Bioprospecting *Melocanna baccifera* Roxb. – a phytotechnological approach

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DOCTOR OF PHILOSOPHY

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Dedicated to my
parents

Contents

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DECLARATION

I hereby declare that the work presented in the thesis titled “**Bioprospecting *Melocanna baccifera* Roxb. - a phytotechnological approach”** has been carried out by me under the supervision of **Prof. M.N.V. Prasad, Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad - 500 046,** and that this work has not been submitted for any other degree or diploma to any University or Institute.

3/8/2011

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CERTIFICATE

This is to certify that the research work in the thesis titled “**Bioprospecting *Melocanna baccifera* Roxb. - a phytotechnological approach”** has been carried out by **Mr. H. Lalhruaitluanga** under my supervision for the full period prescribed under the Ph.D ordinance of this University and that this work has not been submitted for any other degree or diploma to any University or Institute.

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H. Lalhruaitluanga

Abbreviations

AC	Activated carbon
b	Bonding energy
BIS	Bureau of Indian Standard
CC	Column Chromatography
C_e	Concentration of sorbate in solution
CEC	Capillary Electro-Chromatography
COI	Cycloeucalenol Obtusifoliol Isomerase
CTAB	Hexadecyl tri-methyl ammonium bromide
CVD	Cardiovascular disease
DMAPP	Dimethylalkyl diphosphate
DMEM	Dulbecco’s modified eagle medium
DMSO	Dimethyl sulphoxide
dNTPs	Deoxynucleotide triphosphates
D-R	Dubinin-Radushkevich
EDS	Energy-dispersive X-ray spectroscopy
EDTA	Ethylenediaminetetraacetic acid
EI	Electron Impact
ELSD	Evaporative Light Scattering Detection
EPA	Environmental Protection Agency
EPR	Electron paramagnetic resonance
ER	Endoplasmic reticullum
EXAFS	Extended X-ray Absorption Fine Structure Spectroscopy
FBS	Fetal Bovine Serum
FID	Flame Ionization Detection
FPP	Farnesyl pyrophosphate
FT-IR	Fourier Transformed Infrared Spectroscopy
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrocopy
HMGR	3-hydroxymethyl-3-glutaryl coenzyme A reductase
HPLC	High Performance Liquid Chromatography
hs-CRP	C-reactive protein
IDL	Intermidiate density lipopropein
IL-6	Interleukin-6
IPP	Isopentenyl pyrophosphate
ISSR	Inter-Simple-Sequence-Repeats
K_f	Adsorption capacity
LB	Liebermann-Burchard
LB	Luria Bertani
LDL	Low-density lipoprotein
MBAC	*Melocanna baccifera* activated charcoal
MBRC	*Melocanna baccifera* raw charcoal
MS	Mass Spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMR	Nuclear Magnetic Resonance
NPC1L1	Niemann–Pick C1-like 1
NTSYS	Numerical Taxonomy and Multivariate Analysis System

OPA	Operon primer A
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PS	Phytosterol
q_e	Sorbate uptake by the sorbent
q_{max}	Maximum adsorption capacity
RAPD	Random Amplified Polymorphic DNA
R_f	Retension factor
SEM	Scanning Electron Microscope
SFE	Supercritical Fluid Extraction
SFF	Supercritical Fluid Fractionation
SMT2	$C24^{1}$-methyltransferase
TBE	Tris-borate-EDTA
TLC	Thin-Layer Chromatography
TNF-α	Tumor Necrosis Factor-alpha
TSS	Toxic Shock Syndrome
UBC	University of British Columbia
UPGMA	Unweighted Pair-Group Method with Arithmetical average
XPS	X-ray photoelectron spectroscopy
XRD	X-Ray Diffraction

1 General introduction

1.1. Bioprospecting

Bioprospecting is the study of plant, microbe and animal resources of commercial value. It is a rapidly developing field wherein researchers across disciplines are involved in building data base of the commercially potential bioresources (Fig 1**.** This involves the collection and study of biological samples from plants, animals and microbes, as well as gathering information from indigenous peoples and the knowledge, innovations, and customary practices of local communities associated with those plants, microbes and animals resources; for purposes of research, product development, conservation or industrial or commercial application, and includes investigative research and sampling, but does not include customary uses of resources and derivatives.

Several bioprospecting strategies of bioresources have been initiated at global level for the benefit of human welfare by various scientists. Bioprospection is intended for economic uses (e.g., new drugs discovery, crops, industrial products etc). Bioprospection of under explored/unexplored plant resources have been relevant for the improvement of the rural/tribal community and economy. The scientific and indigenous knowledges improved the bioprospecting of bio-resources. The bioprospecting of plant and living organisms for industrial products, pharmaceuticals etc purposes are useful not only for scientific industries engaged in R & D but also for the country and the indigenous community, who gain from ownership of these resources and expect good compensation for biological resource use, especially after the Convention on Biological Diversity (CBD) in 1992. Before 1992, biological resources were considered a common heritage of humankind, as scientists could take samples from anywhere in the world without any specific permission. But now, the convention clearly establishes the control and sovereignty of local agency over the biological resources and its diversity (Kumar and Tarui, 2004).

Bioprospecting collaborations between scientists, companies and the countries supplying the raw material and knowledge offer not only the revenue resource for

“Bioprospecting”

…is the study of plant, microbe and animal resources of commercial value. It is a rapidly developing field wherein researchers across disciplines are involved in building data base of the commercially potential bioresources.

Figure 1. Biopropecting of *Melocanna baccifera* – its importance for non-timber building material, rich folklore, environmental remediation, protection of mountain ecology, nutraceuticals and value addition value.

under-developed countries, but also opportunities for the society for better education and employment avenues. Many studies have suggested that if the bioprospecting search is based on the information and knowledge from local people, then the value of the bioprospecting benefits will be higher (Martin, 2001).

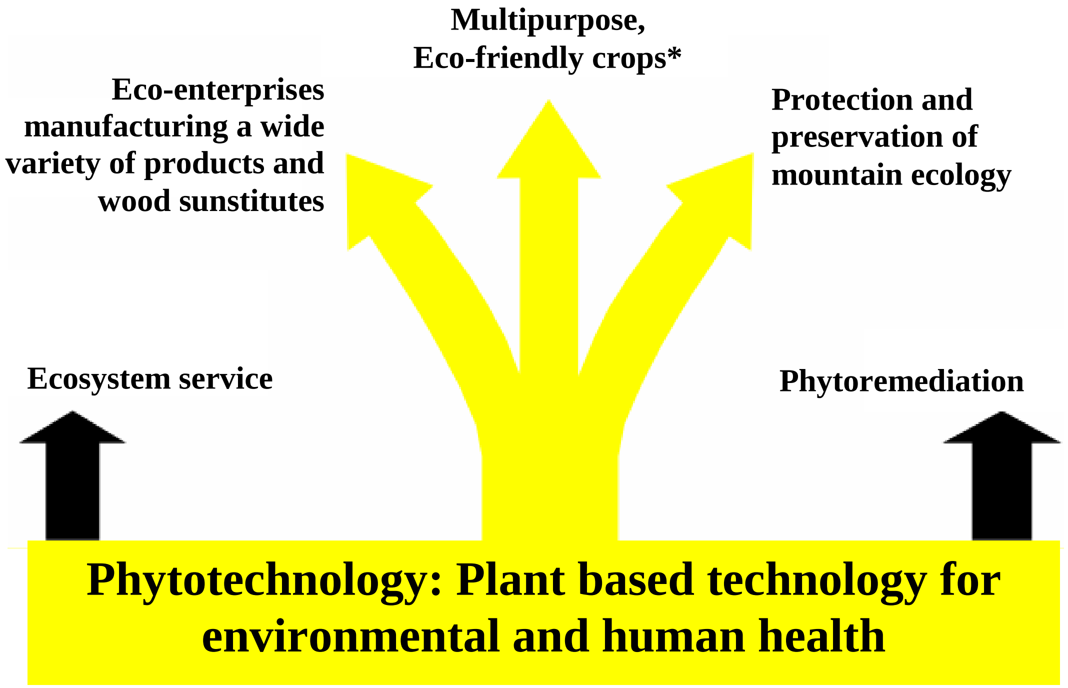
1.2. Phytotechnology

Phytotechnologies are plant based technologies for environmental and human health. Phytoremediation, ecosystem services and multipurpose eco-friendly crops for protection of catchment and watershed management, protection and preservation of mountain ecology and co-generation of economic phytoproducts is the scope of this emerging area (Fig 2). It is an emerging technology that has the potential to treat a wide range of contaminants for a lower cost than traditional technologies. This technology uses various types of plants and plant products to degrade, extract, contain, or immobilize contaminants in soil and water. Phytotechnology has been used for remediation of chlorinated solvents, metals, explosives and propellants, pesticides, polycyclic aromatic hydrocarbons, radionuclides, and petroleum hydrocarbon compounds.

While Phytotechnologies generally are applied in situ, ex situ applications (e.g., hydroponics systems) are possible. Typical organic contaminants, such as petroleum hydrocarbons, gas condensates, crude oil, chlorinated compounds, pesticides, and explosive compounds, can be addressed using plant-based methods. Phytotechnologies also can be applied to typical inorganic contaminants, such as heavy metals, metalloids, radioactive materials, and salts.

The term phytotechnology describes the application of science and engineering to examine problems and provide solutions involving plants. The term itself is helpful in promoting a broader understanding of the importance of plants and their beneficial role within both societal and natural systems. A central component of this is the use of plants as living technologies that provide services in addressing environmental issues.

Phytotechnology provides numerous advantages for supporting sustainable water quality sanitation, and environmental conservation. Studies and application of phytotechnology for water sanitation and conservation are still limited in Asian



*Protection of catchment, regulation of water flow, recharge of water table, conservation flora and fauna etc; support to developmental activities such as roads, bridges, hydel projects and human settlements

Figure 2. Phytotechnologies are plant based technologies for environmental cleanup, human health including production of value additions

countries. More efforts should be undertaken to explore scientific information and the use of this technology. Furthermore, some phytotechnology applications involving the use of plants for housing, food, forage and sources of medicine can create employment. This is particularly important in developing countries.

1.3. Study area and bamboo forest of Mizoram

Mizoram is located at the extreme southern part of the north eastern India. It lies between 21^{o}58’ to 24^{o}30’ N latitude and 92^{o}15’ to 93^{o}29’ E longitude covering an area of 21,081 sq. km. The width of the state from east to west is 121 km and the length from north to south is 277 km. Mizoram is flanked by Manipur state and Cachar District of Assam in the northern site, Chin Hills of Myanmar in the east, Chittagong Hills of Bangladesh and Tripura state in the west and Arakan Hill ranges of Myanmar in the southern site. The entire terrain is mostly mountainous and hilly with precipitous slopes forming deep gorges culminating into several streams and water falls. Almost all the hill ranges traverse in the north-south direction. The state enjoys a sub-tropical humid climate (temperature being 11^{o}C to 32^{o}C) with high rainfall (2000 mm to 3500 mm) coming from south-west monsoon. The climatic condition is inductive to the vigorous growth of vegetation (Lalramnghinglova, 2003).

Forests and forestry constitute dominant feature of the state’s landscape, economy and environment. Bamboo is one of the major constituent of the state forest vegetation. The distribution of bamboo in Mizoram is shown in Fig 3. According to the forest survey of India, state of forest report 2003, the forests covered 87.42% out of 21,081 sq. kms of the total geographical area of the state. But due to the age-old traditional practice of shifting agriculture of jhuming, uncontrolled spread of wild fire, unregulated grazing and land allotment to private hand and individuals, the area has already been degraded. Such depleted and partly degraded forest could not meet the growing demands of timber and other forest produce in the state. The landscape, bamboo forest and slash and burn agriculture of jhuming is shown in Fig 4.

Bamboo forests are evergreen forests in tropical and subtropical regions, occupying about 1.5–2% of the world’s total forest area (Zhuang et al., 2011). Bamboo forests can mitigate climate change, in order to mitigate climate change, more carbon should be sequestered in forest ecosystems (Zhou et al., 2011). Bamboo

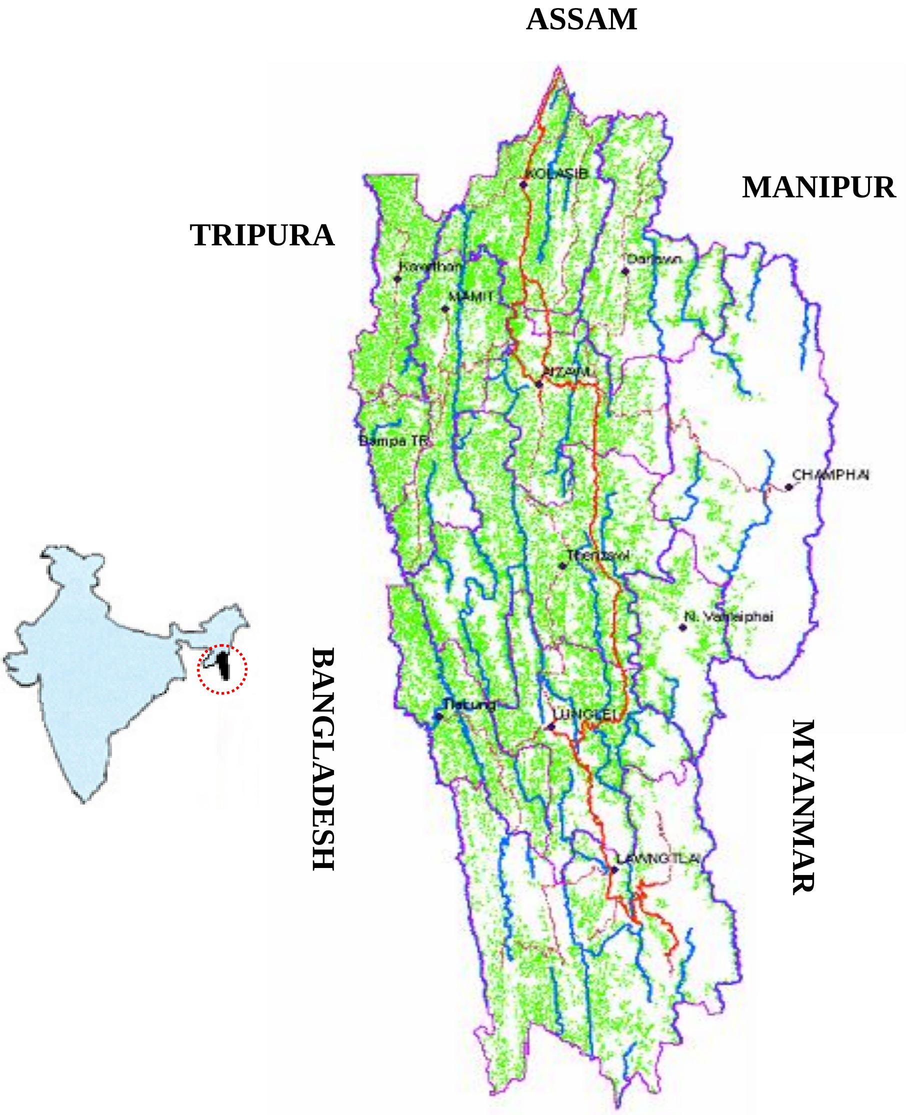


Figure 3. Distribution of bamboo in Mizoram state of India; green patches represent bamboo forest, out of these 95% are contributed by *Melocanna baccifera*. Green patch is sparely populated at the northeast side of Mizoram.

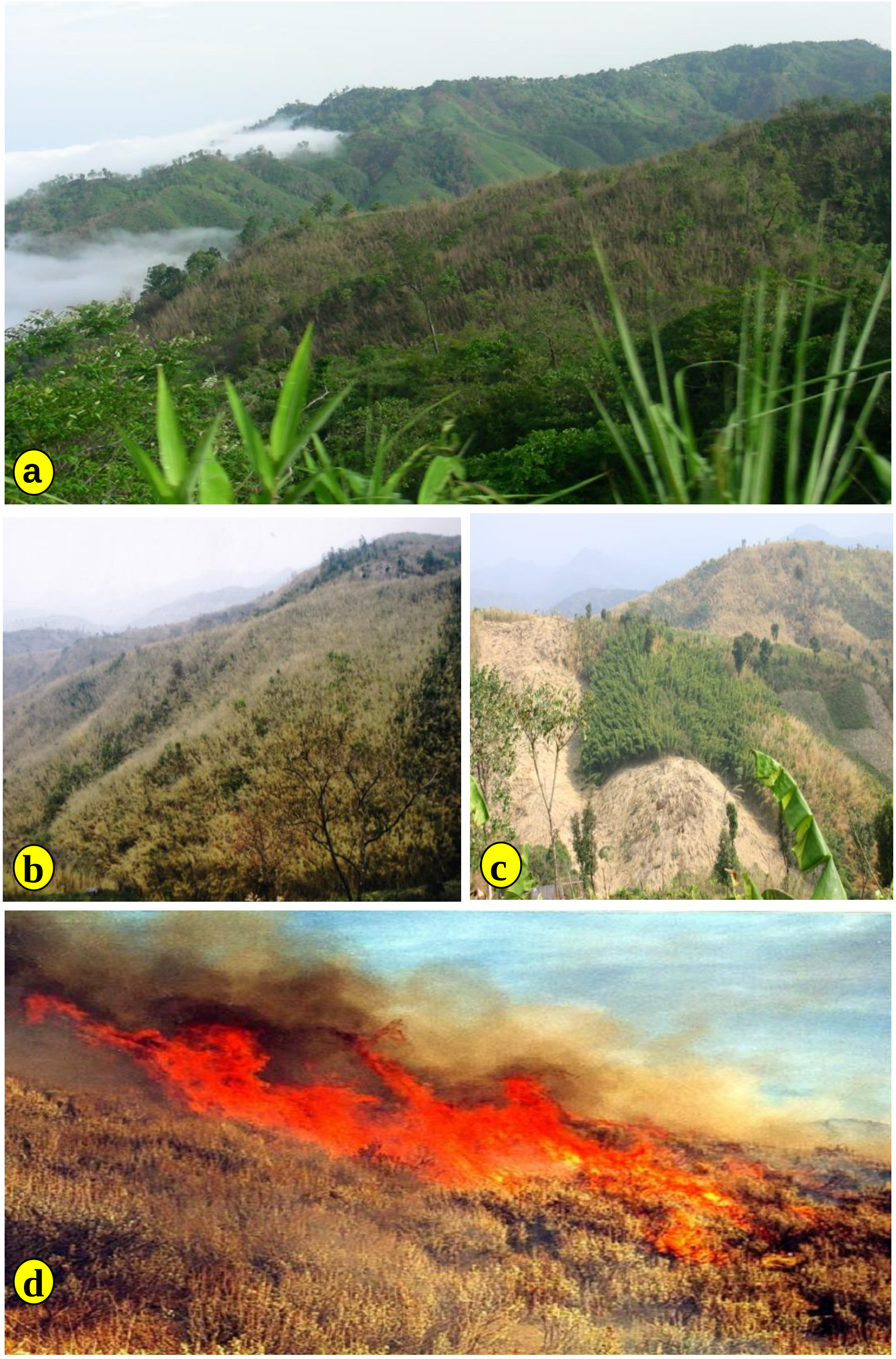


Figure 4. a) Landscape of Mizoram showing hilly and mountainous area; b) Bamboo forest of Mizoram; c) Destruction of bamboo forest for jhuming; d) Slash and burn system of jhuming at the slope of mountain

is very efficient in the conversion of solar energy and can also be easily managed. Bamboo forests contribute greatly to carbon stock, as an important carbon sink on earth. In total, there are 22 million ha of bamboo forests in the world (making up about 1% of the tropical and subtropical woody forests in the world. It is apparent that bamboo is an excellent wood substitute, with increasing production, while the world forest is shrinking (Cao et al., 2011). Due to their biological characteristics and growth habits, bamboo forests have ecological and environmental functions in soil erosion control, water conservation, land rehabilitation, and carbon sequestration (Zhou et al., 2005)

A better understanding of local bamboo harvesting and management systems, as well as of the traditional knowledge about the species, is needed to give forest time to rejuvenate without hampering the livelihood of its dependants. Traditional living and lifestyle of Mizo society, to a large extent, is dependent on bamboo for its variety of uses and bamboo has much to offer by way of contributing to socio-economic advancement of modern Mizo society. The ecofriendly Bamboo crop has immense potential in improving rural economy, industrial development and a sound economic base for the state on sustained basis. Bamboo forests are found mainly along the river banks and abandoned jhumland as a dominant secondary vegetation. Both the clump forming and non-clump forming bamboos are available in most part of Mizoram except in the higher altitudes of the eastern parts of Mizoram. There are 22 species of bamboo, that have been distinctly identified in the forest of Mizoram

1. *Bambusa Khasiana* (Rawte)
2. B. longispiculata (Rawthing)
3. *B. oliveriana* (Talan)
4. *B. pallida*
5. *B. tulda* (Rawthing)
6. *B. vulgaris* (Vairua)
7. *Chimonobambusa collosa* (Phar),
8. *Dendrocalamus hamiltonii* (Phulrua)
9. *D. hookeri* (Rawlak/Rawkhauh)
10. *D. longispathus* (Rawnal)
11. *D. sikkimensis* (Rawmi)
12. *D. strictus* (Tursing)
13. *Dinochloa compactiflora* (=*Melocalamus compactiflorus*) (Sairil)
14. *Gigantochloa albociliata* (Rawthing tial)
15. ***Melocanna baccifera* (Mautak)**
16. *Pseudoschyum polymarphum* (Chal)

17. *Racemobamboos mannii* (=*Neomicrocalamus mannii*) (Saiman)
18. *Schizostachyum capitatum* (Rawngal),
19. *S. dulloa* (Rawthla)
20. *Sinarundinaria griffithiana* (Lik),
21. *S. longispiculata*
22. *S. intermedia* (Lik)

Melocanna baccifera is the most dominant forest resource of the state which fetches the government considerable revenue. The survey result indicated that *Melocanna baccifera* is the dominant species contributing to 77% of the total bamboo coverage. Bamboo, being a multipurpose, eco-friendly crop abundantly available, yet an underutilized natural resource, needs to be managed and exploited for sustainable use. Bamboo is conceived as a thrust area in the industrial development of Mizoram for the economic and ecological security of the people. This precious resource needs to be fully tapped as an Industrial raw material, as substitute for wood in rural/urban housing, engineering works, handicrafts, furniture and value addition through export. Undoubtedly bamboo can revolutionize the economy of the state ensuring employment opportunities to a large number of people. Extension and awareness about bamboo sector development will be given a renewed thrust. With its far-reaching potential for economic growth, bamboo would be ideal for ensuring a steady source of income, while simultaneously facilitating forest conservation.

2 Problems and prospects

2.1. Problems

Mizoram has abundant natural bamboo resources. Bamboo represents a vast untapped major resource of Mizoram state whose full ecological and economic potentials remain underutilized; it needs to be recognized, developed and promoted in a manner ensuring ecological security for all-round sustainable development of the State and well- being of its people. These bamboo forest areas of Mizoram constitute 14% of India’s total bamboo area i.e. 8.96 million hectare. Bamboo occurs in the lower storey of tropical evergreen and moist deciduous forests along the banks of rivers in the riverside forests and in the valleys with humid conditions. Bamboo is found from 400 metres to 1500 metres elevation but is rare in the eastern region of the state, particularly in Champhai, due to high altitude. Mizoram is covered with terrain hill and mountains hence transportation system in some places are under developed, due to these factors, all the bamboo forests cannot be utilised to a large scale in proper manner.

Melocanna baccifera (Mautak) is the most abundant bamboo, contributing about 95% of the growing stock of bamboo, while other important species are *Dendrocalamus hamiltonii* (Phulrua), *Dendrocalamus longispathus* (Rawnal), *Bambusa tulda* (Rawthing), *B.longispiculata* (Rawthing chi) and *Arundinaria callosa* (Phar). Barring Mautak, other species are found in a small proportion, yet are important for value added products.

The bamboo forests area covers about 31% of geographical area. *Melocanna baccifera* is the most abundant and most economic bamboo in Mizoram. It belongs to the family of *Poaceae* and sub-family of Bambusoideae, contributing 95% of the growing stock of bamboo in Mizoram (Anonymous, 1999). Flowering of this bamboo takes place in 48-50 years cycles. Sudden increases in rodent population have been observed to coincide with bamboo flowering. The reason for the increase of the rat population has not yet been investigated. The whole part of the bamboo dies out after flowering. *M. baccifera* flowering and fruiting are shown in Fig 5. Bamboo flowering and death lead to decline in nutrient status and microbial population (Rai, 2011). Natural stands of *M. baccifera* subjected to excessive human pressure (collection of

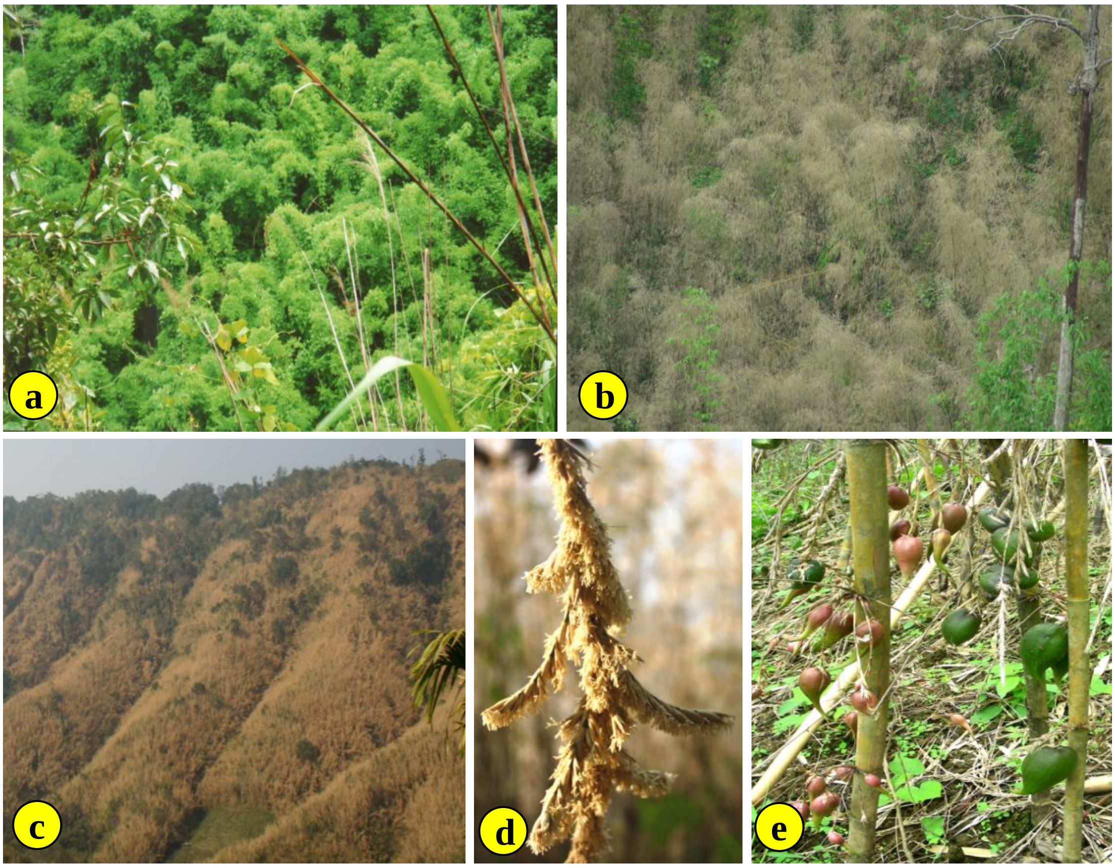


Figure 5. *Melocanna baccifera* forest; a) before flowering; b) after flowering; c) gregarious flowering of *M. baccifera*; d) *M. baccifera* flower; e) *M. baccifera* fruiting

young shoots for vegetables and mature culms) in Mizoram due to high demand of young shoots for vegetables and mature culms for other uses, the culm and young shoot density rate decreased significantly in the stand subjected to these excessive biotic pressures (Lalnunmawia, 2008). Jhum cultivation (Slash and burn agriculture) has become unproductive owing to frequent exposure of soil as is evident from the reduction of jhum cycle from 20-25 years to 4-5 years. Soil, moisture, vegetation, and bio-diversity loss has rendered large area refractory and unsuitable for jhuming. Presently jhum cultivation is practiced by burning standing bamboo which in itself is a great loss in terms of revenue.

A few species of bamboo are edible and are also of medicinal value. In north-east India, *M. baccifera* is a commercially available edible species, consumed either raw or processed as a traditional food item because of its exotic taste and flavour. The people do not have much knowledge about the composition of this food item. Therefore, nutraceutical composition of this precious food should be evaluated for value addition. This species naturally growing throughout the hill forests of Bangladesh, Myanmar and North East (Mizoram, Assam, Arunachal, Meghalaya and eastern part of Tripura, Nagaland, Manipur) of India.

Protection and preservation of mountain ecology; protecting the mountain slopes, watershed management bamboo forests play a significant role. Therefore, Sustainable development and value addition chain of products from bamboo resources through scientific validation would strengthen the economy and revenue of the State.

2.2. Prospects

The Government of Mizoram, through various programs established bamboo plantations on hill slopes and jhum fallows to ensure a continuous supply of high quality raw material and at the same time exploring the possibility of producing bamboo shoots which can be obtained. *M. baccifera* and other bamboos are sold within Mizoram and outside the neighbouring states for paper and other industries through Bamboo Development Agency and organizations of the Government of Mizoram.

The people of the Mizoram use this potential resource in many ways (details are provided in pages 165-183). Charcoal production is one of them. Bamboo charcoal has application in food, pharmaceutical and chemical industries. Bamboo charcoal is obtained by thermal decomposition of bamboo culm at a high temperature in charcoal kiln. These charcoals are mainly used for domestic fuel. This charcoal has to be investigated for industrial application especially in the field of environmental problem, so that this plant would be more valuable.

Bamboo shoots are low in fat and calories but rich in fibre with about 90% water. In India, bamboo shoots, either processed or raw, have a high demand in the markets of the north-east along with other areas like Mumbai, Bangalore, Chennai and Delhi. Being a lesser known food product, *M. baccifera* young shoot processing and research has potential to be developed as an innovative and promising enterprise.

Bamboo being a multipurpose, eco-friendly crop abundantly available, yet an underutilized natural resource, needs to be managed and exploited for sustainable use. Bamboo is conceived as a thrust area in the Industrial Development of Mizoram for the economic and ecological security of the people. This precious resource needs to be fully tapped as an Industrial raw material, as substitute for wood in rural/urban housing, engineering works, handicrafts, furniture and value addition through export. Undoubtedly Bamboo can revolutionize the economy of the State ensuring employment opportunities to a large number of people. Production and utilization of bamboo resource coupled with research and development, extension and awareness will strengthen the economy of the Mizoram State.

In view of the foregoing subject matter *supra vide*, the following objectives have been investigated in my thesis:

2.3. Objectives

1. *Melocanna baccifera* charcoals for removal of heavy metals from aqueous solutions through biosorption.
2. Extraction, quantification and purification of phytosterols from *Melocanna baccifera* young shoot.
3. Anti-cancer and anti-bacterial properties of *M. baccifera* young shoot extract.
4. Genetic similarity assessment of *M. baccifera* growing in Mizoram state of India by using RAPD and ISSR markers.
5. Economic importance and traditional uses of *M. baccifera* Roxb. in Mizoram

3 *Melocanna baccifera* charcoals for removal of heavy metals from aqueous solutions through biosorption

3.1. Introduction

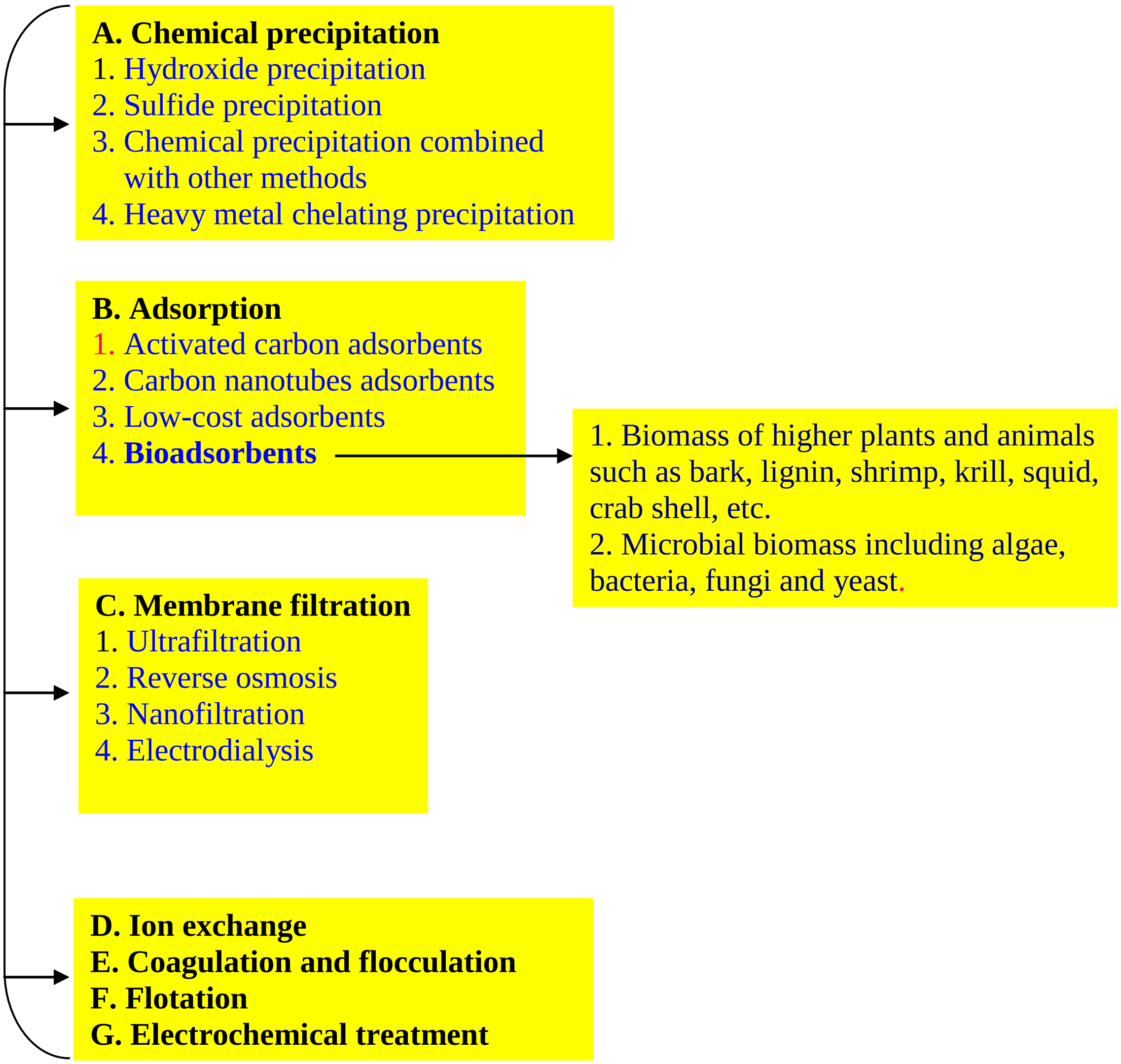
Toxic heavy metal ions get introduced to the aquatic streams by means of various industrial activities such as mining, refining ores, fertilizer industries, tanneries, batteries, paper industries, pesticides etc. and poses a serious threat to environment (Momcilovic et al., 2011; Dong et al., 2010; Celik and Demirbas, 2005; Pasticakova, 2004). The major toxic metal ions hazardous to humans as well as other forms of life are Fe, Se, Cr, V, Cu, Co, Ni, Cd, Hg, As, Pb, Zn etc. According to the World Health Organisation (WHO) (2004, 2006), among the most toxic metals are cadmium, chromium, copper, lead, mercury and nickel. These heavy metals are of specific concern due to their toxicity, bio-accumulation tendency and persistency in nature (Garg et al., 2007). Heavy metal pollution is a major problem leading to the public health hazards and environmental degradation. Heavy metals are non-mutable (Dahiya et al., 2008; Isik, 2008; Salem and Allia, 2008). Therefore, heavy metal removal from wastewater is a priority area of research for the protection of the environment and human health.

The development biosorption technique with a low-cost adsorbents and their tech-economic feasibility for wastewater and water treatment are currently extensively investigated (Wu et al., 2010; Ofomaja et al., 2010; Miretzky et al., 2010). Traditional methods used for the removal of heavy metals from the environment are in general expensive and potentially risky due to the possibility of the generation of hazardous by-products. To remove heavy metals from the wastewater and water, physical and chemical methods have been proposed and applied, but in general, these methods are commercially impractical, either because of high operating costs or the difficulty in treating the solid wastes generated (Silver, 1991; Ag-west Biotech, 1998). The use of conventional methods for removal of metals ions from aqueous solutions include chemical precipitation, ion exchanger, chemical oxidation/reduction, reverse osmosis, electro dialysis, evaporative recovery and ultra filtration, solvent extraction, membrane processes, evaporation and coagulation etc (Tofighy and Mohammadi, 2011; Fu and Wang, 2011; Febrianto et al., 2009; Wang and Chen, 2009;

Bhattacharya et al., 2006; Xiangliang et al., 2005). Nevertheless these techniques have disadvantages including incomplete metal removal, high consumption of reagent and energy, low selectivity, high capital and operational cost and generation of secondary wastes that are difficult to be disposed off (Aksu, et al., 2002; Eren et al., 2009) and ineffective, when metal ions present in wastewater is at a low concentration (1-100 mg/L) (Volesky, 2000; Arica et al., 2001). An alternative method like adsorption gained important credibility due to high removal of heavy metals to very low concentration. The advantages of adsorption over conventional treatment methods include: high efficiency in detoxifying very dilute effluents, minimization of chemical and biological sludge, no additional nutrient requirement; these advantages have served as the primary incentives for developing full-scale adsorption processes to clean up heavy-metal pollution. Schematic diagram showing important strategies for removal of heavy metals is shown in Fig. 6.

3.2. Achievements over the past decade

Although many biological materials bind heavy metals, only those with sufficiently high metal-binding capacity and selectivity for heavy metal are suitable for use in a full-scale adsorption process. The first major challenge for the adsorption field was to select the most promising types of adsorbent from an extremely large pool of readily available and inexpensive biomaterials. A large number of adsorbents have been tested for their metal-binding capacity under various conditions. Although several proprietary adsorption processes (such as AlgaSORB^{TM} and AMT-Bioclaim^{TM}) were developed and commercialized in the early 1990s, a lack of understanding of the mechanism underlying the metal-sorption process has hindered adequate assessment of process performance and limitations. The next real challenge for the field of adsorption was to identify the mechanism of metal uptake by dead adsorbent. Several possible mechanisms for metal adsorption have been scrutinized. Not all of the potentially applicable adsorbent has yet been systematically examined; a substantial body of evidence has been collected that identifies ion exchange as the principal mechanism of metal adsorption.



A. Chemical precipitation
1. Hydroxide precipitation
2. Sulfide precipitation
3. Chemical precipitation combined with other methods
4. Heavy metal chelating precipitation

B. Adsorption
1. Activated carbon adsorbents
2. Carbon nanotubes adsorbents
3. Low-cost adsorbents
4. **Bioadsorbents**

1. Biomass of higher plants and animals such as bark, lignin, shrimp, krill, squid, crab shell, etc.
2. Microbial biomass including algae, bacteria, fungi and yeast.

C. Membrane filtration
1. Ultrafiltration
2. Reverse osmosis
3. Nanofiltration
4. Electrodialysis

D. Ion exchange
E. Coagulation and flocculation
F. Flotation
G. Electrochemical treatment

Figure 6. Important strategies for removal of heavy metals in wastewater (Fu and Wang 2011)

3.3. Biosorption

Biosorption is the removal of substances (compounds, metal ions, organic etc) by inactive, non-living, as well as excreted and derived products materials (materials of biological origin) due to high attractive forces present between the two (Volesky and Holan, 1995). Biosorption consists of several mechanisms, mainly adsorption, ion exchange, chelating and diffusion through cell walls and membrances, which differ depending on the species used, the origin and processing of the biomass and solution chemistry (Haluk and Yetis, 2001; Churchill et al., 1995). Adsorption is one of the processes, which is being widely used for treatment of low soluble contaminants in water. The term adsorption refers to a process wherein a substance is concentrated at a solid surface from its liquid surroundings. It is now customary to differentiate between two types of adsorption. If the attraction between the solid surface and the adsorbed molecules is physical in nature, the adsorption is referred to as physical adsorption (physiosorption). Generally, in physical adsorption the attractive forces between adsorbed molecules and the solid surface are van der Waals forces and they being weak in nature result in reversible adsorption. On the other hand if the attraction forces are due to chemical bonding, the adsorption process is called chemisorption. In view of the higher strength of the bonding in chemisorption, it is difficult to remove chemisorbed species from the solid surface. Ion exchange is basically a reversible chemical process wherein an ion from solution is exchanged for a similarly charged ion attached to an immobile solid particle. Ion exchange (LeVan et al., 1997) shares various common features along with adsorption, in application in batch and fixed-bed processes and they can be grouped together as‘‘sorption processes’’for a unified treatment to have high water quality. Ion exchange has been fruitfully used too for the removal of colours.

3.4. Sorption

The most important chemical removal process in aqueous solution is sorption, which results in short-term retention of long-term immobilization of several classes of contaminants. Sorption is transfer of ions from water to the adsorbent i.e. from solution phase to the solid phase. Sorption actually describes a group of processes, which includes adsorption and precipitation reactions. In heavy metals are adsorbed solid particles by either cation exchange or chemisorption. Cation exchange involves the physical attachment of cations (positively charged ions) to the surfaces of adsorbent by electrostatic attraction. The heavy metals once adsorbed on to the adsorbent will remain as metal atoms, unlike organic pollutants, which will ultimately decompose. Their speciation may change with time as the conditions change (Tipping and Hurley, 1992; Groudev et al., 1999; Batty et al., 2002; Wiebner et al., 2005). Many constituents of wastewater and run off exist as cations, including most of the trace metals such as Cu, Zn, Pb, Ni and Cd. Chemisorption represents a stronger and more permanent form of bonding than cation exchange. The adsorption capacity by cation exchange or non-specific adsorption depends upon the physico-chemical environment of the medium, the properties of the metals concerned and the concentration and properties of other metals and soluble ligands present (Alloway, 1992; Alloway and Ayre, 1993; Thomas et al., 1996; Matagi et al., 1998). The adsorption of metals varies with the fluctuation of pH in the out flow water (Machemer and Wildeman, 1992). According to Wood (1990) the precipitated hydroxides also act as absorption sites for phyto-toxic metals present in the water.

Living as well as dead (metabolically inactive) biological materials have been sought to remove metal ions. It was found that various functional groups present on their cell wall offer certain forces of attractions for the metal ions and provide a high efficiency for their removal (Ashkenazy et al., 1997; Kuyucak and Volesky, 1988). The mechanisms of uptake by living materials (bioaccumulation) and removal by dead ones (sorption) are entirely different. Use of dead materials has several advantages because there is no need of growing, no growth media is required and these materials are available as wastes or by-products. Biomass from algae (Hamdy, 2000; Seki and Suzuki, 1998), fungi (Guibal et al., 1992; Kapoor et al., 1999), bacteria (Ozturk, 2007), seaweeds (Elangovan et al., 2008; Murphy et al., 2008), some higher plants (Joshi et al., 2003; Rahman et al., 2005), all of these have been effectively and successfully utilized in metal removal studies.

Many adsorbents are used in this field for the removal of heavy metals, which included adsorbent from bacteria, fungi, algae, agricultural wastes, aquatic weeds, carbon/charcoal from different sources, etc.

3.5. Activated charcoal/carbon

Commercial activated carbon is a preferred adsorbent for the removal of micro-pollutants from the aqueous phase; however, its widespread use is restricted due to high associated costs. To decrease treatment costs, attempts have been made to find alternative activated carbon precursors. High surface areas can be obtained using either physical or chemical activation; however, combined treatment might enhance the surface properties of the adsorbent, therefore increasing its adsorption capacity. Activated carbon (AC) is known as very effective adsorbent due to its highly developed porosity, large surface area, variable characteristics of surface chemistry and high degree of surface reactivity (Rodrigues-Reinoso, 1997; Bansal et al., 1988). Generally, the physical activation requires high temperature and longer activation time as compared to chemical activation, however, in chemical activation the activated charcoal needs a thorough washing due to the use of chemical agents. The product formed by either of the methods is known as activated carbon/charcoal and normally has a very porous structure with a large surface. The removal of heavy metals by activated carbon is economically favourable and technically easy (Khezami and Capart, 2005). Therefore, activated carbons are widely used to treat waters contaminated with heavy metals. The applicability of activated charcoal for water treatment has been reported in various literatures (Gupta et al., 1997; Srivastava et al., 1996; Stenzel et al., 1997). A compilation of some studies on different sources of activated carbons for adsorption of heavy metals is presented in Table. 1

3.6. Mechanism of heavy metals removal

Adsorption applications have derived their usefulness through the metal binding capacities. Adsorption is made possible by the ability of adsorbent materials to accumulate heavy metals from wastewater through physico-chemical uptake pathways. Metal adsorption is the removal of metal ions by inactive, non-living biomass due to highly attractive forces present between the adsorbent and adsorbate (Volesky and Holan, 1995). Due to the interaction of several factors on specific adsorbents, it is almost impossible to propose a general mechanism. Metal ions are attracted and bound to the biomass by a complex process that comprises of a number of mechanisms like adsorption on the surface and pores, chemisorption by ion-exchange, surface precipitation, complexation and chelation, entrapment in capillaries, spaces of polysaccharide network, physical adsorption, etc., (Chojnacka et al., 2005; Crist et al., 1981; Kuyucak and Volesky, 1989; Miretzky et al., 2006; Muraleedharan and Venkobachar, 1990; Murphy et al., 2009; Tsezos and Mattar,

Table 1: Different sources of activated charcoal/carbon used in heavy metals adsorption

Activated carbon/charcoal	**Heavy metal**	**Reference**	**Year**
Potato peel	Cu(II)	Moreno-Piraján et al	2011
Palm shell	Cu(II)	Issabayeva et al	2010
Tunisian olive-waste cakes	Cu(II)	Baccar et al	2009
Pecan shell	Cu(II)	Klasson et al.,	2009
Hazelnut husks	Cu(II) and Pb(II)	Imamoglu and Tekir	2008
Walnut shell	Cu(II)	Kim et al	2001
Cola edulis shell	Pb(II)	Eba et al	2011
Enteromorpha prolifera	Pb(II)	Li et al	2010
Carica papaya seed	Pb(II) and Cu(II)	Omeiza et al	2010
Pumpkin seed shell	Pb(II)	Okoye et al	2010
Polygonum orientale	Pb(II)	Wang et al	2010
Euphorbia rigida	Pb(II)	Gerçel et al	2007
Coconut shell	Pb(II)	Goel et al	2005
Nut shell	Cd(II)	Tajar et al	2009
Olive stone	Cd(II)	Kula et al	2008
Ceiba pentandra hulls	Cd(II)	Rao et al	2006
Corn-stalks	Cd(II)	Youssef et al	2004
Coconut coirpith	Cd(II)	Kadirvelu et al	2003
Walnut shell	Hg(II)	Zabihi et al	2010
Organic sewage sludge	Hg(II)	Zhang et al	2005
Sago waste	Hg(II)	Kadirvelu et al	2004
Bagasse pith	Hg(II)	Krishnan et al	2003
Syzygium jambolanum	Cr(VI)	Muthukumaran et al	2011
Tamarind wood	Cr(VI)	Acharya et al	2009
Olive bagasse	Cr(VI)	Demiral et al	2008
Coconut activated carbon	Cr(VI)	Liu et al	2007
Hevea Brasilinesis sawdust	Cr(VI)	Karthikeyan et al	2005
Lotus stalks	Ni(II)	Huang et al	2011
Melocanna baccifera	Ni(II) and Zn(II)	Lalhruaitluanga et al	2011
Apricot	Ni(II)	Erdogan et al	2005
Almond husk	Ni(II)	Hasar et al	2003
Coconut shell	Zn(II)	Yanagisawa et al	2010
Olive pulp	Zn(II)	Galiatsatou et al	2002

1996; Veglio and Beolchini, 1997; Yang and Volesky, 1999). The actual mechanism of metal adsorption is still not fully understood (Wang and Chen, 2006). One of the important mechanisms of heavy metals removal is ion exchange. It is a reversible chemical reaction where an ion within a solution is replaced by a similarly charged ion attached onto an immobile solid particle (Han et al., 2006). Ion exchange was later ruled out as the most plausible mechanism by data for the adsorption energy value, E, obtained from the D-R isotherm model. Due to the complexity of the biomaterials used, it is quite reasonable that at least some of these mechanisms are acting simultaneously to varying degree depending on the biosorbent and the solution environment (Gavrilescu, 2004). The Pb(II) removal mechanism by *Rhodotorula glutinis* (Cho and Kim, 2003) involved direct biosorptive interaction with the biomass through ion exchange and precipitation by phosphate released from the biomass. The interaction between Pb(II) ions and the functional groups on the surface of the fungus *Aspergillus parasiticus*'s cell wall is also believed to occur by a combination of ion exchange and complexation processes (Akar et al., 2007). Chen and Wang (2008) investigated the interaction between zinc and *Saccharomyces cerevisiae* by employing a combination of SEM-EDX and XAFS methods. The existence of both covalent and ionic bonds between Zn(II) and the available functional groups on a yeast cell surface was verified by displacement of H^+ ,K^+, Mg^{2+}, Ca^{2+} and Na^+ ions during zinc uptake. The release of K^+, Mg^{2+}, Ca^{2+}, and Na^+ from the biosorbent during heavy metal ion attachment was taken as evidence that ion exchange was the dominant mechanism. The existence of an ion-exchange mechanism during Pb(II), Ni(II), Cu(II), and Cd(II) biosorption by olive stone waste was also reported by Fiol (2006). Metal sequestration during biosorption adheres to complex mechanisms that mainly include ionic interactions, formation of complexes between metal cations and ligands contained within the cell wall biopolymer structure and precipitation on the cell wall matrix. Raize et al. (2004) proposed biosorption mechanisms of different heavy metals by brown marine macro-algae. In their study, Cd(II) ions were attached to chemical groups containing oxygen, carbon, nitrogen and sulfur. In addition, the binding process did not alter the magnesium and calcium concentration in solution, which indicates that ion exchange might not be the main mechanism for Cd(II) biosorption on the brown marine macro-algae *Sargassum vulgaris*. Another frequently encountered metal binding mechanism is chelation. Chelation can be properly defined as a firm binding of a metal ion with an organic molecule (ligand) to form a ring structure. As mentioned before, various functional groups including carboxylate, hydroxyl, sulfate, phosphate amides, and amino groups can be considered responsible for metal sorption. Among these groups, the amino group is the most effective for removing heavy metals, since it does not merely chelate cationic metal ions but also adsorbs anionic metal species through electrostatic interaction or hydrogen bonding (Deng and Ting, 2005). The presence of various functional groups and their complexation with heavy metals during adsorption process has been reported by different researchers using spectroscopic techniques (Ahluwalia and Goyal, 2005; Garg et al., 2007; Tarley and Arruda, 2004). The structural changes were also studied using spectroscopic techniques like FT-IR, XPS, Raman microscopy, EDS, XRD, EPR, etc. (Nakbanpote et al., 2007). Each one can reveal certain information and thus can contribute to explain the mechanism of adsorption.

3.7. Factors affecting biosorption

The investigation of the efficacy of the metal sorption by the adsorbent is essential for the industrial application. The efficiency is strongly influenced by the physico-chemical characteristics of the solutions, e.g., pH, metal concentration, time, etc. A large portion of biosorption studies has been devoted to investigating this relationship.

3.7.1. Influence of pH

pH is one of the most crucial factor in heavy metals biosorption process. It significantly influences the solution chemistry of the metals and the dissociation site on the surface of adsorbent, e.g., hydrolysis, complexation by organic and/or inorganic ligands, redox reactions, and precipitation, as well as the speciation and biosorption availability for heavy metals (Guo et al., 2008; Komy et al., 2006; Ghodbane et al., 2008). In a particular pH range, most metal sorption is enhanced with pH, increasing to a certain value followed by a reduction on further pH increase. In principle, the dependence of metal uptake on pH can be associated with both the surface functional groups on the adsorbent as well as the metal chemistry of the solution (Sheng et al., 2004). Adsorbents in general are considered to contain various functional groups like hydroxyl, carboxyl, sulphydryl etc. With the change in pH of solution, the behaviour of each of these functional group changes. For example, the ionisation constants of various carboxyl groups have been reported to be around 3-4 (Eccles and Hunt, 1986). In highly acidic pHs, these are protonated and act as positively charged species (Gardea-Torresdey et al., 1990). Deprotonation of these functional groups occur on increasing pH and these behave as negatively charges moieties. It starts attracting the positively charged metal ions and there is a competition between hydrogen ions and positively charged metal ions. The "winner" can be estimated through the amount of metal adsorbed at a certain pH value. As the pH is increased from highly acidic to slightly acidic region, the positive character of adsorbent is converted to negative one.

The solution chemistry is also influenced by pH. In acidic pHs, metal ions are generally positively charged and are attracted by negatively charged on the adsorbent. When the pH is increased, the amount of OH^- ions is increased in the solution. Metal ions react with these OH^- ions and are precipitated as metal hydroxide at some pH value. In general, metal ions are precipitated out in alkaline pH range and do not contribute towards the adsorption. This indicated that the effect of pH has upper limit to be studied. For example, cadmium is present as free Cd^+ species along the whole acid pH range. Above pH 7.5, it starts to precipitate as $Cd(OH)_2$ and thus no more available for adsorption (Basualto et al., 2006).

3.7.2. Influence of temperature

Depending on the structure and surface functional groups of a biosorbent, temperature has an impact on the adsorption capacity, to a certain extent. It is well known that a temperature change alters the adsorption equilibrium in a specific way determined by the exothermic or endothermic nature of a process. Quite a number of biosorption studies have been performed concerning the effect of temperature on isotherms, metal uptake and also with respect to biosorption thermodynamics parameters (Malkoc and Nuhoglu, 2007; Gupta and Rastogi, 2008; Park et al., 2005). The uptake of metal ions increased with higher temperature (Shen and Duvnjak, 2004). A more enhanced level of uptake in parallel with a temperature rise resembles the nature of a chemisorption mechanism (endothermic process). In contrast, the opposite behaviour for the temperature effect on adsorption capacity was obtained baker's yeast that higher temperature leads to a lower adsorption capacity (Padmavathy, 2008).

3.7.3. Effect of initial metal concentration

The initial heavy metal concentration can alter the metal removal efficiency through a combination of factors, i.e., the availability of specific surface functional groups and the ability of surface functional groups to bind metal ions (especially at high concentrations). The initial concentration acts as a driving force to overcome mass transfer resistance for metal ion transport between the solution and the surface of the biomass (Arief et al., 2008). At a fixed biosorbent dose, pH and temperature, the equilibrium sorption capacity improved with higher initial ion concentration. The ion removal was highly concentration dependent. The increase in the biosorbent's loading capacity as a function of metal ion concentration was believed to be due to a high driving force for mass transfer. For most cases with low initial ion concentration, the sorption capacity or metal uptake was enhanced with higher initial ion concentrations. However, the opposite phenomenon occurred when starting with high initial ion concentration. This opposite tendency was caused by saturation of the available active sites on surface functional groups, thus preventing further metal ion uptake.

3.7.4. Effect of adsorbent dosage

Adsorbent dosage is an important parameter because it determines the capacity of an adsorbent for a given initial concentration of the adsorbate. By increasing the adsorbent dose, the adsorption efficiency increases even though the amount adsorbed per unit mass decreases. In principle, with more adsorbent present, the available adsorption sites or functional groups also increase and the amount of adsorbed heavy metal ions is also increased, which brought about improved adsorption efficiency. In some cases, with increase in adsorbent dose, the adsorption capacity was lesser at a higher adsorbent dose. This is due to overlapping and aggregation of adsorption sites resulting in the decrease of the surface area available to metal ions was observed (Amarasinghe and Williams, 2007; Rahman and Islam, 2009).

Many factors can affect biosorption. The type and nature of the biomass or derived product can be very important. Physical and chemical treatments such as boiling, drying, autoclaving and mechanical disruption will all affect binding properties while chemical treatments such as alkali treatment often improve biosorption capacity, especially evident in some fungal systems (Gadd, 2009).

3.8. Biosorption equilibrium models and isotherms–Assessment of sorption performance

Examination and preliminary testing of solid-liquid sorption system are based on two types of investigations: a) equilibrium batch sorption tests; b) dynamic continuous-flow sorption studies.

Batch equilibrium sorption studies can provide useful information on relative adsorbent efficiencies and important physico-chemical factors that affect adsorption. The equilibrium of the adsorption process is often described by fitting the experimental points with models usually used for the representation of isotherm adsorption equilibrium. (Gadd et al., 1988). Flow and other continuous systems are more complex, but many column studies use breakthrough curves to assess sorbent efficiency that occur when column contents become saturated with the sorbate (Volesky, 2001; White et al., 1995).

A variety of models have been used to describe adsorption isotherm. The widely accepted linear adsorption equilibrium isotherm models for a single solute system are:

3.8.1. Langmuir isotherm

The Langmuir adsorption isotherm describes the surface as homogeneous assuming that all binding sites possess an equal affinity for the adsorbate, adsorption is limited to formation of a monolayer. There is no interaction between adsorbed species and the number of adsorbed species does not exceed the total number of surface sites (Langmuir, 1916). The adsorption isotherm equation is given as:

$$q_e = q_{max}\, bC_e/1+bC_e \text{ (non-linear form)} \qquad (1)$$

$$C_e/q_e = 1/q_{\max}b + C_e/q_{\max} \text{ (linear form)} \tag{2}$$

where q_{e} is the equilibrium value of sorbate uptake by the sorbent, C_e is the concentration of sorbate in solution (mg/L) at equilibrium, $q_{\max}$ (mg/g) is the maximum adsorption capacity corresponding to complete monolayer coverage and b (L/mg) is an affinity parameter related to the bonding energy of the sorbate species to the surface.

3.8.2. Freundlich isotherm

The Freundlich isotherm defines adsorption to heterogeneous surfaces i.e. surfaces possessing adsorption sites of varying affinities. A monolayer formation accompanied by interaction between adsorbed molecules (Freundlich, 1907).

$$q_{\mathrm{e}} = K_f\, C_e^{\,1/n} \text{ (non-linear)} \tag{3}$$

$$\log q_{\mathrm{e}} = \log K_f + (1/n) \log C_e \text{ (linear)} \tag{4}$$

where C_e (mg/L) is the equilibrium concentration of the sorbate, q_e (mg/g) is the amount of metal ions adsorbed per specified amount of adsorbent at equilibrium, K_f (mg/g) and '*n*' are constants which are adsorption capacity and intensity of adsorption, respectively.

3.8.3. Dubinin-Radushkevich isotherm

In order to explain the nature of sorption processes as physical, chemical or ion exchange sorption, Dubinin-Radushkevich (D-R) isotherm model was used (Dubinin-Radushkevich, 1947).

$$\mathrm{In} q_e = \mathrm{In} X_m - \beta\varepsilon^2 \tag{5}$$

where q_e (mol/g) is the metal ion concentration on the adsorbent at equilibrium, X_m (mol/g) is the maximum adsorption capacity, β is the activity coefficient related to the mean free energy of adsorption and ε is Polanyi potential. The Polanyi potential (ε) can be expressed as,

$$\varepsilon = RT\ \mathrm{In}\ (1 + 1/C_e) \tag{6}$$

where R (J/mol) is the universal gas constant, T is the absolute temperature in Kelvin and C_e (mol/L) is the metal ion concentration in the solution at equilibrium.

The activity coefficient β was further used to calculate the adsorption mean free energy 'E' (kJ/mol).

$$E = 1/\sqrt{-2\beta} \tag{7}$$

E is the free energy for the transfer of one mole of metal ions from the infinity in the solution to the surface of the adsorbent.

3.9. Adsorption kinetics

With the scope of the literature review, two kinetic models, namely pseudo-first-order and pseudo-second-order equations have been widely used to describe adsorption data (Ho et al., 2000). In most adsorption kinetic studies, both pseudo-first-order and pseudo-second-order kinetic equations have been commonly employed in parallel, and one is often claimed to be better than another according to marginal difference in correlation coefficient.

3.9.1. Pseudo-first-order Kinetic equation

The Pseudo-first-order equation or the so-called Lagergren model (Lagergren, 1898) for solid/liquid systems of adsorption stated that the rate is proportional to the number of unoccupied sites. It is expressed as:

$$\log(q_e - q_t) = \log q_e - k_1 t/2.303 \tag{8}$$

where q_e and q_t (mg/g) are the amounts of cadmium adsorbed on the adsorbent at equilibrium and at any time t, in mg/g, respectively. k_1 (/min) is the rate constant of pseudo-first-order adsorption. The slopes and intercepts of plot of log ($q_e - q_t$) versus t were used to calculate the first-order rate constant k_1 and equilibrium adsorption capacity q_e.

3.9.2. Pseudo-second-order Kinetic equation

The Pseudo-second-order (Ho and McKay, 1999) assumes that the rate of sorption is proportional to the square of the number of unoccupied sites. It is expressed as:

$$t/q_t = 1/k_2 q_e^2 + t/q_e \qquad (9)$$

where k_2 is the equilibrium rate constant of pseudo-second-order adsorption (g/mg/min). The slopes and intercepts of plots t/q_t *vs* t is used to calculate the pseudo-second-order rate constants k_2 and q_e.

The shape of graph and comparison of experimental and calculated q_e values can help in deciding which kinetic model is followed by adsorption system. Another important factor that determined a decision is the correlation coefficient of determination R^2. The kinetic models showing higher R^2 value is followed by adsorption system.

3.10. Adsorption of lead(II), cadmium(II), Nickel(II), Zinc(II) and Copper(II) using Melocanna baccifera charcoals

Industrial wastewater and effluents containing toxic heavy metals pose serious problems to human health and the environment. Beyond the permissible limits, essential metals like Ni, Zn, Cu etc. are also toxic. So it is necessary to remove these excess of essential metals from aqueous substrates. Chemical precipitation, reverse osmosis, ion exchange, membrane technologies and other available methods are cost-prohibitive and often impracticable in remote regions when heavy metal contaminants originate from geogenic sources. In such situations, adsorption is one of the feasible and convenient alternatives available to water treatment. This process is based on the binding of metal ions by the various functional groups present on the adsorbent.

According to the ranking of metal interested priorities, Pb (II) is one of the most interesting heavy metal for removal and/or recovery considering the combination of environmental risk and reserve depletion (Volesky, 2001). This metal is widely used in many industrial applications, such as storage battery manufacturing, painting pigment, fuels, photographic materials, explosive manufacturing, coating, automobile, aeronautical and steel industries (Jalali et al., 2002; Igbal and Edyvean, 2004; Sekhar et al., 2004; Slania et al., 2004). Lead pollution results from textile dyeing, ceramic and glass industries, petroleum refining, battery manufacture and mining operations (Aksu, 1998). Lead is a highly toxic and cumulative poison, accumulates mainly in bones, brain, kidney and muscles. Lead poisoning in human causes severe damage to kidney, nervous system, reproductive system, liver and brain (Naiyaa et al., 2009). In drinking water, even a low concentration may cause anemia, encephalopathy, hepatitis and nephritis syndrome (Lo et al., 1999). The permissible limit for Pb(II) in waste water as set by Environmental Protection Agency (EPA) is 0.05 mg L^{-1} and that of Bureau of Indian Standard (BIS) is 0.1 mg L^{-1} (Anonymous, 1981) and in drinking water intended for drinking, as set by EU, USEPA and WHO are 0.010, 0.015 and 0.010 mg L^{-1} respectively (Bhattacharjee et al., 2003; Balarial et al., 2008) . It is therefore, essential to remove Pb(II) from wastewater before disposal.

Cadmium is one of the toxic heavy metal present in this effluent. Cadmium does not degrade into harmless end products in the metabolism and accumulates in the food chain, thereby posing a great danger to living organisms (Çay et al., 2004). It is recognized as carcinogen for human, accumulated in the human body, causing erythrocytes destruction, nausea, salivation, diarrhea and muscular cramps, renal degradation, chronic pulmonary problems and skeletal deformity (Dinesh and Singh, 2002). The main source of cadmium in waste waters is discharging of waste stream from metallurgical alloying, ceramics, metal plating, photograph, pigment works, phosphate fertilizers, textile printing industries, mining and sewage sludge (Ecekenfelder, 1989). The permissible limit for cadmium in drinking water is 0.01 mg/L. According to World Health Organization (WHO), the permissible concentration value of cadmium is 0.005 mg/L.

Nickel(II) is found in wastewater due to geogenic source (e.g. discharges from mining), electroplating, pigments and ceramic industries, battery and accumulator manufacturing (Parab et al., 2006). Nickel is widely used in plating plants, steel factories, Ni batteries and in the production of some alloys (Ajmal et al., 2000). The most common adverse health effect of nickel in human is an allergic reaction; large amounts of nickel can cause lung and nasal sinus cancers (Wang et al., 2007), damage to lungs, gastrointestinal distress, e.g., nausea, vomiting, diarrhea, pulmonary fibrosis, renal edema, and skin dermatitis (Akhtar et al., 2004). In India, nickel in drinking water should not exceed 0.01 mg/L and for discharge of industrial wastewater it should not be more than 2.0 mg/L (Kadirvelu et al., 2001).

Zinc is an essential element for life. But too much zinc can be harmful to health. Zinc mostly enters into the environment as a result of human activities such as mining, purifying zinc, lead and cadmium ores, steel production, insecticides, coal burning and burning of wastes (Bhattacharya et al., 2006). Symptoms of zinc toxicity include irritability, muscular stiffness, loss of appetite and nausea. The recommended level of zinc in drinking water is 5 mg/L.

Copper, a commonly used heavy metal, which is found in many useful applications in our life, is quite harmful if discharged into natural water resources (Oztürk et al., 2004). Acute copper poisoning after ingestion may show systematic effects such as hemolysis, liver and kidney damage, and fewer with influenza syndrome, irritation of upper respiratory tract, gastrointestinal disturbance with vomiting, diarrhea and a form of contact dermatitis (Rengaraj et al., 2004). World Health Organization recommended a maximum acceptable concentration of Cu(II) in drinking water of 1.5 mg/L (Rao et al., 2006) and according to U.S. EPA in 1991, the limited concentration of copper in public drinking water at the consumer's tap is 1.3 mg/L (Pontius, 1996).

Bamboo is the most abundant natural vegetation in the state of Mizoram, India. The bamboo forest area of Mizoram constitutes 14% of the total India's bamboo area. Out of these *Melocanna baccifera* is the most economical and abundant, contributing about 95% of the growing stock of bamboo. The communities of the region use this potential resource for housing, mat-ply production, handicraft, medicines and charcoal production. This raw charcoal is mainly used for domestic purposes. Activated charcoal was prepared from *Meloccana baccifera* raw charcoal by KOH treatment in order to make better use of this abundant material. Bamboo charcoal is obtained by thermal decomposition of bamboo culm at a high temperature in charcoal kiln. These charcoals are mainly used for domestic fuel. The motivation of the study is to investigate industrial application of this bamboo charcoal in the field of environmental problem, so that this plant would be more valuable for the community.

Charcoal from different source of bamboos is used for removal of Cadmium(II)(Wang et al., 2010), chemical oxygen demand (COD) and color of cotton textile mill wastewater (Ahmad and Hameed, 2010), dye (Ip et al., 2008), methylene blue (Hameed at al., 2007), Acid Yellow 117 and Acid Blue 25 (Chan et al., 2008), Nitrate-nitrogen (Mizuta et al., 2004), heavy metals (Wang et al., 2008), quinoline (Zhu et al., 2010), Benzene and Toluene (Chuang et al., 2008). Activated carbons play an important role in many areas of modern science and technology such as purification of liquids and gases, separation of mixtures, and catalysis (Kenneth, 2002). Adsorption of activated carbon is governed by the chemical nature of the aqueous phase, the solid phase, and the chemical nature of the adsorbing organic (Kim et al., 2005). It is well known that carbon (or charcoal) materials can be activated through physical (H_2O, CO_2, air, etc.) or chemical methods ($ZnCl_2$, H_2PO, K_2CO_3 etc). Alkaline metal compounds, usually KOH, are used for the activation of coal precursors or chars (Illan-Gomez et al., 1996; Ahmadpour and Duong, 1996). There is a growing interest in alkaline hydroxide activation process and KOH has been found to be one of the most effective compounds in the production of activated carbons (Evans et al., 1999). The use of activated carbon is considered to be the appropriate technology for removing low-solubility contaminants in water treatment, including trace metals (Derbyshire et al., 2000; Bansal and Goyal, 2005). Charcoal is a carbonaceous material with profuse porosity and internal surface area (Lalhruaitluanga et al., 2010).

Therefore, the present study was performed to activate *M. baccifera* raw charcoal by KOH activation. *M. baccifera* raw charcoal (MBRC) and activated charcoal (MBAC) was used as an adsorbent for the removal of heavy metals such as Pb(II), Cd(II), Ni(II), Zn(II) and Cu(II) from aqueous solutions.

3.11. Materials and Methods

3.11.1. Production of *M. baccifera* charcoal

M. baccifera charcoal was produced by thermal decomposition of the culm inside the charcoal kiln. The oxygen supply inside the charcoal kiln was minimal and maintained in order to prevent burning of the culm to ash and the culms are supplied in 2 to 3 feets depending on the kiln size which can be accommodated. Therefore, under the control of oxygen supply, charring of the culm occurs. The charring period takes place 2 to 4 h. These charcoals are mainly used for domestic purposes by the local people. The bamboo charcoal was milled with a blender and sieved to particles of 100 mesh screen (Fig. 7).

3.11.2. Activation of *M. baccifera* charcoal

Activation was done by potassium hydroxide (Himedia) treatment with various concentrations (20, 40, 50, 60, and 80% w/w). The concentrations of KOH were selected as follow:

KOH (gm) = Desire percentage of KOH x 100 ml/% purity of the compound

A charcoal powder with a mass concentration of 40 g/100 mL was in contact with the above various concentrations of chemical reagents for 24 h by mixing on an orbital shaker (Orbitek, Scigenics biotech, Chennai, India) at a speed of 150 rpm, then heated at 700^{o}C. The activated charcoal was thoroughly washed several times with deionized water. Activated charcoal was separated from KOH solutions by centrifugation at a speed of 10,000 rpm for 10 min using Remi centrifuge (C-20, India), oven-dried at 110^{o}C for 1 to 2 days, cooled at room temperature and stored in an air-tight container for further use. This is the activated charcoal, henceforth named MBAC. The MBAC (0.1 g) pre-treated with various concentrations of KOH and 0.1 g of the MBRC was mixed with 100 mL heavy metals solution for 6 h on the orbital shaker at 29±1^{o}C. The supernatants were separated from the biomass by centrifuging at 10,000 rpm for 8 min. The concentrations of metal in the solutions were analyzed.

3.11.3. Adsorbates

All the chemicals used in this study were of analytical reagent (AR) grade. Stock solution of each heavy metal (1000 mg/L) was prepared by weighing 1.598 g of $Pb(NO_3)_2$, 2.103 g of $Cd(NO_3)_2$, 4.955 g of $Ni(NO_3)_2$, 4.397 g of $ZnSO_4$ and 3.929 g of $CuSO_4$. Initial solutions with different concentrations of metals were prepared by proper dilution from stock of 1000 mg/L of heavy metals solutions. Standard solutions of each heavy metal from Sisco Research Laboratories (India) were used for calibration of atomic absorption spectrometer.

3.11.4. Adsorbent and its characters

The samples were gold sputter coated and the scanning electron microscope (SEM) micrographs were taken by using a XL30 ESEM, Philips, USA to understand the surface morphology of the MBRC and MBAC. Crystal structure analysis of raw charcoal and after activation of raw charcoal was taken by using x-ray diffraction (XRD-PW1830, Philips). FTIR spectrometer (JASCO FTIR-5300) was employed to determine the nature of surface functional groups in which are present on the surface of MBRC and MBAC, before and after the adsorption of metal ions, at room temperature over a spectral wave number range of 400-4000 cm^{-1}. The physical characters of charcoals such as surface area, average pore volume, micropore volume and average pore diameter were taken by using Micromeritics-ASAP 2020-Surface Area and Porosity Analyser-USA.

3.11.5. Batch mode adsorption experiments

The adsorption of heavy metal ions onto the MBRC and MBAC was investigated in batch mode adsorption process. All batch experiments were carried out in 250 ml conical flasks containing 100 mL of each metal solution.

The effect of pH on the sorption capacity of MBRC and MBAC of heavy metal ions removal was evaluated in the range of 2.0-7.0. The pH of each metal solution was adjusted to the required pH value by using 1N H_2SO_4 or 1N NaOH. Then 0.1 g of dried adsorbent was added into the metal solution. The reaction mixture was shaken for 6 h at 150 rpm, $29\pm1^{o}C$. Similarly, adsorbent dose (100-500 mg), contact time (15-360 min), and Initial metal concentration (50-90 mg/L for lead; 5-30 mg/L for cadmium; 50-70 mg/L for nickel; 30-70 mg/L for zinc and 80.0-120.0 mg/L for copper) for raw and activated charcoals were also conducted. At the end of the experiment, the solutions were separated from the adsorbent by centrifuging at 10,000 rpm for 8 min. The concentrations of metal in the solutions were analyzed using Flame Atomic Adsorption Spectrometer (GBC 932 plus, Australia). The wavelength of 283.3 nm for Pb(II); 288 nm for Cd(II); 341.5 nm for Ni(II); 213.9 nm for Zn(II) and 222.6 nm for Cu(II) were used for the analysis of the metal ions. The instrument was calibrated with a standard solution within a linear range and a correlation coefficient (R^2) of 0.995 to 1.0 was obtained. In order to reproduce the results, the experiments were conducted in triplicate and the average values were used for the data analysis.

3.12. Results and Discussion

3.12.1. KOH activation of *M. baccifera* charcoal

The activation with various concentrations of KOH to increase adsorption capacity onto charcoal biomass was investigated. Treatment with 50% KOH concentration show highest percentage of adsorption in case of Ni(II) and Cu(II) i.e. 56.21% and 59.78% adsorption, respectively whereas 60% KOH treatment show highest percentage of adsorption in case of Pb(II), Cd(II) and Zn(II). The percentage of adsorption by their corresponding controls i.e. MBRC are 19.4%, 51.39%, 4.01%, 14.3% and 12.8% for Pb(II), Cd(II), Ni(II), Zn(II) and Cu(II), respectively as shown in Fig. 8. KOH activation process is actively researched. In general the chemical reaction between KOH and carbon material can be written as follows (Otowa and Shiraish, 1992; Guo et al., 2000).

$$4KOH + C \longrightarrow K_2CO_3 + K_2O + 2\,H_2 \qquad (10)$$

Additional reactions do take place during the process of activation as shown:

$$2KOH \longrightarrow K_2O + H_2O \qquad (11)$$

$$C + H_2O \longrightarrow H_2 + CO \qquad (12)$$

$$CO + H_2O \longrightarrow H_2 + CO_2 \qquad (13)$$

$$K_2O + CO_2 \longrightarrow K_2CO_3 \qquad (14)$$

$$K_2O + H_2 \longrightarrow 2K + H_2O \qquad (15)$$

$$K_2O + C \longrightarrow 2K + CO \qquad (16)$$

$$K_2CO_3 + 2C \longrightarrow 2K + 3CO \qquad (17)$$

$$K_2O + SiO_2 \longrightarrow K_2SiO_3 \qquad (18)$$

KOH reacts with carbon at high temperatures to form K_2CO_3 and the decomposition product K_2O along with the evolution of hydrogen. The steam generated in (step 11) reacts with amorphous carbon, CO is released as shown in (step 12) leading to formation of pores. K_2O formed during the process of KOH activation can easily infiltrate into the charcoal. K_2O and K_2CO_3 are reduced to K by carbon resulting in carbon gasification with a subsequent emission of CO (step 16 and 17), leading to the formation of pores. All these carbon losses contribute to the creation of porous network in the carbon material forming micropore. The effectiveness of KOH activation can be attributed to the ability of K to form intercalation compounds with carbon easily. In addition, Also K atoms that intercalate into the lamella of the carbon crystallites widen the space between the adjacent carbon layers (intercalation phenomenon), resulting in an increase in the value of specific surface area (Ji et al., 2007). The reaction (18) is important in the activation process where silica in the carbon disappears through the above reactions and pore structure is formed. Pictorial representation of the formation of micropore in the carbon upon activation with KOH was shown in Fig. 9.

3.12.2. Adsorbent and its characterization

SEM micrographs of the charcoal biomass revealed that the surface was highly porous in nature; this increased the surface area for metal adsorption. The pore size of heavy metals loaded activated charcoal was larger in size than the raw charcoal as shown in Fig. 10. The larger pore size in the activated charcoal can increase surface area.

Functional groups characterization on the surface of charcoals is carried out using FTIR. The FTIR spectrum of MBRC and MBAC are shown in Fig. 11. The MBAC spectra show 3476 cm^{-1} indicating the present of hydroxyl group (-OH). This hydroxyl group is absent in MBRC. Hydroxyl group may be added from the KOH solution to the MBAC during activation. Spectra of 1709 cm^{-1} and 1735 cm^{-1} on the charcoals indicated the present carbonyl group. Spectra of 1572 cm^{-1} and 1585 cm^{-1} indicated the presence of carboxyl group (-COOH). The peak of carboxyl groups are increased after KOH activation i.e in MBAC. Spectra of 1379 cm^{-1}, 1388 cm^{-1} and 1440 cm^{-1} on the charcoals indicated the presence of $–CH_3$ wagging. 1109 cm^{-1} and 1167 cm^{-1} assigned the presence of -C-O and -C-C stretching (Roepe et al., 1988) on the charcoal surface. 1011 cm^{-1} and 1022 cm^{-1} spectra indicated the presence of -C-O-H stretch, $-CH_2$ mode (Robert et al., 2005). Some bands below 1000 cm^{-1} is called fingerprint zone. The fingerprint zone could be attributed to the phosphate or sulphur groups (Xu and Liu, 2008).

