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STUDIES ON NUTRITIONAL AND ANTI-NUTRITIONAL COMPOSITION OF *BAMBUSA MULTIPLEX* (LOUR.) RAEUSCH. EX SCHULT

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ABSTRACT

Bambusa multiplex, a multipurpose ornamental and commercial bamboo species used for hedges, construction, basketing and as handicraft material. The shoots are edible and consumed in Southeast Asia and in North eastern regions of India. As earlier investigations does not emphasize on finding out the harvesting time, an attempt has been made to find out the harvesting time to obtain quality shoots. The bamboo shoots were harvested on different days (7-30 days after emergence from ground) and analysed for chemical, nutritional and anti-nutritional components. The shoots harvested at various time intervals showed variation in nutritional composition with an overall decrease in protein and increase in dietary fiber and carbohydrate content. All the nutritional elements except calcium showed decreased content with shoot maturity. The optimum harvesting age for *B. multiplex* shoots were found to be 7- 10 days with high nutritional content and anti-nutritional component, cyanide was found to be completely absent.

KEYWORDS: ***Bambusa multiplex*, shoots, chemical composition, nutritional content, anti-nutritional component, harvesting age.**

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INTRODUCTION

Bamboo is an enduring, versatile and highly renewable resource in the Indian socio-economic- cultural- ecological- climatic-functional context known for their multiple uses[1]. A bamboo shoot is the new tender growth of young culm from the rhizome apex having compressed internodes which are protected by numerous leathery sheaths. They rank among the five most popular healthcare foods in the World[2]. The edible shoots are considered as ideal vegetable, being low in calories, high in dietary fiber and rich in mineral elements additionally packed with numerous phytonutrients and hence known as "power food" [3]. They contain many secondary metabolites which are used as precursors in pharmaceutical industries [4]. In industrially important bamboo species success have been made in tissue culture techniques for commercial cultivation [5-7]. The bamboo shoots possess number of health benefits like improving appetite and digestion, controlling weight loss, curing cardiovascular disease and show antioxidant [8], anti-inflammatory [9-10] and anti-cancer [11] properties. The bamboo shoots are known to protect neurons from oxidative stress and show anti-fatigue activity [12]. The bamboo shoots are available for a short period, being seasonal, perishable and are consumed raw, dried, canned, boiled and fermented forms [13]. At present, over two million tonnes of bamboo shoots are consumed in the World annually [14]. In the International market, China earns 6500 million rupees annually from export of edible shoots, USA imports around 44,000 tonnes accounting for 14.5% of the World's total import, out of which 30,000 tonnes are canned bamboo shoots from Taiwan, Thailand and China [13]. Though, India has second largest bio-resource of bamboo species after China, but systematic steps have not been taken to exploit the immense potential of the valuable resource available in India, except being used as vegetable by the rural people. The nutritional value of edible shoots of bamboo species has been carried out by several workers [15-19].

Besides having nutritional content, the bamboo shoots also contain anti-nutritional elements that are toxic and require removal before human consumption [20]. The anti-nutritional elements are cyanogenic glycosides which are nitrogenous phytoanticipins[21] and are used by various plants in defense mechanism against the predators [22]. However, subsequent processing such as chopping of tender shoots into small pieces, partial drying of fresh shoots, boiling in water/salt water and draining or keeping shoots in hot water / water for a week at ambient temperature helps to remove the cyanide concentration [22]. Studies have shown that the cyanogenic glycosides of bamboo shoots are hazardous resulting in acute cyanide poisoning in human, bird, fish, wildlife and livestock [23-25]. The cyanogenic glycosides are assessed using various methods [26-30]. These methods are highly complex and expensive. The present study reports an improvised method for cyanide determination by spectrophotometric method. *Bambusa multiplex* (Lour.) Raeusch. ex Schult. commonly known as 'Hedge Bamboo' is indigenous to China and Japan and cultivated in many Asian countries such as India, Sri Lanka, Myanmar, Malaya, Bangladesh [31]. It is an ornamental and commercial bamboo species largely used for hedges, construction, basketing and handicrafts materials. The shoots of this bamboo species are edible and consumed in Southeast Asian countries such as Taiwan [32, 2] and in North-eastern regions of India such as Meghalaya and Manipur [2], where they serve as popular ingredient of local cuisine. However, there still exists paucity of information on the detailed nutritional and anti-nutritional constituents in this species. Hence, the present work undertaken deals with assessment of the chemical composition and harvesting period of *B. multiplex* shoots.

