

# *Researcher*

ISSN 1553-9865

**Volume 1 - Issue 4 (Cumulated No. 4), August 15, 2009**



Marsland Press, New York, the United States

# Researcher

ISSN: 1553-9865

The *Report and Opinion* is an international journal with a purpose to enhance our natural and scientific knowledge dissemination in the world under the free publication principle. Papers submitted could be reviews, objective descriptions, research reports, opinions/debates, news, letters, and other types of writings that are nature and science related. All manuscripts submitted will be peer reviewed and the valuable papers will be considered for the publication after the peer review. The Authors are responsible to the contents of their articles.

**Editor-in-Chief:** Hongbao Ma

**Associate Editors-in-Chief:** Shen Cherng, Jingjing Z Edmondson

**Editors:** George Chen, Qiang Fu, Mark Hansen, Mary Herbert, Deng-Nan Horng, Wayne Jiang, Chuan Liang, Mark Lindley, Margaret D. Ma, Mike Ma, Yongsheng Ma, Da Ouyang, Xiaofeng Ren, Ajaya Kumar Sahoo, Shufang Shi, Tracy X Qiao, Pankaj Sah, George Warren, Qing Xia, Yonggang Xie, Lijian Yang, Jenny Young, Tina Zhang, Ruanbao Zhou, Yi Zhu

**Web Design:** Jenny Young

## Introductions to Authors

### 1. General Information

**(1) Goals:** As an international journal published both in print and on internet, this journal is dedicated to the dissemination of fundamental knowledge in all areas of nature and science. The main purpose of the journal is to enhance our knowledge spreading in the world under the free publication principle. It publishes full-length papers (original contributions), reviews, rapid communications, and any debates and opinions in all the fields of nature and science.

**(2) What to Do:** This journal provides a place for discussion of scientific news, research, theory, philosophy, profession and technology - that will drive scientific progress. Research reports and regular manuscripts that contain new and significant information of general interest are welcome.

**(3) Who:** All people are welcome to submit manuscripts in any fields of nature and science.

**(4) Distributions:** Web version of the journal is freely opened to the world, without any payment or registration. The journal will be distributed to the selected libraries and institutions for free. For the subscription of other readers please contact with: [sciencepub@gmail.com](mailto:sciencepub@gmail.com).

**(5) Advertisements:** The price will be calculated as US\$400/page, i.e. US\$200/a half page, US\$100/a quarter page, etc. Any size of the advertisement is welcome.

### 2. Manuscripts Submission

**(1) Submission Methods:** Electronic submission through email is encouraged and hard copies plus an IBM formatted computer diskette would also be accepted.

**(2) Software:** The Microsoft Word file will be preferred.

**(3) Font:** Normal, Times New Roman, 10 pt, single space.

**(4) Indent:** Type 4 spaces in the beginning of each new paragraph.

**(5) Manuscript:** Don't use "Footnote" or "Header and Footer".

**(6) Cover Page:** Put detail information of authors and a short title in the cover page.

**(7) Title:** Use Title Case in the title and subtitles, e.g. "Debt and Agency Costs".

**(8) Figures and Tables:** Use full word of figure and table, e.g. "Figure 1. Annual Income of Different Groups", Table 1. Annual Increase of Investment".

**(9) References:** Cite references by "last name, year", e.g. "(Smith, 2003)". References should include all the authors' last names and initials, title, journal, year, volume, issue, and pages etc.

#### Reference Examples:

**Journal Article:** Hacker J, Hentschel U, Dobrindt U. Prokaryotic chromosomes and disease. *Science* 2003;301(34):790-3.

**Book:** Berkowitz BA, Katzung BG. Basic and clinical evaluation of new drugs. In: Katzung BG, ed. Basic and clinical pharmacology. Appleton & Lance Publisher. Norwalk, Connecticut, USA. 1995:60-9.

**(10) Submission Address:** [sciencepub@gmail.com](mailto:sciencepub@gmail.com), Marsland Press, 525 Rockaway PKWY, #B44, Brooklyn, New York 11212, the United States, 718-404-5362.

**(11) Reviewers:** Authors are encouraged to suggest 2-8 competent reviewers with their name and email.

### 2. Manuscript Preparation

Each manuscript is suggested to include the following components but authors can do their own ways:

**(1) Title page:** including the complete article title; each author's full name; institution(s) with which each author is affiliated, with city, state/province, zip code, and country; and the name, complete mailing address, telephone number, facsimile number (if available), and e-mail address for all correspondence.

**(2) Abstract:** including Background, Materials and Methods, Results, and Discussions.

**(3) Key Words.**

**(4) Introduction.**

**(5) Materials and Methods.**

**(6) Results.**

**(7) Discussions.**

**(8) Acknowledgments.**

**(9) Correspondence to.**

**(10) Submission date**

**(11) References.**

#### **Journal Address:**

Marsland Press,  
525 Rockaway PKWY, #B44,  
Brooklyn, New York 11212, the United States,  
718-404-5362.

[sciencepub@gmail.com](mailto:sciencepub@gmail.com)

<http://www.sciencepub.org>; <http://www.sciencepub.org>

# Researcher

Volume 1 – Issue 4 (Cumulated No. 4), August 15, 2009, ISSN 1553–9865

[Cover Page](#), [Introduction](#), [Contents](#), [Call for Papers](#), [All papers in one file](#)

Welcome to send your manuscript(s) to: [marslandresearcher@gmail.com](mailto:marslandresearcher@gmail.com)

## CONTENTS

No. / Titles / Authors	page
<a href="#">1. Quadratic and Linear Models for Predicting the Hardness of Heat Affected Zone in Air Cooled Cast Iron Weldment in Relation to the HAZ Hardness of Aluminum and Mild Steel Weldments Cooled in Same Media</a> C. I. Nwoye	1-6
<a href="#">2. Model for Quantitative Analysis of Dissolved Haematite Relative to the Initial Solution pH during Leaching of Iron Oxide Ore in Oxalic Acid</a> C. I. Nwoye, P. C. Agu, C. C. Nwakwuo, G. C. Obasi and C. Nlebedim	7-14
<a href="#">3. Taxonomic studies on the family Pteridiaceae Ching and Pteridaceae Ching (Pteridophyta) in Uttarakhand</a> Mamta Rani, Y.P.S. Pangtey, Lalit M. Tewari, Sanjay Kumar, Jeevan Singh Jalal, Anita Martolia, Kanchan Upreti and Tapan Nailwal	15-41
<a href="#">4. Evaluation of metal pollutants in medicinal plants of Pakistan</a> Ismat naeem, Abida Taskeen, Nadia Arif , Hifsa Mubeen and Tahira Mughal	42-49
<a href="#">5. Effects of dose-related levels of powdered <i>Stachytarpheta jamaicensis</i> Vahl leaves on body weight and liver functions of albino rats</a> E.A Ogie-Odia, M.N Ilechie, A.H Erhenhi and E. F Oluowo	50-55
<a href="#">6. <i>In vitro</i> Rapid clonal propagation of <i>Phyllanthus urinaria</i> Linn. (Euphorbiaceae) – A Medicinal Plant</a> Kalidass C, and Mohan V.R	56-61
<a href="#">7. Effect of <i>Azotobacter</i> and Nitrogen on Seed Germination and Early Seedling Growth in Tomato</a> P. Mahato, Anoop Badoni and J. S. Chauhan	62-66
<a href="#">8. The impact of genetic variability and smoking habits on the prevalence of periodontitis among adults</a> Faten S. Bayoumi, Fathya. M. Metwaly, Goma M.Hind and E. H. A. Abouel-Ezz..	67-73
<a href="#">9. Investigating the Optimum Operating Conditions of Some Process Parameters during Leaching of Iron Oxide Ore in Sulphuric Acid Solution</a> C. I. Nwoye, C. C. Nwakwuo and O. O. Onyemaobi	74-82
<a href="#">10. Forest Products Of Ehor Forest Reserve In Uhumwode Local Government Area, Edo State</a> Jane Ihenyen; Okoegwale E.E. and Mensah J.K.	83-89
<a href="#">11. Review On Analysis Of Bisphenol A Diglycidylether (Badge) In Canned Food</a> Abida Taskeen and Ismat Naeem	90-92

Marsland Press, 525 Rockaway PKWY, Brooklyn, New York 11212, The United States; (347) 321-7172; [editor@sciencepub.net](mailto:editor@sciencepub.net)

<http://www.sciencepub.org>

[editor@sciencepub.net](mailto:editor@sciencepub.net)

**Emails:** [marslandresearcher@gmail.com](mailto:marslandresearcher@gmail.com); [editor@sciencepub.net](mailto:editor@sciencepub.net); [sciencepub@gmail.com](mailto:sciencepub@gmail.com)

---

© 2009. Marsland Press

## Quadratic and Linear Models for Predicting the Hardness of Heat Affected Zone in Air Cooled Cast Iron Weldment in Relation to the HAZ Hardness of Aluminum and Mild Steel Weldments Cooled in Same Media

Chukwuka Ikechukwu Nwoye

Department of Materials and Metallurgical Engineering, Federal University of Technology, P.M.B 1526,  
Owerri, Nigeria.

[chikeyn@yahoo.com](mailto:chikeyn@yahoo.com)

**Abstract:** Successful attempt has been made to derive quadratic and linear models for predicting the HAZ hardness of air cooled cast iron weldment in relation to the combined and respective values of HAZ hardness of aluminum and mild steel welded and cooled under the same conditions. It was discovered that the general model;  $\theta = [2.9774\beta - \gamma]/2 + \sqrt{[(\gamma - 2.9774\beta)/2]^2 - \gamma\beta}$  predicts the HAZ hardness of cast iron weldment cooled in air as a function of the HAZ hardness of both aluminum and mild steel welded and cooled under the same conditions. The linear models;  $\theta = 2.2391\gamma$  and  $\theta = 1.7495\beta$  on the other hand predict the HAZ hardness of cast iron weldment cooled in air as a function of the HAZ hardness of aluminum or mild steel welded and cooled under the same conditions. Re-arrangement of the general model also resulted to the evaluation of the corresponding HAZ hardness in aluminum and mild steel weldments. It was found that the validity of the model is rooted on the fractional expression;  $\gamma/2.9774\theta + \gamma/2.9774\beta + \theta/2.9774\beta = 1$  since the actual computational analysis of the expression was also equal to 1, apart from the fact that the expression comprised the three metallic materials. The respective deviations of the model-predicted HAZ hardness values  $\theta$ ,  $\gamma$ , and  $\beta$  from the corresponding experimental values  $\theta_{exp}$ ,  $\gamma_{exp}$ , and  $\beta_{exp}$  was less than 0.003% indicating the validity and reliability of the model. [Researcher. 2009;1(4):1-6]. (ISSN: 1553-9865).

**Keywords:** Model, Hardness, Heat Affected Zone, Cast Iron Weldments, Aluminum, Mild Steel.

### 1. Introduction

It has been reported [1, 2] that several processes and methods of arc welding exist, for example carbon-arc welding, atomic hydrogen welding, shielded metal arc welding, plasma arc welding, electroslag welding, etc. Arc welding has been described [3] to involve the process where by the heat generated by the electric arc is maintained in most cases between the electrodes and the work piece. The quantity of heat required for melting the base metal in the vicinity of the arc and also the electrode is supplied by the arc. In arc welding, some of the processes utilize consumable electrodes which serve to strike an arc onto the work pieces, and they melt to provide the weld metal. In recent times, advancement has been made in such joining processes as adhesives, mechanical fasteners, brazing soldering [4]. However, welding remains the most important metal joining process.

Arc welding is the most widely used fusion welding process. It produces smooth welding surfaces and utilizes both direct and alternating current. Oxidation is minimal as weld metal is completely shielded from the atmosphere. The process is excellent welding low carbon, medium carbon and alloy steels. The arc is quiet, discomfort from glare or fume is minimal, and is applicable in fabricating vessels, boilers and pipes, etc. Disadvantages of the process include need for very high current for welding operations and formation of a crater in the molten metal of the work piece arising from the pressure produced by the stream of ions flowing from the cathode [2]. Electrodes are the elements of an arc lamp or furnace between which an arc is struck. They are filler materials which a joining engineer should be able to match with the parent material to avoid failure [1]. Uncoated electrodes produce an atmosphere of oxygen and nitrogen, so that the oxides and nitrides formed may be in the weld metal, thus impairing ductility and impact toughness in the weld. The situation is avoided by use of coated electrodes, which contains slag and so form a fluid covering over the weld [2]. In this case, stabilization of the arc is achieved by including materials which would produce ionization and consequently may be welded by the metallic arc process. In welding carbon and low carbon steels, coated electrodes are used especially for low carbon steels but for alloy steels in which martensite occurrence is likely on cooling and formation of hydrogen embrittlement expected, the electrode coating must be free from hydrogen forming cellulose [5].

Cracking of weldment has been found [6] to be one of the reasons for low mechanical properties such as hardness and impact strength in welded parts. Adjacent to the immediate welded area or fusion zone is the heat affected zone [6]. The mechanical property of main importance in HAZ is the hardness since it

gives an indication of the degree of embrittlement there. Studies [7] have shown that the heat affected zone hardness produced by any given welding operation depends on the cooling rate experienced by the HAZ. Too rapid rate of cooling favours the formation of hard and brittle martensite in all the sub zones of the HAZ or increases the martensite region in size relative to the other regions. The presence of martensite in the HAZ results in a very high hardness value for the heat affected zone. Slow cooling favours a better microstructure needed for engineering applications. Also, the more rapid the quenching rate, the greater the HAZ hardness.

Available literatures shows that much has been done on different joining processes and methods, but no emphasis has been placed on the derivation of models for prediction or evaluation of the hardness of the heat affected zone (HAZ) in weldments cooled in different media; evaluation of the hardness of HAZ cooled in a particular medium as a function of the hardness of HAZ from the same material but cooled in different media. Researches carried out on HAZ; its cooling and mechanical properties have not addressed the issue of predicting or evaluating the hardness of the HAZ of a material cooled in a particular medium by simple substitution of the value of the hardness of HAZ from the same material, but cooled in different media. The hardness of HAZ in aluminum, cast iron and mild steel cooled in kerosene was found to be exactly the same as the hardness value of the same materials cooled in groundnut oil [8]. This implies that

$$H_G = H_K \quad (1)$$

Where

$H_G$  = Hardness of HAZ cooled in groundnut oil

$H_K$  = Hardness of HAZ cooled kerosine

Nwoye [8] reported that 8-10% less hardness than that from water occurs when kerosine or groundnut oil is used as quenchant for HAZ. He discovered that quenching the HAZ with kerosine or groundnut oil gives approximately 8-10.7% more hardness than that from quenching with air. He found that palm oil gave the lowest hardness and cooling rate on the HAZ.

The present study aims at deriving quadratic and linear models for predicting the hardness of the heat affected zone (HAZ) in cast iron weldment cooled in air, as a function of the respective and combined values of HAZ hardness of aluminum and mild steel welded and cooled under the same conditions.

## 2. Materials and methods

Aluminum, mild steel and cast iron were cut and welded using the shielded metal arc welding technique and the hardness of the HAZ (cooled in air maintained at room temperature) tested. The hardness of the HAZ is as presented in Table 2. The full details of the experimental procedures and equipment used are presented in the previous report [8]. Table 1 shows the welding current and voltage used.

**Table1: Variation of materials with welding current and voltage [8].**

Materials	Current Type	Welding current (A)	Welding Voltage (V)
Aluminum	Direct (d.c)	120	280
Cast Iron	Alternating (a.c)	180	220
Mild Steel	Alternating (a.c)	180	220

**Table 2: Hardness of HAZ in weldments [8].**

Materials	HAZ Hardness (VHN)
Aluminum	368
Cast Iron	824
Mild Steel	471

## 3. Model formulation

Experimental data obtained from research work [8] carried out at Metallurgical and Materials Engineering Department of Federal University of Technology, Owerri were used for this work. Results of the

experiment as presented in the report [8] and used for the model formulation are as shown in Table 2. Computational analysis of the experimental data [8] shown in Table 2 resulted in Table 2.

**Table 3: HAZ Hardness ratio between aluminum, mild steel, and cast iron weldments cooled in air.**

$\gamma/\theta$	368/824	0.4466
$\theta/\beta$	824/471	1.7495
$\gamma/\beta$	368/471	0.7813

Table 3 shows that the hardness of HAZ in cast iron weldment cooled in air is a function of the hardness of HAZ in aluminum and mild steel weldment also cooled in air, hence

$$\text{Therefore } \gamma = 0.4466\theta \quad (2)$$

$$\theta = 2.2391\gamma \quad (3)$$

$$\theta = 1.7495\beta \quad (4)$$

$$\gamma = 0.7813\beta \quad (5)$$

Also from Table 3,

$$\left(\frac{\gamma}{\theta}\right) + \left(\frac{\theta}{\beta}\right) + \left(\frac{\gamma}{\beta}\right) = 0.4466 + 1.7495 + 0.7813 \quad (6)$$

$$\left(\frac{\gamma\beta + \gamma\theta + \theta^2}{\theta\beta}\right) = 2.9774 \quad (7)$$

$$\gamma\beta + \gamma\theta + \theta^2 = 2.9774\theta\beta \quad (8)$$

Dividing both sides of equation (8) by  $2.9774\theta\beta$

$$\left(\frac{\gamma}{2.9774\theta}\right) + \left(\frac{\gamma}{2.9774\beta}\right) + \left(\frac{\theta}{2.9774\beta}\right) = 1 \quad (9)$$

Also from equation (8)

$$\theta^2 + \gamma\theta - 2.9774\theta\beta + \gamma\beta = 0 \quad (10)$$

$$\theta^2 + (\gamma - 2.9774\beta)\theta + \gamma\beta = 0 \quad (11)$$

Solving the quadratic equation in equation (8) for the value of  $\theta$

$$\theta^2 + (\gamma - 2.9774\beta)\theta = -\gamma\beta \quad (12)$$

Adding square of the half of the coefficient of  $\theta$  to both sides of equation (12)

$$\theta^2 + (\gamma - 2.9774\beta)\theta + \left[\frac{\gamma - 2.9774\beta}{2}\right]^2 = -\gamma\beta + \left[\frac{\gamma - 2.9774\beta}{2}\right]^2 \quad (13)$$

$$\left[\frac{\theta + \gamma - 2.9774\beta}{2}\right]^2 = -\gamma\beta + \left[\frac{\gamma - 2.9774\beta}{2}\right]^2 \quad (14)$$

$$\left[\frac{\theta + \gamma - 2.9774\beta}{2}\right] = \sqrt{\left[\left(\frac{\gamma - 2.9774\beta}{2}\right)^2 - \gamma\beta\right]} \quad (15)$$

$$\theta = -\left[\frac{\gamma - 2.9774\beta}{2}\right] + \sqrt{\left[\left(\frac{\gamma - 2.9774\beta}{2}\right)^2 - \gamma\beta\right]} \quad (16)$$

$$\theta = \left[\frac{2.9774\beta - \gamma}{2}\right] + \sqrt{\left[\left(\frac{\gamma - 2.9774\beta}{2}\right)^2 - \gamma\beta\right]} \quad (17)$$



The derived model is equation (17)

Where

$\gamma$  = Model-predicted hardness of HAZ in aluminum weldment cooled in air (VPN)

$\beta$  = Model-predicted hardness of HAZ in mild steel weldment cooled in air (VPN)

$\theta$  = Model-predicted hardness of HAZ in cast iron weldment cooled in air (VPN)

#### 4. Boundary and initial conditions

The welding was carried out under atmospheric condition. After welding, weldments were also maintained under atmospheric condition. Welding current and voltage used are 180A and 220V respectively. SiO<sub>2</sub>-coated electrodes were used to avoid oxidation of weld spots. The coolants used were maintained at 25<sup>0</sup>C (room temperature). Volume of coolants used; 1000cm<sup>3</sup>. No pressure was applied to the HAZ during or after the welding process. No force due to compression or tension was applied in any way to the HAZ during or after the welding process. The sides and shapes of the samples are symmetries.

#### 5. Model validation

The derived model was validated by evaluating the model-predicted values of HAZ hardness in cast iron weldment  $\theta$  cooled in air and comparing them with the corresponding values obtained from the experiment  $\theta_{exp}$  [8]. Following re-arrangement of the model equation; (17), the values of  $\gamma$  and  $\beta$  were also evaluated as;

$$\gamma = \left( \frac{2.97740\beta - \theta}{\beta + \theta} \right)^2 \quad (18)$$

$$\beta = \left( \frac{\gamma\theta + \theta^2}{2.97740 - \gamma} \right) \quad (19)$$

and compared with their respective corresponding experimental values  $\gamma_{exp}$  and  $\beta_{exp}$  to further establish the validity of the model. The model-predicted values of  $\theta$ ,  $\gamma$  and  $\beta$  are shown in Table 5. The general model was also validated by solving the derived quadratic expression (equation (11)) for the value of  $\theta$  using the conventional general formular;  $x = [-b \pm \sqrt{(b^2 - 4ac)}]/2a$  [9] derived from the quadratic equation;  $ax^2 + bx + c = 0$ . Therefore, for equation (11);  $\theta^2 + (\gamma - 2.9774\beta)\theta + \gamma\beta = 0$ ,  $a = 1$ ,  $b = \gamma - 2.9774\beta$ ,  $c = \gamma\beta$  and  $x = \theta$ . Analysis and comparison between the model-predicted values  $\theta$ ,  $\gamma$ ,  $\beta$  and the respective corresponding experimental values  $\theta_{exp}$ ,  $\gamma_{exp}$ , and  $\beta_{exp}$  reveal deviations of model data from the experimental data. This is attributed to the non-consideration of the chemical properties of the coolant and the physiochemical interactions between the materials (aluminum, mild steel and cast iron) and the coolant which is believed to have played vital roles in modifying the microstructure of the HAZ during the coolant process. These deviations necessitated the introduction of correction factor to bring the model-predicted values to exactly that of the corresponding experimental values.

Deviation (Dv) of the model-predicted HAZ hardness values ( $\theta$ ,  $\gamma$ , and  $\beta$ ) from the corresponding experimental values  $\theta_{exp}$ ,  $\gamma_{exp}$ , and  $\beta_{exp}$  is given by

$$Dv = \left( \frac{M_H - E_H}{E_H} \right) \times 100 \quad (20)$$

Where

$M_H$  = Model-predicted HAZ hardness values

$E_H$  = HAZ hardness values from the experiment [8]

Correction factor (Cf) is the negative of the deviation i.e.

$$Cf = -Dv \quad (21)$$

Therefore

$$Cf = -100 \left( \frac{M_H - E_H}{E_H} \right) \quad (22)$$



Introduction of the value of Cf from equation (22) into the models give exactly the corresponding experimental values  $\theta_{exp}$ ,  $\gamma_{exp}$ , and  $\beta_{exp}$  [8].

### 6. Results and discussion

A comparison of the HAZ hardness values from experiment and those of the model show model values very much within the range of the experimental values. Results of this comparison are presented in Tables 4 and 5. Model values of  $\theta$  evaluated from equations (3) and (4) and tabulated in Table 4 show that the associated equations are valid since all of them gave almost the same corresponding experimental values  $\theta_{exp}$ . The value of  $\gamma$  in equation (5) was evaluated to establish the validity of the model. It was found that the model-predicted  $\gamma$  value was also almost the same as the corresponding experimental value  $\gamma_{exp}$ . This is a clear indication that the HAZ hardness of any of aluminum, mild steel and cast iron weldments cooled in air can be predicted as a function of the HAZ hardness of any of the other two materials, providing each pair was cooled in air. Table 5 also indicates that the model-predicted value of  $\beta$  is approximately the same as the corresponding experimental value.

**Table 4: Comparison of the hardness of HAZ in aluminum, mild steel and cast iron weldments cooled in air as obtained from experiment [8] and as predicted by derived model (each material as a function of 1-material).**

N	Models derived	$M_H$	$E_H$	Dv (%)	Cf (%)
1	$\theta = 2.2391\gamma$	823.9888	824.00	-0.0014	+0.0014
1	$\gamma = 0.7813\beta$	367.9923	368.00	-0.0021	+0.0021
1	$\theta = 1.7495\beta$	824.0145	824.00	+0.0018	-0.0018

**Table 5: Comparison of the hardness of HAZ in aluminum, mild steel and cast iron weldments cooled in air as obtained from experiment [8] and as predicted by derived model (each material as a function of 2-materials).**

N	Models derived	$M_H$	$E_H$	Dv (%)	Cf (%)
2	$\theta = [2.9774\beta - \gamma] / 2 + \sqrt{[(\gamma - 2.9774\beta) / 2]^2 - \gamma\beta}$	824.0079	824.00	+0.001	-0.001
2	$\gamma = [2.9774\theta\beta - \theta^2] / \beta + \theta$	368.0037	368.00	+0.001	-0.001
2	$\beta = [\gamma\theta + \theta^2 / 2.9774\theta - \gamma]$	470.9977	471.00	-0.0005	+0.0005

Where

N = No. of materials constituting the corresponding model as independent variable.

It can also be seen from Table 5 that the model-predicted values of  $\gamma$  and  $\beta$  are also almost the same as the corresponding experimental values of  $\gamma_{exp}$  and  $\beta_{exp}$  respectively. The value of  $\theta$  (824.0079 VPn) evaluated using the general formular for quadratic equation was exactly equal to that predicted by the general model (equation (17)). Tables 4 and 5 indicate that the respective deviations of the model-predicted HAZ hardness values  $\theta$ ,  $\gamma$  and  $\beta$  from those of the corresponding experimental values  $\theta_{exp}$ ,  $\gamma_{exp}$ , and  $\beta_{exp}$  are all less than 0.003% which is quite negligible and within the acceptable model deviation range from experimental results. Furthermore, the values of  $\gamma$  and  $\beta$  (from equations (18) and (19) respectively) evaluated to be approximately equal to the respective corresponding experimental values  $\gamma_{exp}$  and  $\beta_{exp}$  confirm the validity of the model. This also implies that the general model; equation (17) can predict the HAZ hardness of any of aluminum, mild steel and cast iron weldments cooled in air as a function of the HAZ hardness of the other two materials, providing the three materials (aluminum, mild steel and cast iron) constituting the model were cooled in air. Equation (17) is regarded as the general model equation because it comprised the HAZ hardness of all the materials considered for the model formulation. It was found that the validity of the model is rooted on the fractional expression;  $\gamma/2.9774\theta + \gamma/2.9774\beta + \theta/2.9774\beta = 1$  since the actual computational analysis of the expression was also equal to 1 apart from the fact that the expression comprised the three metallic materials. Based on the foregoing, the models in equations (3), (4) and (17) are valid and very useful for predicting HAZ hardness of aluminum, mild steel and cast iron weldments cooled in air depending on the material of interest and the given HAZ hardness values for the other materials. The general model (equation (17)) was also found to give lesser magnitude of deviation from

experimental HAZ hardness values and is therefore preferred to other derived models (equations (3) and (4)). However, the latter models are much useful if the HAZ hardness is expected to be predicted in relation to just one material which could be either aluminum, mild steel or cast iron.

### Conclusion

The derived models;  $\theta = 2.2391\gamma$  and  $\theta = 1.7495\beta$  can predict the HAZ hardness of cast iron weldment cooled in air as a function of the HAZ hardness of aluminum or mild steel welded and cooled under the same conditions. Similarly, the general model;  $\theta = [2.9774\beta - \gamma]/2 + \sqrt{[(\gamma - 2.9774\beta)/2]^2 - \gamma\beta}$  can predict the HAZ hardness of cast iron weldment cooled in air as a function of the HAZ hardness of both aluminum and mild steel welded and cooled under the same conditions. Furthermore, re-arrangement of these models could be done to evaluate the HAZ hardness of aluminum and mild steel respectively as in the case of cast iron. The validity of the model was rooted on the fractional expression;  $\gamma/2.9774\theta + \gamma/2.9774\beta + \theta/2.9774\beta = 1$  since the actual computational analysis of the expression was also equal to 1. The respective deviations of the model-predicted HAZ hardness values  $\theta$ ,  $\gamma$ , and  $\beta$  from the corresponding experimental values  $\theta_{\text{exp}}$ ,  $\gamma_{\text{exp}}$  and  $\beta_{\text{exp}}$  was less 0.003% indicating the reliability and validity of the model.

### Acknowledgement

The authors thank the management of Federal University of Technology, Owerri for providing the equipment used for this work.

### References

- [1] Davies, A. C., (1993). The Science and Practice of Welding, 10<sup>th</sup> Edition, Cambridge University Press, p521.
- [2] Higgins, R. A., (1994). Engineering Metallurgy, Arc Welding Processes, 2<sup>nd</sup> Edition, Edward Arnold Publishers, London. p57.
- [3] Lancaster, J. F., (1993). Metallurgy of Welding 5<sup>th</sup> Edition, London. Chapman and Hall p389.
- [4] Callister, W.D., (Jnr.) (1996). Material Science and Engineering. An introduction, 1<sup>st</sup> Edition, Sydney John Wiley & Sons inc. p852.
- [5] Higgins, R. A., (1977). Engineering Metallurgy Part II, 2<sup>nd</sup> Edition, London, ELBS, p466.
- [6] Nnuka, E. E., Ovat, F. A., and Oseni, M. I., (2008). Effect of Electrode Types on the Mechanical Properties and Structure of Welded Steel Joints. JMME Vol. 3, No. 1.
- [7] Lancaster, J. F., (1987). Metallurgy of Welding: The Mechanical Properties of the Heat Affected Zone, 4<sup>th</sup> Edition, Allen and University, London. p45.
- [8] Nwoye, C. I. (2008). Comparative Studies of the Cooling Ability of Hydrocarbon Based Media and their Effects on the Hardness of the Heat Affected Zone (HAZ) in Weldments JMME Vol.3, No.1
- [9] Stroud, K. A., (1986). Further Engineering Mathematics. 1<sup>st</sup> edition, Macmillan Education, London. p47.

3/22/2009

## Model for Quantitative Analysis of Dissolved Haematite Relative to the Initial Solution pH during Leaching of Iron Oxide Ore in Oxalic Acid

Chukwuka Ikechukwu Nwoye<sup>1\*</sup>, Paulinus Agu<sup>1</sup>, Chris Nwakwuo<sup>2</sup>, Gideon Obasi<sup>3</sup> and Cajetan Nlebedim<sup>4</sup>

<sup>1</sup>Department of Materials and Metallurgical Engineering, Federal University of Technology, Owerri, Nigeria.

<sup>2</sup>Department of Material Science, Oxford University, United Kingdom.

<sup>3</sup>Department of Material Science, Aveiro University Portugal.

<sup>4</sup>Department of Material Science, Cardiff University, Wales, United Kingdom.

[chikeyn@yahoo.com](mailto:chikeyn@yahoo.com)

### Abstract

Model for quantitative analysis of dissolved haematite (relative to the initial solution pH) during leaching of iron oxide ore in oxalic acid solution has been derived. The model;

$$\%Fe_2O_3 = \left( \frac{N}{N_c} \left( \frac{1}{\gamma} \right) \right)$$

was found to calculate the concentration of dissolved haematite being dependent on the values of the initial leaching solution pH measured during the leaching process. The respective positive and negative deviation of the model-predicted values of %Fe<sub>2</sub>O<sub>3</sub> (dissolved) from the corresponding experimental values was found to be less than 11% which is quite within the acceptable range of deviation limit of experimental results. The values of the assumed coefficients of dilution (N) and dissolution of haematite (N<sub>c</sub>) in oxalic acid solution were calculated to be 197.7503 and 700.0618 respectively. [Researcher. 2009;1(4):7-14]. (ISSN: 1553-9865).

**Keywords:** Model, Dissolved Haematite, Solution pH, Oxalic Acid, Iron Oxide Ore.

### 1. Introduction

Past reports [1,2] have shown that at a temperature as low as 25°C, the presence of Fe<sup>2+</sup> significantly enhances the leaching of iron extraction from silica sand. Ferrous oxalate is quickly oxidized by air during dissolution, giving room for an induction period of a few hours unless a strong acidic environment (<pH 1) or an inert atmosphere is maintained. It has been found [3] that maintaining the high level of ferrous oxalate in the leach liquor using an inert gas enhance the reaction kinetics. It is believed that during this process, removal of phosphorus from the iron compound and subsequent dissolution of the phosphorus oxide formed were effected.

It has been reported [4] that the optimum pH for dissolving iron oxide is pH 2.5 – 3.0. The solution pH governs the distribution of various oxalate ions in the leach system. Below pH 1.5, oxalic acid exists mainly as H<sub>2</sub>C<sub>2</sub>O<sub>4</sub>, whereas HC<sub>2</sub>O<sub>4</sub> is the most predominant species at pH 2.5 – 3.0.

Studies [5,6] have shown that the final pH of leaching solution depend on the leaching time, initial pH for the leaching solution and the leaching temperature.

Models for computational analysis of the concentration of dissolved haematite and heat absorbed by oxalic acid solution during leaching of iron oxide ore have been derived [7]. These models are:

$$\%Fe_2O_3 = K (\gamma/\mu) \quad (1)$$

$$Q = K_c \mu \quad (2)$$

Where

%Fe<sub>2</sub>O<sub>3</sub> = Concentration of dissolved haematite in oxalic acid solution.

γ = Final pH of the leaching solution at time t at which %Fe<sub>2</sub>O<sub>3</sub> was obtained.

μ = Weight of iron oxide added into the oxalic acid leaching solution (g)

K = Constant of proportionality associated with haematite dissolution

K<sub>c</sub> = Constant of proportionality associated with heat absorption

Q = Quantity of heat absorbed by oxalic acid solution during the leaching process (J)

Nwoye [7] found that optimization of the weight input of iron oxide ore could be achieved using the model; (%Fe<sub>2</sub>O<sub>3</sub> = K (γ/μ) ) by comparing the concentrations of dissolved haematite at different weights input of the iron oxide ore, with the view to identifying the optimum weight input of iron oxide ore that

gives the maximum dissolution of  $Fe_2O_3$ . The model also indicates that the concentration of haematite dissolved during the leaching process is directly proportional to the final pH of the leaching solution and inversely proportional to the weight input of the iron oxide ore.

It was also found [7] that values of  $Q$  obtained from both the experiment and model ( $Q = K_C \mu$ ) agree to the fact that leaching of iron oxide ore using oxalic acid solution is an endothermic process, hence the absorbed positive heat energy by the leaching solution. The quantity of heat energy absorbed by the oxalic acid solution during the leaching process (as calculated from the model;  $Q = K_C \mu$ ) was found to be directly proportional to the weight input of the iron oxide ore. These results were obtained at initial pH 6.9, average grain size of  $150\mu m$  and leaching temperature of  $30^\circ C$ . The constants of proportionality  $K$  and  $K_C$  associated with the respective derived models were evaluated to be 0.0683 and 66.88 respectively.

Nwoye [8] derived a model for predicting the time for dissolution of pre-quantified concentration of phosphorus during leaching of iron oxide ore in oxalic acid solution as:

$$\tau = \text{Log} \left( \frac{\left( \frac{P^{1/4}}{1.8} \right)}{\text{Log} T} \right) \quad (3)$$

Where

$T$  = Leaching temperature ( $^\circ C$ ) in the experiment [9], taken as specified leaching temperature ( $^\circ C$ ) aiding the expected dissolution of phosphorus .

$N=1.8$ (Dissolution coefficient of phosphorus in oxalic acid solution during leaching of iron oxide ore) determined in the experiment [9].

$P$  = Concentration of dissolved phosphorus (mg/Kg) in the experiment [9], taken as pre-quantified concentration of phosphorus expected to dissolve after a leaching time  $t$  (mg/Kg) in the model.

$\tau$  = Leaching time (sec.) in the experiment [9], taken as time for dissolution of the pre-quantified concentration of phosphorus (hrs) in the model.

The model was found to depend on a range of specified leaching temperatures ( $45-70^\circ C$ ) for its validity. It was found [9] that the time for dissolution of any given concentration of phosphorus decreases with increase in the leaching temperature (up to  $70^\circ C$ ), at initial pH 5.5 and average grain size of  $150\mu m$ .

Nwoye et al. [10] also formulated a model for predicting the concentration of phosphorus removed during leaching of iron oxide ore in oxalic acid solution. It was found to predict the removed phosphorus concentration, with utmost dependence on the final pH of the leaching solution and weight input of the iron oxide ore. The model indicates that the concentration of phosphorus removed is inversely proportional to the product of the weight input of the iron oxide ore and the final pH of the leaching solution. Process conditions considered during the formulation of the model [10] include: leaching temperature of  $25^\circ C$ , initial solution pH 5.5 and average ore grain size;  $150\mu m$ ).

Biological processes for phosphorus removal have also been evaluated based on the use of several types of fungi, some being oxalic acid producing. Anyakwo and Obot [11] recently presented their results of a study on the use of *Aspergillus niger* and their cultural filtrates for removing phosphorus from Agbaja (Nigeria) iron oxide ore. The results of this work [11] show that phosphorus removal efficiencies at the end of the 49 days of the leaching process are 81, 63 and 68% for 5, 100 and 250 mesh grain sizes respectively.

An attempt has been made in the past [12] to leach Itakpe iron oxide ore using oxalic acid solution in order to determine the maximum concentration of phosphorus that is removable. Results of chemical analysis of the ore indicate that the percentage of phosphorus in the ore is about 1.18%, which from all indication is quite high and likely to affect adversely the mechanical properties of the steel involved; hence the need for dephosphorization. It was reported [12] that phosphorus can be removed from iron oxide ore through a process associated with hydrometallurgy. Phosphorus was removed at a temperature of  $25^\circ C$  and initial solution pH 2.5, leading to the dissolution of the phosphorus oxide formed. This involved using acid leaching process to remove phosphorus from the iron oxide ore in readiness for steel making process.

Nwoye [13] derived a model for evaluating the final pH of the leaching solution during leaching of iron oxide ore in oxalic acid solution. The model evaluates the pH value as the sum of two parts, involving the % concentrations of Fe and  $Fe_2O_3$  dissolved. The model can be expressed as;

$$\gamma = 0.5 \left( \frac{K_1}{\% Fe} + \frac{K_2}{\% Fe_2O_3} \right) \quad (4)$$

Where

$K_1$  and  $K_2$  = dissolution constants of Fe and  $Fe_2O_3$  respectively.

$\gamma$  = final pH of leaching solution (after time t).

It was also found that the model [13] could predict the concentration of Fe or  $Fe_2O_3$  dissolved in the oxalic acid solution at a particular final solution pH by taking Fe or  $Fe_2O_3$  as the subject formular. The prevailing process conditions under which the model works include: leaching time of 30mins., constant leaching temperature of 30°C, average ore grain size; 150 $\mu$ m and 0.1M oxalic acid.

Nwoye [14] has reported that the heat absorbed by oxalic acid solution during leaching of iron oxide ore can be predicted using the model he derived which works under the process condition; initial pH 6.9, average ore grain size; 150 $\mu$ m and leaching temperature; 30°C. The model [14] can be stated as

$$Q = K_N \left( \frac{\gamma}{\%Fe_2O_3} \right) \quad (5)$$

Where

Q = Quantity of heat absorbed by oxalic acid solution during the leaching process. (J)

$\gamma$  = Final pH of the leaching solution (at time t).