Crystal structure analysis was recorded using x-ray diffraction. The diffraction profiles were obtained in the scan range (2θ) of 5 - 80°. The XRD patterns of raw and

Figure 7. a) Charcoal Kiln from where *Melocanna baccifera* charcoal is produced and b) *Melocanna baccifera* charcoal powder after milled with a blender

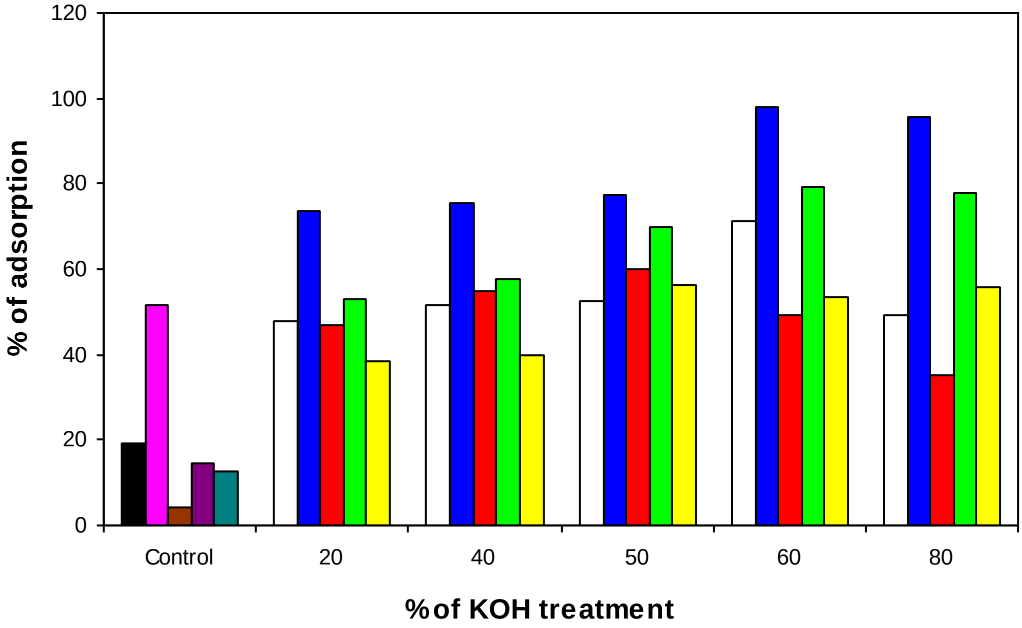


Figure 8. Effect of different concentrations of KOH treatment on adsorption of heavy metals

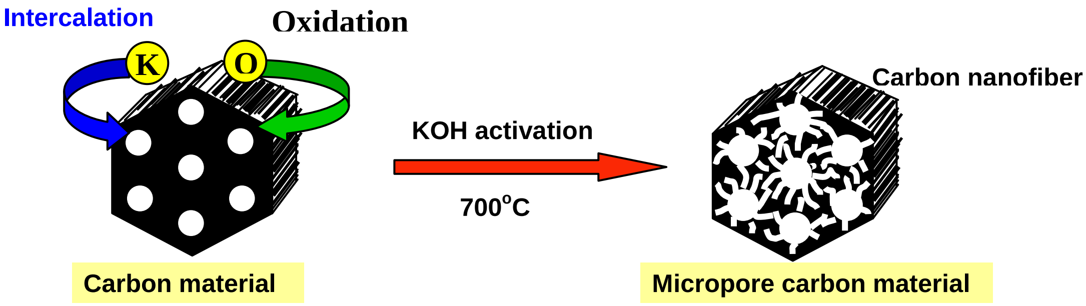


Figure 9. Pictorial representation of the formation of micropore in the carbon upon activation with KOH

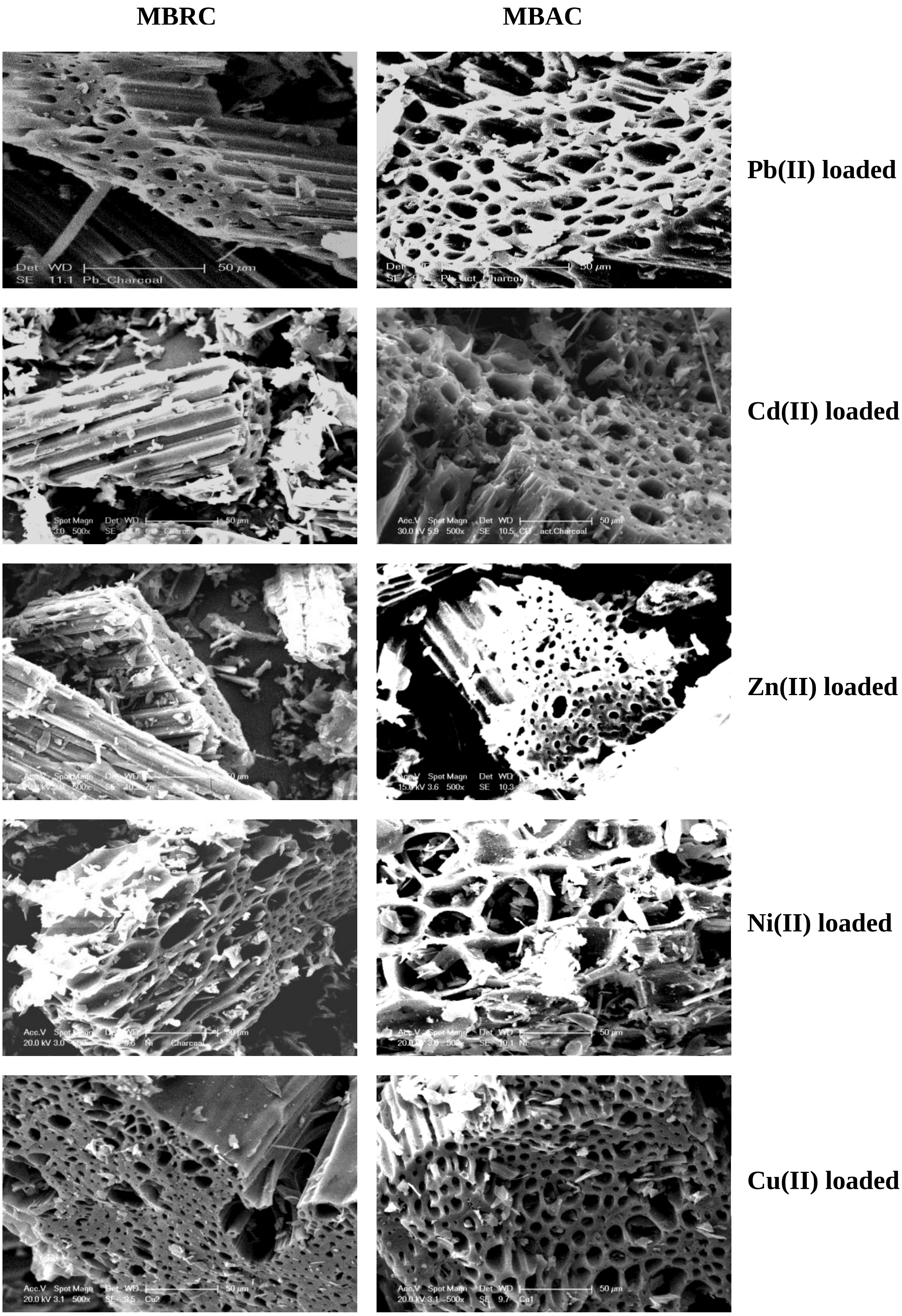


Figure 10. Scanning Electron Microscope (SEM) micrograph of MBRC and MBAC loaded with heavy metals **(**MBRC = Melocanna baccifera raw charcoal, MBAC = Melocanna baccifera activated charcoal**)**

activated charcoals are shown in Fig. 12. The XRD pattern of the charcoal comprises of two broad diffraction peaks centered at 2θ value 22° and 44^o. The broad diffraction peak at a 2θ value of 22° is characteristic of the presence of lignin component. The broadness of the diffraction peak indicates the amorphous nature of the lignin in the char (Rials and Glasser, 1989). Charcoal from lignocellulosic materials were known to comprise of an interwoven network of carbon "ribbons" containing hexagonally arrayed carbon layers – a turbostratic graphitic structure. Precursors containing hexagonally arrayed carbon, as those found in lignin, would readily reform to stable graphitic arrays (Mackay and Roberts, 1982). The broad peaks centered around the 2θ values of 44^o is attributed to diffraction peaks of turbostratic nature of carbon structure (Prahas et al., 2008). The intensity of the peak is higher on MBAC than MBRC, this indicated that the turbostratic nature of carbon structure was improved after activation. Above a 2θ value of 44° a sharp and intense diffraction peak is observed in the XRD profile as a result of silica and other typical mineral matter present in the plant tissues which remain intimately bound with carbon material in the charcoal. After activation of the raw charcoal with KOH, this peak disappeared (in case of MBAC). Therefore, KOH activation of charcoal not only improved turbostratic nature but also removed impurities from activated charcoal. The physical characters of MBRC and MBAC are shown in Table 2.

Table 2. Physical characterization of raw (MBRC) and activated charcoals (MBAC)

Physical characters	**MBRC**	**MBAC**
Surface area	321.64 m^2/g	432.09 m^2/g
Average pore volume	0.154 cm^3/g	0.212 cm^3/g
Micropore volume	0.084 cm^3/g	0.108 cm^3/g
Average pore diameter	10.6 nm	12.7 nm
Particles size	100-160 mess	100-160 mess

3.12.3. Batch mode studies for the removal of heavy metals

3.12.3.1. Effect of pH

Many studies showed that pH is an important factor affecting biosorption of heavy metals. The pH of the solution is one of the most important factors in the study of the adsorption of metal ions on solids. The state of metal ions in solution strongly

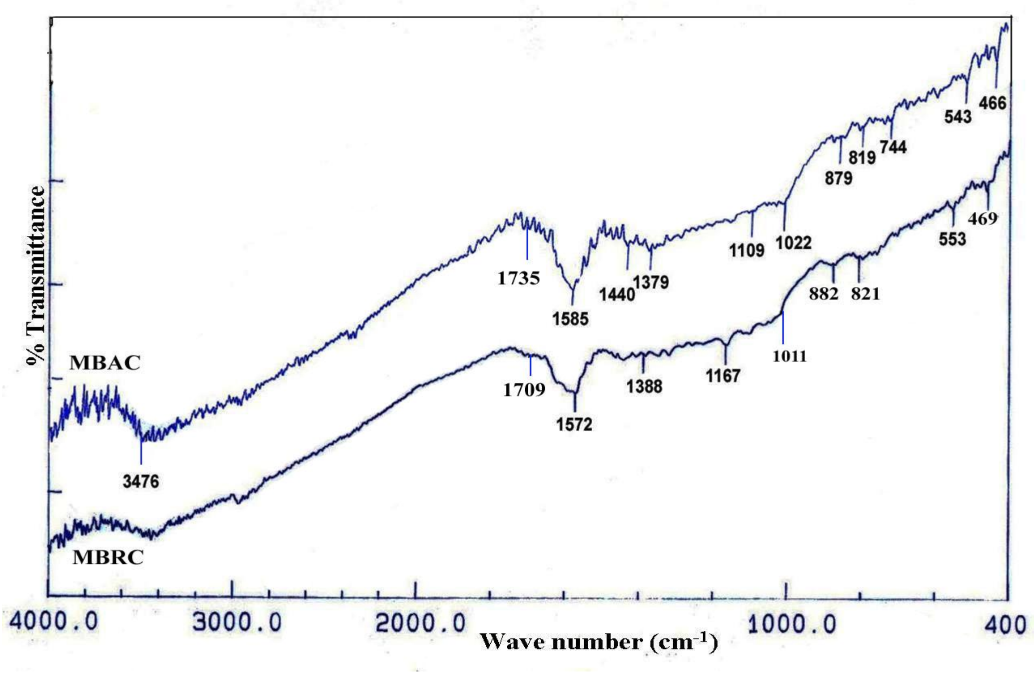


Figure 11. Surface functional groups analysis of MBRC and MBAC using FTIR (Fourier Transformed Infra-red spectroscopy)

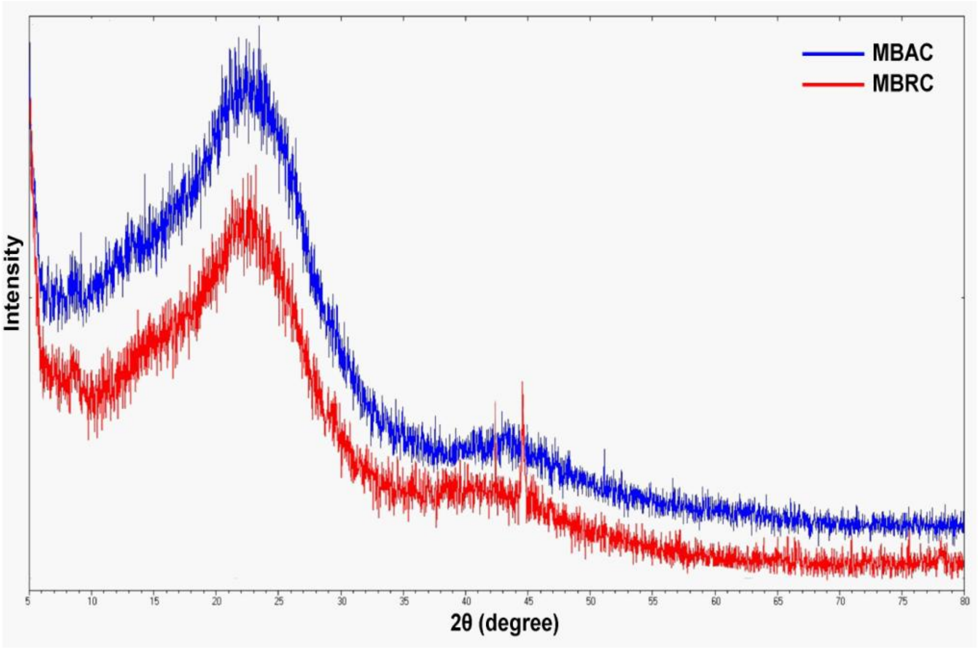


Figure 12. Crystal structure analysis of MBRC and MBAC using x-ray diffraction (XRD)

depends on the pH and the acidity and basicity of the various solutions can influence the composition and the properties of the adsorbent surface. Therefore, in order to determine the optimum pH for metal adsorption, the adsorption was studied at various pH. The adsorption pH was examined in a pH range of 2-6 for Pb(II), 2-7 for Cd(II), Ni(II), Zn(II) and 2-5 for Cu(II) to avoid the formation of their metals hydroxide precipitation. The maximum biosorption of Pb(II) was found to be 16.26% for MBRC whereas for MBAC 84.89% was observed at pH 5 in 60 mg/L of Pb(II) solution. At pH 6, the adsorption yield for Pb(II) was slightly decreased in MBRC and MBAC. Therefore, all the further adsorption experiments were carried out at pH 5. The maximum biosorption of Cd(II) was found to be 97.65% for MBAC and 54.97% for MBRC at pH 5 in 10 mg/L of Cd(II) solution. At pH 6 and 7, there is no significant changes in the adsorption capacity of Cd(II) for both MBRC and MBAC. Therefore, all the adsorption experiments were carried out at pH 5. Ni(II) was 5.92% for MBRC and 49.35% for MBAC at pH 5 in 20 mg/L of Ni(II) solution, Zn(II) was found 14.02% for MBRC and 78.06% for MBAC at pH 6 in 10 mg/L of Zn(II) solution and the maximum adsorption of Cu(II) was found 11.74% for MBRC and 60.25% for MBAC at pH 5 in 30 mg/L Cu(II) solution (Fig. 13). Therefore, all the further adsorption experiments were carried out at their corresponding pH showing maximum adsorption. The increase in sorption of metal with increase in pH value can be explained using the analogy between the reaction of metal hydrolysis and the reaction between binding sites of the sorbent and the metal, where in both reactions, the bond for hydrogen is broken and hydrogen ions are released and substituted by the metal (Tipping, 2002; Pagnanelli et al., 2003). Therefore, the electrostatic attraction plays an important role in metal adsorption (Lugo-Lugo et al., 2009). As the pH increases, metal adsorption increased since ion exchange is more effective when fewer protons are available to compete with the metal for binding sites onto the adsorbent. At low pH, there was a high concentration of H^+ ions, which competed with Pb(II), resulting in decrease of heavy metals adsorption. At higher pH, more divalent cation forms disappear, and more soluble or insoluble hydroxylated forms of metals increases (Comte et al., 2008). The formation of hydroxylated complexes of the metal would also compete with the active sites and as a consequence, the retention would decrease again.

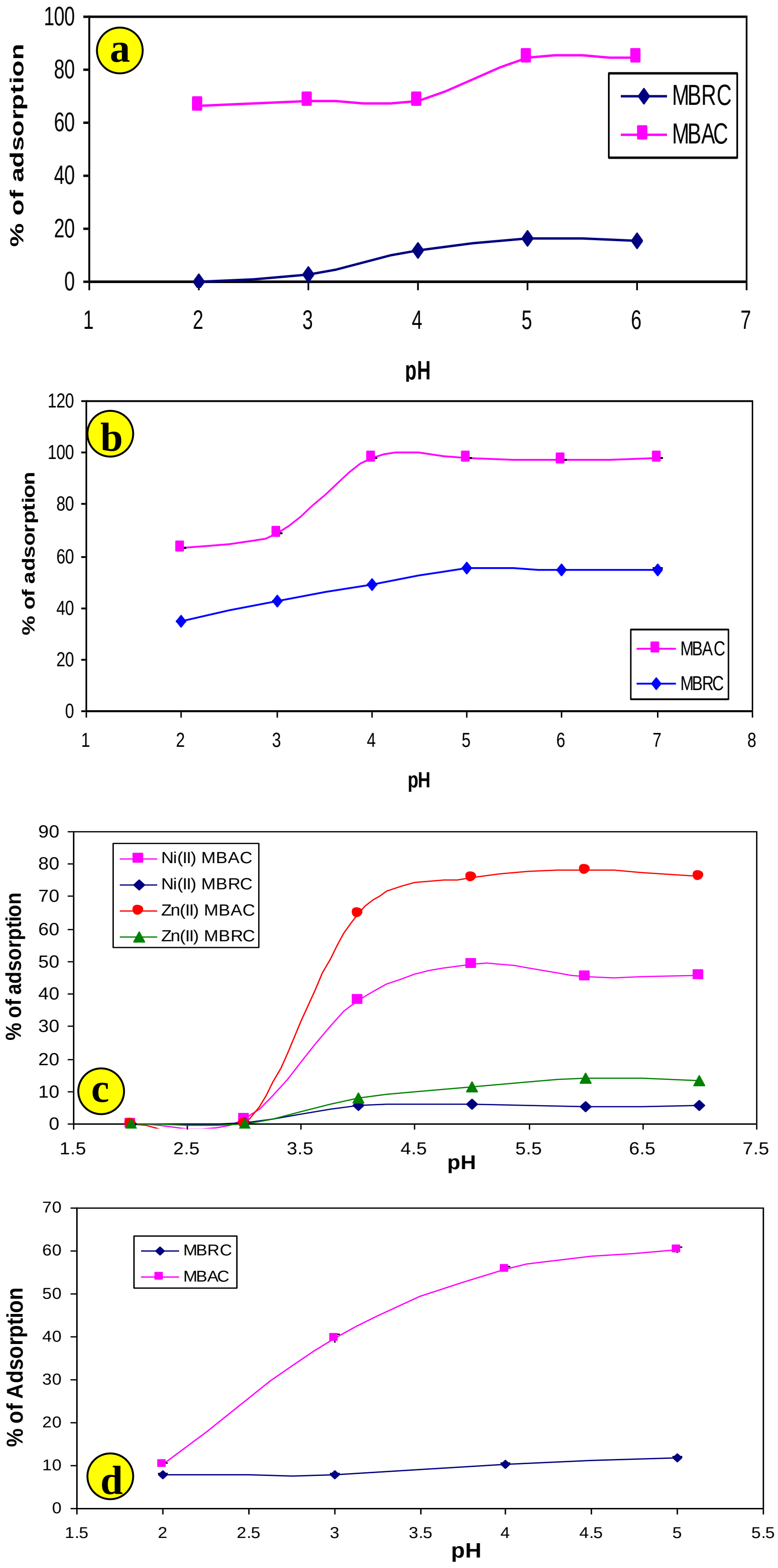


Figure 13. Effect of pH on a) Pb(II); b) Cd(II); c) Ni(II) and Zn(II); d) Cu(II) removal by *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC).

3.12.3.2. Effect of adsorbent dosage

The number of available sites for the adsorption of metal ions also depends on the amount of adsorbent. Adsorption of heavy metals at different adsorbent doses (100 to 500 mg/100mL) was carried out at a constant concentration of metal solutions. The percentage of adsorption of Pb(II) was increased from 14.73% – 81.69% for MBRC. Similarly, the percentage of adsorption was increased from 58.81% – 98.76% for MBAC with increase in adsorbent dose as shown in Fig. 14a. This can be explained as when adsorbent dose increased, more and more surface area will be available which exposed more active sites for binding of metal ions. A similar trend for the effect of adsorbent concentration was also observed and discuss in some literatures (Jayaram et al., 2009; Prasanna Kumar et al., 2006).The influence of adsorbent concentration on the Cd(II) adsorption is shown in Fig. 14b. The adsorptive behaviour of Cd(II) ions on MBAC and MBRC are similar. However, the percentage of adsorption of Cd(II) onto MBAC was higher than that of MBRC. From Fig. 14b, the percentage of adsorption increased with increase in biomass dose. This is due to availability of more active sites for the metal ions to bind. The maximum increase in percentage of adsorption was at 400 mg i.e. 98.32 and 65.42 for MBAC and MBRC respectively, after that the increase in percentage of adsorption was 99.11% and 68.46% at 500 mg for MBAC and MBRC respectively. These are little or no significant changes in adsorption. So 400 mg in 3 mg/L of Cd(II) concentration shows optimum adsorbent dose.

The influence of adsorbent concentration on the metal ions adsorption was investigated from 100 to 500 mg keeping Ni(II) and Zn(II) concentrations 50 mg/L and 30 mg/L, respectively. The percentage of adsorption increased as the adsorbent dose increased. Maximum increase in percentage of adsorption was observed with adsorbent dose of 400 mg/100 mL of Ni(II) and Zn(II) solution onto MBRC and MBAC (Fig. 14c).

The adsorptive behaviour of Cu(II) ions on MBAC and MBRC are similar. However, the percentage of adsorption of Cu(II) onto MBAC was higher than that of MBRC. The maximum increase in percentage of adsorption was at 400 mg i.e. 57.34% and 29.102% for MBAC and MBRC respectively, after that there are little or

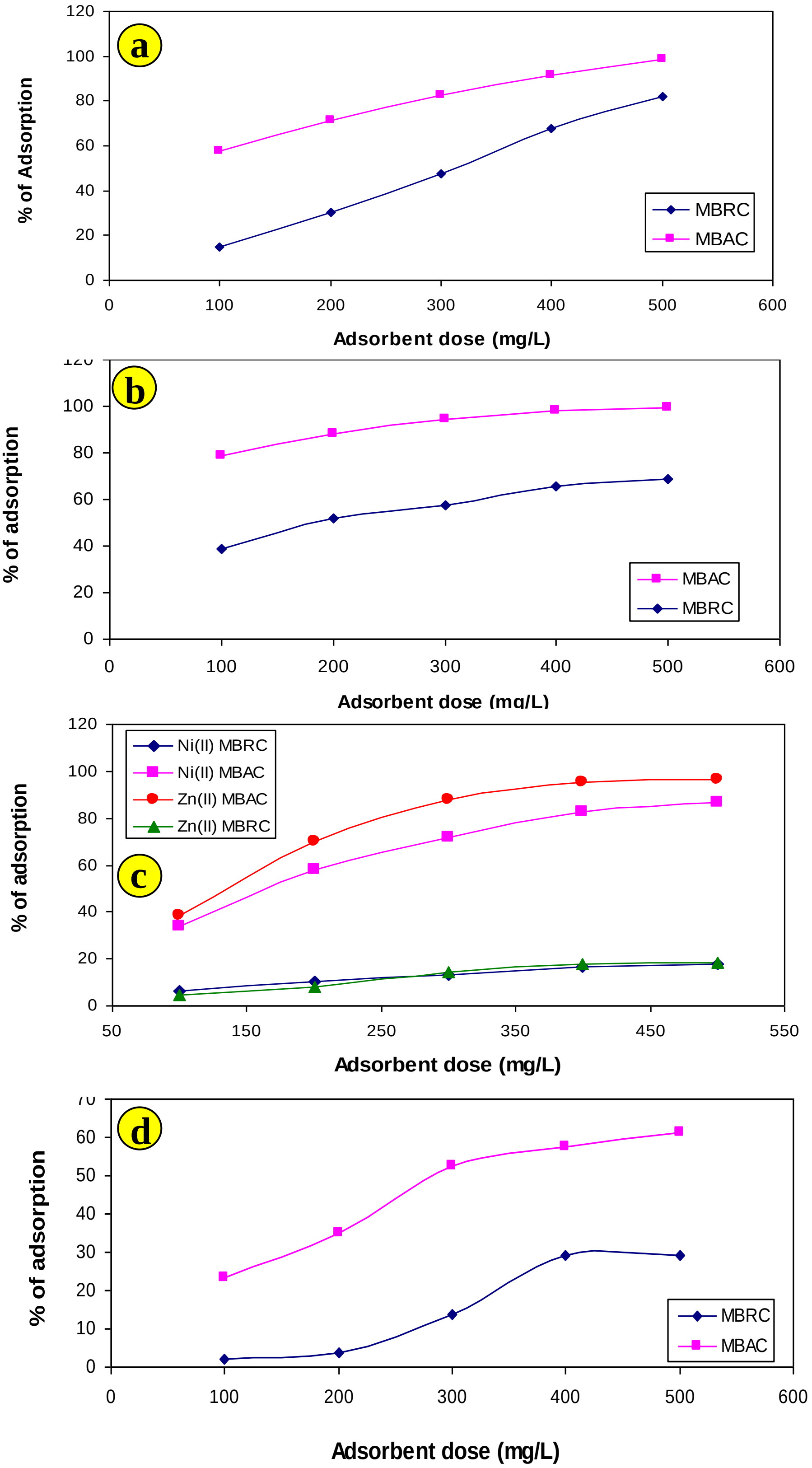


Figure 14. Effect of adsorbent dosage on a) Pb(II); b) Cd(II); c) Ni(II) and Zn(II); d) Cu(II) removal by *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC)

no significant changes in adsorption (Fig. 14d). So 400 mg in 80 mg/L of Cu(II) concentration shows optimum adsorbent dose.

The percentage of adsorption increased with increase in biomass dose. This can be explained that increase in the percentage of removal with the increase in the adsorbent dose is due to the increase in the number of active adsorption sites. The adsorption capacity was lesser at a higher adsorbent dose. This is due to overlapping and aggregation of adsorption sites resulting in the decrease of the surface area available to metal ions.

3.12.3.3. Effect of initial metal concentration

Adsorption experiments of Pb(II) concentration from 50 to 90 mg/L with a fixed adsorbent doses (0.1 g/100 mL) at pH 5 for MBRC and MBAC were performed. For MBRC, Pb(II) removal range from 19.46% to 11.22% whereas for MBAC, Pb(II) removal range from 95.74% and 59.87%. Adsorption experiments of Cd(II) concentration from 5 to 30 mg/L with fixed adsorbent doses at 29^{o}C at pH 5 for MBRC and MBAC were performed (Fig. 15). For MBRC, Cd(II) removal ranges from 68.12% to 36.96% whereas for MBAC, Cd(II) removal ranges from 96.74% and 78.106%. As shown in Fig. 15, the percentage of adsorption decreased from 82.89% to 70.02% for MBAC and 16.51 % to 13.61% for MBRC when initial metal concentrations increased from 50 mg/L to 70 mg/L in case of Ni(II) removal. The percentage of adsorption decreased from 95.31% to 56.17% for MBAC and 14.85% to 6.46% when metal concentrations increased from 30 mg/L to 70 mg/L in case of Zn(II) removal (Fig. 15). Effect of initial metal concentration of Cu(II) concentration from 80 to 120 mg/L with fixed adsorbent for MBRC and MBAC were performed (Fig. 15). With the increase in the initial metal concentration of Cu(II) concentration from 80 to 120 mg/L, Cu(II) removal decreased from 35.17% to 24.12% for MBRC. For MBAC, Cu(II) removal decreased from 62.49% to 45.61%.

As shown in Fig. 15, the percentage of heavy metals adsorption decreased with increase in metals ions concentration and showed little decrease or almost constant in percentage (%) of adsorption at higher concentration for all the heavy metals adsorption. This can be explained that all the adsorbents have a limited number of active sites, which would have become saturated at a certain concentration. For a

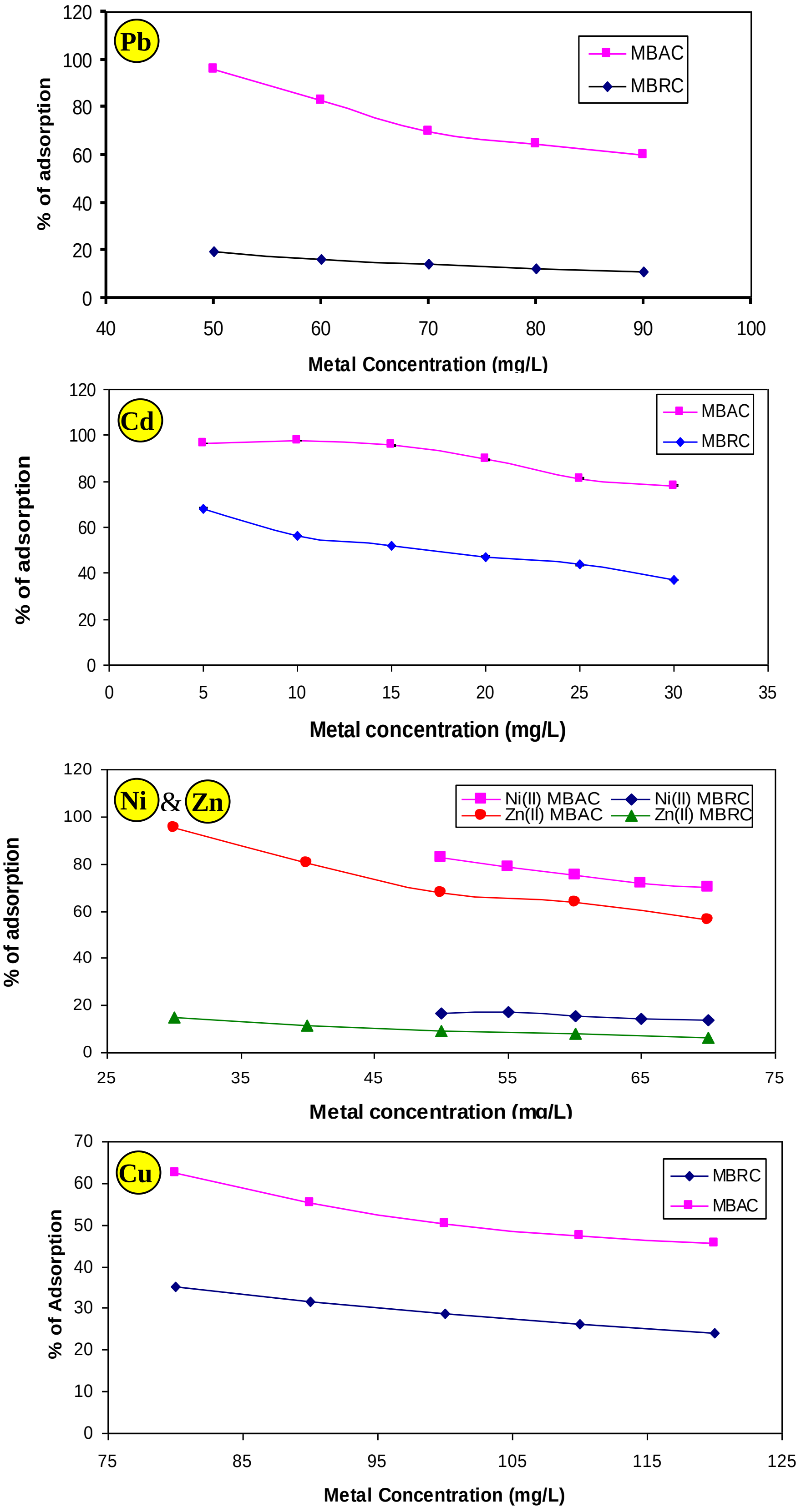


Figure 15. Effect of initial concentration of heavy metals onto the *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC).

fixed adsorbent dose, at the beginning of initial concentration, the heavy metals removal percentage was higher due to availability of more active sites. When the concentration of the metal ion solution becomes higher, the heavy metals removal percentage was lower because the available active sites for adsorption of metal become less. At a higher initial concentration, the ratio of initial number of moles of heavy metals to the available adsorption surface area was higher and as a result adsorption percentage was less. The data obtained from effect of initial concentration were further used to determine the isotherms of heavy metals biosorption.

3.12.3.4. Effect of contact time

The effect of contact time (15-360 min) on the biosorption of lead (60 mg/L) onto MBRC and MBAC (0.1 g) in the solution of pH 5 at 29^{o}C was shown in Fig. 16a. The lead percent adsorption increases with contact time until the equilibrium was attained. Studies conducted on the adsorption kinetics of Pb(II) removal revealed that the majority of metals ions were removed within the first 60-120 min contact with the adsorbents. The equilibrium state was attained at 360 min. It can be seen that the biosorption yield of Pb(II) increases with rise in contact time up to 120 min for MBRC and MBAC. Percentage of maximum biosorption was 13.63% for MBRC and 83.01% for MBAC. Afterwards, there was no significant increase in Pb(II) adsorption. The fast adsorption at the initial stage is probably due to the increased concentration gradient between the adsorbate in solution and adsorbate in adsorbent as there must be increased number of vacant sites available in the beginning (Acharya et al., 2009). The initial fast uptake of Pb(II) is through physical adsorption since adsorption phenomenon tends to attain instantaneous equilibrium. As contact time was increased, more and more functional groups participated in adsorption of the metal ions until it reached equilibrium. After it reached the equilibrium, there was no significant changes in lead concentration in the solution.

The effect of contact time on the adsorption of cadmium (10 mg/L) onto MBRC and MBAC in the solution of pH 5 at 29^{o}C is shown in Fig. 16b. The rate of adsorption is very fast initially and the equilibrium state was reached at 360 min. The maximum increase in adsorption takes place within 30 min. The initial fast sorption may be explained as uptake of Cd(II) through physical adsorption since adsorption phenomenon characteristically tends to attain instantaneous equilibrium (Saeed et al., 2005). The number of active sites in the system is fixed and each active site can adsorb only one ion in a monolayer, therefore metal uptake by the sorbent surface is rapid initially and then decreases as the availability of active sites decreases thus slowing down the transfer of metal ion from solution to adsorbent surface (Jayaram et al., 2009).

The effect of contact time on the adsorption of Ni (50 mg/L) and Zn (30 mg/L) onto MBRC and MBAC is shown in Fig. 16c. The maximum adsorption occurred within 60 min, after which there were no significant changes. The equilibrium was reached at 360 min for Ni and Zn(II) adsorption onto MBRC and MBAC. Metal uptake by the sorbent surface is rapid initially upto 60 min and then decreases as time increases. This can be explained as: initially adsorption of metal ions is increased as contact time is increased due to the availability of more active sites. More and more functional groups participated in adsorption of the metal ions until it reached equilibrium. After it reached the equilibrium, there was no significant changes in Ni(II) and Zn(II) concentrations in the solutions.

The rate of adsorption of Cu(II) is very fast initially and the equilibrium state was reached at 360 min. The maximum increase in adsorption was occured within 120 min. After that the increase in adsorption was almost constant (Fig. 16d). The plot between percentage of adsorption versus time reveals that the rate of percentage removal of Cu(II) ions is initially fast which is probably due to the availability of larger active binding site on the surface of the biomass. As time increases, more and more metal ions are adsorbed onto the surface of the adsorbent until all the active sites was bonded by Cu(II) ions and the adsorption sites become exhausted, the rate of uptake is controlled by the rate of transport from the exterior to the interior sites of the adsorbent particles.

The data obtained from effect of contact time were further used to determine the kinetics of heavy metals biosorption.

3.12.4. Adsorption isotherm studies

Adsorption isotherms of Pb(II), Cd(II), Ni(II), Zn(II) and Cu(II) with a fixed adsorbent doses for MBRC and MBAC were studied using Langmuir, Freundlich and

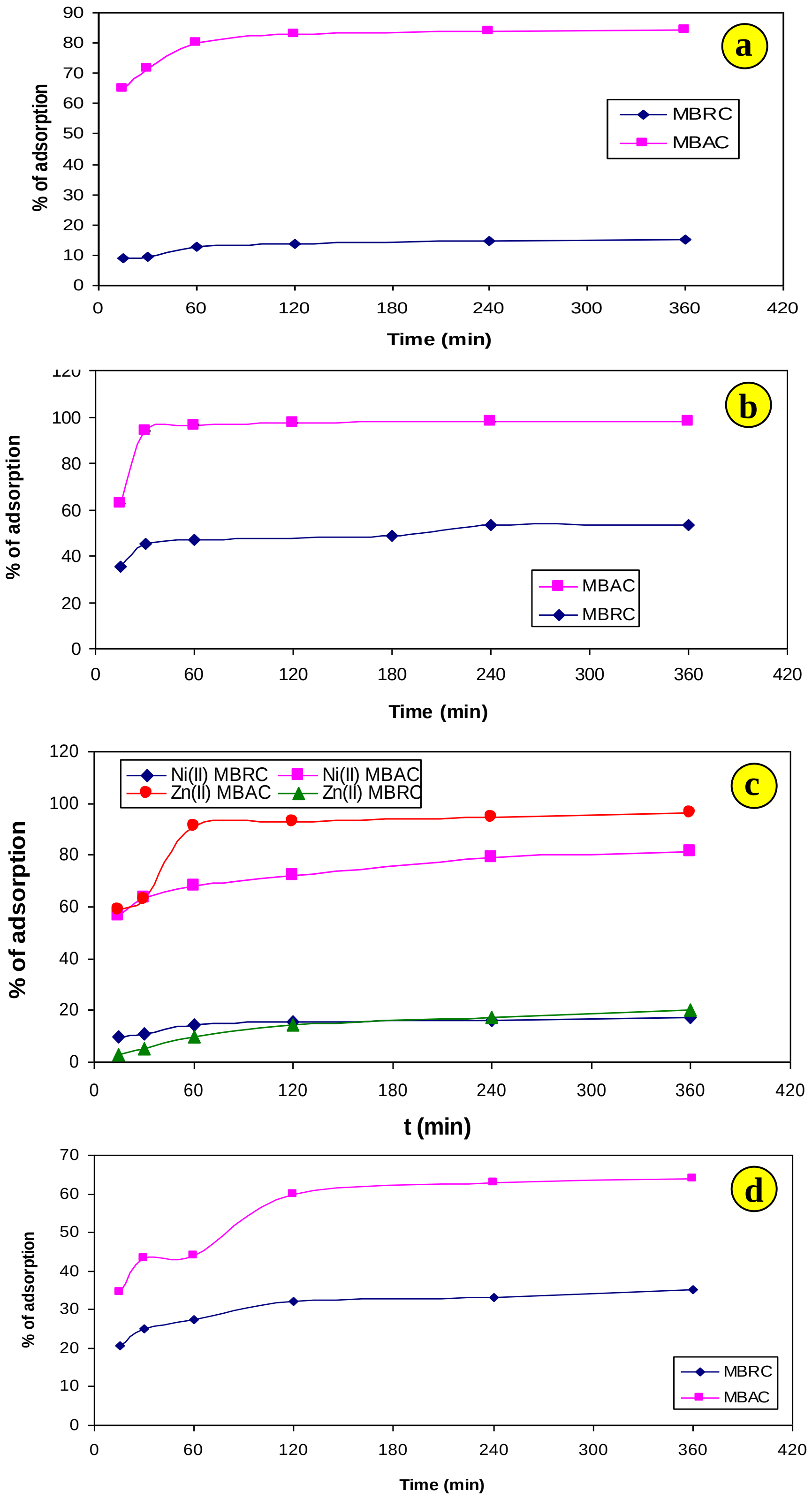


Figure 16. Effect of time on a) Pb(II); b) Cd(II); c) Ni(II) and Zn(II); d) Cu(II) removal by *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC)

Dubinin-Radushkevich (D-R) adsorption isotherms to describe the equilibrium relationships between sorbent and sorbate in solution. Sorption equilibrium is established when the concentration of sorbate in the bulk solution is dynamic balance with that of the interface (Ho and McKay et al., 2000).

The Langmuir linear isotherm mathematical form is as follows:

$$C_e/q_e = 1/q_{\max}b + C_e/q_{\max} \qquad (19)$$

where C_e (mg/L) is the concentration of heavy metals in solution at equilibrium, $q_{\max}$ (mg/g) is the maximum adsorption capacity corresponding to complete monolayer coverage and b (L/mg) is a parameter related to the energy of adsorption, respectively. A graph was plotted C_e/q_e versus C_e gives a straight line with correlation coefficient (R^2) of 0.998, 0.984, 0.995, 0.994 and 0.999 for MBRC and 0.998, 0.991, 0.999, 0.992, 0.991 for MBAC of Pb(II), Cd(II), Ni(II), ZN(II), Cu(II), respectively (Fig. 17). The slopes and intercepts of the graph were used to calculate the $q_{\max}$ and b for each heavy metal (Table 3).

The Freundlich isotherm mathematical formula is given as:

$$\log q_e = \log K_f + (1/n) \log C_e \qquad (20)$$

where C_e (mg/L) is the equilibrium concentration, q_e (mg/g) is the amount of metal ions adsorbed per specified amount of adsorbent at equilibrium, K_f (mg/g) and '*n*' are constants which are adsorption capacity and intensity of adsorption, respectively. A graph was plotted $\log q_e$ versus $\log C_e$. The graph gives a straight line with correlation coefficient (R^2) of 0.855, 0.984, 0.534, 0.244, 0.971 for MBRC and 0.679, 0.849, 0.994, 0.922, 0.921 for MBAC of Pb(II), Cd(II), Ni(II), ZN(II), Cu(II), respectively (Fig. 18). The slopes and intercepts of the graph were used to calculate the n and K_f values (Table 3).

The correlation coefficients of the Langmuir isotherm model were higher than Freundlich isotherm model and the experimental q_e values were also agreed well with the calculated data in Langmuir isotherm model rather than Freundlich isotherm model for MBRC and MBAC for all the heavy metals. The maximum adsorption capacity (q_{max}) value of MBAC was higher than MBRC (Table 3); this is attributed by

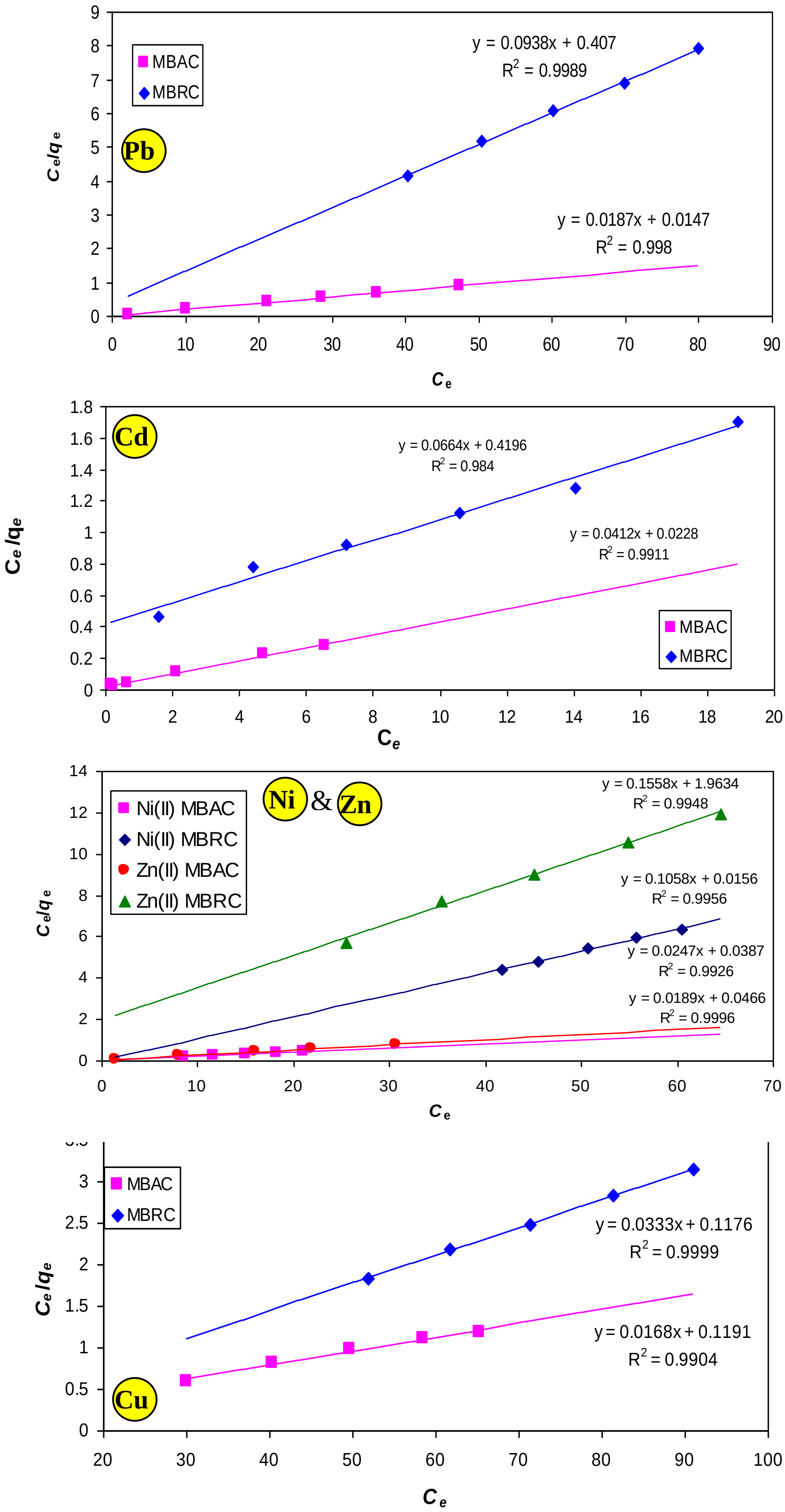


Figure 17. Linearized Langmuir isotherm plot for adsorption of heavy metals onto *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC).

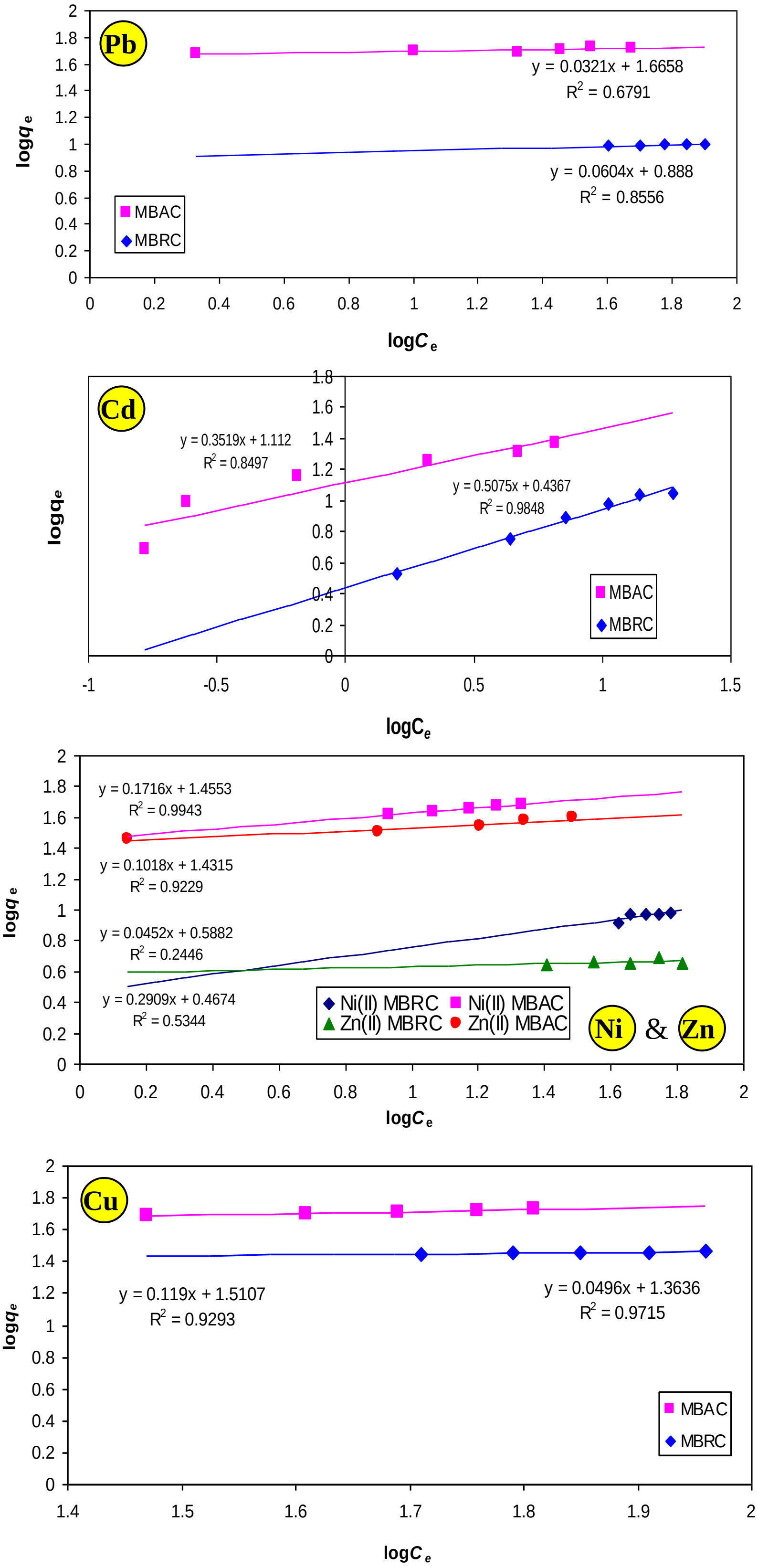


Figure 18. Linearized Freundlich isotherm plot for adsorption of heavy metals onto *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC)

Table 3. Comparison of Langmuir isotherms and Freundlich isotherm parameters with experimental values for the adsorption of heavy metals onto *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC)

Adsorbent	**Pb(II) MBRC**	**Pb(II) MBAC**	**Cd(II) MBRC**	**Cd(II) MBAC**	**Ni(II) MBRC**	**Ni(II) MBAC**	**Zn(II) MBRC**	**Zn(II) MBAC**	**Cu(II) MBRC**	**Cu(II) MBAC**
Experimental value										
q_{max} (mg/g)	10.104	53.88	11.08	23.43	9.53	49.01	4.52	39.32	28.96	54.74
Langmuir isotherm										
q_{max} (mg/g)	10.66	53.760	15.061	24.272	9.45	52.91	4.723	40.485	30.03	59.52
b (L/mg)	0.231	1.317	0.158	1.808	6.783	0.405	9.273	0.638	0.283	0.141
R^2	0.998	0.996	0.984	0.991	0.995	0.999	0.994	0.992	0.999	0.990
Freundlich isotherm										
K_f (mg/g)	7.730	46.320	2.89	14.63	28.53	2.93	3.87	27.01	23.09	32.41
n	16.556	31.153	2.064	4.09	5.82	3.43	22.12	9.82	20.16	8.403
R^2	0.855	0.679	0.984	0.849	0.994	0.534	0.244	0.922	0.971	0.929

larger surface area of MBAC which had exposed several active binding sites and functional groups for metal ions binding. Therefore, the fitness of the adsorption data of heavy metal ions to the Langmuir isotherm implies that the adsorbed metal ions do not interact or compete with each other and that they are adsorbed by forming a monolayer.

Further, the Langmuir isotherm was used to calculate a dimensionless constant called separation factor or equilibrium parameter (R_L) which tells the favorability of adsorption of heavy metals onto the adsorbents. Its mathematical formula was given as:

$$R_L = 1/\ (1+bC_i) \qquad (21)$$

where R_L is a dimensionless separation factor or equilibrium parameter, b is the Langmuir isotherm constant and C_i (mg/L) is the initial concentration of heavy metal ions. The parameters R_L indicate the shape of isotherm as follows: $R_L > 1$ unfavorable; $R_L = 1$ linear; $0 < R_L < 1$ favorable; $R_L = 0$ irreversible. The values of R_L at different concentrations of Pb(II) were calculated for MBRC and MBAC. The calculated R_L values at different concentrations for each heavy metal fall within a favorable range. A graph was plotted R_L versus C_i (Fig. 19). The graph showed that, with increasing initial concentration of metal ion, the R_L values were decreasing. This indicated that adsorption of heavy metal ions at higher concentration were also still favorable for both the biomass.

To account for the adsorption behaviour of the metal ions onto the MBRC and MBAC, the Langmuir type equation related to surface coverage (θ) was used. The equation is given as:

$$KC_i = \theta/1-\ \theta \qquad (22)$$

where K is the adsorption coefficient, C_i is initial concentration of lead ions and θ is the surface coverage. The fraction of biomass surface covered by metal ions was studied by plotting the surface coverage values (θ) versus each heavy metal ions concentration (Fig. 20). The increase in initial metal ion concentration for heavy metals, increased the surface coverage on the MBRC and MBAC until the surface was

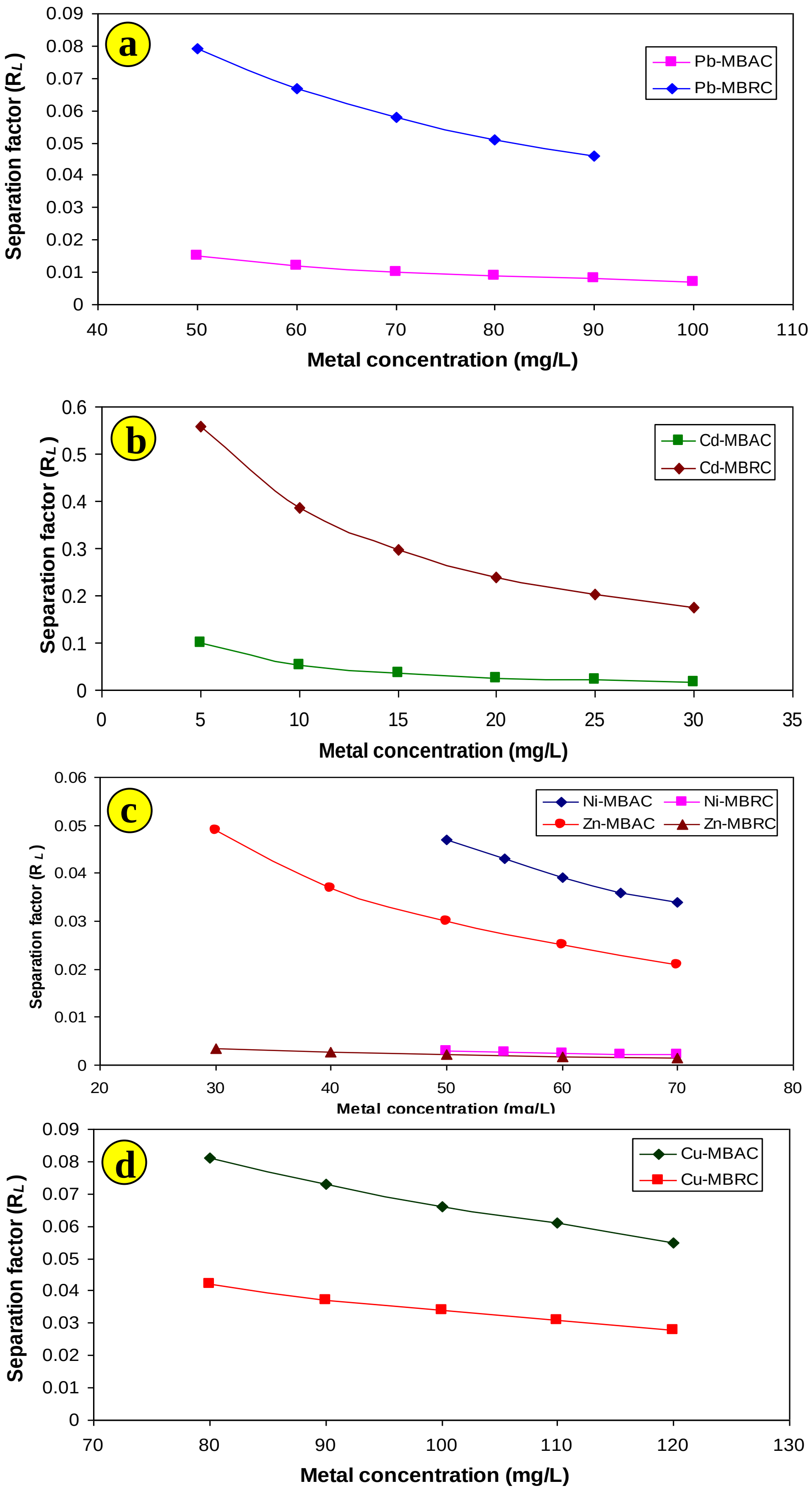


Figure 19. The calculated separation factor (R*L*) for a) Pb(II); Cd(II); c) Ni(II) and Zn(II); d) Cu(II) adsorption plot against metal ions concentrations (mg/L)

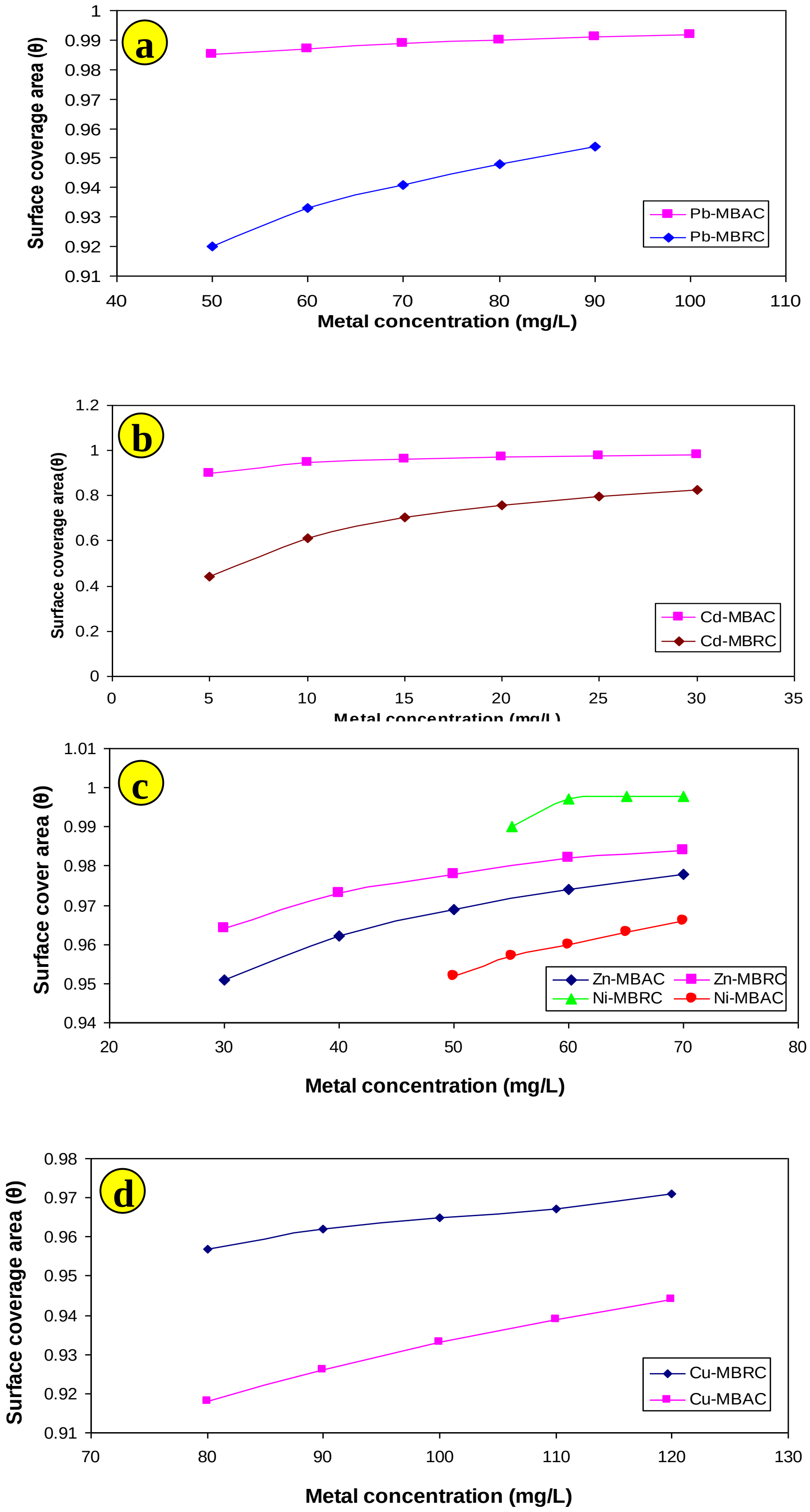


Figure 20. The calculated surface coverage area (θ) for a) Pb(II); Cd(II); c) Ni(II) and Zn(II); d) Cu(II) adsorption plot against metal ions concentrations (mg/L)

nearly fully covered with a monomolecular layer. This indicates that both the adsorbents will be highly effective in removing heavy metal ions from aqueous effluents.

The third isotherm is Dubinin-Radushkevich (D-R) isotherm model which determines the nature of sorption processes as physisorption, chemisorption or ions exchange. The linear form of D-R isotherm equation is:

$$\mathrm{In}q_e = \mathrm{In}X_m - \beta\varepsilon^2 \qquad (23)$$

where q_e (mol/g) is the metal ion concentration on the adsorbent at equilibrium, X_m (mol/g) is the maximum adsorption capacity, β is the activity coefficient related to the mean free energy of adsorption and ε is Polanyi potential. The Polanyi potential (ε) can be expressed as:

$$\varepsilon = RT\ \mathrm{In}\ (1 + 1/\mathrm{C}_e) \qquad (24)$$

where R (J/mol) is the universal gas constant, T is the absolute temperature in Kelvin and C_e (mol/L) is the metal ion concentration in the solution at equilibrium. A graph was plotted between $\mathrm{In}q_e$ versus ε^2 (Fig. 21) and gives a linear relationship with slopes of intercepts of X_m. The activity coefficient β was further used to calculate the adsorption mean free energy 'E' (kJ/mol) which is the free energy for the transfer of 1 mole of metal ions from the infinity to the surface of the adsorbent.

$$E = 1/\sqrt{-2\beta} \qquad (25)$$

based on equations (23) and (25), the isotherm constants, E and β are calculated and presented in Table 4. When one mole of ions is transferred, its value in the range of 1-8 kJ/mole indicates physical adsorption (Onyango et al., 2004), the value of E is in between 8-16 kJ/mol which indicates ion-exchange adsorption (Helfferich et al., 1962), if the value of E is in between 20-40 kJ/mol, it indicated chemisorption (Tahir and Rauf, 2006). Thus, the values of E for each heavy metals adsorption are calculated for MBRC and MBAC and presented in Table 4. Therefore, from the

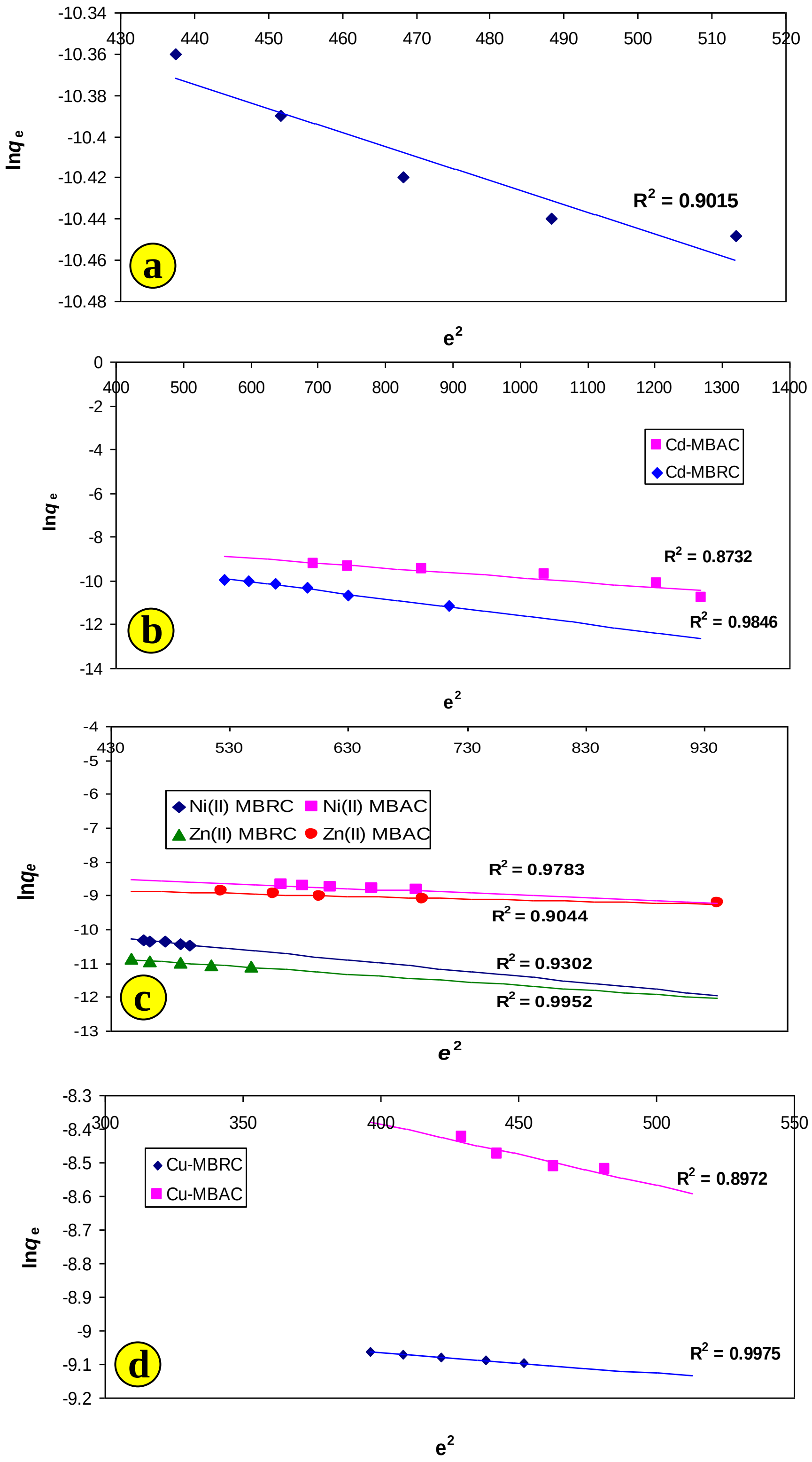


Figure 21. Linearized Dubinin-Radushkevich isotherm plot for adsorption of a) Pb(II); b) Cd(II); c) Ni(II) and Zn(II); d) Cu(II) onto *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC)

(*Note*: Since, Pb(II) adsorption onto MBAC was not fitted in D-R isotherm, the graph was not shown).

Table 4, it seems that chemisortion mechanism is dominating in the adsorption process of heavy metals.

Table 4. Dubinin-Radushkevich isotherm parameters values for the adsorption of heavy metals onto *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC)

Adsorbent	**Dubinin-Radushkevich**		
	E **(kJ/mol)**	β **(kJ^2/mol^2)**	R^2
Pb(II) MBRC	20.83	-0.0012	0.901
Cd(II) MBRC	11.32	-0.0039	0.984
Cd(II) MBAC	15.08	-0.0022	0.873
Ni(II) MBRC	12.13	-0.0034	0.931
Ni(II) MBAC	18.91	-0.0014	0.978
Zn(II) MBRC	14.74	-0.0023	0.995
Zn(II) MBAC	25.00	-0.0008	0.904
Cu(II) MBRC	28.91	-0.0006	0.997
Cu(II) MBAC	16.66	-0.0018	0.897

(Note: Since, Pb(II) adsorption onto MBAC was not fitted in D-R isotherm, the data was not shown).

3.12.5. Kinetics studies

The several kinetic models are needed to determine the data to examine the mechanism of adsorption process. Pseudo-first-order and Pseudo-second-order were used to explain the kinetics of adsorption to investigate the mechanism of biosorption. The Pseudo-first-order Lagergren model for solid/liquid systems of adsorption is expressed as

$$\log(q_e - q_t) = \log q_e - k_1 t/2.303 \qquad (26)$$

where q_e and q_t (mg/g) are the amounts of heavy metals adsorbed on the adsorbent at equilibrium and at any time t, k_1 (min^{-1}) is the rate constant of pseudo-first-order biosorption. A plotted of ($q_e - q_t$) versus t gives a straight line with correlation coefficient (R^2) of 0.963, 0.784, 0.842, 0.966, 0.904 for MBRC and 0.921, 0.817, 0.988, 0.752, 0.968 for MBAC of Pb(II), Cd(II), Ni(II), ZN(II), Cu(II), respectively (Fig. 22). The slopes and intercepts of plot of log were used to calculate the first-order rate constant k_1 and equilibrium adsorption capacity q_e for each heavy metal (Table 5).

The adsorption kinetics data were further analyzed using Ho and McKay's pseudo-second-order kinetic model. It is expressed as:

$$t/q_t = 1/k_2 q_e^2 + t/q_e \tag{27}$$

where k_2 is the equilibrium rate constant of pseudo-second-order adsorption (g/mg /min). A plotted of t/q_t versus t gives a straight line with correlation coefficient (R^2) of 0.998, 0.998, 0.999, 0.987, 0.999 for MBRC and 1.00, 0.999, 0.999, 0.999, 0.997 for MBAC of Pb(II), Cd(II), Ni(II), ZN(II), Cu(II), respectively (Fig. 23). The slopes and intercepts of plots were used to calculate the pseudo-second-order rate constants k_2 and q_e for each metal (Table 5).

From the Table 5, the correlation coefficients of the pseudo-second-order kinetics model for MBRC and MBAC were higher than pseudo-first-order kinetics and the experimental q_e values also agreed well with the calculated data of q_e values from pseudo-second-order rather than pseudo-first-order model for eacg metal. Therefore, it can be concluded that this adsorption system followed a pseudo-second-order reaction rather than pseudo-first-order reaction. This suggests that sorption of the metal ions involve two species, in this case, the metal ion and the adsorbent.

3.12.6. FTIR analysis of functional groups involved in adsorption

The FTIR spectral analysis is important to identify some characteristic functional groups which are responsible of adsorbing metal ions (Ahalya et al., 2005; Sheng et al., 2004; Tarley and Arruda, 2004; Hanif et al., 2007). The FTIR spectra of the adsorbents were recorded in the range of 400-4000 cm^{-1} to obtain the information regarding stretching and bending vibrations of the functional groups which are involved in the adsorption of heavy metal ions. The FTIR spectra of raw charcoal,

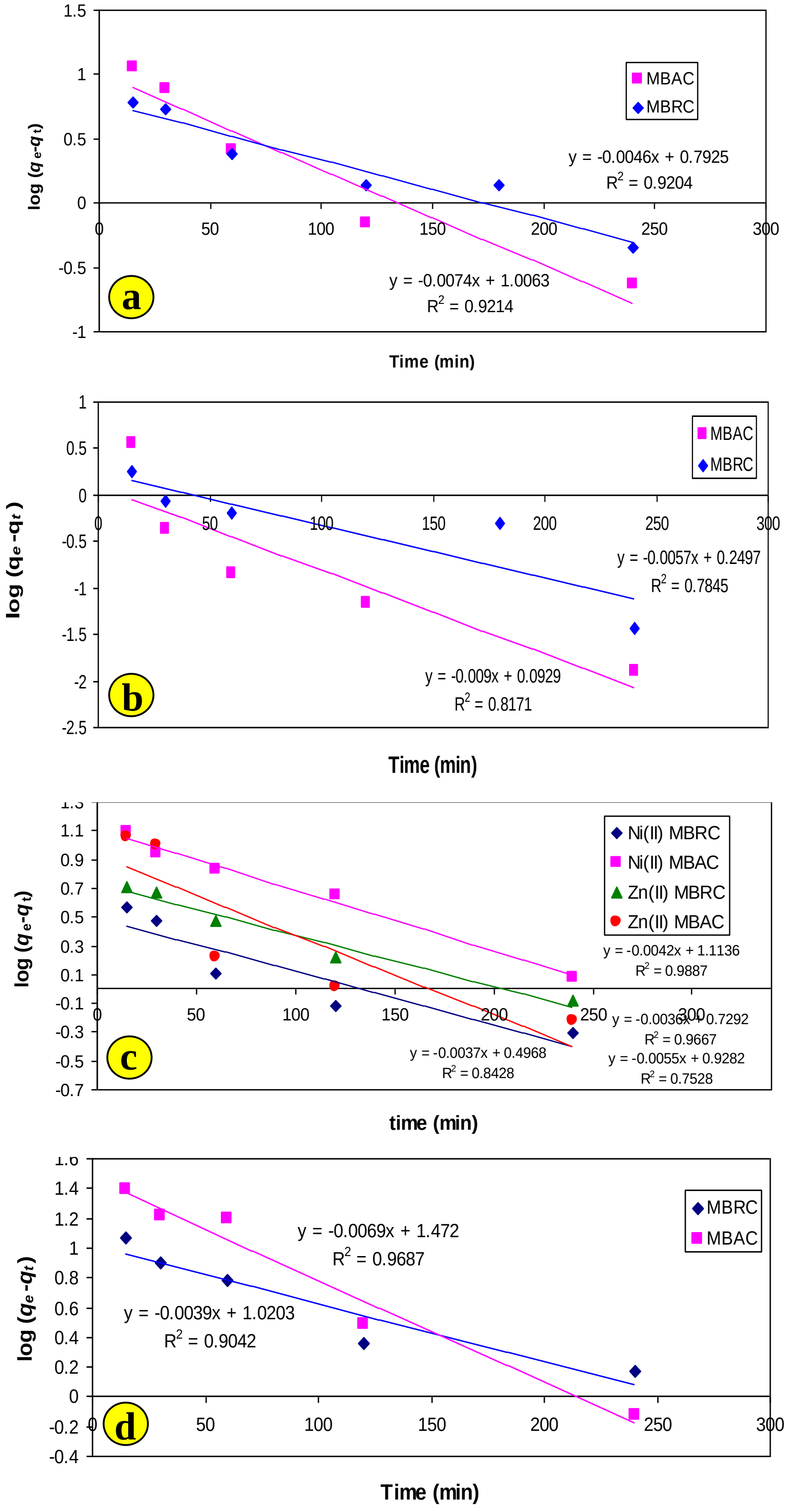


Figure 22. Pseudo-first-order kinetic of a) Pb(II); b) Cd(II); c) Ni(II) and Zn(II); d) Cu(II) removal by MBRC and MBAC

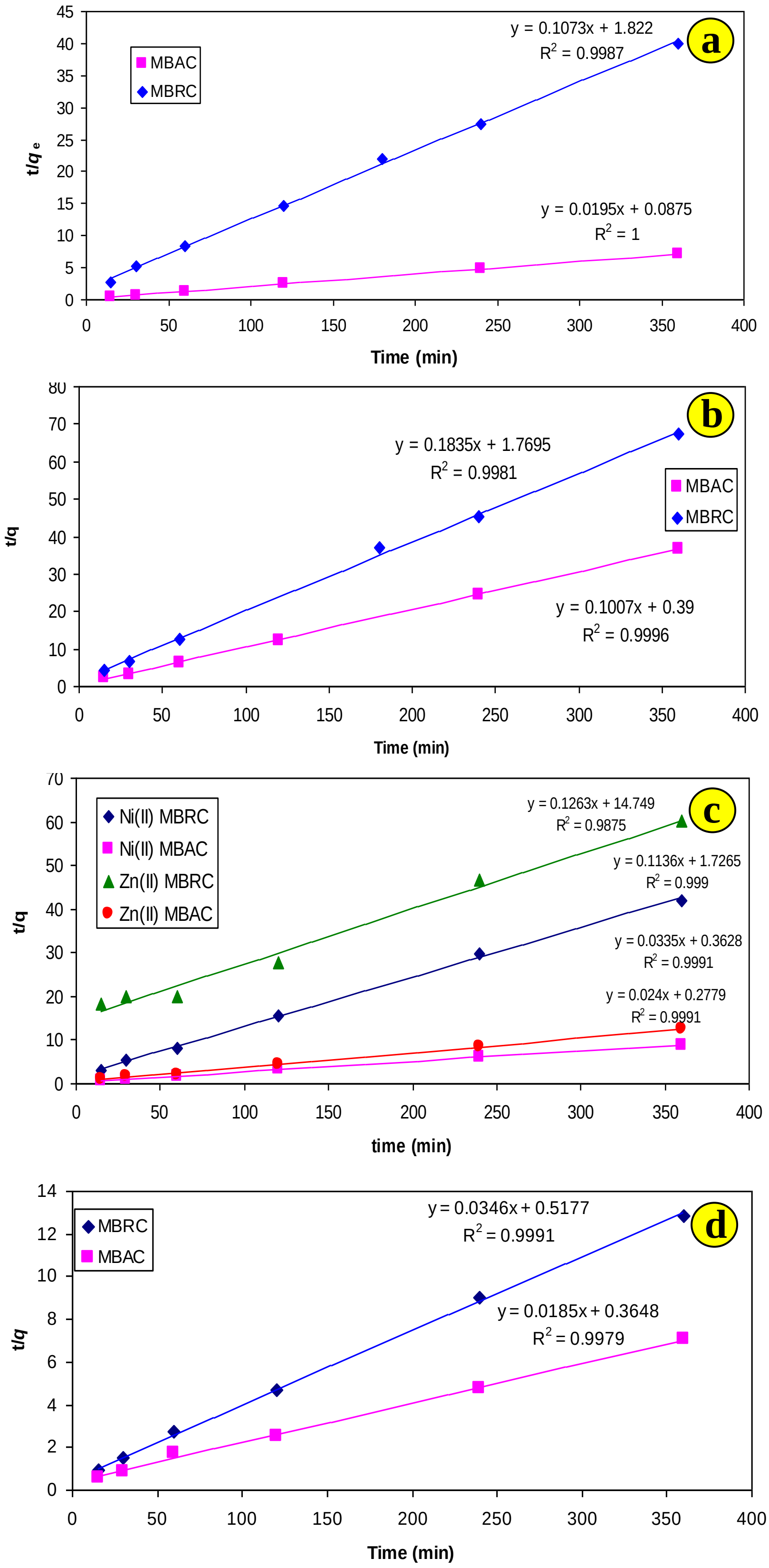


Figure 23. Pseudo-second-order kinetic of a) Pb(II); b) Cd(II); c) Ni(II) and Zn(II); d) Cu(II) removal by MBRC and MBAC

Table 5. Calculation of kinetic parameters of pseudo-second-order and pseudo-first-order model for heavy metals removal

Adsorbent	**Experimental value**	**Second-order-kinetic model**			**First- order-kinetic model**		
	***qe*(mg/g)**	***q*e.cal.(mg/g)**	**k_2 (g/mg min)**	***R^2***	***q*e.cal.(mg/g)**	**k_1 (min^{-1})**	***R^2***
Pb-MBRC	8.99	9.363	6.605 X 10-3	0.998	3.786	17.042 X 10-3	0.963
Pb-MBAC	50.49	51.282	4.345 X 10-3	1.00	10.146	11.515 X 10-3	0.921
Cd-MBRC	5.35	5.449	33.67 X 10-3	0.998	1.777	13.127 X 10-3	0.784
Cd-MBAC	9.79	9.931	10.14 X 10-3	0.999	1.238	20.727 X 10-3	0.817
Ni-MBRC	8.55	8.81	7.46 X 10-3	0.999	6.96	8.52 X 10-3	0.842
Ni-MBAC	40.63	41.66	2.07 X 10-3	0.999	12.98	9.67 X 10-3	0.988
Zn-MBRC	5.98	7.91	1.08 X 10-3	0.987	3.36	8.29 X 10-3	0.966
Zn-MBAC	28.98	29.85	3.09 X 10-3	0.999	8.47	12.66 X 10-3	0.752
Cu-MBRC	28.035	29.06	2.28 X 10-3	0.999	10.47	8.98 X 10-3	0.904
Cu-MBAC	51.03	54.054	93.81 X 10-3	0.997	29.64	15.89 X 10-3	0.968

metals loaded raw charcoal, activated charcoal and metal loaded activated charcoal were plotted to obtain information on the nature of possible interactions between the functional groups and the metal ions. Raw charcoal shows a spectrum of 1709 cm^{-1}, 1572 cm^{-1}, 1388 cm^{-1}, 1167 cm^{-1}, 1011 cm^{-1}, 882 cm^{-1}, 821 cm^{-1}, 553 cm^{-1} and 469 cm^{-1}. Similarly, activated charcoal also shows a spectrum of 3476 cm^{-1}, 1735 cm^{-1}, 1585 cm^{-1}, 1440 cm^{-1}, 1379 cm^{-1}, 1109 cm^{-1}, 1022 cm^{-1}, 879 cm^{-1}, 819 cm^{-1}, 744 cm^{-1}, 543 cm^{-1} and 466 cm^{-1}. After metal loaded onto raw and activated charcoals, the disappearance, decrease in intensity and shifts of these clear bands could be observed. In case of Pb(II) loaded on raw charcoal, band at 1709 cm^{-1} shifted to 1743 cm^{-1}, the band intensity at 1572 cm^{-1} clearly decreased and shifted to 1608 cm^{-1}, 1388 cm^{-1} band disappeared, 1167 cm^{-1} shifted to 1199 cm^{-1}, 1011 cm^{-1} also disappeared, 882 cm^{-1} shifted to 897 cm^{-1}, the spectra of 821 cm^{-1}, 553 cm^{-1}, 469 cm^{-1} disappeared and comparing Pb(II) unloaded MBAC with Pb(II) loaded MBAC, the bands at 3476 cm^{-1} and 1735 cm^{-1} were disappeared, 1585 cm^{-1} band intensity was greatly reduced and shifted to 1595 cm^{-1}, 1440 cm^{-1} band was disappeared, 1379 cm^{-1} was shifted to 1396 cm^{-1}, 1109 cm^{-1} band was disappeared, no significant change was observed at 1022 cm^{-1}, 879 cm^{-1} and 819 cm^{-1} bands were disappeared, 744 cm^{-1} was shifted to 779 cm^{-1}, 543 cm^{-1} and 466 cm^{-1} bands also disappeared (Fig. 24a).

