K., Porobo, A.D., Prakash, C.S (1994) thidiazuron promotes high frequency regeneration system for peanut (*Arachis hypogaea* L.) plant *in vitro* plant cell reports 14:1-5
9. Onwubiko, O. and Mbanaso E.N.A (2006). Micropropagation for the enhancement of food production. In Repositioning Agriculture for Sustainable Millennium Development Goal. Asumugha, G.N., Olojede, A.O., Ikeorgu, J.G., Ano, A.O and Herbert U. (eds) proceedings of the 40th Annual conference of Agriculture Society of Nigeria held in Umudike Abia State, 16th -20th, October 2006.
10. Okigbo, B.N. (1986) Root and tuber in the Africa food crisis. In: Tropical Root crops. Terry E.R., Akoroda, M.O. and Arene, O.B.(eds). Proceedings of the third triennial symposium of Intentional Society for Tropical Root Crops- Africa Branch held in Owerri. Nigeria, 17-23 August, 1986
11. 11. Peng, Z., Salak.P., Johanna, P.K. (2001). Improvement of cassava shoot organogensis by the use of silver nitrate *in vitro* plant cell, Tissue and orgam culture 67(1) 47-54.
12. Preece, J.E.(1987) treatment of the stock plant with plant growth regulators to improve propagation success. Hort science, 22(5) 54-759.
13. Trigiano, N.R. and Grey, J.D. (1996) plant tissue culture concepts and laboratory exercise. CRC press. N.Y 374 PP.

# R: Modern Tool for Scientific Computing

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**Abstract:** The R language and environment, free software, has a wide range of features that make it useful in statistical computing. These features may help the R language become a main tool for statisticians and those needing to analyze their research data. In this paper, we point the readers' attention to this software and discuss some of the advantages and disadvantages of R. [Nature and Science. 2007;5(2):41-43] (ISSN: 1545-0740).

**Key words**: data analysis, graphics, programming, software, statistical computing, statistics

## Introduction

Nowadays, the necessity of using statistical software in research is undeniable. Statistical packages help analyze research data, teach statistical and non-statistical students, save time spent on statistical analysis, and even enable analyses that were not possible earlier (Ramasubramanian, 2002). They did improve quality in teaching statistics: non-statistical students may now find it easier to understand statistics and interpret results; only knowing how to calculate tedious formulae hardly helps them gain a better understanding of a problem.

There are many statistical packages for a researcher to choose from. What criteria should one take into account? First of all, of course, is the demand for analyses; then, functionality and ease of application/use must be considered. Acceptance of software by researchers throughout the world also must be taken into account. Finally, software cost may also be an important consideration; can the scientist, especially in Third World countries, afford it?

The R language and environment (R Development Core Team, 2006) shows promise of becoming a widely used computational tool in various disciplines. There are many features and issues that should make R software of choice in many statistical applications. This paper discusses these issues and shows why R might be an important analytical tool for researchers from both developed and developing countries, irrespective of their field of research.

## Features, advantages and disadvantages of R

The R language, which is based on S language (Becker et al., 1988), is a system of statistical computation and graphics. It provides many useful features, including a wide range of statistical and mathematical functions, models and methods, a programming language, a matrix language, a user-friendly interface, high level graphics, and the like. Various features of R make it useful in classical data analyses as well as in advanced studies that require sophisticated computational tools.

***Developing software.*** In the 10 years of its existence, R has become a tool for mathematicians, statisticians, engineers, biologists, psychologists, and other scientists. Since its inception, a huge team has worked on its development. Besides the basic R software, which is developed by a small core group, there are almost a thousand contributed add-on CRAN packages developed by numerous scientists who are not directly connected with the core R group. The add-on packages constitute a powerful tool for statistical analyses in an extremely wide range of areas of science. While other statistical programs, even the most expensive and popular ones, are rather slow in implementing novelties, R contains a lot of state-of-the-art methods, quite often implemented by their developers (e.g., Mclust and Mclust2, the packages written by Fraley and Raftery (2006), the acknowledged experts in model-based clustering).

***Ease of use.*** At a cursory glance, R does not seem to be very user-friendly. Its environment requires the user to learn how to use its procedures, even for simple analyses. However, for those who may not want to learn R as a programming language and use sometimes quite complex R functions, there is a user-friendly interface in Rcmdr (R commander) package (Fox, 2006). This interface facilitates data handling and gives an opportunity to perform basic statistical analyses, such as, among others, basic parametric and non-parametric hypotheses, analysis of variance, regression analysis, general linear models, or some multivariate analyses (like principal component, factor, and cluster analyses). The user may also make quite interesting, useful graphs. Using Rcmdr seems to be as easy as most of the other statistical packages. Moreover, the R language provides advanced techniques to develop self-implemented procedures, which are necessary in analyses of sophisticated statistical problems and simulation studies.

***Books with R.*** Because R has gained increasing attention in recent years, those who develop statistical methods and those who teach statistics also have started paying attention to this software. As a result, quite a few statistical books base their examples on R. The reader is encouraged to visit the R official web page, http://www.r-project.org, to see a long list of books concerned with R and S-plus. Examples are books by Dalgaard (2002), Faraway (2004), Fox (2002), and Jureckova and Picek (2006). The books from the list given by the R team deal with a wide range of statistical methods, from the introductory statistics and graphics to various advanced methodologies, such as regression; linear, generalized and mixed models; multivariate analysis; survival data; time series; engineering issues; environmental studies; geostatistics; phylogenetics and evolution; and bioinformatics and genomics. Of course, these topics have been discussed in the books mentioned, but R offers a much broader spectrum of possible analyses. This implies that R is not a local, unimportant tool for advanced programmers, but that it is directed to researchers who need to apply statistics to various kinds of data.

***Free Software.*** That R is freeware may not be an important advantage for scientists from developed countries. However, those from developing countries may find it to be an important option when choosing software for their purposes. Of course, it should not be the only argument; linking it with what we have already said about R goes beyond the price issue.

**Conclusion**

As scientists' awareness of necessity of using statistics continues to increase, statistical packages will become more and more popular; soon, using a calculator for statistics (which is still sometimes used) will become obsolete. Among many statistical programmes, the R language and environment has a significant chance of becoming software of choice for researchers representing various scientific disciplines. It has already become a well-known package for statistical and mathematical computing, and in the near future researchers themselves should be able to use R as a tool for statistical analyses.

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**References**

1. Ramasubramanian, V. Impact of statistical software packages on scientific research and statistical education. Current Science 2002;83(6):678.
2. R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org;2006.
3. Becker, R.A., Chambers, J.M., Wilks, A.R. The new S language. Pacific Grove, Ca.: Wadsworth & Brooks;1988.
4. Fraley, C. and A.E. Raftery. MCLUST: Model-Based Clustering/Normal Mixture Modeling. R package version 3.0-0;2006.
5. Fox, J., with contributions from M. Ash, T. Boye, S. Calza, A. Chang, P. Grosjean, R. Heiberger, G. J. Kerns, R. Lancelot, M. Lesnoff, S. Messad, M. Maechler, D. Putler, M. Ristic and P. Wolf. Rcmdr: R

Commander. R package version 1.2-1. http://www.r-project.org, http://socserv.socsci.mcmaster.ca/jfox/Misc/Rcmdr/; 2006.
6. Dalgaard, P. Introductory Statistics with R. Springer;2002.
7. Faraway, J.J. Linear Models with R. Chapman & Hall/CRC, Boca Raton, FL;2004.
8. Fox, J. An R and S-Plus Companion to Applied Regression. Sage Publications, Thousand Oaks, CA, USA;2002.
9. Jureckova, J., and Picek, J. Robust Statistical Methods with R. Chapman & Hall/CRC, Boca Raton, FL;2006.

# To See beyond the Horizon: Structural Equation Modeling

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**Abstract:** This article shows what the greatest strength of Structural Equation Modeling is: that it enables one to study what is impossible to be measured. [Nature and Science. 2007;5(2):44-44] (ISSN: 1545-0740).

**Keywords:** horizon; imagine; magician; structural equation modeling;

I was once told this couldn't be true. I was once told this was the method of magicians. It indeed is: Structural Equation Modeling (Shipley 2002).

To say that Structural Equation Modeling is a method for studying causal systems is not enough. It is a method that enables one to look into these systems like a magician: from data that one has at one's disposal one can say much about what has not been studied at all—variables that have not been measured are included in the system. In other words, one can study what is impossible to be measured. Isn't it what magicians do—show something that is impossible for their audience to imagine?

And this is where the greatest strength of Structural Equation Modeling lies: it goes beyond the horizon.

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**Reference**

1. Shipley, B. *Cause and correlation in biology: A user's guide to path analysis, structural equations and causal inference*. Cambridge University Press, Cambridge. 2002.

# Review of Stem Cell Studies

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**Abstract:** The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Stem Cell is the original of life and all cells come from stem cells. Germline stem cell (GSC) is the cell in the earliest of the cell stage. This article is a review of the stem cell research to introduce the current topics in the stem cell field. [Nature and Science. 2007;5(2):45-65] (ISSN: 1545-0740).

**Key words:** DNA; life; stem cell; universe

## 1. Introduction

The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell" *(*Stedman's Medical Dictionary, 2002).

Stem cell is the origin of an orgnism's life. Stem cells have the potential to develop into all different types of cells in life bodies, tissues and organs. Stem cells can be used to the study of the essential properties of the life, and it can be used in the clinical medicine to treat patients with a variety of diseases (Daar, 2003), and also it gives a hope to let us get the eternal life. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situition or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a tipical and important topic of life science.

The long-term implications of stem cell therapy could be a revolution in medicine. Many diseases are caused by the death of cells vital to the proper functioning of the organs. Heart failure, for example, is often caused by damage to the muscles caused by a blood clot. Stem cells injected into the heart could recreate the heart muscle. Type 1 diabetes is caused by the destruction of the pancreatic cells that make insulin. These cells might be reintroduced as stem cells. Parkinson's disease is caused by a loss of cells. In animal experiments stem cells have been shown to reduce symptoms of the disease.

Some of the most notable recent findings are as follows:

(1) The stemness profile may be determined by approximately 250 genes;

(2) organ-specific stem-cell growth and differentiation are stimulated during the reparative phase following transient injury;

(3) Two bone marrow stem-cell types show a remarkable degree of differentiation potential;

(4) Some organs contain resident marrow-derived stem cells, and their differentiation potential may only be expressed during repair;

(5) The metanephric mesenchyme contains pluripotent and self-renewing stem cells;

(6) Marrow-derived cells invade the kidney and differentiate into mesangial and tubular epithelial cells, and these processes are increased following renal injury;

(7) Epithelial-to-mesenchymal transition generates renal fibroblasts (Oliver, 2004).

Stem cell is totipotent, that means it holds all the genetic information of the living body and it can develop into a mature cell. Stem cell is a single cell that can give rise to progeny that differentiate into any of the specialized cells of embryonic or adult tissue. The ultimate stem cells (fertilized egg) divide to branches of cells that form various differentiated tissues or organs. During these early decisions, each progeny cell retains totipotency. Through divisions and differentiations the embryonic stem cells lose totipotency and gain differentiated function. During normal tissue renewal in adult organs, tissue stem cells give rise to progeny that differentiate into mature functioning cells of that tissue. Stem cells losing

totipotentiality are progenitor cells. Except for germinal cells, which retain totipotency, most stem cells in adult tissues have reduced potential to produce different cells.

The animal body has an unlimited source of stem cells, almost. However, the problem is not in locating these stem cells, but in isolating them from their tissue source.

Five key stem cells have been isolated from human:
(1) Blastocysts;
(2) Early embryos;
(3) Fetal tissue;
(4) Mature tissue;
(5) Mature cells that can be grown into stem cells.

At least, three aspects attract people to be interested in stem cell:
(1) To explore the life mysteries;
(2) To cure disease;
(3) To extend human's life.

**2. Germline Stem Cell (GSC)**

The recently developed testis cell transplantation method provides a powerful approach to studying the biology of the male germline stem cell and its microenvironment, the stem cell niche. For example, the *Drosophila* testis contains an average of nine germline stem cells surrounding a small cluster of nondividing somatic cells known as the hub. Two recent studies have shown that the hub is responsible for creating the germline stem cell niche by secreting a signal that is required by germline stem cells for their self-renewal. Testis is the organ for animal to reproduce the generation. As the new generation always has a young feature for the life, no matter how old the parents are, it is possible for the mature life to use the stem cell coming out from the reproduce organ (germline stem cells) to replace the old cells, to keep the mature body always young. In *Drosophila* germline stem cells of the testis, one centrosome remains anchored to the region of the cortex at the interface between germ cells and somatic hub cells, while the other centrosome migrates to the opposite side to establish mitotic spindle orientation. The orientation of the mitotic spindle ensures that as the stem cell divides, the daughter cell nearest the hub remains in the niche and is marked for self-renewal, whereas the daughter cell farther away from the hub is edged out of the niche and begins to differentiate.

Germline stem cells in *Drosophila* testes that carry a mutation in centrosomin, an integral centrosome component, provide clues as to how the spindle-positioning mechanism may operate. These mutant stem cells display defects in positioning of the centrosomes during interphase, and the resultant mitotic spindles are often misoriented. This is consistent with a direct role for the centrosomes in setting up the division plane, as suggested by the early localization of the centrosomes during interphase. Strikingly, the number of stem cells in the testes of the centrosomin mutant flies increases significantly. These stem cells become crowded around the hub, presumably because of the symmetric divisions of stem cells that have misoriented spindles. It thus appears that in *Drosophila* testes, the balance between stem cell self-renewal and differentiation is not dictated entirely by the amount of available space in the niche; rather, this balance is influenced directly by the orientation of stem cell division.

