THESIS

ON

MANAGEMENT OF WATER HYACINTH BIOMASS ALONG WITH INDUSTRIAL EFFLUENTS THROUGH MUSHROOM CULTURING & VERMICOMPOSTING

Submitted in partial fulfillment of the requirements

For the award of degree of

MASTER OF TECHNOLOGY

in

(ENVIRONMENTAL SCIENCE & TECHNOLOGY)

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JUNE 2010

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CERTIFICATE

This is to certify that the work done in this Thesis report entitled "**Management of water hyacinth biomass along with industrial effluents through mushroom culturing & vermicomposting**" submitted in partial fulfilment of requirement for the award of the **Master of Technology in Environmental Science & Technology** in the department of Biotechnology & Environmental Science, Thapar University, Patiala, is an authentic record of work carried out by me under the guidance of **Dr. A.S Reddy,** Associate Professor**, Thapar University, Patiala.**

The matter embodied in this report has not been submitted in part or full to any other university or institute for the award of any degree.

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ACKNOWLEDGEMENT

I would like to express my gratitude and thank my guide Dr. A.S. Reddy (Associate Professor, Department of Biotechnology & Environmental Sciences, Thapar University, Patiala) for his guidance.

Thanks are also to the other faculty members of Department of Biotechnology and Environmental Sciences for the support provided.

I am also thankful to all the employees of TCIRD especially **Mr. Mukesh Aggarwal** who helped me in the project.

I am also grateful to the authorities of TCIRD, Thapar University, Patiala for allowing to work in the laboratory and use the facilities.

I am also thankful to my friends **Mr. Amit Kumar, Mr. Devender Singh Poonia, Mr. Balpreet Singh, Mr. Ghanshyam Mourya, Ms. Pooja Sharma and Ms. Amita Jain** who helped me in the project.

Finally, I thank all my family members, friends and colleagues who have both directly and indirectly contributed towards successful completion of this project thesis report.

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ABSTRACT

Water hyacinth is one of the fast growing notorious aquatic weed. However, the weed can be effectively used in the wastewater treatment and in the control of eutrophication and pollution of water bodies, provided the weed can be properly managed. Management through harvesting removal though highly desirable has not been successful. The removed biomass usually proves a nuisance. High water content, accumulation of pollutants like heavy metals and apparent presence of some irritant compounds, make the harvested biomass not very useful.

Use of the harvested biomass for mushroom culturing and vermicomposting of the spent substrate is believed to make the use of water hyacinth for wastewater treatment and for the control of pollution of water bodies both feasible and viable. In the present study, use of water hyacinth biomass as substrate along with industrial effluent as supplements in the mushroom culturing and vermicomposting of water hyacinth along with cow dung have been tried. In addition to it, mushroom culturing has also been studied on wheat straw as substrate without adding any supplements for comparison.

Mushroom culturing though successful on wheat straw, was found not successful on water hyacinth biomass. In fact, mycelium growth was found to be much more in water hyacinth bags than on wheat straw. But, pinning and fruiting was not achieved in water hyacinth bags may be due to improper environmental conditions. Vermicomposting was carried out on water hyacinth biomass mixed with cow dung in the ratio of 1:3. Constituents of the water hyacinth biomass (may be heavy metals or certain irritant substances specific to water hyacinth biomass) must not be allowing survival of earthworms especially under the stressed environmental conditions like temperature and humidity. A 50-day study of vermicomposting resulted in the reduction in carbon content, total potassium and increase in calcium. The heavy metal concentration (i.e. Cu, Zn, Ni, Cr, Mg, Pb) is found to be less in vermicomposts than in initial feed mixtures.

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CHAPTER 1

INTRODUCTION

1.1. BACKGROUND

Water hyacinth (*Eichhornia crassipes*) is one of the most intransigent weeds of the world. It has successfully resisted all attempts of eradicating it by chemical, biological, mechanical or hybrid means. Wherever water hyacinth is not controlled, due to limited resources or other reasons, it rapidly covers all the water-bodies and surrounding marshy areas in those regions. At an average annual productivity of 50 dry (ash-free) tonnes per hectare per year, water hyacinth is one of the most productive plants in the world. Such colonization of wetlands leads to rapid decline of the quantity and the quality of water (DO depletion and aging of water bodies) through addition of dead biomass. It also affects aquatic ecosystems health and biodiversity of the water bodies. The only use of water hyacinth that has found world wide acceptance is in treating biodegradable wastewaters (**Tchobanoglous and Burton, 1991**). Also, volatile fatty acids (VFAs) has been extracted which can be used as feed supplement in slurry biogas digesters and solid-feed digesters to generate fuels (**Ganesh *et al*., 2005**). But the quantities of the weed that can be utilized in this manner are very low. Even if these options are gainful, the problem of disposal of 'spent' weed still remains. There is a need of novel technology (or combination of technologies), which is (or are) ecological sound and economically viable, to solve the problem of aquatic weed management and disposal. It can be possible by using water hyacinth biomass as a substrate for growing mushrooms.

Due to the change in consumer's preference, the demand for organic and healthy foods is increasing world over. Mushroom (which falls under the category of healthy, medicinal organic foods) is one of the most delicious food ingredients. Mushroom production is the biggest solid-state fermentation industry in the world. Mushroom production is associated with the generation of large quantities of spent mushroom compost (spent substrate). 1 kg mushroom production results in the generation of about 5 kg spent mushroom compost (SMC) (**Sample *et al*., 2001**). So, the biomass of water hyacinth as a substrate for mushroom production, disposal of spent substrate will continue to remain a haunting problem. In the past decades, potential of earthworms for breaking down organic waste has been explored in depth and many large scale vermicomposting have been developed all over the world with varying success (**Garg *et al*., 2005**). It is a viable, cost-effective and rapid technique for the efficient management of the organic solid wastes. Vermicompost is considered as an excellent product since it is homogenous

and has reduced level of contaminants and tends to hold more nutrients over a longer period, without impacting the environment (**Edwards and Niederer, 1988**).

Also, on one side, tropical soils are deficient in all necessary plant nutrients and on the other side, large quantities of such nutrients contained in domestic wastes and agricultural byproducts are wasted. It is estimated that in cities and rural areas of India nearly 700 million tones organic waste is generated annually which is either burned or land filled (**Bhiday, 1994**). Similarly, large quantities of nutrients are contained in the municipal sewage. Further, organic contamination, necessitates sewage treatment (both for the removal of organic matter and nutrients) prior to disposal into natural water bodies. Otherwise, the water bodies can be eutrophicated and polluted. For many obvious reasons, the conventional treatment technologies are not that successful, at least in developing countries. Use of aquatic plants, like water hyacinth, in the sewage treatment, apparently, holds the key for the problem.

In nature's laboratory, there are many organisms (both micro and macro) with the ability to convert organic waste into valuable resources, which are critical for maintaining soil productivity. Earthworms are the most important among them. These earthworms have been beneficially used in vermicomposting for the stabilization of municipal solid wastes and converting them into vermicompost. This compost is rich in nutrient content and humus and can be used as both fertilizer and soil conditioner. A revolution is in fact unfolding in vermicomposting for achieving quicker and cheaper solutions to several social, economic and environmental problems plaguing the human society from 'waste management' to 'land and soil remediation' and 'safe and sustainable food production' without recourse to dangerous agro-chemicals.

Water hyacinth biomass (harvested from water hyacinth infested natural water bodies), of course after using as substrate in mushroom culturing, can be transformed into compost through vermicomposting route and used in agricultural fields to close the nutrient circulation loop.

1.2. OBJECTIVES

There are mainly two objectives of the thesis:

- To develop protocols for the mushroom culturing of water hyacinth biomass and for the vermicomposting of spent mushroom substrate.
- To understand the fate of selected heavy metals of the water hyacinth biomass during mushroom culturing and vermicomposting.

1.3. OVERVIEW OF THE CONTENTS OF THE REPORT

This thesis report includes five chapters along with references and bibliography:

Chapter 1 (**Introduction):** It highlights the importance of the topic, explicitly states the overview of the thesis report contents, and concludes through stating the importance and usefulness of the thesis.

Chapter 2 (**Review of Literature):** It includes a comprehensive overview on the water hyacinth, mushroom culturing, vermicomposting and vermicomposting of spent mushroom substrate. It also includes the management and utilization of water hyacinth, cultivation technology of mushroom and vermicomposting.

Chapter 3 (**Methodology):** This chapter deals with work elements involved in the thesis including experimental set up built for mushroom cultivation, protocol developed for mushroom cultivation and vermicomposting. It also includes different parameters that require monitoring and also analytical techniques followed during mushroom cultivation and vermicomposting.

Chapter 4 (**Results and Discussions):** This chapter includes the results obtained along with the discussions during thesis.

Chapter 5 (Conclusion): This chapter includes the conclusion derived from the thesis work.

This thesis report concentrates mainly on the oyster mushroom cultivation and vermicomposting of water hyacinth biomass. This covers the idea of management of aquatic weed i.e. water hyacinth. Also, proper literature has been surveyed and compiled up in this report which can be utilized for further study.

CHAPTER 2
REVIEW OF LITERATURE

2.1. INTRODUCTION

Review of literature has been carried out on the following aspects:

- Water Hyacinth
- Mushroom culturing
- Vermicomposting with focus on the spent mushroom substrate

This chapter provides brief overview of the work done in this area.

2.2. WATER HYACINTH

2.2.1. WATER HYACINTH

Eichhornia crassipes (Water hyacinth) is a member of pickerelweed family (*Pontederiaceae*). It is a monocotyledonous, perennial, free floating and most intransigent weeds of the world. Optimum growth of water hyacinth occurs in eutrophic, still or slow-moving fresh water with a pH of 7, a temperature range between 28-30°C, and abundant nitrogen, phosphorus and potassium (**Chadwick and Obeid 1966; Knipling *et al*., 1970**). Weeds can, however, tolerate a wide range of growth conditions and climatic extremes. Good growth can continue at temperatures ranging from 22-35°C and can survive frosting (**Wright and Purcell, 1995**). Although prolonged cold weather may kill plants, the seeds remain viable (**Ueki and Oki, 1979**). Plants can tolerate acidic waters but cannot survive in salt or brackish water (**Penfound and Earle, 1948**).

Fig: 2.1 Water hyacinth growing in a pond.

2.2.2. IMPACTS AND THREATS POSED BY WATER HYACINTH

E. crassipes is a highly competitive plant that is capable of rapid growth and spread. It can displace native species, reduce biodiversity, limit recreation, diminish aesthetic value and decrease water quality and flow. Threats posed by water hyacinth are given below:

1. **Hindrance to water transport:** Access to harbours and docking areas can be seriously hindered by mats of water hyacinth. Canals and freshwater rivers can become impassable as they clog up with densely intertwined carpets of the weed.
2. **Blockage of canals and rivers causing flooding:** Water hyacinth can grow so densely that a human being can walk on it. When it takes hold in rivers and canals it can become so dense that it forms a herbivorous barrage and can cause damaging and dangerous flooding.
3. **Micro-habitat for a variety of disease vectors:** The diseases associated with the presence of aquatic weeds in tropical developing countries are among those that cause the major public health problems: malaria, schistosomiasis and lymphatic filariasis. Some species of mosquito larvae thrive on the environment created by the presence of aquatic weeds.
4. An acre of water hyacinth can weigh more than 200 tons; infestations can be many, many acres in size; mats may double their size in as little as 6-18 days (**Mitchell, 1976**).
5. Water hyacinth mats degrade water quality by blocking the air-water interface and greatly reducing oxygen levels in the water, eliminating underwater animals such as fish (**Penfound & Earle, 1948**).
6. **Increased evapotranspiration:** Various studies have been carried out to ascertain the relationship between aquatic plants and the rate of evapotranspiration compared with evaporation from an open-surfaced water body. The rate of water loss due to evapotranspiration can be as much as 1.8 times that of evaporation from the same surface but free of plants.
7. **Reduction of biological diversity:** Mats eliminate native submersed plants by blocking sunlight, alter immersed plant communities by pushing away and crushing them, and also alter animal communities by blocking access to the water and/or eliminating plants. (**Gowanloch, 1944**).
8. Dense floating rafts of water hyacinth can form on the water's surface, restricting light to the complete exclusion of other native plants and decreasing the air exchange between the water's surface and the atmosphere.
9. Algae, a major component of the base of the food chain, can be shaded out by dense mats of water hyacinth. The resulting decline in algae can disrupt the entire food web in a water body.

2.2.3. CONTROL OF WATER HYACINTH

The complete removal of water hyacinth is impossible for most areas. Where 'eradication' of an infestation has occurred, the effects are usually short-term. Plants and/or seeds are readily transported by currents, boats, fishing nets and possibly animals and birds, and only one or a few plants can result in a new infestation. The seeds are long-lived and germination can continue for up to 20 years. The aim is therefore to manage, rather than eradicate, this weed species. Control methods fall into three main categories: physical, chemical and biological. The application of these methods is not mutually exclusive and 'best practice' is to formulate a management strategy incorporating some or all of these methods, but with reliance on biological control as the most significant component or the long term objective (**Harley *et al*., 1996**).

1. **Physical:** Physical methods include removal by hand, use of equipments such as cranes, mowers or weed harvesters etc. Floating booms and barriers are used to maintain areas free of weed and to reduce the downstream spread of an infestation. The rate of growth and invasion by water hyacinth usually exceeds the rate at which it can be cleared. Reinfestation from plant fragments and/or seeds generally occurs rapidly and the process of removal must be repeated continuously. The material removed from the water should be transported away from the site and disposed of appropriately. Mats of water hyacinth can be enormous and can have a density of up to 200 tonnes per acre (**Harley, Julien and Wright, 1997**). Reducing the nutrients that water hyacinth thrives on will reduce its proliferation. This treatment is more of a preventative measure than a method of eradication. It is costly and cannot deal with very large infestations. It is not suitable for large infestations and is generally regarded as a short-term solution.
2. **Chemical:** Applying herbicides like diquat, glyphosate, amitrole, and the amine and acid formulations of 2,4-D (2,4-dichloreophenoxyacetic acid), applied as foliar sprays to eradicate water hyacinth is the most effective method. **Maclean in 1922** tried a number of inorganic chemicals on water hyacinth. It has been recorded that $CuSO_4$ and $BaCl_2$ at a concentration of 0.018% or more killed the water hyacinth. **Hildebrand in 1946** was successful in killing water hyacinth with 0.1% 2,4-D. The application of these compounds requires skilled operators, strict spray regimes, long term vigilance and frequent reapplication to provide effective, long-term control over the weed and any re-growth. In most situations, chemical control is unacceptably costly in terms of chemicals, equipment, labor and environmental impact. Herbicides can kill non-targeted plants and pollute the water.
3. **Biological:** Biological control is the use of natural enemies to control or reduce pests. Water hyacinth is attacked by a complex of arthropods. Moths, fungi and a variety of weevils are predators of water hyacinth. The two *Neocbetina* species are the most widely distributed of the water hyacinth biological control agents. **Center *et al*., (1999**) suggest that biological and herbicidal controls should be integrated; using herbicides to maintain

water hyacinth infestations below management thresholds but in a manner that conserves biological control agent populations. **Williams *et al*., (2005)** inferred that although weevils likely played a role in the rapid disappearance of water hyacinth in Lake Victoria, the cloudy weather of 1997/1998 was probably a major contributory factor to poor growth and reduction in water hyacinth biomass lake-wide. One major drawback is that it can take a long time to initiate such projects because it can take several years for the insect population to reach a population density sufficient to tackle the pest problem.