$\%Fe_2O_3$  = Concentration of haematite dissolved in oxalic acid solution during the leaching process.

$K_N$  = 4.57 (Haematite dissolution constant in oxalic acid solution) determined in the experiment [14].

Nwoye [14] carried out further work on the model using the same process conditions and observed that on re-arranging the model as;

$$\%Fe_2O_3 = K_N \left( \frac{\gamma}{Q} \right) \quad (6)$$

the concentrations of haematite predicted deviated very insignificantly from the corresponding experimental values. In this case, the value of Q was calculated by considering the specific heat capacity of oxalic acid. Values of heat absorbed by the oxalic acid solution during the leaching of iron oxide ore as predicted by the model [14] agree with the experimental values that the leaching process is endothermic. This is because all the predicted values of the heat absorbed by the oxalic acid solution were positive. The model shows that the quantity of heat absorbed by oxalic acid solution during the leaching process is directly proportional to the final pH of the solution and inversely proportional to the concentration of haematite dissolved.

Model for evaluation of the concentration of dissolved phosphorus (relative to the final pH of the leaching solution) during leaching of iron oxide ore in oxalic acid solution has been derived [15]. It was observed that the validity of the model is rooted in the relationship  $\ln P = N/\alpha$  where both sides of the expression are approximately equal to 4. The model;  $P = e^{(12.25/\alpha)}$  is dependent on the value of the final pH of the leaching solution which varies with leaching time. In all, the positive or negative deviation of the model-predicted phosphorus concentration from its corresponding value obtained from the experiment was found to be less than 22%.

Temperature measured at the reaction sites gives an idea of whether the reaction is speeding up or stopping especially when it is measured consistently.

It has been reported [16] that the temperature of a reaction system plays the major role in controlling the rate of the reaction.

Past report [17] has shown that measurement of the temperature of a reaction system consistently shows whether the reaction involved is endothermic or exothermic.

Nwoye [18] derived a model for the computational analysis of the solution temperature during leaching of iron oxide ore in hydrochloric acid solution. The model is expressed as:

$$T = e^{(8.9055/\gamma)} \quad (7)$$

Where

T = Solution temperature during leaching of iron oxide ore using hydrochloric acid. (°C)

N = 8.9055 (pH coefficient for hydrochloric acid solution during leaching of iron

oxide ore) determined in the experiment [18].

$\gamma$  = Final pH of the leaching solution at the time t when the solution temperature is evaluated.

The model is dependent on the value of the final pH of the leaching solution which was found to also depend on the concentration of iron dissolved in the acid. The prevailed process conditions on which the validity of the model depended on include: initial pH 2.5, leaching time; 30 minutes, leaching temperature; 25°C, average ore grain size; 150 $\mu$ m and hydrochloric acid concentration at 0.1mol/litre.

Nwoye et al [19] derived a model for predicting the concentration of dissolved iron during leaching of iron oxide ore in sulphuric acid solution. The model is stated as;

$$\%Fe = 0.35(\alpha/T)^3 \quad (8)$$

Where

T = Solution temperature at the time t, when the concentration of dissolved iron is evaluated. ( $^{\circ}$ C)

0.35= (pH coefficient for iron dissolution in sulphuric acid solution during the leaching process) determined in the experiment [19].

$\alpha$  = Final pH of the leaching solution at the time t, when the concentration of dissolved iron is evaluated.

The model (formulated at conditions; leaching temperature of 25 $^{\circ}$ C, initial solution pH 5.0 and average grain size; 150 $\mu$ m) is dependent of the final pH and temperature of the leaching solution. The model shows that the concentration of iron dissolved during the leaching process is directly proportional to the third power of the ratio of final leaching and temperature.

The aim of this work is to derive a model for quantitative analysis of dissolved haematite relative to the initial solution pH during leaching of Itakpe (Nigeria) iron oxide ore in oxalic acid solution.

## 2. Model

The solid phase (ore) is assumed to be stationary, contains the un-leached iron remaining in the ore. Hydrogen ions from the oxalic acid attack the ore within the liquid phase in the presence of oxygen.

### 2.1 Model Formulation

Experimental data obtained from research work [20] carried out at SynchroWell Research Laboratory, Enugu were used for this work.

Results of the experiment as presented in report [20] and used for the model formulation are as shown in Table 1.

Computational analysis of the experimental data [20] shown in Table 1, gave rise to Table 2 which indicate that;

$$\gamma \propto \left( \frac{1}{D} \right) \quad (9)$$

$$D \propto \%Fe_2O_3 \quad (10)$$

$$D = 1000\%Fe \quad (11)$$

Introducing constants of proportionality into equations (9) and (10)

$$\gamma = \left( \frac{N}{D} \right) \quad (12)$$

Similarly equation (10) becomes

$$D = N_c (\%Fe_2O_3) \quad (13)$$

Table 1 indicates that D is constant for the concentrations of dissolved iron and haematite Therefore, equating equations (11) and (13);

$$1000\%Fe = N_c (\%Fe_2O_3) \quad (14)$$

$$\left( \frac{\%Fe}{\%Fe_2O_3} \right) = \left( \frac{N_c}{1000} \right) \quad (15)$$

Substituting equation (11) into (12);

$$\gamma = \left( \frac{N}{1000\%Fe} \right) \quad (16)$$

From equation (16),

$$\%Fe = \left( \frac{N}{1000} \right) \times \left( \frac{1}{\gamma} \right) \quad (17)$$

Substituting equation (17) into (15);

$$\left( \frac{N}{1000\gamma\%Fe_2O_3} \right) = \left( \frac{N_c}{1000} \right) \quad (18)$$

Rearranging and evaluating equation (18) for  $\%Fe_2O_3$

$$\%Fe_2O_3 = \left( \frac{N}{N_c} \left( \frac{1}{\gamma} \right) \right) \quad (19)$$

Where  $\gamma$  = Initial pH of the leaching solution at time  $t = 0$ .

N= Constant of proportionality assumed as the coefficient of dilution for oxalic acid solution.

$N_c$ = Constant of proportionality assumed as the dissolution coefficient of haematite in oxalic acid solution.

D= Dilution factor

$\%Fe$  = Concentration of dissolved iron in oxalic acid during the leaching process.

$\%Fe_2O_3$  = Concentration of dissolved haematite in oxalic acid during the leaching process.

Equation (19) is the derived model.

The values of the constants N and  $N_c$  were calculated from equations (12) and (13) respectively (using Table 1) for each of the Samples (A-G) and average value of each constant taken since all samples were subjected to the same experimental process conditions (except initial solution pH). This was done by substituting the values of  $\gamma$ , D and D,  $\%Fe_2O_3$  obtained (after a leaching time of 180mins.) for Samples A-G into equations (12) and (13) respectively.

**Table 1: Variation of concentration of dissolved haematite with initial solution pH.[20]**

Sample Code	$\%Fe_2O_3$	$\%Fe$	D	$\gamma$
A	0.044	0.031	31.166	5.88
B	0.045	0.032	31.633	5.71
C	0.049	0.034	34.012	6.00
D	0.041	0.029	28.500	6.32
E	0.055	0.039	38.591	5.74
F	0.050	0.035	35.168	6.13
G	0.050	0.035	34.745	5.73



**Table 2: Values of assumed coefficients of dilution and dissolution of haematite in oxalic acid.**

Sample Code	N	N <sub>c</sub>
A	183.26	708.3182
B	180.62	702.9556
C	204.07	694.1224
D	180.12	695.1220
E	221.51	701.6545
F	215.58	703.3600
G	199.09	694.9000

Average N = 197.7503 , Average N<sub>c</sub> = 700.0618

### 3. Boundary and Initial Condition

Consider iron ore in cylindrical flask 30cm high containing leaching solution of oxalic acid. The leaching solution is stationary i.e (non-flowing). The flask is assumed to be initially free of attached bacteria. Initially, atmospheric levels of oxygen are assumed. Constant weight 10g of iron oxide ore was used. The range of initial pH of leaching solution used; 5.71-6.32 and leaching time; 180 minutes were used. A constant leaching temperature of 25°C was used. Average ore grain size; 150µm, and oxalic acid concentration; 0.1mol/litre was used. These and other process conditions are as stated in the experimental technique [20]. The boundary conditions are: atmospheric levels of oxygen (since the cylinder was open at the top) at the top and bottom of the ore particles in the liquid and gas phases respectively. At the bottom of the particles, a zero gradient for the liquid scalar are assumed and also for the gas phase at the top of the particles. The leaching solution is stationary. The sides of the particles are taken to be symmetries.

### 4. Model Validation

The formulated model was validated by direct analysis and comparison of %Fe<sub>2</sub>O<sub>3</sub> values predicted by model and the corresponding experimental %Fe<sub>2</sub>O<sub>3</sub> values for equality or near equality. Analysis and comparison between these %Fe<sub>2</sub>O<sub>3</sub> values reveal deviations of model-predicted %Fe<sub>2</sub>O<sub>3</sub> values from the corresponding experimental values. This is believed to be due to the fact that the surface properties of the ore and the physiochemical interactions between the ore and leaching solution which were found to have played vital roles during the leaching process [20] were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model-predicted %Fe<sub>2</sub>O<sub>3</sub> values to those obtained from the experiment (Table 3).

Deviation (Dv) (%) of model-predicted %Fe<sub>2</sub>O<sub>3</sub> values from the corresponding experimental %Fe<sub>2</sub>O<sub>3</sub> values is given by

$$Dv = \left( \frac{Mv - Ev}{Ev} \right) \times 100 \quad (20)$$

Where Mv = Predicted %Fe<sub>2</sub>O<sub>3</sub> values from model  
Ev = %Fe<sub>2</sub>O<sub>3</sub> values obtained from experimental data

Correction factor (Cf) is the negative of the deviation i.e

$$Cf = -Dv \quad (21)$$

Therefore

$$Cf = -100 \left( \frac{Mv - Ev}{Ev} \right) \quad (22)$$

Introduction of the corresponding values of Cf from equation (22) into the model gives exactly the corresponding experimental %Fe<sub>2</sub>O<sub>3</sub> value [20].

### 5. Results and Discussion

The derived model is equation (19). A comparison of the values of %Fe<sub>2</sub>O<sub>3</sub> from the experiment and those from the model shows minimum deviation hence depicting the reliability and validity of the model. This is shown in Table 3. The respective deviations observed were less than 11% which is quite within the acceptable range of deviation of experimental results. The values of the assumed coefficients of dilution

(N) and dissolution of haematite ( $N_c$ ) in oxalic acid were evaluated to be 197.7503 and 700.0618 respectively.

**Table 3: Comparison between concentrations of dissolved iron as predicted by model and as obtained from experiment [20].**

$\%Fe_2O_{3exp}$	$\%Fe_2O_{3M}$	Dv (%)	Cf (%)
0.044	0.0480	+9.09	-9.09
0.045	0.0495	+10.00	-10.00
0.049	0.0471	-3.88	+3.88
0.041	0.0447	+9.02	-9.02
0.055	0.0492	-10.55	+10.55
0.050	0.0461	-7.80	+7.80
0.050	0.0493	-1.40	+1.40

Where

$\%Fe_2O_{3exp}$  =  $\%Fe_2O_3$  values from experiment [20]

$\%Fe_2O_{3M}$  =  $\%Fe_2O_3$  values predicted by model.

## 6. Conclusion

The model calculates the concentration of dissolved haematite relative to the initial solution pH during oxalic acid leaching of Itakpe (Nigeria) iron oxide ore. The respective deviations of the model-predicted  $\%Fe_2O_3$  values from the corresponding experimental  $\%Fe_2O_3$  values were less than 11% which is quite within the acceptable range of deviation limit of experimental results.

Further works should incorporate more process parameters into the model with the aim of reducing the deviations of the model-predicted  $\%Fe_2O_3$  values from those of the experiment.

## Acknowledgement

The authors thank Dr. Ekeme Udoh, a modelling expert at Linkwell Modelling Centre Calabar for his technical inputs. The management of SynchroWell Nig. Ltd. Enugu is also appreciated for permitting and providing the experimental data used in this work.

## References

- [1] Taxiarchour, M., Pnias, D., Doumi, I., Paspaliaris, I., Kontopoulos, A. (1997a) Removal of Iron from Silica Sand by Leaching with Oxalic Acid, *Hydrometallurgy*, 46, 215-227.
- [2] Taxiarchou, M., Parnias, D., Douni, I., Paspaliaris, I., Kontopoulos, A. (1997b) Dissolution of Haematite in Acidic Oxalate Solutions. *Hydrometallurgy*, 44, 287-299.
- [3] Lee, S. O., Tran, T., Park Y.Y., Kim S.J., Kim, M. J. (2006) Study on the Kinetics of Iron Leaching by Oxalic Acid. *Int. J. Miner Process*, 80, 144-152.
- [4] Pnias, D., Taxiarchou, M., Paspaliaris, I., Kontopoulos, A. (1996) Mechanism of Dissolution of Iron Oxides in Aqueous Oxalic Acid. *Hydrometallurgy* 42, 257-265.
- [5] Pinches, A. (1975) Bacterial Leaching of an Arsenic Bearing Sulphide Concentrate. The Institute of Mining and Metallurgy, England, 34.
- [6] Nwoye, C. I. (2008) Ph.D Thesis, Metallurgical and Materials Engineering Department, Federal University of Technology, Owerri, 178.
- [7] Nwoye, C. I. (2008) Model for Computational Analysis of Dissolved Haematite and Heat Absorbed by Oxalic Acid Solution during Leaching of Iron Oxide Ore, *J. Eng. & App. Sc.*, 4, 22-25.
- [8] Nwoye, C. I. (2008) Model for predicting the Time of Dissolution of Pre-quantified Concentration of Phosphorus during Leaching of Iron Oxide Ore in Oxalic Acid, *IJONAS*, 4(3):168-174.
- [9] Nwoye, C. I. (2006) SynchroWell Research Work Report, DFM Unit, No 2561178, 66-83. [13]
- [10] Nwoye, C. I., Agu, P. C., Mark, U., Ikele, U. S., Mbuka, I. E., and Anyakwo, C. N. (2008) Model for Predicting Phosphorus Removal in Relation to Weight of Iron Oxide Ore and pH during Leaching with Oxalic Acid *Inter. J. Nat. Appl. Sc.*, 4(3): 106-112.

- [11] Anyakwo, C. N., and Obot, O.W. (2008) Phosphorus Removal from Nigeria's Agbaja Iron Ore by *Aspergillus niger*, IREJEST 5(1), 54-58.
- [12] Nwoye, C. I. (2003) SynchroWell Research Work Report, DFM Unit, No 2031196, 26-60.
- [13] Nwoye, C. I. (2008) Model for Quantitative Analysis of Dissolved Iron in Oxalic Acid Solution during Leaching of Iron Oxide Ore, Inter. Res. J. Eng. Sc. Tech., 5(1): 37-41.
- [14] Nwoye, C. I. (2008) Model for Computational Analysis of Dissolved Haematite and Heat Absorbed by Oxalic Acid Solution during Leaching of Iron Oxide Ore, J. Eng.& App. Sc., 4, 22-25.
- [15] Model for Evaluation of the Concentration of Dissolved Phosphorus during Leaching of Iron Oxide Ore in Oxalic Acid Solution. JMMCE (in press)
- [16] Ferreira, R.C.H and Burkin, A.R; Acid Leaching of Chalcopyrite. In A.R Burkin; Leaching and Reduction in Hydrometallurgy. The Institution of Mining and Metallurgy, England, 1975 60.
- [17] Ugarte, F J and Burkin, A.R; Mechanism of Formation of Idaite from bornite by Leaching with Ferric Sulphate Solution. In A.R Burkin ; Leaching and Reduction in Hydrometallurgy. The Institution of Mining and Metallurgy, England, 1975, 47.
- [18] Nwoye, C. I., (2006) SynchroWell Research Work Report, DFM Unit, No. 2021196, 28-43.
- [19] Nwoye, C. I., Amara, G. N., and Onyemaobi, O. O. (2008) Model for Evaluating Dissolved Iron during Leaching of Iron Oxide Ore in Sulphuric Acid Solution, Inter. J. Nat. Appl. Sc., 4(2): 209-211.
- [20] Nwoye, C. I., (2006) SynchroWell Research Work Report, DFM Unit, No. 2031178, 75-92.

3/2202009

## Taxonomic studies on the family Pteridiaceae Ching and Pteridaceae Ching (Pteridophyta) in Uttarakhand

Mamta Rani, Y.P.S. Pangtey, Lalit M. Tewari, Sanjay Kumar, Jeevan Singh Jalal, Anita Martolia, Kanchan Upreti and Tapan Nailwal\*

Department of Botany, \*Department of Biotechnology, D.S.B. Campus, Kumaun University, Nainital – 263002, India

[l\\_tewari@rediffmail.com](mailto:l_tewari@rediffmail.com); [jeewansinghjalal@rediffmail.com](mailto:jeewansinghjalal@rediffmail.com); [y\\_pangtey@yahoo.com](mailto:y_pangtey@yahoo.com);  
[sonu\\_monu28@rediffmail.com](mailto:sonu_monu28@rediffmail.com); [tapannailwal@gmail.com](mailto:tapannailwal@gmail.com)

**ABSTRACT:** The present work, 2 families, 2 genera and 15 species i.e. *Pteridium* (1 species) and *Pteris* (14 species) including 1 subspecies have been studied. Some of the taxa of ferns reported earlier from Uttarakhand by previous workers based on wrong identification have been placed under the heading excluded/doubtful species giving only botanical name and the reasons of their being excluded/doubtful species are based on Khullar (1994, 2000, 2001). [Researcher. 2009;1(4):15-41]. (ISSN: 1553-9865).

**Key words:** Pteridaceae, Uttarakhand, Pteridium

### Introduction

The eastern part of the west Himalaya is occupied by the Uttarakhand state and lies between the latitudes 28° 44' and 31° 32' North and longitudes 77° 38' and 81° 1' East. The newly created state of Uttarakhand is situated at the trijunction of Nepal, Tibet and India. In the north, a natural water divide separates it from the Tibet, Kali river defines its eastern border with the kingdom of Nepal, Yamuna-Tons rivers separates it from the state of Himachal Pradesh in the west and southern limit of tarai belt almost demarcates its southern boundary and corresponds almost exactly with the southern limit of the tarai-belts separating it from Saharanpur, Bijnor, Moradabad, Bareilly, Rampur and Pilibhit districts of Uttar Pradesh state. Thus it constitutes a distinct geographical entity of great strategic significance.

The publication of very comprehensive account of plant collections from Kumaun and adjacent parts of Garhwal by Sir Richard Strachey and J.E. Winterbottom between the years 1846-1849 followed the classical works. The original catalogue was published in 1852 in *Atkinson's Gazetteer of Himalayan Provinces and Oudh*. This original catalogue of Strachey and Winterbottom was later revised and supplemented by J.F.Duthie (1906), which is known as *Catalogue of the Plants of Kumaun and of the adjacent portions of Garhwal and Tibet based on the collections made by Strachey and Winterbottom during the years 1846-1849* and on the catalogue originally prepared by Sir Richard Strachey in 1852, by adding the results of previous and subsequent botanical explorations. This catalogue still functions as a milestone for the floristic works including pteridophytes in the part of Himalaya. From this vast area, covering an area of 18,400 km<sup>2</sup>, a total of 30 genera of ferns and 4 genera of fern-allies belonging to 185 species of ferns and 13 species of fern-allies were recorded. However, the nomenclature used is outdated and unsuitable for modern researches and present day needs. Besides, it completely lacks keys, descriptions, figures and ecological notes etc. of the species of pteridophytes listed.

Herbarium specimens of plants drawn are deposited in the Herbarium, Department of Botany, D.S.B. Campus, Kumaun University, Nainital except *Pteris multifida*, *Pteris pellucida* and *Pteris vittata* subsp. *Vermae*, which are included on the basis of published reports only. The figures of the whole plant or frond or part of it are natural size, while for the detailed drawings the following magnifications have been used; dermal appendages (x 17.5), magnified portion (x 10), spores (x 450), part of lamina to show venation and arrangement of sori (x 150), indusia (x 17.5) following Khullar (1994).

**KEY TO FAMILIES**

- A. Lamina 1-2-pinnate, glabrous; veins free or anastomosing;  
indusia formed by incurved lamina margin.....2. **Pteridaceae**
- A. Lamina 2-3-pinnate, hairy throughout; veins free or  
forked; indusia double; inner one obsolete.....1. **Pteridiaceae**

1. Family: **PTERIDIACEAE** Ching, Acta Phytotax. Sin. **13**: 96 (1975).

Rhizome long-creeping, profusely branched, polystelic, densely hairy; hairs light brown, long, weak, straight, broad scales absent. Lamina large, 2-3-pinnate-quadripinnatifid, hairy throughout, hairs pale brown. Sori continuous, marginal, linear; indusia double, one consisting of thin reflexed edge of the lamina, the other thinner, attached just below the receptacle.

Type: *Pteridium* Gled. ex Scop., Fl. Carniolica : 169 (1760), *nom. cons.*

**PTERIDIUM**

*Pteridium* Gled. ex Scop., Fl. Carniolica : 169 (1760), *nom. cons.*

*Circinalis* Gled., Syst. Pl. : 290 (1764).

*Eupteris* Newm., Phytologist **2**: 278 (1845).

*Ornithopteris* (J. Agardh) J. Smith, Hist. Fil. : 297 (1875), *nom. Bernh.*

Type : *Pteridium aquilinum* (L.) Kuhn, Deck. Reis. 3 Bot.:11 (1879).

Rhizome long-creeping, hypogeal, clothed with rather few pale brown hairs, solenostelic. Stipes long. Fronds large. Lamina 2-3-pinnate or quadripinnatifid, continuing apical growth for a considerable period; costules, costa and rachises grooved on the upper surfaces, ultimate pinnules or lobes rather small and narrow; texture firm to subcoriaceous, more or less densely hairy all over; veins free except for a marginal strand. Sori submarginal, linear, borne on the connecting veins, indusiate; indusium double, outer false formed by reflexed margin, the inner true well developed or obsolete. Sporangia with the annulus passing just on one side of the stipe. Spores globose, tetrahedral, trilete, pale brown, nonperinate, exine smooth.

***Pteridium revolutum*** (Bl.) Nakai, Bot. Mag. 39: 109 (1925); Brownsey, Aust.Syst. Bot. **2**: 120 (1989); Fraser-Jenkins, New Sp. Syndrome Indian Pterid. & Ferns Nepal: 217 (1997); Chandra, Ferns India : 109 (2000).

*Pteris rostrata* Bl., Enum. Pl. Jav.: 214 (1820).

*Pteridium aquilinum* (L.) Kuhn var. *wightianum* sensu auct. Ind.; Stewart. 150<sup>th</sup> Ann. Vol. Royal Bot. Calcutta 2: 168 (1942); Loyal & Verma, J. Bombay nat. Hist. Soc. 57: 484 (1960); Pande, Indian For. 99: 50 (1973); Chandra, J. Bombay nat. Hist. Soc. 74: 643 (1979); Awasthi & Sharma, Proc. Indian Acad. Sci. (Pl. Sci.) 89: 310 (1980); Dhir & Sood, Biblioth. Pterid. 2: 36 (1981); Goel & Bhattacharyya, Indian Fern J. 4: 33 (1981); Pangtey et al., Him. Res. & Dev. 1:160 (1982); Bir et al., Pterid. Fl. Garhwal Him.: 32 (1983); Dixit, Census Indian Pterid.: 98 (1984); Pande & Kandpal, Acta Botanica Indica 14 (Suppl.): 118 (1986); Singh et al., Indian J. For. 9: 14 (1986); Khullar et al. in Western Him. 1: 396 (1987); Pande & Pande, Acta Botanica Indica 15: 102 (1987); Pande & Dashila, Indian Fern J. 5: 81 (1988); Pande, Geobios new Reports 8: 106 (1989); Indian Fern J. 7: 165 (1990); Khullar et al., Ferns Nainital 86 (1991); Pangtey et al., New Botanist 18: 221 (1991); I11. Fern Fl. West Him. 1: 254. t. 91 (1994); Chandra, Ferns India: 108 (2000).

*Pteris wightianum* Wall., Nurmer. List no.: 2178 (1829), *nom. nud.*

*Pteridium aquilinum sensu* Mehra, Ferns Muss.: 11 (1939); Chandra, Ferns India: 108 (2000).

*Pteris aquiline sensu* Clarke, Trans. Linn. Soc. Lond. II (Bot.) 1: 468 (1880); Bedd., Handb. Ferns Brit. India : 115 (1883); Duthie, Cat. Pl. Kumaun: 224 (1906); Marten, J. Bombay nat. Hist. Soc. 19: 179 (1909).

Rhizome long-creeping, stout, hairs pale brown, uniseriate, 7-13-celled, acicular, straight. Stipes 40 cm or more, stout, stramineous, hairy but extreme base densely woolly, hairs brown. Stipe becoming sparsely hairy to almost glabrous higher up; rachis sparsely hairy becoming glabrous. Fronds rather distant. Lamina 2-3-pinnate, large, 30-60 (-150) x 30-60 cm; subdeltate, texture subcoriaceous to coriaceous, hairy, hairs white; pinnae 10-15 pairs, alternate, distal pinnae opposite, lower pinnae the largest, 30 x 10 cm; pinnules lanceolate, margin lobed to the costules into numerous short lobes, which are ovate, more or less falcate, apex obtuse, margin entire or lobed in the middle pinnae; pinnules about 6 x 1 cm, margin lobed almost to the costule, about the middle few pairs of pinnae lanceolate, densely lobed to the costa, lobes short, triangular or linear, terminal few pairs of pinnae simple; veins free, simple or forked, hairy, costules and costae grooved on the upper surface densely hairy. Sori marginal, continuous linear, indusiate; indusia double, inner one obsolete, membranaceous. Spores brown, 21.5 – 31.5 x 24.0 – 35.0 µm, exine almost smooth.

**HABITAT:** Grows on forest floor or as a weed in orchards. It forms thickets resulting in a characteristic ground vegetation of oak forest.

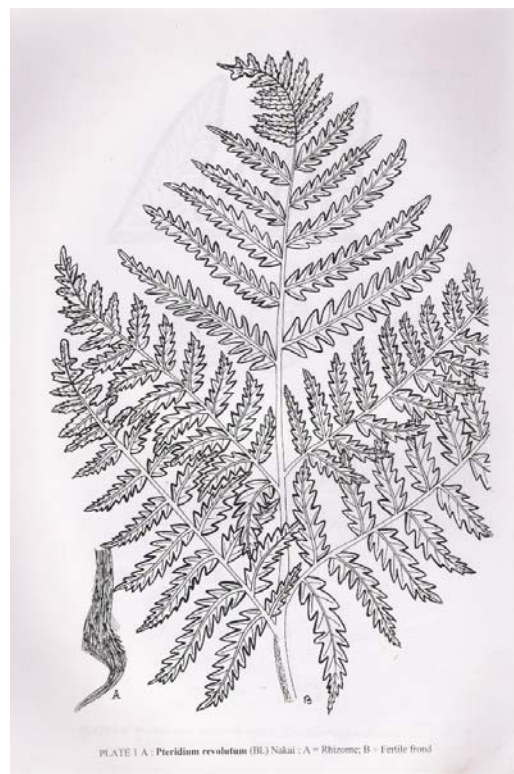
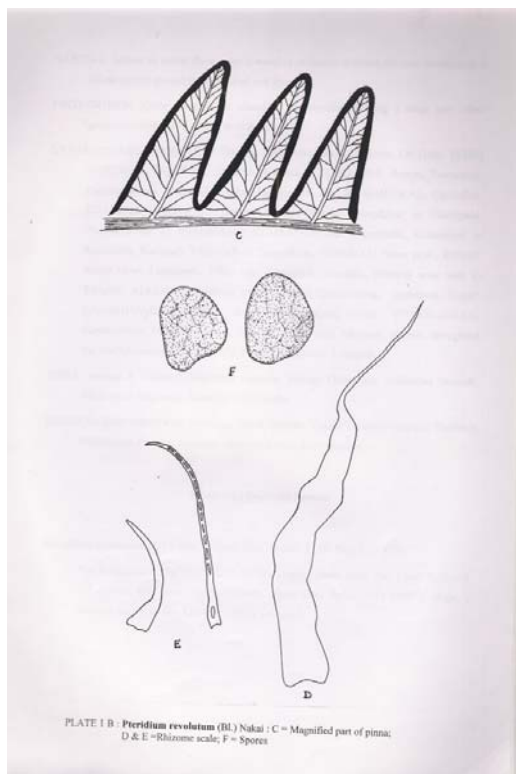
**DISTRIBUTION:** Quite common to abundant and usually covering a large area often gregarious from 1800-2700 m altitude.

**UTTARAKHAND:** DEHRADUN: Deoban, Mussoorie, Jabarkhet forest, Lal Tibba. **TEHRI GARHWAL:** Nag Tibba, Dhanolti, Govana, **UTTARKASHI:** Barkot, Yamunotri, Hanumanchatti, Gangotri, Lanka, Jangalchatti. **PAURI GARHWAL:** Candollea. **CHAMOLI GARHWAL:** Gopeshwar to Chopta, Gobindghat to Ghangaria, Pandukeshwar to Vinayakchatti. **RUDRAPRAYAG:** Guptakashi, Gaurikund to Kedarnath, Kalimath, Ukhimath to Duggalbitta. **NAINITAL:** Naini peak, Kilbury, Snow view, Lariakanta, Tiffin top, Khurpatal, Jeolikote, Bhimtal area, way to Ratighat. **ALMORA:** Ranikhet, Jhuni, Kausani, Dhaulchhina, Jageshwar, Binsar. **BAGESHWAR:** Laharkhet to Dhakuri, Khat, Jhuni. **PITHORAGARH:** Kanalichhina, Didihat, Berinag, Chakori, Gangolihat, Munsiri, Namik, throughout the district (common). **CHAMPAWAT:** Champawat, Lohaghat.

**INDIA:** Jammu & Kashmir; Himachal Pradesh: Sikkim; Darjeeling; Arunachal Pradesh; Manipur; south India.

**GENERAL DISTRIBUTION:** Pakistan; Nepal; Bhutan; China; Taiwan; Vietnam; Thailand; Philippines; Borneo; Sumatra; Java; Sri Lanka; New Guinea.





### Excluded / Doubtful Species

**Pteridium aquilium** (L.) Kuhn, v. Deck. Reis. Ostaf. 3(3) : Bot. 11 (1879).

*Pteris aquilina* L., Sp. Pl. 2 : 1075 (1753); Clarke, Trans. Linn. Soc. Lond. II (Bot.)1: 468 (1880), *pro parte*; Bedd., Handb. Ferns Brit. India : 115 (1883); Hope, J. Bombay nat. Hist. Soc.13 : 455 (1901), *pro parte*.

2. Family: **PTERIDACEAE** Ching, Webbia 35(2): 239 (1982).

Rhizome short, erect or short-creeping, dictyostelic, densely scaly; scales lanceolate, septate hairs absent. Fronds mostly clustered or close together. Lamina 1-2-pinnate, labrous; veins free or anastomosing; costae with small appendages, setae on upper surface. Sori continuous, marginal; indusia formed by incurved lamina, which never reach the costae. Spores mostly tetrahedral, trilete, rarely bilateral, monolete.

### PTREIS

*Pteris* Sp. Pl. 2 : 1073 (1753); Gen. Pl.ed.5 : (1754).

*Campteris* Presl, Tent. Pterid. : 146 (1836).

Type: *Pteris longifolia* L., Sp. Pl. 2: 1074 (1753).

Rhizome short, erect or creeping, scaly, scales generally towards rhizome apex, linear-lanceolate, margin entire or variously lobed or fimbriate. Stipes base generally scaly; rachis grooved on the upper side. Fronds dimorphic or isomorphic. Lamina 1-2(-3)-pinnate, never finely dissected, herbaceous to coriaceous, glabrous, lowest pair of pinnae often forked near the base on basiscopic side resulting in a long pinnule with its lobes similar to other pinnae, terminal pinna similar to lateral ones; veins free or anastomosing to form a series of narrow areolae along the costar or costules; areolae without included veinlets; costae and costules usually with short setae on the upper surface. Sori actually submarginal but as the margin curls back to form the indusium it becomes marginal, linear; indusia formed by reflexed pinna margin. Spores brown, tetrahedral, trilete, rarely bilateral, monolete, nonperinate, exine smooth, tuberculate



or verrucose, the two surfaces have different types of ornamentation, a prominent collar-like ridge girdles the spores.

**KEY TO SPECIES**

- A. Lamina pinnate.....B
- A. Lamina 2-pinnatifid or 2-pinnate.....G
- B. Lowest pinnae never forked.....C
- B. Lowest pinnae forked at least once.....E
- C. Pinnae generally 3 (-7), lower never reduced;  
fronds dimorphic.....10. **P. stenophylla**
- C. Pinnae numerous, lower gradually reduced,  
distant and sterile.....D
- D. Plants tetraploid sexual.....12. **P. vittata**
- D. Plants diploid sexual.....13. **P. vittata** subsp. **Vermae**
- E. Terminal pinna and the pair of pinnae below it  
decurrent on rachis.....7. **P. multifida**
- E. Terminal pinnae and the pair of pinnae below it  
not decurrent on rachis.....F
- F. Fronds on a rhizome few; pinnae many  
(3-10 pairs), broad 0.5-1.0 cm fertile, 1-2 cm sterile.....4. **P. cretica**
- F. Fronds on a rhizome many; pinnae usually 3,  
rarely up to 7 narrow (0.3 cm broad).....5. **P. dactylina**
- G. Rhizome short-creeping; lowest pair of  
pinnae usually not forked; ultimate lobes  
long, facate; sori extend from almost the  
base of the sinus to the apex of the pinna lobe.....6. **P. excelsa**
- G. Rhizome erect; lowest pair of pinnae always  
forked at least once; ultimate lobes linear,  
oblong, short; sori never reaching the sinus  
but restricted to the middle of the pinna lobes.....H
- H. Pinnae few, a large central terminal pinna with usually one, rarely two pairs of  
pinnae.....11. **P. subquinata**
- H. Pinnae many, an apical pinna with 4-15  
pairs of lateral ones.....I
- I. Lamina pinnately compound.....J
- I. Lamina pedately compound.....14. **P. wallichiana**
- J. Stipes cataneous throughout.....K
- J. Stipes castaneous to deeply pinkish.....L

- K. Lateral pinnae up to 5 pairs.....2. **P. asperula**  
K. Lateral pinnae 5-15 pairs.....1. **P. aspericaulis**  
L. Lateral pinnae 2-5 pairs.....9. **P. puberula**  
L. Lateral pinnae not as above.....M  
M. Lateral pinnae deeply lobed.....3. **P. biaurita**  
M. Lateral pinnae unlobed.....8. **P. pellucida**

### 1. **PTERIS ASPERICAULIS**

*Pteris aspericaulis* Wall. Ex Agardh, Recens. So. Gen. Pterid. : 22 (1839); Pande, Indian For. 99: 50 (1973); Chandra, J. Bombay nat. Hist. Soc. 74: 643 (1979); Dixit, Census Indian Pterid.: 68 (1984); Chandra, Ferns India :33 (2000).

*Pteris quadriaurita* Retz. Ver. *Aspericaulis* (Wall. Ex Agardh) Bedd., Dandb. Ferns Brit. India : 111 (1883).

*Pteris pseudoquadriaurita* Khullar, Ill. Fern. Fl. West Him. 1: 272. t. 98 (1994).

Rhizome short, erect, scaly; scales dark-brown, concolorous, linear-lanceolate, margin irregularly dentate- crenate, apex acute. Stipes 25-45 cm long, stramineous or pink or upper surface stramineous, lower pink or almost violet, asperous or glabrous; rachis variable in colour like stipe, glabrous. Lanceolate or ovate-lanceolate; texture subcoriaceous, glabrous; pinnae 4-15 pairs. 10-20 x 1.5-3.0 cm, alternate, lower pinnae shortly stalked, upper sessile, margin deeply lobed to the costa; pinnules many, 0.5-2.0 x 0.3-0.4 cm, linear or oblong-obtuse, size and shape variable, distant or close, apex apiculate to acuminate, infertile apex of fertile lobes almost entire, lowest pair of pinnae always the largest and forked at least once, rarely twice or three times, the next one or two pairs of pinnae may also be forked; veins free, forked or simple, the lowest from either side of costa reaching the sinus but never fusing. Sori indusiate, extent of fertility variable, generally the sorus is present a little above the sinus and not reaching the lobe apex. Spores dark-brown, 31.5-42.0 x 38.5-45.5 µm, exine tuberculate.

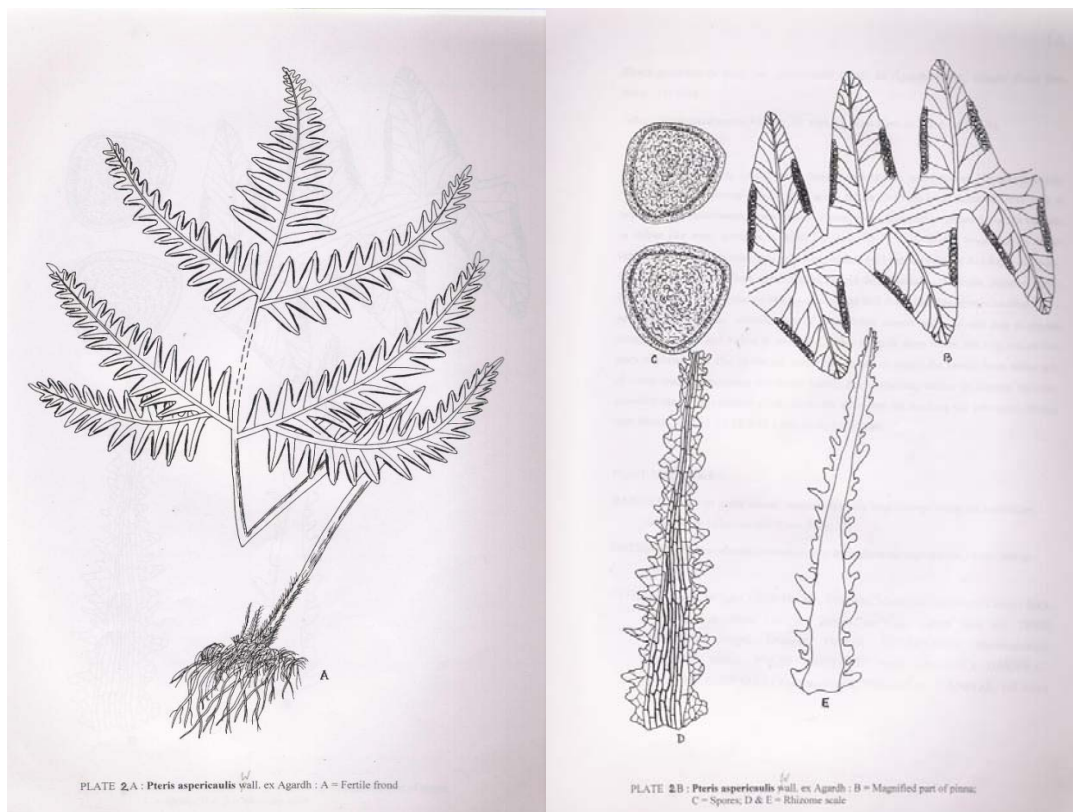
**HABITAT:** Grows on moist shaded localities forming large clumps along the road-sides, way sides or in humus rich forest floor.