In case of Cd(II) loaded on MBRC, 1709 cm^{-1} band disappeared, the band intensity at 1572 cm^{-1} clearly decreased and shifted to 1583 cm^{-1}, 1388 cm^{-1} shifted to 1392 cm^{-1}, 1167 cm^{-1} and 1011 cm^{-1} bands disappeared, 882 cm^{-1} shifted to 884 cm^{-1}, the spectra of 821 cm^{-1}, 553 cm^{-1} disappeared and 469 cm^{-1} shifted to 471 cm^{-1}. In case of Cd(II) loaded on MBAC, the bands at 3476 cm^{-1} and 1735 cm^{-1} were also disappeared, 1585 cm^{-1} band intensity was reduced and shifted to 1589 cm^{-1}, 1440 cm^{-1} band was shifted to 1444 cm^{-1}, 1379 cm^{-1} was disappeared, 1109 cm^{-1} band was shifted to 1114 cm^{-1}, 1022 cm^{-1}, 879 cm^{-1} 819 cm^{-1} 744 cm^{-1} and 543 cm^{-1} bands disappeared and 466 cm^{-1} bands was shifted to 470 cm^{-1} (Fig. 24b).

Comparing Ni(II) unloaded charcoal with Ni(II) loaded MBRC, 1709 cm^{-1} band disappeared, 1572 cm^{-1} band intensity clearly decreased and shifted to 1574 cm^{-1}, 1388 cm^{-1} shifted to 1365 cm^{-1}, band at 1167 cm^{-1} disappeared, 1011 cm^{-1} band shifted to 1012 cm^{-1}, 882 cm^{-1} band disappeared, 821 cm^{-1} shifted to 780 cm^{-1}, the spectra of 553 cm^{-1} and 469 cm^{-1} disappeared. For FTIR analysis of Ni(II) loaded onto MBAC adsorption, the bands at 3476 cm^{-1} and 1735 cm^{-1} also disappeared, 1585 cm^{-1} band intensity was greatly reduced, 1440 cm^{-1} band disappeared, 1379 cm^{-1} was shifted to 1413 cm^{-1}, 1109 cm^{-1} band was shifted to 1172 cm^{-1}, 1022 cm^{-1} band was shifted to 1091 cm^{-1}, there was no significant changes at 879 cm^{-1} and 819 cm^{-1}, 744 cm^{-1} and 543 cm^{-1} bands disappeared and 466 cm^{-1} bands was shifted to 472 cm^{-1} (Fig. 24c).

In case of Zn(II) loaded onto MBRC, the band at 1709 cm^{-1} disappeared, 1572 cm^{-1} band intensity decreased and shifted to 1585 cm^{-1}, 1388 cm^{-1} shifted to 1394 cm^{-1}, 1167 cm^{-1} band disappeared, 1011 cm^{-1} band shifted to 1020 cm^{-1} and 882 cm^{-1}, 821 cm^{-1}, 553 cm^{-1}, 469 cm^{-1} bands disappeared. In case of Zn(II) loaded onto the MBAC, the bands at 3476 cm^{-1} and 1735 cm^{-1} also disappeared, 1585 cm^{-1} band intensity was greatly reduced and shifted to 1593 cm^{-1}, 1440 cm^{-1} and 1379 cm^{-1} bands disappeared, 1109 cm^{-1} band was not changed, 1022 cm^{-1} band disappeared, 879 cm^{-1} was shifted to 875 cm^{-1}, 819 cm^{-1} shifted to 812 cm^{-1}, 744 cm^{-1}, 543 cm^{-1} and 466 cm^{-1} bands disappeared (Fig. 25a).

After Cu(II) loaded onto raw charcoal, the disappearance and shifts of these clear bands could be observed at 1709 cm^{-1} disappear, the band intensity at 1572 cm^{-1} clearly decreased and shifted to 1596 cm^{-1}, 1388 cm^{-1} shifted to 1446 cm^{-1}, 1167 cm^{-1} shifted to 1182 cm^{-1}, 1011 cm^{-1} shifted to 1024 cm^{-1}, the spectra of 882 cm^{-1}, 821 cm^{-1}, 553 cm^{-1} and 469 cm^{-1} also disappeared and Cu(II) loaded onto activated charcoal, the band intensity at 3476 cm^{-1} decreased and shifted to 3481 cm^{-1}, 1735 cm^{-1} disappeared, the band intensity at 1585 cm^{-1} highly decreased and shifted to 1602 cm^{-1}, the band at 1440 cm^{-1} disappeared, 1379 cm^{-1} shifted to 1389 cm^{-1}, 1109 cm^{-1} disappeared, the band at 1022 cm^{-1} shifted to 1035 cm^{-1}, the bands at 879 cm^{-1}, 819 cm^{-1}, 744 cm^{-1}, 543 cm^{-1} and 466 cm^{-1} also disappeared (Fig. 25b).

The decrease of the intensity, disappearance and shifts in the bands after Pb(II), Cd(II), Ni(II), Zn(II) and Cu(II) loaded onto MBRC and MBAC indicated that these functional groups were involved in binding processes on the surface of carbon (Pavasant et al., 2003). The names of functional groups present on the charcoals were mentioned under the sub-heading of adsorbent and its characterization.

The functional groups involved in the adsorption were as follows: a band at 3476 cm^{-1} representing hydroxyl group (-OH). Spectra of 1709 cm^{-1} and 1735 cm^{-1}

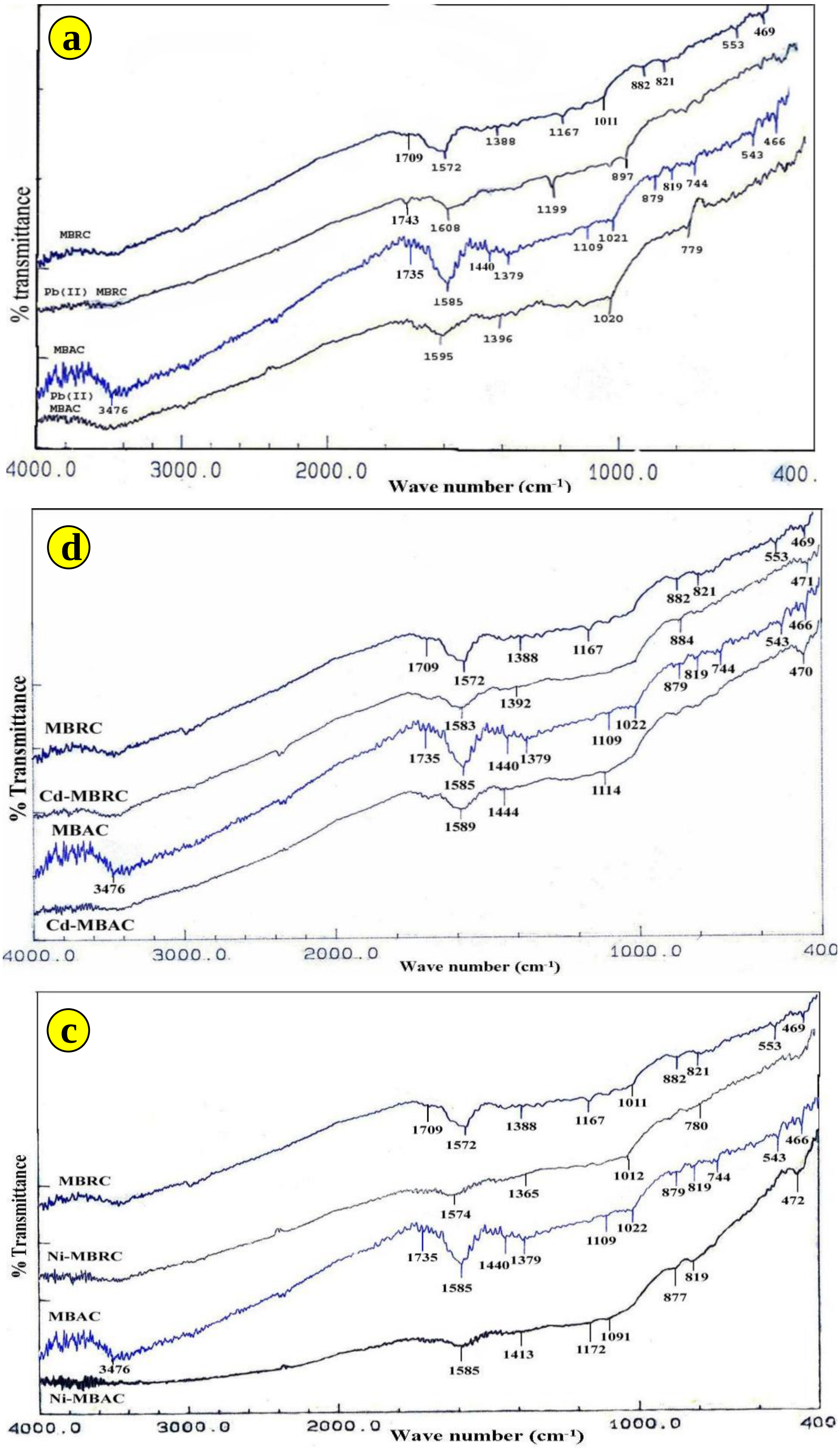


Figure 24. Surface functional groups analysis of MBRC and MBAC involved in a) Pb(II); b) Cd(II) and c) Ni(II) adsorption using FTIR

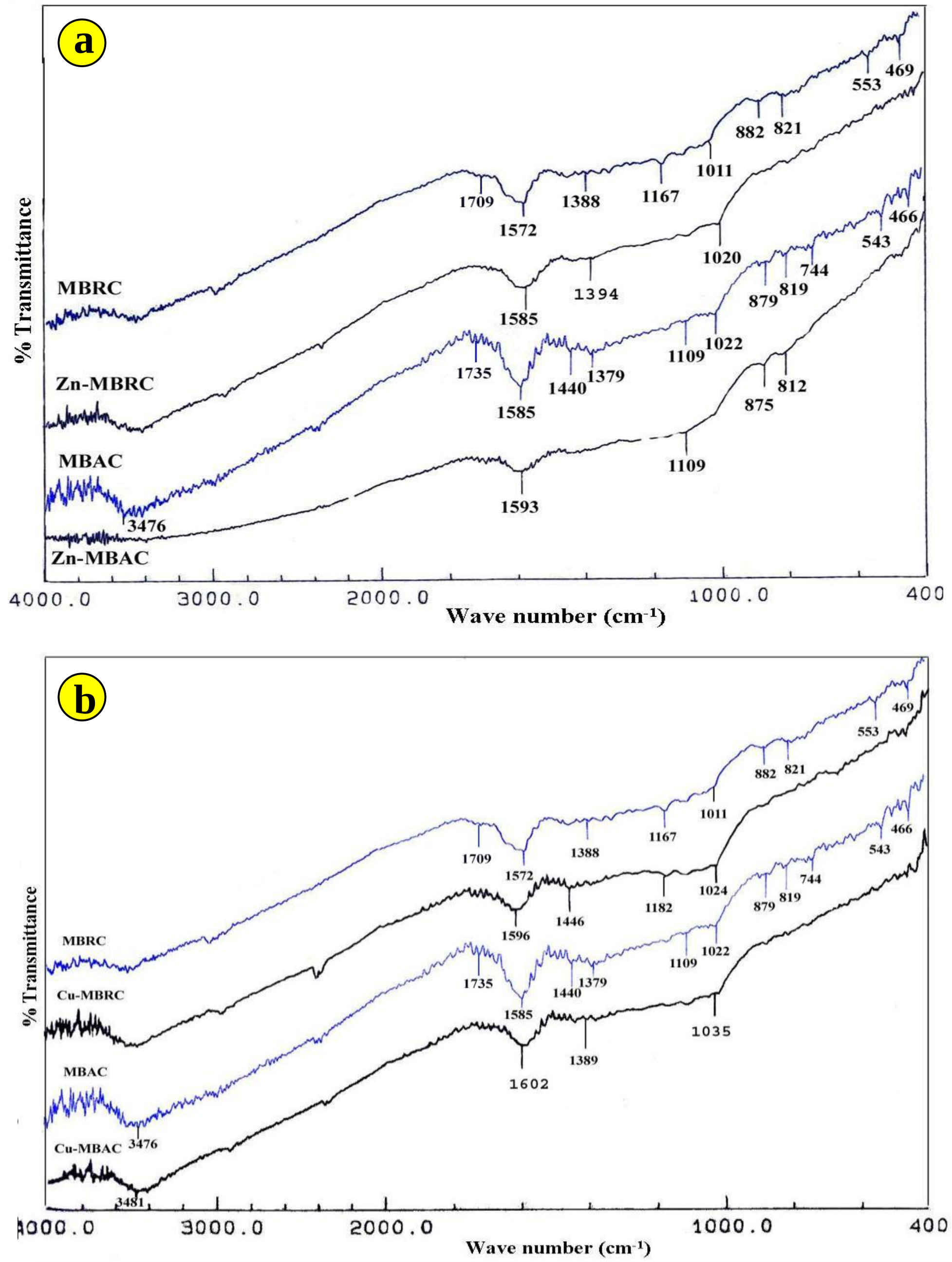


Figure 25. Surface functional groups analysis of MBRC and MBAC involved in a) Zn(II); and b) Cu(II) adsorption using FTIR

indicated the present carbonyl group. Spectra of 1572 cm^{-1} and 1585 cm^{-1} indicated the present of carboxyl group (-COOH). Spectra of 1379 cm^{-1}, 1388 cm^{-1} and 1440 cm^{-1} on the charcoals indicated represent $–CH_3$ wagging. 1109 cm^{-1} and 1167 cm^{-1} assigned the presence of -C-O and -C-C stretching. 1011 cm^{-1} and 1022 cm^{-1} spectra indicated the presence of -C-O-H stretch, $-CH_2$ mode. Some bands below 1000 cm^{-1} is called fingerprint zone. The region below 1000 cm^{-1} is the ‘fingerprint zone’ and the adsorption can not clearly be assigned to any particular vibration because they correspond to complex interacting vibration systems (Akkaya and Ozer, 2005). The fingerprint zone could be attributed to the phosphate or sulphur groups. These functional groups could act as chemical binding agents where C=O, -C-O-H etc could dissociate negatively charged active surface. This meant that these functional groups could attract the positively charged objects such as heavy metal ions (Volesky, 2000).

3.13. Conclusions

Due to intercalation of K^+ ion and oxidation by O^- into the carbon/charcoal during KOH activation of *M. baccifera*, micropore was formed; pore size and surface area were also increased. KOH solutions of 50% and 60% activation of *M. baccifera* charcoal show higher adsorption of heavy metals.

SEM analysis of the charcoals revealed that the carbonaceous surface was highly porous in nature; this increased the surface area for metal adsorption. The pore size of activated charcoal was larger in size than the raw charcoal. XRD analysis of MBRC and MBAC showed that hexagonally arranged carbon structure and these carbons are array in strata called turbostratic nature of carbon. Due to KOH activation, turbostratic nature of carbon was increased and the impurities were also removed. FTIR analysis of MBRC and MBAC showed that different surface functional groups are present and more functional groups i.e hydroxyl groups are added during KOH activation.

In batch mode adsorption studies, the maximum biosorption of Pb(II) was found at pH 5. Maximum adsorption capacity was 10.66 mg/g for MBRC and 53.76 mg/g for MBAC. Maximum increase of biosorption was attained at 120 min for MBRC and MBAC. Afterwards, there was no significant increase in Pb(II) adsorption. The maximum adsorption of Cd(II) was found 15.061 mg/g for MBRC and 24.271 mg/g MBAC at pH 5. Maximum increase in adsorption was attained at 30 min for MBRC and MBAC. The maximum adsorption of Ni(II) was obtained as 9.452 mg/g and 52.91 mg/g for MBRC and MBAC, respectively. Zn(II) $q_{\max}$ value was 4.723 mg/g and 40.485 mg/g for MBRC and MBAC, respectively. The equilibrium of adsorption was attained within 360 min. pH 5 are optimum for Cu(II) removal from the solution. The maximum adsorption capacity of MBRC and MBAC for removal of Cu(II) is 30.02 mg/g and 59.52 mg/g, respectively calculated using Langmuir isotherm.

The rate of adsorption was best described by Pseudo-second-order reaction. This implied that sorption of the metal ions involve two species, i.e, the metal ion and the adsorbent. The adsorption isotherm was best described by Langmuir isotherm for all the heavy metals removal. The FTIR analysis of MBRC and MBAC showed that different functional groups are involved in the adsorption of the metal ions. Maximum removal of heavy metals are Cu > Pb > Ni > Zn > Cd. For Dubinin-Radushkevich isotherm, most of the sorption pattern of MBRC and MBAC followed chemisorption for removal of heavy metals.

From these results, MBRC and MBAC can be efficiently used for the removal of different kinds of heavy metals from the aqueous solutions. *M. baccifera* charcoal powder adsorption process is highly reproducible and efficient. It further paves the way to develop a technology at large scale to remove various heavy metals from the contaminated aqueous medium.

4 Extraction, quantification and purification of phytosterols from *Melocanna baccifera* young shoot extract

4.1. Introduction

Plants contain a broad range of bioactive compounds such as phytochemicals, pharmaceuticals, fragrances and pigments. Plant extracts are widely used in food, pharmaceutical and cosmetics industries. Plant sterol is one of bioactive compound; also called phytosterols and are a group of steroid alcohols naturally occurring in plants and vegetable constituents and are therefore normal constituents of the human diet. It has been reported to include over 250 different sterols and related compounds in various plant and marine materials (Akihisa et al., 1991). The primary sources of phytosterols are vegetables, nuts, fruit and seeds. Plant sterols composition can vary within different species and physiological stage of development. The principal plant sterols are β-sitosterol (24α-ethylcholesterol), campesterol (24α-methylcholesterol) and stigmasterol (Δ^{22}, 24α-ethylcholesterol). In fact, in many food items sitosterol is reported as the most abundant form, followed by campesterol and stigmasterol. Many different minor plant sterols have been reported from various food sources. Ergosterol ($\Delta^{7,\ 22}$, 24α-methyl-cholesterol) is the principal sterol of yeast and occurs in corn, cotton-seed, peanut and linseed oils. Initially, phytosterols are used as pharmacological agents, but with the recognition that they are part of the normal diet, the idea arose of their administration in the form of conventional food products. At first, phytosterols are incorporated into margarine, but with new processes to improve their bioavailability, they are now marketed in fruit juice, ice cream, and other vehicles (Kritchevsky and Chen, 2005). The chemical structures of these sterols are similar to cholesterol; this means that sterol has cholesterol backbone structure and differing in the side chain only. For instance, sitosterol and stigmasterol have an ethyl group at C-24 and campesterol has a methyl group at the same position. The chemical name for cholesterol is 5α-cholesten-3β-ol, which contains one double bond compared to the basic sterol compound 5α-cholestan-3β-ol.

4.2. Chemical structural patterns of sterol

The term “sterol” is traditionally applied by chemists to describe a 3β-monohydroxy compound based upon the tetracyclic-cyclopentanophenanthrene ring system, with methyl substitution at C10 and C13 and a long flexible side chain with 8-10 carbon atoms at the C17 (Fig. 26). The four rings (A, B, C, D) have trans ring junctures, forming a flat α system. The side chain and the two methyl groups at C18 and C19 are angular to the ring structure and above the plane, thus having β stereochemistry (IUPAC, 1989). Moreover, the sterols create planer surfaces at the top and the bottom of the molecules, which allow for multiple hydrophobic interactions the rigid sterol nucleus and the membrane matrix (Nes, 1987; Bloch, 1988). Generally, the hydroxyl group at C3 also has β stereochemistry. The α and β notations are independent of constituents at neighbouring atoms, with α in front of the plane and β behind the plane. The complexity of phytosterols is increased further by steric asymmetry. Addition of alkyl groups in the side chain results in the substituted carbon becoming asymmetric. e.g, soybeans contain two 24-methylcholesterol isomers, campesterol (24α-configuration) and dihydrobrassicasterol (24β-configuration) (Nes et al., 1976). Generally, the α-configuration is more common in higher plants, whereas the β-configuration predominates in algae (Goodwin, 1985). Stereochemistry has not generally been considered in human and animal studies of phytosterols.

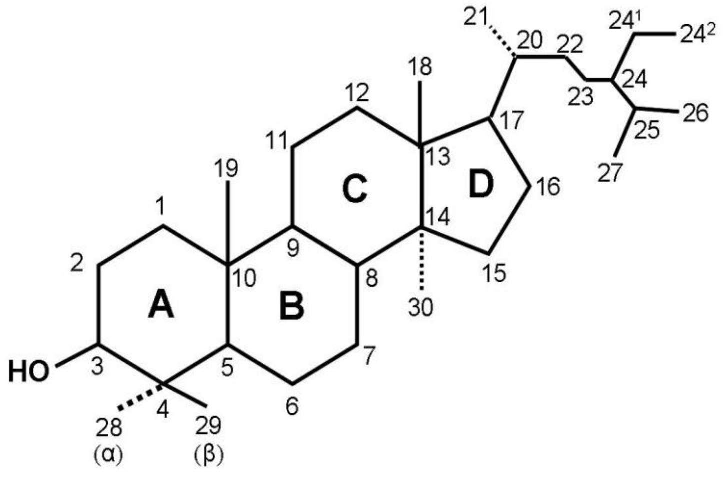


Figure 26. Basic structure of a sterol with standard carbon numbering according to the 1989 IUPAC-IUB recommendation (Moss, 1989)

In plants, sterols are always present as a mixture. Structural variations arise from different substitutions in the side chain and number and position of double bonds in the tetracyclic skeleton (Nes, 1977; Goad, 1991). Plant sterols can be divided on structural and biosynthetical grounds into 4-desmethyl sterols, 4α-monomethyl sterols and 4, 4-dimethyl sterols (Akihisa et al., 1991). The 4α-methyl sterols and 4, 4-dimethyl sterols are plant sterol precursors and exist at lower levels than the terminal products 4-desmethyl sterols (Hartmann and Benveniste, 1987). The 4-desmethyl sterols may be categorized into Δ^5-sterols, Δ^7-sterols and $\Delta^{5,7}$-sterols according to the position and number of double bonds in the B ring (Nes, 1987). Most plant sterols, eg campesterol and sitosterol have a Δ^5 bond and an additional one-carbon or two-carbon substituent in the side chain at C24 and 5α-reduction of that double bond is called stanols. These sterols are reflected in the common names of the compounds. For example, sitosterol is structurally identical to sitostanol except for the double bond at position 5. Similarly, campesterol bears the same relationship to campestanol (Katan et al., 2002). Sterols with the Δ^7 nucleus are frequently encountered in members of some plant families e.g. Caryophyllaceae, Theaceae and Cucurbitaceae (Nes, 1977).

Plant sterols are amphiphilic in nature and occur as membrane constituents. They are primarily encountered in the plasma membrane, the outer membrane of mitochondria and the endoplasmic reticulum, and to a large extent determine the properties of these membranes. Free sterols fit in membranes, because the overall length of free sterols is virtually the same as that of a phospholipids monolayer (Nes, 1987). In bulk the plant sterols are solid. The bigger its side chain, the more hydrophobic a sterol becomes. Thus plant sterols with 28 or 29 carbon atoms are more hydrophobic and have lower micellar solubilities than cholesterol with 27 carbon atoms (Armstrong and Carey, 1987).

4.3. Biosynthesis of sterols

Phytosterols are triterpenes and belong to the vast family of isoprenoids. The plant sterols are products of the isoprenoid biosynthesis pathway. In plants, the sterol biosynthesis pathway represents a sequence of more than 30 enzyme-catalyzed reactions, all associated with membranes (Benveniste, 1986). Isopentenyl pyrophosphate (IPP) serves as the fundamental building block for the biosynthesis of all the terpenoids, including sterols which are C30 triterpenoids. In case of sterols synthesis it has been shown that the IPP derives exclusively from the mevalonic acid pathway, as opposed to the pyruvate/glyceraldehydes-3-phosphate pathway which appears to be responsible for the synthesis of terpenes of plasticidal origin (McCaskill et al., 1998). However, it has been reported that in germinating corn seedlings, mevalonic acid may not be an intermediate in the synthesis of the phytosterols (Guo et al., 1995). With other pathways may be able to direct carbon flux from sugars and amino acids into IPP. This observation throws doubt on the pivotal role of 3-hydroxymethyl-3-glutaryl coenzyme A reductase (HMGR) in the plant sterol pathway (Piironen et al., 2000).

The conversion of IPP into dimethylalkyl diphosphate (DMAPP) by prenyl transferase serves as an important compound for elongation. Farnesyl pyrophosphate synthase converts the metabolite to farnesyl pyrophosphate (FPP), which is the branch point for the synthesis of sesquiterpenes and triterpenes (sterols). Direction into the sterol synthesis pathway is controlled by the activity of squalene synthetase. Sterols synthesis exclusively takes place in cytoplasm. In photosynthetic phyla, squalene oxide is converted into cycloartenol, whereas non-photosynthetic phyla convert squalene oxide into lanosterol finally ergosterol (in fungi) and cholesterol (in mammals) (Hartmann, 1998). The major post-squalene biosynthetic pathway is regulated by critical rate-limiting steps such as the methylation of cycloartenol into cycloeucalenol (Nes and Venkatramesh, 1997) by two different methyl transferase namely C24-methyl transferase (SMT1) and C4-demethylase (SMO1)/4α-decarboxylase/3-ketoreductase. The 9β, 19-cyclopropane ring of cycloeucalenol is opened by cycloeucalenol obtusifoliol isomerase (COI) to form obtusifoliol. The conversion of obtusifol to 4α-methyl ergostatrienol is carried by C14-demethylase and with the help of Δ^{14}-reductase; 4α-methyl ergostatrienol is reduced to 4α-methyl ergostadienol. Isomerisation of 4α-methyl ergostadienol takes place forming 24-methylene Iophenol and at this step branching takes place. In one branch after a sequence of enzyme-catalyzed reactions; methylene Iophenol gives rise to campesterol and brassicasterol. In another branch, methylene Iophenol is converted to 24-ethylidene Iophenol by $C24^1$-methyltransferase (SMT2). After a sequence of enzymatic reactions, 24-ethylidene Iophenol gives rise to sitosterol and stigmasterol (Schaller, 2004). Sterol biosynthesis pathways was shown in Fig. 27

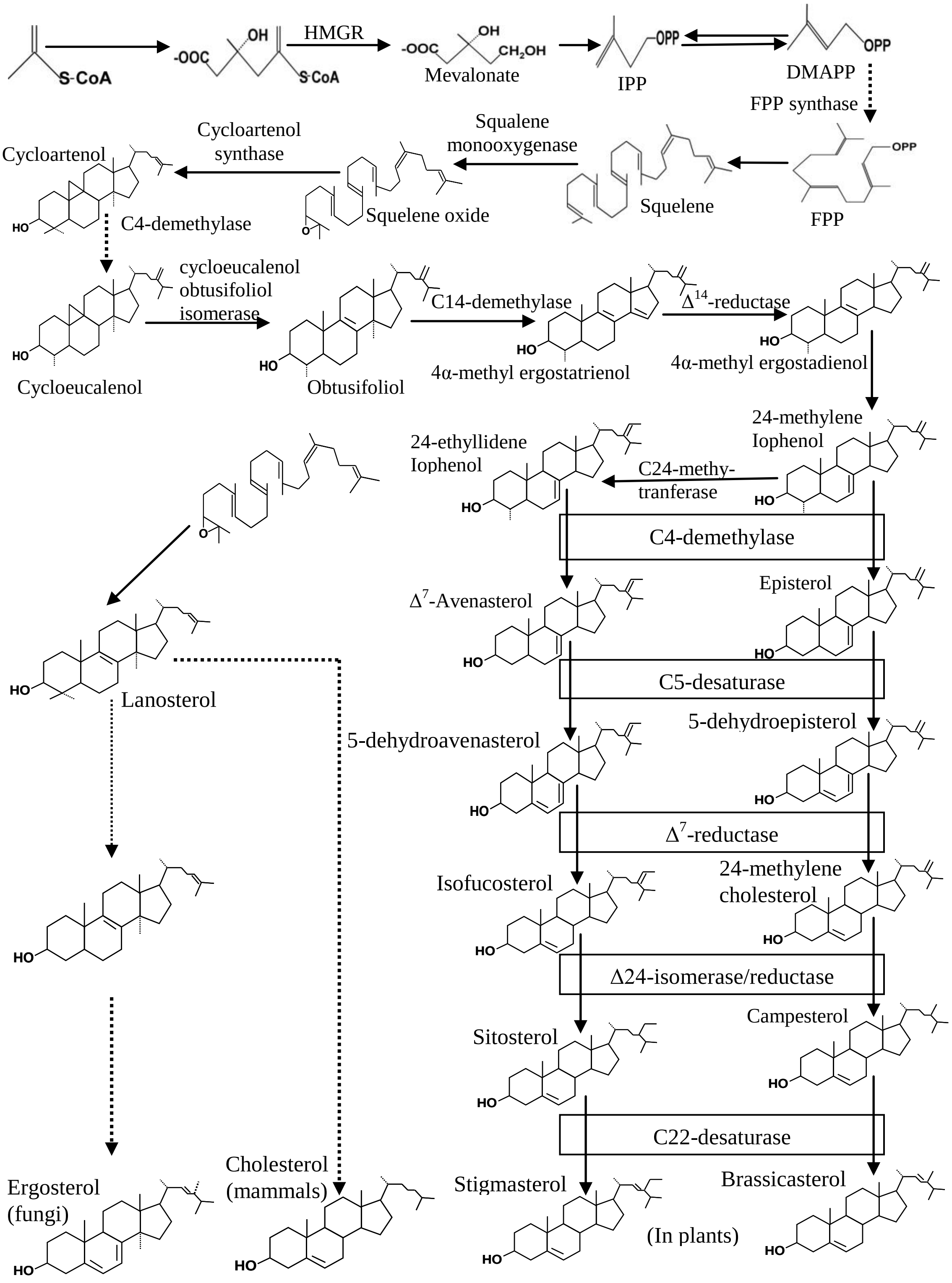


Figure 27. Different sterol biosynthesis pathways in living organisms. The opening of the cyclopropane ring is catalyzed by the cycloeucalenol obtusifoliol isomerase, a step occurring only in photosynthetic phyla. Broken arrows indicate multiple steps.

4.4. Biological functions of sterols in plants

Sterols are membrane components and it regulates membrane fluidity, permeability and probably plays a role in the adaptation of membranes to temperature. This structural role is often described as the ‘bulk’ function, because it is played by significant amounts of sterols and can be fulfilled by virtually any of the compounds. However, which involves the action of a few specific sterols; their involvement in signal transduction events has also been reported in mammalian cells. Sterols can also participate in the control of membrane-associated metabolic processes. A free hydroxyl group in free sterols is an important factor enabling its specific interactions with phospholipids and protein in the membranes (Wojciechowski, 1991). Moreover, sterols are the precursors of a vast array of compounds involved in important cellular and developmental processes in animals (e.g. steroid hormones and bile acids), fungi (e.g. ecdysteroids, antheridiol and oogoniol) and higher plants (e.g. brassinosteroids). Finally, in plants, sterols are substrates for the synthesis of a wide range of secondary metabolites – such as cardenolides, glycoalkaloids, pregnane derivatives and saponins. Saponins have been proposed to play a role in resistance to fungal attack (Osbourn, 1996). Whereas mammalian and fungal cells generally contain one major sterol, cholesterol and ergosterol, respectively, plants have a characteristically complex sterol mixture.

Sterol molecules are incorporated into membranes. The side chain extends into the hydrophobic core and interacts with fatty acid acyl chains of phospholipids and proteins with the free 3-hydroxyl facing the aqueous phase. Thus, they modulate the physical state of bilayers by restricting the motion of fatty acyl chains. Phosphatidylcholine bilayers indicate that all the plant sterols tested are able to regulate membrane fluidity, but with different efficiency (Schuler et al., 1990; Schuler et al., 1991; Krajewsky-Bertrand et al., 1992). Sitosterol and 24-methylcholesterol are the most efficient sterol for restricting the mobility of phospholipid fatty acyl chain. The introduction of a trans-oriented double bond at C22 in the side chain of the sterol molecule significantly reduced its ordering ability, as attested by the comparison of sitosterol and stigmasterol. Such a reduced ordering ability was observed only in the presence of an alkyl group in the side chain, as cholesterol and Δ^{22}-dehycholesterol have similar efficiencies (Krajewsky-Bertrand et al., 1992). Effects triggered by the same range of sterols on the water permeability of phosphatidylcholine bilayers have also been investigated (Schuler et al., 1991). Sitosterol and 24-methylcholesterol appeared to be very active in reducing membrane permeability. In contrast, stigmasterol is much inefficient. Experiments in these artificial membranes have shown a good correlation between acyl chain ordering and water permeability.

Sterols also play a role in cellular differentiation and proliferation. Their accumulation in seeds and oils is likely to provide a reservoir for the growth of new cells and shoots. Active sterol synthesis occurs following seed germination to meet the needs for synthesizing new membranes. The rate of sterol synthesis then gradually decreases with seed maturity (Guo et al., 1995). The role of steryl aster also includes storage and transport; this is supported by their presence in soluble forms, such as in lipoprotein complexes (Wojciechowski, 1991; Dyas and Goad, 1993).

Recent evidence from maize roots experiment suggests that plant sterols are able to modulate the activity of the plasma membrane H^+-ATPase (Grandmoujin-Ferjani et al., 1997). In particular, cholesterol and stigmasterol were found to stimulate proton pumping, especially at a low concentration, whereas all the other sterols tested (e.g. sitosterol and 24- methylcholesterol) behaved as inhibitors. When given at a concentration of 3 μM to intact maize roots, these two sterols are also able to stimulate H^+ secretion (Cerana et al., 1984). Therefore, they might be considered as effective ligands in a similar way that cholesterol is for the Na/K ATPase of animal cells (Cornelius, 1995). Specific sterol molecules might be required by surface receptors and participate in some signal transduction events as in mammalian cells.

4.5. Cellular distribution of sterols

All vegetable foods contain appreciable quantities of phytosterols. In plant free sterols reside predominantly in the plasma membranes of plant cells, like in animal and fungal cells (Hartmann and Benveniste, 1987). The plasma membrane contains both the greatest sterol content in proportion to the amount of protein present and the highest sterol-to-phospholipid molar ratio than the other cell membrane systems. Sterols are present in low amounts in ER (Hartmann and Benveniste, 1987), tonoplast (Yoshida and Uemura, 1986) and mitochondrial membranes (Méance et al., 1976). The sterol molecules content in the envelope of chloroplasts is also a relatively small proportion, but they are absent from thylakoid membranes (Hartmann and Benveniste, 1987). It appears that there is no specific association of an individual sterol molecule with a given membrane compartment, and that all the membranes contain the same sterols in similar proportions. Cholesterol has been found in all the membranes. Cellular concentrations of free sterols and their intracellular distribution are probably tightly regulated, but factors that maintain sterols in the plasma membrane are unclear. Their partitioning between the two leaflets of the plasma membrane and their organization in the lateral plane of the membrane has not been investigated (Hartmann and Benveniste, 1987). The synthesis of sterols in the ER and accumulation in the plasma membrane implies transport between these two membrane compartments. Unfortunately, evidence for sterol domains in the plasma membrane is still lacking.

4.6. Physiological functions of phytosterols

Phytosterols have been regarded as plasma and liver cholesterol-lowering agents and also reduced the severity of atherosclerotic lesions (Peterson, 1951; Peterson et al., 1952) - a disease of the arteries characterized by the deposition of plaques of fatty material on their inner walls. Contrary to popular belief, the use of phytosterols for lowering serum cholesterol levels is not new; sitosterol has been used since the 1950s as a supplement and as a drug for lowering serum cholesterol levels in hypercholesterolemic individuals (Pollak, 1953). These compounds reduce total cholesterol and LDL-cholesterol (low-density lipoprotein) levels through a reduction in cholesterol absorption. Different mechanisms have been suggested to explain the cholesterol-lowering activity of plant sterols (Meguro et al., 2001). In the intestine, sterols are initially solubilized into a micelle form. These micelles interact with brush border cells and are transferred into enterocytes. Sterols are esterified within the enterocyte, assembled into chylomicrons and secreted into the lymphatics. The molecular mechanisms responsible for the transfer into the enterocyte are not fully elucidated. Recently it has been revealed that the Niemann–Pick C1-like 1 (NPC1L1) protein transporter is most likely responsible for the transport of cholesterol and plant sterols from the brush border membrane into the intestinal mucosa (Altmann et al., 2004). Firstly, plant sterols may displace cholesterol from mixed micelles (Child and Kuksis, 1986), because they are more hydrophobic than cholesterol. This replacement causes a reduction of micellar cholesterol concentrations and consequently lowers cholesterol absorption. Furthermore, plant sterols might reduce the esterification rate of cholesterol in the enterocyte (Child and Kuksis, 1983) and consequently the amount of cholesterol excreted via the chylomicrons. In response to the decrease in cholesterol absorption, cholesterol synthesis is increased (Gylling and Miettinen, 1994), also, LDL receptor mRNA and protein expression increases (Plat and Mensink, 2001). This will not only increase clearance of LDL, but also of IDL (intermidiate density lipopropein). As IDL is the precursor for LDL, this cause lowering of LDL production, the higher LDL receptor expression and the higher endogenous cholesterol synthesis together result in an average reduction of LDL cholesterol of up to 14% (Mensink, 1998). The decrease of total serum cholesterol is completely accounted by a reduction in LDL.

Recent evidence indicates that consumption of phytosterols, apart from lowering cholesterol levels, also provides protection against certain cancers such as anti-cancer activities to breast cancer cells (Awad et al., 2000), colon cancer cells (Rao and Janezic, 1992) and prostrate cancer cells (Gregg, 2001). This is discussed in more detail in the next part

The anti-inflammatory effects of phytosterols were also reported. Pro-inflammatory cytokines include interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). In addition, high-sensitivity C-reactive protein (hs-CRP), the prototypic marker of inflammation, has been proven to be reliable and important markers of CVD (cardiovascular disease) risk. The anti-inflammatory effects of PS were demonstrated in a study with a reduced-calorie, PS-enriched orange juice beverage which reduced CRP by 12% (Devaraj et al., 2006**)**. Further, a recent in vitro study showed that stigmasterol inhibits several pro-inflammatory as well as matrix degradation mediators, which may be due in part to the inhibition of the nuclear factor kappaB (NF-kappaB) pathway (Gabay et al., 2010**)**.

4.7. Commercial suppliers of sterols product

Depending on the manufacturer, the commercial product may be a mixture of the extracted sterols, a mixture of free sterols and stanols, sterol and stanol esters or stanol esters. A list of commercial suppliers is given in Table 6.

Table 6. Commercial suppliers of phytosterols and phytostenols for human health (Cantrill, 2008)

Manufacturer	**Brand name phytosterol products**
Raisio Plc	Benecol ®
Cognis	Vegapure ®
Archer Daniels Midland Co	CardioAid TM
Cargill Inc.	Corowise TM
Triple Crown	Prolocol TM
Pharmaconsult Oy Ltd	Multibene ®
Teriaka Ltd.	Diminicol TM
Forbes Medi-Tech Inc.	Reducol TM
Arboris	AS-2 TM
PrimaPharm B.V.	Beta sitosterol
Fenchem Enterprises Ltd.	Cholevel TM
DRT	Phytopin ®
DDO Processing LLC	Nutraphyl TM
Degussa Food Ingr. GmbH	Cholestatin TM
Phyto-Source LP	Phyto-S-Sterol TM
Lipofoods	Lipophytol TM
Enzymotech Ltd.	CardiaBeat TM

4.8. Methods for extraction, separation and purification

Evaluation of phytosterols mixed with a diversity of other non-saponifiable components in food lipids of complex sample matrices is a formidable task and requires reliable analytical techniques for the extraction, isolation, separation, purification, detection and quantitative data analyses. Isolation and enrichment of sterols from plant tissues or oilseeds entails initial solvent extraction, soxhlet extraction, supercritical fluid extraction (SFE), or supercritical fluid fractionation (SFF) followed by various clean-up and chromatographic procedures. For subsequent characterization and quantification of sterol compounds, the crude isolate can be purified and separated by a wide variety of chromatographic techniques including column chromatography (CC), gas chromatography (GC), thin-layer chromatography TLC), normal-phase high-performance liquid chromatography (HPLC), reversed-phase (HPLC) and capillary electro-chromatography (CEC). The sterols can be detected with flame ionization detection (FID), UV detection (UV), evaporative light scattering detection (ELSD), infrared detection (IR), nuclear magnetic resonance detection (NMR) and mass spectrometry (MS). With the advent of sophisticated column technologies, complex mixtures of sterols can be efficiently separated.

Most common methods used for the extraction of lipids are also eligible for phytosterol extraction. Isolation techniques depend largely on the nature of the sample source and vary among solid and liquid samples. Sterols from plant-derived tissues and oilseeds can be isolated by solvent extraction with chloroform–methanol (Evershed et al., 1987), hexane (Abidi et al., 1999; Mohamed and Awatif, 1998; Moreau et al., 1996), methylene chloride (Akihisa et al., 1994; Akihisa et al., 1992) or acetone (Claasen et al., 2000) followed by saponification and chromatographic purification for obtaining enriched total sterols. Mixing of solvent with homogenized materials can be achieved by shaking the mixtures in heated sealed tubes for 1–18 h (Moreau et al., 1996) or by refluxing with a soxhlet extractor (Heupel, 1985).

M. baccifera was commercially available edible species in Mizoram. *M. baccifera* is commercially available edible species, consumed either raw or processed because of its exotic taste and flavour. Young bambooshoots are low in fat and calories but rich in fibre with about 90% water. In India, young bamboo shoots, either processed or raw, have a high demand in the markets of Mizoram and other north-east part of India. The community use this potential resource for food. *M. baccifera* young shoot markets in Mizoram are shown in Fig. 28

Sterols have many applications in medicine, cosmetics and as a food additive taken to lower cholesterol. As a food ingredient or additive, phytosterols have cholesterol-lowering properties, reducing cholesterol absorption in intestines (Ostlund et al., 2003). Phytosterols are considered potential raw material for partial synthesis of pharmaceutically active steroids (Martin, 1977). The increasing demand for steroidal drugs has resulted in the depletion of many resources. Hence, an alternative for the starting material is necessary. The analysis of nutraceutical content concerning to phytosterol was not reported in this species. The presence of good amount of phytosterols in bamboo had been reported in the literatures (Srivastava, 1990; arangthem and Srivastava, 1997). Therefore, analysis of phytosterols was carried out in this species.

Figure 28. *M. baccifera* young shoot markets; Bamboo young shoot is an important food source in Mizoram

4.9. Materials and methods

4.9.1. Chemical, stock solutions

All chemicals used are of analytical grade (AR). All the standards phytosterols are purchased from sigma-aldrich. Stock solutions of cholesterol (5 mg/2 mL), sitosterol (95% pure), campesterol (70% pure), stigmasterol (95% pure) and brassicasterol (95% pure) are prepared by weighing 3 mg/2 mL of methanol (HPLC grade). Methanol and Acetonitrile (HPLC grade) are purchased from Merck chemicals, India.

4.9.2. Plant materials

M. baccifera succulent young shoot was collected during July to August (harvesting season) in Mizoram. The young shoot was sliced into 2 to 3 inches and dried at room temperature for 3 weeks. The dried sample was grinded into a fine powder by using grinder and stored in an air tight container for further used.

4.9.3. Extraction of phytosterols

To purify phytosterols, the first step was extraction of phytosterols from the plant sample. Extraction was done in a 100 mL soxhlet extraction flask containing 20g of powder sample using a mixture of benzene, petroleum ether and 2N ethanolic KOH (10 : 5 : 1) as a refluxing solvent (Sarangthem and Srivastava, 1997) for 6 h followed by n- hexane and ethyl acetate (7 : 1) extraction and then the extracted solutions are cooled down and filtered. The organic solvent was evaporated thoroughly at room temperature or using roter evaporator to give a tarry lump, and stored at below -20^{o}C.

4.9.4. Sample preparation

About 10 mg of the extract sample is weighted and then dissolved in 2 mL methanol (HPLC grade) and filtered through 0.45 μm poly-vinylidene-fluoride membrane filter and stored at 4^{o}C for further used.

4.9.5. GC-MS (Gas Chromatography-Mass Spectrocopy)

GC-MS (Gas Chromatography-Mass Spectrocopy) analysis was performed using Shimadzu Europe GCMS-QP5050A. The MS is paired with GC-17A gas chromatograph, which uses Advanced Flow Control (AFC) for rapid, reproducible results equipped with a split/splitless injector; direct connection capillary interface direct probe inlet. The injector operated at condition: injection volume 1 μL. GC-MS analysis of sterols and fatty acids of crude extracted was carried out on a Shimadzu GCMS-QP 5050A equipped with a DB-17 capillary column (0.25 mm × 0.25 μm × 30 m). The carrier gas was helium (purity of 99.9995%) and the splitting ratio was 1 : 30. The oven temperature was programmed from 50^{o}C to 260^{o}C at a rate of 10^{o}C/min. The injection and ion source temperatures were 250^{o}C and 230^{o}C. The spectra were recorded at ionization energy of 70 eV with an electron impact ion source (EI). The scan parameter was from 20 to 500.

Compound identification was done by comparing the NIST library data of the peaks with those reported in literature, mass spectra of the peaks with literature data.

4.9.6. HPLC (High Performance Liquid Chromatography)

The filtered crude extracted is subjected to HPLC (Shimadzu LC-10AT) analysis for further purification and quantification. The separation is made on a reverse phase phenomenex luna 5u C18 column (250 x 4.60 mm 5 micron) with methanol and acetonitrile in the ratio of 9 : 1 act as isocratic mobile phase. Flow rates of 1 mL/min and 0.8 mL/min are tested with the 0.8 mL/min providing better separation. At this flow rate the purified fractions of each phytosterol from chromatography are also collected. The effect of column temperature on resolution of phytosterols is also investigated. Tests at both 28^{o}C and 30^{o}C showed little difference in resolution of the peaks; therefore, the analyses are performed at room temperature, 28^{o}C. The retention time of each plant sterol is determined by comparison with elution times their corresponding standards sterols and the concentrations of each phytosterol in the crude extract is also evaluated by using the area of their standards phytosterols. The C18 column is washed for 1.30 h before and after analysis and 25 μL samples are injected into the HPLC for analysis. β-sitosterol and stigmasterol spectra have an absorption maximum at 210 nm (Huang et al., 2007). Therefore, the detection of plant sterols was performed at 210 nm using shimadzu SPD-10A UV-VIS detector. The spectrum bands are viewed using spinchrome software.

4.9.7. TLC (Thin-layer Chromatography)

In order to confirm the purity of each sterol fraction collected. The fractions are subjected to TLC analysis using TLC silica plates (silica gel 60G 20 x20 cm thickness 0.25 mm) and n-hexane and ethyl acetate in the ratio of 7 : 1 as a mobile phase. Each individual sterol is confirmed by using the R_f value of their corresponding standard compounds on the TLC plate. After development the plate was sprayed with Liebermann-Burchard reagent, incubated at 37°C for colour development and the sterols spots were identified using their corresponding reference standards spots.

4.9.8. Liebermann-Burchard (LB) test

The total phytosterols content and the presence of phytosterols in the plant sample are confirmed by using LB test. In the preparation of the LB reagent, a mixture containing the following is necessary: glacial acetic acid (30%); acetic anhydride (60%) and sulphuric acid (10%) by volume were prepared in ice cold water according to the directions of Schoenheimer and Sperry (1934). Anhydrous sodium sulphate is also added to the mixture to a concentration of 2%.

Add 5 mL of the LB reagent. Then add 0. 2 mL of water, 0.2 mL of experimental plant sample and 0.2 mL of standard (cholesterol) slowly along the edges of the corresponding tubes. Blank is also prepared with LB reagent and water. Mixed by gently swirling and incubated at 37°C for 15 min. Read the OD at 620 nm. Caculate the total sterols content using the following formula (Kim and Goldberg, 1969)

$$\mathrm{Conc}_{\mathrm{unknown}} = \mathrm{Conc}_{\mathrm{standard}} \times \mathrm{OD}_{\mathrm{unknown}}/\mathrm{OD}_{\mathrm{standard}}$$

4.10. Results and Discussion

4.10.1. GC-MS analysis

Mass spectrometry is valuable tools for phytosterols identification. Electron impact (EI) GC-MS has been extensively employed to identify free phytosterols (Moreau et al., 2002). GC-MS analysis is used by Chevy et al (2005) for determination of cholesterol, lathosterol, 8-dehydrocholesterol and 7-dehydrocholesterol from amniotic fluid. Another use of GC-MS is determination of phytosterols from sea buckthorn (Li et al., 2007). At the present, gas chromatography coupled with mass spectrometry remains one of the most sensitive and specific analytical techniques.

The GC-MS chromatogram of sterols and fatty acid is shown in Fig 29. The chromatogram shows the presence 1 to 17 peaks at different retention times. Each peak is analysed, electron impact mass spectrum and their major fragment ions are revealed. This is the so called "target". This target compound spectrum identification was done by comparing the NIST library data of the peaks with those already reported in literature, mass spectrum of the peaks with literature data. Therefore, five possible compounds which may likely represent the peak are given by GC-MS, which is called "hit". The spectrum peak of 7, 14, 15 , 16 and 17 along with their hits are shown from Fig. 30 – 34. The remaining spectrum peaks are also analysed and possible compounds of fatty acids and plant sterols which may be present in the extract were listed in Table 7.

4.10.2. HPLC analysis

Standard compounds of sterols were purchased according to sterols presence revealed by GC-MS in order to confirm these plant sterols. Analysis of plant sterols by HPLC depends on sufficient appropriate separation and detection of the compounds. Separation of the compounds by HPLC requires a specific ratio of solvents at an appropriate flow rate through a column containing a nonpolar stationary phase. Therefore, the HPLC conditions tested were column type, solvents and ratios and flow rate. Both C8 and C18 columns have been used for the HPLC separation of phytosterols. The reversed-phase column separates plant sterols according to chain length as well as the number and location of double bonds and functional groups. The more polar compounds elute first when analyzed with a reversed column. Holen (1985) found that the phytosterols were retained to a greater degree on the C18 column than on the C8 column. The C18 column was typically the column of choice as indicated in the literature and was therefore chosen for this study.

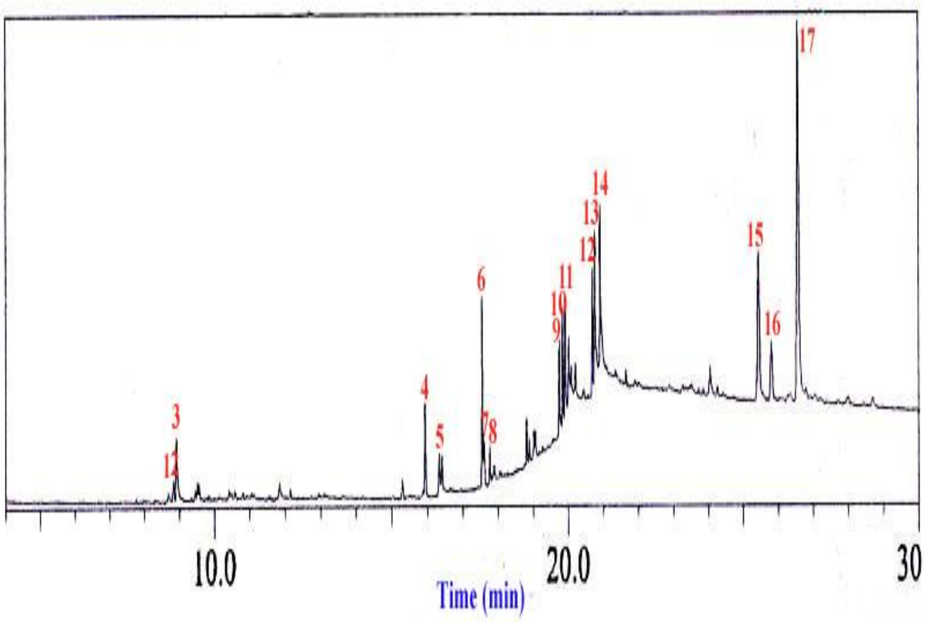


Figure 29. GC-MS chromatogram of *M. baccifera* young shoot extract showing 1 to 17 spectrum peaks

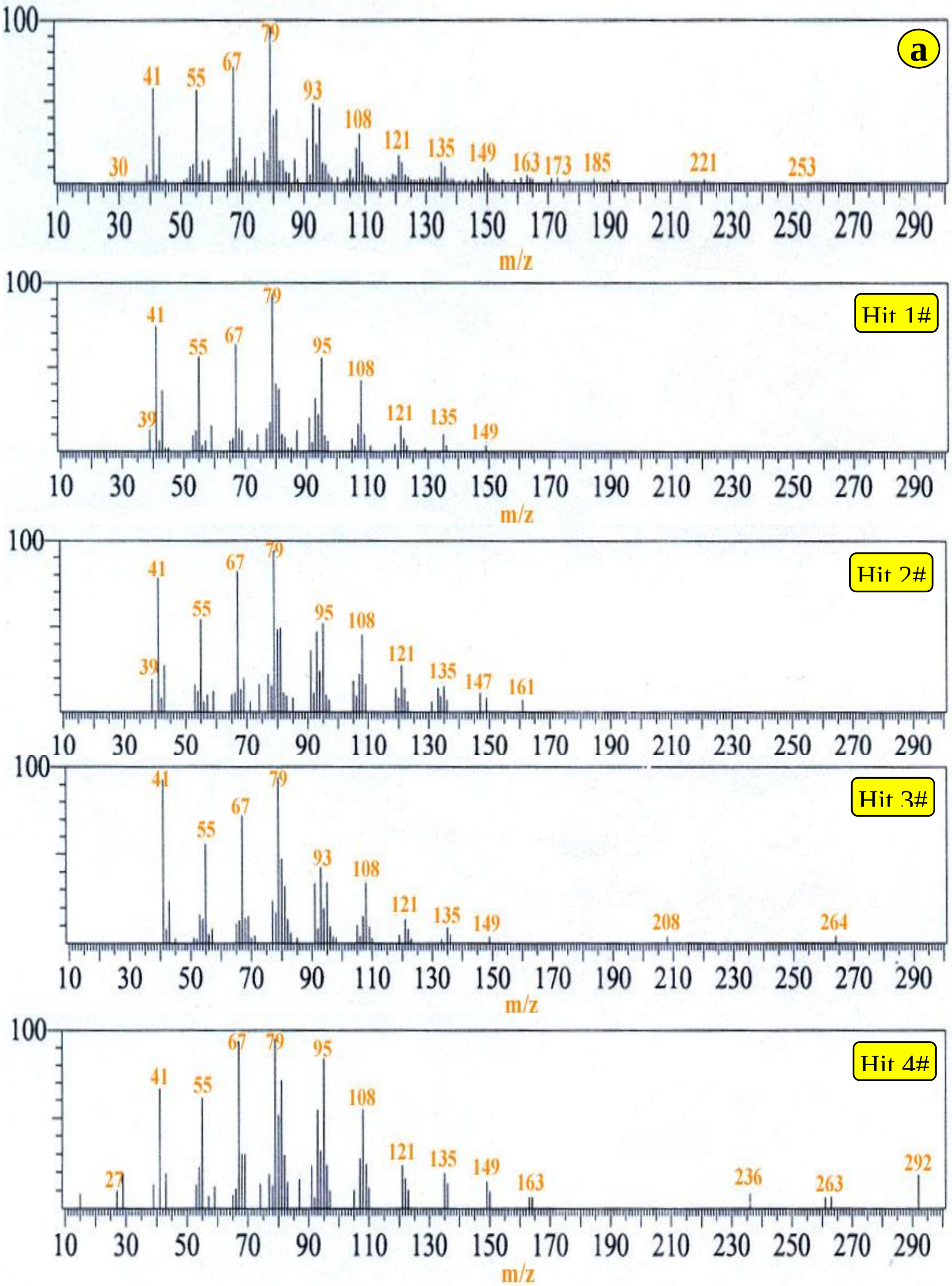


Figure 30. Spectrum fragment structure of peak number 7 along with its target and hits; a) Target; Hit 1# 11,14,17-Eicosatrienoic acid, methyl ester; Hit 2# Methyl (Z)-5,11,14,17-eicosatetraenoate; Hit 3# 9,12,15-Octadecatrien-1-ol; Hit 4# Linolenic acid, methyl ester

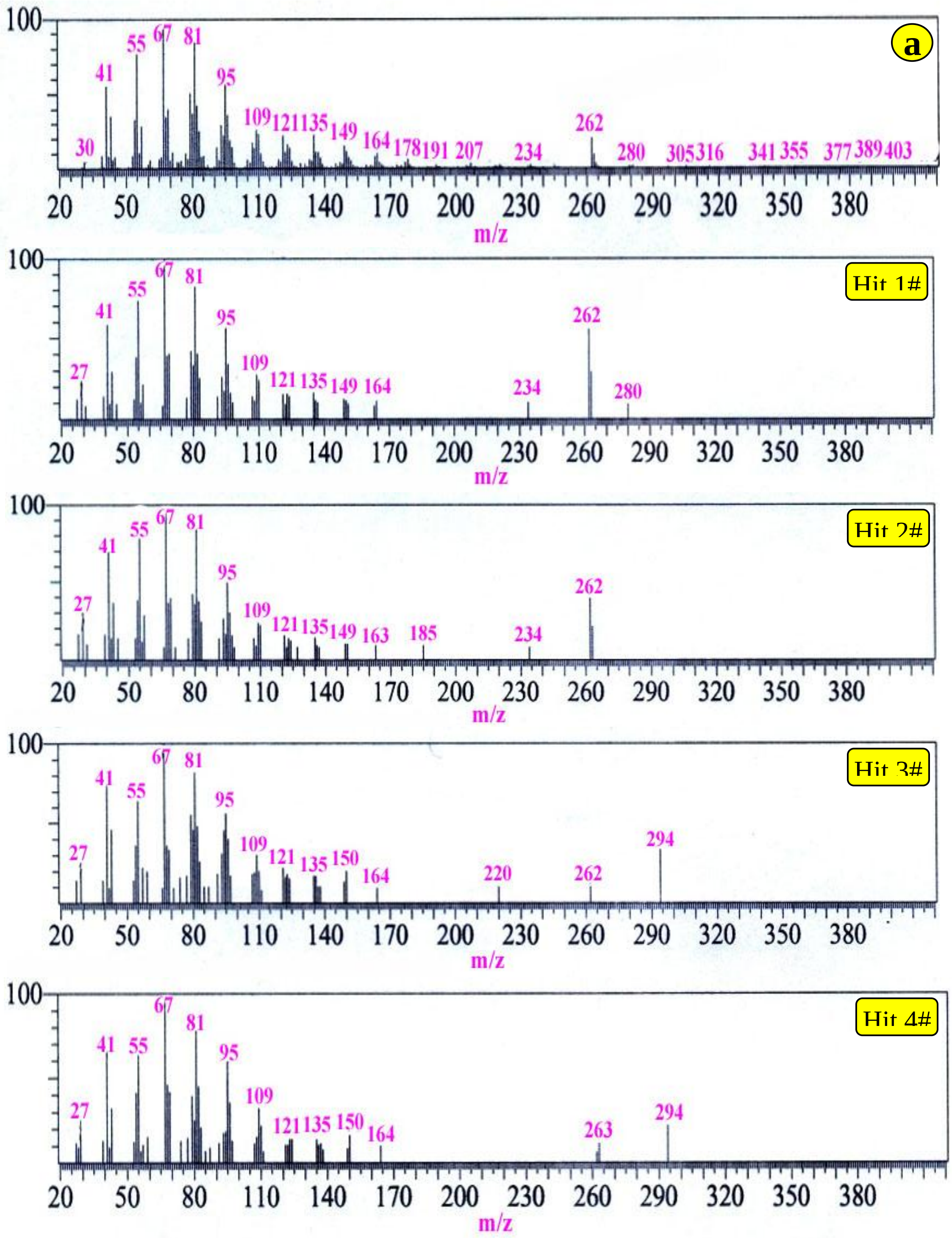


Figure 31. Spectrum fragment structure of peak number 14 along with its target and hits; a) Target; Hit 1# :9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester; Hit 2# 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester; Hit 3# 6,9-Octadecadienoic acid, methyl ester; Hit 4# 8,11-Octadecadienoic acid, methyl ester

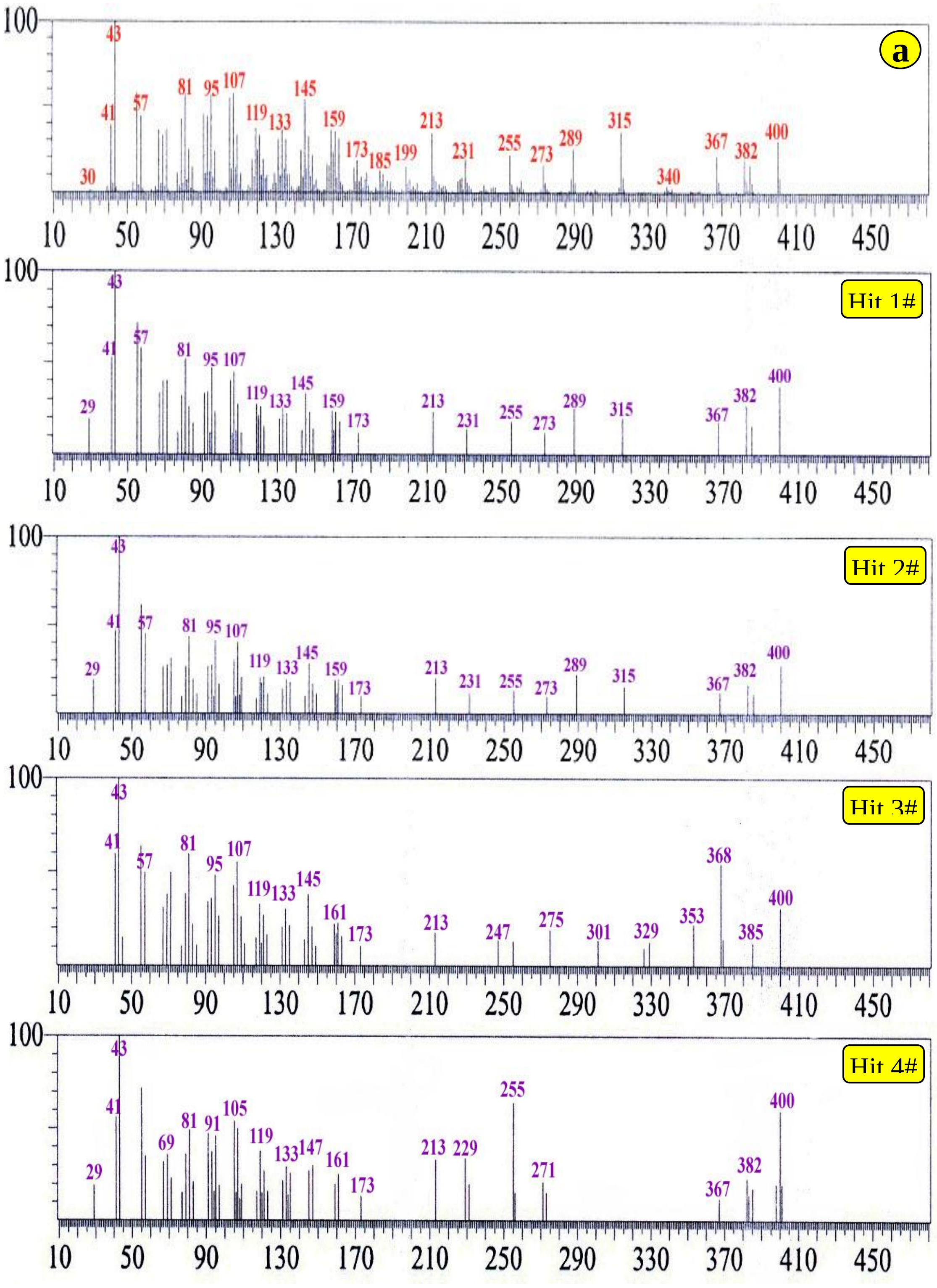


Figure 32. Spectrum fragment structure of peak number 15 along with its target and hits; a) Target; Hit 1# Ergost-5-en-3-ol; Hit 2# Campestero; Hit 3# Isocholesteryl methyl ether; Hit 4# Ergost-7-en-3-ol.

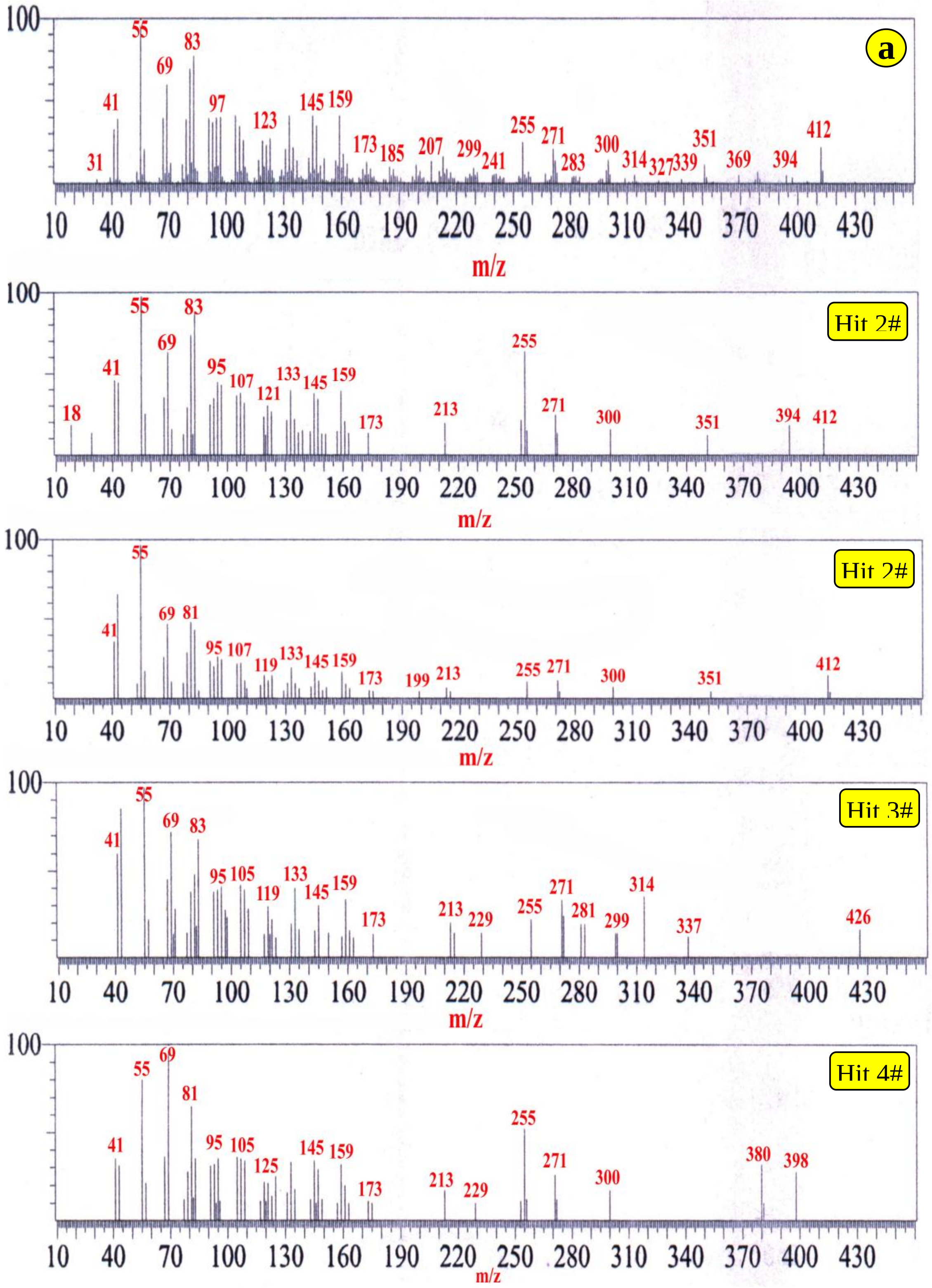


Figure 33. Spectrum fragment structure of peak number 16 along with its target and hits; a) Target; Hit 1# Stigmasterol; Hit 2# Stigmasta-5,22-dien-3-ol; Hit 3# Gorgost-5-en-3-ol; Hit 4# Ergosta-5,22-dien-3.beta.-ol

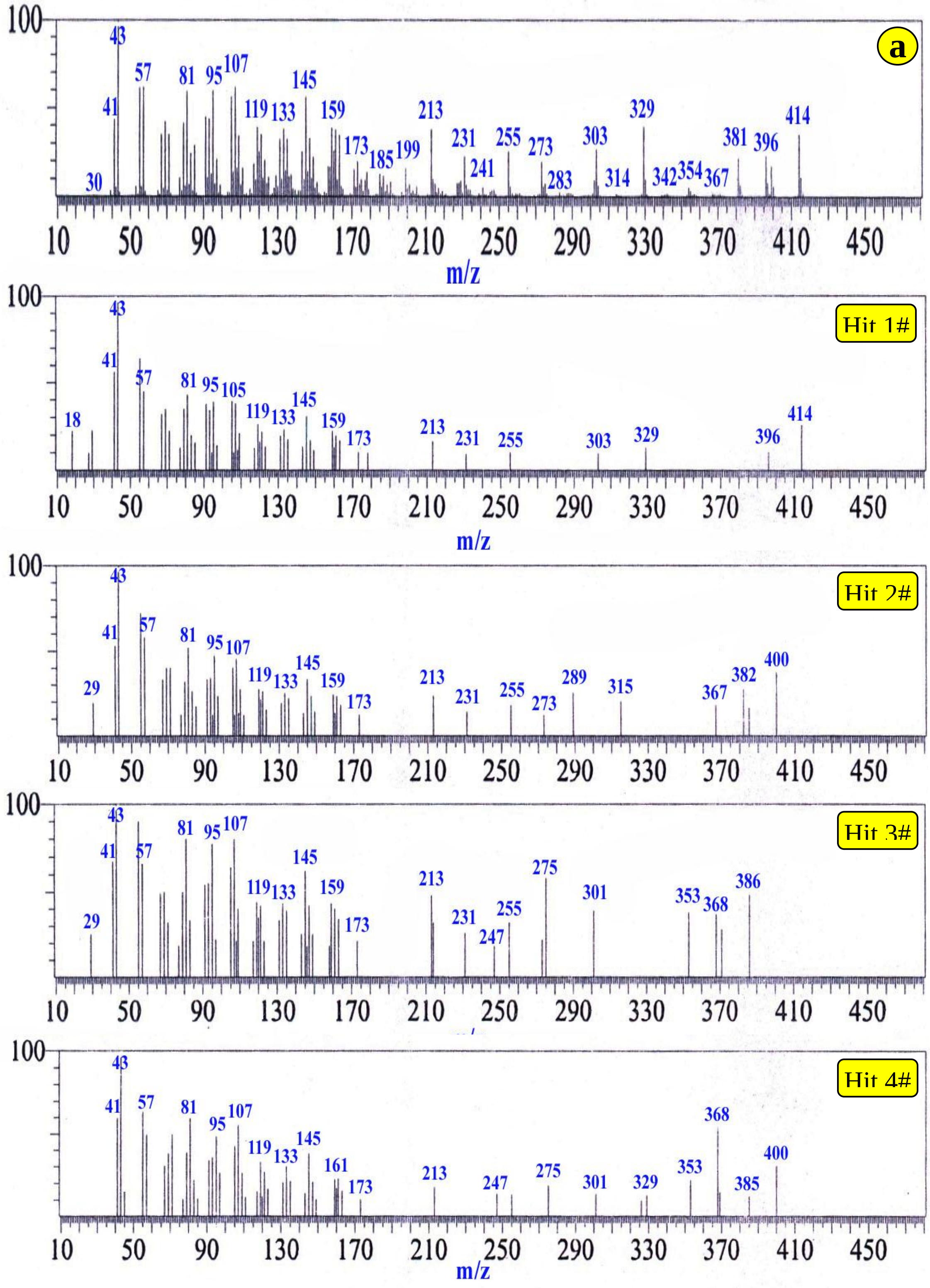


Figure 34. Spectrum fragment structure of peak number 17 along with its target and hits; a) Target; Hit 1# beta.-Sitosterol; Hit 2# Ergost-5-en-3.beta.-ol; Hit 3# Cholest-5-en-3.beta.-ol; Hit 4# Isocholesteryl methyl ether

Table 7. GC-MS spectrum peaks of 1 to 17 are analysed and possible compounds of fatty acids and plant sterols which may be presence in the extract

Compounds	**Mol. formula**	**CAS (Chemicals Abstracts Service) umber**	**Mol. weight**
Fatty acids			
Benzenemethanol, 4-hydroxy	$C_7H_8O_2$	623-05-2	124
Benzenemethanol, 3-hydroxy	$C_7H_8O_2$	620-24-6	124
2,4-Diisocyanato-1-methylbenzene	$C_9H_6N_2O_2$	584-84-9	174
2,6-Diisocyanatotoluene	$C_9H_6N_2O_2$	91-08-7	174
4,6-Dimethyl-2H-chromen-2-one	$C_{11}H_{10}O_2$	14002-89-2	174
Benzaldehyde, 4-hydroxy	$C_7H_6O_2$	123-08-0	122
Benzaldehyde, m-hydroxy	$C_7H_6O_2$	100-83-4	122
Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	112-39-0	270
Methyl isoheptadecanoate	$C_{18}H_{36}O_2$	6929-04-0	284
Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	5129-60-2	270
Pentadecanoic acid	$C_{15}H_{30}O_2$	1002-84-2	242
Palmitic acid	$C_{16}H_{32}O_2$	57-10-3	256
Stearic acid	$C_{18}H_{36}O_2$	57-11-4	284
Myristic acid	$C_{14}H_{28}O_2$	544-63-8	228
Arachic acid	$C_{20}H_{40}O_2$	506-30-9	312
9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	2462-85-3	294
8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	56599-58-7	294
10,13-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	56554-62-2	294
Linolenic acid, methyl ester	$C_{19}H_{32}O_2$	301-00-8	292
11,14,17-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	55682-88-7	320
Methyl (Z)-5,11,14,17-eicosatetraenoate	$C_{21}H_{34}O_2$	59149-01-8	318
9,12,15-Octadecatrien-1-ol	$C_{18}H_{32}O$	506-44-5	264
Stearaldehyde	$C_{18}H_{36}O$	638-66-4	268
Myristaldehyde	$C_{14}H_{28}O$	124-25-4	212
Palmitaldehyde	$C_{16}H_{32}O$	629-80-1	240
Cyclohexaneethanamine	$C_{10}H_{21}N$	101-40-6	155
Ethanamine, 2,2'-oxybis[N,N-dimethyl]	$C_8H_{20}N_2O$	3033-62-3	160
1-Heptadecanamine, N,N-dimethyl	$C_{19}H_{41}N$	3002-57-1	283
Octadecanoic acid, 3-oxo-, methyl ester	$C_{19}H_{36}O_3$	14531-34-1	312
Eicosanoic acid, 2,3-bis [(trimethylsilyl) oxy] propyl ester	$C_{29}H_{62}O_4Si_2$	55517-94-7	530
1,3-Dipalmitin trimethylsilyl ether	$C_{38}H_{76}O_5Si$	53212-95-6	640
Hexadecanoic acid, 3-[(trimethylsilyl) oxy] propyl ester	$C_{22}H_{46}O_3Si$	56630-48-9	386
Ribose, 2-deoxy-bis (thioheptyl)-dithioacetal	$C_{19}H_{40}O_3S_2$	123390-22-7	380
Tridecanoic acid, 3-hydroxy-, ethyl ester	$C_{15}H_{30}O_3$	107141-15-1	258
Tetracosanoic acid, 3-oxo-, methyl ester	$C_{25}H_{48}O_3$	14531-37-4	396
1-n-Hexadecylindan	$C_{25}H_{42}$	55334-29-7	342

Cyclopropane, 1,1-dichloro-2,2,3,3-tetramethyl	$C_7H_{12}Cl_2$	3141-45-5	166
3-n-Butylthiophene-1,1-dioxide	$C_8H_{12}O_2S$	142076-45-7	172
9,12-Octadecadienoyl chloride, (Z,Z)- $$ Linoleoyl chloride	$C_{18}H_{31}ClO$	7459-33-8	298
9,12-Octadecadienoic acid	$C_{21}H_{38}O_4$	2277-28-3	354
9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{38}O_4$	3443-82-1	354
2-cis,cis-9,12-Octadecadienyloxy	$C_{20}H_{38}O_2$	17367-08-7	310
6,9-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	56599-55-4	294
8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	56599-58-7	294
Phytosterols			
Ergost-5-en-3.beta.-ol	$C_{28}H_{48}O$	4651-51-8	400
Campesterol	$C_{28}H_{48}O$	474-62-4	400
Isocholesteryl methyl ether	$C_{28}H_{48}O$	29944-53-4	400
Ergost-7-en-3-ol	$C_{28}H_{48}O$	26047-31-4	400
Stigmasterol	$C_{29}H_{48}O$	83-48-7	412
Gorgost-5-en-3-ol	$C_{30}H_{50}O$	29782-65-8	426
Brassicasterol	$C_{28}H_{46}O$	474-67-9	398
Cholest-5-en-3-ol	$C_{27}H_{46}O$	57-88-5	386
beta.-Sitosterol	$C_{29}H_{50}O$	83-46-5	414

The plant sterols of brassicasterol, stigmasterol, campesterol and sitosterol were analyzed. Of the four phytosterols, brassicasterol with a chemical formula of $C_{28}H_{46}O$ and two double bonds eluted first, followed by stigmasterol, campesterol and β-sitosterol, which had the highest molecular weight and two double bonds. This pattern of phytosterol elution by HPLC was also reported in the literature (Warner and Mounts, 1990). Reference standards were used to determine the retention time of plant sterols. Reference standards were injected singly and retention times for the four phytosterols were brassicasterol-17.29 min; stigmasterol-18.69 min; campesterol-19.87 min; β-sitosterol-21.36 min. The HPLC chromatogram of reference standards and crude extract of *M. baccifera* with their retention times were shown in Fig. 35.