MATERIALS AND METHODS

The germplasm consisting of three genotypes of *B. multiplex* were procured from Bambusetum, Kerala Forest Research Institute, Velupaddam, Kerala, India and were maintained in the Departmental garden of

Biotechnology, Bangalore University, Bengaluru under uniform conditions. The plant material was authenticated by Bamboo taxonomist Dr. Muktesh Kumar MS, KFRI, Kerala, India. The young fresh succulent *B. multiplex* shoots collected from the maintained germplasm (2 year old) were harvested at different days (7-30) after emergence from tground during the onset of rainy season (May-June). The collection of material was done during morning hours to reduce the water loss due to transpiration and evaporation.

Physical properties

Measurements on length, circumference, fresh weight, percentage of the peel and edible portion of *B. multiplex* shoots were recorded. After peeling off of the sheaths, shoots were washed under running tap water to remove adhering soil particles. The inner soft portion of shoots were analyzed for the nutritional and anti-nutritional composition to determine suitable age for harvesting bamboo shoots.

Chemical, nutritional and anti-nutritional composition

The moisture and ash content were determined by AOAC method [33]. The moisture content was determined gravimetrically by subjecting the powdered shoots to oven drying at 105±3ºC for 5 hours. The ash content was gravimetrically determined by incinerating the sample using Muffle furnace. The crude protein was determined using Kjeldahl method [34]. The fat content was determined after extraction with petroleum ether using Soxhlet apparatus [34]. The dietary fiber was determined by Enzymatic Gravitational method [34]. The carbohydrate content was estimated by Colorimetric method using anthrone reagent [35]. The mineral content of bamboo shoots were determined using Atomic Absorption Spectrophotometer. Potassium content was determined by Wet Digestion method through Flame photometer and phosphorous was estimated Colorimetrically by Molybdenum Blue method. Acidity value was determined titrimetrically and Ascorbic acid content was determined by titrimetric method using 3% metaphosphoric acid [36-37]. The hydrogen cyanide was estimated by Pyridine barbituric acid Spectrophotometric method [38].

Data Analysis

All the experimental data were derived from triplicates each containing three sets. The results were expressed as mean ±SD of three independent experiments.

RESULTS AND DISCUSSION

The time of harvest determines the quality and quantity of the product. Hence, a proper harvesting practice maintains the quality to a greater extent. The bamboo shoots when harvested early offers small sized shoots besides having high cyanogens content rendering it unfit for consumption. Late harvesting make the bamboo shoots woody and tough. Generally, the bamboo shoots are harvested when they attain a height of about 38-45cm from the ground level (4-9 days old) [3]. The bamboo shoots are harvested during morning or evening hours to avoid greater field heat [3]. For harvesting of bamboo, strong and healthy shoots are selected. The shoots are cut above 1-2 inch from the ground, just above the neck of rhizome, cutting to make a curve upwards away from the rhizome [3].

Physical properties

The physical attributes tested to ascertain quality parameters of *B. multiplex* shoots are shown in Table 1. Ten shoots were taken at random for all the parameters tested and average values of these parameters were considered. The shoots harvested of 7-10 days old showed an average length of 30 cm, circumference of 8.50 cm, weighing 350 gm, peel of 69% and edible portion of 35%. Correspondingly the shoots harvested at 10-20 days and 20-30 days showed an average length of 70 cm and 110 cm, circumference of 14 cm and 19. cm, weighing 720 gm and 950 gm, peel of 80% and 92% and edible portion of 65% and 85% respectively. The increase in edible portion at 10-20 days and 20-30 days in *B. multiplex* is attributed to high moisture content.