**DISTRIBUTION:** An extremely common fern throughout the region from 1800-2400 m altitude.

**UTTARAKHAND:** DEHRA DUN: Deoban, Chakrata, Mussoorie (all over), Camel's back, Jabarkhet, Lal Tibba, Company garden, *en route* Kemty falls etc. **TEHRI GARHWAL:** Magra, Dhanolti, Deolsari. **UTTARKASHI:** Hanumanchatti, Brahmshal, Barkot. **PAURI GARHWAL:** Pauri. **CHAMOLI GARHWAL:** Gobindghat. **RUDRAPRAYAG:** Gaurikund, Vinayakchatti, **NAINITAL:** (all over), Snow view, land's end, Cheena peak range, Kilbury, Iriakanta, Dhobighat etc. **ALMORA:** Almora, Lodhia, Binsar, Jageshwar, Ranikhet. **BAGESHWAR:** Jalat, Phurkia, Dhakuri, Sikhar, Bageshwar. **PITHORAGARH:** Above Maghar, Deochula, Dhaj, Thalkedar, Didihat, Berinag, Munsiri etc. (common). **CHAMPAWAT:** Lohaghat, Champawat.

**INDIA:** Jammu & Kashmir (rare); Himachal Pradesh; Sikkim; Darheeling; Karnataka; Takil Nadu; Kerala.

**GENERAL DISTRIBUTION:** Nepal; Tibet; China (Yunnan); Thailand; N Myanmar.



## 2. PTERIS ASPERULA

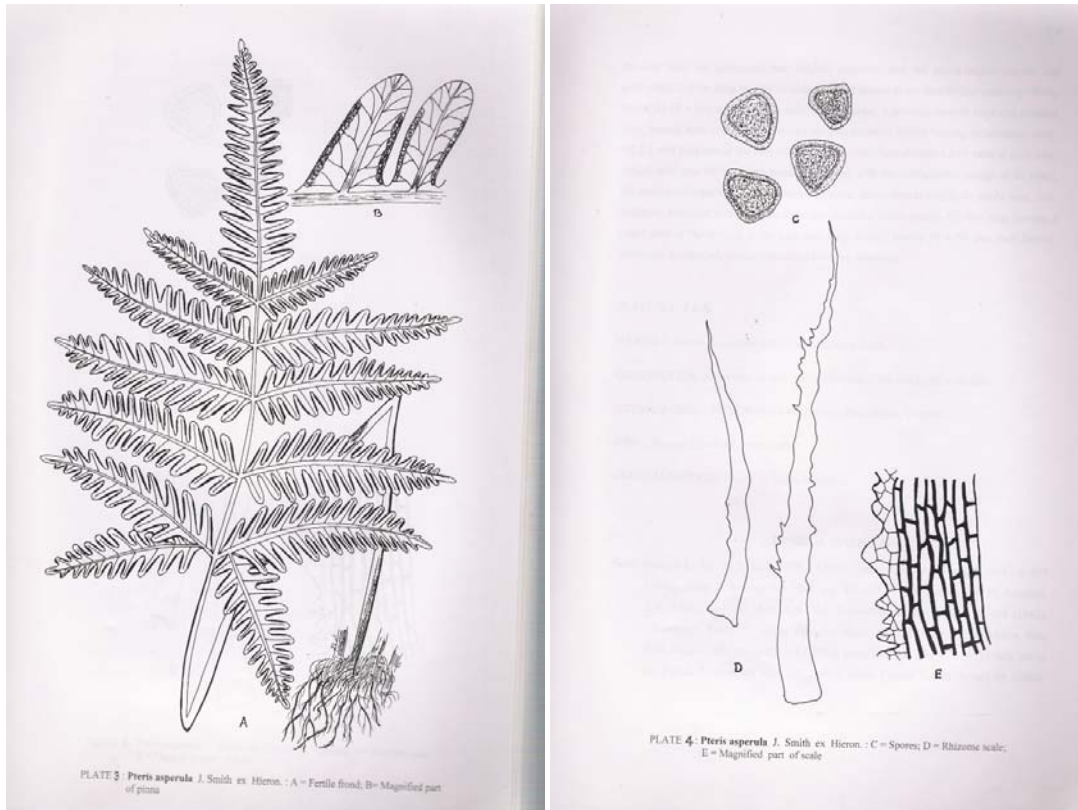
*Pteris asperula* J. Smith ex Hieron., Hedwigia 55 : 361 (1941); Dixit, Census Indian Pterid. : 69 (1984); Pande, Indian Fern J. 7: 157 (1990); Chandra, Ferns India : 33 (2000).

*Pteris quadriaurita* Retz. Var. *setigera* Hook. in Bedd. Handb. Ferns Brit. India : 11 (1883).

*Pteris quadriaurita* Retz. Var. *hamulosa* Wall. Ex Bedd., Handb. Ferns Brit. India Suppl.: 23 (1892).

*Pteris quadriaurita* Retz. Var. *asperula* (J. Smith ex Hieron.) Bedd., Handb. Ferns Brit. India Suppl.: 24 (1892).

Rhizome suberect, 3-7 cm thick, unbranched. Stipe 15-45 cm long, pale reddish brown when dry, with median groove shallow and broad, glabrous upwards at maturity. Fronds  $\pm$  spreading. Lamina 20-30 x 15-25 (-30) cm, bipinnate, with the rachis glabrous and bearing 5-10 pairs of subopposite lateral obliquely placed pinnae and a similar terminal one, basal lateral pinna more spaced and short pitiolate but others subsessile to sessile, basal one or two pairs larger than others, 20 x 2-3 (-4) cm, bearing with basiscopic basal half pinnae like lobes which are similar to and as large as lateral pinnae; pinnules ablong, with almost truncate base but acroscopic base slightly narrowed and the apical region narrow and acuminate, 2-3 cm long, margin of pinnule lobed almost to the midrib into narrowly oblong lobes, 12-18 x 3-4 mm and with nearly parallel sides, a pellucid smooth edge and rounded, apex, lamina thin, coriaceous, glabrous on both surfaces, midrib bearing an obliquely erect, 1.0-1.5 mm long seta at the base of main lateral veins, lateral veins 12-15 pairs in each lobe, forked once near the base with branches merging with the cartilaginous margin of the lobes, the basal basiscopic veins arising from the costa; fertile fronds similar to sterile ones. Sori indusiate, restricted to the margin of the lobes, usually 4-5 mm long, leaving a major part of the margin at the base and apex sterile. Spores 35 x 50  $\mu$ m, dark brown, verrucose, tetrahedral, trilete, sometimes bilateral, monolete.



**HABITAT:** Grows as isolated land on shaded forest floor.

**DISTRIBUTION:** An extremely rare species between 1000 and 1500 m altitude.

**UTTARAKHAND :** PITHORAGARH : Didihat, Debichhina, Kukrouli.

**INDIA:** Eastern Himalaya, South India.

**GENERAL DISTRIBUTION:** SE India, Myanmar.

### 3. PTERIS BIAURITA

*Pteris biaurita* L., Sp. Pl. 2: 1076 (1753); Clarke, Trans. Linn. Soc. Lond. II (Bot.) 1: 469 (1880); Hope, J. Bombay nat. Hist. Soc. 13: 455 (1901); Duthie, Cat. Pl. Kumaun: 224 (1906); Stewart, 150<sup>th</sup> Ann. Vol. Royal Bot. Gard. Calcutta 2: 168 (1942); Chowdhury, Pterid. Fl. Upper Gangetic Plain: 32 (1973); Dhir, Biblioth. Pterid. 1: 49 (1980); Bir et al., Pterid. Fl. Garhwal Him.: 27 (1983); Dixit, Census Indian Pterid.: 69 (1984); Pande & Kandpal, Acta Botanica Indica 14 (Suppl.): 117 (1986); Khullar et al. in Western Him. 1: 363 (1987); Pangtey in Western Him. 1: 396 (1987); Pande & Dashila, Indian Fern J. 5: 80 (1988); Pande, Geobios new Reports 8: 105 (1989); Indian Fern J. 7: 157 (1990); Khullar et al., Ferns Nainital: 77 (1991); Ill. Fern Fl. West Him. 1: 260. t. 92 (1994); Chandra, Ferns India: 34 (2000).

*Pteris nemoralis* Willd., Enum. Pl. Berol.: 1073 (1809); Caroli, Sp. Pl. 5: 386 (1810); Alston & Bonner, Candollea 15: 202 (1956).

*Pteris pectinata* D. Don, Prodr. Fl. Nepal.: 15 (1825).

*Campteria biaurita* (L.) Hook., Gen. Fil.: t. 65 A (1841); Bedd., Ferns South. India: t. 44 (1863); Handb. Ferns Brit. India: 116 (1883)

Rhizome short. Erect, apex scaly; scales brown, bicolorous, margins irregularly fimbriate with a few long projections. Stipes 30-45 (-60) cm long, stramineous but extreme base brown, thick; rachis glabrous. Lamina pinnate, 30-45 (-60) x 25-30 cm; texture herbaceous, glabrous; pinnae 5-10 pairs, 15-25 x 3-5 cm, alternate, short petiolate, lanceolate, margin deeply lobed almost to the costa; lobes 20 pairs, lobes 20 pairs, oblong, apex rounded, margin entire, sinus narrower in sterile, broad in fertile, acroscopic lobes smaller than the basiscopic ones, the lowest pair of pinnae always forked on the basiscopic side opposite the second acroscopic lobe; veins the basiscopic basal veins from each costule anastomosing with the acroscopic basal veins of the next costule to form a more or less curved continuous arch along the costa, many veins arise from arch and go towards the base of the sinus, the remaining veins in the inner lobe free, forked; costae the costules with small setae on the upper surface at their junction. Sori indusiate, margins continuous from the base of sinus and reaching almost to the apex with a very little infertile apex left; indusia continuous curls back at maturity. Spores dark brown, 45.5-49.0 x 49.0-61.5  $\mu\text{m}$ , densely rugose on the distal surface, verrucose on the proximal one.

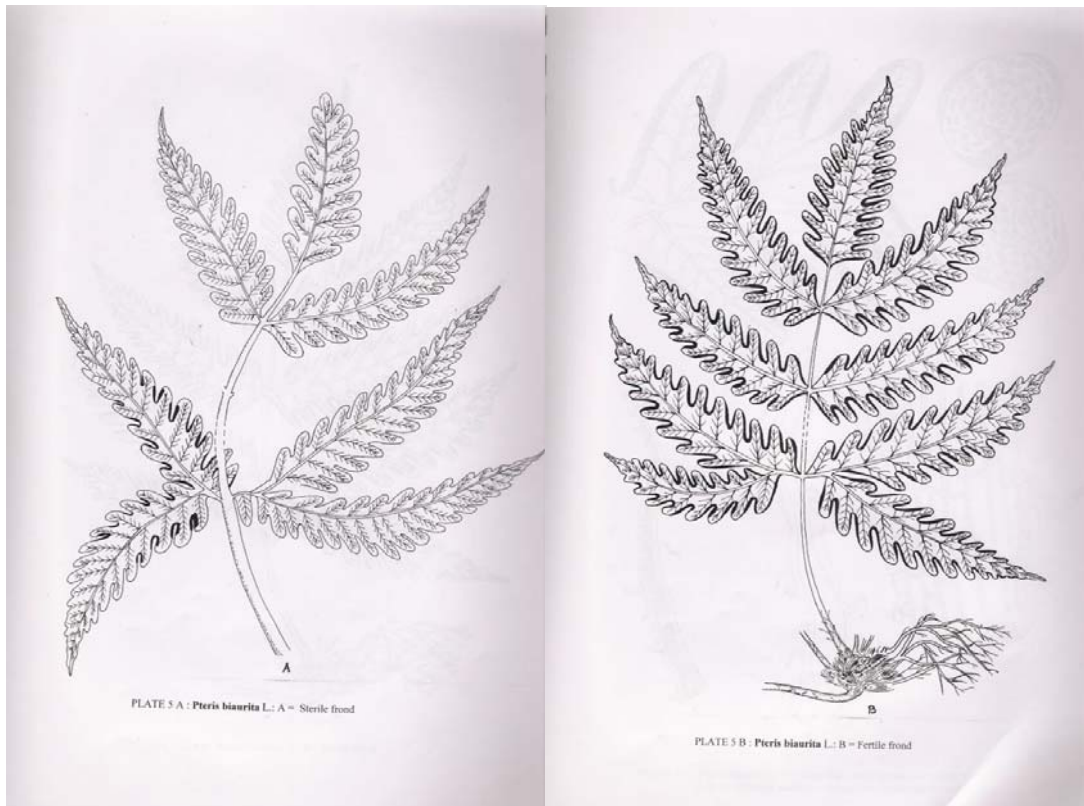
**HABITAT:** Generally grows along the banks of streamlets or in wet places in forested areas.

**DISTRIBUTION:** Quite common in the region between 600 and 1300 m altitude.

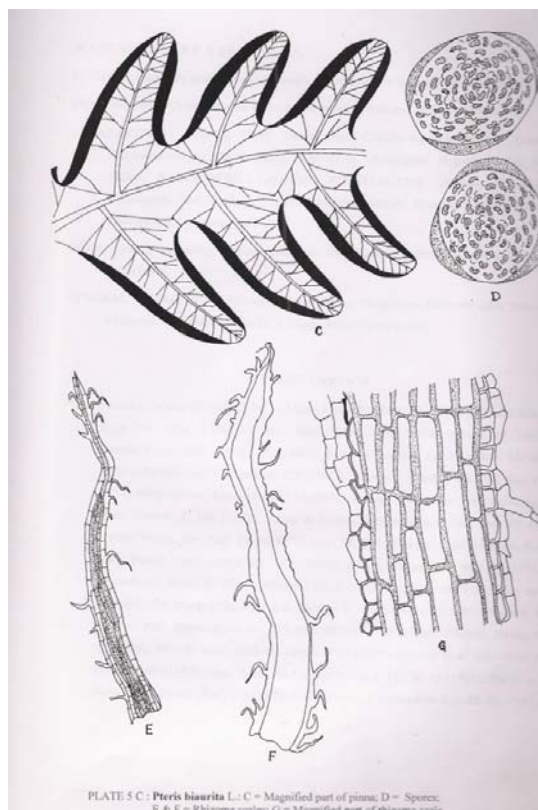
**UTTARAKHAND:** DEHRA DUN: Dehra Dun. **CHAMOLI GARHWAL:** between pandukeshwar to Vinayakchatti. **NAINITAL:** Bhujiaghat, Dogaon; Sattal, Gola valley. **BAGESHWAR:** Loharkhet. **PITHROGARH:** Didihat, Gangolihat, Pithoragarh, Thal, Thalkedar, Pamtori, Malghar, Suklari, Munsiri. **CHAMPAWAT:** Champawat, Lohaghat, Sukhidak.

**INDIA:** Sikkim; Darjeeling; Arunachal Pradesh; Meghalaya; Nagaland; Tripura; central and south India.

**GENERAL DISTRIBUTION:** Nepal; Bhutan; China; Philippines; Malaysia; Java; Borneo; Myanmar; Sri Lanka; Australia; S. Africa; Brazil; West Indies.







#### 4. PTERIS CRETICA

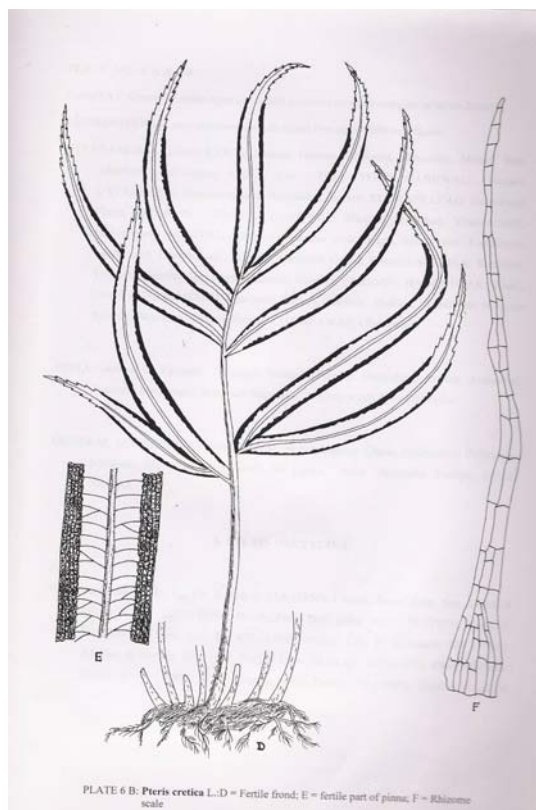
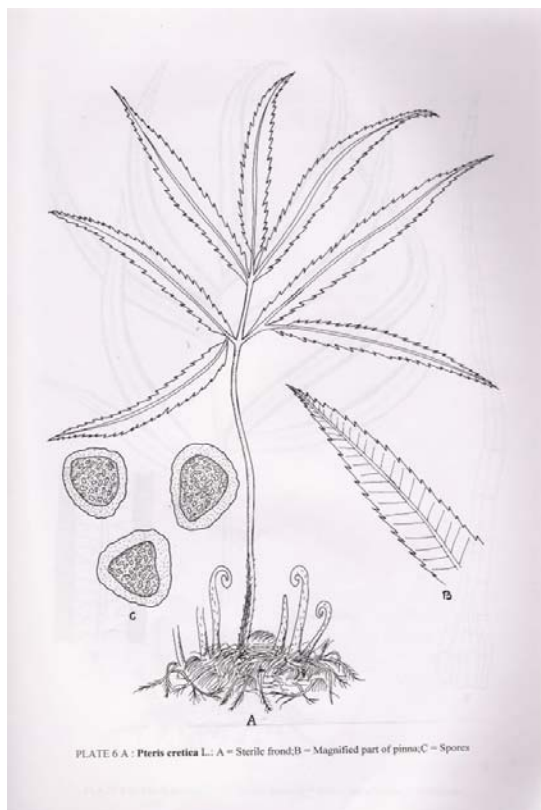
*Pteris cretica* L., Mant. Pl. : 130 (1767); D. Don, Prodr. Fl. Nepal.: 15 (1825); Clarke, Trans. Linn. Soc. Lond. II (Bot.) 1: 462 (1880); Handb. Ferns Brit. India: 106 (1883); Hope, J. Bombay nat. Hist. Soc. 13: 449 (1901); Duthie, Cat. Pl. Kumaun: 224 (1906); Khullar & Mehra, Res. Bull. Panjab Univ. (n.s.) 23: 193 (1972); Pande, Indian For. 99: 50 (1973); Chandra, J. Bombay nat. Hist. Soc. 74: 643 (1979); Verma & Khullar, Fern Gaz. 12: 85 (1980); Dhir, Biblioth. Pterid. 1: 47 (1980); Dhir & Sood, Biblioth. Pterid. 2: 33 (1981); Pangtey et al., Him. Res. & Dev. 1: 160 (1982); Bir et al., Pterid. Fl. Garhwal Him.: 27 (1983); Dixit, Census Indian Pterid.: 69 (1984); Pande & Kandpal, Acta Botanica Indica 14 (Suppl.): 117 (1986); Khullar et al. in Western Him. 1: 363 (1987); Pangtey & Punetha in Western Him. 1: 393 (1987); Khullar et al., J. Cytol. & Genet. 23: 49 (1988); Pande, Geobios new Reports 8: 105 (1989); Indian Fern J. 7: 157 (1990); Pangtey et al., New Botanist 18: 221 (1991); Khullar et al., Ferns Nainital: 77 (1991); Chandra, Ferns India: 35 (2000).

*Pteris nervosa* Thunb., Fl. Jap.: 332 (1784); Ching & S.K. Wu in C.Y.Wu Fl. Xizangica 1: 68 (1983).

*Pycnodoria cretica* (L.) Small, Fl. Florida: 39 cum tab. (1932).

Rhizome short-creeping, thick, scaly; scales dark brown, concolorous, linear-lanceolate, margin entire, apex acuminate. Fronds dimorphic; sterile fronds usually smaller and bending backwards; fertile fronds erect with longer and stronger stipes. Stipes 10-45 cm long, stramineous, glabrous, dark brown towards base, thick, stipe base sparsely scaly; scales small, dark brown, linear-lanceolate, higher up stipe glabrous; rachis stramineous, glabrous. Lamina pinnate, imparipinnate, 30-50 x 10-30 cm; texture herbaceous to subcoriaceous; pinnae 3-5 pairs, 15-20 x 1-2 cm, opposite, petiolate but upper pinnae gradually sessile, lanceolate, margin spinulose serrate, lowest pair of pinnae usually forked at base, sometimes the next pair also be similarly forked. Fertile subcoriaceous; pinnae 8-10 pairs, more in number than sterile ones, opposite, petiolate, upper pinnae subsessile or sessile, lanceolate, margin entire, the infertile apex dentate-serrate, apex acuminate, lower pinnae slightly distant, usually forked once at base;

veins free, simple or forked, costae and costules glabrous. Sori indusiate, marginal, entire, pinnae soriferous except for the infertile apex; indusia membranaceous, continuous. Spores 35.0-45.5 x 42.0-49.0  $\mu\text{m}$ , exine levigate, undulate.



**HABITAT:** Grows in moist open or shaded localities or at the margins or in the forests.

**DISTRIBUTION:** A very common and abundant fern up to 3000 m altitude.

**UTTARAKHAND:** DEHRA DUN: Jaunsar, Deoban, Chakrata, Mussoorie, Mossy falls, Jabarkhet, Company khud, Lal Tibba. **TEHRI GARHWAL:** Deolsari. **UTTARKASHI:** Hanumanchatti, Brahmkhal, Barkot. **RUDRAPRAYAG:** Gaurikund, Phata, Guptakashi. **CHAMOLI GARHWAL:** Mandal, Gwaldam, Vinayakchatti, Gobindchat. **NAINITAL:** (all over), Cheena peak ranges, Snow view, Iriakanta, Land's end, Dorothy seat, Kilbury, Pangtey's Gorge, Bhowali. **ALMORA:** Ranikhet, Taikhet, Almora, Jageshwar, Binsar, Kausani, Mahadev. **BAGESHWAR:** Khati, Dwali. **PITHORAGARH:** Chaubatti, Garaon, Harkote, Didihat, Thalkedar, Narayan Ashram, Munsiri, Hokara, Namik. **CHAMPAWAT:** Champawat.

**INDIA:** Jammu & Kashmir; Himanchal Pradesh; Sikkim; Darjeeling; Assam; Arunachal Pradesh; Meghalaya; Manipur; Nagaland; Tripura; south & central India.

**GENERAL DISTRIBUTION:** Iran; Pakistan; Nepal; Bhutan; China; Philippines; Polynesia; Malaysia; Myanmar; Bangladesh; Sri Lanka; Asia; Australia; Europe; Africa; America.

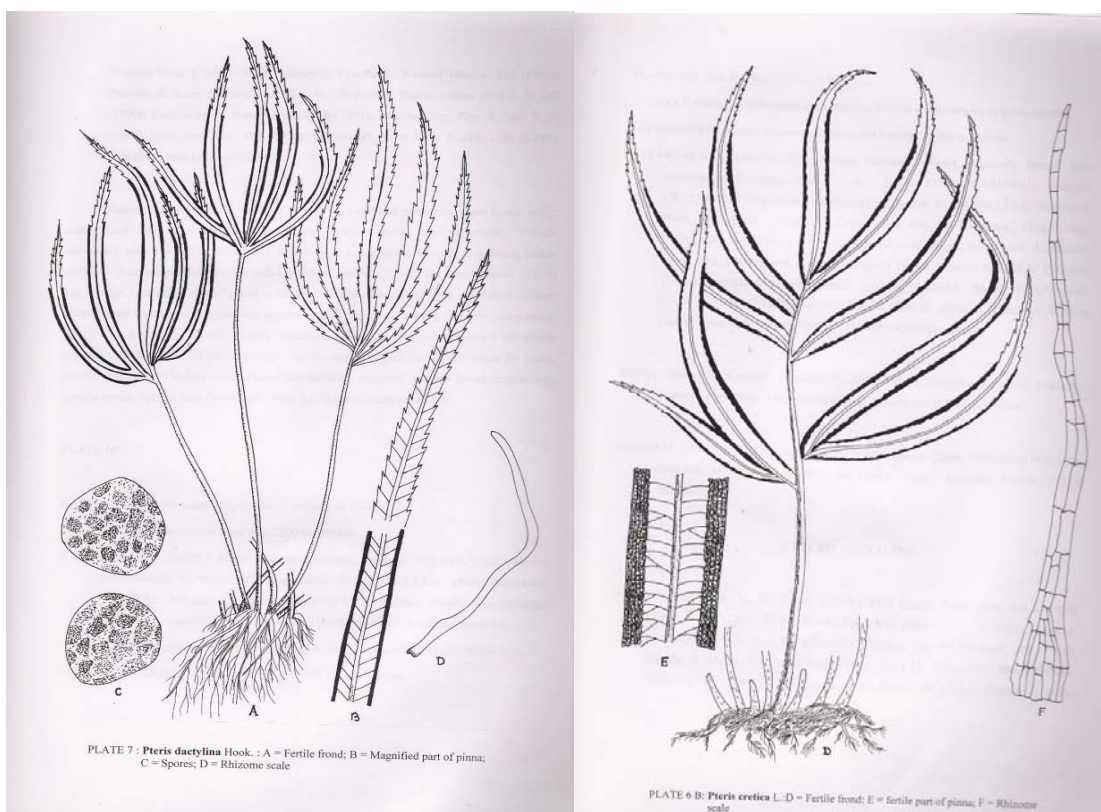
## 5. PTERIS DACTYLINA

*Pteris dactylina* Hook., Sp. Fil. 2: 160. t. 13A (1858); Clarke, Trans. Linn. Soc. Lond. II (Bot.) 1: 463 (1880); Bedd., Handb. Ferns Brit. India: 107. t. 56 (1883); Hope, J. Bombay nat. Hist. Soc. 13: 451 (1901); Duthie, Cat. Pl. Kumaun: 224 (1906) Khullar & Mehra, Res. Bull. Panjab Univ. (n.s.) 23:



193 (1972); Dhir, Biblioth. Pterid.1: 46 (1980); Dixit, Cunsus Indian Pterid.: 69 (1984); Khullar et al. in Western Him. 1: 363 (1987); Pande, Indian Fern J. 7: 157 (1990); Khullar et al., Ferns Nainital: 78 (1991); Him. Bot. Res.: 394 (1991); Ill. Fern Fl. West Him. 1: 265. t. 94 (1994); Chandra, Ferns India: 36 (2000).

Rhizome short-creeping, covered all over by roots and persistent stipes bases, scaly; scales dark brown, concolorous, lanceolate, margin entire, apex acuminate. Fronds dimorphic, only few fertile. Stipes 20 (-30) cm long, stramineous, thin, wiry, glabrous; rachis glabrous. Lamina pinnate; texture subcoriaceous, glabrous, 10-20 cm long; pinnae 1-7 (-9) but usually 3, mostly a central pinna at the apex and two lateral ones that are forked at base, all clustered together and apparently appearing as 5 pinnae all arising from the same point, up to 15.0 x 0.3 cm, shortly stalked, linear-lanceolate, margin crenate-serrate (in sterile pinnae); infertile apex of fertile pinnae sharply toothed, apex elongated; veins far apart, mostly simple, few forked, conspicuous Sori indusiat, marginal; indusia broad, continuous, margin entire, spores dark-brown, 35-49 x 42-64  $\mu$ m, exine reticulate.



**HABITAT:** Grows on moist steep banks of streams or roadsides.

**DISTRIBUTION:** Infrequent from 800-2700 m altitude.

**UTTARAKHAND:** DEHRA DUN: Jaunsar, Chakrata, Deoban. **UTTARKASHI:** between Jankibaichatti to Yamunotri, Jangalchatti. **RUDRAPRAYAG:** above Gaurikund. **NAINITAL:** Nainital, Lariakanta. **BAGESHWAR:** Pindari, Dwali, Sundardhunga. **BAGESHWAR:** Dhaulchhina, Sikhar. **PITHORAGARH:** Bogdwar, Satsiling.

**INDIA:** Himanchal Pradesh; Sikkim; Darjeeling; Assam; Arunachal Pradesh; Meghalaya.

**GENERAL DISTRIBUTION:** Nepal; Bhutan; SW China; Taiwan.

## 6. PTERIS EXCELSA

*Pteris excelsa* Gaud., Freyc. Voy. Bot.: 388 (1827); Clarke, Trans. Linn. Soc. Lond. II (Bot.) 1: 467 (1880); Bedd., Handb. Ferns Brit. India: 114 (1883); Hope, J. Bombay nat. Hist. Soc. 13: 454 (1901); Duthie, Cat. Pl. Kumaun: 224 (1906); Stewart, 150<sup>th</sup> Ann. Vol. Royal Bot. Gard. Calcutta 2: 168 (1942); Khullar & Mehra, Res. Bull. Panjab Univ. (n.s.) 23: 193 (1972); Pangtey et al., Him. Res. & Dev. 1: 160 (1982); Bir et al., Pterid. Fl. Garhwal Him.: 27 (1983); Dixit, Census Indian Pterid.: 70 (1984); Pangtey & Punetha in Western Him. 1: 393 (1987); Pande & Dashila, Indian Fern J. 5: 80 (1988); Pande, Geobios new Reports 8: 105 (1989); Pande & Pande, Vegetos 3: 58 (1990); Khullar et al., Ferns Nainital: 79 (1991); Khullar, Him. Res. & Dev. 7: 62 (1988); Ill. Fern Fl. West Him. 1: 265. t. 95 (1994); Chandra, Ferns India: 36 (2000).

*Pteris terminalis* Wall., Numer. List. no.: 101 (1828), *nom. nud.*

*Pteris excelsa* Gaud. Var. *rotunda* (*routundus*) P. & H. Pande, Indian Fern J. 11: 99 (1994), *nom. nud.*

Rhizome short-creeping, thick, apex scaly; scales brown, concolorous, lanceolate, margin entire, apex acute or acuminate. Stipes 40-100 cm long, generally shorter or equal to lamina, dark-brown to violet, base very dark, thick, base scaly, scales brown, linear-lanceolate, margin entire, higher up stipe glabrous, grooved. Rachis light brown, glabrous, glossy. Lamina 1-2-pinnate, 50-100 x 20-30 cm, subdeltate, texture herbaceous, glabrous; pinnae 4-8 pairs or more, large, 15-30 x 1.5 cm, alternate, petiolate becoming subsessile to sessile in the distal part of lamina, deltate to lanceolate, margin deeply lobed to the base or the lobes becoming free in bipinnate fronds; pinnules (or lobes) many, 5.0-6.0 x 0.7-1.0 cm, linear-lanceolate, subfalcate, the lower base decurrent, margins of sterile lobes, finely crenate-serrate, basiscopic lobe generally longer than the acroscopic one; the lowest pinnae the largest, not forked; veins free forked, rarely a few veinlets anastomose; costae with a few short setae at the base of costule; veins slightly raised on the lower surface. Sori endusiate, marginal, continuous extend from the base to about the apex of the pinnule; indusia somewhat broad, membranaceous, olive-green of olive-brown. Spores brown, 28.5 –35.5 x 35.0-42.0 µm, exine smooth.

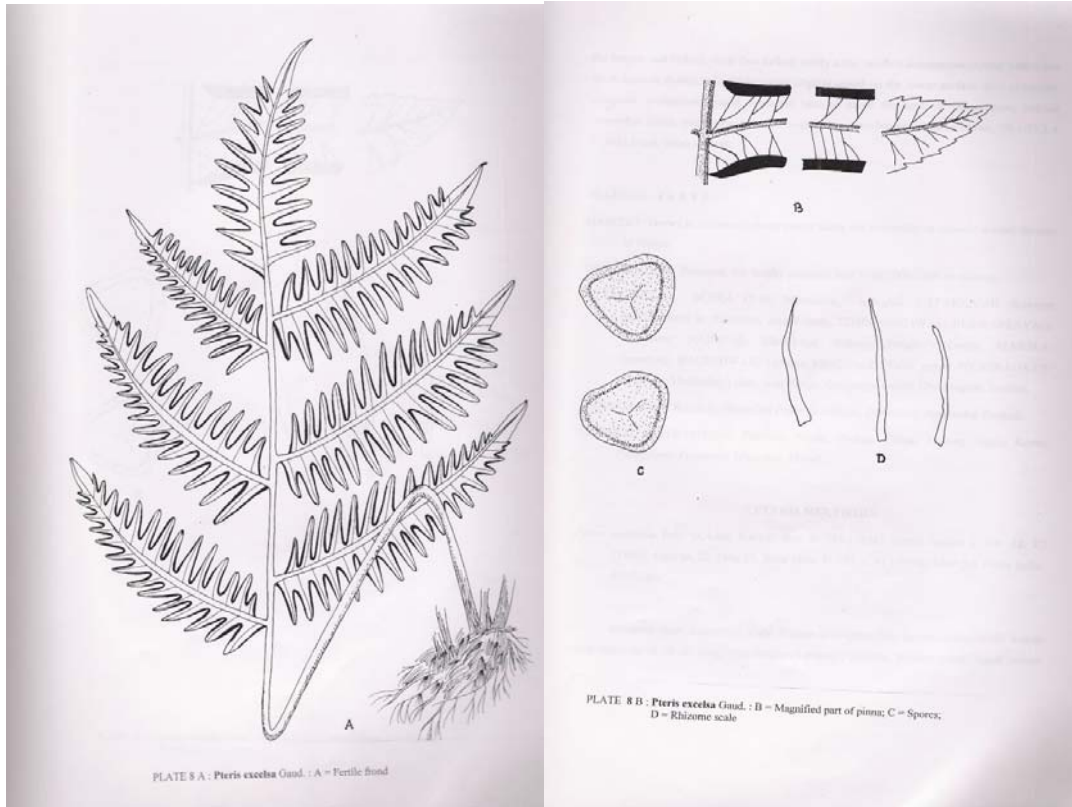
HABITAT: Grows in extremely moist places along the streamlets in densely shaded ravines in forests.

DISTRIBUTION: Frequent, but locally common fern from 2000-2400 m altitude.

UTTARAKHAND: DEHRA DUN: Mussoorie, Dhubighat. UTTARKASHI: between Jankibaichatti to Yamnotri, Jangalchatti. TEHRI GARHWAL. RUDRAPRAYAG: Gaurikund. NAINITAL: Dhubikhud, Kilbury, Pangtey's Gorge. ALMORA: Jageshwar. BAGESHWAR: Turturia, Khati, Jhuni, Pindar gorge. PITHROGARH: Bogdwar, Thalkedar, Lilam, near Pangu, Goriganga valley, Dharamghar, Sandeo.

INDIA: Jammu & Kashmir; Himanchal Pradesh; Sikkim; Darjeeling; Arunanchal Pradesh.

GENERAL DISTRIBUTION: Pakistan; Nepal; Bhutan; China; Taiwan; Japan; Korea; Philippines; Polynesia; Malaysia; Hawaii.



## 7. PTERIS MULTIFIDA

*Pteris multifida* Poir. in Lam. Encycl. Bot. 5: 714 (1804); Singh, Indian J. For. 12: 82 (1989); Khullar, Ill. Fern Fl. West Him. 1: 270. t. 97 (1994); Chandra, Ferns India: 41 (2000).

Rhizome short, ascending, scaly. Fronds dimorphic, thin, papyraceous; sterile fronds with stipes up to 30 cm long, stramineous. Lamina 1-pinnate, broadly ovate; upper pinnae decurrent resulting in a winged rachis on each side; pinnae sharply serrate; fertile pinnae much longer and narrower. Sori marginal, continuous along the margin.

**HABITAT:** Grows along the banks of canals or walls of old gardens.

**DISTRIBUTION:** Rather rare. Adventive plants, presumably an escape from cultivation.

**UTTARAKHAND:** DEHRA DUN: Dehra Dun, New Forest Campus.

**INDIA:** Uttar Pradesh; South India.

**GENERAL DISTRIBUTION:** Pakistan; China; S Korea; Japan; SE United States.

No specimens are seen and the description is given here as given by Singh (1989) and Khullar (1994).

## 8. PTERIS PELLUCIDA

*Pteris pellucida* Presl, *Rel. Haenk*, 1: 55 (1825); Clarke, *Trans. Linn. Soc. Lond. II (Bot.)* 1: 468 (1880); Bedd., *Ferns South. India*: t. 38 (1863); *Handb. Ferns Brit. India*: 106 (1883); Dixit, *Census Indian Pterid.*: 71 (1984); Chandra, *Ferns India*: 41 (2000).

Rhizome short or thick and creeping, densely scaly at the apex; scales lanceolate, pale brown at the periphery, dark at the center, gland tipped, margins with amny multicellular glandular, finger-like

projections. Stipe length variable, up to 45 cm long, more or less uniformly chest-nut brown all over except stramineous or yellowish-brown distal part, sometimes pale brown or stramineous all over, glossy and glabrous. Lamina simply pinnate with ternate apex, c 30 cm long or longer, coriaceous-membranaceous, bright-green; pinnae 3-11, generally 15.0-25.0 x 2.5-4.0 cm long, entire or slightly serrulate at the apex; lateral pinnae sessile, upper ones sometimes decurrent, generally all entire or the lowest pair bifid; veins simple or forked, close. Sori linear marginal except and the base or along the margin of the one third to half of the distal or median part of the pinna excluding the apex, up to 2 mm wide indusiate; indusium dark brown, entire, glabrous, narrow. Spores tetrahedral, trilete, 55 x 55  $\mu$ m, perispore yellowish-brown, exine dark brown, coarsely verrucose.

**HABITAT:** Grows in well shaded dense forests on the banks of streamlets at lower elevation.

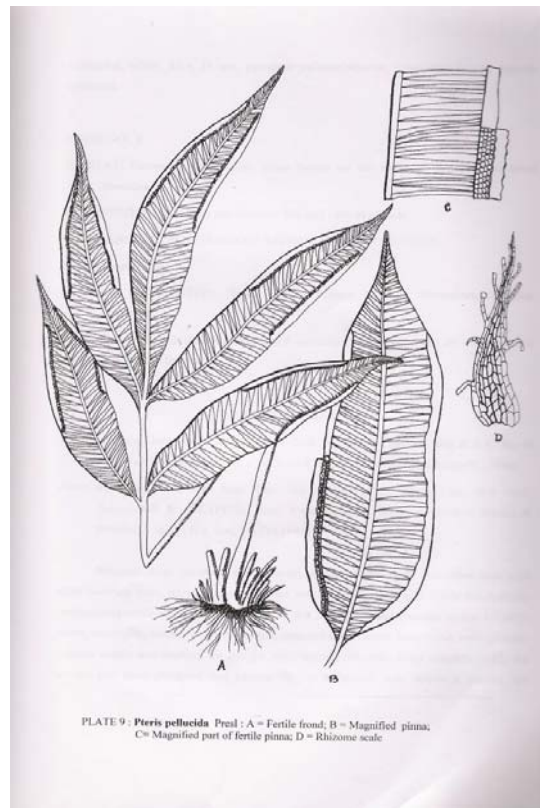
**DISTRIBUTION:** Extremely rare between 600 and 1800 m altitude.