The study of stem cells holds immense promise for furthering our understanding of processes such as embryonic development, adult aging, and tumor formation. This is due to their remarkable ability to self-renew, to produce more stem cells and to differentiate into one or more specialized cell types. The *Drosophila* testis contains an average of nine germline stem cells surrounding a small cluster of nondividing somatic cells known as the hub.

The stem cells that sustain metazoan tissues face a difficult challenge. Each time a stem cell divides--it can divide indefinitely - it risks damage from errors in the duplication and segregation of genetic and cellular material that could stunt its vitality or propel it toward a cancerous state. Normally, each division must be asymmetric to ensure that only one daughter cell differentiates, while the other becomes a stem cell, thus renewing the stem cell population. Yet stem cells safely grow and divide many more times than other cell types, including their own daughters.

Unlike other known animal cell organelles, the two centrosomes inherited by daughter cells at division are not identical. All normal cells initially have one centrosome, comprising a mother and daughter centriole as well as pericentriolar material. The mother centriole contains structures and proteins that are

absent from the daughter centriole, and it nucleates more microtubules than the daughter. During each cell division cycle, the centrosome replicates. The mother centrosome retains the original mother centriole. In contrast, the daughter centrosome undergoes maturation during mitosis and during the $G_1$ phase of the next cell division cycle, converting its inherited daughter centriole into a new mother centriole. Whether this intrinsic asymmetry facilitates asymmetric stem cell division has remained a mystery.

Possible additional roles for programmed centrosome inheritance in stem cells. Aside from their participation in spindle assembly, centrosomes associate with membrane-bound organelles such as the Golgi and recycling endosomes. Centrosomes also regulate cytokinesis by delivering membranes asymmetrically to the cleavage furrow. Is differential centrosome inheritance the long-sought secret of stem cell, function? It should now be possible to determine whether maternal centrosomes are retained by several other well-characterized *Drosophila* stem cells. In male germline stem cells, such behavior seems likely to contribute to the stable asymmetric programming of stem cell and daughter. And it is satisfying to contemplate the possibility that this strategy might also promote stem cells' remarkable stability and longevity.

Extrinsic signals from niches are believed to control stem cell behavior, including self-renewal through interacting with intrinsic factors. However, it remains largely unclear how niche signals regulate their target gene expression in stem cells at the chromatin level. Adenosine triphosphate (ATP)–dependent chromatin remodeling factors control stem cell self-renewal by regulating responses to niche signals. Chromatin remodeling factors are involved in maintaining chromatin structures and modulating gene expression in organisms ranging from yeast to humans.

Male germline stem cells, called spermatogonial stem cells (SSCs) in postnatal mammals, are the foundation of spermatogenesis (the process for spermatozoa production) and, together with oocytes from females, are essential for species continuity. SSCs reside on the basement membrane of the seminiferous tubule in the testis and are almost completely surrounded by somatic Sertoli cells, which form a microenvironment or niche. Within the niche, growth factors and extracellular signals regulate the fate decisions of SSCs either to self-renew or to form daughter cells that will begin the complex differentiation process of spermatogenesis, resulting in mature spermatozoa after about 35 days in the mouse and 64 days in the human. The timing of sequential steps in spermatogenesis is tightly regulated by genes of the germ cell, and Sertoli cells support the differentiation process.

The first step in spermatogenesis is the fate decision of an SSC to produce daughter cells committed to differentiation. There is no known unique biochemical or phenotypic markers for distinguishing SSCs from their initial daughters, called undifferentiated spermatogonia. The availability of a functional transplantation assay and a culture system that allows long-term replication of SSCs made it possible to examine intracellular signals that influence self-renewal and differentiation invitro in a rigorous manner that is not available for most adult stem cells. These studies demonstrated that *Oct 3/4* and *SRY–box-containing gene 2* (*Sox 2*), which regulate *Nanog,* are expressed in SSCs. Stem cell recovery and cryopreservation may be applicable to all mammalian species and could be used to preserve the male germ line of valuable livestock animals, companion animals, and endangered species. Perhaps the most provocative and potentially valuable medical application of SSC research is for prepubertal boys undergoing chemotherapy or irradiation for cancer.

There are many possible future directions to pursue. Three particularly important areas include (1) the further definition of factors and signals that support self-renewal of SSCs, relative to those that initiate differentiation in order to provide a better understanding of this fate decision; (2) the extension of the serum-free culture system to other species, including domestic animals, endangered species, and humans to confirm that self-renewal signals are conserved among mammals and for relevant applications; and (3) the development of methods to allow in vitro differentiation of stem cells to provide mature spermatozoa, which would be enormously valuable in understanding the complex process of spermatogenesis and would have great practical use.

Stem cells are unique cell populations that are able to undergo both self-renewal and differentiation and are found in the embryo, as well as in the adult animal. In the early mammalian embryo, pluripotent embryonic stem cells are derived from the blastocyst stage and have the ability to form any fully differentiated cell of the body. As the embryo develops, stem cells become restricted in their ability to form different lineages (multipotent stem cells). Multipotent stem cells are also found in a wide variety of adult tissues such as bone marrow and brain. However, in the adult animal, the ability of certain stem cells to differentiate can be restricted to only one cell lineage (unipotent stem cells). Examples of mammalian

unipotent stem cells include the stem cells residing in the gut epithelium, the skin, and the seminiferous epithelium of the testis.

Spermatogonial stem cells can generate spermatogenesis when transplanted into the seminiferous tubules of an infertile male. Spermatogonial stem cells exhibit a distinct phenotype such as the high expression of ß-1 and specific light-scattering properties. While the stem cell identity of the $A_s$ spermatogonia has not yet been rigorously demonstrated, their morphology and location in the seminiferous epithelium make them good candidates for being stem cells.

The ability to isolate, culture, and manipulate the germ line stem cell in vitro would allow us to unravel the molecular mechanisms that drive the first steps of spermatogenesis and to characterize the signaling pathways that induce spermatogonial differentiation versus self-renewal. In turn, this could help us understand the origin of certain testicular neoplasias and the causes of male infertility. To look at these issues, an in vitro system in which these cells could be maintained in long-term cultures would be ideal. In the study reported here, we attempted to establish a mouse spermatogonial stem cell line using the large T antigen under the control of an inducible promoter.

In the mammalian testis, the germ line stem cells are a small subpopulation of type A spermatogonia that proliferate and ultimately differentiate into sperm under the control of both endocrine and paracrine factors.

**3. Embryonic Stem Cell**

Embryonic stem cells hold great promise for treating degenerative diseases, including diabetes, Parkinson's, Alzheimer's, neural degeneration, and cardiomyopathies (Bavister, 2005). Embryonic stem cells are derived from the inner cell mass of blastocyst stage embryos. Embryonic stem cells can replicate indefinitely. This makes it feasible to culture the cells on a large scaled for cell transplantation therapy in clinical application. Embryonic stem cells are pluripotent and have the potential to differentiate into all three germ layers of the mammalian body including the germ cells.

In 2003, scientists in Edinburgh have identified the gene that gives foetal stem cells their ability to multiply without limit and never grow old (Hawkes, 2003). The discovery may make it possible to create foetal stem cells from adult cells, and use them to treat diseases. At present the only way to get such cells is to create embryos. This is controversial, especially in the United States where federal research money cannot be used for embryonic research of this kind. The gene, which the team has named Nanog after the mythical Celtic land where nobody grows old, is a regulator that controls the operation of many other genes. It operates only in embryonic stem cells, which are pluripotent (able to develop into any of the body's specialised cells). Nanog's role, according to papers published in the journal *Cell* by the team from Edinburgh University and Nara Institute of Science and Technology in Japan, is to maintain stem cells and to make them grow. Ian Chambers, of the Institute for Stem Cell Research at Edinburgh, said that nanog was a master gene, which "makes stem cells immortal". Unlike specialised cells, that can only divide a limited number of times before they die, embryonic stem cells can go on dividing for ever. This means that a culture of stem cells can be kept alive for transplantation into patients where they will diversify into necessary cells — brain, muscle, liver or skin, for example. For this to be possible, scientists need to understand how it is that stem cells can either divide without limit, or choose instead to differentiate into specialised cells. Nanog appears to be the key. Nanog does not disappear in adult cells, but it lies dormant. This means that if a way could be found to reactivate it, adult cells could be persuaded to become embryonic cells again.

James Thompson, of the University of Wisconsin, told the *Washington Post*: "As we know`more and more about pluripotency, it will probably be possible to reprogramme cells to make stem cells out of any cell in the body. This is an important step in that direction." The Edinburgh paper is published alongside a study from Shinya Yamanaka, from the Nara Institute. The two groups realised that they had discovered the same gene last year and have since collaborated in completing the research. The next step is to work out how Nanog is switched on and off. To achieve that it may be necessary to continue working on embryonic stem cells and watching the process as it happens. British scientists have long argued that while work on adult stem cells is important, understanding how they work still requires the use of embryos. Most of the research so far has been conducted in mice, but humans have an almost identical gene. In one experiment the Edinburgh team inserted the human Nanog gene into embryonic mouse cells, and subjected those cells to conditions that would normally make them turn into specialist cells. The human Nanog gene stopped that process. Embryonic stem (ES) cells can be cultured in conditions that either maintain pluripotency or allow differentiation to the three embryonic germ layers. Heparan sulfate (HS).

**4. Somatic Stem Cell**

Normally to say that somatic stem cells differentiate only into specific tissue cells wherein they reside. However, somatic stem cells can differentiate into cells other than those of their tissue of origin. Adult bone marrow, fat, liver, skin, brain, skeletal muscle, pancreas, lung, heart and peripheral blood possess stem or progenitor cells with the capacity to transdifferentiate. Due to this developmental plasticity, somatic stem cells may have potential in autologous regenerative medicine, circumventing problems like rejection and the ethically challenged use of embryocyte stem cells.

**5. Isolation and Characterization of Stem Cells**

As the example, the following is describing the isolation and characterization of the putative prostatic stem cell, which was done by Bhatt, Brown, Hart, Gilmore, Ramani, George, and Clarke in 2003. The detail methods have been described by Bhatt, Brown, Hart, Gilmore, Ramani, George, and Clarke in the article "Novel method for the isolation and characterization of the putative prostatic stem cell" in the journal Cytometry A in 2003 (Bhatt, 2003).

**5.1 Prostatic tissue collection and culture**

When using human tissue, formal consent by the donator must be obtained before tissue collection. Tissue sections are obtained under sterile conditions. Each individual tissue section is bisected with half being sent for histological analysis for diagnostic evaluation and the remainder used for tissue culture. After then, tissue sections are chopped and placed in collagenase type I at 200 U/ml in RPMI 1640 medium with 2% v/v FCS overnight on a shaking platform at 37°C. The digest is then broken down further by shaking in 0.1% trypsin in PBS with 1% BSA and 1 mM ethylenediaminetetraacetic acid (EDTA) for 15-20 min. The cell suspension is then washed three times in PBS with 1% BSA and 1 mM EDTA before resuspending in RPMI 10% v/v FCS. Prostate epithelial cells are separated from fibroblasts by differential centrifugation (360 g, 1 min without braking). This process produced a supernatant enriched for fibroblasts and a pellet enriched for epithelia. The epithelial cell suspension is then spun on a metrizamide gradient (1.079 g/ml), and the cells are isolated from the interface (Bhatt, 2003).

**5.2 Ber-EP4/$\alpha_2$/CD45 labeling of cells**

Isolated epithelial cells are labeled at ambient temperature with either anti-human integrin $\alpha_2$ monoclonal antibody or Ber-EP4 antibody (8 μg/ml in 1% BSA/PBS) for 30 min before the addition of the secondary antibody, RAMBO (2.6 μg/ml in 1% BSA/PBS) for 30 min. After washing with PBS, the cells are incubated for 20 min in the dark with streptavidin PE-Cy7 (20 μg/ml). Samples are then dual labeled with CD45-FITC (1 μg/ml in 1% BSA/PBS) for 30 min (Bhatt, 2003).

**5.3 Ber-EP4/$\alpha_2$ and Hoechst labeling for flow cytometry**

Isolated epithelial cells are labeled at ambient temperature with anti-human integrin $\alpha_2$ monoclonal antibody (8 μg/ml in 1% BSA/PBS) for 30 min before the addition of the secondary antibody, RAMBO (2.6 μg/ml in 1% BSA/PBS) for 30 min. After washing with PBS, the cells are incubated for 20 min in the dark with streptavidin PE-Cy7 (20 μg/ml). Hoechst staining could be performed by using the protocol for HSC as described by Rupesh, et al (Bhatt, 2003). Briefly, epithelial cells are resuspended in Hoechst buffer (Hanks' balanced salts solution, 10% FCS,`1% D-glucose, and 20 mM HEPES) and warmed to 37°C. Hoechst 33342 is then added to give a final concentration of 2 μM and the cells incubated at 37°C for 2 h. Fifteen min before the end of incubation, the cells are labeled with monoclonal anti-human Ber-EP4 directly conjugated to FITC (8 μg/ml). The cells are then washed in ice-cold Hoechst buffer before resuspending in ice-cold Hoechst buffer containing propidium iodide (PI) at 20 ng/ml (Bhatt, 2003).

**5.4 Flow cytometry isolation of the SP fraction**

Flow cytometry is carried out using a Becton Dickinson FACS Vantage SE flow cytometer. Hoechst 33342 is excited with an argon ion, ultraviolet-enhanced laser at 350 nm, and its fluorescence is measured with a 424/44 BP filter (Hoechst BLUE) and a 675DF20 BP optical filter (Hoechst RED; Omega Optical, Brattleboro VT). A 640 LP dichroic mirror is used to separate the emission wavelengths. PI fluorescence is also measured through the 675DF20 BP (having been excited at 350 nm). A second argon ion laser is used to excite the additional fluorochrome PE-Cy7 at 488 nM. PE-Cy7 is measured using a 787RDF40 (Omega Optical) filter (Bhatt, 2003).