The quantification of these phytosterols was also evaluated using the known concentration of their standards. β-sitosterol gives the highest amount followed by stigmasterol, brassicasterol and campesterol (Table 8). The purified fractions of these sterols were also checked using HPLC and shown in Fig 36 and Fig 37. In this analysis, campesterol was not purified due to presence in low concentration. Comparison of total phytosterol content in *M. baccifera* with other plant sources are given in Table 9.

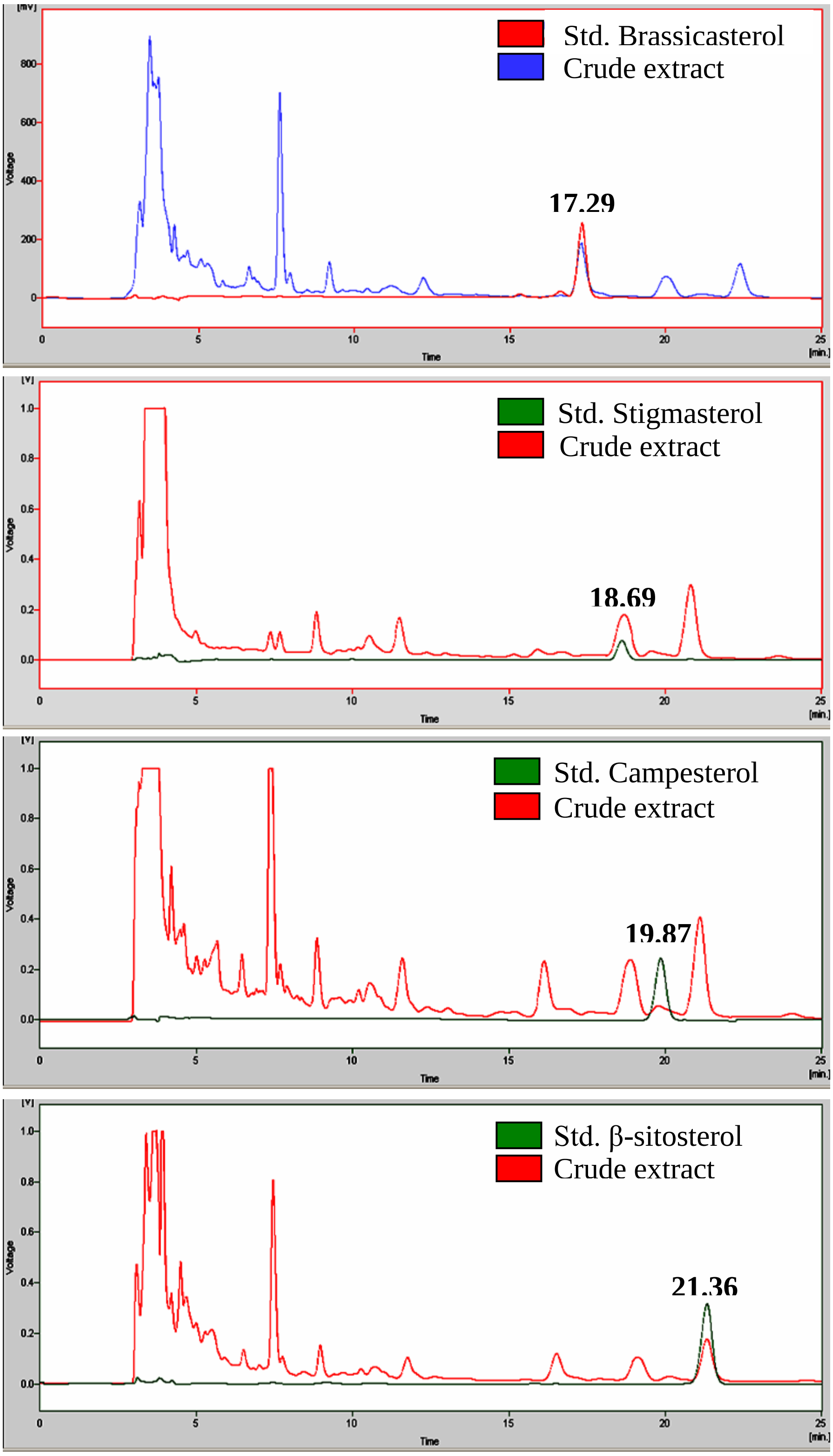


Figure 35. HPLC chromatograms of phytosterols along with standard compounds showing their retention times from *M. baccifera* young shoot extract.

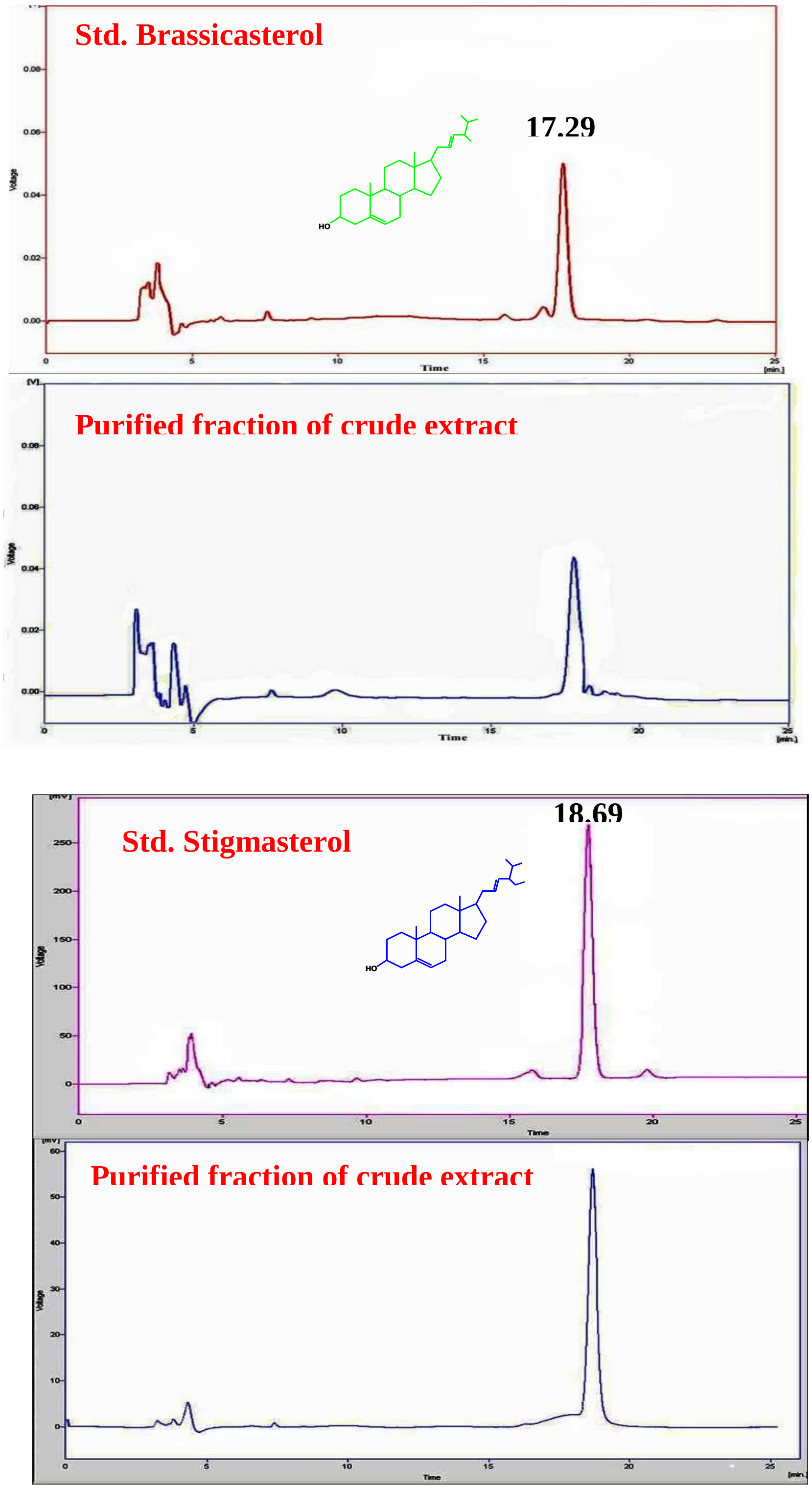


Figure 36. Purification of brassicasterol and stigmasterol from crude extract of *M. baccifera* shoot analysed by HPLC

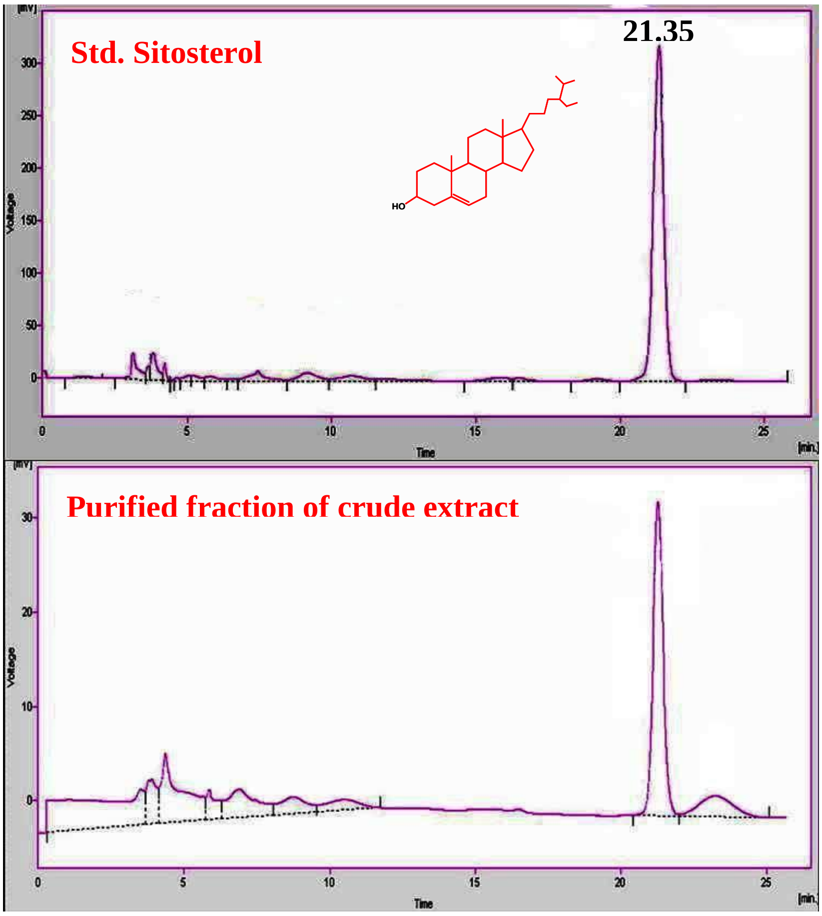


Figure 37. Purification of β-Sitosterol from crude extract of *M. baccifera* shoot analysed by HPLC

Table 8. Quantification of total and different phytosterols from *M. baccifera* young shoot

Compound	**mg/g DW**
Total phytosterols	3.34
β-Sitosterol	1.19
Stigmasterol	0.83
Campesterol	0.26
Brassicasterol	0.79

Table 9. Comparison of Some reported sterol concentrations in selected foods and vegetable oils with *M. baccifera* (United States Department of Agriculture Food Composition, 1999)

Food source	**Total plant sterols content (mg/g)**
Potato	0.05
Tomato	0.07
Pear	0.08
Lettuce	0.1
Carrot	0.12
Apple	0.12
Onion	0.15
Banana	0.16
Fig	0.31
Garbanzo bean	0.35
Pecan	1.08
Kidney bean	1.27
Almond	1.43
Cashew nut	1.58
Soybean	1.61
Peanut oil	2.07
Peanut	2.20
Olive oil	2.21
Soybean oil	2.50
Cottonseed oil	3.24
M. baccifera young shoot	3.34
Safflower oil	4.44
Sesame seed	7.14
Sesame oil	8.65
Corn oil	9.68
Rice bran oil	11.90

4.10.3. TLC analysis of purified fractions

In order to confirm the presence of these phytosterol in the fractions, TLC was run along with the reference standards. The non-polar solvent of n-hexane and ethyl acetate (7 : 1) mixture was used as mobile phase. In the non-polar solvent, the more non-polar compounds travel faster than the polar compounds. Therefore, β-sitosterol has highest R_f value followed by stigmasterol and brassicasterol. The reference standard R_f value of brassicasterol was 3.9 cm; stigmasterol was 4.1 cm and β-sitosterol was 4.3 cm. All these reference standards R_f values were also matching with the tested plant sterols R_f in the fractions (Fig. 38)

4.10.4. Liebermann-Burchard test

All the phytosterols has cholesterol backbone in their structure. Cholesterol structure under strongly acidic conditions of LB mixture-sulphuric acid, glacial acetic acid and acetic anhydride rapidly converts cholesterol to its acetate and sulfate derivatives and undergo sulfonation at a variety of positions, often with skeletal rearrangements. Elimination of an SO_3H group as H_2SO_3 generates a new double bond. Repetition of this desaturation process leads to polyenes and ultimately to aromatic steroids. Linearly conjugated polyene cations can appear blue but form too slowly to account for the LB colour response, whose chemical origin remains unidentified (Xiong et al., 2007). The colour response varies markedly depending on the double bond system (Xiong et al., 2002), other functional groups and the presence of a nonpolar side chain (Brieskorn and Hofmann, 1964). It is notable that the LB reaction produces a colourless at 1 min than changed to blue and green (Xiong et al., 2007).

The total phytosterol content was quantified based on this colour at 620 nm using cholesterol as standard and presented in Table 8. Liebermann-Burchard (LB) test was also carried out to confirm the presence of these phytosterol in the fractions based on colour visualized using cholesterol as standard. Reference standard cholesterol showed green colour after mixing with LB reagent and all the fractions containing different phytosterols also showed green colour (Fig. 39). This confirmed the presence of sitosterol, stigmasterol and brassicasterol in different fractions.

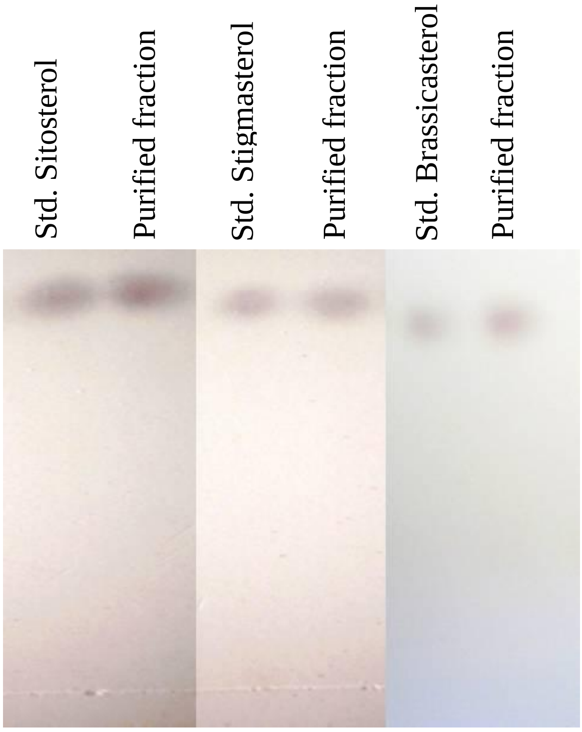


Figure 38. TLC of reference standards and purified fraction of phytosterols

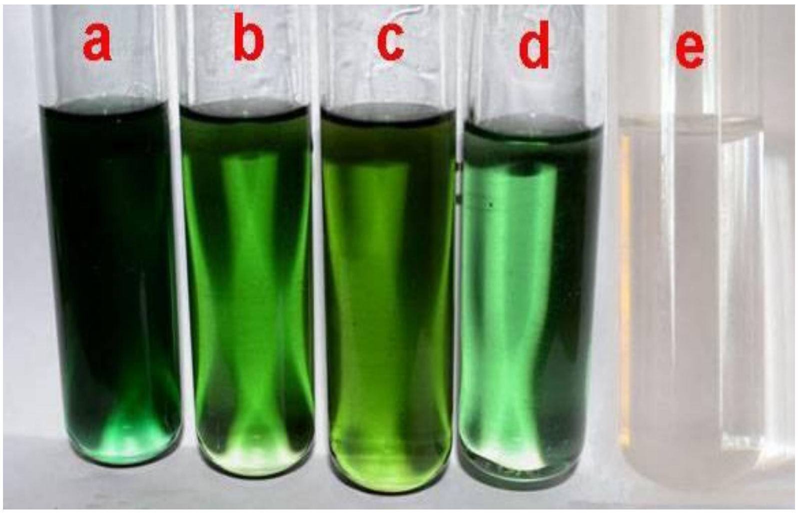


Figure 39. Liebermann-Burchard test: a) +ve control (Std cholesterol); purified fraction of b) sitosterol; c) stigmasterol; d) brassicasterol; e) –ve control

4.11. Conclusions

M. baccifera young shoot is an important food source in Mizoram state of India. The species of this young shoot contain a good amount of phytosterols. Phytosterols in plant was synthesis through mevalonate pathway. Biological important functions of phytosterols in plant includes regulation of membrane fluidity and permeability, phytosterols are also substrates for the synthesis of a wide range of secondary metabolites. It also has a role in signal transduction and in cellular differentiation and proliferation. In plant free sterols reside predominantly in the plasma membranes of plant cells; sterols are present in low amounts in ER and enveloped of chloroplast. Phyiological effects of phytosterols have been regarded as lowering plasma and liver cholesterol. It also has anticancer and anti-inflammatory activity. Many products of phytosterols are available and marketed at the commercial scale. In *M. baccifera* young shoot, different fatty acids and phytosterols are detected. The presence of sitosterol, campesterol, stigmasterol and brassicasterol were confirmed using HPLC, TLC and LB test in the shoot extract. Phytosterols are an important pharmaceutical compound and provide numerous health benefits to humans. Therefore, *M. baccifera* young shoot is also a good source of phytosterol.

5 Anti-cancer and anti-bacterial properties of *Melocanna baccifera* young shoot extract

5.1. Introduction

Plants have a long history of use in the treatment of cancer and microbial infection. Traditional medicines derived from plants may play an essential role in modern health care, principally where satisfactory treatment is not available. Traditional medicines are still very commonly used in India for numerous diseases. For millennia, terrestrial plants have been used as a prominent source of medicines, and their use in this regard in ancient Egypt, India, China and the Arab world has been documented (Sneader, 2005). Investigations into the health maintaining properties of plants have resulted in the identification of a wide array of bioactive compounds that include flavonoids, phenolics, limonoids, carotenoids, coumarins, phytosterols, etc (Vanamala et al., 2006; Patil et al., 2006; Girennavar et al., 2007; Jayaprakhasha and Patil, 2007). The active principles are also used as starting materials for further medicinal chemistry modifications (Butler, 2004; Balunas and Kinghorn, 2005; Koehn and Carter, 2005; Jones et al., 2006). Consumption of fruits and vegetables is associated with the protection or prevention from several ailments such as chronic heart diseases, cancer, diabetes, and osteoporosis, etc (Drewnowski et al., 2000; Bloch et al., 1995; Poulose et al., 2005; Poulose et al., 2006; Deyhim et al., 2006). Drug discovery from plants still provides important new drug leads, many of which are approved or undergo trials for clinical uses against cancer, malaria, alzheimer disease, HIV/AIDS, pulmonary pathologies and other diseases (Butler, 2004; Newman et al., 2003). However, plant drugs present many challenges, including the legal and logistical difficulties involved in the procurement of plant materials (Rosenthal, 2002; Soejarto et al., 2004), the lengthy and costly process of bioassay-guided fractionation and compound isolation (Balunas and Kinghorn, 2005; Jones et al., 2006). et al., 2003). Investigations about natural products have recently regained prominence with the increasing understanding of their biological significance and increasing recognition of the origin and function of their structural diversity. 2005). Over 60% of currently used anticancer agents are derived in one way or another from natural sources including plants, marine organisms and micro-organisms (Cragg et al., 1997; Cragg and Newman, 2005). The search for anti-cancer agents from plant sources started in earnest in the 1950s with the discovery and development of the vinca alkaloids, vinblastine and vincristine, and the isolation of the cytotoxic podophyllotoxins. Plant-derived compounds have played an important role in the development of several clinically useful anti-cancer agents. These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin, and paclitaxel (taxol®). Several promising new agents are in clinical development based on selective activity against cancer-related molecular targets, including flavopiridol and combretastin A4 phosphate, and some agents which failed in earlier clinical studies are stimulating renewed interest (Cragg, and Newman, 2005). Tumor invasion and metastasis still remains as the major cause of treatment failure in malignancy. Metastatic cells have to perform a series of events to reach a distant site for establishing a new colony (Fidler and Hart, 1982; Schirmacher, 1985). If any agent can intercept any of the steps in metastatic cascade, the probability for its clinical trails will be promising. Metastasis may also be controlled by the immuno surveillance and the process succeeds when immune defenses were partially or completely abrogated. A number of medicinal plant extracts that are used in indigenous system of medicine are known to boost the immune system (Davis and Kuttam, 2002). Plant extracts used in traditional therapy are being reviewed for their chemo protective and immunomodulatory activities. Immunomodulators are biological response modifiers; exert their antitumor effects by improving host defense mechanisms against the tumor. They have a direct anticancer effect on tumor cells and also enhance the ability of the host to tolerate damage by toxic chemicals that may be used to destroy the cancer. Certain agents have been shown to possess activity to normalize or modulate path physiological processes and are hence called immunomodulatory agents (Wagner, 1983).

The bacteria resistance against the actual drugs used in traditional therapy caused the escalating development of new antibacterial agents present in a high number of sources such as plant materials (Eloff, 1998). In the last decade, essential oils and various plant extracts have been the focus of great interest from researchers because they represent natural resources. The potential use of these products as an alternative for the treatment of several infectious diseases has been extensively screened. Functional properties such as antimicrobial activity (antibacterial, antifungal and insecticidal) of essential oils and plant extracts are the base of its application in processed food preservation, pharmaceuticals, cosmetics, alternative medicine and natural therapies (Bakkali et al., 2008).

5.2. Bamboo shoot prospects for utilization as a health food

Bamboo is intricately associated with humans from ancient times. Popularly known for their industrial uses, a lesser known fact of bamboos is the usage of its juvenile shoots as a food that can be consumed fresh, fermented, or canned. Bamboo shoots have a long history of being used as a source of both food and medicine in China and Southeast Asia (Bao 2006). Juvenile or new young shoots in bamboos usually develop with the beginning of the monsoon season during which the young edible shoots are harvested. In India, however, despite the fact that it is the 2nd largest producer of bamboos after China, not much importance has been given to the use of bamboo shoots as food due to lack of awareness of the edible characteristics of the shoots. Consumption of tender shoots is confined mainly to the North-eastern states of India where they are part of the traditional cuisine. Canned and preserved bamboo shoots currently dominate international trade, but due to increased consumer demand for non-processed food, it is projected that the share of fresh shoots will significantly increase in the near future. The main nutrients in bamboo shoots are protein, carbohydrates, amino acids, minerals, fat, sugar, fiber, and inorganic salts. The shoots have a good profile of minerals, consisting mainly of potassium (K), calcium (Ca), manganese, zinc, chromium, copper, iron (Fe), plus lower amounts of phosphorus (P), and selenium (Shi and Yang 1992; Nirmala et al., 2007). Fresh shoots are a good source of thiamine, niacin, vitamin A, vitamin B6, and vitamin E (Visuphaka 1985; Xia, 1989; Shi and Yang 1992). They contain 17 amino acids, 8 of which are essential for the human body (Qiu 1992; Ferreira et al., 1995). Fat content is comparatively low (0.26% to 0.94%) and the shoots contain important essential fatty acids. Bamboo shoot has been in use in medicine since time immemorial by the people in various places. With different flavones, glycosides, bamboo shoots have good anti-oxidant, anti-free-radical and anti-aging agents, and can be extracted to make capsules and tablets (Shi and Yang 1992; RFRI 2008). In the traditional system of Ayurveda, the silicious concretions found in the bamboo shoots is called “banslochan” and in the

Indo-Persian and Tibettan system of medicine, it is called as “bamboo manna” and is known to be a good tonic for respiratory disorders. Bambusa arundinacea species is considered as the excellent source of bamboo manna (Puri, 2003).

In South Asian countries, bamboos have been utilized for traditional medicine treatments to relieve hypertension, sweating, and paralysis. However, little scientific evidence has supported such claims until now. Modern research has revealed that bamboo shoots have antioxidant and anti-inflammatory effects (Hu et al., 2000; Lu et al., 2005), anti-microbial and antifungal activities of bamboo shoot pyrolysates (Fujimura et al., 2005), anti-apoptotic activities (Hong et al., 2010) and anti-cancer, anti-bacterial, antiviral activity of bamboo shoot fiber (Shi and Yang, 1992; Fujimura et al., 2005). Bioactive compounds of carotenoids, phenolic compounds (flavonoids, phytoestrogens, phenolic acids), phytosterols and phytostanols, saponins, tocotrienols, organosulfur compounds (allium compounds and glucosinolates), and non digestible carbohydrates (dietary fiber and prebiotics) are also founded in bamboo species (Chongtham et al., 2011). These phytochemicals have good effects to infectious diseases for human health. Plant sterol or phytosterol is a bioactive compound of all vegetable foods. Bamboo shoots are rich in both phenols and phytosterols. Phytosterols possess anticancer effects in addition to their cholesterol lowering activity.

The presence of different phytosterols is also confirmed in *M. baccifera*. Therefore, evaluation of anticancer and antibacterial activities of this young bamboo shoot extract is also carried out to different human cancer cell lines and pathogenic bacteria.

5.3. Cancer cells

Cancer is one of the prominent human diseases in which group of cells display uncontrolled growth, invasion that intrudes upon and destroys adjacent tissues and sometimes metastasis or spreading to other locations in the body via lymph or blood. These three malignant properties of cancers differentiate them from benign tumours, which do not invade or metastasize. It is the second leading cause of death. Researchers divide the causes of cancer into two groups: those with an environmental cause and those with a hereditary genetic cause. Cancers are primarily an environmental disease with 90-95% of cases attributed to environmental factors and 5-10% due to genetics (Anand et al., 2008). Environmental factors include: tobacco, diet and obesity, infections, radiation, stress, lack of physical activity, and environmental pollutants. Cancer cells can also develop because of damage to DNA. Most of the time when DNA becomes damaged, the body is able to repair it. In cancer cells, the damaged DNA is not repaired. People can inherit damaged DNA, which accounts for inherited cancers. A disability in the DNA repair genes can predispose to mutations in the genome and hence to neoplastic transformation. The sooner a cancer is found and treatment begins, the better are the chances for living for many years. Tumours can occur in both mesenchymal cells and epithelial cells. Anti-cancer drugs presently used have been demonstrated to have severe side effects. Alternative system of medicine comprising the use of medicinal plant extracts to cure cancer with minimal side effects is gaining importance.

Anticancer properties of M. baccifera shoot extract are evaluated on three different cancer cell lines:

5.3.1. HepG2 (human liver carcinoma cell line) - is a perpetual cell line which was derived from the liver tissue of a 15 year old Caucasian American male with a well differentiated hepatocellular carcinoma. These cells are epithelial in morphology, have a model chromosome number of 55 and are not tumorigenic in nude mice. The cells secrete a variety of major plasma proteins; e.g., albumin, transferrin and the acute phase proteins fibrinogen, alpha 2-macroglobulin, alpha 1-antitrypsin, transferrin and plasminogen. HepG2 cells have been shown to be Neomycin G418 resistant (400 µg/mL). The cells will respond to stimulation with human growth hormone. With the proper culture conditions, HepG2 cells display robust morphological and functional differentiation with a controllable formation of apical and basolateral cell surface domains (van IJzendoorn et al., 1997). HepG2 cells and its derivatives are also used as a model system for studies of liver metabolism and toxicity of xenobiotics, the detection of cytoprotective, anti (environmental and dietary) genotoxic and cogenotoxic agents, understanding hepatocarcinogenesis, and for drug targeting studies. HepG2 cells are also employed in trials with bio-artificial liver devices.

5.3.2. Oaw42 (human ovarian cancer cell line) - The Oaw42 cell line was established from the ascites of a patient with ovarian cystadenocarcinoma. It has retained the ability to form free floating cysts in vitro, produces extracellular matrix, and shows a defined chemosensitivity pattern. It is a valuable cell line for studies on the biology of human ovarian cancer. The morphology of oaw42 is epithelial and has monolayer growth properties. Chromosome number of 86.

5.3.3. A341 (human skin cancer cell line) – A341 is originated from skin epithelium. It arises from the uncontrolled multiplication of transformed malignant cells. A341 cell line has an extraordinarily high expression of EGF (epidermal growth factor receptor). The skin begins as a small nodule and as it enlarges, the centre and the nodule turns into an ulcer, intermittent bleeding from the tumour, especially on the lip. Usually the tumour presents as an ulcerated lesion with hard, raised edges.

5.4. Bacteria used for testing antimicrobial activity of *M. baccifera* shoot extract

Although the vast majority of bacteria are harmless or beneficial, quite a few bacteria are pathogenic. Some bacteria are only pathogenic under certain conditions, such as a wound that allows for entry into the blood, or a decrease in immune function.For example, *Pseudomonas aeruginosa*, *Mycobacterium avium*, are opportunistic pathogens and cause disease mainly in people suffering from immunosuppression or cystic fibrosis. Each species of pathogenic bacteria has a characteristic spectrum of interactions with its human hosts.

Antibacterial activity of *M. baccifera* shoot extract is evaluated on three different human pathogenic bacteria by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. This assay is also used for *Mycobacterium tuberculosis* detection (Abate et al., 2004) :

5.4.1. *Staphylococcus aureus* - is a facultative anaerobic Gram-positive coccal bacterium. It is frequently part of the skin flora found in the nose and on skin, and in this manner about 20% of the human population are long-term carriers of *S. aureus* (Kluytmans et al., 1997). One of the reasons for this is a carotenoid pigment staphyloxanthin that is responsible for the characteristic golden colour of *S. aureus* colonies. This pigment acts as a virulence factor, with an antioxidant action that helps the microbe evade death by reactive oxygen species used by the host immune system (Clauditz et al., 2006; Liu et al., 2005). *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections and responsible for food poisoning through the production of an enterotoxin. *S. aureus* infections may spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that destroys tissues, and contact with objects such as towels, sheets, clothing, or athletic equipment used by an infected person.

5.4.2. *Pseudomonas aeruginosus* - Pseudomonas aeruginosa is a common bacterium, opportunistic human pathogen that can cause disease in humans as well as animals. It is a Gram-negative, aerobic, rod-shaped bacterium with unipolar motility (Ryan and Ray, 2004). It is found in soil, water, skin flora, and most man-made environments throughout the world. It uses a wide range of organic material for food; in animals, the versatility enables the organism to infect damaged tissues or people with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonizations occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal (Balcht and Smith, 1994). It is also able to decompose hydrocarbons and has been used to break down tarballs and oil from oil spills (Itah and Essien, 2005). P. aeruginosa secretes a variety of pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown).

5.4.3. *Salmonella typhi* – It is gram-negative rod-shaped and belongs to the family Enterobacteriaceae (Giannella, 1996). It is a motile, facultative anaerobe that is susceptible to various antibiotics. S. typhi has a combination of characteristics that makes it an effective pathogen. This species contains an endotoxin typical of Gram negative organisms, as well as the Vi antigen which is thought to increase virulence. It also produces and excretes a protein known as “invasin” that allows non-phagocytic cells to take up the bacterium, where it is able to live intracellularly. It is also able to inhibit the oxidative burst of leukocytes, making innate immune response ineffective.

5.5. Materials and Methods

5.5.1. Chemicals and reagents

Trypsin-EDTA, dulbecco’s modified eagle medium, antibiotic –antimycotic solution, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), LB (luria bertani) broth, streptomycin, ciprofloxine are obtained from himedia, India. FBS (fetal bovine serum)-S. America is purchased from Gibco, Invitrogen. DMSO, n-hexane and ethyl acetate are purchased from Merck chemicals, India.

5.5.2. Preparation of plant extracts

The general organic solvent extraction method was followed. 10 g of roots are shade dried, finely powdered, and extracted in soxhlet apparatus with n-hexane and ethyl acetate (7 : 1) ratio for 8 h. Then, the extract is filtered through filter paper and the filtrate was concentrated by evaporating at 60^{o}C. A paste-like final residue is formed and refrigerated at -20^{o}C until use.

5.5.3. Preparation of samples

Stock solution of 200 mg/mL concentration of plant extract is prepared in DMSO (dimethyl sulfoxide). From the stock solution different concentrations of 1 μg/mL to 700 μg/mL samples are prepared using DMEM (for anticancer analysis) and LB medium (for antibacteria analysis).

5.5.4. Preparation of culture medium

FBS is completely dissolved at room temperature. Then, heated at 60^{o}C for 1 h in water bath. To the 500 mL DMEM solution, add 5.5 mL antibiotic antimycotic solution and 50 mL FBS solution. This is henceforth called complete medium and store at 4^{o}C.

LB (luria bertani) broth is prepared by dissolving 25 g in 1000 mL mili Q water for bacteria culture medium.

5.5.5. Maintenance of human cancer cell lines

Three human cancer cell lines namely; HepG2 (human liver carcinoma cell line), Oaw42 (human ovarian cancer cell line) and A341 (human skin cancer cell line) are obtained from the National Center for Cell Sciences (NCCS) Pune. The growth medium used for the propagation of these cell lines are complete medium. The cell lines are maintained in humidified atmosphere with 5% CO_2 at 37°C. The seeding of the cells are 2 X 10^5 cells/mL of the medium. When the cells reached a density of 1 X 10^6 cells/mL they are sub cultured. Medium is renewed 2 to 3 times per week

5.5.6. Maintenance of bacteria

Three bacteria namely; *Staphylococcus aureus*, *Pseudomonas aeruginosus* and *Salmonella typhi* are obtained from the IMTACH, Chandigard, India. The bacteria are cultured in LB medium at 37°C on an orbital shaker 140 rpm. The bacteria are sub-cultured every week.

5.5.7. MTT assay for cancer cells viability

The complete medium is removed from the cell line culture plate and 1 mL of trypsin-EDTA is added. The trypsin-EDTA is and 1 mL of trypsin-EDTA added again. Incubated at 37°C for 5 to 8 min, the cells should be completely detached from the substratum of the culture plate. Culture medium of 5 mL to 8 mL is added. The culture medium containing cells is transferred to 15 mL falcon tube. Then, the number of cells/mL is counted using haematocrit, 9000 cells/well are seeded in 96 wells plate. The plate is incubated in the CO_2 incubator for 24 h. All the medium is removed by inverting 96 wells plate on the tissue paper. Then different concentrations of plant extract samples of 100 μL are added, incubated for another 48 h at 37°C, positive control is also made i.e, without plant extract. MTT (5 mg/mL in PBS) solution of 20 μL/well is added and incubated for 2 h. Remove all the medium containing plant extract and DMSO solution of 100 μL/well is added. The plate is shaken gently and the absorbance is measured at 570nm single wavelength mode. The experiment is carried out in triplicate and the average value is used for the calculation of percentage of Inhibition. The percentage of inhibition is calculated as:

$$\% \text{ inhibition} = \text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{standard}} / \text{Absorbance}_{\text{control}} \text{ X } 100$$

A graph is plotted with percentage of inhibition on the y-axis versus concentration of plant extract on the x-axis. The concentration of the plant extract that inhibited cell growth by 50% (IC_{50}) is determined from the graph.

5.5.8. MTT assay for bacterial cells viability

The absorbance of an overnight grown culture is measured at 600 nm. The bacterial culture showing 0.600 OD is used in this assay, 0.600 OD of bacterial culture indicated presence of 6 X 10^6 cells/mL approximately. 10 μL/well of this culture is seeded in 96 wells plate and different concentrations of plant extract samples of 90 μL are added, incubated for 24 h at 37^oC, positive control is also made i.e, without plant extract. MTT (5 mg/mL in PBS) solution of 20 μL/well is added and incubated for 1 h. Remove all the medium containing plant extract and DMSO solution of 100 μL/well is added. The plate is shaken gently and the absorbance is measured at 570nm single wavelength mode. The experiment is carried out in triplicate and the average value is used for the calculation of percentage of Inhibition. The percentage of inhibition is calculated as described in the above equation.

A graph is plotted with percentage of inhibition on the y-axis versus concentration of plant extract on the x-axis. The concentration of the plant extract that inhibited bacterial growth by 50% (IC_{50}) is determined from the graph.

The inhibitions of these pathogenic bacteria by antibiotics are also evaluated by using MTT assay.

5.5.9. Morphological assessment of cells

HepG2, Oaw42 and A341 cells are treated with or without their IC_{50} values of *M. baccifera* shoot extract in 6 wells plate, incubated for 24 h, 48 h and 72 h and are examined under phase contrast microscope.

5.6. Results and Discussion

MTT assay is a spectrometric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (eg. DMSO, isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

The effects of *M. baccifera* young shoot extract on human cancer cell growth inhibition is examined on HepG2 (human liver carcinoma cell line), Oaw42 (human ovarian cancer cell line) and A341 (human skin cancer cell line). All these cell lines are submitted to different concentrations of shoot extract for 48 h. *M. baccifera* young shoot extract inhibited the proliferation of these cell lines in a similar fashion. Fig. 40 shows the percentage of inhibition versus the concentration of shoot extract. Concentrations of shoot extract for which each cell line's growth is inhibited by 50% (IC_{50}) are calculated from the graph. The IC_{50} represents the concentration of an inhibitor that is required for 50% inhibition of its target. IC_{50} obtained are 90 μg/mL for HepG2, 134 μg/mL for Oaw42 and 80 μg/mL for A341. These low IC_{50} values confirmed strong anticancer properties of *M. baccifera* young shoot extract. At low concentrations, the extracts inhibited the cancer cell growth. With increased concentrations, the cancer cell growth inhibition is also increased.

To understand how *M. baccifera* shoot extract induced cell death, morphological alterations of HepG2, Oaw42 and A341 cells affected by this extract incubating for 24 h, 48 h and 72 h were examined. Images are captured by phase contrast microscope. In case of HepG2 cell grown in complete medium in the absence of *M. baccifera* shoot extract (i.e. control) found to be polygonal, elongated and well spreading forming monolayer. Treatment with 90 μg/mL (IC_{50}) of shoot extract reduced the number of cells attached to plate, which inhibited the interaction of cells with extracellular matrix. Cell structure integrity was disturbed. The original cell structure was lost and exhibited altered cellular morphology with cytoplasmic shrinkage, membrane blebbing and become rounded. With increase in incubation

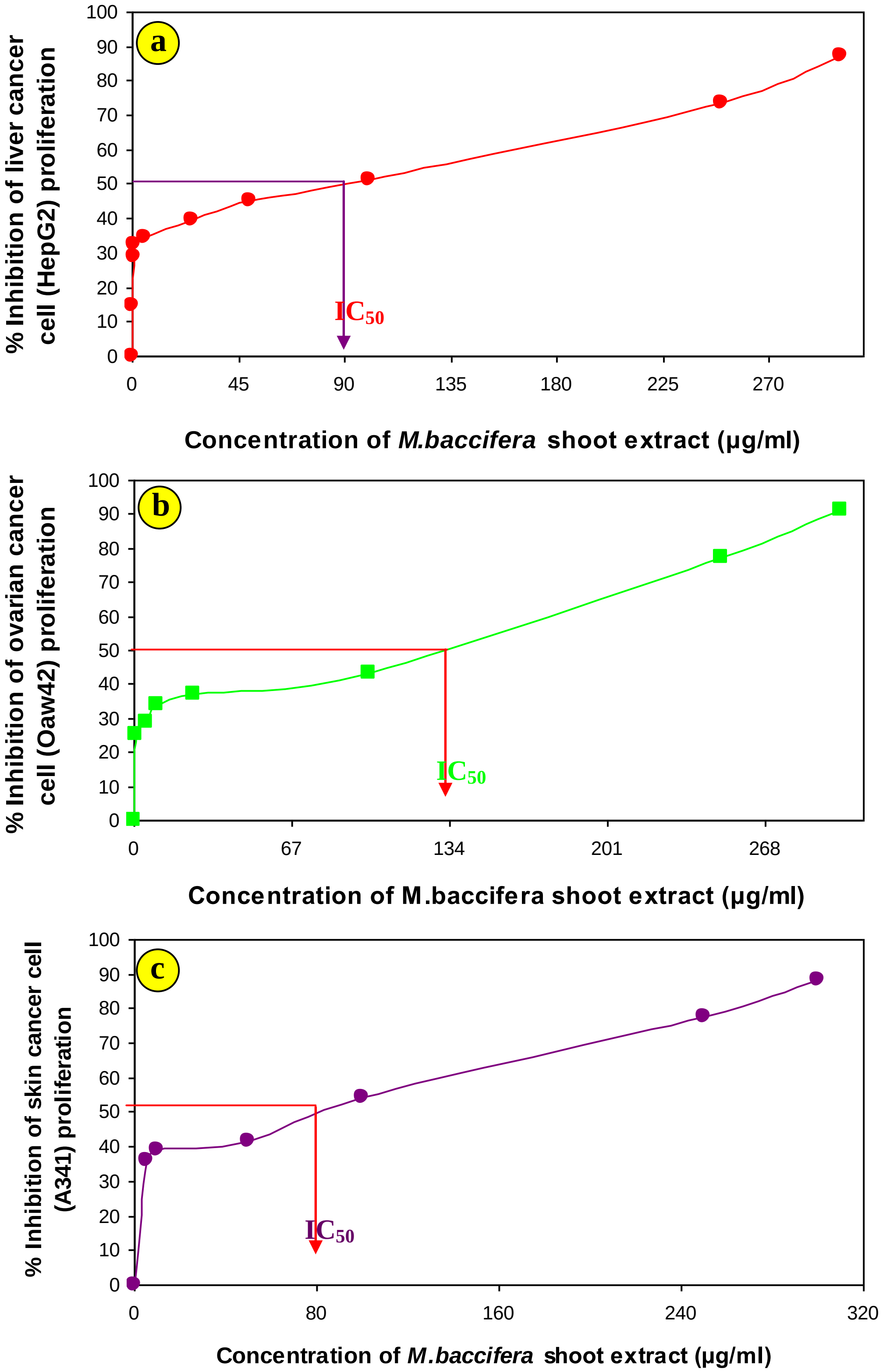


Figure 40. Effect of *M. baccifera* shoot extract on proliferation of a) HepG2; b) Oaw42; c) A341 human cancer cell lines. The cells are cultured in 10% FBS medium and treated with different concentrations of shoot extract for 48 h.

time, this effect is increased and most of the cells become rounded up at higher incubation time (Fig 41). Similar pattern of this effect was also founded in Oaw42 and A341 cells treated with *M. baccifera* as shown in Fig 42 and Fig 43, respectively.

The commercialization of plant having medicinal value has become rapid due to the identification, purification and characterization of active bio-molecules for various therapeutic purposes which have been vital for the present scenariobecause the synthetic drugs exhibit severe side effects due to their mode of action. Hence, "bioprospection" plays an important role in the identification of the novel bio-molecules from the natural resources, especially from plants, having fewer side effects and are cost effective. An active research is required for the identification of the plants which produce active compounds with therapeutic value. In modern times focus on plant research has increased all over the world, increased number of evidences put forward that many plants species have been studied in various indigenous traditional system of medicine. The potential role of various plants in cancer therapy as direct anti cancer agent, chemo preventive agent, and radiosentizer or immunity enhancer has been reported (Dhanhukar et al., 2000). In current years the use of plant extracts is gaining more significance in curing various diseases and recent reports suggest that they are very effective in anticancer, due to the supplementary or synergistic effect of particular compounds of the extract. It is significant to avoid unwanted effects of cytotoxic drugs in cancer chemotherapy without compromising antitumor activity. Majority of plant extracts exhibit antiproliferative activity on cancer cells which is often much better than the effect of their particular bioactive compounds. In cancer research, use of natural drugs instead of synthetic ones for chemoprevention of cancer is rapidly evolving. Medicinal plants being the source of bioactive principles such as polysaccharides, sterols, terpenoids, flavonoids, alkaloids, and pigments were shown to effect the multiplication of cancer cells.

The anticancer properties of *M. baccifera* shoots are due to the presence of its bioactive compounds. One of the important bioactive compounds is phytosterols. The presence of various phytosterols (brassicasterol, stigmasterol, campesterol and sitosterol) is already reported at the previous chapter. In recent years, a great deal of interest has been given to the role of phytosterols in the protection from human

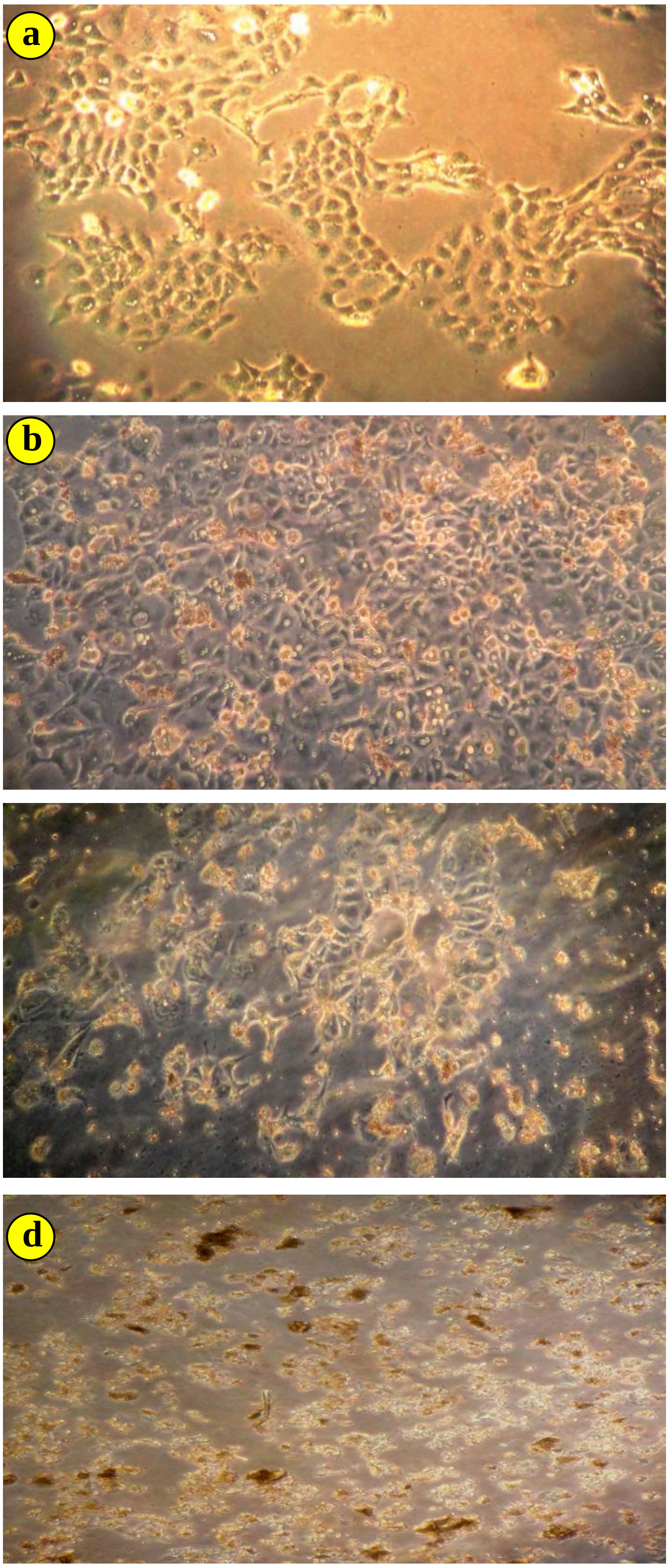


Figure 41. Morphological alterations of HepG2 cells following exposure to *M. baccifera* shoot extract of IC50 concentration for a) Control; b) 24 h; c) 48 and d) 72 h. images are captured by face contrast microscope

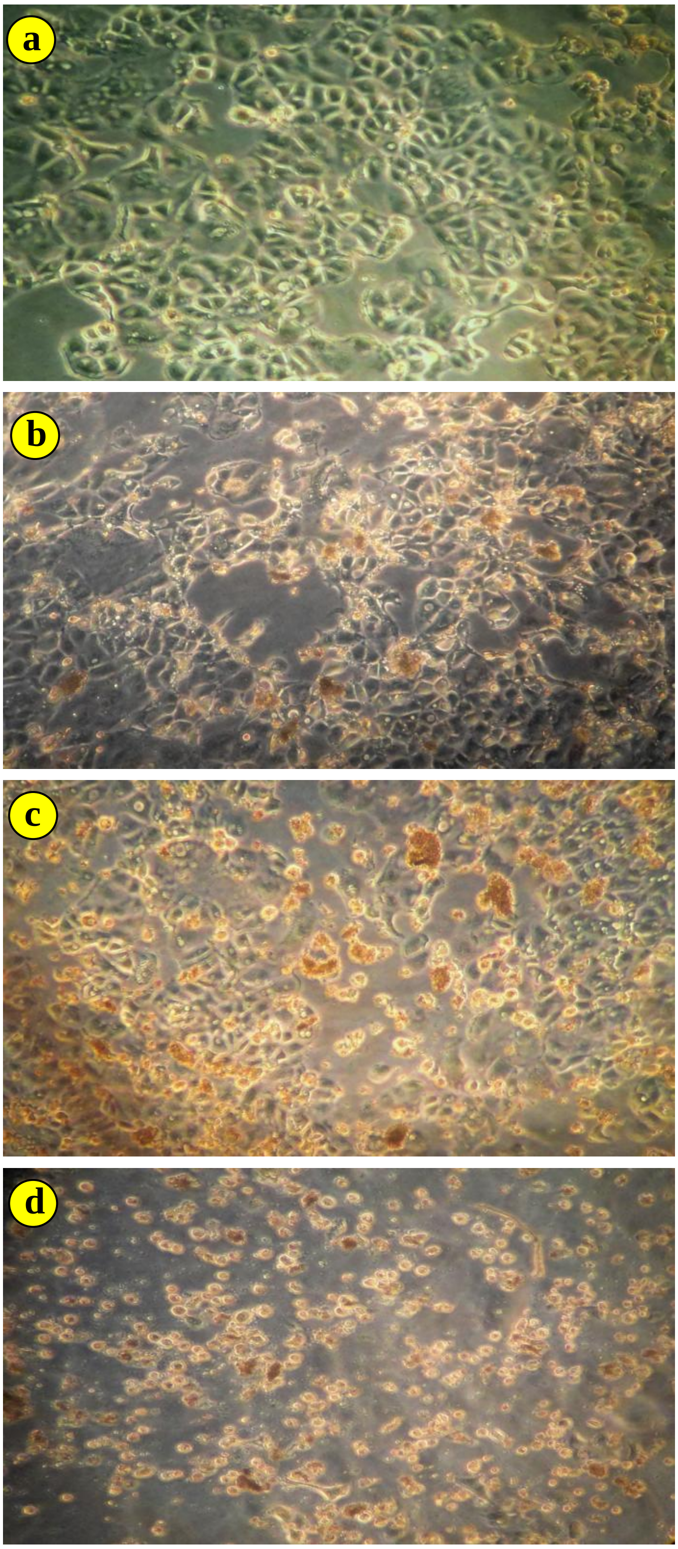


Figure 42. Morphological alterations of Oaw42 cells following exposure to *M. baccifera* shoot extract of IC50 concentration for a) Control; b) 24 h; c) 48 and d) 72 h. images are captured by face contrast microscope

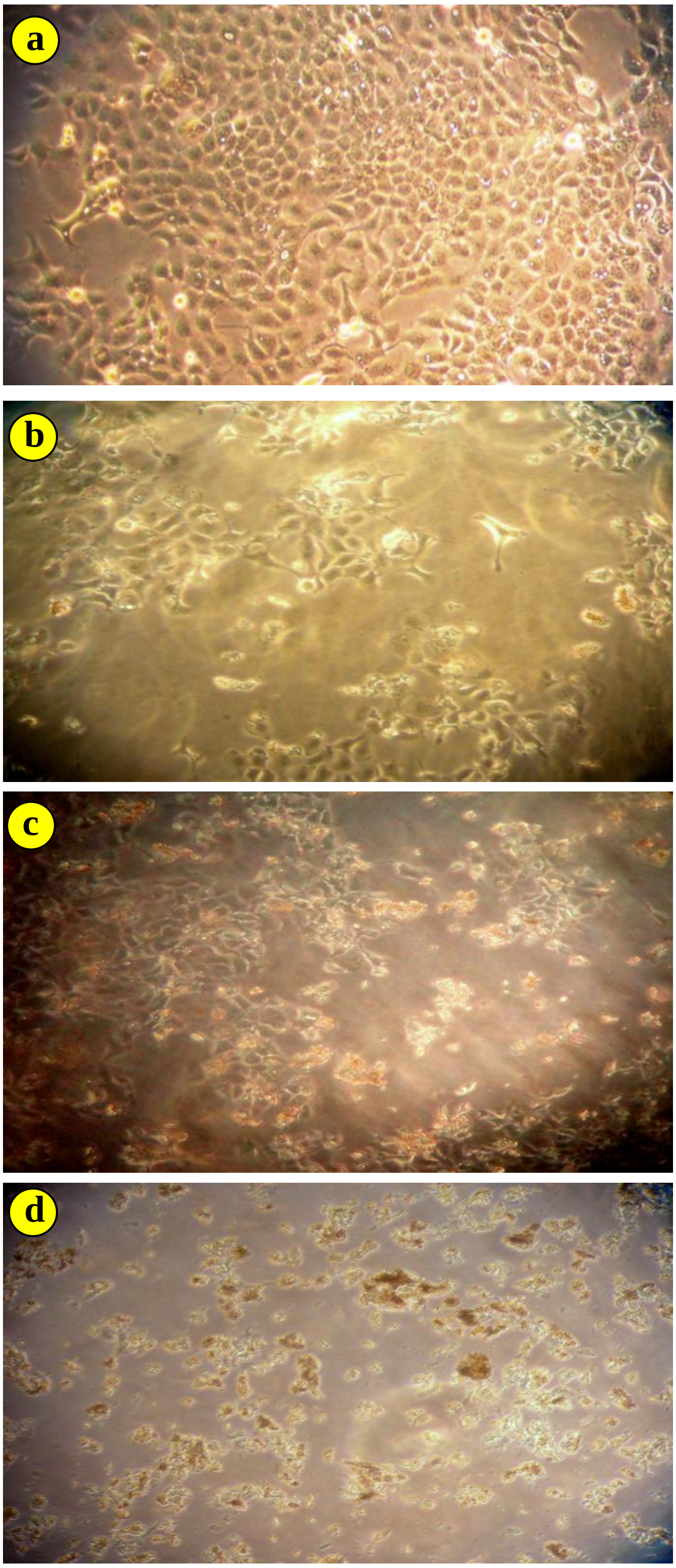


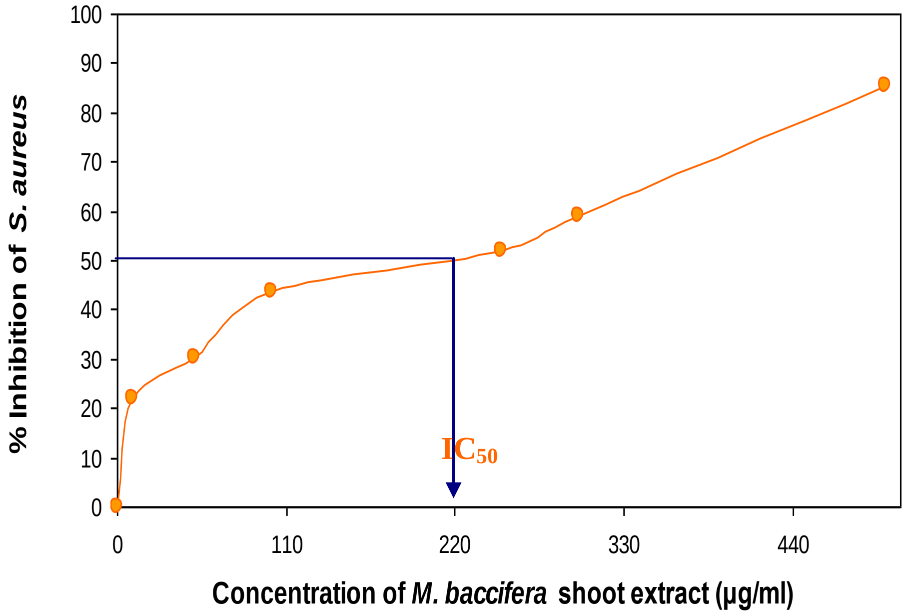
Figure 43. Morphological alterations of A341 cells following exposure to *M. baccifera* shoot extract of IC50 concentration for a) Control; b) 24 h; c) 48 and d) 72 h. images are captured by face contrast microscope

cancer. Epidemiological data suggest that the phytosterols content of the diet is associated with a reduction in the incidence of common cancers including cancers of the lung, stomach, colon, breast, and prostate (Bouic, 2001). The possible mechanism by which dietary phytosterols may be achieving these anti-cancer effects is not yet fully understood. However, it is believed that phytosterols may act through numerous mechanisms of action similar to other anti-carcinogenic compounds. First, the absorbed phytosterols may act on host systems to affect tumour surveillance, or act directly or indirectly on tumours to affect tumour cell biology (Bradford and Awad, 2007; Awad and Fink, 2000**)**. Secondly, phytosterols may have effects that directly inhibit tumour growth, including the slowing of cell cycle progression, the induction of apoptosis, and the inhibition of tumour metastasis (Bradford and Awad, 2007; Awad and Fink, 2000**)**. Thirdly, phytosterols may affect host systems by potentially enabling more robust anti-tumor responses, including the boosting of immune recognition of cancer, influencing hormone-dependent growth of endocrine tumours (Bradford and Awad, 2007; Awad and Fink, 2000**)**. Further, phytosterol consumption may also increase the activity of antioxidant enzymes and thereby reduce oxidative stress, which is related to several diseases and cancers (Woyengo et al., 2009). More research is needed to help explain how phytosterols influences cell growth and tumour development. In addition, the interaction between phytosterols and other beneficial food components that have demonstrated protective effects against cancer need to be examined (Rudkowska, 2010).

Recent evidence indicates that consumption of phytosterols, apart from lowering cholesterol levels, also provides protection against certain cancers such as anti-cancer activities to breast cancer cells (Awad et al., 2007), colon cancer cells (Rao and Janezic, 1992) and prostrate cancer cells (Gregg, 2001). Fas levels and caspase-8 activity, which is needed for extrinsic apoptotic programmed cell death pathway are significantly increased when breast cancer cells is treated with β-sitosterol. These findings indicated that β-sitosterol is an effective apoptosis-promoting agent (Awad et al., 2007). The incorporation of not only sitosterol but also other phytosterols from *M. baccifera* shoots extract may serve as anticancer activities to HepG2, Oaw42 and A341 human cancer cells.

The inhibitory effects of *M. baccifera* young shoot extract on human pathogenic bacteria are examined on *Staphylococcus aureus*, *Pseudomonas aeruginosus* and *Salmonella typhi* along with the inhibitory effect of these bacteria by antibiotic using MTT assay. All these bacteria are submitted to different concentrations of shoot extract for 24 h. Concentrations of shoot extract for which each bacteria growth is inhibited by 50% (IC_{50}) are calculated from the graph. IC_{50} obtained are 220 µg/mL for *Staphylococcus aureus* (Fig. 44), 46 µg/mL for *Pseudomonas aeruginosus* (Fig. 45) and 230 µg/mL for *Salmonella typhi* (Fig. 46). These low IC_{50} values confirmed antibacterial properties of *M. baccifera* young shoot extract.

The data obtained from MTT assay indicated that *P. aeruginosus* is most sensitive than the other bacteria tested. The presence of antimicrobial activity of chitin-binding peptides, *Pp*-AMP1 and *Pp*-AMP2 from bamboo shoot was reported in the literature (Fujimaru et al., 2005). More research is needed to help explain how *M. baccifera* shoot extract influences bacteria cell growth. *M. baccifera* contain many bioactive compounds including different fatty acids, sterols, alkaloids, flavonoids etc. Therefore, they could be responsible for the observed antibacterial activity.



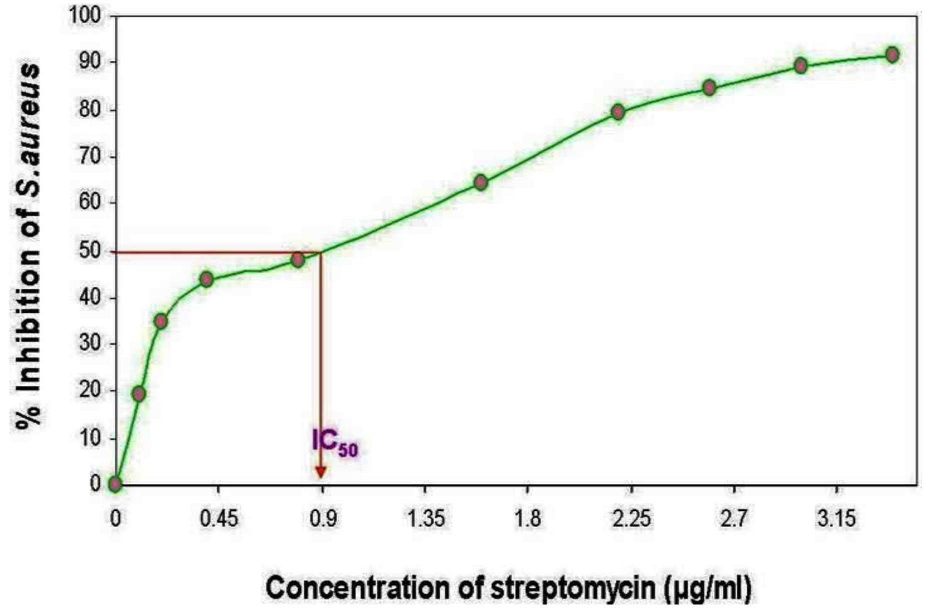
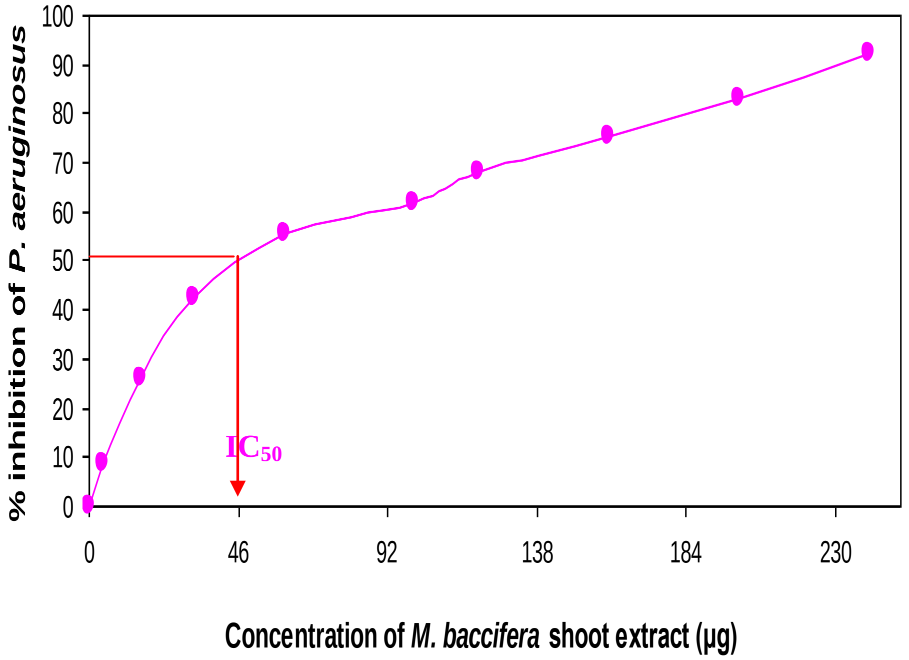


Figure 44. Inhibition of *Staphylococcus aureus* growth by a) *M. baccifera* shoot extract; b) streptomycin. The cells are cultured in LB medium and treated with different concentrations of shoot extract and antibiotic for 24 h



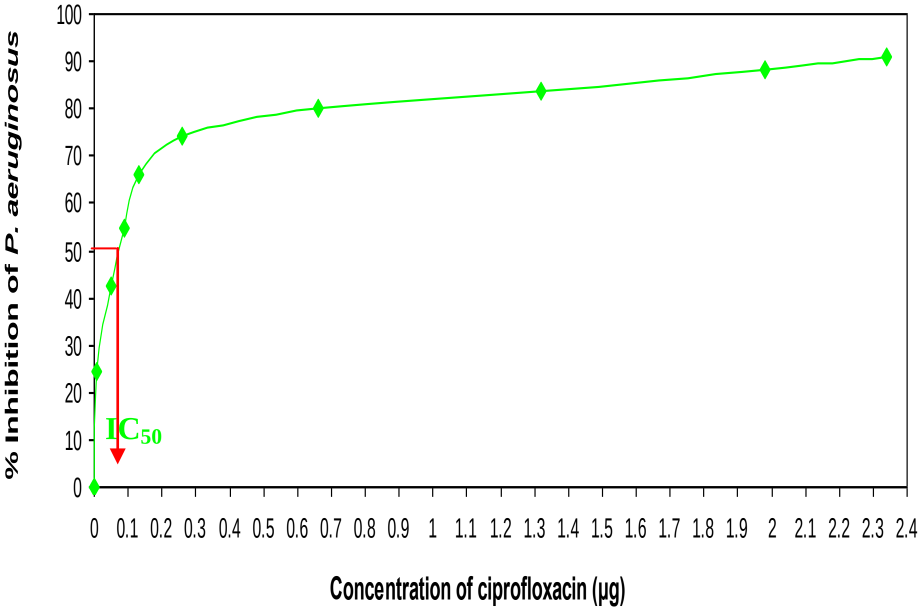


Figure 45. Inhibition of *Pseudomonas aeruginosus* growth by a) *M. baccifera* shoot extract; b) ciprofloxacin. The cells are cultured in LB medium and treated with different concentrations of shoot extract and antibiotic for 24 h.

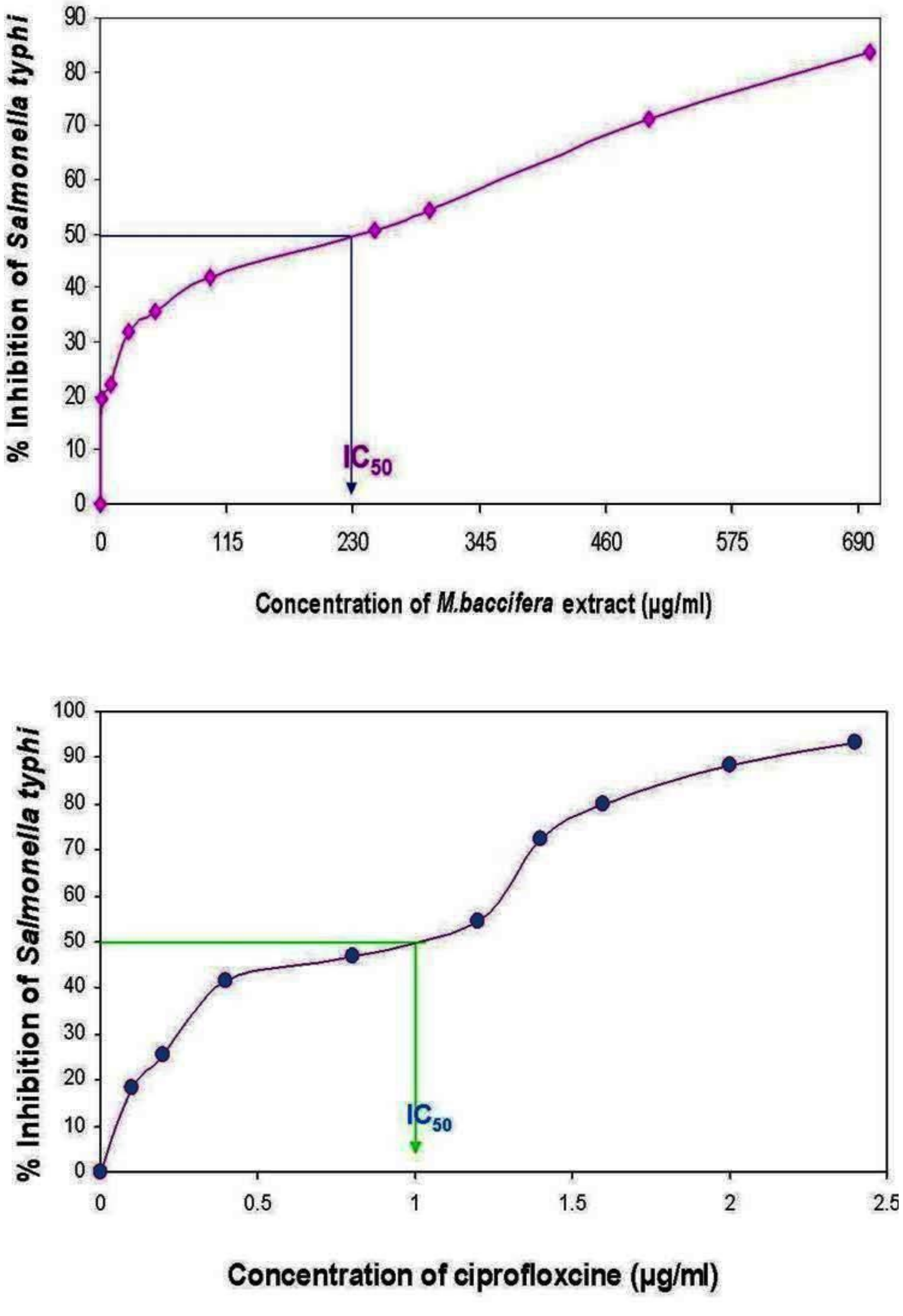


Figure 46. Inhibition of *Salmonella typhi* growth by a) *M. baccifera* shoot extract; b) ciprofloxacin. The cells are cultured in LB medium and treated with different concentrations of shoot extract and antibiotic for 24 h.

5.7. Conclusions

Bamboos have been utilized for traditional medicine from ancient time, but there is no scientific proof. In modern age, it was repored that bamboo has anticancer, antiviral, antibacterial, anti-inflammatory activities due to the presence of biochemical active compounds such as, Plant sterols, flavonoids, alkaloids, etc.

In *M. baccifera*, the young shoot extract exhibited anti-proliferative and antibacterial activity to different human cancer cell lines and bacteria. However, the mechanism underlying its anti-cancer and antibacterial effect is not clear and further investigation is needed. Inhibiting cancer cell growth has been a continuous effort in cancer treatment. A reduction in cell growth and an induction in cell death are two major means to inhibit cancer cell growth. Induction in cell death can be due to apoptosis or necrosis programmed cell death. Due to the presence of phytosterols and some other compounds, *M. baccifera* shoot extract show anti-cancer and anti-bacterial properties to different cancers cell lines and bacteria. Therefore, experiments based on *M. baccifera* shoot extract strongly suggest the potential of this vigorous bamboo shoot extract as a tumor suppressive, cancer and bacterial preventive food supplement.

6 Genetic similarity assessment of *Melocanna baccifera* growing in Mizoram state of India by using RAPD and ISSR markers.

6.1. Introduction

Traditional approaches to the measurement of diversity rely upon the ability to resolve differences in phenotypic traits like growth habit, leaf type, floral morphology, fruit colour and structure etc. The range of characters available may be increased by the use of electron microscopy or phytochemical assays. Although these approaches are extremely powerful, genetic information provided by morphological characters is often limited and expression of quantitative traits is subjected to strong environmental influence. Majority of plant taxa were defined morphologically and in most cases, this is still poor taxonomic description. Previously biochemical methods or isozyme profiles were used for identification of duplicates, assessment of genetic diversity, phylogenetic studies etc. in many plants (Aguirre *et al*., 1999; Fuentes *et al*., 1999). However, the information based on these methods is limited due to their inability to detect low levels of variation. Classification techniques based on the use of molecular markers provide a more accurate and powerful means of analyzing genetic relationships due to its availability in a limited number which results in reduction of time taken in generating information for planning conservation, improvement of genetic resources and investigating diversity analysis (Soltis *et al*., 1992). These methods are being used as complementary strategies to traditional approaches for assessment of genetic diversity, the major advantage being that they analyze the variation at the DNA level, excluding all environment influences (Soller and Beckmann, 1983). Advances in molecular biology technique have provided the basis for uncovering virtually unlimited numbers of DNA markers. Since the mid 1980s, genome identification and selection has progressed rapidly with the help of Polymerase Chain Reaction (PCR) technology. PCR is a molecular biology technique for enzymatically replicating/amplifying small quantities of DNA without using living organism. The amplification of target DNA can be exponential in that every cycle has the potential to double the amount of target DNA from the previous cycle, provided there is sufficient amount of DNA polymerase, primers and deoxynucleotide triphosphates (dNTPs) in the reaction solution. Although the basic protocol of PCR is straight-forward, each application requires optimizing the various parameters for the species to be studied. The discovery of Taq DNA polymerase, the DNA polymerase that is used by the bacterium "*Thermus auquaticus*" for its DNA replication/amplification and thus it is able to operate at the high temperature needed to perform the amplification without denatured, where other DNA polymerases become denatured. Nowadays, thermostable DNA polymerase like Pfu or Vent polymerase was also available. The PCR technique is being used for the screening, characterization and evaluation of genetic diversity (Williams et al., 1990; Welsh and McCleland, 1990). The development of the polymerase chain reaction (PCR) for amplifying DNA led to a revolution in the applicability of molecular methods and arrange of new technologies were developed which can overcome many of the technical limitations. These molecular techniques give a powerful tool to characterize the genetic relationships between species and contributed immensely to the molecular analysis of relatedness among genotypes and focuses more explicitly on particular taxa and attempts to conserve the full range of genetic (allelic) variation within those taxa (Welsh and McCleland, 1990). The assessment of the population structure of the plant species and the understanding of the genetic diversity patterns and relationships are necessary to effectively utilize and preserve these genetic resources. Newer and more powerful molecular techniques that are rapid and require only small quantities of DNA have been developed. Various types of molecular markers have been described in the literature. i.e. allele specific associated primers (ASAP; Gu et al., 1995), allele specific oligo (ASO; Beckmann, 1988), allele specific polymerase chain reaction (AS-PCR; Landegren et at., 1988), amplified fragment length polymorphism (AFLPs; Vos et al., 1995), anchored microsatellite primed PCR (AMP-PCR; Zietkiewicz et al., 1994), anchored simple sequence repeats (ASSR; Wang et al., 1998), arbitrary primed polymerase chain reaction (AP-PCR; Welsh and McClelland, 1990), cleaved amplified polymorphic sequence (CAPS; Akopyanz et al., 1992; Konieczny and Ausubel, 1993), degenerated oligonucleotide primed PCR (DOP-PCR; Telenius et al., 1992), diversity arrays technology (DArT; Jaccoud et al., 2001), DNA amplification fingerprinting (DAF; Caetano-Anolles et al., 1991), expressed sequences tags (EST; Adams et al., 1991), inter-simple sequence repeat (ISSR; Zietkiewicz et al., 1994), inverse PCR (IPCR; Triglia et al., 1988), inverse sequence-tagged repeats (ISTR; Rohde, 1996), micro-satellite primed PCR (MP-PCR; Meyer et al., 1993), multiplexed allele-specific diagnostic assay (MASDA; Shuber et al., 1997), random amplified microsatellite polymorphisms (RAMP; Wu et al., 1994), random amplified microsatellites (RAM; Hantula et al., 1996), random amplified polymorphic DNA (RAPDs; Williams et al.,1990), restriction fragment length polymorphism (RFLP; Botstein et al., 1980), selective amplification of microsatellite polymorphic loci (SAMPL; Morgante and Vogel, 1994), sequence characterized amplified regions (SCAR; Paran and Michelmore, 1993), sequence specific amplification polymorphisms (S-SAP; Waugh et al., 1997), sequence tagged microsatellite site (STMS; Beckmann and Soller, 1990), sequence tagged site (STS; Olsen et al., 1989), short tandem repeats (STR; Hamada et al., 1982), simple sequence length polymorphism (SSLP; Dietrich et al., 1992), simple sequence repeats (SSR; Akkaya et al., 1992), single nucleotide polymorphism (SNP; Jordan and Humphries, 1994), single primer amplification reactions (SPAR; Gupta et al., 1994), single stranded conformational polymorphism (SSCP; Orita et al., 1989), site-selected insertion PCR (SSI; Koes et al., 1995), stranded displacement amplification (SDA; Walker et al., 1992) and variable number tandem repeat (VNTR; Nakamura et al., 1987). Although some of these marker types are very similar (e.g., ASAP, ASO and AS-PCR), some synonymous (e.g., ISSR, RAMP, RAM, SPAR, AMP-PCR, MP-PCR and ASSR; Reddy et al., 2002) and some identical (e.g., SSLP, STMS, STR and SSR),each marker technique has its own advantages and disadvantages, for example, RAPD markers are very quick and easy to develop (because of the arbitrary sequence of the primers) but lack reproducibility (Karp et al., 1997; Hansen et al., 1998; Jones et al., 1999; Virk et al., 2000). AFLP has medium reproducibility but is labour intensive and has high operational and development costs (Karp et al., 1997). Microsatellites are specific and highly polymorphous (Karp et al., 1997; Jones et al., 1999), but they require knowledge of the genomic sequence to design specific primers and, thus, are limited primarily to economically important species. The appropriateness of individual marker system varies depending on the objectives of study and the properties of the species (Karp and Edwards., 1997). The choice of a molecular marker technique depends on its reproducibility and simplicity. The best markers for genome mapping, marker assisted selection, phylogenic studies and crop conservation have low cost and labour requirements and high reliability. Several researches have used RAPD and ISSR markers to assess levels and patterns of genetic variation, genetic relationship and taxonomic identity in plants (Table 10).