**UTTARAKHAND:** PITHORAGARH: Didihat. **CHAMPAWAT:** Chalthi.

**INDIA:** South India.

**GENERAL DISTRIBUTION:** Nepal; Bhutan; Taiwan; Malaysia; Philippines; Malaysia; Myanmar.

This species is included here based on earlier reports only and the description is based on published literature.



## 9. PTERIS PUBERULA

*Pteris puberula* Ching, Bull. Fan Mem. Inst. Biol. Bot. 11: 52 (1941); Ching & S.K. Wu in C. Y. Wu Fl. Xizangica 1: 71. t. 18. f. 5 – 8 (1983); Chandra, Ferns India: 41 (2000).

*Pteris nepalensis* H. Ito in Fl. East. Him.: 466. t. 26 (1966); K. Iwats., Univ. Mus. Univ. Tokyo Bull. 8: 177 (1975); Dixit, Census Indian Pterid.: 71 (1984); Kholia & Punetha, J. Indian Bot. Soc. 74: 184 (1995).

Rhizome short, creeping; scales brown, linear, margin dark, membranaceous with short fimbriate teeth; stipes shining, glabrous, solid, c 35-40 cm long. 1-2 mm dia., reddish; lamina ovate or oblong-ovate, 40-50 cm long and 25-35 cm broad, pinnate; pinnae 4-7 pairs, linear, acuminate, sessile or shortly stalked; pinnules linear, acute, base broad; veins pinnate; veinlets simple and reaching the margin; main costae with setae, basal pinnules (rarely the second pair also) elongated and pinnate on the basiscopic side, otherwise similar, the terminal pinna at the lamina top similar to other pinnae; sori marginal; indusia membranaceous, pale brown, linear, margin entire 1-3 cm long and 0.6-0.8 mm broad; sporangia annular cells 18-20; spores tetrahedral, wings small.

HABITAT: Grows in humus rich forest floor.

DISTRIBUTION: Rather infrequent fern between 2300 and 2700 m altitude.

UTTARAKHAND: KUMAUN: BAGESHWAR: Dwali.

INDIA: Eastern Himalaya: Darjeeling.

GENERAL DISTRIBUTION: Nepal, S. W. China, N. Thailand.



## 10. PTERIS STENOPHYLLA

*Pteris stenophylla* Wall. ex Hook. & Grev., Icon. Fil.: t. 130 (1829); Mehra, Ferns Muss.: 11 (1939); Stewart, 150<sup>th</sup> Ann. Vol. Royal Bot. Gard. Calcutta 2: 168 (1942); Mehra & Verma, Caryologia 13: 619 (1960); Dhir, Biblioth. Pterid. 1: 46 (1980); Dhir & Sood, Biblioth. Pterid. 2: 32 (1981); Bir *et al.*, Pterid. Fl. Garhwal Him.: 28 (1983); Dixit, Census Indian Pterid.: 72 (1984); Khullar *et al.* in Western Him. 1: 364 (1987); Pangtey & Punetha in Western Him. 1: 394 (1987); Pande, Geobios



new Reports 8: 105 (1989); Khullar *et al.*, Ferns Nainital: 80 (1991); Him. Bot. Res.: 394 (1991); Ill. Fern Fl. West Him. 1: 275. t. 99 (1994); Chandra, Ferns India: 45 (2000).

*Pteris digitata* Wall., Numer. List. no.: 91 (1828), *nom. nud.*; *ex* Hope, J. Bombay nat. Hist. Soc. 13: 450 (1901); Duthie, Cat. Pl. Kumaun: 224 (1906).

*Pteris pellucida* Presl var. *stenophylla* (Wall. *ex* Hook. & Grev.) Clarke, Trans. Linn. Soc. Lond. II (Bot.) 1: 463 (1880); Bedd., Handb. Ferns Brit. India: 107 (1883).

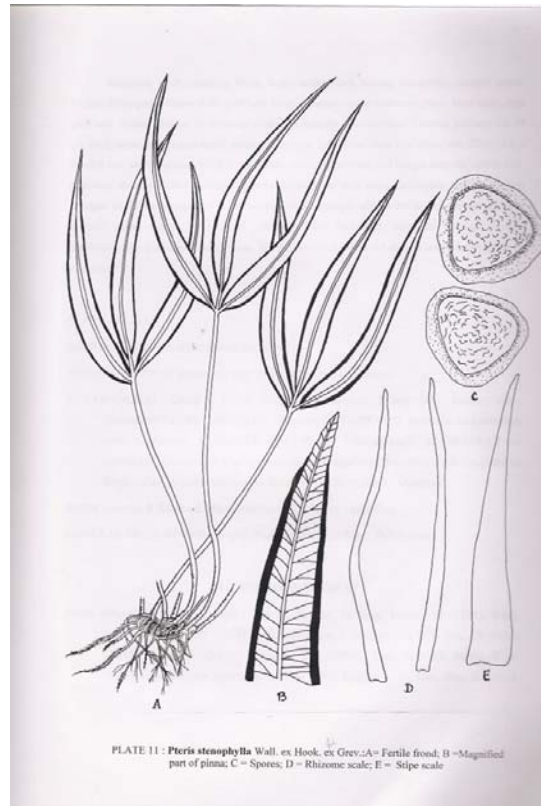
Rhizome short-creeping, thick, scaly; scales dark brown, lanceolate, margin entire. Fronds dimorphic. Stipes 4-25 (-30) cm long, stramineous or brownish, thin, base scaly, rest glabrous, scales similar to rhizome scale but smaller and narrower. Lamina pinnate, 20-30 cm long; texture subcoriaceous, glabrous; pinnae 3-5, sometimes 3 or even one, 20.0-30.0 x 0.5-2.0 cm; sterile pinnae 1.5-2.0 cm, fertile pinnae narrower and longer than the sterile one, opposite, shortly stalked, usually clustered towards the stipe apex, lanceolate, margin slightly toothed, in fertile pinnae the infertile apex small, margin almost entire or coarsely dentateserrate; veins simple or toothed, glabrous. Sori indusiate, marginal; indusia almost continuous from pinnae base to apex. Spores brown, 35-42 x 42-49  $\mu$ m, exine tuberculate or verrucose.

HABITAT: Grows in rather humid locations.

DISTRIBUTION: An infrequent fern from 1000 – 1600 m altitude.

UTTARAKHAND: DEHRA DUN: Chakrata, Mussoorie, Mossy falls, Kempt falls, Jhariapani. TEHRI GARHWAL: Dhanolti. UTTARKASHI: between Jankibaichatti and Yamunotri. CHAMOLI GARHWAL: Vinayakchatti.

RUDRAPRAYAG: Gaurikund, Gaundar to Madhyamaheshwar, Dungalbitta. BAGESHWAR: Bageshwar, Sarju valley, between Dhakuri to Khati. PITHROGARH: Munsiri.



INDIA: Jammu & Kashmir; Himanchal Pradesh; Sikkim; Darjeeling.

GENERAL DISTRIBUTION: Nepal; Bhutan; Thailand; Laos; Philippines.

## 11. PTERIS SUBQUINATA

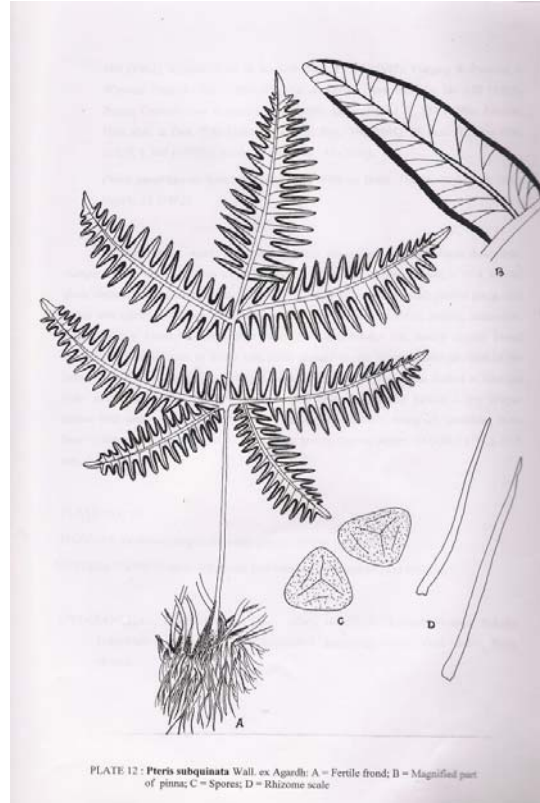
*Pteris subquinata* (Wall. ex Bedd.) Agardh, Recens. So. Gen. Pterid.: 21 (1839); Hope, J. Bombay nat. Hist. Soc. 13: 453. t. 17 (1901); Duthie, Cat. Pl. Kumaun: 224 (1906); Dhir, Biblioth. Pterid. 1: 47 (1980); Dixit, Census Indian Pterid: 72 (1984); Pangtey *et al.*, Him. Res. & Dev. 1: 160 (1982); Khullar *et al.* in Western Him. 1: 364 (1987); Pangtey & Punetha in Western Him. 1: 394 (1987); Punetha & Kholia, New Botanist 16: 120 (1989); Indian Fern J. 7: 158 (1990); Khullar, him. Res. & Dev. 7:62 (1988); Ill. Fern Fl. West Him. 1: 277. t. 100 (1994); Chandra Fern India : 45 (2000).

*Pteris quadriaurita* Retz. var. *subquinata* Wall. ex Bedd., Handb. Ferns Brit. India Suppl.: 23 (1892).

Rhizome short, erect, apex scaly; scales brown, concolorous, linear-lanceolate, margin entire. Stipes 7-20 cm long, stramineous, glabrous. Lamina pinnate, c 15 x 10 cm, short deltate, texture herbaceous, glabrous; pinnae 3 (-5)-6, a terminal central pinna and either one (rarely 2-4) pairs of lateral ones, 7-10 x 3-5 cm, opposite, sessile, lanceolate, margin deeply lobed to costa, lobes many 1.5-3.0 x 0.4-0.5 cm, nearly wqually broad throughout, infertile apex of fertile lobe finely crenate-serrate, lowest basicopic lobe of the lowest lateral pinnae generally enlarged (in young plants) or the pinnae forked at base (in older plants), terminal central pinna the largest; veins free, mostly forked, a few simple costae with small setae at junction with costules. Sori indusiate, marginal, stretching from base to almost the apex; indusia membranaceous, brown. Spores brown, 35.0-38.5 x 39.5-52.5  $\mu$ m, exine smooth.

HABITAT: Grow on slopes or on damp rocks in lime rich areas.

DISTRIBUTION: A rather infrequent fern between 1000 and 1400 m altitude.





UTTARAKHAND: BAGESHWAR: Sarju valley, Bageshwar, Kapkote-Bharari, Takula, Loharkhet, Kharbaggar. PITHORAGARH: Ramaganga valley, Gori valley, Dore, Nolara.

INDIA: Sikkim; Darjeeling.

GENERAL DISTRIBUTION: Nepal; Thailand; Tonkin.

## 12. PTERIS VITTATA

*Pteris vittata* L., Sp. Pl. 2: 1074 (1753); Stewart, 150<sup>th</sup> Ann. Vol. Royal Bot. Gard. Calcutta 2: 168 (1942); Loyal & Veram, J. Bombay nat. Hist. Soc. 57: 484 (1960); Khullar & Mehra, Res. Bull. Punjab Univ. (n. s.) 23: 193 (1972); Awasti & Sharma, Proc. Indian Acad. Sci. (Pl. Sci.) 89: 310 (1980); Bir et al., Pterid. Fl. Garhwal Him.: 28 (1983); Dixit, Census Indian Pterid.: 73 (1984); Pande & Kandpal, Acta Botanica Indica 14 (Suppl.): 117 (1986); Pangtey & Punetha in Western Him. 1: 394 (1987); Khullar et al., J. Cytol. & Genet. 23: 49 (1988); Pande & Dashila, Indian Fern J. 5: 80 (1988); Pande, Geobios Naintal: 80 (1991); Khullar, Him. Res. & Dev. 7: 62 (1988); Him. Bot. Res.: 394 (1991); Ill. Fern Fl. West Him. 1: 279. t. 101 (1994); Chandra, Fern India: 46 (2000).

*Pteris longifolia auct. quoad. Pl. Asia*; D. Don, Prodr. Fl. Nepal.: 15 (1825); Bedd., Ferns South. India: t. 33 (1863); Handb. Ferns Brit. India: 106. t. 55 (1883); Clarke, Trans. Linn. Soc. Lond. II (Bot.) 1: 461 (1880); Hope, J. Bombay nat. Hist. Soc. 13: 148 (1901); Duthie, Cat. Pl. Kumaun: 224 (1906); Marten, J. Bombay nat. Hist. Soc. 19: 180 (1909); Mehra, Ferns Muss.: 11 (1939); Chowdhury, Pterid. Fl. Upper Gangetic Plain: 30 (1973).

*Pycnodoria vittata* (L.) Small, Ferns SE States: 102 cum tab. 468 (1938).

Rhizome creeping, thick, scaly; scales light-brown, concolorous, 0.3-0.5 long, narrow, linear-lanceolate, margin entire, apex acuminate. Stipes length variable, (2-) 3.5-13.5 (-50) cm long, generally much shorter than the lamina, stramineous or light-green or light-brown on drying, thick, scaly and fibrillose; scales abundant at base, light-brown, narrowly linear-lanceolate, margin entire, scales becoming narrower higher up on stipes, fibrils of the same colour as the stipes on stipe but narrower. Lamina pinnate, 6-100 x 1-27 cm, oblong-obovate; texture herbaceous to subcoriaceous, upper surface glabrous; pinnae 20-30 pairs, 0.5-8.0 (-15) x 0.3-0.5 cm, alternate, sessile, linear, base truncate or cordate or slightly auricled, margin of sterile pinnae and infertile apex of fertile pinnae finely dentate-serrate, lower 5-7 pairs of pinnae gradually reduced and distant one usually sterile, apical pinnae variable in size; veins free, simple or usually once forked or 3-4 times forked; costules hairy on lower surface. Sori marginal, continuous from the base of pinnae, stopping at little short of the apex, indusiate; indusia whitish, membranaceous, margin irregularly serrulate. Spores light-brown, 38.5-45.5 x 42.0-54.0  $\mu$ m, non-perinate, exine tuberculate to rugulose on the proximal surface, reticulate on distal surface.

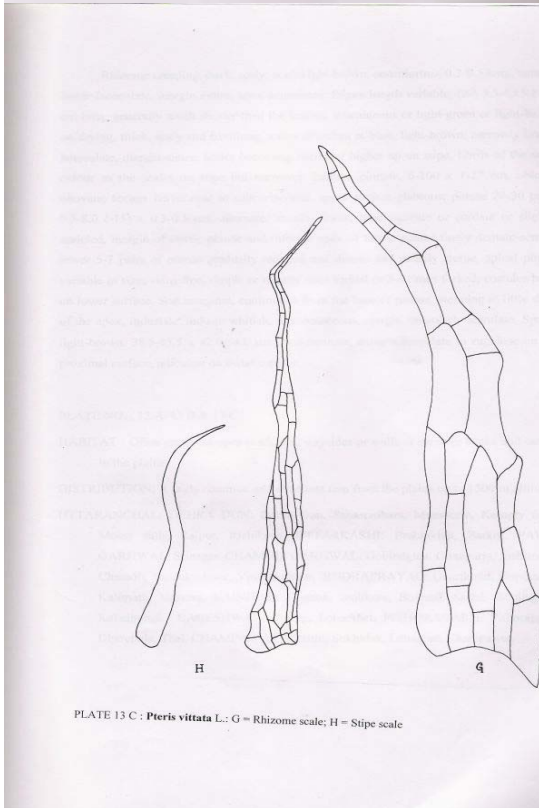
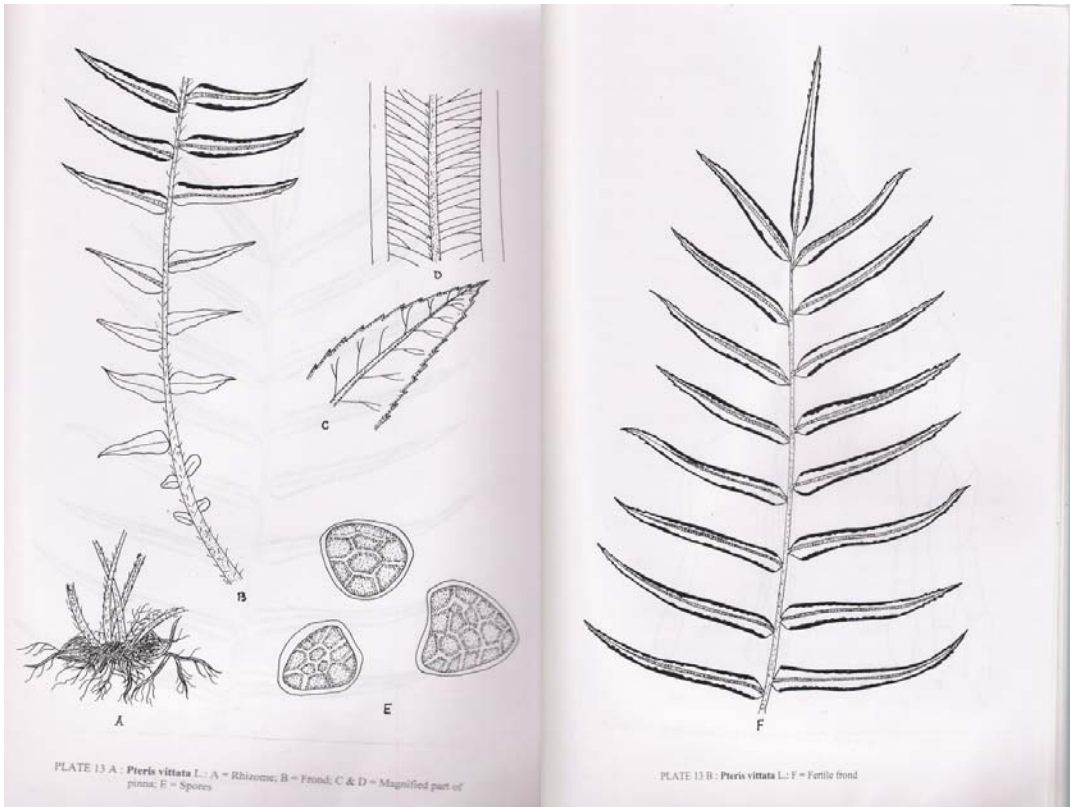
HABITAT: Often grows on open roadsides, waysides or walls or on river banks and canals in the plains.

DISTRIBUTION: A family common and abundant fern from the plains up to 1500 m altitude.

UTTARAKHAND: DEHRA DUN: Dehra Dun, Sahasradhara, Mussoorie, Kempty falls, Mossy falls, Rajpur, Rishikesh. UTTARKASHI: Brahmkhal, Barkot. PAURI GARHWAL: Srinagar. CHAMOLI GARHWAL: Gobinghat, Ghangaria, Joshimath, Chamoli, Pandukeshwar, Vinayakchatti. RUDRAPRAYAG: Gaurikund, Guptkashi, Kalimath, Mastura. NAINITAL: Dogaon, Jeolikote, Bhimtal, Sattal, Bhujuyaghat, Kaladhungi. BAGESHWAR: Suring, Loharkhet. PITHORAGARH: Pithoragarh, Dharchula, Thal. CHAMPAWAT: Chalthi, Sukhidak, Lohaghat, Champawat.

INDIA: Jammu & Kashmir; Himachal Pradesh; Sikkim; Darjeeling; Assam; Arunachal Pradesh; Meghalaya; Nagaland; Manipur; Tripura; Orissa; North; Central, south, western India.

GENERAL DISTRIBUTION: Afghanistan; Pakistan; Nepal; China; Taiwan; Philippines; Australia; Africa; Mediterranean Europe.



### 13. PTERIS VITTATA subsp. VERMAE

*Pteris vittata* L. subsp. *vermae* Fras.-Jenk., New Sp. Syndrome Indian Pterid. & Ferns Nepal.: 231 (1997); Chandra, Ferns India: 47 (2000).

*Pteris vittata* L. forma *brevipinna* Verma, nom. nud.; Khullar *et al.*, Ferns Nainital : 81 (1991); Khullar, III. Fern Fl. West Him. 1: 282 (1994).

Differs from *P. vittata* in having somewhat darker coloured scales, cells of the scales larger and with thicker walls; pinnae narrower, base not widened but broadly and unequally cuneate; indusial flaps broader. Spores light-yellow, somewhat globose and with small papillae present on the exine.

HABITAT: Grows along the waysides and roadsides.

DISTRIBUTION: Collected only once from below Nainital around 600 maltitude.

UTT ARANCHAL: NAINIT AL: Bhujiaghat.

INDIA: Uttarakhand.

GENERAL DISTRIBUTION: China, Sichuan.

### 14. PTERIS WALLICHIANA

*Pteris wallichiana* Agardh, Recens. Sp. Gen. Pterid.:69 (1839); Clarke, Trans. Linn. Soc. Lond. II (Bot.) 1: 469 (1880); Hope, J. Bombay nat. Hist. Soc. 13: 456 (1901); Duthie, Cat. Pl. Kumaun: 224 (1906); Khullar & Mehra, Res. Bull. Panjab Univ. (n.s.) 23: 193 (1972); Dhir, Biblioth. Pterid. 1: 49 (1980); Dixit, Census Indian Pterid.: 73 (1984); Pangtey & Punetha in Western Him. 1: 394 (1987); Khullar *et al.*, J. Cytol. & Genet. 23: 50 (1988); Pande & Pande, Acta Botanica Indica 15: 101 (1987); Pande, Geobios new Reports 8: 106 (1989); Indian Fern J. 7: 159 (1990); Khullar, Him. Res. & Dev. 7: 62 (1988); Him. Bot. Res.: 394 (1991); Ill. Fern Fl. West Him. 1: 282. t. 102 (1994); Chandra, Ferns India: 47 (2000).

*Campteria wallichiana* Moore, Index Fil.: 221 (1861); Bedd., Handb. Ferns Brit. India: 118 (1883).

*Pteris raghavendrae* (*raghavendii*) Chowdhary & Singh, Indian J. For. 12: 163 (1990).

*Pteris x khullarii* Pangtey, Samant & Verma, Fern Gaz. 13: 357 (1990); Khullar, III. Fern Fl. West Him. 1: 268. t. 96 (1994); Chandra, Ferns India: 39 (2000).

Rhizome erect, apex scaly; scales brown, concolorous. Stipes very long up to 2 m, castaneous, becoming paler higher up, thick, extreme base scaly, scales brown, concolorous, rest of stipe glabrous, glossy; rachis of the same colour as the stipe but paler glabrous, glossy. Lamina subpedate, generally 3 or 4-6-partite, two lateral and a central branch, each branch pinnate, large, thin, texture thin, herbaceous, glabrous; pinnae 30-60 x 15-30 cm, opposite, petiolate, lanceolate, each pinna pinnate, lowest pinnae forked at base, terminal pinna as large as the lateral ones; pinnules many, 10-20 pairs, 5-15 x 1-3 cm, alternate, petiolate, lanceolate, margin deeply lobed to the costa, lobes many, linear-lanceolate, margin of sterile lobes .and the infertile apex of fertile lobe serrulate, apex acute or acuminate, the lower pair of acroscopic and basispicopic lobes may sometimes be free, basal pair of veins anastomosing to form a pair of areolae at base of costule, remaining veins free, forked. Sori indusiate, generally occupying only the lower half of the lobe; indusia brown, not opening out maturity. Spores brown, 24.5-35.0 x 35.0-38.0 µm, slightly verrucose.

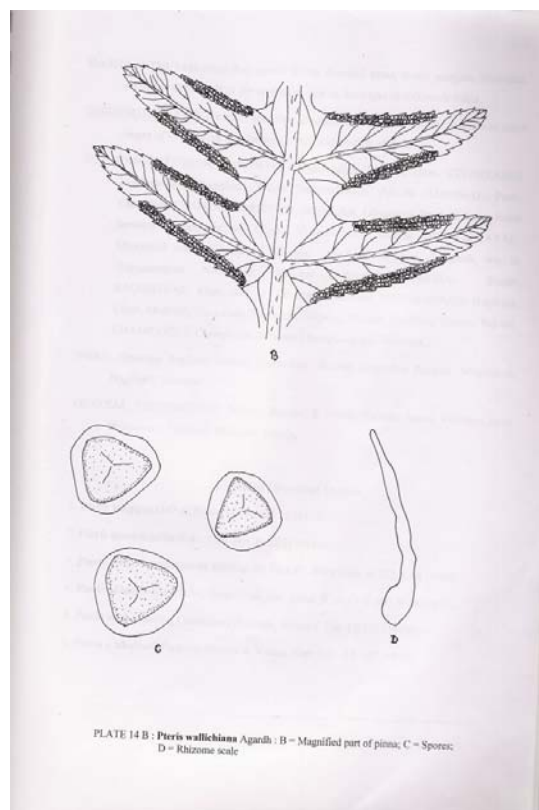
HABITAT: This large-sized fern grows in the forest areas, forest margins, roadsides, waysides, in the beds of dry streamlets and on the edges of cultivated fields.

DISTRIBUTION: Fairly common and often gregarious or abundant in the central and inner ranges

of Uttarakhand hills between 1800 and 2400 m altitude.

UTTARAKHAND: TEHRI GARHWAL: Ganges valley, Pinswar, Urmi. UTTARKASHI: Jankibaichatti, Sianichatti, below Hanumanchatti. PAURI GARHWAL: Pauri, Candeloea. CHAMOLI GARHWAL: Gobindghat, Ghangaria, Pandukeshwar, forest between Gopeshwar to Chota, Vianyakchatti, Mandali. RUDRAPRAYAG: Mandakini valley, Gaurikund, Chopta, Madhyamaheshwar, Tungnath, way to Trijuginarayan. NAINITAL: Nainital (cultivated). ALMORA: Binsar. BAGESHWAR: Khati, way to Dhakuri, Gwaldam. PITHORAGARH: Bogdwar, Lilam, Munsiri, Dharchula, Tawaghat, Girgaon, Didihat, Deochula, Garaon, Suklari. CHAMPAWAT: Champawat, between Champawat and Sukhidak.

INDIA: Himachal Pradesh; Sikkim; Darjeeling; Assam; Arunachal Pradesh; Meghalaya; Nagaland; Manipur.



GENERAL DISTRIBUTION: Nepal; Bhutan; S. China; Taiwan; Japan; Vietnam; Java; Philippines; Thailand; Malaysia; Samoa.

#### Excluded / Doubtful Species

1. *Pteris longipes* D. Don, Prodr. Fl. Nepal.: 15 (1825)
2. *Pteris quadriaurita* Retz., Obs. Bot. 6: 38 (1791).
3. *Pteris pseudoquadriaurita* Khullar, Ill. Fern Fl. West Him. 1: 272. t. 98 (1994).
4. *Pteris subindivisia* Clarke, Trans Linn. Soc. Lond. II (Bot.) 1: 467. t. 56 (1880).
5. *Pteris raghavendrei* Chowdhury & Singh, Indian J. For. 12: 163 (1989).

6. *Pteris x khullarii* Pangtey, Samant & Verma, Fern Gaz. 13: 357 (1990).

### Acknowledgement

The authors are thankful to Vice Chancellor, Kumaun University, Nainital for encouragement and also thankful to Head, Botany Department, D.S.B. Campus for providing facilities.

### REFERENCES

- Awasthi, D. K. & P. C Pande (1984). A note on the phytogeographical distribution of ferns and fern-allies of Almora (W.H.). *J. Bombay nat. Hist. Soc.* **8**: 739-741.
- Awasthi, D. K. & M. P. Sharma (1980). Ecological and phytogeographical observations on the ferns and fern-allies of Nagpur block (Chamoli Garhwal). Western Himalayas. *Proc. Indian Acad. Sci. (Plant Sci.)* **89**: 307-313.
- Beddome, R. H. (1863-1864). *The Ferns of British India*. Madras: Gantz Brothers (1863-1864). 1-38, t. 1-110 (1863); 3-88, t. 111-271 (1864). 2nd edn. (1873). 1-88, t. 1-27. Reprint: Today & Tomorrow's Printers & Publishers. New Delhi (1970).
- Beddome, R. H. (1865-1870). *The Ferns of British India*. Madras: Gantz Brothers. (1865 - 1870) **1**: 1-120 (1865); 121-150 (1866); **2**: 151-210 (1866); 211-255 (1867); 256-300 (1868); 301-330 (1869); 331-354 (1870); Reprint: Oxford and IBH Publication Co., New Delhi (1976).
- Bir, S. S. (1962). Taxonomy of the Indian members of family Aspleniaceae. *Bull. Bot. Surv India* **4**: 1-16.
- Bir, S. S. (1964). Taxonomic notes on some Himalayan ferns. *J. Indian Bot. Soc.* **43**: 556-572.
- Chandra, P. (1979). Ferns of Kedarnath, Madhyamaheshwar and Tungnath. *J. Bombay*
- Ching, R. C. (1940). On the natural classification of the family Polypodiaceae. *Sunyatsenia*. **5**: 202-268.
- Ching, R. C. (1978). The Chinese fern families and genera : systematic arrangement and historical origin. *Acta Phytotax. Sin.* **16**(3): 1-19; **16**(4): 16-37.
- Chowdhery, H. J. & Surendra, Singh (1989). A new species of *Pteris* L. (*Pteridaceae*) : *P. raghavendreei* sp. nov. from India. *Indian. J. For.* **12**: 163-164.
- Chowdhury, N. P. (1973). *The Pteridophytic Flora of the Upper Gangetic Plain*. Navayug Traders, New Delhi.
- Clarke, C. B. (1879). Ferns of north India. *J. Linn. Soc. Lond. Bot.* **17**: 402-404.
- Clarke, C. B. (1880). A review of ferns of northern India. *Trans. Linn. Soc. Lond. II. (Bot.)* **1**: 425-611.
- Copeland, E. B. (1947). *Genera Filicum. The Genera of Ferns*. Chronica Botanica Co., Waltham. Mass. USA.

- Dhir, K. K (1980). Ferns of North Western Himalayas. *Bibliotheca Pteridologia* **1**: 1-158.
- Dhir, K. K. & K. Rani (1978). The genus *Athyrium* Roth in Nainital hills. *J. Bombay nat. Hist. Soc.* **76**: 49-58.
- Dixit, R. D. (1981). The genus *Vittaria* Sm. in India. *J. Econ. Tax. Bot.* **2**: 209-222.
- Dixit, R. D. (1984). *A Census of the Indian Pteridophytes*. Botanical Survey of India, Howrah.
- Dixit, R. D. & A. Das (1979). The genus *Coniogramme* Fee in India. Proc. Indian Acad. Sci (Plant Sci.) **88**: 253-268.
- Duthie, J. F. (1906). *Catalogue of the Plants of Kumaon and of the Adjacent Portions of Garhwal and Tibet, based on the collections made by Strachey and Winterbottom during the years 1846 to 1849 and on the Catalogue originally prepared in 1852 by Sir Richard Strachey*. Lovell Reeve & Co., Limited, London.
- Fraser-Jenkins, C. R. (1978). *Dryopteris stewartii* a new fern from the West Himalaya *Kalikasan Philip. J. Biol.* **7**: 269-274.
- Fraser-Jenkins, C.R. (1984). An introduction to the fern genera of the Indian subcontinent *Bull. Brit. Mus. Nat. Hist. (Bot. Ser.)* **12**: 37-76.
- Fraser-Jenkins, C. R. (1986). A classification of the genus *Dryopteris* (Pteridophyta : Dryopteridaceae). *Bull. Brit. Mus. Nat. Hist. (Bot. Ser.)* **14**: 183-218.
- Gaur, R. D. & P. Painuli (1992). Some little known Pteridophytes from Garhwal Himalaya. *J. Econ. Tax. Bot.* **16**: 345-347.
- Gaur, R. D. & P. Painuli (1993). Some little known Pteridophytes from Garhwal Himalaya. *Indian J. For.* **16**: 173-175.
- Ghosh, S. R. & R. K. Ghosh (1986). *Gymnopteris delavayi* (Baker) Underw. : A new fern record for India. *J. Bombay nat. Hist. Soc.* **82**: 243.
- Goel, A. K. & U. C. Bhattacharyya (1981). Contribution to the Pteridophytic flora of Tehri district (Garhwal). *Indian J. For.* **4**: 30-37.
- Hajra, P. K. & R. D. Dixit (1984). *Woodsia cycloloba* Hand-Mazz. (Woodsiaceae)- A new fern from Nandadevi National Park, Chamoli district, Uttar Pradesh. *Bull. Bot. Surv. India* **26**: 202.
- Holttum, R. E. (1947). A revised classification of leptosporangiate ferns. *J. Linn. Soc. (Bot.)* **58**: 123- 158.
- Hope, C. W. (1899-1904). The ferns of North-Western India. *J. Bombay nat. Hist. Soc.* **12** (2): 315-325 (March 1899); **12** (3): 527-538 (July 1899); **12** (4): 621-633 (Nov.1899); **13** (1): 25-36 (April 1900); **13** (2): 236-251 (July 1900); **13** (3): 443-461 (Jan. 1901); **13** (4): 657-671 (May 1901); **14** (1): 118-127 (Jan. 1902); **14** (2): 252-266 (May 1902); **14** (3): 458-480 (Oct. 1902); **14** (4): 720-749 (Feb. 1903); **15** (1): 67-111 (June 1903); **15** (3): 415-429 (Feb.1904).



- Kaur, S. & N. Punetha (1984). *Arthromeris lungtauensis* Ching: A new record for Western Himalaya (India). *J. Bombay nat. Hist. Soc.* **81**: 737-738.
- Kaur, S. & N. Punetha (1984). A new variety of *Dicranopteris linearis* (Burm. f.) Underw. from India. *J. Indian Bot. Soc.* **63**: 34 -36.
- Kaur, S. & N. Punetha (1985). Some new records of ferns from Kumaun Himalaya. *New Botanist* **12**: 166-168.
- Kaur, S. & S. Chandra (1985). Indian Thelypteroid ferns-their nomenclature, distribution and phytogeography. *New Botanist.* **12**: 83-116.
- Kholia, B. S. & N. Punetha (1993). An enumeration of *Athyrium* Roth species of the Pithoragarh district (West Himalayas). *Higher Plants of Indian Subcontinent* **4**: 243251.
- Kholia, B. S. & N. Punetha (1995). Notes on taxonomy and distribution of some pteridophytes in Kumaun (West Himalaya). *J. Indian Bot. Soc.* **74**: 183-187.
- Khullar, S. P. (1976). Some lesser known ferns from the W. Himalayas-I. *Cheilanthes anceps* var. *brevifrons*. *Amer. Fern J.* **66**: 21-24.
- Khullar, S. P. (1984). The ferns of Western Himalaya- A few additions, corrections and annotations. *Indian Fern J.* **1**: 89-95.
- Khullar, S. P. (1988). The ferns of West Himalaya: An enumeration as in *The Illustrated Fern Flora of West Himalaya. Him. Res. & Dev.* **7**: 59-67.
- Khullar, S. P. (1994). *An Illustrated Fern Flora of West Himalaya*. Vol. I. (Botrychiaceae to Aspleniaceae). International Book Distributors, Dehra Dun.
- Khullar, S. P. (2000). *An Illustrated Fern Flora of West Himalaya*. Vol. II (Onocleaceae to Salviniaceae). International Book Distributors, Dehra Dun.
- Khullar, S.P. (2001). Diversity of the fern wealth of the West Himalaya. **In**: *Plant Diversity of the Himalaya*. eds. P. C. Pande & S. S. Samant, Gyanodaya Prakashan. Nainital. 153-176.
- Khullar, S. P. & S. C. Gupta (1979). On the occurrence of *Polystichum aculeatum* in the Himalaya. *Recent Researches in Plant Sciences* **1**: 66-70.
- Khullar, S. P. & S. C. Gupta (1980). Systematics of the genus *Polystichum* from the Himalayas. *Nova Hedwigia* **32**: 415-444.
- Kumar, S. & M. B. Raizada (1989). Survey and distribution of fern-allies and ferns of Chakrata Forest Division. Uttar Pradesh. *Indian For.* **115**: 578-589.
- Loyal, D. S. & S. C. Verma (1960). Ferns of Nainital. *J. Bombay nat. Hist. Soc.* **57**: 479-490.
- Marten. J. (1909). List of ferns in and around Mussoorie. *J. Bombay nat. Hist. Soc.* **19**: 179-183.
- Mehra, P. N. (1939). *Ferns of Mussoorie*. Punjab University Publication, Lahore.



- Murti, S. K., D. K. Singh & S. Singh (2000). Plant diversity in Lower Gori valley Pithoragarh U.P. (Hydro-Electric Project Area). *Higher Pl. Indian Subcont.* **10**:1-284.
- Naithani, B. D. (1969). Plant collection with Kedarnath Parbat Expedition, 1967. *Bull. Bot. Surv. India* **11**: 224-233.
- Naithani, B. D. (1979). An interesting tree fern *Cyathea spinulosa* Wall. ex Hook. - A new record from Garhwal Himalaya. *Bull. Bot. Surv. India* **21**: 186.
- Nayar, B. K. & Others (1961-1964). Ferns of India I-XV. *Bull. Nat. Bot. Gard. Nos.* 52, 56, 58, 64, 67, 68, 75, 79, 81, 82, 88, 94, 100, 106, 117. Lucknow.
- Nayar, B. K. & S. Kaur (1974). *Companion to R. H. Beddome's Handbook to the Ferns of British India, Ceylon and Malay Peninsula*. Chronica Botanica, New Delhi.
- Pande, P. C. (1973). Pteridophytic flora of Ranikhet. *Indian For.* **99**: 49-53.
- Pande, P. C. (1985). *Peranema cyatheoides* D. Don from Kumaun Himalaya. *J. Indian Bot. Soc.* **64**: 227.
- Pande, P. C. (1989). Contribution to the fern flora of Almora district. *Geobios new Reports* **8**: 103-110.
- Pande, P. C. (1990). A census of Kumaun ferns (North - Western Himalaya). *Indian Fern J.* **7**: 140-195.
- Pande, P. C. & S. S. Bir (1985). Occurrence of *Onychium fragile* Verma & Khullar from Kumaun Himalaya. *J. Bombay nat. Hist. Soc.* **82**: 441.
- Pande, P. C. & P. S. Basera (1988). Pteridophytic flora of Berinag hills (Western Himalaya). *Indian Fern J.* **5**: 150-161.
- Pangtey, Y. P. S. (2001). Further additions to the fern flora of Nainital-II. *Indian. J. Forestry* **25**: 106-107.
- Pangtey, Y. P. S. & S. S. Samant (1987 c). Additions to the Pteridophytic flora of Nainital. *J. Bombay nat. Hist. Soc.* **83**: 683-684.
- Pangtey, Y. P. S. & S. S. Samant (1987 d). Additions to the Pteridophytic flora of Nainital-II. *J. Econ. Tax. Bot.* **11**: 5-6.
- Pangtey, Y. P. S. & S. S. Samant (1987 e). Additions to the Pteridophytic flora of Kumaun and Nainital (Western Himalaya). *J. Bombay nat. Hist. Soc.* **84**: 486-487.
- Panigrahi, G. & S. K. Basu (1980). Three species of *Dryopteris* Adans. (Aspidiaceae) re-interpreted. *Indian. J. Forestry* **3** : 266-271.
- Panigrahi, G. & R. D. Dixit (1969). Studies in Indian Pteridophytes-II. The family Osmundaceae in India. *J. Indian Bot. Soc.* **48**: 90-101.
- Panigrahi, G. & R. D. Dixit (1969). Studies in Indian Pteridophytes-IV. The family Ophioglossaceae in India. *Proc. nat. Inst. Sci. India* **35 B**: 230-266.