Out of the above three mentioned methods, physical method of control is best if we want to use water hyacinth for further utilization like in biogas, alcohol production, and also for mushroom culturing, etc.

2.2.4. POTENTIAL UTILIZATION OF WATER HYACINTH

Water hyacinth contains more than 95% water but due to its fibrous tissue and a high energy and protein content, it can be used for a variety of useful applications. These are:

1. **Water hyacinth as phytoremediation agent:** Water hyacinth is one of the plant species that attracted considerable attention because of its ability to grow in heavily polluted water together with its capacity for metal ion accumulation. It has found world-wide acceptance in treating wastewaters (**Tchobanoglous *et al*., 1989**). Natural wetland systems colonized by water hyacinth serve as "nature's kidneys" for proper effluent treatment to preserve the earth's precious water resources from getting polluted. It has been used for decontaminating inorganic nutrients, toxic metals as well as persistent organic pollutants. It is used for treating number of wastewaters as given below:

   a. Nutritionally rich waste waters: The use of water hyacinth wetlands as biological filters for agricultural runoff (**Reddy *et al*., 1982**) or for polishing the treated dairy effluents (**Tripathi and Upadhyay, 2003**) provides a promising strategy in view of the ample space availability in these areas. In India, water hyacinth-based wastewater treatment plants (pilot/full-scale) are performing well with regards to reduction in BOD, COD and total nitrogen reduction despite poor designing and vegetation management (**Trivedy and Thomas, 2004**). Moreover, suitable combinations of water hyacinth with other aquatic plants such as duckweed and/ or blue-green algae produce superior nutrient removal than water hyacinth alone (**Sinha and Sinha, 2000**).

   b. Industrial effluents: Also, water hyacinth can be successfully utilized in treatment of industrial effluents due to its ability to survive in presence of toxic contaminants. **Jayaweera and Kasturiarachchi (2004)** demonstrated that water hyacinth is a promising candidate for removal of nitrogen and phosphorus from industrial wastewaters. Further, the use of water hyacinth in treatment of pulp and paper mill (**Menon *et al*., 2005**), tannery, textile and electroplating effluents has also been investigated.

c. Metals and other pollutants: Zhu *et al*., (1999) have studied the ability of water hyacinth to take up and translocate six trace elements namely As (V), Cd (II), Cr (VI), Cu (II), Ni (II) and Se (VI) under controlled conditions. Cd, Cr, Cu, Ni and As were more highly accumulated in roots than in shoots whereas Se was accumulated more in shoots than in roots at the most external concentrations. **Roy and Hanninen (1994)** studied the uptake/elimination kinetics and metabolism of a well known pollutant pentachlorophenol (PCP) by water hyacinth. They observed an initial rapid PCP uptake by the plant that reached a nearly steady state between 24 and 48hr. The major by-products of PCP metabolism in the plant were ortho- and para-substituted chlorohydroxyphenols (chlorocatechols and -hydroquinones), -anisoles, and -veratroles along with traces of dechlorinated products of PCP. **Nor (1994)** studied the removal of phenols in the presence of copper and zinc by water hyacinth.

d. Sewage treatment: Water hyacinth in particular is preferred because of its handiness and high productivity especially when grown in sewage (**Rogers *et al*., 1972**). The plant grows luxuriantly in sewage and has an extensive root system that allows it to absorb nutrients directly from sewage. Studies have shown that the plant is very efficient in the removal of very large quantities of nutrients from raw sewage (**Boyd 1970; Stewards, 1970**). The potential of adopting water hyacinth in sewage treatment concluded that the plant survived with normal growth and was efficient in the removal of pollutants in synergy with microorganisms. **Wolverton in 1975** studied effluent sewage from a wastewater lagoon with a retention period of 14 days and observed a 97% reduction in influent and 77% reduction in effluent waste. **McDonald and Wolverton in 1979** obtained 94% reduction in BOD from a facultative waste water lagoon during summer. Others have reported some special attributes of water hyacinth which include its ability to remove coliform bacteria and viruses form diluted sewage and reduction in nutrients like total nitrogen (by 99% in a 2-day retention), ammonia (by 97.9% after 14-day retention), organic compounds and pathogens from water (**Gopal, 1987**).

The use of water hyacinth has found world wide acceptance in treating biodegradable wastewaters (**Tchobanoglous and Burton, 1991**). By planting water hyacinth in a wastewater pond, part of the gaseous oxygen produced by photosynthetic activity of the green leaves is translocated to the stems and roots and to the water body; this oxygen is used by the aerobic and facultative bacteria in biodegrading organic matter contained in the wastewater. Two groups of bacteria normally exists in a water hyacinth pond, namely the suspended bacteria which are present in the liquid portion and the biofilm bacteria which are attached on the surfaces of the roots of the water hyacinth plants and on the side walls and bottom layers of the pond itself.

2. **Alcohol production**: Hemicellulose component of the lignocellulosic biomass is considered as an attractive raw material for the production of fuel ethanol. Relatively high content of hemicellulose (30-55% of dry weight) in the water hyacinth indicates that it could be a good

source of hemicellulose for bioconversion (**Nigam, 2002**). The production of fuel ethanol from biomass is a multistage process that involves prehydrolysis, hydrolysis, fermentation, and distillation.

Saccharification of the water hyacinth and subsequent fermentation of the reducing sugars to alcohol using *Saccharomyces cerevisiae* has been explored (**Abraham and Kurup, 1996**). **Nigam (2002)** also investigated ethanol production from untreated and treated water hyacinth hemicellulose acid hydrolysate using *Pichia stipitis* NRRL Y-7124.

3. **Biogas production**: Conversion of organic matter (usually animal or human waste) to biogas is a well established small and medium scale technology. Better yields of biogas are obtained using mixture of animal waste and water hyacinth (**Kumar, 2005**) and the sludge obtained from mixed feed with better nitrogen, phosphorus and potassium content can be utilized as very good manure. However, use of the water hyacinth for digestion in a traditional digester presents some problems (**Mshandete *et al*., 2004**) such as large digester size, lower biogas conversion efficiency (due to very high water content) and mandatory pre-treatment before digestion (to remove air entrapped in the tissue).

   Attempts have also been directed towards extraction of volatile fatty acids (VFAs) from the water hyacinth to be used as feed-supplement in slurry biogas digesters (**Abbasi and Ramasamy, 1996**), and solid-feed digesters to generate fuel (**Abbasi and Ramasamy, 1999b**).

4. **Compost preparation:** Conventional composting, which is suitable for labor intensive, low capital production can be done by mixing dried plant with ash, soil and some animal manure/organic municipal waste. Vermicomposting of the water hyacinth is more advantageous because the water hyacinth loses its ability to reproduce vegetatively after it has passed through the earthworm gut (**Abbasi and Ramasamy, 1996**). Also, vermicasts produced during vermicomposting are believed to contain enzymes and hormones that stimulate plant growth and discourage pathogens (**Ismail, 1997; Szczeck, 1999**).

5. The fibers from the stems of the water hyacinth plant can be used to make rope, baskets or even good quality paper if blended with waste paper or jute. The potential use of the water hyacinth as a pulp material for producing greaseproof paper has also been successfully investigated (**Goswami and Saikia, 1994**).

6. In India, many natives use the water hyacinth as a medicinal plant mainly to treat the goiter disease (**Oudhia, 1999a, b**). They have optimized the formulations i.e. either fresh water hyacinth, table salt and *Piper longum* mixed in equal quantity (12g mixture/d) or dried and burnt water hyacinth taken with fresh cow urine.

2.3. MUSHROOM CULTURING

Mushrooms, also called 'white vegetables' or 'boneless vegetarian meat'. India has large number of agro-climate regions that offer congenial climate condition for mushroom cultivation. At present 3 mushrooms are being cultivated in India.

Mid-November to Mid-March	: White mushroom (*Agaricus bisporus)*
February to Mid-April	: Oyster mushroom (*Pleurotus sajor-caju)*
Mid-June to Mid-September	: Paddy straw mushroom (*Volvariella volvacea)*
September to November	: Oyster mushroom (*Pleurotus sajorcaju)*

Oyster mushroom can grow at moderate temperature ranging from 22 to 28°C. Therefore, it is suitable for most of the places of India. In north India, the climate conditions prevailing during different seasons can be exploited for growing mushroom throughout the year.

Mushrooms contain proteins, vitamins, fibers and medicines. It contains 20-35% protein (dry weight) which is higher than those of vegetables and fruits and is of superior quality. The niacin content is about ten times higher than any other vegetables. The folic acid present in oyster mushrooms helps to cure anemia. It is suitable for people with hyper-tension, obesity and diabetes due to its low Na: K ratio, starch, fat and calorific value. Alkaline ash and high fiber content makes them suitable for consumption for those having hyperacidity and constipation.

2.3.1. RAW MATERIALS

The main nutritional sources for oyster mushroom are cellulose, hemicelluloses and lignin. C/N ratio is important factor for optimal substrate composition. Oyster mushroom is a white rot fungus that utilizes organic matter containing lignin and cellulose & it includes wastes such as corncobs, cotton waste, sugarcane bagasse and leaves, corn leaves, grasses, rice hulls, and water hyacinth leaves for growing the mushroom together as carbon and nitrogen sources and inorganic K, P, Si, Fe, Mg etc. (**Quimio, 1986**). The substrates used in each region depend upon the availability of agricultural wastes.

Kimenju *et al*., in 2009 evaluated the locally available substrates that are suitable for Oyster mushroom (*Pleurotus ostreatus*) cultivation in Kenya. Ten different substrates namely water hyacinth (*Eichhornia crassipes*), maize cobs, coconut fibre, finger millet straw, banana fibre, sawdust, rice straw, bean straw and wheat straw were tested for their suitability in mushroom production.

Pleurotus ostreatus was cultivated on dry weed plants like *Leonotis* sp*, Sida acuta, Parthenium argentatum, Ageratum conyzoides, Cassia sophera, Tephrosia purpurea and Lantana camara* (**Das *et al*., in 2007**).

2.3.2. OYSTER MUSHROOM CULTIVATION USING WATER HYACINTH

Water hyacinth provides compost with carbohydrates, the basic food stuffs of mushroom nutrition. Water hyacinth is 36% cellulose, 25% pentose and 16% lignin. Cellulose and pentose are carbohydrates which upon break down yield simple sugars. These sugars supply the energy for microbial growth. Lignin, a highly resistant material also found in the heartwood of trees, is changed during composting to a "Nitrogen-rich-lignin-humus-complex", a source of protein. In essence, water hyacinth is a material with the structural and chemical properties ideal for making mushroom compost.

Murugesan with his coworkers in the year 1995, an attempt was made to utilize the water hyacinth (*Eichhornia crassipes)* for oyster mushroom cultivation and a good yield was achieved. Before this, edible mushroom cultivation in paddy straw has been practised in India. This was related to the ideal C/N ratio and low lignin content. This technology could be very cheap and it is also a method of helping to eradicate a troublesome aquatic weed.

Mahmoud in the year 2006 studied the biodegradation of water hyacinth *(Eichhornia crassipes)* without roots by growing *Pleurotus ostreatus* and *P. sajorcaju* and trial for using in production of mushroom spawn. The mushroom delignified the lignocellulose of water hyacinth and produced sugar, protein and organic matter. *Pleurotus ostreatus* and *P. sajorcaju* delignified 26.2Â±2.0 and 30.4Â±3.0% of the plants and utilized most of its hemicellulose after 7 weeks under its growing condition more than in solid-state fermentation. The produced biomass was enriched with mycelia protein, organic matter and reducing sugars, which increased gradually with incubation time. The effect of incubation time and moisture contents on spawn production by using chopped water hyacinth biomass was also studied. 70% moisture content and 14 days of incubation are the optimum conditions for spawn production for both tested *Pleurotus* species.

Kivaisi *et al*., in 2006 identified the performance of *Pleurotus flabellatus* on water hyacinth (*Eichhornia crassipes*) shoots at two different temperature and relative humidity regimes in Tanzania. This study investigated the suitability of water hyacinth as a bulk substrate for growing a newly domesticated local oyster mushroom, *Pleurotus flabellatus*. The performance of the mushroom was investigated under ambient temperature and relative humidity (RH) regimes of 18-25/27-29Â°C and 55-85/78-93%, respectively. The growth cycle of the mushroom was completed in 40 days with three and four flushes respectively. At the higher temperature and RH regime, the mushroom grew faster and the first flush was harvested at the 13th day after substrate inoculation with a Biological Efficiency (B.E.) of 84%, whereas the first harvest was done on the 19th day after inoculation at the lower temperature and RH regime with a B.E. of 53%. Substrate total fibre loss at the end of the growth cycle was in the range of 31-40%, and cellulose the most utilized fraction, decreased by 35-48%. The rates of fibre loss increased over time during the mushroom growth and were highest during the first and second flush during which about 80% of

the total mushroom yields were obtained. Water hyacinth shoots proved to be a good substrate for growing the local oyster mushroom at ambient environmental conditions.

2.3.3. MUSHROOM CULTIVATION TECHNOLOGY

The procedure for oyster mushroom cultivation can be divided into following steps. The whole cycle of mushroom cultivation is shown in figure 2.2.