### 5.5 Cell cycle characterization of SP fraction

Epithelial cells are isolated and all fractions are resuspended in Hoechst buffer and warmed to 37°C. Hoechst 33342 is then added to give a concentration of 2 μM and incubated at 37°C for 45 min. Pyronin Y (250 ng/μl) is added to each tube, and the samples are incubated for 45 min. Monoclonal anti-human Ber-EP4 FITC (8 μg/ml) is added as appropriate 15 min before the end. After this, ice-cold Hoechst buffer is added immediately and the samples are washed then resuspended in ice-cold Hoechst buffer. The samples are analyzed immediately by flow cytometry. Flow cytometry is performed using a modification of the method described above. Cells under study are selected by positive labeling for Ber-EP4 FITC before being analyzed for Hoechst and Pyronin Y staining. These cells are then analyzed by plotting the Hoechst profile on the x-axis and Pyronin Y along the y-axis in a linear scale (Bhatt, 2003).

### 5.6 Cytokeratin phenotype studies

Samples are processed as above, divided into two fractions, and labeled with either cytokeratin 8 or 14 indirectly conjugated to PE-Cy5. Samples are then dual labeled with Ber-EP4 FITC and integrin $\alpha_2$ PE-CY7. Flow cytometry is performed as described and analyzed on forward (FSC) and side (SSC) scatter (Bhatt, 2003).

## 6. Application of Stem Cells in Clinical Medicine

There are over four thousand registered diseases specifically linked to genetic abnormalities. Although stem cells are unlikely to provide powerful treatment for these diseases, they are unique in their potential application to these diseases.

Indeed, in many research projects, scientists have demonstrated that stem cells can be used to replenish or rejuvenate damaged cells within the immune system of the human body and that damaged stem cells can repair themselves and their neighbors. For example, in what is regarded as the first documented case of successful gene-therapy "surgery", scientists at the Necker Hospital for Sick Children in Paris of French succeeded in treating two infants diagnosed with Severe Combined Immunodeficiency Disease, a life-threatening degenerative disease caused by defects on the male (X) chromosome. With the identification of stem cell plasticity several years ago, multiple reports raised hopes that tissue repair by stem cell transplantation could be within reach in the near future (Kashofer, 2005). In cardiovascular medicine, the possibility to cure heart failure with newly generated cardiomyocytes has created the interest of many researchers (Condorelli, 2005). Gene clone techniques can be widely used in the stem cell researches and applications (Ma, 2004).

## 7. Renal Stem Cells

Functional recovery in acute renal failure is well known, and the adult kidney is generally recognized to have the capacity to regenerate and repair. The adult stem cells exist in the kidney, including slow-cycling cells, side population cells, CD133+ cells and rKS56 cells. However, in vivo differentiation of bone marrow-derived cells into renal tubular cells may not occur at all, or is at most a minor component of the repair process. Moreover, it is generally accepted that stem cells and multipotent cells contribute to the regenerative process by producing protective and regenerative factors rather than by directly differentiating to replace damaged cells. Therefore, for clinical regenerative medicine in kidney disease, the focus of stem cell biology will shift from multiple differentiation of cells or cell-therapy to multiple functions of the cells, such as the production of bone morphologic protein-7 and other regenerative factors (Hishikawa and Fujita, 2006).

Adult stem cells have been characterized in several tissues as a subpopulation of cells able to maintain generate, and replace terminally differentiated cells in response to physiological cell turnover or tissue injury. Little is known regarding the presence of stem cells in the adult kidney but it is documented that under certain conditions, such as the recovery from acute injury, the kidney can regenerate itself by increasing the proliferation of some resident cells. The origin of these cells is largely undefined; they are often considered to derive from resident renal stem or progenitor cells. Whether these immature cells are a subpopulation preserved from the early stage of nephrogenesis is still a matter of investigation and represents an attractive possibility. Moreover, the contribution of bone marrow-derived stem cells to renal cell turnover and regeneration has been suggested. In mice and humans, there is evidence that extrarenal cells of bone marrow origin take part in tubular epithelium regeneration. Injury to a target organ can be sensed by bone marrow stem cells that migrate to the site of damage, undergo differentiation, and promote

structural and functional repair. Hematopoietic stem cells are mobilized following ischemia/reperfusion and engrafted the kidney to differentiate into tubular epithelium in the areas of damage. The evidence that mesenchymal stem cells, by virtue of their renoprotective property, restore renal tubular structure and also ameliorate renal function during experimental acute renal failure provides opportunities for therapeutic intervention (Morigi, 2006).

Acute renal failure has 50-80% mortality and treatment options for this life-threatening disease are limited. Stem cells offer an exciting potential for kidney regeneration. This review discusses pathogenesis of acute renal failure resulting from ischemia-reperfusion injury and the role of stem cells in reversing or mitigating this disorder. Specifically, the issues of differentiation of kidney cells from embryonic stem cells and bone marrow stem cells, and whether adult kidney stem/progenitor cells exist in the postnatal kidney are discussed. Evidence to support the conclusion that intra-renal cells, including surviving tubular epithelial cells and potential renal stem/progenitor cells, are the main source for renal regeneration is provided. Future research in selecting the type(s) of stem cells and optimizing the dose, frequency and route of administration of the cells will be fundamental in successful cell replacement therapy in acute renal failure (Lin, 2006).

Repair of inflammatory and/or ischemic renal injury involves endothelial, mesangial and epithelial regeneration. These structures may be rebuilt by resident progenitor cells and bone marrow-derived stem cells. Resident progenitor cells in adult kidney have not yet been conclusively identified. They are likely to be slowly cycling cells located mainly in the outer medulla and renal papilla. In glomerulonephritis with mesangiolysis, mesangial regeneration involves progenitor cells migrating from the juxtaglomerular apparatus and also bone marrow-derived cells. In acute ischemic renal failure, epithelial regeneration of proximal tubules results from the migration, proliferation and differentiation of resident progenitor cells; bone marrow-derived cells may play an accessory role. Molecular mechanisms underlying these repair processes could be targets for new therapeutic approaches (Baud, 2005).

Ischemia causes kidney tubular cell damage and abnormal renal function. The kidney is capable of morphological restoration of tubules and recovery of function. Recently, it has been suggested that cells repopulating the ischemically injured tubule derive from bone marrow stem cells. In GFP chimeras, some interstitial cells but not tubular cells express GFP after ischemic injury. More than 99% of those GFP interstitial cells are leukocytes. In female mice with male bone marrow, occasional tubular cells (0.06%) appeared to be positive for the Y chromosome, but deconvolution microscopy revealed these to be artifactual. In beta-gal chimeras, some tubular cells also appear to express beta-gal as assessed by X-gal staining, but following suppression of endogenous (mammalian) beta-gal, no tubular cells could be found that stain with X-gal after ischemic injury. Whereas there is an absence of bone marrow-derived tubular cells, many tubular cells expressed proliferating cell nuclear antigen, which is reflective of a high proliferative rate of endogenous surviving tubular cells. Upon i.v. injection of bone marrow mesenchymal stromal cells, postischemic functional renal impairment was reduced, but there was no evidence of differentiation of these cells into tubular cells of the kidney. Bone marrow-derived cells do not make a significant contribution to the restoration of epithelial integrity after an ischemic insult. It is likely that intrinsic tubular cell proliferation accounts for functionally significant replenishment of the tubular epithelium after ischemia (Duffield, 2005).

Acute renal failure (ARF) is a common disease with high morbidity and mortality. Recovery from ARF is dependent on the replacement of necrotic tubular cells with functional tubular epithelium. Recent advancement in developmental biology led to the discovery of immature mesenchymal stem cells (MSCs) in bone marrow and several established organs and to the definition of their potential in the recovery from tissue injury (Herrera, 2004).

The kidney has a dramatic capacity to regenerate after injury. Whether stem cells are the source of the epithelial progenitors replacing injured and dying tubular epithelium is an area of intense investigation. Many surviving renal epithelial cells after injury become dedifferentiated and take on mesenchymal characteristics. These cells proliferate to restore the integrity of the denuded basement membrane, and subsequently redifferentiate into a functional epithelium. An alternative possibility is that a minority of surviving intratubular cells possess stem cell properties and selectively proliferate after damage to neighboring cells. Some evidence exists to support this hypothesis but it has not yet been rigorously evaluated. Extratubular cells contribute to repair of damaged epithelium. Bone marrow-derived stem cells have been proposed to contribute to this process but and vast majority of tubular cells derive from an intrarenal source. Interstitial cells may represent another extratubular stem cell niche. It is not clear whether renal stem cells exist in the adult, and if they do where are they located (interstitium, tubule, cortex,

medulla) and what markers can be relied upon for the isolation and purification of these putative renal stem cells (Humphreys, 2006).

The kidney has a dramatic capacity to regenerate after injury. Whether stem cells are the source of the epithelial progenitors replacing injured and dying tubular epithelium is currently an area of intense investigation. Studies from our laboratory and others have supported a model whereby many surviving renal epithelial cells after injury become dedifferentiated and take on mesenchymal characteristics. These cells proliferate to restore the integrity of the denuded basement membrane, and subsequently redifferentiate into a functional epithelium. An alternative possibility is that a minority of surviving intratubular cells possess stem cell properties and selectively proliferate after damage to neighboring cells. Some evidence exists to support this hypothesis but it has not yet been rigorously evaluated. A third hypothesis is that extratubular cells contribute to repair of damaged epithelium. Bone marrow-derived stem cells have been proposed to contribute to this process but our work and work of others indicates that the vast majority of tubular cells derive from an intrarenal source. Recent evidence suggests that interstitial cells may represent another extratubular stem cell niche. The fundamental unanswered questions in this field include whether renal stem cells exist in the adult, and if they do where are they located (interstitium, tubule, cortex, medulla) and what markers can be relied upon for the isolation and purification of these putative renal stem cells. In this review we focus on our current understanding of the potential role of renal and extrarenal stem cells in repair of the adult kidney and highlight some of the controversies in this field (Humphreys, 2006).

The capacity of the kidney to regenerate functional tubules following episodes of acute injury is an important determinant of patient morbidity and mortality in the hospital setting. After severe injury or repeated episodes of injury, kidney recovery can be significantly impaired or even fail completely. Although significant advances have been made in the clinical management of such cases, there is no specific therapy that can improve the rate or effectiveness of the repair process. Recent studies have indicated that adult stem cells, either in the kidney itself or derived from the bone marrow, could participate in this repair process and might therefore be utilized clinically to treat acute renal failure. This review will focus on our current understanding of these stem cells, the controversies surrounding their in vivo capacity to repopulate the renal tubule, and further investigations that will be required before stem cell therapy can be considered for use in the clinical setting (Cantley, 2005).

While it remains unknown whether there is a stem cell in the adult kidney, characterization of the cell populations involved in renal repair and misrepair is allowing a new understanding of the mechanisms that are responsible for renal homeostasis (Oliver, 2004).

Ischemia-reperfusion injury (I/R injury) is a common cause of acute renal failure. Recovery from I/R injury requires renal tubular regeneration. Hematopoietic stem cells (HSC) have been shown to be capable of differentiating into hepatocytes, cardiac myocytes, gastrointestinal epithelial cells, and vascular endothelial cells during tissue repair. The current study tested the hypothesis that murine HSC can contribute to the regeneration of renal tubular epithelial cells after I/R injury (Lin, 2003).

The kidney has the ability to restore the structural and functional integrity of the proximal tubule, which undergoes extensive epithelial cell death after prolonged exposure to ischemia. Small numbers of peritubular endothelial cells to be derived from bone marrow cells that may serve in the repair process (Duffield, 2005).

Renal progenitor tubular cells [label-retaining cells (LRC)] are identified in normal kidneys by in vivo bromodeoxyuridine (BrdU) labeling. In normal and contralateral kidneys, LRC are observed scattering among tubular epithelial cells. After unilateral ureteral obstruction (UUO), the number of the LRC significantly increase, and most of them are positive for proliferating cell nuclear antigen (PCNA). In contrast, PCNA+ cells lacking BrdU label are rarely observed. LRC are not only in tubules but also in the interstitium after UUO. Laminin staining showed that a number of the LRC are adjacent to the destroyed tubular basement membrane. Some tubules, including LRC, lose the expression of E-cadherin after UUO. A large number of cell populations expressed vimentin, heat shock protein 47, or alpha-smooth muscle actin in the UUO kidneys, and each population contained LRC. None of the LRC is positive for these fibroblastic markers in contralateral kidneys. When renal tubules from BrdU-treated rats are cultured in the gel, some cells protruded from the periphery of the tubules and migrated into the gel. Most of these cells are BrdU+. Neither the total content of BrdU in the kidneys nor the number of LRC in bone marrow significantly is changed after UUO. LRC is a cell population that proliferates, migrates, and transdifferentiates into fibroblast-like cells during renal fibrosis (Yamashita, 2005).

**8. Human Embryonic Stem Cell (hESC)**

Scientific progress in human embryonic stem cell (hESC) research and increased funding make it imperative to look ahead to the ethical issues generated by the expected use of hESC for transplantation. Several issues should be addressed now, even though Phase I clinical trials of hESC transplantation are still in the future. To minimize the risk of hESC transplantation, donors of materials used to derive hESC lines will need to be recontacted to update their medical history and screening. Because of privacy concerns, such recontact needs to be discussed and agreed to at the time of donation, before new hESC lines are derived. Informed consent for Phase I clinical trials of hESC transplantation also raises ethical concerns. In previous Phase I trials of highly innovative interventions, allegations that trial participants had not really understood the risk and benefits caused delays in subsequent trials. Thus researchers should consider what information needs to be discussed during the consent process for hESC clinical trials and how to verify that participants have a realistic understanding of the study. Lack of attention to the special ethical concerns raised by clinical trials of hESC transplantation and their implications for the derivation of new hESC lines may undermine or delay progress towards stem cell therapies.