- Pant, P. C. (1986). *Flora of Corbett National Park*. Botanical Survey of India, Howrah.
- Pichi Sermolli, R. E. G. (1977). Tentamen Pteridophytorum genera in taxonomicum ordinem redigendi. *Webbia* **31**: 313-512.
- Price, M. G. (1990). Four new Asian *Loxogramme*. *Amer. Fern J.* **80**: 4-8.
- Punetha, N. (1985). Taxonomic observations on some species of Athyroid ferns from Pithoragarh district of Kumaun Himalaya (western Himalaya). *Indian Fern J.* **2**: 22-31.
- Punetha, N. (1985). Taxonomic observations on some species of Pteris from Pithoragarh district of Kumaun (Western Himalaya). *Indian Fern J.* **2**: 65-72.
- Rao, T. A. (1959). Report on a botanical tour to Milam glacier. *Bull. Bot. Surv. India* **1**: 97-120.
- Rau, M. A. (1961). Flowering plants and ferns of north Garhwal, Uttar Pradesh, India (an account based on collections made during 195 -1959). *Bull. Bot. Surv. India* **3**: 15-25.
- Rawat, G. S. & B. S. Aswal (1984). On the occurrence of tree fern *Cyathea spinulosa* Wall. ex Hook. (Cyatheaceae) in Kumaun Himalaya. *Him. Res. & Dev.* **3**: 44.
- Samant, S. S. & Y. P. S. Pangtey (1990). New records of some ferns for Kumaun Himalaya (Western Himalaya). *J. Bombay nat. Hist. Soc.* **87**: 173-174.
- Samant, S. S. & Y. P. S. Pangtey (1990). Rediscovery of some ferns of Kumaun Himalaya (Western Himalaya). *J. Bombay nat. Hist. Soc.* **87**: 333-334.
- Satija, C. K. & S. S. Bir (1985). Polypodiaceous ferns of India. **In: Aspects of Plant Sciences** **8**: 1-131.
- Satija, C. K., S. S. Bir & D. K. Bhardwaj (1983). Taxonomic revision of the Polypodiaceous genera of India – 7. *Pyrrosia* Mirbel. *Bull. Bot. Surv. India* **25**: 62-89.
- Singh, Surendra (1989). *Pteris multifida* Poir. – A new record for India. *Indian J. For.* **12**: 82.
- Singh, S., U. Choudhery & R. R. Rao (1986). Ferns and fern – allies of Chamoli district. *Indian J. Forestry* **9**: 1-15; 161-167.
- Stewart, R. R. (1942). The ferns of Mussoorie and Dehra Dun. *150th Ann. Vol. Royal Bot. Garden, Calcutta* **2**: 159-172.
- Tewari, L. M., R. S. Rawal & Y. P. S. Pangtey (1992). Ecology of *Drynaria mollis* Bedd. (Drynariaceae) in Kumaun (Western Himalaya). *Geobios new Reports* **11**: 180-183.
- Verma, S. C. & S. P. Khullar (1965). A new western Himalayan species of *Onychium* from *O. contiguum* complex : *O. fragile*. *Nova Hedwigia* **9**: 83-88.

4/10/2009

## Evaluation of metal pollution in medicinal plants.

Ismat naeem, Abida Taskeen\*, Nadia Arif and Hifsa Mubeen

Lahore College for Women University, Lahore  
Jail Road Lahore, Pakistan.  
Tel: 92-42-9203801-9/245  
Email: [paristan12@yahoo.com](mailto:paristan12@yahoo.com)

**Abstract:** In this study following plants were used: *Withinia Coagulas*, *Sarcococca Saligna*, *Cronopus Didymus*, *Senecio Chrysanthamoides*, *Aerva javanica*, *Vinca major*, *Salvadora* (yellow), *Impatiens walleriana*, *Pteris vittata*, *Calotropis procera*, *Eicohornia crassipes*, *Pinus walliachiana*. All these plants have different medicinal properties. 10 metals used in study were Magnesium, Potassium, Chromium, Copper, Nickel, Iron, Arsenic, Cobalt, Lead and Cadmium. It was concluded from the study that lead was present in highest amount among all these plants and it could be dangerous. Of all plants *Pinus walliachiana* contained highest amount of lead 450.60 ppb. Other metals were also present but their concentration was less as compared to lead. [Researcher. 2009;1(4):42-49]. (ISSN: 1553-9865).

## INTRODUCTION

During the past decades, spice and medicinal plants gained a more important role in agronomy production, pharmacy, and exportation because of their increased use as a raw material for the pharmaceutical industry and pharmaceutical preparations and in the everyday life of the general population. In recent years the cultivation of medicinal and aromatic plants has been achieved with increasing interest in Egypt. The interest in our country for these plants is much greater because of the possibility of exportation. From plant nutrition studies, it is known that plants require a certain amount of trace elements that they respond differently to an enhanced or lowered trace element supply, and that, in some cases, agricultural products may be contaminated with toxic heavy metals (Krug, 1986).

There are two major reasons (De Smet, 1992) to monitor levels of toxic metals in medicinal plants. The first reason, contamination of the general environment with toxic metals, has increased (Ali, 1983). The sources of this environmental pollution are quite varied, ranging from industrial and traffic emissions to the use of purification mud and agricultural expedients, such as cadmium-containing dung, organic mercury fungicides, and the insecticide lead arsenate (Schilcher, 1983; Gosselin et al., 1984; Schilcher et al., 1987). The second reason, exotic herbal remedies, particularly those of Asian origin, have been repeatedly reported to contain toxic levels of heavy metals and/or arsenic.

Several investigators have performed several studies on the residual levels of toxic metals in medicinal herbs (Schilcher, 1982; Ali, 1983, 1987; Peters and Schilcher, 1986; Schilcher et al., 1987). Most studies on residual levels of toxic metals in medicinal herbs have focused on lead, cadmium, and mercury (Schilcher, 1985; Ali, 1987; Schilcher et al., 1987).

The accumulation of heavy metals in some desert plants may open a new perspective for application of these species as 'accumulators' of heavy metals to clean-up contaminated soils in arid environments.

## Experimental Procedures

### Sample preparation and analysis:

The samples were dried to constant weight. 1g sample was digested with 20mL of HNO<sub>3</sub> /HCl (Anal grade), and heated until evolution of white fumes. Where necessary more acid mixture was added and the sample digested until evolution of white fumes marking the end of the digestion process. The digests were filtered into standard 50mL volumetric flask and made up to mark with distilled water. This was subsequently analyzed for Pb, Cd, Cu, Cr, Co and Fe by air-acetylene flame atomic absorption spectrometry with Graphite Furnace (Hitachi Z – 3000) by the standard calibration technique.

**Standard Preparation:**

Calibration standards were prepared by dilution of the high purity commercial metal standards (Merck) for atomic absorption analysis.

**Quality control and Quality Assurance:**

Hitachi A-3000 Atomic Absorption Spectrophotometer (graphite furnace) was used for analysis of arsenic. Known standards were used to calibrate the instrument and to keep a good quality control, our goal was to obtain a correlation coefficient value of as close to 1.0 as possible. Adequate quality assurance measures were carried out to ensure reliability of results. Glassware was properly cleaned and reagents (HNO<sub>3</sub>, HClO<sub>4</sub> and distilled water) were of analytical grade. Spikes and blanks were also introduced. Results reported are average of duplicates.

**Results**

**Table 1. Metal ion concentration in *Withinia Coagulas***

Sr.No	Name of metal ion	Symbol	Concentration (ppm)
Metals analyzed by Flame Atomic Absorption Spectroscopy			
1	Magnesium	Mg	0.20±0.2ppm
2	Potassium	K	4.64±0.1ppm
3	Chromium	Cr	0.00±0.3ppm
4	Copper	Cu	0.15±0.3ppm
5	Nickel	Ni	0.11±0.2ppm
6	Iron	Fe	3.25±0.7ppm
Metals analyzed by Graphite Furnace Atomic Absorption Spectroscopy			
7	Lead	Pb	207.25±0.1ppb
8	Copper	Cu	0.92±0.2ppb
9	Cobalt	Co	3.69±0.1ppb
10	Arsenic	As	2.03±0.4ppb

**Table 2. Metal ion concentrations in *Sarcococca Saligna***

No. of metal ions	Name of metal ion	Symbol	Concentration
Metals analyzed by Flame Atomic Absorption Spectroscopy			
1	Magnesium	Mg	0.18±0.2ppm
2	Potassium	K	0.17±0.3ppm
3	Chromium	Cr	0.29±1.2ppm
4	Copper	Cu	0.57±0.1ppm
5	Nickel	Ni	0.12±0.1ppm
6	Iron	Fe	6.72±0.3ppm
Metals analyzed by Graphite Furnace Atomic Absorption Spectroscopy			
7	Lead	Pb	77.56± 0.3ppb
8	Cadmium	Cd	2.05±0.1ppb
9	Cobalt	Co	4.78±0.3ppb
10	Arsenic	As	4.83±0.1ppb

**Table 3. Metal ion concentrations in *Cronopus Didymus*.**

Sr.No	Name of metal ion	Symbol	Concentration
Metals analyzed by Flame Atomic Absorption Spectroscopy			
1	Magnesium	Mg	0.11±0.3ppm
2	Potassium	K	11.2*20±0.2ppm
3	Chromium	Cr	0.00 ppm
4	Copper	Cu	0.94 ± 0.3ppm
5	Nickel	Ni	0.08±0.02ppm
6	Iron	Fe	11.09± 0.2ppm
Metals analyzed by Graphite Furnace Atomic Absorption Spectroscopy			
7	Lead	Pb	183.87 ±0.1ppb
8	Cadmium	Cd	3.33±0.3ppb
9	Cobalt	Co	5.07±1.0ppb
10	Arsenic	As	1.10±0.2ppb

**Table 4. Metal ion concentration in *Senecio Chrysanthamoides*.**

Sr.No	Name of metal ion	Symbol	Concentration
Metals analyzed by Flame Atomic Absorption Spectroscopy			
1	Magnesium	Mg	1.69±0.4ppm
2	Potassium	K	8.83*20±0.6ppm
3	Chromium	Cr	0.36±0.4ppm
4	Copper	Cu	0.31±0.6ppm
5	Nickel	Ni	0.1±0.5ppm
6	Iron	Fe	17.89±0.3ppm
Metals analyzed by Graphite Furnace Atomic Absorption Spectroscopy			
7	Lead	Pb	240.06±0.1ppb
8	Cadmium	Cd	2.85±0.4ppb
9	Cobalt	Co	21.86±0.7ppb
10	Arsenic	As	2.30±0.3ppb

**Table 5. Metal ion concentrations in *Aerva javanica*.**

No. of metal ions	Name of metal ion	Symbol	Concentration
Metals analyzed by Flame Atomic Absorption Spectroscopy			
1	Magnesium	Mg	0.18±0.1ppm
2	Potassium	K	11.93*20±0.4ppm
3	Chromium	Cr	0.38±0.5ppm
4	Copper	Cu	0.07±0.4ppm
5	Nickel	Ni	0.19±0.2ppm
6	Iron	Fe	8.84±0.1ppm
Metals analyzed by Graphite Furnace Atomic Absorption Spectroscopy			
7	Lead	Pb	156.60±0.1ppb
8	Cadmium	Cd	1.54±0.4ppb
9	Cobalt	Co	6.40±0.3ppb
10	Arsenic	As	0.15±0.4ppb

**Table 6. Metal ion concentrations in *Vinca major*.**

Sr.No	Name of metal ion	Symbol	Concentration
Metals analyzed by Flame Atomic Absorption Spectroscopy			
1	Magnesium	Mg	0.80±0.2ppm
2	Potassium	K	3.12*20±0.3ppm
3	Chromium	Cr	0.05±0.3ppm
4	Copper	Cu	0.08±0.5ppm
5	Nickel	Ni	0.12±0.4ppm
6	Iron	Fe	3.43±0.1ppm
Metals analyzed by Graphite Furnace Atomic Absorption Spectroscopy			
7	Lead	Pb	132.08±0.3ppb
8	Cadmium	Cd	1.73±0.5ppb
9	Cobalt	Co	7.94±0.4ppb
10	Arsenic	As	0.16±0.3ppb

**Table 7. Metal ion concentrations in *Salvadora* (yellow)**

No. of metal ions	Name of metal ion	Symbol	Concentration
Metals analyzed by Flame Atomic Absorption Spectroscopy			
1	Magnesium	Mg	0.16±0.3ppm
2	Potassium	K	3.12±0.3ppm
3	Chromium	Cr	0.19±0.3ppm
4	Copper	Cu	0.26±0.4ppm
5	Nickel	Ni	0.14±0.7ppm
6	Iron	Fe	2.20±0.5ppm
Metals analyzed by Graphite Furnace Atomic Absorption Spectroscopy			
7	Lead	Pb	132.08±0.2ppb
8	Cadmium	Cd	2.62±0.4ppb
9	Cobalt	Co	3.30±0.5ppb
10	Arsenic	As	1.25±0.7ppb

**Table 8. Metal ion concentrations in *Impatiens walleriana***

Sr.No	Name of metal ion	Symbol	Concentration
Metals analyzed by Flame Atomic Absorption Spectroscopy			
1	Magnesium	Mg	1.69± 0.2ppm
2	Potassium	K	8.83±0.3ppm
3	Chromium	Cr	0.36±0.1ppm
4	Copper	Cu	0.31±0.2ppm
5	Nickel	Ni	0.1±0.3ppm
6	Iron	Fe	17.89±0.1ppm
Metals analyzed by Graphite Furnace Atomic Absorption Spectroscopy			
7	Lead	Pb	237.97±0.2ppb
8	Cadmium	Cd	2.29±0.3ppb
9	Cobalt	Co	17.49±0.3ppb
10	Arsenic	As	1.35±0.4ppb



**Table 9. Metal ion concentrations in *Pteris vittata***

Sr.No	Name of metal ion	Symbol	Concentration
Metals analyzed by Flame Atomic Absorption Spectroscopy			
1	Magnesium	Mg	0.19±0.3ppm
2	Potassium	K	15.6*20±0.3ppm
3	Chromium	Cr	0.32±0.5ppm
4	Copper	Cu	0.29±0.7ppm
5	Nickel	Ni	0.08±0.3ppm
6	Iron	Fe	14.22±0.1ppm
Metals analyzed by Graphite Furnace Atomic Absorption Spectroscopy			
7	Lead	Pb	152.52±0.3ppb
8	Cadmium	Cd	3.09±0.4ppb
9	Cobalt	Co	8.03±0.2ppb
10	Arsenic	As	3.56±0.4ppb

**Table 10. Metal ion concentrations in *Calotropis procera***

Sr.No	Name of metal ion	Symbol	Concentration in ariel parts	Concentration in roots
Metals analyzed by Flame Atomic Absorption Spectroscopy				
1	Magnesium	Mg	1.58*20±0.4ppm	1.68*20±0.6ppm
2	Potassium	K	50±0.3ppm	3.83±0.3ppm
3	Chromium	Cr	0.36±0.2ppm	0.02±0.5ppm
4	Copper	Cu	0.320.5ppm	0.36±0.4ppm
5	Nickel	Ni	0.1±0.5ppm	0.06±0.3ppm
6	Iron	Fe	14.18±0.6ppm	4.26±0.1ppm
Metals analyzed by Graphite Furnace Atomic Absorption Spectroscopy				
7	Lead	Pb	175.930.1ppb	103.31±0.2pb
8	Copper	Cu	1.23±0.6ppb	2.57±0.5ppb
9	Cobalt	Co	17.580.7ppb	3.44±0.5ppb
10	Arsenic	As	0.150.1ppb	8.04±0.2ppb

**Table 11. Metal ion concentrations in *Eicohhornia crassipes*.**

Sr.No	Name of metal ion	Symbol	Concentration
Metals analyzed by Flame Atomic Absorption Spectroscopy			
1	Magnesium	Mg	0.18±0.5ppm
2	Potassium	K	11.93*20± 0.1ppm
3	Chromium	Cr	0.38±0.3ppm
4	Copper	Cu	0.07±0.4ppm
5	Nickel	Ni	0.19±0.3ppm
6	Iron	Fe	8.84±0.6ppm
Metals analysed by Graphite Furnace Atomic Absorption Spectroscopy			
7	Lead	Pb	156.60±0.5ppb
8	Cadmium	Cd	1.98±0.3ppb
9	Cobalt	Co	6.40±0.1ppb
10	Arsenic	As	0.15±0.3ppb

**Table 12. Metal ion concentrations in *Pinus walliachiana***

No. of metals	Name of metal ion	Symbol	Concentration
Metals analyzed by Flame Atomic Absorption Spectroscopy			
1	Magnesium	Mg	0.90±0.2ppm
2	Potassium	K	25.37±0.2ppm
3	Chromium	Cr	0.00
4	Copper	Cu	0.09±0.1ppm
5	Nickel	Ni	0.13±0.3ppm
6	Iron	Fe	4.25±0.1ppm
Metals analyzed by Graphite Furnace Atomic Absorption Spectroscopy			
7	Lead	Pb	450.60±0.1ppb
8	Cadmium	Cd	25.37 ±0.2ppb
9	Cobalt	Co	15.41±0.2ppb
10	Arsenic	As	2.40±0.1ppb

**Discussion:**

In this study following plants were used: *Withinia Coagulas*, *Sarcococca Saligna*, *Cronopus Didymus*, *Senecio Chrysanthamoides*, *Aerva javanica*, *Vinca major*, *Salvadora* (yellow), *Impatiens walleriana*, *Pteris vittata*, *Calotropis procera*, *Eicohhornia crassipes*, *Pinus walliachiana*. 10 metals used in study were Magnesium, Potassium, Chromium, Copper, Nickel, Iron, Arsenic, Cobalt, Lead and Cadmium.

All these plants have different medicinal properties. In all these plants lead was present in very high concentration which is dangerous.

In *Sarcococca Saligna* and *Withinia Coagulas* only lead was present in slightly high concentration as compared to other metals. However its concentration as compared to other plants was very low.[Table:1,2]. In *Withinia Coagulas* chromium was not present. However potassium was high.[Table:2]. *Cronopus Didymus* also contained a high concentration of lead and potassium only.[Table:3]. In *Senecio Chrysanthamoides* lead, cobalt, potassium and iron were detected at high levels.[Table:4]. *Aerva javanica* potassium, iron and lead were in high amounts [Table:5]. *Impatiens walleriana*, *Salvadora* (yellow)

contained lead in high concentration.[Table:7,8]In *Calotropis procera* magnesium and lead were in high range while other metals were only present in normal amounts[Table:10]

In *Eicohhornia crassipes*, *Vinca major* and *Pteris vittata* potassium and lead were present in high concentration. Other metals were in very low amounts.[Table:6,9,11]. In *Pinus walliachiana* a very high amount of lead was present. Other metals were normal in concentration. [Table: 12]. Pinus has been reported as a source of for many natural products.

Of all plants *Pinus walliachiana* contained highest amount of lead 450.60 ppb.[Table:12].

#### **Conclusion:**

Before using these medicinal plants for remedies of different diseases their metal content should be kept in mind. This is important because otherwise metals may affect us with their harmful effects.

#### **Acknowledgment:**

I want to acknowledge Sir Talib Mehmood, Instrument engineer Central Research Laboratories Lahore College for Women University Lahore, for his cooperation during this research.

#### **References**

1. Ali, S L Bestimmung der pestiziden Rückstände und anderer bedenklicher verunreinigungen-wie toxische Metallspuren — in Arzneipflanzen 2-Mitteilung Bestimmung von toxischen Metallspuren in Arzneidrogen *Pharm Ind* **1983**, 45, 1294–1295.
2. De Smet, P A G M *Adverse Effects of Herbal Drugs*; Springer-Verlag Berlin, **1992**; ISBN 3-540-53100-9.
3. Gosselin R E, Smith, R P, Hodge H C, Braddock J E *Clinical Toxicology of Commerical Products*, 5th ed ; Williams and Wilkins Baltimore, MD, **1984**.
4. Krug, H Gemüseproduktion *Paul Parey* 1986 (cited from Chizzola, R Metallic trace elements in herbs and spices grown in Austria *Acta Horti* **1989**, 249).
5. Peters H, Schilcher H, Schwermetallbelastung von Arzneidrogen und deren Zubereitungen *Planta Med* **1986**, 521–522.
6. Schilcher H, Rückstände und Verunreinigungen bei Drogen und Drogenzubereitungen 19 Mitteilung: Zur Wertbestimmung und Qualitätsprüfung von Drogen *Planta Me* . **1982**, 44, 65–77.
7. Schilcher H, Contamination of natural products with pesticides and heavy metals In *Topics in Pharmaceutical Sciences 1983*; Breimer D, D Speiser P Eds Elsevier Science Publishers Amsterdam, The Netherlands, **1983** pp 417–423.
8. Schilcher H, Rückstandsanalytik bei Drogen und Drogenzubereitungen. *Fresenius' Z Aanl Che* . **1985**, 321, 342–351.
9. Schilcher H, Peters H, Wank H, Pestizide und Schwermetalle in Arzneipflanzen und Arzneipflanzen-zubereitungen *Pharm Ind* **1987**, 49, 203–211.

4/12/2009

## Effects of dose-related levels of powdered *Stachytarpheta jamaicensis* Vahl leaves on body weight and liver functions of albino rats

E.A Ogie-Odia<sup>1\*</sup>, M.N Ilechie<sup>2</sup>, A.H Erhenhi<sup>3</sup> and E. F Oluowo<sup>4</sup>

<sup>1\*</sup>Department of Botany, Ambrose Alli University, PMB 14, Ekpoma, Edo State

<sup>2</sup> Department of Biology Education, Federal College of Education, PMB 1044 Asaba, Delta State

<sup>3</sup> Department of Botany, Delta State University, PMB 1, Abraka, Delta State

<sup>4</sup> Department of Animal and Environmental Biology, University of Benin, PMB 1154, Benin City, Nigeria

\*Email: [effexing@yahoo.com](mailto:effexing@yahoo.com)

**ABSTRACT:** Potential dose-related effect of powdered *S. jamaicensis* L. leaves known for treating different ailment was investigated for changes in the body weights and its effects on the liver of albino rats. In the study, twenty albino rats (male and female) after due acclimatization, were fed different graded mixtures of pellet feedmash i.e 25g, 50g, and 75g mixed with different concentrations of powdered *S. jamaicensis* leaves in the ratio 75g, 50g, and 25g while the control was fed pellet feedmash only. The albino rats were weighed, grouped into fives and divided into four groups of three treatment groups and a control group. The results obtained showed slight variation in the agility, food intake and physical appearances of the rats with mild congestion, fatty changes and necrosis in the liver. Bilirubin levels in all the groups showed slight variation ( $p > 0.05$ ). From the results obtained, it would appear that *S. jamaicensis* does not cause large variations in body weights ( $p > 0.05$ ) but causes a few changes in the liver of albino rats which is not significantly different ( $p > 0.05$ ) from that of the control group. [Researcher. 2009; 1(4):50-55]. (ISSN: 1553-9865).

**Keywords:** Dose-related, *Stachytarpheta jamaicensis*, body weight, liver function, albino rats.

### INTRODUCTION

Many societies respond to their environment in the interpretation of various aspects of life especially concerning ill-health. The fear of illness and death as well as the necessity to feed, of health and protection have led men of all times and under all skies to resort to anything that nature can offer them (Koumare, 1985). *Stachytarpheta jamaicensis* is a common herb of field crops, bush, roadsides and disturbed places in the higher rainfall forest zones of West Africa, occurring from Sierra Leone to Nigeria. It comes from the large Verbanaceae family which comprises about 100 genera and 2,600 species and is widely used by various indigenous people throughout the world. Various researches on *Stachytarpheta jamaicensis* include works carried out by Coimbra (1994), Cruz (1995), Schapoval *et al.*, (1998), Ramos *et al.*, (2001) and Antoun *et al.*, (1999).

The potency of various plants has long been known and recognized from ages past. Some of these plants that exhibit medicinal properties have been known to help in stabilizing different internal organs in animals, while others have had side effects on the organs probably due to the varying amounts or quantity of toxic matter present in such plants.

Toxicity testing in animals is carried out on a new drug to identify potential hazard. It helps in determining the upper limits of administration (Sofowora, 1993). The effect of the toxic material is to test the usefulness in human thereafter. If the effect is low, then there is a chance of possible introduction of such drug material for consumption with a view to effect cure to a potential disease condition. This proves the drug material to be non-toxic and therapeutically safe. The basic premise is that toxic effect caused by a drug is similar in man and other animals (Range *et al.*, 1995). If a chemical (or drug material) produces injury to a tissue, the capacity of the tissue to regenerate or recover will largely determine the reversibility of the effect. Most toxic effects of drugs occur at a predictable (usually short) time after administration (Curtis, 2001). Toxic effects can range from negligible to so severe as to preclude further development of the compound (Range *et al.*, 1995). It should however be noted that the target organ of toxicity is not necessarily the site of accumulation of the chemical (Curtis, 2001).

This study is with a view to test whether the effects of different dose treatments of powdered leaves of *Stachytarpheta jamaicensis* would have any changes in the body weights and liver of albino rats.

## METHODOLOGY

### Preparation of plant extract.

The plant was harvested from Ugbowo area in Benin City, Edo state and identified using texts like Hutchinson and Dalziel (1968) and Handbook on West African weeds” (Akobundu and Agyakwa, 1987). The leaves were washed and air dried under the sun for three (3) days. After drying, it was cleaned off debris and kept in the oven to dry at 40°C for 18 hours. The leaves were plucked off the dried branches and pounded in a mortar to obtain the powdered form. About 3kg of the powdered leaves was weighed and stored in a moisture free airtight container for use.

### Experimental Rats

Twenty albino rats were randomly sampled and kept individually in various cages to curb cannibalism and to allow for close observations. They were allowed to acclimatize for two weeks, during which they were fed with mash before commencement of the experiment. After acclimatization, the rats were divided into four (4) main groups of three (3) treatment groups and one (1) control group .The weights of the rats were taken and tabulated (Table 1).

**Table1. Grouped experimental rats with initial weights.**

Cage	Animal	Weight (gms)
Control	C1 a	151
	C1 b	130
	C1 c	134
	C1 d	169
	C1 e	215
Treatment group 1	T1 a	162
	T1 b	122
	T1 c	150
	T1 d	160
	T1 e	161
Treatment group 2	T2 a	128
	T2 b	131
	T2 c	191
	T2 d	151
	T2 e	141
Treatment group 3	T3 a	170
	T3 b	141
	T3 c	140
	T3 d	171
	T3 e	171

**Table 2. Food intake of experimental rat.**

TREATMENT GROUPS	NO. OF WEEKS					
	1	2	3	4	5	6
CONTROL	←————— Feed Mash only —————→					
TRTM 1	←—— Feed Mash only —→			75g mash +25g powdered <i>S.jamaicensis</i> —→		
TRTM 2	←—— Feed Mash only —→			50g mash + 50g powdered <i>S.jamaicensis</i> —→		
TRTM 3	←—— Feed Mash only —→			25g mash + 75g powdered <i>S.jamaicensis</i> —→		

During the course of treatment, behavioural signs and general appearance such as agility, food consumption, and water consumption were observed. Body weight was measured weekly. The animals were sacrificed under diethyl ether anesthesia (100mg/kg body weight) and the excised liver was stored in sample bottles containing 10% formal saline and Boehing solution. Each bottle was labeled for easy identification.

**Statistical analysis**

The data obtained was expressed as mean +S.D and analyzed as analysis of variance (ANOVA). Statistical significances of the difference of the mean was evaluated using the Students t-test and the differences were considered statistically significant if the p values were less than 0.05 (p<0.05).



**Table 3. Mean body weight of rats.**

Treatment	Dose level (g)	No. of animals	Initial body weight	Final body weight
Control	Feedmash only	5	159.80 ± 15.43	231.25 ± 18.46
Treatment 1	25g <i>S. jamaicensis</i> + 75g feedmash	5	151.00 ± 7.56	190.80 ± 13.17
Treatment 2	50g <i>S. jamaicensis</i> +50g feedmash	5	148.40 ± 11.39	159.00 ± 11.81
Treatment 3	75g <i>S. jamaicensis</i> +25g feedmash	5	158.60 ± 7.39	157.67 ± 11.55

Mean ± S.E (standard error) for 5 determinations.

**Table 4. Analysis of food consumption during experimental period**

Treatment groups	No of animals	Concentration(g) (Feedmash)	Concentration(g) ( <i>S. jamaicensis</i> )	Food consumption
T1	5	75.0	25.0	+++
T2	5	50.0	50.0	++
T3	5	25.0	75.0	+
C1	5	100.0	0	+++

+++ = Good, ++ = Average, + = Fair

T1= 25g powdered *S. jamaicensis* + 75g feedmash  
 T2= 50g powdered *S. jamaicensis* + 50g feedmash  
 T3= 75g powdered *S. jamaicensis* + 255g feedmash  
 C1= 100g Feedmash only

**Table 5: Effect of *Starchytarpehta jamacensis* on bilirubin levels in treated rats.**

Parameter tested	Treatment group 1	Treatment group 2	Treatment group 3	Control
Total Bilirubin (mg/dl)	0.6 ± 0.08	0.6 ± 0.12	0.5 ± 0.1	0.45 ± 0.06
Conjugated bilirubin (mg/dl)	0.3 ± 0.04	0.3 ± 0.06	0.25 ± 0.05	0.63 ± 0.15

Mean ± S.E (standard Error) for 5 determinations.

**Table 6. Physical characteristics/observations of Experimental rats**

Treatment Groups	Agility	Hair loss	Food intake	Eyes
Treatment 1	Normal	None	Normal	Normal
Treatment 2	Slightly reduced in 2 <sup>nd</sup> week	None	Slightly reduced but normal 2 <sup>nd</sup> week	Normal
Treatment 3	Reduced in 2 <sup>nd</sup> and 3 <sup>rd</sup> week	None	Reduced	Normal
Control	Normal	None	Normal	Normal

**Table 7. Observations on the liver.**

PARAMETERS TESTED	CONTROL GROUP	TREATMENT GROUP 1	TREATMENT GROUP 2	TREATMENT GROUP 3
<b>Histology of the liver</b>	Normal	Normal except that T1A had congested blood vessel and T1C had necrosis	Normal	Normal except where T3C showed area of fatty acid change

Treatment group 1 = 25g powdered *S. jamaicensis* + 75g feedmash

Treatment group 2 = 50g powdered *S. jamaicensis* + 50g feedmash

Treatment group 3 = 75g powdered *S. jamaicensis* + 25g feedmash

Control group 1 = 100g Feedmash only

## RESULTS AND DISCUSSION

From the various tables above viz 1, 2, 3, 4, 5, 6, 7 the different results can be clearly seen. Table 1 shows the initial weights of the rats before they were fed the powdered leaves of *Stachytarpheta jamaicensis* while Table 2 shows the feeding mode and pattern administered to the different groups of albino rats. In the course of the experimental periods, four rats in treatment group 3 (T3) were noticed to have loss of agility (Table 6). Increased concentrations of active compounds in plant extracts are not always beneficial and can even promote adverse biological effect (Pepato *et al.*, 2001). Rats in T3 lost some weight probably due to the high dose of powdered leaves given to them (Table 3). It was also noticed that two rats in treatment group 1 and one in treatment group 2 gave birth during the experimental period and also gained weight. Most times, increase in weight can be as a result of pregnancy, which affects the levels of oestrogen and progesterone known to affect both uterine receptivity (Wand and Dey, 2006) influence food intake and energy expenditure (USEPA, 1996). Body weight provides some indication of the general health status of animals. A decrease may be due to the rejection of food or water caused by reduced palatability, treatment induced anorexia or systemic toxicity (Abdulazeez *et al.*, 2009). Possible reasons for the loss in agility and weight may be due to the feeding mode of the experimental rats in question as regards the taste of the powdered concentration. Reduced agility is an indication of disease condition (Brigid *et al.*, 1980). There was no hair loss observed in the rats and the eyes were all normal. Water intake was also normal in all groups (Table 6).

From the results obtained in Table 7, there were generally no significant changes in the liver when compared with the control. The liver of all the groups were normal and within the same range with no significant differences between them ( $p > 0.05$ ). Congested blood vessel and areas of hemorrhage was noticed in the liver of Treatment group I (T1) rat. Fatty change of the liver was present in T3 (Table 7) The Bilirubin level all the rats were within normal range (Table 5) although there was slightly marked variation, as high value of bilirubin is an indication of red blood cell destruction which may consequently result into jaundice. Bilirubin reduction indicates improvement in health conditions (Beck *et al.*, 1994)

## CONCLUSION

It can be concluded from the results that while *Stachytarpheta jamaicensis* may be found to be very useful in the treatment of certain disease conditions, systemic sensitivity is also of note. It would appear that specific tissue sensitivity of *S. jamaicensis* on the liver is opposed to related toxicity as often associated with therapeutic agents. This remark is plausible as the tissues of the liver cells are susceptible to slight damage with the use of the plant.

Further research work would be needed to test the effect of the plant extract on more organs and also to isolate the active ingredients and such possible toxicants that may be present in the plant.

## REFERENCES

- Abdulazeez, M.A., Ameh A.D., Ibrahim S., Ayo J.O and Ambali S.F (2009) Effect of fermented seed extract of *Carica papaya* on litters of female wistar rats (*Rattus norvegicus*) *African journal of Biotechnology* **8 (5)**: 854-857
- Akobundu, I.O and Agyakwa, C.N (1987) A Handbook on West African Tropical Weeds, I.I.T.A Publication, Ibadan, Nigeria 420pp.
- Antoun M.D, Martinez E, Caballero R, Oquendo T, Proctor, G.R., Weislow, O.S., McCloud, T.G., Kiser, R., Stanley. P, Clanton D., (1999). Evaluation of the flora of Puerto Rico for the *in vitro* Cytotoxic and Anti-HIV activities, *Pharmaceutical Biology*. **37(4)**: 277 – 280.
- Beck J.R., Kattan M.V. and Miles B.J. (1994). A critique of the decision analysis for clinically localized prostate cancer. *Journal of Urology*, **152**: 1894 – 1899.
- Brigid .G.L, Curt I.C and Gregory .R (1980) Markers in Human Lymphoid Tumour *In* :Sell S (ed.) Cancer Markers , Humana press, Clifton ,New Jersey pp 90-113
- Cruz, G.L (1995) *Dicionario Das plantas Uteis Do Brasil* ,5<sup>th</sup> Edn. Bertrand; Rio de Janeiro, Brazil. 11p
- Curtis, D.K (2001). Principles of Toxicology and Treatment of Poisoning: In Good and Gilnan,. “The Pharmacological Basic of Therapeutics”, 10<sup>th</sup> Edn. McGraw Hill pp67 – 71.
- Hutchinson, J. and J.M. Dalziel (1968) Flora of West tropical Africa. Vol III Part 1. Crown Agents for Overseas Government and Administrations, Mill bank, London. 828p
- Koumare M (1985). Research into African medicinal plants. Newsletter, *J P* 27.No 11 p1
- Pepato M.T, Folgado V.B.B, Kettlehust I.C and Brunetti I.L (2001) Lack of antidiabetic effect of *Eugenia jambolan* leaf decoction on streptozotocin diabetic rats. *Brazil Journal of Medicine and Biological Research*, **34**:389-395
- Ramos A, Piloto J, Visozo A, Garcia A, Lastra H, Ponce de Leon H. (2001). Mutagenic and antioxidant assessment of *Stachytarpheta jamaicensis* (L) Vahl. *Phytotherapeutic Research*. **15**: 360-363.
- Range H.P., Dale M.M. and Ritter J.M. (1995). Pharmacology, 3<sup>rd</sup> Edn., Churchill Livingstone; USA, 800p.
- Schapoval E.E, Vargas M.R, Chares C.G, Bridi .R, Zuancozzi J.A, Henriques. A.T. (1998) Anti inflammatory and anti-nocieptive activities of extracts and isolated compound from *Stachtarpheta cayennensis*. *Journal of Ethnopharmacology*, **60** : 53-59.
- Sofowora ,A (1993) Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan pp58-196
- United States Environmental Protection Agency (USEPA) (1996) Guidelines for reproductive toxicity risk assessment. *Federal register* **61(212)**; 56274-56322
- Wang H and Dey S.K (2006) Roadmap to embryo implantation: clues from mouse models. *Nat. Rev. Genet.* **7**: 185-199

## ***In vitro* Rapid clonal propagation of *Phyllanthus urinaria* Linn. (Euphorbiaceae) – A Medicinal Plant**

Kalidass C\*, and Mohan V.R  
Ethnopharmacology unit,  
Research Department of Botany,  
V.O.Chidambaram College, Tutuicorin-8.

\*Corresponding author e-mail: [vrn\\_mohan\\_2005@yahoo.com](mailto:vrn_mohan_2005@yahoo.com)

**Abstract:** An efficient micropropagation protocol was developed for the medicinal plant *Phyllanthus urinaria* Linn. (Euphorbiaceae) using nodal segments for axillary shoot proliferation. Maximum multiplication rates was achieved on Ms Media supplemented with 1.0  $\mu$ M BA. Rooting was achieved with 100% of the micro shoots in MS medium with 2.0  $\mu$ M IBA. Regenerated plants were successfully acclimatized and about 80 – 90 % of plantlets survived under ex vitro conditions. [Researcher. 2009;1(4):56-61]. (ISSN: 1553-9865).

**Key words:** *Phyllanthus urinaria* Linn., Euphorbiaceae, Clonal propagation, acclimatization.

### **Introduction**

The genus *Phyllanthus* Linn. (Euphorbiaceae) has between 550 to 750 species and several of them produce useful secondary metabolites which have been extracted from whole plants (Unander 1996). The stems, infusion of leaves and roots of *Phyllanthus* spp are used in folk medicine for treating intestinal infections, diabetes, the hepatitis B virus and disturbances of the kidney and urinary bladder (Calixto *et al.*, 1998). Several compounds such as alkaloids, tannins, flavonoids, lignans, phenols and terpenes have been isolated and identified in various species of *Phyllanthus* and have shown antinociceptive action in mice and other therapeutic activities (Cechinel Filho *et al.*, 1996). Antiviral effects against hepatitis B virus and possibly against the reverse transcriptase of retroviruses have also been reported (Venkateswaran *et al.*, 1987, Thyagarajan *et al.*, 1988, Shead *et al.*, 1992). Pharmacological studies carried out with callus extracts of *P. niruri*, *P. tenellus* and *P. urinaria* have shown antinociceptive properties and the main compounds identified in the extracts were flavonoids, tannins and phenols (Santos *et al.*, 1994). Additional studies on callus and root extracts of these different species have shown the presence of phyllembin, a tannin which has antimicrobial activity, of possible hydrolyzable tannins which inhibited DNA polymerase and reverse transcriptase, of geraniin and its derivatives which showed high activity in the inhibitions of HIV reverse transcriptase and angiotensin-converting enzyme involved in diabetic complications (Ueno *et al.*, 1988, Ogata *et al.*, 1992, Unander 1996).