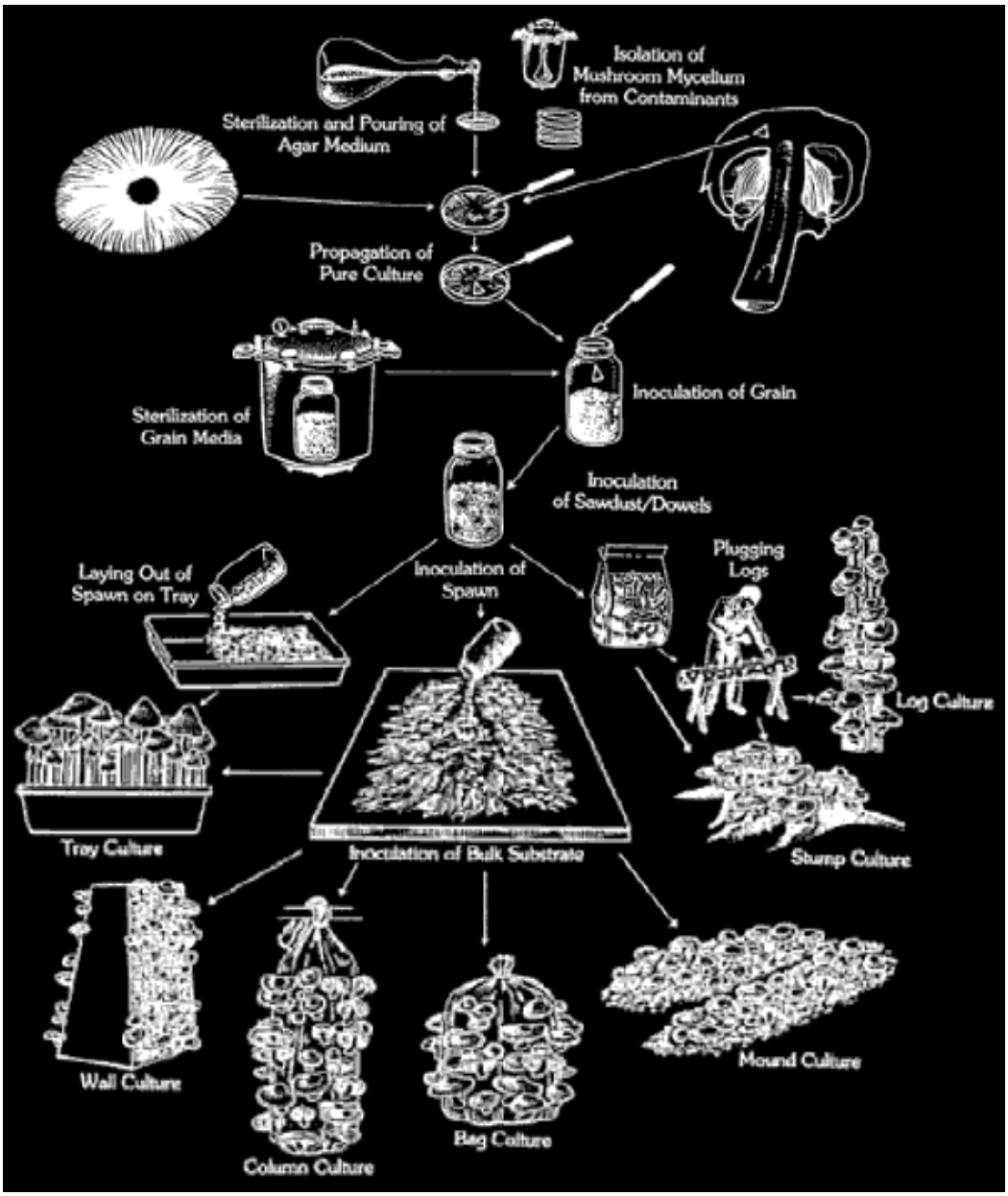


Fig: 2.2 Diagram illustrating overview of general techniques for the cultivation of mushrooms.

Source: Stamets, P. and Chilton, J.S. The Mushroom Cultivator. A Practical guide to growing mushrooms at home.

(i) **Preparation or procurement of spawn:** Mother spawn can be procured from any mushroom research center and primary culture of spawn can be prepared by following procedure: (**Mushroom Growers Handbook**)

1. Select good quality jowar or wheat grains free from pest and moulds.
2. Boil the grains submerged in clean water for 20-30 minutes. When the grains become soft, remove and spread evenly on a cotton cloth to drain out the water and cool the grains.
3. Mix 3% chalk powder (30g/ kg of grain) for adjusting the pH and to keep the grains loose.
4. Fill 250gms of grain in cleaned and dried glucose bottle of 500ml capacity or propylene bags and plug the mouth of the bottle tightly with non-absorbent cotton.
5. Sterilize the bottles in autoclave by exposing to 121°C and 15lbs pressure/sq inch for 20 minutes. After cooling, transfer the bottles to inoculation chamber.
6. Transfer few grains with mycelial growth into sterilized substrate bottle under aseptic condition and plug it with cotton.
7. Shift the inoculated bottles to spawn running room having temperature range of 25-30°C.

(ii) **Substrate preparation:** Substrate mixture of oyster mushroom should supply specific nutrients required for oyster mushroom cultivation.

Nutritious materials required for oyster mushroom are given below:

1. **Organic:**
   a. C source- Cellulose and hemicellulose
   b. N source- Protein and amino nitrogen
2. **Inorganic:** K, P, Si, Fe, Mg, etc.

There are many methods of treatment that are given to substrate before spawning (**Dr. Ajay Singh, Haryana Agro Research and Development Center, Murthal (Sonipat)**). These are:

1. **Hot water Treatment:** Substrate is filled in "jute bag" and soaked in cold water for the whole night. On second day, this bag is dipped in hot water (70-80°C) for 15 to 20 minutes. When it is cooled, then it is spread on the plastic sheet.
2. **Pasteurization:** In this, heap of wet substrate is made on cemented floor. Temperature of room is kept constant with air using blower. It is maintained at 70°C for 6 hours or 40-45°C for 30-40 hours. Turning of the substrate is done after every two days. Then, substrate is left overnight to cool down to 25-30°C and then it is spawned.

3. **Sunlight treatment:** Substrate treatment can also be done using sunlight. Firstly, the substrate is dipped in water for 24 hours. Then, it is spread on floor for 5 hours so that its moisture content is not above 70%. Later on, it is spread under sunlight from 11A.M to 3P.M. and covered with transparent polythene.
4. **Fermentation:** In this method, compost is prepared by fermentation. The substrate is spread on cemented floor or plastic sheet and watering is done for 48 hours until the substrate is not wet. Watering, however, should not be so excessive that the substrate becomes waterlogged and water came out from substrate. Then, supplements are added, covered and kept for 24 hours. Heap of substrate is prepared and kept for a month. Proper turnings are given to it.
5. **Chemical Sterilization:** In this technique, some special type of fungicides is used to kill the microbes. In 200 liter drum or plastic tub (containing about 100 liter water), 12 to 15 kg of substrate is dipped. In bucket having 10 liter water, 7.5gm bavistin and 125ml of formalin are added and mixed properly. Then, this mixture is spread on the substrate and covered with the plastic sheet. After 18 hours, substrate is spread on a cemented floor.
6. **Steam sterilization:** After soaking into the water for 12 hours, the substrate is moved to the pasteurization room. Heat up the room till the temperature reached to 60°C and maintain the temperature by steam injection for 2-3 hours. Cool down the substrate temperature.

(iii) Bagging & Spawning of substrate: The polyethylene bags with a thickness of 0.05-0.1cm and have a 25cm diameter and 80-100cm in length are used for mushroom cultivation. At first, the bags are perforated with arc punch that makes holes of 1-1.2cm in diameter at intervals of 10cm both horizontally and vertically (**Mushroom Growers Handbook**).

Freshly prepared (20-30 days old) grain spawn is best for spawning. Old spawn (3-6 months) stored at room temperature (at 20-300°C) forms a very thick mat like structure due to mycelium aggregation and sometimes young pinheads and fruit bodies start developing in the spawn bottle itself. The spawning should be done in a pre-fumigated room (48hrs with 2% formaldehyde). The quantity of spawn added is generally 2% of compost, but it varies with mushroom's species.

The layer of substrate is put in the bag and then spawn is sprinkled over it. Then, put another layer of substrate and again sprinkle spawn. Repeat this until the bag is full. The last layer of spawn is covered with very shallow layer of substrate. Then, tie the bag's opening tightly.

(iv) Spawn run (Incubation): After inoculation, the bags are incubated in specially arranged room where the microclimate factors such as light, temperature, humidity and ventilation are strictly controlled. *Pleurotus* mushroom need different environmental conditions both for vegetative and reproductive growth. During incubation, appropriate relative humidity is 85-90%, water content of substrate is 65% and optimal temperature required for mycelial growth is 20-

25°C. Mushroom mycelia are quite durable to high concentration of carbon dioxide during incubation. Upon the completion of incubation, pinning induction follows.

(v) Pinning, Fruiting and harvesting: Pinning induction is made by worsening the environment in order that the mycelia can't keep on with their vegetative growth and will therefore convert to a reproductive growth mode, which initiates fruiting formation. Pinning induction includes cold shock, watering and lighting. Once pins come out, growers stop pinning induction and maintain environmental conditions that are favorable to fruiting. Carbon dioxide concentration should be less than 800 ppm in its reproductive growth. Fruit body formation also requires high humidity up to 80-95%, lower temperature of 20-28°C and light of 50-500 lux for primordial formation. Then, mushrooms are harvested. It is not advisable to use knives to cut the fruit bodies at the base because some stumps will be left and these can cause the crops to suffer from infections.

2.3.4. ABNORMALITIES IN FRUITING BODY

The formation and growth of fruiting bodies are sensitive to environmental conditions, such as temperature, humidity, carbon dioxide concentration and moisture content in the mushroom substrate. Improper balance of these factors can induce fruiting body deformations. These are given below: (**Mushroom Growers handbook**)

1. **Temperature and Relative Humidity:** It affects the fruiting bodies shape. Optimal temperature and humidity for fruiting body formation of oyster mushroom are 13-16°C and >80%. High and low temperature indicates >16°C and <12°C, respectively and high and low humidity indicates >80% and <60%.
2. **CO_2 concentration:** High CO_2 concentration is one of the major causes of abnormality in fruiting bodies. Proper ventilation is needed in order to reduce CO_2 concentration. Too much air movement caused by excessive ventilation also induces abnormalities in fruiting body shapes. An increase of CO_2 concentration can decrease cap sizes and increase length of stipes. However, even cap stipes are short at CO_2 concentrations of more than 0.5%.
3. **Substrate moisture content:** Disease usually increases with too much watering on cultivation beds (excessive moisture content). Too little watering reduces yields and induces abnormal shapes in fruiting bodies. Fruiting bodies become brown on dry cultivation beds.

2.4. VERMICOMPOSTING

Vermicomposting can be defined as "a simple process of composting organic matter by certain species of earthworms into a better end product (widely known as vermicompost)". Vermicomposting involves worms feeding on the organic waste and defecating as worm casts (humus-like material known as vermicompost). It can be used to make a positive environment impact by reducing the amount of green/organic and biodegradable waste that finds its way into landfills, incinerators and sometimes the water bodies including oceans. Vermicomposting is different from the standard composting (wherein microorganisms play a major role) and preferred over the latter because of the following reasons:

1. Low maintenance of the process.
2. Faster decomposition.
3. Not requiring manual or mechanical turning (consequently reduced methane emissions).
4. Vermicompost being better than the compost in promoting plant growth and increasing crop yield.
5. Increased water holding capacity of soil with vermicompost application.
6. Production of crops with better taste, luster and lasting quality and without toxic residues.
7. Reduced risk of salinization and acidification.
8. Induced resistance to pests and disease attacks.
9. Applicability at all scales including household level.

The earthworm species commonly used in vermicomposting include:

- *Eisenia andrei* (red tiger worm or red wriggler)
- *Eisenia fetida* (tiger worm) (**Fig. 2.3**)
- *Eudrilus eugeniae* (African nightcrawler)
- *Lumbricus rubellus* (red worm)
- *Perionyx excavatus* (Indian blue worm)

Of the 4 genera, *Eisenia* are the dominant commercial composting earthworms especially in the temperate regions.

Reasons for preferring the *Eisenia spp.* include:

- Rapid consumption of food and higher rates of breeding.
- Capacity to inhabit, consume and breed in a highly nutrient environment.
- Suited to a broad range of climates (0 to 35°C) and environmental conditions.
- Handle rough treatment.

Fig: 2.3 *Eisenia fetida* - The compost worm

Source: http://en.wikipedia.org/wiki/Eisenia_foetida

E. eugeniae and *P. excavatus* are of tropical origin and best suited for indoor or controlled temperature vermicomposting.

2.4.1. CONDITIONS FOR VERMICOMPOSTING

For effective composting, an earthworm needs the following basic things: (**Munroe**)

1. A hospitable living environment (**Bedding**): High moisture absorbency, high porosity, low protein and/or nitrogen content (high C: N ratio). Commonly used bedding materials include horse manure, peat moss, hay, straw, newspapers, paper mill sludge, leaves, etc. Paper-mill sludge (**Elvira *et al*., 1996; 1997**), which has the high absorbency and small particle size, can complement well with materials straw, bark, shipped brush or wood shavings which have high C:N ratios and good bulking properties, as a bedding material.
2. **A food source**: Under ideal conditions, earthworms can consume more than half of their body weight per day. Dairy manures are the most commonly as the feedstock. Rabbit manure is apparently an exception (**Gaddie & Douglas, 1975**).
3. **Adequate moisture**: The bedding must be able to hold sufficient moisture (>50% and ideal moisture level is 70-90%). With the exception of extreme heat or cold, nothing will kill the worms faster than a lack of adequate moisture.
4. **Adequate aeration**: Worms cannot survive anaerobic conditions. High levels of grease in the feedstock or excessive moisture combined with poor aeration can conspire to cut off oxygen supplies and kill the worms. Toxic substances like ammonia can also kill the worms. One should not include meat or greasy wastes in the worm feedstock unless they are pre-composted to break down the oils & fats.
5. **Protection from temperature extremes**: Controlling temperature is vital to the vermicomposting process.

- *Eisenia* can survive low temperatures (as low as 0°C), but don't reproduce and do not consume much food.
- *Eisenia* can survive having their bodies partially encased in frozen bedding and will die only when they are no longer able to consume food.
- Though can survive temperatures in the mid-30s, earthworms prefer a temperature in the 20s (°C). Above 35°C, will cause the worms to leave the area and temperatures above 20°C stimulate reproduction and rapid food consumption.