Increased funding and continued scientific progress have opened a new era in the ethics of human embryonic stem cell (hESC) research. These developments will reframe the ethical debate, which to date has focused on the moral status of the embryo and the acceptability of using embryos for research purposes. Although such philosophical questions have not been resolved, the issue is no longer *if* hESC research should proceed, but rather *how* it should proceed. The rapid pace of research makes it imperative to look ahead to the ethical issues generated by the expected use of hESC for transplantation. Some of these issues should be addressed now, even though Phase I clinical trials of hESC transplantation are still in the future. Crucial issues concerning safety of hESC transplantation and the need to recontact donors of materials used to derive new hESC lines are best resolved when these materials are donated. In addition, informed consent for hESC transplantation Phase I clinical trials will present particular challenges, which will require modification of the usual consent process for clinical trials. Failure to address these ethical issues may delay or preclude clinical trials that will test whether interventions based on hESC are safe and effective.

**8.1 The current Scientific, Ethical, and Policy Context of hESC Research**

New hESC lines are needed if hESCs or their products are to be used for transplantation into humans. The twenty or so hESC lines approved for federally funded studies in 2001 by President Bush were derived using nonhuman feeder cells and serum and express the nonhuman antigen Neu5Gc. Thus, they would probably be immunologically rejected by the recipients unless this problem was remedied. Derivation of new hESC lines will be stimulated by the $3 billion in funding for stem cell research authorized by California voters in 2004. This measure will give priority to funding research that cannot be funded by NIH, which is currently the case for derivation of new hESC lines. Other states and private funders have followed suit in providing nonfederal support for hESC research. Outside of the U.S., hESC research is advancing vigorously. In May 2005, researchers from South Korea reported the derivation of 11 hESC lines using somatic cell nuclear transfer, demonstrating that technical obstacles to developing such stem cell lines can be overcome more readily than expected. In turn, such findings will stimulate further research.

Current ethical and policy guidelines for hESC research focus on the derivation of new hESC lines. In May 2005, a National Academy of Sciences (NAS) panel called for voluntary adoption of ethical guidelines in hESC research. Their recommendations included institutional oversight of hESC research protocols through Embryonic Stem Cell Research Oversight Committees (ESCROs), informed consent from donors of materials for new hESC lines, restrictions on payment to gamete donors, and guidelines for banking stem cells and documentation. The twenty-three NRC recommendations have been endorsed by academic and scientific organizations and adopted as interim regulations for research funded by the state of California. That same month, the FDA issued regulations on screening and testing donors of human cells, tissues, and cellular and tissue-based products (HCT/P). While valuable, these initial efforts do not address crucial ethical issues in clinical trials of hESC transplantation, which have important upstream implications for how hESC lines should be derived, as well as for the conduct of the trials themselves. Our analysis begins with the need both to protect participants in Phase I trials of hESC transplantation and to respect the confidentiality of donors of materials used for derivation of hESC lines. These ethical responsibilities need to be addressed during the initial process of donating materials for new hESC lines. Next we consider challenges confronting informed consent for Phase I trials of hESC transplantation. We present specific recommendations for resolving these ethical issues.

**8.2 Balancing the Need to Protect Participants in Phase I Clinical Trials Against the Need to Respect Donors**

The goal of Phase I clinical trials is to assess the safety and feasibility of the investigational intervention and to determine dosages for subsequent clinical trials. Direct therapeutic benefit, although hoped for, is unlikely in early trials, particularly if the first participants receive low doses. The guiding ethical principle of Phase I studies should be "Do no harm." This ethical responsibility to protect the subjects in Phase I trials has important implications for the derivation of hESC lines. A major safety concern is transmission of infectious agents or serious genetic conditions through transplanted hESC cells or products. The public will expect strong protections against diseases transmitted through hESC transplantation, just as it demands that blood transfusions and solid organ transplants be tested for very rare but serious communicable diseases. The May 2005 FDA regulations addressed possible transmission of communicable diseases by cell-based therapies, setting standards for screening and testing at the time of donation and for tracking transplanted materials back to the original donors. HCT/P must be linked through an identification code to the donor and to pertinent donor medical records. Although these requirements are necessary to protect recipients, we contend that they are not sufficient to adequately protect them.

A broader perspective on protecting recipients of transplanted hESC materials is needed because of several clinical features of hESC transplantation. First, there is likely to be a considerable time period between donation of biological materials used to derive hESC lines and clinical trials involving transplantation of hESCs or products from them. During this period, new risks may become apparent in the donors whose gametes were used to derive the hESC lines. Emerging infectious diseases with long latency periods, such as Creutzfeldt-Jakob Disease (CJD), may be identified, for which testing and screening were not available at time of donation. Polymorphisms and biomarkers associated with risk for specific diseases are being defined at a rapid pace. Second, in hESC transplantation, serious genetic conditions might also be transmitted, some of which may not have been apparent at the time the materials were donated. For instance, after donating, donors may develop cancer or a strong family history of cancer. Third, immunosuppressive drugs, which may be essential after cell transplantation to reduce rejection, will increase the risk of communicable diseases and cancer in recipients. Fourth, if hESC transplantation proves clinically effective, many patients may receive transplantation from a single hESC line over time. Hence many recipients may be at risk for diseases transmitted from donors. In order to safeguard recipients of hESC transplantation, researchers need to recontact persons whose gametes were used to derive the hESC lines at the time of clinical hESC transplantation trials to update information and perhaps do additional testing. Furthermore, if hESC transplantation becomes a proven clinical treatment, periodic updating of the clinical status of donors would be prudent.

How can screening and testing of donors of materials for hESC lines be updated in an ethically acceptable manner? The responsibility to protect hESC transplant recipients from harm must be balanced against a responsibility to respect donors and protect their confidentiality. To resolve these countervailing mandates, researchers will need to obtain permission to recontact donors if hESC cells or materials derived from their gametes or embryos will be used for transplantation. Researchers need to tell donors about the kinds of information or testing that might be requested later and the reasons the information is needed. Such permission for recontact needs to be obtained when materials are donated for research. Without this permission, it would be a serious invasion of privacy to later recontact the donors. Also, donors who had not agreed to be recontacted might object strongly to a subsequent contact, refuse to provide information about their interim medical history, or undergo additional testing. Previous reports on the consent process for donating gametes and embryos for hESC research have not discussed the issue of recontact in depth. Obtaining permission to recontact will undoubtedly complicate the consent process for donating embryos for hESC research. However, permission for recontact will likely minimize the disqualification of hESC lines late in the development process for use in transplantation studies because of inadequate follow-up with donors. Recontacting donors presents logistical challenges because donors may move and contact may be lost. It would be desirable to ask donors to provide contact information for a relative or friends who will know their new address should they move. Confidentiality must be carefully protected because breaches might subject donors to unwanted publicity or even harassment. Concerns that their identities will not be kept confidential may deter some individuals from agreeing to be recontacted. Because of the intense public interest in and contentiousness over hESC research, it would be prudent for researchers and research institutions to develop stringent mechanisms, extending beyond those employed in routine clinical care, in order to assure donors that their identity and contact information remain protected.

Recently, confidentiality of personal health care information has been violated through deliberate breaches by staff, through break-ins by computer hackers, and through loss or theft of laptop computers. Files containing the identities of persons whose gametes were used to derive hESC lines should be protected against such breaches through additional security measures. Any computer storing such files should be locked down in a secure room and password protected, with access limited to a minimum number of individuals on a strict "need-to-know" basis. Entry to the computer storage room should also be restricted by means of a card-key, or equivalent system, that records each entry. Audit trails of access to the information should be routinely monitored for inappropriate access. The files with identifiers should be copy-protected and double encrypted, with one of the keys held by a high-ranking institutional official who is not involved in stem cell research. The computer storing these data should not be connected to the Internet. To protect information from subpoena, investigators should obtain a federal Certificate of Confidentiality. Human factors in breaches of confidentiality should also be considered. Personnel who have access to these identifiers might receive additional background checks, interviews, and training. The personnel responsible for maintaining this confidential database and contacting any donor should not be part of a hESC research team. Funders of hESC research and IRBs or ESCROs that oversee hESC research should ensure that appropriate provisions for recontact and confidentiality are in place. The IRB should review and approve any requests for recontact of donors. The ethical reasons for these provisions are sufficiently compelling that materials donated without explicit permission for recontact should not be used to develop hESC lines for transplantation, lest the safety of recipients or privacy of the donors be compromised.

**8.3 Informed consent for recipients of hESC transplantation in Phase I clinical trials**

Current procedures for obtaining informed consent are likely to be inadequate to address particular issues faced by recipients of hESC transplantation in Phase I clinical trials. Because the matter is complex and any changes in policy will need careful consideration, discussions of the consent process need to begin now. Problems with informed consent commonly occur in clinical trials. Participants in cancer clinical trials commonly expect that they will benefit personally from the trial, even though the primary purpose of Phase I trials is to test safety rather than efficacy. This tendency to view clinical research as providing a personal benefit has been termed the "therapeutic misconception". Analyses of consent forms suggest that such misunderstandings in cancer clinical trials do not reflect information in the consent forms. Indeed, cancer patients seeking therapeutic benefit may decide to enroll in a clinical trial before they meet the research staff, before they learn about the risks and benefits of the study or read a consent form.

One study of the consent process in gene transfer clinical trials found that researchers' descriptions of the direct benefit to participants in Phase I trials commonly were vague, ambiguous, and indeterminate. Some investigators try to balance hope and practical reality, for example believing that "if we've done our job right, they don't expect it, but they hope for it". This study concluded that "there is no clear resolution to the underlying normative question: what should investigators communicate about the potential for direct benefit to subjects in early phase clinical research"? The authors suggested that "this dilemma cannot be addressed by individual PIs alone, but must be acknowledged and openly discussed by the scientific community". Investigators need to determine how to develop ways to present clearly to participants such issues as promising preclinical evidence, the lack of power to detect benefit in Phase I studies, and the clinical significance of surrogate endpoints. In Phase I trials of hESC transplantation, guidelines for describing the likely direct benefits to participants similarly would require wide discussion, not only by scientists but also by public representatives. Participants receiving hESC transplantation in Phase I trials might overestimate the benefits and underestimate the risks for several reasons. The therapeutic misconception may be particularly prominent because the scientific rationale for hESC transplantation and preclinical results may seem compelling. In addition, press accounts of stem cell research, which typically have emphasized its potential to treat currently incurable diseases, may reinforce unrealistic hopes. Participants in Phase I trials may not appreciate that there is a possibility that hESC transplantation might make their condition worse. In previous clinical trials of transplantation of fetal dopamine neurons into persons with Parkinson's, transplanted cells failed to improve clinical outcomes. Indeed, late disabling dyskinesias developed in about 15% of patients receiving transplantation, with some patients needing ablative surgery to relieve these adverse events. Although the transplanted cells localized to the target areas of the brain, engrafted, and functioned to produce the intended neurotransmitters, appropriately regulated physiologic function was not achieved.

Several measures may reduce the therapeutic misconception in recipients of hESC transplantation in Phase I clinical trials. First, researchers should frame their discussions with participants in the context of publicity about the potential for hESC to treat serious diseases. Researchers need to communicate the distinction between the long-term hope for such effective treatments and the uncertainty inherent in any Phase I trial. Participants in Phase I studies need to understand that hESCs have never been tried before in humans for the specific study purposes, that researchers do not know whether they will work as hoped, and that in fact the great majority of participants in Phase I studies do not receive any direct benefit. Second, investigators in hESC clinical trials must discuss a broader range of information with potential participants than in other clinical trials. Informed consent requires researchers to discuss with potential participants information that is pertinent to their decision to volunteer for the clinical trial. Generally, the relevant information concerns the nature of the intervention being studied and the medical risks and prospective benefits. However, in hESC transplantation, non-medical issues may be prominent or even decisive for some participants. Individuals who regard the embryo as having the moral status of a person would likely have strong objections to receiving hESC transplants. Even though this intervention might benefit them medically, these individuals might regard it as collaborating with or taking advantage of an immoral action, and thus tacitly supporting it. Researchers need to appreciate that views of hESC research are not monolithic and may change over time. Indeed, some individuals who are strong advocates of pro-life positions and opponents of abortion regard the blastocyst as a potential person, not an actual person. In this latter view, hESC transplantation is morally acceptable. Researchers in clinical trials of hESC transplantation should inform eligible participants that transplanted materials originated from human embryos and help them to think through the ethical implications and clarify their personal beliefs about this research. The therapeutic misconception and beliefs about the moral acceptability of hESC research may interact in complex ways. It is possible that people who mistakenly believe that hESC Phase I clinical trials will benefit them medically may, in their eagerness to obtain treatment for a serious medical condition, overlook the origin of transplanted material. If they fail to gain clinical benefit from the clinical trial, they may then have second thoughts about their decision to accept such an intervention. Third, and most importantly, researchers should verify that participants have a realistic understanding of the study. The crucial ethical issue about informed consent is not what researchers disclose in consent forms or discussions, but rather what the participants in clinical trials understand. In other contexts, some researchers have ensured that participants understand the key features of the trial by testing their comprehension. In controversial HIV clinical trials in developing countries, where it has been alleged that participants did not understand the trial, some researchers are now testing each participant in such trials to be sure he or she understands the essential features of the research as part of the consent process. Direct assessment of participants' understanding of the study, in contexts where misunderstandings are likely, has also been recommended by several national panels. We urge that such tests of comprehension be routine in clinical trials of hESC transplantation. Controversies about the consent process might lead to delays in clinical trials of cutting-edge interventions. In early clinical trials of organ transplantation, the implantable totally artificial heart, and gene transfer, the occurrence of serious adverse events led to allegations that study participants had not truly understood the nature of the research. In turn, these concerns about consent contributed to delays in subsequent trials. Assessing the comprehension of participants would reduce or preclude post-hoc criticisms that hESC recipients did not understand the essential features of the Phase I trial. To strengthen the informed consent process in trials of hESC transplantation, stakeholders should develop consensus best practice recommendations for informing potential participants about early hESC clinical trials and for assessing participants' comprehension of key features of these trials. These stakeholders include researchers, public representatives, advocacy groups, government officials, and members of institutional hESC oversight committees. Because such consensus guidelines need to be in place by the time such clinical trials are proposed, these stakeholder meetings should be convened now. hESC clinical trials raise other important ethical questions. What kinds of in vit2o studies must be done to characterize hESC and document karyotype, epigenetic status, cell cycle parameters, and differentiation potential? What kinds of preclinical and animal studies should be required before hESC transplantation is attempted in humans? What long-term follow-up of participants should be carried out, and how can data on adverse events be pooled across different protocols? Who will pay for such long-term follow-up, since many Phase I trials will not lead to commercial products? To the extent that disagreements over these questions might delay clinical trials, these issues also need to be addressed in advance. In conclusion, for hESC to fulfill its promise as therapy, a chain of activities needs to be established, including funding, basic science, and clinical trials. Recent events have increased funding and shown that the science may proceed

rapidly. But a chain is only as strong as its weakest link. Attention to ethical issues raised by clinical trials is an essential part of the chain. The issues we have discussed are based on lessons from previous experience with related but not identical fields; invariably other unforeseeable issues will arise. Lack of attention to the special ethical concerns raised by clinical trials of hESC transplantation and their implications for the derivation of new hESC lines may undermine or delay progress towards stem cell therapies (Bernard, 2005).