Table 10. RAPD and ISSR analysis in some of the useful plants

Taxon/Family	Salient finding	Reference
Zinnia elegans	A comparative analysis of the genetic diversity between inbred lines of *Zinnia elegans* using morphological traits and RAPD and ISSR markers	Ye et al., 2008
Houttuynia sp.	Evaluation of genetic relationships in the genus *Houttuynia* Thunb. in China based on RAPD and ISSR markers	Wu et al., 2005
Cashew	Assessment of genetic diversity in cashew germplasm using RAPD and ISSR markers	Thimmappaiah et al., 2009
Benincasa hispida	Genetic diversity of ash gourd [*Benincasa hispida* (Thunb.) Cogn.] inbred lines based on RAPD and ISSR markers and their hybrid performance	Veerendra et al., 2007
Ricinus communis	Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers	Bhavesh et al., 2010
Nelumbo sp.	Genetic diversity and classification of *Nelumbo* germplasm of different origins by RAPD and ISSR analysis	Li et al., 2010
Caldesia grandis	The extent of clonality and genetic diversity in the rare *Caldesia grandis* (Alismataceae): Comparative results for RAPD and ISSR markers	Chen et al., 2006
Vanilla sp.	The extent of genetic diversity among *Vanilla* species: Comparative results for RAPD and ISSR	Praveen et al., 2009
Sorghum bicolor	Evaluation of genetic variability of sorghum (*Sorghum bicolor* L. Moench) in northwestern Morocco by ISSR and RAPD markers	Medraoui et al., 2007
Chrysanthemum morifolium	A preliminary genetic linkage map of chrysanthemum (*Chrysanthemum morifolium*) cultivars using RAPD, ISSR and AFLP markers	Zhang et al., 2010
Nelumbo nucifera	Analyses of genetic relationships in *Nelumbo nucifera* using nuclear ribosomal ITS sequence data, ISSR and RAPD markers	Han et al., 2007
Eichhornia crassipes	Lack of genetic variation of an invasive clonal plant *Eichhornia crassipes* in China revealed by RAPD and ISSR markers	Li et al., 2006
Momordica charantia	Comparative analysis of genetic diversity in Indian bitter gourd (*Momordica charantia* L.) using RAPD and ISSR markers for developing crop improvement strategies	Behera et al., 2008
Citrus sp.	Utility of RAPD, ISSR, IRAP and REMAP	Biswas et al.,

	markers for the genetic analysis of *Citrus* spp.	2010
Broccoli sp.	Cultivar identification and genetic diversity analysis of *broccoli* and its related species with RAPD and ISSR markers	Lu et al., 2009
Zingiber officinale	Genetic diversity analysis of ginger (*Zingiber officinale* Rosc.) germplasm based on RAPD and ISSR markers	Kizhakkayil and Sasikumar, 2010
Oryza granulata	Genetic diversity among and within populations of *Oryza granulata* from Yunnan of China revealed by RAPD and ISSR markers: implications for conservation of the endangered species	Wu et al., 2004
Encephalartos woodii	Diversity in *Encephalartos woodii* collections based on Random Amplified DNA markers (RAPD's) and Inter-Specific Sequence Repeats (ISSR's)	Prakash et al., 2008
Trigonella sp.	Assessment of genetic diversity in *Trigonella foenum-graecum* and *Trigonella caerulea* using ISSR and RAPD markers	Rakhee et al., 2004
Morus sp.	Genetic diversity and relationships in mulberry (genus *Morus*) as revealed by RAPD and ISSR marker assays	Arvind et al., 2004
Jatropha curcas	Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different *Jatropha curcas* genotypes	Gupta et al., 2008
Melilotus sp.	Isozymes, RAPD and ISSR variation in *Melilotus indica* (L.) All and *M. siculus* (Turra) B.G. Jacks. Leguminosae	Abdel-Moneim Ibraheim Aboel-Atta, 2009
Dalbergia sissoo	A comparative analysis of ISSR and RAPD Markers for study of genetic diversity in Shisham (*Dalbergia sissoo*)	Arif et al., 2009
Hordeum vulgare	Inter-simple sequences repeat (ISSR) and RAPD variation among wild Barley (*Hordeum vulgare* subsp. spontaneum) populations from west Turkey.	Tanyolac et al., 2003
Citrullus & *Cucumis* sp.	Analysis based on RAPD and ISSR markers reveals closer similarities among *Citrullus* and *Cucumis* species than with *Praecitrullus fistulosus* (Stocks) Pangalo	Levi et al., 2005
Morus alba	Genetic analysis of *Morus alba* through RAPD and ISSR markers	Srivastava et al., 2004
Barley	Genetic diversity in barley from west China based on RAPD and ISSR analysis	Hou et al., 2005

6.2. Principle and application of RAPD and ISSR markers technique for diversity assessment

Advances in molecular biology techniques have provided the basis for uncovering virtually unlimited numbers of DNA markers. Over the last decade, polymerase chain reaction (PCR) technology has become a widespread research technique to amplify uncharacterized DNA fragments opened many species to general analysis. RAPD primers must meet two basic criteria indicated by Williams et al (1990): a minimum of 40% GC content (50-80% GC content is generally used) and the absence of palindromic sequence (a base sequence that reads exactly the same from right to left as from left to right). Because G-C bond consists of four hydrogen bridges and A-T bond consists of only two, a primer-DNA hybrid with less than 50% GC will probably not withstand the 72^{o}C temperature at which DNA elongation takes place by DNA polymerase. The total number of PCR products (i.e. sum of monomorphic and polymorphic fragments) per primer-template combination theoretically depends on the size of the template genome, but may vary from this depending on PCR conditions, primer sequence, base-mismatching between primer and template, and the competition between potentially amplified sequences during the reaction. The standard RAPD technology uses short synthetic oligonucloetides (10 bases long) as primer to amplify nanogram amounts of total genomic DNA under low stringency by PCR (annealing temperatures<45^{o}C). At an appropriate annealing temperature during the thermal cycle, oligonucleotide primers of random sequence bind several priming sites on the complementary sequences in the template genomic DNA and produce discrete DNA products if these priming sites are within an amplifiable distance from each other. The amplification of nucleic acids with arbitrary primers is mainly driven by the interaction between primer template annealing sites and enzymes and determined by complex kinetic and thermodynamic processes (Caetano-Anolles, 1997). Amplification products are generally separated on agarose gels and stained with ethidium bromide or visualisation and resolution was also achieved by PAGE with silver staining (Weising et al., 1995). In PCR-RAPDs method no prior knowledge of the genome subjected to analysis is required. The size of bands produced by RAPD is usually 200-2000 base pair. The profile of amplified DNA primarily depends on nucleotide sequence homology between the template

DNA and oligonucleotide primer at the end of each amplified product. Nucleotide variation between different sets of template DNAs will result in the presence or absence of bands because of changes in the priming sites. The cause of RAPD polymorphisms can occur due to base substitutions at the primer binding sites or insertion/deletions between two RAPD primers. Therefore, amplification products from the same alleles in a heterozygote differ in length and will be detected as presence and absence of bands in the RAPD profile. The DNA segment is amplified from one locus that is heterozygous or homozygous and RAPD markers are therefore dominant. Two kinds of polymorphism occur: the band may be present or absent, and the brightness (intensity) of the band may be different. Band intensity may result from the copy number or relative sequence abundance (Devos and Gale, 1992) and may serve to distinguish homozygous dominant from heterozygous, as more bright bands are expected for the homozygous. However, some authors (Thormann et al., 1994) found that there was no correlation between copy number and band intensity. The fact that fainter bands are generally less robust in RAPD experiments (Ellsworth et al., 1993; Heun and Helentjaris, 1993) suggests that varying degrees of primer mismatch may account for many band intensity differences. Since the source of band intensity differences is uncertain (copy number or primer mismatch). RAPDs may vary in sequence complexity and include repetitive or low copy sequences. When amplified from eukaryotes they are assumed to be nuclear markers (Heun and Helentjaris, 1993; Lu et al., 1995; Jenczewski et al., 1997). The high incidence of amplified repetitive DNA in RAPD fingerprints is to be expected given its prevalence in eukaryotic genomes. Centromeric and telomeric sequences are likely to be amplified as they contain inverted repeats, which may provide priming sites (Black et al., 1992). Most of the amplicons that have been characterized have been the polymorphic ones, so little is known about the origin of uninformative monomorphic fragments. The limitations of RAPD are its reproducibility and dominant inheritance. The concern about reproducibility of RAPD markers, however could be overcome through choice of an appropriate DNA extraction protocol to remove any contaminants (Micheli et al., 1994), by optimizing the parameters used (Ellsworth et al., 1993; Skroch and Nieuhuis, 1995), by testing several oligonucloetide primers and scoring only reproducible DNA fragments (Kresovich et al., 1992; Yang and Quiros, 1993), and by using appropriate DNA polymerase brand. The other limitation of RAPD markers is that the majority of the alleles segregate as dominant markers, and hence the technique does not allow identifying dominant homozygotes from heterozygotes. The RAPD assays produce fragments from homozygous dominant or heterozygous alleles. No fragment is produced from homozygous recessive alleles because amplification is disrupted in both alleles.

ISSR (Inter-Simple-Sequence-Repeats) is a segment of DNA found between two simple sequence repeats (usually dinucleotide or trinucleotide repeats). ISSR techniques are nearly identical to RAPD techniques except that ISSR primers are designed from microsatellite regions and are longer in length (approximately 14 or more bp) than RAPD primers. The technique uses microsatellites as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly inter simple sequence repeats of different sizes. The primers used can be either unanchored (Meyer et al., 1993; Gupta et al., 1994; Wu et al., 1994) or more usually anchored at 3^l or 5^l end with 1 to 4 degenerate bases extended into the flanking sequences (Zietkiewicz et al., 1994). Anchoring theoretically prevents amplification from within long repeat regions and reduces the number of fragments produced, particularly when primers utilize common repeat motifs such as (CA)n. Successful amplification without anchoring is also common (Borbet and Branchard, 2001; Kochieva et al., 2002). Consequently, ISSR polymorphisms are most likely to represent variation in SSR (Simple Sequence Repeat) flanking sequences and not variation in the repeated itself. Although the presence of microsatellite motifs in the primers biases amplification to repetitive DNA, products will mostly consist of regions located between two microsatellite repeats, the intervening sequences are expected to represent unique sequences (Bornet et al., 2002). ISSR use longer primer (15-30 mers) as compared to RAPD primers (10 mers), which permit the subsequent use of high annealing temperature leading to higher stringency. The annealing temperature depends on the GC content of the primer used and ranges from 45 to 65^{o}C. The amplified products are usually 200-2000 bp long and amenable to dectection by both agarose and polyacrylamide gel electrophoresis. The level of polymorphism has been shown to vary with the detection method used, polyacrylamide gel electrophoresis (PAGE) in combination with radioactivity was shown to be most sensitive, followed by PAGE with $AgNO_3$ staining and then agarose gel with EtBr system of detection (Semagn et al., 2006) ISSR exhibit the specificity of microsatellite markers, but need no sequence information for primer synthesis enjoying the advantage of random markers (Joshi et al., 2000). The technique is simple, quick, need small amount of DNA and radioactive labeling is not necessary. ISSR markers usually show high polymorphism (Kojima et al., 1998). Like RAPD, reproducibility, dominant inheritance and homology of co-migrating amplification products are the main limitations of ISSR. The reproducibility of ISSR amplification products ranged from 86 to 94%, with the maximum being when polyacrylamide gel electrophoresis and $AgNO_3$ staining were used and weak bands excluded from scoring (Moreno et al., 1998). ISSR segregate mostly as dominant markers (Gupta et al., 1994; Tsumura et al., 1996; Ratnaparkhe et al., 1998; Wang et al., 1998), although co-dominant segregation has been reported in some cases (Wu et al., 1994; Akagi et al., 1996; Wang et al., 1998; Sankar and Moore, 2001). There is also a possibility as in RAPD that fragments with the same mobility originate from non-homologous regions (Sanchez et al., 1996). RAPD and ISSR markers have been extensively used for DNA finger-printing (Moreno et al., 1998), genetic diversity studies (S'anchez de la Hoz et al., 1996; Esselman et al., 1999), population genetic studies (Wolfe et al., 1998; Nebauer et al., 1994) and phylogenetic studies (Hess et al., 2000)

Traditionally, morphological characters like growth habit, leaf type and floral morphology have been used to define taxa, this is still a poor taxonomic description. To date, no molecular analysis has been performed to test genetic variation of *M. baccifera* species. *M. baccifera* with green fruit is the most common throughout Mizoram. I would like to analyze whether *M. baccifera* with non-flowering, red fruit and blackish fruit show any variation at genetic level. Therefore, the genetic similarity and phylogenetic relationships within *M. baccifera* using RAPD and ISSR markers was carried out.

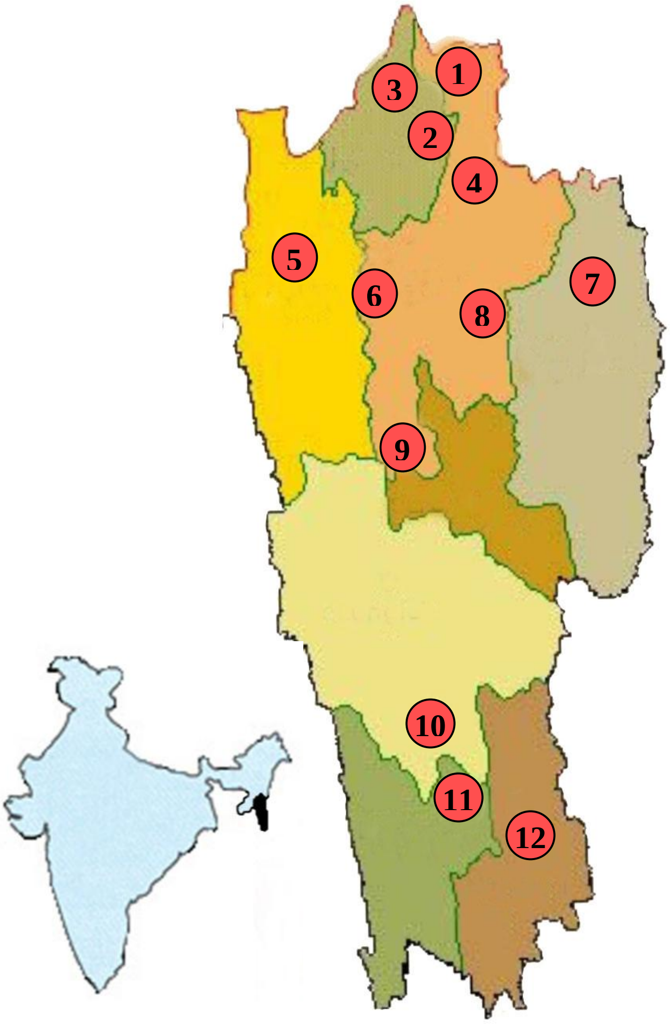
6.3. Materials and methods

6.3.1. Collection of germplasm

Extensive field survey was carried out i.e March-August throughout Mizoram (India) during the flowering of *M. baccifera*. Twelve accessions of *M. baccifera* were collected from the study area. One accession is collected in the form of leave and the remaining 11 accessions are collected in the form of seeds. The places of collection are shown in the map of Fig. 47. It is found that, Variation can be seen at morphological level from the difference in the seeds colour of *M. baccifera* in some accessions (Fig. 48). In order to analyze variation at genetic level in their gene pool, RAPD and ISSR markers are used. All the seeds collected were shown in the experimental field in Hyderabad University campus. The accession names, form of collection and accessions characters are given in Table 11. The distance between the collected accessions was at least 40-50 Km to increase the possibility of detecting the variation potential within each population (Fu et al., 2003).

Table 11. Bamboo (*Melocanna baccifera* Roxb.) accessions collected in different regions of Mizoram

S/N	Accessions name	Form of sample collection	Accessions character
1.	Zohmun	Leaves	Non-flowering culms
2.	N.Hlimen	Seeds	Red colour fruit
3.	Kolasib	Seeds	Green colour fruit
4.	Ratu	Seeds	Green colour fruit
5.	Mamit	Seeds	Green colour fruit
6.	Sairang	Seeds	Green colour fruit
7.	NE. Khawdungsei	Seeds	Green colour fruit
8.	Saitual	Seeds	Green colour fruit
9.	Sialsuk	Seeds	Blackish-brown fruit
10.	Haulong	Seeds	Green colour fruit
11.	Lawngtlai	Seeds	Green colour fruit
12.	Saiha	Seeds	Green colour fruit



1. Zohmun
2. N. Hlimen
3. Kolasib
4. Ratu
5. Mamit
6. Sairang
7. NE. Khawdungsei
8. Saitual
9. Sialsuk

10. Haulong
11. Lawngtlai
12. Saiha

Figure 47. Hydrographic map of the Mizoram, showing the collection sites of *Melocanna baccifera*.

6.3.2. DNA extraction and purification

Genomic DNA of every accession was isolated from the frozen leaf according to the CTAB (hexadecyl tri-methyl ammonium bromide) protocol developed by Doyle and Doyle (1990) with minor modification, as this method is successfully used on a broad range of tissues, fresh or dried, including calli from many species. Here the most convenient plant organ to sample is the leaf and freshly expanded young leaves were preferred to fully mature leaves, as they frequently contain smaller amount of polysaccharides. Young leaf tissue (2 gm) was grounded into a fine powder in liquid nitrogen with a frozen pestle and mortar along with 0.05 gm of PVPP (polyvinyl poly-pyrrolidone) and transferred to preheated 2% CTAB (10 ml) extraction buffer (2% CTAB, 100 mM Tris HCl, pH 8, 20 mM EDTA, 1.4 M NaCl) containing 10 mM β-mercaptoethanol per gram of tissue. The solution mixture was incubated for 60 min at 65^{o}C in a water bath, shaken at regular intervals of time. An equal volume of chloroform: isoamylalcohol (24: 1) was added and centrifugation was carried out at 12000 rpm for 15 min at 4^{o}C. To the supernatant equal volume of phenol: chloroform: isoamylalcohol (25: 24: 1) was added prior to centrifugation at 12000 rpm for 10 min at 4^{o}C. The supernatant was recovered and equal volume of phenol: chloroform: isoamylalcohol (25: 24: 1) was added again. The supernatant was recovered and equal volume of chloroform: isoamylalcohol (24: 1) was added. Centrifugation was carried out at 8000 rpm for 10 min. Equal volume of ice cold isopropanol was added to the supernatant and incubated at -20^{o}C for 60 min followed by centrifugation at 6000 rpm for 6 min. The pellet was collected and washed with 70% ethanol, centrifuged at 6000 rpm for 8 min. The pellet was dried and redissolved in 30 μl of TE buffer containing 2 μl of RNase A (10mg/ml) in order to eliminate RNA contamination. The solution was incubated at 37^{o}C overnight. This was followed by equal volume of phenol: chloroform: isoamylalcohol (25: 24: 1) treatment followed by centrifugation at 8000 rpm for 10 min. To the supernatant equal volume of chloroform: isoamylalcohol (24: 1) was added and centrifuged at 8000 rpm for 10 min. To the supernatant 1/10th volume of 3 M sodium acetate and equal volume of ice-cold isopropanol was added and incubated for 60 min at –20^{o}C to precipitate DNA. Centrifugation was done at 6000 rpm for 10 min. The DNA was washed by adding 70% ethanol, centrifuged at

6000 rpm for 5 min. The pellet was dried and redissolved in appropriate amount of TE buffer. Stored at -20°C for future use.

6.3.3. Quantification of DNA

After isolation of DNA, quantification and analysis of quality are necessary to ascertain the approximate quantity of DNA obtained and the suitability of DNA sample for further analysis. This is important for many applications including digestion of DNA by restriction enzymes or PCR amplification of DNA by genetic markers. The most commonly used methodologies for quantifying the amount of nucleic acid in a preparation are: (i) gel electrophoresis in which an aliquot of the extracted sample was compared with standard DNase like Hind III digested λ DNA, of known concentration and (ii) spectrophotometric analysis. With both methods additional information is gained concerning the quality and purity of the extracted samples obtained (Karp *et al.*, 1998). Agarose gel electrophoresis for DNA quantification and quality analysis is based on the ethidium bromide fluorescent staining of DNA. Ethidium bromide is a fluorescent dye, which intercalates between the stacked bases. The fluorescent yield of the dye: DNA complex is much greater than the unbound dye. UV irradiation at 254nm is absorbed by the DNA and transmitted to the dye and the bound dye itself absorbs radiation at 302nm and 366nm. This energy is retransmitted at 590nm, the reddish-orange region of the visible spectrum. In case of plant genomic DNA, the nucleic acids are electrophoretically separated on a 0.8% agarose gel containing ethidium bromide at a final concentration of 0.5 μg/ml in TBE buffer. Visualized and photographed by using gel documentation system of Uvidoc (Uvitech, Uk) (Fig. 49). DNA isolated from tissues was quantified based on the UV spectrophotometer. Analysis of UV absorption by the nucleotides provides a simple and accurate estimation of the concentration of nucleic acids in a sample. Purines and pyrmidines in nucleic acid show absorption maxima around 260 nm. lf the DNA sample is pure without significant contamination from proteins or organic solvents. The ratio of OD_{260}/OD_{280} should be determined to assess the purity of the sample. A ratio between 1.8-2.0 denotes a pure nucleic acid. A ratio lower than 1.8 indicates the presence of proteins and/or other UV absorbers. A ratio higher than 2.0 indicates that the samples may be contaminated with chloroform or phenol. In either case (<1.8 or >2.0), it is advisable to re-precipitate the DNA.

Figure 48. Accessions of *Melocanna baccifera* showing different accessions characters; (a) Non- flowering accession (green patch) surrounded by brownish-white colour flowering culms, (b) green colour fruit, (c) red colour fruit and (d) blackish brown colour fruit

Figure 49. Purified nucleic acids electrophoretically separated on a 0.8% agarose gel for twelve accessions. HE=N.Hlimen, HL=Haulong, KS=Kolasib, LT=Lawngtlai, MM= Mamit, NK= NE. Khawdungsei, RT=Ratu, SH=Saiha, SR=Sairang, SS=Sialsuk, ST=Saitual, ZH= Zohmun

6.3.4. PCR (Polymerase chain reaction)-RAPD analysis

Forty arbitrary decamer random primers (primer sets of kit OPA and OPC, Operon Technologies, Alameda, CA) with 10-bp oligonucleotides of random sequence were tested on the genomic DNA of two accessions and nine showed reproducible amplified products were chosen for this investigation. These nine primers are used to amplify 12 accessions DNA. Primers used are given in the Table 12. Amplification was performed in a 15µl reaction volume, containing 50µg template DNA, 1x PCR buffer, 0.3mM each dNTP, 2mM of MgCl2, 1.5µM of each primer and l unit of Taq DNA Polymerase (Sigma Aldrich). Amplification was performed in My-gene^{TM} series peltier thermal cycler (Longgene Scientific Instruments Co., Ltd. Hangzhou). Initial denaturation was for 4 mins at 94^{o}C, followed by 45 cycles of 50 sec at 94^{o}C, 1 min at 37^{o}C, 2 mins at 72^{o}C and 10 mins at 72^{o}C for final extension step. The amplified products were separated on 1.2% agarose gels in 1x TBE buffer at 50 V for 3 h and then stained with ethidium bromide. Gels with amplification fragments were visualized and Images were captured using Uvidoc (Uvitech, UK) gel documentation system. GeNei^{TM} medium range DNA ruler was used as molecular marker in the gels as a size reference.

6.3.5. PCR-ISSR analysis

Forty five ISSR primers sets from University of British Columbia (Vancouver, Canada) were used to amplify the genomic DNA of two accessions, and 17 primers were selected (Table 12). Amplification was preformed in a 15µl reaction volume, containing 50ng template DNA. 1X PCR buffer, 0.4 mM each of dNTP, 2mM of $MgCl_2$, 1.0µM each of primer and 1 unit of Taq. DNA polymerase (Sigma Aldrich). Amplification was performed in My-Gene^{TM} series peltier thermal cycle (Longgene Scientific Instruments Co., Ltd. Hangzhou). Initial denaturation was for 7 min at 94^{0}C, followed by 45 cycles of 1min at 94^{0}C, 1min at 54^{0}C, 2min at 72^{0}C and a 10 min final extension step at 72^{0}C. The amplified products were separated on 1.2% agarose gels in 1x TBE buffer at 50 V for 3 h and then stained with ethidium bromide. Gels with amplification fragments were visualized and Images were captured using Uvidoc (Uvitech, UK) gel documentation system. GeNei^{TM} medium range DNA ruler was used as molecular marker in the gels as a size reference.

6.3.6. Data Analysis

The amplified DNA fragments for each accession were scored as present (1) or absent (0); RAPD data generated with nine primers and ISSR data generated with 17 primers were used to compile a binary matrix for cluster analysis. Genetic similarity among accessions was calculated according to Dice similarity coefficient (Dice, 1945). The similarity coefficients were then used to construct a dendrogram using the UPGMA (Unweighted Pair-Group Method with Arithmetical average) cluster analysis (Sneath and Sokal, 1973). Similarity matrices based on RAPD and ISSR markers were compared using the mantel matrix-correspondence test (Mantel, 1967). All procedures were computed with the computer package NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System, Biostatistics, New York, USA, software version 2.02j package). (Rohlf, 1993).

6.4. Results and Discussion

Identification of genetic relationships in *M. baccifera* is very difficult because of the lack of morphological differences. The traditional methods of identifying DNA profiling are largely used for identification because of several limitations of morphological data. In recent years, RAPD and ISSR technology is a rapid and sensitive technique which can be used to estimate relationship between closely, and more distantly accessions.

6.4.1. Polymorphisms detected by RAPD & ISSR markers

The polymorphism of the *M. baccifera* germplasm was high, a total 40 arbitrary RAPD primers were used for screening, out of these 9 primers were selected out for twelve accessions of *M. baccifera* DNA amplification, which produced clear bands for further analysis. The nine arbitrary primers used for RAPD analysis detected a total of 94 fragments, with an average of 10.4 fragments per primers and 98.02% fragments were polymorphic. 45 arbitrary ISSR primers were used for screening, out of these 17 primers were selected. A total of 120 fragments were detected, with an average of 7.05 fragments per primer, and 84.1% fragments were polymorphic (Table 12). The DNA fragments produced by RAPD and ISSR markers were shown in Fig. 50 - 62.

Table 12. The statistical data for 9 RAPD and 17 ISSR primers used for analyzing 12 accessions of *Melocanna baccifera*

Primer	**Sequence**	**Annealing temperature**	**TNB**	**NPB**	**PB (%)**
RAPD					
OPA-05	$5^{/}$-AGGGTCTTG-$3^{/}$	37^0C	5	4	80%
OPA-09	$5^{/}$-GGGTAAGGCC-$3^{/}$	37^0C	8	7	87.5%
OPA-10	$5^{/}$-GTGATCGCAG-$3^{/}$	37^0C	20	19	95%
OPA-17	$5^{/}$-GACCGCTTGT-$3^{/}$	37^0C	20	19	95%
OPC-02	$5^{/}$-GTGAGGCGTC-$3^{/}$	37^0C	7	7	100%
OPC-04	$5^{/}$-CCGCATCTAC-$3^{/}$	37^0C	5	5	100%
OPC-07	$5^{/}$-GTCCCGACGA-$3^{/}$	37^0C	9	8	88.8%
OPC-11	$5^{/}$-AAAGCTGCGG-$3^{/}$	37^0C	11	10	90.9%
OPC-15	$5^{/}$-GACGGATCAG-$3^{/}$	37^0C	9	9	100%
Total			94	88	
Average			10.4	9.7	98.02%
ISSR					
UBC-810	$5^{/}$-GAGAGAGAGAGAGAGAT-$3^{/}$	52^0C	10	9	90%
UBC-813	$5^{/}$-CTCTCTCTCTCTCTCTT-$3^{/}$	55^0C	11	10	90.9%
UBC-814	$5^{/}$-CTCTCTCTCTCTCTCTA-$3^{/}$	52^0C	5	4	80%
UBC-815	$5^{/}$-CTCTCTCTCTCTCTCTG-$3^{/}$	52^0C	4	2	50%
UBC-820	$5^{/}$-GTGTGTGTGTGTGTGTC-$3^{/}$	57^0C	5	5	100%
UBC-821	$5^{/}$-GTGTGTGTGTGTGTGTT-$3^{/}$	55^0C	7	5	71.4%
UBC-822	$5^{/}$-TCTCTCTCTCTCTCTCA-$3^{/}$	55^0C	8	8	100%
UBC-823	$5^{/}$-TCTCTCTCTCTCTCTCC-$3^{/}$	55^0C	8	7	87.5%
UBC-824	$5^{/}$-TCTCTCTCTCTCTCTCG-$3^{/}$	55^0C	4	4	100%
UBC-827	$5^{/}$-ACACACACACACACACG-$3^{/}$	57^0C	8	7	87.5%
UBC-828	$5^{/}$-TGTGTGTGTGTGTGTGA-$3^{/}$	57^0C	5	4	80%
UBC-830	$5^{/}$-TGTGTGTGTGTGTGTGG-$3^{/}$	57^0C	5	4	80%
UBC-846	$5^{/}$-CACACACACACACACART-$3^{/}$	55^0C	8	8	100%
UBC-847	$5^{/}$-CACACACACACACACARC-$3^{/}$	56^0C	9	7	77.7%
UBC-856	$5^{/}$-ACACACACACACACACYA-$3^{/}$	55^0C	7	6	85.7%
UBC-857	$5^{/}$-ACACACACACACACACYG-$3^{/}$	56^0C	12	12	100%
UBC-859	$5^{/}$-TGTGTGTGTGTGTGTGRC-$3^{/}$	56^0C	4	2	50%
Total			120	104	
Average			7.05	6.1	84.1%

Note: - R=A/G; Y=C/T; TNB: total number of bands; NPB: number of polymorphic bands; PB (%): percentage of polymorp

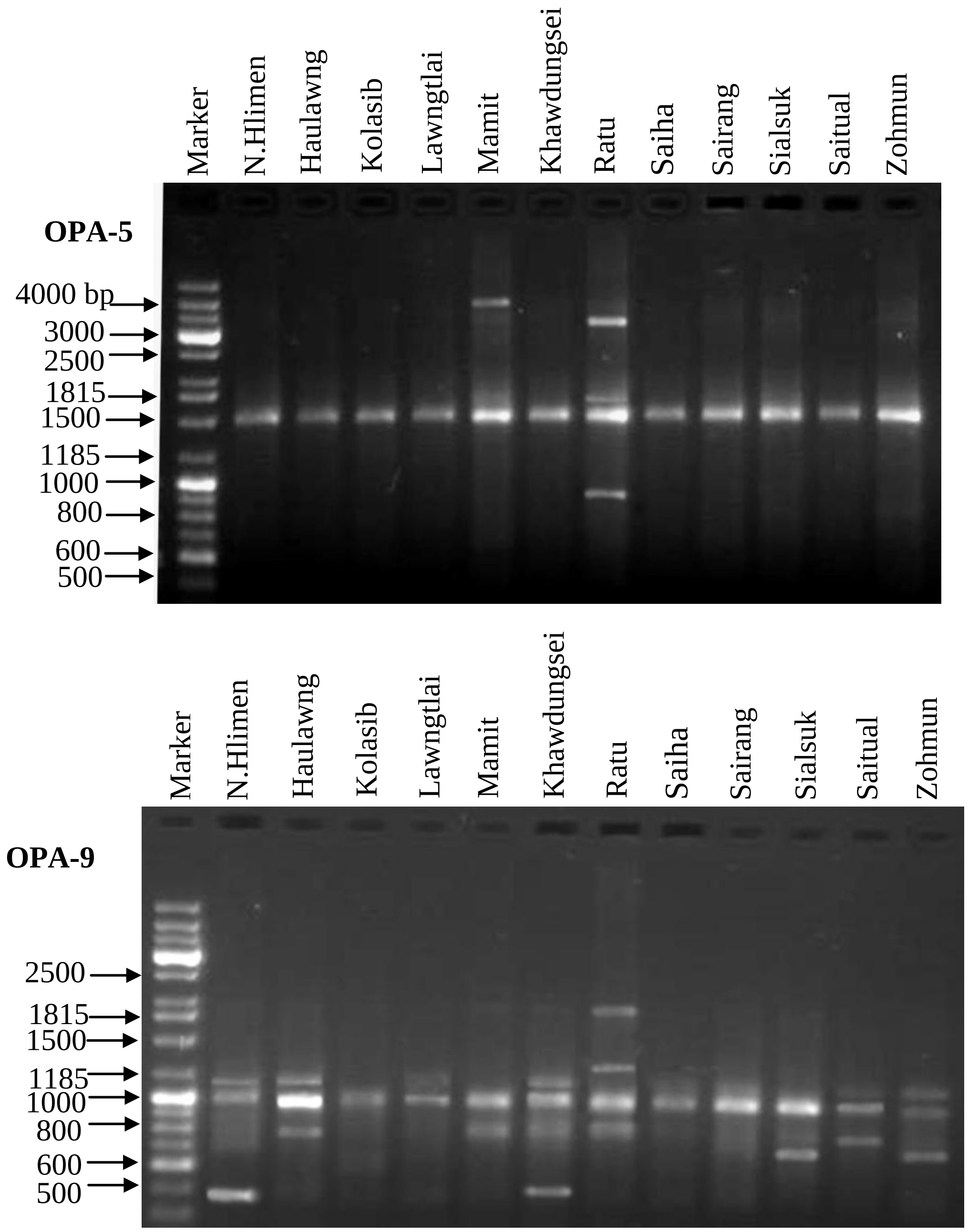


Figure 50. RAPD amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA obtained with arbitrary primers OPA-5 and OPA-9 on 1.2% agarose gel

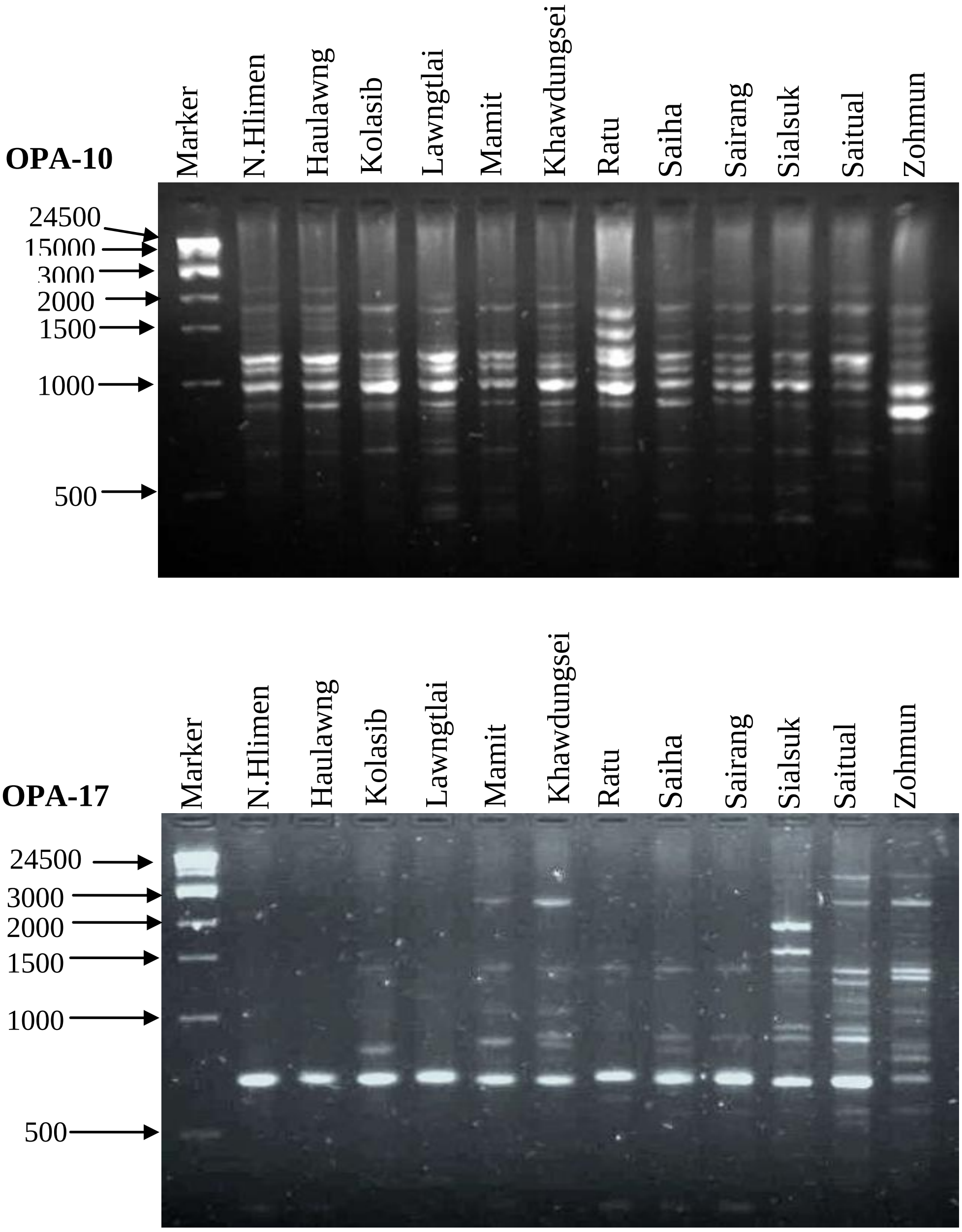


Figure 51. RAPD amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA obtained with arbitrary primers OPA-10 and OPA-17 on 1.2% agarose gel

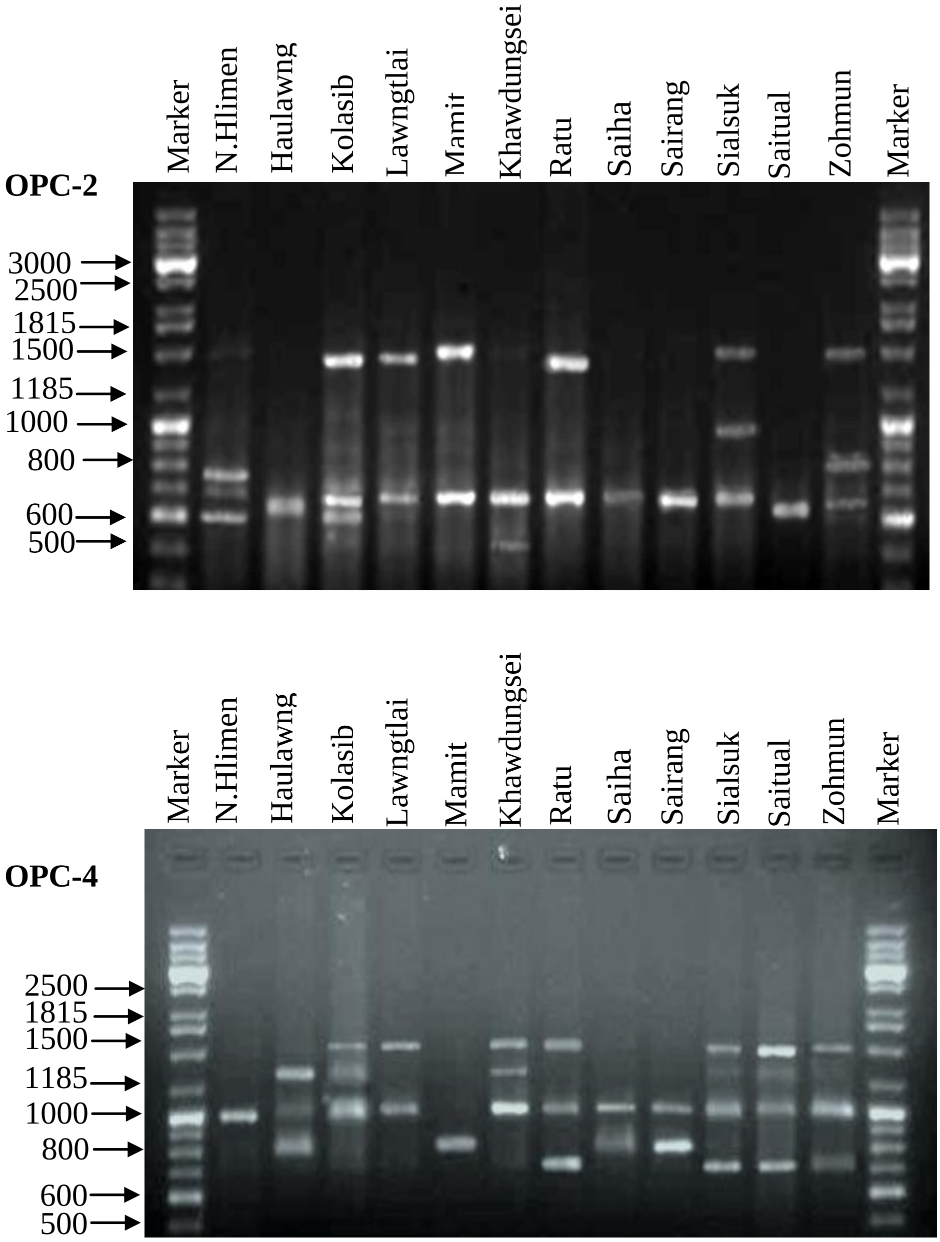


Figure 52. RAPD amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA obtained with arbitrary primers OPC-2 and OPC-4 on 1.2% agarose gel

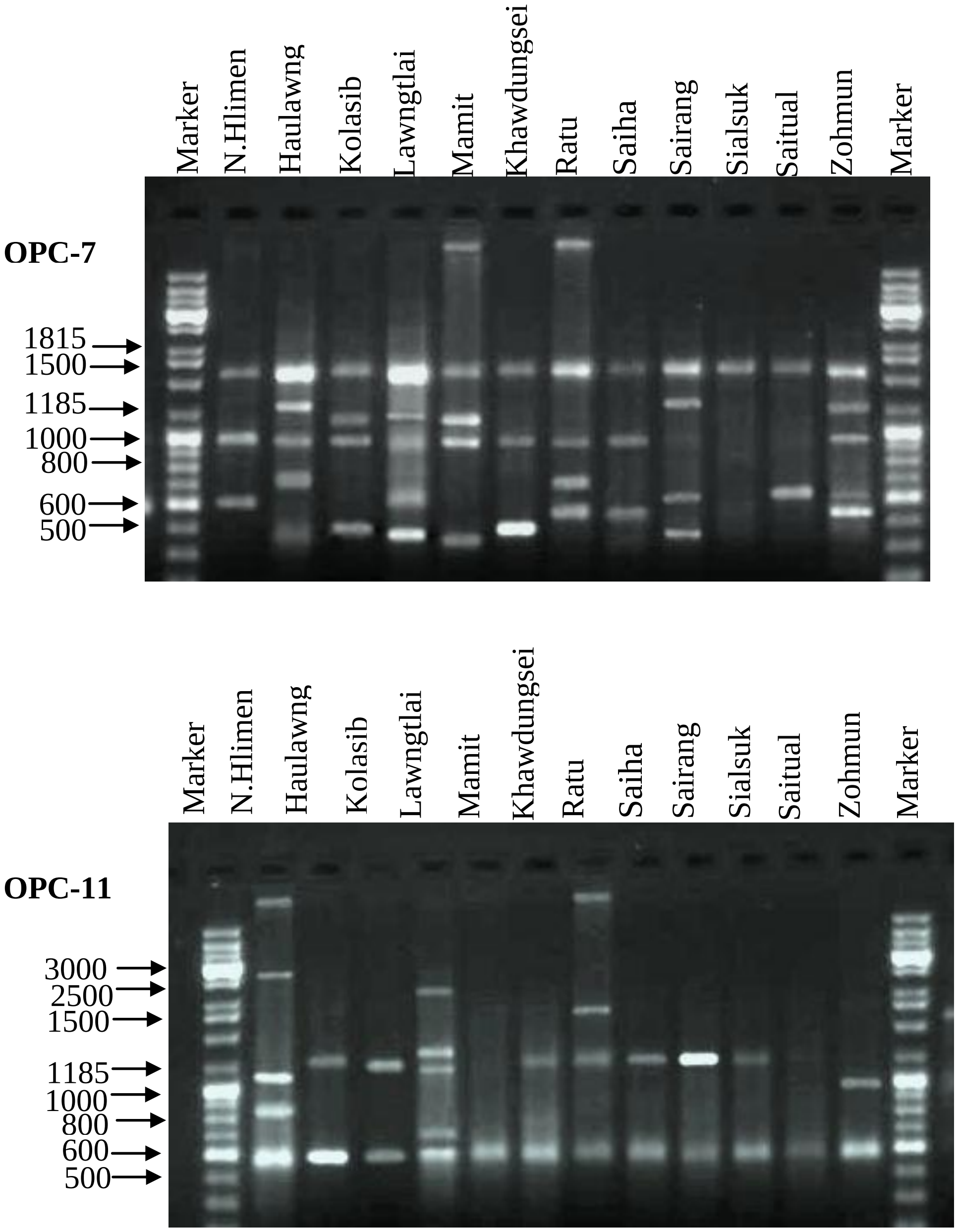


Figure 53. RAPD amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA obtained with arbitrary primers OPC-7 and OPC-11 on 1.2% agarose gel

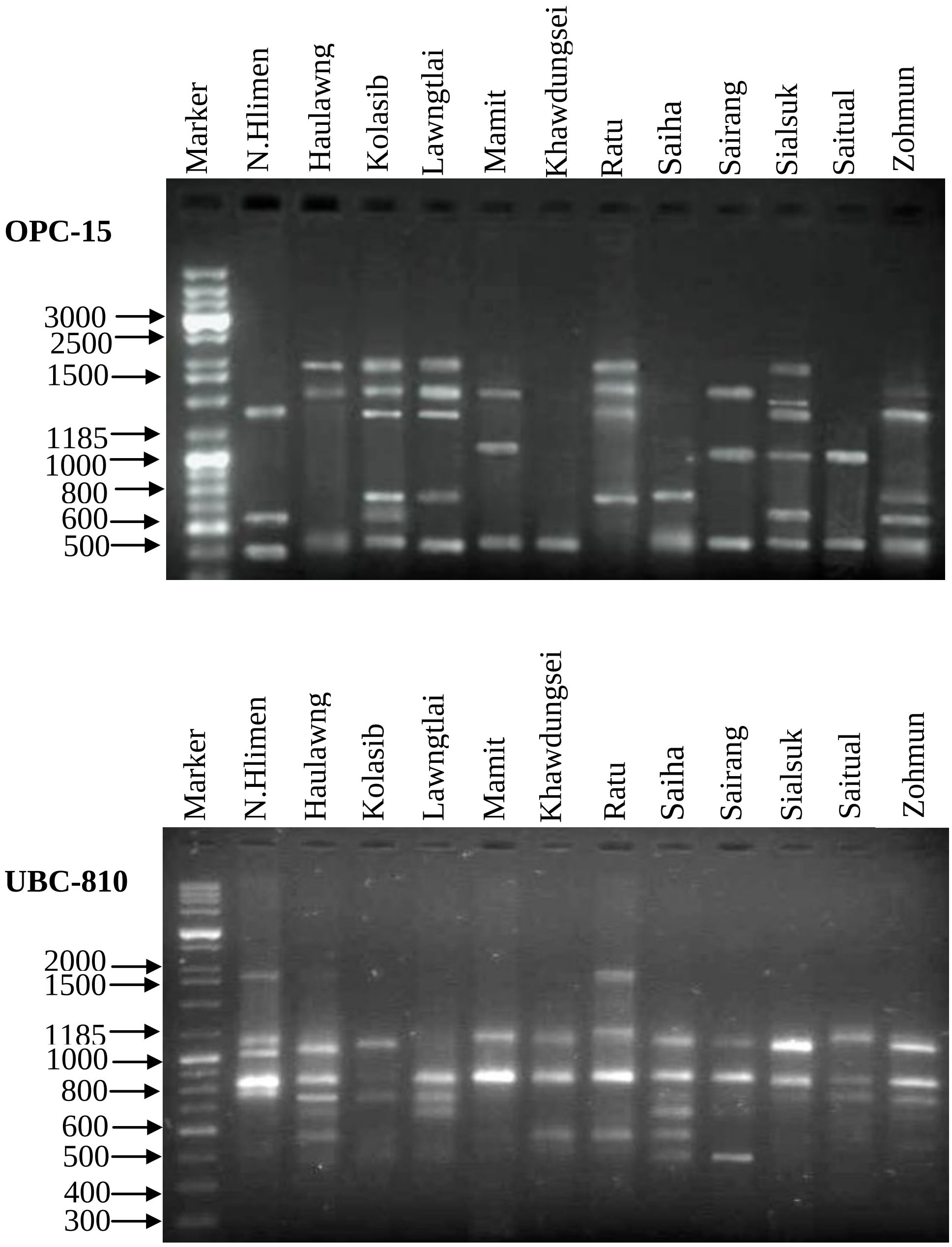


Figure 54. RAPD (OPC-15) and ISSR (UBC-810) amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA on 1.2% agarose gel

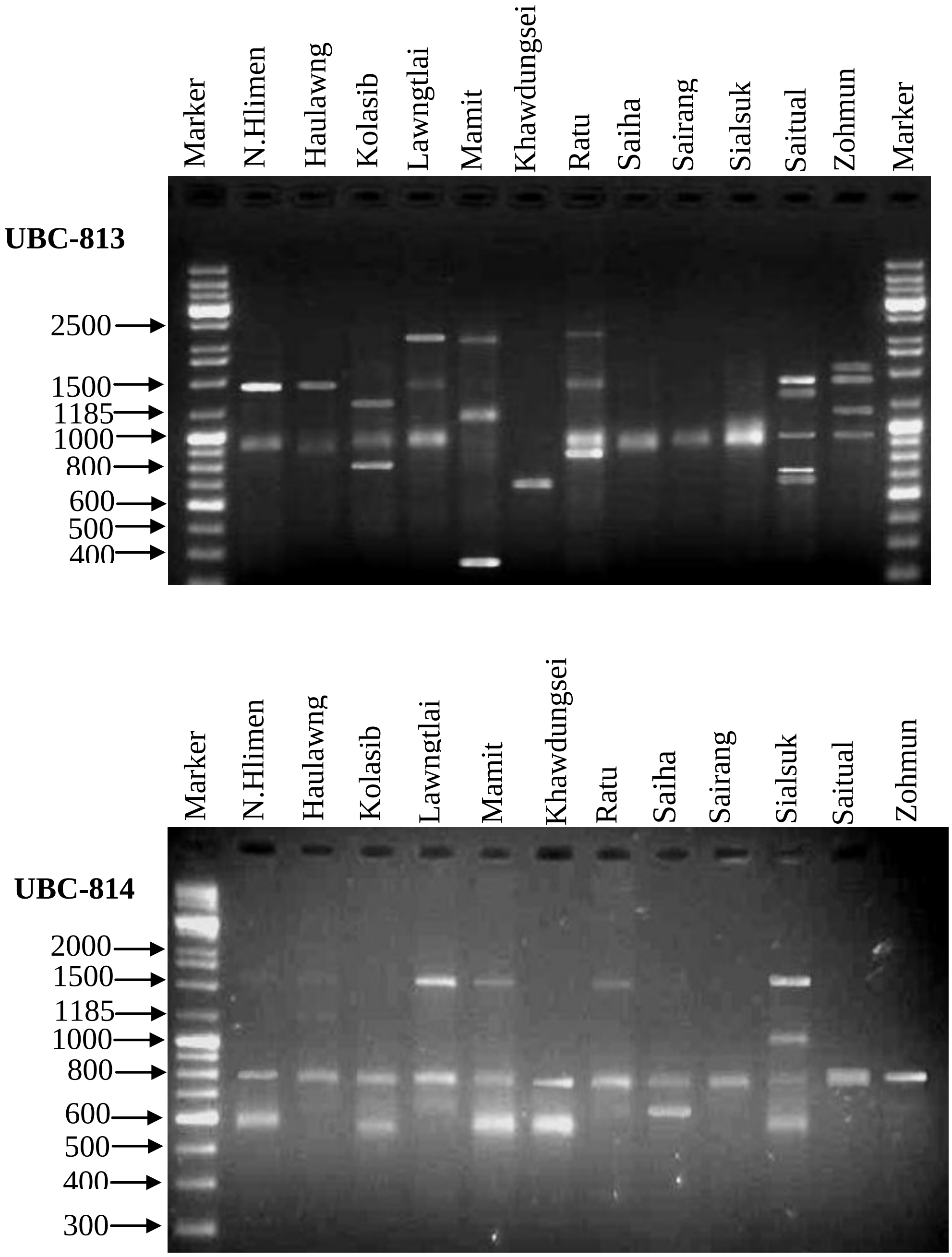


Figure 55. ISSR amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA obtained with primers UBC-813 and UBC-814 on 1.2% agarose gel

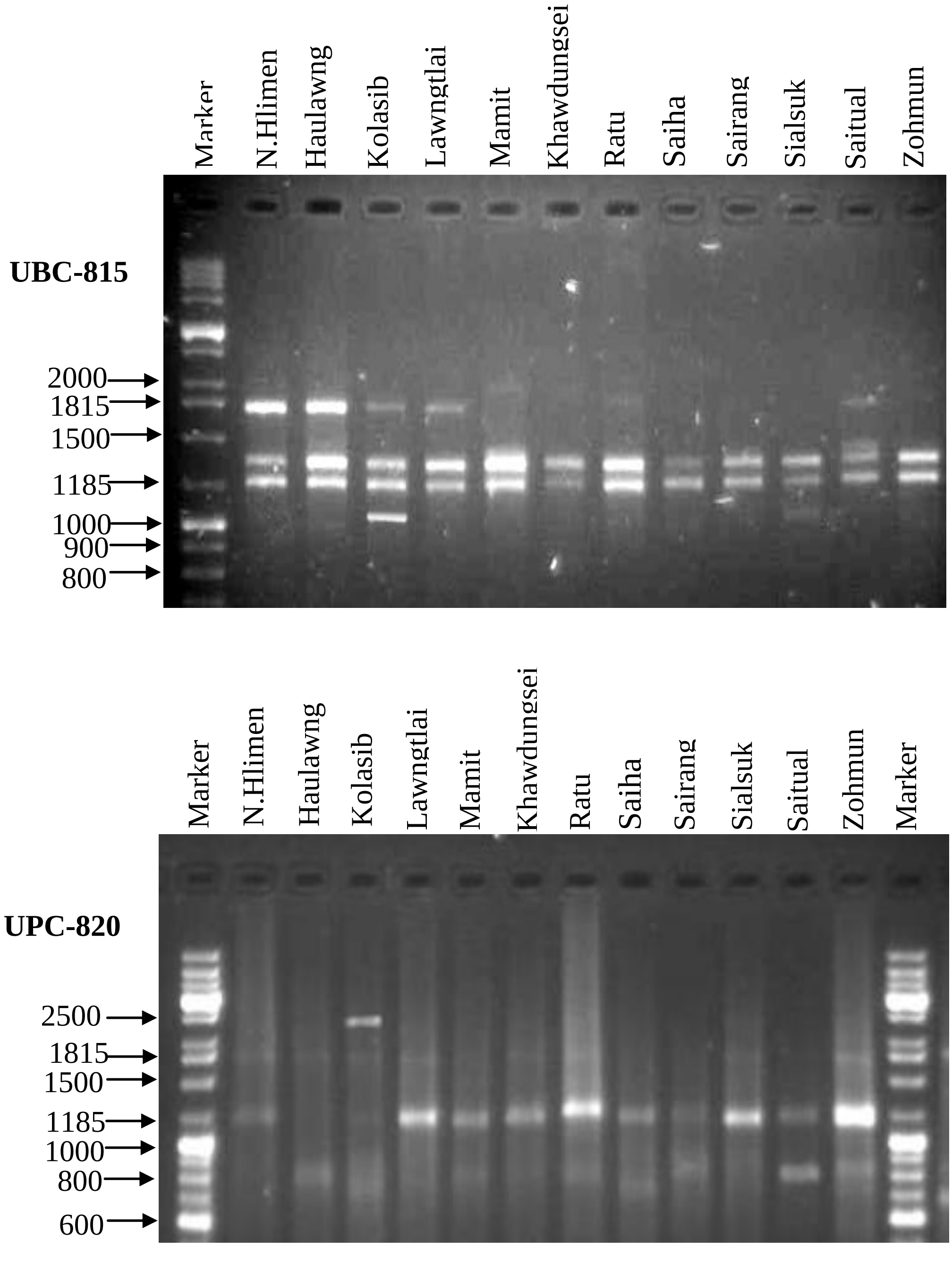


Figure 56. ISSR amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA obtained with primers UBC-815 and UBC-820 on 1.2% agarose gel.

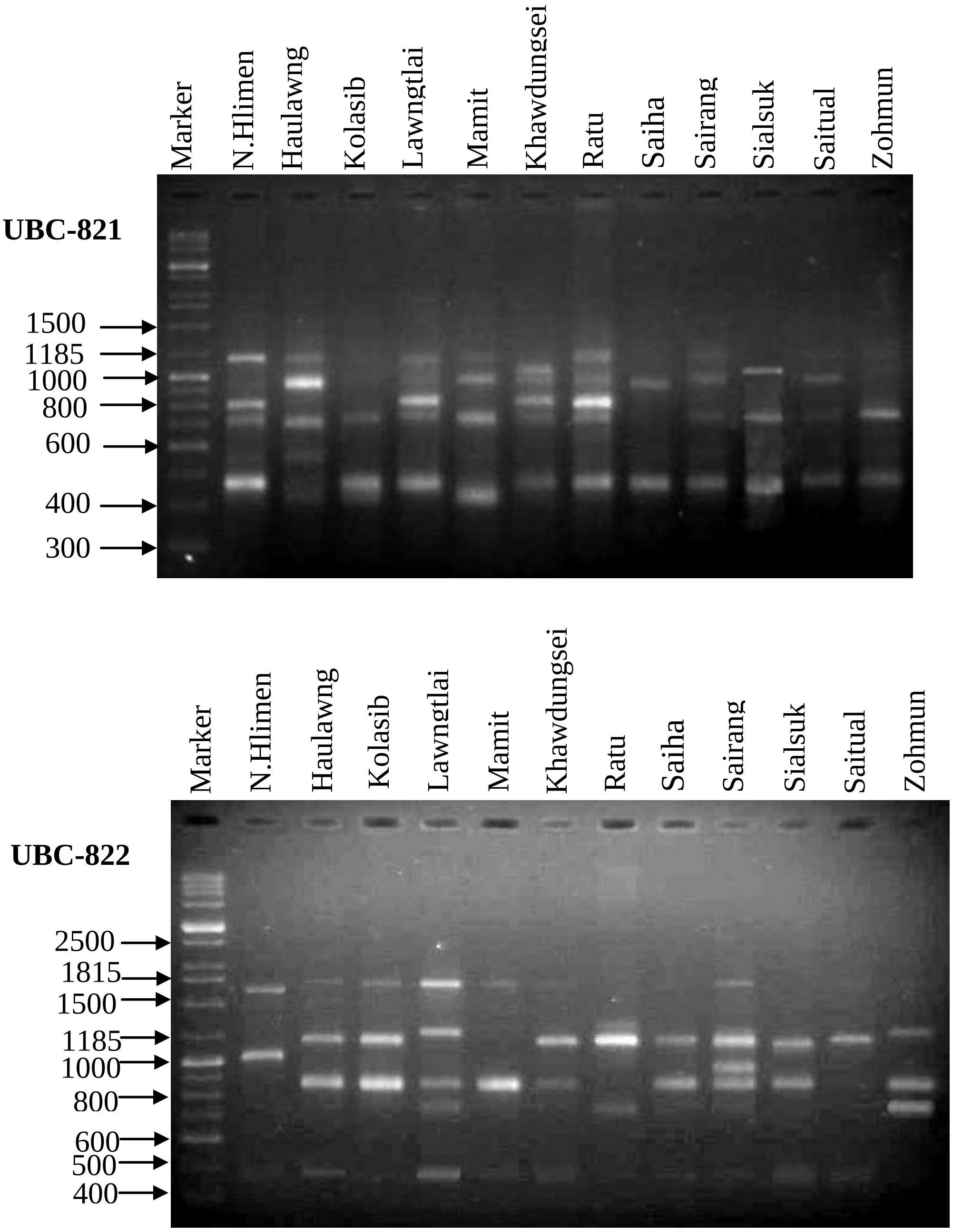


Figure 57. ISSR amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA obtained with primers UBC-821 and UBC-822 on 1.2% agarose gel

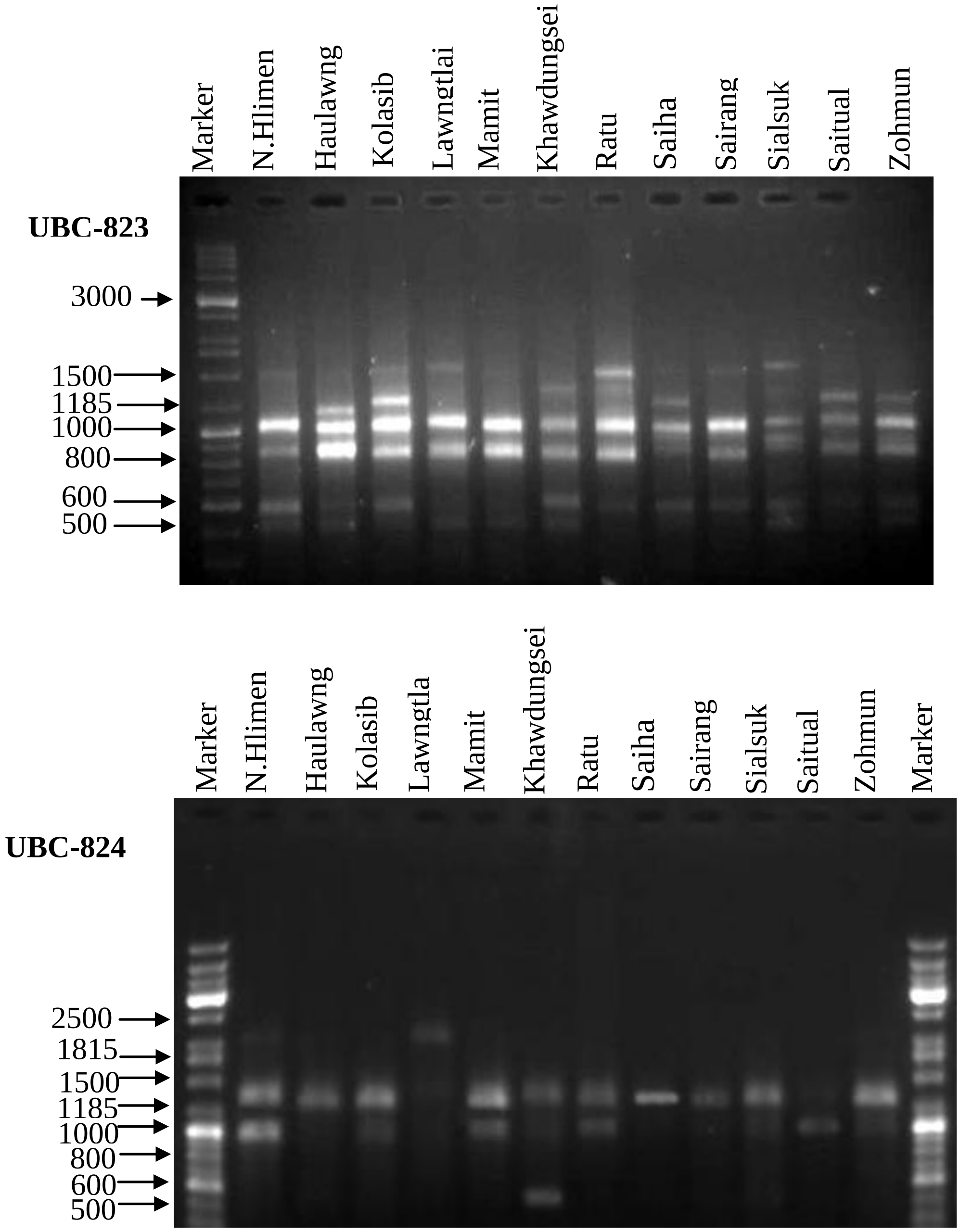


Figure 58. ISSR amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA obtained with primers UBC-823 and UBC-824 on 1.2% agarose gel

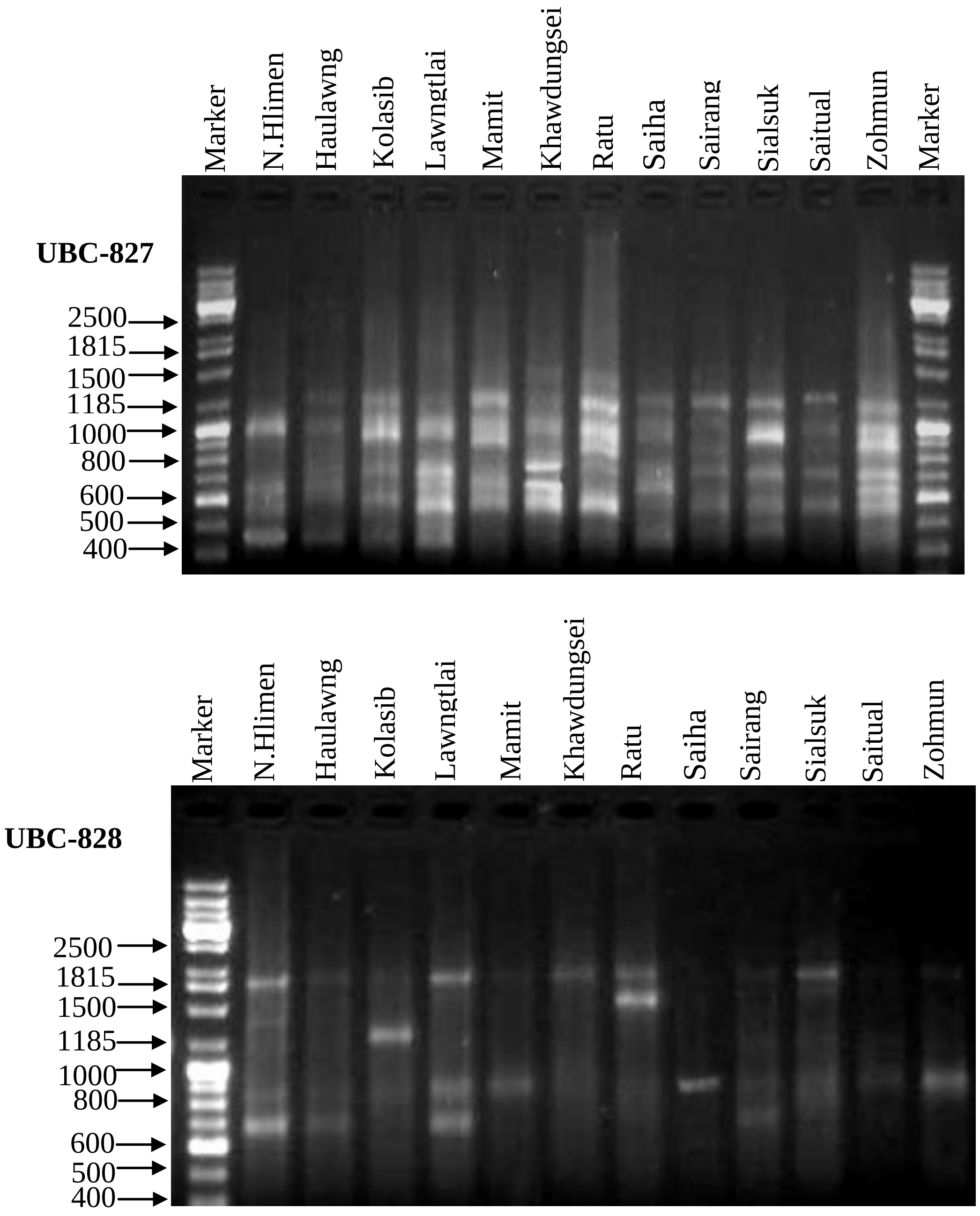


Figure 59. ISSR amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA obtained with primers UBC-827 and UBC-828 on 1.2% agarose gel

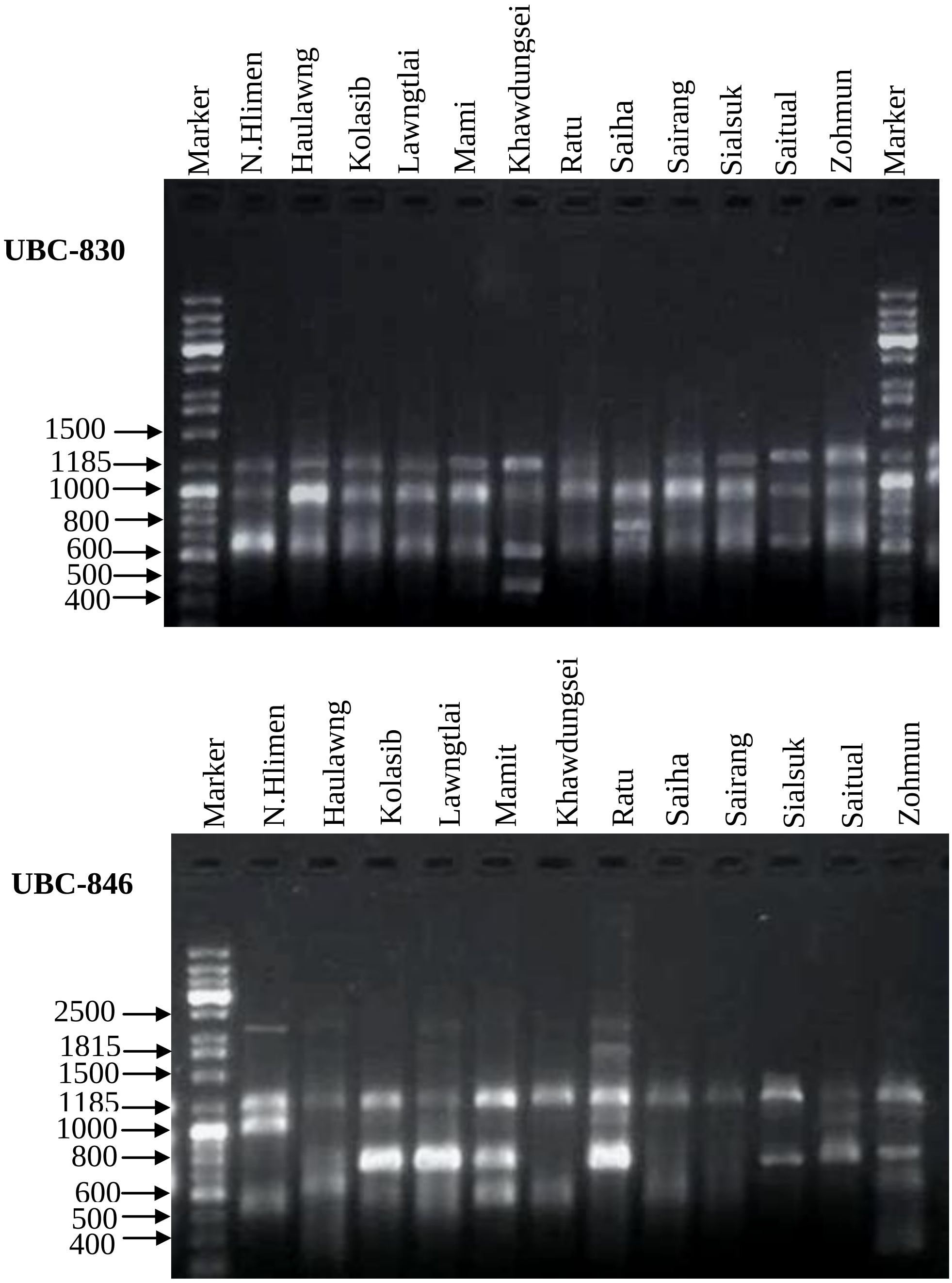


Figure 60. ISSR amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA obtained with primers UBC-830 and UBC-846 on 1.2% agarose gel.

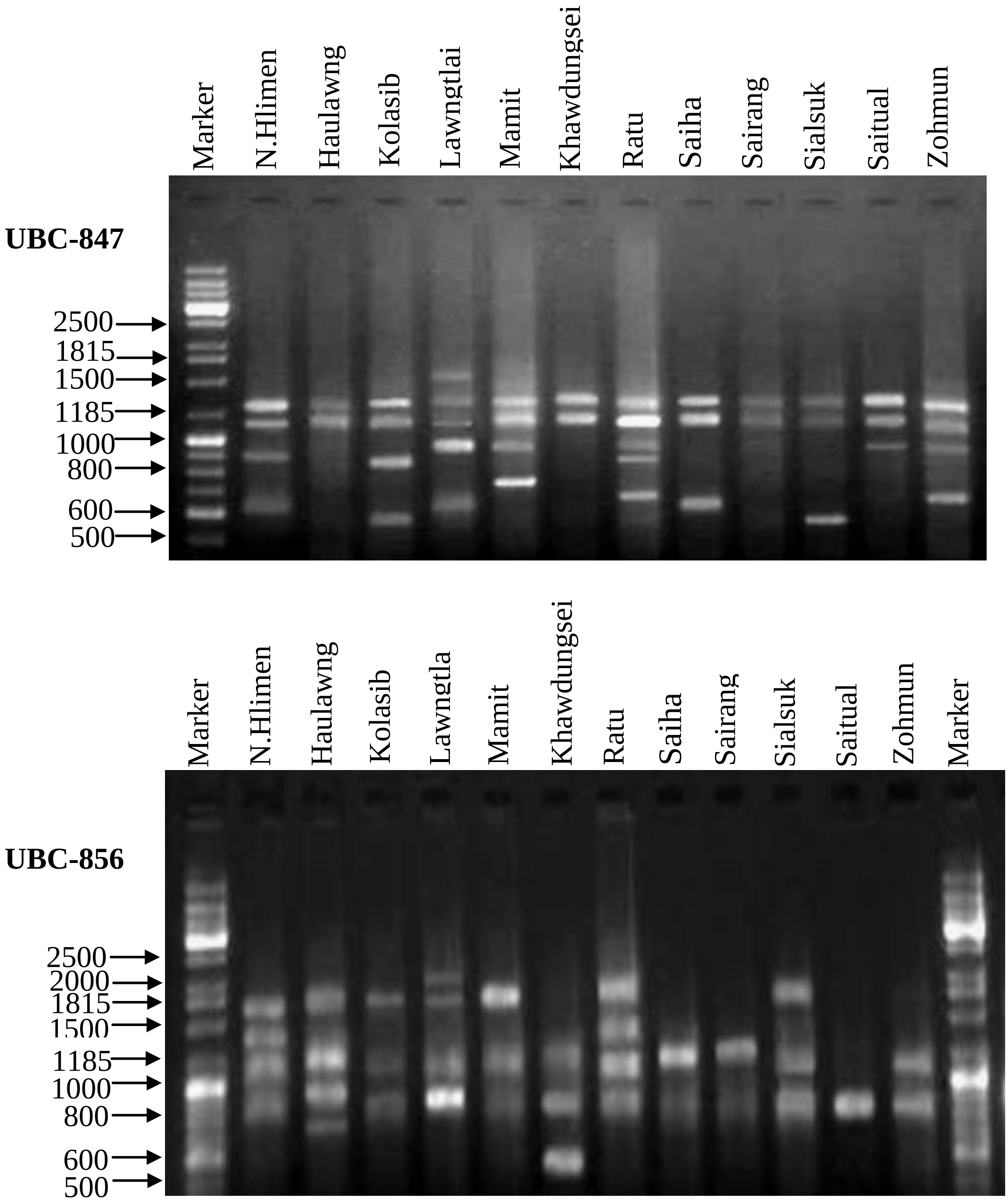


Figure 61. ISSR amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA obtained with primers UBC-847 and UBC-856 on 1.2% agarose gel

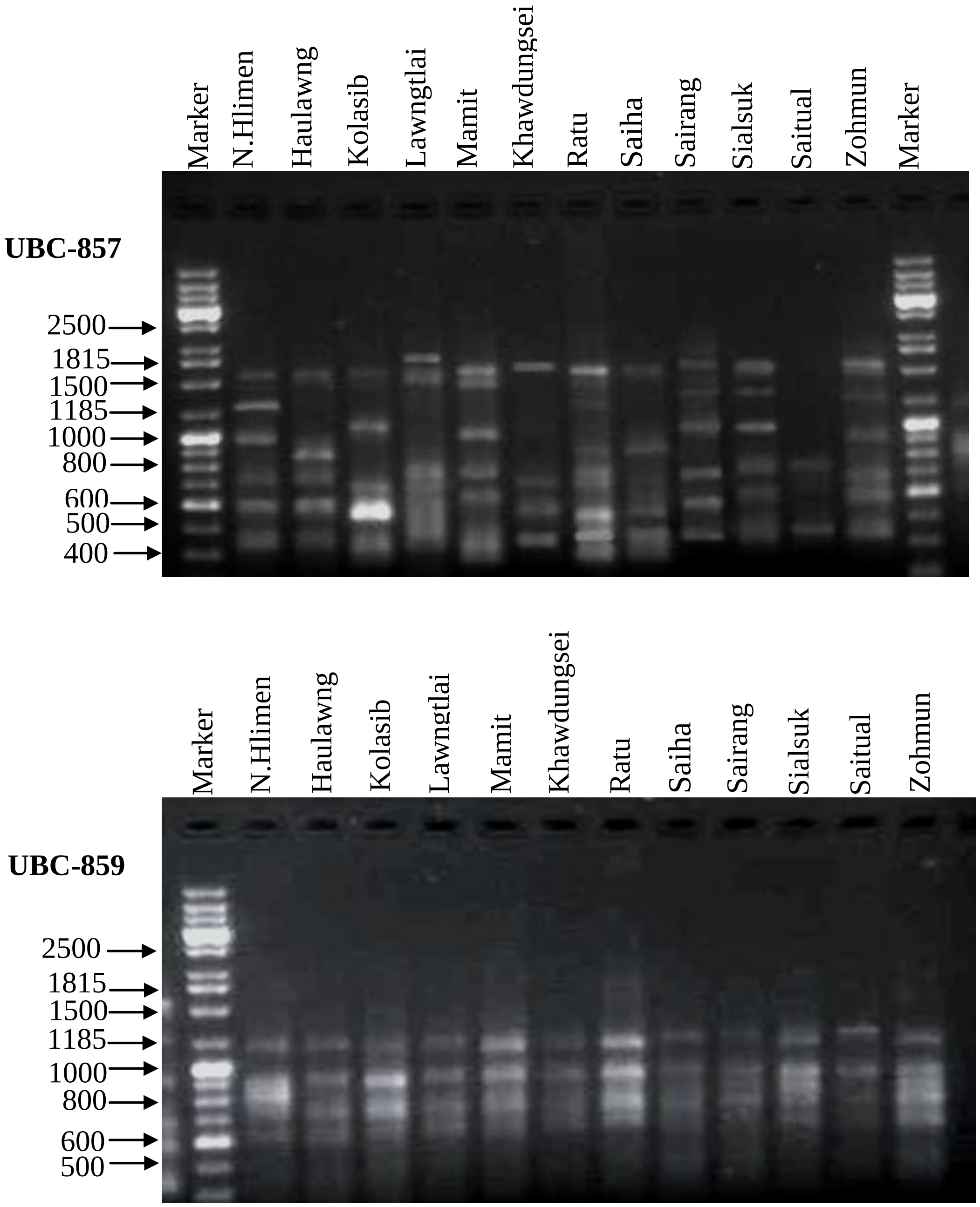


Figure 62. ISSR amplification products generated from 12 different accessions of *Melocanna baccifera* genomic DNA obtained with primers UBC-857 and UBC-859 on 1.2% agarose gel

6.4.2. Genetics similarities base on RAPD and ISSR markers

The genetic similarities values based on RAPD and ISSR markers are: The maximum genetic similarity matrix value is 0.739 between Kolasib and Sialsuk, while the lowest genetic similarity value of 0.514 is between Saitual and Zohmun (Table 13). RAPD and ISSR markers are proved to be an efficient and inexpensive way to provide molecular data. They have been used successfully in determining genetic relationship and used for DNA fingerprinting (Moreno et al., 1998; Blair et al., 1999; Divaret et al., 1999; Gilbert et al., 1999). The polymorphism of the *M. baccifera* germplasm was high. Based on RAPD and ISSR markers data, the percentage of the polymorphic fragments were 98.02% and 84.1% respectively. The reason why the level of polymorphism in *M. baccifera* was high might be that the *M. baccifera* accessions live in the different regions for a rather long time. In the process of the natural selection, the different populations with different genotypes adapted to the different environment and were preserved and the accessions of *M. baccifera* mainly depend on their underground rhizomes as the propagating organs, since the flowering cycle is 48-50 years. Thus the genetic diversity of *M. baccifera* was well conserved; this might lead to the plentiful genetic diversity of *M. baccifera* in this region.