6. **pH**: Worms can survive in a pH range of 5 to 9 (**Edwards, 1998**). But according to many experts, the worms prefer a pH of 7 or slightly higher. The pH of worm beds tends to drop over time. Worms can also adjust the pH of its medium by secreting calcium. For increasing the pH of the bedding calcium carbonate can be added to the bedding or addition of peat moss can be minimized.
7. **Salt content**: Worms are very sensitive to salts (prefer <0.5% salts, **Gunadi *et al*., 2002**). Many types of manures have high salt content (up to 8%). This is not a problem when the manure is used as a feed and applied on the top, where the worms can avoid feeding it until the salts are leached out over time by watering or precipitation (**Gaddie and Douglas, 1975**). Salts problem of the manures can be taken care of by pre-composting them outdoors by simply running water.
8. **Urine content**: Excessive urine will build up dangerous gases in the bedding. **Gaddie and Douglas (1975)** state that the manure from animals raised or fed off in concrete lots contain excessive urine because it cannot drain off into the ground. For vermicomposting, this manure should first be leached.
9. **Other toxic components**: Different feeds can contain different varieties of potentially toxic components. These typically include:
    - De-worming medicines in the manures.
    - Detergent cleansers, industrial chemicals & pesticides in the feeds such as sewage or septic sludge, paper-mill sludge, food processing wastes, etc.
    - Tannins: certain trees (cedar and fir) have high levels of tannins and these can harm worms and even drive them out from the beds (**Gaddie and Douglas, 1975**).

Gunadi *et al*., (2002) point out that pre-composting of wastes can reduce or even eliminate most of these threats. But the pre-composting reduces the nutrient value of the feed and this makes the pre-composting is a definite trade-off.

2.4.2. MECHANISMS OF WORM ACTION

Vermicomposting is a complex mechanical & biochemical transformation of organic matter achieved through the action of earthworms. Earthworms act as aerators, grinders, crushers, chemical degraders and biological stimulators (**Sinha *et al*., 2002**). These decompose organics,

mineralize nutrients, ingest the heavy metals and devour the pathogens (bacteria, fungus, nematodes and protozoa).

The steps involved in the vermicomposting are: (**Nagavallemma *et al*., 2004**)

1. Softening of the waste by the grume excreted in the 'mouth' of the earthworms and going into the 'esophagus', where the waste is neutralized by calcium (excreted by the inner walls of esophagus). From here the waste passed on to the gizzard for further action.
2. In the muscular gizzard, the waste is finely ground (with the aid of stones) into small particles (to a size of 2-4 microns) and passed on to the intestine.
3. In the intestine, the ground and pulped waste is decomposed by the secreted enzymes such as proteases, lipases, amylases, cellulases and chitinases and then absorbed. The gizzard and the intestine work as a 'bioreactor'.
4. Final process in the vermi-processing is 'humification' in which large organic particles are converted into complex amorphous colloids containing 'phenolic' materials. The humified material is excreted as vermicast.

2.4.3. METHODS OF VERMICOMPOSTING

Vermicomposting systems can be either batch systems or continuous systems. In the batch systems bedding and food are mixed and then the earthworms are added. In continuous flow systems earthworms are placed in the bedding, thereupon feed and new bedding are added incrementally on a regular basis.

Four basic types of vermicomposting systems, namely, windrows systems, wedge systems, beds and bins systems and reactor systems, are in use: (**Munroe**)

1. **Windrows**: These systems are relatively inefficient and generally result in an inferior vermicompost product because nutrients are lost through volatilization and leaching (**Edwards, 1998**). These are labour intensive and relatively slower (6-18months) and usually exposed to a broad range of environmental conditions and require large areas of land. In these, organic materials are placed on the ground up to 50 cm in depth in long rows and worms are introduced into the material (**Fig. 2.4**).

Fig: 2.4. Vermicomposting windrows of shredded cardboard and manure.

Source: Munroe, G.: 'Manual of on-farm vermicomposting and vermiculture'. Organic Agriculture Centre of Canada. Available at: http://www.organicagcentre.ca/DOCs/ Vermiculture_FarmersManual_gm.pdf.

2. **Reactor system:** Reactor systems have raised beds with mesh bottoms as shown in fig. 2.5. Feedstocks are added daily in layers on top of the mesh or grate. Finished vermicompost is harvested by scraping a thin layer from just above the grate and then it falls into a chamber below. These systems can be relatively simple and manually-operated or fully-automated with temperature and moisture controls. For maximum efficiency, they should be under cover.

Fig: 2.5. Reactor used in vermicomposting

Source:http://www.swlf.ait.ac.th/UpdData/Presentations/Thai/8%20Vermicomposting%20of%20 Food%20Wastage.pdf.

3. **Wedge system:** This is a modified windrow system housed inside a structure or outdoors, but covered with a compost cover to prevent leaching of nutrients. This system maximizes utilization of space and simplifies harvesting because there is no need to separate worms from vermicompost. Organic materials are applied in layers of 12-18 inches thickness against a finished windrow at a 45°angle. Front-end loaders are usually used to establish the wedge systems of 4-10 feet wide and varied length.
4. **Beds and bins system:** These systems have been extensively used throughout to varying degrees, from home enthusiasts to part-time worm growers to large operations. Outdoor large-scale systems usually require some type of cover to keep out direct sunlight or rain. It's a labor-intensive process to harvest worms and vermicompost by hand.

2.4.4. PROTOCOL OF VERMICOMPOSTING

Vermicomposting involves the following steps: (**Nagavallemma *et al*., 2004**)

1. Cover the bottom with a layer of tiles or polythene sheet.
2. Spread 15–20 cm thick layer of organic waste material on the polythene sheet. Sprinkle rock phosphate powder if available (it helps in improving nutritional quality of compost) on the waste material and then sprinkle cow dung slurry. Allow the material to decompose for 15 to 20 days.
3. When the heat evolved during the decomposition of the materials has subsided (15–20 days after heaping), release earthworms (500 to 700 worms per ft^2) through the cracks developed and cover the top with wire mesh or gunny bag to prevent birds from picking the earthworms.
4. Sprinkle water every three days to maintain adequate moisture and body temperature of the earthworms. When the compost is ready, do not water for 2–3 days to make compost easy for shifting.
5. The vermicompost is ready in about 2 months if agricultural waste is used and about 4 weeks if sericulture waste is used as substrate. The processed vermicompost is black, light in weight and free from bad odor.
6. Pile the compost in small heaps and leave under ambient conditions for a couple of hours to allow all the worms to move down the heap into the bed. Separate upper portion of the heap, and sieve the lower portion of the heap to separate the earthworms. The culture bed contains different stages of the earthworm's life cycle, namely, cocoons, juveniles and adults, and this can be transferred to fresh half decomposed feed material or for feeding fish or poultry. Pack the compost in bags and store the bags in a cool place.
7. Prepare another pile about 20 days before the expected date of removal of the compost and repeat the process by following the same procedure as described above.

2.4.5. COMPOST SEPARATION TECHNIQUES

Vermicomposting reduces volume of the material to 10% of its original volume. The finished material will be brown and earthy-like, and the original bedding will no longer be recognizable. If only the worm casts are required as a fertilizer, any of the following methods can be used for the separation. If wants to separate the worms also then a light separation method or a wire mesh screen can be used for small scale or pilot operations and a mesh screener can used for commercial scale operations. These techniques are: (**Vermicomposting E-report**)

1. **Light Separation**: This method utilizes worm's sensitivity to light and tendency to burrow beneath the surface in order to escape light sources. The finished material is spread on a surface and exposed to a light source. This makes the worms to quickly burrow downwards and allow removal of the surface material as compost. The removed compost may however have un-hatched egg capsules of the worms. The thin bottom layer left behind after the removal of the compost will contain all the worms, and this is added to the new bedding with a fresh supply of feed.
2. **Sideways Separation:** The finished material is moved to one side, whilst fresh bedding mixed with organic waste is placed alongside. Within 7 to 14 days, the worms will migrate from the finished vermicompost into the fresh bedding, and the finished compost can be harvested. Advantage of this method is that it allows hatching of the egg capsules and migration of the young worms into the fresh bedding.
3. **Vertical Separation:** Already composted ready harvest material along with worms is placed in a container. Then a nylon mesh screen is placed on the compost of this container. The mesh screen should be large enough to flatten up the sides of the container overlap at the top. The container is then filled with fresh bedding on top of the mesh screen and fed with organic waste. Over time worms will migrate into the material over the wire mesh since the food source below the mesh is depleted. When the upper part is ready for harvesting, the screen and the upper finished material containing the worms is lifted and placed in another container. Material left behind in the container will have a very high concentration of worm castings and very few worms, hatchlings or capsules. This is harvested as the ready to use vermicompost.
4. **Gradual Transfer:** Here two containers are alternatively used. Feed the first container with organic matter for up to four months. Then start a second container and primed it with fresh bedding and a supply of worms from the first box. To ensure there are enough worms in both the containers, the second can be prepared about a month earlier, adding some worms to this container from the first whenever the first container is fed. Maintain the first container until the second is full. By this time, the first container will contain a very high proportion of fine castings, but very few surviving worms.
5. **Screening:** In this method, once the compost is ready for harvesting, it is manually screened or passed through a rotary screen for separating worms from the finished

compost. This method may be preferred when coarse green waste was incorporated into the bedding. The harvested vermicompost here may contain egg capsules and hatchlings.

2.4.6. VERMICOMPOST

Vermicompost is ready when the material is moderately loose and crumbly and the color of the compost is dark brown. It is granular, light weight, odorless & smelling like good soil and humus rich, having pH in the range of 6.3 to 7.2. Vermicompost (earthworms castings) harvested will have been in the bed in excess of 80 days and is free from live worms and viable eggs. The harvested compost is dried in windrows under cover, blending for quality and screening to obtain a uniform product. However, the product is not sterilized or pasteurized. Despite this, it meets all the stabilization criteria.

Vermicasts quality will vary according to the food source, process used for production and post processing practices. Vermicompost is superior to conventional aerobic compost in a number of ways include the following: (**Munroe**)

1. **Level of plant-available nutrients**: **Atiyeh *et al*., (2000)** reported that the aerobic compost was higher in ammonium, while vermicompost tended to be higher in nitrates (which are the more plant-available form of nitrogen). Also the supply rate of several nutrients, including P, K, S and Mg, is higher in vermicompost.
2. **Level of beneficial microorganisms:** Vermicompost is more than 1000 times richer and beneficially microbially active than the conventional compost.
3. **Ability to stimulate plant growth: Atiyeh *et al*., (2000)** demonstrated consistently that vermicomposted organic wastes have beneficial effects on plant growth independent of nutritional transformations and availability. Whether they are used as soil additives or as components of horticultural soil less media, vermicomposts have consistently improved seed germination, enhanced seedling growth and development and increased plant productivity. Vermicompost includes plant-growth regulators which increase plant growth and yield.
4. **Ability to suppress diseases:** High levels of beneficial microorganisms in vermicompost protect plants by out-competing pathogens and also block their access to plant roots by occupying all the available sites.
5. **Ability to repel pests:** Worm castings sometimes repel hard-bodied pests (**Edwards and Arancon, 2004**). This repellency works all the times, is still remain to be determined. George Hahn, a vermicompost producer in California, claims that his product repels many different insect pests. He feels that this is due to the production of chitinase enzyme by the worms, and this breaks down chitin in the insect's exoskeleton.

2.4.7. WORM BIN TROUBLESHOOTING

Despite our attempts to take excellent care of worms, things just get out of knock in the bin. List of some common problems that arise in vermicomposting with their causes and solutions are shown in Table 2.1.

Table 2.1: Troubleshootings in bin.

S.No.	Problems	Solutions	Causes
1.	Bin smells bad	Overfeeding	Stop feeding for 2 weeks
		Food scraps exposed	Bury food completely
		Bin too wet	Mix in Fluff bedding; leave lid off
		Not enough air	Fluff bedding; clear drainage holes
2.	Bin attracts flies	Food scraps exposed	Bury food completely
		Rotten food	Cover with clean bedding
		Too much food; esp. citrus	Don't overfeed worms
		Black soldier fly larvae	Pick out larvae, add them to backyard compost pile; bury food completely; reduce acidic foods
		Black soldier fly adults	Release from bin
3.	Bin attracts ants, centipedes		Remove centipedes; change bin location
4.	Worms are dying	Bin too wet	Mix in dry bedding; leave lid off
		Bin too dry	Thoroughly dampen bedding
		Extreme temperatures	Move bin to 70–80˚F location
		Not enough air	Fluff bedding, check for blocked vents
		Not enough food	Add more bedding and more food scraps
5.	Warms are crawling away	Bin conditions not right	Leave lid off
		Excess vibrations	Eliminate vibrations
6.	Excess mold	Conditions too acidic	Cut back on acidic foods

7.	Bedding drying out	Too much ventilation	Dampen bedding; keep lid on
8.	Excess drainage	Poor ventilation	Fluff bedding; add dry bedding
		Too much water in food	Cut back on coffee grounds and watery scraps

Source: Worm bin troubleshooting E-report available at: http://www.bae.ncsu.edu/topic /vermicomposting/vermiculture/worm_bin_troubleshooting.pdf

2.4.8. VERMICOMPOSTING OF WATER HYACINTH BIOMASS

Vermicomposting has nowadays attracted much attention due to its simple technology and lack of need for expensive equipments. It has been reported to be a viable, cost-effective and rapid technique for the efficient management of the organic solid wastes.

There are many applications like vermicomposting of different wastes, types of organic substrates (kitchen waste, agro-residues, institutional and industrial wastes) and many more into valuable vermicompost.

Gajalakshmi *et al*., 2000 assessed the two epigeic species (*Eudrilus eugeniae* and *Perionyx excavatus)* and two anecic species *(Lampito mauritii* and *Drawida willsi)* of earthworms in terms of efficiency and sustainability of vermicomposting of water hyacinth and cow dung (6:1 w/w). Trend observed in the mass of vermicasts produced per unit time for the given rate of feed input:

E. eugeniae > *P. excavates* > *L.mauritii* > *D. willsi*

Similar trends were observed for increase in biomass and number of offspring biomass produced, with the exception that in the latter aspect *L.mauritii* was indistinguishable from *D. willsi*.

Then in year 2001, **Gajalakshmi** & his coworkers studied the weed by composting first by a 'high-rate' method and then subjected to vermicomposting in reactors operating at much larger densities of earthworm than recommended: 50, 62.5, 75, 87.5, 100, 112.5, 125, 137.5, and 150 adults of *Eudrilus eugeniae* per litre of digester volume. The worm zoomass in each reactor increased with time and the increase in worm density would cause further increase in the vermicast output. There was also production of offspring in all the reactors during each and every run. The vermicast yield consistently increased with worm density - from the average of 46.6% in 50 worm l^{-1} reactors to 93.4% in 150 worm l^{-1} reactors.