## 9. Selected Protocols for Stem Cell Researches

### 9.1 INFT2 Protocol

Hematologic malignancies (blood cell cancers) in very young children are hard to treat with standard doses of chemotherapy (anti-cancer drugs). Stem cell transplantation (infusion of healthy blood forming cells) has been used but has not always been successful. The best donor of stem cells is a sibling (brother or sister) who is a match (the sibling's cells match the subject's immune type, or HLA type). But few very young children with leukemia have a matched sibling donor. This research study is for those children who do not have a matched sibling donor. In this study, a parent will be the stem cell donor. Using a parent donor (a parent donor is a partial match for the subject's HLA type) increases the risk of graft-versus-host disease (GVHD). GVHD occurs when the donor cells (the graft) recognize that the body tissues of the child (the host) are different. Because severe GVHD can be life-threatening, the parent's stem cells will be filtered using a machine called the CliniMACS system, which removes the cells that cause GVHD. This system has not been approved by the Food and Drug Administration (FDA) and is considered experimental. In addition to the stem cell transplant, parent donor natural killer (NK) cells will be given. NK cells are special cells in the immune system (the body organs and cells that defend the body against infection and disease) that target cancer cells. NK cells may help donor cells to grow and may reduce the chance of GVHD. In this experimental treatment, chemotherapy will be used in addition to the stem cell and NK cell transplants. It is unknown if these treatments will work better than the treatments now being used to treat very young children with hematologic malignancies. **(**Leung, 2007**)**

### 9.2 OPBMT2 Protocol

Malignant osteopetrosis **is a** genetic disease in which cells in the bone tissue (osteoclasts) do not function properly. These cells are unable to perform their biological job of breaking down old bone tissue as new bone tissue is being made. This causes the bone tissue to build up, producing thick bones that do not work properly and causing the child to lose his/her bone marrow space, where red cells, platelets, and white cells are made.

Stem cell transplantation from an allogeneic donor is the only known cure for this disease. Stem cells are immature cells found in the bone marrow that can grow into other kinds of cells. An allogeneic donor is another person who provides the stem cells.

There are three types of donors:

(1) A matched sibling donor (brother or sister) is the ideal treatment, but is not possible for the majority of patients.

(2) A matched unrelated donor may also be used, but finding such a match may take several months. During this time the disease may get worse; the child may need red cell or platelet transfusions as the child may be unable to make these cells and permanent damage to vision and hearing may occur.

(3) A haploidentical parental donor (a mother or father), has not been studied previously as a treatment for malignant osteopetrosis.

This study is designed to use a haploidentical parental donor in the event that a matched sibling donor is unavailable. Using a parental donor would enable transplantation earlier in the disease process than waiting for a matched unrelated donor. This might reduce the chance of the disease getting worse before the transplant is done. With a parental donor, the risk of graft rejection (the patient's body will not accept and allow donated cells to grow) may be greater than the risk of rejection using a matched sibling donor.

The purpose of this study is to learn more about the cause and treatment of malignant osteopetrosis. It is designed to determine if children with malignant osteopetrosis can properly accept a parental donor

transplant and to study the genetic (characteristics carried by genes) factors which cause the disease (Kasow, 2007).

**9.3 SCDHAP Protocol**

Sickle cell disease is a lifelong blood condition that can cause damage to the brain and other organs of the body. Children may develop severe clinical states with recurrent vaso-occlusive crises (VOC) which can cause severe pain, acute chest syndrome (ACS) and/or stroke. Treatment may include blood transfusions which may be required to prevent some of the conditions caused by this disease. Unfortunately, blood transfusions can cause iron overload, which can lead to severe and sometimes fatal complications.

Stem cells are young blood cells that can grow to make new blood cells such as white blood cells that help fight infections, platelets that help the blood to clot, and red blood cells that carry oxygen to the vital organs of the body. These cells may be taken from one individual (donor) and given to another (recipient). These stem cells, when placed in the body of the recipient, travel through the body to the bone marrow space and begin to grow and make new blood cells.

A stem cell transplant has been shown to help, and possibly cure, patients with sickle cell disease. Stem cells taken from a brother or sister may provide bone marrow that is a perfect match (same tissue type) for the recipient. Unfortunately, only about 10-20% of sickle cell patients have a matched sibling donor. Stem cells from partially matched (partial tissue match) family members have been tried with a few children with sickle cell disease. The risk and benefits of these types of transplants are not as well known as transplants using a matched donor. When children with sickle cell disease have no matched brother or sister donor, allogeneic transplants are a possible treatment available for these patients (Paul Woodard, 2007)

**9.4 SCT521 (COG # ASCT0521) Protocol**

Idiopathic pneumonia syndrome is a complication that may occur in children who have had a stem cell transplant. Often patients with pneumonia have a cough and chest pain, are short of breath, or require oxygen to help them breathe. In some transplant patients, pneumonia is caused by a bacteria or virus. However, with idiopathic pneumonia syndrome, pneumonia occurs in the absence of infection. Despite corticosteroids and supportive care, this condition may be fatal.

This research study will use a drug named etanercept. The drug has been approved by the Food and Drug Administration (FDA) for the treatment of certain joint or skin conditions in children over 4 years of age. Etanercept works by blocking the effects of a protein known as Tumor Necrosis Factor (TNF). TNF has been found in lung fluid from patients with idiopathic pneumonia syndrome. TNF may be involved in the development of lung injury in idiopathic pneumonia syndrome. An earlier study has determined`the largest amount of etanercept that can be given without causing bad effects. A small research study has been done with adults and children with idiopathic pneumonia syndrome. Etanercept was found to be safe, and several patients had improvement in their breathing (Madden, 2007).

**10. Brief Descriptions of Stem Cell Facts**

Mouse embryonic stem cells were first discovered in 1981. Since then, they have been an invaluable tool of modern biology and medical research. They have provided models to study diseases, they have brought about the discovery of many genes associated with diseases and they have been used to cure certain human disorders in animal models. After 20 years of exciting research, the mouse embryonic stem cell has helped to establish the value of these cells in *regenerative medicine*, which is the creation of cells or organs to replace tissues lost to disease or injury. The discovery of *human* embryonic stem cells in 1998 triggered important ethical controversy and debate, yet scientists are convinced that they hold enormous potential for clinical applications. Many diseases plaguing the modern world may be improved, or even cured, with therapies using human stem cells. Whether human embryonic stem cells or adult stem cells are used in future therapies will depend on the type of disease or injury. There are specific advantages for each stem cell type. Thanks to the ease of growing them in the laboratory, human embryonic stem cells may one day become the source of artificial organs. Or scientists might one day be able to mobilize one's own adult stem cells to repair tissue damage caused by trauma, disease, and even aging. To reach such goals, both human embryonic and adult stem cells will have to be extensively studied. The complementary information acquired from studying both stem cell types is the key to unlocking their full potential.

**10.1 What Are Stem Cells?**

A stem cell is the base building block of an entire family of cells that make up any organ. A common trait of stem cells is that they can maintain themselves indefinitely in a stem cell state, which is referred to as "self-renewal," while also producing — through division — more specialized cells. For example, the blood stem cell can produce all the cells in the blood, including the red blood cells, white blood cells and platelets.

**10.2 Who Needs Stem Cells?**

Harnessing the power of human stem cells will revolutionize our health, our lives, and our society. In principle, any affliction involving the loss of cells, including many diseases, injuries and even aging, could be treated with stem cells. In the United States alone, more than 100 million people could benefit from therapies derived from stem cell research.

**10.3 Adult Stem Cells**

Adult stem cells are more specialized stem cells living in the majority of tissues and organs in our bodies and generate the mature cell types within that tissue or organ. In tissues where adult stem cells have been found, they are extremely rare and very difficult to isolate. Once isolated, adult stem cells grow poorly in culture, and it is difficult to obtain enough of these cells for use in clinical trials. In addition, access to the tissues harboring these cells is problematic since most human tissue is not easily available. Two readily available sources of human adult stem cells are the bone marrow and the umbilical cord blood. In both these tissues are blood stem cells, as well as other rare types of stem cells, which can produce bone, muscle, blood vessels, heart cells and possibly more.

**10.4 Adult Stem Cells in the Clinic**

The majority of stem cell clinical trials now underway use blood stem cells from the bone marrow or umbilical cord blood to treat blood disorders or diseases, such as leukemia, different types of anemia, systemic lupus, and certain other autoimmune diseases or deficiencies. A handful of clinical trials are evaluating the use of one's own bone marrow stem cells to repair heart tissue and to improve blood flow or to help to repair bone and cartilage. Other adult stem cells being explored for use in the clinic include stem cells in the eye and the skin. Adult stem cells are also thought to play a role in tissue transplants that have been performed for several years. For example, insulin-producing cells for type I diabetes, fetal neurons for Parkinson's disease, and skin for bladder reconstruction have been transplanted successfully. It is possible that in cases where long-term regeneration has been achieved, stem cells contained in these tissues have contributed to regeneration. The widespread use of adult stem cell-derived therapies and treatments is complicated by several factors. First, available human tissue is scarce, with only 6000 donors/year for more than 100 million Americans that could benefit from cellular therapy. Second, immune rejection caused by not using one's own cells or tissue is a problem. On the other hand, using one's own cells or tissue may become a problem for older patients, as evidence has been accumulating that adult stem cells age during the life of the body and lose their potential. Thus, stem cells isolated from a young adult may have a greater potential to produce numerous daughter cells than the cells of an older person.

**10.5 Embryonic Stem Cells**

Human embryonic stem cells are like a blank slate and can produce all the cells of the body. They are obtained from the ICM (inner cell mass) of the blastocyst. The blastocyst is a very early stage of human development, which forms about 5 days after fertilization of an egg. It is approximately 1/10 the size of the head of a pin, almost invisible to the eye, and it has not yet implanted into the uterus.

Once the blastocyst has implanted and a normal pregnancy can be detected, it is too late to derive human embryonic stem cells from the embryo. At the blastocyst stage, organ formation has not started and more specialized cells are not yet present, not even the beginning of the nervous system. To obtain human embryonic stem cells, blastocysts created in culture for *in vitro fertilization* (IVF) treatment by combining sperm and egg in a dish, are used. If they are not implanted into the uterus, the blastocysts are either discarded or frozen for later fertility cycles. They can also be donated to other patients or to research. If not donated, they will stay in the freezer as long as the storage fees are paid, otherwise they will be discarded. Because the cells obtained from the blastocyst have not yet specialized, they are considered highly valuable. They can generate cells that go on to form all the body's tissues and organs.

**10.6 Why Are Embryonic Stem Cells So Valuable?**

While grown in a dish, human embryonic stem cells can maintain their *"stem-cellness"* and provide an unlimited supply of more stem cells, as well as specialized cells that can be used for experiments and for the development of therapies. Apart from their potential to treat or cure diseases, human embryonic stem cells also provide a model to study very early human development and some of the disorders that lead to birth defects and childhood cancers. Many of these disorders develop in early pregnancy and are impossible to study in humans. Also, human embryonic stem cells also can be used to examine the genes that are turned "on" or "off" as stem cells generate more specialized cell types, permitting a unique understanding of the genetics of human development. The specialized cells derived from human embryonic stem cells also can be used to study the effectiveness of potential new drugs to treat diseases. This provides a human cellular model and can reduce animal experimentation and drug development costs. Additionally, embryonic stem cells can be derived from human blastocysts with specific genetic abnormalities. These types of blastocysts are identified through genetic diagnosis during IVF treatment, to screen out genetically abnormal blastocysts, and are usually discarded. The stem cells from them can provide a unique resource to understand genetic diseases and to develop cures. Human embryonic stem cells also could be used to understand the origin or causes of various diseases such as Alzheimer's disease or Parkinson's disease, which are currently unknown. Stem cells derived through *nuclear transfer* (more info below) from patients with such afflictions would provide special tools to study these diseases and possibly develop drugs for treatments.