The gallotannin corilagin, the haemostatic ellagic acid, as well as seven ellagitannins, which have been shown to be active against Epstein–Barr virus DNA polymerase at the micro-molar level (Liu *et al.*, 1999), have been isolated from *Phyllanthus urinaria* Linn. Two new phenolic compounds, namely methyl brevifolin carboxylate and trimethyl ester dihydrochebulic acid, have also been isolated from the same source (Yao and Zuo, 1993). Corilagin has been reported to show bioactivity in various different therapeutic areas such as cardiovascular disease anti-hypertensive (Lin *et al.*, 1993; Cheng *et al.*, 1995) and infectious disease antiviral (Yoon *et al.*, 2000). Therefore, the aim of the current work was to establish consistent micropropagation, for *P. urinaria* Linn. for large scale multiplication of selected genotypes and to explore their potential for secondary metabolite studies.

### **Materials and Methods**

Tender twigs were collected from field grown mature plants of *P. urinaria* L. defoliated and sectioned into 2 - 3 nodal segments. They were washed under continuous flashing of running tap water for 30 min and then treated with a solution of the Savlon (5% v/v) for 10 min and finally surface sterilized with HgCl<sub>2</sub> (0.1% w/v) for 5-10 min. Lastly, the material was washed 3 -5 times with autoclaved distilled water to remove any trace of HgCl<sub>2</sub>.

The shoot tip and nodal segments were excised from the disinfected material and divided into 1.0 - 1.5 cm pieces with at least one node in each explants. The basal medium used for all the experiments was MS (Murashige and Skoog 1962) formulation containing 3% sucrose, 6 - 8 % agar and supplemented with BA and KN, either individually or in different combinations with auxins, IBA, NAA and IAA. The media were adjusted to pH 5.7  $\pm$  0.2 and autoclaved at 1.1 kg/cm<sup>2</sup> for 20 min at 121°C.

Cultures were incubated at  $25 \pm 1^\circ\text{C}$  with a photoperiod of 16 h at 2000 - 3000 lux of cool white fluorescent light. Cultures were initiated in 150 - 25 mm glass tube and subcultured regularly on fresh medium at four-week intervals in 100 ml flasks. The shoots that proliferated from primary explants were isolated and subcultured on fresh medium several times for bulking up shoot culture material. Shoots (3 - 4 cm) were excised from proliferating cultures and implanted on half strength MS supplemented with either of IBA, NAA and IAA (0.1 - 1.0  $\mu\text{M}$ ) for rooting. Rooted shoots were transferred to pots under *ex vitro* condition after proper hardening.

### Results and Discussion

Axillary shoot induction, multiplication and rooting, the effects of cytokinins and auxins on morphogenesis of nodal segment explants are presented in Tables 1, and 2. The effects of cytokinins and auxins at various concentrations on axillary shoot induction from nodal explants are presented in Tables 1. Cytokinins did not promote intensive shoot multiplication and either had no effect or inhibited significantly the number of nodes, shoot length and culture fresh mass in comparison to the controls (Table 1). The proliferation efficiency of nodal explants from mature plants was significantly higher than that of shoot tip explants when evaluated twenty days after proliferation. As a supplement, 1.0  $\mu\text{M}$  BA showed the best performance of proliferation that produced shoots in 100% of cultured explants. Explants produced the highest number of  $2.85 \pm 0.10$  shoots per culture on the medium with  $5.97 \pm 0.13$  cm average length of shoots per culture (Figs. B - D). When the explants were cultured on KN based medium, only 47 - 73% of them proliferated. In this treatment, the highest number of shoots per explants and average shoot lengths were  $5.23 \pm 0.24$  and  $4.60 \pm 0.35$  cm for nodal explants, respectively.

These are the first attempts to establish shoot cultures of this species and the results obtained show that the unusual promotive effects of IBA on shoot culture growth was not only due to the increase in axillary shoot proliferation but also in the number of axillary buds formed in the shoots which can be used as starting plant material for further multiplication. Similar results were obtained for *Leptadenia reticulata* shoot cultures, where IBA, NAA and IAA stimulated significantly the number of nodes per plantlet in comparison to cytokinins (Kalidass *et al.*, 2008). However, for other *Phyllanthus* species, such as *P. tenellus*, *P. niruri*, *P. caroliniensis*, and *P. fraternus* (Catapan 1999, Saradhi and Islamia 1997) and for other Euphorbiaceae species, such as *Excoecaria agallocha* L. (Rao *et al.*, 1998) cytokinins stimulated shoot proliferation.

Root formation was induced in *in vitro* regenerated shoots by culturing them on half strength of MS with 0.1 - 4.0  $\mu\text{M}$  either of IBA, NAA, and IAA. Among the three types of auxin, IBA was found to be most effective at different concentrations tested for root production on cut margins of the shoot (Table 2). Among different concentrations, 2.0  $\mu\text{M}$  IBA was found to be the best for proper rooting in which 100% shoots rooted within three weeks of culture (Fig. D). These findings are in agreement with those observed in other plant species *Phyla nodifolia*, *Leptadenia reticulata* (Bhatt *et al.*, 2006) and *Lins culinaris* Medik (Omran *et al.*, 2008). The *in vitro* derived plants acclimated better under *ex vitro* condition when they were transferred on specially made plastic trays containing coco-peat as potting mix and moistened uniformly at periodic intervals taking special care not to damage the roots. The rest of the procedure, followed from this stage up to their establishment in soil was as usual.

About 80 - 90% of the regenerated plantlets could tolerate and survive under *ex vitro* environment or field conditions. A number of plantlets were lost due to damping off and necrosis during acclimatization in *ex vitro* condition. Loss of regenerants due to such symptoms was also observed in *Eucalyptus tereticornis* (Gill *et al.*, 1993), *Solanum nigrum* (Ara *et al.*, 1993), *Rauvolfia serpentina* (Ilahi 1993) and *Rosa damascena* (Kumar *et al.*, 1995). Through this study a protocol for regeneration of complete plantlets has been established. This is perhaps the first report on *in vitro* plant regeneration of *Phyllanthus urinaria* Linn. The results may be of some importance as a pioneering study on tissue culture of this medicinal plant.

**Table 1. Effect of growth regulators on shoot proliferation and number of shoots per culture from nodal explants. Data (Mean  $\pm$  S.D.) were recorded after four weeks.**

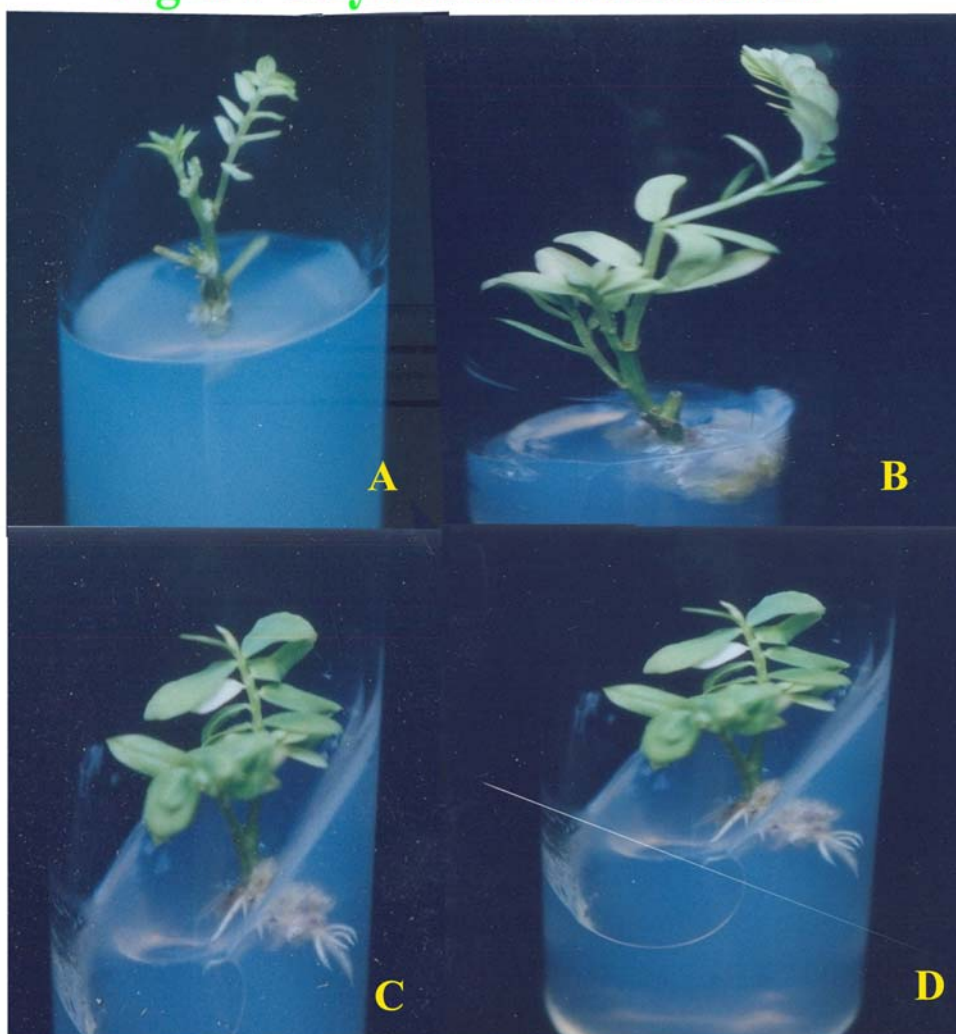
Growth regulators ( $\mu$ M)	% of shoot formation	No. of node per shoots/culture	No. of usable shoots/culture	Av. Length of shoots/culture
<b>BA</b> 0.0	80	0.84 $\pm$ 0.13	1.10 $\pm$ 0.21	1.79 $\pm$ 0.17
0.1	80	1.38 $\pm$ 0.08	2.41 $\pm$ 0.13	3.24 $\pm$ 0.17
0.2	75	1.30 $\pm$ 0.37	1.73 $\pm$ 0.36	4.29 $\pm$ 0.08
0.5	82	2.47 $\pm$ 0.18	2.85 $\pm$ 0.06	4.90 $\pm$ 0.04
1.0	100	2.85 $\pm$ 0.10	3.38 $\pm$ 0.50	5.97 $\pm$ 0.13
2.0	76	2.37 $\pm$ 0.21	1.96 $\pm$ 0.11	3.70 $\pm$ 0.12
3.0	64	1.28 $\pm$ 0.05	1.76 $\pm$ 0.12	2.32 $\pm$ 0.21
5.0	-	-	-	-
<b>Kn</b> 0.1	65	0.89 $\pm$ 0.12	1.25 $\pm$ 0.13	2.70 $\pm$ 0.18
0.2	47	1.10 $\pm$ 0.35	1.00 $\pm$ 0.18	4.26 $\pm$ 0.25
0.5	55	1.80 $\pm$ 0.42	1.62 $\pm$ 0.15	4.60 $\pm$ 0.35
1.0	73	2.12 $\pm$ 0.16	1.95 $\pm$ 0.14	5.23 $\pm$ 0.24
2.0	54	1.58 $\pm$ 0.13	1.96 $\pm$ 0.15	3.50 $\pm$ 0.12
3.0	48	0.90 $\pm$ 0.15	1.76 $\pm$ 0.31	2.10 $\pm$ 0.42
5.0	-	-	-	-

**Table 2. Effect of different concentration and combination of auxins on adventitious root formation from the *in vitro* grown micro-cutting cultured on 1/2 MS medium. Data (Mean  $\pm$  S.D.) were recorded after four weeks.**

Growth regulators (types auxin) ( $\mu$ M)	% of micro cutting rooted	No. of root/micro cutting	Av. Length of root/micro cutting
<b>IBA</b> 1.0	78	1.56 $\pm$ 0.09	1.49 $\pm$ 0.13
2.0	100	2.30 $\pm$ 0.16	1.34 $\pm$ 0.04
3.0	65	3.36 $\pm$ 0.21	1.97 $\pm$ 0.01
4.0	-	-	-
<b>IBA</b> 1.0 + <b>NAA</b> 0.1	70	2.63 $\pm$ 0.24	1.79 $\pm$ 0.17
<b>IBA</b> 2.0 + <b>NAA</b> 1.0	65	1.67 $\pm$ 0.16	0.96 $\pm$ 0.04
<b>IBA</b> 3.0 + <b>NAA</b> 2.0	55	1.48 $\pm$ 0.04	1.98 $\pm$ 0.07
<b>IBA</b> 4.0 + <b>NAA</b> 3.0	-	-	-
<b>IBA</b> 1.0 + <b>IAA</b> 0.5	55	2.30 $\pm$ 0.16	1.59 $\pm$ 0.12
<b>IBA</b> 1.0 + <b>IAA</b> 1.0	65	1.88 $\pm$ 0.09	1.81 $\pm$ 0.15



## Fig. I : *Phyllanthus urinaria* L.



**Figs A-D: Regeneration of plantlets *in vitro* from the nodal explants obtained from field grown *P. urinaria* L. plants. A. Development of shoot induction. B. Elongation of multiple shoots. C. Development of multiple shoots. D. Adventitious root formation on regenerated shoots.**

## References

- Ara, M. Jahan, A. and Hadiuzzaman, S. 1993 *In vitro* plant regeneration from leaf explant of *Solanum sisymbriifolium* Lamk in Bangladesh. *Intl. Plant Tissue Cult. Conf.* (Dhaka, 19 - 21 Dec.) p. 47.
- Bhatt, T., Jain, V., Jeyathirtha, M.G, Banerjee, G. and Mishra, S.H. 2006. *In vitro* regeneration of roots of *Phyla nodifolia* and *Leptadenia reticulata* and comparison of roots from cultured and mature plants secondary metabolites. *Indian J. EExp. Biol.* 40: 1382 – 1386.
- Calixto, J.B., Santos, A.R.S., Cechinel Filho, V. and Yunes, R.A. 1998. A review of the plants of the genus *Phyllanthus*: Their chemistry, pharmacology and therapeutic potential. *Medical Research Review* 4:225-258.
- Catapan, E. 1999. Cultivo *in vitro* e analises fitoquimicas de especies de *Phyllanthus*. Tese de Mestrado, Universidade Federal de Santa Catarina, Florianopolis.
- Cechinel Filho, V., Santos, A.R.S., Campos, R.O.P., Miguel, O.G., Yunes, R.A., Ferrari, F. , Messana, J. and Calixto, J.B. 1996. Chemical and pharmacological studies of *Phyllanthus caroliniensis* in mice. *Journal of Pharmaceutical Pharmacology* 48:1231-1236.
- Cheng, J.T, Lin, T.C and Hsu, F.L. 1995. Anti-hypertensive effect of corilagin in rat. *J. Physiol. Pharmac.* 73: 1425 – 1429.
- Gill, R.I.S, Gill S.S. and GOSAL, S.S. 1993. Vegetative propagation of *Eucalyptus tereticornis* Sm. through tissue culture. *Intl. Plant Tissue Cult. Conf.* (Dhaka, 19-21 Dec.), p. 44.
- Ilahi, I 1993. Micropropagation and biosynthesis of alkaloid by *Rauvolfia serpentina* cell culture. *Intl. Plant Tissue Cult. Conf.* (Dhaka, 19-21 Dec.) p. 21.
- Kalidass, C., Manickam, V.S., and Glory, M. 2008. *In vitro* studies on *Leptadenia reticulata* (Retz.) Wt. & Arn. (Asclepiadaceae). *Indian Jor. of Multidis. Res.* Vol. 4 (2): 221 -225.
- Kumar, S. Choudhary, M.L. and Raghava, S.P.S. 1995. *In vitro* manipulation of *Rosa damascena* Mill for oil contents. 2nd Intl. Plant Tissue Cult. Conf. (Dhaka, 10-12 Dec.), p. 47.
- Lin, T.C, Hsu, F.L and Cheng, J.T. 1993. Anti-hypertensive activity of corilagin and chebulinic acid, tannins from *Lumnitzera racemosa* . *J. Nat. Prod.* 56: 629 – 632.
- Liu, K.C, Lin, M.T, Lee, S.S, Chiou, J.F, Ren, S, and Lien, E.J. 1999. Antiviral tannins from two *Phyllanthus* species. *Planta Med.* 65: 43 – 46.
- Murashige, T. & Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15:473-497.
- Ogata, T., Higuchi,H., Mochida, S., Matsumoto,H., Kato, A., Endo, T., Kaji, A. and Kaji, H. 1992. HIV-1 reverse transcriptase inhibitor from *Phyllanthus niruri*. *AIDS Research Human Retroviruses* 8:1937-1944.
- Omran V.G., Bagheri A. and Moshtaghi N. 2008. Direct *in vitro* regeneration of Lentil (*Lens culinaris* Medik.), *Pakistan Journal of Biological Science*, 11 (18): 2237 – 2242.
- Rao, C.S., Eganathan, P., Anand, A., Balacrishna, P. and Reddy, T.P. 1998. Protocol for *in vitro* propagation of *Excoecaria agallocha* L., a medicinally important mangrove species. *Plant Cell Reports* 17:861-865.
- Santos, A.R.S., Cechinel Filho, V., Viana, A.M., Moreno, F.N., Campos, M.M., Yunes, R.A. and Calixto, J.B. 1994. Analgesic effects of callus culture extracts from selected species of *Phyllanthus* in mice. *Journal of Pharmaceutical Pharmacology* 46:755-759.
- Saradhi, P.P. and Islamia, J. M. 1997. *Phyllanthus fraternus* - A plant with anti-hepatitis viral activity. *Industrial Crop and Products* 6:35-40.
- Shed, A., Vickery, K., Pajkos, A., Medhurst, R., Freiman, J., Dixon, R. and Cossart, Y. 1992. Effects of *Phyllanthus* plant extracts on duck hepatitis B virus *in vitro* and *in vivo*. *Antiviral Research* 18:127-138.
- Thyagarajan, S.P., Subramanian, S., Thirunalasudary, T., Venkastewaran, P.S. and Blumberg, B.S. 1988. Preliminary study: the effect of *Phyllanthus amarus* on chronic carriers of hepatitis B virus. *Lancet* 2:764-766.
- Ueno, H., Horie, S., Nishi, Y., Shogawa, H., Kawasakim, M., Suzuki, S., Hayashi, T., Arisawa, M., Shimizu, M., Yoshizakim, M., Morita, N., Berganza, L.H., Ferro, E. and Basualdo, I. 1988. Chemical and pharmaceutical studies on medicinal plants in Paraguay. Geraniin, an angiotensin-converting enzyme inhibitor from “papaparaimi”, *Phyllanthus niruri*. *Journal of Natural Products* 51:357-359.

- Unander, D.W. 1996. *Phyllanthus* species: *in vitro* culture and production of secondary metabolites. In Biotechnology in agriculture and forestry (Y.P.S. Bajaj, ed.), v.37 Springer-Verlag, Berlin, p.304-318.
- Venkateswaran, P.S., Unander, D.W., Blumberg, B.S., Halbherr, T., Sharager, D., Dahl, L., Kraus, M., Rissinger, C. and Simmons, H.H. 1987. Potential antiviral agents for the treatment of hepatitis B virus and retroviruses. *Scientia Reports* 300-301.
- Yao Q.Q and Zuo C.X. 1993. Chemical studies on the constituents of *Phyllanthus urinaria*. *Acta Pharm. Sin.* 28: 829 – 835.
- Yoon S, Chung T.H and Chung C.C. 2000. Pharmaceutical composition for the treatment of Hepatitis B comprising corilagin or brevifolin. *Patent* WO0027413.

4/21/2009

## Effect of *Azotobacter* and Nitrogen on Seed Germination and Early Seedling Growth in Tomato

P. Mahato, Anoop Badoni\* and J. S. Chauhan

Seed Testing Laboratory  
Department of Seed Science and Technology  
H. N. B. Garhwal University, Srinagar- 246 174, Uttarakhand, India  
\*For correspondence: [annabadoni@yahoo.co.in](mailto:annabadoni@yahoo.co.in)

---

### Abstract:

The present study was carried out to evaluate the response of bio-fertilizer and inorganic fertilizer on germination and growth of tomato plant. Nitrogen (N) was used as inorganic fertilizer and *Azotobacter* was used as bio-fertilizer. Three treatments were used for investigation i.e. Control (T1- only soil), Soil + N (T2) and Soil + *Azotobacter* (T3). The germination was observed higher in T 3 treatment in contrast to other treatments. Generally plant height increased with the advancement of growth stages. The shoot length ( $35.5 \pm 0.8$ ), number of leaves ( $5.6 \pm 0.6$ ), length ( $7.8 \pm 0.8$ ) and width ( $7.5 \pm 0.8$ ) of leaves was reported higher in T 3 treatment followed by T 1 and T 2. The root length was measured after 30 days of sowing. T2 treatment showed higher root length ( $8.5 \pm 0.8$ ) in comparison to all other treatments. The conclusion of the present study is that *Azotobacter* as bio-fertilizer reported better than inorganic fertilizer in relation to seed germination and all plant growth parameters described. [Researcher. 2009;1(4):62-66]. (ISSN: 1553-9865).

### Introduction:

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and widely grown vegetable crop in the world, covering an area of 2.85 million hectares with corresponding production of 77.24 million tones, whereas in India it covers an area of 0.31 million hectares with production of 4.6 million tones (FAO, 1995). Tomato is being cultivated as an important summer season vegetable crop in low and mid hills of Uttarakhand. The crop is quite remunerative and the farmers are getting rich dividends by its cultivation. The fruits are rich source of vitamin A, B and C and minerals like calcium, iron, and phosphorus besides they have been reported quite useful in controlling liver problems, indigestion, arthritis and urinary troubles (Chouhan, 1983).

In the management of tomato crop the application of fertilizers have a major role for germination and growth. Although these fertilizers contribute a lot in fulfilling the nutrient requirement of vegetable crops but their regular, excessive and unbalanced use may lead to health and ecological hazards, depletion of physiochemical properties of the soil and ultimately poor crop yields. The problems of nutrient drain from the soil are becoming so acute that it is beyond the capacity of any single fertilizer to accept the challenge of appropriate nutrient supply. Hence there is a need to think of alternate sources of safe fertilized which may enhance crop yields without having adverse effects on soil properties, the use of bio-fertilizers seen to be a hope in this direction.

Bio-fertilizers are the carrier-based preparations containing mainly effective strains of microorganisms in sufficient number, which are useful for nitrogen fixation. If they are used in association with macronutrients the expected yields per unit area may be much higher. Amongst these nutrients, nitrogen is the only nutrient, which play major role in synthesis of chlorophyll, amino acids and protein building blocks, which is ultimately responsible for higher source to sink ratio. Amongst bio-fertilizers *azotobacter* strains play a key role in harnessing the atmospheric nitrogen through its fixation in the roots. They have been also reported to improve fertility condition of the soil. Therefore, the present investigation was based on the objective, to find out the effect of *azotobacter* and nitrogen on germination and growth of tomato.

### Material and Methods:

Present study was done using following material and method:

**Treatments:**

T-1: Control (only soil),

T-2: Soils + Urea (Inorganic fertilizer)

T-3: Soils + *Azotobacter* (Bio-fertilizer; Population density  $>2 \times 10^9$  (c.f.u./gram), Microbial adjuvant 2%, Microbial media residue inert ingredient 95-97%)

Total number of germinated seeds were counted in all the treatments, at the interval period of five days after sowing and recorded as emergence count / poly bag. For growth study, height of ten randomly selected plants from each treatment was measured with a meter scale from the ground level to the tip of the spike, (shoot length) and mean height was calculated from each treatment. The total number of leaves from ten randomly selected plants from each treatment was counted after twenty days of germination. Length and width of leaves of ten randomly selected plants from each treatment were measured with a meter scale from the end of petiole to the apex of the leaf. Width of the leaves was measured by measuring leaf margins. Root lengths of selected plants from each treatment were measured with a meter scale.

**Result:**

*Germination Counts:* The germination percentage was influenced by different treatments (Table-1; Plate-1). Result showed that the maximum number of seedling emergence was reported in T3 treatment (90%), which contains bio-fertilizer (*Azotobacter*), in contrast to followed by T2 (80%) and T1 (60%).

*Growth Study:*

The shoot length ( $35.5 \pm 0.8$ ), number of leaves ( $5.6 \pm 0.6$ ), length ( $7.8 \pm 0.8$ ) and width ( $7.5 \pm 0.8$ ) of leaves was reported higher in T 3 treatment followed by T 1 and T 2. The root length was measured after 30 days of sowing. T2 treatment showed higher root length ( $8.5 \pm 0.8$ ) in comparison to all other treatments (Table-1; Plate-2).

**Discussion:**

The experiment on the plant tomato (*Lycopersicon esculentum* Mill) is done in natural environmental condition to evaluate the advantage of bio-fertilizer upon the inorganic fertilizer. After getting the results of the experiments it is clear that the bio-fertilizer shows better results than the inorganic fertilizer. Since Green Revolution the inorganic fertilizers are used in large amount to increase the yield of the crops. In all the agriculture sectors of India the use of these fertilizers by farmers is increasing day by day to increase the yield and economy. Using inorganic fertilizers farmers can increase the yield of crops but the soil pollution is also increased with this day by day. The use of inorganic fertilizers is increased 6-8 times from the time of green revolution. These fertilizers not only affect the soil but also influence the characteristics and the product of the crop. Fertility of the soil increases due to the continuous use of the fertilizers but it also reduces the crop productivity. The main reason of reduction in crop productivity is due to soil pollution. Soil pollution is caused due to the use of inorganic fertilizers, pesticides, and other chemicals etc (Badoni, 2006).

*Azotobacter* has long been used in Russia to inoculate seeds or roots of crop plants and increase in yields have been reported from this practice (Mishustin and Naumova, 1962). Jackson *et al.* (1964) found accelerated growth of tomato stem with inoculation of *Azotobacters*. Mishutin (1966) demonstrated that bacterial fertilizers slightly improved yield of a wide range of crop plants, especially vegetable. The yield increases have been reported up to 28.56, 18.25, 19.33 and 55 per cent in case of tomato, potato, cabbage and cucumber respectively. Mehrotra and Lehri (1971) while working at kanpur observed that successful proliferation of *Azotobacter* can be achieved in association with synthetic fertilizers and yield increases up to 50 per cent in cabbage and 62 per cent in brinjal were obtained by the application of *Azotobacter*, however they observed that these increases extremely depend upon the fertility status of the soil and the type of strain used. Vanisht *et al.* (1979) from their experiment conducted t higher germination (69-70%)



compared with 43 per cent in the control. El-Shanshoury *et al.* (1989) conducted experiment in Egypt on tomato in a sandy soil having low content of available nitrogen and phosphorus. The soil was inoculated with *Azotobacter* which resulted in better plants growth and higher nitrogen content in shoots as compared to uninoculated soil. Pandey and kumar (1989) concluded from their experiments at New Delhi that inoculation of *Azotobacter* to without application of nitrogen, phosphorus and potassium had increased the yield per unit area. Martinez *et al.* (1993) in this study carried out at La-Habana, Cuba, reported that soil inoculation with *Azotobacter* increased tomato seed germination by 33-46 per cent, shortened the period between sowing and transplanting by 5-7 days, increased the yield by 38-60 per cent.

Kuksal *et al.* (1977) from their studies on tomato in UP Hills concluded that plants height, fruit and sees yield increased with increasing levels of nitrogen from 60 to 120 kg N ha<sup>-1</sup>. Similarly, Randhawa *et al.* (1977) from Ludhiana reported that maximum plant height of tomato was obtained optimum seed quality from tomato plants which received N @ 120 kg ha<sup>-1</sup> in Russia. On the other hand, Rastogi *et al.* (1978) reported from Solan that 60kg N ha<sup>-1</sup> gave the highest yield of tomato var. Solan Gola. Seth and Choudhry (1978) observed that 90 kg N ha<sup>-1</sup> gave the highest fruit and seed yield. In trails at Moldavian, Russia, Nesterova and Butkevich (1980) obtained highest tomato seed yields when N was applied @ 120 kgha<sup>-1</sup>. Kooner and Randhawa (1983) reported from Ludhianaa that tomato plant growth. Fruit and seed yield increased with rising nitrogen rates and the maximum values were obtained at 200 kg N ha<sup>-1</sup>. Vadivelu (1983) reported from coimbtore that highest seed yields in tomato were obtained with the application of nitrogen @ 100 kg ha<sup>-1</sup>. Olasantan (1991) reported from Ila-Orangun, Nigeria that tomato fruit yield increased with every ascending level of nitrogen and the highest value was obtained at 60 kg N ha<sup>-1</sup>. Eryuce *et al.* (1992) reported from their experiment conducted at Lisbon, Portugal that 1000 seed weight of field grown tomato cv. Rio Grande increased at highest nitrogen level, where as seed germination and seed vigour reduced at this level. Singh and singh (1992) from their studies carried out at Faizabad, concluded that plant height and number of branches per plant increased significantly and maximum values were obtained at 125 kg N ha<sup>-1</sup> in tomato cv. Pusa Ruby. They also reported tht fruits per plant and marketable fruit yield (q ha<sup>-1</sup>) increased in linear fashion with increasing nitrogen levels. Arora *et al.* (1995) reported from Hisar that nitrogen application @ 120 kg N ha<sup>-1</sup> increased seed content of fruits and seed yield per plant and per hectare in tomato cv. Hisar Arun.

In the present study application of bio-fertilizer resulted increase of shoot length and more number of leaves per plant. Similar observations were observed by Martinez *et al* (1993) in case of tomato. Bio-fertilizer application significantly increased the nitrogen uptake in tomato at growth stage. This may be because of better nitrogen fixation as result of accelerated bacterial activity and better root system which might have resulted in more nitrogen accumulation in tomato shoots. Mohandas (1987) and EL-Shanshoury *et al.* (1989) while working with *Azotobacter* in tomato have also obtained similar results. From the results of the experiment it is clear that bio-fertilizer shows better results as compare to that of the inorganic fertilizers. The main advantage of bio-fertilizer is that it does not pollute the soil and also does not show any negative effect to environment and human health.

**Table-1 Germination and Growth observation in Tomato plant using inorganic and organic fertilizers:**

Treatment length	Germination%	Shoot height	No. of leaves per plant	Length of leaves	Width of leaves	Root
T1	60	20.4 ±0.5	3.4 ±0.7	4.0 ±0.7	4.2 ±0.5	7.2 ±0.7
T2	80	29.5 ±0.5	5.4 ±0.6	6.6 ±0.8	5.7 ±0.5	8.5 ±0.8
T3	90	35.5 ±0.8	5.6 ±0.6	7.8 ±0.8	7.5 ±0.8	8.2 ±0.8





*Plate-1 Plant growth after germination on field*



*Plate-2 Comparison of root length between T1, T2 & T3 Treatments*

#### References:

- Arora, SK; Singh, I; Pandita, ML and Banerjee, NK. (1995). Response of tomato to nitrogen fertilization and plant geometry for seed yield. Haryana Journal of Horticultural Science. 24: 49-54.
- Azcon, R and Barea, JM. (1975). Synthesis of auxins, gibberellins and cytokinins by *Azotobacter beijerinckii* related to effects produced on tomato plants. Plants and soil. 43: 609-619.
- Badoni, Anoop (2006). Effect of inorganic fertilizers and organic manure on seed germination and seedling growth of *Solanum melongena* L. under environmental condition. M. Sc. Thesis, Department of Seed Science and Technology, H. N. B. Garhwal University, Srinagar.
- El-Shanshoury, AR; Hassan, MA and Abdal Gaffar, BA. (1989). Synergistic effect of vesicular-arbuscular-mycorrhizas and *Azotobacter* on the growth and the nutrient contents of tomato plants. Phyton Horn. 29: 203-212 (Hort. Abst. 61: 358).
- Grigoryan, GA.(1978). Changes in tomato seed quality in relation to varietal characteristics and fertilization. Biologicheskii Zhurnal Armenii. 30: 21-25 (Hort. Abstr. 48: 9928).
- Hedge, DM and Srinivas, K. (1989). Studies on irrigation and nitrogen requirement of tomato. Indian journal of Agronomy. 34: 157-162.

- Jackson, RM; Brown, ME and Burlingham, SK. (1964). Similar effects on tomato Plants of *Azotobacter* inoculation and application of gibberellins. *Nature*. 192:575.
- Kuksal, PP; Shyam , RD and Yadav, JP. (1977). Effect of different levels of nitrogen and phosphorus on fruit and seed yield of tomato variety Chaubattia Red. *Progressive Horticulture*. 9: 13-20.
- Martinez, R.; Dibut B and Gonzalez , R. (1993). Stimulation of tomato development and yield by inoculation of red ferrallitic with *Azotobacter chroococcum*.
- Mehrotra, CL and Lehri, LK. (1971). Effect of *Azotobacter* inoculation on crop yields. *Jurnal of the Indian Society of Soil Science*. 19: 243-248.
- Mishustin, EN and Naumora, AN. (1962). Bacterial fertilizers their effectiveness and mechanism of action. *Microbiologiya*. 31: 543-555.
- Mohandas, S. (1998). Field response of tomato (*Lycopersicon esculentum* Mill. 'Pusa Ruby') to inoculation with a VA mycorrhizal fungus *Glomus fasciculatum* and with *Azotobacter vinelandii*. *Plant and soil*. 98: 295-297.
- Nesterova, RF and Butkevich, TB .(1980) . Yeild and quality of tomato seeds in relation to mineral nutrition. *Semenovod ovoshuh. Kul'tur Kishinev Moldavian SSR*: 43-48 (Hort. Abst. 51:6279).
- Oampo, JA and Barea, JM. (1978) . Effects of microbial fertilizers on flowering of tomato in the absence of established inocula in the rhizosphere. *Anales de Edafologia Y Agrobiologia*. 37: 315-325 (Hort. Abst. 50: 1104).
- Olasantan, FD. (1991). Response of tomato and okra to nitrogen fertilizers in sole cropping and inter cropping with cowpea. *Journal of Horticultural Science*. 66: 191-199.
- Randhawa, KS; Nandpuri, KS and Singh, D. (1977). The effect of N, P and K fertilization on the growth and yield of tomato cultivar. *Vegetable Science*. 4: 61-65.
- Seth, JN and Chaudhry, B. (1978). Effect of fertilizer and spacing on the fruit and seed yield in tomato. *Progressive Horticulture*. 2: 5-14.
- Singh, AB and Singh, SS. (1992). Effect of various levels of nitrogen and spacing on groeth, yield and quality of tomato. *Vegetable Science*. 19: 1-6.

3/16/2009

## The impact of genetic variability and smoking habits on the prevalence of periodontitis among adults

Faten S. Bayoumi\*, Fathya. M. Metwaly\*\*, Goma M.Hind\*\* and E. H. A. Abouel-Ezz...\*\*\*

• Assistant professor in immune-genetics Department, National Research Center

\*\*Professor in Environmental & Occupational Medicine Department, National Research Center

\*\*Assistant Professor in Environmental & Occupational Medicine Department, National Research Center

\*\*\*Professor in genetic orodental Dep. National Research Center

[fatenbayoumi@yahoo.co.uk](mailto:fatenbayoumi@yahoo.co.uk)

### Abstract

**Aim:** Elucidate the effect of genetic variance of inflammatory mediators expression, the influence of microbial expression, and smoking as a risk factors for periodontitis. **Material & Methods:** Sample of this study composed of 50 smokers & 50 non smoker volunteers (unrelated and of the same ethnic population) with 40-60 years old. Their periodontal status was estimated through periodontal examination (full mouth clinical attachment loss measurement, probing depths, plaque index scores, and bleeding on probing). Isolation and detection of certain oral pathogens; *A.actinomycescomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* was performed. Genotype for bi-allelic IL-1A+4845, IL-1B+3954 gene polymorphisms using mouth wash was detected by PCR based methods. **Results:** There were a significant difference only between the two groups (smokers & non-smokers) as regards to colonization of *A.actinomycescomitans* & not among *Porphyromonas gingivalis* & *Prevotella spp.* There were no significant difference between the overall frequencies of carrying allele 2 of IL-1 A, IL-1B among smoker and non-smokers. The percentage of non smokers having healthy periodontal status was much higher than smokers and the difference was significant. On the other hand, smokers recorded much higher percentage for mild, moderate and severe periodontitis. The difference was statistically significant concerning the percentage of those with severe periodontitis. **Conclusion:** Environmental factors play either a direct (i.e., causative factor) or indirect (modifying factor) role as a risk factor for periodontitis. The association between genetic polymorphism of allele 2 of IL-1 A, IL-1B expression & smoking habits caused a synergistic effect for progression of periodontitis. Smoking initiated *A.actinomycescomitans* growth. [Researcher. 2009;1(4):67-73]. (ISSN: 1553-9865).

**Key words:** genetic polymorphism, periodontitis, Interleukin -1, periodontal pathogens, smoking

### **\*\* Abbreviations:**

*A.actinomycescomitans* = *A.actino.*

CAL = Clinical Attachment loss

Interleukin -1 = IL-1

*Porphyromonas gingivalis* = *P. Gingivalis*

*Prevotella intermedia* = *P. intermedia.*

### **Introduction:**

The oral cavity is vulnerable to external agents as cigarette smoking exposure which causes oral changes in both hard and soft tissues *Susin, et al., (2004).*

Periodontitis is a chronic inflammatory disease initiated by specific bacteria that activate host mechanisms destroying bone and connective tissues which support the teeth. Substantial data supported the current concept that specific bacteria are essential for initiation and progression of chronic periodontitis (*Page et al., 1997*), but the rate of progression and disease severity are determined by host modifiers such as smoking (*Bergstrom, 1989*), diabetes, (*Collin et al., 1998*) and genetic influences. (*Kornman, 2006*)

Smoking has major effects on the host response, but there are also a number of studies recorded some microbiological differences between smokers and non-smokers (*Gomes, et al., 2006*).

The pro-inflammatory cytokine interleukin-1 (IL-1) is a key regulator of the host responses to microbial infection and a major modulator of extracellular matrix catabolism and bone desorption. It has been reported that variations in the IL-1 gene cluster on chromosome 2 are associated with increased susceptibility to severe periodontitis (*Mc Devitt, et al., 2002*). Therefore, a genetic test was being marketed to predict risk for periodontal disease progression (*Higashi, 2002*).

The aim of this study Elucidate the effect of genetic variability including the variance of inflammatory mediators expression ,the influence of microbial expression, and smoking as effects influencing risk for periodontitis.