Gajalakshmi with his coworkers in 2002 conducted six month long trials on different vermireactors fed with one of the following forms of water hyacinth (WH) by the earthworm *Eudrilus eugeniae.*

a) Fresh whole plants

b) Dried whole plants

c) Chopped pieces of fresh plants

d) Spent weed taken from reactors after extracting volatile fatty acids (VFAs)

e) Precomposted fresh weed

f) Precomposted spent weed

The first 4 forms were studied with cow dung in the ratio of 6:1 (weed: cow dung) and also without cow dung. While in last 2 forms, cow dung had been during partial composting and hence, these forms were vermicomposted without further addition of vermicompost. The maximum mass of castings per unit feed mass and time (six month average: 56.2%). This was closely followed by precomposted spent water hyacinth.

E. eugeniae prefers the feed in the following order:

Precomposted spent weed > precomposted chopped weed > spent weed fortified with cow dung > chopped weed fortified with cow dung > spent weed > dried whole plants fortified with cow dung > dried whole plants ~ chopped weed > fresh whole plants with cow dung > fresh whole plants.

The worm zoomass gained with cowdung fortified spent weed was slightly higher than with precomposted water hyacinth, the zoomass gained with fresh chopped water hyacinth was greater than dried water hyacinth.

Kurien *et al*., 2006 assessed the bioconversion potential of two epigeic species (*E. fetida* and *Eudrilus eugeniae*) of earthworms was assessed in terms of efficiency and sustainability of vermicomposting of Taro (*Colocasia esculenta*). In different vermireactors, (run in triplicates), with one of the two species of earthworms and 60 g of 6:1 *Colocasia*: cowdung were given as feed to worms. *E. eugeniae* worms have performed better than *E. fetida* in terms of efficiency of vermiconversion of *Colocasia* (as reflected in the mass of vermicasts produced per unit time for the given rate of feed input. The average mass of earthworms of both species increased by close to two folds in the case of *E. fetida* and two-and-a-half folds in the case of *E. eugeniae*. The earthworm population in reactors with *E. fetida* and *E. eugeniae* increased six times and four times, respectively.

Gupta *et al*., 2007 aimed to investigate the potential of water hyacinth spiked with cow dung into vermicompost. Five vermi-reactors containing water hyacinth and cow dung in different ratios, were run under laboratory conditions for 147 days. All the containers were kept in dark under identical ambient conditions (room temperature 25 $\pm$ 3°C, relative humidity 60-80%). The maximum worm growth was recorded in cow dung alone. Worms grew and reproduced favorably in 25% water hyacinth + 75% cow dung feed mixture. Higher percentage of water hyacinth in the feed mixture retarded the growth and fecundity of the worms used and also

affected the nutritional quality of vermicompost. This might be due to the fact that higher proportion of water hyacinth in the feed mixture made it harder and more tensile, which was not utilized by worms.

In all the vermi-reactors, there was significant decrease in pH, TOC and C:N ratio, but increase in TKN, TK and TAP at the end. The heavy metals content was slightly lower in the vermicomposts than in initial feed mixtures. This decrease in heavy metal concentration could be related to leaching of the cations by excess water hyacinth or accumulation by the earthworms. It was found that WH could be potentially useful as raw substrate in vermicomposting if mixed with up to 25% in cow dung (on dry weight basis).

There are two methods of Vermicomposting i.e., the pit method and the heap method. The efficacy of these two methods was compared under field conditions over three seasons (winter, summer and rainy) by using *E. fetida*. 100 adult earthworms were released in each pit (2 × 0.5 × 0.6m) and heap (2 × 0.6 × 0.5m) containing 80kg dry weight of organic waste. Observations were recorded on the worm population and biomass after 30, 60 and 90 days. In winter season, on an average, 7783 earthworms were found per pit in comparison to 7413 worms per heap. In summer season 6146 worms were counted in each pit, whereas an average of 5631 worms was found in each heap. However in the rainy season, a significantly higher number of earthworms were recorded in the heaps (9160 worms/heap) than in the pits (8945 worms/pit). The data indicate that the pit method is superior to the heap method in winter and summer seasons, whereas in the rainy season, the heap method gave better results. The mean biomass at the end of winter and summer seasons was higher in the pits (2459 g and 1920 g respectively) in comparison to heaps (2298 g in winter and 1796 g in summer). But in the rainy season higher biomass was recorded in the heaps (3219 g/heap) than in the pits (3047 g/pit). (**Saini *et al.*, 2008**)

2.4.9. VERMICOMPOSTING OF SPENT MUSHROOM SUBSTRATE

The residual compost waste generated by the mushroom is known as spent compost. It is an excellent source of humus, although much of its nitrogen content will have been used up by the growing mushrooms. It is a good source of general nutrients (0.7% N, 0.3% P, 0.3% K plus a full range of trace elements), as well as a useful soil conditioner. This is present in large amounts, and raises the question of what can be done with it. It is certainly not desirable to leave it as a possible source of pollution. It is known that there still remains in the spent compost a considerable amount of lignocellulosic material in addition to the mushroom mycelia and also other products formed by the metabolic activities of the mycelium. Thus, the spent compost should be capable of supporting further biological activities, e.g., the growth of another species of edible mushroom; use as fodder for livestock; as a soil conditioner and fertilizer; and also as manure obtained by vermicomposting and also in bioremediation.

According to Dr.P. Muralikkannan, In India, around 20,000 tons of fresh button mushroom are produced annually and out of which about 10-15% of mushroom production is shared by

Tamilnadu and near by state. Approximately, 5 kg of mushroom waste is being generated to produce one kg of fresh mushroom.

Characteristics of Mushroom waste:

1. High Organic matter content.
2. High moisture content.
3. Moderate plant nutrient content.
4. Relatively low bulk density.
5. Unbalance distribution of major plant nutrients.

Mushroom waste cannot be used as such in agriculture. There are many constrains for using in agriculture. These are:

1. High concentration of soluble salts.
2. Due to its chalk content, it is highly alkaline, and should not be used on acid-loving plants, nor should it be applied too frequently, as it will overly raise the soil's pH levels.
3. Mushroom compost may also contain pesticide residues, particularly organochlorides used against the fungus. Chemicals may also have been used to treat the straw, and also to sterilize the compost. Therefore, the organic gardener must be careful regarding the sourcing of mushroom compost.

Constrains in disposal of mushroom waste:

1. Mostly land filled as agriculture waste.
2. Environmental Impact on landfill of SMC.
3. Contamination of groundwater and rivers due to high phosphorous content of SMC.
4. Leaching of nitrate (NO_3^-) and some compounds used for sterilization or as pesticide.

Therefore, the huge amount of SMC is added to the burden of the municipal refuses, especially around the mushroom cultivation complexes. The novel approach is to divert SMC waste from landfill in an environmentally friendly manner to manufacture nutrient rich organic manure for agriculture use.

Advantages of using mushroom waste in composting:

The following are advantages in using mushroom waste as one of alternate raw material source for large scale composting of different organic waste.

1. Improve the quality of compost by increasing high nutrient content.
2. More air filled pore space for better plant growth.
3. Improve water holding capacity.

4. Effective utilization of alternate raw material for compost preparation.
5. Reduces the production cost by 30 to 40% level.

Considering its abundance and remarkable physical & chemical attributes, such as high aeration porosity, water holding capacity and nutrition's, it can be used as one of the source for composting with other raw material to improve the nutritive and quality of finished products. In the past decades, potential of earthworms for breaking down organic waste has been explored in depth and many large scale vermicomposting have been developed all over the world with varying success (**Garg *et al*., 2005**).

In year 2008, a study was conducted by **Tajbakhsh** and his coworkers to assess the potential of earthworms, *Eisenia fetida* and *Eisenia andrei*, in composting different types of organic substances (i.e. SMC, stump and different agro-wastes) and study the quality of vermicompost produced. The experiments were performed in plastic containers (31 cm × 31 cm × 27 cm) with some holes at the bottom. Considering 0.75 kg feed/kg worm/day, for a period of 90 days, 40 matured worms were inoculated to each bin containing 2,700g (dry weight basis) of the proposed wastes. The treatments included SMC alone, SMC with different agro-residues (i.e., stump, potato tomato, pomegranate, fruit and vegetables) in 1:1 ratio and 2:1 ratio. Prior to use the SMC and the agro-residues were characterized. Vermicomposting caused significant reduction in pH (8%), electrical conductivity (41%), total organic carbon (35%), C:N ratio (56%), K (68%), Na (10%) and increased in available macro and micronutrients such as P by 3-fold, N 1.37-fold, B 1.29-fold, S 1.59-fold, Fe 2.1-fold, Cu 1.89-fold, Zn 1.68-fold, Mn 1.2-fold, Ca 1.92-fold and Mg 1.72-fold compared to those of the initial substrate.

CHAPTER 3
METHODOLOGY

3.1. INTRODUCTION

The methodology included the following aspects:

- Understanding the mushroom cultivation and vermicomposting techniques.
- Development of facilities for the experimental mushroom culturing and vermicomposting.
- Spawn preparation.
- Collection of water hyacinth and industrial effluents and its characterization.
- Preparation of compost for mushroom culturing.
- Oyster Mushroom cultivation on the substrate prepared from water hyacinth biomass.
- Vermicomposting of water hyacinth substrate.
- Analysis of heavy metals present in the mushrooms and the vermicompost.
- Process Monitoring.
- Analytical techniques.

Brief overview on the above aspects is given in this chapter.

3.2. UNDERSTANDING THE TECHNIQUES

To understand and practice the mushroom cultivation and vermicomposting techniques, review of literature was done and also practical training on mushroom culturing and vermicomposting was taken. Mushroom culturing and vermicomposting practice was observed in 'HAIC Agro Research and Centre', Murthal (Distt. Sonipat) and nearby farmers doing vermicomposting of cow dung respectively.

3.3. DEVELOPMENT OF FACILITIES

Based on the review and practical training, facilities needed for experimentation was decided. For mushroom culturing, proper environmental conditions like temperature, humidity, etc. are to be maintained. In the light of this the following setup was designed:

3.3.1. SUBSTRATE FERMENTATION CUM MUSHROOM CULTIVATION CHAMBER

A four compartment chamber was designed for this purpose. Each of the compartments is supposed to serve the purpose for each of the stages of mushroom culturing i.e. substrate preparation, mycelium growth, pinning and fruiting & harvesting. Only one chamber could be built. The rest of the chambers are under construction. Each chamber includes the following three sections and a central duct:

- **A hopper bottom section:** It consists of u-tube drain to facilitate draining out the accumulated water without allowing entry of air.
- **A middle biomass holding section:** It consists of perforated sheet for separating the section from the hopper bottom and for supporting the biomass above.
- **A top head space section above the biomass holding section:** Thermometer is attached for measuring temperature of the substrate. It also has a vent for breathing external air as and when required.
- **Central duct:** It connects the top head space section with the bottom air space section. An axial fan fitted within the duct of air for facilitating circulation of air.

Please see the figure 3.1 (a) and (b) for details.

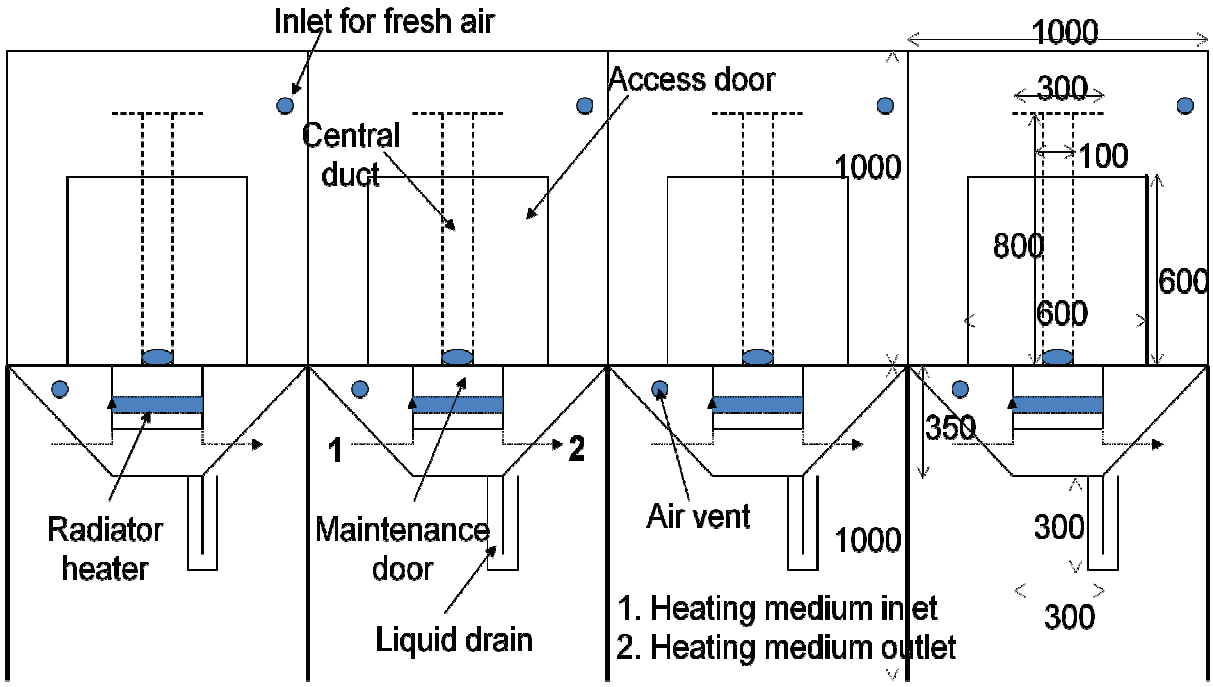


Fig: 3.1 (a) Diagram of Substrate fermentation cum mushroom cultivation chamber.

Fig: 3.1 (b) Substrate fermentation cum mushroom cultivation chamber.

3.3.2. MUSHROOM CULTIVATION ROOM

Mushroom cultivation room was developed to maintain temperature and humidity in the range of 15-27°C and 80-95% respectively. Room was made insulated by using thermocol sheets. Dark room was created by using black charts and curtains. Also, the provision of A.C. and cooler was provided. Jute bags were provided in the bottom to maintain humidity. Please refer figure 3.2 for details.

Fig: 3.2. Mushroom cultivation room**.**

Another room was also developed utilizing the materials available at that stage. This room was developed below the substrate fermentation chamber as shown in figure 3.3 (a, b).

Fig: 3.3. (a) Front view and (b) Inner view of new mushroom cultivation room.