**10.7 Embryonic Stem Cells in the Clinic**

Embryonic stem cells have not yet been used in treating humans. But numerous animal studies have shown that many of the specialized cells derived from them can indeed integrate into damaged tissues and function properly. Thus, diseases such as myocardial infarction, severe immune deficiency, diabetes, Parkinson's disease, spinal cord injury, and demyelination have been successfully treated in animal models. But the pathway from animal models to the clinic is still complex and burdened with obstacles to be overcome. First, not all specialized cells derived from human embryonic stem cells have been shown to integrate into animal tissue and function properly. This can be due to the poor quality of the specialized cells derived in culture, or to a lack of adequate communication between the human cells and the animal environment in which they are placed. Then there is the problem of scaling up to yield enough of the specialized cells to treat a human, since this requires many more cells than to treat a tiny mouse. Such cells will have to be produced under specific conditions to ensure safety for use in patients. Most human embryonic stem cells are still grown on a layer of mouse feeder cells, a potential source of contamination. Last, there's the problem of immune rejection by the patient. While the drugs used in the organ transplantation field to suppress immune rejection have been improved over the years, rejection is still a major problem.

**11. Techniques of Human Embryonic Stem Cells**

**11.1 Nuclear Transfer to Generate Stem Cells**

Immune rejection of transplanted stem cells could be avoided if the therapeutic cells derived from the human embryonic stem cells express a patient's own genes and proteins. A method to generate these types of stem cells is by *nuclear transfer*. The nuclear transfer technique is similar to the process of generating a blastocyst from the fertilization of an egg by a sperm cell; however, in this process the DNA in an egg is exchanged for the DNA from a cell of the patient. The egg is then coaxed to divide in a culture dish into a blastocyst. The human embryonic stem cells derived from this blastocyst will be an identical genetic match to the patient and can provide "customized" replacement cells for any disorder.

**11.2 Blastocyst Develops into a Living Clone**

As long as the blastocyst is not implanted into a uterus, it cannot develop further into a living clone of the patient. If the blastocyst is implanted, it is possible that a live offspring could be born (so-called *reproductive cloning*). But based on animal models of reproductive cloning, the procedure is very inefficient; over 95%of the clones die before birth, and those that do survive have serious genetic and biological problems. Thus, medically it is irresponsible to consider reproductive cloning for humans. It is also morally and ethically unacceptable.

**12. Eternal Life**

The production of functional male gametes is dependent on the continuous activity of germline stem cells. The availability of a transplantation assay system to unequivocally identify male germline stem cells has allowed their in vitro culture, cryopreservation, and genetic modification. Moreover, the system has enabled the identification of conditions and factors involved in stem cell self-renewal, the foundation of spermatogenesis, and the production of spermatozoa. The increased knowledge about these cells is also of great potential practical value, for example, for the possible cryopreservation of stem cells from boys undergoing treatment for cancer to safeguard their germ line

According to Greek mythology, the hapless mortal Tithonus mistakenly asked the goddess Eos to confer eternal life rather than eternal youth, and he thus found himself condemned to immortal decrepitude. A new report suggests that if Tithonus had cut a side deal with Dionysus, the god of wine, he might have fared much better.

The study knits together threads of recent molecular research on aging, the venerable antiaging strategy of calorie restriction, and, surprisingly, the health benefits of moderate tippling. David Sinclair of Harvard Medical School in Boston and colleagues identify several naturally occurring small molecules that extend the life of yeast cells by approximately 70% and offer some protection to cultured human cells exposed to radiation. The molecules activate genes known to extend life span in laboratory animals. They belong to a family of chemicals known as polyphenols, some of which are prominent components of grapes, red wine, olive oil, and other foods.

The work by Sinclair and collaborators at the biotech firm BIOMOL Research Laboratories in Plymouth Meeting, Pennsylvania, including Konrad Howitz, is the latest in an increasingly hot field exploring the molecular biology of calorie restriction, a phenomenon first demonstrated in the 1930s. Laboratory rats fed a limited diet live about 40% longer than normal and are resistant to many chronic illnesses typical of aging. The observations have been replicated in yeast, fruit flies, nematodes, fish, spiders, and mice, with hints from ongoing experiments that they hold true for primates. These findings have fueled interest in understanding how calorie restriction works--and an increasingly spirited search for molecules that might mimic the process without requiring a draconian diet.

Research in the Massachusetts Institute of Technology laboratory of Leonard Guarente, for example, has shown that increasing the activity of a single gene, called *SIR2*, can extend the life span of yeast. And without the gene, calorie restriction doesn't prolong life. The new research shows that certain molecules activate *SIR2* in yeast, as well as an analogous gene, *SIRT1*, in human cells. Sinclair says that preliminary data from experiments in nematodes and fruit flies are "encouraging," in terms of whether similar activation of *SIR*-like genes, known collectively as sirtuins, can occur in those organisms, too. The study "establishes that you can get activation of *SIR2*," says Guarente, who has co-founded a company called Elixir Pharmaceuticals, which is searching for drugs that target the Sir pathway.

Working with colleagues at Harvard, BIOMOL researchers began screening a library of compounds about 2 years ago for molecules that trigger *SIRT1* activity. The initial screen yielded two polyphenols, quercetin (found in apples and tea) and piceatannol. The team then searched for other molecules with similar structures. That canvass yielded another 15 compounds, the most potent of which turned out to be resveratrol, found in grapes and red wine. It increased *SIRT1* activity 13-fold, the team reports online 24 August in *Nature*.

Resveratrol's *SIRT1*-activating power adds another dimension to the work, because it suggests a link to the so-called French paradox, the observation that despite a high-fat diet, people in France suffer about 40% less cardiovascular disease than expected; epidemiologists have linked this effect to the moderate consumption of red wine. Sinclair and colleagues speculate that these benefits may derive from activation of *SIR*-like genes. Increased *SIRT1* activity in human cells seems to blunt the activity of the tumor-suppressor gene *p53*, blocking programmed cell death. Sinclair suggests that the *SIR*-activating compounds buy time for cells to heal themselves rather than commit suicide.

In addition to its immediate implications for aging and life extension, the new work bolsters the notion that there is an evolutionarily conserved mechanism to stall the aging process during times of stress, such as when food is scarce. It also raises the possibility that the sirtuin-activating compounds reflect an interaction between plant and animal species. According to this hypothesis, which Sinclair calls "xenohormesis," plants increase their own production of polyphenols in response to environmental stresses such as drought, and that message of impending crisis may be passed on to animals that eat the plants. "Other unrelated, nonplant species can get chemical clues from the plant world," Sinclair says, "which causes them to mount their own defense response." Alternatively, he adds, the plant compounds may simply be similar to analogous, unidentified molecules in human biology.

Richard Weindruch of the University of Wisconsin, Madison, who is conducting calorie-restriction experiments in monkeys and other animals, applauds the new report but adds, "I think one needs to be very cautious about making dramatic leaps from the yeast model into mammals." He notes that it was unclear, for example, whether resveratrol affected the aging process in the kind of cells in the heart and brain that are particularly susceptible to degeneration with age.

"It's kind of romantic that red wine contains something that could extend your longevity, don't you think?" says Cynthia Kenyon, who researches aging at the University of California, San Francisco, after seeing the data presented at a meeting in Switzerland last week. But the results have not caused Sinclair to renegotiate his relationship with Dionysus. "I'd already increased my red wine consumption prior to this discovery," he confesses with a laugh (Hall, 2003).

**13. Debates on Stem Cell Research**

There are a lot of debates on the stem cell research. There most important concerns for the stem cell research and application are:

(1) As the stem cell has the totipotent property to form any kind of cells, it is very easy for the stem cell to form can cancer cell. Also, it has the potential danger possibility to create some kind of disease that there no way to cure in a current human technological condition. If it loses control, it is danger for the whole human society.

(2) The stem cell can be a potential weapon if it is used by terrorists.

(3) From the religious aspects, it is a critical topic to use stem cell at research, especially to use embryonic stem cell, as the embryo can be considered as the individual living body from some religious spirit. At this point, some religious followers persist that to take cells, tissues and organs from an embryo is same as to take that cells, tissues and organs from an individual alive living body, and destroy the embryo is same as to kill the living individual. It is a big ethnical question in the religious stage.

(4) The stem cell research and application is a critical topic in the political stage, as the politician must consider the public opinions, no matter the politician agree the opinions or not, and no matter the opinions are right or wrong.

(5) Up to now, the technique ability is not enough for the scientists to full control the stem cell research and application, and too many thing we do not know the field.

Stem cell research is a high-tech question and the people involved in this rebates should have certain scientific knowledge on the stem cell. However, it is OK for the politicians or religionists to show their opinions on any topic they are interested in, but not suitable for them to make decisions (or make laws) that will significantly influence the scientific research as this field the politicians or religionists are not specialized. Such as, it is not suitable for the American President George W. Bush to show the power in the stem cell research. It is scientists' job. When politics and science collide, science should do scientific way, rather political way. Major ethical and scientific debates surround the potential of stem cells to radically alter therapies in health care (Williams, 2005).

No matter how much debate the stem cell research and application are facing, nobody can stop these researches and applications. One country or community can stop these researches and applications within its boundary through its political or its religious power, but it has very weak power to control the researches and application outside it boundary. No matter it is United States with its super military power, or United Union through its super international influence ability, or Pope through its super religious power, nobody can stop stem cell research and application right now. There are too many danger things in stem cell exploring, and there are too many benefits from the stem cell facts. The attractions are too strong that are luring people to much.

**14. Stem Cell Glossary**

(1) **Adult stem cells:** Stem cells found in different tissues of the developed, adult organism that remain in an undifferentiated, or unspecialized, state. These stem cells can give rise to specialized cell types of the tissue from which they came, i.e., a heart stem cell can give rise to a functional heart muscle cell, but it is still unclear whether they can give rise to all different cell types of the body.

(2) **Blastocyst:** A very early embryo consisting of approximately 150 cells. It contains the inner cell mass, from which embryonic stem cells are derived, and an outer layer of cells called the trophoblast that forms the placenta.

(3) **Cell line:** Cells that can be maintained and grown in culture and display an immortal or indefinite life span.
(4) **Differentiation:** The process of development with an increase in the level of organization or complexity of a cell or tissue, accompanied with a more specialized function.
(5) **Embryo:** The product of a fertilized egg, from the zygote until the fetal stage.
(6) **Embryonic stem cell:** Also called ES cells, embryonic stem cells are cells derived from the inner cell mass of developing blastocysts. An ES cell is self-renewing (can replicate itself), pluripotent (can form all cell types found in the body) and theoretically is immortal.
(7) **In vitro fertilization:** A procedure where an egg cell and sperm cells are brought together in a dish so that a sperm cell can fertilize the egg. The resulting fertilized egg, called a zygote, will start dividing and after a several divisions, forms the embryo that can be implanted into the womb of a woman and give rise to pregnancy.
(8) **Mesenchymal stem cell:** Also known as bone marrow stromal cells, mesenchymal stem cells are rare cells, mainly found in the bone marrow, which can give rise to a large number of tissue types such as bone, cartilage, fat tissue, and connective tissue.
(9) **Multipotent stem cells:** Stem cells whose progeny are of multiple differentiated cell types, but all within a particular tissue, organ, or physiological system. For example, blood-forming (hematopoietic) stem cells are single multipotent cells that can produce all cell types that are normal components of the blood.
(10) **Nucleus:** A part of the cell, situated more or less in the middle of the cell, which is surrounded by a specialized membrane and contains the DNA of the cell, which is the genetic, inherited material of cells.
(11) **Plasticity:** A phenomenon used to describe a cell that is capable of becoming a specialized cell type of different tissue.
(12) **Pluripotent stem cells:** Stem cells that can become all the cell types that are found in an implanted embryo, fetus, or developed organism.
(13) **Progenitor cell:** An early descendant of a stem cell that can differentiate, but cannot renew itself. By contrast, a stem cell can renew itself (make more stem cells by cell division) or differentiate (divide and with each cell division evolve more and more into different types of cells).
(14) **Regenerative medicine:** Medical interventions that aim to repair damaged organs, most often by using stem cells to replace cells and tissues damaged by aging and by disease.
(15) **Reproductive cloning:** Somatic cell nuclear transfer used for the production of a fetus and delivery of a live offspring that is genetically identical the donor of the somatic cell DNA.
(16) **Somatic cells:** All the cells within the developing or developed organism with the exception of germline (egg and sperm) cells.
(17) **Stem cells:** Cells that have both the capacity to self-renew (make more stem cells by cell division) and to differentiate into mature, specialized cells.
(18) **Therapeutic cloning:** Somatic cell nuclear transfer for the isolation of embryonic stem cells. The embryonic stem cells are derived from the blastocyst (before it becomes a fetus) and can be instructed to form particular cell types (e.g. heart muscle) to be implanted into damaged tissue (e.g. heart) to restore its function. If the stem cells are placed back into the individual who gave the DNA for the somatic cell nuclear transfer, the embryonic stem cells and their derivatives are genetically identical and thus immunocompatible (they will not be rejected).
(19) **Transdifferentiation:** The ability of a particular cell of one tissue, organ or system, including stem or progenitor cells, to differentiate into a cell type characteristic of another tissue, organ, or system; e.g., blood stem cells changing to liver cells.
(20) **Transplantation biology:** The science that studies the transplantation of organs and cells. Transplantation biologists investigate scientific questions to understand why foreign tissues and organs are rejected, the way transplanted organs function in the recipient, how this function can be maintained or improved, and how the organ to be transplanted should be handled to obtain optimal results.
(21) **Umbilical cord stem cells:** Hematopoietic stem cells are present in the blood of the umbilical cord during and shortly after delivery. These stem cells are in the blood at the time of delivery, because they move from the liver, where blood-formation takes place during fetal life, to the bone marrow, where blood is made after birth. Umbilical cord stem cells are similar to stem cells that reside in

bone marrow, and can be used for the treatment of leukemia, and other diseases of the blood. Efforts are now being undertaken to collect these cells and store them in freezers for later use.