The mean level of genetic similarity within the population of *M. baccifera* by using RAPD and ISSR markers is 0.619. This pattern of genetic variation may be caused and maintained by effective gene flow within populations and high fecundity. From this, it is evident that the accessions from different geographical locations exhibited a wide range of genetic distance, which did not show any correlation with geographical distances between the collection sites, negating to ‘spatial autocorrelation’ concept, if the population is more or less continuous allele frequencies will fluctuate with distance due to genetic drift and selection (Sokal et al., 1989). Wright (1965) predicted that every finite population will experience genetic drift. But that the effects of such drift become more pronounced as population size decreases. Population with continually small effective population size will be especially susceptible to the loss and the re-organization of variation by genetic drift (Barrett and Kohn, 1991; Frankel and Soule, 1981).

Table 13. Similarity matrix of *M. baccifera* accessions based on RAPD and ISSR markers generated from Dice estimation of similarity based on the number of shared fragments.

	Zohmun	Haulawng	Kolasib	Lawngtlai	Mamit	Khawdungsei	N. Hlimen	Saiha	Sairang	Sialsuk	Saitual	Ratu
Zohmun	1											
Haulawng	0.643	1										
Kolasib	0.647	0.674	1									
Lawngtlai	0.629	0.631	0.647	1								
Mamit	0.563	0.621	0.638	0.654	1							
Khawdungsei	0.558	0.629	0.658	0.556	0.641	1						
N. Hlimen	0.597	0.619	0.623	0.596	0.586	0.593	1					
Saiha	0.534	0.649	0.653	0.569	0.582	0.644	0.549	1				
Sairang	0.621	0.727	0.692	0.633	0.727	0.631	0.597	0.737	1			
Sialsuk	0.607	0.643	0.739	0.629	0.654	0.674	0.607	0.620	0.708	1		
Saitual	0.514	0.585	0.578	0.548	0.598	0.632	0.542	0.597	0.639	0.658	1	
Ratu	0.565	0.618	0.633	0.585	0.618	0.581	0.587	0.571	0.608	0.627	0.586	1

6.4.3. Phylogenetic analysis base on RAPD and ISSR

Phylogenetic relationships among 12 accessions of *M. baccifera* were analyzed by an UPGMA method. The cluster result indicated that all the 12 accessions could be distinguished by RAPD and ISSR markers, respectively. The cluster analysis of RAPD and ISSR markers showed variation of a geographic distribution as all the accessions could be separated into two major groups. Majority of accessions i.e. 10 out of 12 accessions formed one cohesive cluster and Zohmun (non-flowering) and N. Hlimen (red fruit) are resolved in another cluster. The bigger group was again sub-grouped in two smaller clusters containing Haulong, Saiha, Sairang, Kolasib, Sialsuk and NE. Khawdungsei in cluster I. Lawngtlai, Mamit, Ratu and Saitual in cluster II. It can be observed from the dendrogram that the variation between accessions Kolasib and Sialsuk is the least in cluster I with similarity coefficient of 0.74. It can be further noted that Zohmun and N. Hlimen was the most diverse within the cluster (Fig. 63).

Cluster analysis based on Dice coefficient by RAPD and ISSR markers given in Fig. 63 show two major groups. The accessions collected from Zohmun and Ratu with a relative distance of 40 kms was found in the separated groups. Most geographically closer localities found in separate groups showed that they are genetically distant. This shows that there is no correlation between genetic make up and geographical distances. The genetic uniformity or non-uniformity is reflected by the genetic distance value as described by Das et al., 1997.

On the other hand, the accessions collected from Kolasib and Sialsuk showed closest affinities among the accessions collected, though they are quite distant apart geographically and linked with Haulong, Saiha, Sairang and NE. Khawdungsei to form a well defined cluster. The possible explanation for the accessions diversity found in *M. baccifera* populations is somatic mutation.These bamboo plants can live for a very long time due to this, somatic mutations may occur in their gene pool which may eventually lead to some variations. Some researchers have considered RAPD markers to represent segments of DNA with non-coding regions and to be selectively neutral (Bachmann, 1997; Landergott et al., 2001). Similar opinions have been reported with regard to ISSR markers (Esselman et al., 1999). Some studies have shown that RAPD markers are found throughout the genome and may be associated with functionally important loci (Penner, 1996).

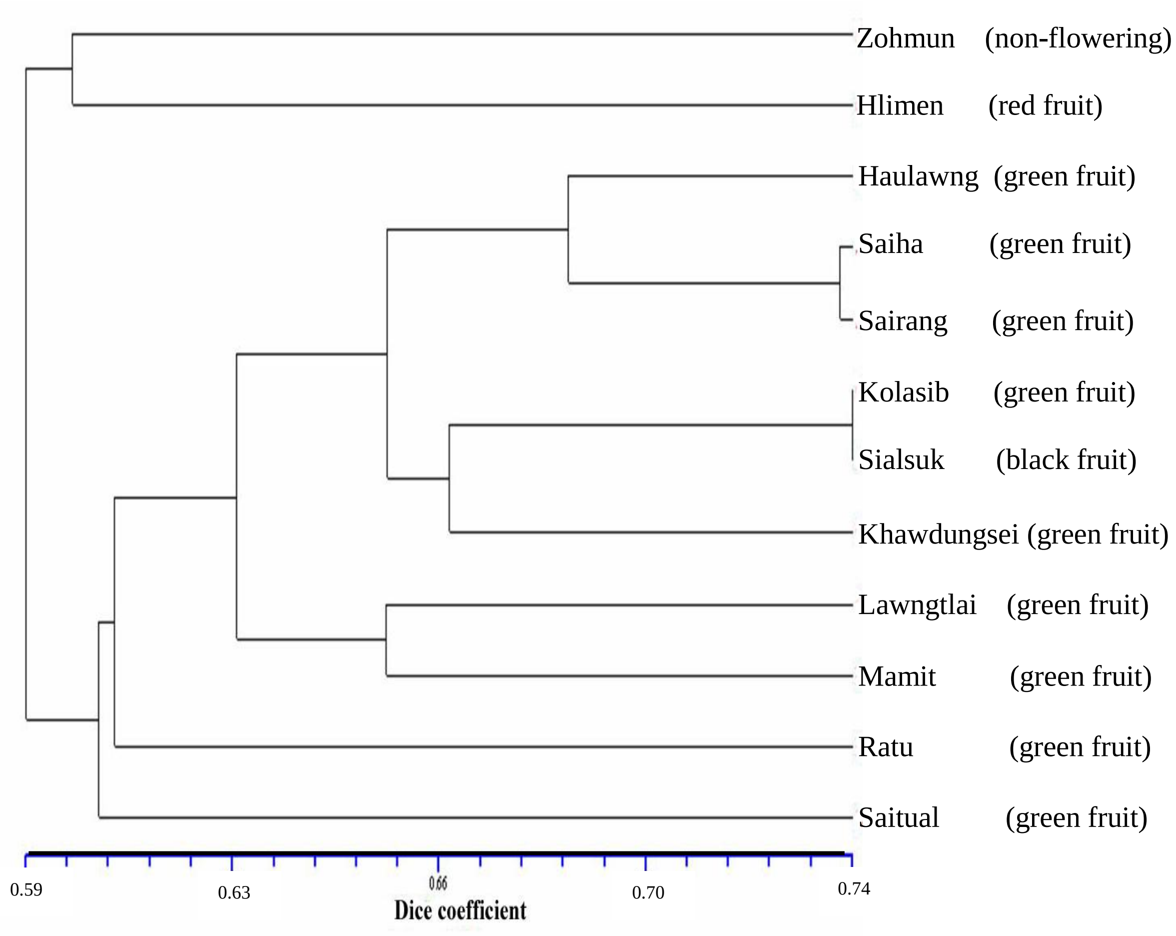


Figure 63. Dendrogram generated using UPGMA method among 12 accessions of *Meloccana baccifera* by RAPD and ISSR markers

6.5. Conclusions

The RAPD and ISSR data indicates that because of high polymorphism generated by each primer, these are sufficiently informative and powerful to assess genetic variability in *Melocanna baccifera*. The presence of unique markers in *Melocanna baccifera* may be due to relatively high rate of mutation in RAPD and ISSR loci and are important to accentuate differences between closely related accessions. These results suggest that RAPD and ISSR markers based analysis may enhance the efficiency of searching for genotypes with unique variants from these germplasm collections. Based on these RAPD and ISSR markers, analysis is more accurate in investigating phylogenetic relationships among populations of single species or closely related species than between less related species. This shows the usefulness of RAPD and ISSR markers in studying variation among accessions, phylogenetic relationships, lineages for preservation and genomic composition of populations. It is likely that RAPD and ISSR target different regions of genome which are subjected to different mechanisms generating genetic variation. Genomic regions sampled by the RAPD and ISSR markers maintain a different evolutionary process under selection. RAPD and ISSR markers were powerful dominant DNA markers; they are effective and promising markers for assessing genetic variation in *M. baccifera* species.

7 Economic importance and traditional uses of *Meloccana baccifera* Roxb. in Mizoram

7.1. Introduction

Bamboo serves multiple functions such as soil and water conservation, and soil erosion control (Fu and Banik 1995). Bamboo generates 30% more oxygen than trees. It helps reduce carbon dioxide gases blamed for global warming. Some bamboo sequesters up to 12 tons of carbon dioxide per hectare, which makes it an efficient replenisher of fresh air. Because of its wide spread root system and large canopy, bamboo greatly reduces rain run off and prevents massive soil erosion. Bamboo helps mitigate water pollution due to its high nitrogen consumption, making it a solution for excess nutrient uptake of waste water from manufacturing, livestock farming and sewage treatment. Bamboo can restore degraded lands. It is a pioneering plant and can be grown in soil damaged by overgrazing and poor agriculture. Proper harvesting does not kill the bamboo plant, so topsoil is held in place. Because of its dense litter on the forest floor it feeds (www.bambooliving.com). Some of the research involves bamboo based agro-forestry systems highlighting the capacity of bamboo in increasing the soil moisture, nutrient and reducing water run off and soil erosion (Ramakrishnan and Toky,1981). Bamboo is harvested almost every day by the people for marketing and household consumption. Being a versatile and renewable resource, bamboo has been over-exploited so much that concerns are being expressed over erosion of this gene pool (Renuka, 1996).

Mizoram is situated at the North Eastern part of India and it is the land of a number of craftsmen and artisans in various crafts. Bamboo related products are the major source of income to the state as well as the people. Bamboo’s most important uses include use for timber or as a raw material for timber or a raw material for paper, pulp, housing and material for handicrafts, besides some minor uses such as leaves for medical purpose (Zhang 1997). So bamboo plays a very important role in the economy of Mizoram. Out of these bamboos, *Meloccana baccifera* is the most important bamboo species. The Mizo’s dexterity in bamboo works is well known. Bamboo has its multifarious uses in turning out various commercial crafts and items of furniture (http://ignca.nic.in/craft155.htm). Bamboo possesses excellent strength properties, especially tensile strength (Sekhar and Gulati 1973). The branch, leaf, culm, rhizome and seeds of *M. baccifera* is used for many items such as Winnowing tray, Rice carrying baskets, Vegetable containers, Carrying and Storing baskets, Stool, Food container for long term preservation and use, Musical instrument etc, (Bhatia et al., 2003). This resource has also been considered valuable for agro-forestry owing to its short gestation period and recurring return (Bhatt et al., 2001). So far as the bamboo in the Mizoram hills are concerned, it is available in large quantities but due to lack of transport accessibility, it has not been utilized to the maximum. However, it seems that the constituted authorities have envisaged the feasibility of introducing in Mizoram better vocational trades in spinning and weaving, hats, baskets, bamboo chairs, tables, teapots, racks, safes, etc as well as bamboo screen cages and umbrella handles.

In this paper, the importance of *M. baccifera* in terms of traditional, economical and ecological services in Mizoram was reported.

7.2. Study area

Field survey was conducted twice in different places of Mizoram (India). The state lies between 21^{o}58’ and 24^{o}35‘ N latitudes and 92^{o}15’ and 93^{o}29’ E longitudes. The state is only 21,087 Sq Kms in area and the ways of people using this bamboo is almost same in different places. Palsang, Zohmun, Ratu, Aizawl, Lunglei and Zonun mat-ply industry were visited for these studies (Fig. 64).

7.3. Methodology

Field survey was carried out at Mizoram State Museum, Department of Art and Culture, Govt. of Mizoram, Department of Forest and Environment, Govt. of Mizoram, different villages in Mizoram, Zonun mat-ply industry, Aizawl and the handicraft center of Hnam Chhantu Pawl and Mizoram Handloom & Handicraft (Aizawl) in order to collect information data’s concerning traditional uses and economic importance of *M. baccifera*.

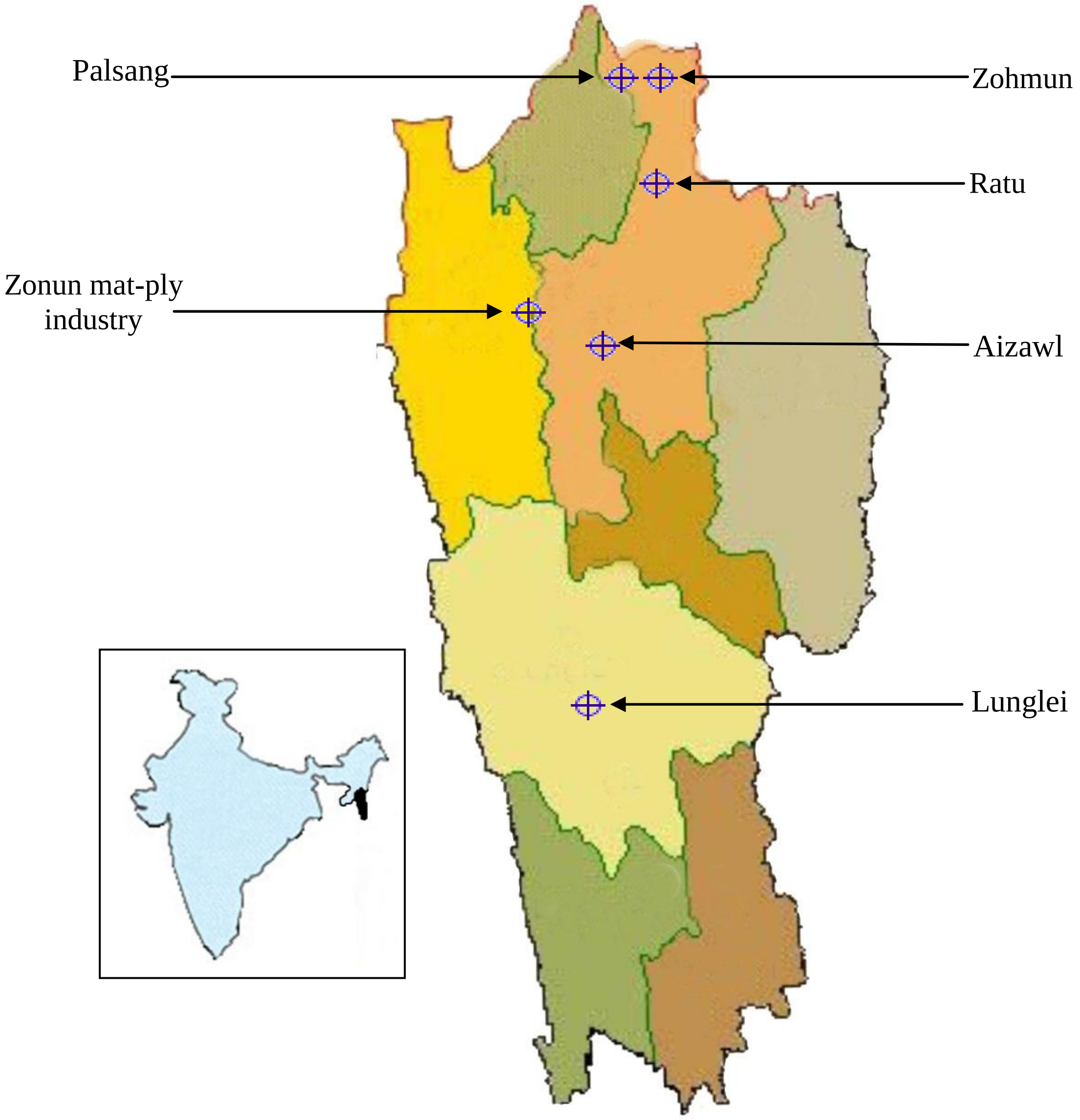


Figure 64. Hydrographic map of the Mizoram**,** showing the survey sites for Traditional uses, Economic importance and Ecological services

7.4. Traditional uses

Some of the traditional items of Mizo people which were made from this bamboo are:

7.4.1. Housing

The short supply of timber and other conventional construction materials accompanied by rising costs make it imperative to increasingly use bamboo for housing (Mathur 1981; Janssen 1987, 1988, 1990; Mishra 1990). Because of its easy availability, workability and low cost, bamboo is employed for columns, purlins, rafters, trusses, as well as walling and roofing (Satter 1995). The importance of bamboo as a construction material, particularly for housing has received renewed attention in recent years. The houses built by the Lushai tribe of Mizoram, predominantly uses different bamboo species and wood in their construction of which *M. baccifera* contributed largest material for this house construction. In the small and medium-sized towns corrugated metal sheets for the roofs was also used, though in the villages, grass thatched roofs were predominately used. Cane is generally used for keeping the joints together and in some cases, iron nails are also used. When the floor of the house is at a much higher level from the ground, a ladder made of wooden log was placed at the intervening space between the floor of the house and the ground. The doors and windows are usually made up of bamboo, but in some cases wooden planks were also used. Houses based on bamboo are shown in Fig. 65.

7.4.2. Baskets

Basketry among the Mizos is a delicate work. They are experts in making different shapes and sizes, in several models like oval, square, flat structures etc. baskets are very useful for carrying grains from field, firewood from the forest, water in bamboo tubes from the rivers etc. The different baskets of the Mizos are presented in Fig. 66(a – e) and these baskets are:

a) Empai/Paiem (woven basket):- The Paiem is a closed-weave carrying basket used by the people of Mizoram. Especially used for carrying food grain and other field products. The Mizo women also use this basket for marketing. The rope-like structure is called ‘hnam’ for carrying Empai.

Figure 65. Mizo typical bamboo house made from *Meloccana baccifera* and other bamboo species

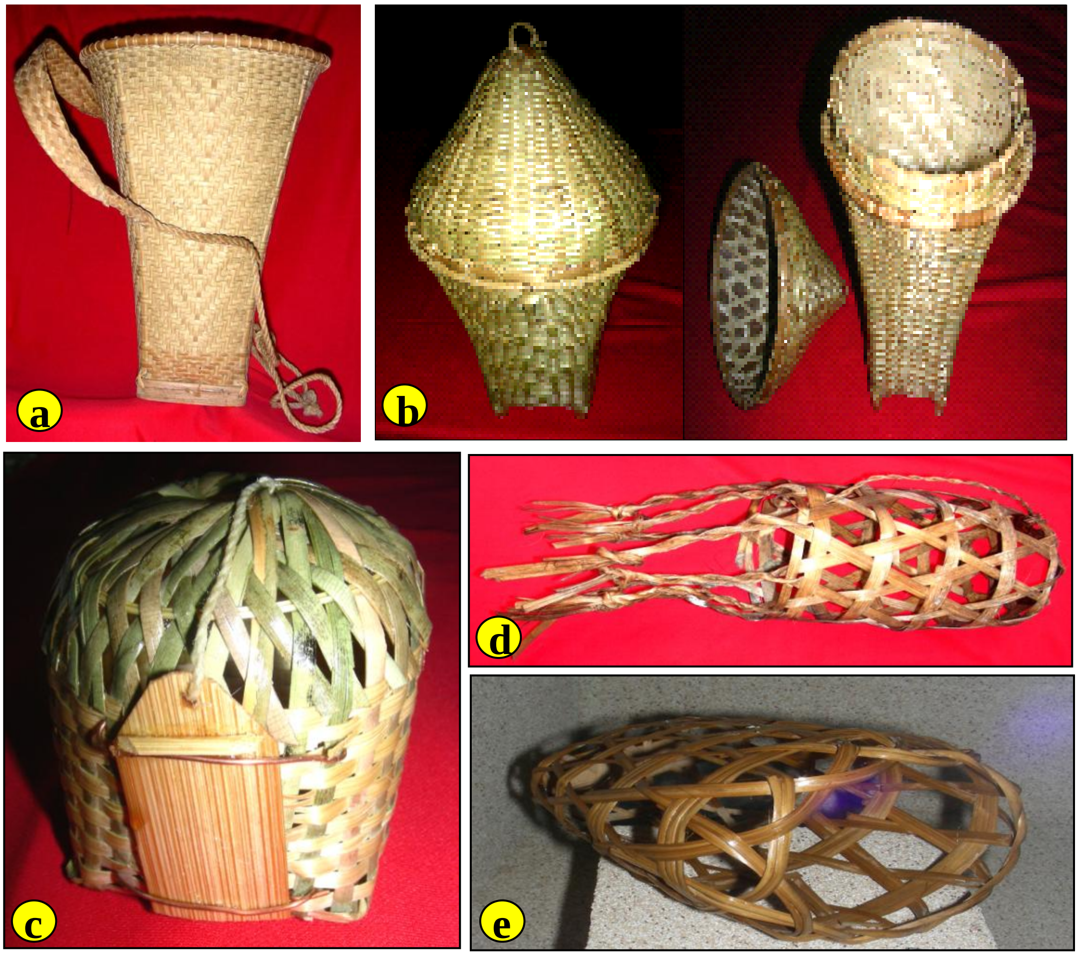


Figure 66. Different Mizo baskets. a) Empai; b) Thul (Storage basket); c) Arbawm (Chicken Basket); d) Ar-awt (Chicken transport basket); e) Tekte

b) Thul: - The thul is a close-weaved storage basket. It is mainly used for storing cloths and garments. It is like a traditional briefcase, has a double–walled structure and the legs are located at the corners of the square base. It has a lid shaped either like a dome shape or a conical shape.

c) Arbawm:- Arbawm is an open-weave basket. It is used as a hen-house at night.

d) Ar-awt:- Ar-awt is used for transporting chicken from one place to another.

e) Tekte:- It is a basket used for keeping yams.

7.4.3. Musical Instruments

The Mizos are sentimental people. They are very fond of music and songs. The uses and functions of musical instruments provide an inexhaustible line of enquiry**.** Some of the musical instruments are shown in Fig. 67(a-e) and these musical instruments are given below:

a) Perhkhuang: - The Perhkhuang is a typical Mizo musical instrument. It can be made from different species of bamboo.

b) Rawchhem: - The rawchhem is another typical Mizo musical instrument. It is also called Mizo bagpipes.

c) Phenglawng: - The Phenglawng is also Mizo traditional musical instrument. It is a Mizo flute.

d) Mizo Tingtang: - The Mizo Tingtang is a typical Mizo guitar, made up of bamboo and some animal leather.

e) Mau Tawtawrawt: - The Mau Tawtawrawt is a typical Mizo trumpet, mainly made from *Dendrocalamus* species and *M. baccifera*.

7.4.4. Snare/Traps:

Snare/Traps are mainly used for killing small animals and birds. They are shown in Fig. 68(a-h) and given as follow:

a) Hnawhtawt: - The Hnawhtawt is a trap for killing rats.

b) Kawlper: - The Kawlper is a trap for small animals.

c) Thangchep: - The Thangchep is a trap for rodent family.

Figure 67. Different Mizo traditional music instruments. a) Perhkhuang; b) Rawchhem (Mizo begpipe); c) Phenglawng (flute); d) Mizo tingtang (Mizo guiter); e) Tawtawrawt (trumpet).

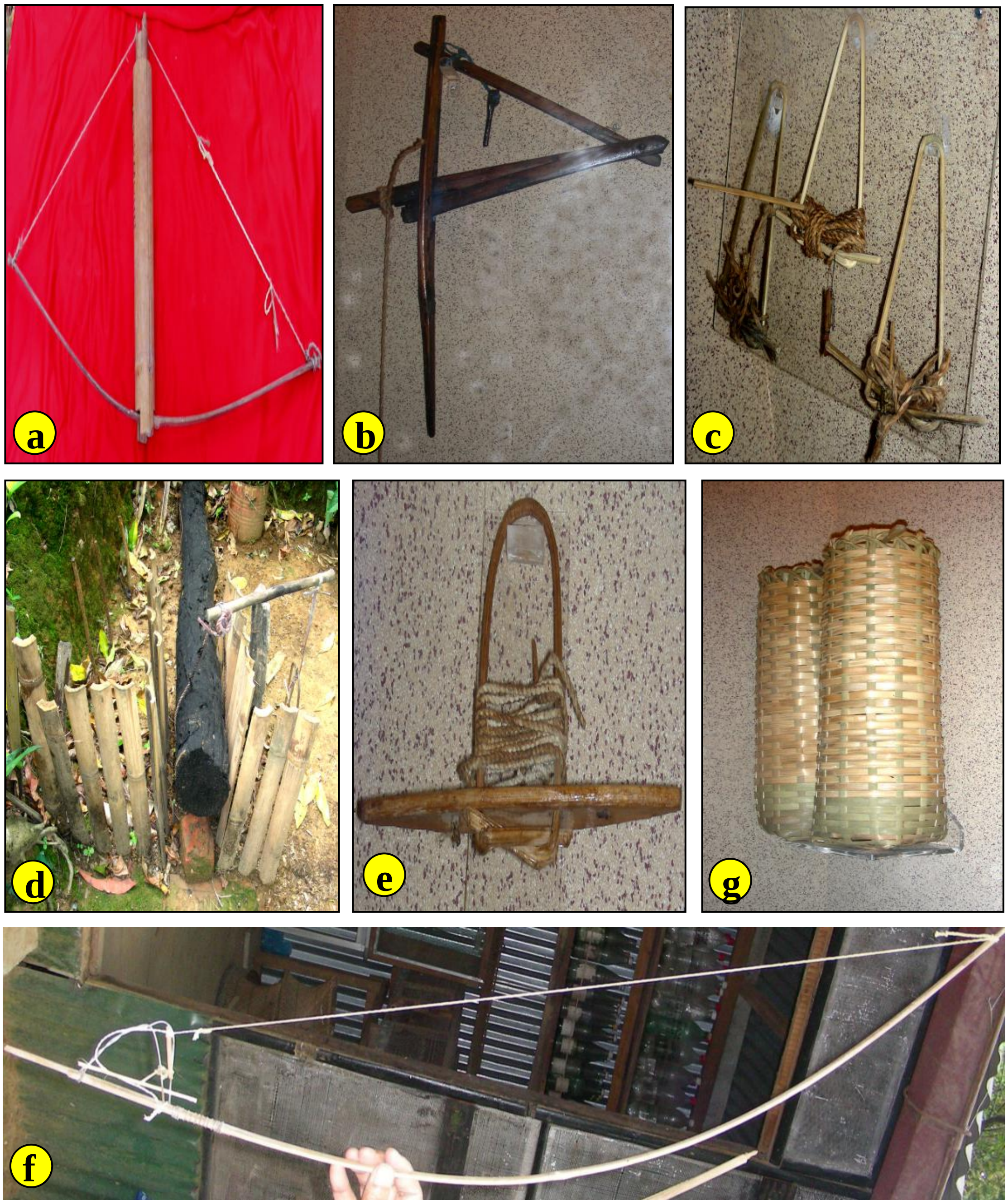


Figure 68. Different Mizo traditional snares/traps. a) Hnawhtawt (Rat trap); b) Kawlper; c) Thangchep; d) Mangkhawng; e) Thangthleng; f) Vaithang; g) Ai-awt (Crap trap).

d) Mangkhawng: - The Mangkhawng is a trap for birds and small animals. It is made from a big log and bamboo.

e) Thangthleng: - The thangthleng is a trap for birds. It is usually placed on a tree.

f) Vaithang: - The Vaithang is a trap for rats.

g) Aiawt: - The Aiawt is a trap for crabs. It is placed in the rivers with bait within the Aiawt.

7.4.5. Agricultural implements

For Agricultural implements, *M. baccifera* was extensively used for making the handles. The bamboo rhizome, culm , combination of culm and rhizome are mostly used as handles for a small Hoe, an Axe, a Spud, Adze, Sickle, Bill hook, Dao (Fig. 69. a-d).

7.4.6. Household items:

Mizo traditional house-hold items are shown in Fig. 70(a-l). These traditional items are:

a) Kho: - It is a flattened shallow container. It is used for keeping vegetables.

b) Mau Fian (Bamboo Spoon):- It is a spoon made usually from *M. baccifera* and *Dendrocalamus* species. It has many uses such as for taking out curry, taking out of liquid or as a scoop for eating.

c) Chingalthlawrbur (Leaching Funnel):- It is a big funnel used for leaching of ash. The watery part which comes out from the leaching funnel is called ‘chingal’ and is used as ‘Soda’.

d) Zu sawrna (bamboo beer funnel):- It is a funnel-like shaped structure. It is used for separation of rice from rice beer.

e) Vaihrik (Sieve):- Vaihrik is a flattened bamboo tray with many pores. It is used as a sieve for separation of small particles from large ones.

f) Thlangra (Winnowing tray):- Thlangra is a flattened bamboo winnowing tray. It is used for sifting and winnowing rice grains after dehusking.

g) Mau Haileng No (Rice beer cup):- It is a bamboo cup and is used for drinking of rice beer and other liquid matters.

Figure 69. Used of *Meloccana beccifera* as handle in Agriculture tools. a) Differents agriculture tools; b) Used of *Meloccana baccifera* rhizome; c) Combination of rhizome and culm; d) Used of culm part only

Figure 70. Traditional household items. a) Kho; b) Mau fian (Bamboo spoon); (C) Chingalthlawr bur (Leaching funnel); d) Zu sawrna (Rice beer funnel); e) Vaihrik (Sieve); f) Thlangra (Winnowing tray); g) Mau haileng no (Rice beer cup); h) Sawhbur (Mortal and Pestle); i) Mau chaicheh (Bamboo tong); j) Bamboo fire-wood; k) Leihlawn (Ladder); l) Tuium.

h) Sawhbur (Mortar & Pestle): - It can be any species of bamboo. Mortar is bamboo and pestle is usually wood. It is used for grinding vegetables and fruits

i) Raw chaicheh (Bamboo tong):- Raw chaicheh is a cross shape of two bamboo sticks. It is used for holding hot materials.

j) Fire wood: - Bamboo can also be used as firewood. It is extensively used as firewood especially in villages and rural areas.

k) Leihlawn (Ladder):- Bamboo is also used for making ladder. *Dendrocalamus* species and *Melocanna baccifera* is the most commonly used.

l) Tuium (Bamboo water tube):- Tuium can be made from any species of bamboos. It is a long tube of bamboo. This is used for transporting and storing of water.

7.4.7. Rain Shield

Siksil is a traditional umbrella worn during rain by Mizo people. It also has two layers. Palm leaves were also inserted between these two layers. This is also made from strips of bamboo woven in an open-hexagonal weave (Fig. 71).

7.4.8. Water Pipes

M. baccifera is used as a pipe for rain water collection from the roof top, for collecting water from the running river and for trip irrigation (Fig. 72, a-d).

7.5. Economic importance:

Bamboos are multipurpose plants, with over a thousand economic applications (Ueda 1981). Bamboo represents a vast untapped major resource of Mizoram State whose full ecological and economic potentials remain underutilized. Out of these bamboos, *M. baccifera* plays the most economical importance for the people; it needs to be recognized, developed and promoted in a manner ensuring ecological security for all round sustainable development of the State and well- being of its people. Bamboo is an essential component of forest eco- system, which is a dominant feature of state's landscape. *M. baccifera* and its related bamboo industries already provide income, food and housing to many people. *M. baccifera* is a versatile multipurpose forest produce that has immense potential in the industries and domestic applications.

Figure 71. Traditional rain sheath. Siksil (umbrella)

Figure 72. Bamboo pipes. a) Pipe for rain water collection from the roof top; b) Collection of water to their door step; c) Collection of water from the running river; d) Collection of water to the reservoir from the river source

Growth of merchantable culms from seeds of *M. baccifera* usually takes 10-12 years in natural condition, but it may attain merchantable size in 4-5 years if properly tended. Inspite of the economic importance of the bamboo there has not been an attempt in promoting bamboo-based industries that has a potential to transform the economy of the people of the Mizoram region. *M. baccifera* being a multipurpose, eco-friendly crop abundantly available, yet an underutilized natural resource, needs to be managed and exploited for sustainable use. *M. baccifera* and other Bamboos were conceived as a thrust area in the Industrial Development of Mizoram for the economic and ecological security of the people. This precious resource needs to be fully tapped as an Industrial raw material, as substitute for wood in rural/urban housing, engineering works, handicrafts, furniture and value addition through export. Undoubtedly *M. baccifera* along with other Bamboos can revolutionize the economy of the State ensuring employment opportunities to a large number of people. At present, only a small portion of bamboo resources are harvested for the purpose of local construction, tiny handloom and handicraft production.

M. baccifera young shoot can be used as food after cooking. However, young shoot is seasonal and so preservation is necessary for storage. There are many methods of preservation. The most common practice in Mizoram is sun drying and drying on fire. *M. baccifera* shoot also has a huge market potential. Young shoot gives opportunities for lively-hood to a large number of people. These young shoots are sold as fresh shoot form and in cans. The shoots of most of the bamboo species in Mizoram are edible and are consumed locally. *M. baccifera* are the most consumed species by the people followed by *Dendrocalamus hamiltonii*, *Dendrocalamus longispathus* and *Bambusa tulda*. On average, *M. baccifera* contributed 53.69% to the total annual bamboo shoot consumption (Bhatt *et al*., 2003). *M. baccifera,* at present, is availed in an unregulated manner by villagers to meet their domestic need free or on payment of royalty.

M. baccifera and other bamboos from Government of Mizoram state Notified Forests are sold under Mahal (mostly bamboo grown at the river side) and Permit System. With a view to manage the bamboo resources in a sustainable manner and to ensure a support base to increasing bamboo yield to meet the local need and export of bamboo and improved bamboo produce, annual bamboo harvest and yield need to be predetermined through silvicultural availability, felling regulation, seasonal requirement, market need and in consultation with all concerned and regulated so that bamboo resources are optimally harvested and used. These bamboos, sold by Mahal systems go to the Paper Mill located at Panchgram, Hailakandi, Assam. A smaller quantity of bamboo sold by Permit system was utilized in the local industries.

As the forest reserve is depleting globally, timber are getting scarce day by day. This is due to long period of time taken by even soft- wood to attain maturity. Bamboo can attain their maturity within a short period of 2-3 years only. So, a substitute, or if that is not possible, an alternative has to be found. Bamboo is the answer for this. *M. baccifera* is extensivelyused for the production of bamboo ply boards**.** Bamboos are sliced into thin strips and these strips are woven together to produce bamboo mats. Bamboo mats and slivers are hot pressed to produce bamboo ply board. Bamboo ply board is very strong and as it can be made water resistant, it can be used for almost all purposes where timbers are now used. It has applications for construction boards etc. Bamboo mat boards and bamboo ply boards can be promoted as wood substitute for the growing construction needs within and outside the State. This will not only result in a value addition to bamboo products but will also be a wood substitute reducing use of timber within the state. Another economical uses of *M. baccifera* was for the production of bamboo charcoal. Bamboo charcoal application includes environment protection, food industry, pharmaceutical industry etc. Output efficiency is 20 % i.e. 100 kg of raw bamboo can produce 20 kgs of bamboo charcoal. During the production of bamboo charcoal in the charcoal klin, the steam and watery portion coming out from bamboo is condensed. This bamboo watery portion is used for organic fertilizer, preservative medium and for relief from pains etc.

M. baccifera has found its place in many items of daily uses. Due to its high tensile strength, it is ideal for various items of daily uses like tea coaster, hanger, flower vases, trays, containers, baskets, mats, frames, furniture, ornaments etc. are made from this bamboo. Due to its high texture, properties, high tensile strength, easy splitting possibility, and other characteristics, it is a good choice of material for handicrafts. There are numerous designs and varieties of handicrafts made out of bamboo. Economic importances of *M. baccifera* are shown in Fig. 73, (a-f) and Fig. 74, (a-l).

M. baccifera bamboo species is an essential component of forest eco- system in Mizoram, which is a dominant feature of the state's landscape. The distribution of bamboo in the North-East region of India is given below.

Bamboo Resource in NE States of India

State	Bamboo growing area (km2)	Bamboo growing stock (mil. tons)
Mizoram	6446	25.26
Assam	8213	13.41
Arunachal	4590	9.84
Manipur	3692	11.47
Meghalaya	3102	4.41
Tripura	939	0.86
Nagaland	758	3.66
Total	30504	54.53

** Data obtained from Dept. of Forest and Environment, Govt. of Mizoram.*

Out of 25.26 million tons of bamboo growing stock 95% is contributed by *M. baccifera*. But this bamboo forest was destroyed by slash and burn system of agriculture. This system of agriculture has become unproductive owing to frequent exposure of soil, increased land-slide and soil erosion, and loss of vegetation, and bio-diversity. This system is practiced by burning standing of these bamboos which in itself is a great loss in term of revenue.

Figure 73. Different economic importance of *M. baccifera*. a) *M. baccifera* Young shoot market; b) Young shoot in a cans form; c) Transported through river and d) Road for paper industries; e) Local *M. baccifera* market; f) Bamboo mat-ply produced mainly from *M. baccifera*

Figure 74. Economic importance in different daily uses and handicrafts produce from *M. baccifera*. a) Watery extract from *M. baccifera* charcoal production; b) Tea coster; c) Hangers; d) Flowers vases; e) Trays; f) Mat; g) Stool; h) Cups; i) Cigarette case; j) Furniture; k) Baskets; l) Ear ring

7.6. Conclusions

Bamboo plays a very important role in the traditional way and economical way of Mizo people. Out of these bamboos, *M. baccifera* is the most important bamboo species. It helps the people in day to day living. Bamboo related products are the major source of income to the state as well as the people. Many people earn their lively-hood from this plant. It also contributes an amount to the state government income. Traditionally, the community of the region uses this resource for making houses, baskets, musical instruments, traps, agricultural implements, household items, rain shield and water pipe. Economically, the community also exploited this abundant resource for food, charcoal production, mat-ply and for paper production.

M. baccifera forests and regrowth areas in critical mountain slopes and around village habitations shall be afforded protection to ensure environmental security (protection of catchments, regulation of water flow, recharge of water table, conservation of flora and fauna etc. and protection of developmental infrastructure like roads, bridges, hydel projects, human settlements, habitations, etc.). Thus *M. baccifera* protected and preserved the mountain ecosystem, it protected rain watersheds, regulated the water flow, recharge the water table and it conserved the flora and fauna etc. and thus it provided environmental security for the people.

8 Summary

Mizoram is one of the northeastern states of India. Mizoram state is a mountainous region with steep gorges covering with different kind of vegetations. Bamboo is the main constituent in the forest vegetation. Different taxa of bamboo (22 genera) are present in the hilly forests of Mizoram. *Melocanna baccifera* is one of the most dominant species. It is a multi-purpose, eco-friendly and non-timber forest product. It has an importance role in traditional and economics of the State and life style of the local community. Flowering of this bamboo species take place in 48 to 50 years cycles accompanied with increased in rat population, the scientific explanation for this phenomenon is still under investigation. The economy of the state is primarily depends on agrarian with majority of the people practicing jhum cultivation (Shifting agriculture or slash and burn agriculture). Jhuming on the mountain is one of the main factors which destroyed the precious bamboo forest. This system of cultivation has become unproductive owing to several ecological problems which ultimately resulted loss of revenue and environmental degradation.

Bamboo charcoal produced from *Melocanna baccifera* is used as domestic fuel by the people. Therefore, detailed laboratory investigations have been carriedout with raw and activated charcoal for removal of lead, cadmium, zinc, nickel and copper through adsorption in aqueous solutions. Several parameters such as effect of pH; effect of adsorbent dosage; effect of metal concentrations; effect of time; isotherms (Langmuir, Freudlich, D-R) and Kinetics (Pseudo-first-order, Pseudo-second-order) have been applied to explain the efficiency of heavy metal removal by these two adsorbents. All the parameters showed that *M. baccifera* raw charcoal and activated charcoal are good adsorbents for the removal of heavy metals from the aqueous solutions which forms the first scientific report.

Tender shoots of *M. baccifera* are dible. The local community considers this as delicious food and nutraceutical. Therefore, phytosterol content was evaluated and characterized. Phytosterols are a group of steroid alcohols naturally occurring in plants. They play a crucial role in maintaining membrane stability and fluidity. They also have an ability to reduce cholesterol level and other pharmaceuticals effect in human. The presence of brassicasterol, stigmasterol, campesterol and β-sitosterols were confirmed using their corresponding standard compounds in tender shoots. Purification of these phytosterols from the extract was carried out using silica gel (60-120 mess) column chromatography. The fractions containing phytosterols were pooled together and further purification was done with HPLC. Presences of different type of phytosterol in the fractions were also confirmed using Liebermann-Burchard test. These investigations confirmed that the tender shoots of *M.baccifera* are a good source of phytosterol.

The consumption of phytosterols, apart from lowering cholesterol levels, also provides protection against certain cancers. The commercialization of medicinal plant resources has become rapid due to the identification, purification and characterization of active bio-molecules for various therapeutic purposes which have been vital for the present scenario. Because, the synthetic drugs exhibit severe side effects along with their mode of action. Hence, “bioprospection” plays an important role in the identification of the novel bio-molecules from the natural resources, especially from plants, having fewer side effects and they are cost effective. Hence, of the objectives of this investigation is aimed at evaluating the anti-cancer potential of *M. baccifera* tender shoot extract. These studies were accomplished by selecting three human cancers cell lines namely liver cancer cells (HepG2), skin cancer cells (A341) and ovarian cancers cells (OAW42) and three pathogenic bacteria namely *Staphylococcus aureus, Pseudomonus aeruginosus, Salmonella typhi* are used for anticancers and antibacterial activities of *M. baccifera* shoot extract. The result showed that a good antiproliferative activity of these cancers cell lines and a good inhibitory effect to these bacteria. Therefore, *Melocanna baccifera* tender shoots possesses anti-cancer and anti-microbial properties.

Different accessions of *M. baccifera* showing variation at morphological level during flowering are collected from different location of Mizoram and genetic similarity assessment among these accessions are carried out using RAPD and ISSR genetic markers. These accessions showed a good similarity except two accessions collected from Zohmun and N. Hlimen.

Traditionally, the community of the region uses *Melocanna baccifera* for shelter, handicraft, medicines, musical instruments, agriculture implements, household items, for making different types of traditional baskets, rain water collection and watershed management. Therefore, bamboo forest in Mizoram needs to be scientifically managed, protected, researched and exploited for sustainable use. Bamboo industry would contribute to industrial and economic development of Mizoram for the economic and ecological security of the people. This valuable resource can be fully utilised as an industrial raw material, for non-timber as wood substitute in rural/urban housing, engineering works, furniture and value addition through export. Undoubtedly Bamboo can change radically the economy of the state ensuring employment opportunities to a large number of the community. Socio-economic services of *M. baccifera* is depicted in Fig. 75.

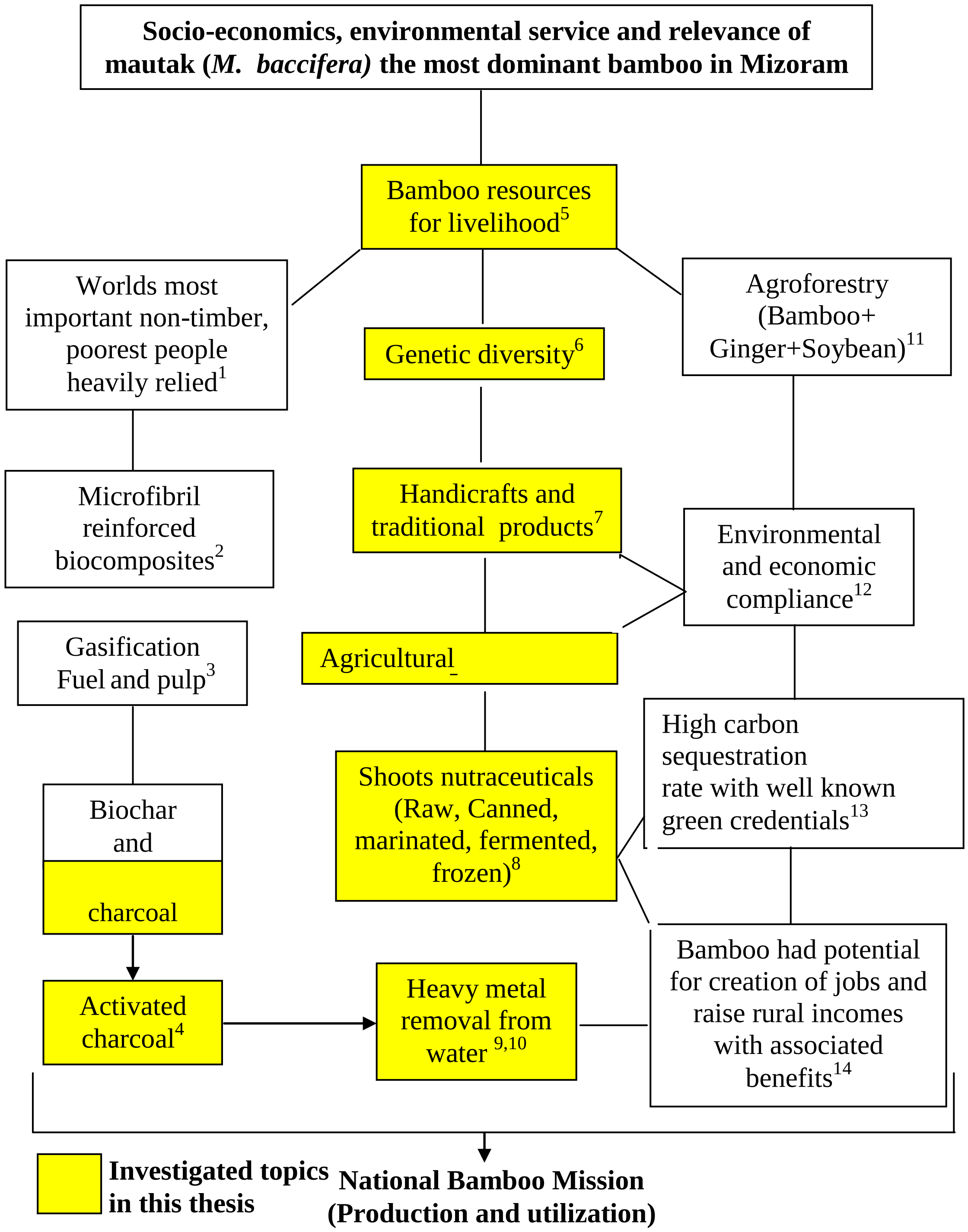


Figure 75. Bamboos for sustainable development and to tackle the MAD Challenge. [Mitigation, Adaptation to and Development] in the face of Climate Change

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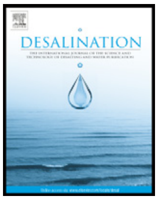

Potential of chemically activated and raw charcoals of *Melocanna baccifera* for removal of Ni(II) and Zn(II) from aqueous solutions

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ABSTRACT

Melocanna baccifera (Bamboo) is the most abundant and economically important non-timber product in the State of Mizoram, India. The community of the region is getting benefited by this bamboo enormously. The notable uses are: tender shoots are edible and delicious preparations are prepared out of bamboo shoots, a potential source of production construction and composite materials for domestic dwellings and an important source of raw material for charcoal production, which is required for a variety of industries. This paper highlights the utility of bamboo charcoal in wastewater treatment. It is known that activated and raw charcoals from different biomass sources have wide range of applications in water treatment. Therefore, activated charcoal was prepared from *M. baccifera* raw charcoal by chemical treatment and used in various experiments to test its functions for removal of heavy metals. To achieve this, batch mode experiments were conducted choosing specific parameters such as pH (2.0–7.0), adsorbent dose (0.1–0.5 g), contact time (15–360 min) and metal ion concentrations (0.03–0.07 g/L). The surface characterization has been carried out using Autosorb-1 instrument (Quanta chrome), SEM and FTIR. From these studies, it is evident that charcoal could be used as adsorbents for removal of Ni(II) and Zn(II) from aqueous solution.

1. Introduction

Industrial wastewater and effluents containing toxic heavy metals pose serious problems to human health and the environment. Beyond the permissible limits, essential metals like Ni, Zn, Cr, Fe etc. are also toxic. So it is necessary to remove these excess of essential metals from aqueous substrates. Chemical precipitation, reverse osmosis, ion exchange, membrane technologies and other available methods are cost-prohibitive and often impracticable in remote regions when heavy metal contaminants originate from geogenic sources. In such situations, adsorption is one of the feasible and convenient alternatives available to water treatment. This process is based on the binding of metal ions by the various functional groups present on the adsorbent.

Nickel(II) is found in wastewater due to geogenic source (e.g. discharges from mining), electroplating, pigments and ceramic industries, battery and accumulator manufacturing [1]. Nickel is widely used in plating plants, steel factories, Ni batteries and in the production of some alloys [2]. The most common adverse health effect of nickel in human is an allergic reaction; large amounts of nickel can cause lung and nasal sinus cancers [3], damage to lungs, gastrointestinal distress, e.g., nausea, vomiting, diarrhea, pulmonary fibrosis, renal edema, and skin dermatitis [4]. In India, nickel in drinking water should not exceed 0.01 mg/L and for discharge of industrial wastewater it should not be more than 2.0 mg/L [5].

Zinc is an essential element for life. But too much zinc can be harmful to health. Zinc mostly enters into the environment as a result of human activities such as mining, purifying zinc, lead and cadmium ores, steel production, insecticides, coal burning and burning of wastes [6]. Symptoms of zinc toxicity include irritability, muscular stiffness, loss of appetite and nausea. The recommended level of zinc in drinking water is 5 mg/L.

Several researchers have utilized a number of materials to remove Ni(II) and Zn(II) including *Citrus reticulate* fruit peel [2], Na-mordenite [3], *Chlorella sorokiniana* [4], wheat straw [7], fibric histosol [8], Turkish fly ashes [9], pine bark and blast furnace [10], black gram husk [11], modified jute fibers [12], Activated carbon [13], amberlite IR-120 synthetic resin [14].

Bamboo is the most abundant vegetation in the state of Mizoram, India. The bamboo forest area of Mizoram constitutes 14% of the total of India's bamboo area (i.e. 8.96 million hectare). *Melocanna baccifera* is the most abundant and economically important one among the bamboos of this region. The communities of the region use this bamboo in many ways, among which charcoal production is one of them. *M. baccifera* charcoal was collected from the state of Mizoram. The use of activated carbon is considered to be the appropriate technology for removing low-solubility contaminants in water treatment, including trace metals [15,16]. Charcoal is a carbonaceous material with profuse porosity and internal surface area [17].

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Several researchers have prepared activated carbon (or charcoal) from bamboo by chemical activation using phosphoric acid for the removal of chemical oxygen demand (COD) and color of cotton textile mill wastewater [18]. Ip et al. [19] produced activated carbons from the natural biomaterial bamboo, using phosphoric acid as the activating agent to adsorb acid red 18 dye from aqueous solution, Hameed et al. [20] prepare activated carbon from bamboo by physiochemical activation with potassium hydroxide (KOH) and carbon dioxide (CO_2) as the activating agents for the adsorption of methylene blue, Liu et al. [21] prepared activated carbon from bamboo by microwave-induced phosphoric acid activation, Hameed and El-Khaiary [22] also prepared activated carbon from bamboo by K_2CO_3 activation and subsequent gasification with CO_2.

As per literature survey, there are no studies on the adsorption of Ni(II) and Zn(II) ions using *M. baccifera* raw charcoal (MBRC) and activated charcoal (MBAC). Therefore in the present study, *M. baccifera* raw and activated charcoals have been used for the removal of individual metal ions, Ni(II) and Zn(II) from aqueous solutions. Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) were used to characterize the surface structure and surface functional groups, respectively. The present study also reports the effect of such factors as chemical pretreatment, initial pH, adsorbent dose, initial metal ion concentration and contact time on the adsorption efficiency of these metal ions from aqueous solutions by MBRC and MBAC. The isotherms of adsorption have been studied and various isotherm models, such as Langmuir, Freundlich and Dubinin–Radushkevich (D–R) models have been tested. Kinetics have also been studied using pseudo-first-order, and pseudo-second-order models, which have been tested with experimental data for their validity.

2. Materials and methods

2.1. Adsorbent and its properties

M. baccifera charcoal was collected from the state of Mizoram. This bamboo charcoal was produced by charring of bundles of bamboo culms (usually 2–3 ft in length) inside the charcoal kiln. Thermal decomposition of *M. baccifera* culm was carried out inside the charcoal kiln. A small opening of air ventilation was made at the charcoal kiln for oxygen circulation during charring of bamboo culm. The oxygen supply should be minimal and maintained so that the culm will not burn away. Under reduced oxygen supply, charring of the culm would take place. The charring operation can take between 2 and 4 h. The adsorbent was milled with a blender and sieved to a particle size of 100 mesh screen. Guo and Lua [23] produced activated carbon using 5, 10, 20 and 30% KOH concentrations. Lozano-Castello et al. [24] used KOH/coal in the ratio of 1:1, 2:1, 3:1, 4:1 or 5:1 in 10 mL of water in order to produce activated carbon. Ajayi and Olawale [25] used 30% KOH concentration for Activation of carbon prepared from *Canarium schweinfurthii* Nutshell. Some researchers observed that at higher concentration of KOH activation, the surface area of charcoal also increased [26,27]. Therefore, the raw charcoal was activated with potassium hydroxide (Himedia) from low to high concentrations i.e., 10, 20, 30, 40, 50, 60, 70 and 80%. The concentrations of KOH were selected as follows:

$$\mathrm{KOH\,(gm)} = \text{Desired percentage of KOH} \times 100\,\mathrm{ml} / \%\,\text{purity of the compound.}$$

In the pretreatment process, the raw charcoal powder (40 g/L) was added to the KOH solution of various concentrations as given above. The mixture was stirred for 24 h at a speed of 150 rpm. Activated charcoal was separated from the activating reagent by centrifugation at a speed of 10,000 rpm for 10 min separated, and thoroughly washed with deionized water, oven-dried at 110 °C for 24 h, cooled at room temperature and stored in an air-tight container for further use. This is the activated charcoal, henceforth named MBAC. The MBAC (0.1 g) was pretreated with various concentrations of potassium hydroxide and 0.1 g of the MBRC were mixed with metals solution for 6 h on the orbital shaker at 29 ± 1 °C. The supernatants were separated from the biomass by centrifuging at 10,000 rpm for 8 min. The concentrations of metals in the solutions were analyzed.

To understand the surface morphology of the MBRC and MBAC, the samples were gold sputter coated and SEM micrographs were taken using a XL30 ESEM, Philips, USA. The specific surface areas of MBRC and MBAC were estimated using N_2 adsorption isotherm at 77 K by BET method using an Autosorb-1 instrument (Quanta chrome). FTIR spectrometer (JASCO FTIR-5300) was employed to determine the presence of surface functional groups in MBRC and MBAC, before and after the adsorption of metal ions, at room temperature over a spectral range of 400–4000 cm^{-1}.

2.2. Adsorbates

All the chemicals used in this study were of analytical grade. A stock solution of nickel (1000 mg/L) was prepared by weighing 4.955 g of $Ni(NO_3)_2.6H_2O$. Similarly, stock solution of zinc was also prepared by weighing 4.397 g of $ZnSO_4.7H_2O$. Initial solutions with different concentrations of Ni(II) and Zn(II) were prepared by proper dilution from stock of 1000 mg/L of Ni(II) and Zn(II) solutions. Standard solution of Ni(II) and Zn(II) from Sisco Research Laboratories (India) were used for calibration of atomic absorption spectrometer.

2.3. Batch adsorption studies

The adsorption of Ni(II) and Zn(II) ions onto the MBRC and MBAC of *M. baccifera* was investigated in batch mode adsorption equilibrium experiments. All batch experiments were carried out in 250 mL conical flasks containing 100 mL metal solution.

The effect of pH on the sorption capacity of MBRC and MBAC for Ni (II) and Zn(II) removal was evaluated in the range of 2.0–7.0. The pH of each metal solution was adjusted to the required pH value by using 1 N HNO_3 or 1 N NaOH. Then 0.1 g of dried adsorbent was added into the metal solution. The reaction mixture was shaken for 6 h at 150 rpm, 29 ± 1 °C, this is enough to attain adsorption equilibrium. Similarly, adsorbent dose (0.1–0.5 g), contact time (15–360 min), and initial metal concentration (30.0–70.0 mg/L) for raw and activated charcoals were also conducted. At the end of the experiment, the solutions were separated from the adsorbent by centrifuging at 10,000 rpm for 8 min. The concentrations of metal in the solutions were analyzed using Flame Atomic Absorption Spectrometer (GBC 932 plus, Australia). The wavelength of 288 nm was used for the analysis of the metal. The instrument was calibrated with a standard solution within a linear range and a correlation coefficient (R^2) of 0.995 to 1.0 was obtained. In order to reproduce the results, the experiments were conducted in triplicate and the average values were used for the data analysis.

2.4. Desorption studies

0.4 g of charcoal was added to 100 mL of solution containing 50 m/L and 30 mg/L of NI(II) and Zn(II) solutions, respectively. After the adsorption studies, the adsorbent was collected by filtration and washed with deionized water twice to remove any residual metal ions from the surface of the adsorbent. To this cleaned adsorbent, 100 mL of 0.1 M HCl was added and stirred for 24 hrs after which it is centrifuged at 10,000 rpm for 8 min. The supernatant was analyzed for estimating the concentration of the metal ions. From this analysis the percentage concentration of desorbed metal ions was calculated as

$$\%\text{Desorption} = \text{amount of metal ions desorbed} \times 100 \,/\, \text{amount of metal ions adsorbed}.$$

3. Results and discussion

3.1. Adsorbent and its characters

The purpose of activation of charcoal is to create pores and develop larger surface area in the carbon material and thereby increase the adsorptive capacity. The adsorption capacity of chemically activated charcoal biomass with various concentrations of KOH was investigated. The KOH concentration of 50% and 60% treatment show highest adsorption of Ni(II) and Zn(II), respectively. (Fig. 1). Among the alkali metal salts, KOH is the most effective activating agent in producing activated carbon materials. The KOH activation was reported to be effective for increasing micropore volume [28,29]. The reaction between KOH and carbon precursor can result in the formation of functional groups such as –OK using oxygen of the alkali salt. The presence of such potassium and oxygen bond in the char leads to the oxidation of cross linking carbon atoms in the adjacent lamella during the process of activation. Surface functional groups are created at the edges of the lamella. As a result of removal of cross linking between adjacent lamella and also the formation of new functional groups on individual lamella, the lamellas of the crystallite are disturbed from their normal form into a slightly wrinkled or folded or puckered form. Also the potassium metal produced in the process of activation, *in-situ*, intercalates in to the lamella of the crystallite. After the activation process, when the carbon material is washed with water, the potassium salts present in the carbon particles are removed by leaching. At the same time, the lamella cannot return to their original state, creating interlayer voids. The lamella remains apart causing porosity and yielding high surface area carbon [30]. Thus during activation with KOH, amorphous carbon as well as silica are removed from the carbon precursor resulting in porous structure and a corresponding increase in the surface area.

SEM micrographs of the charcoal revealed that the surface was highly porous in nature (Fig. 2); this increased the surface area for metal adsorption. The pore size of metals loaded activated charcoal was larger than the metals loaded raw charcoal.

The specific surface area was calculated as 6.26 m^2/g and 222.81 m^2/g for MBRC and MBAC, respectively. The larger surface area after KOH activation may be the reason for larger adsorption capacity of MBAC. This improvement in surface area may lead to exposure of more functional groups. Therefore, more functional groups are available to attract heavy metal ions during adsorption process.

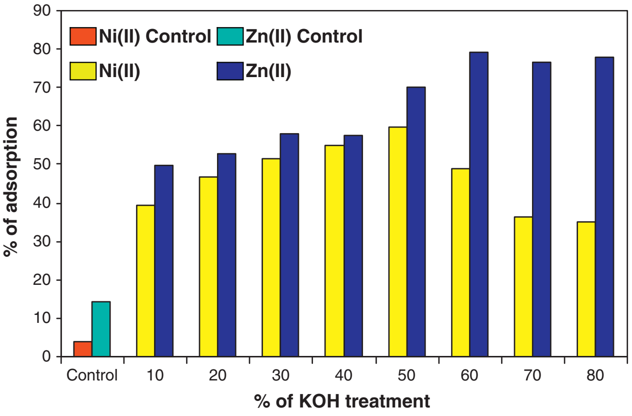

Fig. 1. Effect of pretreatment on *M. baccifera* charcoal by different concentrations of potassium hydroxide for the removal of Ni(II) and Zn(II) ions (Ni(II) concentration = 20 mg/L; Zn(II) concentration = 10 mg/L; adsorbent dose = 0.1 g/100 mL; temperature = 29 ± 1 °C; contact time = 6 h).

The surface functional groups of the adsorbent, besides the porous structure, are of vital importance in understanding the sorption process and the adsorption capacity of an adsorbent. The FTIR spectra of dried unloaded charcoal, the untreated raw charcoal and the activated charcoal both loaded with metal ions were recorded to obtain information on the nature of possible interactions between the functional groups and the metal ions. Dried and unloaded charcoal shows absorption at frequencies at 2351 cm^{-1}, 1585 cm^{-1}, 1398 cm^{-1}, 1016 cm^{-1}, 879 cm^{-1} and 821 cm^{-1}. After adsorption, there are shifts as well as changes in their intensities in the peaks corresponding to the above frequencies. With the Ni(II) loaded raw charcoal, it is seen that the peak at 2351 cm^{-1} shifted to 2391 cm^{-1}, the peak at 1585 cm^{-1} shifted to 1574 cm^{-1}, with a decreased intensity, the peak corresponding to 1398 cm^{-1} shifted to 1365 cm^{-1}, n1016 shifted to 1012 cm^{-1}, n821 cm^{-1} shifted to 780 cm^{-1} and the corresponding peaks for the Ni (II) loaded activated charcoal also show shifts in the frequencies corresponding to 2351 cm^{-1}, the band intensity at 1585 cm^{-1} decreased, 1413 cm^{-1}, band was split into two bands at 1172 cm^{-1} and 1091 cm^{-1}, 877 cm^{-1}, and 819 cm^{-1}. These shifts indicate that there were binding processes taking place on the surface of carbon [31]. The functional groups involved in the adsorption were as follows: a band at 2351 cm^{-1} representing C≡N stretching [32]. The band around 1585 cm^{-1} was assigned to stretching vibration of C=O [33]. The adsorption peak at 1398 cm^{-1} is due to –CH_3 wagging [34]. The band at 1016 cm^{-1} is due to C–O stretching vibration [35] (Fig. 3a).

Similar shift in the absorption frequencies and reduction in the peak intensities were observed for the Zinc loaded charcoals. The shift in some of the frequencies and decrease in intensity of the bands suggest the participation of these functional groups in Zn(II) adsorption (Fig. 3b). The functional groups involved in the adsorption of Zn(II) were as follows: a band at 2924 cm^{-1} representing C–H stretching [36]. The band at 1597 cm^{-1} was assigned to C=O. The band at 1114 cm^{-1} to 1020 cm^{-1} represents C–O stretching vibration. Some bands below 1000 cm^{-1} are called fingerprint zone. The fingerprint zone could be attributed to the phosphate or sulphur groups [37]. These functional groups could act as chemical binding agents where C≡N, C=O etc. could dissociate negatively charged active surface. This meant that these functional groups could attract the positively charge objects such as heavy metal ions [38].

3.2. Effect of pH

Bamboo charcoal can generate a good amount of negative (more precisely, negative-charged) ions. The presence of a large quantity of surface negative charge and a high charge density is also reported by Liang et al. [39].

The pH of the solution is one of the most important factors in the study of the adsorption of metal ions on solids. The state of metal ions in solution strongly depends on the pH. The acidity and basicity of the various solutions can influence the composition and the properties of the adsorbent surface. Therefore, in order to determine the optimum pH for metal adsorption, the adsorption was studied at various pH. Adsorption pH had a range from 2 to 7. At low pH values, the adsorption of heavy metals is zero. As the pH increases, the adsorption of heavy metals also increased and maximum adsorption for Ni(II) occurred at 5.0 pH both with MBRC and MBAC. Whereas, maximum adsorption of Zn(II) took place at 6.0 pH for MBRC and MBAC (Fig. 4).

This adsorption behavior of Ni(II) and Zn(II) ions can be explained thus — at very low pH, higher concentration of H^+ ions compete with metals ions for the adsorption sites of charcoal, resulting in decreased adsorption of Ni(II) and Zn(II) ions. The increase in the adsorption of Ni(II) and Zn(II) ions at the pH range from 2 to 5 and 2 to 6,

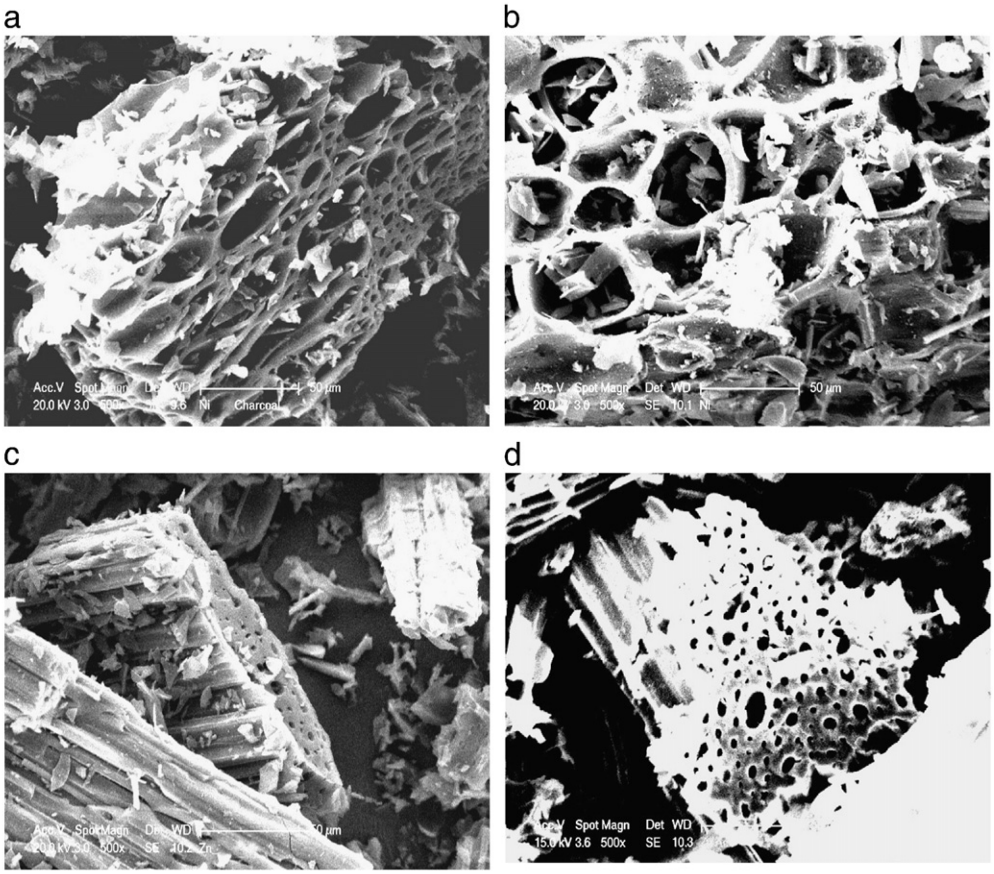

Fig. 2. Scanning electron microscope (SEM) micrograph; a) Ni(II) loaded MBRC, b) Ni(II) loaded MBAC, c) Zn(II) loaded MBRC and d) Zn(II) loaded MBAC.

respectively is due to decrease in the concentration of the competing H^+ ions as pH increased and increase in the adsorption of the metal ions on the adsorption sites of adsorbents.

The decrease in the removal of Ni(II) and Zn(II) ions as the pH increased from 5 to 7 and 6 to 7, respectively might be attributed to the formation of their corresponding hydroxylated form of metals. These hydroxylated forms of metal can also compete with the metal ions at the active sites of the adsorbent thereby decreasing adsorption.

3.3. Optimization of adsorbent quantity

Experiments were carried out to optimize the adsorbent quantity for maximum adsorption of the metal ions. The adsorbent quantity was varied from 0.1 g to 0.5 g keeping the Ni(II) and Zn(II) concentrations constant at 50 mg/L and 30 mg/L, respectively. The percentage of metal adsorption increased with increase in the weight of the adsorbent. Maximum adsorption was observed for the adsorbent weight of 0.4 g/100 mL of Ni(II) and Zn(II) solution onto MBRC and MBAC (Fig. 5). The increased adsorption with increase in the adsorbent amount is due to the increase in the number of active adsorption sites. Beyond 0.4 g, the adsorption capacity decreased which may be due to overlapping and aggregation of adsorption sites resulting in the decrease of the surface area available to metal ions.

3.4. Effect of metal concentration

For a given quantity of adsorbent (0.4 g) the effect of metal concentration on the adsorption is shown in Fig. 6. The percentage of metal ions adsorption decreased with upto certain increase in metal ion concentration after which the decrease was minimal in the case of Ni both for MBAC and MBRC. In the case of Zn (II), the decrease in adsorption is very less with increase in concentration for both the activated and the raw charcoal. This can be explained as; at low metal ion/adsorbent ratios, metal ion adsorption involves higher energy sites. As the metal ion/adsorbent ratio increases, the higher energy sites are saturated and adsorption begins on lower energy sites, resulting in decrease in the adsorption efficiency [40,41].

3.5. Effect of time

The effect of contact time on the adsorption of Ni (50 mg/L) and Zn (30 mg/L) onto MBRC and MBAC is shown in Fig. 7. The maximum adsorption occurred within 60 min, after which there were no significant change in absorption. The equilibrium was reached at 360 min for Ni and Zn(II) adsorption onto MBRC and MBAC. Metal uptake by the sorbent surface is rapid initially up to 60 min and then decreases as time increases. This can be explained as: initially adsorption of metal ions is increased as contact time is increased due to the availability of more active sites. More and more functional groups participated in adsorption of the metal ions until it reached equilibrium. After it reached the equilibrium, there was no significance changes in Ni(II) and Zn(II) concentrations in the solutions was observed.

3.6. Isotherm studies

Ni(II) and Zn(II) uptake capacity onto the MBRC and MBAC at different concentrations of metals on a fixed amount of adsorbent was evaluated using Freundlich and Langmuir adsorption isotherms. The Freundlich isotherm is represented by Eq. (1). The Freundlich isotherm

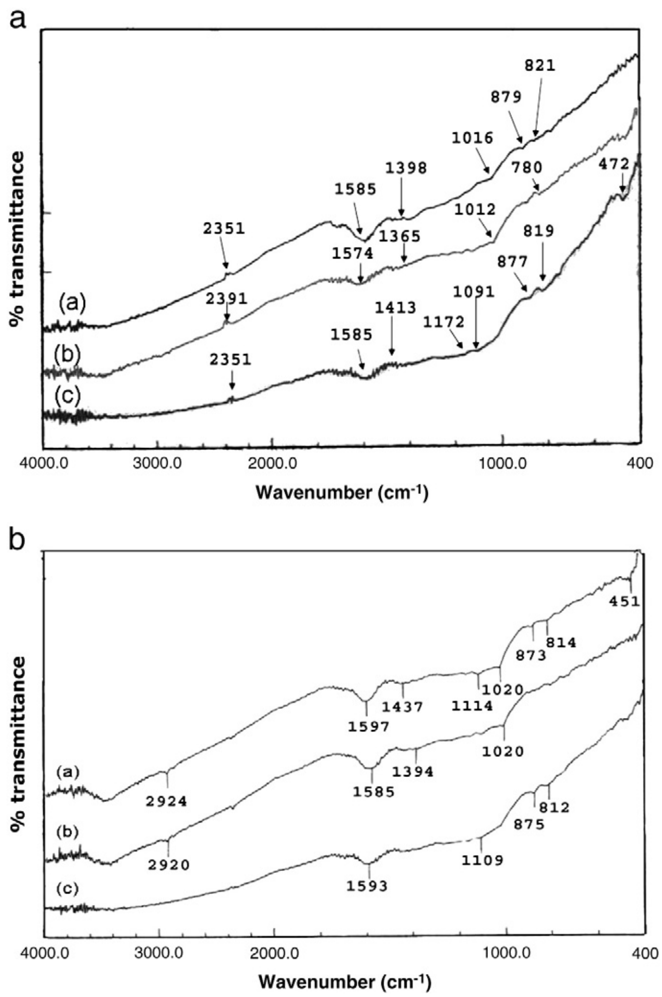

Fig. 3. FTIR spectra of a) a) Ni(II) unloaded charcoal b) Ni(II) loaded *M. baccifera* raw charcoal and c) Ni(II) loaded *M. baccifera* activated charcoal and b) a) Zn(II) unloaded charcoal b) Zn(II) loaded *M. baccifera* raw charcoal and c) Zn(II) loaded *M. baccifera* activated charcoal.

[42] assumes a monolayer sorption with a heterogeneous energetic distribution of active sites, accompanied by interaction between adsorbed molecules

$$\log q_e = \log K_f + (1/n)\log C_e \tag{1}$$

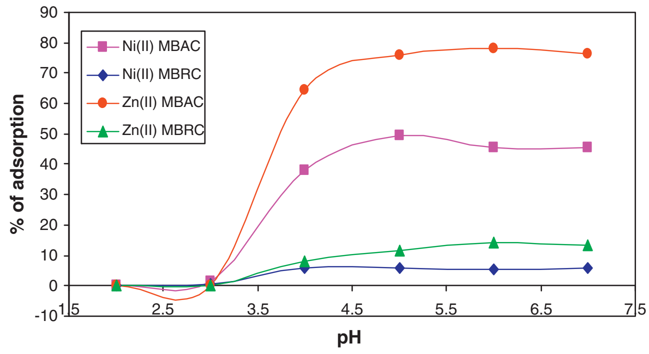

Fig. 4. Effect of pH on Ni(II) and Zn(II) removal by MBRC and MBAC (Ni(II) concentration = 20 mg/L; Zn(II) concentration = 10 mg/L; adsorbent dose = 0.1 g/100 mL; temperature = 29 ± 1 °C; contact time = 6 h).

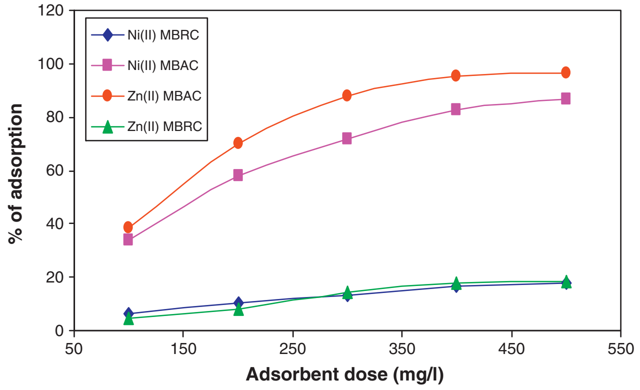

Fig. 5. Effect of adsorbent dosage on Ni(II) and Zn(II) removal by MBRC and MBAC (Ni(II) concentration = 50 mg/L; Zn(II) concentration = 30 mg/L; temperature = 29 ± 1 °C; contact time = 6 h).

where C_e (mg/L) is the equilibrium concentration, q_e (mg/g) is the amount of metal ions adsorbed per specified amount of adsorbent at equilibrium, K_f (mg/g) and '*n*' are constants which are adsorption capacity and intensity of adsorption, respectively. A plot of $\log q_e$ versus $\log C_e$ gives a straight line with correlation coefficient (R^2) of 0.5344 and 0.9943 for Ni(II) onto MBRC and MBAC, respectively and 0.2446 and 0.9229 for Zn(II) onto MBRC and MBAC, respectively (Table 1).