Chemical, nutritional and anti-nutritional composition

The quality evaluation of *B. multiplex* shoots were affirmed in terms of chemical analyses of constituents such as percentage of moisture, ash, protein, carbohydrate, fat, fiber, NDF, ADF, lignin, acidity, besides TSS and ascorbic acid. The values obtained for chemical constituent and nutritional composition of the shoots are shown in Table 2A. The shoots harvested at 7-10 days showed 87.47 % moisture content with ash, protein, carbohydrate , fiber content of 0.97, 3.14, 0.80 and 4.28 % by mass respectively. The fiber constituents like NDF, ADF and lignin showed 6.73, 3.53 and 3.12 % by mass respectively. Fat content was absent in presently studied *B. multiplex* shoots, which contradicts the earlier reports on various bamboo species [15]. The *B. multiplex* presently investigated showed moisture content of 87.47%, which is the lowest value shown in bamboo species as reported earlier. The moisture content in *Dendrocalamus hamiltonii* showed 91.06% [19], *Bambusa nutans* of 94.70%, *B. tulda* of 92.8%, *Melocanna baccifera* of 93.0% [15]. The analysis of ash content (0.97%) in the present species investigated is in close proximity with the values reported in *D. hamiltonii* (0.81 percent) [19], *Dendrocalamus asper* (0.80%), *B. nutans* (0.90%) and *Bambusa vulgaris* (0.80%) [16]. However, higher ash content of 3.70% was recorded in *Teinostachyum wightii* [15]. These variations in chemical content could be attributed to the inherent genetic variation of bamboo species [19]. The protein content (3.14%) in *B. multiplex* on par with other edible bamboo species with *D. hamiltonii* showing 3.50% [19] and *B. tulda* showing 3.40% [15]. The high crude fibre content (4.28%) recorded during the present study was in contrast with the report on *D. hamiltonii* showing low fibre content of 1.50 % [19] ; *D. asper*, *D. strictus, B. tulda* and *B. bambos* showing 0.72%, 0.96%, 0.75% and 0.82%, respectively [39]. In the present study, *B. multiplex* showed TSS value of 7.6º B, acidity of 0.12 % and ascorbic acid content of 1.12 mg/100g . However, higher ascorbic acid value of 5.30 mg/100g was reported in *Dendrocalamus hamiltonii* [19] and 3.0 mg/100g in *D. sikkimensis* [15]. The chemical analyses of *B. multiplex* shoots revealed the potential applications of these shoots as food item due to the presence of rich mineral content like Ca, Na, K, P and Mg in Table 3A. The calcium content (29.4 mg/100g) and selenium 'miracle life element' content (8.1 µg/100g) was also found to be highest when compared to other edible bamboo species [15, 19, 40]. The difference in the values of mineral content could be attributed to the varietal difference and agro-climatic conditions in which the plants are grown [19]. Shoots of *B. multiplex* shoots subjected to late harvesting (10-20 days and 20-30 days of shoot emergence) revealed that, with increased harvesting time the mineral and ash content were decreased with rise in lignin content (Table 3B and 3C). When bamboo gets older, its ability of taking up nutrients from soil is less [23]. The growth period of bamboos are completed within a short period after sprout emergence, but lignification proceeds even after cessation of growth. In *B. multiplex*, late harvesting of the shoots decreased the nutrient elements such as copper, zinc, cobalt, phosphorus, iron and potassium content. The present study also revealed higher calcium content during late harvesting of the shoots compared to early harvesting. In the present study, *B. multiplex* shoots showed complete absence of cyanide content usually detected in traces in the edible bamboos[20]. During the study, simple and efficient method Pyridine barbituric acid spectrophotometric method [38] was used to determine cyanogen content and compared with the conventional method using Picric acid [26]. In this method, hydrogen cyanide measured at 578nm was found to be undetected against the standard cyanide solution, indicating a robust method for cyanide detection in bamboo species.

Table 1
Physical properties of B. multiplex shoots

Harvesting period (days)	Length (cm)	Circumference (cm)	Weight of shoot (gm)	Peel (%)	Edible portion (%)
7-10	30.00± 0.05	8.50± 0.65	350±1.0	69.00±0.05	35.00±0.15
10-20	70.00± 0.08	14.00± 0.90	720± 1.4	80.00± 0.07	65.00± 0.20
20-30	110.00± 0.10	19.00± 0.20	950± 1.7	92± 0.09	85.00± 0.25

All values are in Mean±SD

Table 2 (A)
Chemical and nutritional composition of B. multiplex shoots (7-10 days old)