### **Materials and Methods:**

#### **Subjects:**

Sample of this study composed of 50 smokers & 50 non smokers volunteers (unrelated and of the same ethnic population) with 40-60 years old. Both groups were interviewed and filled a detailed questionnaires for family history, dental, medical as well as smoking habits,. Cigarette consumption was calculated (i.e. mean numbers of packs/day× number of years smoked)

#### **Methods:**

##### **1-Periodontal Examination:**

Criteria for assessment of the severity of periodontitis:

- 1- Mild periodontitis: Mean CAL  $\geq 0.6$  mm to 1.5 mm, no. interproximal sites with CAL  $\geq 3$ mm. No more than 3 missing teeth with the exception of orthodontic purpose, teeth lost as a result of extra oral trauma or extensive decay ,or teeth that were congenitally missing.
- 2- Moderate periodontitis: Mean CAL  $\geq 1.6$  mm to 2.4 mm and  $\leq 8$ mm, interproximal sites with CAL  $\geq 3$ mm distributed through at least 3 quadrants or at 6 teeth. No more than 5 missing teeth with the exception of third molars, teeth extracted for orthodontic purpose, teeth lost as a result of extra oral trauma or extensive decay ,or teeth that were congenitally missing
- 3- Severe periodontitis: Mean CAL  $\geq 2.5$  mm and 1 or more sites in 3 out of 4 quadrants with interproximal sites with CAL  $\geq 5$ mm. No more than 14 missing teeth with the exception of third molars, teeth extracted for orthodontic purpose, teeth lost as a result of extra oral trauma or extensive decay ,or teeth that were congenitally missing

##### **2-Microbiological Examination**

**Sampling :** Paper-point samples were taken from the 4 deepest sub-gingival sites in each quadrant of the dentition (*Mombelli et al., 1991, 1994*). Samples were then placed in 0.9 ml of sterile pre-reduced anaerobically transport fluid(RTF) (*Loesche et al., 1972*) and transferred to the laboratory within 10 minutes.

**Culturing:** The samples were dispersed for 6 sec. with a vortex mixer and 10 fold serially diluted in RTF. Aliquots of 0.1 ml of appropriate dilutions were placed in duplicate onto specific media for different microorganisms in concern.

Samples were grown anaerobically (80% N<sub>2</sub>, 10% H<sub>2</sub>, 10% CO<sub>2</sub>) at 37°C on 5% horse blood agar plates (Oxoid no. 2, Basingstoke, England) enriched with hemin (5 mg/L) and menadione (1 mg/L) for detection of *Porphyromonas gingivalis* and on Trypticase soyserum bacitracin-vancomycin (TSBV) medium in air with 5%CO<sub>2</sub> at 37°C for the selective isolation of *A.actinomycescomitans*. KVLB-2 (kanamycin 75µ g/ml–Vancomycin 2 µ g/ml laked blood agar) for isolation of pigmented and non pigmented *Prevotella spp.*

**Identification:** *Porphyromonas gingivalis* was identified on the basis of Gram stain, anaerobic growth, and the inability to ferment glucose, the production of indole, and a positive hemagglutination test with 3% sheep erythrocytes.

*A.actinomycescomitans* was identified on TSBV plates, based on typical colony morphology and positive catalase reaction. The percentage of the microorganisms of total colony-forming units (CFU) was counted on blood agar plates.

##### **3- PCR based methods:**

###### **a-DNA Isolation from Mouthwash**

DNA from all subjects was isolated according to the method of *de Vries et al. (1996)* as modified and validated for the study of cytokine gene polymorphisms (*Laine et al., 2000*). In short, each individual rinsed out his/her mouth with 10 mL of 0.9% saline for 60 sec. Buccal epithelial cells were centrifuged at 300 x g for 10 min. The pellet was washed twice in 0.9% saline, re-suspended in 100 µL of 50 mM NaOH,

and boiled for 10 min. Samples were neutralized with 14 µL of 1 M Tris (pH 7.5) and centrifuged at 14,000 x g for 3 min. Supernatants were collected and stored at 4°C until analysis.

**b-- Analysis of Polymorphisms in Genes of the IL- 1 A &B**

The bi-allelic polymorphisms at position -889 within the promoter region of the IL-1A gene (McDowell et al., 1995) and at position +3954 (Taq I RFLP) within exon 5 of the IL-1B gene (Bioque et al., 1995), were determined according to previously described methods.

**Results :**

The distribution of isolated microorganisms among examined groups was illustrated in table (1). *Porphyromonas gingivalis* , *Prevotella spp* and *A.actinomycescomitans* colonized (24% ,16%, and 30% of the smokers and 12% , 8% and 6% of non- smokers respectively. There were significant difference between the two groups (smokers & non-smokers) as regards to colonization of Gram (-ve) facultative rods (*A. actinomycescomitans*)& no significant difference between the other two organisms.

The percentage of smokers & non smokers carried allele 2 of IL-1A was more than those carried IL-1B (30%,28%& 10%,0%) respectively, table (2) . The difference was statistically non- significant. All individuals were heterozygous, except 4 were homozygous of whom carried Allele 2 of IL-1A(+4845) polymorphism .

Smokers and non- smoker individuals carried allele 2 of IL-1A and IL-1 B were further divided according to the severity of the periodontitis . The percentage of non smokers having healthy periodontal status (47%) was much higher compared to smokers and the difference was significant. On the other hand, smokers & non smokers recorded nearly a similar percentage among those complained from mild, moderate (15%, 20% & 11.8%,11.8%)with no statistical significant difference .Whereas, statistically significant difference was noted concerning the percentage of individuals in both groups complained from severe periodontitis (65%&29.4% respectively), table (3).

**TABLES**

**Table (1): Percentage of bacterial species in plaque samples of smoker and non smoker individuals examined**

<i>Bacterial species</i>	<i>% of Non Smoker individuals</i>	<i>% of smokers individuals</i>	<i>Z.score</i>	<i>P</i>
<b>Gram (-) facultative rods</b>	6%	30%		
<i>A. actinomycescomitans</i>	(3/50)	(15/50)*	1.96*	0.02
<b>Gram (-) anaerobes</b>	12%	24%	1.3	0.09
<i>Porphyromonas gingivalis</i>	(6/50)	(12/50)		
<b>Gram (-)anaerobes</b>	8%	16%	0.92	0.17
<i>Prevotella intermedia</i>	(4/50)	(8/50)		

**Table (2): Distribution of composite IL-1 genotype of allele 2 carriage of IL-1A(+4845) & IL-1B (+3953)among samples of smokers and non smokers**

<i>Studied samples</i>	<i>IL-1 genotype of allele 2 carriage of IL-1A(+4845) &amp;IL-1B(+3953)</i>		
	<b>IL-1A</b>	<b>IL-1B</b>	<b>Total carriers of allele 2</b>
<b>Non smokers</b>	14/50(28%)	3/50(6%)	17/50(34%)
<b>Smokers</b>	15/50 (30)	5/50(10%)	20/50(40%)
<b>Total studied</b>	29/100 (29%)	8/100(8%)	37/100(37%)

P value ;0.7



**Table(3): Distribution of allele 2 frequency of IL-1A(+4845) & IL-1B (+3953) composite genotype among studied individuals in relation to severity of periodontitis**

Periodontal status	Carriers of of allele 2 of IL-1A & IL-1 of allele			
	Non smokers (n=17)	Smokers N=20)	Z score	P.value
<b>Healthy</b>	(8/17) 47%*	0/20(0%)	3.06	0.001
<b>Mild</b>	(2/17) 11.8%	15%(3/20)	0.2	0.42
<b>Moderate</b>	(2/17) 11.8%	20%(4/20)	0.23	0.4
<b>Severe</b>	(5/17) 29.4%	65%(13/20)*	1.82	0.03

**Discussion:**

Periodontitis is a multifactorial chronic inflammatory disease. However, it is difficult to ascertain the role of the different factors involved in its pathogenesis. Cigarette smoking is associated with increased prevalence and severity of destructive periodontitis in terms of periodontal pocketing, periodontal bone loss, and tooth loss (Gomes,2006).

Microbiological diagnosis, focused on a number of microbial species e.g., *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*). These microorganisms were have been proposed to be a useful tool for the identification of susceptible individuals (Slots and Listgarten, 1988, Maiden et al., 1990 and Haffajee et al., 1994). Substantial data support the current concept that specific bacteria are essential for initiation and progression of chronic periodontitis (Page et al., 1997), but the rate of progression and disease severity are determined by host modifiers such as smoking, diabetes, and genetic influences.

In the present study approximately half of the smokers and third of the non smokers harbor these three microorganisms in their oral cavity. There were significant difference between the two groups (smokers & non-smokers) as regards to colonization of Gram (-ve) facultative rods (*A. actinomycetemcomitans*) & no significant difference between the other two organisms.

Genes who encode inflammatory cytokines are subject to polymorphisms in their regulatory regions that may affect both the level and ratio of cytokines produced in response to exogenous stimuli. These variant alleles are observed in a large percent of the population and are often associated with increased or decreased susceptibility or severity (modifiers) to infectious, immune or inflammatory diseases (Yucesoy et al., 2003).

Axelsson (2002) reported that two factors, smoking and IL-1 genotype, significantly increased the risk of progression of alveolar bone loss and tooth loss due to progressive periodontitis. Moreover, the effect was synergistic: 41% of the IL-1 genotype-positive smokers lost 2 teeth, compared with roughly 11% of those who had only one of the risk factors.

Our results correlated the severity of periodontitis to presence of carriers of allele 2 genotype in the IL-1A and IL-1B genes. A data agreed with Kornman et al., (1997) who reported the same correlation and explained this finding as genetic mechanism by which some individuals, if challenged by bacterial accumulations, may have more vigorous immune-inflammatory response leading to more severe periodontitis. Moreover, Kornman (2006) added that monocytes from individuals homozygous for the IL-1 B +3953 allele 2 produce four-fold more IL-1 β and heterozygous cells produce approximately two-folds more IL-1 β from individuals homozygous for allele 1.

Our results showed that the nature of the host response is determined primarily by genetic factors, environmental and acquired factors (smoking).

The complex interactions that occur between host-response mechanisms and oral pathogens in periodontal disease have made elucidation of genetic factors in disease susceptibility more difficult (Hassell et al., 1995).

**Conclusion :** Environmental factors play either a direct (i.e., causative factor) or indirect (modifying factor) role as a risk factor for periodontitis. The association between genetic polymorphism of allele 2 of IL-1 A, IL-1B expression & smoking habits caused a synergistic effect for progression of periodontitis. Smoking initiated *A. actinomycetemcomitans* growth.



**Authors:**

- **Faten S.Bayoumi:** Immuno-genetics Department, National Research Centre. Cairo Egypt
- **Fatehya.M.Metwaly:** Environmental and Occupational Medicine Department, National Research Centre, Cairo Egypt
- **Hind M.Rashd:** Environmental and Occupational Medicine Department, National Research Centre, Cairo Egypt
- **Eman H.Aboulezz:** Orodental dep. National Research Centre, Cairo Egypt

**\*\* Name of the corresponding author:**

- **Faten S.Bayoumi:**

Assistant professor of microbiology  
Immuno-genetics Department, National Research Centre, Cairo Egypt  
National Research Center

**Address :** Tahrir Street, 12622 Dokki. Giza Egypt

**Tel:** +202-33377164

**Fax:** +202-33370931 & +202-37601877

**Mobile:** +202- 0119051606

**Email:** [fatenbayoumi@yahoo.co.uk](mailto:fatenbayoumi@yahoo.co.uk)

**References:**

**Pociot F., Molvig J., Wogensen L., Worsaae H., and Nerup J (1992):**

A TaqI polymorphism in the human interleukin -1 beta gene correlates with secretion in vitro. European Journal of Clinical Investigation 22,396.

**Axelsson P. (2002):**

Diagnosis and risk prediction of periodontal diseases. Vol 3. Chicago, IL: Quintessence Publishing Co.,:146-63.

**Hassell TM, Harris EL (1995):**

Genetic influences in caries and periodontal diseases. Crit Rev Oral Biol Med 6:319-342.

**Gomes SC, Piccinin FB, Oppermann RV, Susin C, Nonnenmacher CI, Mutters R, Marcantonio RA. 2006**

Periodontal status in smokers and never-smokers: clinical findings and real-time polymerase chain reaction quantification of putative periodontal pathogens Sep;77(9):1483-90

**Slots J, Listgarten MA (1988):**

*Bacteroides gingivalis*, *Bacteroides intermedius* and *Actinobacillus actinomycetemcomitans* in human periodontal diseases. /Clin Periodontol 15:85-93.

**McDowell TL, Symons JA, Ploski R, F0rre 0, Duff GW (1995).**

Agnetic association between juvenile rheumatoid arthritis and a novel interleukin-1 alpha polymorphism. Arthritis RheumJ.38:221-228

**Bioque G, Crusius JBA, Koutroubakis I, Bouma G, Kostense PJ, Meuwissen SGM, et al. (1995):**

Allelic polymorphism in IL-13 and IL-1 receptor antagonist (IL-1RA) genes in inflammatory bowel disease. *Clin Exp Immunol* 102:379-383.

**Maiden MFJ, Carman RJ, Curtis MA, Gillett IR, Griffiths GS, Sterne JAC, et al. (1990).** Detection of high-risk groups and individuals for periodontal diseases: laboratory markers based on the microbiological analysis of subgingival plaque. *Clin Periodontol* 17:1-13.

**Katz, R.S., Premenko-Lanier M., McChesney M.B., Rota P.A. and Bellini W.J. (2002):** Detection of measles virus RNA in whole blood stored on filter paper. *J. Med. Virol.* 67:596-602

**Higashi K.M. (2002):** The Cost-Effectiveness of Interleukin-1 Genetic Testing for Periodontal Disease. *Journal of periodontology*. *J Periodontol*; 73:1474-1484.

**McDevitt M. J., Wang H., Knobelmann C., Michael G., Francesco N., di Giovine S., Timms J., Duff G.W., Kornman K.S. (2002):** Interleukin-1 Genetic Association With Periodontitis in Clinical Practice. *J Periodontol* 2000; 71:156-163

**Susin C, Vecchia CFD, Oppermann RV, Haugejorden O, Albandar JM. (2004):** Periodontal attachment loss in an urban population of Brazilian adults: effect of demographic, behavioral, and environmental risk indicators. *J Periodontol*; 75:1033-41

**Bergstrom J (1989):** Cigarette smoking as risk factor in chronic periodontal disease. *Community Dent Oral Epidemiol* 17:245-247

**Kornman S. (2006):** Interleukin 1 genetics, inflammatory mechanisms, and nutrigenetic opportunities to modulate diseases of aging. *Am J Clin Nutr*; 83(suppl):475S- 83S

**Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. 1997:** Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. *Periodontol* 2000 ; 14:216-48.

**Lange DE, 1986:** Frequency, seriousness and treatment needs in periodontal diseases. *ZWR*; 95(4):402-6.

**Mombelli A, McNabb H, Lang NP (1991). :** Black-pigmenting Gram-negative bacteria in periodontal disease. II. Screening strategies for detection of *P. gingivalis*. *J Periodontol Res* 26:308-313.

**Mombelli A, Gmuir R, Gobbi C, Lang NP (1994). :** *Actinobacillus actinomycetemcomitans* in adult periodontitis. I. Topographic distribution before and after treatment. *J Periodontol* 65:820-826.

**Collin HL, Uusitupa M, Niskanen L, Kontturi-Narhi V, Markkanen H, Koivisto AM, et al. (1998).** Periodontal findings in elderly patients with non-insulin dependent diabetes mellitus. *J Periodontol* 69:962-966.

**de Vries HG, Collee JM, van Veldhuizen MH, Achterhof L, Smit Sibinga CT, Scheffer H, et al. (1996).** Validation of the determination of deltaF508 mutations of the cystic fibrosis gene in over 11 000 mouthwashes. *Hum Genet* 97:334-336.

**Laine ML, Farre MA, Crusius JBA, van Winkelhoff A-J, Pefia AS, (2000):**

The mouthwash: a non-invasive sampling method to study cytokine gene polymorphisms. J Periodontol 71:13 15-1318.

**Albandar JM, Streckfus CF, Adesanya MR, Winn DM.(2000):**

Cigar, pipe, and cigarette smoking as risk factors for periodontal disease and tooth loss  
J. Periodontol. 2000 Dec;71(12):1874-81

2009/24/4

## Investigating the Optimum Operating Conditions of Some Process Parameters during Leaching of Iron Oxide Ore in Sulphuric Acid Solution

Chukwuka Ikechukwu Nwoye\*<sup>1</sup>, Chinedu Chris Nwakwuo<sup>2</sup> and Okechukwu Onyebuchi Onyemaobi<sup>1</sup>

<sup>1</sup>Department of Materials and Metallurgical Engineering, Federal University of Technology, Owerri, Nigeria.

<sup>2</sup>Department of Material Science, Oxford University, United Kingdom.

[chikeyn@yahoo.com](mailto:chikeyn@yahoo.com)

### Abstract

Studies have been carried out to determine the optimum operating conditions of some process parameters during leaching of iron oxide ore in sulphuric acid solution. Results of the investigations show that the optimum leaching temperature, grain size, and initial pH are 55°C, 0.1mm and 6.8 respectively. The optimum initial solution temperature (just before commencement of the leaching process) required for maximum dissolution of Fe was also found to be 28°C. Concentration of dissolved Fe was found to decrease progressively with increase in the weight-input of iron oxide ore due to continuously increased iron ore weight-input - fixed hydrogen ions concentration relationship. [Researcher. 2009;1(4):74-82]. (ISSN: 1553-9865).

**Keywords: Optimum Conditions, Process Parameter, Leaching, Iron Oxide Ore, Sulphuric Acid.**

### 1. Introduction

Studies [1,2] have shown that at a temperature as low as 25°C, the presence of Fe<sup>2+</sup> significantly enhances the leaching of iron extraction from silica sand. Air quickly oxidizes ferrous oxalate during dissolution, giving room for an induction period of a few hours unless a strong acidic environment (<pH 1) or an inert atmosphere is maintained. It has been found [3] that maintaining the high level of ferrous oxalate in the leach liquor using an inert gas enhance the reaction kinetics. It is believed that during this process, removal of phosphorus from the iron compound and subsequent dissolution of the phosphorus oxide formed were effected.

The optimum pH for dissolving iron oxide has been reported [4] to be is pH 2.5 – 3.0. The solution pH governs the distribution of various oxalate ions in the leach system. Below pH 1.5, oxalic acid exists mainly as H<sub>2</sub>C<sub>2</sub>O<sub>4</sub>, whereas HC<sub>2</sub>O<sub>4</sub> is the most predominant species at pH 2.5 – 3.0.

It has been found [5,6] that the final pH of leaching solution depend on the leaching time, initial pH for the leaching solution and the leaching temperature.

Models for computational analysis of the concentration of dissolved haematite and heat absorbed by oxalic acid solution during leaching of iron oxide ore have been derived [7]. These models are:

$$\%Fe_2O_3 = K (\gamma/\mu) \quad (1)$$

$$Q = K_C \mu \quad (2)$$

Where

%Fe<sub>2</sub>O<sub>3</sub> = Concentration of dissolved haematite in oxalic acid solution.

γ = Final pH of the leaching solution at time t at which %Fe<sub>2</sub>O<sub>3</sub> was obtained.

μ = Weight of iron oxide added into the oxalic acid leaching solution (g)

K = Constant of proportionality associated with haematite dissolution

K<sub>C</sub> = Constant of proportionality associated with heat absorption

Q = Quantity of heat absorbed by oxalic acid solution during the leaching process (J)

Nwoye [7] found that optimization of the weight input of iron oxide ore could be achieved using the model; (%Fe<sub>2</sub>O<sub>3</sub> = K (γ/μ) ) by comparing the concentrations of dissolved haematite at different weights input of the iron oxide ore, with the view to identifying the optimum weight input of iron oxide ore that gives the maximum dissolution of Fe<sub>2</sub>O<sub>3</sub>. The model also indicates that the concentration of haematite dissolved during the leaching process is directly proportional to the final pH of the leaching solution and inversely proportional to the weight input of the iron oxide ore.

It was also found [7] that values of Q obtained from both the experiment and model (Q = K<sub>C</sub> μ) agree to the fact that leaching of iron oxide ore using oxalic acid solution is an endothermic process, hence the absorbed positive heat energy by the leaching solution. The quantity of heat energy absorbed by the oxalic acid solution during the leaching process (as calculated from the model; Q = K<sub>C</sub> μ) was found to be directly proportional to the weight input of the iron oxide ore. These results were obtained at initial pH 6.9, average

grain size of 150 $\mu$ m and leaching temperature of 30 $^{\circ}$ C. The constants of proportionality K and K<sub>c</sub> associated with the respective derived models were evaluated to be 0.0683 and 66.88 respectively.

Nwoye [8] derived a model for predicting the time for dissolution of pre-quantified concentration of phosphorus during leaching of iron oxide ore in oxalic acid solution as:

$$\tau = \text{Log} \left( \frac{\left[ \frac{P^{1/4}}{1.8} \right]}{\text{Log}T} \right) \quad (3)$$

Where

T= Leaching temperature ( $^{\circ}$ C) in the experiment [9], taken as specified leaching temperature ( $^{\circ}$ C) aiding the expected dissolution of phosphorus .

N= 1.8 (Dissolution coefficient of phosphorus in oxalic acid solution during leaching of iron oxide ore) determined in the experiment [9].

P = Concentration of dissolved phosphorus (mg/Kg) in the experiment [9], taken as pre-quantified concentration of phosphorus expected to dissolve after a leaching time t (mg/Kg) in the model.

$\tau$  = Leaching time (sec.) in the experiment [9], taken as time for dissolution of the pre-quantified concentration of phosphorus (hrs) in the model.

The model was found to depend on a range of specified leaching temperatures (45-70 $^{\circ}$ C) for its validity. It was found [9] that the time for dissolution of any given concentration of phosphorus decreases with increase in the leaching temperature (up to 70 $^{\circ}$ C), at initial pH 5.5 and average grain size of 150 $\mu$ m.

Nwoye et al. [10] also formulated a model for predicting the concentration of phosphorus removed during leaching of iron oxide ore in oxalic acid solution. It was found to predict the removed phosphorus concentration, with utmost dependence on the final pH of the leaching solution and weight input of the iron oxide ore. The model indicates that the concentration of phosphorus removed is inversely proportional to the product of the weight input of the iron oxide ore and the final pH of the leaching solution. Process conditions considered during the formulation of the model [10] include: leaching temperature of 25 $^{\circ}$ C, initial solution pH 5.5 and average ore grain size; 150 $\mu$ m).

Biological processes for phosphorus removal have also been evaluated based on the use of several types of fungi, some being oxalic acid producing. Anyakwo and Obot [11] recently presented their results of a study on the use of *Aspergillus niger* and their cultural filtrates for removing phosphorus from Agbaja (Nigeria) iron oxide ore. The results of this work [11] show that phosphorus removal efficiencies at the end of the 49 days of the leaching process are 81, 63 and 68% for 5, 100 and 250 mesh grain sizes respectively.

An attempt has been made in the past [12] to leach Itakpe iron oxide ore using oxalic acid solution in order to determine the maximum concentration of phosphorus that is removable. Results of chemical analysis of the ore indicate that the percentage of phosphorus in the ore is about 1.18%, which from all indication is quite high and likely to affect adversely the mechanical properties of the steel involved; hence the need for dephosphorization. It was reported [12] that phosphorus can be removed from iron oxide ore through a process associated with hydrometallurgy. Phosphorus was removed at a temperature of 25 $^{\circ}$ C and initial solution pH 2.5, leading to the dissolution of the phosphorus oxide formed. This involved using acid leaching process to remove phosphorus from the iron oxide ore in readiness for steel making process.

Nwoye et al [13] derived a model for predicting the concentration of dissolved iron during leaching of iron oxide ore in sulphuric acid solution. The model is stated as;

$$\%Fe = 0.35(\alpha/T)^3 \quad (4)$$

Where

T = Solution temperature at the time t, when the concentration of dissolved iron is evaluated. ( $^{\circ}$ C)

0.35= (pH coefficient for iron dissolution in sulphuric acid solution during the leaching process) determined in the experiment [13].

$\alpha$  = Final pH of the leaching solution at the time t, when the concentration of dissolved iron is evaluated.

The model (formulated at conditions; leaching temperature of 25 $^{\circ}$ C, initial solution pH 5.0 and average grain size; 150 $\mu$ m) is dependent of the final pH and temperature of the leaching solution. The model shows that the concentration of iron dissolved during the leaching process is directly proportional to the third power of the ratio of final leaching and temperature.

Nwoye [14] derived a model for evaluating the final pH of the leaching solution during leaching of iron oxide ore in oxalic acid solution. The model evaluates the pH value as the sum of two parts, involving the % concentrations of Fe and Fe<sub>2</sub>O<sub>3</sub> dissolved. The model can be expressed as;

$$\gamma = 0.5 \left( \frac{K_1}{\%Fe} + \frac{K_2}{\%Fe_2O_3} \right) \quad (5)$$

Where

K<sub>1</sub> and K<sub>2</sub> = dissolution constants of Fe and Fe<sub>2</sub>O<sub>3</sub> respectively.

γ = final pH of leaching solution (after time t).

It was also found that the model [14] could predict the concentration of Fe or Fe<sub>2</sub>O<sub>3</sub> dissolved in the oxalic acid solution at a particular final solution pH by taking Fe or Fe<sub>2</sub>O<sub>3</sub> as the subject formular. The prevailing process conditions under which the model works include: leaching time of 30mins., constant leaching temperature of 30°C, average ore grain size; 150µm and 0.1M oxalic acid.

Nwoye [15] has reported that the heat absorbed by oxalic acid solution during leaching of iron oxide ore can be predicted using the model he derived which works under the process condition; initial pH 6.9, average ore grain size; 150µm and leaching temperature; 30°C. The model [15] can be stated as

$$Q = K_N \left( \frac{\gamma}{\%Fe_2O_3} \right) \quad (6)$$

Where

Q = Quantity of heat absorbed by oxalic acid solution during the leaching process. (J)

γ = Final pH of the leaching solution (at time t).

%Fe<sub>2</sub>O<sub>3</sub> = Concentration of haematite dissolved in oxalic acid solution during the leaching process.

K<sub>N</sub> = 4.57(Haematite dissolution constant in oxalic acid solution) determined in the experiment [15].

Nwoye [15] carried out further work on the model using the same process conditions and observed that on re-arranging the model as;

$$\%Fe_2O_3 = K_N \left( \frac{\gamma}{Q} \right) \quad (7)$$

the concentrations of haematite predicted deviated very insignificantly from the corresponding experimental values. In this case, the value of Q was calculated by considering the specific heat capacity of oxalic acid. Values of heat absorbed by the oxalic acid solution during the leaching of iron oxide ore as predicted by the model [15] agree with the experimental values that the leaching process is endothermic. This is because all the predicted values of the heat absorbed by the oxalic acid solution were positive. The model shows that the quantity of heat absorbed by oxalic acid solution during the leaching process is directly proportional to the final pH of the solution and inversely proportional to the concentration of haematite dissolved.

Model for evaluation of the concentration of dissolved phosphorus (relative to the final pH of the leaching solution) during leaching of iron oxide ore in oxalic acid solution has been derived [16]. It was observed that the validity of the model is rooted in the relationship  $\ln P = N/\alpha$  where both sides of the expression are approximately equal to 4. The model;  $P = e^{(12.25/\alpha)}$  is dependent on the value of the final pH of the leaching solution which varies with leaching time. In all, the positive or negative deviation of the model-predicted phosphorus concentration from its corresponding value obtained from the experiment was found to be less than 22%.

Temperature measured at the reaction sites gives an idea of whether the reaction is speeding up or stopping especially when it is measured consistently.

It has been reported [17] that the temperature of a reaction system plays the major role in controlling the rate of the reaction.

Past report [18] has shown that measurement of the temperature of a reaction system consistently shows whether the reaction involved is endothermic or exothermic.



Nwoye [19] derived a model for the computational analysis of the solution temperature during leaching of iron oxide ore in hydrochloric acid solution. The model is expressed as:

$$T = e^{(8.9055/\gamma)} \quad (8)$$

where

T= Solution temperature during leaching of iron oxide ore using hydrochloric acid.(<sup>0</sup>C)

N= 8.9055(pH coefficient for hydrochloric acid solution during leaching of iron oxide ore) determined in the experiment [19].

$\gamma$  = Final pH of the leaching solution at the time t when the solution temperature is evaluated.

The model is dependent on the value of the final pH of the leaching solution which was found to also depend on the concentration of iron dissolved in the acid. The prevailed process conditions on which the validity of the model depended on include: initial pH 2.5, leaching time; 30 minutes, leaching temperature; 25<sup>0</sup>C, average ore grain size; 150 $\mu$ m and hydrochloric acid concentration at 0.1mol/litre.

The aim of this work is to investigate the optimum operating conditions of some process parameters during leaching of Agbaja (Nigerian) iron oxide ore in sulphuric acid solution.

## 2. Materials and Methods

Iron oxide ore collected from different spots at the Agbaja (Nigeria) iron ore deposit was homogenized and then sieved into 0.06, 0.08, 0.1, 0.3, 0.4mm.1000ml of sulphuric acid solution was prepared using 10ml of 0.1M sulphuric acid. The leaching solution was divided into ten and each part placed in a different conical flask for each sample investigated. Investigations on the effects of variation of grain size, mass of iron oxide ore, initial pH and leaching temperature on the dissolution of iron were carried out using 2-24g of iron oxide ore respectively. The initial solution pH and temperatures (just before the commencement of the leaching process) were recorded where necessary. Sodium hydroxide was used to regulate the initial solution pH for investigation to a range 6.63-6.82. Leaching time of 30 minutes was used for all samples. Ten different samples were used for investigation in the case of each variable and average values taken.

## 3. Results and Discussion

Table 1 shows that the ore contains some elements; sulphur and phosphorus which can be oxidized to form gases capable of dissolved in water to produce acids.

**Table 1. Results of chemical analysis of the as-received Agbaja iron oxide ore**

Element/compound	Fe	S	P	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>
(%)	45.6	0.10	0.76	11.91	3.82

### *Effect of leaching temperature on the dissolution of iron in sulphuric acid solution*

Investigation carried out over a leaching temperature range 40-85<sup>0</sup>C shows (Fig.1) that the optimum temperature for maximum dissolution of Fe during the leaching process is 55<sup>0</sup>C. It was also observed that the maximum iron dissolution concentration associated with this temperature is 0.0734%. It was also observed that above this temperature, precipitates of Fe(OH)<sub>3</sub> were formed in the leaching solution, hence the decrease in the dissolution of Fe.

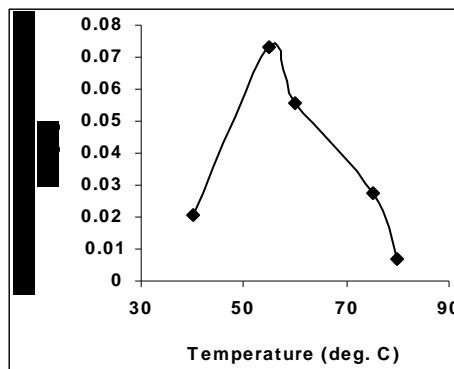


Fig.1-Effect of variation in leaching temperature on the concentration of dissolved iron

***Effect of grain size on the dissolution of iron in sulphuric acid solution***

The effect of grain size on the dissolution of iron was investigated over a range of grain size 0.06-0.4mm. It was found (as in Fig.2) that the optimum grain size for maximum dissolution of Fe during the leaching process is 0.1mm (100µm). This implies that the dissolution of Fe increased with increased grain size up till a size of 0.1mm after which decrease in Fe dissolution was recorded with larger grains. Fig. 2 shows that the maximum dissolution of Fe associated with the optimum grain size (0.1mm) is 0.1222%.

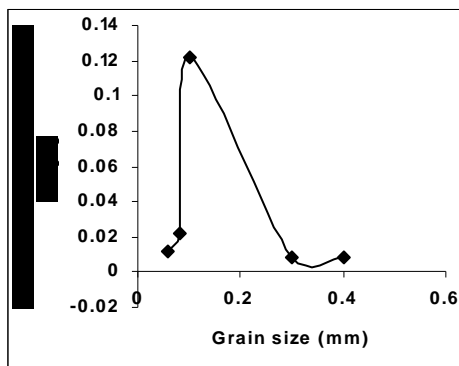


Fig.2-Effect of variation in grain size of iron oxide ore on the concentration of dissolved iron

***Effect of initial solution pH on the dissolution of iron in sulphuric acid solution***

The effect of initial solution pH on the dissolution of iron was ascertained using a range of pH values 6.63-6.82. Comparison of Figs. 3 and 4 show that increase in the initial solution pH (just before commencement of the leaching process) increases the initial solution temperature (just before commencement of the leaching process) and then increases concentration of Fe dissolved in the sulphuric acid solution during the leaching process up to an optimum value; 6.8.

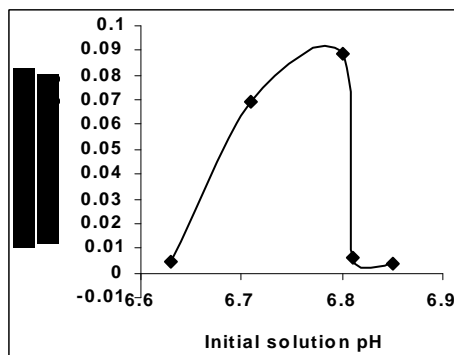
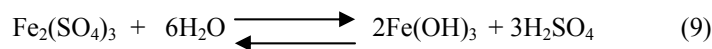


Fig.3-Effect of variation in initial solution pH on the concentration of dissolved iron

It was found that above this optimum pH value, the concentration of dissolved Fe dropped. This is due to the fact that above pH6.8, ferric sulphate becomes hydrolyzed to precipitate Fe(OH)<sub>3</sub> in the sulphuric acid solution in agreement with past experiment by Pinches [5]. This process of hydrolysis is expressed as;



Figs. 3 and 4 show that the maximum initial solution temperature and dissolution of Fe associated with the optimum initial pH are 28°C and 0.0889% respectively.

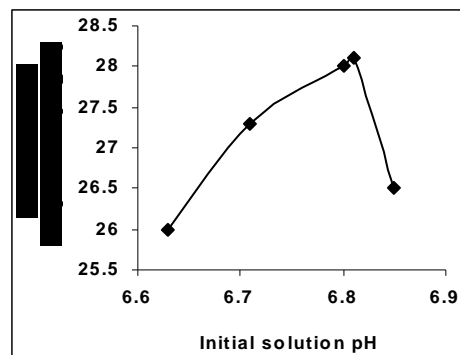


Fig.4-Effect of variation in initial solution pH on the initial solution temperature

Fig.4 shows that the initial solution temperature increases with increase in the initial solution pH up to 6.8. Above this optimum pH value, the initial solution temperature dropped with further increase in the initial solution pH. It is suspected that the increment in the initial solution temperature (as the initial solution pH increases) resulted from the interaction of  $H^+$  and  $OH^-$  to produce more water in the leaching solution i.e



Furthermore, this reaction is believed to be exothermic in nature, hence the temperature increment. Above the optimum pH, implying addition of more  $OH^-$ , the reaction in equation (10) is suspected to become endothermic as a result of absorption of the heat of reaction between  $H^+$  and  $OH^-$  by water already formed, hence the drop in the temperature.

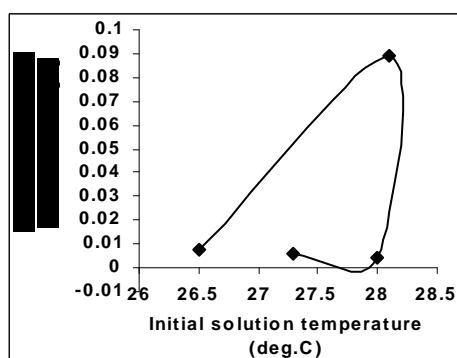


Fig.5. Effect of variation in initial solution temperature on the concentration of dissolved iron

Comparison of Figs. 4 and 5 show that at an initial solution pH6.8, the associated initial solution temperature 28°C which is optimum gives the maximum dissolution of Fe because at the initial stage of the leaching process, increment in the solution temperature translates into an increase in the activation energy required for the commencement of the leaching process. This invariably enhanced Fe dissolution by reducing the chemical resistance to the dissolution process. However, decrease in the initial solution temperature occasioned by further increase in the initial solution pH reduced the activation energy required to ensure progressive dissolution of Fe and this resulted to a decrease in the concentration of Fe dissolved. This is in accordance with studies by King et al. [21].

***Effect of variation in weight-input of iron oxide ore on the dissolution of iron in sulphuric acid solution***

Fig. 6 shows that increase in the weight-input of iron oxide ore decreases the concentration of Fe dissolved. This is because the concentration of the aggressive ions which control the leaching process ( $H^+$ ) is fixed for all weights-input of iron oxide ore. These hydrogen ions attack the ore thereby enhancing the leaching and dissolution of Fe. Increasing the iron oxide ore increases the quantity of ore to be attacked per  $H^+$ . This makes the  $H^+$  unable to react with the whole quantity of ore within the stipulated time; enhancing leaching and dissolution of Fe, instead it reacts with just a part of the larger ore quantity. This results to lower leaching and dissolution of Fe for each increment in weight-input of the ore.

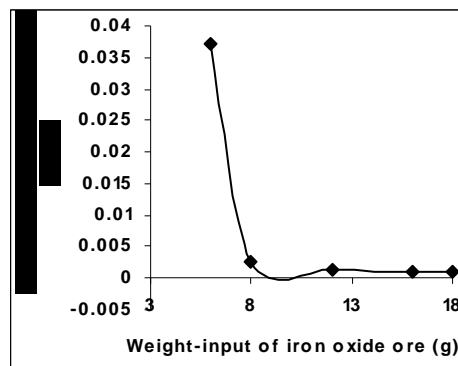


Fig.6-Effect of variation in weight-input of iron oxide ore on the concentration of dissolved iron

Fig.7 shows that the initial solution temperature decreases with increase in the weight-input of iron oxide ore. This is as a result of the absorption of heat present in the leaching solution which resulted from the process in equation (10) following the relationship in Fig. 4. It is strongly believed that as the iron oxide ore is increasingly being added to the leaching solution, the heat present in the solution would continuously be mopped-up, hence decreasing the initial solution temperature.

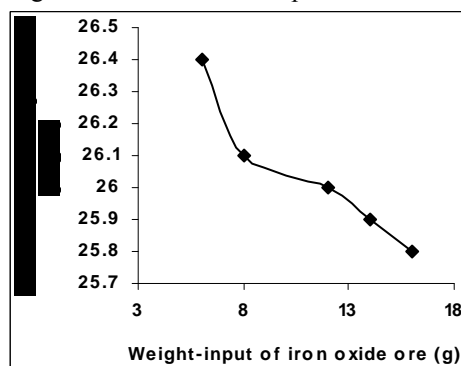


Fig.7-Effect of variation in weight-input of iron oxide ore on the initial solution temperature

It has been found (Fig.8) that increase in the weight of iron oxide ore added to the leaching solution (just before the leaching process) decreases the initial solution pH. It is strongly believed that presence of sulphur and phosphorus in the ore was responsible for the drop in the pH. It is also believed that increased quantities of sulphur and phosphorus dissolved in the leaching solution for each increment in the weight of iron oxide ore added, hence the progressive drop in the pH with increase in weight-input of iron oxide ore.