It was developed in such a way that temperature and humidity can be maintained as desired for oyster mushroom cultivation. This chamber was partitioned in two halves using wooden board. One half is used for mycelium growth and second half is having provision for light for pinning. Initially, it was difficult to maintain temperature. The sides of the chamber bottom were covered initially with jute bags and then with curtains. So, water was sprayed on the jute bags covered with curtains two times a day. But, sometimes the water droplets fall on the polyethylene bags which lead to increase in moisture content. So, instead of spraying water on the curtains, the perforated pipe was made & attached to the three sides of the chamber and provision was made in such a way that water is continuously sprinkled on the curtains.

3.4. SPAWN PREPARATION

The mother spawn of *Pleurotus sajorcaju*, collected from 'HAIC Agro Research and Development Centre, Murthal (Distt. Sonipat), was used for preparing spawn in bottles, which is then kept for use as and when required. Procedure followed for spawn preparation is given below:

1. Good quality of wheat grains free from pest and moulds were selected and boiled in clean water for 20-30 minutes.
2. The boiled soft grains were spread evenly on a cotton cloth for draining water and cooling to room temperature.
3. 3% chalk powder (30g/ kg of grain) was mixed with the grains for adjusting the pH and keeping the grains loose.
4. 250gms of the grains were filled in 500ml capacity bottles and the mouth of the bottle was covered and then sterilized in autoclave at 121°C and 15lbs/sq inch pressure for 20 minutes.
5. After cooling, the bottles were transferred to inoculation chamber and a few grains with mycelial growth from mother spawn culture were transferred into the sterilized bottle under aseptic conditions.
6. Thus, inoculated bottles were then shifted to an incubator maintained at 25-30°C temperature for 15-20 days till the grains in bottle are covered with mycelium growth.

3.5. COLLECTION OF WATER HYACINTH, INDUSTRIAL EFFLUENTS AND COW DUNG AND THEIR CHARACTERIZATION

Fresh water hyacinth plants were collected from a natural drain infested with water hyacinth adjacent to Bhakra Canal, Patiala. The harvested biomass was washed with running water. The plants were cut into pieces of 2-3 cm long pieces and sun dried for 2-3 days. The dried biomass is stored for use as and when required.

The dried water hyacinth biomass (both combined and roots, leaves and shoot separately) was powdered and listed parameters given in table 3.1 were analyzed.

The industrial effluents like lactose mother liquor and spent wash were obtained i.e. lactose mother liquor from dairy industry, Mathura and spent wash from Main distillery, Patiala. These industrial effluents were used as supplements in mushroom culturing. The parameters analyzed for these effluents are listed in table 3.1.

Cow dung was also procured for vermicomposting. Many heavy metals were analyzed in it as listed in table 3.1.

S.No.	Parameters	Pond water	Water hyacinth (combined as well as different parts)	Industrial effluents	Cow dung
1.	BOD & COD	✓	-	✓	-
2.	TSS & TDS	✓	-	-	-
3.	MPN	✓	-	-	-
4.	Acidity & Alkalinity	✓	-	-	-
5.	Ash content	-	✓	-	-
6.	Heavy metals (Zn, Cr, Pb, Ni, Fe, Hg, Cu, Ca, Mg)	✓	✓	✓	✓
7.	Ions (Fl^-, Br^-, Cl^-)	✓	-	✓	-
8.	Phosphate & nitrate	✓	-	✓	-
9.	Sulfate	✓	-	✓	-
10.	Total Kjeldahl	-	-	✓	-

	Nitrogen				
11.	Total organic fraction	-	-	✓	-
12.	Sodium & Potassium	-	-	✓	-

Table: 3.1 List of parameters to be analyzed

3.6. PREPARATION OF COMPOST FOR MUSHROOM CULTURING

Compost for oyster mushroom was prepared using water hyacinth as substrate and industrial effluents as supplements. Dried water hyacinth was soaked in lactose mother liquor or spent wash overnight. Then, the biomass was removed and excess liquor was squeezed out and stacked out in the fermentation chamber for fermentation. Within two days of fermentation, temperature of the material is supposed to rise to 65°C and facilitate pasteurization. In case the temperature of 65°C is not obtained, the fermentated material is supposed to be pasteurized under sunlight. For this, the substrate was packed in transparent polyethylene bag and kept under direct sunlight from 11A.M. to 4P.M. for solar pasteurization. Later on, compost was cooled at room temperature.

3.7. OYSTER MUSHROOM CULTIVATION ON THE WATER HYACINTH SUBSTRATE

Based on the literature review and the experience gained from both training and visits to mushroom culturing sites, the protocol for mushroom cultivation was articulated. In this, water hyacinth is to be used as substrate and lactose mother liquor or spent wash as supplements in place of rice bran etc. the protocol is briefly described below:

3.7.1. PACKAGING AND SPAWNING OF SUBSTRATE

Transparent poly bags of size 50×40 cm were taken, 10-12 holes were punched and the prepared compost was packed. Fresh *Pleurotus sajor-caju* spawn, collected from 'HAIC Agro research and development center', Murthal, Haryana, was added to the compost in bags at 30-40g per kg rate. In the bags, layers of compost alternated with the layers of spawn. Then, mouths of the bags were plugged with cotton and bags were transferred to cultivation room.

3.7.2. INCUBATION

The poly bags were transferred to mushroom cultivation room for incubation. The temperature and humidity to be maintained is in the range of 15-27°C and 80-95% respectively. The spawned compost bags were kept at distance 15cm to 20cm in a dark room until the mycelium has fully penetrated to the bottom of the substrate. The incubation period is about 2 weeks till the mycelium growth completely covered the compost present in the polyethylene bags.

3.7.3. FRUITING

When the mycelium fully colonized the substrate, the bags were opened to initiate fruiting, inside a mushroom house. At the same time, a small amount of light was provided inside the house. Fruiting requires an appropriate temperature range (20-28°C), ventilation, light moisture and humidity (80-95%) and these conditions are ensured in the mushroom culturing room. To provide moisture, daily watering of the substrate was done. A light water mist was frequently used to lower the temperature whenever it crossed 30°C. Doors and windows were kept open, especially at night, to allow the cool night air to enter. Approximately 3 to 4 days time is required for mushroom primordial begin to form. Mature mushrooms will be ready for harvesting within another 2 to 3 days.

3.7.4. HARVESTING AND YIELD

To harvest the mushrooms, they are to be grasped by the stalk and gently twisted and pulled. After harvesting from the top end of the bag, the other end of the bag may be opened to allow fruiting. The two ends are sometimes opened and allowed to fruit at the same time. As long as the substrate appears white, mushrooms will continue to form under adequate environmental conditions. When it appears colorless and soft, the bags are discarded from the house.

3.8. VERMICOMPOSTING OF WATER HYACINTH

Vermicomposting has been done on dried fresh water hyacinth biomass after mixing with cow dung in the ratio of 1:3. Red Earthworms (*Eisenia fetida)* were collected from nearby farmer doing vermicomposting in Patiala. The following steps were followed for vermicomposting. These are given below:

1. The water hyacinth and cow dung mixture was prepared in a tub and left for 3 days.
2. Then, about 250 earthworms were added to the tub containing 10kg of water hyacinth cow-dung mixture and left for composting till most of the biomass is composted.
3. Thorough, sprinkling of water (70-80% moisture content) was maintained.

4. Once the composting is over, vermicompost was harvested by scrapping layer wise from the top of the tub. This will help in separation of earthworms from the compost. The compost recovered was then sieved to separate the earthworms and cocoons.

3.9. ANALYSIS OF HEAVY METALS PRESENT IN MUSHROOM AND VERMICOMPOST

The mushrooms obtained from mushroom culturing of water hyacinth and also the vermicompost and even worms were to be characterized especially for heavy metal content. The mushroom and vermicompost were to be dried in oven at 110°C and then taken for digestion and checked for heavy metal content. The metals analyzed include Zn, Cr, Pb, Ni, Fe, Hg, Cu, Ca and Mg.

3.10. PROCESS MONITORING

The following parameters were monitored during different stages of mushroom cultivation:

1. **Temperature:** Temperature was measured regularly and based on it, thorough adjusting the A.C. temperature and thorough spraying water. During mycelial growth, the temperature was maintained around 20-25°C. During the pin head initiation, temperature is decreased to 13-16°C especially by regular sprinkling of water.

2. **Humidity**: Dry and wet thermometer was put in the room to monitor relative humidity. The humidity at desired level was maintained by desert cooler. Jute bags were also laid. Humidity was maintained around 90% during cultivation and after 15 days of incubation at 95%.

3. **Light:** During initial stages of incubation, total darkness was provided by putting black charts. For initiation of pinning, white light was given for few hours per day.

3.11. ANALYTICAL TECHNIQUES

Different parameters (given in table 3.1) were analyzed for the characterization of substrate (water hyacinth) and also of the supplements (lactose mother liquor and spent wash). After mushroom cultivation and vermicomposting, the products i.e. mushroom, spent substrate and vermicompost and even the worms were analyzed for heavy metal content. The metals analyzed include Zn, Cr, Pb, Ni, Fe, Hg, Cu, Ca and Mg.

S.No.	Parameters	Methods followed	References
1.	pH	Electrometric method	Method no. 4500-H^+ of Standard methods, 20th Edition, 1998
2.	BOD	3-day BOD test	Method no. 5210 of Standard methods, 20th Edition, 1998
3.	COD	Open reflux method	Method no. 5220 of Standard methods, 20th Edition, 1998
4.	MPN	Multiple tube fermentation technique	Method no. 9221 of Standard methods, 20th Edition, 1998
5.	TSS & TDS	Oven drying method	Method no. 2540 D & C respectively of Standard methods, 20th Edition, 1998
6.	Total Kjeldahl nitrogen	Kjeldahl method	Method no. 4500-NH_3 B & C of Standard methods, 20th Edition, 1998
7.	Phosphorous content	Vanadium-Molybdate method	Method no. 4500-P C. of Standard methods, 20th Edition, 1998
8.	Heavy metals analysis	Atomic Absorption Spectroscopy (AAS)	Method no. 3110 of Standard methods, 20th Edition, 1998
9.	Acidity	Titrimetric method	Method no. 2310 of Standard methods, 20th Edition, 1998
10.	Alkalinity	Titrimetric method	Method no. 2320 of Standard methods, 20th Edition, 1998
11.	Sodium & Potassium	Flame Photometric method	Method no. 3500 of Standard methods, 20th Edition, 1998
12.	Sulfate content	Gravimetric method	Method no. 4500 of Standard methods, 20th Edition, 1998

Table: 3.2. Analytical parameters

CHAPTER 4

RESULTS & DISCUSSION

4.1. INTRODUCTION

The results of this study are presented under the following sections:

- Characterization of water hyacinth biomass, industrial effluents and cow dung.
- Preparation of spawn.
- Cultivation of mushrooms and their characterization.
- Preparation of vermicompost and its characterization.

4.2. CHARACTERIZATION OF WATER HYACINTH BIOMASS, INDUSTRIAL EFFLUENTS AND COW DUNG

Characteristics of the water hyacinth biomass and of the industrial waste supplements i.e. lactose mother liquor and spent wash are given in table 4.1 and 4.2 respectively. Heavy metal composition of the cow dung used in the vermicomposting of the water hyacinth biomass is given in table 4.3.

Table: 4.1. Characteristics of the dried water hyacinth biomass.

Composition of dried water hyacinth biomass					
S.No.	**Parameters**	**Root**	**Leaves**	**Stem**	**Combined biomass**
1.	Moisture (%)	NE*	NE	NE	6
2.	Ash (%)	NE	NE	NE	26.79
3.	Biomass (%)	13.27	11.33	75.4	100
4.	Carbon (%)	NE	NE	NE	14.47
5.	Nitrogen (%)	NE	NE	NE	6.88

6.	Protein (%)	NE	NE	NE	43.87
7.	Zinc (ppm)	36.57	36.42	22.98	21.63
8.	Chromium (ppm)	<0.33	<0.33	<0.33	<0.33
9.	Lead (ppm)	11.3	8.34	11.95	10.45
10.	Nickle (ppm)	8.0	5.0	4.9	4.535
11.	Iron (ppm)	3073.1	1878.9	1031.2	1321.63
12.	Mercury (ppm)	0.5	0.34	0.38	0.95
13.	Copper (ppm)	4.5	2.82	1.13	1.52
14.	Calcium (ppm)	10623.5	7754.2	9835.9	18389.22
15.	Magnesium (ppm)	6838.9	6755.8	7125.4	6597.96

NE*: Not Estimated

Table: 4.2. Analysis of supplements.

Analysis of Supplements			
S.No.	**Parameters**	**Lactose Mother Liquor**	**Spent wash**
1.	BOD (mg/l)	1,88,333.33	17,375
2.	COD (mg/l)	2,24,043	23,667
3.	Total organic Fraction (mg/l)	1,78,580	7,760
4.	Organic Nitrogen (mg/l)	709.63	43.512
5.	Ammonical Nitrogen (mg/l)	627.09	16.58
6.	Total Kjeldahl Nitrogen (mg/l)	1336.72	60.09
7.	Sodium (mg/l)	10,648.4	184.69
8.	Potassium (mg/l)	6,064.86	347.55
9.	Sulfate (mg/l)	7,307	401.62
10.	Chloride (mg/l)	38,820	400
11.	Nitrate (mg/l)	2,111	NE
12.	Phosphate (mg/l)	12,750	NE
13.	Fluoride (mg/l)	6,260	NE

14.	Bromide (mg/l)	99	NE
15.	Mercury (µg/l)	0.27	17.70

Table: 4.3. Heavy metal analysis of cow dung.

Heavy metal composition of Cow dung		
S.No.	**Parameters**	**Cow dung**
1.	Zinc (ppm)	61.9
2.	Chromium (ppm)	21.98
3.	Lead (ppm)	<0.05
4.	Nickle (ppm)	<0.30
5.	Iron (ppm)	1500.59
6.	Mercury (ppm)	0.24
7.	Copper (ppm)	7.78
8.	Calcium (ppm)	3230.17
9.	Magnesium (ppm)	3808.78

In addition to this, pond water from where water hyacinth has been collected was also characterized and its characteristics are given in table 4.4.