Parameters	Values
Moisture (%)	87.47±0.49
Ash (%)	0.97±0.08
Protein (%)	3.14±0.18
Fibre (%)	4.28±0.45
NDF (%)	6.73±0.52
ADF (%)	3.53±0.21
Lignin (%)	3.12 ±0.19
TSS (ºB)	7.6±0.58
Acidity (%)	0.12±0.65
Ascorbic acid (mg/100g)	1.12±0.06
Carbohydrate (%)	0.80 ± 0.07
Fat (%)	Nil

All values are in Mean±SD

Table 2 (B)
Chemical and nutritional composition of B. multiplex shoots (10-20 days old)

Parameters	Values
Moisture (%)	94.47±0.39
Ash (%)	0.98±0.07
Protein (%)	1.48±0.24
Fibre (%)	2.82±0.38
NDF (%)	3.57±0.42
ADF (%)	2.35±0.16
Lignin (%)	3.18 ±0.39
TSS (ºB)	6.8±0.45
Acidity (%)	0.10±0.58
Ascorbic acid (mg/100g)	1.10±0.04
Carbohydrate (%)	0.50 ± 0.05
Fat (%)	Nil

All values are in Mean±SD

Table 2 (C)
Chemical and nutritional composition of B. multiplex shoots (20-30 days old)

Parameters	Values
Moisture (%)	95.47±0.42
Ash (%)	0.87±0.08
Protein (%)	2.14±0.10
Fibre (%)	2.28±0.35
NDF (%)	2.73±0.26
ADF (%)	1.53±0.11
Lignin (%)	4.10 ±0.17
TSS (ºB)	5.6±0.36
Acidity (%)	0.09±0.52
Ascorbic acid (mg/100g)	1.09±0.05
Carbohydrate (%)	0.40± 0.45
Fat (%)	Nil

All values are in Mean±SD

Table 3(A)
Nutritional (mineral content) and anti-nutritional composition of B. multiplex shoots (7-10 days old)

Parameters	Values
Calcium (mg/100g)	29.4±3.08
Copper (mg/100g)	1.07±0.2
Magnesium (mg/100g)	20±2.03
Selenium (µg/100g)	8.1± 0.55
Phosphorous (mg/100g)	54.00± 0.14
Potassium (mg/100g)	195±15
Sodium (mg/100g)	3.20±0.04
Iron (mg/100g)	2.5±0.03
Manganese (mg/100g)	1.22±0.23
Cyanide	Undetected

All values are in Mean±SD

Table 3(B)
Nutritional (mineral content) and anti- nutritional composition of B. multiplex shoots (10-20 days old)

Parameters	Values
Calcium (mg/100g)	32.4±2.16
Copper (mg/100g)	1.05±0.10
Magnesium (mg/100g)	21±1.80
Selenium (µg/100g)	7.6± 0.52
Phosphorous (mg/100g)	42.00± 0.12
Potassium (mg/100g)	188±11
Sodium (mg/100g)	2.80±0.02
Iron (mg/100g)	1.6±0.03
Manganese (mg/100g)	1.16±0.11
Cyanide	Undetected

All values are in Mean±SD

Table 3(C)
Nutritional (mineral content) and anti- nutritional composition of B. multiplex shoots (20-30 days old)

Parameters	Values
Calcium (mg/100g)	35.4±3.08
Copper (mg/100g)	1.03±0.24
Magnesium (mg/100g)	16±2.03
Selenium (µg/100g)	7.1± 0.55
Phosphorous (mg/100g)	38.00± 0.14
Potassium (mg/100g)	185±15
Sodium (mg/100g)	2.20±0.04
Iron (mg/100g)	1.32±0.01
Manganese (mg/100g)	1.12.±0.09
Cyanide	Undetected

All values are in Mean±SD

CONCLUSION

The edible usage of *B. multiplex* shoots at present has been restricted only to certain North-eastern regions of India. However, with the advent of multi-cuisine culture and with people willing to explore new culinary frontiers especially in urban areas, the bamboo shoots hold great promise. The present investigation on nutritional analyses in *B. multiplex* shoots revealed high levels of mineral and fiber content, which could be further explored and extended for edible purpose at large. The high fiber content of bamboo shoot helps in lowering the cholesterol content in blood. The minerals like potassium, phosphorus, sodium, magnesium, calcium, etc present in bamboo species including *B. multiplex* are absent in many common vegetables. Due to high