The Langmuir [43] isotherm assumes that a solid surface has a finite number of identical sites which are energetically uniform. According to this model, there is no interaction between adsorbed species, which means that the amount adsorbed has no influence on the rate of adsorption. A monolayer was formed when the equilibrium was attained. Its linear mathematical form is as follows:

$$C_e/q_e = 1/q_{\max}b + C_e/q_{\max} \tag{2}$$

where C_e (mg/L) is the concentration of metal ions in solution at equilibrium, $q_{\max}$ (mg solute/g adsorbent) is the maximum adsorption capacity corresponding to complete monolayer coverage and *b* (L/mg) is a parameter related to the energy of adsorption. A plot of C_e/q_e versus C_e which gives a straight line (Fig. 8). The slopes of the line was used to calculate the $q_{\max}$ values. These calculated $q_{\max}$ values agree well with the theoretical q_e values of Ni(II) and Zn(II) adsorption (Table 1). The fitness of the adsorption data of Ni(II) and Zn(II) ions to the Langmuir isotherm implies that the adsorbed metal ions do not interact or compete with each other and that they are adsorbed by forming a monolayer.

In order to explain the nature of sorption processes as physical, chemical or ion-exchange sorption, Dubinin–Radushkevich (D–R) isotherm model [44] was used

$$\mathrm{In}q_e = \mathrm{In}X_m – \beta\varepsilon^2 \tag{3}$$

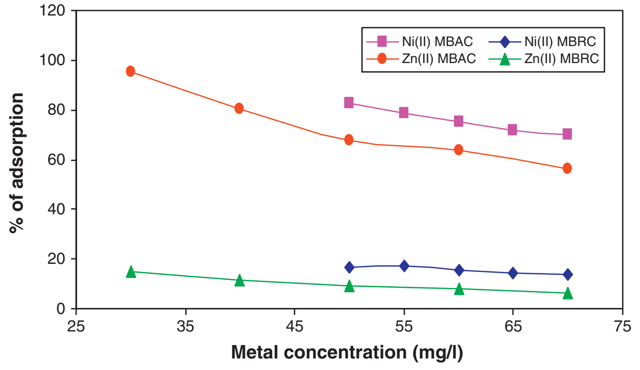

Fig. 6. Effect of initial metal concentration on Ni(II) and Zn(II) removal by MBRC and MBAC (adsorbent dose = 0.4 g/100 mL; temperature = 29 ± 1 °C; contact time = 6 h).

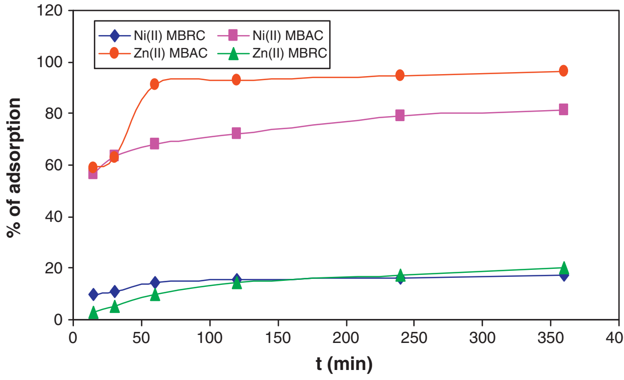

Fig. 7. Effect of time on Ni(II) and Zn(II) removal by MBRC and MBAC (Ni(II) concentration = 50 mg/L; Zn(II) concentration = 30 mg/L; adsorbent dose = 0.4 g/100 mL; temperature = 29 ± 1 °C).

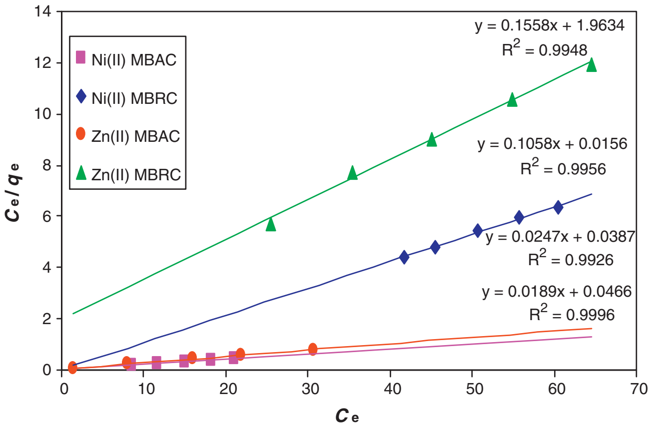

Fig. 8. Linearized Langmuir isotherm plot for adsorption of Ni(II) and Zn(II) onto MBRC and MBAC.

where q_e (mol/g) is the metal ion concentration on the adsorbent at equilibrium, X_m (mol/g) is the maximum adsorption capacity, β is the activity coefficient related to the mean free energy of adsorption and ε is Polanyi potential. The Polanyi potential (ε) can be expressed as,

$$\varepsilon = RT\ln(1+1/C_e) \tag{4}$$

where R (J/mol) is the universal gas constant, T is the absolute temperature in Kelvin and C_e (mol/L) is the metal ion concentration in the solution at equilibrium. A graph was plotted between $\ln q_e$ versus ε^2 gives a linear relationship with slopes of β and intercepts of X_m (figure not shown). The activity coefficient β was further used to calculate the adsorption mean free energy 'E' (kJ/mol) which is the free energy for the transfer of 1 mol of metal ions from the infinity in the solution to the surface of the adsorbent.

$$E = 1/\sqrt{-2\beta} \tag{5}$$

Based on Eqs. (3) and (5), the isotherm constants, E and β are calculated and presented in Table 1. When 1 mol of ions is transferred, its value in the range of 1–8 kJ/mol indicates physical adsorption [45], the value of E between 8 and 16 kJ/mol indicates ion-exchange adsorption [46], if the value of E is between 20 and 40 kJ/mol, it indicates chemisorption [47]. Thus the value of E for Ni adsorption was calculated as 12.13 kJ/mol and 18.91 kJ/mol (Table 1) for MBRC and MBAC, respectively. Similarly, the E values of Zn adsorption were 14.74 kJ/mol and 25.0 kJ/mol for MBRC and MBAC, respectively. So, it seems that ion-exchange mechanism dominates in the adsorption process except in Zn(II) adsorption by MBAC which shows chemisorption. However, the above conclusion for Zn(II) adsorption by MBAC is not very reliable taking into account the low correlation coefficient.

Table 1
Dubinin–Radushkevich, Freundlich and Langmuir isotherms parameters for the adsorption of Ni(II) and Zn(II) onto MBRC and MBAC.

Adsorbent	Ni(II)MBAC	Ni(II)MBRC	Zn(II)MBAC	Zn(II)MBRC
Experimental value q_e (mg/g)	49.01	9.53	39.32	4.52
Dubinin–Radushkevich				
E (kJ/mol)	18.91	12.13	25.00	14.74
β (kJ2/mol2)	−0.0014	−0.0034	−0.0008	−0.0023
R^2	0.978	0.931	0.904	0.995
Freundlich				
K_f (mg/g)	28.53	2.93	27.01	3.87
n	5.82	3.43	9.82	22.12
R^2	0.994	0.534	0.922	0.244
Langmuir				
q_{max} (mg/g)	52.91	9.45	40.485	4.723
b (L/mg)	0.405	6.783	0.638	9.273
R^2	0.999	0.995	0.992	0.994

3.7. Kinetics studies

In order to explain the mechanism of adsorption of Ni(II) and Zn(II), pseudo-first-order and pseudo-second-order are used to explain sorption kinetics of the reaction.

The pseudo-first-order Lagergren model [48] for solid/liquid systems of adsorption states that the rate is proportional to the number of unoccupied sites. It is expressed as:

$$\log(q_e - q_t) = \log q_e - k_1 t/2.303 \tag{6}$$

where q_e and q_t (mg/g) are the amounts of metal ions adsorbed on the adsorbent at equilibrium and at any time t, in mg/g, respectively. k_1 (/min) is the rate constant of pseudo-first-order adsorption. The graph was plotted between log $(q_e - q_t)$ versus t. From the slopes and intercepts of the graph, the constant values of pseudo-first-order were calculated.

The pseudo-second-order [49] assumes that the rate of sorption is proportional to the square of the number of unoccupied sites. It is expressed as

$$t/q_t = 1/k_2 q_e^2 + t/q_e \tag{7}$$

where k_2 is the equilibrium rate constant of pseudo-second-order adsorption (g/mg/min). The slopes and intercepts of plots t/q_t versus t (Fig. 9) were used to calculate q_e and k_2.

The correlation coefficients (R^2) of Ni(II) for the pseudo-second-order kinetics model onto MBRC and MBAC were 0.999 and 0.999 (Fig. 9), respectively and the experimental q_e values were agreed well with the calculated q_e values. Similarly, the correlation coefficients (R^2) of Zn(II) for the pseudo-second-order kinetics model onto MBRC and MBAC were 0.987 and 0.999 (Fig. 9), respectively and the experimental q_e values were also agreed well with the calculated q_e values (Table 2). On the other hand, the correlation coefficients of the pseudo-first-order kinetics model were lower than the pseudo-second-order kinetics model in MBRC and MBAC for Ni(II) and Zn(II) and the calculated q_e values from pseudo-second-order kinetics were agreed well with the experimental q_e values than the pseudo-first-order (Table 2). Therefore, it can be concluded that this adsorption system followed a pseudo-second-order reaction rather than pseudo-first-order reaction. This suggests that sorption of the metal ions involve two species, in this case, the metal ion and the adsorbent.

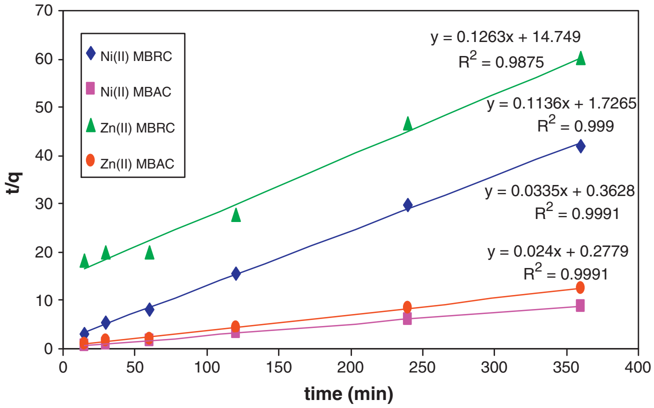

Fig. 9. Kinetic model of pseudo-second-order kinetic on Ni(II) and Zn(II) removal onto MBRC and MBAC.

3.8. Desorption studies

The adsorption studies have shown that for, 40.633 mg/L and 8.551 mg/L Ni(II) ions were adsorbed by 0.4g of MBAC and MBRC respectively. Similarly, MBAC and MBRC adsorbed 28.98 mg/L and 5.98 mg/L Zn(II) ions, respectively. In desorption process, Ni(II) ion was found to desorb 3.340 mg/L after 6 h and 3.369 mg/L after 24 h from MBRC whereas 15.205 mg/L and 15.225 mg/L was desorbed from MBAC after 6 h and 24 h, respectively. This means that only 39.96% 37.42% was desorbed from the adsorbents and 60.04% and 62.58% of Ni(II) was still adsorbed onto MBRC and MBAC, respectively. Similarly, Zn(II) ion of 1.13 mg/L and 1.158 mg/L was desorbed after 6 h and 24 h, respectively from MBRC whereas 1.467 mg/L and 1.477 mg/L was desorbed from MBAC after 6 h and 24 h, respectively, this indicated that 81.12% and 94.91% of Zn(II) are still left in MBRC and MBAC. From this result, it can be seen that the adsorbents can be used for a couple of cycles.

4. Conclusion

The chemically activated charcoal has higher adsorption capacity than the raw charcoal for Ni(II) and Zn(II). This can be attributed by larger surface area of activated charcoal, which was confirmed by BET surface analysis. The scanning electron microscope also shows that activated charcoal has larger pore size than raw charcoal. The FTIR spectra data shows the presence of different functional groups are involved in metal adsorption. The maximum adsorption of Ni(II) was obtained as 9.452 and 52.91 mg/g for MBRC and MBAC, respectively. Zn(II) $q_{\max}$ value was 4.723 and 40.485 mg/g for MBRC and MBAC, respectively. The kinetics of adsorption follows pseudo-second-order reaction. *M. baccifera* charcoal powder adsorption process is highly reproducible and efficient. It can be used at large scale removal various heavy metals from the contaminated aqueous media.

Table 2
Pseudo-first order and pseudo-second-order adsorption kinetic model for the adsorption of Ni(II) and Zn(II) onto MBRC and MBAC.

Adsorbent				
	Ni(II)MBAC	Ni(II)MBRC	Zn(II)MBAC	Zn(II)MBRC
Experimental value q_e (mg/g)	40.63	8.55	28.98	5.98
Pseudo-first-order				
q_e.cal.(mg/g)	12.98	6.96	8.47	5.36
k_1 (/min)	9.67×10^{-3}	8.52×10^{-3}	12.66×10^{-3}	8.29×10^{-3}
R^2	0.988	0.842	0.752	0.966
Pseudo-second-order				
q_e.cal.(mg/g)	41.66	8.81	29.85	7.91
k_2 (g/mg/min)	2.07×10^{-3}	7.46×10^{-3}	3.09×10^{-3}	1.08×10^{-3}
R^2	0.999	0.999	0.999	0.987

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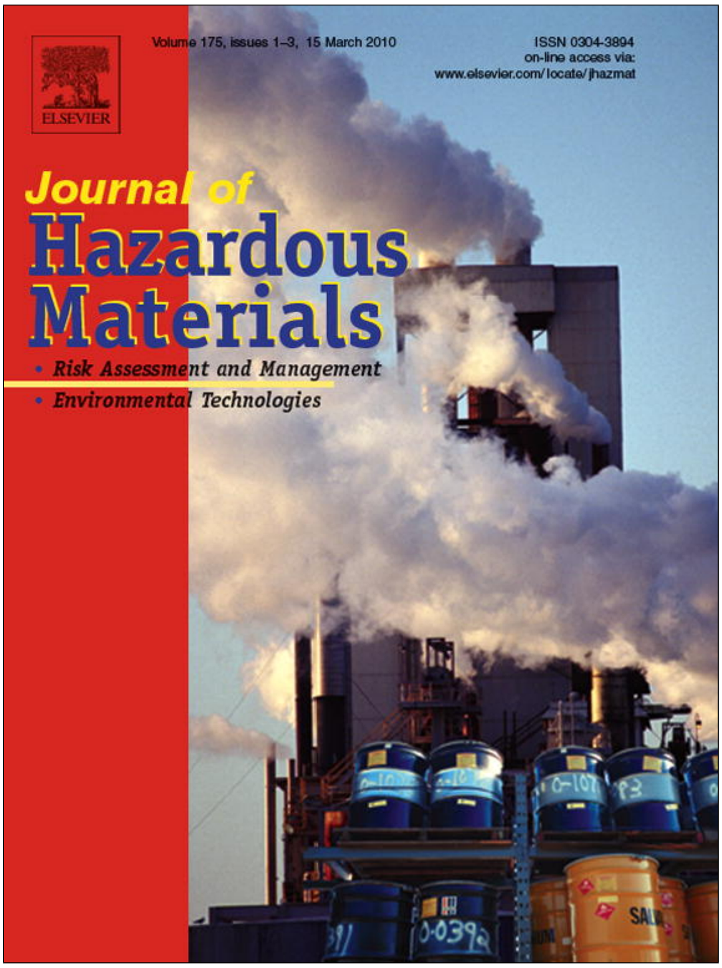

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Lead(II) adsorption from aqueous solutions by raw and activated charcoals of *Melocanna baccifera* Roxburgh (bamboo)—A comparative study

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ABSTRACT

Melocanna baccifera (Poaceae) is the most abundant and economically important non-timber product in state of Mizoram, India. The communities of the region use this potential resource in many ways, charcoal production is one of them. Bamboo charcoal has application in food, pharmaceutical and chemical industries. Activated charcoal was prepared from *M. baccifera* charcoal by chemical pretreatment in order to make better use of this abundant biomass material. Batch experiments were conducted under varying range of pH (2.0–6.0), contact time (15–360 min) and metal ion concentrations (50–90 mg L^{-1}). The optimum conditions for lead biosorption are almost same for *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC)-pH 5.0, contact time 120 min, adsorption capacity q_{max} 10.66 mg g^{-1} and 53.76 mg g^{-1}, respectively. However, the biomass of MBAC was found to be more suitable than MBRC for the development of an efficient adsorbent for the removal of lead(II) from aqueous solutions. FTIR analysis revealed that –OH, C—H bending, C═O stretching vibration and carbonyl functional groups were mainly responsible for Pb(II) biosorption. Thus, this study demonstrated that both the charcoal biomass could be used as adsorbents for the treatment of Pb(II) from aqueous solution.

1. Introduction

Heavy metal ions such as lead, cadmium, mercury, chromium, nickel, zinc and copper are non-biodegradable, can be toxic and carcinogenic even at very low concentrations, and hence, usually pose a serious threat to the environmental and public health [1]. The traditional methods for the treatment of lead and other toxic heavy metal contaminated in wastewaters include complexation, chemical oxidation or reduction, solvent extraction, chemical precipitation, reverse osmosis, ion exchange, filtration, membrane processes, evaporation and coagulation, nevertheless these techniques have disadvantages including incomplete metal removal, high consumption of reagent and energy, low selectivity, high capital and operational cost and generation of secondary wastes that are difficult to be disposed off [2,3]. For these reasons, cost-effective alternative technologies for treatment of metals contaminated waste streams are needed.

According to the ranking of metal interested priorities referred by Volesky [4], Pb(II) is one of the most interesting heavy metal for removal and/or recovery considering the combination of environmental risk and reserve depletion. This metal is widely used in many industrial applications, such as storage battery manufacturing, painting pigment, fuels, photographic materials, explosive manufacturing, coating, automobile, aeronautical and steel industries [5–8]. Lead pollution results from textile dyeing, ceramic and glass industries, petroleum refining, battery manufacture and mining operations [9]. Lead is a highly toxic and cumulative poison, accumulates mainly in bones, brain, kidney and muscles. Lead poisoning in human causes severe damage to kidney, nervous and reproductive systems, liver and brain [10]. In drinking water, even at a low concentration may cause anemia, encephalopathy, hepatitis and nephritis syndrome [11]. The permissible limit for Pb(II) in waste water as set by Environmental Protection Agency (EPA) is 0.05 mg L^{-1} and that of Bureau of Indian Standard (BIS) is 0.1 mg L^{-1} [12] and in drinking water intended for drinking, as set by EU, USEPA and WHO are 0.010, 0.015 and 0.010 mg L^{-1}, respectively [13,14]. It is therefore, essential to remove Pb(II) from wastewater before disposal.

Charcoals biomass is popular adsorbents for air and water purification [15,16]. Charcoal is manufactured initially by thermal decomposition according to the following reaction:

$$C_6H_{12}O_6 \underset{500-700\,^{\circ}C}{\longrightarrow} 6C + 6H_2O$$

The use of activated carbon is considered to be the best currently available technology for removing low-solubility contaminants in water treatment, including trace metals [17,18]. Charcoal was a carbonaceous material that has highly developed porosity and internal surface area.

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Melocanna baccifera belongs to a family of Poaceae. The bamboo forest area of Mizoram constitutes 14% of the total of India's bamboo area. *M. baccifera* is the most abundant, contributing about 95% of the growing stock of bamboo. The communities of the region use this potential resource for food, shelter, furniture, handicraft, medicines, charcoal production etc.

Bamboo charcoal application includes environment protection, food, pharmaceutical, chemical and metallurgical industries. A surface area of pores of bamboo charcoal is wider than wood charcoal. So ability of cleaning water of bamboo charcoal is more than wood charcoal.

So, the present study was performed to compare and evaluate *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC) as adsorbents for the removal of Pb(II) from aqueous solution by systematic evaluation of the parameters involved such as pH, Pb(II) concentration and time. Furthermore, the Redlich–Peterson, Langmuir–Freundlich, Langmuir, Freundlich adsorption isotherms and pseudo-first-order and pseudo-second-order reaction were applied to calculate isotherm parameters and to study the kinetics of adsorption. Fourier transform infrared spectroscopy (FTIR) was also used to elucidate the adsorption.

2. Experimental

2.1. Biosorbent

M. baccifera charcoal was collected from the state of Mizoram (North-East India). This bamboo charcoal was produced by stacking of many bamboo culms (usually 2–3 feet in length) inside the charcoal kiln. Thermal decomposition of *M. baccifera* culm was carried out inside the charcoal kiln. During charcoal production, a small opening of air ventilation was made at the charcoal kiln for oxygen circulation. The oxygen supply should be reduced so that the culm will not burn away. Under reduced oxygen supply, charring of the culm was take place. The charring operation can take between 2 and 4 h. The charcoal biomass was milled with a blender and sieved to a particular size.

2.2. Activation of M. baccifera charcoal

Chemical activation was carried out using phosphoric acid (Qualigens) and potassium hydroxide (Himedia) at various concentrations (20, 40, 60 and 80%, $\mathrm{w\,w^{-1}}$). In the activation system, the charcoal powder with a mass concentration of $40\,\mathrm{g\,L^{-1}}$ was in contacted with the above various concentrations of chemical reagents, respectively, for 24 h by shaking on an orbital shaker with a speed of 150 rpm. Centrifugation was done at a speed of 10,000 rpm for 10 min using Remi centrifuge C20 in order to separated activated charcoal from the chemical solutions. The activated charcoal was thoroughly washed with deionised water, oven-dried at 110 °C for 24 h, cooled at room temperature and stored in an air-tight container. 0.1 g of the MBRC and the MBAC pretreated by the above various concentrations of potassium hydroxide and phosphoric acid were mixed with $70\,\mathrm{mg\,L^{-1}}$ of Pb(II) solution for 6 h on the orbital shaker at $29 \pm 1\,^{\circ}\mathrm{C}$. The supernatants were separated from the biomass by centrifuging at 10,000 rpm for 8 min. The concentrations of metal in the solutions were analyzed.

2.3. Preparation of stock solution of metal

All chemicals used in this study were analytical grade. Stock solution of lead was prepared using $\mathrm{Pb(NO_3)_2}$ in double distilled water. Different desire concentrations of Pb(II) were prepared by diluting $1000\,\mathrm{mg\,L^{-1}}$ of the stock solution. Standard solution of Pb(II) ($1000\,\mathrm{mg\,L^{-1}}$) for atomic adsorption spectrophotometer was obtained from Sisco Research Laboratories (India). Acid and base solutions (1N $\mathrm{HNO_3}$ and 1N NaOH) were used for pH adjustments.

2.4. Scanning electron microscope (SEM) analysis

In order to characterize the surface structure of activated charcoal and raw charcoal, SEM (XL30 ESEM, Philips, USA) analysis was carried out.

2.5. Batch mode adsorption studies

The biosorption of Pb(II) ions on the MBRC and MBAC of *M. baccifera* was investigated in batch mode sorption equilibrium experiments. All batch experiments were carried out in 250 mL conical Erlenmeyer flasks containing 100 mL metal solution.

The effect of pH on the sorption capacity of MBRC and MBAC for Pb(II) was evaluated in the range of 2.0–6.0. The pH of each metal solution was adjusted to the required pH value by using 1N $\mathrm{HNO_3}$ or 1N NaOH. Then 0.1 g of dried adsorbent was added to the metal solution. The reaction mixture was shaken on an orbital shaker for at 150 rpm, 29 °C for 6 h, which was enough to attain adsorption equilibrium. Similarly, adsorbent dosage (0.1–0.5 g), Pb(II) concentration ($50–90\,\mathrm{mg\,L^{-1}}$), contact time (15–360 min) and for raw and activated charcoals were also conducted. At the end of the experiment, the solutions were separated from the biomass by centrifuging at 10,000 rpm for 8 min. The concentrations of metal in the solutions were analyzed using Flame Atomic Adsorption Spectrometer (GBC 932 plus, Australia). The wavelength of 283.3 nm was used for the analysis of the metal. The instrument was calibrated with a standard solution within a linear range and a correlation coefficient (R^2) of 0.995–1.0 was obtained. In order to reproduce the results, the experiments were conducted in triplicate and the average values were used in this data analysis.

2.6. Desorption experiments

For the desorption study, 0.1 g biomass was in contacted with 100 mL of Pb(II) solution ($60\,\mathrm{mg\,L^{-1}}$). After adsorption experiment, the biomass was collected by filtration and washed with deionised water for two times to remove excess Pb(II) residual on the surface. Then it was in contacted with 100 mL of 0.1 M HCl. The mixture was shaken for 6 h and then centrifuged at 10,000 rpm for 8 min. supernatant was collected and metal analysis was carried out. The experiment was carried out five cycles.

The percentage of desorption values were obtained as

$$\%\ \text{of Desorption} = \frac{\text{amount of metal ions desorbed}}{\text{amount of metal ions adsorbed}} \times 100$$

2.7. FTIR analysis

In order to determine the functional groups involved in metal adsorption, the unloaded biomass, MBRC-Pb(II) loaded and MBAC-Pb(II) loaded with $50\,\mathrm{mg\,L^{-1}}$ Pb(II) ions were analyzed using Fourier Transform Infrared Spectroscopy (JASCO FTIR-5300) in a range of $400–4000\,\mathrm{cm^{-1}}$.

3. Results and discussion

3.1. Properties of charcoal

Composition of bamboo charcoal is carbon 44.40%, hydrogen 5.17%, oxygen 43.00% and ash 7.43%. Ash in the charcoal powder was not required. The pH of the bamboo charcoal was 7.85. The average diameter of the pores was 6.77 μm and 11.72 μm for raw

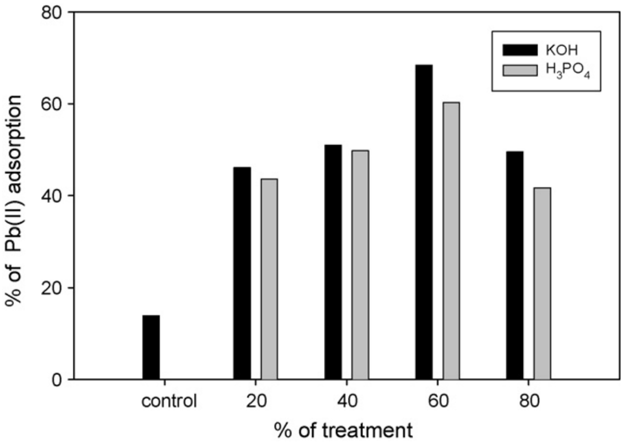

Fig. 1. Effect of pretreatment on *M. baccifera* charcoal by different concentrations of phosphoric acid and potassium hydroxide for the removal of Pb(II) ions.

and activated charcoal, respectively, and the particular size of raw and activated charcoals powder was 100 meshes.

3.2. Activation of *M. baccifera* charcoal

Changes in the sorption capacity of the biomass which was chemically pretreated with different concentrations of KOH and H_3PO_4 were investigated. The effect of chemical pretreatment on the adsorption of Pb(II) ions by MBRC and MBAC was shown in Fig. 1. Data obtained from activation studies showed that 60% KOH pretreatment shows highest percentage of adsorption, i.e. 68.42% in 70 mg L^{-1} of Pb(II) solution.

In case of pretreatment with acidic solutions of H_3PO_4, percentage of adsorption capacity was slightly decreased in comparison with that of alkali activation, 60.27% was the highest percentage of adsorption obtained with 60% phosphoric acid pretreatment. 60% KOH treated activation was used for further experiments since it shows highest percentage of adsorption.

Some researchers reported that alkali pretreatments of biomass enhanced the adsorption capacity in comparison with raw biomass [19–22]. Remaining alkalinity can cause hydrolysis of various metals, which may improve adsorption capacity of biomass. In case of pretreatment with an alkali solution, hydrolysis reactions can occur. The hydrolysis reactions can lead to the formation of more carboxylate (–COO) and alcohol (–OH) groups in the pretreated biomass, which enhances the cationic biosorption [23].

An increased in biosorption capacity was observed in case of pretreatment with an acidic solution, which cause protonation of biomass surface, may be attributed to the exchange of bound hydrogen ions with heavy metal ions.

3.3. Scanning electron microscope (SEM) analysis

SEM micrographs of the charcoal biomass revealed that the surface was highly porous in nature; this increased the surface area for metal adsorption. The pore size of Pb(II) loaded activated charcoal was larger in size than the raw charcoal as shown in Fig. 2.

3.4. Effect of pH

Many studies showed that pH is an important factor affecting biosorption of heavy metals [24,25]. The adsorption pH was examined in a range of 2–6 to avoid the formation of lead hydroxide precipitation and the results were presented in Fig. 3. The maximum biosorption of Pb(II) was found 16.26% for MBRC whereas for MBAC 84.89% was observed at pH 5 in 60 mg L^{-1} of Pb(II) solution. At pH 6, the adsorption yield for Pb(II) was slightly decreased in MBRC and MBAC. Therefore, all the biosorption experiments were carried out at pH 5. The increased in sorption of metal with increased in pH value can be explained using the analogy between the reaction of metal hydrolysis and the reaction between binding sites of the sorbent and the metal, where in both reactions, the bond for hydrogen is broken and hydrogen ions are released and substituted by the metal [26,27]. Therefore, the electrostatic attraction plays an important role in metal adsorption [28]. As the pH increases, metal adsorption increased since ion exchange is more

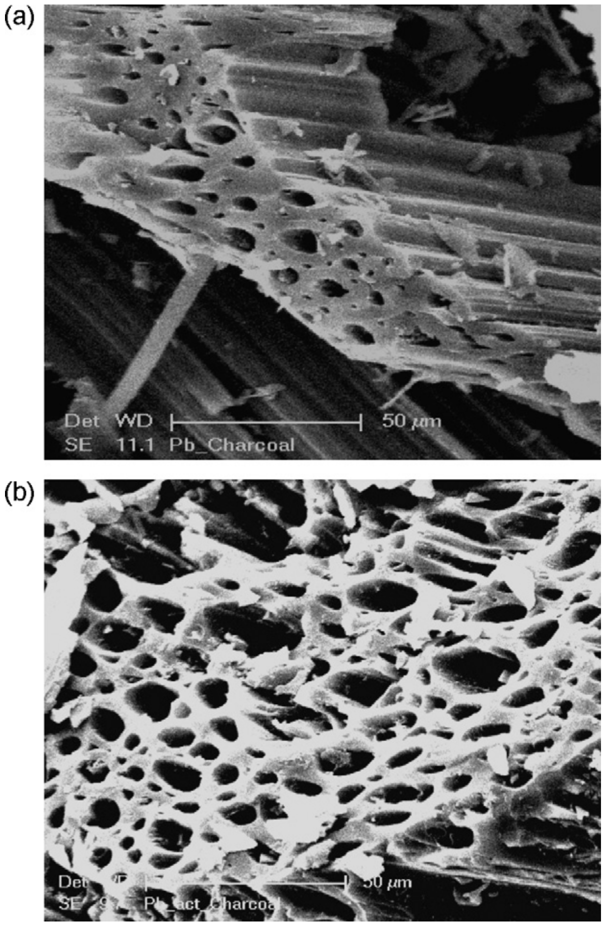

Fig. 2. Scanning electron microscope (SEM) micrograph: (a) Pb(II) loaded *M. baccifera* raw charcoal and (b) Pb(II) loaded *M. baccifera* activated charcoal.

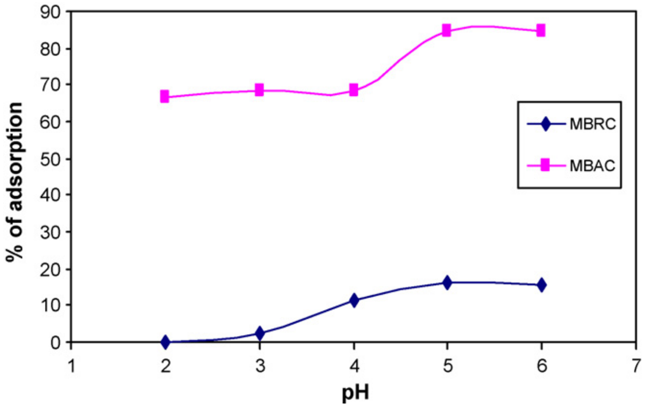

Fig. 3. Effect of pH on Pb(II) removal by *M. baccifera* raw charcoal (MBRC) and *Melocanna baccifera* activated charcoal (MBAC).

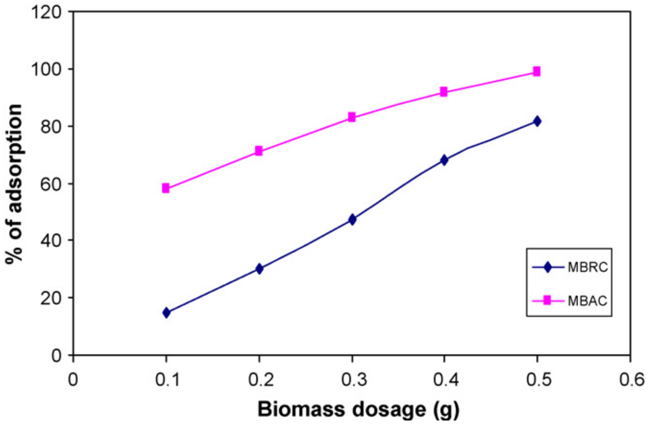

Fig. 4. Effect of adsorbent dosage on Pb(II) removal by *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC).

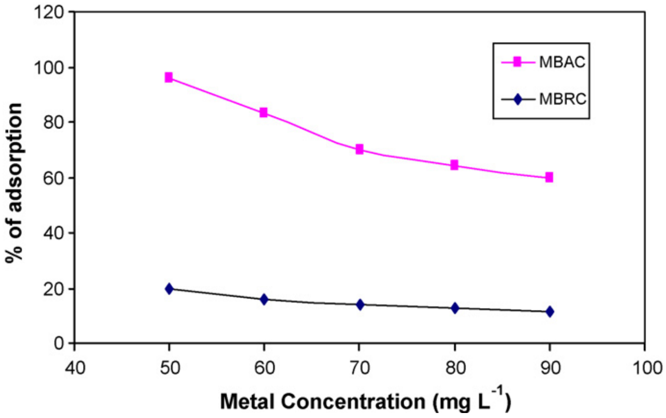

Fig. 5. Effect of initial concentration of Pb(II) concentration 50, 60, 70, 80 and 90 mg L^{-1} onto the *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC).

effective when fewer protons are available to compete with the metal for binding sites onto the adsorbent. At low pH, there was a high concentration of H^+ ions, which competed with Pb(II), resulting in decreased of Pb(II) adsorption. At higher pH, more divalent cation forms disappear, and more soluble or insoluble hydroxylated forms of lead increases [29], the formation of hydroxylated complexes of the metal would also compete with the active sites and as a consequence, the retention would decrease again [30].

3.5. *Effect of adsorbent dosage*

Adsorption of Pb(II) at different adsorbent doses (0.1–0.5 g/100 mL) was analyzed at a constant concentration of metal solution. The percentage of adsorption of Pb(II) was increased from 14.731 to 81.695% for MBRC. Similarly, the percentage of adsorption was increased from 58.81 to 98.76% for MBAC with increased in adsorbent dose as shown in Fig. 4. This can be explained as adsorbent dose increased, more and more surface area will be available which exposed more active sites for binding of metal ions. A similar trend for the effect of adsorbent concentration was also observed and discuss in some literatures [31,32].

3.6. *Effect of initial metal concentration*

Adsorption experiments of Pb(II) concentration from 50 to 90 mg L^{-1} with a fixed adsorbent doses (0.1 g/100 mL) at pH 5 for MBRC and MBAC were performed. For MBRC, Pb(II) removal range from 19.46 to 11.22% whereas for MBAC, Pb(II) removal range from 95.74 to 59.87%. As shown in Fig. 5, the percentage of Pb(II) adsorption decreased with increased in Pb(II) ions concentration and showed little decreased or almost constant in percentage(%) of adsorption at higher concentration. This can be explained that all the biosorbents has a limited number of active sites, which would has become saturated at a certain concentration. Although decreased in percentage (%) of adsorption, the equilibrium adsorption capacity of the biomass increased with increasing Pb(II) ions concentration. The initial concentration provides an important driving force to overcome all mass transfer resistance of metal ions between the aqueous and solid phases, hence a higher initial concentration of metal ion may increase the adsorption capacity [33]. The data obtained from effect of initial concentration were further used to determine the isotherms of Pb(II) biosorption.

3.7. *Effect of contact time*

The effect of contact time (15–360 min) on the biosorption of lead (60 mg L^{-1}) onto MBRC and MBAC (0.1 g) in the solution of pH 5 at 29 °C was shown in Fig. 6. The lead percent adsorption increases with contact time until the equilibrium was attained. Studies conducted on the adsorption kinetics of Pb(II) removal revealed that the majority of metals ions were removed within the first 60–120 min contact with the adsorbents. The equilibrium state was attained at 360 min. It can be seen that the biosorption yield of Pb(II) increases with rise in contact time up to 120 min for MBRC and MBAC. Percentage of maximum biosorption was 13.63% for MBRC and 83.01% for MBAC. Afterwards, there was no a significant increase in Pb(II) adsorption. The fast adsorption at the initial stage is probably due to the increased concentration gradient between the adsorbate in solution and adsorbate in adsorbent as there must be increased number of vacant sites available in the beginning [34]. The initial fast uptake of Pb(II) is through physical adsorption since adsorption phenomenon tends to attain instantaneous equilibrium. As contact time was increased, more and more functional groups participated in adsorption of the metal ions until it reached equilibrium. After it reached the equilibrium, there was no a significance changes in lead concentration in the solution. The data obtained from effect of contact time were further used to determine the kinetics of Pb(II) biosorption.

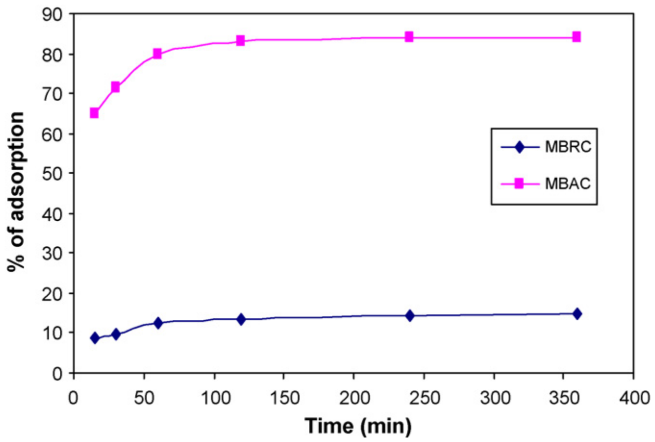

Fig. 6. Effect of contact time on Pb(II) removal by *M. baccifera* raw charcoal (MBRC) and *M. baccifera* activated charcoal (MBAC).

Table 1
Comparison of the pseudo-first-order and pseudo-second-order adsorption rate, the experimental and calculated q_e values of MBRC and MBAC.

Adsorbent	Experimental value	First-order-kinetic model			Second-order-kinetic model		
	q_e (mg g^{-1})	q_e cal. (mg g^{-1})	k_1 (min^{-1})	R^2	q_e cal. (mg g^{-1})	k_2 (g mg^{-1} min^{-1})	R^2
MBRC	8.99	3.786	11.515×10^{-3}	0.963	9.363	6.605×10^{-3}	0.998
MBAC	50.49	10.146	17.042×10^{-3}	0.921	51.282	4.345×10^{-3}	1.00

3.8. Kinetics studies

The several kinetic models are needed to determine the data to examine the mechanism of adsorption process. Pseudo-first-order and pseudo-second-order were used to explain the kinetics of adsorption to investigate the mechanism of biosorption. The Pseudo-first-order Lagergren model [35] for solid/liquid systems of adsorption is expressed as

$$\log(q_e - q_t) = \log\ q_e - k_1 \frac{t}{2.303} \tag{1}$$

where q_e and q_t (mg g^{-1}) are the amounts of lead adsorbed on the biomass at equilibrium and at any time t, in mg g^{-1}, respectively. k_1 (min^{-1}) is the rate constant of pseudo-first-order biosorption. The model is based on the assumption that the rate is proportional to the number of unoccupied sites.

The slopes and intercepts of plot of log ($q_e - q_t$) versus t were used to calculate the first-order rate constant k_1 and equilibrium adsorption capacity q_e. The adsorption kinetics data were further analyzed using Ho and McKay's pseudo-second-order kinetic model [36]. The model is based on the assumption that the rate of sorption is proportional to the square of the number of unoccupied sites. It is expressed as

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{2}$$

where k_2 is the equilibrium rate constant of pseudo-second-order adsorption (g mg^{-1} min^{-1}). The slopes and intercepts of plots t/q_t versus t were used to calculate the pseudo-second-order rate constants k_2 and q_e.

The comparison of the results with the correlation coefficients for both MBRC and MBAC were presented in Table 1. The correlation coefficients of the pseudo-second-order kinetics model for MBRC and MBAC were 0.998 and 1.0 respectively and the experimental q_e values were also agreed well with the calculated data. On the other hand, the correlation coefficients of the pseudo-first-order kinetics model were lower than pseudo-second-order kinetics model in MBRC and MBAC. Therefore, it can be concluded that this adsorption system followed a pseudo-second-order reaction rather than pseudo-first-order reaction.

3.9. Isotherms studies

Adsorption isotherms of Pb(II) concentration from 50 to 90 mg L^{-1} with a fixed adsorbent doses (0.1 g/100 mL) at pH 5 of MBRC and MBAC were studied. Redlich–Peterson (R–P), Langmuir–Freundlich, Freundlich and Langmuir adsorption isotherms were applied to describe the equilibrium relationships between sorbent and sorbate in solution. Sorption equilibrium is established when the concentration of sorbate in the bulk solution is dynamic balance with that of the interface [37].

The R–P equation contains three parameters and incorporates the features of the Langmuir and Freundlich isotherms [38]. The R–P isotherm can be described as follows:

$$q_e = \frac{K_{RP} C_e}{1 + a_{RP} C_e^{\beta}} \tag{3}$$

where K_{RP}, a_{RP} and β are the R–P parameters. β lies between 0 and 1. For $\beta = 1$, the R–P equation convert to Langmuir form. When K_{RP} and a_{RP} are much greater than unity, the equation can transform Freundlich form.

The Langmuir–Freundlich isotherm equation is characterized by the heterogeneity factor, n, and employed to describe the heterogeneity system. The isotherm can be expressed as

$$q_e = \frac{K_{LF} C_e^n}{1 + a_{LF} C_e^n} \tag{4}$$

where K_{LF} is the Langmuir–Freundlich constant (L mg^{-1}). a_{LF} is the affinity coefficient (L mg^{-1}) and n is the heterogeneity coefficient.

The Freundlich isotherm [39] assumes that a monolayer sorption with a heterogeneous energetic distribution of active sites, accompanied by interaction between adsorbed molecules. Its mathematical formula is given as

$$\log\ q_e = \log\ K_f + \left(\frac{1}{n}\right) \log\ C_e \tag{5}$$

where C_e (mg L^{-1}) is the equilibrium concentration, q_e (mg g^{-1}) is the amounts of metal ions adsorbed per specified amount of adsorbent at equilibrium, K_f (mg g^{-1}) and 'n' are constants which are adsorption capacity and intensity of adsorption, respectively. A graft was plotted log q_e versus log C_e. The graft gives a straight line with correlation coefficient (R^2) of 0.855 and 0.679 for MBRC and MBAC, respectively. The slopes and intercepts of the graft were used to calculate the n and K_f values (Table 2).

The Langmuir [40] isotherm assumes that solid surface has a finite number of identical sites which are energetically uniform. According to this model, there is no interaction between adsorbed species, which mean that the amount adsorbed has no influence on the rate of adsorption. A monolayer was formed when the equilib-

Table 2
Redlich–Peterson, Langmuir–Freundlich, Freundlich and Langmuir isotherms parameters for the adsorption of Pb(II) onto MBRC and MBAC.

	Adsorbent	
	MBAC	MBRC
Experimental value		
q_e (mg g^{-1})	53.883	10.104
Redlich–Peterson		
K_{RP} (L g^{-1})	30.03	4.374
a_{RP} ((L mg^{-1})β)	−1.5	−0.319
β	0.849	0.828
R^2	0.9987	0.9993
Langmuir–Freundlich		
K_{LF}	106.382	14.771
a_{LF}	0.789	1.024
n	0.043	0.1704
R^2	0.6322	0.6063
Freundlich		
K_f (mg g^{-1})	46.320	7.730
n	31.153	16.556
R^2	0.679	0.855
Langmuir		
q_{max} (mg g^{-1})	53.760	10.66
b (mg^{-1})	1.317	0.231
R^2	0.996	0.998

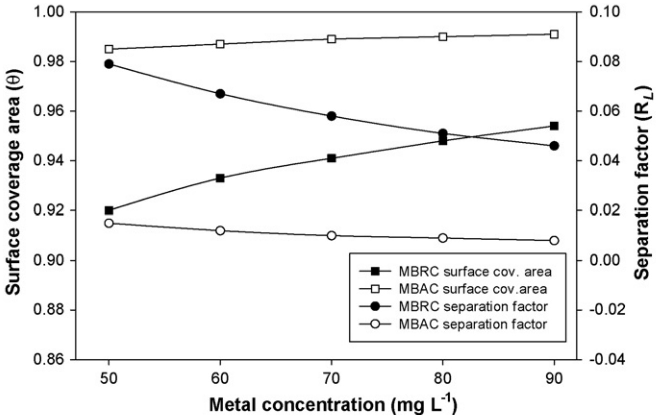

Fig. 7. The calculated separation factor (R_L) and surface coverage (θ) for Pb(II) adsorption plot against Pb(II) ions concentrations (mg L^{-1}).

rium was attained. Its mathematical form is as follow:

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} b} + \frac{C_e}{q_{\max}} \tag{6}$$

where C_e is the concentration of Pb(II) in solution(mg L^{-1}) at equilibrium, $q_{\max}$ (mg solute g^{-1} adsorbent) is the maximum adsorption capacity corresponding to complete monolayer coverage and b (mg^{-1}) is a parameter related to the energy of adsorption, respectively. A graft was plotted C_e/q_e versus C_e gives a straight line with correlation coefficient (R^2) of 0.998 and 0.996 for MBRC and MBAC respectively. The slopes and intercepts of the graft were used to calculate the $q_{\max}$ and b (Table 2). Further the obtained $q_{\max}$ compared with other biosorbents and found to be MBAC is efficient biosorbent (Table 3).

Further, the Langmuir isotherm was used to calculate a dimensionless constant called separation factor or equilibrium parameter (R_L) which tell the favorability of adsorption of Pb(II) onto the biomass. Its mathematical formula was given as:

$$R_L = \frac{1}{1 + bC_i} \tag{7}$$

where R_L is a dimensionless separation factor or equilibrium parameter, b is the Langmuir isotherm constant and C_i (mg L^{-1}) is the initial concentration of Pb(II) ions. The R_L values between 0 and 1 indicated a favorable isotherm. The values of R_L at different concentrations of Pb(II) were calculated for MBRC and MBAC. The calculated R_L values at a different concentrations of Pb(II) fall within a favorable range. A graft was plotted R_L versus C_i (Fig. 7). The graft showed that, with increasing initial concentration of metal ion, the R_L values were decreasing. This indicated that adsorption of Pb(II) ions at higher concentration were also still favorable for both the biomass.

To account for the adsorption behaviour of the metal ions onto the MBRC and MBAC, the Langmuir type equation related to surface coverage (θ) was used. The equation is given as

$$KC_i = \frac{q}{1 - \theta} \tag{8}$$

where K is the adsorption coefficient, C_i is initial concentration of lead ions and θ is the surface coverage. The fraction of biomass surface covered by metal ions was studied by plotting the surface coverage values (θ) versus Pb(II) ions concentration (Fig. 7). As shown in the figure the increase in initial metal ion concentration for Pb(II) increased the surface coverage on the MBRC and MBAC until the surface was nearly fully covered with a monomolecular layer. This indicates that both the biomass will be highly effective in removing Pb(II) ions in aqueous effluent.

R–P parameter (Table 2) describes the adsorption of metal ions. The values of β were closer to unity than 0, which implied that the isotherm was more approaching the Langmuir than the Freundlich isotherm. The Langmuir–Freundlich isotherm confirm the heterogeneity of sorbent surface since $0 < n < 1$. The correlation coefficients of the R–P and Langmuir isotherm model were higher than Freundlich isotherm model for MBRC and MBAC and the experimental q_e values were also agreed well with the calculated data in Langmuir isotherm model rather than Freundlich isotherm model for MBRC and MBAC, respectively. The maximum adsorption capacity (q_{max}) value of MBAC was higher than MBRC; this is attributed by larger surface area of MBAC which had exposed several active binding sites and functional groups for metal ions binding. Therefore, the monolayer coverage process of heavy metal onto charcoals powder was approved by the best fit of equilibrium data in R–P and Langmuir rather than Freundlich isotherm expressions.

3.10. Desorption/reused studies

Adsorption-desorption cycle of Pb(II) was repeated five times in order to show the reusability by saturation of MBRC and MBAC with Pb(II) ions (60 mg L^{-1}) at each cycle. The Pb(II) ions was desorbed from the adsorbents with 0.1 M HCl. More than 77% and 63% were desorbed from MBAC and MBRC respectively. The adsorption capacities of the adsorbents did not noticeably change (only a maximum of 7% and 8% changes was observed) during the repeated adsorption-desorption process (Fig. 8). This result showed that the test adsorbent could be reused without significant losses in its initial adsorption capacity.

Metalliferous adsorbent from different experiments has been pooled (a few milligrams only) and sent to local hazardous waste disposal site.

Table 3
Comparison of biosorption capacity of Pb(II) by MBRC and MBAC and other found in the literatures.

Adsorbent	Biosorption capacity (mg g^{-1})	pH	Reference
Penicillium simplicissimum	76.90	5.0	[46]
Melocanna baccifera act. charcoal	53.76	5.0	This study
Crushed concrete fines	37.0	–	[47]
Cephalosporium aphidicola	36.91	5.0	[48]
Mustard husk	30.48	6.0	[49]
Caulerpa lentillifera	28.7	5.0	[50]
Coconut shell activated carbon	21.88	–	[51]
Powder activated carbon	20.7	5.0	[52]
Coir (*Coco nucifera*)	18.9	–	[53]
Waste bakers yeast in ethanol	17.49	5.0	[54]
Fly ash	15.08	6.0	[55]
Lichen (*Cladonia furcata*)	12.3	5.0	[56]
Melocanna baccifera charcoal	10.66	5.0	This study

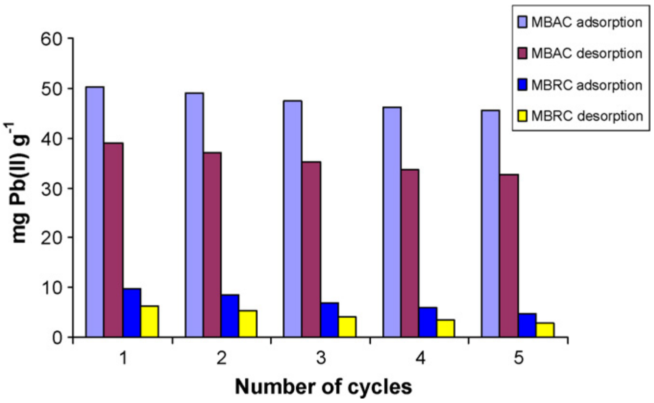

Fig. 8. Adsorption–desorption study of Pb(II) removal.

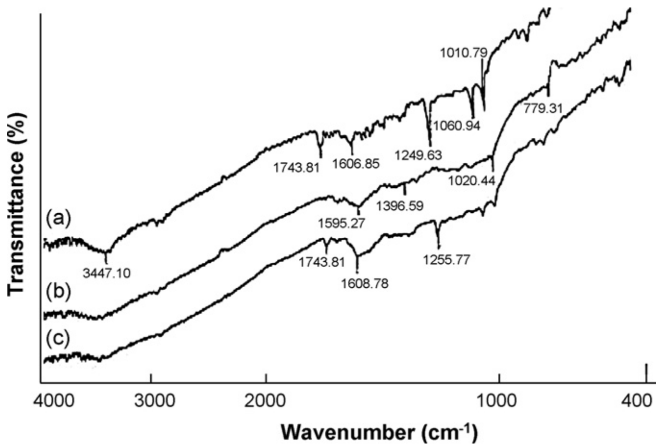

Fig. 9. FTIR spectra of (a) Pb(II) unloaded charcoal, (b) Pb(II) loaded *M. baccifera* activated charcoal and (c) Pb(II) loaded *M. baccifera* raw charcoal.

3.11. FTIR analysis

The FTIR spectral analysis is important to identify some characteristic functional groups which are responsible of adsorbing metal ions [41–44]. The FTIR spectra of dried unloaded charcoal, Pb(II)-loaded charcoal and Pb(II)-loaded activated charcoal were examined to determine the possible interactions between the functional groups and the metal ions and presented in Fig. 9. The FTIR spectra of the adsorbents were recorded in the range of 400–4000 cm^{-1} to obtain the information regarding stretching and bending vibrations of the functional groups which are involved in the adsorption of metal ions. The spectrum of dried unloaded charcoal exhibits a broad band at 3447.10 cm^{-1} due to the presence of hydroxyl groups on the biomass surface. The peak located 1743.21 cm^{-1} is due to the presence of carbonyl functional group. The peak at 1606.85 cm^{-1} is due to stretching vibration of C=O. The adsorption peak at 1259.63–1010.79 cm^{-1} are due to C—H bending and C—O stretching vibration.

Comparing Pb(II) unloaded charcoal with Pb(II) loaded *M. baccifera* raw charcoal and Pb(II) loaded *M. baccifera* activated charcoal. The peak at 3447.10 cm^{-1} was disappeared in Pb(II) loaded activated charcoal and Pb(II) loaded raw charcoal. 1743.21 cm^{-1} peak was disappear in Pb(II) loaded activated charcoal and the peak size was reduced in Pb(II) loaded raw charcoal. The peak at 1606.63 cm^{-1} was shifted to 1595.27 cm^{-1} in Pb(II) loaded activated charcoal and 1608.78 in Pb(II) loaded raw charcoal. The peak at 1259.63 cm^{-1} was shifted to 1396.59 cm^{-1} in Pb(II) loaded activated charcoal and 1255.77 in Pb(II) loaded raw charcoal, also greatly reduced in size. The peak at 1010.79 cm^{-1} was shifted to 1020.44 cm^{-1} in Pb(II) loaded activated charcoal and reduced in size in Pb(II) loaded raw charcoal. The region below 1000 cm^{-1} is the 'fingerprint zone' and the adsorption can not clearly be assigned to any particular vibration because they correspond to complex interacting vibration systems [45]. The relevance explanation of a shift in the spectra, reduction and disappearance of the peaks were that there is an effect of chemical pretreatment and metal biosorption on the functional groups.

4. Conclusion

The effect of chemical pretreatment on the adsorption of Pb(II) ions by MBRC and MBAC showed that 60% KOH pretreatment shows highest percentage of adsorption. The maximum biosorption of Pb(II) was found at pH 5. Maximum adsorption capacity was 10.66 mg g^{-1} for MBRC and 53.76 mg g^{-1} for MBAC. Maximum biosorption was attained at 120 min for MBRC and MBAC. Afterwards, there was no a significant increase in Pb(II) adsorption. The rate of adsorption was best described by pseudo-second-order reaction. The adsorption isotherm was best described by Langmuir isotherm. The FTIR analysis of MBRC and MBAC showed that different functional groups are involved in the adsorption of the metal ions. From these results, MBRC and MBAC can be efficiently used for the removal of Pb(II) from the aqueous solution.

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Full Length Research Paper

Comparative results of RAPD and ISSR markers for genetic diversity assessment in *Melocanna baccifera* Roxb. growing in Mizoram State of India

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The genetic similarity among 12 accessions of *Melocanna baccifera* from Mizoram in India was tested using RAPD and ISSR markers. RAPD and ISSR DNA profile shows high polymorphism of DNA fragments. Genetic similarity among accessions was calculated according to Dice similarity coefficient; the mean level of genetic similarity with populations of *M. baccifera* by using RAPD and ISSR markers were 0.600 and 0.650, respectively. The similarity coefficients were then used to construct a dendrogram using the UPGMA cluster analysis. Cluster analysis base on Dice coefficient shows two major groups. Mantel test revealed the different distribution pattern of the polymorphism between RAPD and ISSR markers and the correlation coefficient (r) was found as r = 0.1902.

Key words: *Melocanna baccifera*, ISSR, RAPD, genetic similarity.

INTRODUCTION

The economy of India and so also of many Asian countries depends on bamboos and their uses are not only in domestic items but also in rural housing and raw materials to several industries. In the state of Mizoram (India), bamboo forest covers 12,54,400 ha, out of the total area of 21,090 sq.km. These bamboo forest areas of Mizoram constitute 14% of the total India's bamboo area, that is, 8.96 million ha. Non-clump forming bamboo *Melocana baccifera* is the most abundant and most economical bamboo in Mizoram, contributing 95% of the growing stock of bamboo. It belongs to members of subfamily Bambusoideae of family Poaceae and flowering is at a regular interval of 48 years, the culm die after flowering. Extensive field survey was carried out during the flowering of this bamboo. It was found that, there was a difference in the seeds colour of *M. baccifera* in some accessions (shown in Figure 1). In order to analyze variation in their gene pool, some accessions were collected. The places of collection were shown in the map of Figure 2. Traditionally, morphological characters like growth habit, leaf type and floral morphology have been used to define taxa (Nayak et al., 2003) this is still poor taxonomic description. To date no molecular analysis has been performed to test genetic relationship of *M. baccifera* period. The random amplified polymorphic DNA (RAPD) marker technique is quick, easy and required less time. This detects nucleotide sequence polymorphisms using a single primer of arbitrary nucleotide sequence (Williams et al., 1990), inter-simple sequence repeat (ISSR) permits detection of polymorphisms in inter-microsatellite loci, using a primer designed from dinucleotide or trinucleotide simple sequence repeats. RAPD and ISSR markers have been extensively used for DNA finger-printing (Moreno et al., 1998), genetic diversity studies (S'anchez de la Hoz et al., 1996; Esselman et al., 1999), population genetic studies (Wolfe et al., 1998; Nebauer et al., 1999) and phylogenetic studies (Hess et al., 2000)

The objectives of the present research are to study the genetic similarity within *M. baccifera,* to compare RAPD and ISSR markers for the molecular characterization of *M. baccifera* and to study phylogenetic relationships in *M. baccifera*.

MATERIALS AND METHODS

Collection

An extensive field survey was carried out, that is, from March-

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Figure 1. Accessions of *Melocanna baccifera* showing different accessions characters; (a) non-flowering accession (green patch) surrounded by brownish-white colour flowering culms, (b) green colour fruit, (c) red colour fruit and (d) blackish brown colour fruit.

August 2007 throughout Mizoram (India) during the flowering of *M. baccifera*. Twelve accessions of *M. baccifera* were collected from the study area. One accession was collected in the form of leaf and the remaining 11 accessions were collected in the form of seeds. All the seeds collected were sown in the experimental field in Hyderabad University campus. The accession names, form of collection and morphological characteristics are given in Table 1.

DNA extraction

Genomic DNA of every sample was isolated from the frozen leaf according to the CTAB protocol of Doyle and Doyle (1990) with minor modification.

RAPD analysis

Forty arbitrary decamer primer sets of Operon Kits A and C (India) were tested on the genomic DNA of two accessions and nine primers showed reproducible amplified products were chosen for this investigation. Primers used are given in the Table 2. Amplification was performed in a 15 µl reaction volume, containing 50 µg template DNA, 1x PCR buffer, 0.3 mM each dNTP, 2 mM of $MgCl_2$, 1.5 µM of each primer and l unit of Taq DNA Polymerase (Sigma Aldrich). Amplification was performed in My-gene^{TM} series peltier thermal cycler (Longgene Scientific Instruments Co., Ltd. Hang zhou). Initial denaturation was for 4 min at 94°C, followed by 45 cycles of 50 s at 94°C, 1 min at 37°C, 2 min at 72°C and 10 min at 72°C for final extension step. The amplified products were

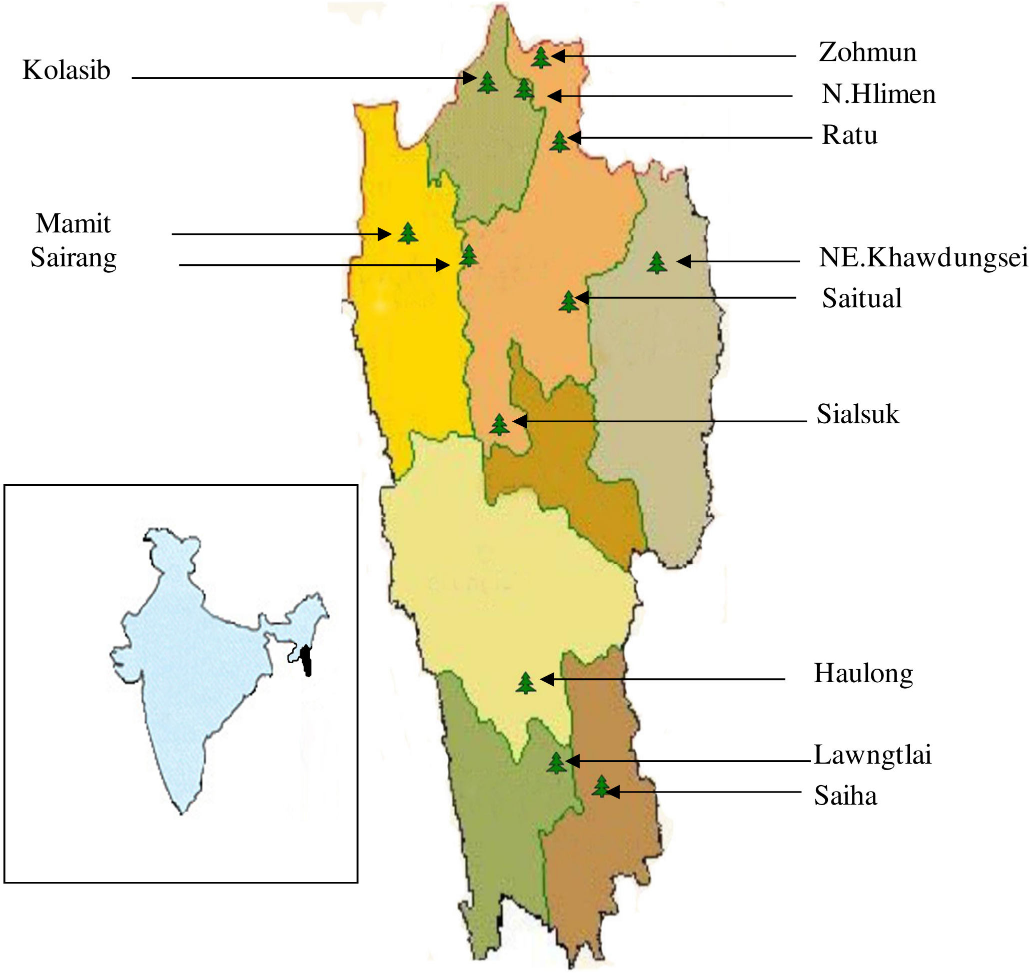

Figure 2. Hydrographic map of the Mizoram, showing the collection sites of *Melocanna baccifera*.

Table 1. Bamboo (*Melocanna baccifera* Roxb.) accessions collected in different regions of Mizoram.

S/N	Accessions name	Sample	Accessions character
1.	N.Hlimen	Seeds	Culm bears red colour fruit
2.	Haulong	Seeds	Culm bears green colour fruit
3.	Kolasib	Seeds	Culm bears green colour fruit
4.	Lawngtlai	Seeds	Culm bears green colour fruit
5.	Mamit	Seeds	Culm bears green colour fruit
6.	NE. Khawdungsei	Seeds	Culm bears green colour fruit
7.	Ratu	Seeds	Culm bears green colour fruit
8.	Saiha	Seeds	Culm bears green colour fruit
9.	Sairang	Seeds	Culm bears green colour fruit
10.	Sialsuk	Seeds	Culm bears blackish-brown colour fruit
11.	Saitual	Seeds	Culm bears green colour fruit
12.	Zohmun	Leaves	Non-flowering culms

separated on 1.2% agarose gels and stained with ethidium bromide. Images were captured using Uvidoc (Uvitech, UK) gel documentation system.

ISSR analysis

Forty five ISSR primers sets from University of British Columbia

Table 2. The statistical data for 9 RAPD and 17 ISSR primers used for analyzing 12 accessions of *M. baccifera*.

Primer	Sequence	Annealing temperature	TNB	NPB	PB(%)
RAPD					
OPA-05	$5^/$-AGGGTCTTG-$3^/$	37^0C	5	4	80%
OPA-09	$5^/$-GGGTAAGGCC-$3^/$	37^0C	8	7	87.5%
OPA-10	$5^/$-GTGATCGCAG-$3^/$	37^0C	20	19	95%
OPA-17	$5^/$-GACCGCTTGT-$3^/$	37^0C	20	19	95%
OPC-02	$5^/$-GTGAGGCGTC-$3^/$	37^0C	7	7	100%
OPC-04	$5^/$-CCGCATCTAC-$3^/$	37^0C	5	5	100%
OPC-07	$5^/$-GTCCCGACGA-$3^/$	37^0C	9	8	88.8%
OPC-11	$5^/$-AAAGCTGCGG-$3^/$	37^0C	11	10	90.9%
OPC-15	$5^/$-GACGGATCAG-$3^/$	37^0C	9	9	100%
Total			94	88	
Average			10.4	9.7	98.02%
ISSR					
UBC-810	$5^/$-GAGAGAGAGAGAGAGAT-$3^/$	52^0C	10	9	90%
UBC-813	$5^/$-CTCTCTCTCTCTCTCTT-$3^/$	55^0C	11	10	90.9%
UBC-814	$5^/$-CTCTCTCTCTCTCTCTA-$3^/$	52^0C	5	4	80%
UBC-815	$5^/$-CTCTCTCTCTCTCTCTG-$3^/$	52^0C	4	2	50%
UBC-820	$5^/$-GTGTGTGTGTGTGTGTC-$3^/$	57^0C	5	5	100%
UBC-821	$5^/$-GTGTGTGTGTGTGTGTT-$3^/$	55^0C	7	5	71.4%
UBC-822	$5^/$-TCTCTCTCTCTCTCTCA-$3^/$	55^0C	8	8	100%
UBC-823	$5^/$-TCTCTCTCTCTCTCTCC-$3^/$	55^0C	8	7	87.5%
UBC-824	$5^/$-TCTCTCTCTCTCTCTCG-$3^/$	55^0C	4	4	100%
UBC-827	$5^/$-ACACACACACACACACG-$3^/$	57^0C	8	7	87.5%
UBC-828	$5^/$-TGTGTGTGTGTGTGTGA-$3^/$	57^0C	5	4	80%
UBC-830	$5^/$-TGTGTGTGTGTGTGTGG-$3^/$	57^0C	5	4	80%
UBC-846	$5^/$-CACACACACACACACART-$3^/$	55^0C	8	8	100%
UBC-847	$5^/$-CACACACACACACACARC-$3^/$	56^0C	9	7	77.7%
UBC-856	$5^/$-ACACACACACACACACYA-$3^/$	55^0C	7	6	85.7%
UBC-857	$5^/$-ACACACACACACACACYG-$3^/$	56^0C	12	12	100%
UBC-859	$5^/$-TGTGTGTGTGTGTGTGRC-$3^/$	56^0C	4	2	50%
Total			120	104	
Average			7.05	6.1	84.1%

R = A/G; Y = C/T; TNB = total number of bands; NPB = number of polymorphic bands; PB (%) = percentage of polymorphic band.

(Vancouver, Canada) were used to amplify the genomic DNA of two accessions and 17 primers were selected (Table 2). Amplification was preformed in a 15 µl reaction volume, containing 50 ng template DNA. 1X PCR buffer, 0.4 mM each of dNTP, 2 mM of $MgCl_2$, 1.0 µM each of primer and 1 unit of Taq. DNA polymerase (Sigma Aldrich). Amplification was performed in My-Gene^{TM} series peltier thermal cycle (Longgene Scientific Instruments Co., Ltd. Hangzhou). Initial denaturation was for 7 min at 94^0C, followed by 45 cycles of 1 min at 94^0C, 1 min at 54^0C, 2 min at 72^0C and a 10 min final extension step at 72^0C. The amplified products were separated on 1.2% agarose gels and stained with ethidium bromide. Images were captured using Uvidoc (Uvitech, UK) gel documentation system.

Data analysis

The amplified DNA fragments for each accession were scored as present (1) or absent (0). Data generated by nine RAPD and 17 ISSR primers were used to compile a binary matrix for cluster analysis. Genetics similarity among accessions was calculated according to Dice similarity coefficient (Dice, 1945). The similarity co-efficients were then used to construct a dendrogram using the UPGMA (Unweighted Pair-Group Method with Arithmetical average) cluster analysis (Sneath and Sokal, 1973). Similarity matrices based on RAPD and ISSR markers were compared using the mantel matrix-correspondence test (Mantel, 1967). All procedures were computed with the computer package NTSYS-pc

(Numerical Taxonomy and Multivariate Analysis System, Biostatistics, New York, USA, software version 2.02j package) (Rohlf, 1993).

RESULTS

Identification of genetic relationships in *M. baccifera* is very difficult because of the lack of morphological differences. The traditional methods of identifying DNA profiling are largely used for identification because of several limitations of morphological data. In recent years, RAPD and ISSR technology is a rapid and sensitive technique which can be used to estimate relationship between closely, and more distantly accessions.

Polymorphisms detected by RAPD and ISSR markers

The polymorphism of the *M. baccifera* germplasm was high. A total of 40 arbitrary RAPD primers were used for screening, out of these 9 primers were selected out for twelve accessions of *M. baccifera* DNA amplification, which produced clear bands for further analysis. The nine arbitrary primers used for RAPD analysis detected a total of 94 fragments, with an average of 10.4 fragments per primers and 98.02% fragments were polymorphic. 45 arbitrary ISSR primers were used for screening, out of these 17 primers were selected. A Total of 120 fragments were detected, with an average of 7.05 fragments per primer and 84.1% fragments were polymorphic. The DNA fragments produced by RAPD and ISSR markers were shown in Figure 3.

Genetics similarities base on RAPD and ISSR markers

The genetic similarities values based on RAPD and ISSR markers were different. The maximum genetic similarity by RAPD was 0.872 between Saiha and Sairang, while the lowest genetic similarity of 0.430 was between Mamit and N. Hlimen (Table 3). The maximum genetic similarity observed by ISSR was 0.769 between Kolasib and Sialsuk, while the lowest genetic similarity of 0.534 was between Saitual and N. Hlimen.

Phylogenetic analysis based on RAPD and ISSR

The phylogenetic relationships among 12 accessions of *M. baccifera* were analyzed by an UPGMA method (Figure 4). The cluster result indicated that all the 12 accessions could be distinguished by RAPD and ISSR markers, respectively. But the relationship among the accessions revealed by RAPD analysis were somewhat different from that revealed by the analysis of ISSR data. The cluster analysis of RAPD marker showed variation of a geographic distribution as all the accessions could be separated into two major groups. Majority of accessions, that is, 11 out of 12 accessions formed one cohesive cluster and the one N. Hlimen was resolved in another cluster. The bigger group was again sub-grouped in two smaller clusters containing Haulong, Saiha and Sairang in cluster I and Kolasib, Lawngtlai, Mamit, NE. Khawdungsei, Saitual, Sialsuk, Ratu and Zohmun in cluster II. It can be observed from the dendrogram that the variation between accessions Saiha and Sairang was the least in cluster I with similarity coefficient of 0.87. It can be further noted that N. Hlimen was the most diverse accession within the cluster.

The dendrogram obtained from UPGMA cluster analysis of genetic distance based on 17 ISSR markers is shown in Figure1b; the cluster analysis resolved 12 accessions into two major groups. Only Saiha accession form group I and the group II contained other eleven accessions. Group II could be further divided into 3 clusters. Cluster I consist of Saitual, NE. Khawdungsei and Ratu accessions. Cluster II consists of Sairang, Mamit, Zohmun, Sialsuk and Kolasib accessions and cluster III consists of Haulong, Lawngtlai, N. Hlimen. It can be observed from the dendrogram, the variation between Kolasib and Sialsuk accessions was the least with similarity coefficient of 0.77. Saiha was the most diverse accession.

Comparison of RAPD and ISSR markers

It is important to understand that different markers have different properties and will reflect different aspects of genetic diversity (Karp et al., 1995). RAPD (William et al., 1990) and SSR (Zietkiewicz et al., 1994) markers have advantages and disadvantages for assessing genetic diversity. In this work, the RAPD and ISSR surveys among 12 accessions of *M. baccifera* revealed 98.02 and 84.1% of polymorphic bands, respectively (nearly same). Mantel test revealed the different distribution patterns of the polymorphism between RAPD and ISSR markers and the correlation coefficient(r) was found as r = 0.1902.

DISCUSSION

RAPD and ISSR markers are proved to be efficient and inexpensive way to provide molecular data. They have been used successfully in determining genetic relationship and used for DNA fingerprinting (Moreno et al., 1998; Blair et al., 1999; Divaret et al., 1999; Gilbert et al., 1999). Researchers who have compared RAPD and ISSR methods have found that ISSR markers exhibit higher level of polymorphism or reproductivity compared

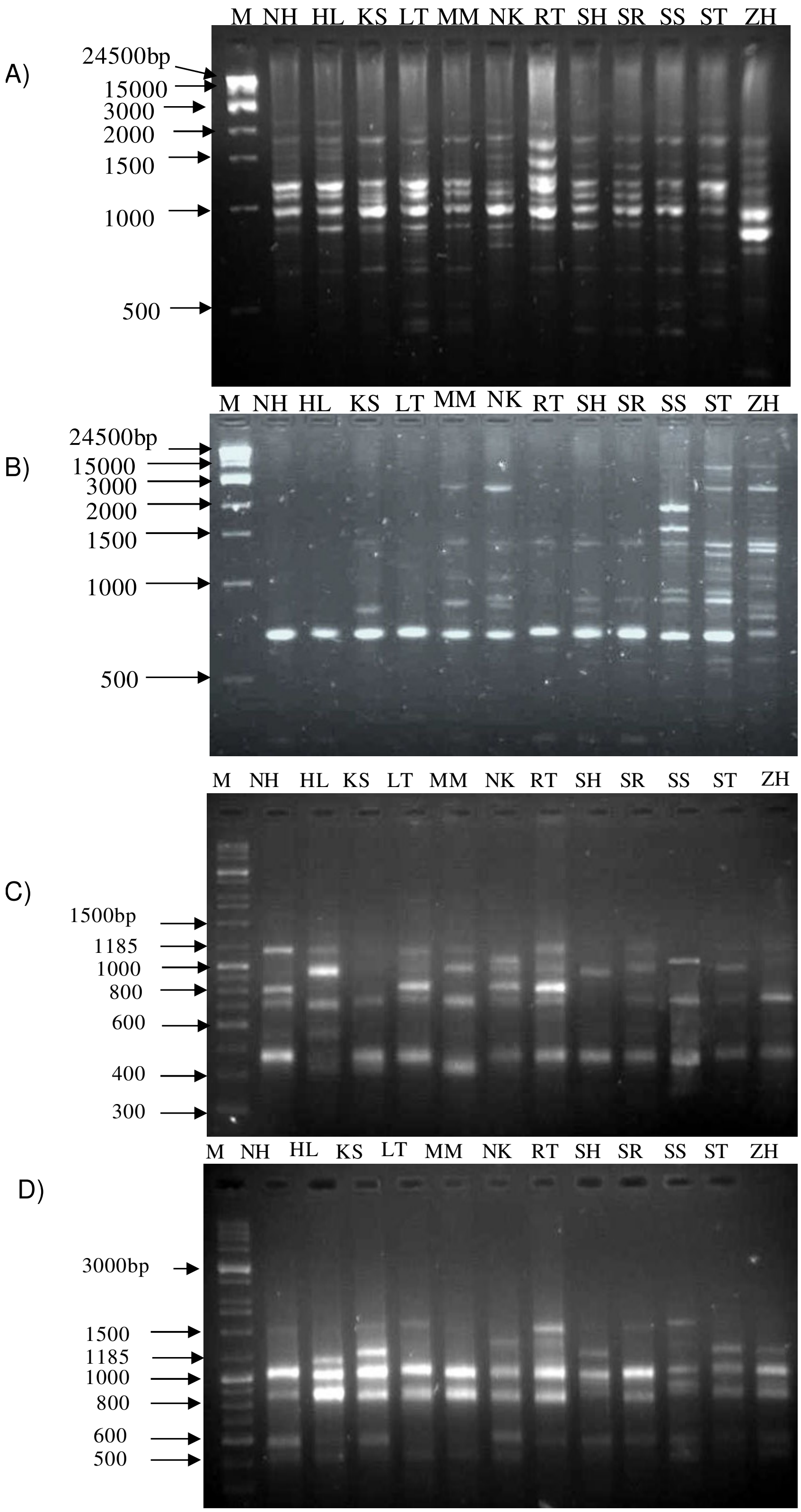

Figure 3. (A) RAPD bands amplified by the primer OPA-10. (B) RAPD bands amplified by the primer OPA-17. (C) ISSR bands amplified by the primer UBC-821. (D) ISSR bands amplified by the primer UBC-823. M = marker, NH = N.Hlimen, HL = Haulong, KS = Kolasib, LT = Lawngtlai, MM = Mamit, NK = NE. Khawdungsei, RT = Ratu, SH = Saiha, SR = Sairang, SS = Sialsuk, ST = Saitual, ZH = Zohmun.

Table 3. (A) Similarity matrix of *M. baccifera* accessions based on RAPD marker, and (B) similarity matrix of M. baccifera based on ISSR marker generated from Dice estimation of similarity based on the number of shared fragments.