Table: 4.4. Analysis of pond water

Analysis of pond water		
S.No.	**Parameters**	**Water Sample**
1.	pH	7.23
2.	BOD (mg/l)	16.56
3.	COD (mg/l)	147.35
4.	TSS (mg/l)	201
5.	TDS (mg/l)	1756
6.	MPN (c.f.u./100ml)	1.4×10^6
7.	Acidity (mg/l as $CaCO_3$)	Absent

8.	Alkalinity (mg/l as $CaCO_3$)	676.56
9.	Zinc (ppm)	0.03
10.	Chromium (ppm)	<0.33
11.	Lead (ppm)	0.23
12.	Nickle (ppm)	<0.3
13.	Iron (ppm)	<0.24
14.	Mercury (µg/l)	<0.2
15.	Copper (ppm)	0.046
16.	Calcium (ppm)	47
17.	Magnesium (ppm)	56.45
18.	Fluoride (ppm)	1.43
19.	Bromide (ppm)	0.54
20.	Nitrate (ppm)	0.45
21.	Chloride (ppm)	133.71
22.	Phosphate (ppm)	9.06
23.	Sulfate (ppm)	84.87

4.3. PREPARATION OF SPAWN

The mother spawn of *Pleurotus sajorcaju*, obtained from 'HAIC Agro Research and Development Centre, Murthal (Sonipat)', was used for preparing spawn on wheat grain in bottles, which is then kept in storage for use as and when required for mushroom culturing. Different stages of spawn preparation are photographed and shown in figure 4.1.

(a) Mother spawn culture of *Pleurotus sajorcaju* (b) Boiled wheat grain with $CaSO_4$ and K_2SO_4

(c) Spawn prepared and ready for use

Fig: 4.1 Stages of spawn preparation

4.4. CULTIVATION OF MUSHROOMS & THEIR CHARACTERIZATION

Water hyacinth was harvested from a natural drain adjacent to Bhakra canal, Patiala, chapped into 1 to 2 inch size pieces, sun dried and kept in storage for use in the substrate preparation (figure 4.2).

(a) Water hyacinth from nearby pond

(b) Cutter used for water hyacinth

(c) Dried water hyacinth

Fig: 4.2. Stages showing collection of water hyacinth.

Dried Water hyacinth biomass soaked in lactose mother liquor was fermentated. The material was left for fermentation for 6 days expecting for the temperature to rise to pasteurization temperature. But the desired temperature of 65°C was not achieved. Only raise to 42°C was achieved. After the 6^{th} day, the fermented substrate was pasteurized under the sunlight through wrapping in polyethene bag and keeping under the direct sunlight on concrete floor for 5 hours from 11A.M. to 4 P.M. Figure 4.3 includes pictures on the events associated with the substrate fermentation and pasteurization.

(a) Water hyacinth undergoing fermentation (b) Solar pasteurization

Fig: 4.3 Substrate undergoing fermentation and pasteurization.

Capacity of the fermentation chamber was much more than the quantity of substrate being fermented. Further, compact packing of the biomass could not be achieved because of the excessive depth of the chamber. Further, circulation rate of air was probably higher and this might have dissipated most of the metabolic heat. All these might have resulted in not attaining the pasteurization temperature. Painted wooden sawdust board might have inhibited specially the fungal activity and slowed down the fermentation process. The alloy sheet used in the chamber construction for holding the substrate, through metal leaching might also have affected the fermentation process. Provisions for maintaining humidity could not be effectively used and even this might have contributed to the delayed fermentation and not attaining the temperature.

Heavy metal composition of the substrate prepared from the water hyacinth biomass prior to use in mushroom culturing is shown in table 4.5.

Table: 4.5. Heavy metal composition of the substrate prepared from water hyacinth

Heavy metal composition of the prepared substrate		
S.No.	**Parameters**	**Compost of water hyacinth mixed with lactose mother liquor**
1.	Zinc (ppm)	12.30
2.	Chromium (ppm)	5.33
3.	Lead (ppm)	0.58
4.	Nickle (ppm)	1.38

5.	Iron (ppm)	740.89
6.	Mercury (ppm)	0.05
7.	Copper (ppm)	1.03
8.	Calcium (ppm)	5202.79
9.	Magnesium (ppm)	6256.17

Polyethylene bags were filled with the substrate (inoculated with spawn) and left in the mushroom cultivation room. Proper mycelial growth is expected usually within 2 weeks. This showed no significant mycelial growth even after 20 days (see figure 4.4). This could be because of our inability to maintain desired humidity levels in the culture room. The A.C. used for maintaining the temperature was actually reducing the humidity levels in the culture room. The humidity levels were later corrected through using a desert cooler. Further, curtains were hanged in the room and they were wetted with water twice a day for maintaining the humidity. New substrate bags inoculated with the spawn were successfully grown in the mushroom culture room within the two weeks time.

Figure: 4.4. Mycelial growth within 20 days.

Please see figure 4.5 for pictures showing substrate with full mycelial growth and pinning.

(a) Mycelium growth in bag. **(b)** Bags kept for pinning.

Fig: 4.5. Pictures showing substrate with full mycelial growth and bags kept for pinning.

The bags with fully mycelial growth were opened and shifted to mushroom cultivation room for facilitating pinning and fruiting. Within 4 days, pinning was expected and after another one week harvestable mushrooms may be drawn from the bags. Although good mycelium growth was found in water hyacinth containing bags. But, pinning and fruiting could not be obtained. This might be due to heavy metal content present in water hyacinth which was restricting the pinning and further fruiting. Parallel to the mushroom culturing on water hyacinth biomass treated with lactose mother liquor, mushroom culturing on wheat straw was also carried out as control. Mushroom culturing on the wheat straw was found much easier and the substrate does not require addition of any supplements. Please see figure 4.6 for pictures relating to different stages of mushroom cultivation on wheat straw as substrate.

(a) Mycelium growth

(b) Pinning head formation

(c) Fruiting body formation

(d) Harvestable mushrooms

Fig: 4.6. Pictures showing stages of mushroom cultivation using wheat straw as substrate.

Then, the mushrooms were harvested from the wheat straw containing bags and were characterized. The composition of the mushroom is shown in table 4.6.

Table: 4.6. Composition of the mushroom obtained by using wheat straw as substrate

Composition of the mushroom		
S.No.	**Parameters**	**Mushroom**
1.	Ash content (%)	96.52
2.	Carbon (%)	11.24
3.	Nitrogen (%)	3.2
4.	Protein (%)	20.1
5.	Zinc (ppm)	57.75
6.	Chromium (ppm)	<0.33
7.	Lead (ppm)	0
8.	Nickle (ppm)	0.38
9.	Iron (ppm)	61.83
10.	Mercury (ppm)	5.87
11.	Copper (ppm)	16.02
12.	Calcium (ppm)	4055.07
13.	Magnesium (ppm)	1340.17
14.	Potassium (ppm)	19874.84

4.5. PREPARATION OF VERMICOMPOST & ITS CHARACTERIZATION

The substrate for vermicomposting was prepared by mixing water hyacinth with cow dung in the 1:3 ratio. The prepared substrate has the metal composition given in table 4.7.

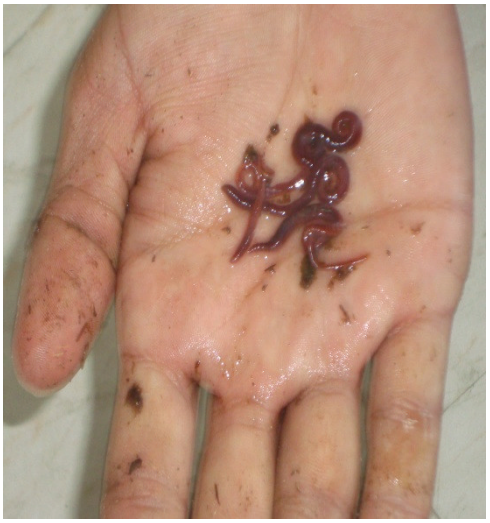
Table: 4.7. Composition of the substrate for vermicomposting.

Composition of water hyacinth and cow dung mixture used in vermicomposting		
S.No.	**Parameters**	**Before vermicomposting**
1.	Ash (%)	96.23%
2.	Moisture (%)	22%
3.	Organic carbon (%)	11.12%
4.	Nitrogen (%)	2.505%
5.	Protein (%)	15.98%
6.	Zinc (ppm)	107.33
7.	Chromium (ppm)	9.91
8.	Lead (ppm)	2.71
9.	Nickle (ppm)	5.10
10.	Iron (ppm)	2976.5
11.	Mercury (ppm)	0.07
12.	Copper (ppm)	9.76
13.	Calcium (ppm)	12683
14.	Magnesium (ppm)	10441.56
15.	Potassium (ppm)	31730.18
16.	Phosphorous (%)	0.22

Vermicomposting was done in plastic tubs. As control, only cow dung was also vermicomposted in a separate tub. Pictures of different stages of vermicomposting are shown in figure 4.7.

The earthworms added were found missing within two days from the water hyacinth-cow dung mixture. This happened with each of the four cases of earthworm's replenishment (fresh addition). The control tub with only cow dung as substrate however was found to have the earthworms but the earthworms found were not growing and small in size. This indicates that the earthworms were stressed by the summer (May and June months) environmental conditions even in cow dung. Constituents of the water hyacinth biomass (may be heavy metals or certain irritant substances specific to water hyacinth biomass) must not be allowing survival of earthworms especially under the stressed environmental conditions like temperature and humidity.

(a) Mixture of cow dung and water hyacinth



(b) *Eisenia fetida* to be added in tub

(c) Vermicompost

Fig: 4.7. Stages showing vermicomposting in a plastic tub.

The characteristics of compost obtained by vermicomposting for about 50 days is given in table 4.8.

Table: 4.8. Composition of the 50 days compost obtained by vermicomposting.

Vermicompost		
S.No.	**Parameters**	**After vermicomposting**
1.	Ash (%)	50.94
2.	Moisture (%)	24
3.	Organic carbon (%)	10
4.	Nitrogen (%)	0
5.	Protein (%)	0
6.	Zinc (ppm)	69.51
7.	Chromium (ppm)	<0.33
8.	Lead (ppm)	2.08
9.	Nickle (ppm)	0.46
10.	Iron (ppm)	3422.56
11.	Mercury (ppm)	0.86
12.	Copper (ppm)	7.93
13.	Calcium (ppm)	12812.21
14.	Magnesium (ppm)	7138.76
15.	Potassium (ppm)	19016.78
16.	Phosphorous (%)	0.12

Higher proportion of water hyacinth in the feed mixture made it harder, which was not easily utilized by earthworms. Total organic carbon reduces in vermicompost than in original compost. This is due to the loss of carbon as CO_2. A comparison of the results obtained by vermicomposting after about 50 days showed that there is an increase in calcium but a decrease in potassium in the vermicomposts than in the initial feed mixtures. Also, the heavy metal content (i.e. Cu, Zn, Ni, Cr, Mg, Pb) in the vermicomposts was slightly lesser than in the initial feed mixtures. The decrease in the heavy metal concentration could be due to accumulation of earthworms.

CHAPTER 5
CONCLUSION

The protocols developed from the literature have been put to test through actually preparing the substrate and culturing the mushrooms. Preparation of the substrate required slight modification to the protocol wherein after fermentation, solar pasteurization was introduced. Culturing of mushrooms could be successfully completed till mycelium growth. But pinning could not be initiated through providing the conditions indicate in the protocol. There is need to find the conditions that indicate the pinning. Time constraints have not allowed carrying out work and find the conditions and accordingly modify the protocol for mushroom culturing.

The protocol for vermicomposting of the spent mushroom substrate could not be tested because of the non-availability of the spent substrate. Instead, the protocol was tried on fresh sun dried water hyacinth biomass mixed with cow-dung in the 1:3 ratio. The composting could not be done successfully. The worms were failing to inhabit and use the substrate. This result we were anticipating. It is reported in the literature that water hyacinth biomass is not a preferred substrate for vermicomposting. Further, the prevailing summer conditions might leave the worms stressed.

The fate of heavy metals of the water hyacinth biomass could be completed only up to that extent to which the mushroom culturing and vermicomposting could be completed. The heavy metal's fate can be better understood once all the protocols are improved, made functional and standardized. On the basis of the experience obtained from the successive trial runs, the protocols should be improved for making them functional. Once it becomes functional, the trial runs should be repeated for the refinement and standardization of the protocols.

Creation of facilities wherein temperature, humidity, carbon dioxide and light conditions can be controlled are important for carrying out this work. Further most of the work (specially the trial runs) may have to be planned for the winter months. That is, the water hyacinth biomass which is available during summer months should be harvested, dried and stored in sufficient quantities for use during winter months in the experimentation.

REFERENCES

Abbasi, S.A and Ramasamy, EV. (1999b): 'Anaerobic digestion of high solid waste'. In: Proceedings of eight national symposium on environment IGCAR, Kalpakkam, India. 220-224.

Abbasi, S.A. and Ramasamy, E.V. (1996): 'Utilization of biowaste solids by extracting volatile fatty acids with subsequent conversion to methane and manure'. In: Proceedings of the 12th international conference on solid waste technology and management. Philadelphia. 4C1-4C8.

Abraham, M. and Kurup, G.M. (1996): 'Bioconversion of tapioca (*Manihot esculenta*) waste and water hyacinth (*Eichhornia crassipes*)- influence of various physicochemical factors'. J. Ferment. Bioeng. 82: 259-63.

Atiyeh, R.M., Subler, S., Edwards, C.A., Bachman, G., Metzger, J.D. and Shuster, W. (2000): 'Effects of vermicomposts and composts on plant growth in horticultural container media and soil'. In Pedo biologia. 44: 579-590.

Bhiday, M.R. (1994): 'Earthworms in agriculture'. Indian Farming. 43(12): 31-34.

Boyd, C.E. (1970): 'Vascular aquatic plants for mineral nutrient removal from polluted waters'. Econ. Bot. 24: 95-103.

Center, T.D., Dray, F.A., Jubinsky, G.P. and Grodowitz, M.J. (1999): 'Biological control of water hyacinth under conditions of maintenance management: Can herbicides and insects be integrated?' Environ. Manage. 23: 241-56.

Chadwick, M.J. and Obeid, M. (1966): 'A comparative study of the growth of *Eichhornia crassipes* Solms and *Pistia stratiotes* L. in water-culture'. J. Ecol. 54: 563-575.

Das, N. and Mukherjee, M. (2007): 'Cultivation of *Pleurotus ostreatus* on weed plants'. Biores. Tech. 98: 2723-2726.

Edwards, C.A. (1998): 'The use of earthworms in the breakdown and management of organic wastes'. In: Edwards, C.A. (ed) Earthworm Ecology. St. Lucie Press, Boca Raton. 327-354.

Edwards, C.A. and Arancon, N. (2004): 'Vermicomposts suppress plant pest and disease attacks'. In Rednova news: http://www.rednova.com.

Edwards, C.A. and Niederer, A. (1988): 'The production and processing of earthworms protein'. Earthworms in waste and in environment. SPB Academic Publishing, The Netherlands. 169-180.