**(22)** **Zygote:** The cell that results from the union of sperm and egg during fertilization. Cell division begins after the zygote forms.

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**References**

1. Baud L, Haymann JP, Bellocq A, Fouqueray B. Contribution of stem cells to renal repair after ischemia/reperfusion. Bull Acad Natl Med. 2005;189(4):635-43.
2. Bavister BD, Wolf DP, Brenner CA. Challenges of primate embryonic stem cell research. Cloning Stem Cells 2005;7(2):82-94.
3. Bernard Lo, Patricia Zettler, Marcelle I. Cedars, Elena Gates, Arnold R. Kriegstein, Michelle Oberman, Renee Reijo Pera, Richard M. Wagner, Mary T. Wuerth, Leslie E. Wolf, Keith R. Yamamoto. A New Era in the Ethics of Human Embryonic Stem Cell Research. Stem Cells. http://www.StemCells.com. http://stemcells.alphamedpress.org/cgi/reprint/2005-0324v1.pdfn 2005.
4. Bhatt RI, Brown MD, Hart CA, Gilmore P, Ramani VAC, George NJ, Clarke NW. Novel method for the isolation and characterisation of the putative prostatic stem cell. Cytometry A. 2003;54(2):89-99.
5. Cantley LG. Adult stem cells in the repair of the injured renal tubule. Nat Clin Pract Nephrol. 2005;1(1):22-32.
6. Condorelli G, Peschle C. Stem cells for cardiac repair: state of the art. Front Biosci 2005;10:3143-50.
7. Daar AS, Sheremeta L. The science of stem cells: ethical, legal and social issues. Exp Clin Transplant. 2003;1(2):139-46.
8. Duffield JS, Bonventre JV. Kidney tubular epithelium is restored without replacement with bone marrow-derived cells during repair after ischemic injury. Kidney Int. 2005;68(5):1956-61.
9. Duffield JS, Park KM, Hsiao LL, Kelley VR, Scadden DT, Ichimura T, Bonventre JV. Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. J Clin Invest. 2005;115(7):1743-55.
10. Hall SS. LONGEVITY RESEARCH: In Vino Vitalis? Compounds Activate Life-Extending Genes. *Science* 2003;301(5637):1165.
11. Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G. Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. Int J Mol Med. 2004;14(6):1035-41.
12. Hishikawa K, Fujita T. Stem cells and kidney disease. Hypertens Res. 2006;29(10):745-9.
13. Humphreys BD, Duffield JD, Bonventre JV. Renal stem cells in recovery from acute kidney injury. Minerva Urol Nefrol. 2006;58(1):13-21.
14. Kashofer K, Bonnet D. Gene Therapy Progress and Prospects: Stem cell plasticity. Gene Ther. 2005 (Epub ahead of print).
15. Kimberly Kasow. OPBMT2 Protocol: Allogeneic Hematopoietic Stem Cell Transplantation for Children Affected with Malignant Osteopetrosis - A Pilot Study. http://www.stjude.org/protocols/0,2881,450_2331_17072,00.html. 2007.
16. Kwang-Soo Kim. Stem cell research continues in Korea beyond the Hwang scandal. Stem Cells. http://www.StemCells.com. http://stemcells.alphamedpress.org/cgi/reprint/2007-0089v1.pdf. 2007.
17. Lin F, Cordes K, Li L, Hood L, Couser WG, Shankland SJ, Igarashi P. Hematopoietic stem cells contribute to the regeneration of renal tubules after renal ischemia-reperfusion injury in mice. J Am Soc Nephrol. 2003;14(5):1188-99.
18. Lin F. Stem cells in kidney regeneration following acute renal injury. Pediatr Res. 2006;59(4 Pt 2):74R-8R.
19. Ma H. Technique of Animal Clone. Nature and Science 2004;2(1):29-35.
20. Morigi M, Benigni A, Remuzzi G, Imberti B. The regenerative potential of stem cells in acute renal failure. Cell Transplant. 2006;15 Suppl 1:S111-7.

21. Nigel Hawkes. Scientists find the secret of eternal life for stem cells. http://www.timesonline.co.uk/tol/news/uk/article1137674.ece
22. Oliver JA. Adult renal stem cells and renal repair. Curr Opin Nephrol Hypertens. 2004;13(1):17-22.
23. Paul Woodard. SCDHAP Protocol: ematopoietic Stem Cell Transplantation (HSCT) for Patients with Sickle Cell Disease and Prior Stroke or Abnormal Transcranial Doppler Ultrasound (TCD) using Reduced Conditioning and T-Cell-Depleted Hematopoietic Stem Cells from Partially Matched Family Donors - Phase I Study. http://www.stjude.org/protocols/0,2081,450_2327_18472,00.html. 2007.
24. Renee Madden. SCT521 (COG # ASCT0521) Protocol: Soluble Tumor Necrosis Factor Receptor: Enbrel (Etanercept) for the Treatment of Acute Non-Infectious Pulmonary Dysfunction (Idiopathic Pneumonia Syndrome) Following Allogeneic Stem Cell Transplantation. http://www.stjude.org/protocols/0,2881,450_2333_5873,00.html. 2007.
25. Spradling AC, Zheng Y. *Science* 26 January 2007;315(5811):469 – 470.
26. Stedman's Medical Dictionary. The American Heritage®. Houghton Mifflin Company. http://dictionary.reference.com/search?q=stem%20cell. 2002.
27. Wallenfang MR, Matunis E. *Science.* 2003;301(5639):1490 – 1491.
28. Williams D. Stem cells in medical techonology. Med Device Technol 2005;16(3):9-11.
29. Wing Leung. INFT2 Protocol: HLA - Nonidentical Stem Cell and Natural Killer Cell Transplantation for Children Less than 2 Years of Age with Hematologic Malignancies. http://www.stjude.org/protocols/0,2881,450_2330_11129,00.html. 2007.
30. Yamashita S, Maeshima A, Nojima Y. Involvement of renal progenitor tubular cells in epithelial-to-mesenchymal transition in fibrotic rat kidneys. J Am Soc Nephrol. 2005;16(7):2044-51.

# Wave Guide Astronomical Experiments for One Way Light Speed Isotropy Measurements

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**Abstract:** There is a rather small number of experiments designed to test one way light speed isotropy. The reason for the small number is the higher degree of difficulty in imagining and conducting such experiments. A very good analysis of such experiments is given by C.M.Will in [1]. The small number of such experiments[10-12] enhances the concern when one of them is proven incorrect. In the following paper we will analyze the experiment conducted by Gagnon[2]. Our paper is a rather unusual and unique one: in the first part of the paper we will show that while the experimental method is valid, the theory behind the experiment is flawed. In the second half of the paper we will show how the corrected theoretical foundation can be used to recover this very valuable experiment. By correcting the theoretical foundation we managed to build a sound foundation of future one way light speed isotropy experiments based on astronomical observations. [Nature and Science. 2007;5(2):66-71] (ISSN: 1545-0740).

**Keywords**: One way light speed isotropy measurement, waveguide theory

## 1. Introduction

The Gagnon paper makes clever use of the Earth's revolution around the Sun and of the Earth's diurnal rotation. Let $v_R$ represent the Earth revolution speed. Let $\Sigma_0(\xi,\psi,\zeta)$ represent a reference frame centered in CMBR. Let $\Sigma(x,y,z)$ represent the frame centered in the center of the Earth and let $\Sigma'(x',y',z')$ represent the slowly rotating reference frame of the lab (fig 1). Two waveguides, A and B of different cutoff frequencies are aligned with the z-axis. A certain difference of phase $\Delta\phi$ is predicted by the test theory used by Gagnon , namely the "Generalized Galilean Theory" GGT [2,3,7,8] between the two waveguides. The transformations between $\Sigma$ and $\Sigma_0$, for the infinitesimal portion of the trajectory where the coordinate axes are parallel such that the motion between $\Sigma$ and $\Sigma_0$ appears to be a translation along z are shown below:

$$\begin{aligned} \xi &= x \\ \psi &= y \\ \zeta &= \gamma(z - vt) \\ \tau &= \gamma^{-1} t \\ \gamma &= \frac{1}{\sqrt{1-\frac{v^2}{c^2}}} \end{aligned} \qquad (1.1)$$

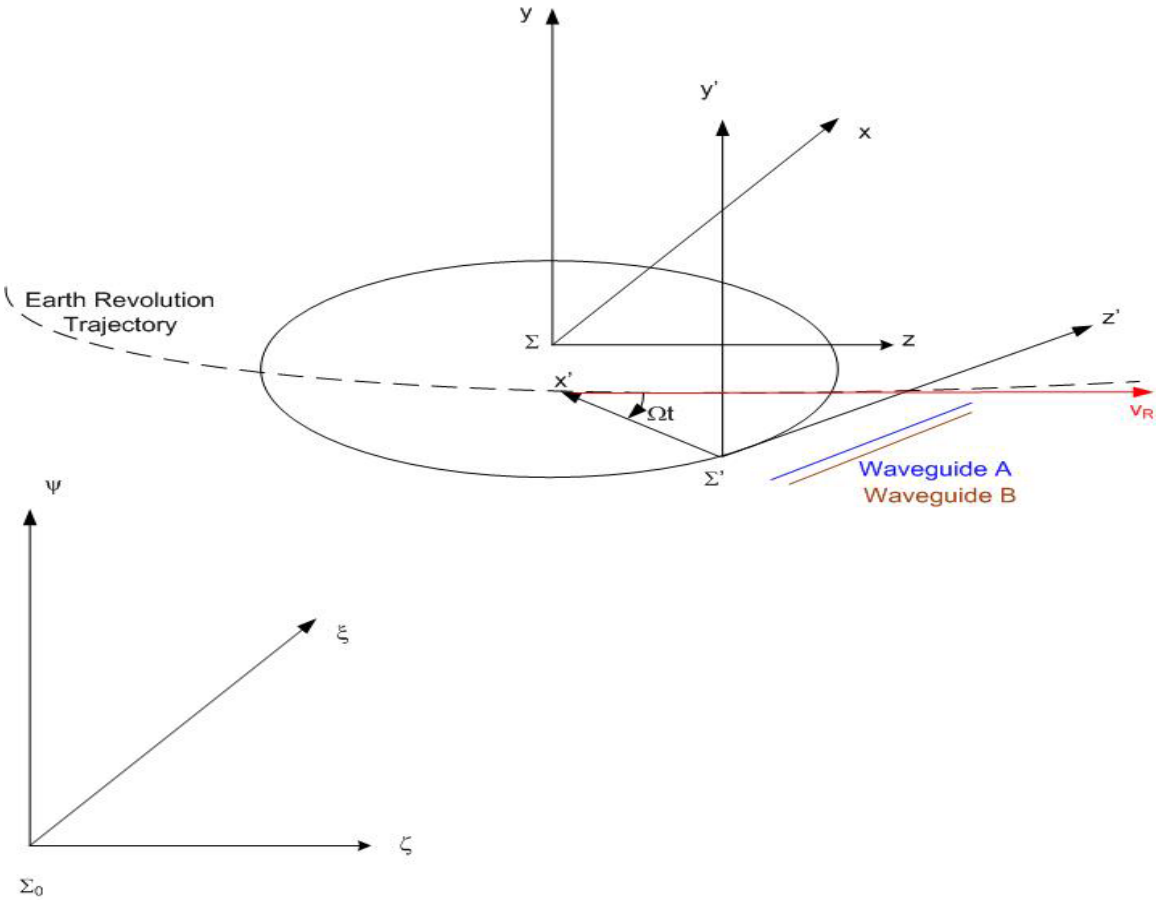


Figure 1. The Gagnon experiment setup

**2. Analysis of the Gagnon paper – the error discovery process**

In the following we will assume that the longitudinal axis of the waveguide is the z-axis, with x-axis perpendicular on it such that x and z determine a plane parallel with the Earth's equatorial plane and with y pointing to one of the poles.

According to the authors, one of them (T.Chang)[3] has derived the wave equation "in a reference frame moving with absolute velocity v", i.e. in the lab frame Σ:

$$\nabla^2 E + \frac{2}{c^2} < v, \nabla \frac{\partial E}{\partial t} > -(1 - \frac{v^2}{c^2}) \frac{1}{c^2} \frac{\partial^2 E}{\partial t^2} = 0 \qquad (2.1)$$

where <,> means the dot product and $E=E(x,y,z,t)=u_xE_x+u_yE_y+u_zE_z$

The authors proceed by looking only at the component along the z-axis. From wave theory we know that the solution is of the form:

$$E_z=X(x)Y(y)e^{i(kz-\omega t)} \qquad (2.2)$$

with the boundary condition $E_z(x=0)=E_z(x=a)=E_z(y=0)=E_z(y=b)=0$ (2.3)

Let X(x) and Y(y) be two functions continuous with continuous second order derivatives.