(A)	NH	HL	KS	LT	MM	NK	RT	SH	SR	SS	ST	ZH
NH	1											
HL	0.597	1										
KS	0.567	0.696	1									
LT	0.542	0.608	0.724	1								
MM	0.430	0.625	0.656	0.656	1							
NK	0.542	0.695	0.724	0.583	0.656	1						
RT	0.513	0.657	0.657	0.526	0.507	0.578	1					
SH	0.500	0.677	0.711	0.580	0.631	0.677	0.545	1				
SR	0.507	0.741	0.677	0.615	0.700	0.646	0.521	0.872	1			
SS	0.500	0.586	0.693	0.564	0.575	0.666	0.585	0.647	0.647	1		
ST	0.485	0.579	0.550	0.527	0.626	0.722	0.473	0.612	0.646	0.692	1	
ZH	0.512	0.545	0.623	0.550	0.533	0.575	0.476	0.485	0.493	0.534	0.525	1

(B)	NH	HL	KS	LT	MM	NK	RT	SH	SR	SS	ST	ZH
NH	1											
HL	0.660	1										
KS	0.701	0.673	1									
LT	0.683	0.672	0.623	1								
MM	0.626	0.647	0.635	0.654	1							
NK	0.579	0.597	0.606	0.549	0.640	1						
RT	0.656	0.626	0.615	0.666	0.661	0.618	1					
SH	0.538	0.659	0.625	0.585	0.556	0.606	0.560	1				
SR	0.691	0.742	0.707	0.666	0.760	0.630	0.672	0.696	1			
SS	0.696	0.686	0.769	0.691	0.723	0.680	0.643	0.638	0.762	1		
ST	0.534	0.615	0.623	0.604	0.638	0.627	0.596	0.626	0.674	0.681	1	
ZH	0.615	0.691	0.678	0.642	0.690	0.607	0.650	0.646	0.725	0.747	0.650	1

with RAPD markers (Fang and Roose, 1997; Esselman et al., 1999; Parsons et al., 1997; Qian et al., 2001). The result of the present study showed that ISSR markers were not more informative than RAPD markers, however, both of the markers worked effectively. The polymorphism of the *M. baccifera* germplasm was high. Based on RAPD and ISSR markers data, the percentage of the polymorphic fragments were 98.02 and 84.1% respectively. The reason why the level of polymorphism in *M. baccifera* was high might be that the accessions are located in the different region for rather long time. In the process of the natural selection, the different populations with different genotypes adapted to the different environment were preserved and the accessions of *M. baccifera* mainly depend on their underground rhizomes as the propagating organs, since the flowering cycle is 48 - 50 years. Thus the genetic diversity of *M. baccifera* was well conserved; this might lead to the abundant genetic diversity of *M. baccifera* in this region.

The mean level of genetic similarity with populations of *M. baccifera* by using RAPD and ISSR markers are 0.600 and 0.650, respectively. This pattern of genetic diversity may be caused and maintained by effective gene flow within populations and high fecundity. From this, it is evident that the accessions from different geographical locations exhibited a wide range of genetic distance, which did not show any correlation with geographical distances between the collection sites, negating to ‘spatial autocorrelation’ concept. If the population is more or less continuous, allele frequencies will fluctuate with distance due to genetic drift and selection (Sokal et al., 1989). Wright (1965) predicted that every finite population will experience genetic drift. But that the effects of such drift become more pronounced as population size decreases. Population with continually small effective population size will be especially susceptible to the loss and the re-organization of variation by genetic drift (Barrett and Kohn, 1991; Frankel and Soule, 1981).

Cluster analysis based on Dice coefficient by RAPD markers given in Figure 1a show two major groups. The accessions collected from Zohmun and N.Hlimen were a relative distance of 30 km was found in separate groups. Though most geographically closer localities found in separate groups showed that they are genetically distant.

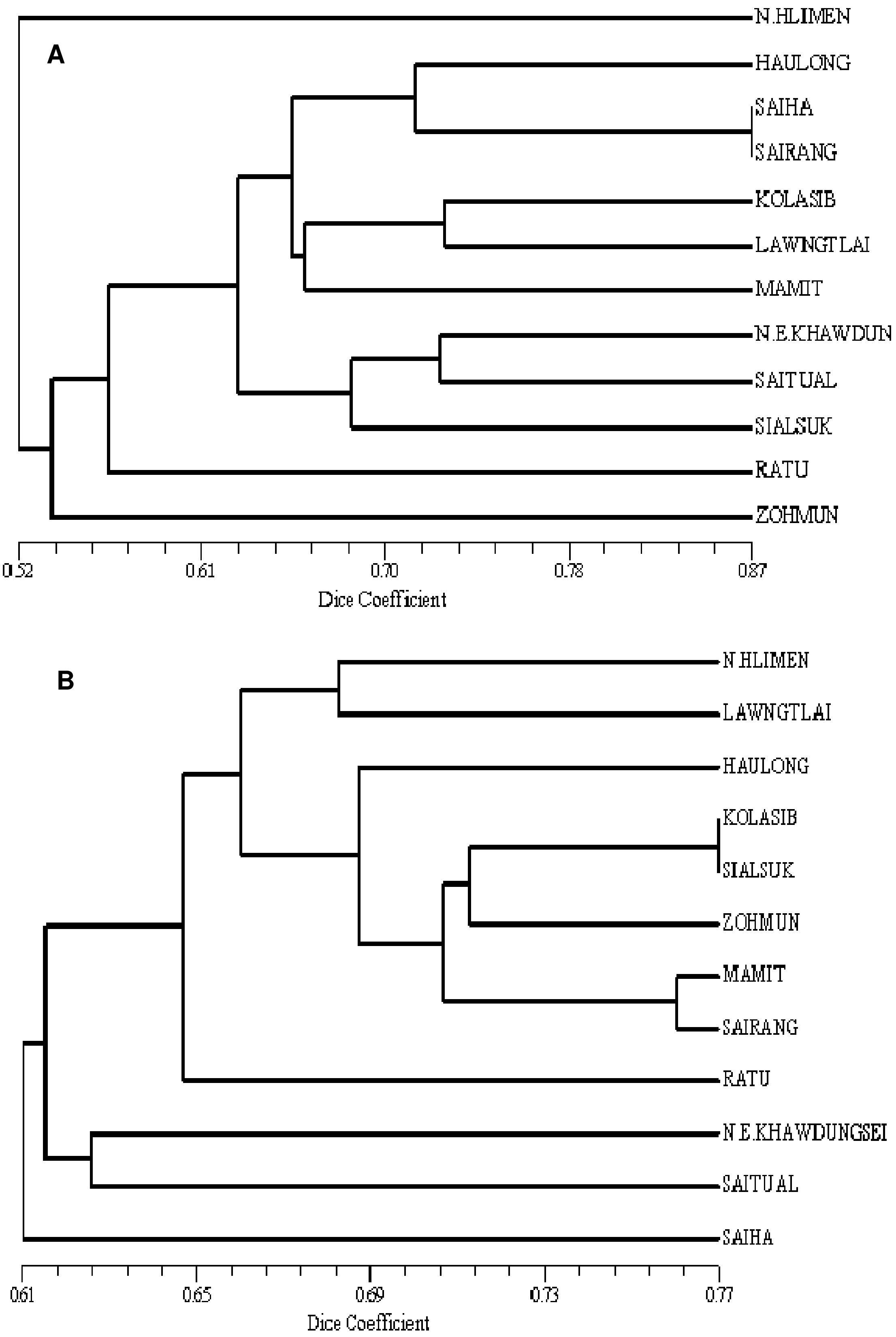

Figure 4. (A) Dendrogram generated using UPGMA method illustrating the genetic diversity relationships among 12 accessions of *M. baccifera* by RAPD marker. (B) Dendrogram generated using UPGMA method illustrating the genetic diversity relationships among 12 accessions of *M. baccifera* by ISSR marker.

This shows that there is no correlation between genetic make up and geographical distances. The genetic uniformity or non-uniformity is reflected by the genetic distance value as described by Das and Mukherjee, (1997.)

Similarly, from cluster analysis based on Dice coefficient by ISSR markers shown in Figure 4b, two main groups were observed. The accessions collected from Kolasib and Sialsuk showed closest affinities; exhibit a genetic identity of 0.77. Though they are quite distant apart geographically and link with Zohmun, Mamit and Sairang to form a well defined cluster. The possible explanation for the accessions diversity found in *M. baccifera* populations is somatic mutation. This bamboo plants can live very long and somatic mutations may occur in their gene pool which may eventually lead to some variations. Some researchers have considered RAPD markers to represent segments of DNA with non-coding regions and to be selectively neutral (Bachmann, 1997; Landergott et al., 2001). Similar opinions have been reported with regard to ISSR markers (Esselman et al., 1999). Some studies have shown that RAPD markers are found throughout the genome and may be associated with functionally important loci (Penner, 1996).

The RAPD and ISSR surveys between 12 accessions of *M. baccifera* revealed 98.02 and 84.1% of polymorphic bands, respectively. The results obtained from cluster analysis based on RAPD and ISSR data sets were different. This was also reflected in the correlation coefficient calculated for the elements of RAPD and ISSR similarity matrices by using Mantel test. Although the value of correlation coefficient between RAPD and ISSR markers was significant as $r = 0.1902$, it shows a very poor fit between two markers, according to its interpretation ($0.7 \le r < 0.8$ poor fit; $r < 0.7$ very poor fit). This inferred that the two sets of markers explore genetic variation differently. It is likely that RAPD and ISSR target different regions of genome which are subjected to different mechanisms generating genetic variation. Genomic regions sampled by the RAPD and ISSR markers maintain a different evolutionary process under selection. In summary RAPD and ISSR markers were a powerful dominant DNA markers, they are effective and promising markers for assessing genetic variation in *M. baccifera* species.

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Flowering of *Melocanna baccifera* (Bambusaceae) in northeastern India

Flowering of bamboo is a botanical enigma. The factors responsible for this are still not clearly established. Bamboos flower only once and die after flowering to regenerate from seeds[1]. The strange phenomenon of simultaneous flowering in bamboo clumps in vast areas is called gregarious flowering and causes ecological havoc. The bamboo clumps die after flowering and it takes a few years before bamboo plants produce seeds again, leaving bare, exposed soil which could be disastrous in mountainous regions. This would lead to food scarcity, since several animals depend on this plant. The second factor is that rats feed on the flowers and seeds of the dying bamboo tree. This activates a rapid birth rate among the rodents, which leads to the huge rat population feeding on agricultural crops in the fields and granaries, thus leading to famine. This had happened in Mizoram[2] in the late 1950s.

Melocanna baccifera is a sympodial bamboo growing to about 20 m height Unlike other sympodial bamboos, the rhizomes are long and so rather than growing as compact clumps, *M. baccifera* produces groves of widely spaced culms, more akin to those of large monopodial bamboos. It is an aggressive colonizer and often forms the dominant vegetation on the tropical and subtropical hill slopes on which it grows. It is naturally distributed in a swathe cutting south to north from southwestern Myanmar through western central and northern Myanmar and the Chittagong hill tracts of the eastern to the northeastern states of India, where it represents between 60 and 95 regional bamboo resources.

The first recorded flowering of *M. baccifera* was in 1863; various periods of vegetative growth prior to flowering have been noted in different locations and there is a wealth of reports that give a reliable picture of the flowering cycle of this species. A study by the Jorhat-based Rain Forest Research Institute (RFRI) has estimated that the 'gregarious flowering' of *M. baccifera* or Muli bamboo will occur in several northeastern states in 2004, over an area of 18,000 km^2. The states identified are Mizoram (Figure 1 *a–d*), Tripura, Manipur, Meghalaya and parts of Assam. According to the estimate of INBAR[3], flowering of *M. baccifera* occurred in approximately 1.76 m ha in the northeastern states of India during 2004–08 and about 26 mt of bamboo will flower and die. Millions of people who depend directly or indirectly on Muli bamboo for their livelihood will suffer, and if famine strikes the whole populace of the region could be at risk. The neighbouring regions of Bangladesh and Myanmar could also be equally affected.

The large fruits of Muli bamboo are also eaten by non-human consumers and the sudden enormous increase in their availability will cause a rapid explosion in the rat populations, whose numbers are related to the availability of food (Figure 1 *e* and *f*). The rats not only devastate the naturally regenerating seeds and seedlings, and thereby reduce the regeneration rate, but also destroy other crops and stored grains. Such famines have occurred in the Lushai hills of Assam (as Mizoram was known before its independence)[4], in 1815, 1863, 1911 and 1959. Throughout the North East, the flowering-induced famine in 1959 claimed 10,000 to 15,000 lives, and flowering in 1881 also claimed a similar number[5].

During December 2005, we observed the flowering of *M. baccifera* in Nongpoh (91°52′E, 25°53′N) in the East Khasi Hills, Meghalaya.

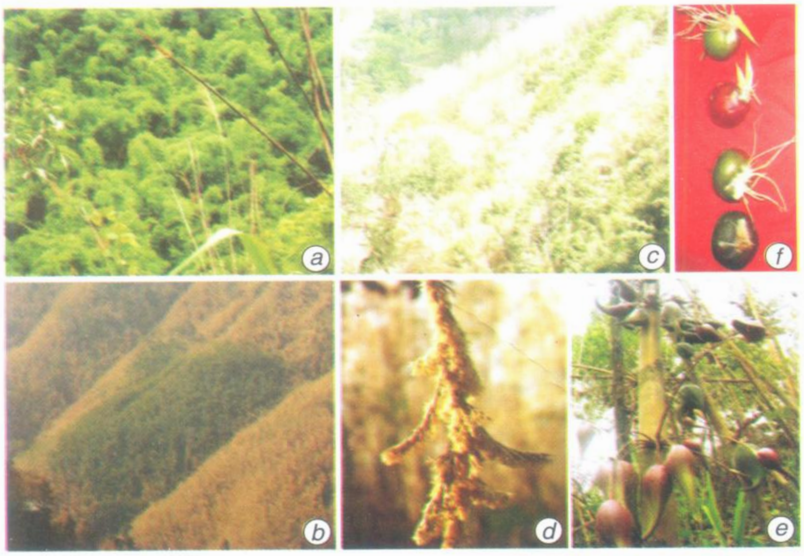

Figure 1. ***a***, *Melocanna baccifera* without flowers in Mizoram. ***b***, *M. baccifera* – Green patch without flowers which is sourrounded by flowered plants (in pale colour). ***c***, Profusely flowering *M. baccifera*. ***d***, Enlarged flower of *M. baccifera*. ***e***, Fruits attached to culms. ***f***, Germinating seeds from fruits.

Recent reports about the flowering of *M. baccifera* are not from Mizoram alone, but in the huge forested areas across the other northeastern states of Tripura, Manipur and southern Assam, which has attracted national and international attention. In Mizoram, the phenomenon is known as 'Mautam', literally meaning 'bamboo dying' ('mau' meaning bamboo and 'tam' meaning to die)[5–8].

As most bamboo flowering is unpredictable, for no species is sufficient information available to state unequivocally that flowering at such long, fixed intervals is the norm for that species. Only for *M. baccifera* there is reasonable evidence to suggest that the patterns observed are likely to be representative of the species[3]. The present observation confirms that the said prediction can be true in case of bamboo flowering for the above-mentioned species.

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Biosorption of lead from aqueous solution by seed powder of *Strychnos potatorum* L.

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ABSTRACT

In the present study, Pb(II) removal efficiency of *Strychnos potatorum* seed powder (SPSP) from aqueous solution has been investigated. Batch mode adsorption experiments have been conducted by varying pH, contact time, adsorbent dose and Pb(II) concentration. Pb(II) removal was pH dependent and found to be maximum at pH 5.0. The maximum removal of Pb(II) was achieved within 360 min. The Lagergren first-order model was less applicable than pseudo-second-order reaction model. The equilibrium adsorption data was fitted to Langmuir and Freundlich adsorption isotherm models to evaluate the model parameters. Both models represented the experimental data satisfactorily. The monolayer adsorption capacities of SPSP as obtained from Langmuir isotherm was found to be 16.420 mg/g. The FTIR study revealed the presence of various functional groups which are responsible for the adsorption process.

1. Introduction

Heavy metal pollution is an environmental problem of global concern. The discharge of metals is increasing continuously as a result of industrial activities and technological development, posing threat to the environment and public health because of their toxicity, accumulation through food chain and persistence. Lead (Pb) has been classified as a toxic heavy metal that can cause serious damage to the liver, brain, kidney, reproductive and nervous system. Severe exposure to Pb(II) has been associated with sterility, stillbirths, abortion and neonatal deaths [1–3]. The major source of Pb(II) pollution in natural waters is due to discharge of waste from acid battery manufacturing, metal plating and finishing, printing, metallurgical alloying, lead mining, ceramics and glass industries [4,5]. The presence of lead in drinking water even at below permissible concentration may cause anaemia, encephalopathy, hepatitis and nephritic syndrome [6]. The permissible limit (mg/l) for Pb(II) in wastewater given by Environmental Protection Agency (EPA) is 0.05 mg/l and by Bureau of Indian Standards (BIS) is 0.1 mg/l [7]. The health hazards due to the presence of Pb(II) in water are of extreme concern to the public, government and industry [8]. The removal of Pb(II) from wastewaters by traditional processes includes its precipitation with lime or alkali hydroxide, coagulation, electrolytic deposition, reverse osmosis and ion exchange. These methods are expensive and have significant disadvantages such as generation of metal bearing sludge or wastes, incomplete metal removal and the disposal of secondary waste.

Recently attention has been drawn to the development of alternative methods like biosorption which uses organic materials as biosorbents. Since the last decade, biosorption or sorption of contaminants by sorbents of natural origin has gained important credibility due to its good performance and low cost of these complexing materials. Due to high uptake capacity and very cost-effective source of raw materials, biosorption is a progression towards a perspective method. Various plant materials viz. waste tea leaves [9], sphagnum moss peat [10], sago waste [11], *Medicago sativa* [12], peat [13], *Quercus ilex* leaf, stem and root phytomass [14], sawdust [15], rice polish [16], *Azadirachta indica* leaf powder [8], *Caladium bicolor* biomass [17], *Oryza sativa* husk [18], maize bran [19], palm shell activated carbon [20], olive pomace [21], maize leaf [22], saw dust [23], coconut and seed hull [24], have been studied for Pb removal from aqueous system.

Strychnos potatorum L. (Loganiaceae) is a moderate sized tree found in Southern and central parts of India, Srilanka and Burma. Seeds are widely used in Ayurvedic and traditional medicine. Apart from its medicinal properties the seed powder is being used for clearing muddy water by the rural community. They are reported to be very effective as coagulant aids. This property is attributed because of the presence of polyelectrolyte, proteins, lipids, carbohydrates and alkaloids containing the –COOH and free –OH surface groups in the seed [25–28]. Having established the coagulating

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properties of the *S. potatorum* seeds there has been a recent interest in the metal binding property [28,29]. The aim of the present study is to determine the efficiency of *S. potatorum* seed powder (SPSP) in removal of heavy metal Pb(II). The influence of pH, biomass dose, contact time and initial Pb(II) concentration on biosorption of Pb(II) ions were studied in aqueous solutions.

2. Materials and methods

2.1. Preparation of Pb(II) stock solution

All the chemicals used in the experiments were of analytical grade. Stock Pb(II) solution (1000 mg/l) was prepared by dissolving 1.5984 g of $Pb(NO_3)_2$ (Qualigens Fine Chemicals, Mumbai; minimum assay 99%) in 100 ml of Milli-Q (Millipore) water and the final volume was made quantitatively to 1000 ml using Milli-Q water. Pb(II) solutions of different concentrations were prepared by adequate dilution of the stock solution with Milli-Q water. pH of the solutions was adjusted with 1 N HNO_3 or 1 N NaOH. All the glassware and polypropylene flasks used were washed with 10% (v/v) HNO_3 and rinsed several times with deionized distilled water.

2.2. Plant material

S. potatorum L. seeds (SPSP) were collected from forests of Andhra Pradesh, India and were dried at 40 °C for 2 days in hot air oven. Seeds were made into powder in Clotech 1093 sample mill, sieved to give a fraction of 100 mesh screen and used as a biosorbent.

2.3. Batch experiments

Batch mode experiments were conducted at 28 °C temperature by shaking 0.100 g of adsorbent (SPSP) in 100 ml of Pb(II) solution of desired concentration in 250 ml glass conical flasks. The flasks were agitated on a rotary shaker at 150 rpm for 6 h to ensure equilibrium. The influence of pH (2.0, 3.0, 4.0, 5.0 and 6.0), contact time (15, 30, 60, 120, 240 and 360 min.), biomass (0.100, 0.150, 0.200 and 0.250 g) and initial Pb(II) concentration (20, 30, 40, 50 and 60 mg/l) were evaluated during the present study. At the end of the experiment, the conical flasks were removed from the shaker and the solutions were separated from the biomass by centrifugation at 10,000 × rpm for 5 min. Metal concentrations were measured using flame atomic absorption spectrometer (GBC 932 plus, Australia). The wavelength used for the analysis of the metal in this study was 283.3 nm. The instrument was calibrated within the linear range of analysis and a correlation coefficient (R^2) of 0.995–1.000 was obtained for the calibration curve. The instrument was periodically checked throughout the analysis with known standards. To check for the reproducibility, all the experiments were repeated thrice and each individual experiment in turn was carried out in triplicates and for data analysis the average value was computed which is mean ± SD value.

2.4. Desorption of the adsorbed Pb(II)

After saturation of the SPSP with 20 mg/l of Pb(II), in order to remove the bound metal ions from the SPSP was washed several times with deionized distilled water to remove any excess of Pb(II). It was treated with 100 ml of 0.1 M HCl and equilibrated by shaking for 2 h and then centrifuged at 5 min for 10,000 rpm. Supernatants were collected and metal analysis was carried out.

2.5. Fourier transform infrared (FTIR) analysis

In order to determine the functional groups responsible for metal uptake and to detect vibration frequency changes in the biosorbent the untreated SPSP and pretreated with 20 mg/l Pb(II) were analyzed using a Fourier transform infrared spectrometer (NICOLET 5700 – FTIR).

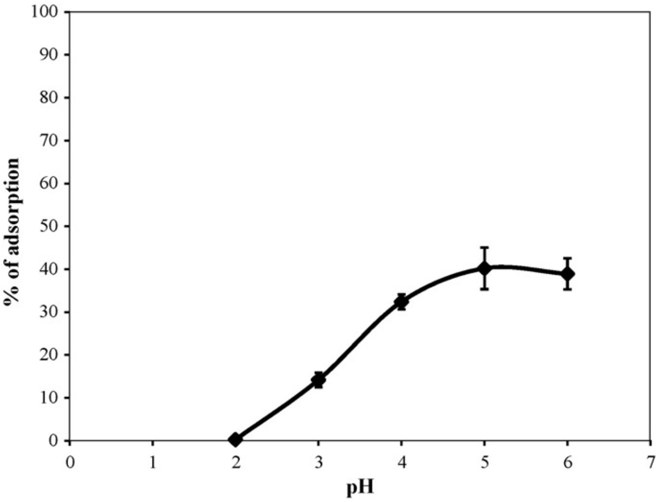

Fig. 1. Effect of pH on Pb(II) removal by SPSP (initial Pb(II) concentration = 20 mg/l; adsorbent dose = 0.100 g/100 ml, temperature = 28 °C, contact time = 360 min, data is the mean ± S.D. of the three independent experiments).

3. Results and discussion

3.1. Effect of pH on metal ion binding

The pH of the aqueous solution is an important controlling parameter in the adsorption process [30]. In order to study the effect of pH on Pb(II) adsorption onto SPSP, pH of solution was varied from 2.0 to 6.0. From Fig. 1, it is observed that the adsorption of Pb(II) varies with pH and there is a gradual increase in Pb(II) uptake as the pH value increases from 2.0 to 6.0. The maximum uptake of these ions was obtained at pH 5.0 (40.21%) at 6 h. At pH below 4.0, an uptake of Pb(II) was less, probably because the H_3O^+ ions may compete with the metal ions for the exchange sites in the sorbent. One of the reasons for the metal ions adsorption behavior of the biosorbent is that the SPSP contains a large number of active functional groups [25–28] as well as on the nature of the metal ions in solution. When pH is increased (above pH 6.5), the Pb(II) ions get precipitated due to hydroxide anion forming a lead hydroxide precipitate. Similar results were reported for adsorption of Pb(II) on *A. indica* leaf powder [8]. For this reason, the optimum pH was selected to be 5.0 for further experiments.

3.2. Effect of biomass

Biomass is a significant factor to be considered for effective metal sorption. When Pb(II) removal at different adsorbent doses (0.100 to 0.250 g/l) was studied at pH 5.0 while keeping the volume and concentration of the metal solution constant. The results have been presented in Fig. 2. It is evident that percentage adsorption of Pb(II) ion increased with increase in adsorbent dose. The resulting effect can be easily explained by an increase in surface area (more availability of active adsorption sites) with the increase in biosorbent mass. Similar behavior for the effect of sorbent concentrations on metal sorption capacity was observed and discussed in the literature for a variety of sorbents and metals [31–34].

3.3. Effect of contact time

The effect of contact time on the adsorption of Pb(II) at 20 mg/l is shown in Fig. 3. The rate of adsorption is very fast initially

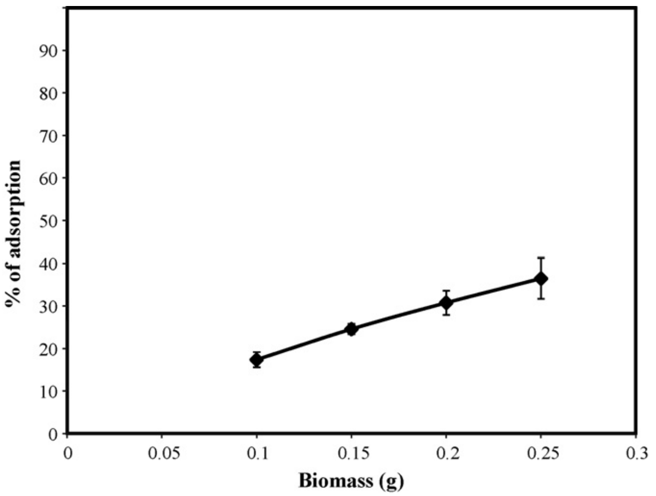

Fig. 2. Effect of adsorbent dose on Pb(II) removal by SPSP (initial Pb(II) concentration = 60 mg/l; adsorbent dose = 0.100, 0.150, 0.200 and 0.250 g/100 ml, temperature = 28 °C, contact time = 360 min, pH 5.0, data is the mean ± S.D. of the three independent experiments).

and maximum removal of Pb(II) occurs at 360 min. The initial fast sorption may be explained as uptake of Pb(II) through physical adsorption since adsorption phenomenon characteristically tends to attain instantaneous equilibrium [35]. The number of active sites in the system is fixed and each active site can adsorb only one ion in a monolayer therefore metal uptake by the sorbent surface is rapid initially and then decreases as the availability of active sites decreases thus slowing down the transfer of metal ion from solution to adsorbent surface. The rate of metal removal is of great significance for developing adsorbent based water technology [36]. The ability of SPSP to adsorb maximum amount of Pb(II) at 360 min indicates that it is an effective biosorbent for the removal of Pb(II) from wastewater.

3.4. Adsorption kinetics

In order to investigate the mechanism of biosorption of Pb(II) by SPSP and the potential rate-controlling steps, such as mass transport and chemical reactions, kinetic models were used to test experimental data. In this study two different kinetic models were used to adjust the experimental data of Pb(II) biosorption on SPSP. In general, the pseudo-first-order model derived by Lagergren [37] has found wide application.

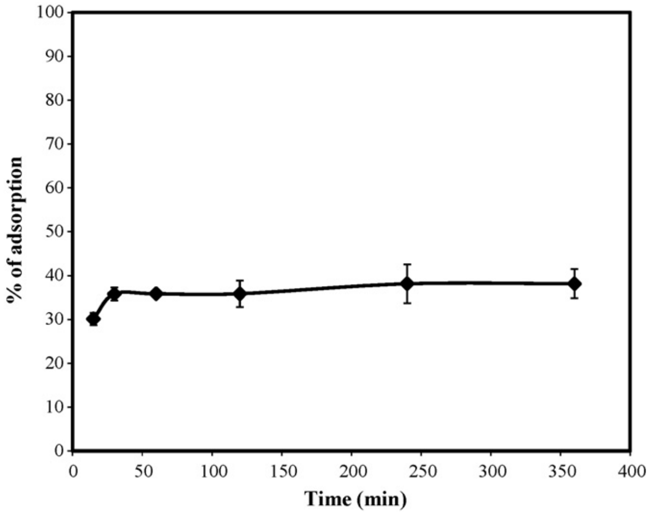

Fig. 3. Effect of time on Pb(II) removal by SPSP (initial Pb(II) concentration = 20 mg/l; adsorbent dose = 0.100 g/100 ml, temperature = 28 °C, contact time = 15, 30, 60, 120, 240 and 360 min, pH 5.0, data is the mean ± S.D. of the three independent experiments).

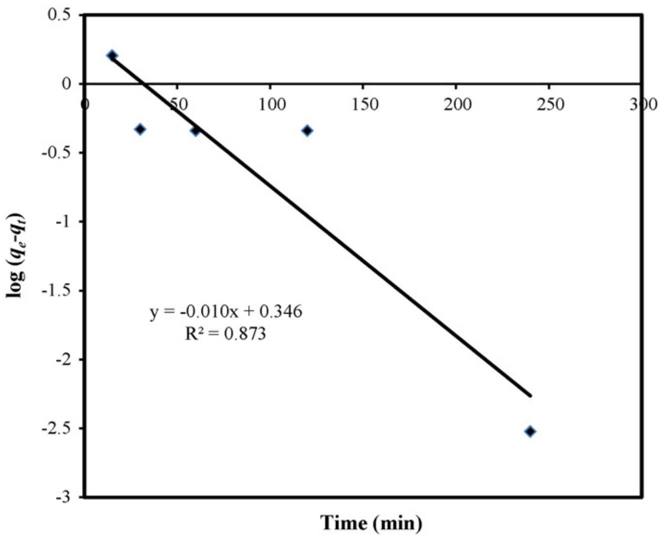

Fig. 4. Pseudo-first-order (Lagergren model) sorption kinetics plot of Pb(II) onto SPSP.

The pseudo-first-order Lagergren model is expressed as Eq. (1):

$$\log(q_e - q_t) = \left(\frac{-k_1, ads}{2.303}\right) t + \log q_e \qquad (1)$$

Where q_e (mg/g) and q_t are the amounts of adsorbed metal ions on the biosorbent at the equilibrium and at any time t, respectively; k_1, ads (min^{-1}) is the Lagergren rate constant of the first-order biosorption. q_e and k_1, ads can be calculated from the slopes and the intercept of the plot $\log(q_e - q_t)$ versus t (Fig. 4). The Lagergren first-order rate constant k_1 and q_e determined from the model indicates that this model has failed to estimate q_e since the experimental value of q_e differs from estimated one.

Several authors [8,38,39] have shown that pseudo-second-order kinetics can also very well describe these interactions in certain specific cases. The pseudo-second-order model is based on the assumption that biosorption follows a second-order mechanism. So, the rate of occupation of adsorption sites is proportional to the square of the number of unoccupied sites. The second-order model is expressed as Eq. (2):

$$\frac{t}{q_t} = \left(\frac{1}{q_e}\right) t + \frac{1}{k_2, ads \cdot q_e^2} \qquad (2)$$

Where k_2, ads is the rate constant of second-order biosorption (g/mg/min). q_e and k_2, ads can be calculated from the slope and the intercept of the plot t/q_t versus t (Fig. 5). It is important to notice that it is not necessary to estimate the experimental value of q_e for the application of such a model. The coefficient of correlation for second-order kinetic model was 0.9991 and the estimated value of q_e also agreed with the experimental data. Both factors suggest that the sorption of Pb(II) ions followed the second-order kinetic model, indicating that the rate-limiting step was a chemical biosorption process between Pb(II) and SPSP. Similar conclusions were found by Ho and McKay [40] and they reported that most of the sorption systems follow a pseudo-second-order kinetic model. The values of regression coefficient, rate constants of pseudo-first-order Lagergren model and pseudo-second-order parameters are given in Table 1.

Table 1
Comparison between adsorption rate constant, q_e estimated and coefficient of correlation associated to the Lagergren pseudo-first-order and to pseudo-second-order kinetic models.

Biosorbent	Experimental value	First-order kinetic model			Second-order kinetic model		
	q_e (mg/g)	q_e, cal. (mg/g)	$K_{1\ ads}$ (1/min)	R^2	q_e, cal. (mg/g)	K_2 (g/mg/min)	R^2
SPSP	7.628	2.218	0.0251	0.8734	7.7041	0.0288	0.9991

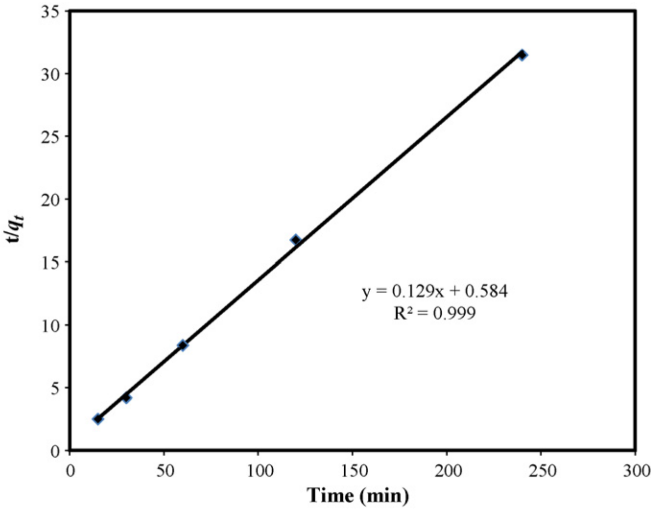

Fig. 5. Pseudo-second-order sorption kinetics plot of Pb(II) onto SPSP.

3.5. Effect of metal concentration

The rate of adsorption is a function of the initial concentration of metal ions, which makes it an important factor to be considered for effective biosorption [41]. The percentage removal of Pb(II) at different metal concentrations using SPSP is presented in Fig. 6. When the initial Pb(II) concentrations were increased from 20–60 mg/l, the percentage of adsorption slightly decreased (38.14–21.08%). This may be due to saturation of active adsorption sites on SPSP. Thus the adsorbent can be utilized effectively for the removal of Pb(II) from waste water at low concentrations of lead.

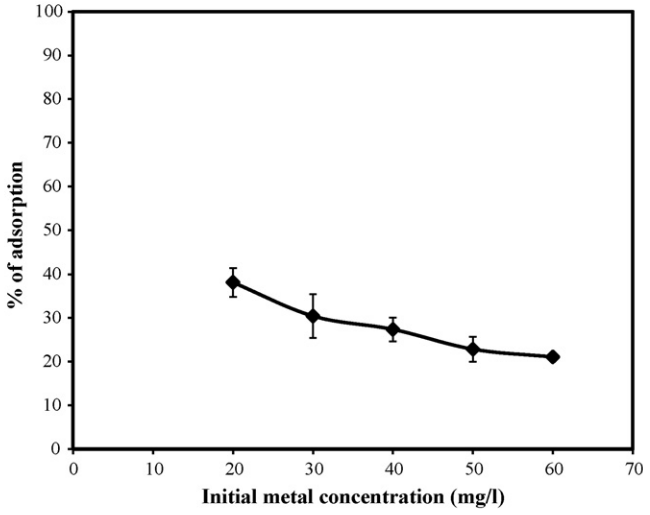

Fig. 6. Effect of different concentrations of Pb(II) (Pb(II) concentration = 20, 30, 40, 50 and 60 mg/l; adsorbent dose = 0.100 g/100 ml, temperature = 28 °C, contact time = 360 min, pH 5.0, data is the mean ± S.D. of the three independent experiments).

3.6. Adsorption isotherms

The Pb(II) uptake capacity of the SPSP at different concentrations (20–60 mg/l) on a fixed amount of adsorbent (0.100 g) at pH 5.0 has been evaluated using the Langmuir and Freundlich adsorption isotherms.

The Freundlich isotherm is represented by the following Eq. (3) [42]:

$$\log q_e = \log K_f + \left(\frac{1}{n}\right) \log C_e \qquad (3)$$

Where C_e is the equilibrium concentration (mg/l); q_e is the amounts adsorbed per specified amount of adsorbent (mg/g) at equilibrium, K_f and n are constants which are adsorption capacity (mg/g) and intensity of adsorption, respectively. Linear plots of $\log q_e$ versus $\log C_e$ (Fig. 7) show that the adsorption followed Freundlich model. K_f and n were calculated from the intercept and slope of the plots. The values of constants are given in Table 2. According to Kadirvelu and Namasivayam [43], 'n' values between 1 and 10 represent beneficial adsorption.

Langmuir model is commonly used for liquid phase adsorption which assumes that the uptake of metal ions occurs on a homogeneous surface by monolayer adsorption without any interaction between adsorbed ions. The Langmuir isotherm is expressed as Eq. (4) [44]:

$$\frac{C_e}{q_e} = \frac{C_e}{X_m} + \frac{1}{q_m K_L} \qquad (4)$$

Where, q_e is the amount of Pb(II) adsorbed at equilibrium (mg/g), C_e is the equilibrium concentration (mg/l), X_m and K_L are Langmuir constants related to the adsorption capacity and energy of adsorption, respectively. The linear plot of C_e/q_e versus C_e (Fig. 8) shows that the Pb(II) removal by SPSP obeys the Langmuir model too. X_m and K_L were calculated from the slope and intercept of the plot and are presented in Table 2. On the basis of correlation coeffi-

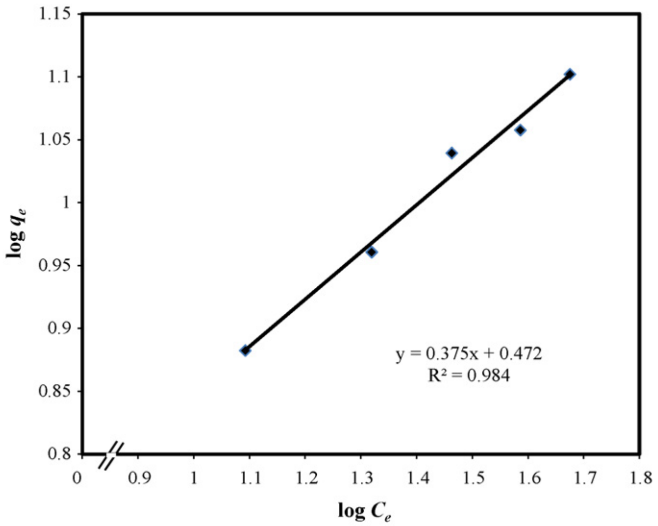

Fig. 7. Linearized Freundlich isotherm plot for adsorption of Pb(II) by SPSP.

Table 2
Langmuir and Freundlich isotherm parameters for Pb(II) adsorption by SPSP.

Biosorbent	Experimental value	Langmuir isotherm parameters			Freundlich isotherm parameters		
	q_e (mg/g)	X_m (mg/g)	K_L (1/mg)	R^2	K_f (mg/g)	n	R^2
SPSP	12.651	16.420	0.065	0.990	2.969	1.259	0.984

cients, it can be observed in Table 2 that the experimental data was better fitted to the Langmuir equation than that of the Freundlich equation.

3.7. Separation factor (R_L)

The shape of the Langmuir isotherm can be used to predict whether a sorption system is favourable or unfavourable in a batch adsorption process [39,45]. Accordingly, the essential characteristics of Langmuir isotherm can be expressed in terms of a dimensionless constant called the separation factor or equilibrium parameter, R_L that is expressed as Eq. (5):

$$R_L = \frac{1}{(1 + K_L C_i)} \tag{5}$$

Where R_L is a dimensionless equilibrium parameter or separation factor, K_L constant from Langmuir equation and C_i is initial metal ion concentration.

The parameter, R_L, indicates the shape of the isotherm and nature of the sorption process as given below:

R_L value	Type of isotherm
$R_L > 1$	Unfavorable isotherm
$R_L = 1$	Linear isotherm
$R_L = 0$	Irreversible isotherm
$0 < R_L < 1$	Favourable isotherm

The values of R_L for Pb(II) were calculated and plotted against initial metal concentration. The data (Fig. 9) showed that, the sorption of Pb(II) on the SPSP increased as the initial metal concentration increased from 20 to 60 mg/l, indicating that adsorption is even favourable at the higher initial metal ion concentrations. The sorption process was favourable for Pb(II) at all the tested concentrations investigated.

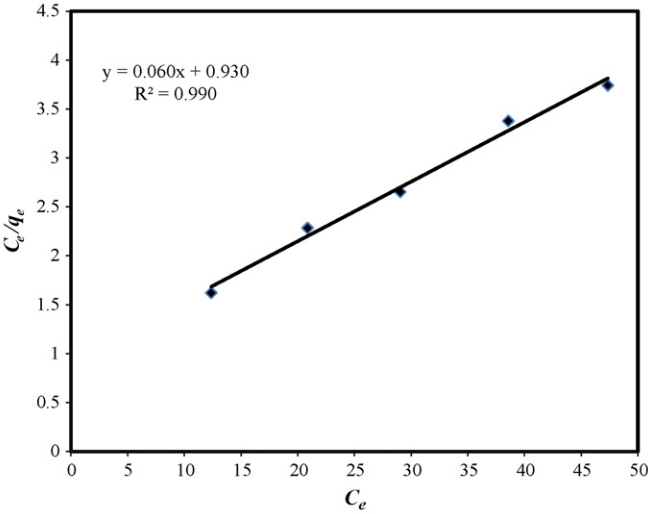

Fig. 8. Linearized Langmuir isotherm plot for adsorption of Pb(II) by SPSP.

3.8. Surface coverage values (θ)

To account for the adsorption behaviour of the metal ions on the SPSP, the Langmuir type equation related to surface coverage was used. The Eq. (6) is expressed as follows:

$$KC_i = \frac{\theta}{1 - \theta} \tag{6}$$

Where K is the adsorption coefficient, C_i is initial concentration and θ is surface coverage. The fraction of biomass surface covered by metal ions was studied by plotting the surface coverage values (θ) against metal ions concentration. The data is presented in Fig. 9. The figure shows that increase in initial metal ion concentration for Pb(II) increased the surface coverage on the biomass until the surface was nearly fully covered with a monomolecular layer. Further examination of Fig. 10 reveals that the surface coverage ceased to vary significantly with higher concentrations of Pb(II) and the reaction rate became independent of the Pb(II) concentration. The overall adsorption process indicates that the biomass will be highly effective in removing Pb(II) ions in aqueous effluent.

3.9. Thermodynamic equilibrium constant (K_c^0)

The thermodynamic equilibrium constant (K_c^0) at different Pb(II) concentrations using SPSP as adsorbent was obtained at 28 °C. The Eq. (7) is expressed as follows:

$$K_c^0 = \frac{C_q}{C_e} \tag{7}$$

Where K_c^0 is the equilibrium constant, C_q is concentration of Pb(II) on the adsorbent at equilibrium in mg/l and C_e is the equilibrium concentration of Pb(II) in solution in mg/l. The values obtained are presented in Table 3.

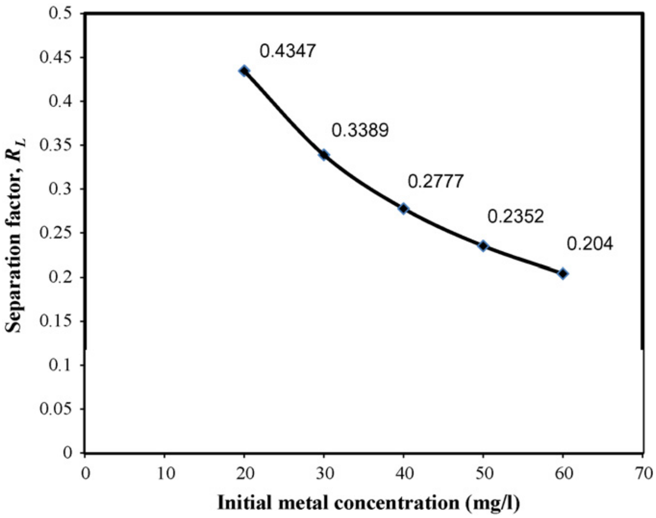

Fig. 9. The calculated separation factor (R_L) for Pb(II) as a function of metal ion concentration (mg/l).

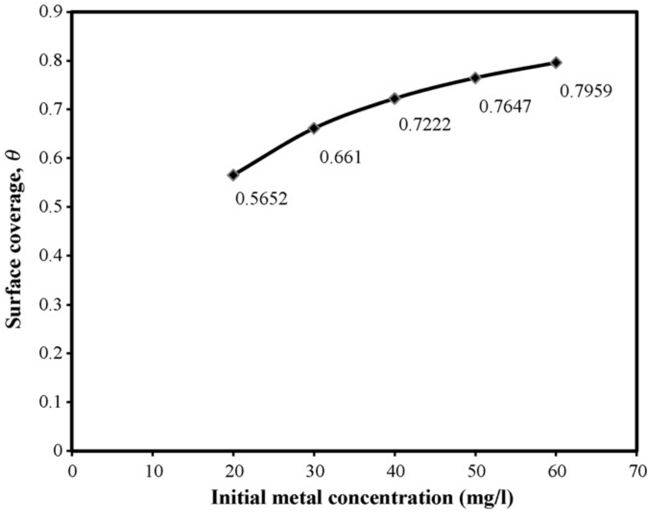

Fig. 10. A plot of surface coverage (θ) against Pb(II) concentration (mg/l).

3.10. *Gibbs free energy (ΔG°)*

The Gibbs free energy (ΔG°) for the adsorption process for each Pb(II) concentration was obtained. The Eq. (8) is expressed as follows:

$$\Delta G^{\circ} = -RT \ln K_c^0 \tag{8}$$

Where *R* is the gas constant and *T* is the absolute temperature in Kelvin. The values obtained are presented in Table 3. The Gibbs free energy indicates the spontaneity of the adsorption process, where higher negative values reflect a more energetically favorable adsorption process. The negative ΔG° values obtained for different Pb(II) concentrations in this study confirm the feasibility of the adsorbent and spontaneity of adsorption.

3.11. *Desorption studies*

Sorption of solute on any sorbent can either be by physical bonding, ion-exchange or combination of both. If the adsorption is by physical bonding then the loosely bound solute can be easily desorbed with distilled water in most cases. However, if the mode of sorption is by chemical bonding or ion-exchange or combination of both, then the desorption can be effected by stronger desorbents like acid or alkali solutions.

Attempts were made to desorb Pb(II) from SPSP using 0.1 M HCl, 80% of recovery was achieved. The results indicated that adsorption is due to ion-exchange mechanism. The re-use of SPSP can not be possible due to pastry nature after imbibed in water.

3.12. *FTIR spectral analysis*

The FTIR spectral analysis is important to identify some characteristic functional groups, which are responsible of adsorbing metal ions [41,46–48]. In order to determine which functional groups were responsible for metal uptake, an FTIR spectrum of SPSP was performed in the range of 400–4000 cm^{-1} to obtain information regarding vibrational frequency changes. The FTIR spectra of control and Pb(II) treated are presented in Fig. 11. The peaks around 3378.67 cm^{-1} are indicative of the existence of broad, intermolecular hydrogen bonded, O–H stretch. The peaks observed at 2917.77 cm^{-1} can be assigned to the stretching vibration of C–H. The peaks around 1643.05 cm^{-1} are due to the amide I, C = O stretching vibration of carboxylic acid. The peaks around 1419.35 are due to the stretching vibration of C–H and peaks at 1060.65 cm^{-1} can be assigned to the C–O stretching vibration of alcohols and carboxylic acids. All these peaks in the sample after adsorption show an absorbance substantially lower than those in the control and small differences in the frequency bands, suggesting the participation of these functional groups in the adsorption of Pb(II) by SPSP.

Table 3
Thermodynamic equilibrium constant (K_c^0) and Gibbs free energy ΔG° (kj mol^{-1}) for the adsorption of Pb(II) by SPSP.

C_i (mg/l)	Equilibrium constant (K_c^0)	Gibbs free energy ΔG° (kj mol^{-1})
20	0.616	−1.541
30	0.437	−1.093
40	0.376	−0.940
50	0.296	−0.740
60	0.267	−0.668

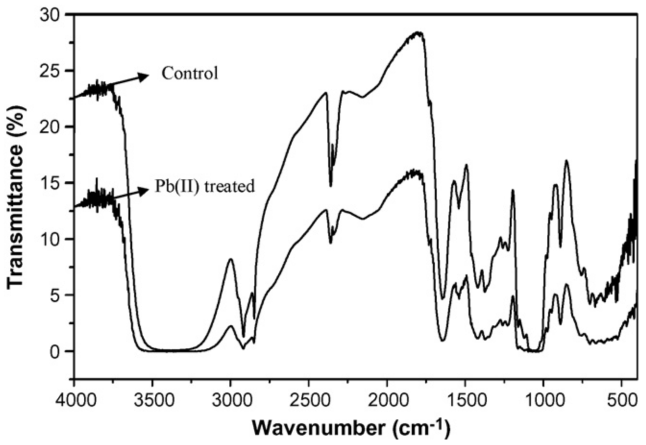

Fig. 11. FTIR spectra of SPSP control and after Pb(II) sorption.

4. Conclusion

SPSP can be used as natural coagulant to clarify turbid water and a reference on usage of these seeds is available in *Sushruta Samhita* [49]. Hence *S. potatorum* seed powder was selected for studying Pb(II) removal from aqueous solution. The following conclusions can be drawn from present study.

The removal of Pb(II) process was affected by pH, biomass dose, contact time and metal concentration. Maximum removal of Pb(II) on SPSP was at pH 5.0. The equilibrium of metal ion removal was attained at 360 min. The overall adsorption rate was best described by pseudo-second-order kinetic model. The data obtained from this study was best described by Langmuir and Freundlich isotherm models. Adsorption of Pb(II) on the SPSP was studied by FTIR and different functional groups involved in the adsorption process were identified. As reproducibility is a fundamental requirement in scientific experiments, our present investigation on removal of Pb(II) by using SPSP adsorption process is highly reproducible, more efficient, cost effective and can further pave the way to develop a technology at large scale to remove various heavy metals from the contaminated aqueous medium.

Acknowledgement

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Traditional Uses, Economic Importance and Ecological Services of *Meloccana baccifera* Roxb. in Mizoram, India

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ABSTRACT

The North Eastern Hill (NE) region of India possesses the largest species of bamboos in India. Among seven states of NE India, Mizoram has the largest bamboo-covered forest area. The bamboo forest area of Mizoram constitutes 14% of the total of India's bamboo area i.e. 8.96 million ha. Non-clump forming bamboo *Melocanna baccifera* is the most abundant, contributing about 95% of the growing stock of bamboo. Traditional living and lifestyle of the Mizo society, to a large extent, is dependent on bamboo for its variety of uses and this bamboo has much to offer by way of contributing to socio-economic advancement of modern Mizo society. The communities of the region use this potential resource for food, shelter, furniture, handicraft, medicines, musical instruments, agriculture implements and household items. *M. baccifera* and other bamboos from the Government of Mizoram state Notified Forests are sold under Mahal and Permit System outside and within the state; this helps the state income to a large extent. *M. baccifera* is extensively used for the production of bamboo ply boards. Many people earn their livelihood from this plant. It also contributes an amount to the state government income. *M. baccifera* forests and regrowth areas in critical mountain slopes and around village habitations shall be afforded protection to ensure environmental security.

Keywords: bamboo, bamboo mat-ply, environmental security, food, handicraft, Mizo society

INTRODUCTION

Mizoram is situated in the North Eastern part of India and is the land of a number of craftsmen and artisans in various crafts. Bamboo-related products are the major source of income to the state as well as the people. Bamboo's most important uses include for timber or a raw material for timber or a raw material for paper, pulp, housing and material for handicrafts besides some minor uses such as leaves for medical purpose (Zhang 1997). So bamboo plays a very important role in the economy of Mizoram. Out of these bamboos, *Meloccana baccifera* is the most important bamboo species. Mizo's dexterity in bamboo work is well known. Bamboo has multifarious uses in turning out various commercial crafts and items of furniture (http://ignca.nic.in/craft155.htm). Bamboo possesses excellent strength properties, especially tensile strength (Sekhar and Gulati 1973). The branch, leaf, culm, rhizome and seeds of *M. baccifera* are used for many items such as winnowing trays, rice carrying baskets, vegetable containers, carrying and storing baskets, stools, food containers for long term preservation and use, musical instruments, etc. (Bhatia *et al*. 2003). So far, as the bamboo in the Mizoram hills are concerned, it is available in large quantities but due to the lack of transport accessibility, it has not been utilized to the maximum. However, it seems that the Mizoram authorities have envisaged the feasibility of introducing in Mizoram better vocational trades in spinning and weaving, hats, baskets, bamboo chairs, tables, teapots, racks, safes, etc. as well as bamboo screen cages and umbrella handles.

In this paper, the importance of *M. baccifera* in terms of traditional, economical and ecological services in Mizoram are reported.

STUDY AREA

A field survey was conducted twice in different parts of Mizoram (India). The state lies between 21°58′ and 24°35′ N latitudes and 92°15′ and 93°29′ E longitudes. The state is only 21,087 km^2 in area and the ways of people using this bamboo is almost same in different places. Palsang, Zohmun, Ratu, Aizawl, Lunglei and Zonun mat-ply industries were visited for these studies (**Fig. 1**).

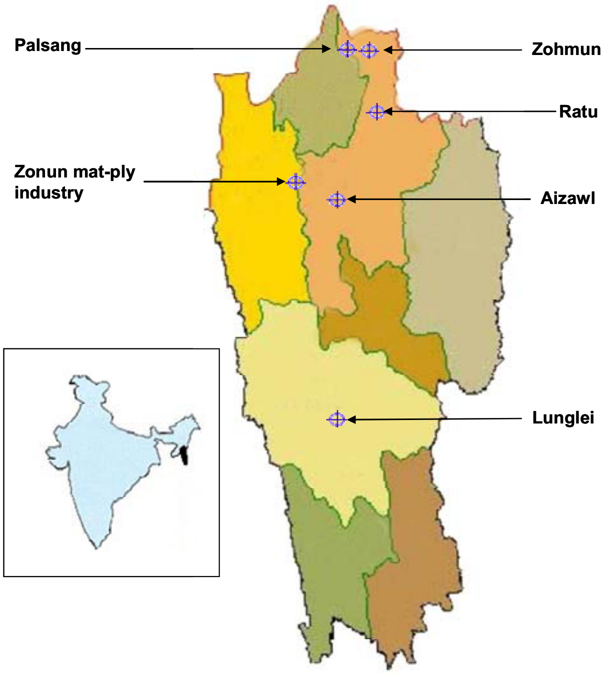

Fig. 1 Hydrographic map of the Mizoram showing the survey sites for traditional uses, economic importance and ecological services.

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Original Research Paper

METHODOLOGY

A field survey was carried out at Mizoram State Museum, Department of Art and Culture, Govt. of Mizoram, Department of Forest and Environment, Govt. of Mizoram, different villages in Mizoram, Zonun mat-ply industry, Aizawl and the handicraft center of Hnam Chhantu Pawl and Mizoram Handloom and Handicraft (Aizawl) in order to collect information and data concerning traditional uses and the economic importance of *M. baccifera*. Field surveys were also conducted in different districts of Mizoram State where *M. baccifera* is grows profusely on hilly terrain. Shifting agriculture on these hill slopes is very common. Therefore, the ecological services of *M. baccifera* have been being investigated.

TRADITIONAL USES

Some of the traditional items made from *M. baccifera* for common dat to day use of Mizo tribe are listed below:

Housing

The short supply of timber and other conventional construction materials accompanied by rising costs make it imperative to increasingly use bamboo for housing (Mathur 1981; Janssen 1987, 1988, 1990; Mishra 1990). Because of its easy availability, workability and low cost, bamboo is employed for columns, purlins, rafters, trusses, as well as walling and roofing (Satter 1995). The importance of bamboo as a construction material, particularly for housing has received renewed attention in recent years. The houses built by the Lushai tribe of Mizoram, predominantly uses different bamboo species and wood in their construction, with *M. baccifera* making the largest material contribution. In small and medium-sized towns corrugated metal sheets for roofs are also used, though in villages, grass thatched roofs are predominantly used. Cane is generally used for keeping the joints together and in some cases, iron nails are also used. When the floor of the house is much higher than the ground, a ladder made of wooden logs is placed at the intervening space between the floor of the house and the ground. The doors and windows are usually made up of bamboo, but in some cases wooden planks are also used. Houses based on bamboo are shown in **Fig. 2**.

Fig. 2 Mizo typical bamboo house made from *Meloccana baccifera* and other bamboo species.

Baskets

Basketry among the Mizo's is a delicate work. They are experts in making different shapes and sizes, in several models like oval, square, flat structures, etc. baskets are very useful for carrying grains from the field, firewood from the forest, water in bamboo tubes from rivers, etc. Different baskets of Mizo's are presented in **Fig. 3A-E**, including:

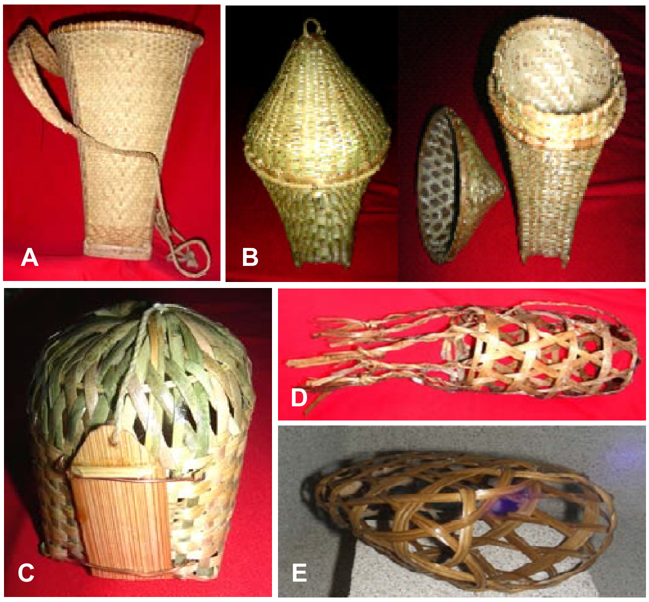

Fig. 3 Different Mizo baskets. (**A**) Empai. (**B**) Thul (storage basket). (**C**) Arbawm (chicken basket). (**D**) Ar-awt (chicken transport basket). (**E**) Tekte.

a) Empai/Paiem (woven basket): a closed-weave carrying basket used by the people of Mizoram. Especially used for carrying food grain and other field products. The Mizo women also use this basket for marketing. The rope-like structure is called 'hnam' for carrying the Empai.
b) Thul: a close-weave storage basket. It is mainly used for storing cloths and garments. It is like a traditional briefcase, has a double-walled structure and legs located at the corners of the square base. A lid is shaped either as a dome or cone.
c) Arbawm: an open-weave basket. It is used as a hen-house at night.
d) Ar-awt: used for transporting chicken from one place to another.
e) Tekte: a basket used for keeping yams.

Musical instruments

The Mizo's are sentimental people. They are very fond of music and song. The uses and functions of musical instruments provide an inexhaustible line of inquiry. Some of the musical instruments are shown in **Fig. 4A-E**, including:

a) Perhkhuang: a typical Mizo musical instrument. It can be made from different species of bamboo.
b) Rawchhem: another typical Mizo musical instrument. It is also called Mizo bagpipes.
c) Phenglawng: also a Mizo traditional musical instrument. It is a Mizo flute.
d) Mizo Tingtang: a typical Mizo guitar made up of bamboo and some animal leather.
e) Mau Tawtawrawt: a typical Mizo trumpet mainly made from *Dendrocalamus* species and *M. baccifera*.

Snare/Traps

Snare/Traps are mainly used for killing small animals and birds. They are shown in **Fig. 5A-H**:

a) Hnawhtawt: a trap for killing rats.
b) Kawlper: a trap for small animals.
c) Thangchep: a trap for the rodent family.
d) Mangkhawng: a trap for birds and small animals. It is made from a big log and bamboo.
e) Thangthleng: a trap for birds. It is usually placed on a tree.

Fig. 4 Different traditional musical instruments. (**A**) Perhkhuang. (**B**) Rawchhem (bagpipe). (**C**) Phenglawng (flute). (**D**) Mizo tingtang (guitar). (**E**) Tawtawrawt (trumpet).

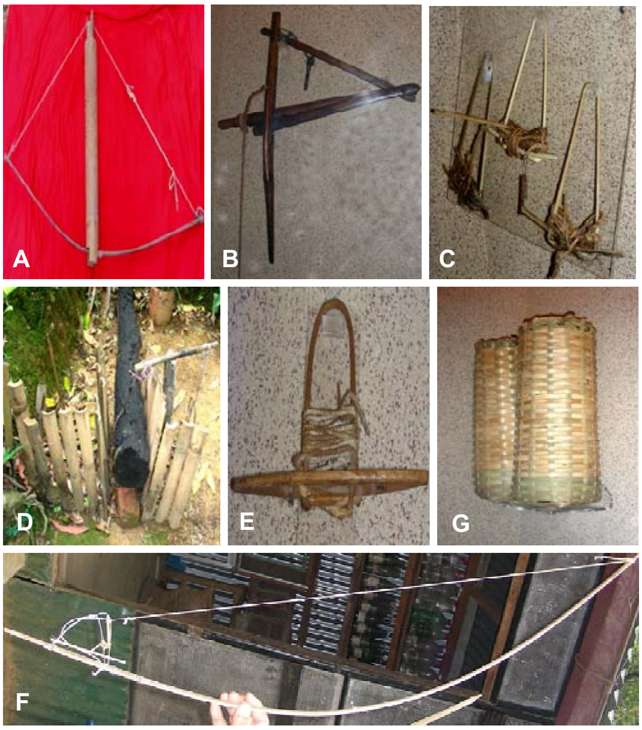

Fig. 5 Different Mizo traditional snares/traps. (**A**) Hnawhtawt (rat trap). (**B**) Kawlper. (**C**) Thangchep. (**D**) Mangkhawng. (**E**) Thangthleng. (**F**) Vaithang. (**G**) Ai-awt (crab trap).

f) Vaithang: a trap for rats.
g) Aiawt: a trap for crabs. It is placed in rivers with bait within the Aiawt.

Agricultural implements

M. baccifera is extensively used as a handle of agricultural implements. The bamboo rhizome, culm, or combination of culm and rhizome are mostly used as handles for small hoes, axes, spuds, adzes, sickles, bill hooks, and daos (**Fig. 6A-D**).

Fig. 6 Use of *Meloccana beccifera* as a handle in agriculture tools. (**A**) Different agriculture tools. (**B**) Use of *Meloccana baccifera* rhizome. (**C**) Combination of rhizome and culm. (**D**) Use of culm part only.

Household items

Mizo traditional house-hold items are shown in **Figs. 7A-L**:

a) Kho: a flattened swallow container. It is used for keeping vegetables.
b) Mau Fian (bamboo spoon): a spoon made usually from *M. baccifera* and *Dendrocalamus* species. It has many uses, for taking out curry, taking out liquid or as a scoop for eating.
c) Chingalthlawrbur (leaching funnel): a big funnel used for leaching ash. The watery part that comes out from the leaching funnel is called 'chingal' and is used as 'soda'.
d) Zu sawrna (bamboo beer funnel): a funnel shaped-like

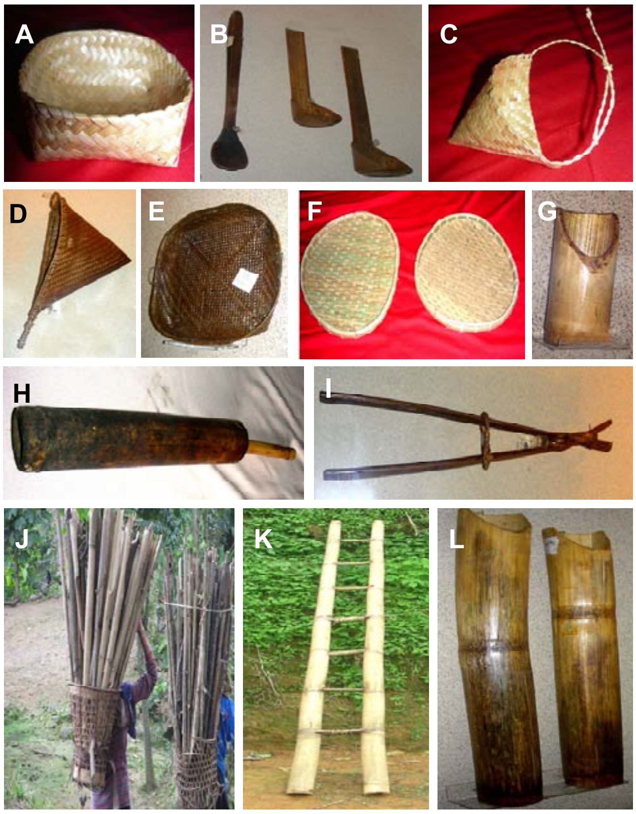

Fig. 7 Traditional household items. (**A**) Kho. (**B**) Mau fian (bamboo spoon). (**C**) Chingalthlawr bur (leaching funnel). (**D**) Zu sawrna (rice beer funnel). (**E**) Vaihrik (sieve). (**F**) Thlangra (winnowing tray). (**G**) Mau hai-leng no (rice beer cup). (**H**) Sawhbur (mortar and pestle). (**I**) Mau chai-cheh (bamboo tong). (**J**) Bamboo fire-wood. (**K**) Leihlawn (ladder). (**L**) Tuium.

structure used for separation of rice from rice beer.

e) Vaihrik (sieve): a flattened bamboo tray with many pores used as a sieve for cleaning food grains and cereal powder.

f) Thlangra (winnowing tray): a flattened bamboo winnowing tray used for sifting and winnowing rice grains after dehusking.

g) Mau Haileng No (rice beer cup): a bamboo cup used for drinking rice beer and other liquids.

h) Sawhbur (mortar and pestle): It can be any bamboo species. Mortar is bamboo and pestle is usually wood. It is used for grinding vegetables and fruits.

i) Raw chaicheh (bamboo tong): a cross shape of two bamboo sticks used for holding hot materials.

j) Fire wood: bamboo can also be used as firewood, used extensively in village and rural areas.

k) Leihlawn (ladder): used for making ladders, the most common being *Dendrocalamus* spp. and *M. baccifera*.

l) Tuium (bamboo water tube): a long bamboo tube that can be made from any species of bamboos and used for transporting and storing water.

Rain shield

Siksil is a traditional umbrella worn during rain by Mizo people. It also has two layers. Palm leaves are also inserted between these two layers. This is also made from strips of bamboo woven in an open-hexagonal weaved pattern (**Fig. 8**).

Fig. 8 Traditional rain sheath. Siksil (umbrella).

Water pipes

M. baccifera is used as pipe for rain water collection from the roof top, for collecting water from the running river and for drip irrigation (**Fig. 9A-B**).

Fig. 9 Bamboo pipes. (**A**) Pipe for rain water collection from the roof top. (**B**) Collection of water from running river.

ECONOMIC IMPORTANCE

Bamboos are multipurpose plants, with thousands of economic applications (Ueda 1981). Bamboo represents a vast untapped major resource of Mizoram State whose full ecological and economic potentials remain underutilized. Out of these bamboos, *M. baccifera* plays the greatest economical importance for the people; it needs to be recognized, developed and promoted in a manner ensuring ecological security for all round sustainable development of the State and well-being of its people. Bamboo is an essential component of a forest eco- system, which is a dominant feature of the state's landscape. *M. baccifera* and its related bamboo industries already provide income, food and housing to many people. *M. baccifera* is a versatile multipurpose forest produce that has immense potential in industries and domestic applications. Growth of merchantable culms from the seeds of *M. baccifera* usually takes 10-12 years in natural conditions, but it may attain a merchantable size in 4-5 years if properly tended. In spite of the economic importance of the bamboo there has not been any attempt to promote the bamboo-based industry which has the potential to transform the economy of the people of the Mizoram region. *M. baccifera* being a multipurpose, eco-friendly crop abundantly available, yet an underutilized natural resource, needs to be managed and exploited for sustainable use. *M. baccifera* and other bamboos were conceived as a thrust area in the Industrial Development of Mizoram for the economic and ecological security of the people. This precious resource needs to be fully tapped as an industrial raw material, as a substitute for wood in rural/urban housing, engineering works, handicraft, furniture and value addition through export. Undoubtedly *M. baccifera*, along with other bamboos, can revolutionize the economy of the state ensuring employment opportunities to a large number of people. At present, only a small portion of bamboo resources are harvested for the purpose of local construction, tiny handloom and handicraft production.

M. baccifera young shoots can be used as food after cooking. However, young shoots are seasonal and so preservation is necessary for storage, for which there are many methods. The most commonly practiced one in Mizoram is sun drying and drying on fire. *M. baccifera* shoots also have a huge market potential. Young shoots provide opportunities for the livelihood of a large number of people. These young shoots are sold in fresh shoot form and in cans. The shoots of most bamboo species in Mizoram are edible and are consumed locally. *M. baccifera* are the most consumed species by the people followed by *Dendrocalamus hamiltonii*, *Dendrocalamus longispathus* and *Bambusa tulda*. On average, *M. baccifera* contributes 53.69% to the total annual bamboo shoot consumption (Bhatt *et al*. 2003). *M. baccifera*, at present, is availed in an unregulated manner by villagers to meet their domestic need free or on payment of some royalty to the Mizoram State Government.

M. baccifera and other bamboos from the Government of Mizoram state Notified Forests are sold under Mahal (mostly bamboo grown at the river side) and Permit System supported by local village bodies with a view to manage the bamboo resources and to provide livelihood to the villagers. The State Forest Department is also involved in this action plan to ensure sustainable management to increase bamboo yield to meet the local and export requirement. Felling regulation, market requirement in consultation with all concerned parties (State Government and Villagers) is being practiced so that bamboo resources are optimally harvested and used. These bamboos, sold by Mahal systems go to the Paper Mill located at Panchgram, Hailakandi in the neighbouring State of Assam, India. A smaller quantity of bamboo is sold by the Permit system was utilized in local industries and also for domestic needs.

As forest reserves are being depleted globally, timber is getting scarce day by day. This is due to a long period taken by even softwood to attain maturity. Bamboo can attain maturity within a short period (only 2-3 years). So, a sub-

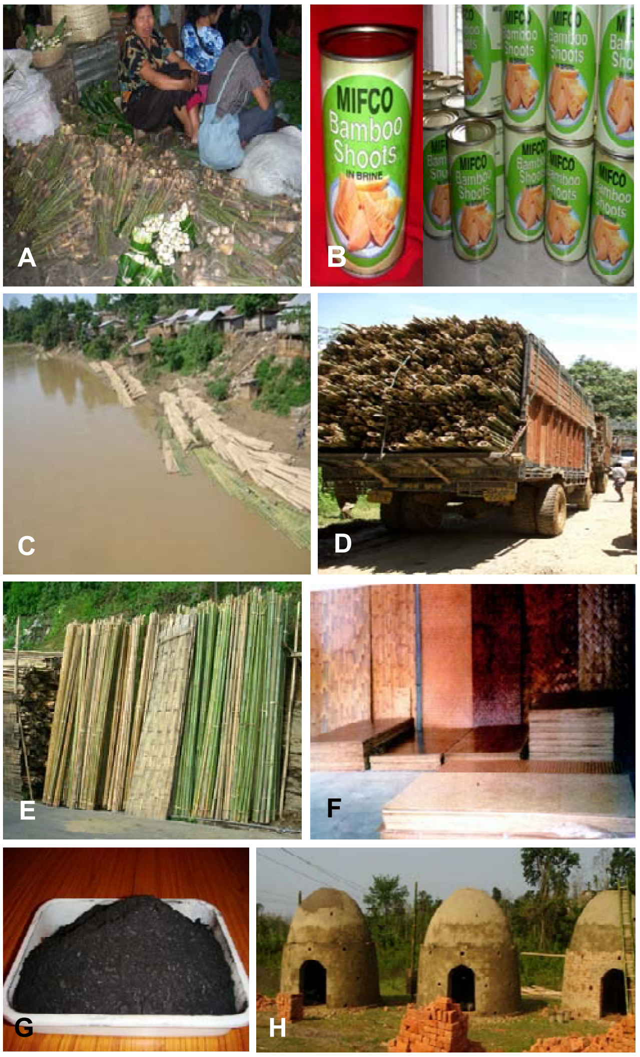

Fig. 10 Different economic importance of *M. baccifera*. (**A**) *M. baccifera* young shoot market. (**B**) Young shoot in canned form. (**C**) Transported through river and (**D**) road for paper industries. (**E**) Local *M. baccifera* market. (**F**) Bamboo mat-ply produced mainly from *M. baccifera*. (**G**) *M. baccifera* charcoal powder. (**H**) Charcoal kiln.

Fig. 11 Economic importance in different daily uses and handicrafts produce from *M. baccifera*. (**A**) Watery extract from *M. baccifera* charcoal production. (**B**) Tea coaster. (**C**) Hangers. (**D**) Flowers vases. (**E**) Trays. (**F**) Mat. (G) Stool. (**H**) Cups. (**I**) Cigarette case. (**J**) Furniture. (**K**) Baskets. (**L**) Ear ring.

stitute, or if that is not possible an alternative has to be found. Bamboo is an answer. *M. baccifera* is extensively used for the production of bamboo ply boards. Bamboo are sliced into thin strips and these strips are woven together to produce bamboo mats. Bamboo mats and slivers are hot pressed to produce bamboo ply board. Bamboo ply board (**Fig. 10F**) is very strong and can be made water resistant, allowing it to be used for almost all purposes where timbers are now used. It has applications for construction boards, etc. Bamboo mat boards and bamboo ply boards can be promoted as wood substitute for growing construction needs within and outside the state. This will not only result in a value addition to bamboo products but will also be a wood substitute reducing the use of timber within the state.

Another economical use of *M. baccifera* is for the production of bamboo charcoal (**Fig. 10G**) whose application includes environment protection, food industry, pharmaceutical industry, etc. Output efficiency is 20% i.e. 100 kg of raw bamboo can produce 20 kg of bamboo charcoal. During the production of bamboo charcoal in the charcoal kiln (**Fig. 10H**), the steam and watery portion coming out from bamboo is condensed. This bamboo watery portion is used for organic fertilizer, preservative medium and for relief from pains, etc.

M. baccifera has found its place in many items of daily uses. Due to its high tensile strength, it is ideal for various items of daily uses like tea coasters, hangers, flower vases, trays, containers, baskets, mats, frames, furniture, ornaments, etc. are made from this bamboo. Due to its high texture, properties, high tensile strength, easy splitting possibility, and other characteristics, it is a good choice of material for handicrafts. There are numerous designs and varieties of handicrafts made out of bamboo. The economic uses of *M. baccifera* are shown in **Fig. 10A-H** and **Fig. 11A-L**.

ECOLOGICAL SERVICES

Bamboo also serves multiple ecological functions such as soil and water conservation, and soil erosion control (Fu and Banik 1995). Bamboo generates 30% more oxygen than trees (www.bambooliving.com). It helps reduce CO_2 gases blamed for global warming. Some bamboo sequesters up to 12 tons of CO_2/ha, which makes it an efficient replenishes of fresh air. Bamboo is a natural water control barrier. Because of its wide spread root system and large canopy, bamboo greatly reduces rain run off and prevents massive soil erosion. Bamboo helps mitigate water pollution due to its high nitrogen consumption, making it a solution for excess nutrient uptake of waste water from manufacturing, livestock farming and sewage treatment. Bamboo can restore degraded lands. It is a pioneering plant and can be grown in soil damaged by overgrazing and poor agriculture. Proper harvesting does not kill the bamboo plant, so topsoil is held in place. Because of its dense litter on the forest floor it feeds (www.bambooliving.com). *M. baccifera* bamboo species is an essential component of the forest ecosystem in Mizoram, which is a dominant feature of the state's landscape. The distribution of bamboo in the North-East region of India is given in **Table 1**.

Out of 25.26 million tons of bamboo growing stock 95% is contributed by *M. baccifera*. But this bamboo forest is destroyed by slash and burn system of agriculture. This system of agriculture has become unproductive owing to frequent exposure of soil, increased land-slides and soil erosion, and loss of vegetation and bio-diversity **Fig. 12A-B**.

Table 1 Bamboo resources in NE States of India (2007-2008).

State	Bamboo growing area (km^2)	Bamboo growing stock (million tons)
Mizoram	6446	25.26
Assam	8213	13.41
Manipur	3692	11.47
Arunachal	4590	9.84
Meghalaya	3102	4.41
Nagaland	758	3.66
Tripura	939	0.86
Total	30504	54.53

Data obtained from Dept. of Forest and Environment, Govt. of Mizoram.

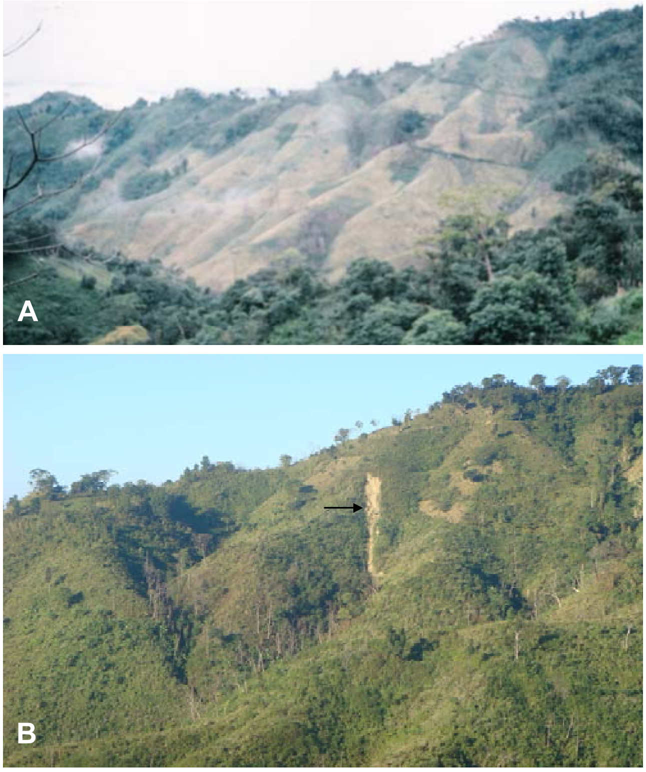

Fig. 12 Ecological services. (**A**) Slash and burn system of agriculture at the mountain slope of a bamboo forest. (**B**) Destruction of bamboo forest results in soil erosion and landslides, indicated by an arrow.

This system is practiced by burning stands of these bamboos which in itself is a great loss in term of revenue. Thus *M. baccifera* protects and preserves the mountain ecosystem and rain watersheds, regulates water flow, recharges the water table and conserves the flora and fauna, etc. while providing environmental security for the local people.

CONCLUSION

Bamboo plays a very important role in the traditional and economical ways of Mizo people. Out of these bamboos, *M. baccifera* is the most important bamboo species and helps the people in day-to-day life. Bamboo-related products are the major source of income to the state as well as the people. Many people earn their livelihood from this plant. It also contributes an amount to the state government income. *M. baccifera* forests and regrowth areas in critical mountain slopes where abandoned slash and burn agriculture sites are located are ecologically restored. Often, these slash and burn agriculture sites are located in close proximity to village habitations. Thus, *M. baccifera* to ensure environmental security (protection of catchments, regulation of water flow, recharge of water table, conservation of flora and fauna etc. and protection of developmental infrastructure like roads, bridges, human settlements, habitations, etc.).

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