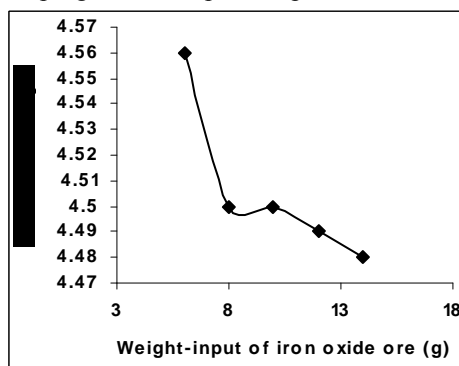


Fig.8-Effect of variation in weight-input of iron oxide ore on the initial solution pH

It was found that increase in the initial solution temperature resulted to increased dissolution of Fe in the leaching solution (Fig.9). This increment in the initial solution temperature is believed to have translated into an increase in the activation energy required for the commencement of the leaching process in

agreement with previous report [21]. This invariably enhanced Fe dissolution by reducing the chemical resistance to the dissolution process. Fig.9 shows that maximum initial solution temperature resulted to maximum Fe dissolved concentration. This is also in accordance with studies [21] by King et al.

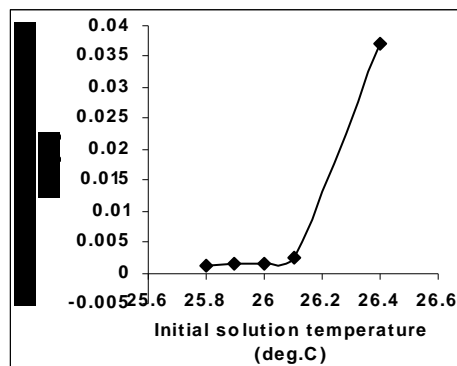


Fig.9-Effect of variation in initial solution temperature on the concentration of dissolved iron

### Conclusion

Following results of investigations carried out to determine the optimum operating conditions of some process parameters during leaching of iron oxide ore in sulphuric acid solution, it is concluded that the optimum leaching temperature, grain size, and initial pH are 55°C, 0.1mm and 6.8 respectively. The optimum initial solution temperature required for maximum dissolution of Fe was also found to be 28°C. Concentration of dissolved Fe was found to decrease progressively with increase in the weight-input of iron oxide ore. Concentration of dissolved Fe was found to decrease progressively with increase in the weight-input of iron oxide ore due to continuously increased iron ore weight-input - fixed hydrogen ions concentration relationship.

### Acknowledgement

The authors are grateful to the management of SynchroWell Nig. Ltd. Enugu for providing the equipment used for this work.

### References

- [1] Taxiarchour, M., Panias, D., Doumi, I., Paspaliaris, I., Kontopoulos, A. (1997a) Removal of Iron from Silica Sand by Leaching with Oxalic Acid, *Hydrometallurgy*, 46, 215-227.
- [2] Taxiarchou, M., Parnias, D., Douni, I., Paspaliaris, I., Kontopoulos, A. (1997b) Dissolution of Haematite in Acidic Oxalate Solutions. *Hydrometallurgy*, 44, 287-299.
- [3] Lee, S. O., Tran, T., Park Y.Y., Kim S.J., Kim, M. J. (2006) Study on the Kinetics of Iron Leaching by Oxalic Acid. *Int. J. Miner Process*, 80, 144-152.
- [4] Panias, D., Taxiarchou, M., Paspaliaris, I., Kontopoulos, A. (1996) Mechanism of Dissolution of Iron Oxides in Aqueous Oxalic Acid. *Hydrometallurgy* 42, 257-265.
- [5] Pinches, A. (1975) Bacterial Leaching of an Arsenic Bearing Sulphide Concentrate. The Institute of Mining and Metallurgy, England, 34.
- [6] Nwoye, C. I. (2008) Ph.D Thesis, Metallurgical and Materials Engineering Department, Federal University of Technology, Owerri, 178.
- [7] Nwoye, C. I. (2008) Model for Computational Analysis of Dissolved Haematite and Heat Absorbed by Oxalic Acid Solution during Leaching of Iron Oxide Ore, *J. Eng. & App. Sc.*, 4, 22-25.
- [8] Nwoye, C. I. (2008) Model for predicting the Time of Dissolution of Pre-quantified Concentration of Phosphorus during Leaching of Iron Oxide Ore in Oxalic Acid, *IJONAS*, 4(3):168-174.
- [9] Nwoye, C. I. (2006) SynchroWell Research Work Report, DFM Unit, No 2561178, 66-83. [13]
- [10] Nwoye, C. I., Agu, P. C., Mark, U., Ikele, U. S., Mbuka, I. E., and Anyakwo, C. N. (2008) Model for Predicting Phosphorus Removal in Relation to Weight of Iron Oxide Ore and pH during Leaching with Oxalic Acid, *Inter. J. Nat. Appl. Sc.*, 4(3): 106-112.
- [11] Anyakwo, C. N., and Obot, O.W. (2008) Phosphorus Removal from Nigeria's Agbaja Iron Ore by *Aspergillus niger*, *IREJEST* 5(1), 54-58.
- [12] Nwoye, C. I. (2003) SynchroWell Research Work Report, DFM Unit, No 2031196, 26-60.

- [13] Nwoye, C. I., Amara, G. N., and Onyemaobi, O. O. (2008) Model for Evaluating Dissolved Iron during Leaching of Iron Oxide Ore in Sulphuric Acid Solution, *Inter. J. Nat. Appl. Sc.*, 4(2): 209-211.
- [14] Nwoye, C. I. (2008) Model for Quantitative Analysis of Dissolved Iron in Oxalic Acid Solution during Leaching of Iron Oxide Ore, *Inter. Res. J. Eng. Sc. Tech.*, 5(1): 37-41.
- [15] Nwoye, C. I. (2008) Model for Computational Analysis of Dissolved Haematite and Heat Absorbed by Oxalic Acid Solution during Leaching of Iron Oxide Ore, *J. Eng. & App. Sc.*, 4, 22-25.
- [16] Nwoye, C. I. (2009). Model for Evaluation of the Concentration of Dissolved Phosphorus during Leaching of Iron Oxide Ore in Oxalic Acid Solution. *JMMCE* (in press)
- [17] Ferreira, R.C.H and Burkin, A.R; Acid Leaching of Chalcopyrite. (1975). In A.R Burkin; *Leaching and Reduction in Hydrometallurgy*. The Institution of Mining and Metallurgy, England, 1975 60.
- [18] Ugarte, F. J and Burkin, A. R. (1975). Mechanism of Formation of Idaite from bornite by Leaching with Ferric Sulphate Solution. In A.R Burkin ; *Leaching and Reduction in Hydrometallurgy*. The Institution of Mining and Metallurgy, England, 1975, 47.
- [19] Nwoye, C. I., (2006) SynchroWell Research Work Report, DFM Unit, No. 2021196, 28-43.
- [20] King, J. A., Burkin, A. R., Ferreira, R. C. H. (1975) Leaching of Chalcocite by Acidic Ferric Chloride Solutions. In A. R. Burkin; *Leaching and Reduction in Hydrometallurgy*. The Institution of Mining and Metallurgy, England, 1975, 39.

5/28/2009



## Forest Products Of Ehor Forest Reserve In Uhunmwode Local Government Area, Edo State

Jane Ihenyen., Okoegwale, E.E. and Mensah, J.K.

Department of Botany, Ambrose Alli University, Ekpoma, Edo State, Nigeria

[inejanet@yahoo.com](mailto:inejanet@yahoo.com)

**ABSTRACT:** Timber and non-timber forest products of Ehor Forest Reserve in Uhunmwode Local Government Area of Edo state, Nigeria was evaluated in this study. A total of 257 uses were identified, distributed into sixteen groups according to their ethnobotanical uses. Many of them had multipurpose uses. Medicinal plants accounted for the highest number of species 62 (24.90%) which explains the importance the rural dwellers place on herbal treatment. This was followed by timber plants with 54 species (22.10%), food producing plants/vegetables 26 species (10.44%) and chewing sticks 18 (7.23%). All merchantable timber species within the various compartments had been harvested leaving the reserve with only wildlings. A number of food and timber trees as well as plants which provide fuel wood were under threat of extinction because of over exploitation. Since a significant proportion of the Edo state economy is derived from the forest products, harvesting of the forest products should be carried out sustainably in order to conserve them for future use. [Researcher. 2009; 1(4):83-91]. (ISSN: 1553-9865).

**Key words:** Forest products, Timber, Non-timber forest products, Medicinal plants.

### INTRODUCTION

The forest is a haven of diverse renewable natural resources. The British Commonwealth Forest Terminology defined forest products as “all materials obtained from a forest estate including earth, stone, gravel and sometimes minerals. It is classified as major which usually are timber, small wood and firewood; and minor such as all forest products other than the major forest products including grasses, fruits, leaves, bark, exudates, animal products, soil and sometimes minerals” (Okoro, 2002).

Isichei (1995) classified timber as the main forest product while others are considered as minor. Cunningham *et al.* (2005) is of the view that since firewood accounts for about half of all wood harvested worldwide on which a third of the people of the world depend as their principal source of fuel, it should be grouped with timber as a major product while other forest products are minor. Research into the exploitation of non-timber forest products has shown that their exploitation is competing fast with that of timber hence what is major and minor is subjective.

Forest products in various forms like medicines, food plants, materials for household items, fuel wood, plant yielding dyes, resins, fibres, alcoholic drinks, toxins and gum, chewing sticks for oral hygiene and fodder plants are the generally accepted minor forest products and these occur in greater variety in the forest than the timber. According to Osemeobo and Ujor (1999) they also have shorter frequency of harvest cycle, smaller yield per unit area in the forest and higher monetary value per unit weight.

Forest products form a significant natural resource component of the poor people particularly in the rural areas. Edo state in particular is regarded as rich in forest resources. Wood based industries hold second place in the provision of employment opportunities with about 14% of the total employees in the state engaged in it. Other industries generating employment from forest products are: traditional craft industries like carving, cane work, raffia work, fuel wood gathering and sale, charcoal production, mortar and pestle products, sponge production and sale, chewing sticks production, mat weaving industry, canoe carving, broom making, tooth picks, pencil and slate production, bee keeping/ honey production (Azeke, 2002).

In view of the significant role played by forest products in the economy of Edo state, the focus of this paper is to take inventory of the floral forest product in Ehor Forest Reserve, Uhunmwode Local Government Area of Edo state, Nigeria.

## MATERIALS AND METHODS

### STUDY SITE:

Ehor Forest Reserve occupies an area of 7,680 hectares in Uhumwode Local Government Area of Edo state, Nigeria. It is located between latitudes 6° 34'N and 6° 38'N and longitudes 5° 54'E and 5° 58'E fifty-six kilometers north of the state capital, Benin-City. It is divided into forty-eight compartments of 160 hectares each. Farming is commonly practiced within the reserve which is situated in the lowland rainforest zone. Though there are no settlements within the reserve, it is surrounded by nine villages viz: Ohe, Eguaholor, Egbisi, Ugieghudu, Uhi, Iriwe, Erhue, Evbowe and Ekudo. It had a sizeable number of timber species which made it attractive to logging companies. Cassava is the most commonly encountered crop in the reserve. Apart from timber, other non-timber forest products like fuelwood, chewing sticks, medicinal plants, construction and weaving materials, vegetable and other food materials are also exploited from the reserve.

### SURVEY METHOD

Three compartments of 160 hectares each were sampled for timber and non-timber products. The compartments sampled are 81 on the western end, 95 centrally located and 112 at the eastern end. This is to have an adequate representation of the whole forest reserve. Sampling was done by laying out sample plots of 30 × 30 metres in each of the compartments studied according to the method of Inegbedion 2008. Inventory was taken of all the timber and non-timber plants within the compartments. Information on their utilization was provided by the three local plant enumerators recruited for such purpose and by reference to Gill (1992), Osemeobo and Ujor (1999), Aiyeloja and Ajewole (2006).

## RESULTS

A list of the forest products and their ethnobotanical uses are presented in Table 1. They are classified into the underlisted sixteen groups based on their uses: 3 species of food wrappers (1.2%), 55 species of timber (22.10%), 2 species of fibre producing plant (0.80%), 6 species of dye plant (2.41%), 5 species of fodder plant (2.01%), 26 species of food plant (10.44%), 4 species of toxin producing plants (1.61%), 51 species of fuel wood (20.48%), 18 species of chewing stick (7.23%), 9 species of plants used in producing household items (3.61%), 62 species of medicinal plants (24.90%), 1 species of plant used for cultural rites (0.40%), 6 species of gum producing plants (2.41%), 3 species of latex producing plants (1.20%), 5 species of plants used in charcoal production (2.01%), 5 species of plants for house construction and agricultural implements (2.01%).

A number of them have multiple uses, for example *Azela africana* can be used as timber, the leaves serve as fodder and vegetables; the stem and branches as fuelwood and charcoal production while its twig is used as chewing stick. *Albizia zygia* is another important forest product used as timber, fodder, food, fuel wood, chewing stick and its exudates can be used as gum.

*Elais guineensis* produces fibre, palm oil for cooking, kernel oil for cosmetics, household items like brooms, beverages like palm wine which is also used for medicinal purposes and it is an important component of items used in traditional ceremonies while the rachis from the palm frond is used for house construction in the villages.

**Table 1: Timber and non- timber forest products in Ehor Forest Reserve and their uses.**

S/N	Plant Species	Food Wrapper	Timber	Fibre	Dye	Fodder	Food	Toxin	Fuel Wood	Chewing stick	Household Item	Medicinal	Cultural rites	Gum	Latex	Charcoal	Agric & Constr
1	<i>Afzelia africana</i>		√			√	√		√	√						√	
2	<i>Aframomium melegueta</i>						√					√					
3	<i>Agaricus species</i>						√										
4	<i>Albizia ferruginea</i>		√					√				√					
5	<i>Albizia lebbeck</i>		√		√							√					
6	<i>Albizia zygia</i>		√			√	√		√	√				√			
7	<i>Allanblackia floribunda</i>		√							√		√					
8	<i>Alstonia boonei</i>	√	√						√			√					
9	<i>Amphimas pterocarpoides</i>																
10	<i>Angylocalyx zenkeri</i>																
11	<i>Anonidium mannii</i>				√				√			√					
12	<i>Anopyxis klianeana</i>																
13	<i>Anthonotha macrophylla</i>																
14	<i>Antiaris africana</i>		√						√								
15	<i>Antiaris welwitschii</i>		√									√					
16	<i>Antracaryon micraster</i>						√										
17	<i>Bambusa vilgaris</i>																√
18	<i>Baphia nitida</i>				√		√		√	√		√				√	
19	<i>Berlinia grandiflora</i>		√														
20	<i>Blighia sapida</i>		√				√		√			√					
21	<i>Bombax brevicuspe</i>		√														
22	<i>Bosqueia angolensis</i>		√						√			√					
23	<i>Brachystegia nigerica</i>		√											√			
24	<i>Calamus mannan</i>										√						
25	<i>Calamus calamus</i>										√						
26	<i>Canarium schweinfurthii</i>		√				√		√			√					
27	<i>Carpolobia lutea</i>						√			√							

S/N	Plant Species	Food Wrapper	Timber	Fibre	Dye	Fodder	Food	Toxin	Fuel Wood	Chewing stick	Household Item	Medicinal	Cultural rites	Gum	Latex	Charcoal	Agric & Constr
28	<i>Ceiba pentandra</i>		√	√		√	√		√			√					
29	<i>Celtis mildbraedii</i>																
30	<i>Celtis zenkeri</i>		√						√								
31	<i>Chrysophyllum albidum</i>						√					√					
32	<i>Chrysophyllum delevoyi</i>		√				√					√					
33	<i>Cleistopholis patens</i>		√														
34	<i>Cola acuminata</i>						√					√					
35	<i>Combretodendron macrocarpum</i>		√						√			√					
36	<i>Cordia millenii</i>		√														
37	<i>Costus afer</i>										√	√					
38	<i>Cylicodiscus gabunensis</i>		√						√			√					
39	<i>Dacryodes edulis</i>		√				√		√			√					
40	<i>Daniellia ogea</i>		√						√							√	
41	<i>Desplatsia subericarpa</i>																
42	<i>Diospyros alboflavescens</i>		√														
43	<i>Diospyros dendo</i>		√							√							
44	<i>Diospyros mesipiliiformis</i>		√			√			√	√		√					√
45	<i>Distemonanthus benthamianus</i>		√						√			√					
46	<i>Elaeis guineensis</i>			√			√				√	√					√
47	<i>Entandrophragma angolense</i>		√					√	√					√			
48	<i>Fagara macrophylla</i>		√						√	√		√		√			
49	<i>Funtumia elastica</i>		√						√			√			√		
50	<i>Garcinia kola</i>									√		√					
51	<i>Gossweilodendron balsamiferum</i>		√						√								
52	<i>Guarea cedrata</i>		√						√								
53	<i>Guibourtia species</i>											√					
54	<i>Hannoa klaineana</i>		√				√		√			√					
55	<i>Hevea brasiliensis</i>								√						√		
56	<i>Homalium aylmeri</i>																
57	<i>Hunteria umbellata</i>											√					

S/N	Plant Species	Food Wrapper	Timber	Fibre	Dye	Fodder	Food	Toxin	Fuel Wood	Chewing stick	Household Item	Medicinal	Cultural rites	Gum	Latex	Charcoal	Agric & Constr
58	<i>Hymenostegia afzelii</i>		√				√			√		√					
59	<i>Irvingia gabonensis</i>		√				√					√					
60	<i>Irvingia grandifolia</i>		√				√										
61	<i>Khaya grandifoliola</i>		√											√			
62	<i>Khaya ivorensis</i>		√														
63	<i>Lannea welwitschii</i>											√					
64	<i>Lentinus tuber-regium</i>						√					√					
65	<i>Lonchocarpus griffonianus</i>																
66	<i>Lophira alata</i>		√									√					
67	<i>Lova trichilioides</i>		√														
68	<i>Maesobotrya bateri</i>																
69	<i>Maesopsis eminii</i>											√					
70	<i>Mansonia altissima</i>		√														
71	<i>Megaphrynium macrostachyum</i>	√					√				√						
72	<i>Memeylon blakeoides</i>									√		√					
73	<i>Milicia excelsa</i>		√									√			√		
74	<i>Musanga cacropioides</i>		√				√		√	√		√					
75	<i>Myrianthus arboreus</i>						√		√	√		√					
76	<i>Nauclea diderrichii</i>		√						√			√				√	
77	<i>Nesogordonia papaverifera</i>		√							√		√					
78	<i>Newbouldia laevis</i>					√						√	√				
79	<i>Okoubaka aubrevillei</i>																
80	<i>Olax subscorpioidea</i>									√		√					
81	<i>Pachyelasma tessmannii</i>		√						√								
82	<i>Pallisota hirsute</i>											√					
83	<i>Panda oleasa</i>																
84	<i>Pausinystalia macroceras</i>								√			√					
85	<i>Pentaclethra macrophylla</i>		√				√		√			√					
86	<i>Pentadesma butyracea</i>											√					
87	<i>Pierreodendron africanum</i>																

S/N	Plant Species	Food Wrapper	Timber	Fibre	Dye	Fodder	Food	Toxin	Fuel Wood	Chewing stick	Household Item	Medicinal	Cultural rites	Gum	Latex	Charcoal	Agric & Constr
88	<i>Piptadeniastrum africanum</i>		√						√			√					
89	<i>Polyalthia suaveolens</i>											√					
90	<i>Polyceratocarpus parviflorus</i>																
91	<i>Pterocarpus osun</i>		√		√				√			√					
92	<i>Pycnanthus angolensis</i>		√									√					
93	<i>Rauwolfia vomitoria</i>							√	√	√		√					
94	<i>Ricinodendron heudelotti</i>		√									√					
95	<i>Rothmania hispida</i>				√				√			√					
96	<i>Spathodea campanulata</i>											√					
97	<i>Staudtia stiptata</i>																
98	<i>Sterculia oblonga</i>		√														
99	<i>Sterculia tragacantha</i>											√		√			
100	<i>Strombosia postulate</i>		√						√	√						√	
101	<i>Tabernaemontana pachysiphon</i>								√			√					
102	<i>Terminalia ivorensis</i>		√						√			√					
103	<i>Tetrorchidium didymostemon</i>											√					
104	<i>Thaumatococcus danielli</i>	√					√				√	√					
105	<i>Trichilia lanata</i>																
106	<i>Trichilia priuriana</i>											√					
107	<i>Triplochiton scleroxylon</i>		√						√								
108	<i>Uvariopsis dioica</i>							1									
109	<i>Xylopia aethiopica</i>						√			√		√					
	Total	3	55	2	6	5	24	4	51	18	9	62	1	6	3	5	3
	Total (%)	1.17	21.40	0.28	2.33	1.95	9.34	1.56	19.84	7.00	3.50	24.12	0.39	2.33	1.17	1.95	1.17



## DISCUSSION

The forest products reported here were among those earlier reported by Gill and Okoegwale (1991) and Osemeobo and Ujor (1999) to occur in the Nigerian forests. From the forests of Osun State, Nigeria. Aiyeloja and Ajewole (2006) reported *Garcinia kola*, *Aframomium melagueta*, *Agaricus* species, *Irvingia gabonensis*, *Baphia nitida*, *Thaumatococcus danielli*, *Cola nitida*, *Megaphrynium macrostachyum*, *Alstonia boonei*, *Rauwolfia vomitoria*, *Pycnanthus angolensis*, *Milicia excelsa* and *Newboldia laevis*. All these species in addition to others were also encountered at the Ehor Forest Reserve.

Some timber and food plants like *Cordia millenii*, *Dacryodes edulis*, *Garcinia kola*, *Irvingia gabonensis*, *Khaya grandifoliola*, *Nauclea diderichii*, *Rauwolfia vomitoria*, *Terminalia ivorensis* and *Xylopiya aethiopica* were represented by only one stand. This indicated that they have been overexploited and may go into extinction from the forest reserve if adequate measures are not taken to conserve them. Unsustainable harvesting of these products play a great role in their depletion from the forest. According to Osemeobo and Ujor (1999) poor harvesting of plants lead to: reduction in fruiting patterns and in quantity of fruits and surface area of crowns resulting in decreased photosynthesis thereby causing a die-back in plants and finally death of some plants harvested in the dry season due to water stress and bush burning.

The non-timber forest product sector is growing very rapidly, perhaps faster than the timber industry and it is expected to grow more in future not only in Nigeria but worldwide. *Manilkara zapota* is a non-timber forest species found in most of the surviving forests in Central America, producing latex which is a source of chicle used in chewing gum (Kellman and Tackaberry, 1997). Latex producing trees like *Hevea brasiliensis*, *Funtumia elastica* and *Milicia excelsa* recorded in this study could be screened for such purposes. These same plants could also be screened to be utilized as either diesel fuel or high quality liquid fuel which will serve as an alternative source of energy for running our vehicles or for domestic use. According to Shukla and Chandel (2008) a large number of species of Euphorbiaceae to which *Hevea brasiliensis* belongs secrete latex containing about 30% hydrocarbon and may serve as important source of hydrocarbon.

Forest medicines are very popular with rural dwellers particularly in Edo state; hence the State has a board for the control of traditional medicine practices. Plant medicines are generally the first recourse for rural households. When this fails, they either turn to traditional healers or western-type medicines (Azeke 2002). Most of the forests in Edo State are very rich in medicinal plants. This explains why the highest number of species put in figures used as medicinal plants were more than other ethnobotanical uses. Generally, a large number of forest plants have medicinal value hence Abu and Adebisi (2002) regarded the forest as the richest drugstore.

Most of the timber species encountered were wildlings because this reserve had been stripped of merchantable trees by logging companies exploiting the forest reserve. Despite that the timber species diversity of the reserve can be said to be reasonable. These timber species and those from other forest reserves provide the raw materials to feed the numerous saw mills in Edo State thereby providing employment for thousands of youths and a significant proportion of those affected by the wave of retrenchments in the public service (Azeke, 2002).

The food plants encountered in this study either produce fruits e.g. *Chrysophyllum albidum*; vegetables-*Myrianthus arboreus*; spices *Xylopiya aethiopica*; mushrooms *Agaricus sp.* Seeds for preparing soup like *Irvingia gabonensis*; oil and alcohol beverage *Elaeis guineensis*. Osemeobo and Ujor (1999) classified food plants into seven groups based on what they produce.

Different parts of the eighteen species of plants identified in this study used for chewing sticks, are useful. For some like *Fagara macrophylla* and *Garcinia Kola*, the roots are used while for others like *Carpolobia lutea*, *Olax subscorpioidea*, *Hymenostegia afzelii* and *Nesogordonia papaverifera*, the twigs are used (Gills, 1992).

Fifty-one species of fuelwood and five species of plant used for charcoal production were identified. Fuel wood is one of the most important product of the forest because the majority of the rural dwellers depend on it for their energy source particularly for cooking. According to Cunningham (2005), it accounts for almost half of all wood harvested world wide and it is the main source of energy for one third of the human race. As a result of the pressure on the traditional fuelwood species, some of the well known timber species are also used either as fuel wood or for charcoal production e.g *Afzelia africana*, *Daniellia ogea*, *Nauclea diderrichii* and *Strombosia postulata*. *Thaumatococcus danielli* was one of the food wrappers found in Ehor Forest Reserve producing little berries at its base which contain proteinaceous sweeteners. These berries are believed to hold man's key to alternative sweeteners particularly for diabetic patient (Isichei, 1995). Other food wrappers in the reserve were leaves of *Alstonia boonei* and *Megaphrynium macrostachyum*.

The stalk of *T.danielli* and *M.macrostachyum* and the stem of *Calamus calamus*, *C.mannan*, *Costus afer* and the frond of *Elaeis guineensis* are used in the production of household items like native trays, cane furniture, baskets, brooms e.t.c.

The dye plants in the reserve produce dye from the leaves e.g *Pterocarpus osun* and *Ruthmania hispida* while *Baphia nitida* produces it from the bark. The dye is extracted and used to decorate the body during traditional ceremonies in the villages. It is also used to dye textile on a small scale.

Other uses to which the forest product can be employed include house construction with the rachis of *Elaeis guineensis* and *Bambusa vilgaris*; fibre production from the fruit of *Elaeis guineensis* and *Ceiba pentandra*; fodder from the leaves of *Azelia africana*, *Albizia zygia*; *C.pentandra*, *Diospyros Mesipiliformis* and *Newboldia laevis*. The latter is also used for cultural rites.

Of the twenty-seven gum producing plants enumerated by Soladoye (1977) to occur in the Nigerian forests, six were encountered in Ehor Forest Reserve. These were *Albizia zygia*, *Fagara macrophylla*, *Entandrophragma angolense*, *Brachystegia nigerica*, *Khaya grandifoliola* and *Sterculia tragacantha*. He also found that these plants produced gum in abundance which could be harnessed for export to earn foreign exchange for the country.

*Albizia ferruginea*, *Entandrophragma angolense* and *Rauwolfia vomitoria* produced toxins used for fishing.

*Diospyros mesipiliformis* is used for the construction of agricultural and household implements like hoe handle, pestle and mortar while the rachis of *Elaeis guineensis* and *Banabusia vilgaris* stems are used as poles for house construction in the villages.

The surrounding villages of Ehor Forest Reserve depend on these enumerated plant species for their livelihood and provide additional income by collecting and selling these forest products in the markets. Conservation of these products should be a priority of governments at all levels in Edo State.

## CONCLUSION

Attention has been drawn to the variety of forest products available in Ehor Forest Reserve and their uses. These products contribute significantly to the economy of Edo state so they should be exploited with caution to avoid their depletion from the forests.

## REFERENCES

1. Abu, J.E and Adebisi, L.A (2002): A review of traditional forest uses. In : *Forest, people and the environment* ( Popoola L Ed.) Proceedings of the 2<sup>nd</sup> National Workshop organized by FANCONSULT and Edo State chapter of Forestry Association of Nigeria held in Benin-City, Edo State, 5-6 September, 2002.
2. Aiyeloja, A.A and Ajewole, O.I (2006): Non-timber forest products marketing in Nigeria. A case study of Osun State. *Educational Research and Reviews* 1(2):52-58
3. Azeke I.E (2002) : Forestry contribution to Edo State economy. In: *Forest, people and the environment* (Popoola L Ed.) Proceedings of 2<sup>nd</sup> National workshop organized by FANCONSULT and Edo State chapter of Forestry Association of Nigeria held in Benin-City, Edo state, 5-6 September, 2002.
4. Cunningham, P.W, Cunningham, M.A and Saigo, B. (2005): *Environmental Science: A global concern*. 8<sup>th</sup> Edition. McGraw Hill 600p.
5. Gill, L.S (1992): *Ethnomedical uses of plants in Nigeria*. University of Benin press, Benin-city 276p
6. Gill, L.S & Okoegwale, E.E.(1991): Nigerian timber 1: Their physical properties and possible End-uses. *Wood News* 1(3): 39 – 41
7. Inegbedion, J.O (2008) *Timber Resource Status of Ehor Forest Reserve, Ubumwode Local Government Area of Edo state*, Ph.D thesis. Ambrose Alli University, Ekpoma. Nigeria 113p
8. Isichei, A.O. (1995): *Omo biosphere reserve, current status, utilization of biological Resources and sustainable management*. South-South corporation programme on environmentally sound socio-economic development in the humid tropics. Working paper No 11.
9. Kellman, M and Tackaberry, R (1997): *Tropical environments- the functioning and management of Tropical ecosystems*. Routledge publication London and New York. 594p
10. Okoro, S.P.A. (2002) Forest produce in Edo State. In: *Forest, people and the environment*. (Popoola L. Ed.) Proceedings of 2<sup>nd</sup> National Workshop organized by FANCONSULT and Edo State chapter of

Forestry Association of Nigeria held in Benin-City, Edo State, 5-6 September, 2002.

11. Osemeobo, G.J and Ujor, G. (1999). *The non-wood forest products of Nigeria*. EC-FAO partnership programme (1998-2000). 42p
12. Shukla, R. S and Chandel, P. S. (2008): *A Text book of Plant Ecology including Ethnobotany and Soil Science*. Chand and company Limited. Ram Nagar, New Delhi. 544p.
13. Soladoye, M.O (1977): Taxanomic studies of some Gum yielding Trees in Nigeria. *The Nigerian Journal of Forestry* 7(1):34-38

## Review On Analysis Of Bisphenol A Diglycidylether (Badge) In Canned Food

Abida Taskeen and Ismat Naeem \*

Department of Chemistry, Lahore College for Women University, Lahore, Jail Road Lahore, Pakistan.

Telephone: 92-42-9203801-9/245; Email: [ismat4\\_naeem@yahoo.co.in](mailto:ismat4_naeem@yahoo.co.in)

**ABSTRACT: Objective:** Diglycidyl ether of bisphenol A (DGEBA) is constantly discharged at trace levels in food packed in metal cans with PVC lining. This represents a cause for concern because of potential effects of bisphenol a to human health. We compiled data on the analysis of diglycidyl ether of bisphenol A (DGEBA) in canned food published in the last 10 years. **Data sources:** Google Scholar, Pubmed and Medline, Journal of agriculture and food chemistry website, EHP website were used to search for articles published in peer-reviewed journals written in the English language since 1999. **Data extraction:** Information on diglycidyl ether of bisphenol A (DGEBA) concentrations in canned food the source of contamination, method used for analysis, detection limits, year of publication and method of analysis was extracted. **Conclusions:** Detrimental effects of diglycidyl ether of bisphenol A (DGEBA) on human beings are possible with the constant exposure through canned food. Indirect impact on human health from canned food cannot be ruled out when considering the potential risk of diglycidyl ether of bisphenol A (DGEBA). [Researcher. 2009;1(4):90-92]. (ISSN: 1553-9865).

**KEYWORDS:** Canned food, Bisphenol A, Food, Diglycidyl ether of bisphenol A (DGEBA)

### INTRODUCTION

Glycidyl ethers are basic components of epoxy resins which have been commercially available. Bisphenol A diglycidyl ether and its oligomers are major components of epoxy resins. Other glycidyl ethers, including phenyl glycidyl ether, are frequently incorporated into epoxy resin systems as reactive modifiers. Epoxy resins based on bisphenol A diglycidyl ether are widely used in protective coatings, including paints, in reinforced plastic laminates and composites, in tooling, casting and moulding resins, in bonding materials and adhesives, and in floorings and aggregates. Occupational exposure to bisphenol A diglycidyl ether may occur during its production, during the production of epoxy products and during various uses of epoxy products, but data on exposure levels are sparse [IARC, 1989].

John E. Biles et al [1999] reported migration of the diglycidyl ether of bisphenol A (DGEBA) to food from can coatings. Derivatives of DGEBA were also determined in some foods. Levels of DGEBA in the foods range from non-detected (<0.3 ppb) to 50 mg/kg. It was determined by liquid-liquid extraction or solid-phase extraction coupled with high-pressure liquid chromatography with fluorescent detector. Theobald A et al [2000] quantified levels of bisphenol-F-diglycidyl ether (BFDGE) as part of a European survey on the migration, into oil from canned fish, of residues of epoxy resins. The analysis was performed using reverse phase HPLC with fluorescent detector. BFDGE could be detected in 12% of the fish, 24% of the cans and 18% of the lids. Only 3% of the fish contained BFDGE in concentrations above 1 mg/kg.

Hammarling L et al [2000] investigated the presence of BADGE and the chlorohydroxy compounds (BADGE.HCl and BADGE.2HCl) in various kinds of canned foods by HPLC with fluorescent detector. BADGE was found in levels up to 5.1 mg/kg in the food. The results indicated that the migration of BADGE.HCl and BADGE.2HCl, compounds with almost no data on toxicity, implies a greater problem than BADGE.H<sub>2</sub>O and BADGE.2H<sub>2</sub>O.

Berger U et al [2001] investigated a reversed phase high performance liquid chromatographic method combined with fluorescent and mass spectrometric detector in series was presented for the separation and quantification of bisphenol A diglycidyl ether (BADGE) and novolac glycidyl ether (NOGE) derivatives in food can coatings, tuna and oil. The highest values found were 20 micrograms/g in tuna and 43 micrograms/g in the oil phase.

Simoneau C et al [2002] investigated the migration of bisphenol-A-diglycidyl-ether (BADGE) into vegetable oil from processed and non-processed cans as a function of the process treatment and the temperature of storage. The results revealed that temperature processing had the largest effect on migration of BADGE. Storage temperature also affected migration from non-processed cans. The results of migration at higher temperatures were also correlated to the potential degradation of BADGE from oxidation products.

Leepipatpiboon N et al [2005] developed and validated a gradient reversed-phase liquid chromatographic method with fluorescence detection for bisphenol-A-diglycidyl ether (BADGE), bisphenol-F-diglycidyl ether (BFDGE). The method detection limits were 0.72-4.20 ppb and the method quantitation limits were 2.40-14.85 ppb. The validation data indicate excellent precision, acceptable recovery, and good robustness. This supports a good potential to further develop a standard method for the determination of migrations from interior can coatings into foodstuffs.

Petersen H et al [2008] elucidated the fate of BADGE and identified Food proteins as the main reaction partner with BADGE. The hydrolysis and hydrochlorination derivatives subject to European legislation make up only a fraction of the totally migrated BADGE.

## REFERENCES

1. IARC (1989) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 47,
2. Some Organic Solvents, Resin Monomers and Related Compounds, Pigments and Occupational
3. Exposures in Paint Manufacture and Painting, Lyon, pp. 237–261
4. John E. Biles,\* Kevin D. White, Timothy P. McNeal, and Timothy H. Begley. Determination of the Diglycidyl Ether of Bisphenol A and Its Derivatives in Canned Foods. *J. Agric. Food Chem.*, 1999, 47 (5), pp 1965–1969.
5. Theobald A, Simoneau C, Hannaert P, Roncari P, Roncari A, Rudolph T, Anklam E. Occurrence of bisphenol-F-diglycidyl ether (BFDGE) in fish canned in oil. *Food Addit Contam.* 2000 Oct;17(10):881-7
6. Hammarling L, Gustavsson H, Svensson K, Oskarsson A. Migration of bisphenol-A diglycidyl ether (BADGE) and its reaction products in canned foods. *Food Addit Contam.* 2000 Nov;17(11):937-43.
7. Berger U, Oehme M, Girardin L. Quantification of derivatives of bisphenol A diglycidyl ether (BADGE) and novolac glycidyl ether (NOGE) migrated from can coatings into tuna by HPLC/fluorescence and MS detection. *Fresenius J Anal Chem.* 2001 Jan 2;369(2):115-23.
8. Simoneau C, Theobald A, Roncari P, Hannaert P, Anklam E. Time-temperature study of the kinetics of migration of BADGE (bisphenol-A-diglycidyl-ether) into a fatty medium. *Food Addit Contam.* 2002;19 Suppl:73-8.
9. Leepipatpiboon N, Sae-Khow O, Jayanta S. Simultaneous determination of bisphenol-A-diglycidyl ether, bisphenol-F-diglycidyl ether, and their derivatives in oil-in-water and aqueous-

- based canned foods by high-performance liquid chromatography with fluorescence detection. *J Chromatogr A*. 2005 May 6;1073(1-2):331-9.
10. Petersen H, Biereichel A, Burseg K, Simat TJ, Steinhart H. Bisphenol A diglycidyl ether (BADGE) migrating from packaging material 'disappears' in food: reaction with food components. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 2008 Jul;25(7):911-20.



# *Researcher*

ISSN: 1553-9865

The international academic journal, “*Researcher*” (ISSN: 1553-9865), is registered in the United States, and invites you to publish your papers.

All authors are welcome to submit manuscripts in all fields and the manuscripts will be processed in a professional peer review. After the peer review, *Researcher* will make the best efforts to publish all the valuable works as soon as possible. Any valuable papers that describe natural phenomena and existence or any reports that convey scientific research and pursuit are welcome, including both natural and social sciences. Papers submitted could be reviews, objective descriptions, research reports, opinions/debates, news, letters, and other types of writings that are nature and science related.

This journal will be no charge for the manuscript contributors. If the author needs a hard copy of the journal, it will be charged for US\$80/issue to cover the printing and mailing fee. Here is a new avenue to publish your outstanding reports and ideas. Please also help spread this to your colleagues and friends and invite them to contribute papers to the journal. Let's work together to disseminate our research results and our opinions.

Papers in all fields are welcome.

Email submission: [sciencepub@gmail.com](mailto:sciencepub@gmail.com)

Online submission: [Manuscript Submission](#); [Manuscript Submission](#) (Chinese)

**For more information, please visit:**

<http://www.sciencepub.org>

<http://www.sciencepub.net>

Marsland Press  
525 Rockaway PKWY, #B44  
Brooklyn, New York 11212, the United States  
Telephone: 718-404-5362  
[sciencepub@gmail.com](mailto:sciencepub@gmail.com)  
<http://www.sciencepub.net>  
<http://www.sciencepub.org>

# Researcher

ISSN 1553-9865

Marsland Press  
525 Rockaway PKWY, #B44  
Brooklyn, New York 11212, the United States  
Telephone: 718-404-5362  
[sciencepub@gmail.com](mailto:sciencepub@gmail.com)  
<http://www.sciencepub.org>  
<http://www.sciencepub.net>

ISSN 1553-9865



9 771553 986042