potassium content in shoots of *B. multiplex*, they can be considered as heart protective vegetable. The high Selenium content in the species studied act as antioxidant agent which helps to prevent cellular damage from free radicals, regulate thyroid function and play significant role in the immune system. During the study, the absence of cyanide content reduces the procedure for processing *B. multiplex* shoots. The present study also serves as basis for developing food products using the shoots of *B. multiplex*. This species studied has vast potential for developing into novel, innovative and promising product of food industry. The plant is edible having rich protein and fiber content with no fat content and thus can be recommended for cardiac and diabetic patients. This paper throw light on usage of *B. multiplex* shoots as food resource with a holistic perspective.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Borah D., Pathak KC., Dera B., Neog D.,Borah K. Utilization aspects of Bamboo and its market value. Indian Forester, 423-427, (2008).
2. Chongtham N., Bisht MS., Harrongbam S. Nutritional Properties of Bamboo Shoots: Potential and Prospects for Utilization as a Health Food. Compr Rev Food Sci and Food Saf, 10(3):153-169, (2011).
3. Rai S. Edible Bamboo Shoot- A Review. Bulletin of Arunachal Forest Research, 23 (1& 2): 39-44, (2007)
4. Sarangthem K., Singh TN. Microbial bioconversion of metabolites from fermented succulent bamboo shoots into phytosterols. Curr Sci, 84:1544-1547, (2003a)
5. Goyal AK., Pradhan S., Basistha BC., Sen A. Micropropagation and assessment of genetic fidelity of *Dendrocalamus strictus* (Roxb.) nees using RAPD and ISSR markers. 3 Biotech, DOI: 10.1007/s13205-014-0244-7, (2014).
6. Babitha B., Rathore TS., Thara Saraswathi KJ. *In vitro* propagation of *Bambusa vulgaris* cv. Wamin by axillary shoot proliferation. Int J Fund Appl Sci, 3(4): 53-58, (2014).
7. Arya ID., Kaur B., Arya S. Rapid and mass propagation of economically important Bamboo *Dendrocalamus hamiltonii*. Ind J Energy, 1(1): 11–16, (2012)
8. Goyal AK., Middha SK., Sen A. *In vitro* antioxidative profiling of different fractions of *Dendrocalamus strictus* (Roxb.) nees leaf extracts. Free Rad Antiox, 1(2):42–48, (2011)
9. Hu CH., Zhang Y., David DK. Evaluation of antioxidant and prooxidant activities of bamboo *Phyllostachys nigra* var. *henonis* leaf extract *in vitro.* J. Agric Food Chem, 48(8): 3170-3176, (2000).
10. Lu BY., Wu XQ., Tie X., Zhang Y. Toxicology and safety of antioxidant leaves. Part I: acute and subchronic toxicity studies on antioxidant of bamboo leaves. Food Chem Toxicol, 43(5): 783-92, (2005)
11. Shi QT., Yang KS. Study on relationship between nutrients in bamboo shoots and human health. Proceedings of the International Symposium on Industrial Use of Bamboo. International Timber Organisation and Chinese Academy of Forestry, Beijing, China: Bamboo and its Use. 338-346, (1992).
12. Akao Y., Seki N., Nakagawa Y., Yi H., Matusumoto K., Funaoka M., Maruyama W., Nozawa Y. A highly bioactive lignophenol derivative from bamboo lignin exhibit a potent activity to suppress apoptosis induced by oxidative stress in human neuroblastoma SH- SYSY cells. Bio and Med Chem, 12: 4791-801, (2004)