The problem is now reduced to finding the solution for the differential equation

$$0 = Y\frac{d^2X}{dx^2} + X\frac{d^2Y}{dy^2} + XY[-k^2 + 2\frac{v_z\omega}{c^2}k + (1-\frac{v^2}{c^2})\frac{\omega^2}{c^2}] - i\frac{2\omega}{c^2}(v_xY\frac{dX}{dx} + v_yX\frac{dY}{dy}) \quad (2.4)$$

with the boundary conditions:

$$X(0) = X(a) = 0$$
$$Y(0) = Y(b) = 0$$

where: $v^2 = v_x{}^2 + v_y{}^2 + v_z{}^2$

Gagnon simply cancelled the real part of (2.4) obtaining an incorrect solution. The correct solution is derived below:

$$\text{Let C}= -k^2 + 2\frac{v_z\omega}{c^2}k + (1-\frac{v^2}{c^2})\frac{\omega^2}{c^2} \quad (2.5)$$

Assuming $XY \neq 0$ we can divide expression (2.4) by XY:

$$-C = (\frac{1}{X}\frac{d^2X}{dx^2} - \frac{2i\omega v_x}{c^2}\frac{1}{X}\frac{dX}{dx}) + (\frac{1}{Y}\frac{d^2Y}{dy^2} - \frac{2i\omega v_y}{c^2}\frac{1}{X}\frac{dY}{dy}) \quad (2.6)$$

Since X(x) is a function only of x and Y(y) is a function only of y and since the left hand of (2.6) is a constant it results immediately that :

$$\frac{1}{X}\frac{d^2X}{dx^2} - \frac{2i\omega v_x}{c^2}\frac{1}{X}\frac{dX}{dx} = -\alpha$$
$$\frac{1}{Y}\frac{d^2Y}{dy^2} - \frac{2i\omega v_y}{c^2}\frac{1}{X}\frac{dY}{dy} = -\beta \quad (2.7)$$

i.e. two differential equations of degree two with imaginary coefficients.

$$\frac{d^2X}{dx^2} - \frac{2i\omega v_x}{c^2}\frac{dX}{dx} + \alpha X = 0 \quad (2.8)$$

must have a solution of the type $X(x)=e^{irx}$ (2.9)
producing the characteristic equation:

$$-r^2 + 2\frac{v_x}{c}\frac{\omega}{c}r + \alpha = 0 \quad (2.10)$$

$$r_{1,2} = \frac{v_x}{c}\frac{\omega}{c} \pm \sqrt{(\frac{v_x}{c}\frac{\omega}{c})^2 + \alpha} \quad (2.11)$$

$$X(x)=C_1e^{ir_1x}+C_2e^{ir_2x} = e^{ix\frac{v_x}{c}\frac{\omega}{c}}(C_1e^{ix\sqrt{\alpha+(\frac{v_x}{c}\frac{\omega}{c})^2}} + C_2e^{-ix\sqrt{\alpha+(\frac{v_x}{c}\frac{\omega}{c})^2}}) \quad (2.12)$$

$0=X(0)=C_1+C_2$ implies $C_2=-C_1$ (2.13)

$$X(x)= e^{ix\frac{v_x}{c}\frac{\omega}{c}}C_1(e^{ix\sqrt{\alpha+(\frac{v_x}{c}\frac{\omega}{c})^2}} - e^{-ix\sqrt{\alpha+(\frac{v_x}{c}\frac{\omega}{c})^2}}) \quad (2.14)$$

$$0=X(a)=e^{ia\frac{v_x}{c}\frac{\omega}{c}}C_1(e^{ia\sqrt{\alpha+(\frac{v_x}{c}\frac{\omega}{c})^2}}-e^{-ia\sqrt{\alpha+(\frac{v_x}{c}\frac{\omega}{c})^2}}) \quad (2.15)$$

$$0=2i\sin(a\sqrt{\alpha+(\frac{v_x}{c}\frac{\omega}{c})^2}) \quad (2.16)$$

$$a\sqrt{\alpha+(\frac{v_x}{c}\frac{\omega}{c})^2}=m\pi \quad (2.17)$$

$$\alpha=(\frac{m\pi}{a})^2-(\frac{v_x}{c}\frac{\omega}{c})^2 \quad (2.18)$$

Analogously:

$$Y(y)=e^{iy\frac{v_y}{c}\frac{\omega}{c}}C_3(e^{iy\sqrt{\beta+(\frac{v_y}{c}\frac{\omega}{c})^2}}-e^{-iy\sqrt{\beta+(\frac{v_y}{c}\frac{\omega}{c})^2}}) \quad (2.19)$$

$$\beta=(\frac{n\pi}{b})^2-(\frac{v_y}{c}\frac{\omega}{c})^2 \quad (2.20)$$

$$-k^2+2\frac{v_z\omega}{c^2}k+(1-\frac{v^2}{c^2})\frac{\omega^2}{c^2}=C=-(\alpha+\beta) \quad (2.21)$$

$$k^2-2\frac{v_z\omega}{c^2}k-(1-\frac{v^2}{c^2})\frac{\omega^2}{c^2}+(\alpha+\beta)=0 \quad (2.22)$$

$$k^2-2\frac{v_z\omega}{c^2}k-(1-\frac{v^2}{c^2})\frac{\omega^2}{c^2}+(\frac{m\pi}{a})^2+(\frac{n\pi}{b})^2-(\frac{v_x}{c}\frac{\omega}{c})^2-(\frac{v_y}{c}\frac{\omega}{c})^2=0 \quad (2.23)$$

$$k^2-2\frac{v_z\omega}{c^2}k-(1-\frac{v_z^2}{c^2})\frac{\omega^2}{c^2}+\frac{\omega_{mn}^2}{c^2}=0 \text{ where} \quad (2.24)$$

$$\frac{\omega_{mn}^2}{c^2}=(\frac{m\pi}{a})^2+(\frac{n\pi}{b})^2 \quad (2.25)$$

Solving (2.24) for k we obtain:

$$k(\omega,v_z)=\frac{v_z}{c}\frac{\omega}{c}\pm\frac{1}{c}\sqrt{\omega^2-\omega_{mn}^2} \quad (2.26)$$

k is a real number if and only if $\omega\geq\omega_{mn}$, $\omega_{mn}$ is the "cutoff pulsation" below which k becomes imaginary and the wave attenuates instead of propagating properly to the end of the waveguide. Waveguide theory[4] uses the pulsation $\omega=2\pi f$ rather than the frequency f.

**3. Physical interpretation of the results**

$$E_z=X(x)Y(y)Re\{e^{i(kz-\omega t)}\}=B\sin(\frac{m\pi}{a}x)\sin(\frac{n\pi}{b}y)\cos(kz+\frac{v_x}{c}\frac{\omega}{c}x+\frac{v_y}{c}\frac{\omega}{c}y-\omega t) \quad (3.1)$$

Remembering that there is a second waveguide in the experiment, driven at the same pulsation ω but with a very different "cutoff" pulsation $\omega_{pq}$, we can write immediately the electrical field:

$$E_z'=B'\sin(\frac{p\pi}{a'}x)\sin(\frac{q\pi}{b'}y)\cos(k'z+\frac{v_x}{c}\frac{\omega}{c}x+\frac{v_y}{c}\frac{\omega}{c}y-\omega t) \quad (3.2)$$

where :

$$\frac{\omega_{pq}^{\ 2}}{c^2} = (\frac{p\pi}{a'})^2 + (\frac{q\pi}{b'})^2 \tag{3.3}$$

$$k'(\omega, v_z) = \frac{v_z}{c}\frac{\omega}{c} \pm \frac{1}{c}\sqrt{\omega^2 - \omega_{pq}^{\ 2}} \tag{3.4}$$

We have enough degrees of freedom in selecting the geometries of the wave guides such that:

$$B'\sin(\frac{p\pi}{a'}x)\sin(\frac{q\pi}{b'}y) = B\sin(\frac{m\pi}{a}x)\sin(\frac{n\pi}{b}y) = E_0 \tag{3.5}$$

Therefore:

$$\begin{aligned} \mathrm{E_z} &= E_0\cos(kz + \frac{v_x}{c}\frac{\omega}{c}x + \frac{v_y}{c}\frac{\omega}{c}y - \omega t) = E_0\cos(a_{mn}z + \frac{v_x}{c}\frac{\omega}{c}x + \frac{v_y}{c}\frac{\omega}{c}y + \frac{v_z}{c}\frac{\omega}{c}z - \omega t) \\ \mathrm{E_z'} &= E_0\cos(k'z + \frac{v_x}{c}\frac{\omega}{c}x + \frac{v_y}{c}\frac{\omega}{c}y - \omega t) = E_0\cos(a_{pq}z + \frac{v_x}{c}\frac{\omega}{c}x + \frac{v_y}{c}\frac{\omega}{c}y + \frac{v_z}{c}\frac{\omega}{c}z - \omega t) \end{aligned} \tag{3.6}$$

where:

$$\begin{aligned} a_{mn} &= -\frac{1}{c}\sqrt{\omega^2 - \omega_{mn}^{\ 2}} \\ a_{pq} &= -\frac{1}{c}\sqrt{\omega^2 - \omega_{pq}^{\ 2}} \end{aligned} \tag{3.7}$$

The phase difference between $E_z$ and $E_z$' is:

$$\Delta\Phi = (\mathrm{a_{mn}} - \mathrm{a_{pq}})z \tag{3.8}$$

We now consider the simple transformation from Σ to Σ':

$$\begin{aligned} z &= (z' - R\cos(\Omega t))\cos(\Omega t) - (x' - R\sin(\Omega t))\sin(\Omega t) + R\cos(\Omega t) \\ x &= (z' - R\cos(\Omega t))\sin(\Omega t) + (x' - R\sin(\Omega t))\cos(\Omega t) + R\sin(\Omega t) \end{aligned} \tag{3.9}$$

where R is the Earth radius. The phase difference does not depend on the Earth revolution speed. Let L be the common length of the two waveguides In the lab frame Σ' the phase difference is determined by setting z'=L and x'=0 in (3.9) resulting into $z = (L + R)\cos(\Omega t) - R\cos(2\Omega t)$ and :

$$\Delta\Phi'(t) = (\mathrm{a_{mn}} - \mathrm{a_{pq}})[(L + R)\cos(\Omega t) - R\cos(2\Omega t)] \tag{3.10}$$

Formula (3.10) shows the predicted GGT variation of phase difference in the lab frame Σ', expressed as a function of time. A quick sanity check shows that (3.10) is a-dimensional since $a_{mn}$, $a_{pq}$ have dimensions of ω/c, that is, inverse of length.

**4. Extensions to standard cavities**

The standard experiments employ precision machined orthogonal cavities. We can easily extend the formalism described in the previous paragraph to orthogonal cavities by simply swapping the roles of x and z in (3.6):

$$\begin{aligned} \mathrm{E}_z &= E_0 \cos(a_{mn} z + \frac{v_x}{c}\frac{\omega}{c}x + \frac{v_y}{c}\frac{\omega}{c}y + \frac{v_z}{c}\frac{\omega}{c}z - \omega t) \\ \mathrm{E}_z' &= E_0 \cos(a_{pq} x + \frac{v_x}{c}\frac{\omega}{c}x + \frac{v_y}{c}\frac{\omega}{c}y + \frac{v_z}{c}\frac{\omega}{c}z - \omega t) \\ \Delta\Phi &= a_{mn} z - a_{pq} x \end{aligned} \qquad (4.1)$$

In the lab frame Σ' z'=L and x'=0 so $z = (L+R)\cos(\Omega t) - R\cos(2\Omega t)$ and $x = (L+R)\sin(\Omega t) - R\sin(2\Omega t)$ so:

$$\Delta\Phi'(t) = \mathrm{a}_{\mathrm{mn}}[(L+R)\cos(\Omega t) - R\cos(2\Omega t)] - \mathrm{a}_{\mathrm{pq}}[(L+R)\sin(\Omega t) - R\sin(2\Omega t)] \qquad (4.2)$$

**5. Conclusions**

The Gagnon experiment is one in a very short series[5,6] of measurements of one way light speed isotropy. The experiment is extremely original and the experimental method based on waveguides is original and valid. We have uncovered some errors in the theoretical underpinnings. With the proper corrections, the experiment becomes a very valuable tool in proving the isotropy of light speed. During the process we have derived the correct theory for using waveguides as a means of detecting one way light speed anisotropy.

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**References**

1. C.M.Will "*Clock Synchronization and isotropy of one-way speed of light*", Phys.Rev. D, **45**, 2 (1992)
2. D.R.Gagnon, D.G.Torr, P.T.Kolen, T.Chang "*Guided-wave measurement of the one-way speed of light*", Phys.Rev. A, **38**, 4 (1988)
3. T.Chang , "Maxwell's equations in anisotropic space", Phys.Lett, **70A**, 1 (1979)
4. S. Wentworth, Fundamentals of Electromagnetics with Engineering Applications (p338-355)
5. T.Krisher, L.Maleki, G.Lutes, L.Primas, R.Logan, J.Anderson, C.Will, Phys. Rev. D, **42**, 2, (1990)
6. S. Herrmann, A. Senger, E. Kovalchuk, H. Müller, A. Peters: *"Test of the isotropy of the speed of light using a continuously rotating optical resonator"*, Phys. Rev. Lett. **95**, (2005)
7. T. Chang, D. Torr, "Dual properties of spacetime under an alternative Lorentz transformation", Found. Of Phys. Lett, **1**, 4, (1988)
8. T.Chang, D.Torr, D.Gagnon, "A modified Lorentz theory as a test theory of special relativity", ", Found. Of Phys. Lett, **1**, 4, (1988)
9. R. Mansouri, R.Sexl, "*A test theory of special relativity: I. Simultaneity and clock synchronization* ",Gen.Relativ.Gravit. , **8**,(1977)
10. S.Schiller, P.Antonini, M.Okhapkin "A precision test of the isotropy of the speed of light using rotating cryogenic optical cavities" Phys. Rev. Lett. 95, 150401 (2005)
11. Lipa, J. A., Nissen, J. A., Wang, S., Stricker, D. A., and Avaloff, D. "A New Limit on Signals of Lorentz Violation in Electrodynamics" Phys. Rev. Lett. 90, 060403 (2003)
12. Wolf, P., Bize, S., Clairon, A., Santarelli, G., Tobar, M. E., and Luiten, A. N. "Improved Test of Lorentz Invariance in Electrodynamics" Phys. Rev. D 70, 051902(R) (2004)
13. A.Sfarti, "One Way Light Speed Measurement – Experimental Proof of Light Speed Isotropy", Proceedings of the XI-th Marcel Grossman Conference, Berlin (2006)
14. A.Sfarti, "Corrected Theory of the Reenactments of the Michelson-Morley Experiment in Non-Vacuum Media", Rom.J.Phys, **52**, 5-6, (2007)

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