Elvira, C., Goioechea, M., Sampedro, L., Mato, S. and Nogales, R. (1996): 'Bioconversion of solid paper-pulp mill sludge by earthworms'. In Biores. Tech., Great Britain. 173-177.

Elvira, C., Sampedro, L., Benitez, E. and Nogales, R. (1997): 'Vermicomposting of sludges from paper mill and dairy Industries with *Eisenia andrei*: A Pilot-Scale Study'. In Biores. Tech., Great Britain. 205-211.

Gaddie, R.E. and Douglas, D.E. (1975): 'Earthworms for ecology and profit'. Scientific Earthworm Farming. Bookworm Publishing Company. 1: 180.

Gajalakshmi, S., Ramasamy, E.V. and Abbasi, S.A. (2000): 'Potential of two different forms of two epigeic and two anecic earthworm species in vermicomposting of water hyacinth'. Biores. Tech. 76: 171-181.

Gajalakshmi, S., Ramasamy, E.V. and Abbasi, S.A. (2001): 'Assessment of sustainable vermiconversion of water hyacinth of different reactor efficiencies employing *Eudrilus Eugenia*'. Biores. Tech. 80: 131-135.

Gajalakshmi, S., Ramasamy, E.V. and Abbasi, S.A. (2001): 'High rate composting-Vermicomposting of water hyacinth (*Eichhornia crassipes*, Mart. Solms)'. Centre for Pollution Control and Energy Technology, Pondicherry University, Kalapet.

Gajalakshmi, S., Ramasamy, E.V. and Abbasi, S.A. (2002): 'Vermicomposting of different forms of water hyacinth by the earthworm *Eudrilus eugeniae*'. Biores. Tech. 82: 165-169.

Ganesh, P.S, Ramasamy, E.V., Gajalakshmi, S. and Abbasi, S.A. (2005): 'Extraction of volatile fatty acids (VFAs) from water hyacinth using inexpensive contraptions, and the use of the VFAs as feed supplement in conventional biogas digesters with concomitant final disposal of water hyacinth as vermicompost'. Biochem. Engg. J. 27: 17-23.

Garg, V.K. and Kaushik, P., (2005): 'Vermistabilization of textile mill sludge spiked with poultry droppings by epigeic earthworm *Eisenia fetida'*. Biores. Tech. 96: 1063-1071.

Gopal, Brij. (1987): 'Water hyacinth, Aquatic pant studies series'. Elesvier.

Goswami, T. and Saikia, C.N. (1994): 'Water hyacinth-a potential source of raw material for greaseproof paper'. Biores. Tech. 50: 235-238.

Gowanloch, J.N. (1944): 'The economic status of water-hyacinth in Louisiana'. La. Conserv. 2: 3-8.

Gunadi, B., Blount, C. and Edwards, C.A. (2002): 'The growth and fecundity of *E. fetida* (Savigny) in cattle solids pre-composted for different periods'. In Pedo biologia. 46: 15-23.

Gupta, R., Mutiyar, P.K., Rawat, N.K., Saini, M.S. and Garg, V.K. (2007): 'Development of a water hyacinth based vermireactor using an epigeic earthworm *Eisenia foetida'*. Biores. Tech. 98: 2605-2610.

Harley, K.L., Julien, M.H. and Wright, A.D. (1996): 'Water hyacinth: a tropical worldwide problem and methods for its control'. In: Brown H, GW Cussans, MD Devine, SO Duke, C

Fernandez-Quintanilla, A Helweg, RE Labrada, M Lanedes, P Kudsk and JC Streibig (eds.). Proceeding of the 2nd International Weed Control Congress, Copenhagen, Denmark. 639-644.

Harley, L.S., Julien, M.H. and Wright, A.D. (1997): 'Water Hyacinth: A Tropical World wide problem and methods for its Control'. Proceedings of the 1st meeting of the International Water Hyacinth Consortium. World Bank.

Hildebrand, E.M. (1946): 'Herbicidal action of 2,3-dichlorophenoxyacetic acid on the water hyacinth, *Eichhornia crassipes*'. Sci. 103: 477-479.

http://en.wikipedia.org/wiki/Eisenia_foetida.

http://www.swlf.ait.ac.th/UpdData/Presentations/Thai/8%20Vermicomposting%20of%20Food%20Wastage.pdf.

Ismail, S.A. (1997): 'Vermicology the biology of earthworms'. Hyderabad: Orient Longman. 92.

Jayaweera, M.W and Kasturiarachchi, J.C. (2004): 'Removal of nitrogen and phosphorus from industrial wastewaters by phytoremediation using water hyacinth (*Eichhornia crassipes* (Mart.) Solms)'. Water Sci. Tech. 50: 217-25.

Kimenju, J.W., Oderò, G.O.M., Mutitu, E.W., Wachira, P.M., Narla, R.D. and Muiru, W.M. (2009): 'Suitability of Locally Available Substrates for Oyster Mushroom (*Pleurotus ostreatus*) Cultivation in Kenya'. Asian J. of Plant Sci. 2009.

Kivaisi, A.K., Magingo, F.S.S. and Mamiro, B. (2003): 'Performance of *Pleurotus flabellatus* on water hyacinth (*Eichhornia crassipes*) shoots at two different temperature and relative humidity regimes. Tanzania J. of Sci. 29 (2): 11-18.

Knipling, E.D., West, S.H. and Haller, W.T. (1970): 'Growth characteristics, yield potential, and nutritive content of water hyacinth'. Soil & Crop Society. 30: 51-63.

Kumar, S. (2005): 'Studies on efficiencies of bio-gas production in anaerobic digesters using water hyacinth and night-soil alone as well as in combination'. Asian J. Chem. 17: 934-8.

Kurien, J. and Ramasamy, E.V. (2006): 'Vermicomposting of Taro (*Colocasia esculenta*) with two epigeic earthworm species'. Biores. Tech. 97: 1324-1328.

Maclean, K. (1922). Agric. J. Ind. 17: 23-40.

Mahmoud, Y.A.G. (2006): 'Biodegradation of water hyacinth by growing *Pleurotus ostreatus* and *P. sajor-caju* and trial for using in production of mushroom spawn. Acta Alimentaria. 35(1): 63-72.

McDonald, R.C. and Wolverton, B.C. (1979): 'Upgrading facultative waste water lagoons with vascular aquatic plants'. J. Water Poll. Cont. Fed: 51: 305-313.

Menon, S., Salasa, A., Shinu, M. and Meera, V. (2005): 'Treatment of paper mill effluent with water hyacinth'. Pollut. Res. 24: 175-176.

Mitchell, D.S. (1976): 'The growth and management of *Eichhornia crassipes* and *Salvinia* spp. in their native environment and in alien situations'. In: Varshney CK, Rzoska J, editors, Aquatic weeds in Southeast Asia. The Hague: Dr. W. Junk b.v., Publishers. 396.

Mshandete, A., Kivaisi, A., Rubindamayugi, M. and Mattiasson, B.O. (2004): 'Anaerobic batch codigestion of sisal pulp and fish wastes'. Biores. Tech. 95: 19-24.

Munroe, G: 'Manual of on-farm vermicomposting and vermiculture'. Organic Agriculture Centre of Canada. Available at: http://www.organicagcentre.ca/DOCs /Vermiculture_FarmersManual_gm.pdf.

Murugesan, A.G., Vijayalakshmi, G.S., Sukumaran, N. and Mariappan, C. (1995): Utilization of water hyacinth for oyster mushroom cultivation'. Biores. Tech. 51(1): 97-98.

Nagavallemma, K.P., Wani, S.P., Stephane, L., Padmaja, V.V., Vineela, C., Babu Rao, M. and Sahrawat, K.L. (2004): 'Vermicomposting: Recycling wastes into valuable organic fertilizer'. Global Theme on Agro-ecosystems Report no. 8. Patancheru, Andhra Pradesh: International Crops Research Institute for the Semi-Arid Tropics. 20.

Nigam, J.N. (2002): 'Bioconversion of water hyacinth (*Eichhornia crassipes*) hemicellulose acid hydrolysate to motor fuel ethanol by xylose-fermenting yeast'. J. Biotech. 97: 107-16.

Nor, Y.M. (1994): 'Phenol removal by *Eichhornia crassipes* in the presence of trace metals'. Water Res. 28: 1161-1166.

Oudhia, P. (1999a): 'Medicinal weeds in rice fields of Chhattisgarh (India)'. Int. Rice Res. Notes: 24-40.

Oudhia, P. (1999b): 'Studies on allelopathy and medicinal weeds in chickpea fields'. Int. Chickpea Pigeonpea Newsletter. 6: 29-33.

P.K., Rawat, N.K., Saini, M.S. and Garg, V.K. (2007): 'Development of a water hyacinth based vermireactor using an epigeic earthworm *Eisenia foetida'*. Biores. Tech. 98: 2605-2610.

Parija, P. (1934): 'Physiological investigations on water hyacinth, *Eichhornia crassipes*, in Orissa with notes on some other aquatic weeds'. Indian J. Agr. Sci. 4: 399-429.

Penfound, W.T. and Minyard, V. (1947): 'Relation of light intensity to effect of 2,4-dichlorophenoxyacetic acid on water hyacinth and kidney bean plants'. Bot. Gaz. 109: 231-234.

Penfound, W.T., Earle, T.T. (1948): 'The biology of the water hyacinth'. Ecol. Monogr. 18: 449-72.

Quimio, T.H. (1986): 'Cultivation of Volvariella volvacea, the straw mushroom'. In: Guide to low cost mushroom cultivation in the tropics. University of the Phillipines at Los Banos, Laguna. 26-36.

Reddy, K.R., Campbell, K.L., Graetz, D.A. and Portier, K.M. (1982): 'Use of biological filters for treating agricultural drainage effluents'. J. Environ. Qual. 11: 591-595.

Rogers, H.H. and Davis, D.E. (1972): 'Nutrient removal by water hyacinth'. Weed Sci. 20: 101-100.

Roy, S. and Hanninen, O. (1994): 'Pentachlorophenol: uptake/elimination kinetics and metabolism in an aquatic plant, *Eichhornia crassipes'*. Environ. Toxicol. Chem. 13: 763-73.

Saini, V.K., Sihag, R.C., Sharma, R.C., Gahlawat, S.K. and Gupta, R.K. (2008): 'Relative efficacy of two methods of vermicomposting for biodegradation of organic wastes'. Int. J. Env. & waste mgmt. 2 (1/2).

Sample, K.T., Reid, B.J. and Fermor, T.R. (2001): Impact of composting strategies of the treatment of soils contaminated with organic pollutants: a review. Environ. Pollut. 112: 269-283.

Sinha, A.K. and Sinha, R.K. (2000): 'Sewage management by aquatic weeds (water hyacinth and duckweed): economically viable and ecologically sustainable biomechanical technology'. Environ. Educ. Inf. 19: 215-26.

Sinha, R.K., Herat, S., Agarwal, S., Asadi, R. and Carretero, E. (2002): 'Vermiculture technology for environmental management: Study of the action of the earthworms *Eisenia fetida*, *Eudrilus euginae* and *Perionyx excavatus* on biodegradation of some community wastes in India and Australia'. The Environmentalist. 22(2): 261-268.

Steward, K.K. (1970): 'Nutrient removal potential of various aquatic plants'. Hyacinth Control J. 1: 19-22.

Szczeck, M.M. (1999): 'Suppressiveness of vermicompost against fusarium wilt of tomato'. J. Phytopathol Phytopathologische Zeitschrift. 47: 155-61.

Tajbakhsh, J., Abdoli, M.A., Goltapeh, E.M., Alahdadi, I. and Malakouti, M.J. (2008): 'Recycling of spent mushroom compost using earthworms *E. fetida* and *E. andrei*'. Environmentalist 28: 476–482.

Tchobanoglous, G. and Burton, F.L. (1991): Wastewater engineering treatment, disposal and reuse (Metcalf & Eddy, Inc.). Mc Graw-Hill, Inc., New York.

Tchobanoglous, G., Maitski, F.K., Thomson, K. and Chadwick, T.H. (1989): 'Evolution and performance of city of San Diego pilot scale aquatic wastewater treatment system using water hyacinth'. J. WPCF. 61: 11-2.

Tripathi, B.D. and Upadhyay, A.R. (2003): 'Dairy effluent polishing by aquatic macrophytes'. Water Air Soil Pollut. 143: 377-85.

Trivedy, R.K. and Thomas, S. (2004): 'Review of performance data of few pilot and full scale aquatic weed based waste treatment plants from India'. Asian J. Microbio. Biotech. Environ. Sci. 6: 157-62.

Ueki, K. and Oki, Y. (1979): 'Seed production and germination of *Eichhornia crassipes* in Japan'. Proceedings of the 7th Asian-Pacific Weeds Science Society Conference. 257-260.

Vermicomposting. E-report available at: http://www.worms.com/worm-pdfs/what%20 vermi-composting.pdf.

Williams, A.E., Duthie, H.C., Hecky, R.E. (2005): 'Water hyacinth in Lake Victoria: why did it vanish so quickly and will it return?' Aquat. Bot.: 81: 300-14.

Wolverton, B.C. (1975): 'Water Hyacinth for Removal of Phenols from pollution waters'. NASA Tech. Mem. TM-X-72722.

Worm bin troubleshooting E-report available at: http://www.bae.ncsu.edu/topic /vermicomposting/vermiculture/worm_bin_troubleshooting.pdf

Wright, A.D. and Purcell, M.F. (1995): '*Eichhornia crassipes* (Mart.) Solms-Laubach'. In the Biology of Australian weeds eds. R.H. Groves, R.C.H. Shepard and R.G. Richardson, R.G. and F.J. Richardson, Melbourne. 111-121.

Zhu, Y.L, Zayed, A.M., Qian J.H, de Souza, M. and Terry, M. (1999): 'Phytoaccumulation of trace elements by wetland plants: II. Water hyacinth'. J. Environ. Qual. 28: 339-44.

BIBLIOGRAPHY

Charudattan, R., Labrada, R., Center, T.D. and Kelly, C. Strategies of water hyacinth control. Report of a panel of experts meeting 11-14 September, 1995. Florida, USA.

Cultivation of oyster mushrooms. College of Agricultural Sciences, Agricultural Research and Cooperative Extension.

Literature Review of Worms in Waste Management. Vol. 1 (2007) Second Edition.

Mushroom growers handbook 1. Oyster Mushroom Cultivation. Mush world.

Singh, A. Mushroom Production technology. HAIC Haryana Agro Research & Development Centre. Murthal, Sonipat.

Stamets, P. and Chilton, J.S. The Mushroom Cultivator. A Practical guide to growing mushrooms at home.