13. Pandey AK., Ojha V. Standardization of harvesting age of bamboo shoots with respect to nutritional and anti-nutritional components. J. Forestry Res, 24(1): 83-90, (2013)
14. Yang Q., Duan Z., Wang Z., He K., Sun Q., Peng Z. Bamboo resources, utilization and *ex situ* conservation in Xishuangbanna, South-eastern China. J Forest Resource, 19: 79-83, (2008)
15. Bhatt BP., Singh A., Singh K. Nutritional values of some commercial edible bamboo species of North Eastern Himalayan region, India. J. Bamboo Rattan, 4: 896-898, (2005)
16. Kumbhare V., Bhargava A. Effect of processing on nutritional value of central Indian Bamboo shoots. Part-1. J. Food Sci. Tech., 44: 29-31,(2007)
17. Nirmala C., David E., Sharma ML.Changes in nutrient components during ageing of emerging juvenile Bamboo shoots. Int. J. Food sci. Nut. 53:1-7, (2007)
18. Nirmala C., Sharma ML., David E. A comparative study of nutrient components of freshly emerged, fermented and canned bamboo shoots of *Dendrocalamus giganteus* Munro. J Am Bamboo Soc., 2: 33-39,(2008)
19. Sood S., Shivaniwalia., Mahesh Gupta., Anil Sood.Nutritional Characterization of Shoots and Other Edible products of an Edible Bamboo – *Dendrocalamus hamiltonii*. Curr. Research in Nutrit. And Food Sci., 1(2): 169-176, (2013)
20. Sarangthem K., Nabakumar Singh. Fermentation decreases the antinutritional content in bamboo shoots Int.J.Curr.Microbiol.App.Sci, 2(11): 361-369, (2013)
21. Zagrobelny M., Bak S., Rassmusen AV., Jorgensen B. Cyanogenic glucosides and plant – insect interaction. Phytochem, 65: 293-306, (2004)
22. Choudhary D., Sahu JK., Sharma GD., Bamboo shoot: Microbiology, Biochemistry and Technology of fermentation – a review. Indian J.Tradit. Knowle, 11(2): 242-249, (2012)
23. Rosenthal GA., Janzen DH., Ed.. Herbivores: Their interaction with Secondary plant metabolites, Academic Press, Inc: New York, London, 387-412, (1979)
24. Ballantyne B ., Marrs TC, Ed. Clinical and experimental toxicology of cyanides, IOP publishing, Wright, Bristol, 41-126 (1987)
25. Yamamoto K., Yamamoto Y., Hattori H., Samori T. Effects of routes of administration on the cyanide concentration distribution in the various organs of cyanide- intoxicated rats. Tohuke J. Exp. Med, 137: 73-78, (1982)
26. Bradbury MG., Egan SV.,Bradbury JH. Determination of all forms of cyanogens in cassava roots and cassava products using picrate paper kits. J Sci. Food Agric., 79: 593-601, (1999)
27. Haskins FA., Gortz HJ., Hill RM. Colorimetric determination of cyanide in enzyme-hydrolyzed extracts of dried sorghum leaves. J. Agric. Food Chem, 36: 775-778, (1988)
28. Palmer IS., Olson OE., Halverson AW., Miller R., Smith C. Isolation of factors in linseed oil meal protective against chronic selenosis in rats. J. Nut, 110: 145-150, (1980)
29. Schwarzmaier U., Uber die cyanogenese von *Bambusa vulgaris* und *B. guadua*. Chemisch Berichte, 109: 3379-3389, (1976)
30. Schwarzmaier U., Cyanogenesis of *Dendrocalamus*: taxiphyllin. Phytochem, 16: 1599-1600, (1977)
31. Seethalakshmi KK., Muktesh Kumar MS. Sankara Pillai M Sarojam N Ed.Bamboos of India: A Compendium, BRILL publisher: 59-60, (1998)
32. ERG. Report on process, market and business opportunity report on edible bamboo shootprepared by Engineering resources Group, Bangalore in association with CPF, FRESH and Delphi, Engineering Resource Group, 4-7, (2003)(Internet). Available from: http://www.bambootech.org/files/ERG%20%report%20on%20shoots.pdf.2004

33. AOAC Official Methods of Analysis, Association of Official Analytical Chemists, 11thEdn, Washington DC, USA, (2005)
34. AOAC Official Methods of Analysis. Association of Official Analytical Chemists, 11th Edn, Washington DC, USA, (1990)
35. Sadasivam S and Manickam A, Biochemical methods for agricultural Science, Wiey Eastern Limited: New Delhi, 11-12, (1992).
36. Mazumdar BC., Majumder K., Methods on new physico-chemical Analysis of Fruits, College of Agriculture, Calcutta University, Vol. IV, 108-109 (2003)
37. Anderson JM, Ingram JSI, Ed. Tropical Soil Biology and Fertility: A Handbook of Methods, 2nd Edn CAB International:Wallingford, (1993)
38. Method 426, Determination of Cyanide emission from stationery source, State of California Air Resource Board., 1-22, (1987).